อิทธิพลของความเป็นกรดเบสและอุณหภูมิในกระบวนการไซลาไนเซชันต่อปริมาณการปล่อยฟลูออไรด์และคุณสมบัติเชิงกลของอะคริลิกเรซินชนิดบ่มด้วยความร้อนที่ผสมสารอัดแทรกที่ปล่อยฟลูออไรด์เคลือบไซเลน

นางสาวณัฏฐา นครชัย

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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ปีการศึกษา 2558

THE EFFECT OF PH AND TEMPERATURE OF SILANIZATION PROCESS ON THE AMOUNT OF FLUORIDE RELEASED AND MECHANICAL PROPERTIES OF HEAT-CURED ACRYLIC RESIN MIXING WITH SILANIZED FLUORIDE RELEASING FILLER

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ณัฏฐา นครชัย : อิทธิพลของความเป็นกรดเบสและอุณหภูมิในกระบวนการไซลาไนเซชันต่อปริมาณการปล่อยฟลูออไรด์และคุณสมบัติเชิงกลของอะคริลิกเรซินชนิดบ่มด้วยความร้อนที่ผสมสารอัดแทรกที่ปล่อยฟลูออไรด์เคลือบไซเลน (THE EFFECT OF PH AND TEMPERATURE OF SILANIZATION PROCESS ON THE AMOUNT OF FLUORIDE RELEASED AND MECHANICAL PROPERTIES OF HEAT-CURED ACRYLIC RESIN MIXING WITH SILANIZED FLUORIDE RELEASING FILLER) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. ทพ. ดร. แมนสรวง อักษรนุกิจ, 53 หน้า.

การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาผลของการปรับ pH ของสารละลายและอุณหภูมิที่ใช้ในกระบวนการไซลาไนเซชันต่อปริมาณการปล่อยฟลูออไรด์และคุณสมบัติเชิงกลของอะคริลิกเรซินชนิดบ่มด้วยความร้อนที่ผสมสารอัดแทรกที่ปล่อยฟลูออไรด์เคลือบไซเลน กลุ่มที่ทดสอบผลของการปรับ pH ของสารละลาย แบ่งเป็น 4 กลุ่ม ดังนี้ กลุ่มที่ไม่เคลือบไซเลน กลุ่มที่ปรับ pH ของสารละลายเป็นกรด กลุ่มที่ไม่ปรับ pH ของสารละลายและกลุ่มที่ไม่ผสมสารอัดแทรกเป็นกลุ่มควบคุม มีตัวอย่างกลุ่มละ 8 ตัวอย่างสำหรับการทดสอบกำลังดัดขวาง และกลุ่มละ 6 ตัวอย่างสำหรับการปล่อยฟลูออไรด์ตามลำดับ กลุ่มที่ทดสอบผลของอุณหภูมิที่ทำให้สารอัดแทรกแห้งภายหลังกระบวนการไซลาไนเซชัน แบ่งเป็น 4 กลุ่ม ดังนี้ กลุ่มที่ใช้อุณหภูมิ 25 50 110 องศาเซลเซียส และกลุ่มที่ไม่ผสมสารอัดแทรกเป็นกลุ่มควบคุม จำนวนตัวอย่างเท่ากับตัวอย่างที่ใช้ทดสอบผลของการปรับ pH เตรียมขนาดของชิ้นตัวอย่างและทดสอบกำลังดัดขวางตามมาตรฐาน ISO 20795-1: 2013 ใช้ฟลูออไรด์อิเลกโตรดในการวัดปริมาณฟลูออไรด์ ในช่วงสัปดาห์ที่1 วัดปริมาณฟลูออไรด์ทุกวัน สำหรับสัปดาห์ที่ 2-8 วัดปริมาณฟลูออไรด์สัปดาห์ละ 1 ครั้ง ผลการศึกษาของการปรับ pH ของสารละลาย พบว่ากลุ่มที่ไม่ปรับ pH ของสารละลายให้ค่ากำลังดัดขวางมากกว่ากลุ่มที่ปรับ pH ของสารละลายเป็นกรดและกลุ่มที่ไม่เคลือบไซเลนอย่างมีนัยสำคัญทางสถิติ (p<0.05) แต่กลุ่มที่ไม่เคลือบไซเลนปล่อยฟลูออไรด์ได้ดีกว่ากลุ่มที่ปรับ pH ของสารละลายเป็นกรดและกลุ่มที่ไม่ปรับ pH ของสารละลายอย่างมีนัยสำคัญทางสถิติ (p<0.05) สำหรับผลการศึกษาของการปรับอุณหภูมิที่ทำให้สารอัดแทรกแห้งภายหลังกระบวนการไซลาไนเซชันพบว่า กลุ่มที่ใช้อุณหภูมิ 50 องศาเซลเซียส ปล่อยฟลูออไรด์ได้มากกว่ากลุ่มที่ใช้อุณหภูมิ 25 องศาเซลเซียสอย่างไม่มีนัยสำคัญทางสถิติ (p>0.05) โดยที่ค่ากำลังดัดขวางไม่แตกต่างกันอย่างมีนัยสำคัญทางสถิติ (p<0.05) แต่กลุ่มที่ใช้อุณหภูมิ 110 องศาเซลเซียสให้ค่ากำลังดัดขวางลดลงอย่างมีนัยสำคัญทางสถิติ (p<0.05)

ภาควิชา	ทันตกรรมประดิษฐ์	ลายมือชื่อนิสิต	....
สาขาวิชา	ทันตกรรมประดิษฐ์	ลายมือชื่อ อ.ที่ปรึกษาหลัก	....
ปีการศึกษา	2558		

5575805132 : MAJOR PROSTHODONTICS

KEYWORDS: ACRYLIC RESIN / FLEXURAL STRENGTH / FLEXURAL MODULUS / PH / SILANE COUPLING AGENT

NATHA NAKORNCHAI: THE EFFECT OF PH AND TEMPERATURE OF SILANIZATION PROCESS ON THE AMOUNT OF FLUORIDE RELEASED AND MECHANICAL PROPERTIES OF HEAT-CURED ACRYLIC RESIN MIXING WITH SILANIZED FLUORIDE RELEASING FILLER. ADVISOR: ASSOC. PROF. MANSUANG ARKSORNNUKIT, Ph.D., 53 pp.

This study aimed to evaluate the effect of an acidic-adjusted pH of solvent and drying temperature in silanization on the amount of fluoride released and mechanical properties of heat-cured acrylic resin containing a silanized fluoride-releasing filler. The experimental groups (pH effect) were divided into 4 groups; non-silanized, acidic-adjusted pH, non-adjusted pH, and no filler as control. The experimental groups (temperature effect) were also divided into 4 groups; T25, T50, T110, and no filler as control. For fluoride measurement, each specimen was placed in deionized water which was changed every day for 7 days, every week for 7 weeks and measured. The flexural strength and flexural modulus were evaluated after aging for 48 hours, 1, and 2 months. For pH effect, two-way ANOVA indicated significant differences among groups, storage times, and its interaction in fluoride measurement and flexural modulus. For flexural strength, there was significant difference only among groups. Acidic-adjusted pH of solvent in silanization enhanced the amount of fluoride released from acrylic resin, while non-adjusted pH of solvent exhibited better flexural strength of acrylic resin. For temperature effect, two-way ANOVA indicated significant differences among groups, storage times, and its interaction in fluoride measurement and flexural modulus. For flexural strength, there was significant difference on two main factors. The flexural strength of all groups at 2 months were comparable to 48 hours storage time. T50 was suggested for silanization of fluoride releasing filler before mixing with heat-cured acrylic resin as it increased the amount of fluoride released.

Department:	Prosthodontics	Student's Signature	....
Field of Study:	Prosthodontics	Advisor's Signature	....
Academic Year:	2015		

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Chapter I

Introduction

Background and Rationales

From the estimation of prospected Thai populations from 2000 to 2030, the senior population continuously increases due to the improvement of life quality[1]; meanwhile, the disease and/or illness of this population has been shifted from acute to chronic disease. In dentistry, chronic oral diseases such as dental caries and periodontal disease have been considered to be main cause of tooth losses in the elderly[2], especially the abutment tooth adjacent to denture[3,4], resulting in the increasing number of the denture wearer.

Fluoride is an anticariogenic substance which inhibits demineralization and promotes remineralization of teeth[5]. After prolonged exposure to fluoride solution, the enamel lesion can be arrested or reversed[6,7]. Moreover, *in vitro* studies reported that fluoride released from restorative materials could effectively protect the enamel adjacent to restorations from demineralization[8,9].

Two different approaches for the development of fluoride-releasing materials have been reported. These include the fluoride-releasing filler systems and additive fluoride salts[10]. Among the fluoride-releasing dental materials, glass ionomer has been considered to be the most effective material to prevent dental caries around restorations[9]. Glass ionomer can release fluoride ions from both matrix and glass particles[11]. According to the advantages of glass ionomer, it has been used as a fluoride releasing filler in several materials[10,12,13]. Recently, an *in vitro* study, indicated that fluoride release and recharge characteristics were assessed from adding surface pre-reacted glass ionomer filler into denture base resins. The results showed direct correlation between the amount of fluoride-releasing filler and fluoride releasing amount. The 20wt% surface pre-reacted glass ionomer in denture base materials was suggested due to the reason of the mechanical property[14]. Although various additive substances increase anticariogenic effect of acrylic resin, adverse effect of fluoridated filler is the decrease in mechanical properties of the materials[10,15].

Silane coupling agent is considered to bond filler particles and resin matrix polymer. The most common silane coupling agent used in dental composite resins is γ – methacryloxypropyltrimethoxysilane (MPS)[16]. Factors influencing on the silanization reaction are, for example, pH and temperature of drying process. These factors might affect the quality of silane layers; thereafter, affect the mechanical properties and fluoride releasing amount of fluoride releasing acrylic resin. However, the effect of pH and temperature of silanization process on fluoride releasing amount and mechanical properties of acrylic resin materials is not clearly elucidated.

Objectives

1. To investigate the effect of pH and temperature of silanization process on the amount of fluoride released from heat-cured acrylic resins mixing with silanized fluoride releasing filler.
2. To evaluate the effect of pH and temperature of silanization process on the flexural strength and the flexural modulus of heat-cured acrylic resins mixing with silanized fluoride releasing filler.

Hypotheses

$H1_0$: There would be no significant differences of the amount of fluoride released, the flexural strength and the flexural modulus of heat-cured acrylic resins mixing with silanized fluoride releasing filler under different pH of solvent in silanization process.

$H1_a$: There would be significant differences of the amount of fluoride released, the flexural strength and the flexural modulus of heat-cured acrylic resins mixing with silanized fluoride releasing filler under different pH of solvent in silanization process.

$H2_0$: There would be no significant differences of the amount of fluoride released, the flexural strength and the flexural ~~modulus~~ of heat-cured acrylic resins mixing with silanized fluoride releasing filler under different temperatures in silanization process.

$H2_a$: There would be significant differences of the amount of fluoride released, the flexural strength and the flexural modulus of heat-cured acrylic resins mixing with silanized fluoride releasing filler under different temperatures in silanization process.

Keywords

Acrylic resin
Flexural modulus
Flexural strength
Fluoride releasing
pH
Silane coupling agent
Temperature

Expected Benefits

The knowledge of the present study is to control the amount of the exchanged fluoride between the acrylic resin material and the intraoral environment by the manipulation of silane coupling agent. The control of intraoral fluoride level would result in the therapeutically anticariogenic level of heat-cured acrylic resin for reducing the incidence of dental caries in the patients with removable prostheses without compromising the mechanical properties of the acrylic resin.

Chapter II

Literature review

Basic knowledge of glass ionomer

Fluoride has anticariogenic effect comprising of preventing demineralization and promoting remineralization of tooth structure. The *in vitro* studies suggested that the fluoride concentrations of at least 0.19 ppm could prevent the demineralization of sound enamel, and the higher concentrations of fluoride were required to prevent the progression of artificial caries-like lesions[17,18].

With the advantages of fluoride, several dental restorative materials have been developed fluoride discharge ability, for example, glass ionomer, fluoride releasing sealant, fluoride releasing resin composite, compomer and giomer. Among these materials, glass ionomer is able to release and recharge the greatest amount of fluoride[13,19,20]. The major advantages of glass ionomer are a high amount and long-termed of fluoride released[21-23]. They also acts as a fluoride reservoir[24,25], which is beneficial in preventing demineralization of enamel and dentin. Fluoride released from glass ionomer affects tooth structure both adjacent to and distanced from the restoration which is caries susceptible[26].

Glass ionomer was developed by Wilson and Kent in the early 1970s. The setting mechanism is occurred via acid-base reaction between fluoroaluminosilicate glass particles and polyacrylic acid. The glass particles mainly contain aluminium oxide and silicon dioxide which play the role as skeletal structure of glass ionomer. Fluoride does not exist in the skeletal structure; therefore, it can diffuse through the glass without deteriorating effect of both physical and mechanical properties of glass ionomer[27].

Regarding the acid-base reaction of glass ionomer as shown in Figure 1, after the glass particles are attacked by the acidic protons of the polyacrylic acid, the positive ions (Ca^{2+} and Al^{3+}) and the negative ions (F^-) leach out, and then the positive ions react with carboxyl groups of polyacrylic acid to form the insoluble polyacid salt matrix including unreacted negative ions. Only the surface layer of the glass particles reacts with the acid, but not the glass core[27].

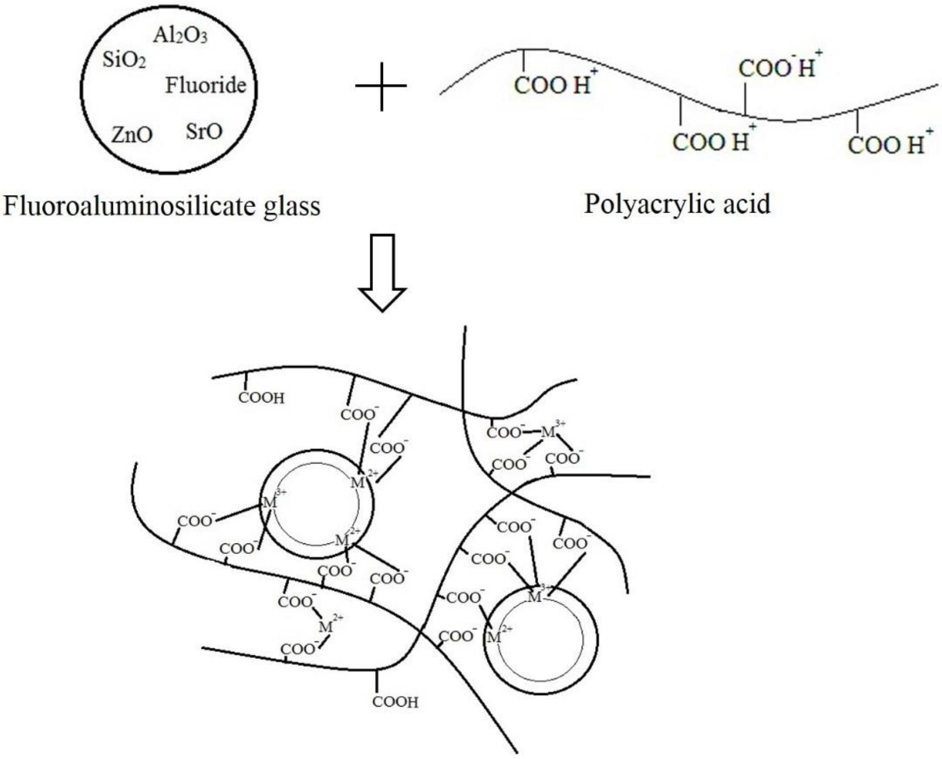


Figure 1 The structure of fluoroaluminosilicate glass particles

Fluoride releasing mechanism of glass ionomer comprises of two processes. The former mechanism is a short-term reaction due to the rapid solubility of fluoride ion from the outer surface into the solution, and the latter mechanism is steadier than the former mechanism due to a sustained diffusion of fluoride through the bulk materials. The glass ionomer materials release fluoride ions from both the insoluble polyacid salt matrix and the reacted glass particles; however, the discharge fluoride amount of both origins is greater than that of the glass core only, even though the kinetic reactions of fluoride discharge are almost similar[11]. Regarding the recharge ability of glass ionomer, the glass particles showed less amount of fluoride uptake than the set glass ionomer[28].

Factors influencing on the fluoride released rate of the glass ionomer include initial concentration of fluoride in the glass particles, powder/liquid ratio and mixing technique. The higher initial fluoride content in glass or the lower powder/liquid ratio exhibit, the greater the amount of fluoride released[24,29]. The mixing technique of glass ionomer enhances the reaction between glass particles and polyacrylic acid and increasing the volume fraction of the cement matrix resulting on the amount of fluoride released. The amount of fluoride from the hand-mixed method was lower than that from the mechanical trituration technique[30]. Moreover, the rate of fluoride released from glass ionomer is under the influence of experimental factors such as storage media[31], pH and temperature of the solution[32,33]. The previous study investigated the effect of storage media, water, artificial saliva and acidic solution, on the quantitative data of fluoride released. The glass ionomer in the condition of acidic solution showed the greatest amount of fluoride released, followed by water and artificial saliva, respectively. The different results among the storage media could be explained by the difference between diffusion gradient and presence of the interfere component in storage media[34]. The *in vitro* study reported the positive correlation between the environmental temperature at the temperature of 4, 37 and 55°C and the amount of released and recharged fluoride from glass ionomer[33].

Fluoride releasing acrylic resin

Regarding the fluoride releasing mechanism of the acrylic resin containing fluoride releasing particles, water in environmental fluid penetrates into the acrylic resin materials, resulting in the solubility of the fluoride releasing particles; thereafter, the fluoride ions leach out to the environment outside the materials. The releasing rate of fluoride is under influenced of factors, *i.e.* the solubility of the fluoride releasing particles and the diffusion of water. Considering other materials such as composite resins, the density and hydrophobicity of resin matrix polymer and in particularly polysiloxane layers between filler particles and resin matrix play the major role on controlling the diffusion of water[35,36]. These factors might effect on the acrylic resin containing fluoride releasing particles.

Numerous efforts have achieved the anticariogenic properties of the acrylic resins by adding fluoride salts, surface pre-reacted glass ionomer filler (S-PRG filler) into acrylic resin materials. Srithongsuk et al. added NaF, CaF_2 and glass ionomer particles into orthodontic acrylic plates and investigated their amount of fluoride released. The acrylic resin plates with additive CaF_2 particles could prolong the amount of fluoride released, followed by the acrylic resin plates with glass ionomer paticles and that with NaF particles, respectively[10].

Al-Bakri et al. added various amount of fluoridated glass fillers (0%, 1%, 2.5%, 5%, and 10% by weight) into PMMA denture base material. They investigated the amount of fluoride released and flexural properties of modified PMMA and found that the specimens contained 2.5%, 5%, and 10% glass fillers released significantly greater amount of fluoride compared with 1% glass fillers and control groups[12]. Unfortunately, the flexural strength of the specimens decreased as glass filler uploading increased.

The S-PRG filler was recently developed as an alternative fluoride releasing particle and added into many kinds of materials such as fluoride releasing sealant[37], giomer[19] and adhesive materials[38]. The fillers added into the denture base acrylic resin materials, resulted in good fluoride releasing and recharging properties and efficiently prevented demineralization of bovine root dentin[14,39].

Although various additive substances are able to promote anticariogenic effect of acrylic denture base materials, a major drawback is the decrease of their mechanical properties due to the absence of bonding between filler and matrix[10,15,40].

Silane coupling agents and silanization process

The general formula of silane coupling agent is $R\text{-}Si\text{-}(OR')_{3,}$ for instant the structural formula of MPS as shown in Figure 2. The non-hydrolyzable group (R), methacrylate group, which can chemically bond with materials containing C=C bonds, whereas the hydrolyzable alkoxy group (R’) can chemically bond with hydroxyl group of inorganic substrate, such as silica surface[41].

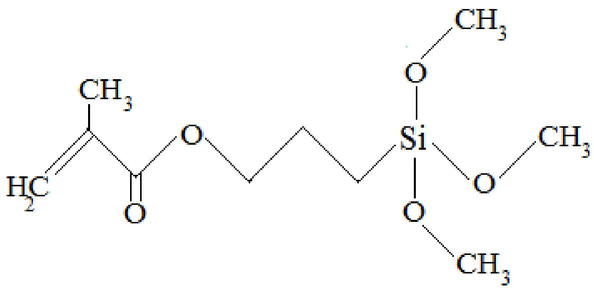


Figure 2 The structure formula of γ-methacryloxypropyltrimethoxysilane (MPS)

Silane coupling agents have been considered to promote the adhesion between inorganic fillers and polymer matrixs. The MPS is commonly used due to the compatibility between the methacrylate group of MPS and polymetylmethacrylate (PMMA)[22]. The silane coupling agents have been widely used to improve the mechanical property of acrylic resin[42].

The silane coupling agents are also considered for ionic exchange regulation of the fillers. A previous study reported the effect of various concentrations of MPS on the fluoride releasing rate of bisphenol-A-glycidyl methacrylate/Triethylene-glycol-dimethacrylate (bis-GMA/TEGDMA) resins with silanized NaF particles. The data showed that the 10 wt% of silane coated group showed the greatest water penetration and fluoride releasing rate, followed by the 5 and 1 wt% group, respectively[43].

The silanization reaction is shown in Figure 3. The reaction generally consists of two main reactions: hydrolysis and condensation reactions. Alkoxy group of silane molecules are hydrolyzed by water presented in an aqueous solution or moisture on substrate surface, and formed silanol groups. The silanol groups are consequently condensed, and performed siloxane bond (Si-O-Si) during drying process. The silanol groups are both horizontally condensed among silane molecules to form polymeric siloxane structures, and vertically condensed with substrate surfaces[44].

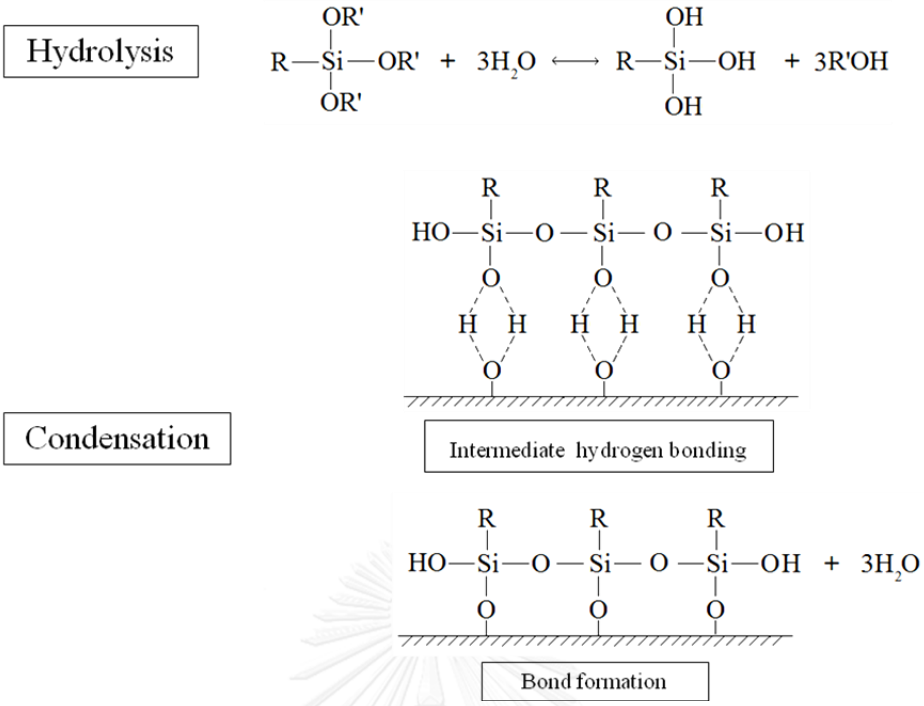
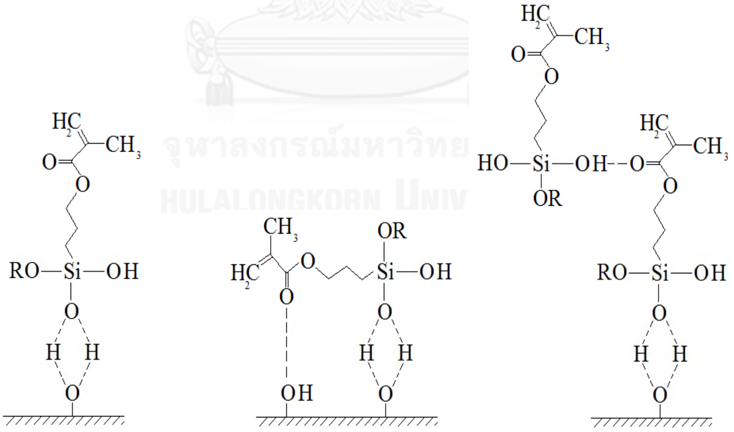


Figure 3 Hydrolysis and condensation reactions of silanization reaction on a substrate

The factors affected the quality of silanization process

The factors affected the quality of silanization process are type, structure[45] and amount[41,46] of silane coupling agents, type of solvents[47], pH of solutions and drying temperature[48]. The previous study revealed that the structure of silane coupling agent affected the ethanol/water absorption behavior of the nano silica-filled composite resins. The fillers coated with MPS absorbed the amount of ethanol/water less than the fillers coated with the octyltrimethoxysilane (OTMS), which had no methacrylate group, and cannot covalently bond with the dimethacrylate monomers[45]. Additionally, the rate of hydrolysis reaction correlated to the molecular weight of alkoxy groups. A study on the comparison of hydrolysis reaction rate between the lower molecular weight methoxysilane and the greater molecular weight ethoxysilane reported that the hydrolysis reaction rate of the methoxysilane was faster than that of ethoxysilane[49].

The orientation of silane molecules on the substrate surface is also under the influence of the amount of silane coupling agent. A study hypothesized three possible binding forms of MPS on the substrate surfaces containing oxide layer, for example silica: 1) hydrogen bonding between silanol groups and silica, 2) hydrogen bond between silanol groups and carbonyl groups (C=O) and 3) multilayer formation through hydrogen bond as shown in Figure 4[16,41,46]. The lower amount of silane illustrated the main hydrogen bond types in the form 1 and 2, whereas the greater amount of silane illustrated the main hydrogen bond types in the form 3. Not only the amount of silane affected the binding form of silane adsorbed on the substrate surface, but also the formation of polymetric silane molecules via the presence of isolated hydroxyl groups[16]. Regarding the high concentration of silane, the multi-silane structure trends to be the major molecules found on the substrate surface. The multi-silane structure consists of two layers: “chemisorbed silane layer” which covalently bond to substrate surface and “physisorbed silane layer” which has no covalent bonds with the substrate surface. The presence of physisorbed silane layer weakens the composite resins because of no bonds between the layers to the surrounding polymer[16,41]. The physisorbed silane layer are bonded to the chemisorbed silane layer by the presence of hydrogen bonds, and is easily washed out by an organic solvent[50].



Form 1 Form 2 Form 3

Figure 4 Three possible binding forms of MPS on the substrate surfaces

Type of solvent is one of the factors affecting the quality of silanization process. A previous study reported the effect of various polarity of solvent during silanization reaction on the bond strength between PMMA and alumina. The MPS in ethanol group exhibited greater shear bond strength than the MPS in isopropanol and toluene groups, respectively[47].

The pH of solution also affects the quality of silanization process by modifying the rate of hydrolysis; however, the pH of solutions showed no effect on flexural strength of experimental composite resins[48]. The hydrolysis in acidic condition induced lower amount of self-condensation silane molecules than that of basic condition[51]. Nishiyama et al. investigated the effect of pH on an adsorption behavior of a silane coupling agent deposited onto silica using gel permeation chromatography. Fourteen mg/ml of silane coupling agent, MPS, was dissolved in 70/30 mixture solution of ethanol and deionized water. The pH of solution was adjusted with hydrochloric acid resulted in 10^{-4}, 10^{-2}, and 10^{-1} mole/liter. Following hydrolysis for 1 hour, 10 g of the colloidal silica was added into 150 ml of the solution. The colloidal silica was dried at room temperature for 14 days. The results showed that acid concentration affected the molecular weight of adsorbed silane species. In non-acidic condition, the dimer and trimer species were the main adsorbed silane species. However, the larger molecules, the tetramer and octamer, were produced in acidic condition[52].

Nishiyama et al. studied an influence of adsorption characteristics of silane on the hydrolytic stability of silane at the silica-matrix interface using gel permeation chromatography and cross-polarization/magic-angle spinning silicon 29 nuclear magnetic resonance (CP/MAS ^{29}Si n.m.r.). The hydrolytic stability of silane before and after water degradation were studied by means of tensile test. The results showed that under acidic conditions, the molecular weight of deposited silane was higher than that of non-acidic conditions. After degradation in hot water (90°c), the tensile bond strength under acidic conditions decreased dramatically. This might be due to the different architecture of chemisorbed silane which influenced bond strength of silane multilayer. In acidic conditions, most of chemisorbed silane had no silanol groups left, thus physisorbed silane bond with chemisorbed silane with weaker van der Waals' force instead of hydrogen bond[50].

Additionally, the quality of silane process is under the influence of temperature of drying process. Several researchers reported that the higher temperature up to 100 $^{\circ}$C increased the bond strength between the composite resin to the fiber post, and the bond strength between resin cement to feldspathic ceramic[53-55]. Arksornnukit et al. reported that the high temperature promoted two condensation processes, the condensation reaction among silanol groups on the one hand and the condensation reaction between silanol groups of silane and hydroxyl groups of substrate surface on the other hand. Moreover, the high temperature increased the polymerization of the unsaturated double bonds among methacrylate groups of silane molecules, led to a decrease of functional groups bonding to the PMMA[48].

Nishiyama et al. investigated the effect of heat treatment on the adsorption behavior of silane onto the silica surface by using gel permeation chromatography (GPC), elemental analysis and CP/MAS^{29}Si NMR. They also observed hydrolytic stability of silane multilayer, before and after degradation, by using tensile test. They found that the amount of the chemisorbed silane onto the silica surface was constant until the heat treatment temperature reached 80°C. For heat treatment at 110°C, the amount of chemisorbed silane dramatically increased and high molecular weight physisorbed silane decreased which indicated that physisorbed silane was forcefully condensed on the chemisorbed silane molecules and formed chemisorbed multilayers. The bond strength gradually decreased by increasing the heat treatment temperature. This was attributed to the chemisorbed silane molucules at high temperature did not have silanol groups to allow hydrogen bonding with physisorbed silane molecules[56].

Chapter III

The pH effect of solvent in silanization on fluoride released and mechanical properties of heat-cured acrylic resin containing fluoride-releasing filler

Introduction

Tooth loss is a major oral health concern in public health system[57]. The middle-aged to elderly population, who had missing teeth, require prostheses to improve masticatory function, esthetics, and phonetics. Several prostheses such as dental implants, fixed partial dentures, and removable dentures are to replace the missing teeth depending on each patient's oral condition and socioeconomic status. The removable denture is a typical choice for patients which can replace multiple missing teeth, easy to fabricate, minimum preparation of adjacent teeth and low cost. The removable denture is commonly made of heat-cured acrylic resin, polymethylmethacrylate based material, which provides good mechanical properties and esthetic. However, removable denture requires wide coverage around the adjacent teeth and soft tissue, which is prone to plaque accumulation, periodontal disease and dental caries[58,59].

To prevent dental caries around the abutment teeth, the denture wearer has been recommended to take extensively oral care in combination with fluoride supplement such as fluoridated mouthrinse and toothpaste[60]. One possible method is to add fluoride-releasing filler into denture base such as fluoride salts[61], fluoridated glass fillers[12] and surface pre-reacted glass-ionomer filler[14]. Denture base containing S-PRG filler exhibited good fluoride releasing and recharging properties and efficiently prevented demineralization of bovine root dentin[14,39]. Although various additive substances increase anticariogenic effect of acrylic resin, adverse effect of fluoridated filler is a decrease in mechanical properties of the materials[12,15,61].

Silane coupling agent, a bonding agent between filler particles and resin matrix polymer, has been widely used to improve the mechanical properties of the acrylic resin[42]. The common silane coupling agent used in dental composite resins is γ-methacryloxypropyltrimethoxysilane (γ-MPS)[16]. Furthermore, the silane coupling agents are also considered for ionic exchange regulation of the fillers[35,43]. Bisphenol-A-glycidyl methacrylate/triethylene-glycol-dimethacrylate (bis-GMA/TEGDMA) resin containing silanized NaF particles released a small amount of fluoride for a long duration while the resin containing non-silanized NaF particles released a large amount of fluoride at the initial stage and terminated in a short duration. Unfortunately, there were no mechanical properties reported in the previous study[43].

The silanization reaction consists of two main reactions: hydrolysis and condensation reactions. Alkoxy groups of silane molecules are hydrolyzed by water presented in an aqueous solution or moisture on substrate surface, and formed silanol groups. The silanol groups consequently condense, and form silane multilayers deposited on the filler surface during drying process[16]. Silane multilayers consist of chemisorbed and physisorbed silane layers. The difference between these two layers is that chemisorbed silane is adsorbed on the filler surface via covalent bond, while physisorbed layer is adsorbed on the chemisorbed silane via hydrogen bond and van der Waals force[50,52].

Numerous factors affect the silanization process and silane multilayers such as type of silane[45], amount of silane[42,62,63] and drying temperature[48]. The pH of solvent is an interesting factor which influences the quality of silanization by modifying the rate of hydrolysis and condensation reaction. Adjusted pH of the solvent by acidic solution was reported to accelerate the hydrolysis of the methoxy groups in the silane molecules and decelerate the inter-molecular condensation of the silane resulting in low-molecular weight species of silane molecules[51,64]. However, the effect of acidic-adjusted pH of solvent in silanization process on fluoride releasing amount and mechanical properties of acrylic resin materials is not clearly elucidated.

Therefore, the objective of the present study was to investigate the effect of the adjusted pH of solvent in silanization process on the amount of fluoride released, flexural strength, and flexural modulus of heat-cured acrylic resin containing a silanized fluoride-releasing filler. The null hypotheses were that there would be no significant differences on the amount of fluoride released, the flexural strength and the flexural modulus of heat-cured acrylic resin containing a silanized fluoride-releasing filler under different pH of solvent in silanization process.

Materials and Methods

Materials used in this study are listed in Table 1. The composition of each material is provided by the manufacturers.

Table 1 Materials used in this study and their compositions

Material	Manufacturer	Batch number	Compositions
Fluoride releasing filler - G018-090	Schott AG, Landshut, Germany	TX22490	Al_2O_3 (20%), SiO_2 (30%), Fluoride (20 %), SrO (20%), ZnO (10%), Na_2O (<5%), P_2O_5 (<5%) (average particle size 1 μm)
Heat-cured acrylic resin - Triplex hot	Ivoclar Vivadent AG, Schaan, Liechtenstein	SHBD3265V	Powder: Polymethylmethacrylate, dibenzoyl peroxide (95%) Liquid: Methyl methacrylate (50-100%), Ethylene dimethacrylate (2.5-10%)
Silane coupling agent - 3-trimethoxysilyl propyl methacrylate	Sigma-Aldrich Co., St.Louis, MO, USA	R66910	Methacryloxypropyl trimethoxysilane (MPS)

Silanization of fluoride releasing filler

G018-090, a fluoride-releasing filler with the average particle size of 1 μm, was silanized as received. Two mass percent of MPS, calculated from the amount of G018-090, was added in 70 vol% ethanol aqueous solution which was prepared by diluting 99.9% ethanol (J.T.Baker, Avantor Performance Materials, Center Valley, PA, USA) with deionized water. The solvent without pH adjustment, pH 6.0±0.5, was designated as a non-adjusted pH group. For an acidic-adjusted pH group, the pH of solvent was adjusted with glacial acetic acid (Carlo Erba Reagenti S.P.A., Lombardia, Italy) until pH4 was achieved by using a pH meter (ORION420A, Orion Research Inc., Beverly, MA, USA). Both solutions were allowed to hydrolyze in the plastic jar for 30 minutes at room temperature. G018-090 filler was added into the solution and manually triturated until the solvent was almost completely evaporated. Consequently, the silanized G018-090 filler was left dry at room temperature for 14 days.

<u>Specimen preparation for fluoride released and mechanical properties test</u>

Thirty-two disc-shaped (Ø5x2 mm) and ninety-six bar-shaped (64x10x 3.3 mm) wax patterns were prepared using a stainless steel mold for fluoride released and mechanical properties test, respectively. The wax patterns were placed in the denture flask filled with plaster for a lost wax technique. The 20 mass% silanized G018-090 fillers of acidic-adjusted pH group, non-adjusted pH group and non-silanized group were homogeneously mixed with liquid monomer using a vortex mixer (VTX-3000L, LMS Co. Ltd., Tokyo, Japan) for 10 minutes. The acrylic resin with no filler content was also served as control group. Acrylic resin powder and liquid monomer were mixed at a powder/liquid ratio of 23.4 g/ 10 ml. The specimens were fabricated using the conventional method. The flasks were placed in the water bath, heated up to 100°C and let boil for additional of 45 minutes. After the flasks were cooled to room temperature, the specimens were deflasked, gently removed the excess using a low-speed handpiece and a carbide bur, and polished using 600, 1,000, and 2,000-grit silicon carbide papers, respectively. The specimens were measured using a digital micrometer (minimum reading: 0.001 mm, Digimatic Micrometer, Mitutoyo Corp., Kanagawa, Japan).

<u>The amount of fluoride released test</u>

Thirty-two specimens were divided into 4 groups (n=8). Each specimen was stored in a 2 ml of deionized water container and placed in an orbital shaker incubator (SI50, Stuart Scientific, Lancashire, UK) at 37°C and 130 rounds per minute. The solutions were kept and changed daily during the first week and once a week thereafter for 7 weeks. The amount of fluoride released into deionized water was measured using a fluoride ion selective electrode (QI518C, QIS, Oosterhout, Netherlands) connected with a pH/Ion meter (Model SL518, Select Biosciences, Sudbury, England). The machine was calibrated using the standard curve method with standard fluoride solutions of 0.02, 0.2, 2, and 20 parts per million (ppm) (Merck, Damstadt, Germany). The 0.5 ml of solution was adjusted with an ionic strength using a 50 µl of total ionic strength adjustment buffer solutions (TISAB III, Thermo Fisher Scientific, Waltham, MA, USA). The average amount of fluoride released ($\mu g/cm^2$) of each group was calculated.

Mechanical properties test

The flexural strength and flexural modulus of the specimens were determined by three-point bending test according to ISO 20795-1:2013[65]. Twenty-four specimens in each group were divided into three subgroups (n=8) and stored in 37°C deionized water for 48 hours, 1 month, and 2 months before testing. A universal testing machine (EZ-SX, Shimadzu, Kyoto, Japan) was used at a crosshead speed of 5 mm/min with a 50-mm-span support. The flexural strength and flexural modulus were calculated using the built-in software following the equations below.

$$\text{Flexural strength (MPa)} = 3Fl / 2bh^2$$

$$\text{Flexural modulus (GPa)} = Fl^3 / 4bh^3d$$

Where, F=the load at specimen failure (N), l=the distance between the span supports (mm), b=the width of the sample (mm), h=the thickness (mm), d=the deflection corresponding to the load F (mm).

Scanning electron microscope (SEM) observation

After three point bending test, the fractured bar-shaped specimens were selected, gold sputter coated, and observed using SEM (Quanta 250, FEI Company, Eindhoven, Netherlands) to examine the fractured surface under 20kV acceleration voltage and magnification of 10,000x.

Statistical analysis

For pH effect, the amounts of fluoride released were analyzed by two-way repeated measures analysis of variance (ANOVA) with groups (acidic-adjusted pH, non-adjusted pH, and non-silanized) and storage times (1-7 days and 2-8 weeks) as main factors, followed by Tukey HSD's post hoc comparison test ($p<0.05$). The flexural strength and flexural modulus were analyzed by two-way ANOVA with groups (acidic-adjusted pH, non-adjusted pH, non-silanized, and control) and storage times (48 hours, 1 month, and 2 months) as main factors, followed by Tukey HSD's post hoc comparison test ($p<0.05$).

Results

Table 2 Two-way repeated measures ANOVA of the amount of fluoride released and two-way ANOVA of the flexural properties of experimental groups for two main factors and interaction

Parameter	Source	Type III Sum of Squares	df	Mean Square	F	Sig.
The amount of fluoride released	Group	490.184	2	245.092	5519.299	<0.001
	Storage time	197.179	13	15.168	680.502	<0.001
	Group x Storage time	104.108	26	4.004	213.179	<0.001
Flexural strength	Group	33031.413	3	11010.471	773.061	<0.001
	Storage time	68.700	2	34.350	2.412	0.096
	Group x Storage time	5.409	6	0.902	0.063	0.999
Flexural modulus	Group	8.196	3	2.732	1393.712	<0.001
	Storage time	0.149	2	0.074	37.996	<0.001
	Group x Storage time	0.036	6	0.006	3.063	0.009

The results of two-way repeated measures ANOVA of the amount of fluoride released are shown in Table 2. The two main factors and its interaction demonstrated significant differences. Due to the amount of fluoride released from specimens of control group was less than detectable limit, the data were excluded from statistical analysis. Specimens of non-silanized group released the greatest amount of fluoride. The amount of fluoride released of the acidic-adjusted pH group was significantly greater than that of non-adjusted pH group in all storage time except for day 1 and 2, which showed no significant differences. Specimens of all groups released the greatest amount of fluoride on day 1. After that, the amount of fluoride released of non-silanized group gradually decreased, while both silanized groups, acidic-adjusted, and non-adjusted pH groups maintained a constant level of fluoride as presented in Figure 5.

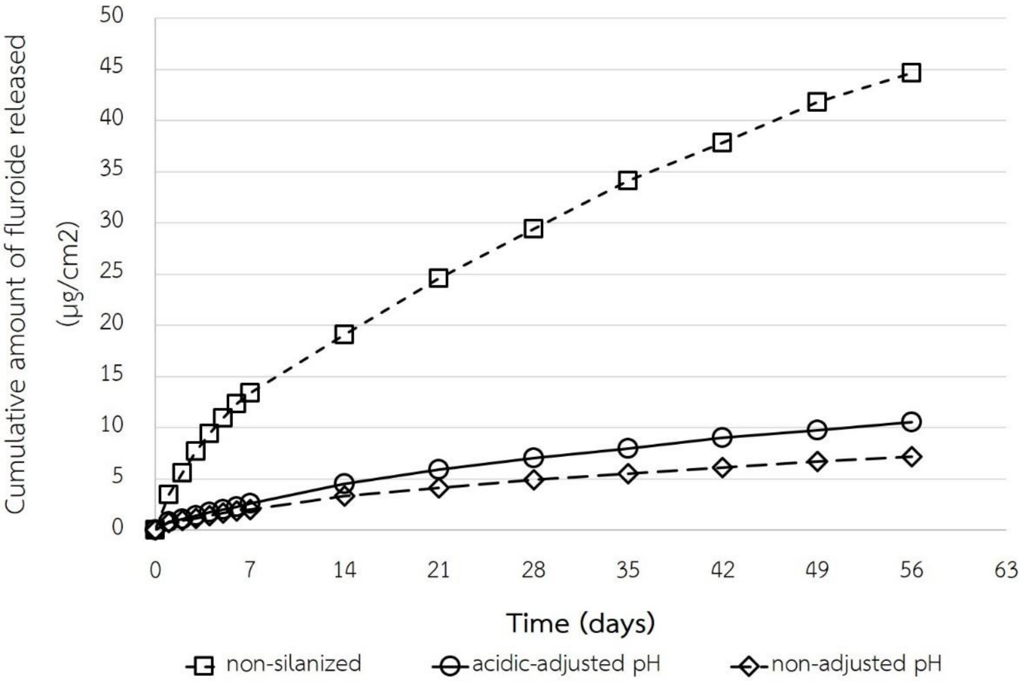


Figure 5 Cumulative amount of fluoride released of the experimental groups

The results of two-way ANOVA of flexural strength and flexural modulus are shown in Table 2. The flexural strength demonstrated significant differences only among groups. The flexural modulus showed significant differences on two main factors and its interaction. The flexural strength and flexural modulus are shown in Figure 6 and 7, respectively. At all storage times, the flexural strength significantly decreased as followed: control > non-adjusted pH > acidic-adjusted pH > non-silanized group. For flexural modulus, there were no significant differences between acidic-adjusted pH and non-adjusted pH group at each storage times. Both groups were significantly greater than non-silanized and control group. After 1 month of water storage, the flexural modulus of all groups significantly increased except control group, which did not change at all storage times.

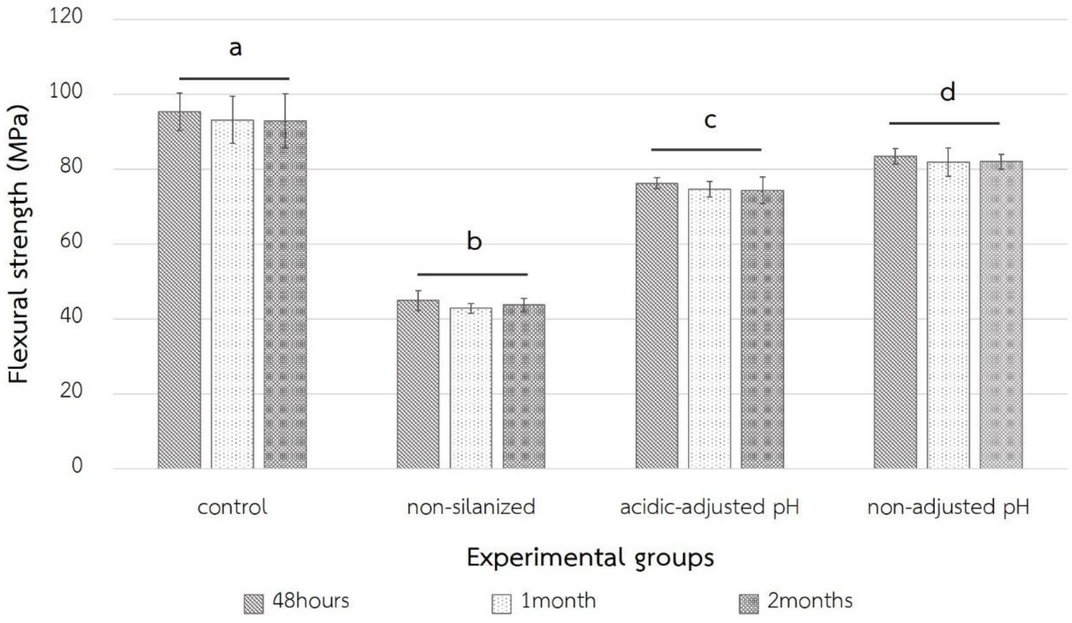


Figure 6 Flexural strength of the experimental groups.

Bars with the same letters were not significantly different ($p<0.05$)

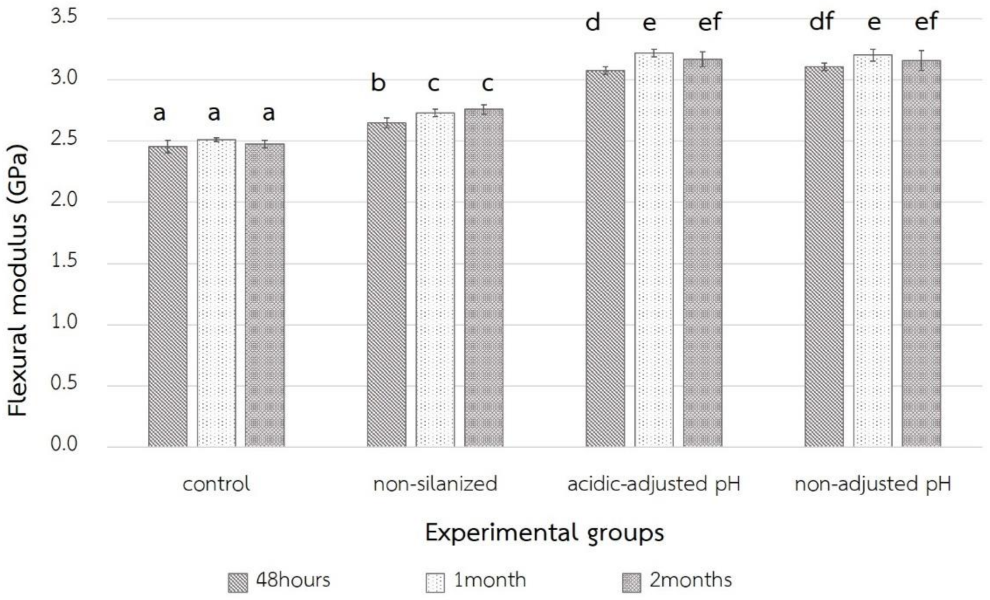


Figure 7 Flexural modulus of the experimental groups.

Bars with the same letters were not significantly different ($p<0.05$)

The SEM images of fracture surfaces from all groups are shown in Figure 8. A different between non-silanized and both silanized samples was apparently observed. Non-silanized sample demonstrated that filler surface could easily be seen without resin matrix coverage especially at 1 and 2 months. In contrast, coverage of resin matrix was observed in both silanized samples. However, the differences between silanized samples were not obviously seen.

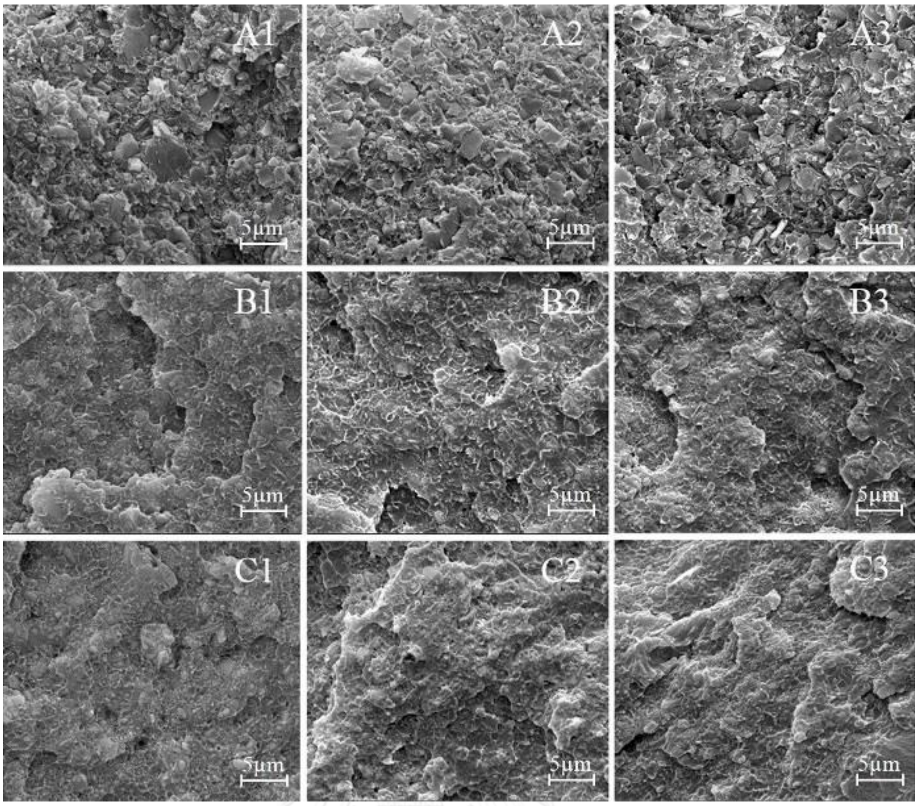


Figure 8 SEM images of the experimental groups (10,000x);

A. non-silanized, B. acidic-adjusted pH, and C. non-adjusted pH. The number after the uppercase letter represents the storage time of the specimens in 37°C deionized water; 1-stored for 48 hours, 2-stored for 1 month, 3-stored for 2 months

Discussion and conclusion

The present study aimed to investigate the effect of pH of solvent in silanization process on the amount of fluoride released, flexural strength, and flexural modulus of heat-cured acrylic resin containing silanized G018-090 filler. Statistical analysis showed significant differences on the amount of fluoride released, flexural strength, and flexural modulus. Thus, the null hypotheses were rejected.

The amount of fluoride released from materials into aqueous solution had been examined by several methods, including ion-selective electrode and ion chromatography. Of these methods, ion-selective electrode was simple, cost effective, and suitable for investigating numerous samples. However, the limitation of this method was that a very low concentration of fluoride ions could not be measured[13].

The amount of fluoride released of non-silanized group was significantly greater than both silanized group. Generally, the amount of fluoride released from resin matrix was regulated by two factors; solubility of fluoridated filler itself[43,61] and the diffusion of water into the resin[12,35,43]. For the latter factor, silane coupling agent played an important role to control this factor by creating hydrophobic polysiloxane layer around the fillers resulting in slow water penetration and fluoride released[36]. Furthermore, it could be assumed that silane coupling agent could modulate a constant sustainable level of fluoride released, since both silanized groups did not exhibit an initial fluoride burst effect as demonstrated in the non-silanized group.

The amount of fluoride released of acidic-adjusted pH group was greater than that of non-adjusted pH group. This result might attribute to acetic acid, which was added into the solvent of acidic-adjusted pH group. During silanization process, acid could react with G018-090 filler, fluoroaluminosilicate glass, by forming a thin polysalt matrix layer around the filler particle. The matrix layer which consisted of calcium- and aluminium-acetates and fluoride ions, could be easily penetrated by water. Diffusion of fluoride ions from the surface of matrix layer into an aqueous solution might be easier than that of glass filler surface alone. However, this matrix layer might interfere the formation of silane multilayers at the filler surface, which led to less hydrolytic stability of silane layers. This finding was in agreement with a previous study that reported the amount of fluoride released of acid-washed glass was greater than that of unwashed glass particle[11].

The mechanisms of fluoride in caries prevention included the inhibition of demineralization, enhancement of remineralization, and reduction of microbial metabolism. The degree of prevention seemed to relate to fluoride concentration. Although the amount of fluoride released in the present study was obviously lower than other commercial glass ionomer, as previously reported[23], the effectiveness of fluoride for caries prevention still remained. The low concentration of fluoride, ranged from 0.024-0.154 ppm, reduced demineralization of enamel surface[66]. This concentration corresponded to the amount of fluoride released of both silanized groups on day 1-7, which ranged from 0.033-0.123 ppm and 0.055-0.137 ppm for non-adjusted pH and acidic-adjusted pH group, respectively. The amount of fluoride released in the present study could be sufficient to prevent enamel demineralization even from a small sample size. Additionally, silanized groups were effective in modulating a constant level of fluoride released for an extended period, which provided a low concentration of fluoride in oral cavity and promoted long term anticariogenic activity.

While improving an anti-cariogenic property of the heat-cured acrylic resin, it was also important to maintain the mechanical properties of acrylic resin. Silane coupling agent promoted bonding between filler and resin matrix resulted in higher flexural strength of silanized groups than that of non-silanized group. Flexural strength and flexural modulus of all experimental groups were greater than 65 MPa and 2 GPa which were recommended by ISO 20795-1 specification[65] except flexural strength of non-silanized group. The result of the lower strength might be explained by poor dispersion of filler particles in resin matrix of non-silanized group, which led to the filler agglomeration, and the lack of bonding at the filler-resin matrix interfaces, as shown in SEM images of non-silanized group. Although the filler was treated with silane before mixing with heat-cured acrylic resin, the flexural strength of acidic-adjusted pH and non-adjusted pH groups were lower than that of control group. G018-090 filler was functioned as a fluoride source which had hydrophilic properties to promote water uptake and fluoride released. Addition of this filler into acrylic resin might decrease the flexural strength in contrast to the other reinforcing fillers such as silica and alumina filler[40,42]. Another reason to explain why the flexural strength of acrylic resin decreased after adding silanized G018-090 filler might attribute to an excessive filler fraction, which created weak points and impaired the flexural strength of acrylic resin[67].

The flexural strength of the non-adjusted pH group was significantly greater than that of acidic-adjusted pH group. This result was in agreement with the find out of previous studies[48,62]. This result might attribute to the different silane molecular species deposited on the fillers surface under different conditions. Acidic condition accelerated hydrolysis reaction and stabilized silanol groups as monomers, dimers and low molecular weight oligomers. During condensation, the solvent began to evaporate resulting in pH change and a decrease in volume of solvent. Due to pH of solvent could not be maintained at 4, the unstable silanol groups of silane molucules could easily self-condense with each other and produced high-molecular weight species of silane molecules[52]. Since the oligomeric silane species occupied large area on filler surface, the chemisorbed amount of silane decreased. The formation of physisorbed layer occurred after saturation of chemisorbed layer. By using an equal amount of silane in silanization process, the amount of physisorbed silane was greater in acidic condition than that in non-acidic condition. Physisorbed layer consisted of unhydrolyzed or partially hydrolyzed silane molecules and polysiloxane oligomers, which bound with each other by hydrogen bond or van der Waals force. This layer might be susceptible to hydrolytic degradation than chemisorbed layer[63]. Furthermore, self-condensation of silanol groups in acidic condition produced high-molecular weight species of silane molecules resulting in the chemisorbed silane species which were almost no silanol groups left. The remaining silanol groups could bond to the physisorbed silane with hydrogen bond. This indicated that the weak van der Waals force was existed between physisorbed silane and chemisorbed silane species instead of hydrogen bond[50].

On the other hand, the silane molecular species deposited on the fillers surface in the non-acidic condition were different from that in the acidic condition. The molecular weight of silane molecules in non-acidic condition was lower than that in acidic condition, resulted in the greater amount of chemisorbed silane layer. Additionally, most of chemisorbed silane molecules still had the silanol groups which could form hydrogen bond with physisorbed silane molecules. This might increase hydrolytic stability between the chemisorbed and physisorbed layer[52].

After 2 months of water immersion, the flexural strength slightly decreased, but not significantly different. This contradicted to the previous study which reported the deterioration of flexural strength with increasing storage time[61]. This might attribute to the differences in type of fluoride-releasing filler and the effectiveness of silanization process. The non-silanized fillers, NaF and $CaF_{2,}$ used in the previous study could release fluoride ions and led to voids in the resin matrix since these fillers were water-soluble salt. The fluoroaluminosilicate glass used in this study mainly contained aluminium oxide and silicon dioxide which played the role as skeletal structure of glass filler. Fluoride ions were not a part of skeletal structure, therefore, it could diffuse through the glass without deteriorating effect of both physical and mechanical properties[23]. Moreover, silane coupling agent, which promoted bonding between filler particles and polymer matrix, could increase hydrolytic stability and provide protection for leaching out of filler particles. Therefore, the mechanical properties could be retained for a longer period of time.

From the result of this study, the pH of solvent did not affect flexural modulus. This finding suggested that modulus of the specimens was not influenced by silane molecular species deposited on the fillers surface. The more important factors for improving modulus might be shape of fillers[68], fillers content, and distribution[14]. The modulus of the specimens contained G018-090 filler increased significantly after 1 month of water storage. Two possible reasons might be explained for this result. First, even though material used in the present study was heat-cured acrylic resin, the presence of fillers decreased degree of conversion by impaired mobility of free radicals molecules of resin matrix[69]. A greater amount of unreacted monomer of experimental groups still remained more than that of control group. This monomer could polymerize in later period so call ‘post-curing process’. Post-curing process could improve degree of conversion resulting in an increasing of mechanical properties[70]. Secondly, unreacted monomer could act like a plasticizer in denture base polymer. After immersion, unreacted monomer could leach out into the water resulted in increasing the stiffness of the specimens[71].

The findings of this study demonstrated that heat-cured acrylic resin contained 20 mass% of silanized G018-090 filler could sustainably release sufficient amount of fluoride for preventing the demineralization of sound enamel, while the mechanical properties of the acrylic resin could be maintained. The addition of non-silanized G018-090 filler to acrylic resin exhibited high burst amount of fluoride released with drastically decrease in flexural strength. Furthermore, adjusting pH of solvent in silanization process could regulate the constant sustainable level of fluoride released which was beneficial to long term anticariogenic activity. Therefore, a novel heat-cured acrylic resin would be an alternative material for controlling caries incidence in patient with removable prosthesis and high caries risk population. However, the present study was an *in vitro* study, the longitudinal observation in clinical outcomes required further investigation.

With the limitation of the present study, it might be concluded that acidic-adjusted pH of solvent had an effect on the amount of fluoride released and flexural strength of heat-cured acrylic resin, but not for flexural modulus. Acidic-adjusted pH of solvent in silanization could enhance the amount of fluoride released from acrylic resin, while non-adjusted pH of solvent exhibited better flexural strength of acrylic resin.

Chapter IV

The temperature effect in silanization on fluoride released and mechanical properties of heat-cured acrylic resin containing fluoride-releasing filler

Introduction

From the result of pH effect of solvent, we found that non-adjusted pH of solvent in silanization process resulted in a better flexural strength compared to the acidic-adjusted pH. Although the amount of fluoride released from non-adjusted pH group was sufficient to decrease demineralization of sound enamel, the higher amount of fluoride was required to prevent further demineralization of an early carious lesion in enamel. The previous study found that the concentration of fluoride as low as 0.19 ppm could prevent the dissolution of sound enamel. For the early carious lesion, the concentration of fluoride ranged from 2.1-10.1 ppm resulted in partial remineralization at outer enamel surface, while the demineralization was still occurred simultaneously at the inner surface[18].

Since the silanization process depended on many factors, modifying the other factor apart from pH of solvent might improve the fluoride released property of silanized G018-090. Drying temperature is an interesting factor which influences the quality of silanization. Heat treatment during drying process was reported to promote two condensation processes; the condensation among the silanol groups of silane and the condensation between silanol groups of silane and surface hydroxyl groups of silica filler[41]. The application of heat treatment at 50°C and 80°C increased the flexural strength of composite resin containing silanized silica filler[48]. In addition, bis-GMA/TEGDMA resin containing silanized NaF particles with heat treatment at 130°C for 3 hours released fluoride for a long duration, while the amount of fluoride from the silanized NaF particles without heat treatement terminated within a short period[43]. However, the effect of drying temperature in silanization process on fluoride releasing amount and mechanical properties of acrylic resin materials is not clearly elucidated.

Therefore, the objective of the present study was to investigate the effect of drying temperature in silanization process on the amount of fluoride released, flexural strength, and flexural modulus of heat-cured acrylic resin containing a silanized fluoride-releasing filler. The null hypotheses were that there would be no significant differences on the amount of fluoride released, the flexural strength and the flexural modulus of heat-cured acrylic resin containing a silanized fluoride-releasing filler under different drying temperature in silanization process.

Materials and Methods

Silanization of fluoride releasing filler

G018-090 was also silanized with 2 mass% of MPS in non-adjusted pH of 70 vol% ethanol aqueous solution. The filler was manually triturated until the solvent was almost completely evaporated. Then, the filler was divided into 3 subgroups and dried at temperature 50°C and 110°C using a hot air oven (UM500, Memmert GmbH and Co., Schwabach, Germany) for 3 hours. Another group was dried at room temperature (25°C) for 14 days.

Specimen preparation for fluoride released and mechanical properties test

The experimental groups were divided into 4 groups; T25, T50, T110 and control group (no filler). The specimens for testing the amount of fluoride released and mechanical properties were prepared and tested as mentioned above.

Statistical analysis

For statistical analysis, the amounts of fluoride released were analyzed by two-way repeated measures analysis of variance (ANOVA) with groups (T25, T50, and T110) and storage times (1-7 days and 2-8 weeks) as main factors, followed by Tukey HSD's post hoc comparison test ($p<0.05$). The flexural strength and flexural modulus were analyzed by two-way ANOVA with groups (T25, T50, T110, and control) and storage times (48 hours, 1 month, and 2 months) as main factors, followed by Tukey HSD's post hoc comparison test ($p<0.05$).

Results

Table 3 Two-way repeated measures ANOVA of the amount of fluoride released and two-way ANOVA of the flexural properties of experimental groups for two main factors and interaction (temperature effect)

Parameter	Source	Type III Sum of Squares	df	Mean Square	F	Sig.
The amount of fluoride released	Group	1.337	2	0.669	24.834	<0.001
	Storage time	47.362	13	3.643	755.593	<0.001
	Group x Storage time	1.066	26	0.041	9.461	<0.001
Flexural strength	Group	3646.415	3	1215.472	53.429	<0.001
	Storage time	180.022	2	90.011	3.957	0.023
	Group x Storage time	113.18	6	18.863	0.829	0.551
Flexural modulus	Group	7.842	3	2.614	1068.097	<0.001
	Storage time	0.058	2	0.029	11.8	<0.001
	Group x Storage time	0.036	6	0.006	2.437	0.032

The results of two-way repeated measures ANOVA of the amount of fluoride released are shown in Table 3. The two main factors and its interaction demonstrated significant differences. Due to the amount of fluoride released from specimens of control group was less than detectable limit, the data were excluded from statistical analysis. The amount of fluoride released of T50 and T110 was slightly greater than that of T25 in all storage time. Specimens of all groups released the greatest amount of fluoride on day 1. After that, the amount of fluoride released was maintained at a constant level as presented in Figure 9.

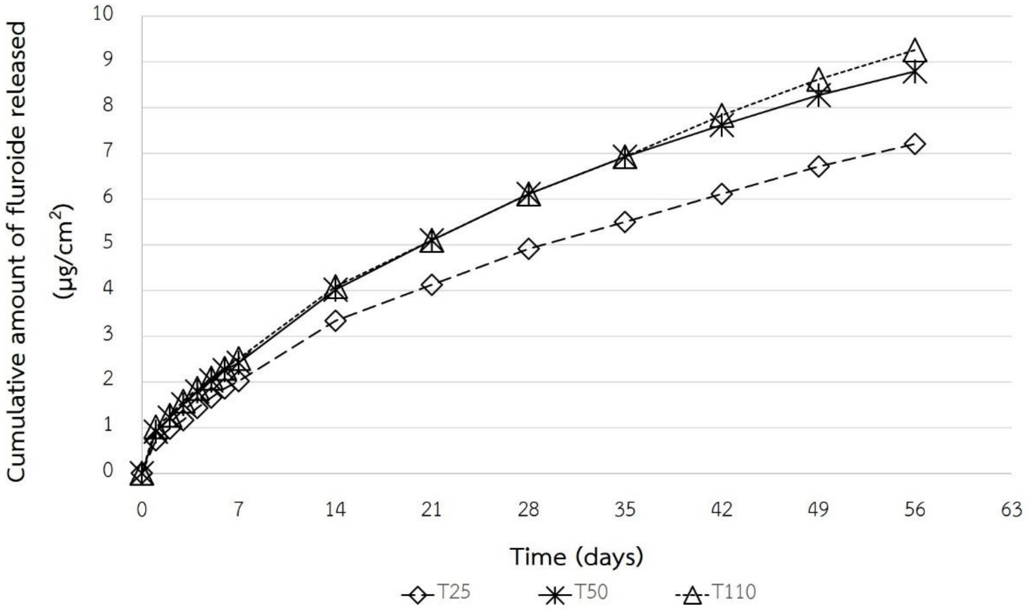


Figure 9 Cumulative amount of fluoride released of the experimental groups (temperature effect)

The results of two-way ANOVA of flexural strength and flexural modulus are shown in Table 3. The flexural strength demonstrated significant differences on two main factors. The flexural modulus showed significant differences on two main factors and its interaction. The flexural strength and flexural modulus are shown in Figure 10 and 11, respectively. The flexural strength of T25 was significantly greater than that of T110. There were no significant differences between the flexural strength of T25 and T50. The flexural strength of all groups at 2 months were comparable to 48 hours storage time. For flexural modulus, there were no significant differences between T25, T50, and T110 at each storage times. The flexural modulus of T25, T50, and T110 were greater than that of control group.

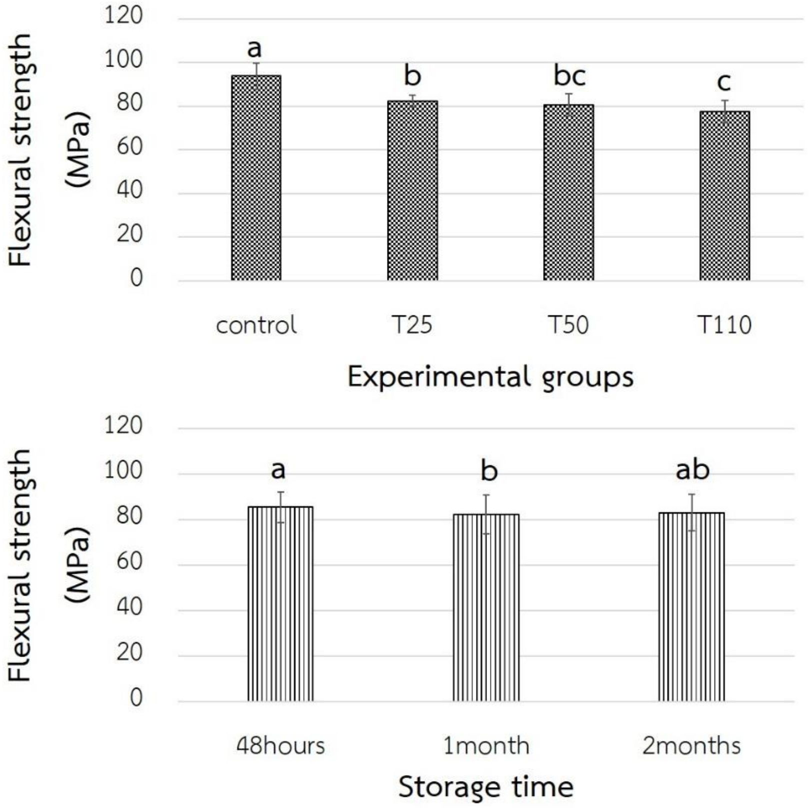


Figure 10 Flexural strength of the experimental groups (temperature effect)

Bars with the same letters were not significantly different (p<0.05)

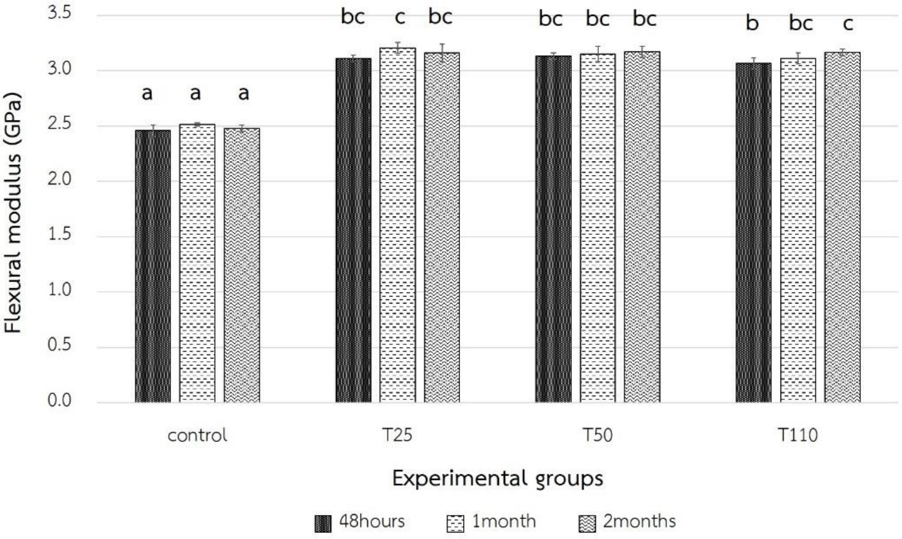


Figure 11 Flexural strength of the experimental groups (temperature effect).

Bars with the same letters were not significantly different (p<0.05)

Discussion and conclusion

The present study aimed to investigate the effect of temperature in silanization process on the amount of fluoride released, flexural strength, and flexural modulus of heat-cured acrylic resin containing silanized G018-090 filler. Statistical analysis showed significant differences on the amount of fluoride released, flexural strength, and flexural modulus. Thus, the null hypotheses were rejected.

The amount of fluoride released of T50 and T110 was greater than that of T25. The rising temperature accelerated self condensation reactions, resulted in the high molecular weight of physisorbed silane molecules. The higher molecular weight of silane molecules, the larger was the free volume and steric hindrance of molecular species[52]. In addition, the high temperature in drying process induced an evaporation of water, alcohol, and other by-products that remained in the pores of the network[72]. Both of free volume from the hindrance structure and the pores of the network might enhance water penetration into the filler, resulted in the higher amount of fluoride released.

The higher concentration of fluoride of T50 and T110 prevented deminerization of sound enamel. Sub-ppm concentration of fluoride was insufficient to inhibit the progressive demineralization of early carious lesion in enamel[18]. However, to provide the optimal protection for sound enamel demineralization, the application of low level of fluoride at frequent intervals was more effective than high level of fluoride a few times. In this study, we also found that drying temperature during silanization process, either T25, T50, or T110, showed no alteration on the constant fluoride-releasing effect of silane.

. There were no significant differences between the flexural strength of T25 and T50, while T110 was lesser than that of T25. This result was in agreement with the previous study[48]. The drying temperature in silanization process affected adsorption behavior of silane multilayers onto the filler surfaces.

The amount of chemisorbed layer on surface of silica was formed to be constant until the drying temperature reached 80°C[56]. Normally, the only limited amount of silane molecule formed as chemisorbed monolayer on filler surface while the excess amount of silane formed physisorbed layer onto the chemisorbed one. After raising heat treatment temperature up to 110°C, the amount of chemisorbed silane dramatically increased. This was probably due to the heat driven self-condensation of hydrolyzed physisorbed silane molecules, resulting in the higher molecular weight of physisorbed silane molecules. Then, these high molecular weight of physisorbed silane were forcefully condensed on the previously chemisorbed silane molecules and formed chemisorbed multilayers[56]. This unorganized silane structure with bulk steric hindrance on the filler surface might decrease the flexural strength of heat-cured acrylic resin.

The reduction of flexural strength could also be explained by the hydrolytic stability of silane multilayer. Silane multilayer, comprised of chemisorbed and physisorbed layers, was linked mostly by hydrogen bonding rather van der Waals attractions[50]. When drying temperature was up to 110°C, only a few silanol group was left on chemisorbed silane molecule, leading to the formation of weak van der Waals attraction rather than strong hydrogen bonding[56]. In addition, the high temperature could induce the polymerization among vinyl groups of silane molecules which might reduce the flexural strength of heat-cured acrylic resin[48].

In conclusion, the drying temperature at 50°C was suggested for silanization of fluoride releasing filler before mixing with heat-cured acrylic resin as it increased the amount of fluoride released.

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APPENDIX

Table 4 Two-way repeated measures ANOVA of the amount of fluoride released (pH effect)

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
group	Sphericity Assumed	490.184	2	245.092	5519.299	.000
	Greenhouse-Geisser	490.184	1.083	452.421	5519.299	.000
	Huynh-Feldt	490.184	1.127	434.958	5519.299	.000
	Lower-bound	490.184	1.000	490.184	5519.299	.000
Error(group)	Sphericity Assumed	.622	14	.044		
	Greenhouse-Geisser	.622	7.584	.082		
	Huynh-Feldt	.622	7.889	.079		
	Lower-bound	.622	7.000	.089		
time	Sphericity Assumed	197.179	13	15.168	680.502	.000
	Greenhouse-Geisser	197.179	2.132	92.495	680.502	.000
	Huynh-Feldt	197.179	3.092	63.763	680.502	.000
	Lower-bound	197.179	1.000	197.179	680.502	.000
Error(time)	Sphericity Assumed	2.028	91	.022		
	Greenhouse-Geisser	2.028	14.922	.136		
	Huynh-Feldt	2.028	21.647	.094		
	Lower-bound	2.028	7.000	.290		
group * time	Sphericity Assumed	104.108	26	4.004	213.179	.000
	Greenhouse-Geisser	104.108	2.259	46.084	213.179	.000
	Huynh-Feldt	104.108	3.390	30.708	213.179	.000
	Lower-bound	104.108	1.000	104.108	213.179	.000
Error(group*time)	Sphericity Assumed	3.419	182	.019		
	Greenhouse-Geisser	3.419	15.814	.216		
	Huynh-Feldt	3.419	23.732	.144		
	Lower-bound	3.419	7.000	.488		

Table 5 Two-way ANOVA of the flexural strength of experimental groups (pH effect)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	33105.522[a]	11	3009.593	211.308	.000
Intercept	522150.000	1	522150.000	36660.910	.000
Time	68.700	2	34.350	2.412	.096
group	33031.413	3	11010.471	773.061	.000
Time * group	5.409	6	.902	.063	.999
Error	1196.386	84	14.243		
Total	556451.908	96			
Corrected Total	34301.908	95			

Table 6 Two-way ANOVA of the flexural modulus of experimental groups (pH effect)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	8.381[a]	11	.762	388.682	.000
Intercept	795.284	1	795.284	405701.444	.000
Time	.149	2	.074	37.996	.000
group	8.196	3	2.732	1393.712	.000
Time * group	.036	6	.006	3.063	.009
Error	.165	84	.002		
Total	803.829	96			
Corrected Total	8.546	95			

Table 7 Two-way repeated measures ANOVA of the amount of fluoride released (temperature effect)

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
group	Sphericity Assumed	1.337	2	.669	24.834	.000
	Greenhouse-Geisser	1.337	1.428	.937	24.834	.000
	Huynh-Feldt	1.337	1.691	.791	24.834	.000
	Lower-bound	1.337	1.000	1.337	24.834	.002
Error(group)	Sphericity Assumed	.377	14	.027		
	Greenhouse-Geisser	.377	9.993	.038		
	Huynh-Feldt	.377	11.835	.032		
	Lower-bound	.377	7.000	.054		
time	Sphericity Assumed	47.362	13	3.643	755.593	.000
	Greenhouse-Geisser	47.362	1.902	24.905	755.593	.000
	Huynh-Feldt	47.362	2.592	18.274	755.593	.000
	Lower-bound	47.362	1.000	47.362	755.593	.000
Error(time)	Sphericity Assumed	.439	91	.005		
	Greenhouse-Geisser	.439	13.312	.033		
	Huynh-Feldt	.439	18.142	.024		
	Lower-bound	.439	7.000	.063		
group * time	Sphericity Assumed	1.066	26	.041	9.461	.000
	Greenhouse-Geisser	1.066	1.807	.590	9.461	.004
	Huynh-Feldt	1.066	2.398	.444	9.461	.001
	Lower-bound	1.066	1.000	1.066	9.461	.018
Error(group*time)	Sphericity Assumed	.788	182	.004		
	Greenhouse-Geisser	.788	12.646	.062		
	Huynh-Feldt	.788	16.783	.047		
	Lower-bound	.788	7.000	.113		

Table 8 Two-way ANOVA of the flexural strength of experimental groups (temperature effect)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3939.617[a]	11	358.147	15.743	.000
Intercept	670251.473	1	670251.473	29462.545	.000
group	3646.415	3	1215.472	53.429	.000
time	180.022	2	90.011	3.957	.023
group * time	113.180	6	18.863	.829	.551
Error	1910.939	84	22.749		
Total	676102.028	96			
Corrected Total	5850.556	95			

Table 9 Two-way ANOVA of the flexural modulus of experimental groups (temperature effect)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	7.935[a]	11	.721	294.774	.000
Intercept	850.374	1	850.374	347471.378	.000
group	7.842	3	2.614	1068.097	.000
time	.058	2	.029	11.800	.000
group * time	.036	6	.006	2.437	.032
Error	.206	84	.002		
Total	858.515	96			
Corrected Total	8.141	95			

VITA

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