ผลของสารเอทานอลภายใต้คลื่นอัลตราโซนิกในการช่วยลดปริมาณมอนอเมอร์ที่ตกค้างและยังคงคุณสมบัติการทนต่อแรงดัดโค้งของอะคริลิกเรซินที่ใช้รองฐานฟันปลอมแบบแข็งชนิดบ่มด้วยตัวเอง

นางสาวณัฏฐา กอบนิธิกุลวงศ์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาทันตกรรมประดิษฐ์ ภาควิชาทันตกรรมประดิษฐ์
คณะทันตแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2559

EFFECT OF ETHANOL SOLUTIONS UNDER ULTRASONIC WAVE ON RESIDUAL MONOMER REDUCTION AND FLEXURAL PROPERTIES IN AUTOPOLYMERIZING HARD RELINE ACRYLIC RESINS

Miss Nattha Kobnithikulwong

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Prosthodontics
Department of Prosthodontics
Faculty of Dentistry
Chulalongkorn University
Academic Year 2016

Thesis Title	EFFECT OF ETHANOL SOLUTIONS UNDER ULTRASONIC WAVE ON RESIDUAL MONOMER REDUCTION AND FLEXURAL PROPERTIES IN AUTOPOLYMERIZING HARD RELINE ACRYLIC RESINS
By	Miss Nattha Kobnithikulwong
Field of Study	Prosthodontics
Thesis Advisor	Associate Professor Chairat Wiwatwarrapan

Accepted by the Faculty of Dentistry, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

.....Dean of the Faculty of Dentistry
(Assistant Professor Suchit Poolthong, Ph.D.)

THESIS COMMITTEE

.....Chairman
(Assistant Professor Prarom Salimee, Ph.D.)

.....Thesis Advisor
(Associate Professor Chairat Wiwatwarrapan)

.....Examiner
(Assistant Professor Wacharasak Tumrasvin, Ph.D.)

.....External Examiner
(Assistant Professor Vanthana Sattabanasuk, Ph.D.)

ณัฏฐา กอบนิธิกุลวงศ์ : ผลของสารเอทานอลภายใต้คลื่นอัลตราโซนิกในการช่วยลดปริมาณมอนอเมอร์ที่ตกค้างและยังคงคุณสมบัติการทนต่อแรงดัดโค้งของอะคริลิกเรซินที่ใช้รองฐานฟันปลอมแบบแข็งชนิดบ่มด้วยตัวเอง (EFFECT OF ETHANOL SOLUTIONS UNDER ULTRASONIC WAVE ON RESIDUAL MONOMER REDUCTION AND FLEXURAL PROPERTIES IN AUTOPOLYMERIZING HARD RELINE ACRYLIC RESINS) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. ชัยรัตน์ วิวัฒน์วรพันธ์, 85 หน้า.

อะคริลิกเรซินที่ใช้รองฐานฟันปลอมแบบแข็งชนิดบ่มด้วยตัวเองนั้นมีข้อดีอยู่มาก แต่ก็พบว่ามีข้อเสียหลายประการต่อผู้ป่วยด้วย เนื่องมาจากมอนอเมอร์ที่หลงเหลือภายหลังปฏิกิริยาการเกิดพอลิเมอร์ มีผลต่อสมบัติทางกายภาพของวัสดุและยังเป็นอันตรายต่อเนื้อเยื่ออ่อนภายในช่องปากด้วย ดังนั้นมีงานวิจัยก่อนหน้านี้หลายงาน ศึกษาหาวิธีลดปริมาณมอนอเมอร์ที่หลงเหลือในอะคริลิกเรซิน ได้แก่ การแช่น้ำในเครื่องทำความสะอาดอัลตราโซนิกหรือในสารละลายเอทานอล เป็นต้น วัตถุประสงค์ของการศึกษานี้เพื่อหาความเข้มข้นที่เหมาะสมของสารละลายเอทานอลภายใต้คลื่นอัลตราโซนิกที่มีผลลดปริมาณมอนอเมอร์ที่หลงเหลือ และยังคงไม่เปลี่ยนแปลงคุณสมบัติการทนต่อแรงดัดโค้งของวัสดุ โดยเตรียมชิ้นงานผลิตภัณฑ์ Unifast Trad (UF) และ Kooliner (KL) อย่างละ 48 ชิ้น ตามคำแนะนำของบริษัทในแต่ละผลิตภัณฑ์ ทำการแบ่งโดยการสุ่มเป็น 8กลุ่ม กลุ่มละ 6 ชิ้น ได้แก่ กลุ่มควบคุมลบ (NC) กลุ่มควบคุมบวก โดยนำไปแช่ในน้ำ 50 องศาเซลเซียส 60 นาที (PC1) และแช่ในน้ำ 55 องศาเซลเซียส 10 นาที (PC2) กลุ่มที่เหลือคือ นำไปแช่ในสารละลายเอทานอลในความเข้มข้นต่างๆ ดังนี้ 10, 20, 30, 40 และ 50 เปอร์เซ็นต์ ในอ่างอัลตราโซนิกที่มีความถี่ 40 กิโลเฮิรตซ์ ที่อุณหภูมิ 55 องศาเซลเซียส 5 นาที ทำการตรวจหาปริมาณมอนอเมอร์ที่หลงเหลือด้วยวิธีโครมาโตกราฟีเหลวสมรรถนะสูงตามวิธีการมาตรฐานสากลหมายเลข 20795-1 (2013) เตรียมชิ้นงานทั้ง 2 ผลิตภัณฑ์ อย่างละ 80 ชิ้น ตามคำแนะนำของบริษัทผู้ผลิต และทำการแบ่งโดยการสุ่มทั้งหมด 8 กลุ่ม ดังที่กล่าวไว้ข้างต้น กลุ่มละ 10 ชิ้นแล้วนำมาทดสอบคุณสมบัติด้านความแข็งแรงดัดโค้งด้วยหัวกด 3 ตำแหน่ง ตามวิธีการมาตรฐานสากลหมายเลข 20795-1 (2013) ทำการวิเคราะห์ข้อมูลที่ได้ทางสถิติด้วยการวิเคราะห์ความแปรปรวนทางเดียวและ Tukey HSD ($p<0.05$) ผลการทดลอง พบว่า ในวัสดุ UF การแช่ในสารละลายเอทานอลที่มีความเข้มข้น 40 เปอร์เซ็นต์ภายใต้คลื่นอัลตราโซนิก และในวัสดุ KL การแช่ในสารละลายเอทานอลที่มีความเข้มข้น 10 เปอร์เซ็นต์ภายใต้คลื่นอัลตราโซนิก ที่อุณหภูมิ 55 องศาเซลเซียส 5 นาที มีประสิทธิภาพสูงที่สุดในการลดมอนอเมอร์ที่ตกค้างและยังคงคุณสมบัติทางด้านความแข็งแรง

ภาควิชา ทันตกรรมประดิษฐ์ ลายมือชื่อนิสิต .....

สาขาวิชา ทันตกรรมประดิษฐ์ ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

ปีการศึกษา 2559

5775805332 : MAJOR PROSTHODONTICS
KEYWORDS: AUTOPOLYMERI / HPLC / RESIDUAL MONOMER / ULTRASONIC / ETHANOL

NATTHA KOBNITHIKULWONG: EFFECT OF ETHANOL SOLUTIONS UNDER ULTRASONIC WAVE ON RESIDUAL MONOMER REDUCTION AND FLEXURAL PROPERTIES IN AUTOPOLYMERIZING HARD RELINE ACRYLIC RESINS. ADVISOR: ASSOC. PROF. CHAIRAT WIWATWARRAPAN, 85 pp.

Autopolymerizing hard reline acrylic resins have not only have many advantages but also have disadvantages to patients. According to residual monomer (RM) content after polymerization, RM affect materials' physical properties and also have harmful effects to soft tissue in oral cavity. Therefore, there are previous researches studied in reducing the RM content in acrylic resins such as water immersion in ultrasonic cleanser or immersion in ethanol solutions. The purpose of this study was to determine the appropriate concentration of ethanol under ultrasonic wave, affecting the reduction of RM content and maintain their flexural properties. Forty-eight specimens of Unifast Trad (UF) and forty-eight specimens of Kooliner (KL) were prepared according to their manufacturers' instructions and divided randomly into eight groups (N=6): NC: Negative control; PC1 and PC2 (positive control); water immersion at 50°C 60 min and 55°C 10 min respectively; The others were immersed in ethanol solutions of 10, 20, 30, 40 and 50% with ultrasonic bath (40 kHz) at 55°C 5 min. RM were determined by HPLC following ISO 20795-1 (2013). Eighty specimens of UT and eighty specimens of KL were prepared according to their manufacturers' instructions, and also divided into the same as the above eight groups to test 3-point flexural properties following ISO 20795-1 (2013)(N=10). Statistical analysis was performed using one-way ANOVA and Tukey HSD at $p<0.05$. Immersion UF in 40% ethanol solution under ultrasonic bath (40 kHz) at 55°C for 5 min and immersion KL in 10% ethanol solution under ultrasonic bath (40 kHz) at 55°C for 5 min are chosen to be a proper method to reduce RM effectively and maintain their flexural properties respectively.

Department: Prosthodontics Student's Signature .....
Field of Study: Prosthodontics Advisor's Signature .....
Academic Year: 2016

ACKNOWLEDGEMENTS

This research paper was supported by the Ratchadaphiseksomphot Endowment. (2014) Fund of Chulalongkorn University (CU-57-071-AS). First, I am thankful to Associate Professor Chairat Wiwatwarrapan who is my advisor, he provided all his great effort in advising me and concerned in this research. In addition, I would like to thank Faculty of Dentistry, Chulalongkorn University and all stuffs for helping me to succeed this project. Finally, I would like to thank my family for their support and encouragement.

CONTENTS

Page

Page

Page

LIST OF TABLES

LIST OF FIGURES

CHAPTER I INTRODUCTION

Background and rational

The development of acrylic resins has been used in dentistry since 1936 (1). Heat-cured acrylic resins were used in making dentures while autopolymerizing acrylic resins were used as relining, repair or temporary crown materials in prosthodontics. The autopolymerizing acrylic resins are more convenient to patients than heat-cured acrylic resins because there is no need for processing in a laboratory, resulting in a reduced waiting time. The autopolymerizing system has many disadvantages. Unpleasant monomer odor, potential for sensitization and irritation of oral soft tissue by the monomers and heat generation during polymerization are areas of clinical concern.

Some residual monomers leach out of the acrylic resin and sometimes cause a soft tissue reaction (2, 3). Their leached concentrations are potentially high enough to irritate and response to mucosal tissues. For example, residual methyl methacrylate monomer can be present in acrylic resins used for dental appliances and can leach from these resins into water (4, 5).

Nowadays, there are several methods of reducing the residual monomer (RM) in acrylic resins such as immersion in hot water (5-7), microwave irradiation (7), ultrasonic cleaner (8) and ethanol solutions (9).

Ultrasonic waves have been used in different fields such as SONAR in underwater world, ultrasonic medical imaging, and industrial cleaning that was reported to be a method to increase the extraction rate of organic substances by the effect of sonication (10). The ultrasonic sensing can probe into objectives nondestructively because it can spread through any kinds of media including solids, liquids and gases except vacuum.

Ethanol is a versatile solvent, miscible with water and with many organic solvents. Ethanol molecules can swell and penetrate into the space in polymers for promoting reduce the RM content (9). Thus ethanol is considered as a practical solution to remove RM content from acrylic resins.

The aim of this study was to find the proper concentration of ethanol solution under ultrasonic wave that can reduce the RM content effectively and remain the flexural properties of autopolymerizing hard reline acrylic resins.

Research objective

To determine the optimal concentration of ethanol solution under ultrasonic wave for reducing RM content in autopolymerizing hard relining acrylic resins and maintaining their flexural properties.

Research hypothesis

Hypothesis 1

$H1_0$: there is no significant difference in mean of RM content of each material between all experimental groups of postpolymerization treatment and that of the control group. ($p \geq 0.05$)

$H1_1$: there is a significant difference in mean of RM content of each material between at least one experimental group of postpolymerization treatment and that of the control group. ($p < 0.05$)

Hypothesis 2

$H2_0$: there is no significant difference in mean of RM content of each material between all groups of postpolymerization treatment in various ethanol concentrations under ultrasonic wave and that of the group of water immersion at 50°C 60 min. ($p \geq 0.05$)

$H2_1$: there is significant difference in mean of RM content of each material between at least one group of postpolymerization treatment in various ethanol concentrations under ultrasonic wave and that of the group of water immersion at 50°C 60 min. ($p < 0.05$)

Hypothesis 3

$H3_0$: there is no significant difference in mean of RM content of each material between all groups of postpolymerization treatment in various ethanol concentrations under ultrasonic wave and that of the group of water immersion at 55°C 10 min. ($p \geq 0.05$)

$H3_1$: there is significant difference in mean of RM content of each material between at least one group of postpolymerization treatment in various ethanol concentrations under ultrasonic wave and that of the group of water immersion at 55°C 10 min. ($p < 0.05$)

Hypothesis 4

$H4_0$: there is no significant difference in mean of flexural strength of each material between all experimental groups of autopolymerizing hard reline acrylic resins after postpolymerization treatment and that of the control group. ($p \geq 0.05$)

$H4_1$: there is a significant difference in mean of flexural strength of each material between at least one of experimental groups of autopolymerizing hard reline acrylic resins after postpolymerization treatment and that of the control group. ($p < 0.05$)

Hypothesis 5

$H5_0$: there is no significant difference in mean of flexural strength of each material between all experimental groups of autopolymerizing hard reline acrylic resins after postpolymerization treatment in various ethanol concentrations under ultrasonic wave and that of the group of water immersion at 50°C 60 min. ($p \geq 0.05$)

$H5_1$: there is a significant difference in mean of flexural strength of each material between at least one of experimental groups of autopolymerizing hard reline acrylic resins after postpolymerization treatment in various ethanol concentrations under ultrasonic wave and that of the group of water immersion at 50°C 60 min. ($p < 0.05$)

Hypothesis 6

$H6_0$: there is no significant difference in mean of flexural strength of each material between all experimental groups of autopolymerizing hard reline acrylic resins after

postpolymerization treatment in various ethanol concentrations under ultrasonic wave and that of the group of water immersion at 55°C 10 min. ($p \geq 0.05$)

$H6_1$: there is a significant difference in mean of flexural strength of each material between at least one of experimental groups of autopolymerizing hard reline acrylic resins after postpolymerization treatment in various ethanol concentrations under ultrasonic wave and that of the group of water immersion at 55°C 10 min. ($p < 0.05$)

Hypothesis 7

$H7_0$: there is no significant difference in mean of flexural modulus of each material between all experimental groups of autopolymerizing hard reline acrylic resins after postpolymerization treatment and that of the control group. ($p \geq 0.05$)

$H7_1$: there is a significant difference in mean of flexural modulus of each material between at least one of experimental groups of autopolymerizing hard reline acrylic resins after postpolymerization treatment and that of the control group. ($p < 0.05$)

Hypothesis 8

$H8_0$: there is no significant difference in mean of flexural modulus of each material between all experimental groups of autopolymerizing hard reline acrylic resins after postpolymerization treatment in various ethanol concentrations under ultrasonic wave and that of the group of water immersion at 50°C 60 min. ($p \geq 0.05$)

$H8_1$: there is a significant difference in mean of flexural modulus of each material between at least one of experimental groups of autopolymerizing hard reline acrylic resins after postpolymerization treatment in various ethanol concentrations under ultrasonic wave and that of the group of water immersion at 50°C 60 min. ($p < 0.05$)

Hypothesis 9

$H9_0$: there is no significant difference in mean of flexural modulus of each material between all experimental groups of autopolymerizing hard reline acrylic resins after postpolymerization treatment in various ethanol concentrations under ultrasonic wave and that of the group of water immersion at 55°C 10 min. ($p \geq 0.05$)

$H9_1$: there is a significant difference in mean of flexural modulus of each material between at least one of experimental groups of autopolymerizing hard reline acrylic resins after postpolymerization treatment in various ethanol concentrations under ultrasonic wave and that of the group of water immersion at 55°C 10 min. ($p<0.05$)

Scope of the research

1. This research is an *in vitro* study
2. Two commercial autopolymerizing hard reline acrylic resins used in this study are Unifast Trad (UF) and Kooliner (KL)
3. Investigator in this study only is one person

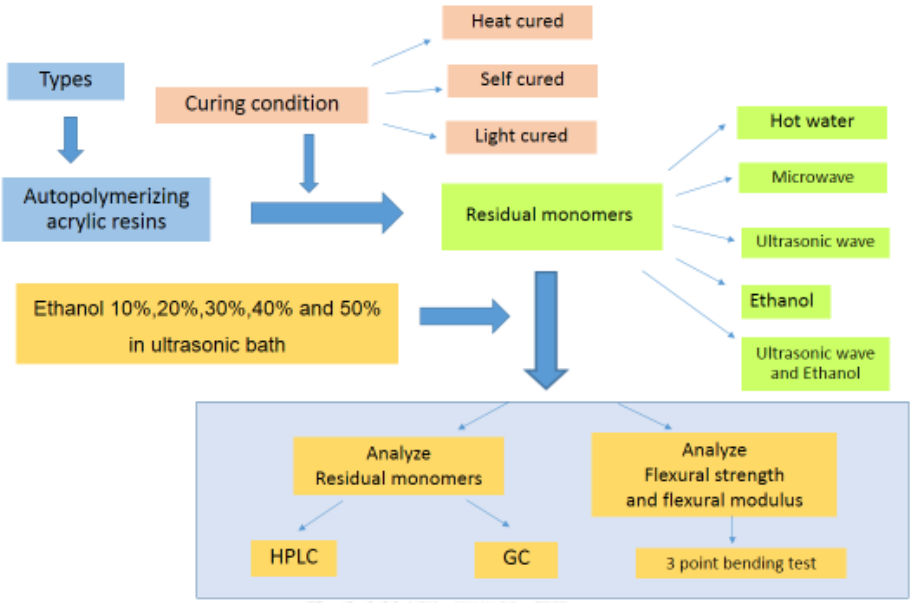
Limitation

This research was studied in 2 materials of autopolymerizing hard reline acrylic resins; UF and KL are determined *in vitro*. If the results appeared effectively, we will apply this method including other autopolymerizing hard reline acrylic resins to decrease RM content for patients in dental offices.

Keywords

- Autopolymerizing acrylic resins
- Residual monomer
- Ethanol
- Ultrasonic wave
- HPLC
- Flexural properties

Conceptual framework



Expected benefits

The application of ethanol solutions under ultrasonic wave as postpolymerization treatment in autopolymerizing hard reline acrylic resins will help patients save time in clinic by reducing RM content rapidly.

CHAPTER II LITERATURE REVIEW

Denture Base Polymers

Denture base is a prosthetic device constructed to replace the missing teeth that are supported by surrounding hard and soft tissues. There is evidence that dentures are used as treatment in prosthodontics around 700 B.C. in Dentistry (11). Therefore, there is development in the quality of dental materials to improve in esthetics, function and comfort for patients (11).

In 1936, Walter Bauer found polymerization of acrylic resins. Then, the acrylic resins have become popular as denture base material in clinical fabrication. Poly (methyl methacrylate) is still use in denture base because of its esthetics, ease of repair and saving cost (12). Denture base polymers are classified into five types as shown in *Table 1*. (13)

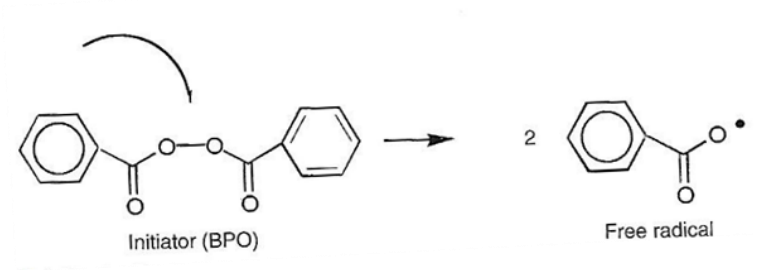
Table 1: Classification of denture base polymers according to ISO 1567

Type	*Class*	*Description*
1	1	Heat-processing polymers, powder and liquid
1	2	Heat-processed (plastic cake)
2	1	Auto-polymerized polymers, powder and liquid
2	1	Auto-polymerized polymers (powder and liquid pour type resins)
3	-	Thermoplastic blank or powder
4	-	Light-activated materials
5	-	Microwave-cured materials

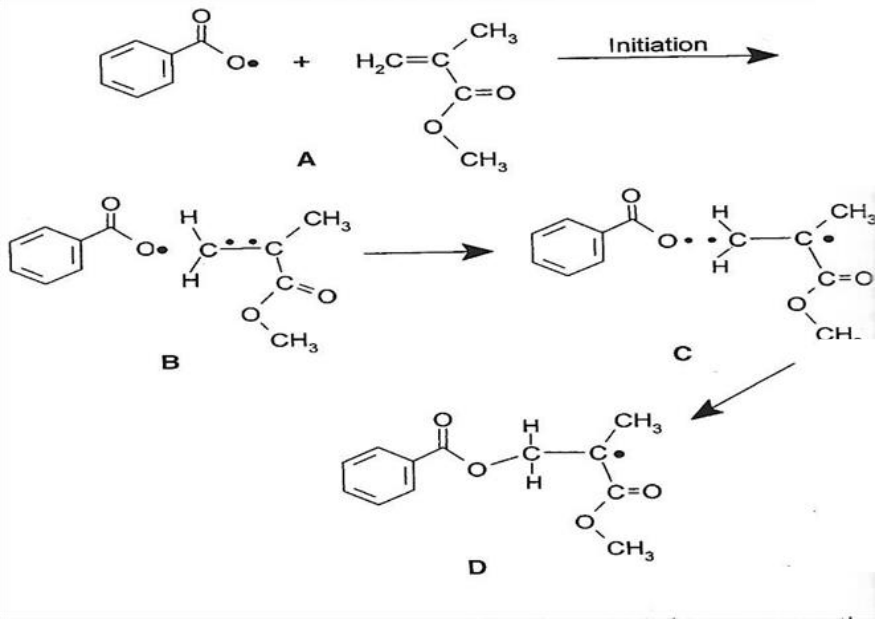
Polymer-monomer interaction

Acrylics are polyesters based on acrylic acid (propenoic acid, $CH_2=CHCO_2H$) formed from the polymerization of an alkyl acrylate ester. As the polymerization progresses, there is the conversion of MMA into poly-MMA during which the conversion of monomer molecules into the polymers (14). There are 4 stages in the addition

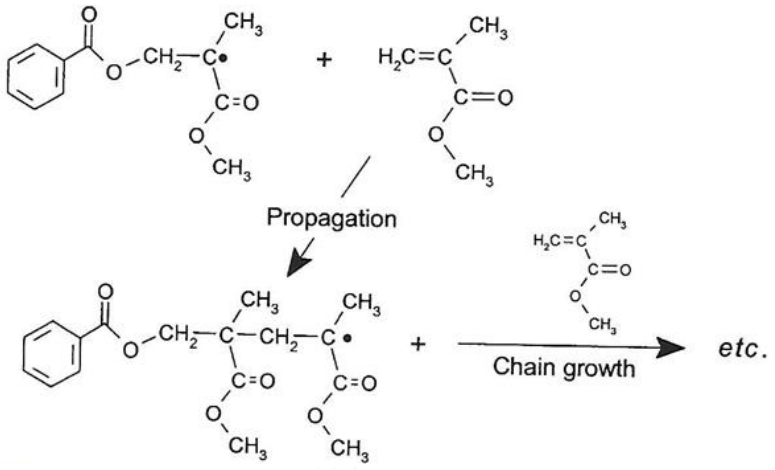
polymerization chain reaction: induction, propagation, chain transfer and termination (15).



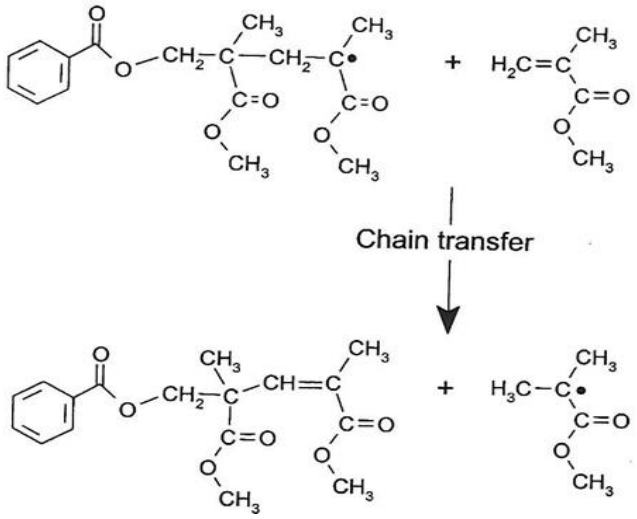
Activation of benzoyl peroxide (BPO). During activation, the bond is broken between the two oxygen atoms and the pair of electron is split between the two fragments.



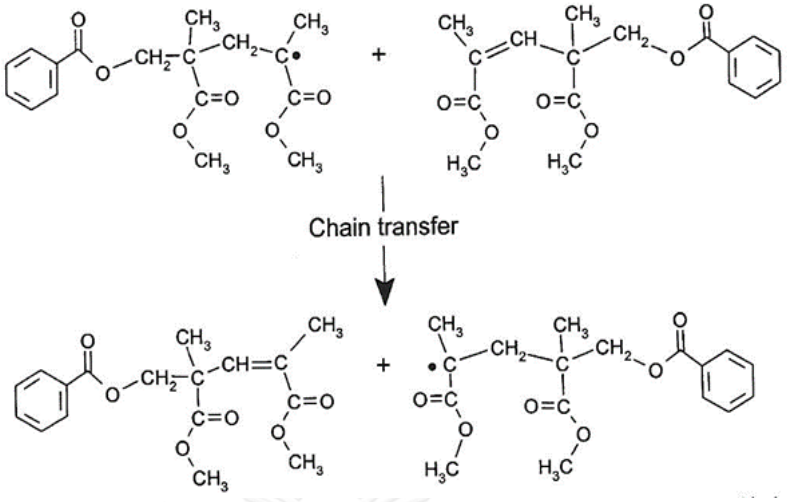
Initiation of a methyl methacrylate molecule. The unpaired electron of the free radical accesses the methyl methacrylate molecule (A and B), the electron in the double bond (C=C) is attracted to the free radical to form an electron pair and covalent bond between the free radical and the monomer molecule (C and D). Finally, the new molecule a free radical is made from the remaining unpaired electron (D).



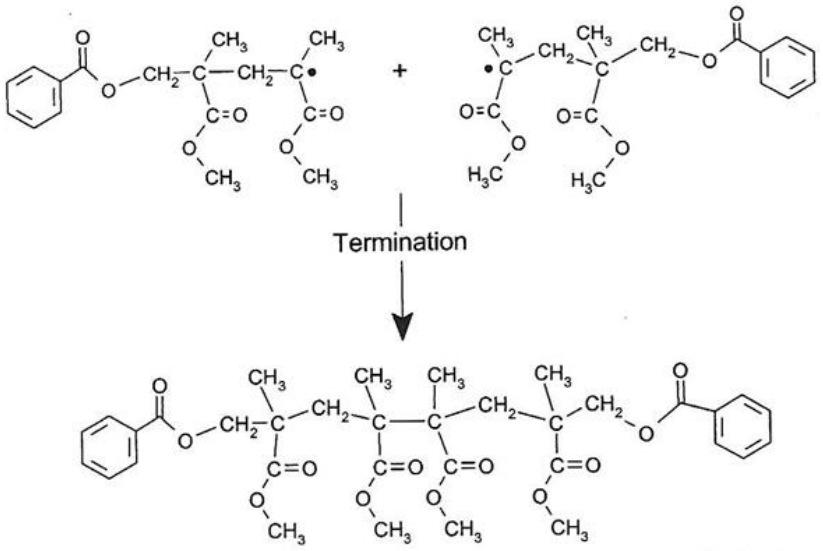
Propagation and chain growth. The free electron interacts with the double bond (C=C) of the methyl methacrylate molecule. A new, longer free radical is formed.



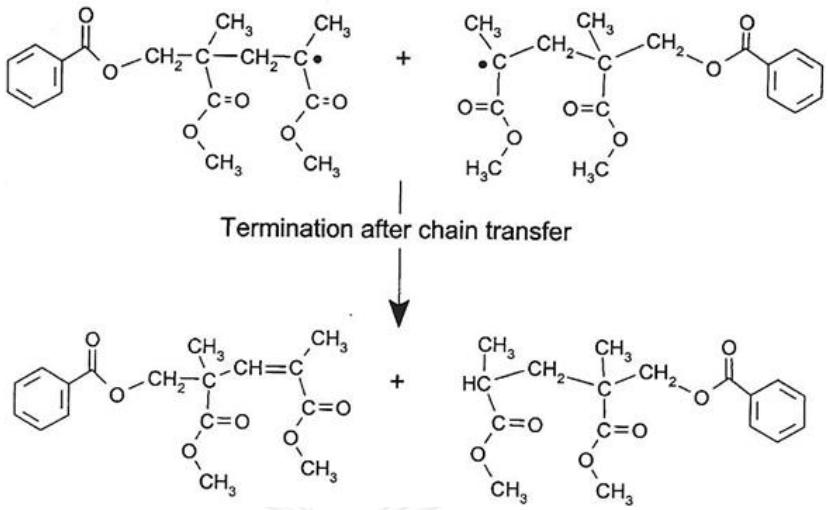
A free radical accesses a methyl methacrylate molecule and devotes a hydrogen atom to the methyl methacrylate molecule then chain transfer occurs.



Another type of the chain transfer can occur when a propagating chain interacts with the passivated segment that was formed.



Two free radicals interact and form a covalent bond then the termination occurs.



When two free radicals access each other, a new double bond may be formed on the molecule that devotes a hydrogen atom to the other free radical (15).

The unreacted (residual) monomer is considered undesirable. Because it effects on mechanical properties and irritating soft tissue.

$$M_j^* + M_1 \longleftrightarrow M_{j+1}^*$$

From above equation, we have known that free-radical polymerization systems are thermodynamic equilibrium. According to Lung CY and Darvell BW's study (2007) (16), they found that time-temperature response surface for the equilibration of PMMA with MMA. So now we can expect the 'residual monomer' in denture base acrylic resins.

Acrylic resins

Acrylic resins were first used in dentistry in the 1940s (11). Owing to their advantages in the clinic, they are used for different applications including temporary crowns, custom trays and baseplates for denture construction (17).

Physical properties of acrylic resins are hard, brittle and glassy polymers composed of long chains of small repeating units (18).

Dental acrylics resins are divided in 3 types, based on the factor that initiates the reaction: chemical-, heat- and light-activated (19). Heat-activated materials were use a hot water bath or microwave oven while light-activated materials were use with wavelength 460-480 nm (peak 470 nm) as an energy source. In chemical activated acrylic resins, action is set when the tertiary aromatic amine in liquid portion activates the benzoyl peroxide in powder portion to produce free radicals (20).

Autopolymerizing hard reline acrylic resins

In Unifast Trad, it composed of pre-polymerized PMMA powder particles, which are mixed with MMA monomer (21). Initiator is a peroxide such as a benzoyl peroxide (13). A cross-linking agent have been added to hard reline materials to improve mechanical properties in craze resistance and stiffness (22). Hydroquinone is added as an inhibitor to prevent premature polymerization (17). Typically, a tertiary aromatic amine is added to induce the reaction, producing free radicals. The inhibitor in the liquid destroys the free radicals that are initially produced, and working time results. In Kooliner, it composed of pre- polymerized poly ethyl methacrylate (PEMA) powder particles, which are mixed with isobutyl methacrylate (IBMA) of monomer (9).

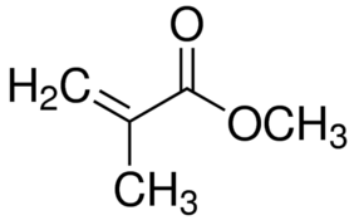


Figure 1. Methyl methacrylate structure

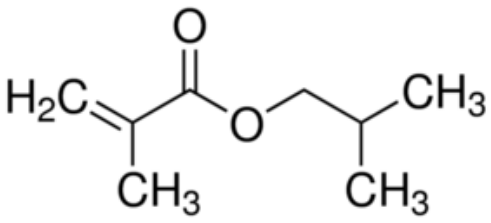


Figure 2. Isobutyl methacrylate structure

Disadvantages of autopolymerizing hard reline acrylic resins

Although autopolymerizing hard reline acrylic resins not only have a lot of advantages but also have disadvantages. As noted by Hickey and Zarb (23), these materials cause lesions in the mouth (i.e. clinical burns on the mucosa) and another problem regarding in this material includes color change (24).

During polymerization of acrylic resins, the conversion of monomer to polymer is incomplete and some unreacted monomer, called residual monomer (RM), remained in space between polymer chains (25, 26). The conversion of heat-polymerizing materials is better than the conversion of autopolymerizing materials. Thus, there is less RM in heat-cured PMMA than in chemical-cured PMMA (27).

An oxidation product of the residual MMA monomer can form formaldehyde, which can leach from the resins (6, 28). Formaldehyde has proven to be cytotoxic as much lower concentrations than MMA (6, 29). This compound is suspected to be a strong irritant to the mucous membranes even at low concentrations.

Allergic reaction

Allergy is a hypersensitive reaction to an allergen. Allergic reactions are divided in two groups: immediate and delayed. Immediate allergic reactions often result from various foods and drugs. Acrylic resins are involved in the delayed or contact allergy type, stomatitis venenata, which described by Nealey and Del Rio as a contact allergy from a prosthesis device of self-curing acrylic resin (30). MMA monomer causes an allergic reaction on contact with skin or oral mucosa. After the polymerization reaction, various amounts of the MMA monomer remain in the acrylic resin. Nowadays, there are relatively few reports of hypersensitivity to dental agents in the literature. Nonetheless, it is pertinent to address this problem, particularly with MMA, and to find a mode of treatment for such cases.

Residual monomer (RM)

After polymerization, the level of RM are leached depending on the time (31, 32). The highest amount of RM are released within 24 h after being processed (33). Then, a slow and moderate release is seen over a long period of time. It is a belief that tissue sensitivity to acrylic resins depends on the level of RM (3). Additionally, RM is a plasticizer which affect the mechanical properties (34, 35)

Different kinds of compounds including RM content, such as MMA, and additives such as hydroquinone (HQ), benzoyl peroxide (BP), N, N-dimethyl-p-toluidine (DMPT), etc. are eluted from acrylic polymers. These compounds diffuse into saliva and come into contact with the oral mucosa, which can lead to denture stomatitis. These lesions exhibit redness or burning sensations, often occurring in the areas beneath the dentures (3).

Methods for reducing residual monomer

Because of the toxicity of RM content, several methods have been proposed to reduce the RM and the degradation products of acrylic resins, including: immersion in hot water (5-7), microwave irradiation (7) and ultrasonic cleaner (8), the latter of which promotes the reduction of MMA content by the effect of cavitation i.e. the formation and collapsing of microscopic vacuum bubbles (8). Ultrasonic treatment has been used to enhance protein extraction from autoclaved soybean flakes (10). In addition, ultrasonic treatment can also promote polymerization (36). In recent years, Neves et al. reported that ethanol solutions can help increasing biocompatibility (9), showing that ethanol solutions and high temperature reduced the monomer content and the cytotoxicity of acrylic reline resins (9). Ethanol solutions have been used in order to accelerate solubility and increase leaching of organic solvents. Bettencourt et al. showed that ethanol promotes the leaching of RM from the polymer matrix of bone cements used in joint arthroplasty (9, 37).

Ultrasonic wave

Ultrasonic sensing techniques are widely used in various fields of engineering and basic science, especially for industrial and medical applications. Ultrasonic sensing can probe inside objectives nondestructively because it can spread through any kinds of media including solids, liquids and gases except vacuum. There are two types of ultrasonic waves: bulk (fundamental) waves that expand inside of an object, and guided wave that expand near the surface or together with the interface of an object (38).

Ultrasonic wave has been used in different fields such as SONAR in underwater world, ultrasonic medical imaging, and industrial cleaning that was reported to be a method to increase the extraction rate of organic substances by the effect of sonication (10). Recently, Kuijpers et al. found that ultrasonic treatment can also promote chemical reactions through ultrasonic polymerization (36). In dentistry, ultrasonic wave is also usually used for cleaning and scaling.

Ethanol

Ethanol is a clear, colorless liquid with an agreeable odor. It is an alcohol, a group of chemical compounds whose molecules contain a hydroxyl group, –OH, bonded to a carbon atom. Ethanol melts at –114.1°C, boils at 78.5°C, and has a density of 0.789 g/ml at 20°C. Its low freezing point has made it useful as a fluid in thermometers for temperatures below –40°C, the freezing point of mercury, and for other low-temperature purposes, such as for antifreeze in automobile radiators (39). Additionally, ethanol is used as solvent to extract compounds from polymer matrix of bone cements used in joint arthroplasty (9, 37).

Methods for determination of residual monomer

Over the years, Gas chromatography (GC) (40) and High-performance liquid chromatography (HPLC) (8, 9, 41) have been used for the determination of RM content from denture base resins.

High-performance liquid chromatography (HPLC) has been recommended to be a suitable method of determining and evaluating low RM values. This method also allows for comparison under identical conditions (42).

High performance liquid chromatography (HPLC)

HPLC was developed in the late 1960s and early 1970s (43). At present, HPLC is generally used in biochemistry and analysis to separate, identify, and quantify active compounds. It is widely applied in various areas, including pharmaceutical and chemical industries (44).

The main section of HPLC is a column that holds packing material (stationary phase), and a mobile phase which is moved through the column by a pump. A detector shows the retention times of the molecules. The retention time, i.e. the time that solvents come out of the end of the column. Depends on the interactions between the stationary phase, the molecules are analyzed and the solvents are used. Solvents used including combinations of water or organic liquids; the most common are methanol and acetonitrile. The solvents, additives and gradient rely on the stationary phase and the analyses. The gradient is separated depending on the difference of mobile phase component during the analysis.

CHAPTER III METHODOLOGY

Target population

Two autopolymerizing hard reline acrylic resins, Unifast Trad (UF) and Kooliner (KL)

Sample

Disk-shaped acrylic resins

Table 2: Materials under evaluation in the study

Product	Manufacturer	P/L ratio (g/mL)	Composition	Curing cycle
Unifast Trad (UF)	GC America Inc., Alsip, IL, USA	2/1	P: PMMA L: MMA	2 minutes
Kooliner (KL)	GC America Inc., Alsip, IL, USA	1.4/1	P: PEMA L: IBMA	10 minutes

P: powder; L: liquid; PMMA: poly (methyl methacrylate); MMA: methyl methacrylate; PEMA: poly (ethyl methacrylate); IBMA: isobutyl methacrylate

Instruments

1. Circular mold recommended by ISO 20795-1(2013)
2. Rectangle mold recommend by ISO 20795-1(2013)
3. Hydraulic pressure machine ‘EWG 5414’ (Kavo, NC, USA)
4. Freezer -20°C (Sanyo, Osaka, Japan)
5. Ultrasonic cleaner at 40 kHz (GT SONIC, Meizhou, China)
6. Ethanol (Merck, Darmstadt, Germany)
7. Polishing machine ‘Nano 2000T’ (Pace Technologies, Tucson, USA)

8. Metallographic grinding paper No.500 and 1200 (TOA, Bangkok, Thailand)
9. Digital scale ‘Satorius BP1105’ (Sartorius, Goettingen, Germany)
10. Magnetic stirrer (Diligent, Bangkok, Thailand)
11. PTFE coated magnetic stirring bar (Cowie Technology, Middlesbrough, UK)
12. Centrifugal machine ‘Avanti J-E’ (Beckman Coulter, CA, USA)
13. HPLC system (Shimadzu Corporation, Kyoto, Japan)
14. HPLC column (Merck Millipore, Darmstadt, Germany)
15. Micropipet (Labnet, New Jersey, USA)
16. Volumetic flask 5mL,10mL, 1L (Duran, Wertheim, Germany)
17. Digital Vernier calipers (Mitutoyo, Kawasaki, Japan)
18. Tetrahydrofuran (Merck KGaA, Darmstadt, Germany)
19. Methanol (Merck KGaA, Darmstadt, Germany)
20. Incubator (Contherm Scientific Ltd., Korokoro, New Zealand)
21. Universal Testing Machine (Shimadzu, Kyoto, Japan)

Residual monomers determination

Sample preparation to analyze RM and postpolymerization treatments

1. All specimens were prepared from stainless steel mold as recommended by ISO 20795-1.(2013) (45) (disk-shaped, a diameter of 50±1 mm, depth 3±0.1 mm with a flat cover)

Figure 3. Circular mold recommended by ISO 20795-1 (2013)

2. Forty-eight specimens of each material were prepared following the recommendations of the manufacturers (powder to liquid ratio is 2.0 g : 1 mL for Unifast Trad and 1.4 g : 1 mL for Kooliner). The mixture was placed into a metal mold. The metal mold was pressed in a hydraulic press at 300 kPa.

Figure 4. Hydraulic Pressure machine

3. The specimens were then kept in a dark place for 24±5 h. All specimens were carefully wet ground with 500 and 1200-grit silicon carbide paper to remove excess material until a thickness of 2.0±0.1 mm by digital vernier calipers (Mitutoyo, Kawasaki, Japan).

Figure 5. Polishing machine (Nano 2000T)

4. After polishing, the specimens of each material (Unifast Trad; UF and Kooliner; KL) were randomly divided into 8 groups as shown in *Table3*.

5. Each specimen was placed in the center of source of ultrasonic wave. Above the source of ultrasonic wave 5 cm by tying the thread with a cover of ultrasonic cleaner. (*Figure 6.*)

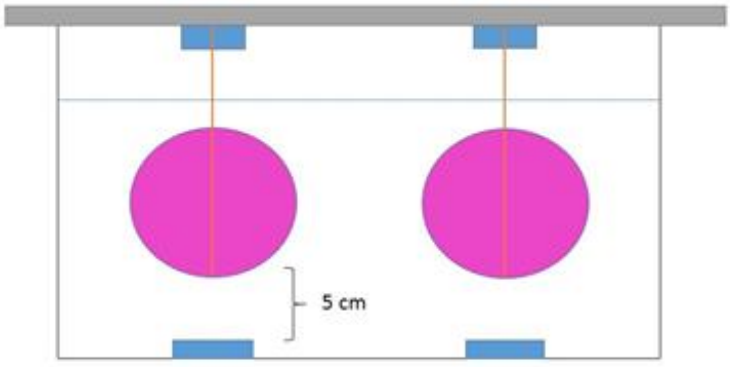


Figure 6. The placement of specimens in the ultrasonic cleaner

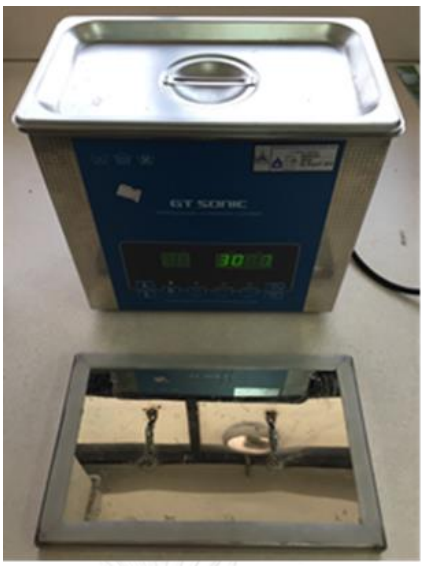


Figure 7. Ultrasonic cleaner (40 kHz)

Solution preparation for extract RM

-Solution A

Tetrahydrofurane (THF) solution was prepared by weight approximately 0.02 g of hydroquinone into a 1L volumetric flask. THF was added until the total volume was 1L.

-Solution B

Methanol solution was prepared by weight approximately 0.02 g of hydroquinone into a 1L volumetric flask. Methanol was added until the total volume was 1L.

Solution preparation for calibration curve

-Solution C

Methanol / Tetrahydrofurane solution was prepared by mixing one volume part of solution A and four volume parts of solution B.

Table 3: Description of experimental groups.

Group	Method to reduce RM	Time	Code		Amount of samples
			UF	KL	
1	Control	-	UFNC	KLNC	6
2	Immersion in water 50°C	60 min	UFPC1	KLPC1	6
3	Immersion in water 55°C	10 min	UFPC2	KLPC2	6
4	Immersion in ethanol 10% and ultrasonic wave 55°C	5 min	UF10%E	KL10%E	6
5	Immersion in ethanol 20% and ultrasonic wave 55°C	5 min	UF20%E	KL20%E	6
6	Immersion in ethanol 30% and ultrasonic wave 55°C	5 min	UF30%E	KL30%E	6
7	Immersion in ethanol 40% and ultrasonic wave 55°C	5 min	UF40%E	KL40%E	6
8	Immersion in ethanol 50% and ultrasonic wave 55°C	5 min	UF50%E	KL50%E	6

Residual monomer extraction procedure

1. After the postpolymerization treatment, all specimens were broken into small pieces in order to prepare three samples with a weight of 650 mg for each sample.

Figure 8. The specimen was broken into small pieces

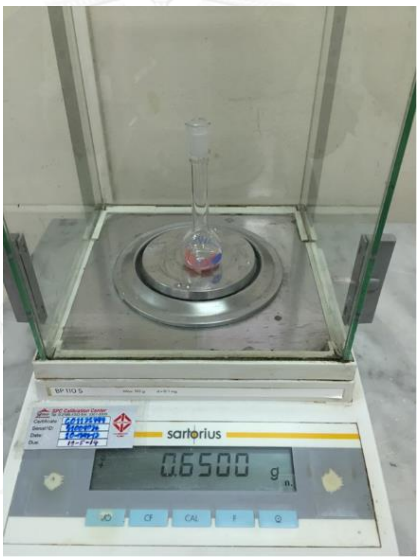


Figure 9. Each sample was weighed by the Sartorius BP1105 digital scale

2. For each sample, 10 ml of solution A was added as an extraction solvent. The sample solutions were magnetically stirred by using a clean 3-mm polytetrafluoroethylene-coated for magnetic stirring bar for 72±2 h.

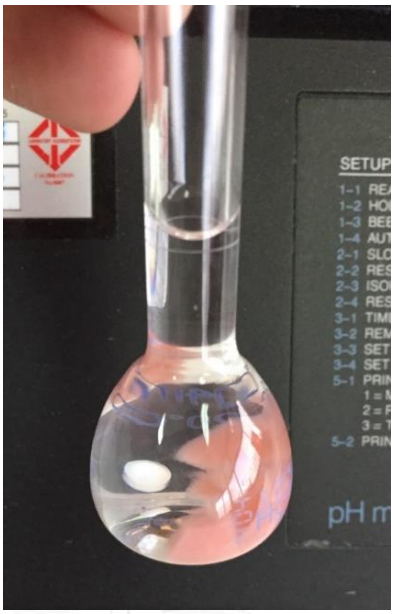


Figure 10. Solution A was added in the sample with magnetic stirring bar

Figure 11. Magnetic stirrer (Diligent, Bangkok, Thailand)

3. To precipitate the dissolved polymer, 8 ml of solution B was added to 2 ml of each of the previously prepared samples to final volume.

4. Each sample was shaken then laid down 20 min. Five mL of the clear solution from each flask was transferred to glass centrifugation tubes, centrifuged at 3000 rpm for 15 min at 25 °C (Avanti J-E, Beckman Coulter, CA, USA).

5. Determined each sample that there was no remaining polymer in the solution by filter. Each sample was transferred to vial 2 mL to assess RM content.

Figure 12. Centrifugal machine (Beckman Coulter, CA, USA)

Figure 13. The clear final sample to analyze by HPLC

6. The supernatant of each sample was analyzed by a high performance liquid chromatography (HPLC) system (Shimadzu, Kyoto, Japan. Prominence system; RP-18-Lichrospher-Merck column; mobile phase of water/methanol (34:66); flow rate of 0.8 ml/min; UV light wave length 205 nm detector).

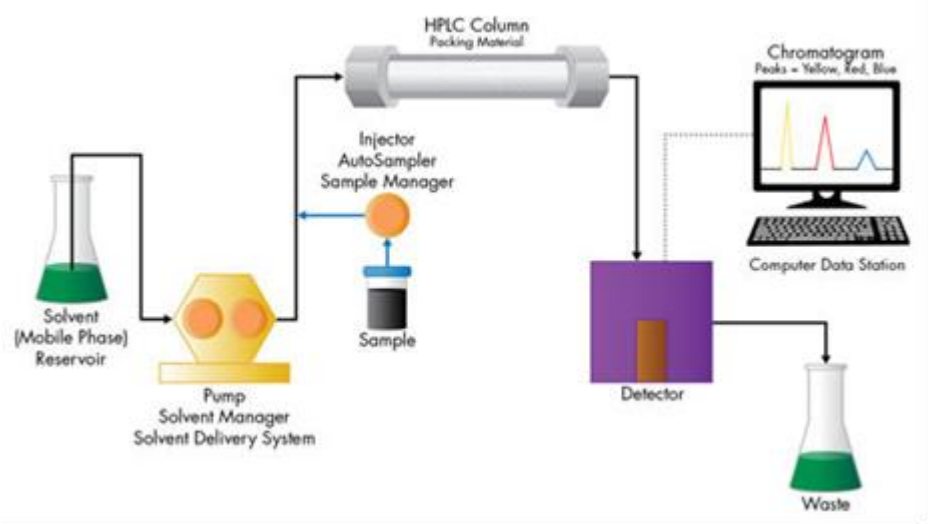


Figure 14. HPLC system

Method for Calibration curve

Calibration solutions for HPLC were prepared by making at least 5 standard solutions with various concentrations of MMA for Unifast Trad and IBMA for Kooliner (i.e. 6 mg, 60 mg, 150 mg, 300 mg and 400 mg by weight) into a glass flask of 5 ml. Solution C was added until the total volume is 5 ml. Then, 100 µl of each calibration solution was transferred into a glass flask of 10 ml. Solution C was added until the total volume was 10 ml.

The mass of MMA or IBMA was recorded and the final concentrations were calculated in percent of mass. If the MMA or IBMA content of the sample solutions did not fit within the extreme MMA or IBMA concentrations of the calibration graph, additional calibration points were made.

Residual monomer determination

The standard curve was used to determine the concentration in microgram of MMA (1) and IBMA (2), C per milliliter of analyzed sample solution.

$$f(x) = (2.21898*10^{7})\, x + 305459 \quad (1)$$

$$f(x) = (1.70872*10^{7})\, x + 685739 \quad (2)$$

These were standard curves. Where $f(x)$ was absorbance area of MMA or IBMA by UV detector and x= MMA or IBMA concentration.

The quantity of residual monomers (μg) in 1 g of each sample was calculated according to the following equation:

$$M = [C \times (10/2)^{*} \times 10^{**}] \quad (3)$$

Where ***** was the tetrahydrofuran amount and ****** was the methanol amount used for extraction.

$$\text{Residual monomer (\% mass fraction)} = (M/ M_{sample}) \times 100 \quad (4)$$

Where M_{sample} is the mass of sample, in micrograms.

Flexural properties determination

Sample preparation

1. All specimens were prepared from stainless steel mold as recommended by ISO 20795-1. (2013) (45) (a length of 64 mm, a width of 10.0±0.2 mm, and a height of 3.3±0.2 mm)
2. Eighty specimens of each material were prepared following the recommendations of the manufacturer (powder to liquid ratio is 2.0 g : 1mL for Unifast Trad and 1.4 g : 1 mL for Kooliner) and the mixture was placed into a metal mold.

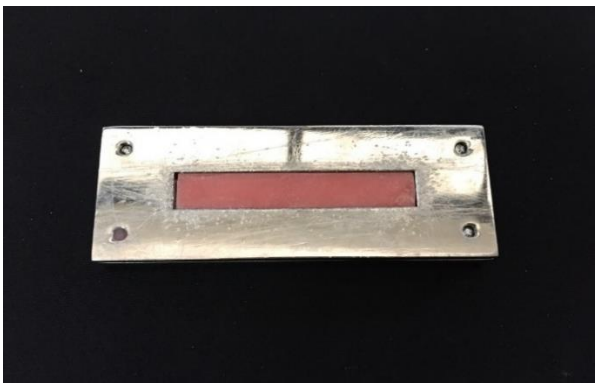


Figure 15. The rectangle mold recommended by ISO 20795-1

3. All specimens were carefully ground with 500 and 1200-grit silicon carbide paper to remove excess material. Before testing, the specimens were stored in water at 37±2°C for 50±2 h, as recommended by ISO 20795-1 (2013) (45).

Flexural strength and Flexural modulus test

1. The average of individual measures of each specimen (width and thickness) was measured by a digital vernier caliper. Inserted value in the software before testing.

2. Using a Shimadzu universal testing machine, a 3-point bending test was performed at a crosshead speed of 5 mm/min with a 50 mm distance between the supports.

The fracture load was recorded in Newton (N). The flexural strength was expressed in MPa and calculated using the following form

$$FS = \frac{3 * W * L}{2 * b * d^2} \quad (5)$$

Where FS was the flexural strength, W was the maximum load before fracture (N), L was the distance between the supports (50 mm), b was the width of the specimen (mm), and d was the thickness of the specimen (mm).

The flexural modulus was calculated using the following formula:

$$E = \frac{F_1 * L^3}{4 * b * d^3 * h} \quad (6)$$

Where F_1 was the load, in N, at a point in the straight line portion (with the maximum slope) of the load/deflection curve, h was the deflection, in mm, at load F_1 (L, b, d are as previously defined).

Statistical analysis

The data of RM content and flexural properties in Unifast Trad and Kooliner were analyzed using One-sample Kolmogorov-smirnov and Levene test to evaluate distribution and variances respectively. If the data were normally distribution and equal variance. The mean values of properties of experimental groups were compared by one-way analysis of variance (ANOVA) followed by Tukey HSD at a significance level α = 0.05. Statistical analyses were performed using SPSS software (SPSS ver.17, IBM, New York, NY, USA).

CHAPTER IV RESULTS

The retention time of MMA and IBMA were 2.499 and 2.364 min respectively. Concentrations of standard solutions were injected into HPLC system to create the chromatograms of standard solutions of MMA and IBMA (*Figure 16. and 19.*) and the chromatograms of sample solutions of MMA and IBMA. (*Figure 18. and 21.*) The standard curve was calculated from HPLC chromatograms. The RM content was determined from standard calibration curve. (*Figure 17. and 20.*)

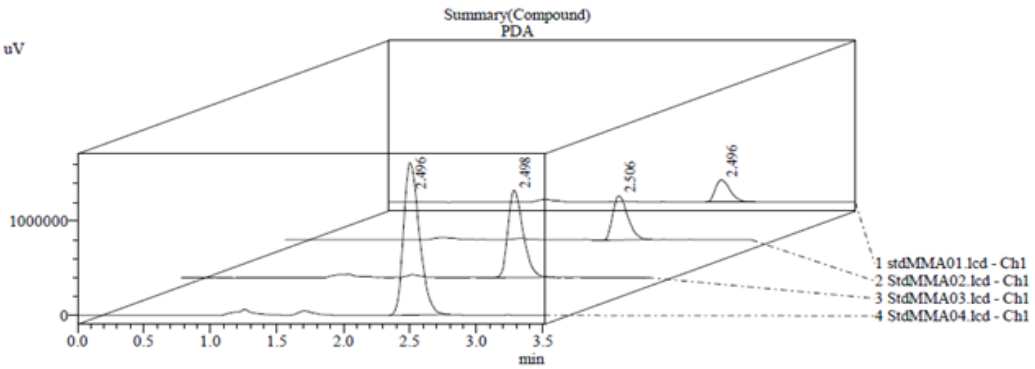


Figure 16. Chromatogram of standard solutions of MMA

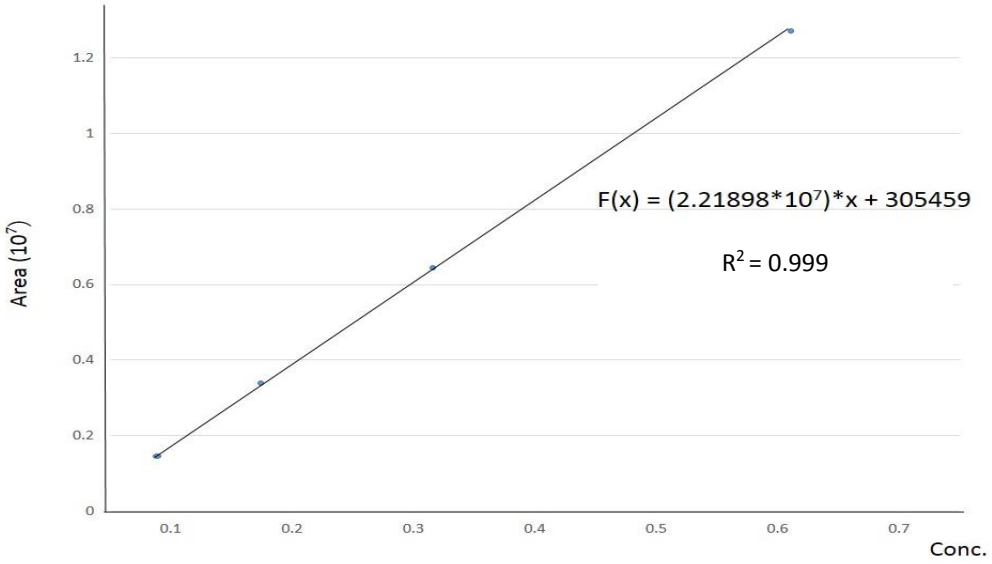


Figure 17. Standard calibration curve of MMA

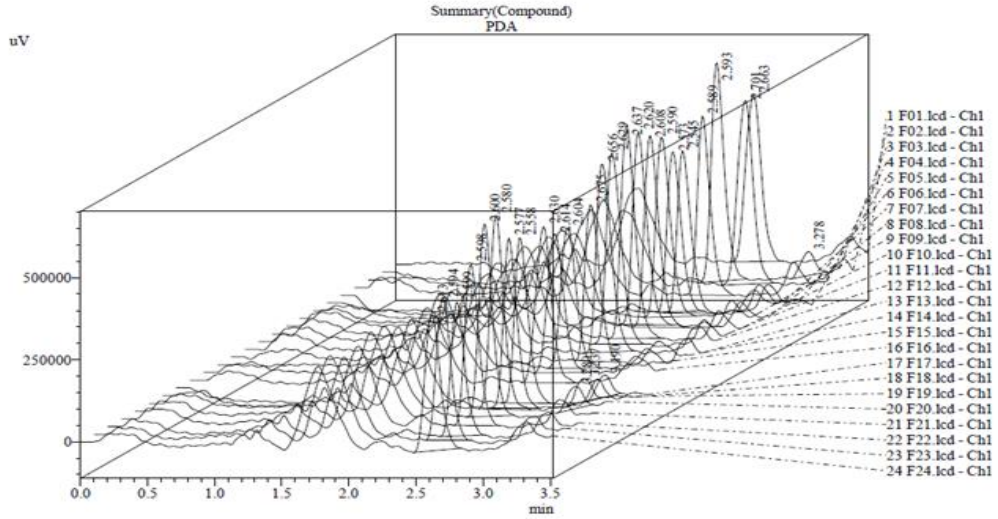


Figure 18. Chromatogram of sample solutions of MMA

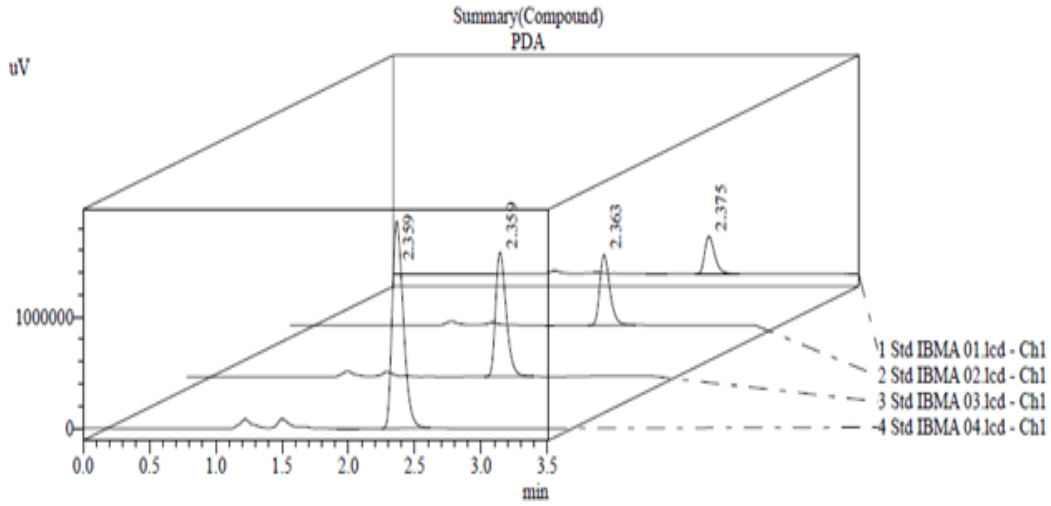


Figure 19. Chromatogram of standard solutions of IBMA

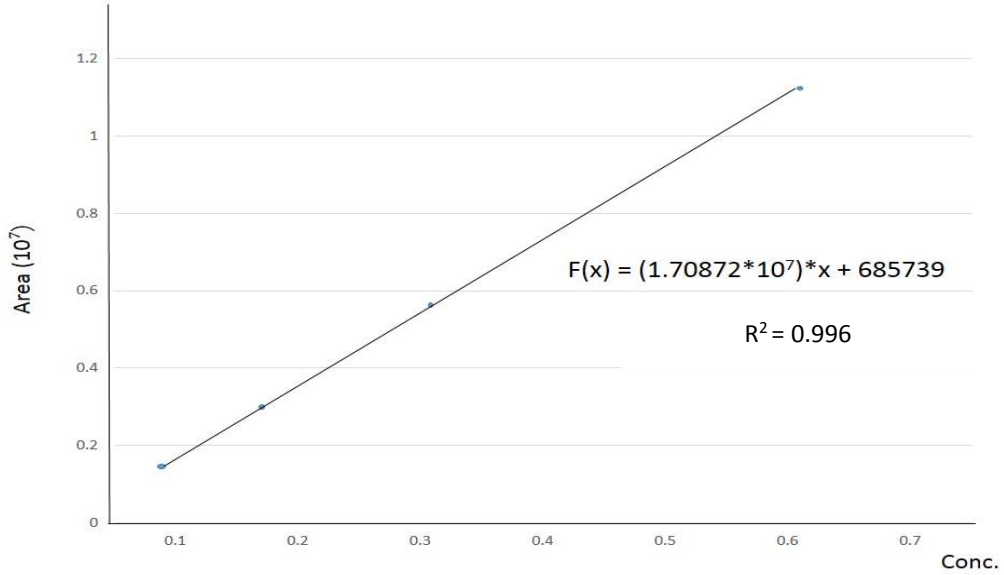


Figure 20. Standard calibration curve of IBMA

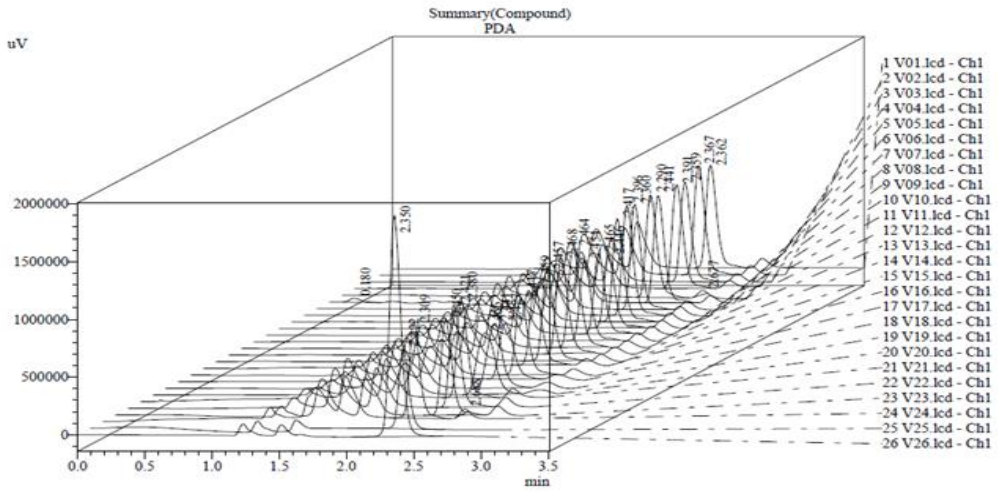


Figure 21. Chromatogram of sample solutions of IBMA

The mean of RM content of Unifast Trad and Kooliner are shown in *Table 4. and 5.*respectively.

Table 4: Means and standard deviations of residual monomer content of the experimental groups in Unifast Trad (UF)

Group	Postpolymerization treatment	Time	Code	RM(mg%)
1	Control	-	UFNC	3.087 ± 0.132
2	Immersion in water 50°C	60 min	UFPC1	2.134 ± 0.125
3	Immersion in water 55°C	10 min	UFPC2	2.508 ± 0.153
4	Immersion in ethanol 10% and ultrasonic wave 55°C	5 min	UF10%E	2.204 ± 0.098
5	Immersion in ethanol 20% and ultrasonic wave 55°C	5 min	UF20%E	2.360 ± 0.185
6	Immersion in ethanol 30% and ultrasonic wave 55°C	5 min	UF30%E	1.643 ± 0.139
7	Immersion in ethanol 40% and ultrasonic wave 55°C	5 min	UF40%E	1.255 ± 0.128
8	Immersion in ethanol 50% and ultrasonic wave 55°C	5 min	UF50%E	1.164 ± 0.101

Table 5: *Means and standard deviations of residual monomer content of the experimental groups in Kooliner (KL)*

Group	Postpolymerization treatment	Time	Code	RM(mg%)
1	Control	-	KLNC	2.258 ± 0.407
2	Immersion in water 50°C	60 min	KLPC1	1.556 ± 0.327
3	Immersion in water 55°C	10 min	KLPC2	2.012 ± 0.282
4	Immersion in ethanol 10% and ultrasonic wave 55°C	5 min	KL10%E	1.685 ± 0.117
5	Immersion in ethanol 20% and ultrasonic wave 55°C	5 min	KL20%E	1.573 ± 0.040
6	Immersion in ethanol 30% and ultrasonic wave 55°C	5 min	KL30%E	1.837 ± 0.207
7	Immersion in ethanol 40% and ultrasonic wave 55°C	5 min	KL40%E	1.617 ± 0.169
8	Immersion in ethanol 50% and ultrasonic wave 55°C	5 min	KL50%E	1.375 ± 0.211

Part 1: No postpolymerization groups (UFNC and KLNC) were used as control

One-way ANOVA of RM content evaluation of both materials indicated significant differences among experimental groups. ($p<0.05$) (*Figure 22. and 23.*) All postpolymerization treatment groups of the UF demonstrated decrease of RM content significantly when compared with the UFNC group. The UF50%E group showed the maximum reduction of RM content, followed by the UF40%E group which did not significant different compared with the UF50%E group. The UF30%E group had less RM reduction significantly when compared with the UF50%E and UF40%E groups. The

least RM reduction was showed in UF10%E group which no significant difference when compared with that in the UF20%E group. The KLPC1, KL10%E, KL20%E, KL40%E and KL50%E groups demonstrated decrease of RM content significantly when compared with the KLNC group. There was no significant difference of RM content in the KL10%E, KL20%E and KL40%E groups. Additionally, the KL50%E group showed the RM content was less than the KL30%E group significantly.

One-way ANOVA of flexural strength data of both materials demonstrated no significant differences between postpolymerization treatment groups and NC group. ($p \geq 0.05$) (*Figure 24. and 25.*) In Unifast Trad, there was no significant difference of flexural strength in postpolymerization treatment in ethanol solutions under ultrasonic wave groups. In Kooliner, there was no significant difference of flexural strength in the KL10%E, KL20%E, KL30%E and KL40%E groups. The KL50%E group showed less flexural strength when compared with the KL20%E group significantly. Flexural modulus data of both materials demonstrated significant differences among experimental groups. ($p<0.05$) (*Figure 26. and 27.*) Flexural modulus of the UFPC1 and UFPC2 groups were significantly increased when compared with the UFNC group. There was no significant difference in flexural modulus between UFNC group and groups of postpolymerization treatment in ethanol solutions under ultrasonic wave. Additionally, there was no significant difference in flexural modulus in the UF20%E, UF30%E, UF40%E and UF50%E groups. The UF10%E group showed the maximum of flexural modulus when compared with the other groups of postpolymerization treatment in ethanol solutions under ultrasonic wave. In Kooliner, the KL10%E group was significantly increased in flexural modulus when compared with the KLNC group. There is no significant difference of flexural modulus in postpolymerization treatment in ethanol solutions under ultrasonic wave groups.

Part 2: The Immersion in water 50°C 60 min groups (UFPC1 and KLPC1) were used as control

One-way ANOVA of RM content evaluation of both materials indicated significant differences among groups of postpolymerization treatment in ethanol solutions under ultrasonic wave and group of immersion in water 50°C 60 min. ($p<0.05$)

(*Figure 22. and 23*.) Compared with the UFPC1 group, the UF10%E and UF20%E groups showed no significant reduction of RM content, while the UF30%E, UF40%E and UF50%E groups showed significant reduction of RM content. The UF50%E group showed the maximum reduction of RM content, followed by the UF40%E group which did not significant difference in RM content compared with the UF50%E group. In KL, all groups of the postpolymerization treatment in ethanol solutions under ultrasonic wave demonstrated no significant difference in RM content compared with the KLPC1 group.

Compared with UFPC1 and KLPC1 groups, all groups of postpolymerization treatment in ethanol solutions under ultrasonic wave demonstrated no significant differences in flexural strength. One-way ANOVA of flexural modulus data of both materials demonstrated significant differences among groups of the postpolymerization treatment in ethanol solutions under ultrasonic wave and group of immersion in water 50°C 60 min. ($p<0.05$) (*Figure 26. and 27.)* In Unifast Trad, the flexural modulus of all groups of postpolymerization treatment in ethanol solutions under ultrasonic wave, except the UF10% group were significant decreased when compared with that of UFPC1 group. In Kooliner, there was no significant difference in flexural modulus between all groups of postpolymerization treatment in ethanol solutions under ultrasonic wave groups and KLPC1 group.

Part 3: The Immersion in water 55°C 5 min groups (UFPC2 and KLPC2) were used as control

One-way ANOVA of RM content evaluation of both materials indicated significant differences among groups of postpolymerization treatment in ethanol solutions under ultrasonic wave and group of immersion in water 55°C 5 min ($p<0.05$) (*Figure 22. and 23*.) Compared with the UFPC2 group, the UF20%E group showed no significant reduction of RM content, while the UF10%E, UF30%E, UF40%E and UF50%E groups showed significant reduction of RM content. The UF50%E group showed the maximum reduction of RM content, followed by the UF40%E group which did not significant difference compared with the UF50%E group. Compared with that of KLPC2 group, only KL50%E group was significantly different in RM content.

One-way ANOVA of flexural strength data of both materials demonstrated significant differences. (*Figure 24. and 25.*) The flexural strength of the UF groups except UF40%E and UF50%E groups showed significant decreased when compared with that of UFPC2 group. Compared with KLPC2 group, all KL groups of postpolymerization treatment in ethanol solutions under ultrasonic wave demonstrated no significant differences in flexural strength. One-way ANOVA of flexural modulus data of both materials demonstrated significant differences among groups of the postpolymerization treatment in ethanol solutions under ultrasonic wave and group of immersion in water 55°C 5 min.($p<0.05$) (*Figure 26.and 27.*) The flexural modulus data of all UF groups of postpolymerization treatment in ethanol solutions under ultrasonic wave, except the UF10%E group were significant decreased compared with that of UFPC2 group. Compared with the KLPC2 group, the flexural modulus of only the KL10%E and KL20%E groups were significant differences but that of the KL30%E, KL40%E and KL50%E groups were not significant differences in flexural modulus.

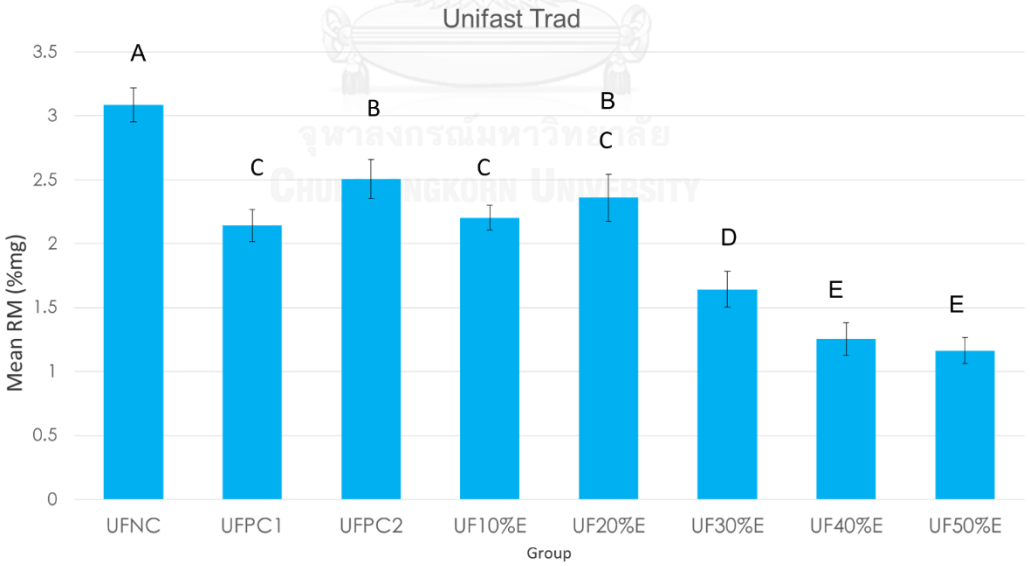


Figure 22. Means and standard deviations of residual monomer content in all postpolymerization treatment groups in Unifast Trad. Identical letters denote no significant differences among groups. ($p \geq 0.05$)

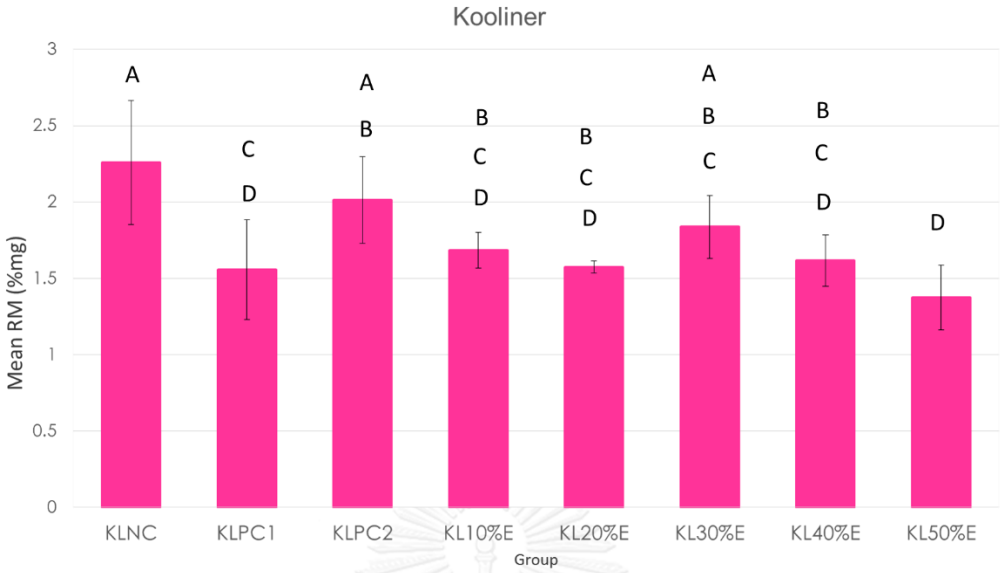


Figure 23. Means and standard deviations of residual monomer content in all postpolymerization treatment groups in Kooliner. Identical letters denote no significant differences among groups. (p≥0.05)

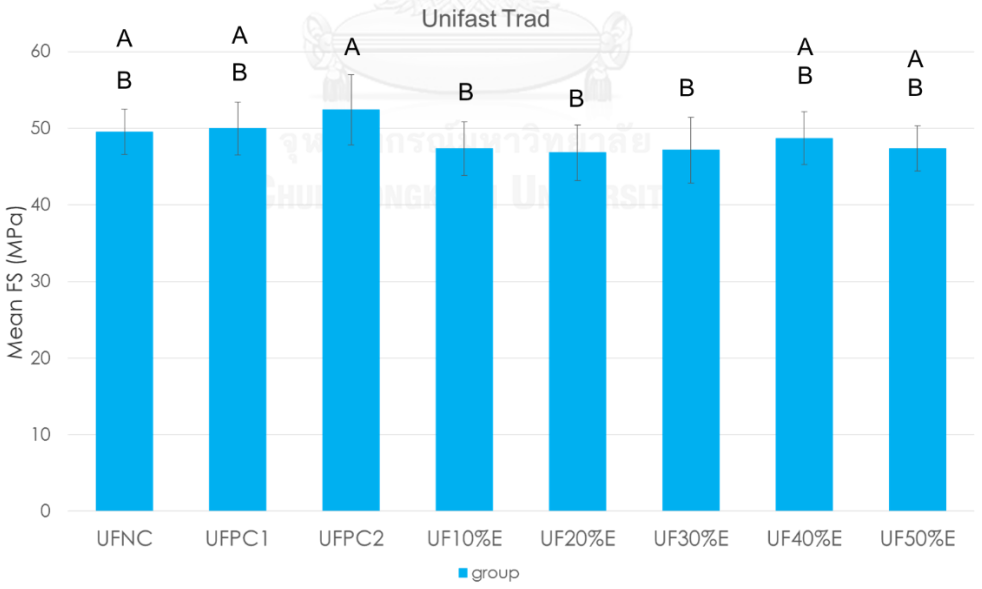


Figure 24. Means and standard deviations of flexural strength in all postpolymerization treatment groups in Unifast Trad. Identical letters denote no significant differences among groups. (p≥0.05)

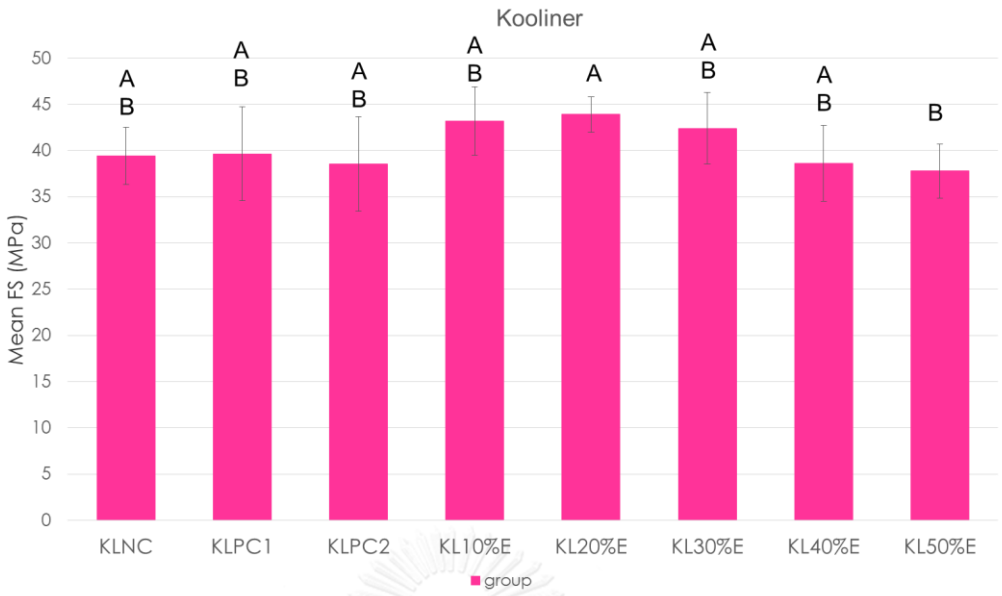


Figure 25. Means and standard deviations of flexural strength in all postpolymerization treatment groups in Kooliner. Identical letters denote no significant differences among groups. (p≥0.05)

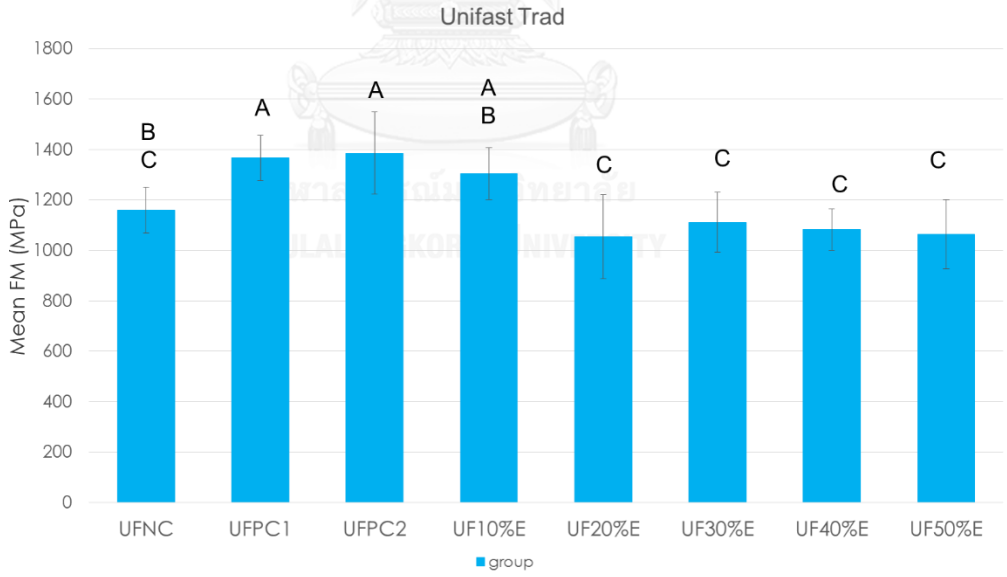


Figure 26. Means and standard deviations of flexural modulus in all postpolymerization treatment groups in Unifast Trad. Identical letters denote no significant differences among groups. (p≥0.05)

Figure 27. Means and standard deviations of flexural modulus in all postpolymerization treatment groups in Kooliner. Identical letters denote no significant differences among groups. (p≥0.05)

CHAPTER V DISCUSSION AND CONCLUSION

In the present study, the effect of concentrations of ethanol solution under ultrasonic wave as postpolymerization treatment was evaluated in order to reduce RM content in autopolymerizing hard reline acrylic resins and maintain their mechanical properties. The RM content affects the mechanical properties of resins and causes of mucosal damage. Additionally, the oxidation of methacrylate groups and the decomposition of oxygen-methacrylate copolymer chains have attributed to formation of formaldehyde, which known potent allergen (6). The maximum allowable RM content in autopolymerizing acrylic resins is 4.5 mg%, defined by ISO 20795-1 (45). The RM amount of all experimental groups passed this threshold value.

The result revealed that all postpolymerization treatment groups significantly reduced more RM content than the NC group. Hence, the first hypothesis was rejected. When all groups of postpolymerization treatment in ethanol solutions under ultrasonic wave compared with group of immersion in water 50°C 60 min (UFPC1 and KLPC1), there were significant differences in RM content of UF10%E-50%E groups. By contrast, there was no significant difference in mean of RM content between the KL10%E-50%E groups and KLPC1 group. Hence, the second hypothesis was rejected in Unifast Trad but accepted in Kooliner. When all groups of postpolymerization treatment in ethanol solutions under ultrasonic wave compared with group of immersion in water 55°C 10 min (UFPC2 and KLPC2), there were significant differences in RM content between at least one group of postpolymerization treatment with variation of ethanol solutions under ultrasonic bath and group of immersion in water 55°C 10 min. Thus, the third hypothesis was rejected.

There was no significant difference in flexural strength between all postpolymerization treatment groups and NC group. In addition, there was no significant difference in flexural strength between groups of postpolymerization treatment with ethanol solutions under ultrasonic wave and group of immersion in water 50°C 60 min. Thus, the fourth and fifth hypothesis were accepted. When all

groups of postpolymerization treatment in ethanol solutions under ultrasonic wave compared with group of immersion in water 55°C 10 min, there were significant differences of flexural strength in UF10%E-30%E groups. By contrast, there was no significant difference between the KL10%E-50%E groups and KLPC2 group. Thus the sixth hypothesis were rejected in Unifast Trad but accepted in Kooliner.

There were significant differences in flexural modulus between experimental groups and NC group in both of materials. Thus, the seventh hypothesis was rejected. When all groups of postpolymerization treatment in ethanol solutions under ultrasonic wave compared with group of immersion in water 50°C 60min (UFPC1 and KLPC1), the flexural modulus were decreased in UF20%E-50%E groups but there was no significant difference with KLPC1 group. So, the eighth hypothesis was rejected in Unifast Trad but accepted in Kooliner. The last hypotheses was also rejected because the flexural modulus were decreased in UF20%E-50%E groups and increased in KL10%E and KL20%E groups significantly when all groups of postpolymerization treatment in ethanol solutions under ultrasonic wave compared with groups of UFPC2 and KLPC2 respectively.

According to ISO, there are 2 methods to evaluate RM content; gas chromatography (GC) and high performance liquid chromatography (HPLC) (45). HPLC method was chosen in this study as this method was more sensitive and precise than GC method (46). Moreover, the cost of HPLC method was cheaper than that of GC to identify the compounds. This study focus on autopolymerizing hard reline acrylic resins; Unifast Trad and Kooliner that composed of MMA and IBMA monomers respectively. Both of them are monofunctional monomers. As the polymerization progresses, the high viscosity of polymer mixture decreases the monomer mobility resulting in unreacted methacrylate groups of monomers remain as RM content that can leach out from the polymerized material. Part of the bifunctional (dimethacrylate) monomers react only to one of the double bond of methacrylate group, resulting in pendant molecules, which remain bound within the polymer network, and are not free to leach. This may help explain why the RM levels of hard reline resins with monofunctional monomers, even though they all had the similar degree of conversion (47). Thus, postpolymerization treatment will help reducing RM of hard reline resins

with monofunctional monomers distinctly. Especially, the results in Unifast Trad reduced RM content more than Kooliner since the lower molecular weight monomer is more liable to leach from the polymer matrix than high molecular weight monomer (48). The molecular weight of MMA and IBMA are 100.18 g/mol and 142.19 g/mol respectively. Ultrasonic wave was used to be a postpolymerization treatment in autopolymerizing acrylic resins in the study of Charasseangpaisarn T. and Wiwatwarrapan C. (8) They recommended using ultrasonic wave at low frequencies (40 kHz) at 50°C for 5 min to reduce waiting time for patients because the RM content from this method was not significant differences from that of immersion in water 50°C for 60 min. High temperature has also been considered an important element in the postpolymerization treatment of acrylic resins since it seems to help furthering consumption of RM content during polymerization (26, 49). Ethanol molecules penetrate into the space between polymer chains. Ethanol molecules also swell to the polymer matrix, promoting the RM diffusion from the polymer. Moreover, the study of Neves C. et al. (2013) found that postpolymerization treatment with 50% of ethanol solution at 55±2°C 10 min promoted reducing the RM content significantly more reducing the RM content than immersion in water 55±2°C 10 min. Nevertheless, high concentration of ethanol solution does not only enhance reducing RM content but also increase the size of the inner porous, promoting changes on resins network structure (9). They found that ethanol 70% showed more reduction in RM content but they also found this group produced internal weakness of the material. In this study, we believe that proper concentration of ethanol, temperature and ultrasonic frequencies can reduce RM content effectively in autopolymerizing hard reline acrylic resins and also save waiting time for patients. Therefore, the purpose of this study to determine the proper concentration of ethanol under ultrasonic waves to decrease RM content in autopolymering hard reline acrylic resins and maintain their mechanical properties. In this study we also test flexural strength and flexural modulus to evaluate mechanical properties. The replacement of RM molecules with solvent molecules has been related to plasticizing effect. The stronger effect from the temperature of postpolymerization significantly increase in flexural strength of acrylic resins (50). Determining the most effective postpolymerization treatment should be the group

that reduces the RM content effectively while the flexural properties of the group that be chosen are not negative effects.

When compared with no postpolymerization treatment (UFNC, KLNC)

All postpolymerization treatment more reduced RM content in Unifast Trad significantly than NC group and there was no difference in mechanical properties. Immersion in water 50°C for 60 min (UFPC1) has been recommended for several years to reduce RM content in autopolymerizing acrylic resins (6). According to waste of waiting time, several studies have been searched for the method that reduce RM content properly and decrease waiting time. In addition, groups of postpolymerization treatment in ethanol solutions under ultrasonic bath showed the more concentration of ethanol used to treatment, the more RM content released. Because of the correlation between the chemistry of a solvent and monomer solubility, Hildebrand solubility parameter (δ), liquid which are similar values of δ seem to be miscible (9). The δ of the MMA and IBMA is 8.91 $(cal/cm^3)^{1/2}$ and 8.2 $(cal/cm^3)^{1/2}$ respectively, which closer to the ethanol than to the water. The δ of ethanol and water is 12.92 $(cal/cm^3)^{1/2}$ and 23.4 $(cal/cm^3)^{1/2}$ respectively (51). The UF50%E group was the most effective reducing RM content. The RM content in UF40%E group was not significantly differences in UF50%E group. However, we also considered in the flexural properties, there was no significant difference between UF40%E and UFNC groups. In Unifast Trad, the UF40%E group was the most effective postpolymerization treatment to reduce RM content and maintain flexural properties when compared with the group of no postpolymerization treatment.

The KLPC1, KL10%E, KL20%E, KL40%E and KL50%E groups reduced RM significantly when compared with the group of no postpolymerization treatment. The KL10%E group was chosen owing to efficiency to decrease RM content and maintain the flexural properties. Using less concentration of ethanol solution can save cost in clinic and avoid internal weakness in material. Even the efficiency of releasing RM in KL10%E group was not different from immersion in water 50°C for 60min group but

KL10%E group reduced more waiting time for patients and the flexural properties were not showed negative results.

When compared with immersion in water 50°C for 60 min (UFPC1, KLPC1)

In Unifast Trad, there was no significant difference in RM content among UF10%E, UF20%E and UFPC1 groups. When compared with UFPC1 group, the UF10%E group was chosen to be the method that decrease the same level of RM content as the UFPC1 group. This method was recommended to reduce RM in autopolymerizing acrylic resins (6) while the UF10%E group wasted less waiting time. Moreover, the UF10%E and UFPC1 groups had no significant difference in flexural properties. Focus on ethanol solution, we can save cost in clinic by using as least as possible of ethanol concentration to reduce RM content properly.

When all groups of postpolymerization treatment in ethanol solutions under ultrasonic wave compared with KLPC1 group, there was no significant difference in reducing the RM content between the postpolymerization and KLPC1 groups. The KL10%E group was chosen because its reduction of RM content was the same level as that of KLPC1 group, while KL10%E group maintained the flexural properties of the resins and reduced more waiting time. The KL10%E group was also the cheapest group of immersion in ethanol solution under ultrasonic wave.

When compared with immersion in water 55°C for 10min (UFPC2, KLPC2)

Immersion in water 55°C for 10min (UFPC2) has already proven to be an effective postpolymerization treatment to reduce RM content (7). In Unifast Trad, when compared with UFPC2 group, all experimental groups reduced RM content significantly except the UF20%E group. The UF10%E group was chosen to be a method that reduced RM properly when all groups of polymerization treatment in ethanol solution under ultrasonic wave groups compared with UFPC2 group. Even the flexural strength of UF10%E group was less than UFPC2 but flexural modulus was considered only the UF10%E group that still keeping the same level of flexural modulus as UFPC2. Thus, high concentration of ethanol solution may affect flexural modulus.

The KL10%E group can reduce RM as same level as KL20%E, KL40%E and KL50%E groups but there was no difference from KLPC2 group. We interested in less concentration of ethanol solution that reduced RM content properly. The KL10%E group was chosen when compared with that of KLPC2 group. The flexural strength of KL10%E group was also showed no significant difference from that of KLPC2 group. Moreover, the flexural modulus of KL10%E group was more than that of KLPC2 group due to compensated by increasing duration of heat in KL10%E group that promoted mechanical properties of the materials (9). To compared with previous study of Neves C. et al., they recommended that immersion in 50% ethanol solution 55±2°C for 10min (9). This study found that postpolymerization with ethanol solutions under ultrasonic wave reduced RM effectively owing to less concentration of ethanol and waiting time were used than previous study. Additionally, the flexural properties were maintained.

According to their benefits, autopolymerizing hard reline acrylic resins are commonly used for denture fabrication in the clinic. The results of the present study can be applied in the dental office to reduce the RM content exposure to patients. Moreover, this treatment is easy to achieve with simple equipment in a dental office and only requires a short amount of time. However, this study did not investigate other mechanical properties after postpolymerization treatment with ethanol solution under ultrasonic wave. Future studies should focus on this topic to confirm our results in autopolymerizing hard reline acrylic resins.

Conclusion

Under our experimental conditions, a postpolymerization treatment with ethanol solutions under ultrasonic wave is another method to reduce RM content of autopolymerizing hard reline acrylic resins while this method keeps their flexural properties.

-When compared with no postpolymerization treatment group.

In Unifast Trad, immersion in 40% ethanol solution under ultrasonic bath (40 kHz) at 55°C for 5min was chosen to be the most effective postpolymerization treatment because this method reduced more RM content than previous studies

effectively. Additionally, this method maintained their flexural properties and saved time in clinic.

In Kooliner, immersion in 10% ethanol solution under ultrasonic bath (40 kHz) at 55°C for 5min was chosen because this method reduced RM content as same level as immersion in water 50°C for 60min and 55°C for 10min but this method had less waiting time for patient.

-When compared with immersion in water at 50°C for 60min

In Unifast Trad, immersion in 10% ethanol solution under ultrasonic bath (40 kHz) at 55°C for 5min was chosen because this method had less waiting time to reduce RM content in the same level of immersion in water 50°C for 60min and no negative effect in flexural properties.

In Kooliner, immersion in 10% ethanol solution under ultrasonic bath (40 kHz) at 55°C for 5min was chosen because this method reduced RM content as same level as immersion in water 50°C for 60min but this method had less waiting time for patient.

-When compared with immersion in water at 55°C for 10min

In Unifast Trad, immersion in 10% ethanol solution under ultrasonic bath (40 kHz) at 55°C for 5min was chosen because this method had less waiting time to reduce RM content in the same level of immersion in water 55°C for 10min and no negative effect in flexural properties.

In Kooliner, immersion in 10% ethanol solution under ultrasonic bath (40 kHz) at 55°C for 5min was chosen because this method reduced RM content as same level as immersion in water 55°C for 10min but this method had less waiting time for patient.

REFERENCES

1. Ardelean L, Bortun C, Podariu A, Rusu L. Some alternatives for classic thermopolymerisable acrylic dentures. Materiale Plastice 2012;49:30-3.

2. Giunta JL, Graucer I, Zablotsky N. Allergic contact stomatitis caused by acrylic resin. J Prosthet Dent 1979;42:188-90.

3. Weaver RE, Goebel WM. Reactions to acrylic resin dental prostheses. J Prosthet Dent 1980;43:138-42.

4. Staffford GD, Brooks SC. The loss of residual monomer from acrylic orthodontic resins. Dent Mater 1985;1:135-8.

5. Lamb DJ. Loss into water of residual monomer from autopolymerizing dental acrylic resin. biomaterials 1982;3:155-9.

6. Tsuchiya H, Hoshino Y, Tajima K, Takagi N. Leaching and cytotoxicity of formaldehyde and methylmethacrylate from acrylic resin denture base materials J Prosthet Dent 1994;71:618-24.

7. Urban VM, Machado AL, Oliveira RV, Vergani CE, Pavarina AC, Cass QB. Residual monomer of reline acrylic resins. Effect of water-bath and microwave post-polymerization treatments. Dent Mater 2007;23:363-8.

8. Charasseangpaisarn T, Wiwatwarrapan C. The effect of various frequencies of ultrasonic cleaner in reducing residual monomer in acrylic resin. Ultrasonics. 2015;63:163-7.

9. Neves C, Lopes L, Ferrão H, Miranda J, Castro M, Bettencourt A. Ethanol Postpolymerization Treatment for Improving the Biocompatibility of Acrylic Reline Resins. BioMed Res Int 2013;1:1-9.

10. Wang LC. Ultrasonic extraction of proteins from autoclaved soybean flakes. J Food Sci 1975;40:549-51.

11. Ranu T, Saurabh G, Samarth A. Denture base materials: From past to future. Indian J Dent Sci 2010;2:33-9.

12. Rajul V, Romesh S. Denture base materials: some relevant properties and their determination. Int J Dent Oral Health 2015;1:4.

13. McCabe JF, Walls A. Applied Dental Materials. New Jesey: Willey-Blackwell; 2008.

14. Haroon R, Zeeshan S, Fahim V. Allergic effects of the residual monomer used in denture base acrylic resins. Eur J Dent 2015;9:615-9.

15. Anusavice KJ, Shen C, Rawls HR. Phillips' science of dental materials 12th ed. Amsterdam: Elsevier; 2012.

16. Lung CY, Darvell BW. Methyl methacrylate monomer–polymer equilibrium in solid polymer. Dent Mater 2007;23:88-94.

17. Stewart M, Bagby M. Clinical aspects of dental materials, theory, practice and case. Philadelphia: Lippincott Williams &Wilkins; 2013.

18. Cooper SL, Visser SA, Hergenrother RW, Lamba NK. Polymer. In: Ratner BC, Hoffman AS, Schoen FJ, Lemons JE, editors. Biomaterials Science: An introduction to materials in medicine. 2nd ed. London: Elsevier Academic Press; 2004. p. 68

19. Bettencourt AF, Neves CB, Almeida MS, Pinheiro LM, Oliveira SA, Lopes LP, et al. Biodegradation of acrylic based resins: A review. Dent Mater 2010;26:e171-80.

20. Carol H, Eakle W, Bird W. Dental Materials: Clinical Applications for Dental Assistants and Dental Hygienists. 2 ed. St. Louise, United State: Saunders Elsevier; 2011. p. 222-3.

21. Osada T, Ishimoto T, Aoki T, Suzuki Y, Takishin N, Ohkubo C, et al. Bending strengths and hardness of autopolymerized acrylic resin. Int Chin J dent 2010;10:1-5.

22. Stafford GD, Bates JF, Huggett R, Handley RW. A review of the properties of some denture base polymers. J Dent 1980;8:292-306.

23. Hickey JC, Zarb GA. Boucher’s prosthodonric treatment for edentulous patients. St. Louis: The CV Mosby Co.; 1980.

24. Smith DC. Recent developments and prospects in dental polymers. J Prosthet Dent 1962;12:1066-78.

25. Çelebi N, Yüzügüllü B, Canay Ş, Yücel Ü. Effect of polymerization methods on the residual monomer level of acrylic resin denture base polymers. Polym Adv Technol 2008;19:201-6.

26. Vallitu PK, Ruyter IE, Buykuilmaz S. Effect of polymerization temperature and time on the residual monomer content of denture base polymers. J oral Sci 1998;106:588-93.

27. Ruyter IE, Øysaed H. Conversion in denture base polymers. J Biomed Mater Res 1982;16:741-54.

28. Tsuchiya H, Hoshino Y, Kato H, Takagi N. Flow injection analysis of formaldehyde leached from denture-base acrylic resins. J Dent 1993;21:240-3.

29. Lopes MS, Azevedo Á, Teixeira A, Aires DB, Lordelo J, Mongiovi DP. Cytotoxicity of acrylic based resin compounds in a human gingival fibroblast cell line. Rev Port Estomatol Med Dent Cir Maxilofac 2013;54:87-90.

30. Nealey ET, Del Rio CE. Stomatitis venenata: reaction of a patient to acrylic resin. J Prosthet Dent 1969;21:480-4.

31. Sofou A, Tsoupi I, Emmanouil J, Karayannis M. HPLC determination of residual monomers released from heat-cured acrylic resins. Anal Bioanal Chem 2005;381:1336-46.

32. Urban VM, Cass QB, Oliveira RV, Giampaolo ET, Machado AL. Development and application of methods for determination of residual monomer in dental acrylic resins using high performance liquid chromatography. Biomed Chromatogr 2006;20:369-76.

33. Kedjarune U, Charoenworaluk N, Koontongkaew S. Release of methyl methacrylate from heat-cured and autopolymerized resins: cytotoxicity testing related to residual monomer. Aust Dent J 1999;44:25-30.

34. Azzarri MJ, Cortizoa MS, Alessandrini JL. Effect of the curing conditions on the properties of an acrylic denture base resin microwave-polymerised. J Dent 2003;31:463-8.

35. Dogan A, Bek B, Çevik NN, Usanmaz A. The effect of preparation conditions of acrylic denture base materials on the level of residual monomer, mechanical properties and water absorption. J Dent 1995;23:313-8.

36. Kuijpers MWA, Jacobs LJM, Kemmere MF, Keurentjes JTF. Influence of CO2 on ultrasound-induced polymerizations in high-pressure fluids. AIChE 2005;51:1726-32.

37. Bettencourt A, Calado A, Amaral J, Vale FM, Rico JM, Monteiro J, et al. The effect of ethanol on acrylic bone cement. Int J Pharma 2002;241:97-102.

38. Ihara I. Ultrasonic sensing : fundamentals and its applications to nondestructive evaluation. Lecture notes electrical engineering. 21. New York: Springer; 2008. p. 287-305.

39. Shakhashiri. Ethanol: Science Fun. N.; 2009. Available from: <http://scifun.chem.wisc.edu/chemweek/pdf/ethanol.pdf>.

40. Golbidi F, Asghari G. The level of residual monomer in acrylic denture base materials. Res J Bio Sci 2009;4:244-9.

41. Vallitu PK, Miettinen V, Alakuijara P. Residual monomer content and its release into water from denture base materials. Dent Mater 1995;11:338-42.

42. Shim JS, Watts DC. Residual monomer concentrations in denture-base acrylic resin. Dent Mater 1999;15:296–300.

43. Beckett AH, Stenlake JB. Practical pharmaceutical chemistry: Part II 4th ed. London: A&C Black; 1988.

44. Martin M, Guiochon G. Effects of high pressures in liquid chromatography. J Chromatogr A 2005;1090:16-38.

45. ISO. 20795-1 Dentistry Base Polymers part 1: Denture Base Polymers. International Standards Organization: Geneva, 2013.

46. Phillips DL, Tebbett IR, Bertholf RL. Comparison of HPLC and GC-MS for Measurement of Cocaine and Metabolites in Human Urine. J Anal Toxicol 1996;20:305-8.

47. Urban VM, Machado AL, Vergani CE, Jorge EG, Santoes LPS, Leite ER, et al. Degree of conversion and molecular weight of one denture base and three reline resins submitted to post-polymerization treatment. Mat Res 2007;10:191-7.

48. Jones DW, Sutow EJ, Hall GC, Tobin WM, Graham BS. Dental soft polymers: plasticizer composition and leachability. Dent Mater 1988;4:1-7.

49. Araújo PH, Sayer C, Poço JG, Giudici R. Techniques for reducing residual monomer content in polymers: a review. Polym Eng Sci 2002;42:1442-68.

50. Ozkomur A, Fortes CB. Effects of postpolymerization microwave irradiation on provisional dental acrylics: physical and mechanical properties. J Appl Biomater Funct Mater 2016;14:e302-6.

51. Belmares M, Blanco M, Goddard III WA, Ross RB, Caldwell G, Chou SH, et al. Hildebrand and Hansen solubility parameters from molecular dynamics with applications to electronic nose polymer sensors. J Comput Chem 2004;25:1814-26.

APPENDIX

Table7. One-Sample Kolmogorov-Smirnov test analysis of the data distribution (RM content in Unifast Trad)

Group	Code	N	Asymp. Sig. (2-tailed)
1	UFNC	6	0.999
2	UFPC1	6	0.997
3	UFPC2	6	0.996
4	UF10%E	6	0.990
5	UF20%E	6	0.996
6	UF30%E	6	0.963
7	UF40%E	6	0.884
8	UF50%E	6	0.996

Table8. The Levene Statistical analysis of RM content in Unifast Trad

Test of Homogeneity of Variances

RM

Levene Statistic	df1	df2	Sig.
.790	7	40	.600

Table9. One-way ANOVA analysis of RM content in Unifast Trad

ANOVA

RM

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	17.960	7	2.566	140.295	.000
Within Groups	.732	40	.018		
Total	18.692	47			

Table10. Multiple comparisons analysis of RM content in Unifast Trad

Multiple Comparisons

Dependent Variable:RM

	(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	control	water50c 60mins	.952833*	.078078	.000	.70326	1.20241
		water55c 10mins	.579167*	.078078	.000	.32959	.82874
		ethanol10	.882500*	.078078	.000	.63292	1.13208
		ethanol20	.726333*	.078078	.000	.47676	.97591
		ethanol30	1.443167*	.078078	.000	1.19359	1.69274
		ethanol40	1.831667*	.078078	.000	1.58209	2.08124
		ethanol50	1.922833*	.078078	.000	1.67326	2.17241
	water50c 60mins	control	-.952833*	.078078	.000	-1.20241	-.70326
		water55c 10mins	-.373667*	.078078	.001	-.62324	-.12409
		ethanol10	-.070333	.078078	.984	-.31991	.17924
		ethanol20	-.226500	.078078	.099	-.47608	.02308
		ethanol30	.490333*	.078078	.000	.24076	.73991
		ethanol40	.878833*	.078078	.000	.62926	1.12841
		ethanol50	.970000*	.078078	.000	.72042	1.21958
	water55c 10mins	control	-.579167*	.078078	.000	-.82874	-.32959
		water50c 60mins	.373667*	.078078	.001	.12409	.62324
		ethanol10	.303333*	.078078	.008	.05376	.55291
		ethanol20	.147167	.078078	.569	-.10241	.39674
		ethanol30	.864000*	.078078	.000	.61442	1.11358
		ethanol40	1.252500*	.078078	.000	1.00292	1.50208
		ethanol50	1.343667*	.078078	.000	1.09409	1.59324
	ethanol10	control	-.882500*	.078078	.000	-1.13208	-.63292
		water50c 60mins	.070333	.078078	.984	-.17924	.31991
		water55c 10mins	-.303333*	.078078	.008	-.55291	-.05376
		ethanol20	-.156167	.078078	.494	-.40574	.09341
		ethanol30	.560667*	.078078	.000	.31109	.81024
		ethanol40	.949167*	.078078	.000	.69959	1.19874
		ethanol50	1.040333*	.078078	.000	.79076	1.28991

ethanol20	control	-.726333	.078078	.000	-.97591	-.47676
	water50c 60mins	.226500	.078078	.099	-.02308	.47608
	water55c 10mins	-.147167	.078078	.569	-.39674	.10241
	ethanol10	.156167	.078078	.494	-.09341	.40574
	ethanol30	.716833*	.078078	.000	.46726	.96641
	ethanol40	1.105333*	.078078	.000	.85576	1.35491
	ethanol50	1.196500*	.078078	.000	.94692	1.44608
ethanol30	control	-1.443167*	.078078	.000	-1.69274	-1.19359
	water50c 60mins	-.490333*	.078078	.000	-.73991	-.24076
	water55c 10mins	-.864000*	.078078	.000	-1.11358	-.61442
	ethanol10	-.560667*	.078078	.000	-.81024	-.31109
	ethanol20	-.716833*	.078078	.000	-.96641	-.46726
	ethanol40	.388500*	.078078	.000	.13892	.63808
	ethanol50	.479667*	.078078	.000	.23009	.72924
ethanol40	control	-1.831667*	.078078	.000	-2.08124	-1.58209
	water50c 60mins	-.878833*	.078078	.000	-1.12841	-.62926
	water55c 10mins	-1.252500*	.078078	.000	-1.50208	-1.00292
	ethanol10	-.949167*	.078078	.000	-1.19874	-.69959
	ethanol20	-1.105333*	.078078	.000	-1.35491	-.85576
	ethanol30	-.388500*	.078078	.000	-.63808	-.13892
	ethanol50	.091167	.078078	.936	-.15841	.34074
ethanol50	control	-1.922833*	.078078	.000	-2.17241	-1.67326
	water50c 60mins	-.970000*	.078078	.000	-1.21958	-.72042
	water55c 10mins	-1.343667*	.078078	.000	-1.59324	-1.09409
	ethanol10	-1.040333*	.078078	.000	-1.28991	-.79076
	ethanol20	-1.196500*	.078078	.000	-1.44608	-.94692
	ethanol30	-.479667*	.078078	.000	-.72924	-.23009
	ethanol40	-.091167	.078078	.936	-.34074	.15841

Table11. Tukey's HSD analysis of RM content in Unifast Trad

RM

	group	N	Subset for alpha = 0.05				
			1	2	3	4	5
Tukey HSD[a]	ethanol50	6	1.16383				
	ethanol40	6	1.25500				
	ethanol30	6		1.64350			
	water50c 60mins	6			2.13383		
	ethanol10	6			2.20417		
	ethanol20	6			2.36033	2.36033	
	water55c 10mins	6				2.50750	
	control	6					3.08667
	Sig.		.936	1.000	.099	.569	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Table12. One-Sample Kolmogorov-Smirnov test analysis of the data distribution (RM content in Kooliner)

Group	Code	N	Asymp. Sig. (2-tailed)
1	KLNC	6	0.987
2	KLPC1	6	0.811
3	KLPC2	6	0.986
4	KL10%E	6	0.960
5	KL20%E	6	0.900
6	KL30%E	6	0.861
7	KL40%E	6	0.996
8	KL50%E	6	0.811

Table13. The Levene Statistical analysis of RM content in Kooliner

Test of Homogeneity of Variances

percent_monomers

Levene Statistic	df1	df2	Sig.
2.094	7	40	.067

Table14. One-way ANOVA analysis of RM content in Kooliner

ANOVA

percent_monomers

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.390	7	.484	8.009	.000
Within Groups	2.419	40	.060		
Total	5.809	47			

Table15. Multiple comparison analysis of RM content in Kooliner

Multiple Comparisons

Dependent Variable: percent_monomers

	(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	control	water50c60m	.702500*	.141978	.000	.24867	1.15633
		water55c10m	.246167	.141978	.666	-.20766	.70000
		ethanol10	.572833*	.141978	.005	.11900	1.02666
		ethanol20	.685500*	.141978	.001	.23167	1.13933
		ethanol30	.420833	.141978	.086	-.03300	.87466
		ethanol40	.641167*	.141978	.001	.18734	1.09500
		ethanol50	.882833*	.141978	.000	.42900	1.33666
	water50c60m	control	-.702500*	.141978	.000	-1.15633	-.24867
		water55c10m	-.456333*	.141978	.048	-.91016	-.00250
		ethanol10	-.129667	.141978	.983	-.58350	.32416
		ethanol20	-.017000	.141978	1.000	-.47083	.43683
		ethanol30	-.281667	.141978	.505	-.73550	.17216
		ethanol40	-.061333	.141978	1.000	-.51516	.39250
		ethanol50	.180333	.141978	.904	-.27350	.63416

water55c10m	control	-.246167	.141978	.666	-.70000	.20766
	water50c60m	.456333*	.141978	.048	.00250	.91016
	ethanol10	.326667	.141978	.318	-.12716	.78050
	ethanol20	.439333	.141978	.064	-.01450	.89316
	ethanol30	.174667	.141978	.918	-.27916	.62850
	ethanol40	.395000	.141978	.128	-.05883	.84883
	ethanol50	.636667*	.141978	.001	.18284	1.09050
ethanol10	control	-.572833*	.141978	.005	-1.02666	-.11900
	water50c60m	.129667	.141978	.983	-.32416	.58350
	water55c10m	-.326667	.141978	.318	-.78050	.12716
	ethanol20	.112667	.141978	.993	-.34116	.56650
	ethanol30	-.152000	.141978	.959	-.60583	.30183
	ethanol40	.068333	.141978	1.000	-.38550	.52216
	ethanol50	.310000	.141978	.383	-.14383	.76383
ethanol20	control	-.685500*	.141978	.001	-1.13933	-.23167
	water50c60m	.017000	.141978	1.000	-.43683	.47083
	water55c10m	-.439333	.141978	.064	-.89316	.01450
	ethanol10	-.112667	.141978	.993	-.56650	.34116
	ethanol30	-.264667	.141978	.582	-.71850	.18916
	ethanol40	-.044333	.141978	1.000	-.49816	.40950
	ethanol50	.197333	.141978	.856	-.25650	.65116
ethanol30	control	-.420833	.141978	.086	-.87466	.03300
	water50c60m	.281667	.141978	.505	-.17216	.73550
	water55c10m	-.174667	.141978	.918	-.62850	.27916
	ethanol10	.152000	.141978	.959	-.30183	.60583
	ethanol20	.264667	.141978	.582	-.18916	.71850
	ethanol40	.220333	.141978	.775	-.23350	.67416
	ethanol50	.462000*	.141978	.043	.00817	.91583
ethanol40	control	-.641167*	.141978	.001	-1.09500	-.18734
	water50c60m	.061333	.141978	1.000	-.39250	.51516
	water55c10m	-.395000	.141978	.128	-.84883	.05883
	ethanol10	-.068333	.141978	1.000	-.52216	.38550
	ethanol20	.044333	.141978	1.000	-.40950	.49816
	ethanol30	-.220333	.141978	.775	-.67416	.23350
	ethanol50	.241667	.141978	.686	-.21216	.69550
ethanol50	control	-.882833*	.141978	.000	-1.33666	-.42900
	water50c60m	-.180333	.141978	.904	-.63416	.27350
	water55c10m	-.636667*	.141978	.001	-1.09050	-.18284
	ethanol10	-.310000	.141978	.383	-.76383	.14383
	ethanol20	-.197333	.141978	.856	-.65116	.25650
	ethanol30	-.462000*	.141978	.043	-.91583	-.00817
	ethanol40	-.241667	.141978	.686	-.69550	.21216

Table16. Tukey's HSD analysis of RM content in Kooliner

percent_monomers

	group	N	Subset for alpha = 0.05			
			1	2	3	4
Tukey HSD[a]	ethanol50	6	1.37533			
	water50c60m	6	1.55567	1.55567		
	ethanol20	6	1.57267	1.57267	1.57267	
	ethanol40	6	1.61700	1.61700	1.61700	
	ethanol10	6	1.68533	1.68533	1.68533	
	ethanol30	6		1.83733	1.83733	1.83733
	water55c10m	6			2.01200	2.01200
	control	6				2.25817
	Sig.		.383	.505	.064	.086

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

Table17. One-Sample Kolmogorov-Smirnov test analysis of the data distribution (flexural properties in Unifast Trad)

Group	Code	N	Asymp. Sig. (2-tailed)(FS)	Asymp. Sig. (2-tailed)(FM)
1	UFNC	10	0.760	0.993
2	UFPC1	10	0.944	0.863
3	UFPC2	10	0.944	0.999
4	UF10%E	10	0.757	0.958
5	UF20%E	10	0.492	0.831
6	UF30%E	10	0.556	0.840
7	UF40%E	10	0.436	0.981
8	UF50%E	10	0.918	0.949

Table18. The Levene Statistical analysis of flexural strength in Unifast Trad

Test of Homogeneity of Variances

FS

Levene Statistic	df1	df2	Sig.
.724	7	72	.652

Table19. The Levene Statistical analysis of flexural modulus in Unifast Trad

Test of Homogeneity of Variances

FM

Levene Statistic	df1	df2	Sig.
.940	7	72	.482

Table20. One-way ANOVA analysis of flexural strength in Unifast Trad

ANOVA

FS

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	256.294	7	36.613	2.773	.013
Within Groups	950.728	72	13.205		
Total	1207.022	79			

Table21. One-way ANOVA analysis of flexural modulus in Unifast Trad

ANOVA

FM

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1354122.984	7	193446.141	12.734	.000
Within Groups	1093748.437	72	15190.951		
Total	2447871.420	79			

Table22. Multiple comparison analysis of flexural strength in Unifast Trad

Multiple Comparisons

Dependent Variable:FS

	(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	control	water50c60m	-.449500	1.625088	1.000	-5.52271	4.62371
		water55c10m	-2.874500	1.625088	.643	-7.94771	2.19871
		ethanol10	2.203200	1.625088	.874	-2.87001	7.27641
		ethanol20	2.728100	1.625088	.701	-2.34511	7.80131
		ethanol30	2.366700	1.625088	.827	-2.70651	7.43991
		ethanol40	.844800	1.625088	1.000	-4.22841	5.91801
		ethanol50	2.150400	1.625088	.887	-2.92281	7.22361
	water50c60m	control	.449500	1.625088	1.000	-4.62371	5.52271
		water55c10m	-2.425000	1.625088	.809	-7.49821	2.64821
		ethanol10	2.652700	1.625088	.729	-2.42051	7.72591
		ethanol20	3.177600	1.625088	.519	-1.89561	8.25081
		ethanol30	2.816200	1.625088	.666	-2.25701	7.88941
		ethanol40	1.294300	1.625088	.993	-3.77891	6.36751
		ethanol50	2.599900	1.625088	.749	-2.47331	7.67311
	water55c10m	control	2.874500	1.625088	.643	-2.19871	7.94771
		water50c60m	2.425000	1.625088	.809	-2.64821	7.49821
		ethanol10	5.077700*	1.625088	.050	.00449	10.15091
		ethanol20	5.602600*	1.625088	.020	.52939	10.67581
		ethanol30	5.241200*	1.625088	.038	.16799	10.31441
		ethanol40	3.719300	1.625088	.314	-1.35391	8.79251
		ethanol50	5.024900	1.625088	.054	-.04831	10.09811

ethanol10	control	-2.203200	1.625088	.874	-7.27641	2.87001
	water50c60m	-2.652700	1.625088	.729	-7.72591	2.42051
	water55c10m	-5.077700*	1.625088	.050	-10.15091	-.00449
	ethanol20	.524900	1.625088	1.000	-4.54831	5.59811
	ethanol30	.163500	1.625088	1.000	-4.90971	5.23671
	ethanol40	-1.358400	1.625088	.990	-6.43161	3.71481
	ethanol50	-.052800	1.625088	1.000	-5.12601	5.02041
ethanol20	control	-2.728100	1.625088	.701	-7.80131	2.34511
	water50c60m	-3.177600	1.625088	.519	-8.25081	1.89561
	water55c10m	-5.602600*	1.625088	.020	-10.67581	-.52939
	ethanol10	-.524900	1.625088	1.000	-5.59811	4.54831
	ethanol30	-.361400	1.625088	1.000	-5.43461	4.71181
	ethanol40	-1.883300	1.625088	.941	-6.95651	3.18991
	ethanol50	-.577700	1.625088	1.000	-5.65091	4.49551
ethanol30	control	-2.366700	1.625088	.827	-7.43991	2.70651
	water50c60m	-2.816200	1.625088	.666	-7.88941	2.25701
	water55c10m	-5.241200*	1.625088	.038	-10.31441	-.16799
	ethanol10	-.163500	1.625088	1.000	-5.23671	4.90971
	ethanol20	.361400	1.625088	1.000	-4.71181	5.43461
	ethanol40	-1.521900	1.625088	.981	-6.59511	3.55131
	ethanol50	-.216300	1.625088	1.000	-5.28951	4.85691
ethanol40	control	-.844800	1.625088	1.000	-5.91801	4.22841
	water50c60m	-1.294300	1.625088	.993	-6.36751	3.77891
	water55c10m	-3.719300	1.625088	.314	-8.79251	1.35391
	ethanol10	1.358400	1.625088	.990	-3.71481	6.43161
	ethanol20	1.883300	1.625088	.941	-3.18991	6.95651
	ethanol30	1.521900	1.625088	.981	-3.55131	6.59511
	ethanol50	1.305600	1.625088	.992	-3.76761	6.37881
ethanol50	control	-2.150400	1.625088	.887	-7.22361	2.92281
	water50c60m	-2.599900	1.625088	.749	-7.67311	2.47331
	water55c10m	-5.024900	1.625088	.054	-10.09811	.04831
	ethanol10	.052800	1.625088	1.000	-5.02041	5.12601
	ethanol20	.577700	1.625088	1.000	-4.49551	5.65091
	ethanol30	.216300	1.625088	1.000	-4.85691	5.28951
	ethanol40	-1.305600	1.625088	.992	-6.37881	3.76761

Table23. Multiple comparison analysis of flexural modulus in Unifast Trad

Multiple Comparisons

Dependent Variable:FM

	(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	control	water50c60m	-207.556900*	55.119780	.008	-379.63031	-35.48349
		water55c10m	-226.301900*	55.119780	.003	-398.37531	-54.22849
		ethanol10	-144.646800	55.119780	.164	-316.72021	27.42661
		ethanol20	105.322100	55.119780	.548	-66.75131	277.39551
		ethanol30	47.718400	55.119780	.988	-124.35501	219.79181
		ethanol40	76.345800	55.119780	.861	-95.72761	248.41921
		ethanol50	95.180500	55.119780	.670	-76.89291	267.25391
	water50c60m	control	207.556900*	55.119780	.008	35.48349	379.63031
		water55c10m	-18.745000	55.119780	1.000	-190.81841	153.32841
		ethanol10	62.910100	55.119780	.945	-109.16331	234.98351
		ethanol20	312.879000*	55.119780	.000	140.80559	484.95241
		ethanol30	255.275300*	55.119780	.000	83.20189	427.34871
		ethanol40	283.902700*	55.119780	.000	111.82929	455.97611
		ethanol50	302.737400*	55.119780	.000	130.66399	474.81081
	water55c10m	control	226.301900*	55.119780	.003	54.22849	398.37531
		water50c60m	18.745000	55.119780	1.000	-153.32841	190.81841
		ethanol10	81.655100	55.119780	.815	-90.41831	253.72851
		ethanol20	331.624000*	55.119780	.000	159.55059	503.69741
		ethanol30	274.020300*	55.119780	.000	101.94689	446.09371
		ethanol40	302.647700*	55.119780	.000	130.57429	474.72111
		ethanol50	321.482400*	55.119780	.000	149.40899	493.55581
	ethanol10	control	144.646800	55.119780	.164	-27.42661	316.72021
		water50c60m	-62.910100	55.119780	.945	-234.98351	109.16331
		water55c10m	-81.655100	55.119780	.815	-253.72851	90.41831
		ethanol20	249.968900*	55.119780	.001	77.89549	422.04231
		ethanol30	192.365200*	55.119780	.018	20.29179	364.43861
		ethanol40	220.992600*	55.119780	.004	48.91919	393.06601
		ethanol50	239.827300*	55.119780	.001	67.75389	411.90071
	ethanol20	control	-105.322100	55.119780	.548	-277.39551	66.75131
		water50c60m	-312.879000*	55.119780	.000	-484.95241	-140.80559
		water55c10m	-331.624000*	55.119780	.000	-503.69741	-159.55059
		ethanol10	-249.968900*	55.119780	.001	-422.04231	-77.89549
		ethanol30	-57.603700	55.119780	.966	-229.67711	114.46971
		ethanol40	-28.976300	55.119780	.999	-201.04971	143.09711
		ethanol50	-10.141600	55.119780	1.000	-182.21501	161.93181
	ethanol30	control	-47.718400	55.119780	.988	-219.79181	124.35501
		water50c60m	-255.275300*	55.119780	.000	-427.34871	-83.20189
		water55c10m	-274.020300*	55.119780	.000	-446.09371	-101.94689
		ethanol10	-192.365200*	55.119780	.018	-364.43861	-20.29179
		ethanol20	57.603700	55.119780	.966	-114.46971	229.67711
		ethanol40	28.627400	55.119780	1.000	-143.44601	200.70081
		ethanol50	47.462100	55.119780	.989	-124.61131	219.53551

	ethanol40	control	-76.345800	55.119780	.861	-248.41921	95.72761
		water50c60m	-283.902700*	55.119780	.000	-455.97611	-111.82929
		water55c10m	-302.647700*	55.119780	.000	-474.72111	-130.57429
		ethanol10	-220.992600*	55.119780	.004	-393.06601	-48.91919
		ethanol20	28.976300	55.119780	.999	-143.09711	201.04971
		ethanol30	-28.627400	55.119780	1.000	-200.70081	143.44601
		ethanol50	18.834700	55.119780	1.000	-153.23871	190.90811
	ethanol50	control	-95.180500	55.119780	.670	-267.25391	76.89291
		water50c60m	-302.737400*	55.119780	.000	-474.81081	-130.66399
		water55c10m	-321.482400*	55.119780	.000	-493.55581	-149.40899
		ethanol10	-239.827300*	55.119780	.001	-411.90071	-67.75389
		ethanol20	10.141600	55.119780	1.000	-161.93181	182.21501
		ethanol30	-47.462100	55.119780	.989	-219.53551	124.61131
		ethanol40	-18.834700	55.119780	1.000	-190.90811	153.23871

Table24. Tukey's HSD analysis of flexural strength in Unifast Trad

FS

			Subset for alpha = 0.05	
	group	N	1	2
Tukey HSD[a]	ethanol20	10	46.79840	
	ethanol30	10	47.15980	
	ethanol10	10	47.32330	
	ethanol50	10	47.37610	47.37610
	ethanol40	10	48.68170	48.68170
	control	10	49.52650	49.52650
	water50c60m	10	49.97600	49.97600
	water55c10m	10		52.40100
	Sig.		.519	.054

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.000.

Table25. Tukey's HSD analysis of flexural modulus in Unifast Trad

FM

	group	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	ethanol20	10	1053.81210		
	ethanol50	10	1063.95370		
	ethanol40	10	1082.78840		
	ethanol30	10	1111.41580		
	control	10	1159.13420	1159.13420	
	ethanol10	10		1303.78100	1303.78100
	water50c60m	10			1366.69110
	water55c10m	10			1385.43610
	Sig.		.548	.164	.815

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.000.

Table26. One-Sample Kolmogorov-Smirnov test analysis of the data distribution (flexural properties in Kooliner)

Group	Code	N	Asymp. Sig. (2-tailed)(FS)	Asymp. Sig. (2-tailed)(FM)
1	KLNC	10	0.979	0.911
2	KLPC1	10	0.997	0.979
3	KLPC2	10	0.992	0.968
4	KL10%E	10	0.756	0.847
5	KL20%E	10	0.990	0.971
6	KL30%E	10	0.910	0.945
7	KL40%E	10	0.984	0.986
8	KL50%E	10	0.988	1.000

Table27. The Levene Statistical analysis of flexural properties in Kooliner

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
FS	1.578	7	72	.156
FM	1.611	7	72	.146

Table28. One-way ANOVA analysis of flexural properties in Kooliner

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
FS	Between Groups	390.111	7	55.730	3.124	.006
	Within Groups	1284.241	72	17.837		
	Total	1674.351	79			
FM	Between Groups	1341760.483	7	191680.069	4.297	.001
	Within Groups	3211490.517	72	44604.035		
	Total	4553251.000	79			

Table29. Multiple comparison analysis of flexural strength in Kooliner

Multiple Comparisons

Dependent Variable		(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
FS	Tukey HSD	control	water50c60m	-.223600	1.888739	1.000	-6.11988	5.67268
			water55c10m	.886600	1.888739	1.000	-5.00968	6.78288
			ethanol10	-3.757200	1.888739	.496	-9.65348	2.13908
			ethanol20	-4.487800	1.888739	.269	-10.38408	1.40848
			ethanol30	-2.982700	1.888739	.761	-8.87898	2.91358
			ethanol40	.809600	1.888739	1.000	-5.08668	6.70588
			ethanol50	1.622700	1.888739	.989	-4.27358	7.51898
		water50c60m	control	.223600	1.888739	1.000	-5.67268	6.11988
			water55c10m	1.110200	1.888739	.999	-4.78608	7.00648
			ethanol10	-3.533600	1.888739	.575	-9.42988	2.36268
			ethanol20	-4.264200	1.888739	.331	-10.16048	1.63208
			ethanol30	-2.759100	1.888739	.825	-8.65538	3.13718
			ethanol40	1.033200	1.888739	.999	-4.86308	6.92948
			ethanol50	1.846300	1.888739	.976	-4.04998	7.74258

water55c10m	control	-.886600	1.888739	1.000	-6.78288	5.00968
	water50c60m	-1.110200	1.888739	.999	-7.00648	4.78608
	ethanol10	-4.643800	1.888739	.230	-10.54008	1.25248
	ethanol20	-5.374400	1.888739	.100	-11.27068	.52188
	ethanol30	-3.869300	1.888739	.458	-9.76558	2.02698
	ethanol40	-.077000	1.888739	1.000	-5.97328	5.81928
	ethanol50	.736100	1.888739	1.000	-5.16018	6.63238
ethanol10	control	3.757200	1.888739	.496	-2.13908	9.65348
	water50c60m	3.533600	1.888739	.575	-2.36268	9.42988
	water55c10m	4.643800	1.888739	.230	-1.25248	10.54008
	ethanol20	-.730600	1.888739	1.000	-6.62688	5.16568
	ethanol30	.774500	1.888739	1.000	-5.12178	6.67078
	ethanol40	4.566800	1.888739	.249	-1.32948	10.46308
	ethanol50	5.379900	1.888739	.099	-.51638	11.27618
ethanol20	control	4.487800	1.888739	.269	-1.40848	10.38408
	water50c60m	4.264200	1.888739	.331	-1.63208	10.16048
	water55c10m	5.374400	1.888739	.100	-.52188	11.27068
	ethanol10	.730600	1.888739	1.000	-5.16568	6.62688
	ethanol30	1.505100	1.888739	.993	-4.39118	7.40138
	ethanol40	5.297400	1.888739	.110	-.59888	11.19368
	ethanol50	6.110500*	1.888739	.037	.21422	12.00678
ethanol30	control	2.982700	1.888739	.761	-2.91358	8.87898
	water50c60m	2.759100	1.888739	.825	-3.13718	8.65538
	water55c10m	3.869300	1.888739	.458	-2.02698	9.76558
	ethanol10	-.774500	1.888739	1.000	-6.67078	5.12178
	ethanol20	-1.505100	1.888739	.993	-7.40138	4.39118
	ethanol40	3.792300	1.888739	.484	-2.10398	9.68858
	ethanol50	4.605400	1.888739	.239	-1.29088	10.50168
ethanol40	control	-.809600	1.888739	1.000	-6.70588	5.08668
	water50c60m	-1.033200	1.888739	.999	-6.92948	4.86308
	water55c10m	.077000	1.888739	1.000	-5.81928	5.97328
	ethanol10	-4.566800	1.888739	.249	-10.46308	1.32948
	ethanol20	-5.297400	1.888739	.110	-11.19368	.59888
	ethanol30	-3.792300	1.888739	.484	-9.68858	2.10398
	ethanol50	.813100	1.888739	1.000	-5.08318	6.70938
ethanol50	control	-1.622700	1.888739	.989	-7.51898	4.27358
	water50c60m	-1.846300	1.888739	.976	-7.74258	4.04998
	water55c10m	-.736100	1.888739	1.000	-6.63238	5.16018
	ethanol10	-5.379900	1.888739	.099	-11.27618	.51638
	ethanol20	-6.110500*	1.888739	.037	-12.00678	-.21422
	ethanol30	-4.605400	1.888739	.239	-10.50168	1.29088
	ethanol40	-.813100	1.888739	1.000	-6.70938	5.08318

Table30. Multiple comparison analysis of flexural modulus in Kooliner

FM	Tukey HSD	control	water50c60m	-142.878900	94.450024	.798	-437.73380	151.97600
			water55c10m	26.902200	94.450024	1.000	-267.95270	321.75710
			ethanol10	-334.692800*	94.450024	.015	-629.54770	-39.83790
			ethanol20	-290.674700	94.450024	.056	-585.52960	4.18020
			ethanol30	-234.473200	94.450024	.220	-529.32810	60.38170
			ethanol40	-52.451100	94.450024	.999	-347.30600	242.40380
			ethanol50	-40.816800	94.450024	1.000	-335.67170	254.03810
		water50c60m	control	142.878900	94.450024	.798	-151.97600	437.73380
			water55c10m	169.781100	94.450024	.624	-125.07380	464.63600
			ethanol10	-191.813900	94.450024	.469	-486.66880	103.04100
			ethanol20	-147.795800	94.450024	.769	-442.65070	147.05910
			ethanol30	-91.594300	94.450024	.977	-386.44920	203.26060
			ethanol40	90.427800	94.450024	.979	-204.42710	385.28270
			ethanol50	102.062100	94.450024	.959	-192.79280	396.91700
		water55c10m	control	-26.902200	94.450024	1.000	-321.75710	267.95270
			water50c60m	-169.781100	94.450024	.624	-464.63600	125.07380
			ethanol10	-361.595000*	94.450024	.006	-656.44990	-66.74010
			ethanol20	-317.576900*	94.450024	.026	-612.43180	-22.72200
			ethanol30	-261.375400	94.450024	.120	-556.23030	33.47950
			ethanol40	-79.353300	94.450024	.990	-374.20820	215.50160
			ethanol50	-67.719000	94.450024	.996	-362.57390	227.13590
		ethanol10	control	334.692800*	94.450024	.015	39.83790	629.54770
			water50c60m	191.813900	94.450024	.469	-103.04100	486.66880
			water55c10m	361.595000*	94.450024	.006	66.74010	656.44990
			ethanol20	44.018100	94.450024	1.000	-250.83680	338.87300
			ethanol30	100.219600	94.450024	.963	-194.63530	395.07450
			ethanol40	282.241700	94.450024	.071	-12.61320	577.09660
			ethanol50	293.876000	94.450024	.051	-.97890	588.73090
		ethanol20	control	290.674700	94.450024	.056	-4.18020	585.52960
			water50c60m	147.795800	94.450024	.769	-147.05910	442.65070
			water55c10m	317.576900*	94.450024	.026	22.72200	612.43180
			ethanol10	-44.018100	94.450024	1.000	-338.87300	250.83680
			ethanol30	56.201500	94.450024	.999	-238.65340	351.05640
			ethanol40	238.223600	94.450024	.203	-56.63130	533.07850
			ethanol50	249.857900	94.450024	.157	-44.99700	544.71280
		ethanol30	control	234.473200	94.450024	.220	-60.38170	529.32810
			water50c60m	91.594300	94.450024	.977	-203.26060	386.44920
			water55c10m	261.375400	94.450024	.120	-33.47950	556.23030
			ethanol10	-100.219600	94.450024	.963	-395.07450	194.63530
			ethanol20	-56.201500	94.450024	.999	-351.05640	238.65340
			ethanol40	182.022100	94.450024	.537	-112.83280	476.87700
			ethanol50	193.656400	94.450024	.456	-101.19850	488.51130

	ethanol40	control	52.451100	94.450024	.999	-242.40380	347.30600
		water50c60m	-90.427800	94.450024	.979	-385.28270	204.42710
		water55c10m	79.353300	94.450024	.990	-215.50160	374.20820
		ethanol10	-282.241700	94.450024	.071	-577.09660	12.61320
		ethanol20	-238.223600	94.450024	.203	-533.07850	56.63130
		ethanol30	-182.022100	94.450024	.537	-476.87700	112.83280
		ethanol50	11.634300	94.450024	1.000	-283.22060	306.48920
	ethanol50	control	40.816800	94.450024	1.000	-254.03810	335.67170
		water50c60m	-102.062100	94.450024	.959	-396.91700	192.79280
		water55c10m	67.719000	94.450024	.996	-227.13590	362.57390
		ethanol10	-293.876000	94.450024	.051	-588.73090	.97890
		ethanol20	-249.857900	94.450024	.157	-544.71280	44.99700
		ethanol30	-193.656400	94.450024	.456	-488.51130	101.19850
		ethanol40	-11.634300	94.450024	1.000	-306.48920	283.22060

Table30. Tukey's HSD analysis of flexural strength in Kooliner

FS

			Subset for alpha = 0.05	
	group	N	1	2
Tukey HSD[a]	ethanol50	10	37.79440	
	water55c10m	10	38.53050	38.53050
	ethanol40	10	38.60750	38.60750
	control	10	39.41710	39.41710
	water50c60m	10	39.64070	39.64070
	ethanol30	10	42.39980	42.39980
	ethanol10	10	43.17430	43.17430
	ethanol20	10		43.90490
	Sig.		.099	.100

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.000.

Table31. Tukey's HSD analysis of flexural modulus in Kooliner

FM

	group	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	water55c10m	10	858.36010		
	control	10	885.26230	885.26230	
	ethanol50	10	926.07910	926.07910	926.07910
	ethanol40	10	937.71340	937.71340	937.71340
	water50c60m	10	1028.14120	1028.14120	1028.14120
	ethanol30	10	1119.73550	1119.73550	1119.73550
	ethanol20	10		1175.93700	1175.93700
	ethanol10	10			1219.95510
	Sig.		.120	.056	.051

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.000.

VITA

Miss Nattha Kobnithikulwong was born on Sunday 28th August 1988 in Bangkok, Thailand. She has two elder sisters. She graduated from Raywadee school in Primary School and TriumUdom Suksa school in High-school. Then, she graduated the degree of Doctor of Dental Surgery (D.D.S.) from Faculty of Dentistry, Chulalongkorn University in 2012. After that she has worked as a dentist at Dental department in Huai Thalaeng Hospital, Nakhon Ratchasima, Thailand for 1 year. Then she is permitted to attend the course of Master of Science in Prosthodontics, Department of Prosthodontics, Faculty of Dentistry, Chulalongkorn University.