

MATERIALS AND METHODS

This research is an experimental laboratory research.^{10,11} Bovine incisive were obtained from animal slaughtering house at Jl. Pegirian Surabaya. After cleaning, tooth was soaked in physiological salt solution and kept in refrigerator (temperature 4⁰ C). Pure bovine type I collagen and HEMA pure liquid were purchased from Sigma Chemical, St. Louis, USA. Appliances that were used: desiccators with vacuum faucet (China), hygrometer (Haar. Synth. Hygro, Germany), air suction (Schuco, USA), and FTIR (Jasco FT / IR 5300, Japan).

To arrange 90 % humidity, 150 cc of water were putted at the bottom part of desiccators then put hygrometer that have been calibrated. At this condition humidity which seen at hygrometer was range from 94 to 95%. Hereafter from faucet located above desiccators, air pumped out by air suction until reach humidity 90% and closed the faucet. For humidity 80%, air inside the desiccators were pumped out until reach humidity 80%. To quicken the process, put silica gel that activated before in the desiccators. Same process was done for 70 % and 60 % humidity.

To make KBr pellets, 300 mg KBr powder (bromide kalium) was prepared, then 10 µl (10,7 mg weight) of HEMA (1,07 g/ml) were dropped using micro-pipette. KBr powder and HEMA (310,7 mg) were crushed with mortal and pestle. Some part of this mixture (50 mg) were put into KBr die appliance then were compressed and vacuuming by compressor until 10 ton. This vacuuming and compressing process was done in 5-10 minutes. The obtained result was a transparent pellet (form like thin tablet). The pellets were observed into Fourier Transform Infrared Spectroscopy (FTIR) appliance. These samples were control samples. Room humidity was 65% and room temperature was 25⁰ C ± 2⁰ C.

To make a mixture of collagen sample and HEMA, 10 µl (10,7 mg) HEMA was dropped to collagen (2 mg), then KBr powder were added till the weight of the mixtures were 310,7 mg. The mixture of this materials were crushed, 50 mg of them were put into KBR die, then compressed and vacuumed until 10 ton. The pellets were observed into FTIR appliance.

To make sample for the group of treatment by different humidity, 2 mg collagen fibers were packed into desiccators with hygrometer appliance that have been calibrated. Then desiccator's humidity was arranged to 60%, 70%, 80% and 90%.

As soon as released from desiccators, HEMA and KBr powder were added on collagen until 310,7 mg in weight. The mixtures were crushed, 50 mg of them were, compressed and vacuumed until 10 ton and pellets were made in the same method, which has been explained above. In this study, each sample group was conducted until three times research (n = 3 to each group). The measuring was conducted at peak value of carbonyl absorbance band (C=O) of treatment group which was compared to control group. The method to calculate peak value of carbonyl absorbance band (P) as follows:⁹

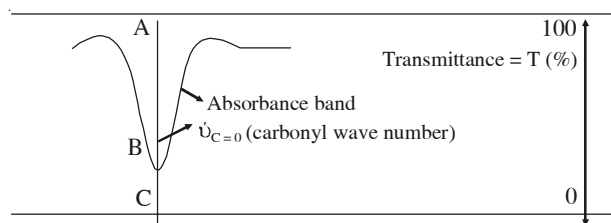


Figure 1. Peak of the HEMA carbonyl absorbance band.

Description:

$P = (BC / AB) \times 100$; AB, BC measured in centimeters.

This calculation is accurate enough and can be trusted if absorbance band intensity at T: 30-60%.

Figure 2 and 3 shows peak value of carbonyl absorbance band at various humidity:

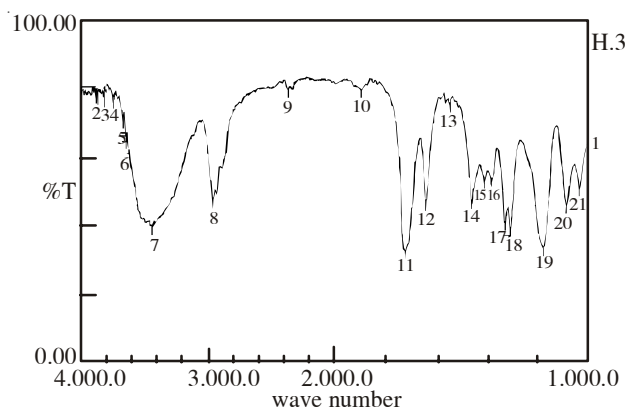


Figure 2. Infrared Spectrum of pure HEMA (KBr pellet)
Peak value of carbonyl absorbance band is 46.1 (number 11)
Wave number (C=O) at 1720 cm⁻¹, T value: ± 80%.

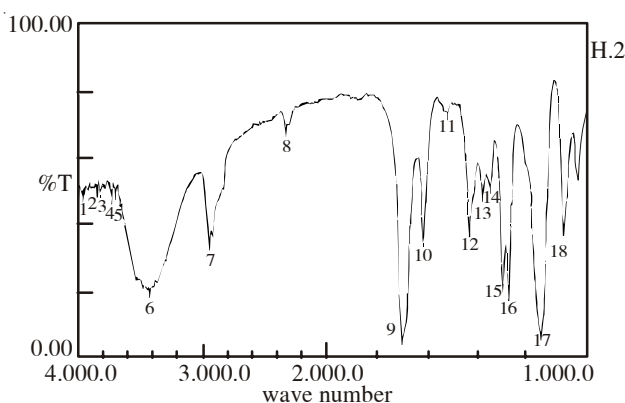


Figure 3. Infrared Spectrum of HEMA + Collagen (KBr pellet)
Peak value of carbonyl absorbance band is 9.8 (number 9)
Wave number (C=O) at 1720 cm⁻¹, T value: ± 50%

The effect of humidity on peak value of HEMA carbonyl absorbance band

Adioro Soetojo

Department of Conservative Dentistry
Faculty of Dentistry Airlangga University
Surabaya – Indonesia

ABSTRACT

Bond strength between 2-Hydroxyethyl methacrylate HEMA-based dentin bonding agent and dentin collagen had proved by presence of the chemical interaction. This union involved the carbonyl group on HEMA resin with amino group on dentin collagen following produce an amide chain. However, this bond strength influence by humidity condition of dentin collagen and HEMA resin. The aim of this study is to measure the effect of humidity on peak value of the HEMA carbonyl absorbance band. The bond strength between by HEMA bonding agent and dentine collagen was analyzed in different humidity, e.g. in 60%, 70%, 80%, and 90% humidity. Control experiment was done in ambient room humidity that is 65%. Chemical bond that formed between HEMA and dentine collagen was carried out by mixing pure HEMA with bovine type-I collagen which had been stored in different humidity. The ester carbonyl group of HEMA will react with the amino group of collagen, produced an amide bonding, which is observed by measuring the IR spectrum absorbance of the ester carbonyl group of HEMA in KBr method. The decrease of the carbonyl group absorbance indicates the more chemical bonds formed. By measuring the absorbance of C=O ester in different humidity, it showed that the greatest number of chemical bonds resulted when the experiment was done in 70% humidity condition.

Key words: humidity, FTIR, HEMA, carbonyl group, type-I dentine collagen

Correspondence: Adioro Soetojo, c/o: Bagian Konservasi Gigi, Fakultas Kedokteran Gigi Universitas Airlangga. Jln. Mayjend. Prof. Dr. Moestopo No. 47 Surabaya 60132, Indonesia.

INTRODUCTION

The 2-hydroxyethyl methacrylate (HEMA) is often used as liquid base of dentine bonding.^{1,2,3} Several reports claims that HEMA has a good physical and chemical characteristic.⁴⁻⁷ The 2-hydroxyethyl methacrylate bind to dentine surface by chemical or mechanical bond. Chemical bond of HEMA to dentine collagen is covalent bond or primary bond between atoms. Covalent bond between nitrogen atom of collagen and carbon atom of resin aldehyde will form in chemical interaction.³ Xu *et al.*⁴ reported that by F-T Raman spectroscopy, if there are new bonding, for example C-N of C=O at ester group of HEMA, hence will yield product of amide [C(O)NH].

Clinically, one of total etched technique step is wash and dries the dentin surface. The purpose of washing step is to discard salt formed as reaction of acid etched materials and dentin minerals. The drying procedure will remove excess water that are used for washing dentin surface after etching process.^{1,2,3} Many researchers said that at the time of drying procedure, the dentin surface should neither too dry nor wet.^{4,7} If the surface is too dry, the dentin collagen will collapse. However, if the surface is too wet, there are many water molecules around collagen hence yield hydrogen bond between water and amino collagen. With this phenomenon, dentin bonding agents will not bond to

dentin collagen network carefully. So we need an optimal condition (optimal humidity) on dentin surface to obtain a maximal bonding between dentin bonding materials and dentin collagen.

Besnault and Attal⁸ expressing that at 50% and 60% humidity, bond strength of bonding materials at dentine is higher than at 80% and 90% humidity. At the minimum humidity, the amount of water around dentine is optimal. Water molecules can form hydrogen bond with collagen peptide, and then break hydrogen bond between molecules of fibril collagen. At drying procedure, fibrils will re-expansion again after collapse.

Kemp⁹ reported that HEMA carbonyl when reacting with amino collagen group, peak reduction of the mixture ester carbonyl and HEMA with collagen will happened at infra red spectrum. It can be assumed that there are many groups of carbonyl ester bonding with amino if there is a high peak reduction of HEMA carbonyl absorbance band. This means chemical bond between HEMA and collagen become stronger.

The purpose of this research is to know the effect of humidity on peak value of the HEMA carbonyl absorbance band. In knowing, the smallest value of HEMA carbonyl absorbance band will also know the chemical bond strength between HEMA and dentine collagen maximally at optimal humidity.

RESULTS

Principally, the peak value of HEMA carbonyl absorbance band will decreased if many groups of HEMA carbonyl bind with groups of amino at dentin collagen,. This matter designate that many groups of carbonyl are bonding with groups of amino, thus it means chemical bond between HEMA and collagen is strong.

Mean value of the HEMA carbonyl absorbance band and standard deviation were shown at table 1.

Table 1. Mean value of peak of HEMA carbonyl absorbance band at various humidity

Humidity	N	$\bar{X}$ (mean)	SD
HEMA	3	47.5667 ^a	5.6448
60%	3	21.2000 ^b	8.8606
70%	3	9.5000 ^c	2.9614
80%	3	29.0000 ^{bdf}	6.3553
90%	3	32.2667 ^{de}	3.2083
65%	3	19.7333 ^{bcd}	6.6214

Description:

N = amount of sample

$\bar{X}$ = mean value of peak HEMA carbonyl absorbance band

SD = standard deviation

ANOVA F = 14.257, $p > 0.001$

Superscript with different letter showed there significances at $\alpha = 0.05$

Pure HEMA have peak carbonyl absorbance band equal to 47.5 ± 5.6 , then the peak value decreased at 60%, 65%, and 70% humidity, the value was 21.2 ± 8.8 ; 19.7 ± 6.6 and 9.5 ± 2.9 respectively. This result mean at the lowest humidity, the bonding between HEMA carbonyl group and amino collagen group will increase. In the other hand, bonding between HEMA carbonyl group and amino collagen decreased at 80% and 90% humidity, where the peak of absorbance band was at 29.0 ± 6.3 and 32.3 ± 3.2 .

To know that the measurement data chemically is normal, Normal Distribution test was done. P value of entire group treatment of 60–90% humidity shows the higher number than 0.05 ($p > 0.05$). This means group data attempt of chemical bond at 60–90% humidity is normal distribution.

Next, homogeneity test was conducted to prove whether the treatment chemical sample group is homogenous, hence from calculation obtained by value of $p = 0.460$ ($p > 0.05$) meaning chemical sample group at 60–90% humidity was homogeneous.

By ANOVA Test, effect of 60–90% humidity to chemical bond between HEMA and dentine collagen shows the significant difference for each sample group ($p < 0.05$). The significance of each chemical sample group at 60–90% humidity was calculated with LSD Test.

The result has shown a significant difference between peak value of pure HEMA carbonyl absorbance band and HEMA carbonyl absorbance band at 60% humidity ($p < 0.05$). There is a significant difference between pure

HEMA and group at 70%, 80%, 90%, and 65%. There was no significant difference between group at 60% and 65%, and 80% humidity ($p > 0.05$).

There was a significant difference between group at 70% humidity and sample group of 60%, 80%, and 90% humidity ($p < 0.05$), but if compared with group at 65% humidity (control), there was no significant difference.

Furthermore, there was a significant difference between group at 80% humidity and 70% humidity, but there was no difference significance between group at 80% humidity and group of humidity 60%, 90%, and 65% (control). Group at 90% humidity showed a significant difference if compared with group at 60% and 65%.

DISCUSSION

Some expert expressed that chemical interactions between HEMA and dentine collagen is through reaction between HEMA carbonyl group and amino dentine collagen group. Bonding between amino collagen (NH_2) group and HEMA carbonyl group is only some of all chemical bond that possibly happened. This reaction form the new formation that are an amide bonding and also the side effect in the form of etilen-glikol.⁴

Renzo and Ellis¹² stated chemical bond between dentin and HEMA happened as result of reaction between resin and collagen function group, for example carboxylate, hydroxyl, amine group, or amide. Could also happened complex reaction with ion of Ca^{++} of hydroxy-apatite.

Wang and Spencer¹³ that investigating chemical interaction between HEMA and Bis-GMA at dentin surface, obtained that penetrating of HEMA into dentin surface which forming hybrid layer can reach until 10 μm . At this depth, penetration of HEMA is decreased until remain 8.85%. They used spectroscopy of micro-Raman and absorbance band which are observed was carbonyl absorbance band (1720 cm^{-1}), C=C phenyl (1609 cm^{-1}), group of CH_2 (1453 cm^{-1}) and others.

Bonding of HEMA resin at dentine collagen also depend on to the number of hydrogen bonds.⁶ If there are any resistance of carboxylic acid dissociation or amino collagen group hence will improve hydrogen bond between HEMA resin and dentine collagen. Previously, they studied the absorption of HEMA resin at dentine collagen characteristically by C-NMR.¹² The reduction of T_1 value of HEMA carbonyl ester carbon is bigger than at other carbon, which estimated will form hydrogen bond between HEMA carbonyl ester and dentine collagen. The amount of HEMA concentrations in solution and HEMA adsorption into collagen has correlation with tensile bond strength of resin at dentine.

In present chemically research, pure HEMA is used to directly knowing the role of HEMA to chemical bond with pure collagen. Mean average peak value of pure HEMA carbonyl absorbance band was equal to 47.5 ± 5.6 . Thus at 60%, 65%, and 70% humidity, the peak value decreased

significantly to 21.2 ± 8.8 , 19.7 ± 6.6 , and 9.5 ± 2.9 . It means that the amount of HEMA carbonyl group, which is bonding with amino collagen group, is increase at 60%, 65%, and 70% humidity. The tensile bond strength of HEMA with collagen also increase and the highest tensile bond strength obtained at 70 % humidity. At 70% humidity the water condition around fibril is very optimal in causing chemical interaction between HEMA and collagen. Water could make collagen to re-expansion, thus the fibril are more active and permeable to HEMA resin.

At 80% and 90% humidity, the chemical bond between HEMA resin and collagen was decreased significantly (peak value of carbonyl absorbance band at 80 % humidity: 29.0 ± 6.3 ; 90 % humidity: 32.3 ± 3.2). The increasing amount of water molecules around collagen will block the penetration of HEMA materials to collagen fibril. The increasing of water amount around collagens will also increase hydrogen bonding between water and HEMA carbonyl group. The increase of this hydrogen bonding will block the bonding between HEMA and collagen, so it will decrease the amount of HEMA carbonyl group which is bonding to amino collagen and also decreasing the tensile bond strength. This situation is according to statement of Nakabayashi and Pashley.⁷

The difficulty to obtain good sample was also explained by Wang and Spencer.¹³ They said that absolute intensity of Raman absorbance band depend on some factors: softness of sample surface, focusing position, detection depth, fluorescence biological component, equipments stability, and the power of laser-ray. For each sample, the result of absorbance band intensity will differ at the time of measurement, even for the same area of spot at one sample.

This research demonstrated that 70% humidity is the most optimal humidity to get maximal chemical bond between HEMA carbonyl group and dentine collagen amino.

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