Determination of BisGMA by ethanol release from dental composites

Nicolás Cohn-Inostroza¹
Camila De la Rosa-Varela², PhD
Ignacio Moreno-Villoslada³

¹²School of Dentistry, Faculty of Medicine, Universidad Austral de Chile, Valdivia, Chile
³Laboratory of Polymers, Institute of Chemical Sciences, Faculty of Science, Universidad Austral de Chile, Valdivia, Chile

Corresponding Author
Nicolás Cohn Inostroza
Laboratorio de Polímeros
Instituto de Ciencias Químicas
Facultad de Ciencias
Universidad Austral de Chile
Campus Isla Teja
Valdivia - CHILE
Fono: +56998011877
Email: nscohn@gmail.com

Abstract
Photopolymerized dental composite resins are periodically used in the field of dentistry, but the rate of polymerization ranges from 55% to 75%. Therefore BisGMA the unreacted monomers in the polymer network reduces clinical longevity of these resins. These substances can penetrate the dentin, may irritate soft tissue and promote allergic reactions. It has been found that the release of these substances have cytotoxic, genotoxic, mutagenic and estrogenic effects.

Objectives: The development of an analytical method for detection of BisGMA, using molecular absorption spectrophotometry UV-vis and high performance liquid chromatography (HPLC) in ethanol and acetonitrile, and study the BisGMA solubility using HPLC at different ethanol concentrations.

Methods: Three coins of dental composite (3 mm diameter and 1.5 mm thick) are immersed in 1.5 ml of ethanol 12% vol/vol, 35% vol/vol and 50% vol/vol, stored in an Eppendorf tube. Emulating alcohol intake. After 1 and 22 days, removed composites and proceeds to measure the solutions in HPLC.

Results: HPLC obtained concentrations between 1.15 E-7 [M] and 4.84 E-4 [M] of BisGMA from a dental composite resin.

Conclusions: The release of BisGMA from the organic matrix of dental composite resin is alcoholic dependent. The release of BisGMA concentrations in the alcoholic described except 12% vol/vol, saturate the β- estrogen receptor, which according to the literature cause an exoestrogenic effect.

Introduction
Photopolymerized dental composite resins are regularly used in dentistry. The dental industry has created different formats in the manufacture of dental composites, since the modification of the organic polymer matrix, to the modification of the inorganic filler, through microparticles and nanoparticles mixed, influencing the physicochemical characteristics of the resins. The matrix is based on organic resins Dimethacrylate / Acrylate linking agent attached to one of the inorganic filler, the silane, all this together with initiators, activators and inhibitors. The monomers most commonly used for the preparation of a dental resin are 2,2-bis [p-(2′-hydroxy-3′-metacriloxipropl oxy) phenyl] propane (BisGMA), 3 - [4 - [2 - [4 - (oxiran-2-ylmethoxy) phenyl] propan-2-yl] phenoxy] propane-1, (BisGA) and 2 - [2 - [2 - (2-methylprop-2-enolox) ethoxy] ethoxy]-methylprop-2-ene-1-2 2-enoate (TEGDMA). They are usually mixtures of these monomers, the selection of which has a high reactivity influence on mechanical properties, polymerization shrinkage, absorption and adsorption having the polymer to solvents and enzymes present in the oral cavity. The rate of polymerization of the dental composite resin ranges from 55% to 75% (Mozner,
Therefore the unreacted monomers in the polymer network reduce clinical longevity of these resins. Furthermore the aqueous environment that degrades resin has enzymes and solvents from the diet as ethanol, releasing unreacted monomers. These substances released from the resin have two possibilities to affect the stomatognathic system, penetrating the dentin to reach the pulp, or the mouth portion irritating oral mucosa and probably promote allergic reactions (Tosic, 2004). It has been found that the release of BisGMA and Bisphenol A, has an effect on the release of oxygen free radicals resulting in cytotoxic, mutagenic and genotoxic effects (Wozniak, 2005). Since 1997, various authors Nicolas Olea and suggest that the BisGMA and bisphenol A, are exoestrogen agents, estrogen receptor may be present in the oral cavity, these compounds can act as a ligand to β-estrogen receptor, with a saturation limit in the detection of Bisphenol A, exclusively by adhesion receptors of approximately 2.3 ppm (Mejía AE et al, 2009). The controversy that arose at that time was that these exoestrogenics agents increase serum estrogen, associating them with early puberty and even a potential factor in the development of cancer cases. That is why it is necessary to determine the nature and concentration of the substances that are secreted into the oral cavity during the consumption of solvents such as alcohol. The determination of the concentration of the diluted residual monomers from a polymerization of a dental composite is usually performed by high performance liquid chromatography (HPLC).

Objectives
Develop an analytical method for the detection of BisGMA by molecular absorption spectrophotometry UV-vis and high performance liquid chromatography (HPLC). Study BisGMA solubility in organic solvents: acetonitrile and ethanol by UV-vis spectrophotometry. Develop an analytical method for the detection of concentrations of BisGMA from dental composite resin discs in ethanol of 12 °, 35 ° and 50 °, emulating drinking, using high performance liquid chromatography (HPLC).

Materials and Methods
HPLC Equipment:

Solvents:
Acetonitrile (Merck® HPLC grade), ethanol (Merck® HPLC grade), Milli Q water, HPLC grade water.

UV-vis spectrophotometer:
UV-vis spectrophotometer (Helios) Volumetric glass, micropipettes Eppendorf ®.

Reagents:
2,2-bis [p-(2'-hydroxy-3'-methacyrloxy propoxy) phenyl] propane (BisGMA) (Aldrich No. 436 909). composites: Ceram • X duo ® (A3) (Dentsply), Te-Econom ® (A2) (Ivoclar Vivadent).

Methodology of the development of dental composite specimens:
It uses two brands of dental composites: Te - Econom ® (A2) (Ivoclar Vivadent) and Ceram • X Duo ® (D3) (Dentsply), whose difference lies in the type of inorganic filler portion, being microparticles and nanoparticles respectively. The samples are 1.5 mm thick and 3 mm in diameter, are polymerized for 40 seconds, then the specimens were polished with diamond bur wheel high speed, fine grain.

Methodology in UV-vis spectrophotometry:
To make the spectral study, the samples of dental composite resin Te-Econom ® (A2) (Ivoclar Vivadent), immersed in 1.5 ml of ethanol at 100 ° in an Eppendorf tube for 30, 60, 120 minutes and 24 hours. Through molecular absorption spectrophotometry UV-visible, determining the wavelength at which the molecule absorbs BisGMA in acetonitrile and ethanol. Subsequent to this calibration curves were performed in both non-polar media, to determine the of Lambert-Beer law (A = ε • c • l) BisGMA concentration present in the sample.

Methodology in HPLC:
It determines the mobile phase between acetonitrile and water (CH3CN/H2O), which is made by combining these eluents to observe the sharper peak (Fig. 1).

The determination of retention time of BisGMA molecule, observing is performed to the wavelength absorbed versus the analyte retention time [l / t (min)]. Later a calibration curve of standard dilutions in ethanol of BisGMA at concentrations: 0.5, 2.5, 5, 10, 15 and 20 ppm, to
determine the concentration of this compound to make the measurement of the solution in HPLC. Three specimens, in triplicate, were used dental composite Duo ® Ceram • X (D3) (Dentsply), which are immersed in ethanol 12% vol/vol, 35% vol/vol and 50% vol/vol (HPLC grade) in a 1.5 ml Eppendorf tube. The composites are removed after 1 day and 22 days, and proceed to measure in HPLC, in Eppendorf tubes remains the inorganic filler of the dental composites.

Results

Results in UV-vis spectrophotometry:
BisGMA as compound of two aromatic rings with highly hydrophobicity, so it is soluble in nonpolar solvents such as ethanol. Scanning performed in UV-vis spectrophotometer in two peaks of UV absorption: 285 nm and 278 nm. Calibration curves were obtained R² 0.987 in ethanol for 285 nm and R² 0.984 to 278 nm (Figures 2 & 3).

Figure 4 shows the absorption spectrum of the dental composite resin Te-Econom ® (A2) (Ivoclar Vivadent) according to the method described.

According to the equation of the calibration curve of BisGMA in ethanol Y = 3642X + 0.043, we can calculate the concentrations of BisGMA in the 285 nm ranging from 1.14 E-4 [M] up to 2.69 E-4 [M] (Table 1). But at 320 nm can be seen that there is another absorption peak (Fig. 4), but it ignores the nature of this, since the absorption spectrum shows a peak BisGMA not at 320 nm (see Fig. 5).

Results in HPLC
In the first place the measurements consist in determining HPLC mobile phase, the clearer representation is seen with a ratio of 72:28 CH3CN/H2O. The retention time for BisGMA is 6.3 minutes, at 205, 275 and 290 nm, (Fig. 1). The calibration curve with standard of BisGMA throws a Y = 111 641X + 64406.7 with R² 0.99. (Fig. 6)

Concerning the influence of ethanol degree according to immersion time, at 24 hours, the calibration curve with standard of BisGMA throws a Y = 3642X + 0.043, we can calculate the concentrations of BisGMA in the 285 nm ranging from 1.14 E-4 [M] to 2.69 E-4 [M] (Table 2) and at 320 nm can be seen that there is another absorption peak (Fig. 4), but it ignores the nature of this, since the absorption spectrum shows a peak BisGMA not at 320 nm (see Fig. 5).

Discussion
The photopolymerization process of dental composite resins is never complete, monomers / comonomers may remain in the composite after the curing process (Ferracane, 2006). Covalent bonds BisGMA ester can be enzymatic hydrolysis degraded. Besides degradation and release into the environment of oral cavity unreacted monomers are enhanced by solvents from the diet as alcohol (Geurtsen, 1998). The chemicals changes from oxidation to hydrolytic attack or cutting functional groups of the polymer chain, this occurs if it absorbs water from the composites. The hydrolysis of the esters bonds can form BisGMA methacrylate (MA) and MA products resulting in the loss of one or two methacrylic acid groups respectively (Ferracane, 2006). Also in aqueous solution can hydrolyze the siloxane bonds (Xiao et al., 1998; Lateef et al., 2002) but the products associated with the breaking of this link have not been identified. Los groups, ethers, esters, hydroxyls in the resins and urethanes are susceptible to hydrolytic degradation. In the experiment performed in UV-vis spectrophotometry, it is observed that there is a release of the organic matrix of dental composite, but there is a peak at 320 nm, which does not correspond to the peak of BisGMA, is why it is necessary to use HPLC, this time, occupying a more current as the second resin Ceram • X Duo ® (A3) (Dentsply). In the release of BisGMA in different alcoholic degree, it can be seen that the influence of alcoholic of 12 ° in the dental composite independent of exposure time, would not produce a significant release of BisGMA to reach the saturation limit β-estrogenic receptor equivalent to 2.3 ppm (4.42 E-6 [M]) as compared to the influence of alcohol in degrees 35° and 50 °.

Conclusion
BisGMA releasing from the organic matrix of a dental composite resin is alcoholic degree dependent.

The release of BisGMA concentrations in the alcoholic described except 12 °, saturate the β-estrogen receptor, which according to the literature cause an exoestrogenic effect.

ACKNOWLEDGMENTS
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Figure 1: Chromatogram 1

Figure 2: Calibration curve Bis GMA 278 nm in ethanol

\[ y = 4001.4x + 0.0816 \]

\[ R^2 = 0.984 \]
Figure 3: Calibration curve Bis GMA 285 nm in ethanol

\[ y = 3642.4x + 0.0439 \]
\[ R^2 = 0.9874 \]

Figure 4: Composite absorption spectrum in ethanol 100º
Figure 5: Absorption spectrum of BisGMA

Table 1: Concentration of BisGMA released.

<table>
<thead>
<tr>
<th>Time of immersion</th>
<th>Absorbance</th>
<th>Concentration (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hours</td>
<td>1.025</td>
<td>2.69E-4</td>
</tr>
<tr>
<td>2 hours</td>
<td>0.687</td>
<td>1.76E-4</td>
</tr>
<tr>
<td>1 hour</td>
<td>0.541</td>
<td>1.36E-4</td>
</tr>
<tr>
<td>30 min.</td>
<td>0.459</td>
<td>1.14E-4</td>
</tr>
</tbody>
</table>

Figure 6: Standard calibration curve BisGMA
<table>
<thead>
<tr>
<th>Sample name</th>
<th>Retention Time (min)</th>
<th>Area</th>
<th>Concentration (ppm)</th>
<th>Concentration (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Composite 24 hours in Ethanol 12% vol/vol</td>
<td>6,871</td>
<td>1399</td>
<td>0,06</td>
<td>1,15E-07</td>
</tr>
<tr>
<td>Composite 24 hours in Ethanol 35% vol/vol</td>
<td>6,848</td>
<td>144344</td>
<td>7,16</td>
<td>1,37E-05</td>
</tr>
<tr>
<td>Composite 24 hours in Ethanol 50% vol/vol</td>
<td>6,855</td>
<td>913392</td>
<td>76,04</td>
<td>1,46E-04</td>
</tr>
</tbody>
</table>

**Table 2: Influence of the degree of Ethanol as 24 hours.**

<table>
<thead>
<tr>
<th>Sample name</th>
<th>Retention Time (min)</th>
<th>Area</th>
<th>Concentration (ppm)</th>
<th>Concentration (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Composite 22 days in Ethanol 12% vol/vol</td>
<td>6,757</td>
<td>9456</td>
<td>0,76</td>
<td>1,46E-06</td>
</tr>
<tr>
<td>Composite 22 days in Ethanol 35% vol/vol</td>
<td>6,773</td>
<td>708278</td>
<td>57,67</td>
<td>1,09E-04</td>
</tr>
<tr>
<td>Composite 22 days in Ethanol 50% vol/vol</td>
<td>6,782</td>
<td>2879801</td>
<td>252,18</td>
<td>4,84E-04</td>
</tr>
</tbody>
</table>

**Table 3: Influence of the degree of Ethanol by time 22 days.**