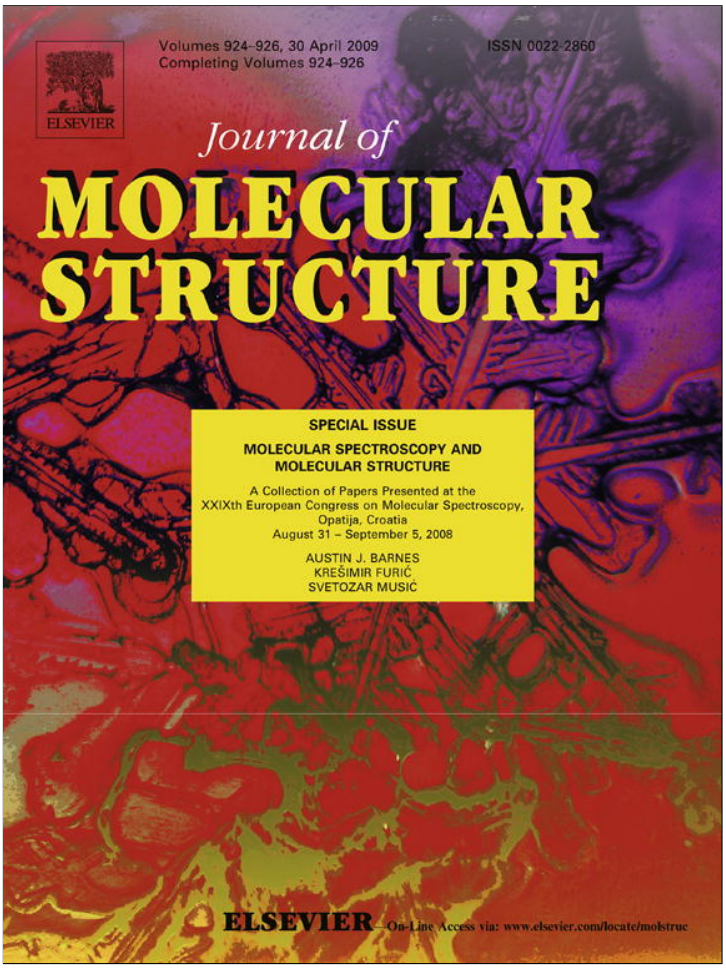

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Detection of HEMA in self-etching adhesive systems with high performance liquid chromatography

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ABSTRACT

One of the factors that can decrease hydrolytic stability of self-etching adhesive systems (SEAS) is 2-hydroxymethylmethacrylate (HEMA). Due to hydrolytic instability of acidic methacrylate monomers in SEAS, HEMA can be present even if the manufacturer did not include it in original composition. The aim of the study was to determine the presence of HEMA because of decomposition by hydrolysis of methacrylates during storage, resulting with loss of adhesion strength to hard dental tissues of the tooth crown. Three most commonly used SEAS were tested: AdheSE ONE, G-Bond and iBond under different storage conditions. High performance liquid chromatography analysis was performed on a Nucleosil C_{18}-100 5 μm (250 × 4.6 mm) column, Knauer K-501 pumps and Wellchrom DAD K-2700 detector at 215 nm. Data were collected and processed by EuroCrom 2000 HPLC software. Calibration curves were made related eluted peak area to known concentrations of HEMA (purchased from Fluka). The elution time for HEMA is 12.25 min at flow rate 1.0 ml/min. Obtained results indicate that no HEMA was present in AdheSE ONE because methacrylates are substituted with methacrylamides that seem to be more stable under acidic aqueous conditions. In all other adhesive systems HEMA was detected.

1. Introduction

Most demanding challenge in restoring damaged tooth crown with composite material is to achieve long-lasting bonding interface between composite and hard dental tissue. That interface is created by application of dentin adhesive systems (DAS). We have to create hybrid layer as surface that can be bonded to composite material [1]. As Van Meerbeek explained, connection of DAS to dentin can be realized through physical absorption, chemical connection with dentin and permeation of the dentin surface [2].

Etch and rinse DAS remove the smear layer using phosphoric acid followed by application of functional monomers in one or multi step protocol [3]. Because of the problem with over etch phenomenon, possible failure to impregnate demineralised dentin and simplification of bonding procedure, self aching DAS are developed. They were created by dissolving acid methacrylate monomers in water-HEMA (hydroxymethylmethacrylat) mixtures [4]. Bonding system is relayed on simultaneous etching with acid methacrylate and impregnation of etched surface [5,6]. De Munck et al. and Goracci et al. along with several other studies claim that self etch adhesives (SEA) have lower bonding strength to enamel [7,8] compared to total etching adhesives (TEA). However, studies performed by Shimida et al., Pilecki et al. and Kelsey et al. reported no significant difference in bonding strength to enamel [9–11].

In order to simplify the application and reduce application time some manufacturers created all in one, single bottle SEA. Saltz et al. introduced to literature the concept that ester bonds and ester groups of HEMA can undergo hydrolysis in acidic condition of SEA [12]. Such adhesive systems can reduce their bonding properties during storage time. That was the reason for some manufacturers to produce DAS without HEMA in their composition (eg, G-Bond, GC, Tokyo, Japan; iBond, Heraeus Kulzer, Dormagen, Germany; AdheSE One, Ivoclar Vivadent, Schaan, Liechtenstein).

The aim of the study was to investigate possibility that during the different condition of storage, during the hydrolysis of esters in acidic condition of SEA, HEMA can occur regardless that it was not originally present in composition.

2. Experimental

In order to find the presence of HEMA reversed phase high-performance liquid chromatography (RP-HPLC) was performed. RP-HPLC was used to separate components of a mixture by using a variety of chemical interactions between the substance being analyzed and the chromatography column. RP-HPLC operates on the principle of hydrophobic interactions, which result from repulsive forces between a polar eluent, the relatively non-polar analyte, and

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the non-polar stationary phase. The binding of the analyte to the stationary phase (such as silica) is proportional to the contact surface area around the non-polar segment of the analyte molecule upon association with the ligand in the aqueous eluent. The time at which a specific sample elutes is called the retention time and it is unique for any characteristic sample given. The use of pressure increases the speed giving the components less time to diffuse within the column resulting with the chromatogram. Common solvents used include any miscible combinations of water or various organic liquids as methanol. Water may contain buffers or salts to assist in the separation of the analyte components. Entire procedure is described by Bentrop et al. and Spahl and Budzikiewiez [13,14].

Adhesive systems were stored at different conditions as can be seen in Table 1.

Samples of SEA were introduced in small volume to the stream of mobile phase and are retarded by specific chemical or physical interactions with the stationary phase as it traverses the length of the column. RP-HPLC was performed on a Nucleosil C_{18}-100 5 μm (250 × 4.6 mm) column, Knauer K-501 pumps and Wellchrom DAD K-2700 detector at 215 nm (Fig. 1).

Data were collected and processed by EuroCrom 2000 HPLC software. Samples of adhesives (~70 mg) were dissolved in 2.0 ml of methanol and filtered; 20 μl were injected and isocraticaly eluted with 20% aqueous methanol for 15 min, followed by gradient to 100% methanol in 5 min. Calibration curves were made related eluted peak area to known concentrations of HEMA (purchased from FLUKA, Milano, Italy). The elution time for HEMA is 12.25 min at flow rate 1.0 ml/min (see Table 2).

3. Results

Results obtained by the RP-HPLC are shown in Table 3. According to chromatograms all adhesive systems except AdheseOne create certain amount of HEMA. Percentage of HEMA is increasing in relation to storage time and temperature raise.

Representative chromatograms for HEMA and tested adhesives are shown in Fig. 2.

4. Discussion

According to the data obtained in this study, during different storage conditions all DAS had different amount of HEMA except AdheSE One.

Surprisingly, even new bottles of iBond and G-Bond had revealed certain amounts of HEMA (iBond 0.07% and G-Bond 0.14%).

In iBond, after the bottle was opened and stored for 5 month at the room temperature (rt) SEA was partially polymerized or dried with 0.13% presence of HEMA, despite the manufacturers instructions that it can be left outside the refrigerator until empty or expired. At the same storage conditions, G-bond revealed greater amount of HEMA (0.22%), but no polymerization of the specimen was present. This finding can be explained with the fact that iBond uses only acetone as a solvent, which evaporates quicker than other solvents used in adhesive systems.

Fig. 1. Knauer K-501 pumps and Wellchrom DAD K-2700 detector at 215 nm.

Table 1
Storage conditions of adhesive systems.

Adhesive	Storage conditions
G-bond	New bottle
	Opened bottle, 2 months room temperature (rt), 3 months 4 °C
	Opened bottle, 5 months rt
	Unopened bottle, 3 months 38 °C
iBond	New bottle
	Opened bottle, 5 months rt
	Unopened bottle, 3 months 38 °C
AdheSE® One	New bottle
	Opened bottle, 5 months rt
	Unopened bottle, 3 months 38 °C

Table 2
Composition of SDA according to manufacturers.

	AdheSE® One	G-Bond	iBond™ GI
Manufacturer	Ivoclar Vivadent	GC	Heraeus Kulzer
Composition	Derivates of bis-acrylamide	Acetone	Acetone
		Distilled water	Water
	Water		
		4-Methacryloxyethyltrimellitate anhydride	Methacrylate monomers
	Bis-methacrylamide dihydrogen phosphate		
		Urethane dimethacrylate	Glutaraldehyde
	Amino acid acrylamide		
		Triethyleneglycol dimethacrylate	
	Hydroxy alkyl methacrylamide		
	Silicon dioxide		
	Catalysts		
	Stabilizers		
pH value	1.5	2.25	1.7

Table 3
Results of HEMA presence.

Adhesive	Storage conditions	HEMA content
G-bond	New bottle	0.14%
G-bond	Opened bottle, 2 months rt, 3 months 4 °C	0.15%
G-bond	Opened bottle, 5 months rt	0.22%
G-bond	Unopened bottle, 3 months 38 °C	0.31%
iBond	New bottle	0.07%
iBond	Opened bottle, 5 months rt	0.13%
iBond	Unopened bottle, 3 months 38 °C	Partially polymerized or dried – (Completely dried or polymerized)

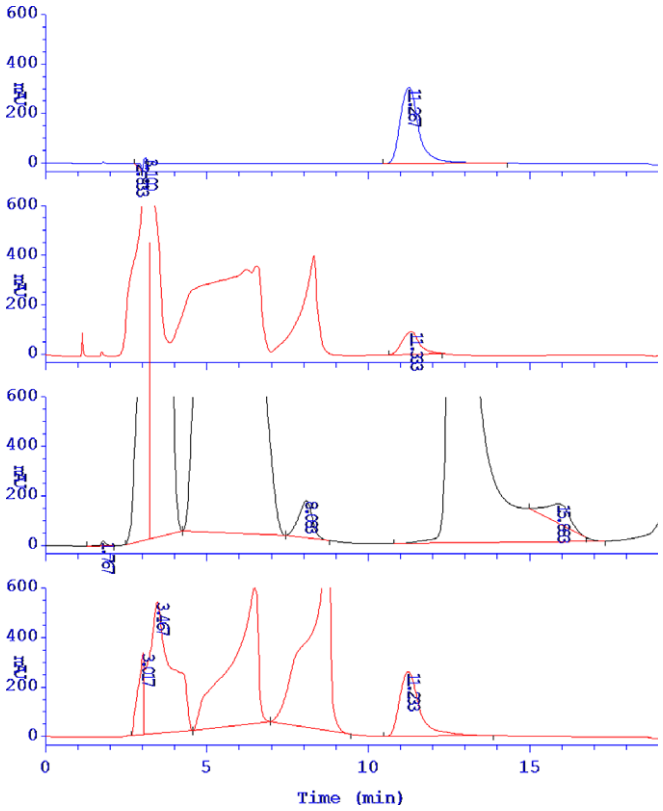

Fig. 2. Chromatograms of the tested samples 1. HEMA 2. iBond 3. AdheSE One 4. G-Bond.

The greatest amount of HEMA was found in an unopened bottle of G-Bond stored for 3 months at 38 °C (0.31%), whereas iBond was completely dried or polymerized and it was not possible to perform analysis on HEMA content. Therefore, it was also unsuitable for clinical use. Our results indicate that greater temperature favors the formation of HEMA in SEA. Also, according to Saltz et al. [12], hydrolysis of methacrylate monomers cannot be prevented even if SEA were stored in refrigerator.

Increase of storage temperature for G-Bond had more influence of HEMA formation than the storage time. The amount of HEMA created in SDA was greater for shorter periods of time under greater temperature than increased storage time on lower temperatures.

The fact that the adhesive systems had their own storage period before the clinical use, can explain the presence of HEMA in freshly opened bottles.

Acidic monomers in SEA supply certain amount of hydronium ions, due to dissociation of hydroxy groups, which are sufficient enough to act as a catalyst inducing hydrolysis of HEMA and phase separation in adhesive systems as Nishiyama explained [4]. Two phases occur, water-soluble and water-insoluble [4,15]. Degradation rate of HEMA depend on pH value and storage temperature of SEA systems [16]. If the pH value is below 2 ester portion in the functional methacrylate used in SDA becomes hydrolysed [12].

Because of HEMA alterations under acid conditions recently several adhesive systems appeared on the market without HEMA in their original condition. But Saltz et al. claim that ester bonds in acid methacrylate monomers and methacrylate co monomers can be attacked leading to decreasing the concentration of functional monomers in SDA [12].

Presence of HEMA in the samples can be explained by the fact that during methacrylate degradation HEMA can occur in degradation process. Degradation will be present in single bottle SEA as long as methacrylate based functional monomers are present [16].

Degradation products are partly nonpolymerizable. As the result, loss of adhesion properties can appear [12,17]. Exposure to relatively low concentration of HEMA for a prolonged time result in cell death, possibly as a consequence of DNA damage, as Samuelsen et al. claim in their study [18]. The odontoblastic secretory activity or odontoblast cells can be affected. According to Schweikl et al., the presence of monomers in dental pulp can modify appropriate repair response like reactionary dentinogenesis [19].

Methacrylamide monomers had proven to be more stabile under acidic conditions than methacrylate monomers [4]. That explains the absence of HEMA in AdheSE One in every examined condition of storage, since monomers in AdheSE One are not esters but amides in origin.

For the analysis of HEMA content in the pulp samples Hamid and Hume have used Resolve Silica C18 column and 30% methanol as mobile phase [20]. We found that on Nucleosil C_{18}-100 5 μm column 20% methanol as the mobile phase ensures good separation of HEMA and other components of tested adhesives.

5. Conclusion

Based on the findings presented in this paper, SEA with methacrylic monomers are expected to be less effective than SDA using methacrylamidic monomers. Affectivity of these SDA will decrease due to increased storage temperature and storage time.

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