MASTER THESIS

Polymer based adhesives for tooth restorations. Monomer leakage and degradation.

Marianne Nygårdsvoll

Supervisors:

Ulf Ørtengren
Einar Jensen

UNIVERSITETET I TROMSØ
Det Medisinske Fakultet
Institutt for Klinisk Odontologi

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This master thesis is based on work performed at Department of Clinical Odontology (IKO). In addition, the GC-MS analysis were performed at Department of Pharmacy, Natural Products and Medicinal Chemistry Research Group from August 2008 to May 2010. Supervisor Ulf Ørtengren, IKO. Co-Supervisor Einar Jensen, Department of Pharmacy.

Abstract:

Bonding materials are essential for modern restorative dentistry (i.e composite restorations and composite cements).

Leakage from composite resin-based materials has been investigated and it is shown that 5-10 % of the residual monomers are released within the first seven days from the moment the restauration is made.

Studies has also stated that monomer leakage can be a problem for patients and dental personal, and allergies can occur. Only a few studies have been focused on the degradation and erosion process of bonding materials. Since bonding agents are insufficiently studied concerning leakage of monomers, the aim of this study was to investigate leakage of monomers from bonding materials after light curing by using Gas Chromatography - Mass Spectrophotometry (GC-MS). The MS-instrument was equipped with an Electron Ionization (EI) and a Chemical Ionisation (CI) ion source that enabled to choose the most sensitive and selective method for the different compounds. Compounds with polar functional groups (i.e. –OH groups) were derivatized and analysed as their trimethyl silyl ethers. The light cured materials were immersed in water and in ethanol. In both cases the analysis demonstrated the leakage of several different compounds, even compounds that were not listed in the Material Safety Data Sheets (MSDS) of the actual bonding material.

Content:

Key words:

Dentistry, Adhesive, GC-MS, Dentin Bonding, Monomer, Leakage

Definition of words:

Activator	A chemical substance reacting with an initiator producing free radicals
Adhesive	Substance that promote adhesion of one substance or material to an adherent
Chromatography	A common name for separation methods based upon a continuous distribution of the analytes between a mobile phase and a stationary phase
Dentin bonding agent	A thin layer of resin between conditioned dentin (more hydrophilic substrate) and the resin matrix (hydrophobic) of a composite
Eluent	A solvent used to carry the components of a mixture through a stationary phase
Elution	A process in which solutes are washed through a stationary phase by the movement of a mobile phase
Inhibitor	A chemical added to resin systems to provide increased working time and extended storage life by minimizing spontaneous polymerization (e.g. monomethyl hydroquinone)
Initiator	A free radical-forming chemical used to start the polymerization reaction (e.g. camphoroquinone)
Primer	In dentistry. A methacrylate based resin with hydrophilic properties. Often of low-viscosity promoting bonding to dentin
Solute	A homogeneous mixture composed of two or more substances.
Solvent	A liquid, solid or gas that dissolves another solid, liquid or gaseous solute, resulting in a solution.

1. Introduction:

Methacrylic resin based adhesives *i.e.* bonding materials are crucial for an adhesion between Composite Resin-based Materials (CRM) and tooth substances *i.e.* enamel and dentin. The monomers used in the adhesives have a complex chemistry with a variety of methacrylate based monomers, used in order to achieve an adequate bonding between the CRM and enamel/dentin. In this respect, especially dentin, constitute a difficult adherent for a sufficient adhesion since dentin consists of both organic and inorganic substances (*i.e.* collagen and hydroxyapatite). In addition the water content is high (Table 1). To achieve a proper adhesion, the methacrylates used for that purpose are therefore of amphiphilic as well as pure hydrofobic nature [1-4].

2-hydroxyethyl methacrylate (2-HEMA) is a common component in most bonding agents. It is an amphiphilic monomer that prevents collapse of collagen and increases the wettability. Due to its nature it is assumed to increasing the bond strength [2, 5]. Studies have shown that HEMA is able to diffuse through dentin. This is due to HEMAs low molecular weight and its degree of hydrophilicity [2, 5, 6].
HEMA diffusion increases with decreasing remaining dentin in primary teeth. With increasing age, the dentinal tubulies in permanent teeth become narrower and sclerotic. Dentin sclerosis lowers dentin permeability, but some diffusion of substances towards the pulp can still take place. Physiological differences between primary dentin in young and old teeth may also alter the diffusion of the residual monomer [6].
The polymerizable matrix of dentin bonding agents is also composed of many other monomers, such as Bis-glyciddimethacrylate(bis-GMA),Triethyleneglycoldimethacrylate (TEGDMA), 2-Hydroxyethyl methacrylate (HEMA) and Urethane dimethacrylate (UDMA). These monomers, when used in dentin bonding agents, are not biologically inert. They may diffuse through dentin causing inflammation and adverse reactions [6].
Bonding materials often consists of a primer and an adhesive and also include other different substances than monomers such as activators, initiators and inhibitors. The primer is an amphiphilic, low viscosity methacrylic based resin with added solvents promoting adhesion between the dentin surface and the adhesive, changing the dentin surface energy and making the surface more hydrophobic [3]. The adhesive is a more high viscosity methacrylic based liquid that, in case of tooth restorations, promotes adhesion of the primer wetted tooth surface to the hydrofobic composite. The adhesive often contain difunctional more hydrophobic methacrylates [3].
The other substances added primers and adhesives are for example initiators, a free radical-forming chemical used to start the polymerization reaction after exposure to energy (e.g chemical or light)

together with an activator. Inhibitors is a substance added to methacrylate based resin systems to provide increased working time, extending storage life and minimizing spontaneous polymerization [7].

Polymerized dental polymer-based materials (e.g. Composite, dentin adhesives etc.) are not resistant to degradation and erosion [1-4] because of incomplete polymerization and influence of fluids [2, 8-10]. Contemporary dentin adhesives have been criticized for their degree of hydrophilicity and absorbtion of a significant amount of water. Compared to more hydrophobic resins, the ability of dentin adhesives to absorb water can lower the stiffness of the material [11].

Hydrophilic resins are able to achieve high immediate bond strength to dentin, but *in vivo* and *in vitro* studies have shown that resin-dentin interfaces become weaker with time. The resin-dentin bonds are instable and has been attributed to the porous nature of hybrid layer that behaves as a permeable structure sensitive to slow water hydrolysis [12]. The instable bonds between resin to- dentin is assumed to be a result of this behaviour. Hydrolysis of resin-dentin bonds involves degradation of hybridized dentin, the methacrylate based resin and collagen fibrils, taking place due to water absorption and polymer-breakdown [12]. Hydrolysis is due to water absorption and polymer-breakdown [9]. It is important though to differentiate between hydrolysis and "non-breaking" effect of water such as swelling of polymer-network due to water absorption or leakage of inorganic substances from the polymer [13]. Methacrylate resin based dental materials are able to absorb water and chemicals from the environment. The sorption capacity of the bonding material and/or CRM is dependent on the hydrophilicity of the monomers used, the density of the three dimentional polymer network and filler loading. Due to solubility, release of components into the surrounding environment also occur [12]. Degradation of the organic matrix within the polymer (i.e. depolymerization) will occur when the polymers within the matrix are cleaved into smaller molecules and/or into the original monomers [9]. The resulting substances can be eluted from the material (i.e. erosion) [14, 15].

The hydrophilicity of acid-etched dentin matrices is due to the presence of water. After acid-etching of the dentin, water is replacing the volume of dentin that earlier was occupied by minerals. Adhesive monomers must displace water from the collagen fibrils before an intimate contact with the collagen fibrils can be established [11]. Also the hydrophilicity of the monomers used is crucial for achievment of a proper bonding. If to much water is absorbed by the monomers in the primer the result could actually be a weaker bonding and degradation of collagen [10].

Table 1: Composition of Enamel and Dentin

	Enamel wt%	Enamel vol%	Dentin wt%	Dentin vol%
Mineral	97	92	70	45
Organic	1	2	20	33
Water	1	6	10	22

Dentin has a relatively high organic component, and a higher water content compared with enamel.

Erosion is defined as the process when degradation products, residual monomers and/or inorganic products (*i.e.* filler particles) are eluted from the material, often resulting in weight loss. The degree of degradation and erosion of the polymer matrix depends on the chemical composition within and the cross-linking of the polymer matrix, and in addition, the degree of conversion as well as the environmental impact and the time factor [3, 4, 16]. Several studies have investigated the leakage of organic products eluted from CRM [3, 4, 11, 15, 16, 17]. It has been estimated that approximately 5-10 % of the residual monomers in dental composite resin materials will be released within the first seven days from the moment the restoration is made [4]. However, with respect to adhesive materials, only a few studies have focused on the degradation and erosion processes of bonding materials [11, 16]. Attention has primarily been drawn to the bonding capacity of adhesives to tooth substance (e.g. Dentin). Since the bonding materials consists of different monomers, many with hydrophilic properties, water absorption and degradation of the material will be enhanced [12]. As a result, monomers and organic substanses could diffuse into the oral cavity causing different types of side-effects. In addition the bonding capacity and the mechanical properties of the material can be affected. Therefore analysis of eluted substances is of great interest.

1.1 Gas Chromatography:

Gas Chromatography (GC) is one of several chromatographic methods. GC is a very powerful tool with respect to separate low molecular weight organic compounds with none or few polar functional groups. In GC the mobile phase is a gas, usually nitrogen, hydrogen or helium. The most commonly used separation columns in GC are Wall Coated Open Tubular (WCOT) columns. These columns are usually made of a 25 – 30 m long fused silica tubing with an internal diameter of 0,25 mm. The inside wall is covered with the stationary phase of the column. There is a large selection of different stationary phases. When using WCOT-columns helium is the preferred mobile phase. Helium gives the best compromise between safety and retaining separation power.

1.2 Mass spectrometry:

Mass Spectrometry (MS) is a powerful tool for structure elucidation, identification and quantification of atoms and molecules. A standard MS-instrument consists of an inlet, an ion source, a mass filter and a detector.
The inlet is used to introduce the sample to the MS-instrument. The ion source converts neutral molecules into ions, and the mass filter separates different ions from each other according to their mass to charge (m/z) ratio. The ions are detected in the detector and the results are usually presented as a plot of intensity of the different ions against m/z ratio. There is a large selection of different inlets, ion sources and mass filters.

1.3 Combined Gas Chromatography – Mass Spectrometry (GC-MS):

GC-MS combines the high separation power of GC with the ability of MS to give simultaneous identification and sensitive and selective quantification of organic compounds. The most commonly used ion sources in a GC-MS instrument are Electron Ionization (EI) and Chemical Ionization (CI) ion sources. One of the advantages of EI sources is that they usually give information about the molecular weight (MW) of a compound. But since the EI process involves transfer of high energy (≈ 70 eV) to the molecules a certain fraction of the molecule will fragment into different smaller ions. The result is that different organic compounds create specific “fingerprints”. The fingerprints are collected in large libraries and by comparing spectra of an “unknown” with spectra in the libraries compounds can be easily identified. However, many organic compounds have a very intensive fragmentation and information about the MW is not obtained. In such cases CI-ion sources are to be preferred. Here only small amounts of energy (3-4 eV) are transferred to the molecules and information about MW is easily obtained.

1.4 Substances with polar functional groups:

When substances contain certain polar functional groups they are no longer suited for analysis by GC-MS. Typical functional groups that creates such problems are -OH, -COOH, $-CONH_2$ and $-NH_2$ groups. Using derivatization agents to derivatize the functional group can often solve the problem. Hydroxyl groups are often converted to their trimethylsilyl ethers, and the product is very well suited for GC-MS analysis.

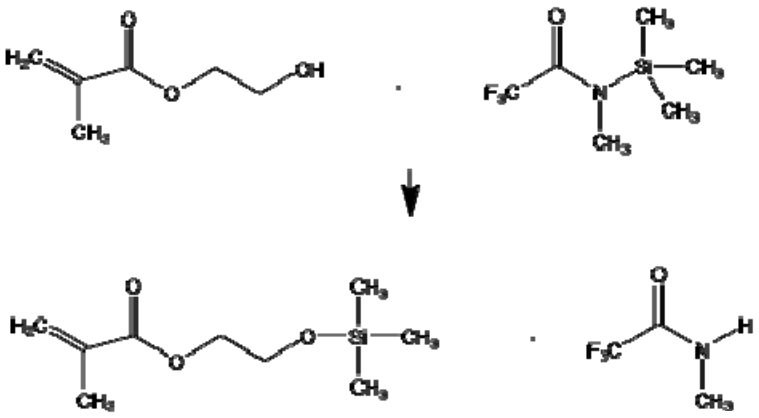

Figure 1
Derivatization of HEMA (2-Hydroxymethyl methacrylate) with MSTFA (*N*-Methyl-*N*-(trimethylsilyl) trifluoracetamide.

As seen above, derivatization of HEMA with MSTFA result in the TMS ether of HEMA, which is a much less polar molecule than HEMA itself. There is also one another advantage with the product. The MW of HEMA is 130, a fairly low MW which is not so well suited for MS analysis. The MW of the product is 202, which facilitates more selective analysis by GC-MS.

Bonding agents have been extensively studied in terms of bonding capacity. In addition, morphological studies on treated tooth surfaces have been conducted. Still, studies on degradation and leakage processes of bonding agents have not been extensively performed to the authors knowledge despite the fact that the polymerisation is poorer than in composite resin-based materials with increased risk of leakage of residual monomers. In addition, degradation will occur in polymer materials especially when amphiphilic monomers and monomers with ether linkages are included.

For analysis of organic leakage products from bonding material GC-MS is a valuable tool and further knowledge of degradation process of these materials could therefore be added.

2. Hypothesis:

Leakage of residual organic substances and degradation products from bonding materials do occur and type of substance eluted may effect the properties of the material. In addition eluted substances may also give adverse effects of patients.

3. Aim:

The aim of the study was to identify compounds eluted from cured bonding materials stored in water and in ethanol.

4. Materials and methods:

A commercial, clinical often used bonding agent (Adper Scotchbond 1 XT, 3M/ESPE, Seefeld, Germany) was tested concerning leachability of organic substances. The content of the material is given in Table 2. Qualitative analysis of water and ethanol samples was performed with full scan and Selected Ion Monitoring (SIM) GC-MS analysis. The identification of the compounds eluted from the cured materials was obtained by comparing the retention times and mass spectra of these compounds with retention times and mass spectra of reference substances, and information from the Material Safety Data Sheets from the manufacturer (Table 2).

Table 2: Composition of Adper Scotchbond 1 XT (3M/ESPE) according to the Material Safety Data Sheets

Name of component and CAS#	**Mass (Da)**	**Wt (%)**
Ethanol 16-17-5	46,04	25-35
Silantreated silica (nanofiller) ---	---	10-20
Bisfenol-A-diglycidyleterdimethacrylate 1565-94-2	512,24	10-20
2-hydroxyethylmethacrylate (HEMA) 868-77-9	130,10	5-15
2-hydroxy-1,3-dimethakryloxypropane 1830-78-0	228,10	5-10 %
Copolymer of acryl – and itachonic acid 25948-33-8	72,02 130,02	5-10
Diurethanedimethacrylate (UDMA) 72869-86-4	470,26	1-5
Water 7732-18-5	18,01	< 5

CAS = Chemical Abatracts Service. Chemical identification codes for chemical compounds

4.1 Preparation of the samples:

A total of 8 samples were prepared for the GC-MS – analysis. A split mould made in Polyethylene, with the inner dimension of 5 mm Ø and 1mm depth was used. The mould was cleaned with 96% ethanol before application of the bonding agent. The bonding agent was placed in the mould, using the manufactures distribution bottle. After 60 seconds, a sheet of polyethylene was immediately placed on top to avoid oxygen inhibition. The time of 60 seconds before placing the sheet on top was motivated to facilitate evaporation of the solvent in the bonding agents.

The material was light cured for 20 seconds according to the manufactures instructions using a LED curing device with an 11 mm Ø light tip (Bluephase, Ivoclar, Schaan, Liechtenstein). The intensity of curing device was regularly controlled using an ordinary testing device (Bluephase meter, Ivoclar, Schaan, Liechtenstein) and the intensity was measured to 880±10 mW/cm^2.

The samples was controlled visually and any excess removed by a scalpel directly. Caution was taken to avoid contamination during the procedures performed. After adjustment, the samples was transferred to and stored in cleaned polyethylene containers. Controlled MQ water was used as storage media for 4 samples (3 ml/sample), and ethanol 96 % for 4 (3 ml/sample). The samples was stored in the closed containers for 7 days in 37 ±1 °C.

After the storage described above, the samples stored in water were frozen (-18 ± 1 ^oC) and those stored in ethanol was put in a refrigerator (5±1^oC) until analysis of the eluates were performed.

4.2 Preparation of samples for GC-MS:

Four parallels of ethanol samples were analysed. The solvent (3 ml) for each sample were evaporated down to 0,5 ml. Since some of the compounds that might elute from the bonding material are better suited for GC-MS analysis as their TMS-ethers, an aliquot (150 µl) of 2 of the 4 samples were transferred to glass vials and the derivatization agent MSTFA was added to these samples. The vials were closed with a screw cap and submitted to 60 ^{o}C for 2 hours. That procedure made it possible to perform analysis optimised both for derivatized compounds and non-dericatized compounds.

50 µl I.S diluted + 950 µl EtAc was added to each of the four water-samples. The EtAc was collected, and two more extractions with 2 ml EtAc was done for each sample. 6 ml EtAc (extract from each sample) was evaporated to 0,5 ml on 60^{o}C. 150 µl from each sample was added to 2 glassvials. 10 µl MSTFA was added to one of the vials, and heated on 60 ^{o}C for two hours. Since aqueous samples are not compatible with GC-MS analysis, the analytes were extracted into ethyl acetate (EtAc). One ml of EtAc containing internal standard was added to the water samples and the organic fraction was collected. The water samples were further extracted two times with 2 ml EtAc. The EtAc fractions were pooled and evaporated down to 0,5 ml before analysis on GC-MS. Internal standard were used to correct for errors that might occur during the extraction and evaporation. The reagenses, solvents and equipment used for the GC-MS analysis are given in Table 3, and the internal standard used are given in Table 4.

Table 3: Reagenses, solvent and equipment for the GC-MS analysis

Chemicals, substances and instruments	**Producent**
Argon (AR)	Yara, Norway
Diclormethane	VWR International, Norway
96 % Ethanol	Institution of Analythical Chemistry, UIT
Ethylacetat (EtAc)	Merck, Germany
GC-MS (Agilent Technologies 6890N Network GC system combined with a Waters MicromassQuattro micro GC Mass Spectrometer. Agilent Technologies 7683 Autosampler and 7683B series injector	Waters Corp., Milford, Massachusetts, U.S.A
Glassvials for GC-MS analysis locked with PTFE/Silicon septum	Walters
Helium (He)	Yara, Norway
Methan (CH_3)	Hydrogass Norway
methanol	Merck, Darmstadt, Germany
MSTFA + 1% TMCS (N-metyl-trimetylsilyltrifluoroacetamid + 1% trimetylklorosilan)	QB Perbio; Pierce, Toronto, Canada
n-hexane	Merck, Darmstadt, Germany

Table 4: Internal standard used in the experiment.

Component	**Abberivation**	**Monoisotopic Mass (Da)**	**Molecular formula**
2-Hydroxy-ethylmethacrylate	HEMA	130,06	$C_6H_{10}O_3$
Benzene iodide	BI	203,94	C_6H_5I
2-Hydroxy-ethylmethacrylate trimethylsilyl ether	HEMA TMS	202,10	$C_8H_{18}O_3SI$
Hydrokinon monomethylether	MEHQ	124,05	$C_7H_8O_2$
Hydro quinone	HQ	110,04	$C_6H_6O_2$
Mequinol trimethylsilyl ether	MEHQ TMS	254,12	$C_{12}H_{22}O_2S_2$
Ethyleneglycol dimethacrylate	EGDMA	198,09	$C_{10}H_{14}O_4$
Camforokinon	CQ	166,10	$C_{10}H_{14}O_2$
Methylhydrokinone	MHQ	124,05	$C_7H_8O_2$
Hydro quinone-trimethylsilyl ether	HQ-TMS	254,12	$C_{12}H_{22}O_2Si_2$
Methylhydrokinone-trimethylsilyl ether	MHQ-TMS	268,13	$C_{13}H_{24}O_2Si_2$
Butylert hydroxytoluen	BHT	220,18	$C_{15}H_{24}O$

Diethyleneglycoldimethacrylate	DEGDMA	242,12	$C_{12}H_{18}O_5$
Benzoic acid, 4-(dimethylamino) ethyl ester	DMABEE	193,11	$C_{11}H_{15}NO_2$
Ethane-1,2-diylbis[oxyethane-2,1-diyl) bis(2- methylpropanoat	IS-TH	290,17	$C_{14}H_{26}O_6$
Triethyleneglycol dimethacrylate	TEGDMA	286,14	$C_{14}H_{22}O_6$
Trimethylolpropane trimethacrylate	TMPTMA	338,17	$C_{18}H_{26}O_6$
Oxybenzone	HMBP	228,08	$C_{14}H_{12}O_3$
Oxybenzone trimethylsilyl ether	HMBT TMS	300,12	$C_{17}H_{20}O_3Si$
Tetraethyleneglycol dimethacrylate	TEEGDMA	330,17	$C_{16}H_{26}O_7$

4.3 Analytical methods:

The analyses were performed by using a Gas Chromatography/Mass Spectrophotometry (GC-MS) (Agilent Technologies 6890N Network GC system combined with a Waters Micromass Quattro micro GC Mass Spectrometer. The instrument was further equipped with an Agilent Technologies 7683 Autosampler and 7683B series injector. For instrument control and treatment of the data processed, a MassLynx v4.1 (Waters Corp., Milford, Massachusetts, U.S.A) was used. The column used for the chromatographic separation was a “wall-coated open tubular (WCOT) low bleed fused silica” capillary column with the length of 30 m, 0,25 mm inner diameter and film-thickness of 0,25 μm (DB-5MS, J&W Scientific). Helium with 60 kPa pressure was used as carrier-gas at an injection temperature of 250°C and "purge pressure" of 50kPa after 2 minutes.

Separation of the different compounds was achieved by using the following temperature programe on the GC-column oven. The starting temperature was 50 ˚C and was held for 2 minutes. The temperature was then raised by 10 ˚C/min until 120 ˚C and held at this temperature for 1 minute. The temperature was further increased by 20 ˚C/min until 240 ˚C and kept at his temperature for 5 minutes.

In different analysis the compounds were ionised either by EI or CI. When EI was used, 70 eV electrons were used to ionise the compounds. When CI was used, methane was used as the ionization gas.

5. Results:

By comparing ions and retention times of standards with ions detected and retention times obtained when analysing the samples, 4 organic compounds were identified both in water and ethanol (table 5 and figure 2-6).

In addition, one peak with retention time and appearance of mass spectrum that did not correspond to the retention time and spectrum of any of the standards was found. However, the obtained results indicated that this compound could be itaconic acid. Also, according to the MSDS the actual bonding material do contain itaconic acid.

When the analyses results were compared with the MSDS from the manufacturer, 2 substances out of the 5 recored (including Itaconic acid) were given by the manufacturer. The other substances (CQ, DEGDMA and TEGDMA) were not given.

Table 5: Detected analytes from Adper Scotchbond 1 XT and internal standard

Component	Retention time (min)	Detected in water	Detected in ethanol
HEMA	8,0	X	X
CQ	12,8	X	X
DEGDMA	15,4	X	X
TEGDMA	17,3	X	X

To enhance the certainty of the results, two of substances (i.e. CQ, DEGDMA) was also analysed by using chemical ionisation. The CI give intense molecular ions thereby providing more reliable identifications than if only EI had been used (Table 6).

Table 6: Characteristic and intense fragments in GC-MS, EI and CI

Component	Moleculeion (m/z)	EI (m/z)	CI (m/z)
CQ	166	95a, 138	139, 167[a]
DEGDMA	242	69[a], 113	69[a], 113[a]
HEMA	130	69[a], 87	69[a], 131
TEGDMA	286	69[a], 113	69[a], 113[a]

The substance CQ (initiator) found in the eluates was not given in the Material Safety Data Sheets for the material analysed. The substance was, however, expected to be found, because of its importance in the polymerisation reaction activated by light. This component are likely to be present in less than 1 % in the material, and for this reason not obligatory to be denoted in the MSDS due to the present EU-regulation (93/42-EEC)

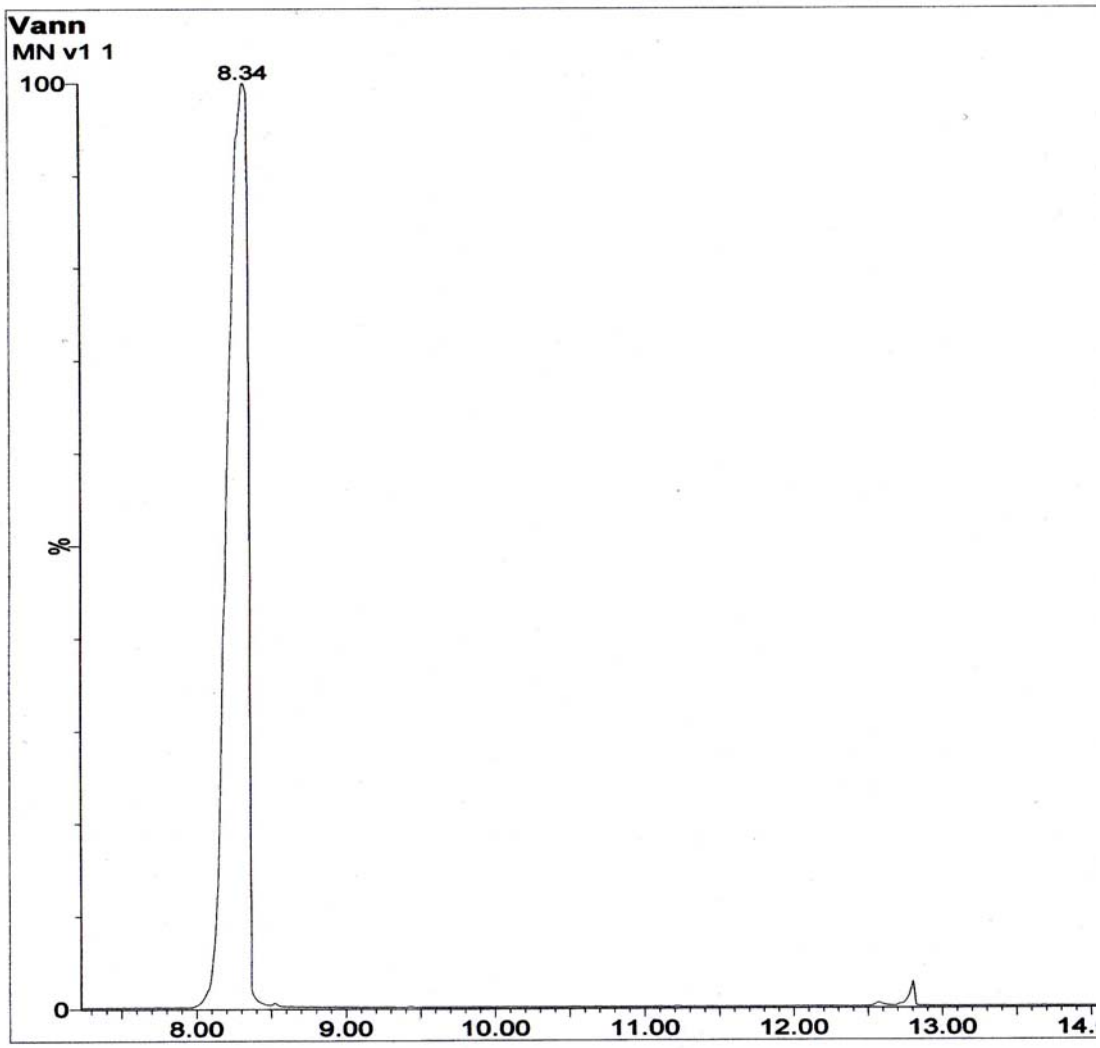

Figure 2

Analysis of water extract. The Chromatogram showes HEMA eluting 8,4 minutes.

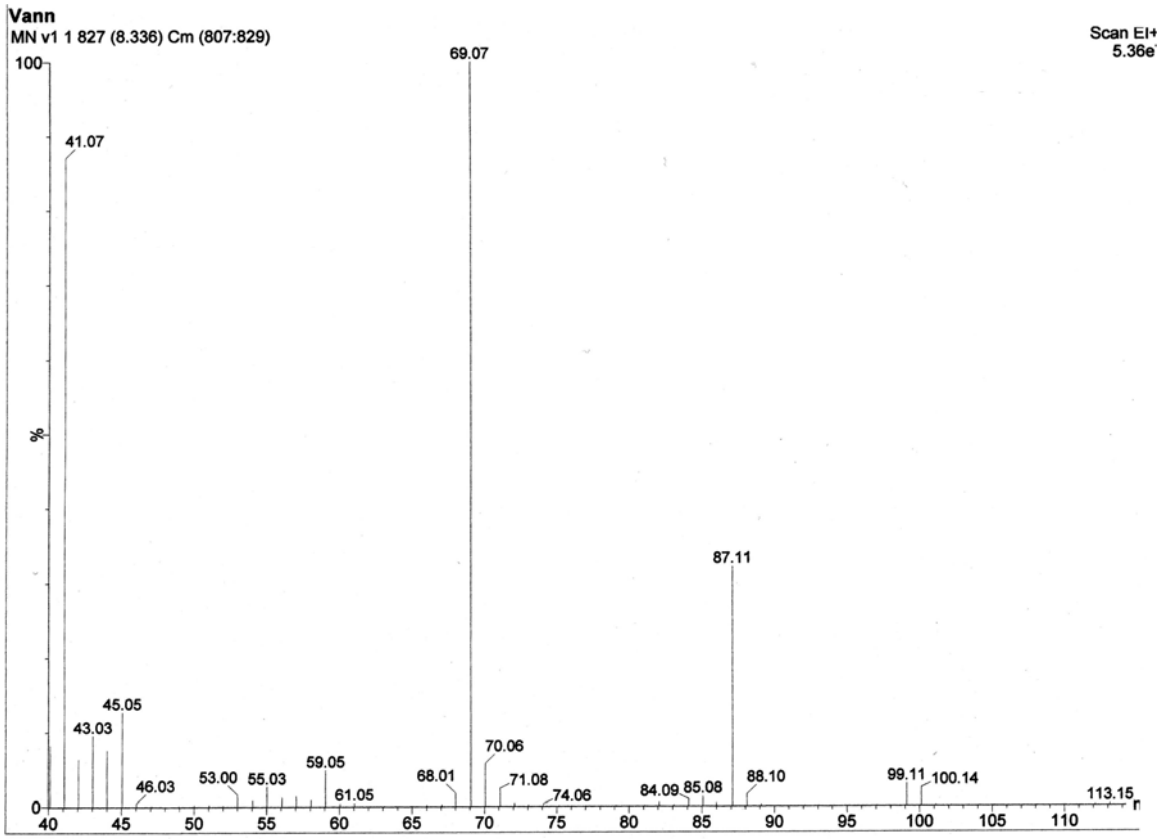

Figure 3

The full scan spectrum of the peak with retention time 8,4 minutes. The obtained spectrum clearly shows the presence of HEMA in the extract.

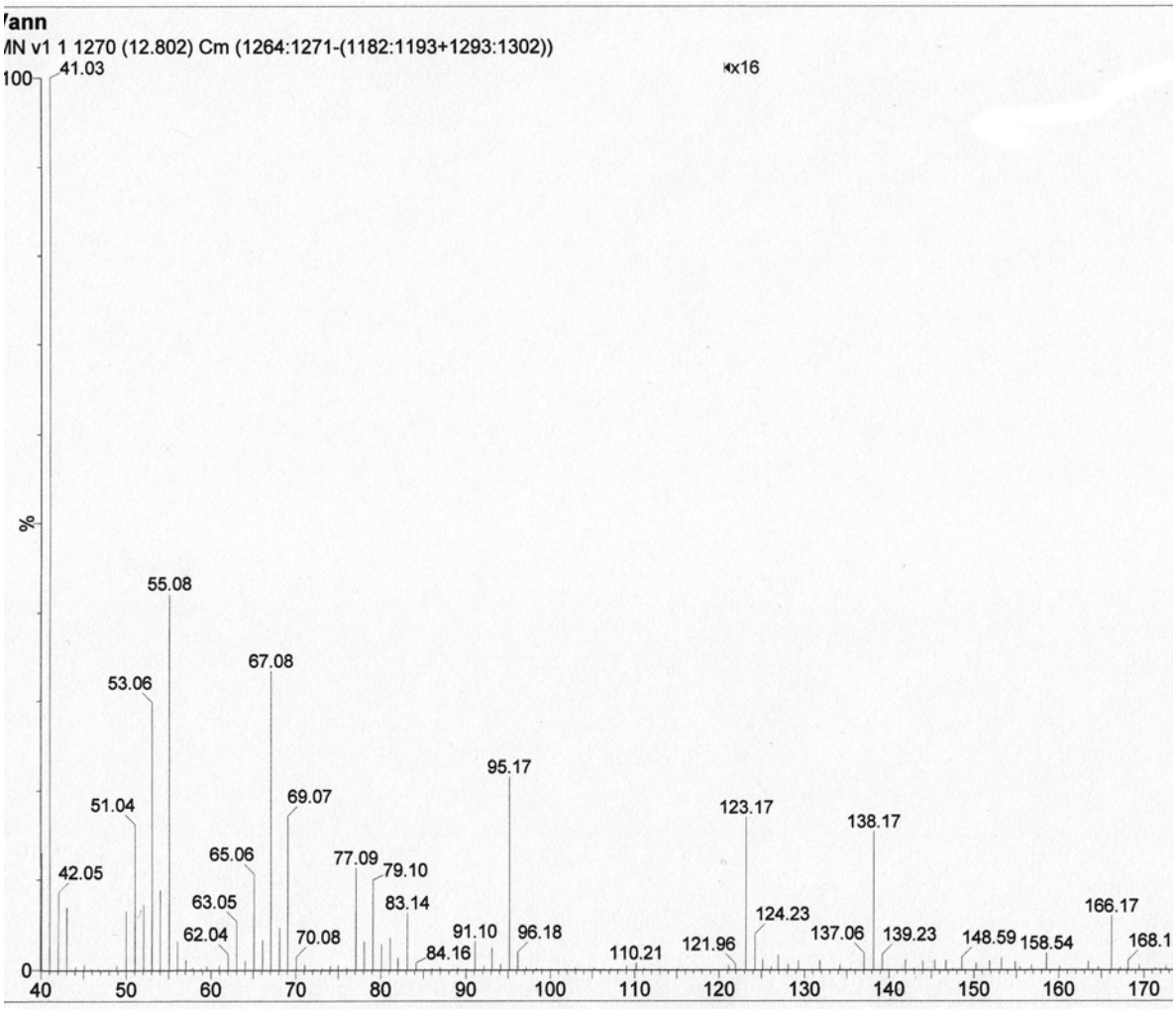

Figure 4

Analysis of water extract. The full scan spectrum clearly shiws the presence of CQ in the extract.

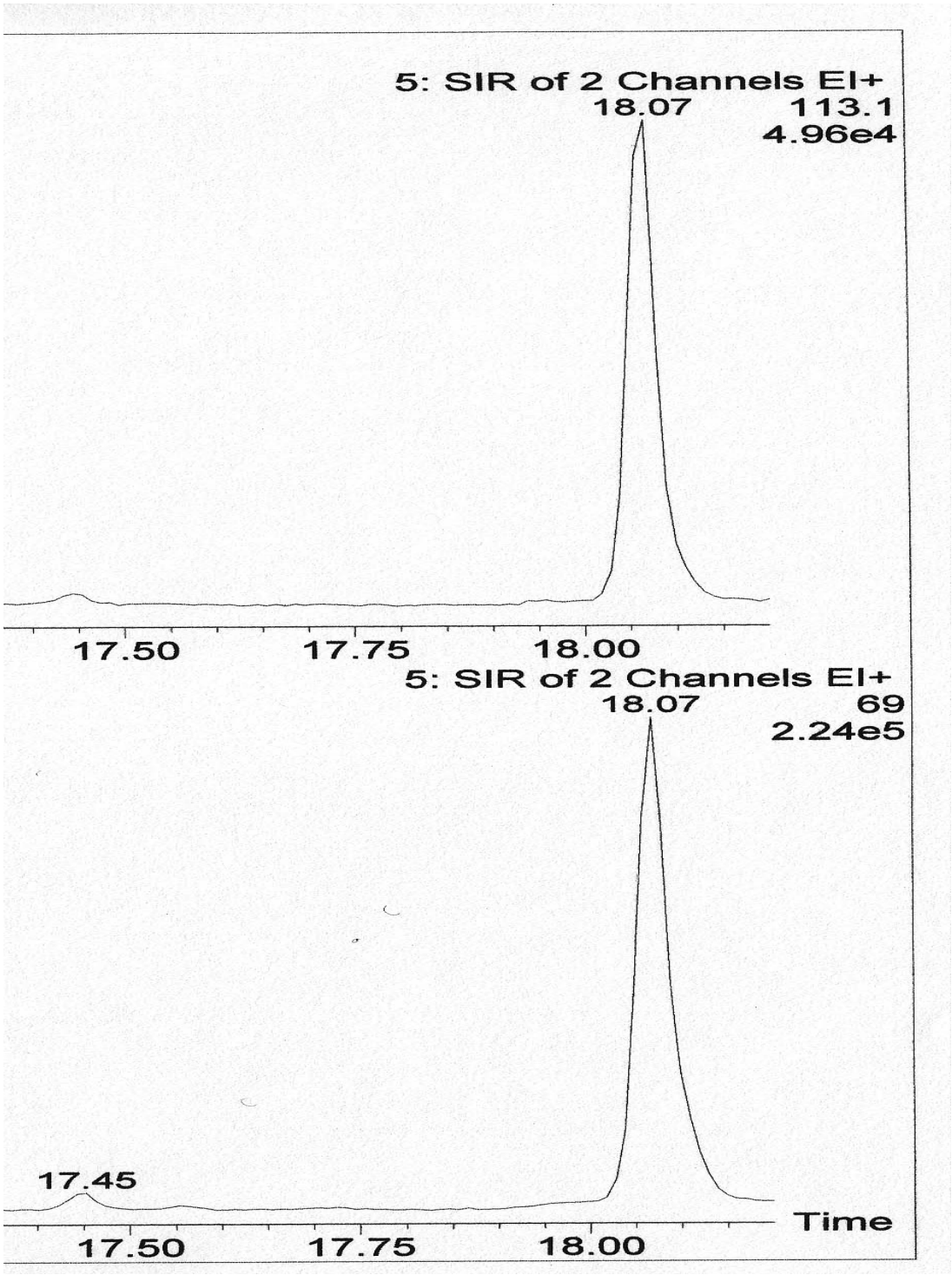

Figure 5

Analysis of Etanol samples. Identification of TEGDMA by Selected Ion Recording (m/z = 113 and m/z = 69) and retention time.

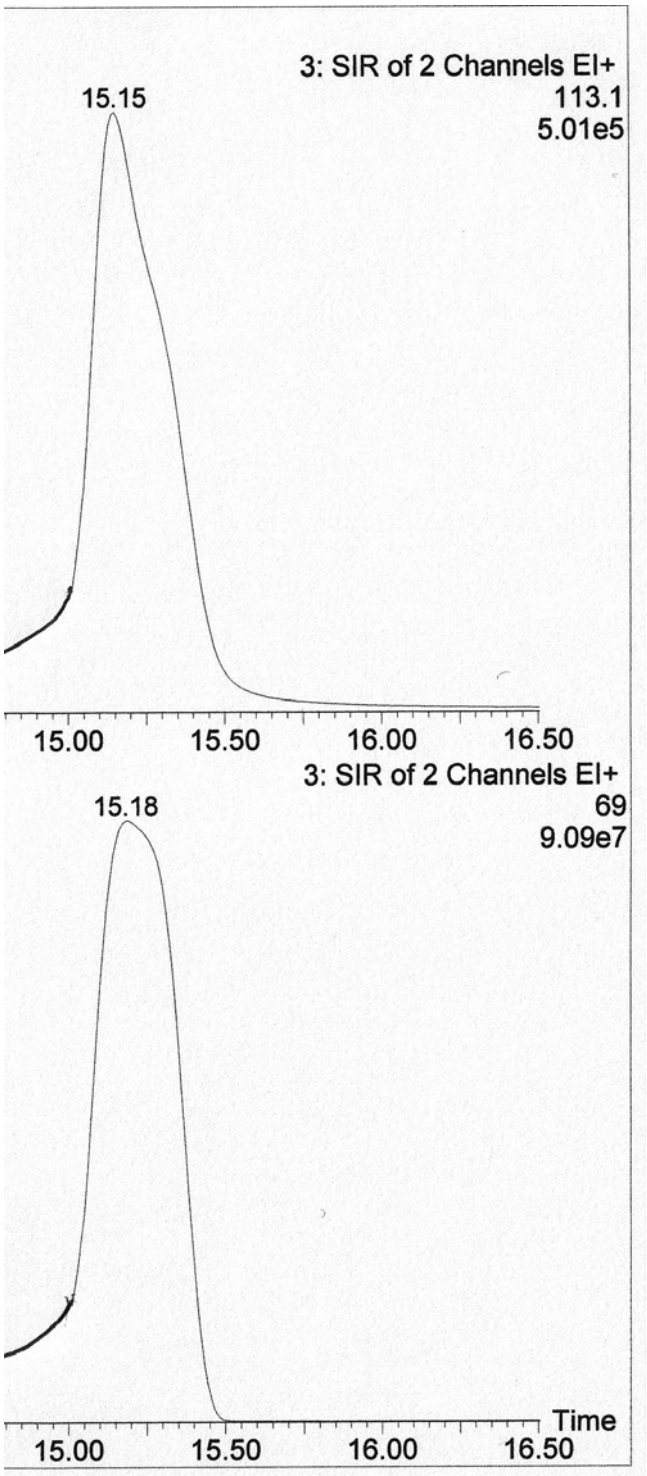

Figure 6

Identification of DEGDMA by Singel Ion Recording (m/z =69 and m/z = 113) and retention time.

6. Discussion:

The results demonstrated the presence of four different compounds in the samples analysed. Hence, leakage of monomers from cured bonding materials does occur and the hypothesis was accepted.

6.1: The analytical method

The findings of the present study were based upon analysis using combined gas chromatography-mass spectrometry (GC-MS). Also other studies of organic leachables from resin-based dental restorative materials have been based upon the use of GC-MS as analysis equipment [11-15]. However, in these studies only underivatized compounds and only EI ionization have been used. Such procedures could have some disadvantages. Compounds with polar functional groups seems not to be well suited for GC-MS analysis, because the chromatography could be difficult to interpret and intensive fragmentation with low intense ions are of limited diagnostic value [13-15]. In the present study derivatisation of these compounds with MSTFA was used to obtain the trimethyl silyl ethers of the actual compounds. This procedure has several advantages. The chromatography is improved; the molecular ion is more abundant and has an increase in molecular weight by 73 mass units, which gives a method more sensitive and more selective towards the different compounds. Even after derivatization and when using EI ionization some compounds might still give low abundance molecular ions. This is due to the high-energy transfer to the molecules when EI is used. By using CI as ionisation technique, the abundance of the molecular ion is increased. The molecular ion is the most important diagnostic ion with respect to quantification and identification of compounds. Hence, our method has contributed to more reliable identification of leachables from dental materials.

GC-MS is not suitable for analyzation of large polare molecules. Derivatisation can be used for improval of molecules vaporisation and temperature stability, and thereby give better chromatography. Some of the findings in the present study were based upon analyzing the substances as their TMS-ethers. This represents a great advantage compared to methods earlier used in the analysis of leachables from dental materials. Not only is the chromatography improved, in addition, derivatization gives a higher MW and a more abundant molecular ion. Low intense and low MW ions are much less selective than higher MW and more abundant molecular ions. In the present samples it was discovered a substance with retention time and mass spectrum that could not be compared by any of the reference substances available. By interpreting the obtained spectrum and using information in the MSDS of the bonding material it could be conclude that this

compound might be itaconic acid.

But, the reference substance is not always available. A reason for this can be that the substance is not existing. This makes a certain identification of a substance difficult, and the identification can sometimes be impossible. If a reference substance is non-excisting, it can still be possible to identify the structure of the substance by interprete the masspectra to get the needed information of the main structure. The largest masses are more specific than the small masses, and examining of the largest masses will be the most specific to examine. Resent research has showed that substances used in dental materials easily fragments by using EI and gives small or none molecule ions. It was also found that quantitative analysis has to be based on measuring fragments with low masses [13]. The use of CI gives a more sensitive fragmentation of the molecule, and fragmentation is taking place with higher masses (table 6).

6.2: Eluted substances.

Five different components were detected in the eluates from the bonding samples using ethanol and water as storage media. Ethanol and water are common used storage media in leakage studies [1, 11, 14, 15]. The reason for their use is that the organic substances used in CRM and bonding materials are polar and are therefore to be extracted in polar solvents. Ethanol is a more potent solvent than water due to its more polar nature and low viscosity compare to water and often used to decrease the extraction time when study leakage for polar polymer materials (e.g. methacrylate based materials)[16]. Therefore unreacted organic molecules taking longer time to extract in water can be eluted faster using ethanol [18]. In the present study, however, no difference was found concerning the media used. This could be due to the reasonable long storage time used (7 days) and the fact that bonding materials is expected to have a low crosslinkage and conversion degree that enhance diffusion of the solvent and unreacted organic products through the material [19, 20**].**
Leakage of monomers are a diffusion controlled process and depending on the cross-linkage of the network and the hydrophilicity of the monomers [19]. Water or ethanol will enter the network and result in a swelling making it easier for unreacted substances to elute.
The conversion degree of bonding materials, especially those containing solvents that evaporate slowly is consider to be low ((i.e. water, ethanol) [20]. That will result in a polymer with shorter chains and lower degree of cross-linkage). In addition the high amount of HEMA, a monofunctional molecule will also lower the cross-linkage degree. The bonding forces between the chains will therefore be affected negatively and the material more sensitive to solvents and elution of residual products.

GS-MS is not suitable for larger molecules due to fragmentation during the analysis process making identification difficult to perform, therefore Bis-GMA and UEDMA as given in the MSDS were not expected to be found in the present study because of their size. Larger molecule will also diffuse slower through the polymer network than smaller molecules [21]. Even though bonding materials have a reasonable low degree of cross linkage, seven days are to short time to detect Bis-GMA and UEDMA.

HEMA was expected to be found in the eluates since it is a small molecule and amphiphilic and was also given by the manufacturer as one of the monomers in the material tested (figure 2 and 3). HEMA is considered as an essential important amphiphilic monomer with many qualities and is commonly present in dental adhesives [22]. It is a monofunctional methacrylate monomer with a hydroxylgroup. Methacrylates that contain both hydrophilic (-OH group) and hydrophobic groups (e.g. methacrylate groups) are supposed to increase the adhesive strength of resins to the dentin [23]. These properties and low molecular weight increases the wetting properties of the adhesive, and also enhances the penetration efficacy into the demineralized dentin. HEMA also promote diffusion of other monomers increasing the entanglement with dentinal components and augment the formation of the hybrid layer [22]. It has been reported that HEMA also has a positive influence on the bond strength to dentin due to preventing collagen collapse and is thought to be an essential part of the polymer network after curing [24]. A bonding without HEMA would probably give a weak bonding between the CRM and the tooth substance. A consequence would be negatively affected longevity of the CRM restoration in the oral environment [22].
HEMA is a quite small molecule (130 MW) and due to its size and amphiphility, its ability to diffuse through the polymer network is enhanced [19]. Because of its role in forming the hybrid layer, creating a proper bond between the dentin and the composite restoration, leakage of HEMA may affect the bond strength over time and the longevity of the restoration.
Due to its ability to diffuse, unreacted HEMA could also affect the pulp [25]. Since no leakage studies have been performed on dentin bonding agents except from the present study to the knowledge of the author, no results are to be compared with concerning the leakage of HEMA and the supposed effects of such a leakage. Still, studies on longevity of bonding agents could support the results of the present study where decreased mechanical properties of the adhesive layer and failure of the restoration could be due to a significant amount of leakage of HEMA [26].

CQ found in the present study, is the most used initiator in lightcuring dental polymer resin based

materials, applied in concentrations of 0,2-1 % (figure 4). The initiator absorb light with wavelength of 400-500 nm and promote the monomers to react, forming a cross-linkage network. Materials with light-induced polymerization contain a photoinitiator (e.g CQ) and co-initiator (e.g. DMABEE). Photones from the lightsource is exiting the electrones in the initiator to make free radicals [27**]**. The initiator, however, will not be consumed and are therefore relatively easy to be eluted from the polymerized material [28]. Therefore, even though its content in the bonding material tested as for other polymer resin based materials used in dentistry, are small, it could be detected as a leakage product. The finding of CQ in the present study is also supported by results from Michelsen et al 2003, 2006 [17, 15]. CQ found in eluates, were not given by the manufacturer, probably due to the fact of its low content in the material tested and the regulations mentioned before.

TEGDMA and DEGDMA are monomers with low molecular weight (figure 5 and 6). The monomers are used to lower the viscosity of a high viscosity resin matrix to ensure incorporation of a significant amount of filler particles in composite resin materials. In addition the cross-linkage of the materials will be increased. A bonding material without a monomer like this would probably not make a good binding to the CRM and weaken the filling.

Of the other monomers given by the manufacturer in the MSDS for Adper Scotch Bond XT (i.e. Copolymer of acryl – itachonic acid and 2-hydroxy-1,3- dimethakryloxypropane) only itaconic acid was probably found. Since no reference substance was analysed for validity, the observation is somewhat uncertain, still the spectra was clear and therefore the probability of itaconic acid in the eluates are quite plausible.
The reason for not detecting the other monomers could be due to molecular size and/or fragmentation of the substances.

6.3: Clinical considerations

Water and ethanol is polar liquids playing an essential role in the leaking of polar monomers (e.g methacrylates). Uncured monomers in a bonding material have a tendency to leak when it is surrounded by an polar medium, (e.g. water). The liquid available will diffuse into the polymer network, swelling it and facilitate for breakdown of the network due to cleavage of the chains. The polymer chains are long strong chains of monomers, with covalent cross-linkages holding the network close together. As the fluid will start degrading the polymers by breakage of the ester-linkage in the chains, this network will fall apart. A consequence of this can be decreased longevity of the bonding material, and weaker bonding to the tooth substance and the CRM. Even toward the pulp, bonding material is exposed to water.
When a CRM is cured, cross-linkage between the polymer chains is formed and the material is shrinking toward its centre. Due to that process a gap between the walls of the tooth and the material may be created. A weak bonding of the CRM to the tooth substance can contribute to that gap formation between the tooth and the filling material increasing the risk for marginal leakage and enhance problems like marginal staining and secondary caries. Due to marginal leakage bacteria could enter the gap between restoration and tooth, and secondary caries lesions may therefore occur. In addition, less bonding capacity and /or secondary caries will facilitate an earlier loss of the CRM and cause problems for the patient in form fractured restoration and/or deep caries that can give the patient pain and cause for more extensive treatment (e.g root-canal treatment, crown treatment or in worse case extract the tooth).

Itaconic acid is a carboxylic acid facilitating bonding to unorganic material in the tooth substance (i.e. hydroxapatite). Itaconic acid is often used in Glass Ionomer Restoratives, where its intention is to increase the materials chemical bonding to the tooth substance. It is plausible to think that the manufactures intention is likewise for the bonding material studied and therefore itaconic acid was used in a copolymer. In the MSDS, the manufacturer stated that the bonding material analysed consisted of 5-10 wt % copolymer of acryl – and itachonic acid. For this reason it was expected to find itachonic acid in the eluates. If itaconic acid are released its bonding capacity to calcium in dentin and enamel could be negatively affected since the copolymer chain are cleft.

The clinical consequences of the leakage of CQ are probably limited because the amount is limited so the effect of the material are supposed to be less although allergy towards CQ has been reported [29].

Apart from suspected decreased longevity of bonded CRM restorations, allergy type IV as a side

effect could occur due to after exposure to uncured substances from polymer resin based materials (e.g. bonding materials). The prevalence of allergic effects caused by resin-based materials among patient is, however, low. In a study made by Kanerva *et al.* 2-hydroxyethyl methacrylate provoked most of the reactions caused by (meth)acrylates among the population studied (2.8%) [30]. Development of allergies is not dose dependent. When a person suffers from allergy toward a specific substance the dose seem too be of little or no relevance, small amounts of allergen is enough to create allergy. As earlier mentioned, the information about ingredients present in quantities less than 1% weight are not required for MSDS. Components in smaller amounts than this can though cause allergic effects, and for this reason it is important to identify the components in the material [31]. As known, HEMA is a potent monomer causing allergy, mostly in dental personnel when uncured [32]. Still, since HEMA is a potent allergen, development of Type IV allergy may occur if a patient is exposed to CRM or bonding materials containing uncured HEMA due to poor curing, leakage of residual monomers or degradation [23].
Astma (allergy type I) caused by inhalation of methacrylates have also reported [33]. Still, it is not likely that this should be a problem for the patient because HEMA in saliva is not volatile. The problem though with a slow release of monomers from a restorative material is a long term exposition for the patient and that may for some individuals cause allergy after a long time exposure [34]. Jaakola et al (2007) found that long time exposure to methacrylate created type IV allergy in the respiratory tract in dental personnel. The risk cannot be rolled out that this also could be the case for some patient.

Therefore, identification of components leaking out from a polymer resin based material used in dentistry and the amount of the components eluted is important to identify for evaluation of the materials biocompatibility and also for an estimation of the quality and longevity of a dental polymer based restoration. Further studies on the subject of degradation of bonding agent are therefore also needed.

7. Conclusion:

Within the limitations of the present study the following conclusions were drawn.

Leakage of monomers and other organic additives did occur from the bonding material tested in water and ethanol.

The leakage of monomers could negatively affect the longevity of the adhesion between the restoration and the tooth.

HEMA as a known potent allergen was found in the eluates.

The monomers TEGDMA and DEGDMA found was not included in the MSDS.

CI analysis performed in the present study confirmed that this technique due to its higher sensitivity is a reliable method for qualitative analysis for organic products eluted from dental bonding material.

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