THE DEVELOPMENT OF CHITOSAN BUCCAL PATCH FORMULATION FOR VERAPAMIL HYDROCHLORIDE

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ABSTRACT

Verapamil hydrochloride is a drug from the calcium antagonist group. Verapamil is widely used in the treatment of coronary heart disease, arterial hypertension, certain supraventricular tachyarrhythmias and obstructive hypertropic cardiomyopathy. In oral administration, there is an extensive metabolism in the liver. Therefore, its bioavailability is only 10 to 30%. The buccal mucosa is an alternative route for systemic delivery; this route avoids the hepatic first-pass metabolism and gastrointestinal degradation. In this study, a verapamil hydrochloride buccal patch was developed by using crab chitosan and chitin whiskers as a mucoadhesive and absorption enhancer. In vitro mucoadhesive study found that the increasing concentration of crab chitosan induced increasing mucoadhesive properties of a buccal patch as compared to HEC, (Hydroxy Ethyl Cellulose), due to the cationic charge of chitosan interacting with an anionic charge of surface mucosa. In addition, the chitin whiskers compound, which is nonionic, reduced the mucoadhesiveness. In vitro penetration study found that the crab chitosan gave higher drug penetration than HEC and the standard solution within 2 to 4 hr. The incorporation of chitin whiskers reduced the drug penetration. The six formulations, when arranged from high to low drug penetration were ratio of chitin whiskers and crab chitosan of 1:4, 1:2. 1.5:1, and crab chitosan concentrations of 1.0%, 1.5% and 2.0%. Increasing concentration of crab chitosan from 1.0 to 2.0% reduced the drug penetration, whereas reduction of concentration of chitin whiskers from 60 to 20 ml or increasing concentration of crab chitosan from 0.6 to 1.2% in a mixture of chitin whiskers and chitosan induced drug penetration. This result suggests that the concentration of crab chitosan used as an absorption enhancer has an optimal concentration. These results implied that there was a good linear relationship between the concentration of crab chitosan and chitin whiskers and the percent of drug penetration via porcine buccal mucosa. In conclusion, the crab chitosan and chitin whiskers influenced the mucoadhesive properties and drug penetration of a verapamil hydrochloride buccal patch.

KEY WORDS: VERAPAMIL/ CHITOSAN/ CHITIN WHISKERS/ BUCCAL PATCH

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บทคัดย่อ

เวอราพามิล ไฮโครคลอไรด์เป็นยาในกลุ่มยาต้านแคลเซียม ซึ่งใช้เป็นยารักษาโรคหัวใจและหลอดเลือด ความดันโลหิตสูงและหัวใจเต้นผิดจังหวะ เมื่อบริหารยานี้โดยการรับประทานจะถูกเมตาบอลิซึมในปริมาณมากที่ตับ ทำให้ระดับยาชีวสมมูลเหลือเพียง 10 ถึง 30% ดังนั้นการส่งผ่านยาทางกระพุ้งแก้มจึงเป็นทางเลือกใหม่อีกทางหนึ่ง เนื่องจากยาที่ให้ทางนี้จะเข้าสู่กระแสเลือดได้โดยตรง ในการทดลองนี้ได้ตั้งตำรับแผ่นแปะเพื่อนำส่งยาเวอราพามิล ไฮโดรคลอไรด์โดยใช้ไคโตซานและไคติน วิสเกอร์เป็นสารยึดเกาะกับเนื้อเยื่อกระพุ้งแก้มและช่วยเพิ่มการดูดซึมของยา ในการทดสอบการยึดเกาะกับเนื้อเยื่อกระพุ้งแก้มหมู พบว่าแผ่นแปะไคโตซานที่ได้จากเปลือกปูมีการยึดเกาะกับเนื้อเยื่อกระพุ้งแก้มได้ดีกว่าไฮดร็อกซีเอทิลเซลลูโลส นอกจากนี้ในแผ่นแปะที่เป็นส่วนผสมระหว่างไคโตซานที่ได้จากเปลือกปูกับไคติน วิสเกอร์ ซึ่งเป็นสารที่ไม่มีประจุ พบว่าเมื่อไคติน วิสเกอร์มีปริมาณมากขึ้น แผ่นแปะจะยึดเกาะกับเนื้อเยื่อได้ลดลง เนื่องจากประจุบวกของไคโตซานที่จับกับประจุลบที่ผิวหน้าของเนื้อเยื่อ ตามทฤษฎีจะมีจำนวนลดลง ส่วนในการทดสอบการซึมผ่านของยาผ่านทางกระพุ้งแก้มหมู พบว่า แผ่นแปะไคโตซานที่ได้จากเปลือกปูทำให้ยาซึมผ่านได้มากกว่าไฮดร็อกซีเอทิลเซลลูโลส และสารละลายมาตรฐานภายใน 2 ถึง 4 ชม. โดยเรียงลำดับปริมาณยาที่ซึมผ่านได้จากมากไปน้อยได้ดังนี้ ส่วนผสมของไคติน วิสเกอร์กับไคโตซานที่ได้จากเปลือกปูในอัตราส่วน 1:4. 1:2, 1.5:1, ไคโตซานที่ได้จากเปลือกปูในความเข้มข้น1.0%, 1.5% และ 2.0% ตามลำดับ ซึ่งจะเห็นได้ว่าปริมาณไคโตซานที่เพิ่มขึ้นจาก 1.0% ถึง 2.0% มีผลให้ยาซึมผ่านได้ลดลง แต่ในส่วนผสมของไคติน วิสเกอร์กับไคโตซานให้ผลตรงกันข้ามคือ การเพิ่มขึ้นของไคโตซานจาก 0.6% ถึง 1.2% หรือการลดปริมาณไคติน วิสเกอร์ จาก 60 มล. ถึง 20 มล. มีผลเพิ่มการซึมผ่านของยา ดังนั้นการใช้ไคโตซานที่ได้จากเปลือกปูเป็นสารช่วยการซึมผ่านของยาควรใช้ในปริมาณที่เหมาะสม กล่าวโดยสรุป ไคโตซานและไคติน วิสเกอร์มีอิทธิพลต่อคุณสมบัติในการยึดเกาะกับเนื้อเยื่อและปริมาณการซึมผ่านของยา ในสูตรตำรับแผ่นแปะไคโตซานเพื่อนำส่งยาเวอราพามิล ไฮโดรคลอไรด์ผ่านทางกระพุ้งแก้ม

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LIST OF ABBREVIATIONS

°C	=	Degree Celcius (Centigrade)
A	=	Absorbance
cm	=	Centimeter
Da	=	Dalton
et al.	=	Et alii, and others
FTIR	=	Fourier Transmission Infared
g	=	Gram
HPLC	=	High performance liquid chromatography
hr	=	Hour
i.e.	=	Id est, that is
kg	=	Kilogram
mm	=	Milimeter
ml	=	Milliliter
min	=	Minute
µl	=	Microliter
µm	=	Micrometer
pH	=	The negative logarithm of the hydrogen ion concentration
%DD	=	Percent degree of deacetylation
rpm	=	Round per minute
SD	=	Standard deviation
UV	=	Ultraviolet
[η]	=	Intrinsic viscosity

CHAPTER I
INTRODUCTIONS

Verapamil [2,8-bis (3,4-dimethoxyphenyl)-6-methyl-2-isopropyl-6-azaoctanitrile] was the first calcium antagonist introduced into therapy in the early 1960s (1). The pioneering work of Fleckentstein and colleagues demonstrated that the negative inotropic effects on myocardial cells and vasodilating effects of these drugs were brought about by inhibition of calcium function in the excitation-contraction coupling. It also coined the term 'calcium antagonists' to characterize the principle mode of action of these drugs (1).

Verapamil is widely used in the treatment of coronary heart disease, arterial hypertension, certain supraventricular tachyarrhythmias and obstructive hypertropic cardiomyopathy. This drug is extensively metabolized and less than 5% of unchanged drug is eliminated in urine. Its systemic plasma clearances are high and dependent on liver blood flow. The liver is the major site of verapamil biotransformation and first-pass metabolism, since its hepatic extraction ratio is 0.8. Therefore, bioavailability of verapamil is only 10 to 30% despite almost complete oral absorption (1). Verapamil is metabolized mainly in human liver to 12 metabolites (2, 3). Only one metabolite, norverapamil has pharmacological activity. It shows 20% efficacy with verapamil in regard to the vasodilatant effect, but is devoid of antiarrhytmic activity (3).

Verapamil is highly bound (90%) to plasma protein in man. Verapamil, like several other basic drugs, is also bound to α_1-acid glycoprotein and albumin (1).

The oral mucosa can be subdivided according to the major regions in the oral cavity, a so-called non-keratinized area consisting of the floor of the mouth (sublingual), the buccal mucosa (cheeks), the soft palatal mucosa and the inner side of the lips, whereas a keratinized area comprising the gum (gingival) and the hard palatal mucosa (4, 5, 6, 7). The mucous membranes have a total area of 200 cm^2 (8) and show differences in structure, thickness and blood flow depending on their location within the oral cavity (9).

The buccal route offers certain advantages over other routes of drug administration such as: (i) fast absorption and rapid onset of action, (ii) avoidance of hepatic metabolism by first-pass effect, (iii) better stability of the drug because of avoidance of gastrointestinal drug degradation, (iv) high patient compliance, and finally (v) ease of administration and/or removal from the site of application (5, 10). However, buccal delivery has certain disadvantages as well, such as: (i) dilution of the drug by the saliva so that part of the dose is no longer available for absorption, (ii) controlled or sustained release of the drug is not achieved as the drug resides for short time periods and can be removed by drinking, eating, and even speaking, and (iii) the buccal mucosa acts as a barrier for drugs, especially the macromolecular drugs (10, 11).

Buccal mucosa consists of a non-keratinized stratified squamous epithelium, 500 to 600 μm in thickness, supported by a connective tissue lamina propria (4, 9, 12, 13). The major routes involved in drug transport across buccal mucosa were the transcellular and paracellular pathways, however, for many hydrophilic drugs, the buccal permeation was mainly through paracellular way by passive diffusion (6, 13, 14). In addition, the superficial layers of the epithelium were also reported to be the major penetrative barrier of substances (13, 15). Nonetheless, in case of low drug permeability across buccal epithelium, penetration enhancers were successfully used in order to overcome the major limitation to drug delivery (12).

To improve buccal delivery of drugs, several new dosage forms have been developed such as tablets/lozenges (including lyophilized and bioadhesive), laminated systems and patches, hydrogels, ointments, adhesive films (4, 8, 9). However, adhesive patches are preferred over other dosage forms because they are flexible and can be formulated in any shape. The size of the patches may vary from 1 to 15 cm^2. Small patches are more convenient and comfortable to wear (8, 11).

Adhesive patches may be monolithic or multilayered reservoir devices or matrix type for either systemic or local drug delivery. A backing is applied to control the direction of drug release and minimize deformation and disintegration of the device during its residence in the mouth (11).

The advantages of the unidirectional patch are (i) retention of the delivery system can be extended to 12 hrs or more, (ii) drug can be delivered across a specific,

localized region of the oral cavity, (iii) the local environment at the selected site and (iv) permits co-administration of permeability enhancers which will modify absorption in a well defined area. (v) irritation or damage to the mucosa by the drug or its excipients will be restricted to the limited area (8, 16). The disadvantages of these systems are that they use only a small mucosal area and the backings have to be removed by the patient after drug administration (8).

Chitin is the second most abundant polysaccharides in nature, cellulose being the most abundant. Chitin is found in the exoskeleton of crustacea, insects, and some fungi. (10).

Chitin whiskers are used as a reinforce polymers in order to improve or modify certain mechanical properties of the host matrix for specific applications (17). Because the building blocks of a chitin whiskers nanocomposite are of nanoscales, they have an enormous interfacial area, and therefore there are a lot of interfaces between the two intermixed phases compared to usual microcomposites (18).

Suspensions of chitin whiskers are prepared by acid hydrolysis of chitin obtained from crab shells, shrimps, squid pen and *Riftia* tubes. The objective of this treatment is to dissolve away regions of low lateral order so that the water-insoluble, highly crystalline residue may be converted into a stable suspensoids by subsequent vigorous mechanical shearing action. The chitin whiskers consist of slender parallelepiped rods (17).

Chitosan molecule is a poly(1→4) β-linked 2-amino-2-deoxy-D-glucose and is obtained by the alkaline deacetylation of chitin (10).

In 1963, Rudall narrated the presence of 3 different polymorphs of chitin to be α, β and γ. The chitin accounted from crab and shrimp shells has an α-structure, in which the chitin's chain arranged in an anti-parallel with strong intra- and intermolecular hydrogen bonding. In the other hand chitin accounted from squid pen has a β-structure, which the chitin's chain arranged in a parallel with weak hydrogen bonding (19, 20).

The word chitosan refers to a large number of polymers, which differ in their degree of *N*-deacetylation (40–98%) and molecular weight (50,000–2,000,000 Da). These two characteristics are very important to the physicochemical properties of the chitosans and hence, they have a major effect on the biological properties (10).

Chitosan is a weak base with a p*K* value of the D-glucosamine residue of about 6.2–7.0 and, therefore, is insoluble at neutral and alkaline pH values. It does, however, make salts with inorganic and organic acids such as hydrochloric acid, acetic acid, glutamic acid, and lactic acid. In acidic medium, the amine groups of the polymer are protonated resulting in a soluble, positively charged polysaccharide that has a high charge density (one charge for each D-glucosamine unit). Chitosan can form gels by interacting with different types of divalent and polyvalent anions (10, 21).

Because chitosan has favorable properties such as biodegradability (10, 22), biocompatibility (9, 10) and antifungal/ antimicrobial properties (6), It is attracted a lot of attention in the pharmaceutical and medical fields (10). In addition, chitosan has low oral toxicity with an LD_{50} in rats of 16 g/kg (10).

The pharmaceutical requirements of chitosan are: particle size <30 mm, density between 1.35 and 1.40 g/ cm^3, pH 6.5–7.5, insoluble in water, and partially soluble in acids (10).

An ideal buccal delivery system should stay in the oral cavity for a few hours and release the drug in a unidirectional way towards the mucosa in a controlled or sustained release fashion. Mucoadhesive polymers will prolong the residence time of the device in the oral cavity (10, 23). Chitosan is an excellent polymer to be used as a film-forming (24) due to its mucoadhesive properties (6, 10, 25, 26, 27) and as an absorption enhancer (6, 10, 13, 25).

The present study is designed to evaluate a developed chitosan buccal patch to deliver a hydrophilic drug. Verapamil hydrochloride is selected as a model drug due to its well-characterized physicochemical properties. The buccal patch will be characterized by measuring the *in vitro* mucoadhesive, drug release behavior and permeation study. Because of similarity to the non-keratinized human mucosa, the porcine buccal mucosa will be chosen for *in vitro* mucoadhesive and permeation study (4, 6, 12).

CHAPTER II
LITERATURE REVIEW

CALCIUM ANTAGONISTS

Verapamil, the prototypes drug of the so-called calcium antagonists was introduced for the treatment of angina pectoris more than 30 years ago. However, it was not until the early 1970s that the mechanism of action of this group of drugs was elucidated, leading to the recognition of their therapeutic potential. The pioneering work of Fleckentstein and colleagues demonstrated that the negative inotropic effects on myocardial cells and vasodilating effects of these drugs were brought about by inhibition of calcium function in the excitation-contraction coupling. It also coined the term 'calcium antagonists' to characterize the principle mode of action of these drugs (1).

In contrast to skeletal muscle, myocardial and smooth muscle are much more susceptible to variations in extracellular calcium concentrations. Thus, the degree of contractile activation of these cells is much more dependent on the availability of calcium from extracellular sources that enter the cell during excitation. Within the conducting tissues of the heart, the excitation of the sinoatrail and atrioventricular nodes are mediated by the slow channel, for which calcium is the major charge carrier. Calcium antagonists exert their pharmacological effects (diminishing myocardial oxygen consumption resulting in a reduction in high-energy phosphate consumption; coronary and peripheral vasodilatation, slowing of the sinus node discharge; prolongation of sinus node recovery time; and lengthening of the atrioventricular conduction time) by inhibiting the calcium inward current through slow channels in these tissues. These properties constitute the pharmacological basis for the established therapeutic efficacy of calcium antagonists in the treatment of ischaemic heart disease, arterial hypertension, hypertrophic obstructive cardiomyopathy and certain cardiac arrhythmias (1).

The chemical structures of calcium antagonists differ substantially, and differ include phenylalkylamine (verapamil), dihydropyridines (nifedipine), and

benzothiazepines (diltiazem). The differences in chemical structure may account for the differences observed within this group of drugs in their relative potencies on the myocardium blood vessels and cardiac pacemakers. Whereas verapamil and diltiazem prolong atrioventricular conduction and are effective drugs for the treatment of supraventricular tachyarrhythmias, nifedipine does not exert any substantial antiarrhythymic effects *in vivo* (1).

VERAPAMIL

Structure

Verapamil was the first calcium antagonist introduced into therapy in the early 1960s (1). It was a phenylalkylamine L-type calcium channel blocker. The chemical structure is [2,8-bis (3,4-dimethoxyphenyl)-6-methyl-2-isopropyl-6-azaoctanitrile] (1) and the chemical formula is C_{27} H_{38} N_2 O_4 (MW = 454.6) (28). Its molecule contains an asymmetric carbon (Figure 1) (29).

Physical properties

Verapamil is viscous pale yellow oil, whereas the hydrochloride salt is a nearly white crystalline powder, practically odourless and bitter taste. Verapamil hydrochloride melts in the range 140°C to 144°C. A 5% w/v solution of verapamil hydrochloride has a pH in the range 4.5 to 6.5 (28). Verapamil has ionization constant (pKa) of 8.6 (30). Verapamil hydrochloride is soluble 1 in 20 of water, sparingly soluble in ethanol, soluble 1 in 1.5 of chloroform and practically insoluble in ether (28).

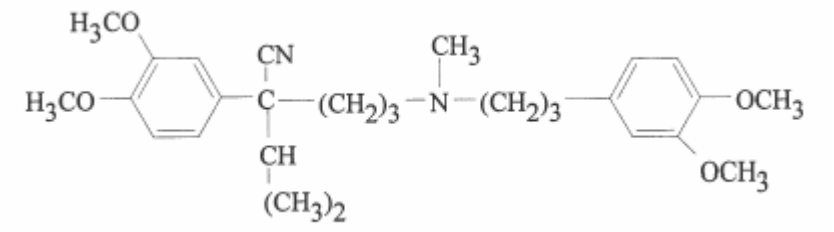


Figure 1. Structure of verapamil (29).

Stability

Verapamil hydrochloride, in the solid state, is both thermally and photochemically stable, although it should be stored under protection form light. It is also stable in neutral, acidic and basic solution. Aqueous solutions of verapamil

hydrochloride (0.5 mg/ml) were stable for 105 days at 50°C (corresponding to about 4.5 years at 25°C). Maximum stability was shown in the pH range 3.2 to 5.6. The inclusion of phosphate buffer in the solutions ad variation of their ionic strengths had minimal effect on stability. In addition, solutions of verapamil hydrochloride can be sterilized by heating in an autoclave (28).

Oral absorption

Verapamil is approximately 90% absorbed form the gastrointestinal tract (2).

Metabolism

This drug is extensively metabolized in the liver to at least 12 metabolites of which norverapamil has been shown to have some activity (2, 3).

Cleavage of the C-N-C bond by N-dealkylation, preferentially at the C-atom belonging to the shorter side chain, is the main metabolic step. Verapamil and its N-dealkylated metabolites are then further metabolized by O-demethylation. Studies in dogs have demonstrated that norverapamil has about 20% of the vasodilating activity of verapamil, whereas the other N-dealkylated metabolites are devoid of any vasodilating effect. Although the O-demethylated metabolite of verapamil exhibits the same potency as the parent drug, its contribution to the overall pharmacological effect is negligible since this metabolite is present in plasma as the glucoronide which has no pharmacological activity. None of the metabolites possesses significant dromotropic effects on atrioventricular conduction (1).

Distributions

Volume of distribution of verapamil is approximately 5.0 l/kg. Verapamil is highly (90%) bound to plasma protein in man (2). The fraction unbound is in order of 8.7 to 16% and is not concentration-dependent as no difference in the fraction unbound was observed at concentrations ranging from 35 to 1557 μg/l (1). Verapamil, like several other basic drugs, is also bound to α_1-acid glycoprotein and albumin. It can be displaced from its plasma binding sites *in vitro* by certain basic drugs, e.g. lidocaine, diazepam, propranolol and disopyramide (1). The fraction unbound of the l-isomer (0.11) was approximately twice that of the d-isomer (0.064) (1).

Eliminations

About 70% of a dose in excreted by the kidneys in the form of its metabolites but about 16% is also excreted in the bile into the faces. Less than 4% is excreted unchanged. Verapamil crosses the placenta and is excreted in breast milk (2).

Pharmacokinetics

1. **Pharmacokinetics following single intravenous and oral doses**

Following intravenous administration, verapamil exhibits multicompartmental characteristics and its pharmacokinetics is best described by an open 2- or 3-compartment model. Terminal elimination half-lives in healthy subjects range from 3 to 7 hours. Total systemic plasma clearances are high and dependent on liver blood flow. The liver is the major site of verapamil biotransformation and first-pass metabolism, since its hepatic extraction ratio is 0.8 was determined by direct measurement of plasma verapamil concentration in hepatic arterial and venous blood (31). Therefore, bioavailability of verapamil is only 10 to 35% despite almost complete (90%) oral absorption (1). In addition, verapamil is very effective in terminating paroxysmal supraventricular tachvarhythmias after intravenous administration of 5 to 10 mg, whereas an oral dose of 80 to 160 mg was required to obtain comparable clinical effects (1).

2. **Pharmacokinetics and bioavailability during multiple dose administration**

Compared with single-dose oral administration, a significant increase in bioavailability and decrease in oral clearance has been reported with multiple dose verapamil administration. Observed steady-state concentrations were, on average, 2 to 3 times higher than the predicted steady-state concentrations using pharmacokinetic data obtained from single oral dose studies (1). The reduction in clearance and increased bioavailability of verapamil is probably due to saturation of first-pass metabolism (1).

3. **Stereoselective disposition of verapamil**

The clinically available formulations of verapamil are racemic mixtures of equal amounts of the dextro (d)- and levo (l)-isomers. Following intravenous administration, the values for clearance and volume of distribution of the l-isomer were substantially higher than those of the d-isomer, whereas the terminal elimination half-lives were nearly identical (1). Thus, following oral administration of racemic

verapamil, bioavailability of the d-isomer (50%) was 2.5 times greater than that of l-isomer (20%). Whereas the d- to l-verapamil plasma concentration ratio following oral administration was 4.5 $\pm$ 1.2 (Mean $\pm$ SD) (32), the ratio following intravenous administration was approximately 2 (1). l-verapamil has been shown to have a negative dromotropic effect on atrioventricular conduction 8 to 10 times greater than d-verapamil in man (32). Therefore, if the same plasma concentrations are achieved following oral and intravenous administration of verapamil, the negative dromotropic effect will be less with the oral preparation (1).

Indications

Verapamil slows conduction through the increased ventricular node, and thus slows the increased ventricular response rate that occurs in atrial fibrillation and flutter. Cardiac output is also reduced. A decrease in both coronary and peripheral vascular resistance together with a sparing effect on myocardial intracellular oxygen consumption appears to be the modes of action in angina. The decrease in peripheral vascular resistance may explain the antihypertensive effect of verapamil. It is used in the control of supraventricular arrhythmias, in the management of angina pectoris and in the treatment of hypertension (30).

Adverse effects

Adverse effects of verapamil on heart include bradycardia, atrioventricular block, worsening heart failure, and transient asystole. These effects are more common with parenteral than with oral therapy. The most troublesome non-cardiac adverse effect is constipation. Other adverse effects include hypotension, dizziness, flushing, and headaches (30).

THE ORAL MUCOSA

Structure

The oral mucosa can be subdivided according to the three major regions in the oral cavity; a masticatory mucosa covers the gum (gingival) and the hard palatal mucosa. It consists of keratinized epithelium that closely resembles the epidermis of the skin in its pattern of maturation and is usually tightly attached to underlying structures by a collageneous tissue. Lining mucosa, so-called non-keratinized area,

consists of the floor of the mouth (sublingual), the buccal mucosa (cheeks), the soft palatal mucosa and the inner side of the lips. The non-keratinized epithelium is attached by a loose, elastic connective tissue to underlying structures and is more permeable than keratinized epithelium. Finally, specialized mucosa is found on the dorsum of the tongue (4, 6), as presented in Figure 2. The mucus membranes show differences in structure, thickness and blood flow depending on their location within the oral cavity (9). The buccal and sublingual tissues are the primary focus for drug delivery via the oral mucosa because they are more permeable than the tissue in other regions of the mouth (8). The surface area of the oral mucosa (200 cm^2) is relatively small compared with the gastrointestinal tract (350000 cm^2) and skin (20000 cm^2) (8). However, the oral mucosa membranes have direct access to the systemic circulation via capillaries and venous drainage. Thus, drugs that are absorbed through the oral mucosa directly enter the systemic circulation, bypassing the gastrointestinal tract and first-pass metabolism in the liver (8).

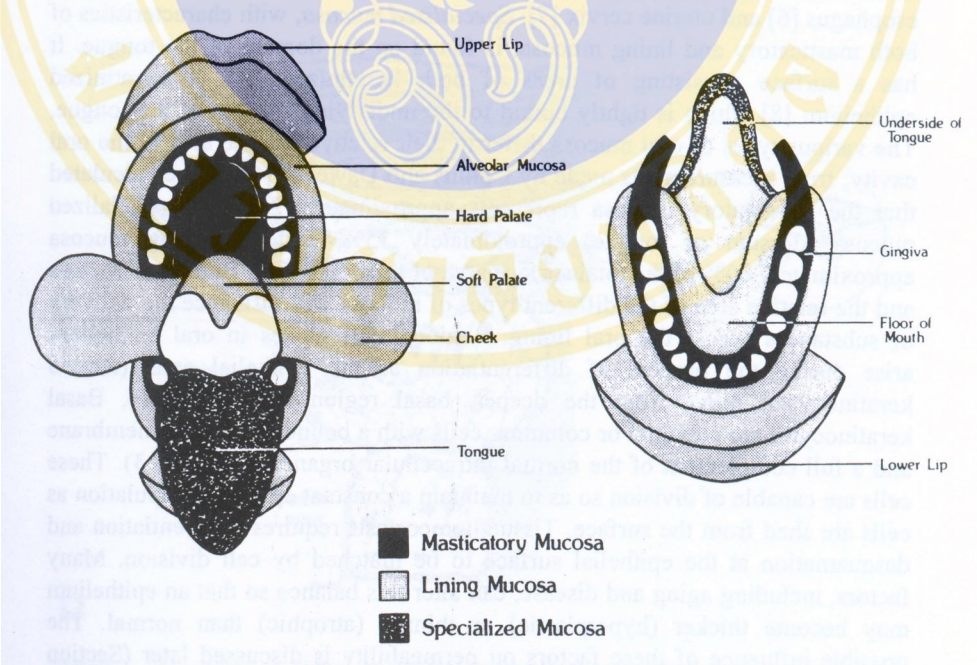


Figure 2. Diagram of the location in the oral cavity of the three major types of oral mucosa; masticatory mucosa, lining mucosa and specialized mucosa (4).

Oral mucosa drug delivery

To improve oral mucosa delivery of drugs, several new dosage forms have been developed such as solutions, tablets/lozenges (including lyophilized and bioadhesive), chewing gum, solution sprays, laminated systems and patches, hydrogels, adhesive films, hollow fibres and microspheres (4, 8, 9).

BUCCAL MUCOSA

Structure

Buccal mucosa consists principally of two components: the epithelium and the underlining connective tissue (9). The interface between these two layers is formed by the basement membrane (4).

The buccal epithelium is a non-keratinized stratified squamous layer of cells, 500 to 600 μm in thickness, composed of strata of different cell types with varying degrees of maturity. The physiological structure of buccal mucosa is illustrated in Figure 3 (6). The upper most superficial region is comprised of flattened compact layers of differentiated cells, about 150 μm thick. Epithelial cohesion in the superficial layers is ensured by the lipid and glycolipid contents extruded from the cellular membrane coating granules (MCG) (6, 9), that are spherical or oval organelles (100 to 300 nm in diameter) found in the prickle-cell layer (4, 11), forming the permeability barrier (6, 9). Deeper into the epithelium lies the malpighian layer, which contains cells at various stages of differentiation. Here, cells are less flattened and are loosely held together by desmosomes and there is less tortuosity as compared to upper layers (9). The predominant barrier to drug diffusion resides approximately within the outermost one-third of the epithelium (11), about 50 μm (9). The rapid turnover of the non-keratinized epithelial cell (about 13 days) relative to the skin is the important implications for healing and the rate of recovery of the tissue for damage (4), as presented in Table 1.

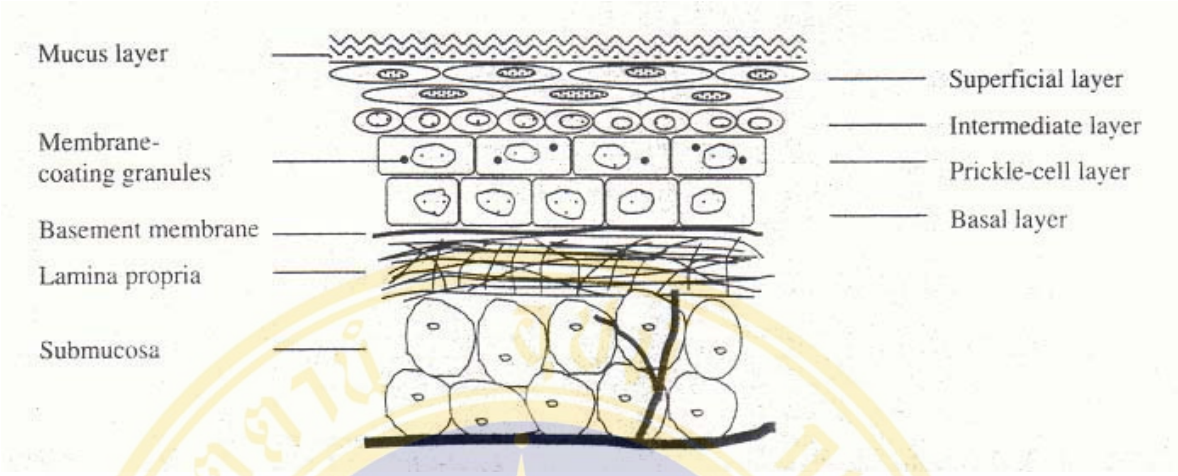


Figure 3. Schematic representation of physiological structure of buccal layer (6).

Table 1. Thickness and turnover rate time for human oral epithelium and epidermis (4).

	Mean thickness (μm)	Median turnover time (days)
Epidermis	120	27
Hard palate	310	24
Buccal mucosa	580	13
Floor of mouth mucosa	190	20

The basement membrane (BM) is a continuous layer of extracellular materials, forming a boundary between the basal layer of the epithelium and the connective tissues of the lamina propria and the submucosa. It can be subdivided into the lamina lucida, the lamina densa, and a sublayer of proteinaceous fibrous matrix (11), 1 to 2 μm in thickness (9). The BM functions include providing adherence between the epithelium and underlying connective tissues, mechanical support for the epithelium, and a barrier to the passage of cells and some large molecules (9, 11).

The connective tissues consist of lamina propria and submucosa. The lamina propria is a continuous sheet of connective sheet composed of blood capillaries and nerve fibers serving the oral mucosa. Vascular drainage from the oral mucosa is principally b the lingual, facial and retromandibular veins. These veins open into the internal jugular vein and thus avoid first-pass metabolism (11). The lamina propria is

not generally thought to function as a barrier. Its structure is insufficiently dense to exclude even relatively large molecules, and its hydrated matrix should facilitate the passage of hydrophilic penetrants (9).

The mucus layer covers the epithelium surface and serves to lubricate and protect as well as to act as a wetting agent. Mucin is a group of glycoproteins composed of oligosaccharide side chains attached to a protein core. Three-quarters of the protein core are heavily glycosylated and impart a gel-like characteristic to mucus. The remaining nonglycosylated groups are involved in cross-linking via disulfide bonds among mucin molecules. Mucus is negatively charged at physiological saliva pH of 5.8 to 7.4 because of the presence of sialic acids (pKa of 2.6) and ester sulfates at the terminals of some pendant oligosaccharide side chains (6).

Advantages and disadvantages

In the present, all possible routes of drug administration are investigated for the suitable route of drug delivery. The gastrointestinal (GI) route has some disadvantages because it presents a hostile environment. Drugs administered by this route are subjected to acid hydrolysis and extensive gut and/ or hepatic “first-pass” metabolism. The parenteral route has direct access to the systemic circulation and therefore produces effective plasma levels. However, this route is associated with pain on administration, resulting in poor patient compliance; in addition, the formulation needs to be sterile. The transdermal route is limited to potent, lipophilic compounds and does not rapidly attain the required blood levels. Furthermore, the dermal epithelium is less permeable than oral mucosa. Various absorptive mucosae have been identified and investigated for systemic drug delivery i.e. nasal, ocular, pulmonary, rectal, vaginal, buccal and sublingual. The nasal mucosa consists of microvilli and has a rich blood supply. Large individual variations in mucus secretion and turnover, negative effects of drugs and/ or excipients on the ciliary cells and pathological changes of the nasal mucosa with long-term treatment limit extensive utilization of this mucosa. The rectal route has met with variable patient acceptance and depending on the site of administration; the drug may undergo a first-pass effect. The vaginal route I is gender specific. The sublingual route is more suitable for the delivery of drugs which require a rapid onset of action. This route is more permeable than buccal route, is not suitable for retentive systems because of the physicial

structure and mobility of this area and the dosage form being continuously bathed by saliva (11).

The buccal route offers certain advantages over other routes of drug administration such as: (i) fast absorption and rapid onset of action, (ii) avoidance of hepatic metabolism by first-pass effect, (iii) better stability of the drug because of avoidance of gastrointestinal drug degradation, (iv) high patient compliance, and finally (v) ease of administration and/or removal from the site of application (5, 10). However, buccal delivery has certain disadvantages as well, such as: (i) dilution of the drug by the saliva so that part of the dose is no longer available for absorption, (ii) controlled or sustained release of the drug is not achieved as the drug resides for short time periods and can be removed by drinking, eating, and even speaking, and (iii) the buccal mucosa acts as a barrier for drugs, especially the macromolecular drugs (10, 11).

Route of drug transport

The cellular structure of the buccal mucosa suggests that there are 2 permeability barriers. The intercellular spaces and cytoplasm are essentially hydrophilic in character and become a transport barrier for lipophllic compounds mainly because the solubility of a lipophilic compound in this environment is low. In contrast, the cell membrane is lipophilic and the penetration of a hydrophilic compound into the cell membranes is low due to a low partition coefficient. Thus, closely compacted cell membranes become obstacles that hydrophilic compounds have to move around (14).

The coexistence of the hydrophilic and lipophilic regions in the buccal mucosa suggests that there are 2 routes for drug transport, i.e., the paracellular and the transcellular routes (Figure 4). All compounds can use these 2 routes simultaneously, except that one route is usually preferred over the other depending on the physicochemical properties of the diffusant (14).

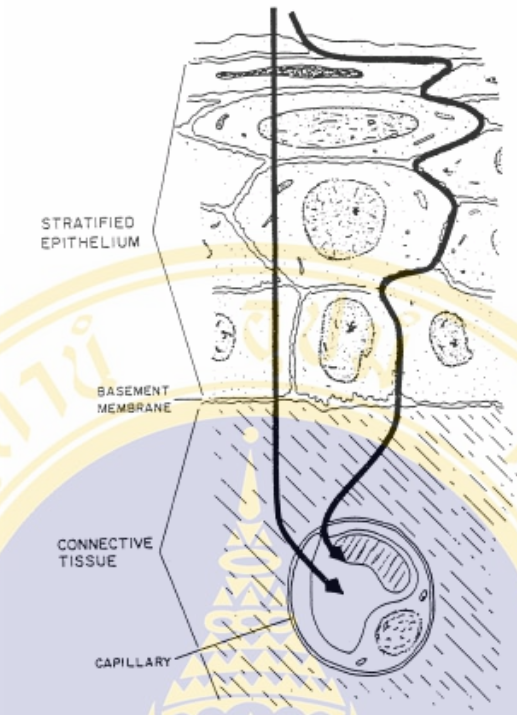


Figure 4. Schematic diagram of 2 possible drug transport routes, i.e., the paracellular and the transcellular routes, in the buccal mucosa (9).

1. **The paracellular route**

This route is the primary route for hydrophilic compounds because it is difficult for a hydrophilic compound to penetrate into the lipophilic cell membrane thus the intercellular space is the preferred route for drug transport. In this case, the limited surface area of the intercellular space and the tortuous pathways within the area are the main limitations for this route (14).

2. **The transcellular route**

For lipophilic compounds, because the surface area for the transcellular route is large, the partition coefficient are high and the pathlength for transcellular movement is relatively short, the permeability of lipophilic compounds across the epithelial cell is typically high (14).

Nonetheless, in case of low drug permeability across buccal epithelium, penetration enhancers were successfully used in order to overcome the major limitation to drug delivery (12).

Principles of drug absorption (33, 34)

For most drugs, absorption via the buccal mucosa is a passive diffusion process and can be described by Fick's first law

$$J = \frac{DK_p}{h} C_D \quad (1)$$

Where J is the flux of the drug absorption defined as the amount of drug moving across the buccal mucosa per unit time and area, D is the diffusion coefficient of the drug inside the buccal mucosa, K_p is the partition coefficient between the oral mucosa and the buffer solution, h is the effective pathlength of the oral mucosa across which the drug must traverse and C_D is drug concentration in the donor chamber.

It is impractical to measure diffusion coefficient, partition coefficient and pathlength separately. However, the permeability coefficient, P, which is defined as:

$$P = \frac{DK_p}{h} \quad (2)$$

is easily obtained. In a typical diffusion experiment, the amount of drug (A) moving across the tissue at time, t, is determined by measuring drug concentration on the receiver side. The surface area of the tissue (S) (the opening of the diffusion cell) and the initial drug concentration in the donor chamber are known and remain constant during in the course of the experiment. Thus, the flux is:

$$J = PC_D = \frac{A}{St} \quad (3)$$

and the amount of drug transported at time t is:

$$A_T = PSC_D t \quad (4)$$

Since the total amount of the drug (A_T) on the donor side is:

$$A_T = V_D C_D \quad (5)$$

Where V_D is the volume of the donor chamber, the percent of drug transported (T%) at time t is:

$$T\% = \frac{A}{A_T} = \frac{PS}{V_D} t \quad (6)$$

A plot of T% versus t will give a slope of $\frac{PS}{V_D}$ and from this slope the permeability coefficient can be obtained.

Equation (6) shows how to determine the permeability coefficient a steady state. Practically, however, almost all diffusion studies do not begin at steady state, and as a matter of fact, steady state is theoretically never reached because the donor and receiver drug concentration are continuously changing. If the concentration changes are small, we can assume that the donor drug concentration is constant and the receiver drug concentration is zero. Under the assumption, the diffusion process can be described in one dimension by Fick's second law:

$$A = (SK_p hC_D)\left[\frac{Dt}{h^2} - \frac{1}{6} - \frac{2}{\pi^2}\sum_{n=1}^{\infty}\frac{(-1)^n}{n^2}e^{\frac{-Dn^2\pi^2 t}{h^2}}\right] \quad (7)$$

As time increase, the series term in equation (7) goes to zero and equation (7) transforms to equation (8) and (9):

$$A = \frac{SDK_p C_D}{h}\left[t - \frac{h^2}{6D}\right] \quad (8)$$

$$T\% = \frac{A}{A_T} = \frac{PS}{V_D}\left[t - \frac{h^2}{6D}\right] \quad (9)$$

Equation (9) shows that a plot of percent transported versus time has a slope of $\frac{PS}{V_D}$ and an x-axis intercept of $\frac{h^2}{6D}$, which is also called the lag time.

BUCCAL DRUG DELIVERY

In 1981, Ishida et al., being interested in buccal delivery attempted to develop an oral insulin dosage form that would solve the problem of injection. The system was a core of cacao butter, insulin, and additive surrounded by a layer of hydroxypropyl cellulose-H and Carbopol-934 (6, 35).

The term bioadhesion can be defined as the ability of a material (synthetic or natural) to stick (adhere) to a biological tissue for extended periods of time (23). The phenomenon of bioadhesion can be visualized as a two-step process. The first step involves the initial contact between polymer and the biological tissue. The second step is the formation of secondary bonds due to noncovalent interactions.

Buccal mucosa presents a relatively smooth and immobile surface for the placement of a bioadhesive dosage form. The amount of drug that can be incorporated is limited by the size limitation of the buccal dosage form. In general, a drug with a daily requirement of 25 mg or less is suitable for buccal delivery. Drugs with short half-lives, requiring sustained and controlled delivery, with poor aqueous solubility, which are sensitive to enzymatic degradation, may be successfully delivered across the buccal mucosa. The dosage forms developed for this purpose include adhesive tablets, adhesive patches, adhesive gels and adhesive ointment. Adhesive tablets and patches can be formulated to release the drug unidirectionally or multidirectionally by varying the extent and permeability of the backing (Figure 5) (11).

However, adhesive patches are preferred over other dosage forms because they are flexible and can be formulated in any shape. The size of the patches may vary from 1 to 15 cm^2. Small patches are more convenient and comfortable to wear (8, 11).

Adhesive patch

Adhesive patch can be of the reservoir or the matrix type. Formulations of the reservoir type are surrounded by a polymeric membrane, which controls the release rate. Reservoir systems present a constant release profile provided (i) that the polymeric membrane is rate limiting and (ii) that an excess amount of drug is present in the reservoir. In a matrix type system, the drug is uniformly dispersed in the polymer and drug release is controlled by the matrix. Drug molecules dispersed in the polymer have to dissolve in the medium and then diffuse through the polymer network. Therefore, a drug dispersion and drug depletion zone always exists in the matrix. A thin hydrodynamic diffusion layer also exists at the interface of the drug and the matrix. A matrix system may result in a constant release profile only at early times when the drug depletion zone is rather insignificant (11).

Adhesive patches may be monolithic or multilayered reservoir devices or matrix type for either systemic or local drug delivery. The two main manufacturing processes to prepare adhesive patches are solvent casting and direct milling. A backing is applied to control the direction of drug release and minimize deformation and disintegration of the device during its residence in the mouth. Preparation of adhesive patches by the solvent casting method involves casting volumes of

appropriately prepared aqueous solutions of polymer (for drug-free patches) or of a drug/mixture onto a backing layer sheet mounted on top of a stainless steel plate by means of a frame. The assembly may be dried by perfusing with a thermostated stream of water or air-drying. The temperature is typically selected according to the formulation excipients. Upon complete drying, the laminated may be cut into the desired shape and size using a suitable punch and a die set (11).

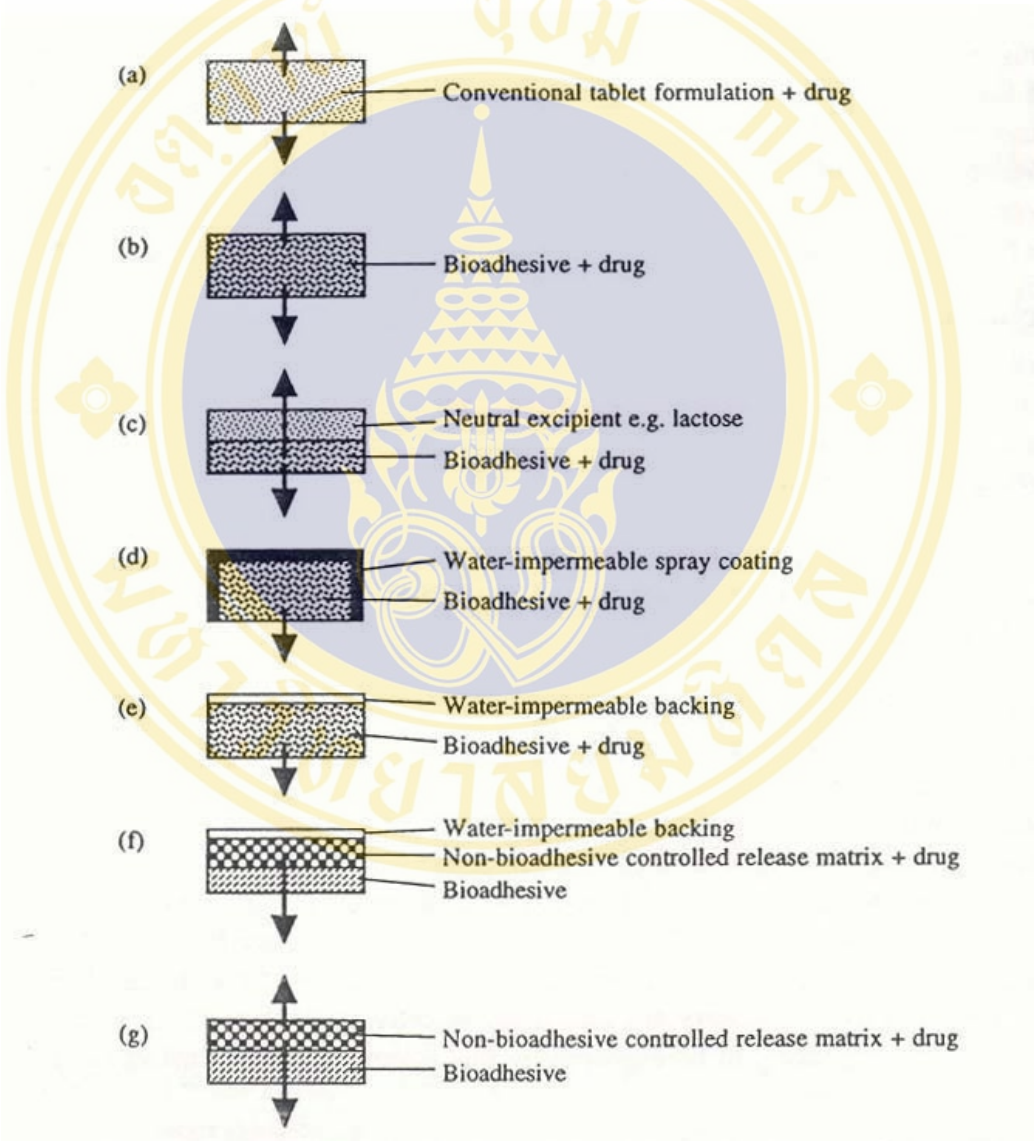


Figure 5. Schematic representation of possible designs of buccal delivery devices for systemic delivery (16).

In the preparation of adhesive patches by direct milling on a two-roll mill, the drug and the bioadhesive are homogenously mixed with or without the aid of a

solvent. The polymer/drug mixture may then be compressed to its desired thickness and patches of appropriate size may be cut or punched out. The polymer/drug mixture prepared with a solvent may require an additional drying step by air or in an oven (11).

The advantages of the unidirectional patch are (i) retention of the delivery system can be extended to 12 hrs or more, (ii) drug can be delivered across a specific, localized region of the oral cavity, (iii) the local environment at the selected site and (iv) permits co-administration of permeability enhancers which will modify absorption in a well defined area. (v) irritation or damage to the mucosa by the drug or its excipients will be restricted to the limited area (8, 16). The disadvantages of these systems are that they use only a small mucosal area and the backings have to be removed by the patient after drug administration (8).

CHITIN WHISKERS

Chitin is the second most abundant polysaccharides in nature, cellulose being the most abundant. Chitin is found in the exoskeleton of crustacea, insects, and some fungi. The main commercial sources of chitin are the shell wastes of shrimp, lobster, krill, and crab. In the world, several million tons of chitin are harvested annually (10).

Native chitin is highly crystalline and depending on its origin it occurs in three forms identified as α-, β- and γ-chitin. In the former, all chains are arranged in an antiparallel fashion with strong intermolecular hydrogen bonding. It is the dominant and stable form since it constitutes arthropod cuticles and mushroom cellular walls. Chitin has been known to form microfibrillar arrangements embedded in a protein matrix and these microfibrils have diameters ranging from 2.5 to 2.8 nm. Crustacean cuticles possess chitin microfibrils with diameters as large as 2.5 nm. Chitin can be used for medical applications since it is safe for the human body (17).

Chitin whiskers are used as a reinforce polymers in order to improve or modify certain mechanical properties of the host matrix for specific applications (17). Because the building blocks of a chitin whiskers nanocomposite are of nanoscales, they have an enormous interfacial area, and therefore there are a lot of interfaces between the two intermixed phases compared to usual microcomposites (18).

Suspensions of chitin whiskers are prepared by acid hydrolysis of chitin obtained from crab shells (17, 36), squid pen (37), shrimp and *Riftia* tubes (38). The whole treatment of chitin whiskers from Riftia tubes is reported in Figure 6. The object of this treatment is to dissolve away regions of low lateral order so that the water-insoluble, highly crystalline residue may be converted into a stable suspenoids by subsequent vigorous mechanical shearing action. Figure 7 shows a transmission electron micrograph of a dilute suspension of hydrolyzed crab shell chitin. The suspension contains chitin fragments consisting of both individual microcrystals and associated or collapsed microcrystals. These chitin fragments consist of slender rods with sharp points that have a broad distribution in size (17).

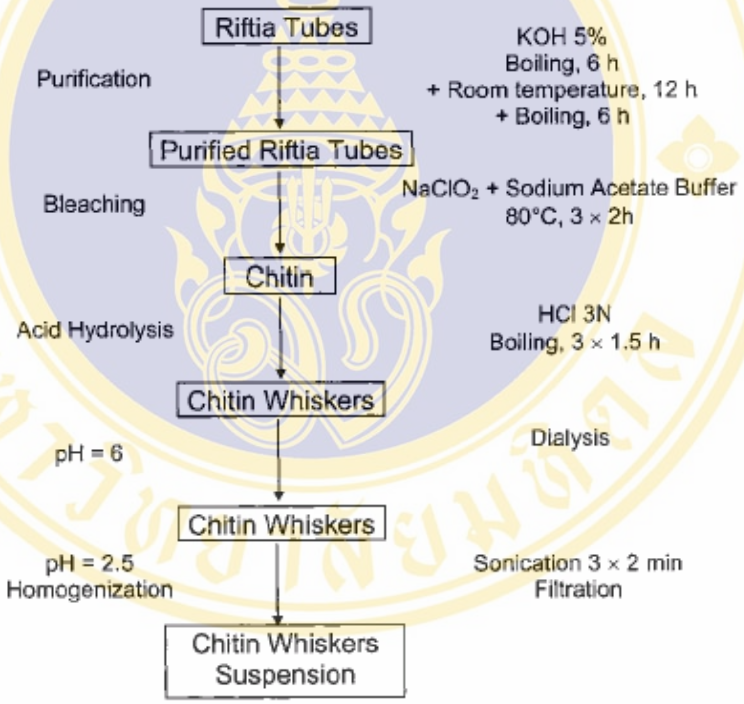


Figure 6. Chemical and mechanical treatments of Riftia tubes for the preparation of chitin whiskers (38).

The averages of length (L) and diameter (d) of crab shell chitin whiskers are about 240 and 15 nm, respectively. The average aspect ratio (L/d) of crab shell chitin whiskers is 16. These dimensions are closed to those reported for chitin whiskers obtained from squid pen (L = 50-300 nm, d = 10 nm, L/d = 15) but much lower than

those observed for chitin whiskers obtained from *Riftia* tubes (L = 0.5-10 μm, d = 18 nm, L/d = 120) (17).

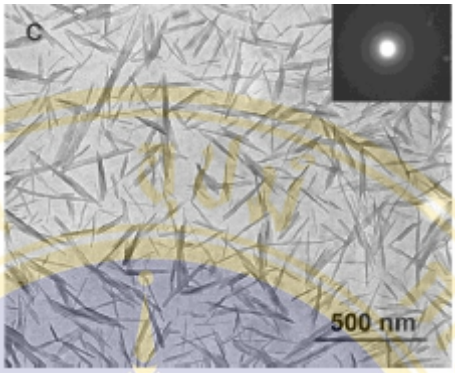


Figure 7. Transmission electron micrograph of a dilute suspension of crab shell chitin whiskers (17).

CHITOSAN

Chitosan was discovered by Rouget in 1859. The treatment of chitin with hot and concentrated potassium hydroxide produced a new substance dissolved in dilute acidic solution and he named "modified chitin". The "modified chitin" was studied again by Hoppe- Seyler, et al. and renamed chitosan (19, 20).

In 1963, Rudall narrated the presence of 3 different polymorphs of chitin to be α, β and γ. The chitin accounted from crab and shrimp shells has an α-structure, in which the chitin's chain arranged in an anti-parallel with strong intra- and intermolecular hydrogen bonding. In the other hand chitin accounted from squid pen has a β-structure, which the chitin's chain arranged in a parallel with weak hydrogen bonding (19, 20).

Chitosan molecule is a poly(1→4) β-linked 2-amino-2-deoxy-D-glucose and is obtained by the alkaline deacetylation of chitin (10). Chitin and chitosan are similar to cellulose in that they are comprised of linear chained molecules of β-(1,4)-linked glycans. Chemical structures for chitin and chitosan compared with cellulose are shown in Figure 8.

Mechanisms of chitosan

The mechanism of interaction between chitosan and the mucus layer has been reported previously. The mucus layer is made up of a network of mucin molecules,

which are linear, flexible and negatively charged due to the sialic acid and sulphate residues present on the mucin molecule (39). The binding and absorption enhancing effects of chitosans on epithelial cells are through their positive charges. The chitosan is able to interact with the opening mechanism of the tight junctions as seen by a decrease in ZO-1 proteins and the change in the cytoskeleton protein F-Actin from a filamentous to a globular structure, which are followed by enhanced transport through the paracellular pathway (25, 40).

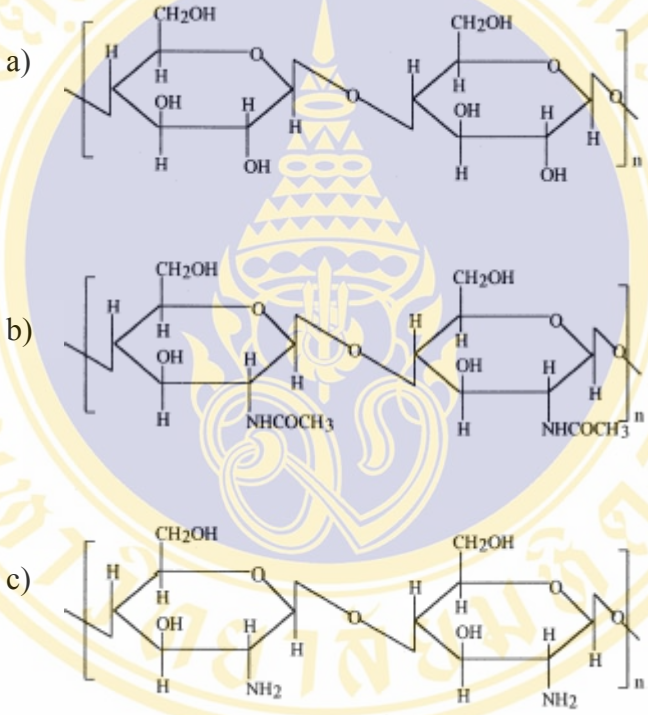


Figure 8. Structures of (a) cellulose (poly(1,4-β-D-glucose)), (b) chitin (poly(1,4-2-acetamido-2-deoxy-β-D-glucose)) and (c) chitosan (poly(1,4-2-amino-2-deoxy-β-D-glucose)).

Besides, chitosan has mucoadhesive properties, which are probably mediates through ionic interactions described above. Mucoadhesive are expected to increase the residence time. In addition, they provide intimate contact between a dosage form and the absorbing tissue, which may result in high drug concentration in a local area

and hence high drug flux through the absorbing tissue. Furthermore, the intimate contact may increase the total permeability of high molecular weight drugs (39).

Due to the fact that chitosan has a large number weight, exhibits a positive charge and demonstrates film forming ability and gelation characteristics, the material has been extensively used in food process (41), agriculture (41, 42), pharmaceutical, cosmetic, dental, hair care product, ophthalmic applications, waste-water treatment, membrane and microcapsule etc (10, 41). Physicochemical properties of chitosan such as rheological properties, antimicrobial and antifungal activity, immuno adjuvant activity (10, 41), wound healing and blood coagulant (6, 43), enzyme-binding activity (10, 41) and the structure and charge density of chitosans are important for the absorption enhancement of hydrophilic compounds across mucosal tissues (25).

Solubility of chitosan

The solubility of chitosan in water depends on the balance between the electrostatic repulsions coming from the protonated amine functions and the hydrogen bonding due to the free amino groups ($-NH^2$). In water at neutral pH, chitosan is not soluble due to it has a free amine form. At acidic pH, chitosan is soluble due to the amino groups are protonated to form cationic amine groups ($-NH^{3+}$) (44).

Chitosan salts are soluble in water, the solubility of chitosan is depending on the degree of deacetylation and the pH of solution. Chitosans with low degree of deacetylation ($\leq$40%) are soluble up to a pH of 9, whereas highly deacetylated chitosans ($\geq$85%) are soluble only up to a pH of 6.5 (10).

It is also interesting that, depending on the acid used to dissolve chitosan, the salt formation is only complete with strong mineral acids such as hydrochloric or nitric acid. This is a potential for the preparation of chitosan solutions which are both not too much acidic and bring the possibility to deliver free acid (45). Moreover, chitosan is soluble in organic acids like acetic, formic and propionic acid. Acetic acid is commonly used as a reference for solubility of chitosan (46).

Viscosity and rheology of chitosan

Chitosan behaves as a pseudoplastic material showing a decrease in viscosity with increase shear, due to high molecular weight and the linear unbranched structure of the molecule (46). Increasing the degree of deacetylation increases the viscosity. This can be explained by the fact that high and low deacetylated chitosans have

different conformations in aqueous solution. Chitosan has an extended conformation with a more flexible chain when it is highly deacetylated, because of the charge repulsion in the molecule. However, the chitosan molecule has a rod-like shape or coiled shape at low degree of deacetylation due to the low charge density in polymer chain (10, 41). The viscosity of chitosan solution is also affected by factors such as concentration and temperature. As the chitosan concentration increases and the temperature decreases, the viscosity increases (10, 47).

The precipitation of chitosan solutions consective to the addition of salts is generally achieved for very high salt concentrations, near the saturation (45). The concentration ratio between chitosan and the acid is of great importance, particularly in the case of mineral and multi-protonic acid (46).

Molecular weight and degree of deacetylation

The determination of the molecular weight distributions, the degree of acetylation, the ionic form of the initial material and the possible ion exchange necessitate knowing in the solution. The lack of consideration of these parameters is certainly the main reason of the discrepancy between the results reported in the literature. The steric exclusion chromatography is possible to deduce the average molecular weights, polydispersity index and the instrinsic viscosity of the sample (45).

The molecular weight of chitosan depends on the processing conditions and grades available within the range 50,000 to 2,000,000 daltons. The degrees of deacetylation (DD) range from 48 to 98% and it has recently been known that the absorption enhancing and toxic effects of chitosan are depending on their chemical composition. The degree of acetylation (DA) and the molecular weight of chitosans determine their absorption enhancing and cytotoxic properties. Chitosan with a low DA (1 to 15%) was active as absorption enhancers at low and high molecular weights but also show clear dose dependent toxicity. Chitosans with high DA (35 to 49%) enhanced the transport of drugs at high molecular weight only. However, these chitosans displayed low toxicity (25).

Ionic interaction and complexation of chitosan

The polycationic character of chitosan is a great interest for various applications involving the formation of polyelectrolyte complexs. Approximately all the naturally occurring surfaces are negatively charged. Thus, when chitosan is added

to aqueous dispersions of mineral, organic or living particles, depending on the concentration, it induces either a flocculation or a stabilization of the particle dispersion. The absorption of chitosan on these surfaces in generally described by Langmuir's law. The change of mechanism of flocculation depends on the molecular weight also. Proteins can be bound to chitosan by hydrogen bonding or Van der Waals interaction. These low energy interactions allow to assuming possible applications of chitosan for fiber coating, allowing an easier dyeing, or as stationary phases of affinity chromatography (45).

Biodegradability and biocompatibility

One of the most beneficial properties of chitosan is its biodegradability. Due to it is composed of glucosamine units found in most mammalian tissues. The path way for breakdown chitosan is enzymatic degradation via chitosanase (48). Alternative pathway is lysozymatic hydrolysis (41). Besides, chitosan can be degraded *in vivo* by lysozome, which is produced from macrophages (44). This mechanism is relevant to wound healing activity, which is one of the most attractive bioactivities of chitosan (41).

Safety and toxicity

Due to chitosan, natural products, is found in abundance in the environment. The chemical structure of chitosan suggests a low order of toxicity. The high molecular weight and apparent lack of enzyme to degrade the β-1,4-glycosidic linkage in the human gastrointestinal tract suggest that ingested chitosan would be excreted unchanged in the feces without significant absorption. This expected lack of absorption would preclude significant systemic toxicity (49).

Chitosan has low oral toxicity with an LD_{50} in rats of 16 g/kg, indicating a lack of acute oral toxicity. Toxicity of chitosan might depend on different factors such as degree of deacetylation, molecular weight, purity, and route of administration (10). Presently chitosan is approved as a food additive in Japan, Italy and Finland (41).

From the reasons which are describe above, chitosan has attracted a lot of attention in the pharmaceutical and medical fields. The pharmaceutical requirements of chitosan are: particle size <30 mm, density between 1.35 and 1.40 g/ cm^3, pH 6.5 to 7.5, insoluble in water, and partially soluble in acids (10).

STRUCTURE OF ORAL MUCOSA IN EXPERIMENTAL ANIMALS

The benefit of using human oral mucosa for drug delivery is that it offers an area of nonkeratinized, which is more permeable than the keratinized oral regions or the skin. However, there are considerable limitations to experimental studies in humans. There are numerous reports of the use of small laboratory animals for permeability studies, including rats, hamsters and guinea pigs. Unfortunately, such choices seriously limit the value of the data obtained for, unlike human; most laboratory animals have an oral lining that is totally keratinized. For example, the rat has a buccal mucosa with a very thick, keratinized, surface layer. The hamster has an extensive cheek pouch that can be easily everted so as to provide access to a large area of mucosa. However, this is a thin, keratinized tissue more closely resembling skin than oral mucosa. The rabbit represents the only laboratory rodent that has nonkeratinzed lining mucosa resembling human tissue. Rabbit buccal mucosa has been well characterized morphologically but the difficulty of access, the small area of nonkeratinized tissue and the abrupt transition to keratinized tissue at the margins limit its values for *in vivo* studies (4).

The oral mucosa of larger experimental animals that has been used for permeability and drug delivery studies include monkeys, dogs and pigs. The monkey has a very similar oral mucosa to that of human, although in the common laboratory species (for example, the Macaque or Rhesus) the oral cavity is smaller and the epithelium generally thinner (Table 2). The permeability coefficient values for water for buccal mucosa that appear to be higher than those obtained for human and pig (Table 2). The high costs of purchasing and maintaining monkeys, and the difficulties of handing them including the risks of infection, make these animals impractical *in vivo* models in most laboratories (4).

Dogs are less expensive and easier to handle than monkeys and have an oral mucosa very similar to that of human although the epithelium is somewhat thinner (4). However, comparative studies indicate that the tissue is more permeable than of human (Table 2).

The considerable similarities between pig and human in term of anatomy, metabolism, disease and wound healing make this a very attractive animal model. The oral mucosa probably resembles that of the human more closely than any other animal

in terms of structure and composition. Measurements of permeability reveal no significant differences from human oral mucosa for water (Table 2). For use *in vivo* studies pigs are relatively inexpensive to maintain although they grow rapidly. Miniature breeds have been used to overcome this problem but are extremely expensive and a more practical solution for short term projects is to work with young (weanling) domestic breeds. For *in vitro* studies it is relatively ease to obtain large amounts of pig oral mucosa from a slaughterhouse (4).

Table 2. Epithelial thickness and permeability constants (Kp) for buccal mucosa from different species of tritiated water (4).

Species	$K_p \times 10^{-7}$ cm/minute (± SD)	Thickness μm (± SD)
Human	579 ± 122	580 ± 90
Pig	634 ± 60	772 ± 150
Monkey	1025 ± 154	271 ± 50
Dog	1045 ± 37	126 ± 20

THE DEVELOPMENT OF CHITOSAN BUCCAL PATCH FOR VERAPAMIL HYDROCHLORIDE

An ideal buccal delivery system should stay in the oral cavity for a few hours and release the drug in a unidirectional way towards the mucosa in a controlled or sustained release fashion. Mucoadhesive polymers will prolong the residence time of the device in the oral cavity (10, 23). Chitosan is an excellent polymer to be used as a film-forming (24) due to its mucoadhesive properties (6, 10, 25, 26, 27) and as an absorption enhancer (6, 10, 13, 25).

CHAPTER III
MATERIALS AND METHODS

MATERIALS

1. Drug

Verapamil hydrochloride (obtained as a gift from Berlin Pharmaceutical Industry Co., Ltd., Thailand)

2. Chemicals and reagents

2.1 Chitosan (from crab shell obtained as a gift from Viriyanun Co., Ltd.)

2.2 Chitosan (from squid pen obtained as a gift from TCA Marketing Co., Ltd.)

2.3 Chitosan (water soluble obtained as a gift from Viriyanun Co., Ltd.)

2.4 Chitin whiskers (from shrimp obtained from Polymer Science Laboratory, The Petroleum and Petrochemical College, Chulalongkorn University)

2.5 Glacial acetic acid (LAB-SCAN Analytical Science, Thailand)

2.6 Disodium hydrogen phosphate anhydrous (Carlo-Erba reagent, MI, Italy)

2.7 Potassium dihydrogen phosphate (Carlo-Erba reagent, MI, Italy)

2.8 Sodium chloride (Carlo-Erba reagent, MI, Italy)

2.9 Ortho-phosphoric acid (BDH Laboratory Supplies, Poole, England)

2.10 Hydrochloric acid (LAB-SCAN Analytical Science, Thailand)

2.11 Acetonitrile (LAB-SCAN Analytical Science, Thailand)

2.12 Triethylamine (S. Tong Chemicals Co., Ltd., Thailand)

2.13 Sterile water for injection (General Hospital Products Public Co., Ltd., Thailand)

2.14 Backing membrane 9733 Scotchpak™ (3M™, MN, U.S.A.)

2.15 Medical double coated tapes 9877 (3M™, MN, U.S.A.)

3. Instruments

3.1 Beaker (50, 100, 250, 600, 1000, 2000 ml)

3.2 Volumetric flask (10, 25, 50, 50, 100, 500, 1000 ml)

3.3 Cylinder (10, 50, 100, 1000 ml)

3.4 Transfer pipette (1, 2, 5 ml)

3.5 Measuring pipette (1, 2 ml)

3.6 Plastic syringe (1, 10 ml)

3.7 Membrane filter, 0.45 μm, diameter 13 mm (Lida Manufacturing Corp., U.S.A.)

3.8 Microdissecting forceps

3.9 Microdissecting scissors

3.10 Modified Franz cell, 15 mm inner orifice: 12 ml volume, 1.77 cm^2 area

3.11 μBondapak™ column (reversed-phase column C18, 10 μm particle size, 300 mm length, 3.9 mm internal diameter)

4. Equipments

4.1 Preparation of chitosan acetate membrane

4.1.1 Labdryer (Werner Mathis AG, Zürich, Switzerland)

4.1.2 Incubator (Clayson Laboratory Apparatus Pty Ltd., U.S.A.)

4.1.3 Micrometer (Combimike® Digit Outside Micrometers, Mitutoyo Mfg. Co., Ltd., Japan)

4.1.4 Sonicator (Bransonic® Model B-1200 EL, U.S.A)

4.2 *In vitro* mucoadhesive study

4.2.1 Texture analyzer (Stable Micro Systems Model TA-XT plus, UK)

4.2.2 pH meter (Accumet® Model Basic AB15, Fisher Scientific Co., U.S.A.)

4.3 *In vitro* drug release rate study

4.3.1 Dissolution tester (Hansan Research® Model SR II6-Flasks dissolution test station, Germany)

4.3.2 UV/ VIS spectrophotometer (Perkin Elmer instruments Model Lambda 35, U.S.A.)

4.4 *In vitro* penetration rate study

4.4.1 Manual skin graft

4.4.2 Sixth-station Franz cell stirrer (Crown Glass Company, INC., New Jersey, U.S.A.)

4.4.3 Circulating waterbath (Thermomix® Model 5BU, B. Braun Melsungen AG, Germany)

4.4.4 High performance liquid chromatography (HPLC) (Shimadzu® Model LC-10AD, Shimadzu corp., Japan)

4.4.5 UV/ VIS detector (Shimadzu® Model SPD-10AV, Shimadzu corp., Japan)

5 Tissue model

Isolated porcine buccal mucosa

METHODS

1. CHARACTERIZATION OF CHITOSAN

Three types of chitosan in this study are from the same batch used in Nosoongnoen (50). The characterization methods used for chitosan in this study are the same as Nosoongnoen (50).

1.1 Determination of chitosan viscosity

The viscosity of chitosan solution (0.25 %w/v) was determined using Ostwald viscometer. The flow time of the chitosan solutions to pass through the capillary measured at 27 ^{O}C was used to calculate absolute viscosity in the term of centipoises unit.

1.2 Determination of intrinsic viscosity (ŋ)

The chitosan solution was adjusted to concentration of 0.05, 0.10, 0.15, 0.25 and 0.35%. The flow time of the chitosan solutions and solvent to pass through the capillary measured at 27 ^{O}C was used to calculate the relative and specific viscosity using Ostwald viscometer and Westphal balance.

The η_{sp}/C and $ln\eta_r/C$ were plotted against the solution concentration (C). The intercept obtained by extrapolation η_{sp}/C and $ln\eta_r/C$ to the zero concentration will present the intrinsic viscosity.

When $\eta_{sp} = \eta_r - 1$; η_{sp} = specific viscosity

η_r = relative viscosity

1.3 Determination of average molecular weight

The average molecular weights were accounted from the measurement of the intrinsic viscosity (η).

In this study, the average molecular weight values were accounted from viscosity using parameter as reported by Robert et al. (51):

$$\eta\ (cm^3/g) = 1.81 \times 10^{-3}\ M^{0.93}$$

1.4 Determination of percent degree of deacetylation

The FTIR spectroscopy was used to calculate percent degree of deacetylation. Powder samples were packed into small aluminium or stainless steel cup. The main objective when preparing a sample is to ensure the highest amount of sample as possible in the foci of the laser beam and collection lens. FTIR spectra of chitosan samples (KBr discs and films) and of the model system (KBr discs) will run in a FT-RAMAN spectrometer. The quantitative values were calculated from integral values fitting the FTIR spectra with software program OPUS version 2.0.

The absorbance (integral value) in the band at 1665 and 2867 cm^{-1} were obtained. Then, the value of A_{1665}/A_{2867} was used to calculate percent degree of deacetylation by comparing to the standard curve which was plotted between values of A_{1665}/A_{2867} against percent degree of deacetylation. The standard samples used in this study were obtained from crab shells and known exactly percent degree of deacetylation.

2. CHARACTERIZATION OF CHITOSAN ACETATE FILM

This study was performed in order to evaluate the film-forming capability and mucoadhesive behavior of chitosan from various sources.

2.1 Preparation of chitosan acetate film

Chitosan was prepared according to Sawayanaga et al. (52). Chitosan from crab shell, chitosan from squid pen and water soluble chitosan of 1.0, 1.5, 2.0, 2.5, 3.0 %w/v concentration were dissolved in 0.55 %v/v glacial acetic acid solution and then standed for 48 hr.

The chitosan acetate solution was casted onto a supporting layer and thickened 2 mm using Labdryer. The film was ovened at 40 °C and air speed 1,800 rpm for 2 hr. Then, it was ovened continuously in incubator at 40 °C for 18 hr. The dried film was cut to the appropriate size and shape.

In this study, 1.5% w/v hydroxyl ethylcellulose (HEC) was used to prepare a control film. According to Nafee et al. (27), 1.5 %w/v of hydroxyl ethylcellulose was dispersed in ¾ the volume of sterile water for injection with continuous stirring and the final volume was adjusted with sterile water for injection. Then it was standed for 48 hr and casted as described above.

2.2 Evaluation of chitosan acetate film

2.2.1 Thickness of chitosan acetate film

The thickness of chitosan acetate film was measured using micrometer.

2.2.2 *In vitro* mucoadhesive study of chitosan acetate film

2.2.2.1 Preparation of porcine buccal mucosa

This method was modified from Artusi et al. (12), a head of a freshly slaughtered pigs weighing 70-100 kg was immediately placed in cold simulated saliva solution (2.38 g Na_2HPO_4, 0.19 g KH_2PO_4 and 8.00 g NaCl in 1000 ml of distilled water at pH 6.8) (53) and transferred to our laboratory within 2 hr. The porcine buccal mucosa which was obtained from buccal tissue (cheek) removed from the pig's head (Figure 9). The buccal mucosa were carefully separated from fat and muscles using

microdissecting forceps and microdissecting scissors, and then were stored at -70 °C until it were used. The thickness of tissue was 1-2 mm.

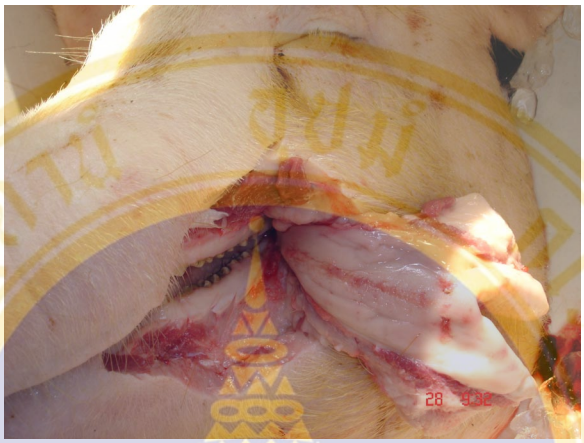


Figure 9. Porcine mucosa was obtained from buccal tissue (cheek) removed from the pig's head.

2.2.2.2 *In vitro* mucoadhesive study

This method was modified from Wong et al. (53), a Texture analyzer equipped with a 5 kg load cell was employed to determine the mucoadhesive property of chitosan acetate film using surface of porcine mucosa. The porcine mucosa was thawed to room temperature before used. The porcine mucosa was fixed to a tissue holder. A circular hole of 14 mm diameter was made on the top of the tissue holder to expose the porcine mucosa for contact with the chitosan film. The whole tissue holder was placed in a beaker filled with simulated saliva solution (37 $\pm$ 0.5 °C). The chitosan film was cut into a circle with a diameter of 10 mm and fixed to a cylinder probe using a double side tape. During measurement, 200 µl of simulated saliva solution was evenly spreaded on the surface of the mucosa. For each sample, the measurements were conducted in 10 replicates. The work of adhesion and peak detachment force was used to study the buccal mucoadhesiveness.

3. CHARACTERIZATION OF CHITIN WHISKERS-CRAB CHITOSAN ACETATE FILM

From result in topic 2, 1.5% w/v crab chitosan was selected to prepare chitin whiskers-chitosan acetate film.

This study was performed in order to evaluate the film-forming capability and mucoadhesive behavior of the mixture of chitin whiskers suspension and crab chitosan acetate solution.

3.1 Preparation of chitin whiskers-crab chitosan acetate film

The ratio of chitin whiskers suspension in water and 1.5 %w/v crab chitosan acetate solution are 1.5:1, 1:1, 1:2, 1:3 and 1:4 were mixed.

The mixture of chitin whiskers suspension and crab chitosan acetate solution were solvent casted onto a supporting layer and thickened 2 mm using Labdryer. The film was ovened at 40 °C and air speed 1800 rpm for 2 hr. Then, it was ovened continuously in incubator at 40 °C for 18 hr. The dried film was cut to the appropriate size and shape.

3.2 Evaluation of chitin whiskers-crab chitosan acetate film

3.2.1 Thickness of chitin whiskers-crab chitosan acetate film

The thickness of chitin whiskers-crab chitosan acetate film was measured using micrometer.

3.2.2 *In vitro* mucoadhesive study of chitin whiskers-crab chitosan acetate film

3.2.2.1 Preparation of porcine buccal mucosa

The porcine buccal mucosa was prepared as described in topic 2.2.2.1.

3.2.2.2 *In vitro* mucoadhesive study

The chitin whiskers-crab chitosan acetate film was studied as described in topic 2.2.2.2.

4 CHARACTERIZATION OF VERAPAMIL HYDROCHLORIDE-CHITOSAN ACETATE BUCCAL PATCH

In order to evaluate the characteristic of verapamil hydrochloride-chitosan acetate buccal patch, film-forming capability, mucoadhesive behavior, drug release rate and penetration rate via porcine buccal mucosa were performed.

4.1 Preparation of verapamil hydrochloride-chitosan acetate buccal patch

The dose of verapamil hydrochloride of 20 mg per 10 x 15 mm of buccal patch was modified from Sawicki and Janicki (54). The 6.67 %w/v of verapamil hydrochloride were dissolved in 0.55 %v/v glacial acetic acid solution and 1.0, 1.5, 2.0 %w/v of crab chitosan or squid pens chitosan were added. Then it was standed for 48 hr.

The verapamil hydrochloride-chitosan acetate solution was casted onto a Scotchpak® 9733 backing membrane coated with a medical adhesive layer (medical double coated tapes 9877) using Labdryer. The film was thickened 2 mm and ovened at 40 °C, air speed 1800 rpm for 2 hr. Then, it was ovened continuously in incubator at 40 °C for 18 hr. The dried film was cut to the appropriate size and shape. The schematic diagram of the patch that modified from Li et al. (55) is shown in Figure 10.

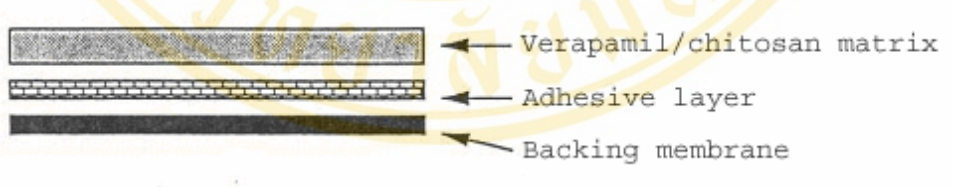


Figure 10. Schematic diagram of verapamil hydrochloride-chitosan acetate buccal patch.

In this study, verapamil hydrochloride-1.5% w/v hydroxyl ethylcellulose (HEC) was used to control buccal patch. For its preparation, the 6.67 % w/v of verapamil hydrochloride was dissolved in water and 1.5 %w/v of hydroxyl ethylcellulose was added. Then it was standed for 48 hr and casted as described above. The dose of verapamil hydrochloride was 20 mg per 10 x 15 mm of buccal patch.

4.2 Evaluation of verapamil hydrochloride-chitosan acetate buccal patch

4.2.1 Thickness of verapamil hydrochloride-chitosan acetate buccal patch

The thickness of verapamil hydrochloride-chitosan acetate buccal patch was measured using micrometer.

4.2.2 *In vitro* mucoadhesive study of verapamil hydrochloride-chitosan acetate buccal patch

4.2.2.1 Preparation of porcine buccal mucosa

The porcine buccal mucosa was prepared as described in topic 2.2.2.1.

4.2.2.2 *In vitro* mucoadhesive study

The verapamil hydrochloride-chitosan acetate buccal patch was studied as described in topic 2.2.2.2.

4.2.3 Content uniformity of verapamil hydrochloride-chitosan acetate buccal patch

4.2.3.1 Standard preparation

Calibration curve of verapamil hydrochloride were established by dissolving an accurately weighed 20 mg of verapamil hydrochloride in 50 ml of 0.01N hydrochloric acid to make 0.4 mg/ml standard stock solution. Dilute the standard stock solution to account the standard solution with 0.01N hydrochloric acid, where the final concentrations were 0.004, 0.008, 0.016, 0.024, 0.032, 0.048 and 0.064 mg/ml. The standard solution was determined using UV/ VIS spectrophotometer at 278 nm. Linearity of standard curve was assessed from the square of correlation coefficient (R^2) which determined by least-square linear regression analysis.

4.2.3.2 Content uniformity study

This method was modified from uniformity of dosage units in verapamil hydrochloride tablets in USP 26 (56), the buccal patch was cut into a rectangle with 10 x 15 mm, which contained about 20 mg of verapamil hydrochloride. Transfered it to a 50-ml volumetric flask, added 25 ml of 0.01N hydrochloric acid and heat on a steam bath for 50 min. Sonicate the heated solution for 10 min, cool, dilute with 0.01N hydrochloric acid to volume, mix and filter. Pipette 1 ml of the filtrate into 10-ml volumetric flask, dilute with 0.01N hydrochloric acid (final concentration 0.04

mg/ml). The amount of verapamil hydrochloride in all samples was determined using UV/ VIS spectrophotometer at 278 and 300 nm. The different values between absorbances at 278 and 300 nm of the sample preparation were ordinated with calibration curve.

4.2.4 *In vitro* drug release rate study of verapamil hydrochloride-chitosan acetate buccal patch

4.2.4.1 Standard preparation

Calibration curve of verapamil hydrochloride were established by dissolving an accurately weighed 20 mg of verapamil hydrochloride in 50 ml simulated saliva solution of pH 6.8 to make 0.4 mg/ml standard stock solution. Dilute the standard stock solution to account the standard solution with simulated saliva solution of pH 6.8, where the final concentrations were 0.004, 0.008, 0.016, 0.024, 0.032, 0.048 and 0.064 mg/ml. The standard solution was determined using UV/ VIS spectrophotometer at 278 nm. Linearity of standard curve was assessed from the square of correlation coefficient (R^2) which determined by least-square linear regression analysis.

4.2.4.2 *In vitro* drug release study

The measurement of verapamil hydrochloride release rate form the buccal patch were performed using a design closely similar to the USP 26th dissolution test apparatus 5 (paddle over disk) (57). The buccal patch was cut into a rectangle with 10 x 15 mm, which contained about 20 mg of verapamil hydrochloride, and sandwiched between the self-fabricated disk assemblies (41.2 mm diameter) made from stainless steel with a sieve opening of approximate 40x40 mesh. The drug release rate study were carried out in 400 ml of simulated saliva solution of pH 6.8 (37$\pm$0.5 °C), 75 rpm. A 5 ml sample were obtained at 5, 10, 20, 30, 45 min and 1, 1.5, 2, 3, 4, 5 hr. Each sample was replaced by an equal volume of simulated saliva solution of pH 6.8 at all time points. All collected samples were determined using UV/ VIS spectrophotometer at 278 and 300 nm. The different values between absorbances at 278 and 300 nm of the collected sample were ordinated with calibration curve.

Subsequently, the residual patch was collected and analyzed for remaining drug. Transfer it to a 25-ml volumetric flask, add 15 ml of 0.01N hydrochloric acid

and heat on a steam bath for 50 min. Sonicate the heated solution for 10 min, cool, dilute with 0.01N hydrochloric acid to volume, mix and filter. Then, all samples were followed as described in topic 4.2.3.

4.2.5 *In vitro* penetration study verapamil hydrochloride-chitosan acetate buccal patch

4.2.5.1 Preparation of porcine buccal mucosa

This method was modified from Artusi et al. (12) and Walker (58), a head of a freshly slaughtered pigs weighing 70-100 kg were immediately placed in cold simulated saliva solution (2.38 g Na_2HPO_4, 0.19 g KH_2PO_4 and 8.00 g NaCl in 1000 ml of distilled water at pH 6.8) and transferred to our laboratory within 2 hr. Porcine buccal mucosa were obtained from buccal tissue (cheek) removed from the pig's head. The buccal mucosa were carefully separated from fat and muscles using microdissecting forceps and microdissecting scissors, and then were stored at -70 °C until it were used. The epithelium was isolated from the underlying tissue and the thickness of mucosa was about 500 µm (12, 15) using manual skin graft (Figure 11).

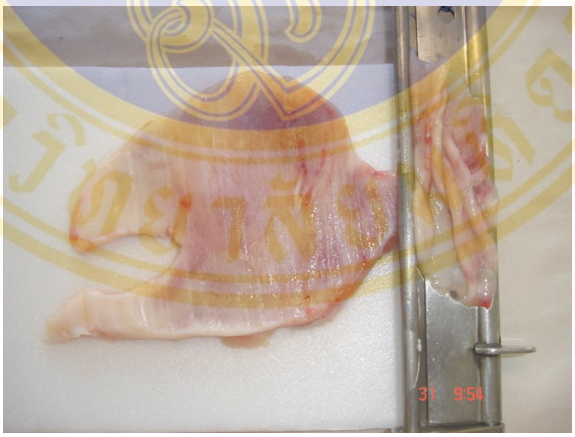


Figure 11. Isolated epithelium of porcine buccal mucosa using manual skin graft.

4.2.5.2 Standard preparation

For receptor compartment (pH 7.4) assay, calibration curve of verapamil hydrochloride were established by dissolving an accurately weighed 25 mg of verapamil hydrochloride in 50 ml phosphate buffer saline of pH 7.4 to make 0.5 mg/ml standard stock solution. Dilute the standard stock solution to account the

standard solution with phosphate buffer saline of pH 7.4, where the final concentrations were 0.005, 0.01, 0.1, 0.2 and 0.3 mg/ml. The standard solution was determined using HPLC.

For donor compartment (pH 6.8) assay, calibration curve of verapamil hydrochloride were established by dissolving an accurately weighed 25 mg of verapamil hydrochloride in 50 ml simulated saliva solution of pH 6.8 to make 0.5 mg/ml standard stock solution. Dilute the standard stock solution to account the standard solution with simulated saliva solution of pH 6.8, where the final concentrations were 0.01, 0.1, 0.2, 0.3 and 0.4 mg/ml. The standard solution was determined using HPLC.

4.2.5.3 *In vitro* penetration study

This method was modified from Artusi et al. (12), a sixth- station Franz cell stirrer system was used for assessment of permeability of the verapamil hydrochloride released from buccal patch. Buccal mucosa was mounted in Franz cell (diffusion area 1.77 cm^2) with the mucosal side facing the donor compartment. Then, donor and receptor compartments were filled with 1 ml and 12 ml of phosphate buffer saline (pH 7.4), respectively. After 30-min equilibration period at 37$\pm$0.5 °C, the receptor was replaced with 12 ml of fresh phosphate buffer saline (pH 7.4) and the donor side was filled with 0.2 ml of the simulated saliva solution (pH 6.8). The buccal patch was cut into a rectangle with 10 x 7.5 mm (diffusion area 0.75 cm^2), which contained about 10 mg of verapamil hydrochloride, and fixed with Scotchpak® 9733 backing membrane coated with an adhesive layer that was cut into a circle with a diameter of 14 mm. Then, it was fixed onto mucosal side of the donor compartment. A 0.3 ml sample were obtained at 0, 5, 10, 15, 20, 25, 30, 45 min and 1, 1.5, 2, 3, 4, 5, 6, 9 hr. Each sample was replaced by an equal volume of phosphate buffer saline (pH 7.4) at all time points. Then, the all residual solution in the donor site was collected at the end of study, rinsed with the simulated saliva solution pH 6.8 and adjusted volume to 5 ml. All collected samples were assayed for the drug concentration by HPLC.

Subsequently, the residual patch was collected and analyzed for remaining drug. Transferred it to a test tube, added 20 ml of 0.01N hydrochloric acid and heated on a steam bath for 50 min. Sonicated the heated solution for 10 min, cool, mixed and filtered. Then, all samples were followed as described in topic 4.2.3.

In this study, verapamil hydrochloride solution was used to the control solution. For its preparation, verapamil hydrochloride was dissolved an accurately weighed 250 mg of verapamil hydrochloride in 5 ml simulated saliva solution of pH 6.8. After 30-min equilibration period, the donor side was replaced with 0.2 ml of the control verapamil hydrochloride solution and followed as described above. Nevertheless, the all of residual solution in the donor site was collected at the end of study , rinse with the simulated saliva solution pH 6.8 and adjusted volume to 10 ml.

4.2.5.3 Determination of verapamil hydrochloride in penetration samples

Concentrations of verapamil hydrochloride in penetration samples were determined by a reversed-phase, high performance liquid chromatography (HPLC) method with UV detection modified from Tassin et al. (59).

4.2.5.3.1 Chromatographic condition

Column:	μBondapak™ column (Reversed-phase column C18, 10 μm particle size, 300 mm length, 3.9 mm internal diameter)
Mobile phase:	0.2 M potassium dihydrogen phosphate, 2 ml/l triethylamine adjusted to pH 3 with 0.2 M ortho-phosphoric acid : acetonitrile (350 ml)
Flow rate:	1.4 ml/min
Injection volume:	10 μl
Detector:	UV spectrophotometric detector (wavelength 278 nm)
HPLC system:	Shimadzu solvent delivery model LC-10AD, a model LC-10AD pump, a model SIL-10A automatic sample injector, a model SPD-10AV UV spectrophotometric detector
Computer integrator:	SPD-10A version 1.3 software LC-10 program
Temparature:	Room temperature (25°C)

4.2.5.3.2 Preparation of mobile phase

0.2 M potassium dihydrogen phosphate was prepared by dissolving 27.218 g of potassium dihydrogen phosphate (KH_2PO_4) with sterile water, added 2 ml of triethylamine and adjusted to 1,000 ml. Then pH of this solution was adjusted to pH 3 with 0.2 M ortho-phosphoric acid. Mixed 650 ml of phosphate buffer solution with 350 ml of acetonitrile, filtered through 0.45 μm nylon membrane filter and then deaerated by ultrasonicator for 15 minutes before used.

4.2.5.3.3 Validation of HPLC method

1. Separation and specificity

Separation and specificity of the analytical methods were assessed in relation to interference peaks from mobile phase, phosphate buffer saline of pH 7.4, simulated saliva solution of pH 6.8 and drug-free chitosan acetate buccal patch constituents by comparing their relation times with verapamil hydrochloride.

2. Linearity

Linearity of standard curve was assessed from the square of correlation coefficient (R^2) which determined by least-square linear regression analysis.

3. The limit of quantitation

The limit of quantitation (LOQ) is defined as the lowest concentration on the standard curve which can be measured with acceptable precision (%CV < 20) and accuracy (%bias < 20)

The limit of quantitation was determined by add various known amount of verapamil hydrochloride in phosphate buffer saline of pH 7.4 or simulated saliva solution of pH 6.8. The lowest concentration of verapamil hydrochloride on the standard curve which still be linearly correlated and measured with acceptable precision (%CV < 20) and accuracy (%bias < 20) was the limit of quantitation of the assay.

4. Precision and accuracy

Precision of the assay procedure was assessed from the percentage of coefficient of variation of analyzed drug concentration. Three different concentrations of verapamil in phosphate buffer saline of pH 7.4 (0.01, 0.1 and 0.3 mg/ml) and three

different concentrations of verapamil in simulated saliva solution of pH 6.8 (0.1, 0.2 and 0.4 mg/ml) were prepared and used to determine within-run (intraday) and between-run (interday) precision. For within-run precision, 5 replicates of each concentration were analyzed within 1 day whereas between-run precision was performed by assaying 2 replicates of each concentration in each day for 5 different days. The percentage of coefficient of variation (%CV) was calculated by the following equation:

$$\%CV = \frac{SD}{\overline{X}} \bullet 100$$

Where: SD = standard deviation

$\overline{X}$ = mean value of verapamil hydrochloride concentration

The accuracy of the analytical method was expressed as the percentage of deviation of measured concentration from target concentration (%bias).

$$\%Bias = \frac{\left| \text{Measured concentration} - \text{Target concentration} \right|}{\text{Target concentration}} \bullet 100$$

The acceptable precision and accuracy were %CV < 15 and %bias < 15.

5 CHARACTERIZATION OF VERAPAMIL HYDROCHLORIDE-CHITIN WHISKERS-CHITOSAN ACETATE BUCCAL PATCH

In order to evaluate the characteristic of verapamil hydrochloride-chitin whiskers-chitosan acetate buccal patch, film-forming capability, mucoadhesive behavior, drug release rate and penetration rate via porcine buccal mucosa were performed.

5.1 Preparation of verapamil hydrochloride-chitin whiskers-chitosan acetate buccal patch

The dose of verapamil hydrochloride was 20 mg per 10 x 15 mm of buccal patch. The verapamil hydrochloride was dissolved in 0.55 %v/v glacial acetic acid solution and 1.5 %w/v of selected chitosan was added. Then it was standed for 48 hr.

The ratios of chitin whiskers suspension in water and verapamil hydrochloride-chitosan acetate solution are 1.5:1, 1:2 and 1:4 were mixed.

The mixture of chitin whiskers suspension and verapamil hydrochloride-chitosan acetate solution were solvent casted onto a Scotchpak® 9733 backing membrane coated with a medical adhesive layer using Labdryer. The film was thickened 2 mm and ovened at 40 °C, air speed 1800 rpm for 2 hr. Then, it was ovened continuously in incubator at 40 °C for 18 hr. The dried film was cut to the appropriate size and shape. The schematic diagram of the patch is shown in Fig.4.

5.2 Evaluation of verapamil hydrochloride- chitin whiskers-chitosan acetate buccal patch

5.2.1 Thickness of verapamil hydrochloride-chitin whiskers-chitosan acetate buccal patch

The thickness of verapamil hydrochloride-chitin whiskers-chitosan acetate buccal patch was measured using micrometer.

5.2.2 *In vitro* mucoadhesive study of verapamil hydrochloride- chitin whiskers-chitosan acetate buccal patch

The verapamil hydrochloride-chitin whiskers-chitosan acetate buccal patch was studied as described in topic 4.2.2.

5.2.3 Content uniformity of verapamil hydrochloride-chitin whiskers-chitosan acetate buccal patch

The verapamil hydrochloride-chitin whiskers-chitosan acetate buccal patch was studied as described in topic 4.2.3.

5.2.4 In vitro drug release rate study of verapamil hydrochloride-chitin whiskers-chitosan acetate buccal patch

The verapamil hydrochloride-chitin whiskers-chitosan acetate buccal patch was studied as described in topic 4.2.4.

5.2.5 *In vitro* penetration study verapamil hydrochloride--chitin whiskers-chitosan acetate buccal patch

The verapamil hydrochloride-chitin whiskers-chitosan acetate buccal patch was studied as described in topic 4.2.5.

6. STATISTICAL ANALYSIS

The data were expressed as mean ± standard deviation (SD). One way analysis of variance (ANOVA) was used to compare the values for each of experimental groups. When the variances were equal, Tukey HSD (Tukey's honestly significant difference) test was used to differentiate the difference between experimental groups. When the variances were unequal, Dunnette's C method was used to differentiate the difference between groups. The p-value of less than 0.05 ($p\text{-value} < 0.05$) was considered to be statistical significant difference. All data are shown in Appendix.

CHAPTER IV
RESULTS

1. CHARACTERIZATION OF CHITOSAN SOLUTION

Three types of chitosan in this study were from the same batch used in Nosoongnoen (59). The apparent viscosity characteristic of chitosan solution from various sources (at concentration of 0.25% w/v), measured with Ostwald viscometer and Westphal balance, is shown in Table 3.

Table 3. Characteristic properties of chitosans from various sources that resulted from Nosoongnoen (59).

Sample	Viscosity (centipoises)	[η] (ml/g)[a]	Mw (g/mole)[b]	% DD[c]
Crab chitosan	871	1.71×10^3	2,666,860	99
Squid pens chitosan	118	0.19×10^3	255,014	91
Water soluble chitosan	113	0.11×10^3	145,106	71

a = Intrinsic viscosity
b = Molecular weight
c = Percent degree of deacetylation

From these results, the apparent viscosity which determined using Oswald viscometer and Westphal balance in terms of centipoises and intrinsic viscosity [η] of chitosan which obtained from crab shell were higher than those obtained from squid pens and water soluble chitosan, respectively. Quantitative FTIR results implied % DD of crab chitosan to be 99% which is higher than % DD found in the case of squid pens (91%) and water soluble chitosan (71%), respectively.

The average molecular weight of chitosan obtained from various sources was calculated from intrinsic viscosity using k and a parameter proposed by Robert et al.

The molecular weight arranged from high to low was crab chitosan, squid pens chitosan and water soluble chitosan, respectively.

2. CHARACTERIZATION OF CHITOSAN ACETATE FILM

The chitosan acetate solution from crab shell chitosan was clear with no color. Therefore, the film was smooth and transparent. The solutions of 1.0, 1.5, 2.0, 2.5 %w/v concentration could be casted to form film, whereas 3.0% was so sticky that it could not spread on supporting layer by Labdryer.

The chitosan acetate solution from squid pens chitosan exhibited yellow liquid with precipitate. Therefore, the film was rough with yellow color. The solutions of 2.0, 2.5, 3.0 %w/v concentration could be casted to form film, whereas 1.0 and 1.5 % were so thin that they could not spread on supporting layer by Labdryer.

The chitosan acetate solution from water soluble chitosan was clear with no color. All concentrations of these chitosan acetate solutions could not be casted to form film because they were so thin that it could not spread on supporting layer by Labdryer.

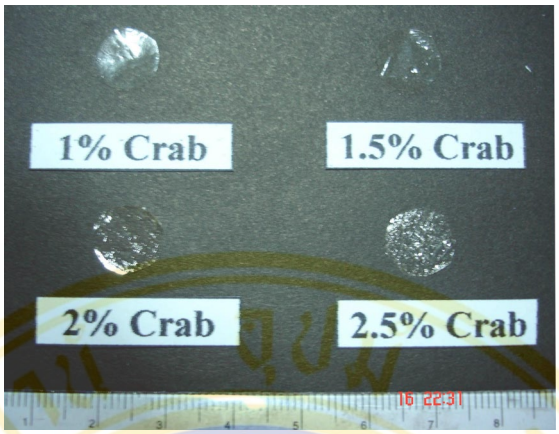
The 1.5% w/v HEC (hydroxyl ethylcellulose) solution was used to prepare a control film. Its solution was clear with no color. Therefore, the film was smooth and transparent. The characteristic of the different films are shown in Figure 12.

2.1 Thickness of chitosan acetate film

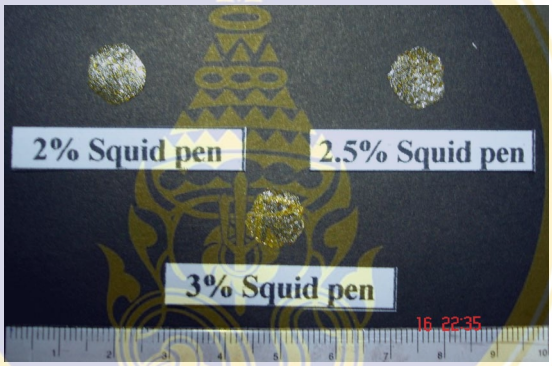
Table 4 shows the average thickness of chitosan acetate film which was measured using micrometer. It was founded that the higher the concentration of both crab chitosan and squid pens chitosan, the higher the thickness of films.

2.2 *In vitro* mucoadhesive study of chitosan acetate film

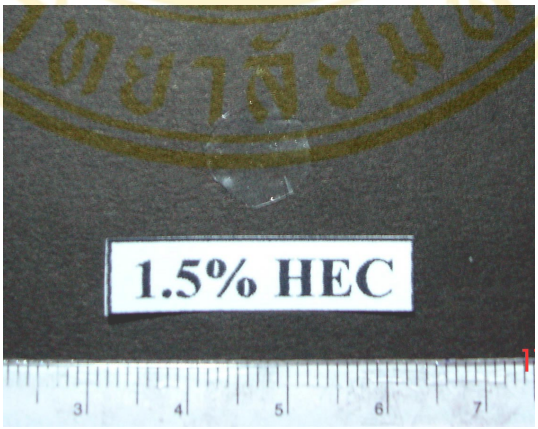
Table 5 shows the average work of adhesion and peak detachment force from chitosan acetate film. In mucoadhesive study, the work of adhesion was determined from the area under the force-distance curve whereas the peak detachment force was the maximum force required to detach the chitosan film from tissue.



a.



b.



c.

Figure 12. Characteristic of the different films **a.** The corresponding concentration of crab chitosan films, **b.** The corresponding concentration of squid pens chitosan films and **c.** The 1.5% hydroxyethylcellulose film.

Table 4. Average thickness of chitosan acetate film from various sources (Mean ± SD, n = 6).

Sample	Concentration (%)	Thickness (mm)
Crab chitosan	1.0	0.02 ± 0.01
	1.5	0.03 ± 0.01
	2.0	0.07 ± 0.01
	2.5	0.09 ± 0.01
Squid pens chitosan	2.0	0.12 ± 0.01
	2.5	0.21 ± 0.01
	3.0	0.29 ± 0.01
Hydroxy ethylcellulose (Control)	1.5	0.02 ± 0.01

Table 5. Average work of adhesion and peak detachment force from chitosan acetate film (Mean ± SD, n = 10).

Sample	Concentration (%)	Work of adhesion (N.mm)	The peak detachment force (N)
Crab chitosan	1.0	0.297 ± 0.075	0.428 ± 0.114
	1.5	0.301 ± 0.086	0.536 ± 0.131
	2.0	0.376 ± 0.069 [a]	0.606 ± 0.094
	2.5	0.576 ± 0.112 [a]	0.898 ± 0.137 [a]
Squid pens chitosan	2.0	0.429 ± 0.110 [a]	0.551 ± 0.129
	2.5	0.611 ± 0.125 [a]	0.940 ± 0.159 [a]
	3.0	0.923 ± 0.156 [a]	1.210 ± 0.178 [a]
HEC (Control)	1.5	0.248 ± 0.104	0.536 ± 0.125

a = Significantly different from 1.5% HEC film ($p<0.05$)

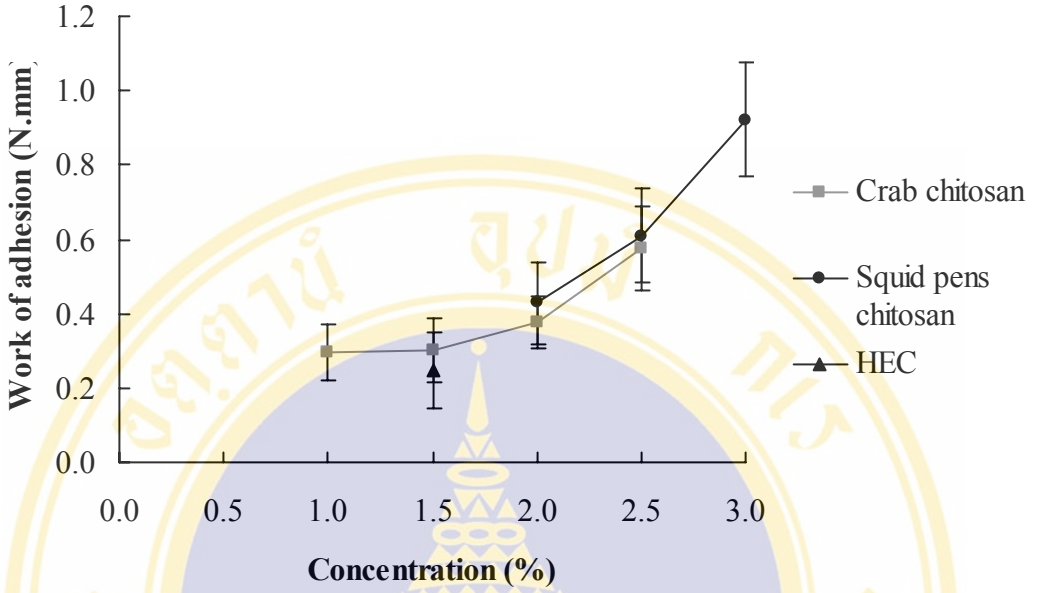


Figure 13. Comparison of work of adhesion (N.mm) from chitosan acetate films (Mean ± SD, n = 10).

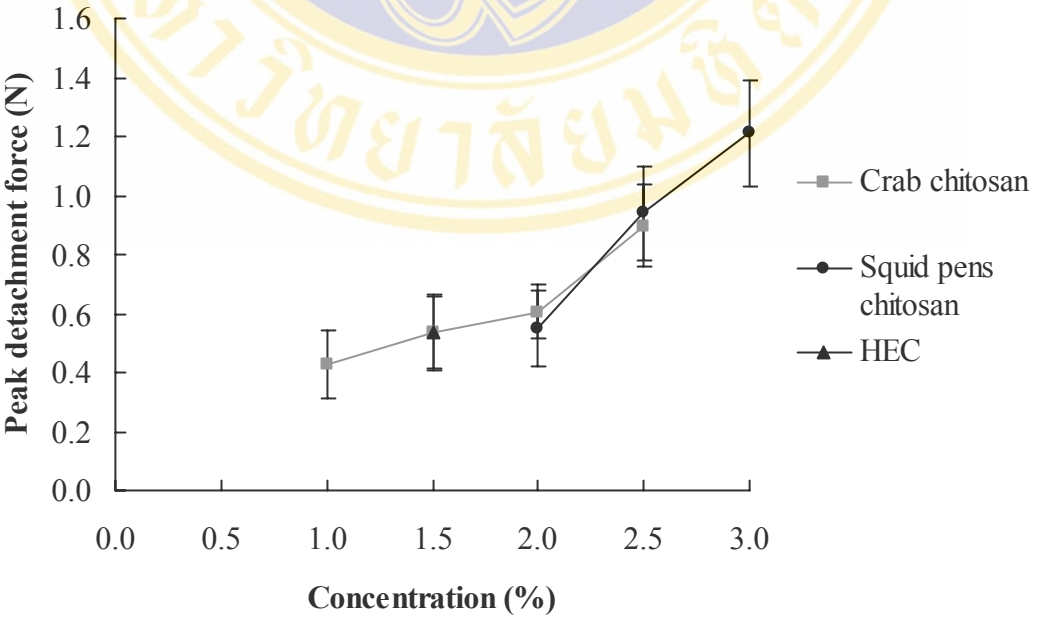


Figure 14. Comparison of peak detachment force (N) from chitosan acetate films (Mean ± SD, n = 10).

The linear regression equation of the curve between the work of adhesion (Y) versus concentration of crab and squid pens chitosan (X) were Y = 0.1825X + 0.0683 and Y = 0.4936X – 0.5799, respectively. They showed a linear relationship with square of correlation coefficient (R^2) of 0.8095 and 0.9771, respectively (Figure 13).

In addition, the linear regression equation of the curve between the peak detachment force (Y) versus concentration of crab and squid pens chitosan (X) was Y = 0.2958X + 0.0994 and Y = 0.6592X - 0.7475, respectively. They showed a linear relationship with R^2 of 0.9024 and 0.9893, respectively (Figure 14).

From results of crab chitosan in Table 5, one-way analysis of variance test (ANOVA) on these works of adhesion indicated that HEC film was significantly lower than 2% and 2.5% crab chitosan ($p<0.05$). At various percent of crab chitosan, the work of adhesion of 2.5% film was significantly higher than 2%, 1.5%, 1% crab chitosan and 1.5% HEC, respectively ($p<0.05$). Nevertheless, these peak detachment forces indicated that 2.5% crab chitosan film was significantly higher than 2% crab chitosan, 1.5% HEC, 1.5% and 1% crab chitosan, respectively ($p<0.05$) and 2% crab chitosan film was significantly different from 1% crab chitosan ($p<0.05$).

In squid pens chitosan, ANOVA on the works of adhesion indicated that differences between 3% squid pen were significantly higher than 2.5%, 2% squid pen and HEC film, respectively ($p<0.05$). The 2.5% squid pen was significantly higher than 2% squid pen and HEC film, respectively ($p<0.05$). The 2 % squid pen was significantly higher than HEC film ($p<0.05$). Whereas, the peak detachment forces indicated that differences between all squid pens film were significant ($p<0.05$), except for HEC film was not significantly different from 2% squid pens.

From the results show in Table 5, Figure 13 and 14, although the increasing of both crab chitosan and squid pens chitosan concentration induced the increasing of the work of adhesion and peak detachment force of film, the crab chitosan was not significant ($p>0.05$).

The comparison between crab and squid pens chitosan film, ANOVA on the works of adhesion indicated that 2.0% squid pens was not significantly different form crab chitosan ($p>0.05$). The 2.5% squid pens was significantly higher than 2.0%, 1.5% and 1.0% crab, repsectively ($p<0.05$). The 3.0% squid pens was significantly higher than all crab chitosan film ($p<0.05$). However, the peak detachment forces

indicated that 2.0% squid pens was significantly lower than 2.5% crab ($p<0.05$). The 2.5% squid pens was significantly higher than 2.0%, 1.5% and 1.0% crab ($p<0.05$). The 3.0% squid pens was significantly higher than all crab chitosan film ($p<0.05$).

The difference of the work of adhesion and peak detachment force between squid pens and crab chitosan film at equal concentration was not significant ($p>0.05$). In addition, the 1.5% crab chitosan acetate solution gave appropriate viscosity to spread and the film exhibited good characterization (smooth and clear film). Therefore, 1.5% w/v crab chitosan was selected to prepare chitin whiskers-chitosan acetate film in topic 3.

3. CHARACTERIZATION OF CHITIN WHISKERS-CRAB CHITOSAN ACETATE FILM

The characteristic of the corresponding ratios of chitin whisker and crab chitosan films are shown in Figure 15.

3.1 Thickness of chitin whiskers-crab chitosan acetate film

Table 6 shows the average thickness of chitin whiskers-crab chitosan acetate film which is measured using micrometer. It was founded that the higher the amount of crab chitosan, the higher the thickness of films.

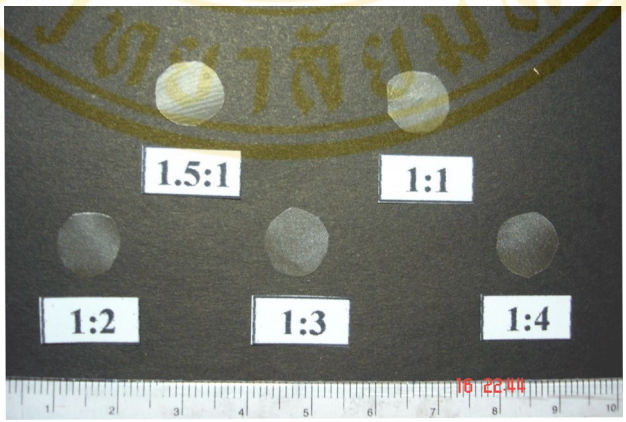


Figure 15. Characteristic of the corresponding ratios of chitin whisker and crab chitosan films.

Table 6. Average thickness of chitin whiskers-crab chitosan acetate film at various ratios (Mean ± SD, n = 6).

Sample	Ratio	Chitin whiskers (ml/100 ml)	Crab chitosan (g/ 100 ml)	Thickness (mm)
Chitin whiskers:	1.5 :1	60	0.60	0.03 ± 0.01
1.5% crab chitosan	1:1	50	0.75	0.05 ± 0.01
	1:2	33	1.00	0.06 ± 0.01
	1:3	25	1.13	0.07 ± 0.01
	1:4	20	1.20	0.07 ± 0.01

3.2 *In vitro* mucoadhesive study of chitin whiskers-crab chitosan acetate film

In mucoadhesive study, the work of adhesion was determined from the area under the force-distance curve whereas the peak detachment force was the maximum force required to detach the film from tissue. The results are presented in Table 7.

The linear regression equation of the curve between the work of adhesion (Y) versus concentration of crab chitosan in chitin whiskers-crab chitosan film (X) was Y = 0.3526 – 0.0036X. It shows a linear relationship with R^2 of 0.9746 (Figure 16).

In addition, the linear regression equation of the curve between the peak detachment force (Y) versus concentration of crab chitosan (X) was Y = 0.3547 – 0.0019X. It shows a linear relationship with R^2 of 0.9355 (Figure 17).

From results in Table 7, ANOVA on these works of adhesion indicated that HEC film was significantly higher than 1.5:1 ($p<0.05$). The 1:3 and 1:4 film were higher than HEC, but they were not significant ($p>0.05$). At various ratios of chitin whiskers and crab chitosan, the 1.5:1 film was significantly lower than 1:3 and 1:4 ($p<0.05$). The 1:1 film was significantly lower than 1:4 ($p<0.05$).

Nevertheless, the peak detachment forces indicated that HEC film was significantly higher than 1:4, 1:3, 1:2, 1:1 and 1.5:1, respectively ($p<0.05$) and the differences between various ratios of chitin-chitosan film were not significantly ($p>0.05$).

From the comparison with results of 1.5% crab chitosan film in Table 5, although the incorporation of the chitin whiskers induced reduction of both the work of adhesion and peak detachment force of film, the difference of the work of adhesion was not significant ($p>0.05$).

Table 7. Average work of adhesion and peak detachment force from the chitin whiskers-crab chitosan acetate films (Mean $\pm$ SD, n = 10).

Sample	Ratio	Work of adhesion (N.mm)	The peak detachment force (N)
Chitin whiskers :	1.5 :1	0.137 $\pm$ 0.073 [a, b]	0.242 $\pm$ 0.081 [a, b]
1.5% crab chitosan	1:1	0.178 $\pm$ 0.078 [b]	0.268 $\pm$ 0.076 [a, b]
	1:2	0.228 $\pm$ 0.078	0.288 $\pm$ 0.088 [a, b]
	1:3	0.250 $\pm$ 0.053	0.298 $\pm$ 0.096 [a, b]
	1:4	0.297 $\pm$ 0.057	0.328 $\pm$ 0.094 [a, b]

a = Significantly different from 1.5% HEC film ($p<0.05$)

b = Significantly different from 1.5% crab chitosan film ($p<0.05$)

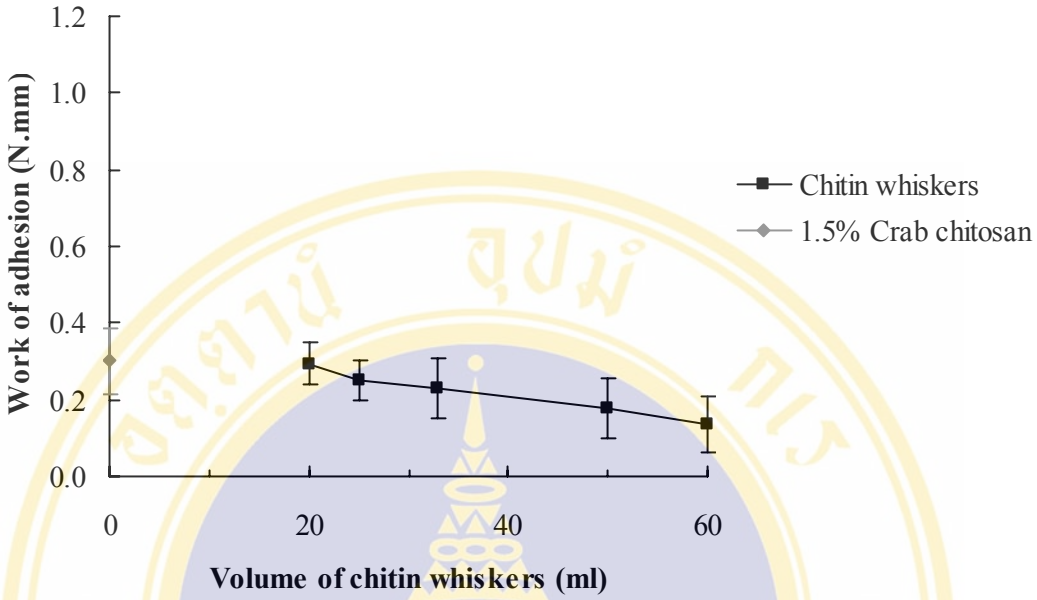


Figure 16. Comparison of work of adhesion (N.mm) from the chitin whiskers-chitosan acetate films (Mean ± SD, n = 10).

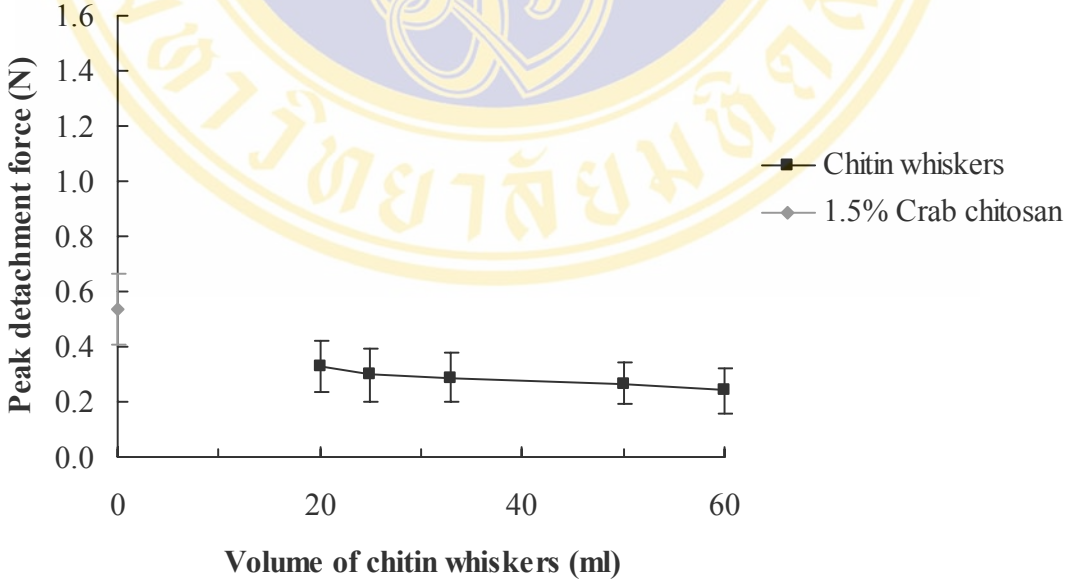


Figure 17. Comparison of peak detachment force (N) from the chitin whiskers-crab chitosan acetate films (Mean ± SD, n = 10).

4 CHARACTERIZATION OF VERAPAMIL HYDROCHLORIDE-CHITOSAN ACETATE BUCCAL PATCH

For chitosan from crab shell, the mixture of verapamil hydrochloride-chitosan acetate solution was clear with no color. The film was smooth and transparent. The solutions of 1.0, 1.5, 2.0 %w/v concentration could be casted to form film, whereas 2.5% was so sticky that it could not spread on Scotchpak® 9733 backing membrane coated with an adhesive layer using Labdryer.

For chitosan from squid pens, the mixture of verapamil hydrochloride-chitosan acetate solution featured yellowish solution with precipitate. Even though it could spread on backing membrane coated with an adhesive layer, it could not form film. Therefore, there was only the verapamil hydrochloride-crab chitosan acetate buccal patch in this study.

For control (hydroxyl ethylcellulose), the mixture of verapamil hydrochloride-1.5% w/v HEC solution was clear with no color. The film was smooth and transparent. The characeteristic of the different films are shown in Figure 18.

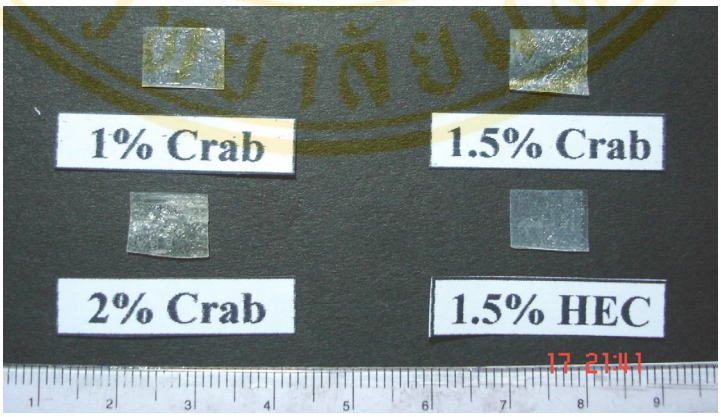


Figure 18. Characteristic of the different verapamil hydrochloride crab chitosan buccal patches.

4.1 Thickness of verapamil hydrochloride-crab chitosan acetate buccal patch

Table 8 shows the average thickness of verapamil hydrochloride-crab chitosan acetate buccal patch which was measured using micrometer. The higher the concentration of crab chitosan, the higher the thickness of films.

Table 8. Average thickness of verapamil hydrochloride-crab chitosan acetate buccal patch (Mean ± SD, n = 6).

Sample	Concentration (%)	Thickness (mm)
Crab chitosan	1.0	0.17 ± 0.01
	1.5	0.19 ± 0.01
	2.0	0.21 ± 0.01
HEC (Control)	1.5	0.17 ± 0.01

4.2 *In vitro* mucoadhesive study of verapamil hydrochloride-crab chitosan acetate buccal patch

In mucoadhesive study, the work of adhesion was determined from the area under the force-distance curve whereas the peak detachment force was the maximum force required to detach the chitosan film from tissue. The results are presented in Table 9.

Table 9. Average work of adhesion and peak detachment force from verapamil hydrochloride-chitosan acetate buccal patch (Mean ± SD, n = 10).

Sample	Concentration (%)	Work of adhesion (N.mm)	The peak detachment force (N)
Crab chitosan	1.0	0.333 ± 0.115	0.562 ± 0.126 [a]
	1.5	0.409 ± 0.130 [a]	0.998 ± 0.136 [a, b]
	2.0	0.454 ± 0.103 [a]	1.127 ± 0.140 [a, b]
HEC (Control)	1.5	0.259 ± 0.109	0.800 ± 0.148

a = Significantly different from 1.5% drug HEC film ($p<0.05$)

b = Significantly different from drug-free crab chitosan film at equal concentration ($p<0.05$)

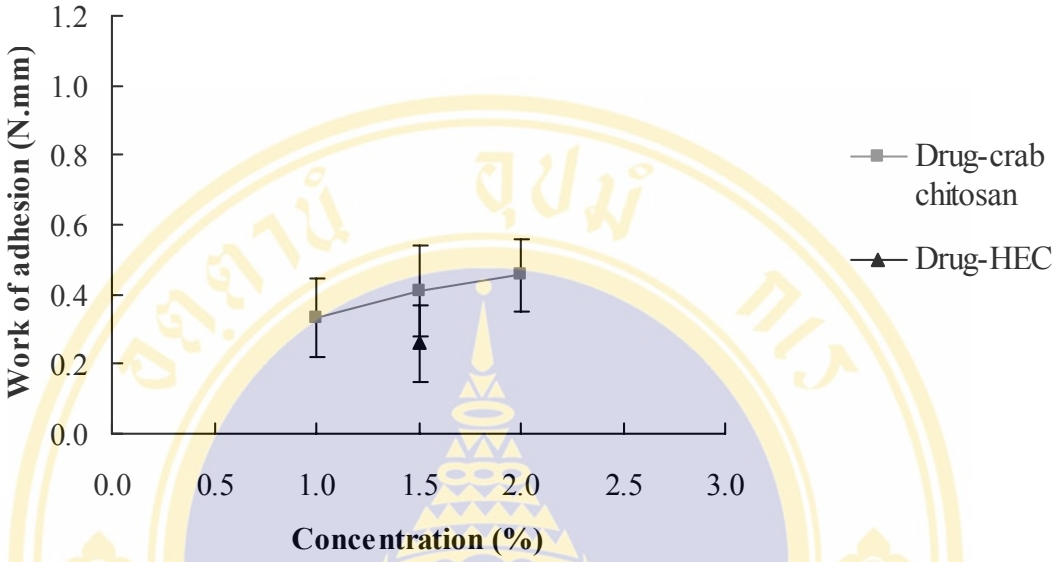


Figure 19. Work of adhesion (N.mm) from the verapamil-crab chitosan acetate buccal patches (Mean ± SD, n = 10).

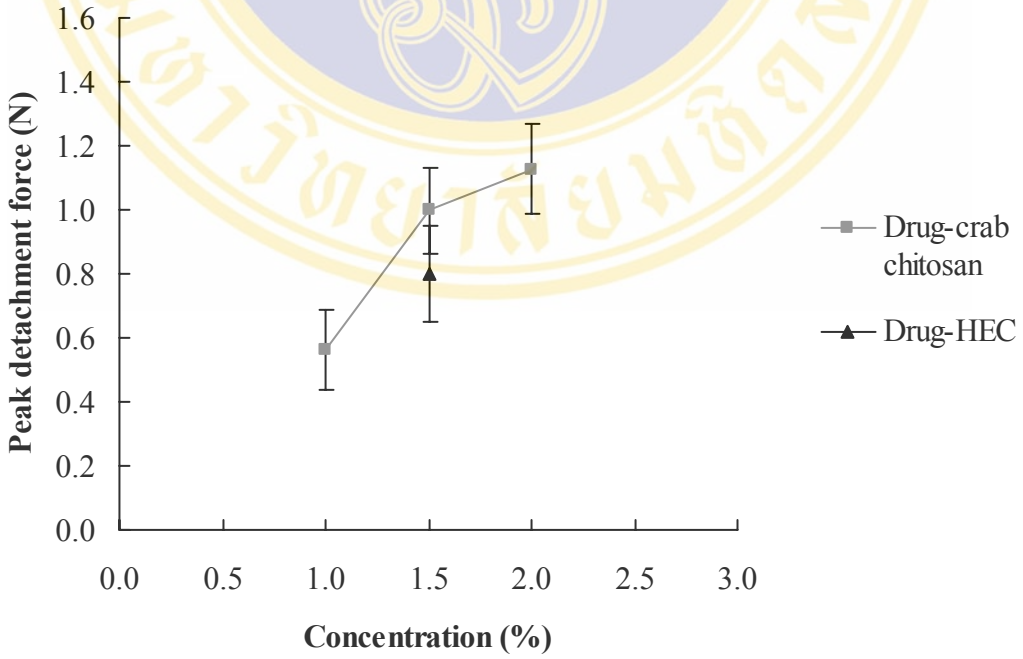


Figure 20. Peak detachment force (N) from the verapamil-crab chitosan acetate buccal patches (Mean ± SD, n = 10).

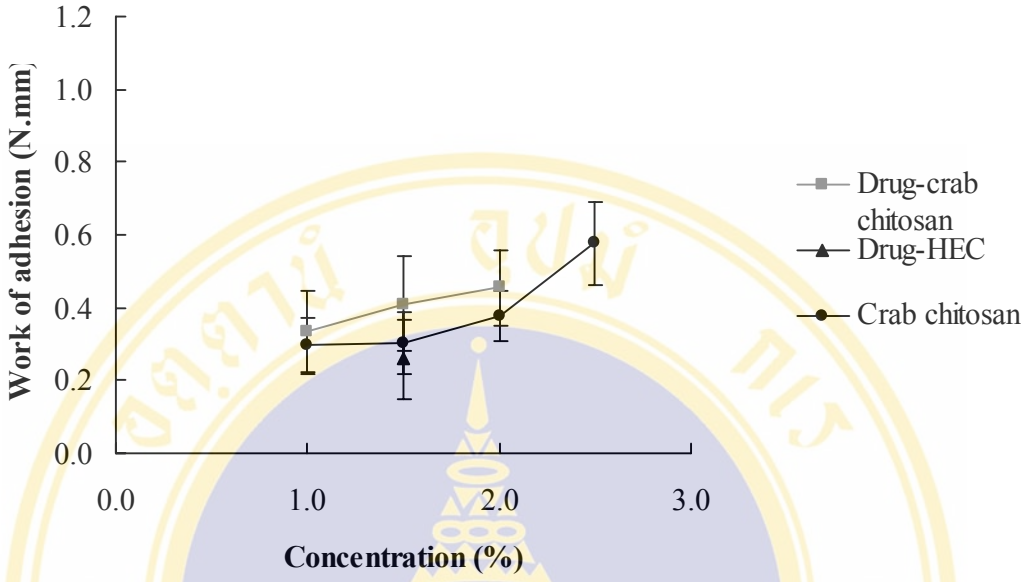


Figure 21. Comparison of the work of adhesion (N.mm) from the verapamil-crab chitosan acetate buccal patches and drug-free crab chitosan films (Mean ± SD, n = 10).

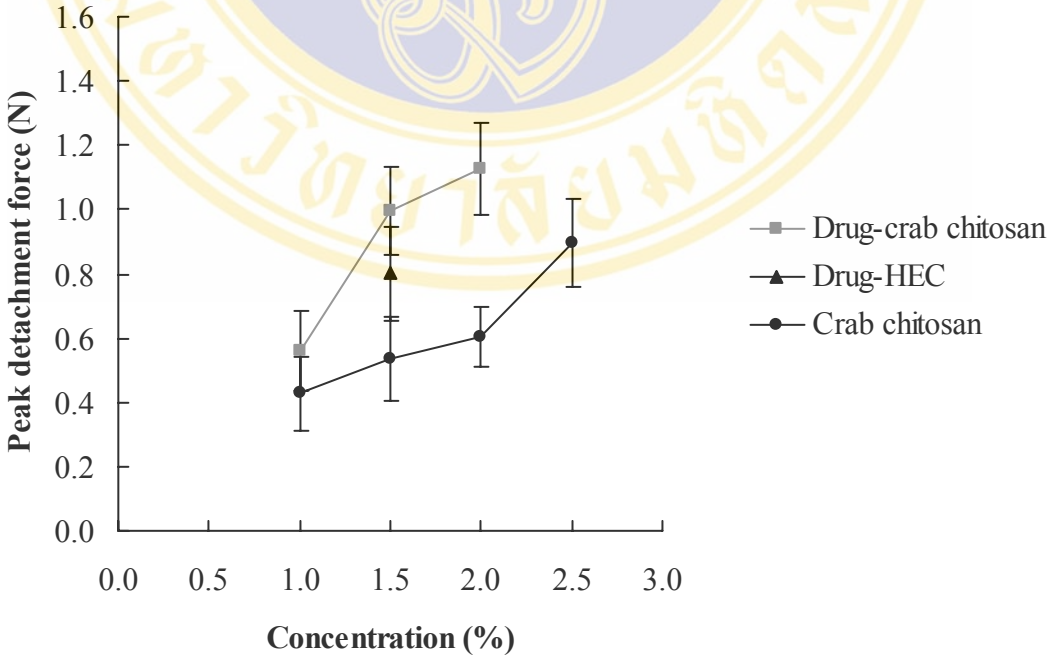


Figure 22. Comparison of the peak detachment force (N) from the verapamil-crab chitosan acetate buccal patches and drug-free crab chitosan films (Mean ± SD, n = 10).

The linear regression equation of the curve between the work of adhesion (Y) versus concentration of crab chitosan (X) was Y = 0.1219X + 0.2159 and it showed a linear relationship with R^2 of 0.9787 (Figure 19).

In addition, the linear regression equation of the curve between the peak detachment force (Y) versus concentration of crab chitosan (X) was Y = 0.5653X + 0.0476 and it showed a linear relationship with R^2 of 0.9106 (Figure 20).

ANOVA on these works of adhesion indicated that the HEC patch was significantly lower than 1.5% and 2.0% crab chitosan, respectively ($p<0.05$) whereas the difference between 1.0%, 1.5% and 2.0% was not significant ($p>0.05$). Nevertheless, the peak detachment forces indicated that the HEC patch was significantly different from all crab chitosan ($p<0.05$) and the 1.0% was significantly lower than 1.5% and 2.0%, respectively ($p<0.05$). However, the differences between 1.5% and 2% was not significant ($p>0.05$).

From the results shown in Table 9, Figure 19 and 20, although the increasing of crab chitosan concentration induced increasing both the work of adhesion and peak detachment force of film, the differences were not significant ($p>0.05$).

From the comparison with results in Table 5, the incorporation of the drug induced the increasing of the work of adhesion and peak detachment at all crab chitosan concentrations but they were not significant ($p>0.05$) (Table 9, Figure 21 and 22).

4.3 Content uniformity of verapamil hydrochloride-crab chitosan acetate buccal patch

Calibration curve

The absorbance of verapamil hydrochloride at corresponding concentrations in 0.01 N hydrochloric acid (0.004, 0.008, 0.016, 0.024, 0.032, 0.048 and 0.064 mg/ml) using UV/ VIS spectrophotometer are presented in Table 10. The linear regression equation of the standard curve between absorbance at 278 nm (Y) versus verapamil hydrochloride concentration (X) was

$$Y = 11.8157X + 0.0027$$

It showed a good linear relationship with square of correlation coefficient (R^2) of 1.0000 (Figure 23).

Table 10. Average absorbance at 278 nm at corresponding verapamil hydrochloride concentration in 0.01 N hydrochloric acid (Mean ± SD, n=3).

Verapamil hydrochloride concentration (mg/ml)	Absorbance at 278 nm
0.004	0.0485 ± 0.0019
0.008	0.0957 ± 0.0038
0.016	0.1930 ± 0.0040
0.032	0.2877 ± 0.0053
0.048	0.3832 ± 0.0053
0.056	0.5685 ± 0.0104
0.064	0.7582 ± 0.0102

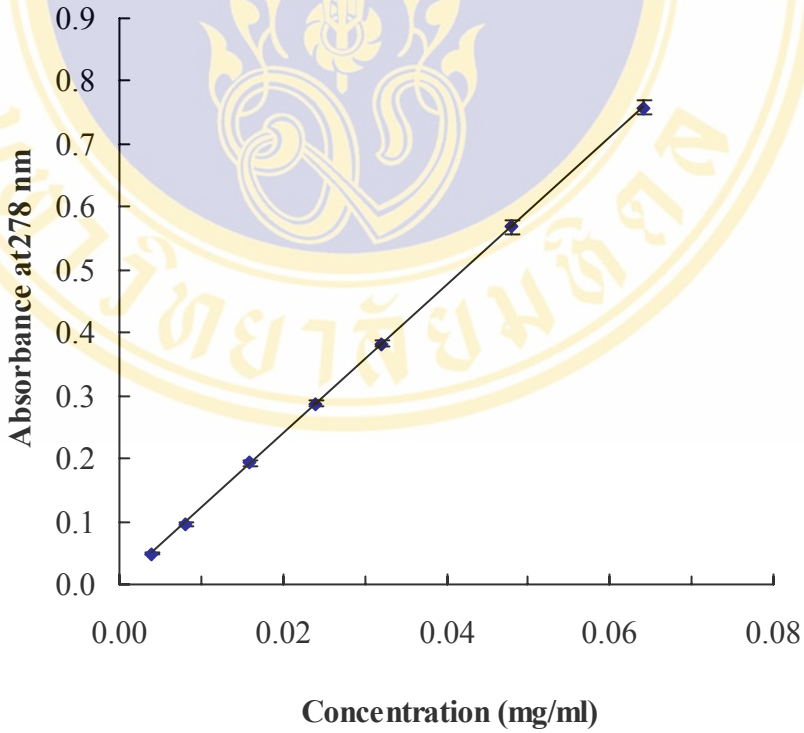


Figure 23. Calibration curve of absorbance at 278 nm versus corresponding verapamil hydrochloride concentration in 0.01 N hydrochloric acid (Mean ± SD, n = 3).

Content uniformity of verapamil hydrochloride in buccal patch

The different values between absorbances at 278 and 300 nm of the sample preparation were ordinated with calibration curve. Calculation of the amount of drug (mg) in the buccal patch was taken by the formula:

$$\text{Amount of drug (mg)} = \text{Conc. from calibration curve}\left(\frac{\text{mg}}{\text{ml}}\right) \bullet \text{Dilution factor}$$

In this test, Dilution factor was 500.

Table 11 shows concentration (mg/ml) and amount of drug (mg) in chitosan buccal patch that was cut into a rectangle with 10 x 15 mm.

Table 11. Average of concentration (mg/ml) and amount of drug (mg) in chitosan buccal patch were determined using UV/ VIS spectrophotometer at 278 and 300 nm. (Mean $\pm$ SD, n = 10).

% Crab chitosan	Concentration of drug (mg/ml)	Amount of drug (mg)	% CV
1	0.0352 $\pm$ 0.0034	17.58 $\pm$ 1.70	9.69
1.5	0.0247 $\pm$ 0.0018	12.35 $\pm$ 0.90	7.27
2	0.0420 $\pm$ 0.0042	20.98 $\pm$ 2.10	10.00
1.5% HEC (Control)	0.0271 $\pm$ 0.0025	13.55 $\pm$ 1.22	9.04

4.4 *In vitro* study of drug release from verapamil hydrochloride-crab chitosan acetate buccal patch

Calibration curve

The absorbance of verapamil hydrochloride at corresponding concentrations in the simulated saliva solution of pH 6.8 (0.004, 0.008, 0.016, 0.024, 0.032, 0.048 and 0.064 mg/ml) using UV/ VIS spectrophotometer is presented in Table 12. The linear regression equation of the standard curve between absorbance at 278 nm (Y) versus verapamil hydrochloride concentration (X) was

$$Y = 11.9442X - 0.0054$$

It showed a good linear relationship with square of correlation coefficient (R^2) of 0.9999 (Figure 24).

Table 12. Average absorbance at 278 nm at corresponding verapamil hydrochloride concentration in the simulated saliva solution of pH 6.8 (Mean ± SD, n=3).

Verapamil hydrochloride concentration (mg/ml)	Absorbance at 278 nm
0.004	0.0485 ± 0.0019
0.008	0.0957 ± 0.0038
0.016	0.1930 ± 0.0040
0.032	0.2877 ± 0.0053
0.048	0.3832 ± 0.0053
0.056	0.5685 ± 0.0104
0.064	0.7582 ± 0.0102

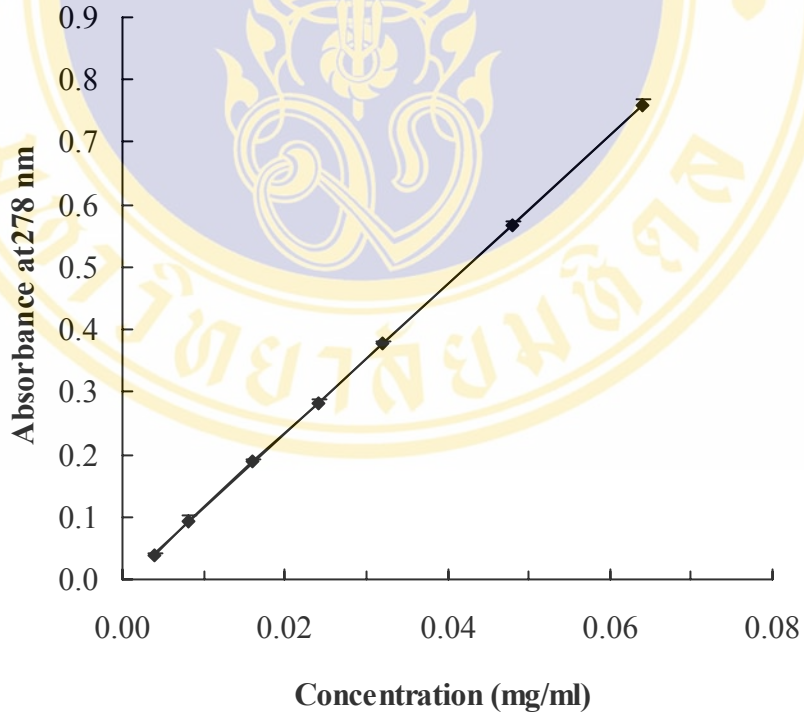


Figure 24. Calibration curve of absorbance at 278 nm versus corresponding verapamil hydrochloride concentration in simulated saliva solution of pH 6.8 (Mean ± SD, n = 3).

Drug release of verapamil hydrochloride from crab chitosan acetate buccal patch

The different values between absorbances at 278 and 300 nm of the sample preparation were ordinated with calibration curve. Calculation of the cumulative amount of drug (mg) that was released from the buccal patch at time t was taken by the formula (55):

$$M_n = C_n \bullet V_t + \sum_{i=1}^{n-1} C_i \bullet V_s$$

where M_n is the amount of drug released at the n^{th} sampling point, C_n is the concentration of drug in the solution sample, V_t is the total volume of medium (400 ml), C_i is the concentration of the sample at the i^{th} sampling time point, and V_s is the sampling volume (5 ml) at each time point.

The average amount of drug (mg) released from crab chitosan buccal patch that was cut into a rectangle with 10 x 15 mm at various times was determined using UV/VIS spectrophotometer at 278 and 300 nm. The result is presented in Table 13.

Then, calculation of the percent of drug release was taken by the formula:

$$\text{Drug release}(\%) = \frac{\text{Amount of drug released}(\text{mg}) \bullet 100}{\text{Amount of drug in patch}(\text{mg})}$$

The average percent of drug release is presented in Table 14. Figure 25, 26, 27 and 28 showed the average percent of verapamil hydrochloride released at various time from buccal patches of 1.0 %, 1.5 %, 2.0 % crab chitosan and 1.5 % hydroxyl ethylcellulose (control). Typical *in vitro* verapamil hydrochloride release-time profile is shown in Figure 29.

From result in Table 14 and Figure 29, the1.0, 1.5 % crab chitosan and control buccal patch released almost (about 96%) all the amount of verapamil hydrochloride in 30, 45 and 120 min, respectively. Whereas 2.0 % crab chitosan patch released only 75% of drug contained in 180 min. The drug released of 1.0 % and 1.5% chitosan patch were higher than that from 1.5% HEC but they were not significantly different ($p>0.05$).

Table 13. Average amount of verapamil hydrochloride (mg) released from crab chitosan buccal patch at various times (Mean ± SD, n = 6).

Time (min)	Amount of verapamil hydrochloride (mg)			
	Sample			
	1.0% Crab	1.5% Crab	2.0% Crab	1.5 % HEC
5	11.48 ± 1.65	7.20 ± 1.52	5.25 ± 0.76	8.30 ± 1.56
10	15.01 ± 2.41	9.45 ± 1.63	6.71 ± 0.76	9.66 ± 1.16
20	16.82 ± 2.61	10.94 ± 1.46	8.64 ± 0.71	10.91 ± 1.01
30	17.42 ± 2.68	11.73 ± 1.40	10.18 ± 0.59	11.68 ± 1.28
45	17.68 ± 2.76	12.24 ± 1.37	11.52 ± 0.93	12.24 ± 1.49
60	17.73 ± 2.81	12.25 ± 1.32	12.65 ± 1.29	12.53 ± 1.71
90	17.71 ± 2.78	12.40 ± 1.43	14.20 ± 2.06	12.78 ± 1.88
120	17.76 ± 2.80	12.44 ± 1.35	15.21 ± 2.63	13.05 ± 1.93
180	17.85 ± 2.77	12.71 ± 1.39	15.84 ± 2.97	13.23 ± 2.10
240	17.90 ± 2.81	12.80 ± 1.37	16.21 ± 3.10	13.39 ± 2.14
300	17.76 ± 2.77	12.62 ± 1.34	16.35 ± 3.35	13.43 ± 2.19

Table 14. Average percent *in vitro* release of verapamil hydrochloride from crab chitosan buccal patch at various times (Mean ± SD, n = 6).

Time (min)	Percent of verapamil hydrochloride			
	Sample			
	1.0% Crab	1.5% Crab	2.0% Crab	1.5 % HEC
5	65.31 ± 9.37	58.31 ± 12.28	25.02 ± 3.62 [a]	61.24 ± 11.50
10	85.39 ± 13.72	76.47 ± 13.23	32.01 ± 3.60 [a]	71.27 ± 8.53
20	95.65 ± 14.84	88.56 ± 11.81	41.21 ± 3.39 [a]	80.52 ± 7.47
30	99.05 ± 15.24	95.00 ± 11.30	48.52 ± 2.82[a]	86.26 ± 9.47
45	100.54 ± 15.69	99.03 ± 11.06	54.90 ± 4.43 [a]	90.36 ± 11.03
60	100.80 ± 15.98	99.22 ± 10.67	60.31 ± 6.16 [a]	92.52 ± 12.63
90	100.72 ± 15.79	100.44 ± 11.61	67.69 ± 9.80 [a]	94.29 ± 13.89
120	100.98 ± 15.93	100.71 ± 10.94	72.53 ± 12.55 [a]	96.33 ± 14.25
180	101.53 ± 15.73	102.92 ± 11.23	75.53 ± 14.18	97.65 ± 15.46
240	101.78 ± 15.98	103.66 ± 11.08	77.26 ± 14.76	98.81 ± 15.83
300	101.02 ± 15.77	102.18 ± 10.83	77.95 ± 15.97	99.15 ± 16.15

a = Significantly different from verapamil hydrochloride-1.5% HEC film at the same time ($p<0.05$)

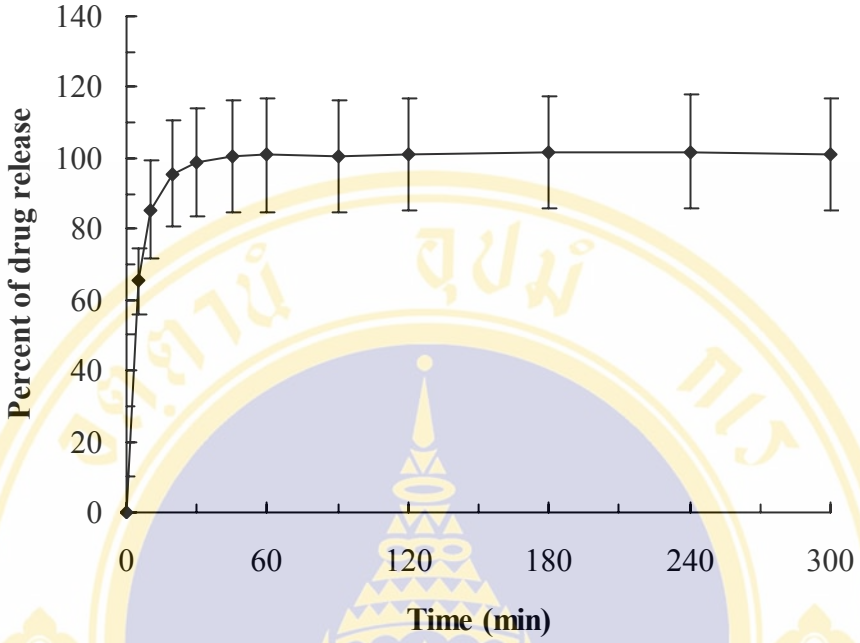


Figure 25. Average percent of verapamil hydrochloride released at various times from 1.0 % crab chitosan buccal patches (Mean ± SD, n = 6).

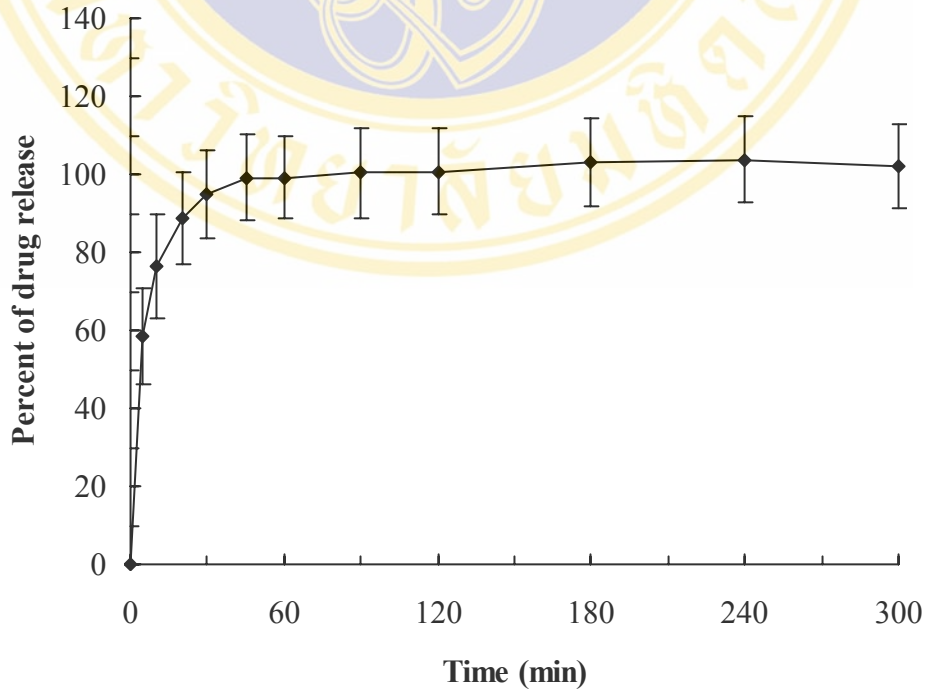


Figure 26. Average percent of verapamil hydrochloride released at various times from 1.5 % crab chitosan buccal patches (Mean ± SD, n = 6).

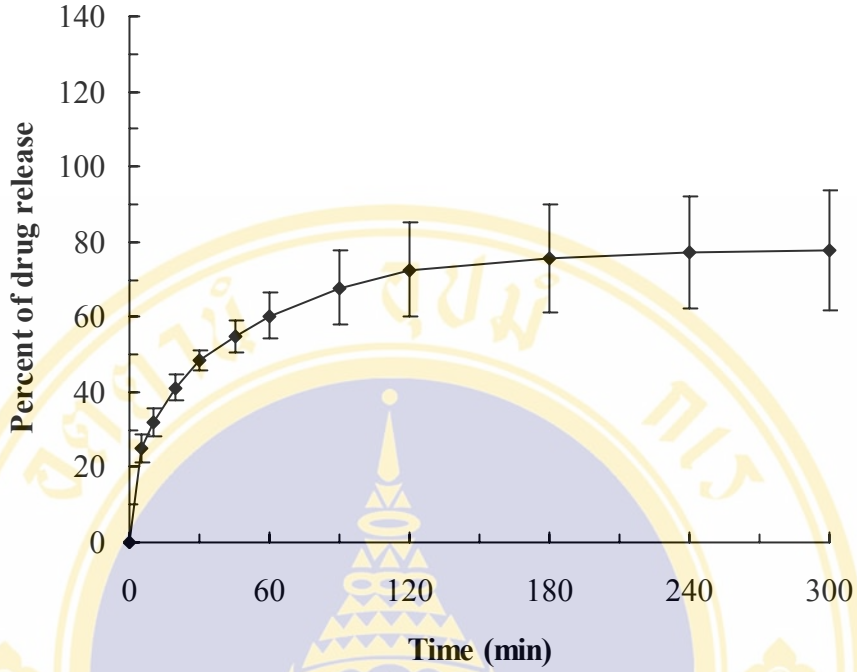


Figure 27. Average percent of verapamil hydrochloride released at various times from 2.0 % crab chitosan buccal patches (Mean ± SD, n = 6).

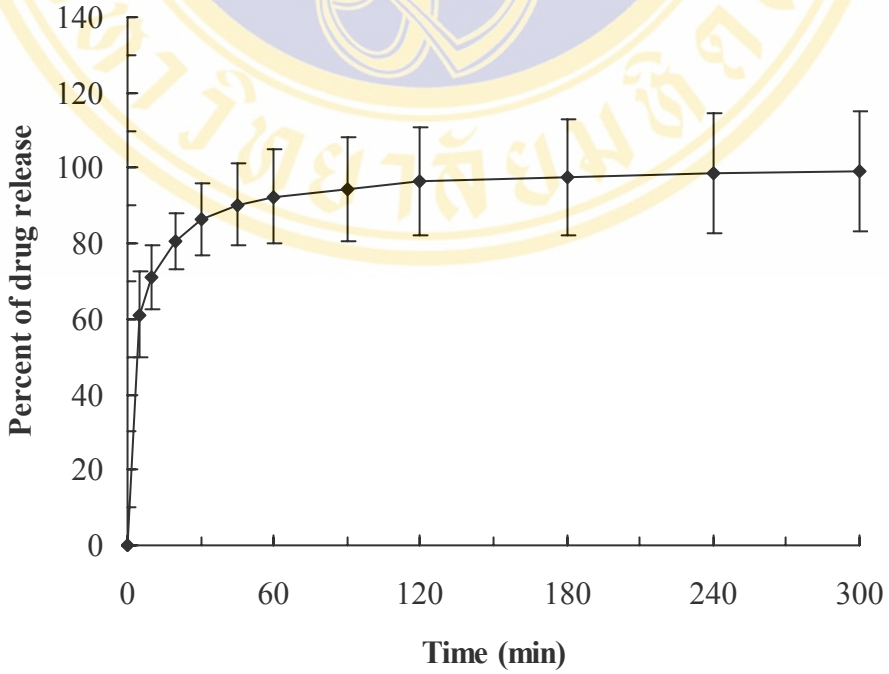


Figure 28. Average percent of verapamil hydrochloride released at various times from 1.5 % HEC buccal patches (Mean ± SD, n = 6).

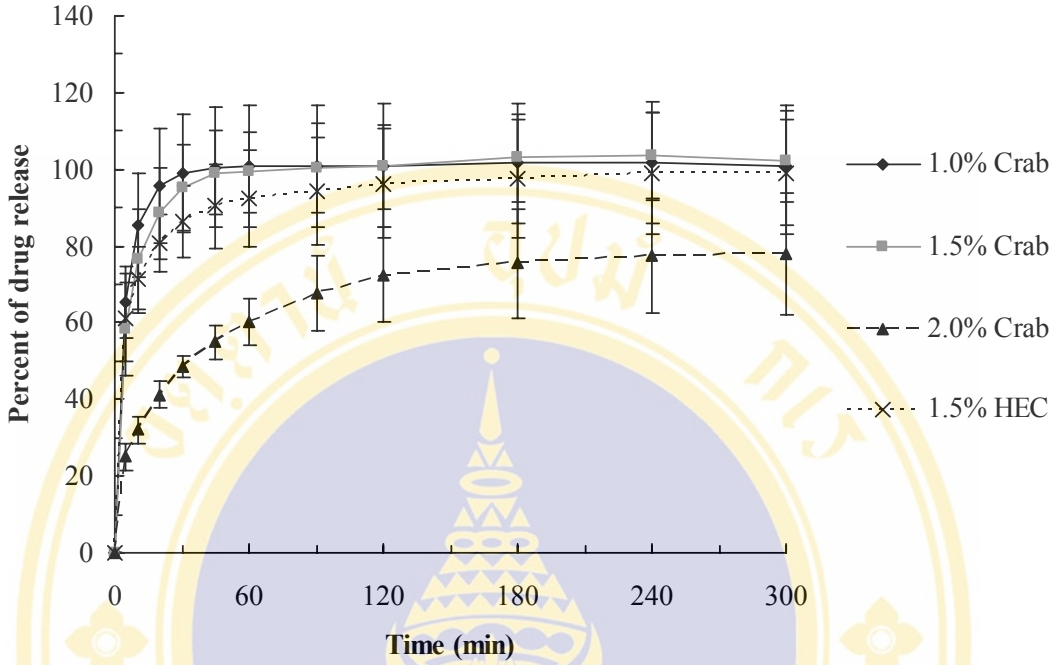


Figure 29. Average percent of verapamil hydrochloride released at various times from different crab chitosan buccal patches (Mean $\pm$ SD, n = 6).

The amount of drug released from 2.0% drug-crab chitosan buccal patch was significantly lower than from 1.0% crab chitosan at 5 to 240 min and 1.5% crab chitosan at 5 to 300 min ($p<0.05$). Nevertheless, the drug release-time profile of 1.0 % and 1.5 % crab chitosan patch were statistically similar ($p>0.05$).

After the last time point, the residual patch was collected and analyzed for drug remained. The different values between absorbances at 278 and 300 nm of the sample preparation were ordinated with calibration curve of the content uniformity. Calculation of the amount of drug (mg) was taken by the formula:

$$\text{Amount of drug}\,(\text{mg}) = \text{Conc. from calibration curve}\left(\frac{\text{mg}}{\text{ml}}\right) \bullet \text{Dilution factor}$$

In this test, Dilution factor was 25.

Then, calculation of the percent of drug remained was taken by the formula:

$$\text{Drug remainder}\,(\%) = \frac{\text{Amount of drug remained}\,(\text{mg}) \bullet 100}{\text{Amount of drug in patch}\,(\text{mg})}$$

The average concentration (mg/ml), amount of drug (mg) and percent of drug remainder are presented in Table 15. From these results, the remainder of drug in 2% crab chitosan buccal patch was higher than 1.5% crab chitosan, 1.5% HEC and 1% crab chitosan, respectively but it was not significantly different from 1.5% crab chitosan ($p>0.05$). In addition, the residual from 1.5% crab chitosan was significantly higher than 1.5% HEC and 1.0% crab chitosan, respectively ($p<0.05$). However, the residual from 1.5% HEC and 1% chitosan were similar ($p>0.05$).

Table 15. Average of concentration (mg/ml), amount of drug (mg) and percent of drug remained in residual buccal patch after the end of drug release study. (Mean $\pm$ SD, n =6).

% Crab chitosan	Concentration of drug (mg/ml)	Amount of drug (mg)	% of drug Remainder	% CV
1	0.0048 $\pm$ 0.0008	0.12 $\pm$ 0.02	0.66 $\pm$ 0.11	16.00
1.5	0.0069 $\pm$ 0.0010	0.17 $\pm$ 0.03	1.39 $\pm$ 0.21 [a]	15.01
2	0.0159 $\pm$ 0.0031	0.40 $\pm$ 0.08	1.90 $\pm$ 0.33 [a]	17.13
1.5% HEC (Control)	0.0041 $\pm$ 0.0007	0.10 $\pm$ 0.02	0.76 $\pm$ 0.14	18.16

a = Significantly different from verapamil hydrochloride-1.5% HEC film ($p<0.05$)

4.5 *In vitro* permeation study of verapamil hydrochloride-chitosan acetate buccal patch

4.5.1 Validation of HPLC method for determination of verapamil hydrochloride in penetration samples

1. Separation and specificity

The representative HPLC chromatograms of phosphate buffer saline of pH 7.4, simulated saliva solution of pH 6.8, verapamil hydrochloride in buffer pH 7.4 and buffer pH 6.8 are shown in Figure 30. Figure 31 shows chromatograms of drug-free chitosan acetate buccal patch, drug-crab chitosan, drug-chitin whiskers-crab chitosan and drug- hydroxy ethylcellulose buccal patch in buffer pH 7.4. Retention time of

verapamil hydrochloride was about 6 minutes. Chromatogram showed no interference peaks from mobile phase, phosphate buffer saline of pH 7.4, simulated saliva solution of pH 6.8 and drug-free chitosan acetate buccal patch constituents.

2. Linearity

For receptor compartment (pH 7.4) assay, the linearity was demonstrated by multiple analyses of phosphate buffer saline of pH 7.4 containing verapamil hydrochloride 0.005, 0.01, 0.1, 0.2 and 0.3 mg/ml. The average (n = 5) peak areas at corresponding concentrations are presented in Table 16. The linear regression equation of the standard curve between absorbance at 278 nm (Y) versus verapamil hydrochloride concentration (X) was

$$Y = 2000000X - 4081.4$$

It showed a good linear relationship with square of correlation coefficient (R^2) of 1.0000 (Figure 32).

For donor compartment (pH 6.8) assay, the linearity was demonstrated by multiple analyses of simulated saliva solution of pH 6.8 containing verapamil hydrochloride 0.01, 0.1, 0.2, 0.3 and 0.4 mg/ml. The average (n = 5) peak areas at corresponding concentrations are presented in Table 17. The linear regression equation of the standard curve between absorbance at 278 nm (Y) versus verapamil hydrochloride concentration (X) was

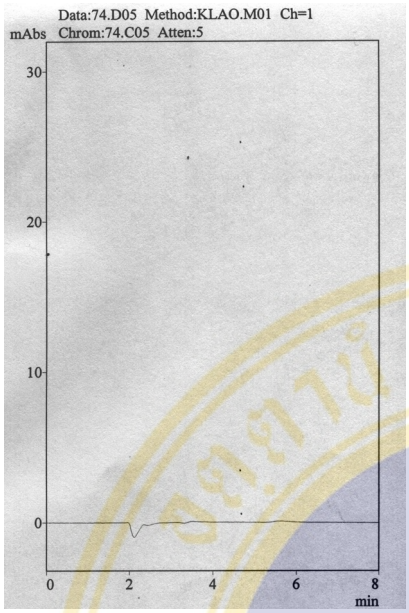
$$Y = 2000000X - 3359.6$$

It showed a good linear relationship with square of correlation coefficient (R^2) of 0.9999 (Figure 33).

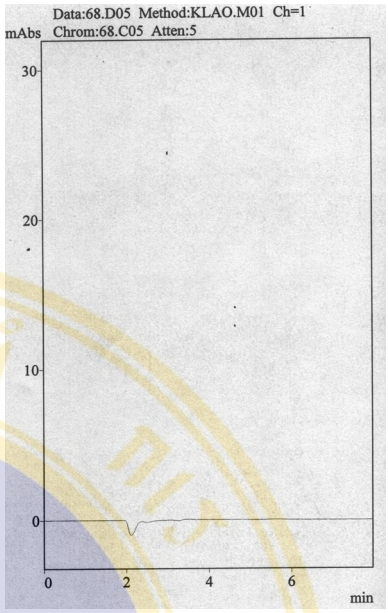
3. The limit of quantitation (LOQ)

For the receptor compartment (pH 7.4) assay, the limit of quantitation was established at 0.0025 mg/ml with coefficient of variation of 4.65 % and bias of 14.16%.

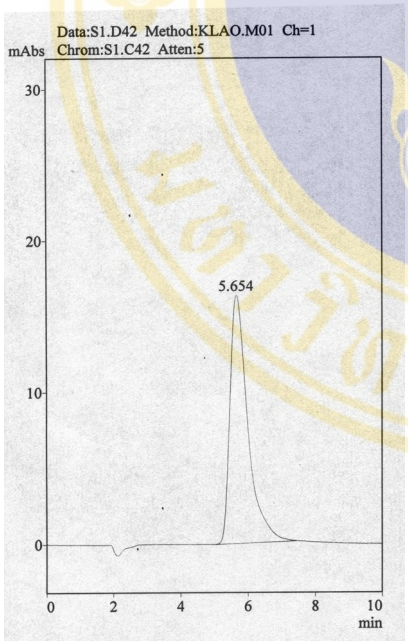
For the donor compartment (pH 6.8) assay, the limit of quantitation was established at 0.0025 mg/ml with coefficient of variation of 4.39 % and bias of 11.68%.



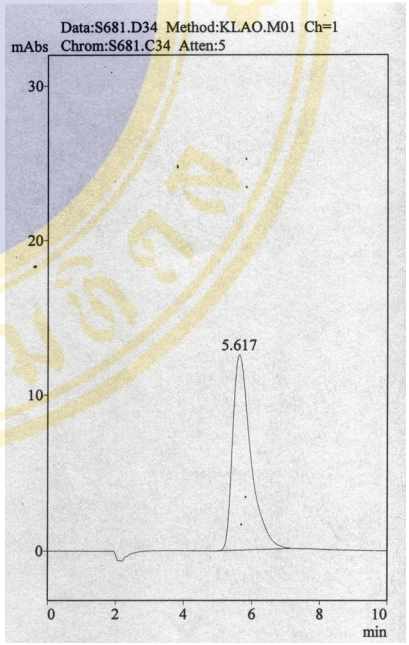
a.



b.



c.



d.

Figure 30. HPLC chromatograms of

a. phosphate buffer saline of pH 7.4
b. simulated saliva solution of pH 6.8
c. verapamil hydrochloride in buffer pH 7.4
d. verapamil hydrochloride in buffer pH 6.8.

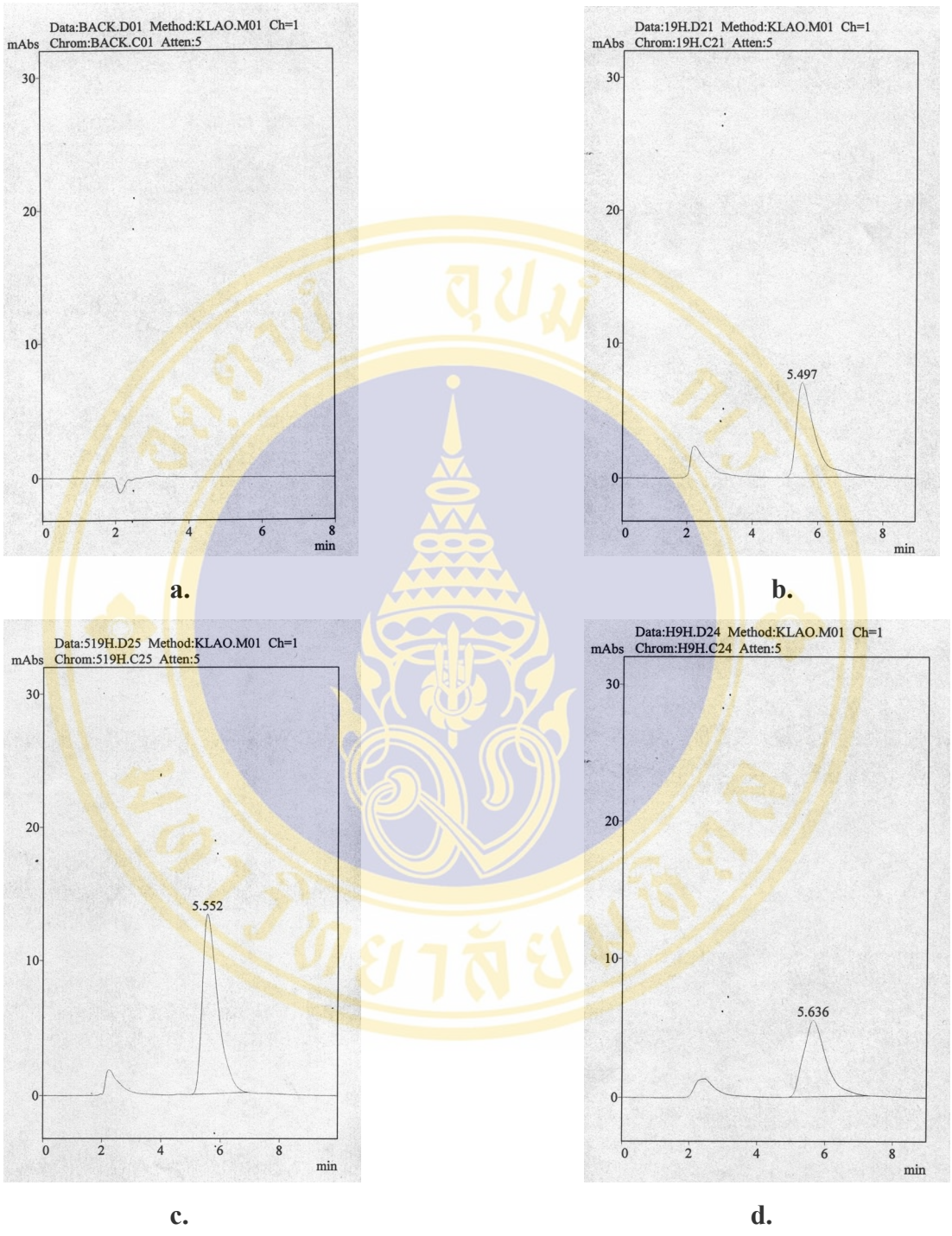


Figure 31. HPLC chromatograms of

a. drug-free chitosan acetate buccal patch in buffer pH 7.4
b. drug-crab chitosan buccal patch in buffer pH 7.4
c. drug-chitin whiskers-crab chitosan buccal patch in buffer pH 7.4
d. drug-hydroxy ethylcellulose buccal patch in buffer pH 7.4.

Table 16. Average peak area at corresponding verapamil hydrochloride concentration in the phosphate buffer saline of pH 7.4 (Mean ± SD, n = 5).

Verapamil hydrochloride concentration (mg/ml)	Peak area	%CV
0.005	5185 ± 95	1.84
0.01	11018 ± 32	0.29
0.1	159011 ± 2144	1.34
0.2	324829 ± 3703	1.14
0.3	486030 ± 9413	1.94

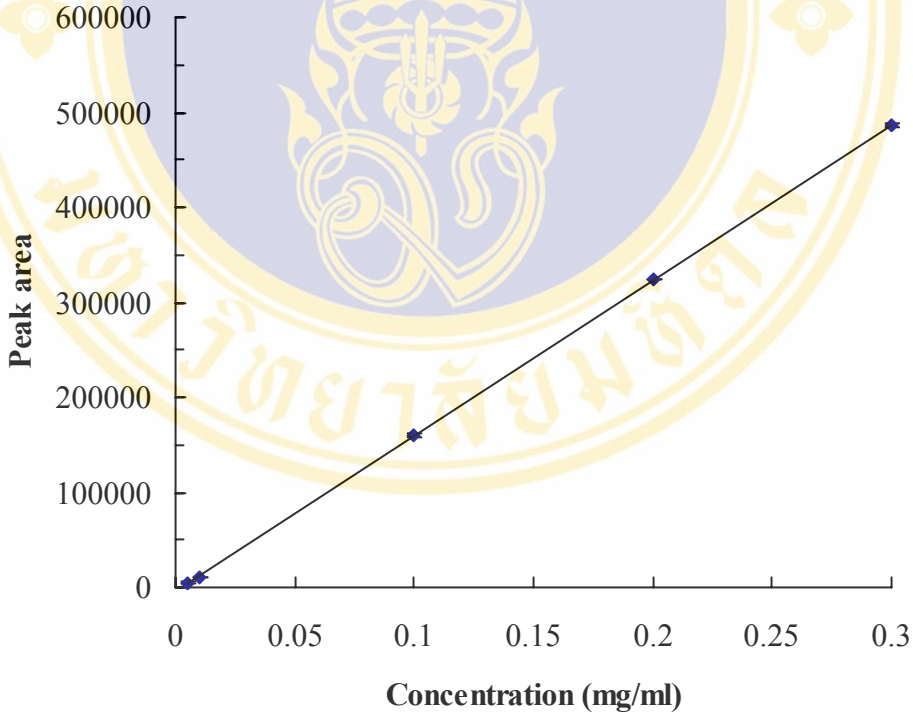


Figure 32. Calibration curve of peak area versus corresponding verapamil hydrochloride concentration in phosphate buffer saline of pH 7.4 (Mean ± SD, n = 5).

Table 17. Average peak area at corresponding verapamil hydrochloride concentration in the simulated saliva solution of pH 6.8 (Mean ± SD, n=5).

Verapamil hydrochloride concentration (mg/ml)	Peak area	%CV
0.01	15387 ± 63	1.28
0.1	229298 ± 4151	1.81
0.2	464445 ± 1528	0.33
0.3	687365 ± 9150	1.33
0.4	917211 ± 14923	1.63

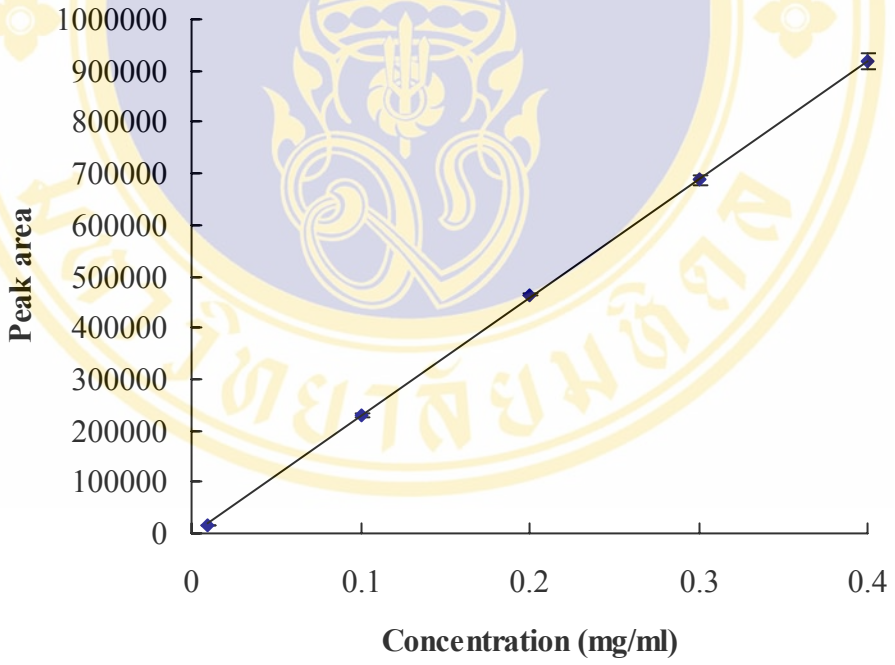


Figure 33. Calibration curve of peak area versus corresponding verapamil hydrochloride concentration in simulated saliva solution of pH 6.8 (Mean ± SD, n = 5).

4. Precision and accuracy

The within-run (intraday) precision and accuracy (expressed as the percentage of bias) of the analytical method are presented in Table 18. The precision was assessed from the percentage of coefficient of variation (%CV). For the receptor compartment (pH 7.4) assay, %CV of each concentration between 0.01 to 0.3 mg/ml ranged from 0.66 to 10.45 and %bias ranged from 7.40 to 13.53. For the donor compartment (pH 6.8) assay, %CV of each concentration between 0.1 to 0.4 mg/ml ranged from 1.14 to 11.05 and %bias ranged from 5.59 to 9.22.

The between-run (interday) precision and accuracy of the analytical method are presented in Table 19. The precision was assessed from the percentage of coefficient of variation (%CV). For the receptor compartment (pH 7.4) assay, %CV of each concentration between 0.01 to 0.3 mg/ml ranged from 2.43 to 6.60 and %bias ranged from 3.79 to 14.28. For the donor compartment (pH 6.8) assay, %CV of each concentration between 0.01 to 0.4 mg/ml ranged from 7.11 to 11.26 and %bias ranged 1.43 from to 2.50.

Table 18. Within-run precision and accuracy of the analytical method for verapamil hydrochloride (Mean $\pm$ SD, n = 5).

Assay	Verapamil concentration (mg/ml)		%CV	%Bias
	Target	Measured		
Receptor	0.01	0.0086 $\pm$ 0.0002	1.88	13.53
(pH 7.4)	0.1	0.0875 $\pm$ 0.0006	0.66	12.53
	0.3	0.2778 $\pm$ 0.0290	10.45	7.40
Donor	0.1	0.1056 $\pm$ 0.0012	1.14	5.59
(pH 6.8)	0.2	0.1875 $\pm$ 0.0059	3.16	6.26
	0.4	0.3631 $\pm$ 0.0401	11.05	9.22

Table 19. Between-run precision and accuracy of the analytical method for verapamil hydrochloride (Mean ± SD, n = 10).

Assay	Verapamil concentration (mg/ml)		%CV	%Bias
	Target	Measured		
Receptor	0.01	0.0086 ± 0.0002	2.43	14.28
(pH 7.4)	0.1	0.0857 ± 0.0030	3.46	14.27
	0.3	0.2886 ± 0.0190	6.60	3.79
Donor	0.1	0.0986 ± 0.0070	7.11	1.43
(pH 6.8)	0.2	0.2150 ± 0.0148	7.22	2.50
	0.4	0.4073 ± 0.0458	11.26	1.81

4.5.2 Penetration of verapamil hydrochloride from crab chitosan acetate buccal patch

The peak area of the sample preparation was ordinated with calibration curve for receptor compartment (pH 7.4). Calculation of the cumulative amount of drug (mg) in the buccal patch that penetrated via porcine buccal mucosa at times t was taken by the formula (55):

$$M_n = C_n \bullet V_t + \sum_{i=1}^{n-1} C_i \bullet V_s$$

where M_n is the amount of penetrated drug at the n^{th} sampling point, C_n is the concentration of drug in the solution sample, V_t is the total volume of medium (12 ml), C_i is the concentration of the sample at the i^{th} sampling time point, and V_s is the sampling volume (0.3 ml) at each time point.

The average amount of drug (mg) from crab chitosan buccal patch (cut into a rectangle with 7.5 x 10 mm) that penetrated via porcine buccal mucosa at various times was determined using HPLC with UV detector. The result is presented in Table 19.

Then, calculation of the percent of drug penetration was taken by the formula:

$$\text{Drug penetration}(\%) = \frac{\text{Amount of drug penetrated (mg)} \bullet 2 \bullet 100}{\text{Amount of drug in patch (mg)}}$$

The average percent of drug penetrated is presented in Table 20. Figure 34, 35, 36, 37 and 38 show the average percent of verapamil hydrochloride penetrated at various time from 1.0%, 1.5%, 2.0% crab chitosan, 1.5% hydroxyl ethylcellulose (control buccal patch) and standard solution (control solution). Typical *in vitro* verapamil hydrochloride penetration-time profile is shown in Figure 39.

Table 20. Average amount of verapamil hydrochloride (mg) penetrated from crab chitosan buccal patch via porcine buccal mucosa at various times (Mean ± SD, n = 6).

Time (hr)	Amount of verapamil hydrochloride (mg)				
	Sample				
	1.0 % Crab	1.5 % Crab	2.0 % Crab	1.5 % HEC	Std solution
0.08	ND	ND	ND	ND	ND
0.17	ND	ND	ND	ND	ND
0.25	ND	ND	ND	ND	ND
0.33	ND	ND	ND	ND	ND
0.42	ND	ND	ND	ND	ND
0.50	ND	ND	ND	ND	ND
0.75	ND	ND	ND	ND	ND
1	0.0465 ± 0.0379	ND	ND	ND	ND
1.5	0.1180 ± 0.0908	0.0428 ± 0.0231	ND	0.0350 ± 0.0198	0.0384 ± 0.0169
2	0.1593 ± 0.0806	0.0841 ± 0.0359	0.1041 ± 0.0387	0.0709 ± 0.0335	0.0877 ± 0.0425
3	0.3599 ± 0.2139	0.1654 ± 0.0803	0.2010 ± 0.0789	0.1436 ± 0.0836	0.2009 ± 0.1769
4	0.5426 ± 0.2648	0.2616 ± 0.1119	0.3873 ± 0.1679	0.2271 ± 0.1308	0.4527 ± 0.1261
5	0.7271 ± 0.3505	0.4044 ± 0.1677	0.5468 ± 0.1747	0.2959 ± 0.1480	0.7209 ± 0.3109
6	0.9636 ± 0.4390	0.5275 ± 0.2042	0.7363 ± 0.3156	0.4063 ± 0.1981	1.0380 ± 0.4149
9	1.5647 ± 0.3657	0.9439 ± 0.1974	1.3087 ± 0.5169	0.7201 ± 0.4019	2.0764 ± 0.5718

ND = not detectable

Table 21. Average percent of verapamil hydrochloride penetrated from crab chitosan buccal patch via porcine buccal mucosa at various times (Mean ± SD, n = 6).

Time (hr)	Percent of verapamil hydrochloride				
	Sample				
	1.0 % Crab	**1.5 % Crab**	**2.0 % Crab**	**1.5 % HEC**	**Std solution**
0.08	ND	ND	ND	ND	ND
0.17	ND	ND	ND	ND	ND
0.25	ND	ND	ND	ND	ND
0.33	ND	ND	ND	ND	ND
0.42	ND	ND	ND	ND	ND
0.50	ND	ND	ND	ND	ND
0.75	ND	ND	ND	ND	ND
1	0.53 ± 0.43	ND	ND	ND	ND
1.5	1.34 ± 1.03	0.69 ± 0.37	ND	0.52 ± 0.29	0.38 ± 0.17
2	1.81 ± 0.92	1.36 ± 0.58	0.99 ± 0.37	1.05 ± 0.49	0.88 ± 0.42
3	4.09 ± 2.43	2.68 ± 1.30	1.92 ± 0.75	2.12 ± 1.23	2.01 ± 1.77
4	6.17 ± 3.01	4.24 ± 1.81	3.69 ± 1.60	3.35 ± 1.93	4.53 ± 1.26
5	8.27 ± 3.99	6.55 ± 2.72	5.21 ± 1.67	4.37 ± 2.19	7.21 ± 3.11
6	10.96 ± 4.99	8.54 ± 3.31	7.02 ± 3.01	6.00 ± 2.92	10.38 ± 4.15
9	17.80 ± 4.16	15.28 ± 3.20	12.48 ± 4.93	10.63 ± 5.93 [a]	20.76 ± 5.72

ND = not detectable

a = Significantly different from verapamil hydrochloride solution ($p<0.05$)

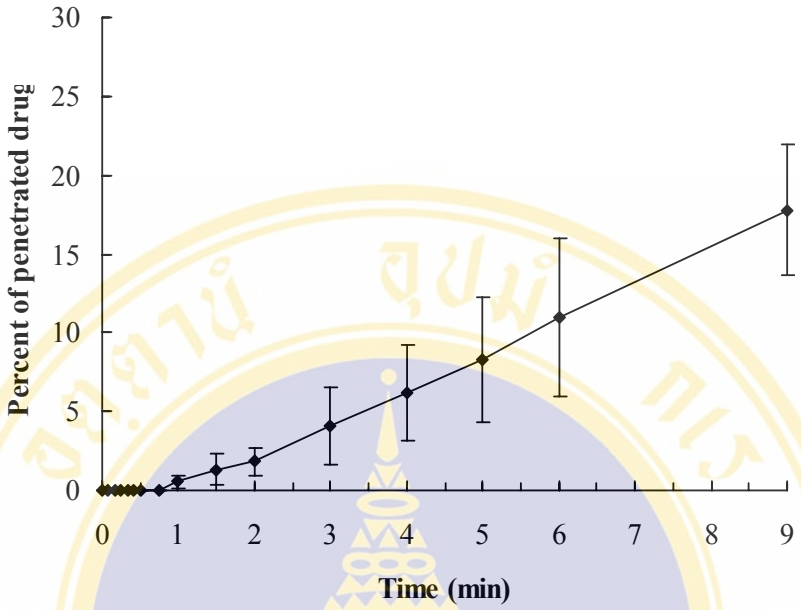


Figure 34. Average percent of verapamil hydrochloride penetrated from 1.0% crab chitosan buccal patches via porcine buccal mucosa at various times (Mean ± SD, n = 6).

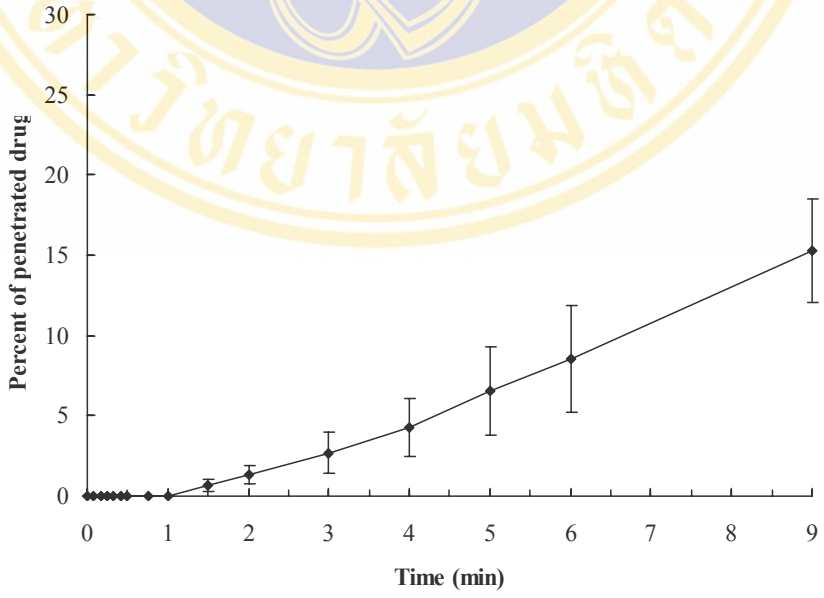


Figure 35. Average percent of verapamil hydrochloride penetrated from 1.5% crab chitosan buccal patches via porcine buccal mucosa at various times (Mean ± SD, n = 6).

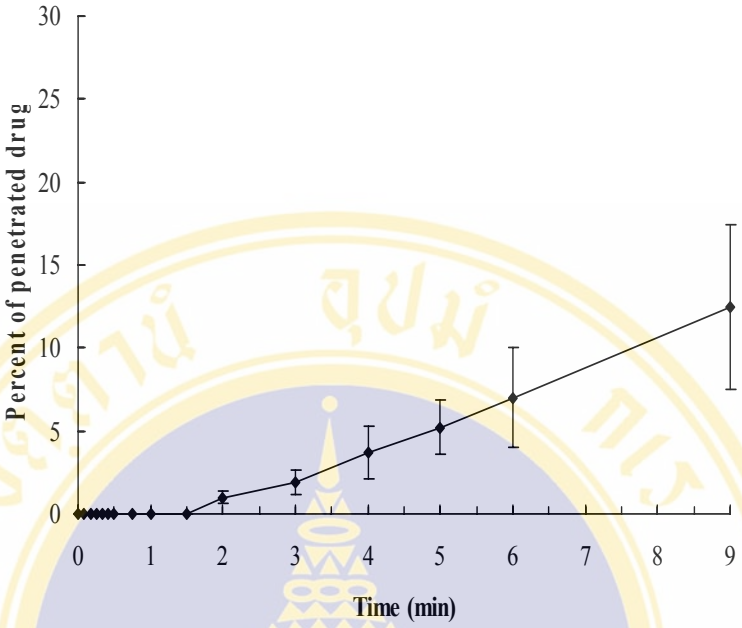


Figure 36. Average percent of verapamil hydrochloride penetrated from 2.0 % crab chitosan buccal patches via porcine buccal mucosa at various times (Mean ± SD, n = 6).

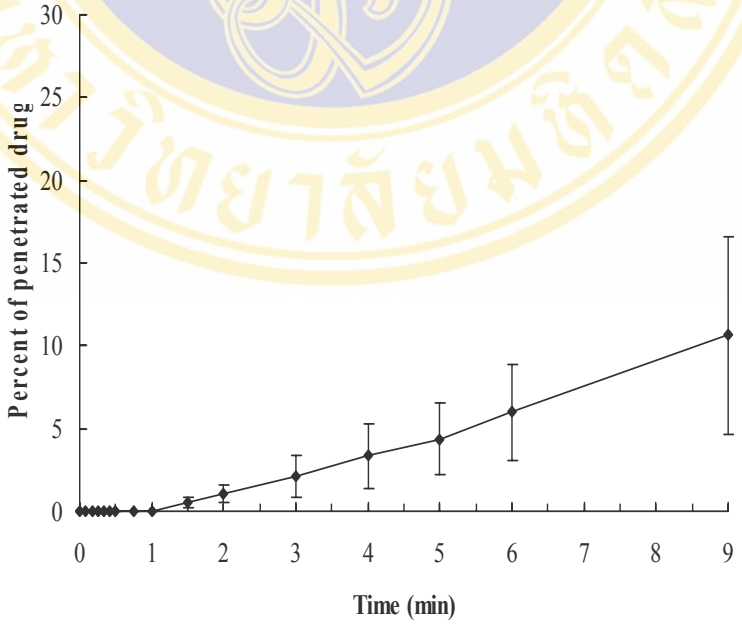


Figure 37. Average percent of verapamil hydrochloride penetrated from 1.5 % HEC buccal patches via porcine buccal mucosa at various times (Mean ± SD, n = 6).

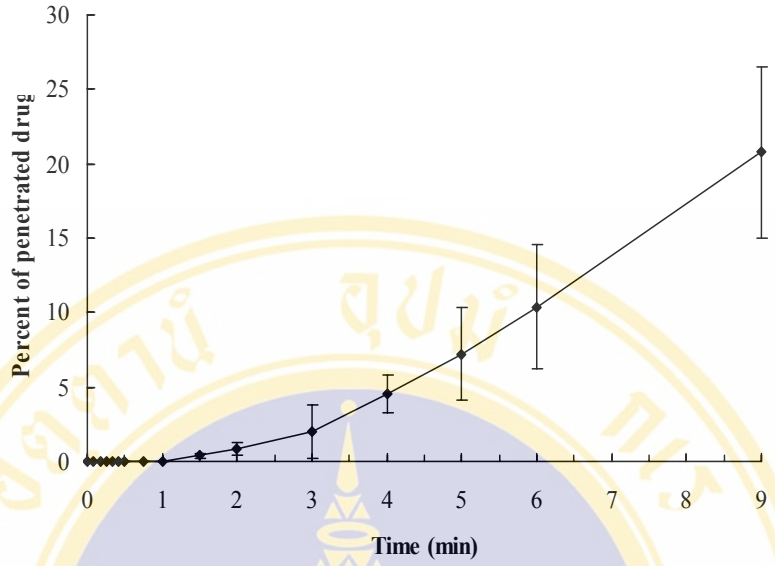


Figure 38. Average percent of verapamil hydrochloride penetrated from standard solution via porcine buccal mucosa at various times (Mean ± SD, n = 6)

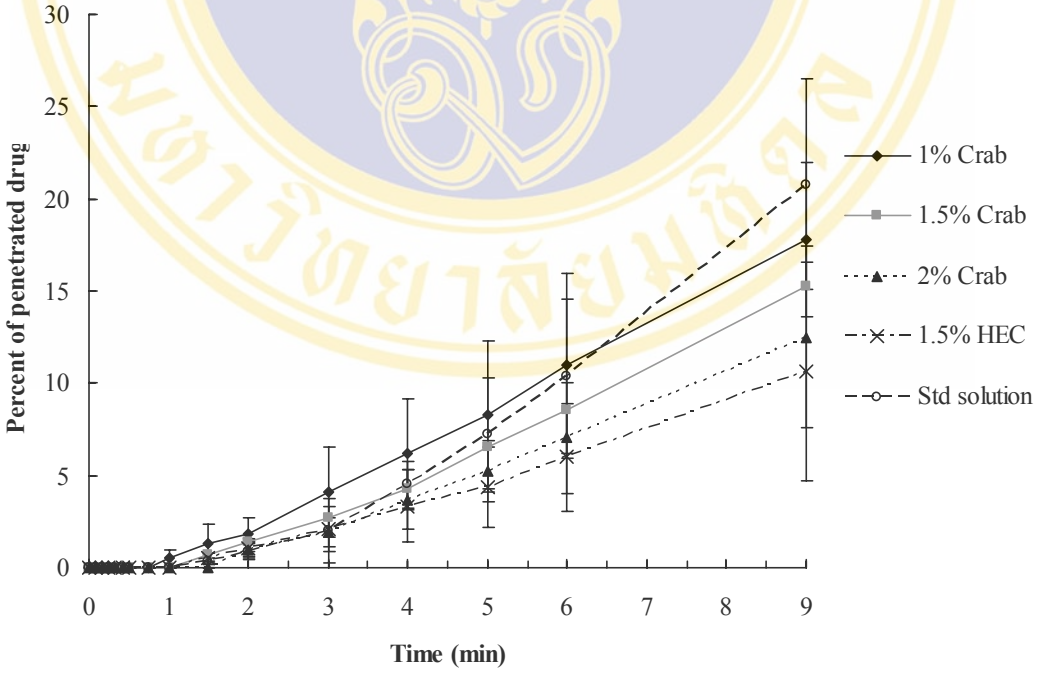


Figure 39. Average percent of verapamil hydrochloride penetrated from different buccal patches via porcine buccal mucosa at various times (Mean ± SD, n = 6).

From result in Table 21 and Figure 39, the amounts of drug penetrated from 1.0, 1.5, 2.0 % crab chitosan, 1.5% HEC buccal patch and standard solution at 9 hr were 17.80 ± 4.16, 15.28 ± 3.20, 12.48 ± 4.93, 10.63 ± 5.93 and 20.76 ± 5.72 % of the contained verapamil hydrochloride, respectively. All three formulations of crab buccal patch were more penetrable than 1.5% HEC despite the fact that they were not significantly different ($p>0.05$). The drug penetration from 1.0% and 1.5% crab chitosan patch was higher than standard solution from 1 to 6 hr and 1.5 to 3 hr, respectively even though they were not significantly different ($p>0.05$). At 9 hr the standard solution was the most penetrable even though it was not significantly different from all three formulation of crab buccal patch ($p>0.05$).

Although the increasing of crab chitosan concentration reduced the peneatration rate, the differences between chitosan concentration was not significant ($p>0.05$).

From modified Fick's second law

$$T\% = \frac{A}{A_T} = \frac{PS}{V_D}\left[t - \frac{h^2}{6D}\right]$$

It shows that a plot of percent of drug penetration versus time has a slope of $\frac{PS}{V_D}$ and x-axis intercept of $\frac{h^2}{6D}$, which is also called the lag time (Table 22).

Table 22. Average slope of a plot of percent of drug penetration versus times (hr) from various verapamil- crab chitosan buccal patches (Mean ± SD, n =6).

% Crab chitosan	Lag time (hr)	Slope	% CV
1.0	1	2.1846 ± 0.5505	25.20
1.5	1.5	1.9541 ± 0.4334	22.18
2.0	2	1.6751 ± 0.6877	41.05
1.5% HEC	1.5	1.3382 ± 0.7312 [a]	54.64
Std solution	1.5	2.7401 ± 0.7819	28.51

a = Significantly different from verapamil hydrochloride solution ($p<0.05$)

From results in Table 22 and Figure 39, the slope of a plot of percent of drug penetration from standard solution versus time was higher than 1.0 %, 1.5 %, 2.0% and 1.5% HEC, respectively. However, the differences between standard solution and various concentration of crab chitosan patch were not significant ($p>0.05$).

After the last time point, the residual solution in the donor site was collected. The peak area was ordinated with calibration curve for donor compartment (pH 6.8). Calculation of the amount of drug remained in donor compartment was taken by the formula:

$$\text{Amount of drug (mg)} = \text{Conc. from calibration curve}\left(\frac{\text{mg}}{\text{ml}}\right) \bullet \text{Dilution factor}$$

In this test, dilution factor of buccal patch was 5 and standard solution was 10. Then, calculation of the percent of drug remained in donor compartment was taken by the formula:

$$\text{Drug remained in Donor (\%)} = \frac{\text{Amount of drug remained (mg)} \bullet 2 \bullet 100}{\text{Amount of drug in patch (mg)}}$$

Table 23. Average of concentration (mg/ml), amount of drug (mg) and percent of drug remained in residual donor compartment after the end of drug penetration study. (Mean $\pm$ SD, n =6).

% Crab chitosan	Concentration of drug (mg/ml)	Amount of drug (mg)	% of drug Remainder	% CV
1	0.1216 $\pm$ 0.0615	0.61 $\pm$ 0.31	6.79 $\pm$ 3.43 [a]	50.60
1.5	0.1448 $\pm$ 0.0528	0.72 $\pm$ 0.26	11.48 $\pm$ 4.19 [a]	36.48
2	0.1591 $\pm$ 0.0821	0.80 $\pm$ 0.41	7.45 $\pm$ 3.84 [a]	51.57
1.5% HEC	0.1446 $\pm$ 0.0426	0.72 $\pm$ 0.21	10.46 $\pm$ 3.08 [a]	29.44
Std solution	0.3937 $\pm$ 0.0739	3.93 $\pm$ 0.74	39.37 $\pm$ 7.39	18.78

a = Significantly different from verapamil hydrochloride solution ($p<0.05$)

The average concentration (mg/ml), amount of drug (mg) and percent of drug remained in donor compartment are presented in Table 23. From these results, the drug remained of standard solution in donor compartment was significantly higher

than that of 1.5% crab chitosan, 1.5% HEC, 2.0 % and 1.0 % crab chitosan buccal patch, respectively ($p<0.05$). The residual donor of 1.5% HEC was not different from all chitosan patches ($p>0.05$) and the differences between various chitosan concentration were not significant ($p>0.05$).

After the last time point, the residual patch was collected and analyzed for drug remainder. The different values between absorbances at 278 and 300 nm of the sample preparation were ordinated with calibration curve of content uniformity. Calculation the amount of drug (mg) was taken by the formula:

$$\text{Amount of drug (mg)} = \text{Conc. from calibration curve}\left(\frac{\text{mg}}{\text{ml}}\right) \bullet \text{Dilution factor}$$

In this test, dilution factor was 20.

Then, calculation of the percent of drug remainder was taken by the formula:

$$\text{Drug remainder (\%)} = \frac{\text{Amount of drug remained (mg)} \bullet 2 \bullet 100}{\text{Amount of drug in patch (mg)}}$$

The average concentration (mg/ml), amount of drug (mg) and percent of drug remainder are presented in Table 24. From these results, the remainder of drug in 1.5% HEC buccal patch was higher than 1.5%, 2% and 1% crab chitosan, respectively. However, they were not significantly different ($p>0.05$).

Table 24 The average of concentration (mg/ml), amount of drug (mg) and percent of drug remained in residual buccal patch after the end of drug penetration study. (Mean $\pm$ SD, n =6).

% Crab chitosan	Concentration of drug (mg/ml)	Amount of drug (mg)	% of drug Remainder	% CV
1	0.0874 $\pm$ 0.0159	1.74 $\pm$ 0.32	9.72 $\pm$ 1.77	18.24
1.5	0.0687 $\pm$ 0.0044	1.38 $\pm$ 0.09	10.91 $\pm$ 1.70	6.41
2	0.1123 $\pm$ 0.0100	2.25 $\pm$ 0.20	10.51 $\pm$ 0.93	8.88
1.5% HEC (Control)	0.0826 $\pm$ 0.0163	1.65 $\pm$ 0.33	11.95 $\pm$ 2.36	19.76

From Figure 40, when percent of drug penetration at 9 hr was plotted against concentration of crab chitosan, it was found that there be a good linear relationship ($Y = 23.1660 - 5.3196X$, $R^2 = 0.9990$). 99.90 % accuracy of drug penetration at 9 hr was

depended on the concentration of crab chitosan. On the other hand, 0.10 % accuracy of drug penetration at 9 hr was depend on other variables.

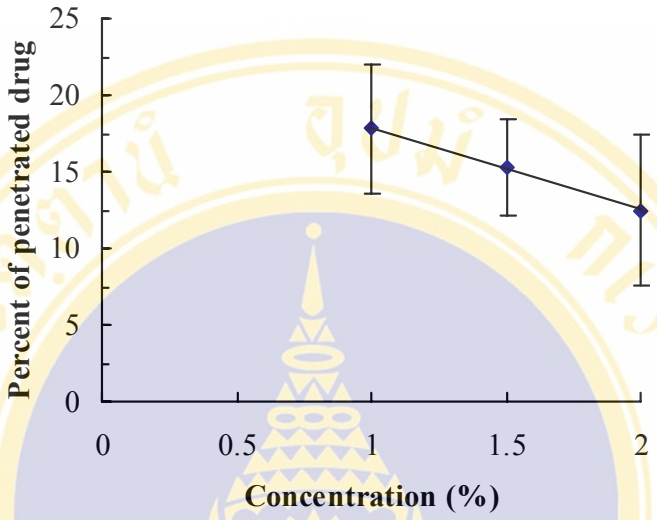


Figure 40. Relationship between percent of drug penetration at 9 hr and concentration of crab chitosan in buccal patch formulation (Mean ± SD, n =6).

5 CHARACTERIZATION OF VERAPAMIL HYDROCHLORIDE-CHITIN WHISKERS-CRAB CHITOSAN ACETATE BUCCAL PATCH

The mixture of verapamil hydrochloride-chitin whiskers-crab chitosan acetate solution was clear with no color. The film was smooth and transparent. The characteristics of the different films are shown in Figure 41.

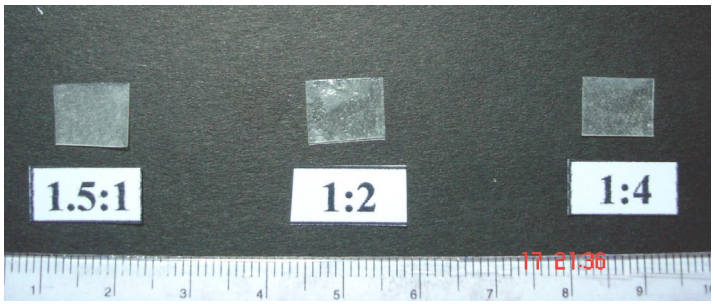


Figure 41. Characteristic of the different verapamil hydrochloride- chitin whiskers and crab chitosan buccal patches.

5.1 Thickness of verapamil hydrochloride-chitin whiskers-crab chitosan acetate buccal patch

Table 25 shows the average thickness of verapamil hydrochloride-chitin whiskers-crab chitosan acetate buccal patch which was measured using micrometer. The higher the concentration of crab chitosan, the higher the thickness of film.

Table 25. Average thickness of verapamil hydrochloride-chitin whiskers-crab chitosan acetate buccal patch (Mean ± SD, n = 6).

Sample	Ratio	Chitin whiskers (ml/100 ml)	Crab chitosan (g/ 100 ml)	Thickness (mm)
Chitin whiskers :	1.5 :1	60	0.60	0.18 ± 0.01
1.5% crab chitosan	1:2	33	1.00	0.20 ± 0.01
	1:4	20	1.20	0.22 ± 0.01

5.2 *In vitro* mucoadhesive study of verapamil hydrochloride-chitin whiskers-crab chitosan acetate buccal patch

In mucoadhesive study, the work of adhesion was determined from the area under the force-distance curve whereas the peak detachment force was the maximum force required to detach the patch from tissue. The results are presented in Table 26.

Table 26. Average work of adhesion and peak detachment force from verapamil hydrochloride-chitin whiskers chitosan acetate buccal patches (Mean ± SD, n = 10).

Sample	Ratio	Work of adhesion (N.mm)	The peak detachment force (N)
Chitin whiskers :	1.5 :1	0.297 ± 0.088 [c]	0.327 ± 0.084 [a, b]
1.5% crab chitosan	1:2	0.318 ± 0.113	0.408 ± 0.095 [a, b]
	1:4	0.376 ± 0.083	0.717 ± 0.139 [b, c]

a = Significantly different from 1.5% drug HEC film ($p<0.05$)

b = Significantly different from 1.5% drug crab chitosan film ($p<0.05$)

c = Significantly different from drug-free chitin whisker-crab chitosan film in equal ratio ($p<0.05$)

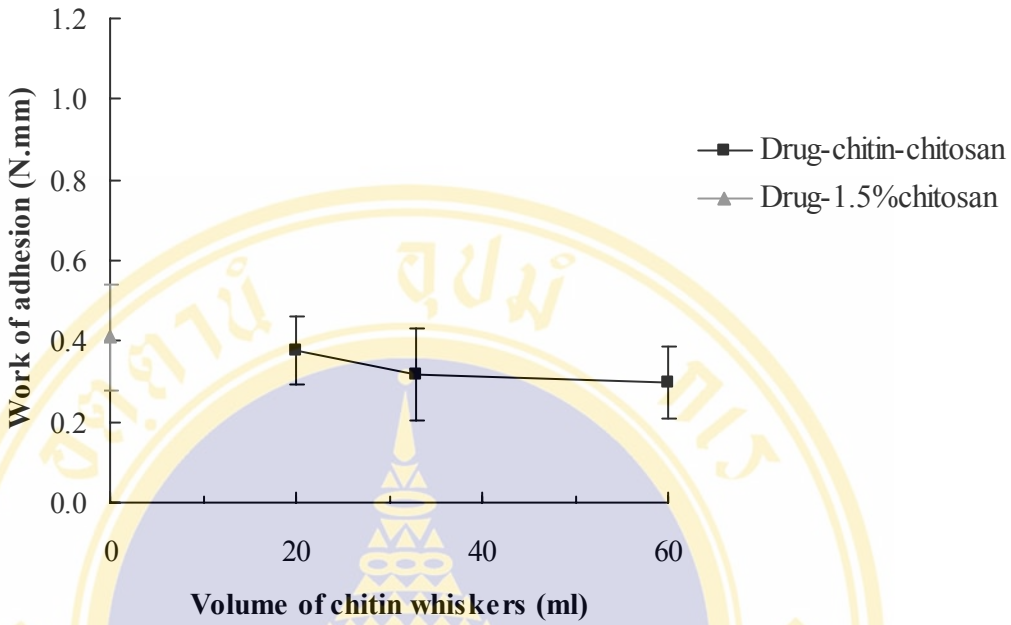


Figure 42. Work of adhesion (N.mm) from the verapamil hydrochloride-chitin whiskers-chitosan acetate buccal patches (Mean ± SD, n = 10).

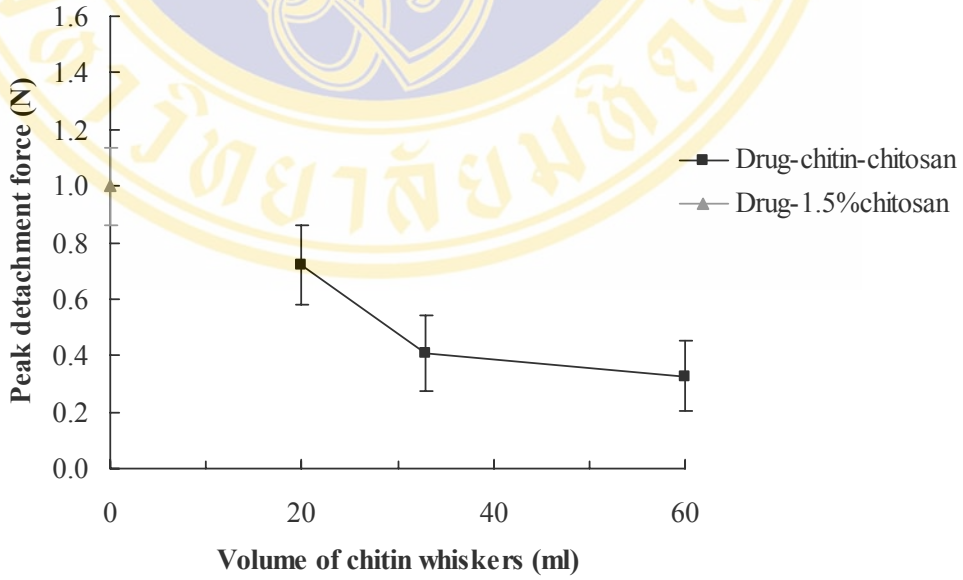


Figure 43. Peak detachment force (N) from the verapamil hydrochloride-chitin whiskers-chitosan acetate buccal patches (Mean ± SD, n = 10).

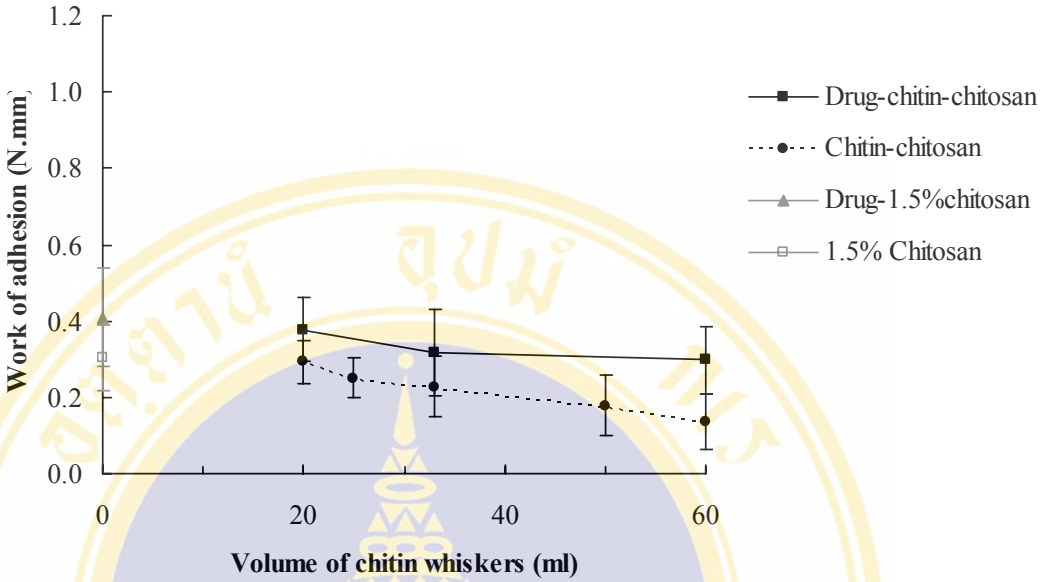


Figure 44. Comparison of the work of adhesion (N.mm) from the verapamil hydrochloride-chitin whiskers-crab chitosan acetate buccal patches and drug-free chitin whiskers-crab chitosan films (Mean ± SD, n = 10).

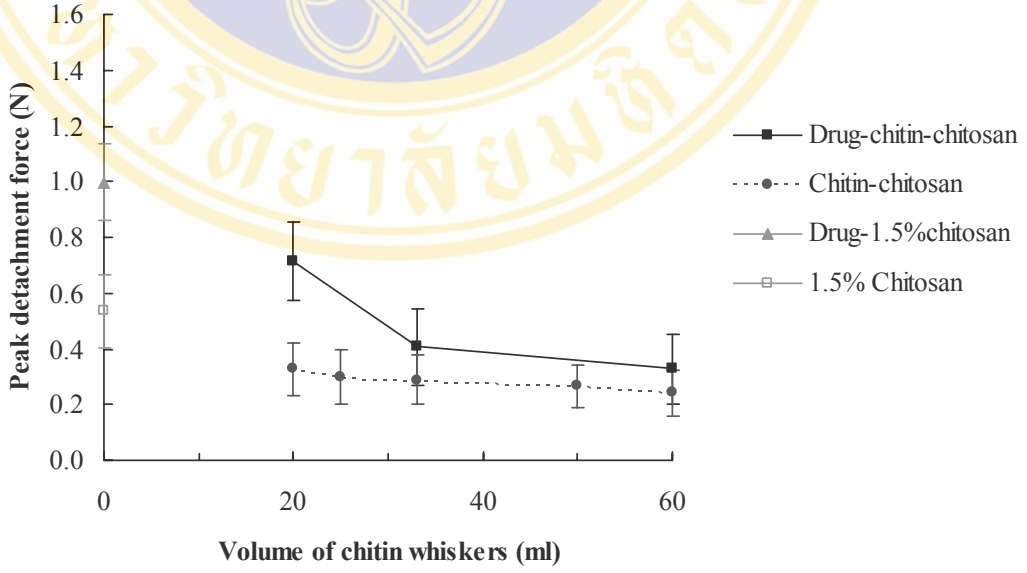


Figure 45. Comparison of the peak detachment force (N) from the verapamil hydrochloride-chitin whiskers-crab chitosan acetate buccal patches and drug-free chitin whiskers-crab chitosan films (Mean ± SD, n = 10).

The linear regression equation of the curve between the work of adhesion (Y) versus concentration of crab chitosan (X) was Y = 0.3390 - 0.0018X and it showed a linear relationship with R^2 of 0.8097 (Figure 42).

In addition, the linear regression equation of the curve between the peak detachment force (Y) versus concentration of crab chitosan (X) was Y = 0.8161 – 0.0088X and it showed a linear relationship with R^2 of 0.7568 (Figure 43).

From Table 26, ANOVA on these works of adhesion indicated that the HEC drug-patch was lower than all chitin whisker-crab chitosan patches but it was not significantly different ($p>0.05$). In addition, the difference of work of adhesion between various volumes of chitin whiskers was not significantly different ($p>0.05$). Nevertheless, the peak detachment forces indicated that the HEC patch was significantly higher than 1:2 and 1.5:1 chitin whisker-crab chitosan ($p<0.05$). The 1:4 drug-patch was significantly higher than 1:2 and 1.5:1 ($p<0.05$), whereas the difference between 1.5:1 and 1:2 was not significant ($p>0.05$).

Comparison with the results of 1.5% crab chitosan-drug patch in Table 9, although the increasing of chitin whiskers induced reduction of both the work of adhesion and peak detachment force of film, the differences of the work of adhesion were not significant ($p>0.05$).

Comparison with the results in Table 7, the incorporation of the drug induced the increasing of both the work of adhesion and peak detachment at equal ratio of chitin and chitosan, the differences were not significant ($p>0.05$) (Figure 44 and 45).

5.3 Content uniformity of verapamil hydrochloride- chitin whiskers-crab chitosan acetate buccal patch

The different values between absorbances at 278 and 300 nm of the sample preparation were ordinated with calibration curve and the amount of drug (mg) was calculated as described in topic 4.3.

Table 27 shows concentration (mg/ml) and amount of drug (mg) in chitin whiskers-chitosan buccal patch that was cut into a rectangle with 10 x 15 mm.

Table 27. Average of concentration (mg/ml) and amount of drug (mg) in chitin whiskers-chitosan buccal patch was determined using UV/ VIS spectrophotometer at 278 and 300 nm. (Mean ± SD, n = 10).

Sample	Ratio	Concentration of drug (mg/ml)	Amount of drug (mg)	% CV
Chitin whiskers :	1.5 :1	0.0350 ± 0.0023	17.49 ± 1.13	6.48
1.5% crab chitosan	1:2	0.0301 ± 0.0025	15.04 ± 1.26	8.37
	1:4	0.0317 ± 0.0027	15.80 ± 1.33	8.39

5.4 *In vitro* study of drug release from verapamil hydrochloride-chitin whiskers-crab chitosan acetate buccal patch

The different values between absorbances at 278 and 300 nm of the sample preparation were ordinated with calibration curve and the cumulative amount of drug (mg) released from the buccal patch at various times was calculated as described in topic 4.4.

The average amount of drug (mg) released from chitin whiskers-crab chitosan buccal patch that was cut into a rectangle with 10 x 15 mm at various times is presented in Table 28.

The average percent of drug (mg) released is presented in Table 29. Figure 46, 47, 48 and 27 show the average percent of verapamil hydrochloride released at various times from buccal patches of 1.5:1, 1:2, 1:4 (ratio of chitin whiskers: crab chitosan) and 1.5% hydroxyl ethylcellulose (control), respectively. Typical *in vitro* verapamil hydrochloride released-time profile is shown in Figure 49.

From result in Table 29 and Figure 49, the 1.5:1, 1:2, 1:4 (ratio of chitin whiskers: crab chitosan) and control buccal patch released almost (about 96%) all the contained verapamil hydrochloride in 30, 45, 90 and 120 min, respectively. The release profile between HEC and all chitin-chitosan patch were statistically similar ($p>0.05$).

Table 28. Average amount of verapamil hydrochloride (mg) released from chitin whiskers-crab chitosan buccal patch at various times (Mean ± SD, n = 6).

Time (min)	Amount of verapamil hydrochloride (mg)			
	Sample			
	1.5 : 1	1 : 2	1 : 4	1.5 % HEC
5	10.25 ± 0.81	8.80 ± 0.37	7.93 ± 0.71	8.30 ± 1.56
10	13.56 ± 0.86	11.03 ± 0.57	10.31 ± 0.96	9.66 ± 1.16
20	16.01 ± 1.04	13.40 ± 0.67	11.94 ± 1.16	10.91 ± 1.01
30	16.79 ± 1.08	14.23 ± 0.85	13.12 ± 1.20	11.68 ± 1.28
45	16.99 ± 1.11	14.91 ± 0.94	14.02 ± 1.46	12.24 ± 1.49
60	17.08 ± 1.11	15.16 ± 1.00	14.54 ± 1.53	12.53 ± 1.71
90	17.16 ± 1.21	15.33 ± 1.05	15.06 ± 1.63	12.78 ± 1.88
120	17.13 ± 1.16	15.45 ± 1.05	15.26 ± 1.63	13.05 ± 1.93
180	17.19 ± 1.12	15.54 ± 1.12	15.41 ± 1.69	13.23 ± 2.10
240	17.23 ± 1.13	15.62 ± 1.04	15.56 ± 1.73	13.39 ± 2.14
300	17.21 ± 1.12	15.50 ± 1.08	15.40 ± 1.71	13.43 ± 2.19

Table 29. Average percent *in vitro* release of verapamil hydrochloride from chitin whiskers-crab chitosan buccal patch at various times (Mean ± SD, n = 6).

Time (min)	Percent of verapamil hydrochloride			
	Sample			
	1.5 : 1	1 : 2	1 : 4	1.5 % HEC
5	58.60 ± 4.62	58.50 ± 2.46	50.17 ± 4.56	61.24 ± 11.50
10	77.53 ± 4.93	73.32± 3.80	65.29 ± 6.07	71.27 ± 8.53
20	91.56 ± 5.94	89.12 ± 4.46	75.58 ± 7.37	80.52 ± 7.47
30	96.02 ± 6.18	94.58 ± 5.67	83.07 ± 7.62	86.26 ± 9.47
45	97.17 ± 6.33	99.12 ± 6.26	88.76 ± 9.22	90.36 ± 11.03
60	97.66 ± 6.36	100.76 ± 6.66	92.05 ± 9.66	92.52 ± 12.63
90	98.13 ± 6.92	101.91 ± 6.95	95.30 ± 10.30	94.29 ± 13.89
120	97.95 ± 6.62	102.69 ± 6.95	96.60 ± 10.35	96.33 ± 14.25
180	98.29 ± 6.43	103.30 ± 7.44	97.53 ± 10.70	97.65 ± 15.46
240	98.51 ± 6.46	103.88 ± 6.91	98.50 ± 10.94	98.81 ± 15.83
300	98.43 ± 6.42	103.05 ± 7.15	97.47 ± 10.80	99.15 ± 16.15

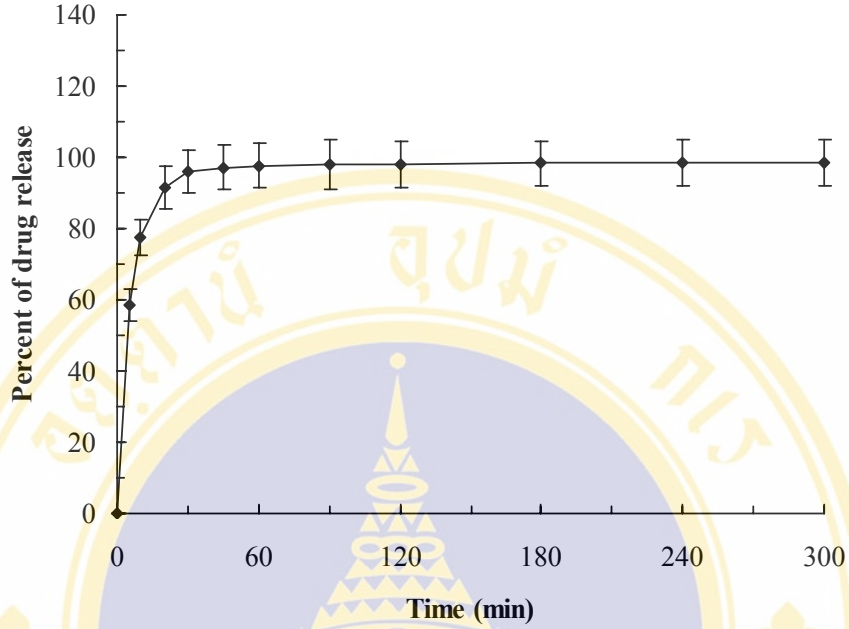


Figure 46. Average percent of verapamil hydrochloride released at various times from verapamil buccal patches which contained 1.5 part of chitin whisker: 1 part of 1.5% crab chitosan (Mean ± SD, n = 6).

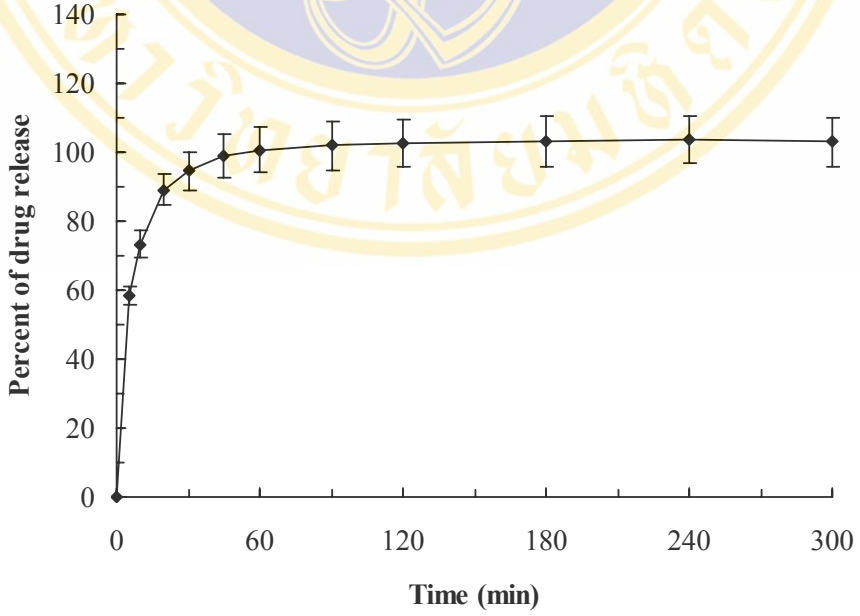


Figure 47. Average percent of verapamil hydrochloride released at various times from verapamil buccal patches which contained 1 part of chitin whisker: 2 part of 1.5% crab chitosan (Mean ± SD, n = 6).

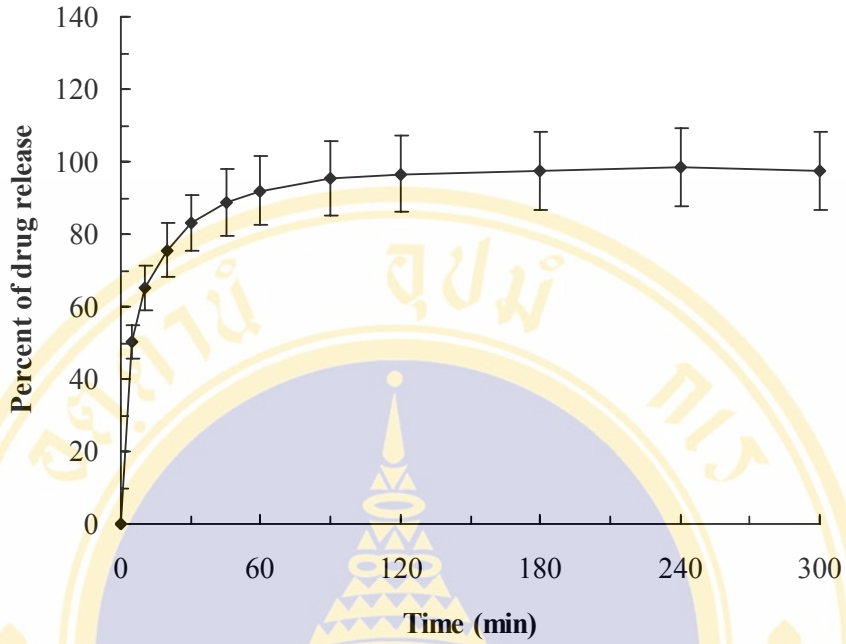


Figure 48. Average percent of verapamil hydrochloride released at various times from verapamil buccal patches which contained 1 part of chitin whisker: 4 part of 1.5% crab chitosan (Mean ± SD, n = 6).

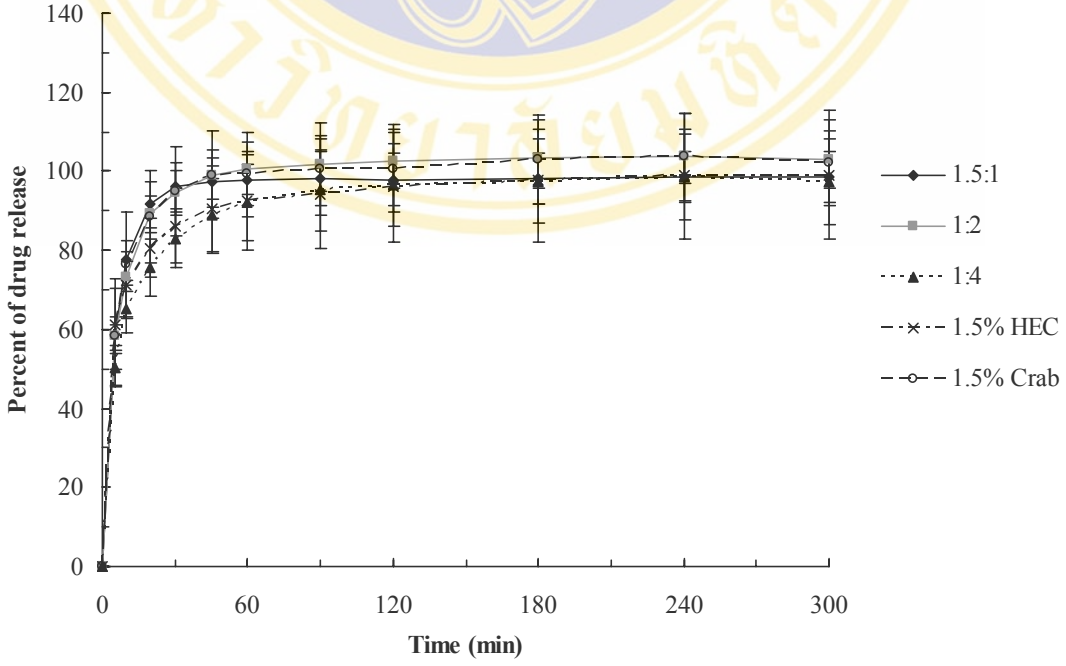


Figure 49. Average percent of verapamil hydrochloride released at various times from different chitin whisker buccal patches (Mean ± SD, n = 6).

The increasing of crab chitosan concentration did not reduce release rate because the release of all chitin-chitosan formulation at all time points were statistically similar ($p>0.05$), except that the 1:4 drug-chitin whiskers-crab chitosan buccal patch was different from 1.5:1 and 1:2 at 20 min ($p<0.05$). The amount of drug released from 1.5% crab chitosan and various chitin whiskers buccal patches were not significantly different ($p>0.05$).

After the last time point, the residual patch was collected and analyzed for the drug remained. Calculation of the drug (mg) remained was performed as described in verapamil-crab chitosan buccal patch.

The average concentration (mg/ml), amount of drug (mg), percent of drug remained are presented in Table 30. From these results, the drug remained in 1.5% HEC buccal patch was significantly lower than 1:2, 1:4 and 1.5:1 chitin whiskers-chitosan, respectively ($p<0.05$). The 1.5:1 was significantly higher than 1:4 and 1:2, respectively ($p<0.05$), whereas the 1:4 and 1:2 patch were not significantly different ($p>0.05$).

Comparison with the results of 1.5% crab chitosan, the residual 1.5% crab chitosan was different from 1.5:1 buccal patch ($p<0.05$) (Table 14, 30 and Figure 49).

Table 30. Average of concentration (mg/ml), amount of drug (mg) and percent of drug remained in residual chitin whisker-crab chitosan buccal patches after the end of drug release study. (Mean $\pm$ SD, n =6).

Sample	Ratio	Concentration of drug (mg/ml)	Amount of drug (mg)	% of drug remainder	% CV
Chitin whiskers :	1.5 :1	0.0127 $\pm$ 0.0014	0.32 $\pm$ 0.04	1.82 $\pm$ 0.20 [a, b]	11.16
1.5% crab chitosan	1:2	0.0065 $\pm$ 0.0010	0.16 $\pm$ 0.02	1.09 $\pm$ 0.16 [a]	15.00
	1:4	0.0087 $\pm$ 0.0015	0.22 $\pm$ 0.04	1.38 $\pm$ 0.24 [a]	17.63
1.5% HEC (Control)	-	0.0041 $\pm$ 0.0007	0.10 $\pm$ 0.02	0.76 $\pm$ 0.14	18.16

a = Significantly different from verapamil-1.5% HEC film ($p<0.05$)

b = Significantly different from verapamil-1.5% crab chitosan film ($p<0.05$)

5.5 *In vitro* permeation study of verapamil hydrochloride-chitin whiskers-chitosan acetate buccal patch

5.5.1 Penetration of verapamil hydrochloride from chitin whiskers-crab chitosan acetate buccal patch

The peak area of the sample preparation was ordinated with calibration curve for receptor compartment (pH 7.4) and the amount of drug (mg) in the buccal patch that penetrated via porcine buccal mucosa at various times was calculated as described in topic 4.5.

The average amount (mg) and percent of drug penetration are presented in Table 31 and 32, respectively. Figure 50, 51, 52, 37 and 38 showed the average percent of verapamil hydrochloride penetration at various times from 1.5:1, 1:2, 1:4 (ratio of chitin whiskers: crab chitosan), 1.5% hydroxyl ethylcellulose (control buccal patch) and standard solution (control solution), respectively. Typical *in vitro* verapamil hydrochloride penetration-time profile is shown in Figure 53.

From result in Table 21, 31 and Figure 53, drug penetrations from 1.5:1, 1:2, 1:4 chitin whiskers-crab chitosan, 1.5% HEC buccal patch and standard solution at 9 hr were 15.62 $\pm$ 5.34, 19.44 $\pm$ 7.18, 20.74 $\pm$ 7.83, 10.63 $\pm$ 5.93 and 20.76 $\pm$ 5.72 % of the contained verapamil hydrochloride, respectively. All three formulations of chitin whiskers-chitosan buccal patch were more penetrable than 1.5% HEC even though they were not significantly different ($p>0.05$), except that the 1:4 was significantly different from HEC at 5 and 6 hr ($p<0.05$).

Even though the drug penetration of chitin-chitosan were not significantly different from standard solution ($p>0.05$), the 1:4, 1:2 and 1.5:1 patch were higher than standard at 1 to 6 hr, 0.5 to 6 hr and 1 to 4 hr, respectively. At 9 hr the standard solution was the most penetrable despite the fact that it was not significantly different from all chitin whiskers-chitosan buccal patches ($p>0.05$).

Although the increasing of chitin whiskers concentration induced the reduction of penetration rate, the penetration rate of 1.5:1, 1:2 and 1:4 were statistically similar ($p>0.05$).

In contrast, comparison with the results of drug-1.5% crab chitosan buccal patch in Table 21 revealed that drug penetration from 1.5% crab chitosan was lower

than 3 formulations of chitin whiskers-chitosan patch but it was not significantly different ($p>0.05$).

Table 31. Average amount of verapamil hydrochloride (mg) penetrated from chitin whiskers-crab chitosan buccal patch via porcine buccal mucosa at various times (Mean ± SD, n = 6).

Time (hr)	Amount of verapamil hydrochloride (mg)				
	Sample				
	1.5 : 1	1 : 2	1 : 4	1.5 % HEC	Std solution
0.08	ND	ND	ND	ND	ND
0.17	ND	ND	ND	ND	ND
0.25	ND	ND	ND	ND	ND
0.33	ND	ND	ND	ND	ND
0.42	ND	ND	ND	ND	ND
0.50	ND	0.0200 ± 0.0039	ND	ND	ND
0.75	ND	0.0353 ± 0.0090	ND	ND	ND
1	0.0439 ± 0.0142	0.0533 ± 0.0158	0.0674 ± 0.0450	ND	ND
1.5	0.0933 ± 0.0207	0.1348 ± 0.0503	0.1713 ± 0.1185	0.0350 ± 0.0198	0.0384 ± 0.0169
2	0.1462 ± 0.0473	0.2282 ± 0.1074	0.2855 ± 0.1856	0.0709 ± 0.0335	0.0877 ± 0.0425
3	0.2706 ± 0.1069	0.3843 ± 0.2137	0.4892 ± 0.2393	0.1436 ± 0.0836	0.2009 ± 0.1769
4	0.4108 ± 0.1090	0.6110 ± 0.3113	0.6977 ± 0.2810	0.2271 ± 0.1308	0.4527 ± 0.1261
5	0.6157 ± 0.2483	0.7987 ± 0.4143	0.9133 ± 0.3470	0.2959 ± 0.1480	0.7209 ± 0.3109
6	0.7746 ± 0.2961	0.9805 ± 0.3986	1.1157 ± 0.4009	0.4063 ± 0.1981	1.0380 ± 0.4149
9	1.3659 ± 0.4671	1.4623 ± 0.5403	1.6386 ± 0.6186	0.7201 ± 0.4019	2.0764 ± 0.5718

ND = not detectable

Table 32 Average percent of verapamil hydrochloride penetrated from chitin whiskers-crab chitosan buccal patch via porcine buccal mucosa at various times (Mean ± SD, n = 6).

Time (hr)	Percent of verapamil hydrochloride				
	Sample				
	1.5 : 1	1 : 2	1 : 4	1.5 % HEC	Std solution
0.08	ND	ND	ND	ND	ND
0.17	ND	ND	ND	ND	ND
0.25	ND	ND	ND	ND	ND
0.33	ND	ND	ND	ND	ND
0.42	ND	ND	ND	ND	ND
0.50	ND	0.27 ± 0.05	ND	ND	ND
0.75	ND	0.47 ± 0.12	ND	ND	ND
1	0.50 ± 0.16	0.71 ± 0.21	0.85 ± 0.57	ND	ND
1.5	1.07 ± 0.24 [b]	1.79 ± 0.67 [a, b]	2.17 ± 1.50	0.52 ± 0.29	0.38 ± 0.17
2	1.67 ± 0.54	3.03 ± 1.43	3.61 ± 2.35	1.05 ± 0.49	0.88 ± 0.42
3	3.10 ± 1.22	5.11 ± 2.84	6.19 ± 3.03	2.12 ± 1.23	2.01 ± 1.77
4	4.70 ± 1.25	8.12 ± 4.14	8.83 ± 3.56	3.35 ± 1.93	4.53 ± 1.26
5	7.04 ± 2.84	10.62 ± 5.51	11.56 ± 4.39 [a,]	4.37 ± 2.19	7.21 ± 3.11
6	8.86 ± 3.39	13.04 ± 5.30	14.12 ± 5.07 [a]	6.00 ± 2.92	10.38 ± 4.15
9	15.62 ± 5.34	19.44 ± 7.18	20.74 ± 7.83	10.63 ± 5.93	20.76 ± 5.72

ND = not detectable

a = Significantly different from verapamil-1.5% HEC buccal patch ($p<0.05$)

b = Significantly different from verapamil hydrochloride solution ($p<0.05$)

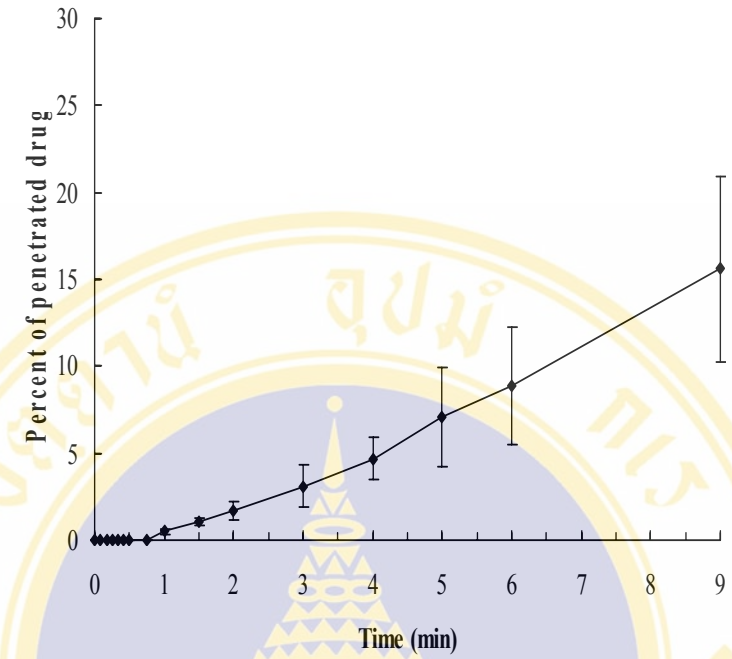


Figure 50. Average percent of verapamil hydrochloride penetrated from buccal patch which contained 1.5 part of chitin whisker: 1 part of 1.5% crab chitosan via porcine buccal mucosa at various times (Mean ± SD, n = 6).

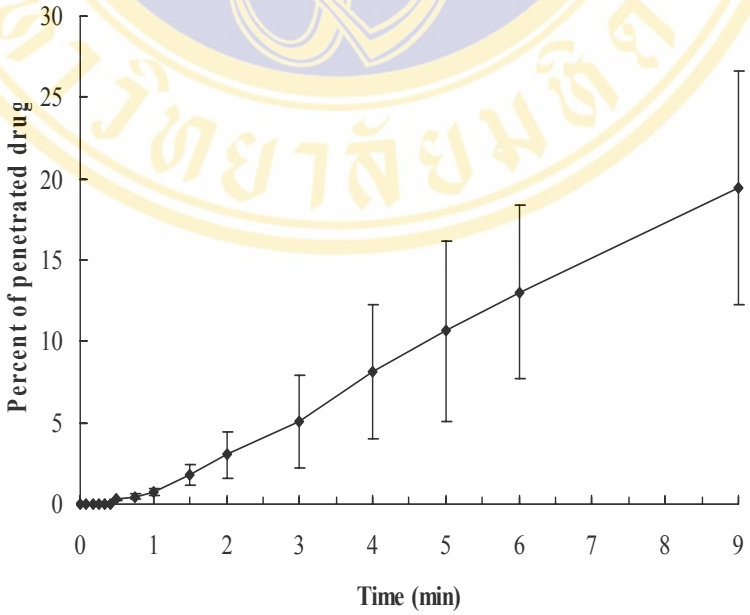


Figure 51. Average percent of verapamil hydrochloride penetrated from buccal patch which contained 1 part of chitin whisker: 2 part of 1.5% crab chitosan via porcine buccal mucosa at various times (Mean ± SD, n = 6).

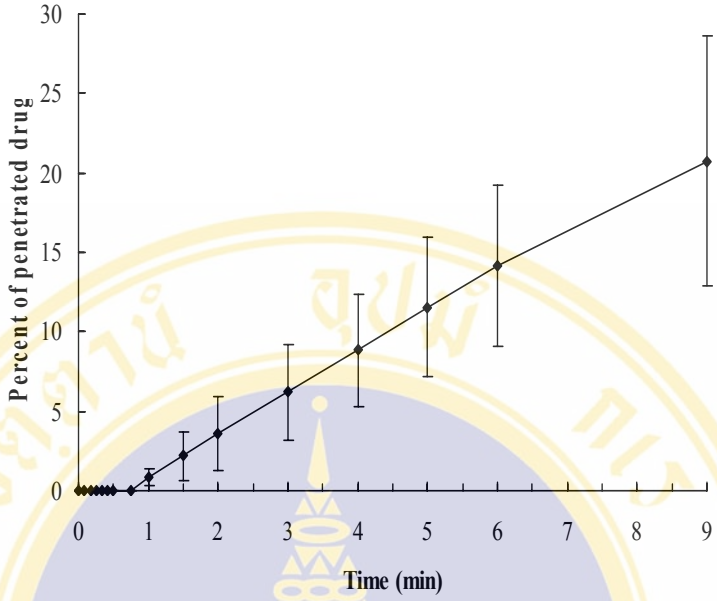


Figure 52. Average percent of verapamil hydrochloride penetrated from buccal patch which contained 1 part of chitin whisker: 4 part of 1.5% crab chitosan via porcine buccal mucosa at various times (Mean ± SD, n = 6).

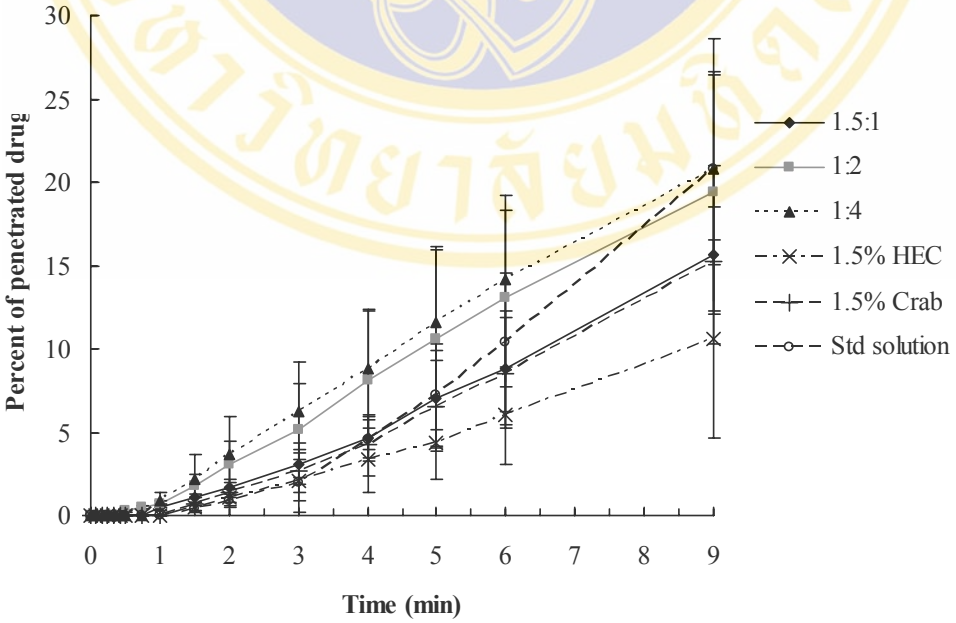


Figure 53. Average percent of verapamil hydrochloride penetrated from different buccal patches via porcine buccal mucosa at various times (Mean ± SD, n = 6).

Table 33. Average slope of a plot of percent of drug penetration versus times from various verapamil-chitin whiskers-crab chitosan buccal patches (Mean ± SD, n =6).

Chitin whisker : chitosan	Lag time (hr)	Slope	% CV
1.5 : 1	1	1.8901 ± 0.6770	35.82
1 : 2	0.5	2.3459 ± 0.9563	40.76
1 : 4	1	2.5167 ± 0.9363	37.20
1.5% HEC	1.5	1.3382 ± 0.7312 [a]	54.64
Std solution	1.5	2.7401 ± 0.7812	28.51

From results in Table 33 and Figure 53, the slope of a plot of percent of drug penetration from standard solution versus time was higher than 1:4, 1:2, 1.5:1 and 1.5% HEC, respectively. However, the differences between standard solution and various chitin whiskers-chitosan patch were not significant ($p>0.05$).

After the last time point, the residual solution in the donor site was collected. The peak area was ordinated with calibration curve for donor compartment (pH 6.8) and the amount of drug remained in donor compartment was calculated as described in topic 4.5.

The average concentration (mg/ml), amount of drug (mg) and percent of drug remained in donor compartment are presented in Table 34. From these results, the drug remained of standard solution in donor compartment was significantly higher than that of 1.5:1, 1:4, 1:2 chitin whiskers-crab chitosan and 1.5% HEC buccal patch, respectively ($p<0.05$). The residual donor of the 1.5:1, 1:2 and 1:4 were statistically similar ($p>0.05$).

The drug remained of 1.5% HEC and 1.5% crab chitosan in donor compartment were lower than 3 formulations of chitin whiskers-chitosan patch even though they were significantly different from 1.5:1 buccal patch ($p<0.05$).

Table 34. Average of concentration (mg/ml), amount of drug (mg) and percent of drug remained in residual donor compartment after the end of drug penetration study. (Mean ± SD, n =6).

Chitin whiskers: chitosan	Concentration of drug (mg/ml)	Amount of drug (mg)	% of drug Remainder	% CV
1.5 : 1	0.3704 ± 0.0466	0.85 ± 0.23	20.78 ± 2.61 [a, b, c]	12.58
1 : 2	0.2356 ± 0.0855	1.18 ± 0.43	15.35 ± 5.57 [b]	36.28
1 : 4	0.3278 ± 0.1055	1.64 ± 0.53	20.35 ± 6.55 [b]	32.16
1.5% HEC	0.1446 ± 0.0426	0.72 ± 0.21	10.46 ± 3.08 [b]	29.44
Std solution	0.3937 ± 0.0739	3.94 ± 0.74	39.37 ± 7.39	18.78

a = Significantly different from verapamil-1.5% HEC buccal patch ($p<0.05$)

b = Significantly different from verapamil hydrochloride solution ($p<0.05$)

c = Significantly different from verapamil-1.5% crab chitosan buccal patch ($p<0.05$)

After the last time point, the residual patch was collected and analyzed for the drug remained. Calculation of the amount of drug remained was performed as described in topic 4.5.

The average concentration (mg/ml), amount of drug (mg) and percent of drug remainder are presented in Table 35. From these results, the remainder of drug in 1.5% HEC buccal patch was higher than 1:4, 1:2 and 1.5:1 chitin whiskers-crab chitosan, respectively ($p<0.05$) even though it was significantly different from 1.5:1 ($p<0.05$). The residual patch of 1.5:1 crab chitosan was significantly lower than 1:4 ($p<0.05$), whereas the 1:2 and 1:4 were statistically similar ($p>0.05$). The residual 1.5% crab chitosan patch was higher than 3 formulation of chitin whiskers-chitosan patch even though it was significantly different from 1.5:1($p<0.05$).

From Figure 54, when percent of drug penetration at 9 hr was plotted against volume of chitin whiskers, it was found that there be a good linear relationship ($Y = 2.5220 - 0.1302X$, $R^2 = 0.9925$). 99.25 % accuracy of drug penetration at 9 hr was depended on the concentration of crab chitosan. On the other hand, 0.75 % accuracy of drug penetration at 9 hr was depend on other variables.

Table 35. Average of concentration (mg/ml), amount of drug (mg) and percent of drug remained in residual buccal patch after the end of drug penetration study. (Mean ± SD, n =6).

Chitin whisker: chitosan	Concentration of drug (mg/ml)	Amount of drug (mg)	% of drug Remainder	% CV
1.5 : 1	0.0487 ± 0.0030	0.97 ± 0.06	5.46 ± 0.34 [a, b]	6.16
1 : 2	0.0637 ± 0.0181	1.27 ± 0.36	8.31 ± 2.36	28.46
1 : 4	0.0763 ± 0.0100	1.53 ± 0.20	9.48 ± 1.24	13.13
1.5% HEC (Control)	0.0826 ± 0.0163	1.65 ± 0.33	11.95 ± 2.36	19.76

a = Significantly different to verapamil-1.5% HEC buccal patch ($p<0.05$)

b = Significantly different to verapamil-1.5% crab chitosan buccal patch ($p<0.05$)

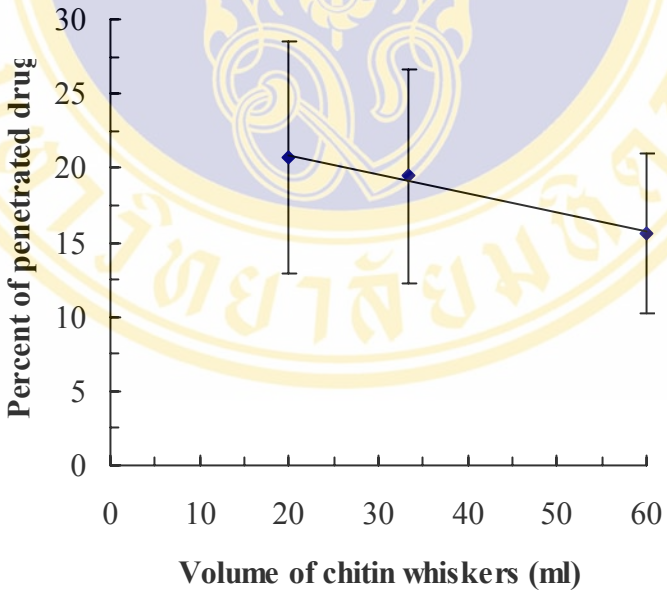


Figure 54. Relationship between percent of drug penetration at 9 hr and volume of chitin whiskers in buccal patch formulation (Mean ± SD, n =6).

CHAPTER V
DISCUSSION

From Table 3, the apparent viscosity and average molecular weight of chitosan which obtained from crab shell were higher than those obtained from squid pens and water soluble chitosan, respectively. The degree of deacetylation of crab chitosan was higher than that of squid pens and water soluble chitosan, respectively.

Chitosan is a linear polysaccharide of a co-polymer of 1,4-linked 2-acetamide-2-deoxy-β-D-glucopyranose (GlcNAc) and 2-amino-β-D-glucopyranose (GlcN). Chitosan is a polycation in acidic pH, with an intrinsic pKa value (independent on degree of acetylation) at approximately 6.5. Due to the polyelectrolyte property of chitosan ($-NH^{3+}$ form), the chitosan solubility in the range of pH 4 is improved. The presence of numerous hydrophilic groups in its structure shows its great affinity toward polar system with a high viscosity. The viscosity of chitosan is enhanced by the electroviscous effects brought about the cationic charges distributed along of the chain (25). %DD of chitosan obtained from squid pens was high with the low viscosity owing to its β-structure, which the chains are arranged in a parallel fashion with relatively weak hydrogen bonding. Thus, it is expected to show higher solubility and less viscosity than that of chitosan from crab and shrimp shells, which have α-structure (19, 20).

Chitosan in solution exists in the form of quasi-globular conformation stabilized by extensive intra-and inter-molecular hydrogen bonding. The hydrogen bonding in chitosan chains due to the presence of amine and hydroxyl groups causes the high viscosity of chitosan solutions. Chitosans with lower degree of deacetylation have lower hydrogen bonding density because of the lower number of amino groups in the polymer chains (10). Intermolecular hydrogen bonding in chitosan is responsible for the film and fiber-forming properties of the polymer. According to Chen et al (61), the degree of deacetylation of chitosan, which will determine the number of the intermolecular hydrogen bonds, was found to affect the rigidity of the polymer film.

The crab chitosan gave more appropriate viscosity to form film than squid pens and water soluble chitosan, respectively. Because chitosan was cationic charge, the increase in the concentration of chitosan increased adhesion to surface of buccal mucosa which exhibited anionic charge at physiological pH. Therefore, both crab and squid pens chitosan film showed higher mucoadhesive than 1.5% hydroxy ethylcellulose (HEC) film which was nonionic polymer (Table 5). The results suggested that chitosan was a suitable polymer to be used for mucoadhesive buccal patch. It could increase residence time in oral cavity. It provides intimate contact between a dosage form and the absorbing tissue, which may result in high drug concentration in a local area and hence high drug flux through the absorbing tissue (40). The incorporation of verapamil hydrochloride in chitosan solution changed the characteristic of film forming, especially the squid pens which has weak hydrogen bond. This result suggested that there was the ionic interaction between drug and cationic charge of chitosan. In addition, the incorporation of drug induced the increasing of mucoadhesive properties of crab chitosan film (Figure 20, 21).

In the characteristic of chitin whiskers film, increasing the concentration of chitin whiskers reduced the mucoadhesiveness due to a nonionic characteristic of chitin whiskers (Table 7).

Because the drug release profile of chitosan, chitin whiskers-chitosan and HEC were similar (Table 14 and 29), chitosan might not have an influence on the release rate of verapamil hydrochloride in buccal patch. It suggested that the chitosan film did not retard the release of drug. However, increasing the concentration of chitosan reduced the release rate of drug even though they were not significantly different ($p>0.05$).

From the in vitro penetration study (Table 21 and 32), the crab chitosan and chitin whiskers-chitosan patch were more permeable than HEC at all selected time (9 hr). They penetrated faster than standard solution within 2 hr. The results suggested that the crab chitosan is an absorption enhancer. The mechanism of action was proposed to be a combination of mucoadhesion and an effect on tight junctions with the epithelium (41, 42).

The incorporate of chitin whiskers reduced the penetration of drug. This result suggested that chitin whiskers might not be an absorption enhancer. From

Figure 40 and 54, a good linear relationship was found from a plot of percent of drug penetration at 9 hr against amount of crab chitosan and volume of chitin whiskers. The comparison with concentration of crab chitosan in chitin-chitosan mixture were increased from 0.6, 1.0 to 1.2%, they increased the penetration rate, respectively. In contrast, the concentration of crab chitosan film were increased form 1.0, 1.5 to 2.0%, they reduced the penetration rate, respectively. In addition, the 1.0% crab chitosan patch was closely similar to 1.0% chitosan in chitin-chitosan buccal patch (or 1:2 of chitin whiskers and crab chitosan) whereas the drug penetration in 1.5% crab chitosan was lower than 1.2% crab chitosan in chitin-chitosan buccal patch (or 1:4 of chitin whiskers and crab chitosan). Thus, the concentration of chitosan in buccal patch that used as an absorption enhancer should be in the range of optimal concentration. From this result suggest that it is about 1.2% of crab chitosan. However, this optimal concentration is suitable with surface area of buccal mucosa in this study (1.77 cm^2).

In this study, the result of absorption enhancer function of chitin whiskers was not clear because the ratio of chitin whiskers and chitosan could not be expended due to the fact that chitin whiskers was in suspension form in water, which exhibited very low viscosity. It can then be concluded that the spreadability of the mixture on Labdryer machine was limited by its viscosity. Moreover, utilizing chitin whiskers powder instead of that in suspension form was also impractical because it was not soluble in water, rather it was suspended in chitosan solution. The texture of the film was rough which can be ranked as bad characteristic of the film.

With respect to the crab chitosan buccal patch, likewise, the concentration of crab chitosan in buccal patch could not be expanded as well owing to the limitation of solution viscosity in terms of film spreadability. In addition, the high concentration of crab chitosan solution resulted in the precipitation of chitosan because it was already saturated in 0.55% glacial acetic acid solution. The higher concentration of glacial acetic acid solution could solve this problem. Nevertheless, the higher concentration of acid solution produced lower pH that can cause damage at the buccal mucosa.

From the results described above, the appropriate chitosan for buccal patch formulation should contain strong hydrogen bond, be soluble in neutral pH, have high viscosity (MW is about 10^6), and comprise no color and no precipitant. These properties altogether reflect good characteristic of the film.

However, in vitro penetration study has high variation that for example porcine buccal mucosa from several pigs and the viability of tissue. Therefore, the in vivo penetration and bioavailability study in pigs or human volunteers should be investigated in the future.

CHAPTER VI
CONCLUSION

From the results of mucoadhesive evaluation, squid pens chitosan gave the higher mucoadhesive properties than crab chitosan, HEC and mixture of chitin whiskers and crab chitosan, respectively. Chitosan is a good mucoadhesive polymer as comparing to hydroxyl ethyl cellulose (HEC) due to cationic charge of chitosan in acidic pH whereas HEC and chitin whiskers are nonionic polymers. It is believed that the interaction between the cationic charge of polymer film and anionic charge of the interface of buccal mucosa has an influence on the mucoadhesive property of film.

From the results of penetration evaluation, the percent of drug penetration at the corresponding concentration of crab chitosan within 2 to 4 hr was in the range of 0.99 to 6.17 %. Drug penetrated from standard solution and 1.5% HEC within 2 to 4 hr were in the range of 0.88 to 4.53 % and 1.05 to 3.35 %, respectively. Therefore, crab chitosan gave the higher drug penetration than standard solution and HEC patch with in the effective times. Due to the maximum of buccal drug retention and absorption is approximately 4 to 6 hr, meal intake and/ or drinking may require the removal of delivery device (11). In addition, the incorporation of chitin whiskers reduced the drug penetration. The percent of drug penetration at the corresponding ratio of chitin whiskers and crab chitosan within 2 to 4 hr was in the range of 1.67 to 8.83 %. It should be noted that, the concentration of chitosan in buccal patch which was used as an absorption enhancer should be in the range of optimal concentration. There were good linear relationships between concentration of polymers (i.e., crab chitosan and chitin whiskers) and the percent of drug penetration. The results implied that, the crab chitosan and chitin whiskers has an influence on the penetration of verapamil hydrochloride in buccal patch via porcine buccal mucosa.

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APPENDIX

APPENDIX

STATISTICS

THE LIST OF ABBREVIATION FOR *IN VITRO* MUCOADHESIVE STUDY

0 = 1.5 % hydroxyethylcellulose (HEC) film

1 = 1.0 % crab chitosan acetate film

15 = 1.5 % crab chitosan acetate film

2 = 2.0 % crab chitosan acetate film

25 = 2.5 % crab chitosan acetate film

32 = 2.0 % squid pens chitosan acetate film

325 = 2.5 % squid pens chitosan acetate film

33 = 3.0 % squid pens chitosan acetate film

151 = The film was contained 1.5 part of chitin whiskers suspension and 1 part of 1.5% crab chitosan acetate solution

11 = The film was contained 1 part of chitin whiskers suspension and 1 part of 1.5% crab chitosan acetate solution

12 = The film was contained 1 part of chitin whiskers suspension and 2 part of 1.5% crab chitosan acetate solution

13 = The film was contained 1.5 part of chitin whiskers suspension and 3 part of 1.5% crab chitosan acetate solution

14 = The film was contained 1.5 part of chitin whiskers suspension and 4 part of 1.5% crab chitosan acetate solution

20 = Verapamil hydrochloride-1.5 % HEC buccal patch

21 = Verapamil hydrochloride-1.0 % crab chitosan acetate buccal patch

215 = Verapamil hydrochloride-1.5 % crab chitosan acetate buccal patch

22 = Verapamil hydrochloride-2.0 % crab chitosan acetate buccal patch

2151 = Verapamil hydrochloride-1.5 part of chitin whiskers suspension and 1 part of 1.5% crab chitosan acetate solution

212 = Verapamil hydrochloride-1.5 part of chitin whiskers suspension and 2 part of 1.5% crab chitosan acetate solution

214 = Verapamil hydrochloride-1.5 part of chitin whiskers suspension and 4 part of 1.5% crab chitosan acetate solution

220 = Verapamil hydrochloride solution

1. *In vitro* mucoadhesive study of crab chitosan film

Oneway

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
adhesion	0	10	.24820	.103972	.032879	.17382	.32258	.128	.526
	1	10	.29710	.074596	.023589	.24374	.35046	.200	.408
	2	10	.37580	.068452	.021647	.32683	.42477	.229	.470
	15	10	.30130	.085776	.027125	.23994	.36266	.174	.399
	25	10	.57640	.111937	.035397	.49633	.65647	.435	.729
	Total	50	.35976	.145569	.020587	.31839	.40113	.128	.729
peakforce	0	10	.53620	.124518	.039376	.44713	.62527	.386	.733
	1	10	.42850	.114064	.036070	.34690	.51010	.283	.619
	2	10	.60630	.094174	.029780	.53893	.67367	.449	.735
	15	10	.53590	.131333	.041531	.44195	.62985	.367	.744
	25	10	.89800	.136864	.043280	.80009	.99591	.700	1.152
	Total	50	.60098	.198185	.028028	.54466	.65730	.283	1.152

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
adhesion	1.441	4	45	.236
peakforce	.384	4	45	.819

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
adhesion	Between Groups	.670	4	.167	20.447	.000
	Within Groups	.369	45	.008		
	Total	1.038	49			
peakforce	Between Groups	1.264	4	.316	21.542	.000
	Within Groups	.660	45	.015		
	Total	1.925	49			

Post Hoc Tests

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
adhesion	0	1	-.048900	.040471	.747	-.16390	.06610
		2	-.127600*	.040471	.023	-.24260	-.01260
		15	-.053100	.040471	.685	-.16810	.06190
		25	-.328200*	.040471	.000	-.44320	-.21320
	1	0	.048900	.040471	.747	-.06610	.16390
		2	-.078700	.040471	.309	-.19370	.03630
		15	-.004200	.040471	1.000	-.11920	.11080
		25	-.279300*	.040471	.000	-.39430	-.16430
	2	0	.127600*	.040471	.023	.01260	.24260
		1	.078700	.040471	.309	-.03630	.19370
		15	.074500	.040471	.364	-.04050	.18950
		25	-.200600*	.040471	.000	-.31560	-.08560
	15	0	.053100	.040471	.685	-.06190	.16810
		1	.004200	.040471	1.000	-.11080	.11920
		2	-.074500	.040471	.364	-.18950	.04050
		25	-.275100*	.040471	.000	-.39010	-.16010
	25	0	.328200*	.040471	.000	.21320	.44320
		1	.279300*	.040471	.000	.16430	.39430
		2	.200600*	.040471	.000	.08560	.31560
		15	.275100*	.040471	.000	.16010	.39010
peakforce	0	1	.107700	.054172	.288	-.04623	.26163
		2	-.070100	.054172	.696	-.22403	.08383
		15	.000300	.054172	1.000	-.15363	.15423
		25	-.361800*	.054172	.000	-.51573	-.20787
	1	0	-.107700	.054172	.288	-.26163	.04623
		2	-.177800*	.054172	.016	-.33173	-.02387
		15	-.107400	.054172	.291	-.26133	.04653
		25	-.469500*	.054172	.000	-.62343	-.31557
	2	0	.070100	.054172	.696	-.08383	.22403
		1	.177800*	.054172	.016	.02387	.33173
		15	.070400	.054172	.693	-.08353	.22433
		25	-.291700*	.054172	.000	-.44563	-.13777
	15	0	-.000300	.054172	1.000	-.15423	.15363
		1	.107400	.054172	.291	-.04653	.26133
		2	-.070400	.054172	.693	-.22433	.08353
		25	-.362100*	.054172	.000	-.51603	-.20817
	25	0	.361800*	.054172	.000	.20787	.51573
		1	.469500*	.054172	.000	.31557	.62343
		2	.291700*	.054172	.000	.13777	.44563
		15	.362100*	.054172	.000	.20817	.51603

*. The mean difference is significant at the .05 level.

Homogeneous Subsets

adhesion

Tukey HSD[a]

group	N	Subset for alpha = .05		
		1	2	3
0	10	.24820		
1	10	.29710	.29710	
15	10	.30130	.30130	
2	10		.37580	
25	10			.57640
Sig.		.685	.309	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.000.

peakforce

Tukey HSD[a]

group	N	Subset for alpha = .05		
		1	2	3
1	10	.42850		
15	10	.53590	.53590	
0	10	.53620	.53620	
2	10		.60630	
25	10			.89800
Sig.		.288	.693	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.000.

2. *In vitro* mucoadhesive study of squid pen chitosan film

Oneway

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
adhesion	0	10	.24820	.103972	.032879	.17382	.32258	.128	.526
	32	10	.42900	.109667	.034680	.35055	.50745	.268	.637
	33	10	.92260	.155839	.049281	.81112	1.03408	.712	1.128
	325	10	.61050	.124531	.039380	.52142	.69958	.464	.799
	Total	40	.55258	.279448	.044185	.46320	.64195	.128	1.128
peakforce	0	10	.53620	.124518	.039376	.44713	.62527	.386	.733
	32	10	.55110	.129182	.040851	.45869	.64351	.345	.731
	33	10	1.21030	.177850	.056241	1.08307	1.33753	.926	1.525
	325	10	.94010	.158565	.050143	.82667	1.05353	.679	1.184
	Total	40	.80943	.319948	.050588	.70710	.91175	.345	1.525

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
adhesion	2.054	3	36	.124
peakforce	.153	3	36	.927

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
adhesion	Between Groups	2.482	3	.827	52.836	.000
	Within Groups	.564	36	.016		
	Total	3.046	39			
peakforce	Between Groups	3.192	3	1.064	47.833	.000
	Within Groups	.801	36	.022		
	Total	3.992	39			

Post Hoc Tests

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
adhesion	0	32	-.180800*	.055960	.013	-.33151	-.03009
		33	-.674400*	.055960	.000	-.82511	-.52369
		325	-.362300*	.055960	.000	-.51301	-.21159
	32	0	.180800*	.055960	.013	.03009	.33151
		33	-.493600*	.055960	.000	-.64431	-.34289
		325	-.181500*	.055960	.013	-.33221	-.03079
	33	0	.674400*	.055960	.000	.52369	.82511
		32	.493600*	.055960	.000	.34289	.64431
		325	.312100*	.055960	.000	.16139	.46281
	325	0	.362300*	.055960	.000	.21159	.51301
		32	.181500*	.055960	.013	.03079	.33221
		33	-.312100*	.055960	.000	-.46281	-.16139
peakforce	0	32	-.014900	.066696	.996	-.19453	.16473
		33	-.674100*	.066696	.000	-.85373	-.49447
		325	-.403900*	.066696	.000	-.58353	-.22427
	32	0	.014900	.066696	.996	-.16473	.19453
		33	-.659200*	.066696	.000	-.83883	-.47957
		325	-.389000*	.066696	.000	-.56863	-.20937
	33	0	.674100*	.066696	.000	.49447	.85373
		32	.659200*	.066696	.000	.47957	.83883
		325	.270200*	.066696	.001	.09057	.44983
	325	0	.403900*	.066696	.000	.22427	.58353
		32	.389000*	.066696	.000	.20937	.56863
		33	-.270200*	.066696	.001	-.44983	-.09057

*. The mean difference is significant at the .05 level.

Homogeneous Subsets

adhesion

Tukey HSD[a]

group	N	Subset for alpha = .05			
		1	2	3	4
0	10	.24820			
32	10		.42900		
325	10			.61050	
33	10				.92260
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.000.

peakforce

Tukey HSD[a]

group	N	Subset for alpha = .05		
		1	2	3
0	10	.53620		
32	10	.55110		
325	10		.94010	
33	10			1.21030
Sig.		.996	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.000.

3. *In vitro* mucoadhesive study between crab chitosan film and squid pen chitosan film

3.1 The work of adhesion

Oneway

Descriptives

adhesion

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
0	10	.24820	.103972	.032879	.17382	.32258	.128	.526
1	10	.29710	.074596	.023589	.24374	.35046	.200	.408
2	10	.37580	.068452	.021647	.32683	.42477	.229	.470
15	10	.30130	.085776	.027125	.23994	.36266	.174	.399
25	10	.57640	.111937	.035397	.49633	.65647	.435	.729
32	10	.42900	.109667	.034680	.35055	.50745	.268	.637
33	10	.92260	.155839	.049281	.81112	1.03408	.712	1.128
325	10	.61050	.124531	.039380	.52142	.69958	.464	.799
Total	80	.47011	.235330	.026311	.41774	.52248	.128	1.128

Test of Homogeneity of Variances

adhesion

Levene Statistic	df1	df2	Sig.
2.496	7	72	.024

ANOVA

adhesion

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.540	7	.506	43.612	.000
Within Groups	.835	72	.012		
Total	4.375	79			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: adhesion

Dunnett C

(I) group	(J) group	Mean Difference (I-J)	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
0	1	-.048900	.040466	-.20433	.10653
	2	-.127600	.039365	-.27880	.02360
	15	-.053100	.042624	-.21682	.11062
	25	-.328200*	.048311	-.51376	-.14264
	32	-.180800	.047788	-.36435	.00275
	33	-.674400*	.059242	-.90194	-.44686
	325	-.362300*	.051301	-.55935	-.16525
1	0	.048900	.040466	-.10653	.20433
	2	-.078700	.032016	-.20167	.04427
	15	-.004200	.035947	-.14227	.13387
	25	-.279300*	.042537	-.44268	-.11592
	32	-.131900	.041942	-.29300	.02920
	33	-.625500*	.054635	-.83535	-.41565
	325	-.313400*	.045905	-.48972	-.13708
2	0	.127600	.039365	-.02360	.27880
	1	.078700	.032016	-.04427	.20167
	15	.074500	.034703	-.05879	.20779
	25	-.200600*	.041492	-.35997	-.04123
	32	-.053200	.040881	-.21022	.10382
	33	-.546800*	.053825	-.75354	-.34006
	325	-.234700*	.044937	-.40730	-.06210
15	0	.053100	.042624	-.11062	.21682
	1	.004200	.035947	-.13387	.14227
	2	-.074500	.034703	-.20779	.05879
	25	-.275100*	.044595	-.44639	-.10381
	32	-.127700	.044028	-.29681	.04141
	33	-.621300*	.056252	-.83736	-.40524
	325	-.309200*	.047818	-.49287	-.12553
25	0	.328200*	.048311	.14264	.51376
	1	.279300*	.042537	.11592	.44268
	2	.200600*	.041492	.04123	.35997
	15	.275100*	.044595	.10381	.44639
	32	.147400	.049555	-.04294	.33774
	33	-.346200*	.060676	-.57925	-.11315
	325	-.034100	.052951	-.23748	.16928
32	0	.180800	.047788	-.00275	.36435
	1	.131900	.041942	-.02920	.29300
	2	.053200	.040881	-.10382	.21022
	15	.127700	.044028	-.04141	.29681
	25	-.147400	.049555	-.33774	.04294
	33	-.493600*	.060260	-.72506	-.26214
	325	-.181500	.052474	-.38305	.02005
33	0	.674400*	.059242	.44686	.90194
	1	.625500*	.054635	.41565	.83535
	2	.546800*	.053825	.34006	.75354
	15	.621300*	.056252	.40524	.83736
	25	.346200*	.060676	.11315	.57925
	32	.493600*	.060260	.26214	.72506
	325	.312100*	.063082	.06980	.55440
325	0	.362300*	.051301	.16525	.55935
	1	.313400*	.045905	.13708	.48972
	2	.234700*	.044937	.06210	.40730
	15	.309200*	.047818	.12553	.49287
	25	.034100	.052951	-.16928	.23748
	32	.181500	.052474	-.02005	.38305
	33	-.312100*	.063082	-.55440	-.06980

*. The mean difference is significant at the .05 level.

3.2 The peak detachment force

Oneway

Descriptives

peakforce

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
0	10	.53620	.124518	.039376	.44713	.62527	.386	.733
1	10	.42850	.114064	.036070	.34690	.51010	.283	.619
2	10	.60630	.094174	.029780	.53893	.67367	.449	.735
15	10	.53590	.131333	.041531	.44195	.62985	.367	.744
25	10	.89800	.136864	.043280	.80009	.99591	.700	1.152
32	10	.55110	.129182	.040851	.45869	.64351	.345	.731
33	10	1.21030	.177850	.056241	1.08307	1.33753	.926	1.525
325	10	.94010	.158565	.050143	.82667	1.05353	.679	1.184
Total	80	.71330	.286049	.031981	.64964	.77696	.283	1.525

Test of Homogeneity of Variances

peakforce

Levene Statistic	df1	df2	Sig.
.400	7	72	.899

ANOVA

peakforce

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5.143	7	.735	40.029	.000
Within Groups	1.321	72	.018		
Total	6.464	79			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: peakforce
Tukey HSD

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0	1	.107700	.060586	.637	-.08144	.29684
	2	-.070100	.060586	.941	-.25924	.11904
	15	.000300	.060586	1.000	-.18884	.18944
	25	-.361800*	.060586	.000	-.55094	-.17266
	32	-.014900	.060586	1.000	-.20404	.17424
	33	-.674100*	.060586	.000	-.86324	-.48496
	325	-.403900*	.060586	.000	-.59304	-.21476
1	0	-.107700	.060586	.637	-.29684	.08144
	2	-.177800	.060586	.081	-.36694	.01134
	15	-.107400	.060586	.640	-.29654	.08174
	25	-.469500*	.060586	.000	-.65864	-.28036
	32	-.122600	.060586	.474	-.31174	.06654
	33	-.781800*	.060586	.000	-.97094	-.59266
	325	-.511600*	.060586	.000	-.70074	-.32246
2	0	.070100	.060586	.941	-.11904	.25924
	1	.177800	.060586	.081	-.01134	.36694
	15	.070400	.060586	.940	-.11874	.25954
	25	-.291700*	.060586	.000	-.48084	-.10256
	32	.055200	.060586	.984	-.13394	.24434
	33	-.604000*	.060586	.000	-.79314	-.41486
	325	-.333800*	.060586	.000	-.52294	-.14466
15	0	-.000300	.060586	1.000	-.18944	.18884
	1	.107400	.060586	.640	-.08174	.29654
	2	-.070400	.060586	.940	-.25954	.11874
	25	-.362100*	.060586	.000	-.55124	-.17296
	32	-.015200	.060586	1.000	-.20434	.17394
	33	-.674400*	.060586	.000	-.86354	-.48526
	325	-.404200*	.060586	.000	-.59334	-.21506
25	0	.361800*	.060586	.000	.17266	.55094
	1	.469500*	.060586	.000	.28036	.65864
	2	.291700*	.060586	.000	.10256	.48084
	15	.362100*	.060586	.000	.17296	.55124
	32	.346900*	.060586	.000	.15776	.53604
	33	-.312300*	.060586	.000	-.50144	-.12316
	325	-.042100	.060586	.997	-.23124	.14704
32	0	.014900	.060586	1.000	-.17424	.20404
	1	.122600	.060586	.474	-.06654	.31174
	2	-.055200	.060586	.984	-.24434	.13394
	15	.015200	.060586	1.000	-.17394	.20434
	25	-.346900*	.060586	.000	-.53604	-.15776
	33	-.659200*	.060586	.000	-.84834	-.47006
	325	-.389000*	.060586	.000	-.57814	-.19986
33	0	.674100*	.060586	.000	.48496	.86324
	1	.781800*	.060586	.000	.59266	.97094
	2	.604000*	.060586	.000	.41486	.79314
	15	.674400*	.060586	.000	.48526	.86354
	25	.312300*	.060586	.000	.12316	.50144
	32	.659200*	.060586	.000	.47006	.84834
	325	.270200*	.060586	.001	.08106	.45934
325	0	.403900*	.060586	.000	.21476	.59304
	1	.511600*	.060586	.000	.32246	.70074
	2	.333800*	.060586	.000	.14466	.52294
	15	.404200*	.060586	.000	.21506	.59334
	25	.042100	.060586	.997	-.14704	.23124
	32	.389000*	.060586	.000	.19986	.57814
	33	-.270200*	.060586	.001	-.45934	-.08106

*. The mean difference is significant at the .05 level.

Homogeneous Subsets

peakforce

Tukey HSD[a]

group	N	Subset for alpha = .05		
		1	2	3
1	10	.42850		
15	10	.53590		
0	10	.53620		
32	10	.55110		
2	10	.60630		
25	10		.89800	
325	10		.94010	
33	10			1.21030
Sig.		.081	.997	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.000.

4. *In vitro* mucoadhesive study of chitin whiskers-crab chitosan film

Oneway

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
adhesion	0	10	.24820	.103972	.032879	.17382	.32258	.128	.526
	11	10	.17790	.078144	.024711	.12200	.23380	.069	.293
	12	10	.22800	.077571	.024530	.17251	.28349	.092	.351
	13	10	.25020	.052624	.016641	.21256	.28784	.159	.322
	14	10	.29420	.056578	.017892	.25373	.33467	.213	.376
	15	10	.30130	.085776	.027125	.23994	.36266	.174	.399
	151	10	.13710	.073354	.023196	.08463	.18957	.070	.310
	Total	70	.23384	.092210	.011021	.21186	.25583	.069	.526
peakforce	0	10	.53620	.124518	.039376	.44713	.62527	.386	.733
	11	10	.26770	.075896	.024000	.21341	.32199	.150	.389
	12	10	.28800	.088320	.027929	.22482	.35118	.162	.424
	13	10	.29830	.095630	.030241	.22989	.36671	.131	.456
	14	10	.32800	.094537	.029895	.26037	.39563	.168	.479
	15	10	.53590	.131333	.041531	.44195	.62985	.367	.744
	151	10	.24160	.081459	.025760	.18333	.29987	.126	.375
	Total	70	.35653	.151507	.018108	.32040	.39265	.126	.744

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
adhesion	.573	6	63	.750
peakforce	1.240	6	63	.298

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
adhesion	Between Groups	.212	6	.035	5.937	.000
	Within Groups	.375	63	.006		
	Total	.587	69			
peakforce	Between Groups	.945	6	.157	15.514	.000
	Within Groups	.639	63	.010		
	Total	1.584	69			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: adhesion

Tukey HSD

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0	11	.070300	.034493	.402	-.03475	.17535
	12	.020200	.034493	.997	-.08485	.12525
	13	-.002000	.034493	1.000	-.10705	.10305
	14	-.046000	.034493	.834	-.15105	.05905
	15	-.053100	.034493	.720	-.15815	.05195
	151	.111100*	.034493	.031	.00605	.21615
11	0	-.070300	.034493	.402	-.17535	.03475
	12	-.050100	.034493	.771	-.15515	.05495
	13	-.072300	.034493	.368	-.17735	.03275
	14	-.116300*	.034493	.021	-.22135	-.01125
	15	-.123400*	.034493	.011	-.22845	-.01835
	151	.040800	.034493	.898	-.06425	.14585
12	0	-.020200	.034493	.997	-.12525	.08485
	11	.050100	.034493	.771	-.05495	.15515
	13	-.022200	.034493	.995	-.12725	.08285
	14	-.066200	.034493	.476	-.17125	.03885
	15	-.073300	.034493	.351	-.17835	.03175
	151	.090900	.034493	.133	-.01415	.19595
13	0	.002000	.034493	1.000	-.10305	.10705
	11	.072300	.034493	.368	-.03275	.17735
	12	.022200	.034493	.995	-.08285	.12725
	14	-.044000	.034493	.860	-.14905	.06105
	15	-.051100	.034493	.755	-.15615	.05395
	151	.113100*	.034493	.027	.00805	.21815
14	0	.046000	.034493	.834	-.05905	.15105
	11	.116300*	.034493	.021	.01125	.22135
	12	.066200	.034493	.476	-.03885	.17125
	13	.044000	.034493	.860	-.06105	.14905
	15	-.007100	.034493	1.000	-.11215	.09795
	151	.157100*	.034493	.000	.05205	.26215
15	0	.053100	.034493	.720	-.05195	.15815
	11	.123400*	.034493	.011	.01835	.22845
	12	.073300	.034493	.351	-.03175	.17835
	13	.051100	.034493	.755	-.05395	.15615
	14	.007100	.034493	1.000	-.09795	.11215
	151	.164200*	.034493	.000	.05915	.26925
151	0	-.111100*	.034493	.031	-.21615	-.00605
	11	-.040800	.034493	.898	-.14585	.06425
	12	-.090900	.034493	.133	-.19595	.01415
	13	-.113100*	.034493	.027	-.21815	-.00805
	14	-.157100*	.034493	.000	-.26215	-.05205
	15	-.164200*	.034493	.000	-.26925	-.05915

*. The mean difference is significant at the .05 level.

Multiple Comparisons

Dependent Variable: peakforce

Tukey HSD

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0	11	.268500*	.045050	.000	.13130	.40570
	12	.248200*	.045050	.000	.11100	.38540
	13	.237900*	.045050	.000	.10070	.37510
	14	.208200*	.045050	.000	.07100	.34540
	15	.000300	.045050	1.000	-.13690	.13750
	151	.294600*	.045050	.000	.15740	.43180
11	0	-.268500*	.045050	.000	-.40570	-.13130
	12	-.020300	.045050	.999	-.15750	.11690
	13	-.030600	.045050	.993	-.16780	.10660
	14	-.060300	.045050	.831	-.19750	.07690
	15	-.268200*	.045050	.000	-.40540	-.13100
	151	.026100	.045050	.997	-.11110	.16330
12	0	-.248200*	.045050	.000	-.38540	-.11100
	11	.020300	.045050	.999	-.11690	.15750
	13	-.010300	.045050	1.000	-.14750	.12690
	14	-.040000	.045050	.973	-.17720	.09720
	15	-.247900*	.045050	.000	-.38510	-.11070
	151	.046400	.045050	.945	-.09080	.18360
13	0	-.237900*	.045050	.000	-.37510	-.10070
	11	.030600	.045050	.993	-.10660	.16780
	12	.010300	.045050	1.000	-.12690	.14750
	14	-.029700	.045050	.994	-.16690	.10750
	15	-.237600*	.045050	.000	-.37480	-.10040
	151	.056700	.045050	.868	-.08050	.19390
14	0	-.208200*	.045050	.000	-.34540	-.07100
	11	.060300	.045050	.831	-.07690	.19750
	12	.040000	.045050	.973	-.09720	.17720
	13	.029700	.045050	.994	-.10750	.16690
	15	-.207900*	.045050	.000	-.34510	-.07070
	151	.086400	.045050	.477	-.05080	.22360
15	0	-.000300	.045050	1.000	-.13750	.13690
	11	.268200*	.045050	.000	.13100	.40540
	12	.247900*	.045050	.000	.11070	.38510
	13	.237600*	.045050	.000	.10040	.37480
	14	.207900*	.045050	.000	.07070	.34510
	151	.294300*	.045050	.000	.15710	.43150
151	0	-.294600*	.045050	.000	-.43180	-.15740
	11	-.026100	.045050	.997	-.16330	.11110
	12	-.046400	.045050	.945	-.18360	.09080
	13	-.056700	.045050	.868	-.19390	.08050
	14	-.086400	.045050	.477	-.22360	.05080
	15	-.294300*	.045050	.000	-.43150	-.15710

*. The mean difference is significant at the .05 level.

Homogeneous Subsets

adhesion

Tukey HSD[a]

group	N	Subset for alpha = .05		
		1	2	3
151	10	.13710		
11	10	.17790	.17790	
12	10	.22800	.22800	.22800
0	10		.24820	.24820
13	10		.25020	.25020
14	10			.29420
15	10			.30130
Sig.		.133	.368	.351

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.000.

peakforce

Tukey HSD[a]

group	N	Subset for alpha = .05	
		1	2
151	10	.24160	
11	10	.26770	
12	10	.28800	
13	10	.29830	
14	10	.32800	
15	10		.53590
0	10		.53620
Sig.		.477	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.000.

5. *In vitro* mucoadhesive study of verapamil hydrochloride-crab chitosan buccal patch

Oneway

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
adhesion	0	10	.24820	.103972	.032879	.17382	.32258	.128	.526
	1	10	.29710	.074596	.023589	.24374	.35046	.200	.408
	2	10	.37580	.068452	.021647	.32683	.42477	.229	.470
	15	10	.30130	.085776	.027125	.23994	.36266	.174	.399
	20	10	.25910	.108499	.034310	.18148	.33672	.132	.437
	21	10	.33250	.115040	.036379	.25021	.41479	.205	.592
	22	10	.45450	.102515	.032418	.38116	.52784	.252	.618
	215	10	.40930	.129985	.041105	.31631	.50229	.234	.657
	Total	80	.33473	.118046	.013198	.30846	.36099	.128	.657
peakforce	0	10	.53620	.124518	.039376	.44713	.62527	.386	.733
	1	10	.42850	.114064	.036070	.34690	.51010	.283	.619
	2	10	.60630	.094174	.029780	.53893	.67367	.449	.735
	15	10	.53590	.131333	.041531	.44195	.62985	.367	.744
	20	10	.80020	.147581	.046669	.69463	.90577	.603	1.094
	21	10	.56160	.126119	.039882	.47138	.65182	.395	.782
	22	10	1.12700	.140460	.044417	1.02652	1.22748	.907	1.389
	215	10	.99770	.136541	.043178	.90002	1.09538	.812	1.173
	Total	80	.69918	.264818	.029608	.64024	.75811	.283	1.389

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
adhesion	.674	7	72	.693
peakforce	.507	7	72	.827

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
adhesion	Between Groups	.373	7	.053	5.279	.000
	Within Groups	.727	72	.010		
	Total	1.101	79			
peakforce	Between Groups	4.364	7	.623	38.162	.000
	Within Groups	1.176	72	.016		
	Total	5.540	79			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: adhesion
Tukey HSD

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0	1	-.048900	.044953	.957	-.18923	.09143
	2	-.127600	.044953	.102	-.26793	.01273
	15	-.053100	.044953	.935	-.19343	.08723
	20	-.010900	.044953	1.000	-.15123	.12943
	21	-.084300	.044953	.572	-.22463	.05603
	22	-.206300*	.044953	.000	-.34663	-.06597
	215	-.161100*	.044953	.013	-.30143	-.02077
1	0	.048900	.044953	.957	-.09143	.18923
	2	-.078700	.044953	.655	-.21903	.06163
	15	-.004200	.044953	1.000	-.14453	.13613
	20	.038000	.044953	.990	-.10233	.17833
	21	-.035400	.044953	.993	-.17573	.10493
	22	-.157400*	.044953	.017	-.29773	-.01707
	215	-.112200	.044953	.214	-.25253	.02813
2	0	.127600	.044953	.102	-.01273	.26793
	1	.078700	.044953	.655	-.06163	.21903
	15	.074500	.044953	.714	-.06583	.21483
	20	.116700	.044953	.174	-.02363	.25703
	21	.043300	.044953	.978	-.09703	.18363
	22	-.078700	.044953	.655	-.21903	.06163
	215	-.033500	.044953	.995	-.17383	.10683
15	0	.053100	.044953	.935	-.08723	.19343
	1	.004200	.044953	1.000	-.13613	.14453
	2	-.074500	.044953	.714	-.21483	.06583
	20	.042200	.044953	.981	-.09813	.18253
	21	-.031200	.044953	.997	-.17153	.10913
	22	-.153200*	.044953	.023	-.29353	-.01287
	215	-.108000	.044953	.256	-.24833	.03233
20	0	.010900	.044953	1.000	-.12943	.15123
	1	-.038000	.044953	.990	-.17833	.10233
	2	-.116700	.044953	.174	-.25703	.02363
	15	-.042200	.044953	.981	-.18253	.09813
	21	-.073400	.044953	.729	-.21373	.06693
	22	-.195400*	.044953	.001	-.33573	-.05507
	215	-.150200*	.044953	.027	-.29053	-.00987
21	0	.084300	.044953	.572	-.05603	.22463
	1	.035400	.044953	.993	-.10493	.17573
	2	-.043300	.044953	.978	-.18363	.09703
	15	.031200	.044953	.997	-.10913	.17153
	20	.073400	.044953	.729	-.06693	.21373
	22	-.122000	.044953	.135	-.26233	.01833
	215	-.076800	.044953	.682	-.21713	.06353
22	0	.206300*	.044953	.000	.06597	.34663
	1	.157400*	.044953	.017	.01707	.29773
	2	.078700	.044953	.655	-.06163	.21903
	15	.153200*	.044953	.023	.01287	.29353
	20	.195400*	.044953	.001	.05507	.33573
	21	.122000	.044953	.135	-.01833	.26233
	215	.045200	.044953	.972	-.09513	.18553
215	0	.161100*	.044953	.013	.02077	.30143
	1	.112200	.044953	.214	-.02813	.25253
	2	.033500	.044953	.995	-.10683	.17383
	15	.108000	.044953	.256	-.03233	.24833
	20	.150200*	.044953	.027	.00987	.29053
	21	.076800	.044953	.682	-.06353	.21713
	22	-.045200	.044953	.972	-.18553	.09513

*. The mean difference is significant at the .05 level.

Multiple Comparisons

Dependent Variable: peakforce

Tukey HSD

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0	1	.107700	.057160	.566	-.07074	.28614
	2	-.070100	.057160	.921	-.24854	.10834
	15	.000300	.057160	1.000	-.17814	.17874
	20	-.264000*	.057160	.000	-.44244	-.08556
	21	-.025400	.057160	1.000	-.20384	.15304
	22	-.590800*	.057160	.000	-.76924	-.41236
	215	-.461500*	.057160	.000	-.63994	-.28306
1	0	-.107700	.057160	.566	-.28614	.07074
	2	-.177800	.057160	.052	-.35624	.00064
	15	-.107400	.057160	.570	-.28584	.07104
	20	-.371700*	.057160	.000	-.55014	-.19326
	21	-.133100	.057160	.293	-.31154	.04534
	22	-.698500*	.057160	.000	-.87694	-.52006
	215	-.569200*	.057160	.000	-.74764	-.39076
2	0	.070100	.057160	.921	-.10834	.24854
	1	.177800	.057160	.052	-.00064	.35624
	15	.070400	.057160	.920	-.10804	.24884
	20	-.193900*	.057160	.024	-.37234	-.01546
	21	.044700	.057160	.994	-.13374	.22314
	22	-.520700*	.057160	.000	-.69914	-.34226
	215	-.391400*	.057160	.000	-.56984	-.21296
15	0	-.000300	.057160	1.000	-.17874	.17814
	1	.107400	.057160	.570	-.07104	.28584
	2	-.070400	.057160	.920	-.24884	.10804
	20	-.264300*	.057160	.000	-.44274	-.08586
	21	-.025700	.057160	1.000	-.20414	.15274
	22	-.591100*	.057160	.000	-.76954	-.41266
	215	-.461800*	.057160	.000	-.64024	-.28336
20	0	.264000*	.057160	.000	.08556	.44244
	1	.371700*	.057160	.000	.19326	.55014
	2	.193900*	.057160	.024	.01546	.37234
	15	.264300*	.057160	.000	.08586	.44274
	21	.238600*	.057160	.002	.06016	.41704
	22	-.326800*	.057160	.000	-.50524	-.14836
	215	-.197500*	.057160	.020	-.37594	-.01906
21	0	.025400	.057160	1.000	-.15304	.20384
	1	.133100	.057160	.293	-.04534	.31154
	2	-.044700	.057160	.994	-.22314	.13374
	15	.025700	.057160	1.000	-.15274	.20414
	20	-.238600*	.057160	.002	-.41704	-.06016
	22	-.565400*	.057160	.000	-.74384	-.38696
	215	-.436100*	.057160	.000	-.61454	-.25766
22	0	.590800*	.057160	.000	.41236	.76924
	1	.698500*	.057160	.000	.52006	.87694
	2	.520700*	.057160	.000	.34226	.69914
	15	.591100*	.057160	.000	.41266	.76954
	20	.326800*	.057160	.000	.14836	.50524
	21	.565400*	.057160	.000	.38696	.74384
	215	.129300	.057160	.329	-.04914	.30774
215	0	.461500*	.057160	.000	.28306	.63994
	1	.569200*	.057160	.000	.39076	.74764
	2	.391400*	.057160	.000	.21296	.56984
	15	.461800*	.057160	.000	.28336	.64024
	20	.197500*	.057160	.020	.01906	.37594
	21	.436100*	.057160	.000	.25766	.61454
	22	-.129300	.057160	.329	-.30774	.04914

*. The mean difference is significant at the .05 level.

Homogeneous Subsets

adhesion

Tukey HSD[a]

group	N	Subset for alpha = .05		
		1	2	3
0	10	.24820		
20	10	.25910		
1	10	.29710	.29710	
15	10	.30130	.30130	
21	10	.33250	.33250	.33250
2	10	.37580	.37580	.37580
215	10		.40930	.40930
22	10			.45450
Sig.		.102	.214	.135

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.000.

peakforce

Tukey HSD[a]

group	N	Subset for alpha = .05		
		1	2	3
1	10	.42850		
15	10	.53590		
0	10	.53620		
21	10	.56160		
2	10	.60630		
20	10		.80020	
215	10			.99770
22	10			1.12700
Sig.		.052	1.000	.329

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.000.

5 *In vitro* release of verapamil hydrochloride-crab chitosan buccal patch

5.1 The percent of drug release at 5 to 300 min

Oneway

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
release5	20	6	61.240534	11.5015975	4.6955075	49.170347	73.310720	39.3274	70.7201
	21	6	65.308232	9.3655727	3.8234790	55.479667	75.136798	52.2618	80.7354
	22	6	25.018930	3.6186739	1.4773174	21.221365	28.816496	20.7879	30.3037
	215	6	58.309909	12.2786343	5.0127315	45.424273	71.195546	41.8920	72.6400
	Total	24	52.469401	18.7613748	3.8296496	44.547168	60.391635	20.7879	80.7354
release10	20	6	71.271426	8.5302238	3.4824493	62.319505	80.223347	54.0570	76.5905
	21	6	85.386292	13.7248998	5.6031669	70.982893	99.789691	64.7997	107.7802
	22	6	32.013494	3.6008328	1.4700338	28.234651	35.792336	27.8705	36.9412
	215	6	76.473459	13.2315027	5.4017384	62.587849	90.359070	58.0879	95.7548
	Total	24	66.286168	23.0823376	4.7116624	56.539351	76.032984	27.8705	107.7802
release20	20	6	80.520024	7.4722177	3.0505201	72.678412	88.361636	70.9420	90.5652
	21	6	95.652086	14.8444548	6.0602233	80.073786	111.230386	70.0202	115.1905
	22	6	41.212414	3.3861159	1.3823760	37.658904	44.765925	38.0816	45.7705
	215	6	88.555322	11.8066949	4.8200630	76.164955	100.945688	71.5513	107.7320
	Total	24	76.484962	23.5688796	4.8109774	66.532697	86.437227	38.0816	115.1905
release30	20	6	86.256046	9.4687592	3.8656048	76.319192	96.192899	78.6233	99.7838
	21	6	99.054906	15.2436164	6.2231803	83.057712	115.052100	72.5723	117.5153
	22	6	48.524803	2.8233162	1.1526140	45.561915	51.487692	46.3223	53.6296
	215	6	95.000966	11.2982822	4.6125044	83.144146	106.857786	79.1004	114.1500
	Total	24	82.209180	22.7259413	4.6389134	72.612857	91.805504	46.3223	117.5153
release45	20	6	90.362334	11.0287980	4.5024879	78.788320	101.936347	79.1767	104.0783
	21	6	100.5385	15.6903967	6.4055776	84.072425	117.004548	72.8221	117.7184
	22	6	54.904694	4.4258125	1.8068304	50.260089	59.549299	49.5563	62.6337
	215	6	99.127523	11.0619995	4.5160424	87.518667	110.736380	83.2892	116.9713
	Total	24	86.233259	21.6347867	4.4161824	77.097690	95.368829	49.5563	117.7184
release60	20	6	92.521150	12.6255254	5.1543492	79.271474	105.770826	79.7004	108.6176
	21	6	100.8025	15.9830335	6.5250461	84.029384	117.575714	72.4926	118.8204
	22	6	60.313022	6.1597228	2.5146963	53.848789	66.777255	53.5710	69.6986
	215	6	99.219544	10.6726141	4.3570765	88.019322	110.419765	84.7527	115.6349
	Total	24	88.214066	20.1010478	4.1031092	79.726138	96.701994	53.5710	118.8204
release90	20	6	94.293788	13.8940703	5.6722305	79.712856	108.874721	79.2815	110.1576
	21	6	100.7152	15.7945492	6.4480977	84.139853	117.290579	72.5861	118.0522
	22	6	67.688610	9.7982668	4.0001257	57.405960	77.971261	57.8998	79.6043
	215	6	100.4366	11.6061897	4.7382071	88.256700	112.616598	85.0561	118.9822
	Total	24	90.783566	18.4051307	3.7569316	83.011761	98.555371	57.8998	118.9822
release120	20	6	96.326179	14.2446585	5.8153575	81.377327	111.275031	80.3020	111.9973
	21	6	100.9769	15.9271205	6.5022197	84.262450	117.691426	72.4226	118.2090
	22	6	72.534232	12.5525615	5.1245618	59.361126	85.707337	60.0608	85.9034
	215	6	100.7053	10.9419504	4.4670325	89.222380	112.188126	87.1673	117.7990
	Total	24	92.635651	17.4252170	3.5569075	85.277627	99.993674	60.0608	118.2090
release180	20	6	97.653315	15.4637430	6.3130467	81.425112	113.881518	80.5823	113.5966
	21	6	101.5320	15.7306141	6.4219963	85.023699	118.040233	73.2558	118.1793
	22	6	75.525054	14.1812913	5.7894879	60.642702	90.407407	60.9236	88.6929
	215	6	102.9193	11.2283051	4.5839364	91.135888	114.702655	87.6655	119.5390
	Total	24	94.407402	17.4587205	3.5637464	87.035231	101.779573	60.9236	119.5390
release240	20	6	98.810046	15.8262645	6.4610454	82.201400	115.418692	81.4725	114.7299
	21	6	101.7810	15.9670450	6.5185188	85.024604	118.537377	73.1368	119.0273
	22	6	77.259941	14.7556347	6.0239627	61.774852	92.745030	61.3413	92.2070
	215	6	103.6641	11.0825406	4.5244282	92.033719	115.294545	88.0760	120.3349
	Total	24	95.378777	17.3556792	3.5427132	88.050117	102.707438	61.3413	120.3349
release300	20	6	99.148742	16.1488519	6.5927412	82.201561	116.095922	82.7795	115.4238
	21	6	101.0163	15.7658334	6.4363745	84.471065	117.561520	72.8993	118.0040
	22	6	77.954804	15.9667806	6.5184109	61.198695	94.710913	61.5239	94.8450
	215	6	102.1747	10.8253924	4.4194479	90.814167	113.535272	86.4675	116.9236
	Total	24	95.073640	17.1689550	3.5045983	87.823826	102.323453	61.5239	118.0040

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
release5	2.155	3	20	.125
release10	1.232	3	20	.324
release20	1.096	3	20	.374
release30	1.685	3	20	.202
release45	1.578	3	20	.226
release60	1.243	3	20	.321
release90	.556	3	20	.650
release120	.501	3	20	.686
release180	.876	3	20	.470
release240	1.110	3	20	.369
release300	1.498	3	20	.246

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
release5	Between Groups	6176.449	3	2058.816	21.454	.000
	Within Groups	1919.302	20	95.965		
	Total	8095.751	23			
release10	Between Groups	10008.388	3	3336.129	29.709	.000
	Within Groups	2245.881	20	112.294		
	Total	12254.269	23			
release20	Between Groups	10641.040	3	3547.013	33.223	.000
	Within Groups	2135.279	20	106.764		
	Total	12776.318	23			
release30	Between Groups	9590.536	3	3196.845	27.942	.000
	Within Groups	2288.238	20	114.412		
	Total	11878.773	23			
release45	Between Groups	8216.579	3	2738.860	21.491	.000
	Within Groups	2548.893	20	127.445		
	Total	10765.472	23			
release60	Between Groups	6459.658	3	2153.219	15.198	.000
	Within Groups	2833.541	20	141.677		
	Total	9293.199	23			
release90	Between Groups	4425.110	3	1475.037	8.764	.001
	Within Groups	3366.113	20	168.306		
	Total	7791.223	23			
release120	Between Groups	3314.296	3	1104.765	6.022	.004
	Within Groups	3669.383	20	183.469		
	Total	6983.678	23			
release180	Between Groups	2941.742	3	980.581	4.820	.011
	Within Groups	4068.817	20	203.441		
	Total	7010.559	23			
release240	Between Groups	2698.208	3	899.403	4.253	.018
	Within Groups	4229.843	20	211.492		
	Total	6928.051	23			
release300	Between Groups	2372.409	3	790.803	3.589	.032
	Within Groups	4407.371	20	220.369		
	Total	6779.779	23			

Post Hoc Tests

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
release5	20	21	-4.0676985	5.6558256	.888	-19.897994	11.762597
		22	36.2216035*	5.6558256	.000	20.391308	52.051899
		215	2.9306247	5.6558256	.954	-12.899670	18.760920
	21	20	4.0676985	5.6558256	.888	-11.762597	19.897994
		22	40.2893019*	5.6558256	.000	24.459007	56.119597
		215	6.9983231	5.6558256	.611	-8.831972	22.828618
	22	20	-36.221603*	5.6558256	.000	-52.051899	-20.391308
		21	-40.289302*	5.6558256	.000	-56.119597	-24.459007
		215	-33.290979*	5.6558256	.000	-49.121274	-17.460684
	215	20	-2.9306247	5.6558256	.954	-18.760920	12.899670
		21	-6.9983231	5.6558256	.611	-22.828618	8.831972
		22	33.2909788*	5.6558256	.000	17.460684	49.121274
release10	20	21	-14.114866	6.1181169	.130	-31.239085	3.009353
		22	39.2579327*	6.1181169	.000	22.133714	56.382152
		215	-5.2020330	6.1181169	.830	-22.326252	11.922186
	21	20	14.1148655	6.1181169	.130	-3.009353	31.239085
		22	53.3727983*	6.1181169	.000	36.248579	70.497017
		215	8.9128325	6.1181169	.481	-8.211387	26.037051
	22	20	-39.257933*	6.1181169	.000	-56.382152	-22.133714
		21	-53.372798*	6.1181169	.000	-70.497017	-36.248579
		215	-44.459966*	6.1181169	.000	-61.584185	-27.335747
	215	20	5.2020330	6.1181169	.830	-11.922186	22.326252
		21	-8.9128325	6.1181169	.481	-26.037051	8.211387
		22	44.4599658*	6.1181169	.000	27.335747	61.584185
release20	20	21	-15.132062	5.9655658	.084	-31.829300	1.565176
		22	39.3076097*	5.9655658	.000	22.610372	56.004848
		215	-8.0352977	5.9655658	.545	-24.732536	8.661940
	21	20	15.1320623	5.9655658	.084	-1.565176	31.829300
		22	54.4396720*	5.9655658	.000	37.742434	71.136910
		215	7.0967645	5.9655658	.640	-9.600474	23.794003
	22	20	-39.307610*	5.9655658	.000	-56.004848	-22.610372
		21	-54.439672*	5.9655658	.000	-71.136910	-37.742434
		215	-47.342907*	5.9655658	.000	-64.040146	-30.645669
	215	20	8.0352977	5.9655658	.545	-8.661940	24.732536
		21	-7.0967645	5.9655658	.640	-23.794003	9.600474
		22	47.3429074*	5.9655658	.000	30.645669	64.040146
release30	20	21	-12.798860	6.1755400	.196	-30.083803	4.486082
		22	37.7312422*	6.1755400	.000	20.446300	55.016185
		215	-8.7449208	6.1755400	.504	-26.029863	8.540022
	21	20	12.7988602	6.1755400	.196	-4.486082	30.083803
		22	50.5301024*	6.1755400	.000	33.245160	67.815045
		215	4.0539395	6.1755400	.912	-13.231003	21.338882
	22	20	-37.731242*	6.1755400	.000	-55.016185	-20.446300
		21	-50.530102*	6.1755400	.000	-67.815045	-33.245160
		215	-46.476163*	6.1755400	.000	-63.761106	-29.191220
	215	20	8.7449208	6.1755400	.504	-8.540022	26.029863
		21	-4.0539395	6.1755400	.912	-21.338882	13.231003
		22	46.4761629*	6.1755400	.000	29.191220	63.761106

*. The mean difference is significant at the .05 level.

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
release45	20	21	-10.176153	6.5177871	.422	-28.419023	8.066717
		22	35.4576397*	6.5177871	.000	17.214769	53.700510
		215	-8.7651897	6.5177871	.547	-27.008060	9.477681
	21	20	10.1761530	6.5177871	.422	-8.066717	28.419023
		22	45.6337927*	6.5177871	.000	27.390922	63.876663
		215	1.4109632	6.5177871	.996	-16.831907	19.653834
	22	20	-35.457640*	6.5177871	.000	-53.700510	-17.214769
		21	-45.633793*	6.5177871	.000	-63.876663	-27.390922
		215	-44.222829*	6.5177871	.000	-62.465700	-25.979959
	215	20	8.7651897	6.5177871	.547	-9.477681	27.008060
		21	-1.4109632	6.5177871	.996	-19.653834	16.831907
		22	44.2228295*	6.5177871	.000	25.979959	62.465700
release60	20	21	-8.2813994	6.8720941	.631	-27.515953	10.953154
		22	32.2081281*	6.8720941	.001	12.973575	51.442681
		215	-6.6983935	6.8720941	.765	-25.932947	12.536160
	21	20	8.2813994	6.8720941	.631	-10.953154	27.515953
		22	40.4895276*	6.8720941	.000	21.254974	59.724081
		215	1.5830060	6.8720941	.996	-17.651547	20.817559
	22	20	-32.208128*	6.8720941	.001	-51.442681	-12.973575
		21	-40.489528*	6.8720941	.000	-59.724081	-21.254974
		215	-38.906522*	6.8720941	.000	-58.141075	-19.671968
	215	20	6.6983935	6.8720941	.765	-12.536160	25.932947
		21	-1.5830060	6.8720941	.996	-20.817559	17.651547
		22	38.9065216*	6.8720941	.000	19.671968	58.141075
release90	20	21	-6.4214278	7.4901193	.826	-27.385794	14.542938
		22	26.6051782*	7.4901193	.010	5.640812	47.569544
		215	-6.1428608	7.4901193	.844	-27.107227	14.821505
	21	20	6.4214278	7.4901193	.826	-14.542938	27.385794
		22	33.0266060*	7.4901193	.001	12.062240	53.990972
		215	.2785669	7.4901193	1.000	-20.685799	21.242933
	22	20	-26.605178*	7.4901193	.010	-47.569544	-5.640812
		21	-33.026606*	7.4901193	.001	-53.990972	-12.062240
		215	-32.748039*	7.4901193	.002	-53.712405	-11.783673
	215	20	6.1428608	7.4901193	.844	-14.821505	27.107227
		21	-.2785669	7.4901193	1.000	-21.242933	20.685799
		22	32.7480391*	7.4901193	.002	11.783673	53.712405
release120	20	21	-4.6507590	7.8202544	.932	-26.539152	17.237634
		22	23.7919473*	7.8202544	.030	1.903554	45.680340
		215	-4.3790741	7.8202544	.943	-26.267467	17.509319
	21	20	4.6507590	7.8202544	.932	-17.237634	26.539152
		22	28.4427062*	7.8202544	.008	6.554313	50.331099
		215	.2716849	7.8202544	1.000	-21.616708	22.160078
	22	20	-23.791947*	7.8202544	.030	-45.680340	-1.903554
		21	-28.442706*	7.8202544	.008	-50.331099	-6.554313
		215	-28.171021*	7.8202544	.009	-50.059415	-6.282628
	215	20	4.3790741	7.8202544	.943	-17.509319	26.267467
		21	-.2716849	7.8202544	1.000	-22.160078	21.616708
		22	28.1710213*	7.8202544	.009	6.282628	50.059415

*. The mean difference is significant at the .05 level.

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
release180	20	21	-3.8786511	8.2349025	.965	-26.927618	19.170316
		22	22.1282604	8.2349025	.063	-.920706	45.177227
		215	-5.2659570	8.2349025	.918	-28.314924	17.783010
	21	20	3.8786511	8.2349025	.965	-19.170316	26.927618
		22	26.0069115*	8.2349025	.024	2.957945	49.055878
		215	-1.3873058	8.2349025	.998	-24.436273	21.661661
	22	20	-22.128260	8.2349025	.063	-45.177227	.920706
		21	-26.006912*	8.2349025	.024	-49.055878	-2.957945
		215	-27.394217*	8.2349025	.016	-50.443184	-4.345251
	215	20	5.2659570	8.2349025	.918	-17.783010	28.314924
		21	1.3873058	8.2349025	.998	-21.661661	24.436273
		22	27.3942173*	8.2349025	.016	4.345251	50.443184
release240	20	21	-2.9709444	8.3962722	.984	-26.471575	20.529686
		22	21.5501050	8.3962722	.080	-1.950525	45.050735
		215	-4.8540858	8.3962722	.937	-28.354716	18.646544
	21	20	2.9709444	8.3962722	.984	-20.529686	26.471575
		22	24.5210494*	8.3962722	.039	1.020419	48.021680
		215	-1.8831414	8.3962722	.996	-25.383772	21.617489
	22	20	-21.550105	8.3962722	.080	-45.050735	1.950525
		21	-24.521049*	8.3962722	.039	-48.021680	-1.020419
		215	-26.404191*	8.3962722	.024	-49.904821	-2.903561
	215	20	4.8540858	8.3962722	.937	-18.646544	28.354716
		21	1.8831414	8.3962722	.996	-21.617489	25.383772
		22	26.4041909*	8.3962722	.024	2.903561	49.904821
release300	20	21	-1.8675513	8.5706579	.996	-25.856276	22.121174
		22	21.1939376	8.5706579	.095	-2.794787	45.182662
		215	-3.0259783	8.5706579	.984	-27.014703	20.962747
	21	20	1.8675513	8.5706579	.996	-22.121174	25.856276
		22	23.0614889	8.5706579	.062	-.927236	47.050214
		215	-1.1584269	8.5706579	.999	-25.147152	22.830298
	22	20	-21.193938	8.5706579	.095	-45.182662	2.794787
		21	-23.061489	8.5706579	.062	-47.050214	.927236
		215	-24.219916*	8.5706579	.047	-48.208641	-.231191
	215	20	3.0259783	8.5706579	.984	-20.962747	27.014703
		21	1.1584269	8.5706579	.999	-22.830298	25.147152
		22	24.2199159*	8.5706579	.047	.231191	48.208641

*. The mean difference is significant at the .05 level.

Homogeneous Subsets

release5

Tukey HSD[a]

group	N	Subset for alpha = .05	
		1	2
22	6	25.018930	
215	6		58.309909
20	6		61.240534
21	6		65.308232
Sig.		1.000	.611

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

release10

Tukey HSD[a]

group	N	Subset for alpha = .05	
		1	2
22	6	32.013494	
20	6		71.271426
215	6		76.473459
21	6		85.386292
Sig.		1.000	.130

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

release20

Tukey HSD[a]

group	N	Subset for alpha = .05	
		1	2
22	6	41.212414	
20	6		80.520024
215	6		88.555322
21	6		95.652086
Sig.		1.000	.084

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

release30

Tukey HSD[a]

group	N	Subset for alpha = .05	
		1	2
22	6	48.524803	
20	6		86.256046
215	6		95.000966
21	6		99.054906
Sig.		1.000	.196

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

release45

Tukey HSD[a]

group	N	Subset for alpha = .05	
		1	2
22	6	54.904694	
20	6		90.362334
215	6		99.127523
21	6		100.5385
Sig.		1.000	.422

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

release60

Tukey HSD[a]

group	N	Subset for alpha = .05	
		1	2
22	6	60.313022	
20	6		92.521150
215	6		99.219544
21	6		100.8025
Sig.		1.000	.631

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

release90

Tukey HSD[a]

group	N	Subset for alpha = .05	
		1	2
22	6	67.688610	
20	6		94.293788
215	6		100.4366
21	6		100.7152
Sig.		1.000	.826

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

release120

Tukey HSD[a]

group	N	Subset for alpha = .05	
		1	2
22	6	72.534232	
20	6		96.326179
215	6		100.7053
21	6		100.9769
Sig.		1.000	.932

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

release180

Tukey HSD[a]

group	N	Subset for alpha = .05	
		1	2
22	6	75.525054	
20	6	97.653315	97.653315
21	6		101.5320
215	6		102.9193
Sig.		.063	.918

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

release240

Tukey HSD[a]

group	N	Subset for alpha = .05	
		1	2
22	6	77.259941	
20	6	98.810046	98.810046
21	6		101.7810
215	6		103.6641
Sig.		.080	.937

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

release300

Tukey HSD[a]

group	N	Subset for alpha = .05	
		1	2
22	6	77.954804	
20	6	99.148742	99.148742
21	6	101.0163	101.0163
215	6		102.1747
Sig.		.062	.984

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

5.2 The residual patch

Oneway

Descriptives

repatch

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
20	6	.756393	.1373307	.0560650	.612273	.900512	.6341	.9620
21	6	.683080	.1092768	.0446121	.568401	.797759	.5571	.8544
22	6	1.897432	.3250196	.1326887	1.556345	2.238519	1.6039	2.3050
215	6	1.388461	.2083862	.0850733	1.169773	1.607148	1.0142	1.6548
Total	24	1.181341	.5439988	.1110433	.951631	1.411052	.5571	2.3050

Test of Homogeneity of Variances

repatch

Levene Statistic	df1	df2	Sig.
3.121	3	20	.049

ANOVA

repatch

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5.907	3	1.969	43.790	.000
Within Groups	.899	20	.045		
Total	6.806	23			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: repatch

Dunnett C

(I) group	(J) group	Mean Difference (I-J)	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
20	21	.0733128	.0716486	-.191064	.337690
	22	-1.1410391*	.1440471	-1.672560	-.609518
	215	-.6320680*	.1018860	-1.008018	-.256118
21	20	-.0733128	.0716486	-.337690	.191064
	22	-1.2143519*	.1399876	-1.730894	-.697810
	215	-.7053808*	.0960609	-1.059837	-.350924
22	20	1.1410391*	.1440471	.609518	1.672560
	21	1.2143519*	.1399876	.697810	1.730894
	215	.5089711	.1576190	-.072629	1.090572
215	20	.6320680*	.1018860	.256118	1.008018
	21	.7053808*	.0960609	.350924	1.059837
	22	-.5089711	.1576190	-1.090572	.072629

*. The mean difference is significant at the .05 level.

6 *In vitro* penetration of verapamil hydrochloride-crab chitosan buccal patch

6.1 The percent of drug release at 2 to 9 hr

Oneway

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
penetrate2	20	6	1.046669	.4943527	.2018186	.527878	1.565460	.6016	1.9643
	21	6	1.811673	.9163939	.3741163	.849977	2.773370	1.0534	3.4999
	22	6	.992764	.3685679	.1504672	.605976	1.379552	.4594	1.3578
	215	6	1.362390	.5805831	.2370220	.753105	1.971674	.9107	2.3266
	220	6	.876900	.4249895	.1735012	.430901	1.322899	.3836	1.5073
	Total	30	1.218079	.6461257	.1179659	.976812	1.459347	.3836	3.4999
penetrate3	20	6	2.119067	1.2341677	.5038469	.823888	3.414247	1.0988	4.3546
	21	6	4.093064	2.4326670	.9931321	1.540137	6.645991	2.4893	8.8121
	22	6	1.916145	.7525225	.3072160	1.126421	2.705869	1.1084	2.7847
	215	6	2.678656	1.2997088	.5306039	1.314695	4.042616	1.0605	4.5005
	220	6	2.009072	1.7685803	.7220199	.153061	3.865084	.4119	5.3156
	Total	30	2.563201	1.6997268	.3103262	1.928512	3.197889	.4119	8.8121
penetrate4	20	6	3.352379	1.9302085	.7880043	1.326749	5.378009	1.8246	6.9437
	21	6	6.171949	3.0123832	1.2298003	3.010647	9.333251	4.0257	12.0618
	22	6	3.693035	1.6008622	.6535493	2.013033	5.373037	2.0415	6.0027
	215	6	4.236493	1.8112957	.7394584	2.335655	6.137332	1.6958	7.1908
	220	6	4.526627	1.2613525	.5149450	3.202919	5.850335	2.2681	5.8204
	Total	30	4.396097	2.1160506	.3863362	3.605950	5.186243	1.6958	12.0618
penetrate5	20	6	4.368723	2.1853237	.8921547	2.075367	6.662080	2.3200	8.3597
	21	6	8.269878	3.9871430	1.6277443	4.085628	12.454128	5.6169	15.9894
	22	6	5.214057	1.6658117	.6800648	3.465895	6.962220	3.2287	7.2025
	215	6	6.548163	2.7163009	1.1089252	3.697580	9.398746	3.9112	9.9175
	220	6	7.209114	3.1088605	1.2691870	3.946565	10.471663	3.3443	12.8535
	Total	30	6.321987	2.9971652	.5472050	5.202828	7.441147	2.3200	15.9894
penetrate6	20	6	5.998249	2.9241186	1.1937664	2.929574	9.066923	3.2541	10.7661
	21	6	10.960053	4.9931093	2.0384283	5.720107	16.200000	7.0997	20.7720
	22	6	7.021130	3.0095944	1.2286618	3.862754	10.179506	2.7607	11.1328
	215	6	8.541281	3.3072864	1.3501940	5.070497	12.012065	5.2565	12.8582
	220	6	10.380325	4.1485678	1.6936457	6.026670	14.733980	5.1907	17.3205
	Total	30	8.580208	3.9890245	.7282929	7.090681	10.069734	2.7607	20.7720
penetrate9	20	6	10.631846	5.9324199	2.4219003	4.406153	16.857539	4.9550	19.7859
	21	6	17.797929	4.1593883	1.6980632	13.432919	22.162939	13.6014	25.6297
	22	6	12.478355	4.9287576	2.0121569	7.305941	17.650769	8.4436	21.6653
	215	6	15.284075	3.1962771	1.3048747	11.929788	18.638362	11.8008	20.6797
	220	6	20.763731	5.7183903	2.3345231	14.762648	26.764813	12.7476	29.6246
	Total	30	15.391187	5.8526117	1.0685358	13.205786	17.576588	4.9550	29.6246

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
penetrate2	1.107	4	25	.375
penetrate3	1.095	4	25	.381
penetrate4	.567	4	25	.689
penetrate5	.614	4	25	.656
penetrate6	.208	4	25	.931
penetrate9	.662	4	25	.624

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
penetrate2	Between Groups	3.418	4	.855	2.459	.072
	Within Groups	8.688	25	.348		
	Total	12.107	29			
penetrate3	Between Groups	19.661	4	4.915	1.916	.139
	Within Groups	64.122	25	2.565		
	Total	83.783	29			
penetrate4	Between Groups	28.679	4	7.170	1.772	.166
	Within Groups	101.174	25	4.047		
	Total	129.852	29			
penetrate5	Between Groups	58.051	4	14.513	1.792	.162
	Within Groups	202.456	25	8.098		
	Total	260.507	29			
penetrate6	Between Groups	108.017	4	27.004	1.910	.140
	Within Groups	353.440	25	14.138		
	Total	461.457	29			
penetrate9	Between Groups	394.824	4	98.706	4.123	.011
	Within Groups	598.515	25	23.941		
	Total	993.339	29			

Post Hoc Tests

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
penetrate2	20	21	-.7650043	.3403623	.196	-1.764605	.234596
		22	.0539051	.3403623	1.000	-.945695	1.053505
		215	-.3157209	.3403623	.883	-1.315321	.683879
		220	.1697689	.3403623	.987	-.829831	1.169369
	21	20	.7650043	.3403623	.196	-.234596	1.764605
		22	.8189094	.3403623	.147	-.180691	1.818510
		215	.4492833	.3403623	.682	-.550317	1.448884
		220	.9347732	.3403623	.075	-.064827	1.934373
	22	20	-.0539051	.3403623	1.000	-1.053505	.945695
		21	-.8189094	.3403623	.147	-1.818510	.180691
		215	-.3696261	.3403623	.812	-1.369226	.629974
		220	.1158638	.3403623	.997	-.883736	1.115464
	215	20	.3157209	.3403623	.883	-.683879	1.315321
		21	-.4492833	.3403623	.682	-1.448884	.550317
		22	.3696261	.3403623	.812	-.629974	1.369226
		220	.4854899	.3403623	.617	-.514110	1.485090
	220	20	-.1697689	.3403623	.987	-1.169369	.829831
		21	-.9347732	.3403623	.075	-1.934373	.064827
		22	-.1158638	.3403623	.997	-1.115464	.883736
		215	-.4854899	.3403623	.617	-1.485090	.514110
penetrate3	20	21	-1.9739968	.9246422	.237	-4.689552	.741558
		22	.2029218	.9246422	.999	-2.512633	2.918477
		215	-.5595883	.9246422	.973	-3.275144	2.155967
		220	.1099948	.9246422	1.000	-2.605560	2.825550
	21	20	1.9739968	.9246422	.237	-.741558	4.689552
		22	2.1769186	.9246422	.161	-.538637	4.892474
		215	1.4144085	.9246422	.554	-1.301147	4.129964
		220	2.0839916	.9246422	.193	-.631564	4.799547
	22	20	-.2029218	.9246422	.999	-2.918477	2.512633
		21	-2.1769186	.9246422	.161	-4.892474	.538637
		215	-.7625101	.9246422	.920	-3.478065	1.953045
		220	-.0929270	.9246422	1.000	-2.808482	2.622628
	215	20	.5595883	.9246422	.973	-2.155967	3.275144
		21	-1.4144085	.9246422	.554	-4.129964	1.301147
		22	.7625101	.9246422	.920	-1.953045	3.478065
		220	.6695831	.9246422	.949	-2.045972	3.385138
	220	20	-.1099948	.9246422	1.000	-2.825550	2.605560
		21	-2.0839916	.9246422	.193	-4.799547	.631564
		22	.0929270	.9246422	1.000	-2.622628	2.808482
		215	-.6695831	.9246422	.949	-3.385138	2.045972
penetrate4	20	21	-2.8195702	1.1614565	.141	-6.230619	.591478
		22	-.3406562	1.1614565	.998	-3.751705	3.070392
		215	-.8841144	1.1614565	.939	-4.295163	2.526934
		220	-1.1742480	1.1614565	.848	-4.585296	2.236800
	21	20	2.8195702	1.1614565	.141	-.591478	6.230619
		22	2.4789140	1.1614565	.237	-.932134	5.889962
		215	1.9354558	1.1614565	.472	-1.475593	5.346504
		220	1.6453222	1.1614565	.623	-1.765726	5.056371
	22	20	.3406562	1.1614565	.998	-3.070392	3.751705
		21	-2.4789140	1.1614565	.237	-5.889962	.932134
		215	-.5434582	1.1614565	.990	-3.954507	2.867590
		220	-.8335918	1.1614565	.950	-4.244640	2.577457
	215	20	.8841144	1.1614565	.939	-2.526934	4.295163
		21	-1.9354558	1.1614565	.472	-5.346504	1.475593
		22	.5434582	1.1614565	.990	-2.867590	3.954507
		220	-.2901336	1.1614565	.999	-3.701182	3.120915
	220	20	1.1742480	1.1614565	.848	-2.236800	4.585296
		21	-1.6453222	1.1614565	.623	-5.056371	1.765726
		22	.8335918	1.1614565	.950	-2.577457	4.244640
		215	.2901336	1.1614565	.999	-3.120915	3.701182

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
penetrate5	20	21	-3.9011549	1.6429888	.156	-8.726402	.924092
		22	-.8453340	1.6429888	.985	-5.670581	3.979913
		215	-2.1794399	1.6429888	.678	-7.004687	2.645807
		220	-2.8403909	1.6429888	.435	-7.665638	1.984856
	21	20	3.9011549	1.6429888	.156	-.924092	8.726402
		22	3.0558209	1.6429888	.364	-1.769426	7.881068
		215	1.7217150	1.6429888	.831	-3.103532	6.546962
		220	1.0607640	1.6429888	.966	-3.764483	5.886011
	22	20	.8453340	1.6429888	.985	-3.979913	5.670581
		21	-3.0558209	1.6429888	.364	-7.881068	1.769426
		215	-1.3341059	1.6429888	.924	-6.159353	3.491141
		220	-1.9950569	1.6429888	.743	-6.820304	2.830190
	215	20	2.1794399	1.6429888	.678	-2.645807	7.004687
		21	-1.7217150	1.6429888	.831	-6.546962	3.103532
		22	1.3341059	1.6429888	.924	-3.491141	6.159353
		220	-.6609510	1.6429888	.994	-5.486198	4.164296
	220	20	2.8403909	1.6429888	.435	-1.984856	7.665638
		21	-1.0607640	1.6429888	.966	-5.886011	3.764483
		22	1.9950569	1.6429888	.743	-2.830190	6.820304
		215	.6609510	1.6429888	.994	-4.164296	5.486198
penetrate6	20	21	-4.9618046	2.1708374	.183	-11.337275	1.413666
		22	-1.0228812	2.1708374	.989	-7.398352	5.352589
		215	-2.5430323	2.1708374	.767	-8.918503	3.832438
		220	-4.3820765	2.1708374	.287	-10.757547	1.993394
	21	20	4.9618046	2.1708374	.183	-1.413666	11.337275
		22	3.9389234	2.1708374	.388	-2.436547	10.314394
		215	2.4187723	2.1708374	.798	-3.956698	8.794243
		220	.5797282	2.1708374	.999	-5.795742	6.955198
	22	20	1.0228812	2.1708374	.989	-5.352589	7.398352
		21	-3.9389234	2.1708374	.388	-10.314394	2.436547
		215	-1.5201511	2.1708374	.955	-7.895621	4.855319
		220	-3.3591952	2.1708374	.543	-9.734666	3.016275
	215	20	2.5430323	2.1708374	.767	-3.832438	8.918503
		21	-2.4187723	2.1708374	.798	-8.794243	3.956698
		22	1.5201511	2.1708374	.955	-4.855319	7.895621
		220	-1.8390441	2.1708374	.913	-8.214514	4.536426
	220	20	4.3820765	2.1708374	.287	-1.993394	10.757547
		21	-.5797282	2.1708374	.999	-6.955198	5.795742
		22	3.3591952	2.1708374	.543	-3.016275	9.734666
		215	1.8390441	2.1708374	.913	-4.536426	8.214514
penetrate9	20	21	-7.1660833	2.8249241	.114	-15.462522	1.130355
		22	-1.8465095	2.8249241	.964	-10.142948	6.449929
		215	-4.6522298	2.8249241	.483	-12.948669	3.644209
		220	-10.131885*	2.8249241	.011	-18.428324	-1.835446
	21	20	7.1660833	2.8249241	.114	-1.130355	15.462522
		22	5.3195738	2.8249241	.352	-2.976865	13.616013
		215	2.5138535	2.8249241	.898	-5.782585	10.810292
		220	-2.9658016	2.8249241	.830	-11.262240	5.330637
	22	20	1.8465095	2.8249241	.964	-6.449929	10.142948
		21	-5.3195738	2.8249241	.352	-13.616013	2.976865
		215	-2.8057203	2.8249241	.856	-11.102159	5.490719
		220	-8.2853754	2.8249241	.050	-16.581814	.011063
	215	20	4.6522298	2.8249241	.483	-3.644209	12.948669
		21	-2.5138535	2.8249241	.898	-10.810292	5.782585
		22	2.8057203	2.8249241	.856	-5.490719	11.102159
		220	-5.4796551	2.8249241	.324	-13.776094	2.816784
	220	20	10.1318849*	2.8249241	.011	1.835446	18.428324
		21	2.9658016	2.8249241	.830	-5.330637	11.262240
		22	8.2853754	2.8249241	.050	-.011063	16.581814
		215	5.4796551	2.8249241	.324	-2.816784	13.776094

*. The mean difference is significant at the .05 level.

Homogeneous Subsets

penetrate2

Tukey HSD[a]

group	N	Subset for alpha = .05
		1
220	6	.876900
22	6	.992764
20	6	1.046669
215	6	1.362390
21	6	1.811673
Sig.		.075

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

penetrate3

Tukey HSD[a]

group	N	Subset for alpha = .05
		1
22	6	1.916145
220	6	2.009072
20	6	2.119067
215	6	2.678656
21	6	4.093064
Sig.		.161

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

penetrate4

Tukey HSD[a]

group	N	Subset for alpha = .05
		1
20	6	3.352379
22	6	3.693035
215	6	4.236493
220	6	4.526627
21	6	6.171949
Sig.		.141

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

penetrate5

Tukey HSD[a]

group	N	Subset for alpha = .05
		1
20	6	4.368723
22	6	5.214057
215	6	6.548163
220	6	7.209114
21	6	8.269878
Sig.		.156

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

penetrate6

Tukey HSD[a]

group	N	Subset for alpha = .05
		1
20	6	5.998249
22	6	7.021130
215	6	8.541281
220	6	10.380325
21	6	10.960053
Sig.		.183

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

penetrate9

Tukey HSD[a]

group	N	Subset for alpha = .05	
		1	2
20	6	10.631846	
22	6	12.478355	12.478355
215	6	15.284075	15.284075
21	6	17.797929	17.797929
220	6		20.763731
Sig.		.114	.050

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

6.2 The slope of a plot of percent of drug penetration versus time (hr)

Oneway

Descriptives

slope

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
20	6	1.338245	.7311779	.2985021	.570921	2.105569	.6316	2.4523
21	6	2.184606	.5505046	.2247425	1.606887	2.762325	1.6694	3.2518
22	6	1.675141	.6877020	.2807532	.953442	2.396840	1.0981	2.9492
215	6	1.954114	.4333569	.1769172	1.499334	2.408894	1.4770	2.6790
220	6	2.740105	.7811779	.3189146	1.920309	3.559901	1.6327	3.9575
Total	30	1.978442	.7722259	.1409885	1.690088	2.266796	.6316	3.9575

Test of Homogeneity of Variances

slope

Levene Statistic	df1	df2	Sig.
.593	4	25	.671

ANOVA

slope

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6.750	4	1.688	4.002	.012
Within Groups	10.543	25	.422		
Total	17.294	29			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: slope

Tukey HSD

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
20	21	-.8463614	.3749353	.192	-1.947498	.254775
	22	-.3368960	.3749353	.895	-1.438033	.764241
	215	-.6158690	.3749353	.486	-1.717006	.485268
	220	-1.4018596*	.3749353	.008	-2.502996	-.300723
21	20	.8463614	.3749353	.192	-.254775	1.947498
	22	.5094654	.3749353	.658	-.591671	1.610602
	215	.2304923	.3749353	.971	-.870645	1.331629
	220	-.5554982	.3749353	.583	-1.656635	.545639
22	20	.3368960	.3749353	.895	-.764241	1.438033
	21	-.5094654	.3749353	.658	-1.610602	.591671
	215	-.2789730	.3749353	.944	-1.380110	.822164
	220	-1.0649636	.3749353	.061	-2.166100	.036173
215	20	.6158690	.3749353	.486	-.485268	1.717006
	21	-.2304923	.3749353	.971	-1.331629	.870645
	22	.2789730	.3749353	.944	-.822164	1.380110
	220	-.7859906	.3749353	.253	-1.887127	.315146
220	20	1.4018596*	.3749353	.008	.300723	2.502996
	21	.5554982	.3749353	.583	-.545639	1.656635
	22	1.0649636	.3749353	.061	-.036173	2.166100
	215	.7859906	.3749353	.253	-.315146	1.887127

*. The mean difference is significant at the .05 level.

Homogeneous Subsets

slope

Tukey HSD[a]

group	N	Subset for alpha = .05	
		1	2
20	6	1.338245	
22	6	1.675141	1.675141
215	6	1.954114	1.954114
21	6	2.184606	2.184606
220	6		2.740105
Sig.		.192	.061

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

6.3 The residual drug in donor compartment

Oneway

Descriptives

redonor

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
20	6	10.460345	2.9577215	1.2074847	7.356406	13.564283	7.5008	15.0725
21	6	6.787511	3.3407993	1.3638756	3.281557	10.293465	2.5695	11.9765
22	6	7.449342	3.7632177	1.5363272	3.500087	11.398597	1.5213	11.2685
215	6	11.478028	4.0536921	1.6549129	7.223939	15.732117	7.5954	18.4022
220	6	39.365356	7.2253255	2.9497268	31.782842	46.947870	32.2027	50.3125
Total	30	15.108116	13.1569268	2.4021152	10.195239	20.020994	1.5213	50.3125

Test of Homogeneity of Variances

redonor

Levene Statistic	df1	df2	Sig.
1.949	4	25	.133

ANOVA

redonor

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4506.494	4	1126.623	54.846	.000
Within Groups	513.543	25	20.542		
Total	5020.037	29			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: redonor

Tukey HSD

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
20	21	3.6728337	2.6167233	.631	-4.012146	11.357813
	22	3.0110028	2.6167233	.778	-4.673977	10.695983
	215	-1.0176832	2.6167233	.995	-8.702663	6.667297
	220	-28.905012*	2.6167233	.000	-36.589991	-21.220032
21	20	-3.6728337	2.6167233	.631	-11.357813	4.012146
	22	-.6618309	2.6167233	.999	-8.346811	7.023149
	215	-4.6905169	2.6167233	.400	-12.375497	2.994463
	220	-32.577845*	2.6167233	.000	-40.262825	-24.892866
22	20	-3.0110028	2.6167233	.778	-10.695983	4.673977
	21	.6618309	2.6167233	.999	-7.023149	8.346811
	215	-4.0286859	2.6167233	.548	-11.713666	3.656294
	220	-31.916014*	2.6167233	.000	-39.600994	-24.231035
215	20	1.0176832	2.6167233	.995	-6.667297	8.702663
	21	4.6905169	2.6167233	.400	-2.994463	12.375497
	22	4.0286859	2.6167233	.548	-3.656294	11.713666
	220	-27.887328*	2.6167233	.000	-35.572308	-20.202349
220	20	28.9050116*	2.6167233	.000	21.220032	36.589991
	21	32.5778454*	2.6167233	.000	24.892866	40.262825
	22	31.9160144*	2.6167233	.000	24.231035	39.600994
	215	27.8873285*	2.6167233	.000	20.202349	35.572308

*. The mean difference is significant at the .05 level.

Homogeneous Subsets

redonor

Tukey HSD[a]

group	N	Subset for alpha = .05	
		1	2
21	6	6.787511	
22	6	7.449342	
20	6	10.460345	
215	6	11.478028	
220	6		39.365356
Sig.		.400	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

6.4 The residual patch

Oneway

Descriptives

repatch

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
20	6	11.946445	2.3607616	.9637769	9.468977	14.423912	9.2290	14.9665
21	6	9.720413	1.7726336	.7236746	7.860148	11.580677	7.0981	11.1774
22	6	10.513258	.9337282	.3811929	9.533371	11.493146	8.9561	11.3553
215	6	10.905502	.6993764	.2855192	10.171552	11.639453	10.0561	12.1088
Total	24	10.771405	1.6914305	.3452618	10.057176	11.485633	7.0981	14.9665

Test of Homogeneity of Variances

repatch

Levene Statistic	df1	df2	Sig.
7.869	3	20	.001

ANOVA

repatch

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	15.420	3	5.140	2.040	.141
Within Groups	50.382	20	2.519		
Total	65.802	23			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: repatch

Dunnett C

(I) group	(J) group	Mean Difference (I-J)	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
20	21	2.2260322	1.2052265	-2.221148	6.673213
	22	1.4331864	1.0364236	-2.391127	5.257499
	215	1.0409424	1.0051801	-2.668085	4.749970
21	20	-2.2260322	1.2052265	-6.673213	2.221148
	22	-.7928459	.8179322	-3.810944	2.225253
	215	-1.1850898	.7779629	-4.055705	1.685525
22	20	-1.4331864	1.0364236	-5.257499	2.391127
	21	.7928459	.8179322	-2.225253	3.810944
	215	-.3922440	.4762660	-2.149624	1.365136
215	20	-1.0409424	1.0051801	-4.749970	2.668085
	21	1.1850898	.7779629	-1.685525	4.055705
	22	.3922440	.4762660	-1.365136	2.149624

7 *In vitro* mucoadhesive study of verapamil hydrochloride-chitin whiskers-crab chitosan buccal patch

7.1 The work of adhesion from *in vitro* mucoadhesive study of verapamil hydrochloride-chitin whiskers-crab chitosan buccal patch

Oneway

Descriptives

adhesion

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
12	10	.22800	.077571	.024530	.17251	.28349	.092	.351
14	10	.29420	.056578	.017892	.25373	.33467	.213	.376
20	10	.25910	.108499	.034310	.18148	.33672	.132	.437
151	10	.13710	.073354	.023196	.08463	.18957	.070	.310
212	10	.31810	.112571	.035598	.23757	.39863	.200	.515
214	10	.37620	.083509	.026408	.31646	.43594	.234	.489
215	10	.40930	.129985	.041105	.31631	.50229	.234	.657
2151	10	.29670	.087631	.027711	.23401	.35939	.121	.436
Total	80	.28984	.120245	.013444	.26308	.31660	.070	.657

Test of Homogeneity of Variances

adhesion

Levene Statistic	df1	df2	Sig.
1.409	7	72	.215

ANOVA

adhesion

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.507	7	.072	8.207	.000
Within Groups	.635	72	.009		
Total	1.142	79			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: adhesion
Tukey HSD

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
12	14	-.066200	.042010	.763	-.19735	.06495
	20	-.031100	.042010	.995	-.16225	.10005
	151	.090900	.042010	.386	-.04025	.22205
	212	-.090100	.042010	.397	-.22125	.04105
	214	-.148200*	.042010	.016	-.27935	-.01705
	215	-.181300*	.042010	.001	-.31245	-.05015
	2151	-.068700	.042010	.728	-.19985	.06245
14	12	.066200	.042010	.763	-.06495	.19735
	20	.035100	.042010	.990	-.09605	.16625
	151	.157100*	.042010	.008	.02595	.28825
	212	-.023900	.042010	.999	-.15505	.10725
	214	-.082000	.042010	.521	-.21315	.04915
	215	-.115100	.042010	.128	-.24625	.01605
	2151	-.002500	.042010	1.000	-.13365	.12865
20	12	.031100	.042010	.995	-.10005	.16225
	14	-.035100	.042010	.990	-.16625	.09605
	151	.122000	.042010	.087	-.00915	.25315
	212	-.059000	.042010	.852	-.19015	.07215
	214	-.117100	.042010	.114	-.24825	.01405
	215	-.150200*	.042010	.014	-.28135	-.01905
	2151	-.037600	.042010	.986	-.16875	.09355
151	12	-.090900	.042010	.386	-.22205	.04025
	14	-.157100*	.042010	.008	-.28825	-.02595
	20	-.122000	.042010	.087	-.25315	.00915
	212	-.181000*	.042010	.001	-.31215	-.04985
	214	-.239100*	.042010	.000	-.37025	-.10795
	215	-.272200*	.042010	.000	-.40335	-.14105
	2151	-.159600*	.042010	.007	-.29075	-.02845
212	12	.090100	.042010	.397	-.04105	.22125
	14	.023900	.042010	.999	-.10725	.15505
	20	.059000	.042010	.852	-.07215	.19015
	151	.181000*	.042010	.001	.04985	.31215
	214	-.058100	.042010	.862	-.18925	.07305
	215	-.091200	.042010	.381	-.22235	.03995
	2151	.021400	.042010	1.000	-.10975	.15255
214	12	.148200*	.042010	.016	.01705	.27935
	14	.082000	.042010	.521	-.04915	.21315
	20	.117100	.042010	.114	-.01405	.24825
	151	.239100*	.042010	.000	.10795	.37025
	212	.058100	.042010	.862	-.07305	.18925
	215	-.033100	.042010	.993	-.16425	.09805
	2151	.079500	.042010	.561	-.05165	.21065
215	12	.181300*	.042010	.001	.05015	.31245
	14	.115100	.042010	.128	-.01605	.24625
	20	.150200*	.042010	.014	.01905	.28135
	151	.272200*	.042010	.000	.14105	.40335
	212	.091200	.042010	.381	-.03995	.22235
	214	.033100	.042010	.993	-.09805	.16425
	2151	.112600	.042010	.146	-.01855	.24375
2151	12	.068700	.042010	.728	-.06245	.19985
	14	.002500	.042010	1.000	-.12865	.13365
	20	.037600	.042010	.986	-.09355	.16875
	151	.159600*	.042010	.007	.02845	.29075
	212	-.021400	.042010	1.000	-.15255	.10975
	214	-.079500	.042010	.561	-.21065	.05165
	215	-.112600	.042010	.146	-.24375	.01855

*. The mean difference is significant at the .05 level.

Homogeneous Subsets

adhesion

Tukey HSD[a]

group	N	Subset for alpha = .05			
		1	2	3	4
151	10	.13710			
12	10	.22800	.22800		
20	10	.25910	.25910	.25910	
14	10		.29420	.29420	.29420
2151	10		.29670	.29670	.29670
212	10		.31810	.31810	.31810
214	10			.37620	.37620
215	10				.40930
Sig.		.087	.397	.114	.128

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.000.

7.2 The peak detachment force from *in vitro* mucoadhesive study of verapamil hydrochloride-chitin whiskers-crab chitosan buccal patch

Oneway

Descriptives

peakforce

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
12	10	.28800	.088320	.027929	.22482	.35118	.162	.424
14	10	.32800	.094537	.029895	.26037	.39563	.168	.479
20	10	.80020	.147581	.046669	.69463	.90577	.603	1.094
151	10	.24160	.081459	.025760	.18333	.29987	.126	.375
212	10	.40820	.094778	.029971	.34040	.47600	.267	.553
214	10	.71730	.139040	.043968	.61784	.81676	.549	.896
215	10	.99770	.136541	.043178	.90002	1.09538	.812	1.173
2151	10	.32750	.084184	.026621	.26728	.38772	.193	.458
Total	80	.51356	.287357	.032127	.44961	.57751	.126	1.173

Test of Homogeneity of Variances

peakforce

Levene Statistic	df1	df2	Sig.
2.255	7	72	.039

ANOVA

peakforce

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5.631	7	.804	64.869	.000
Within Groups	.893	72	.012		
Total	6.523	79			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: peakforce

Dunnett C

(I) group	(J) group	Mean Difference (I-J)	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
12	14	-.040000	.040912	-.19714	.11714
	20	-.512200*	.054388	-.72110	-.30330
	151	.046400	.037995	-.09954	.19234
	212	-.120200	.040967	-.27755	.03715
	214	-.429300*	.052089	-.62937	-.22923
	215	-.709700*	.051424	-.90722	-.51218
	2151	-.039500	.038584	-.18770	.10870
14	12	.040000	.040912	-.11714	.19714
	20	-.472200*	.055423	-.68508	-.25932
	151	.086400	.039463	-.06517	.23797
	212	-.080200	.042332	-.24280	.08240
	214	-.389300*	.053169	-.59352	-.18508
	215	-.669700*	.052517	-.87142	-.46798
	2151	.000500	.040030	-.15325	.15425
20	12	.512200*	.054388	.30330	.72110
	14	.472200*	.055423	.25932	.68508
	151	.558600*	.053306	.35385	.76335
	212	.392000*	.055464	.17896	.60504
	214	.082900	.064119	-.16338	.32918
	215	-.197500	.063579	-.44171	.04671
	2151	.472700*	.053728	.26633	.67907
151	12	-.046400	.037995	-.19234	.09954
	14	-.086400	.039463	-.23797	.06517
	20	-.558600*	.053306	-.76335	-.35385
	212	-.166600*	.039520	-.31839	-.01481
	214	-.475700*	.050959	-.67143	-.27997
	215	-.756100*	.050278	-.94922	-.56298
	2151	-.085900	.037044	-.22818	.05638
212	12	.120200	.040967	-.03715	.27755
	14	.080200	.042332	-.08240	.24280
	20	-.392000*	.055464	-.60504	-.17896
	151	.166600*	.039520	.01481	.31839
	214	-.309100*	.053212	-.51348	-.10472
	215	-.589500*	.052561	-.79138	-.38762
	2151	.080700	.040087	-.07327	.23467
214	12	.429300*	.052089	.22923	.62937
	14	.389300*	.053169	.18508	.59352
	20	-.082900	.064119	-.32918	.16338
	151	.475700*	.050959	.27997	.67143
	212	.309100*	.053212	.10472	.51348
	215	-.280400*	.061624	-.51710	-.04370
	2151	.389800*	.051400	.19238	.58722
215	12	.709700*	.051424	.51218	.90722
	14	.669700*	.052517	.46798	.87142
	20	.197500	.063579	-.04671	.44171
	151	.756100*	.050278	.56298	.94922
	212	.589500*	.052561	.38762	.79138
	214	.280400*	.061624	.04370	.51710
	2151	.670200*	.050725	.47537	.86503
2151	12	.039500	.038584	-.10870	.18770
	14	-.000500	.040030	-.15425	.15325
	20	-.472700*	.053728	-.67907	-.26633
	151	.085900	.037044	-.05638	.22818
	212	-.080700	.040087	-.23467	.07327
	214	-.389800*	.051400	-.58722	-.19238
	215	-.670200*	.050725	-.86503	-.47537

*. The mean difference is significant at the .05 level.

8 *In vitro* release of verapamil hydrochloride-chitin whiskers-crab chitosan buccal patch

8.1 The percent of drug release at 5 min

Oneway

Descriptives

release5

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
20	6	61.240534	11.5015975	4.6955075	49.170347	73.310720	39.3274	70.7201
212	6	58.498031	2.4561894	1.0027351	55.920419	61.075644	53.8595	60.7394
214	6	50.172664	4.5566305	1.8602366	45.390774	54.954555	42.5448	56.1117
215	6	58.309909	12.2786343	5.0127315	45.424273	71.195546	41.8920	72.6400
2151	6	58.601196	4.6197988	1.8860249	53.753014	63.449377	52.8397	65.9587
Total	30	57.364467	8.4663125	1.5457301	54.203094	60.525840	39.3274	72.6400

Test of Homogeneity of Variances

release5

Levene Statistic	df1	df2	Sig.
4.347	4	25	.008

ANOVA

release5

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	422.725	4	105.681	1.595	.207
Within Groups	1655.949	25	66.238		
Total	2078.675	29			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: release5

Dunnett C

(I) group	(J) group	Mean Difference (I-J)	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
20	212	2.7425024	4.8013820	-16.518264	22.003269
	214	11.0678697	5.0505714	-9.192521	31.328261
	215	2.9306247	6.8684254	-24.622097	30.483346
	2151	2.6393380	5.0601266	-17.659384	22.938060
212	20	-2.7425024	4.8013820	-22.003269	16.518264
	214	8.3253673	2.1132813	-.152071	16.802805
	215	.1881223	5.1120402	-20.318851	20.695096
	2151	-.1031644	2.1360168	-8.671806	8.465477
214	20	-11.067870	5.0505714	-31.328261	9.192521
	212	-8.3253673	2.1132813	-16.802805	.152071
	215	-8.1372450	5.3467707	-29.585841	13.311351
	2151	-8.4285317	2.6490697	-19.055287	2.198224
215	20	-2.9306247	6.8684254	-30.483346	24.622097
	212	-.1881223	5.1120402	-20.695096	20.318851
	214	8.1372450	5.3467707	-13.311351	29.585841
	2151	-.2912867	5.3557975	-21.776094	21.193520
2151	20	-2.6393380	5.0601266	-22.938060	17.659384
	212	.1031644	2.1360168	-8.465477	8.671806
	214	8.4285317	2.6490697	-2.198224	19.055287
	215	.2912867	5.3557975	-21.193520	21.776094

8.2 The percent of drug release at 10, 20, 30, 45, 60 and 90 min

Oneway

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
release10	20	6	71.271426	8.5302238	3.4824493	62.319505	80.223347	54.0570	76.5905
	212	6	73.323111	3.7975663	1.5503499	69.337810	77.308413	66.2664	76.3718
	214	6	65.285276	6.0705960	2.4783105	58.914576	71.655976	54.8629	72.3302
	215	6	76.473459	13.2315027	5.4017384	62.587849	90.359070	58.0879	95.7548
	2151	6	77.527883	4.9290965	2.0122952	72.355114	82.700653	73.0158	85.3987
	Total	30	72.776231	8.6832952	1.5853456	69.533836	76.018627	54.0570	95.7548
release20	20	6	80.520024	7.4722177	3.0505201	72.678412	88.361636	70.9420	90.5652
	212	6	89.122200	4.4585558	1.8201978	84.443233	93.801167	81.8927	93.2418
	214	6	75.576175	7.3689990	3.0083812	67.842884	83.309465	63.1522	84.2910
	215	6	88.555322	11.8066949	4.8200630	76.164955	100.945688	71.5513	107.7320
	2151	6	91.558902	5.9361046	2.4234046	85.329342	97.788462	85.9837	100.7239
	Total	30	85.066525	9.4910174	1.7328148	81.522520	88.610529	63.1522	107.7320
release30	20	6	86.256046	9.4687592	3.8656048	76.319192	96.192899	78.6233	99.7838
	212	6	94.583429	5.6697177	2.3146526	88.633426	100.533433	86.7718	101.1209
	214	6	83.070851	7.6199158	3.1108176	75.074240	91.067462	70.9854	91.9598
	215	6	95.000966	11.2982822	4.6125044	83.144146	106.857786	79.1004	114.1500
	2151	6	96.024215	6.1778043	2.5220781	89.541007	102.507423	90.2943	105.1386
	Total	30	90.987102	9.4050903	1.7171267	87.475183	94.499020	70.9854	114.1500
release45	20	6	90.362334	11.0287980	4.5024879	78.788320	101.936347	79.1767	104.0783
	212	6	99.123298	6.2633523	2.5570029	92.550313	105.696283	91.1428	106.3131
	214	6	88.760984	9.2224636	3.7650550	79.082603	98.439366	73.9254	99.3722
	215	6	99.127523	11.0619995	4.5160424	87.518667	110.736380	83.2892	116.9713
	2151	6	97.173361	6.3296544	2.5840706	90.530796	103.815925	91.1983	106.1075
	Total	30	94.909500	9.5351869	1.7408790	91.349003	98.469997	73.9254	116.9713
release60	20	6	92.521150	12.6255254	5.1543492	79.271474	105.770826	79.7004	108.6176
	212	6	100.7591	6.6605749	2.7191683	93.769294	107.748984	93.1505	108.4965
	214	6	92.050640	9.6618904	3.9444503	81.911108	102.190172	77.5908	103.6625
	215	6	99.219544	10.6726141	4.3570765	88.019322	110.419765	84.7527	115.6349
	2151	6	97.655187	6.3553608	2.5945652	90.985645	104.324729	91.6019	106.9001
	Total	30	96.441132	9.5266615	1.7393225	92.883818	99.998446	77.5908	115.6349
release90	20	6	94.293788	13.8940703	5.6722305	79.712856	108.874721	79.2815	110.1576
	212	6	101.9107	6.9526105	2.8383914	94.614395	109.207030	93.6557	110.3487
	214	6	95.297413	10.2971960	4.2038127	84.491168	106.103657	80.7398	107.6311
	215	6	100.4366	11.6061897	4.7382071	88.256700	112.616598	85.0561	118.9822
	2151	6	98.125283	6.9242978	2.8268328	90.858678	105.391888	91.3266	108.4337
	Total	30	98.012769	10.0069693	1.8270143	94.276105	101.749433	79.2815	118.9822

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
release10	2.289	4	25	.088
release20	.604	4	25	.663
release30	.557	4	25	.696
release45	.736	4	25	.576
release60	1.693	4	25	.183
release90	2.405	4	25	.076

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
release10	Between Groups	569.554	4	142.388	2.201	.098
	Within Groups	1617.035	25	64.681		
	Total	2186.589	29			
release20	Between Groups	1089.052	4	272.263	4.468	.007
	Within Groups	1523.251	25	60.930		
	Total	2612.303	29			
release30	Between Groups	836.803	4	209.201	3.026	.036
	Within Groups	1728.413	25	69.137		
	Total	2565.216	29			
release45	Between Groups	594.923	4	148.731	1.821	.156
	Within Groups	2041.751	25	81.670		
	Total	2636.674	29			
release60	Between Groups	374.888	4	93.722	1.038	.407
	Within Groups	2257.073	25	90.283		
	Total	2631.961	29			
release90	Between Groups	253.715	4	63.429	.598	.667
	Within Groups	2650.329	25	106.013		
	Total	2904.044	29			

Post Hoc Tests

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
release10	20	212	-2.0516850	4.6433251	.992	-15.688534	11.585164
		214	5.9861505	4.6433251	.700	-7.650698	19.622999
		215	-5.2020330	4.6433251	.794	-18.838882	8.434816
		2151	-6.2564571	4.6433251	.665	-19.893306	7.380391
	212	20	2.0516850	4.6433251	.992	-11.585164	15.688534
		214	8.0378356	4.6433251	.434	-5.599013	21.674684
		215	-3.1503480	4.6433251	.959	-16.787197	10.486501
		2151	-4.2047721	4.6433251	.892	-17.841621	9.432077
	214	20	-5.9861505	4.6433251	.700	-19.622999	7.650698
		212	-8.0378356	4.6433251	.434	-21.674684	5.599013
		215	-11.188184	4.6433251	.146	-24.825032	2.448665
		2151	-12.242608	4.6433251	.094	-25.879456	1.394241
	215	20	5.2020330	4.6433251	.794	-8.434816	18.838882
		212	3.1503480	4.6433251	.959	-10.486501	16.787197
		214	11.1881836	4.6433251	.146	-2.448665	24.825032
		2151	-1.0544241	4.6433251	.999	-14.691273	12.582425
	2151	20	6.2564571	4.6433251	.665	-7.380391	19.893306
		212	4.2047721	4.6433251	.892	-9.432077	17.841621
		214	12.2426076	4.6433251	.094	-1.394241	25.879456
		215	1.0544241	4.6433251	.999	-12.582425	14.691273
release20	20	212	-8.6021758	4.5066639	.339	-21.837668	4.633316
		214	4.9438494	4.5066639	.806	-8.291643	18.179341
		215	-8.0352977	4.5066639	.405	-21.270790	5.200194
		2151	-11.038878	4.5066639	.135	-24.274370	2.196614
	212	20	8.6021758	4.5066639	.339	-4.633316	21.837668
		214	13.5460252*	4.5066639	.043	.310533	26.781517
		215	.5668781	4.5066639	1.000	-12.668614	13.802370
		2151	-2.4367024	4.5066639	.982	-15.672194	10.798790
	214	20	-4.9438494	4.5066639	.806	-18.179341	8.291643
		212	-13.546025*	4.5066639	.043	-26.781517	-.310533
		215	-12.979147	4.5066639	.057	-26.214639	.256345
		2151	-15.982728*	4.5066639	.012	-29.218220	-2.747235
	215	20	8.0352977	4.5066639	.405	-5.200194	21.270790
		212	-.5668781	4.5066639	1.000	-13.802370	12.668614
		214	12.9791471	4.5066639	.057	-.256345	26.214639
		2151	-3.0035804	4.5066639	.962	-16.239072	10.231912
	2151	20	11.0388782	4.5066639	.135	-2.196614	24.274370
		212	2.4367024	4.5066639	.982	-10.798790	15.672194
		214	15.9827276*	4.5066639	.012	2.747235	29.218220
		215	3.0035804	4.5066639	.962	-10.231912	16.239072
release30	20	212	-8.3273837	4.8005740	.432	-22.426052	5.771285
		214	3.1851946	4.8005740	.962	-10.913474	17.283863
		215	-8.7449208	4.8005740	.384	-22.843589	5.353748
		2151	-9.7681694	4.8005740	.279	-23.866838	4.330499
	212	20	8.3273837	4.8005740	.432	-5.771285	22.426052
		214	11.5125783	4.8005740	.149	-2.586090	25.611247
		215	-.4175370	4.8005740	1.000	-14.516205	13.681131
		2151	-1.4407857	4.8005740	.998	-15.539454	12.657883
	214	20	-3.1851946	4.8005740	.962	-17.283863	10.913474
		212	-11.512578	4.8005740	.149	-25.611247	2.586090
		215	-11.930115	4.8005740	.126	-26.028784	2.168553
		2151	-12.953364	4.8005740	.083	-27.052032	1.145304
	215	20	8.7449208	4.8005740	.384	-5.353748	22.843589
		212	.4175370	4.8005740	1.000	-13.681131	14.516205
		214	11.9301153	4.8005740	.126	-2.168553	26.028784
		2151	-1.0232486	4.8005740	1.000	-15.121917	13.075420
	2151	20	9.7681694	4.8005740	.279	-4.330499	23.866838
		212	1.4407857	4.8005740	.998	-12.657883	15.539454
		214	12.9533640	4.8005740	.083	-1.145304	27.052032
		215	1.0232486	4.8005740	1.000	-13.075420	15.121917

*. The mean difference is significant at the .05 level.

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
release45	20	212	-8.7609645	5.2175994	.464	-24.084383	6.562454
		214	1.6013492	5.2175994	.998	-13.722069	16.924768
		215	-8.7651897	5.2175994	.464	-24.088608	6.558229
		2151	-6.8110270	5.2175994	.690	-22.134445	8.512391
	212	20	8.7609645	5.2175994	.464	-6.562454	24.084383
		214	10.3623137	5.2175994	.301	-4.961105	25.685732
		215	-.0042252	5.2175994	1.000	-15.327644	15.319193
		2151	1.9499376	5.2175994	.996	-13.373481	17.273356
	214	20	-1.6013492	5.2175994	.998	-16.924768	13.722069
		212	-10.362314	5.2175994	.301	-25.685732	4.961105
		215	-10.366539	5.2175994	.301	-25.689957	4.956880
		2151	-8.4123761	5.2175994	.504	-23.735795	6.911042
	215	20	8.7651897	5.2175994	.464	-6.558229	24.088608
		212	.0042252	5.2175994	1.000	-15.319193	15.327644
		214	10.3665389	5.2175994	.301	-4.956880	25.689957
		2151	1.9541628	5.2175994	.996	-13.369256	17.277581
	2151	20	6.8110270	5.2175994	.690	-8.512391	22.134445
		212	-1.9499376	5.2175994	.996	-17.273356	13.373481
		214	8.4123761	5.2175994	.504	-6.911042	23.735795
		215	-1.9541628	5.2175994	.996	-17.277581	13.369256
release60	20	212	-8.2379890	5.4858277	.571	-24.349159	7.873181
		214	.4705103	5.4858277	1.000	-15.640660	16.581681
		215	-6.6983935	5.4858277	.739	-22.809564	9.412777
		2151	-5.1340370	5.4858277	.880	-21.245207	10.977133
	212	20	8.2379890	5.4858277	.571	-7.873181	24.349159
		214	8.7084992	5.4858277	.519	-7.402671	24.819670
		215	1.5395955	5.4858277	.999	-14.571575	17.650766
		2151	3.1039520	5.4858277	.979	-13.007218	19.215122
	214	20	-.4705103	5.4858277	1.000	-16.581681	15.640660
		212	-8.7084992	5.4858277	.519	-24.819670	7.402671
		215	-7.1689037	5.4858277	.690	-23.280074	8.942267
		2151	-5.6045472	5.4858277	.843	-21.715718	10.506623
	215	20	6.6983935	5.4858277	.739	-9.412777	22.809564
		212	-1.5395955	5.4858277	.999	-17.650766	14.571575
		214	7.1689037	5.4858277	.690	-8.942267	23.280074
		2151	1.5643565	5.4858277	.998	-14.546814	17.675527
	2151	20	5.1340370	5.4858277	.880	-10.977133	21.245207
		212	-3.1039520	5.4858277	.979	-19.215122	13.007218
		214	5.6045472	5.4858277	.843	-10.506623	21.715718
		215	-1.5643565	5.4858277	.998	-17.675527	14.546814
release90	20	212	-7.6169238	5.9445536	.705	-25.075313	9.841466
		214	-1.0036243	5.9445536	1.000	-18.462014	16.454765
		215	-6.1428608	5.9445536	.838	-23.601250	11.315529
		2151	-3.8314944	5.9445536	.966	-21.289884	13.626895
	212	20	7.6169238	5.9445536	.705	-9.841466	25.075313
		214	6.6132995	5.9445536	.798	-10.845090	24.071689
		215	1.4740630	5.9445536	.999	-15.984326	18.932452
		2151	3.7854294	5.9445536	.968	-13.672960	21.243819
	214	20	1.0036243	5.9445536	1.000	-16.454765	18.462014
		212	-6.6132995	5.9445536	.798	-24.071689	10.845090
		215	-5.1392365	5.9445536	.907	-22.597626	12.319153
		2151	-2.8278701	5.9445536	.989	-20.286260	14.630519
	215	20	6.1428608	5.9445536	.838	-11.315529	23.601250
		212	-1.4740630	5.9445536	.999	-18.932452	15.984326
		214	5.1392365	5.9445536	.907	-12.319153	22.597626
		2151	2.3113664	5.9445536	.995	-15.147023	19.769756
	2151	20	3.8314944	5.9445536	.966	-13.626895	21.289884
		212	-3.7854294	5.9445536	.968	-21.243819	13.672960
		214	2.8278701	5.9445536	.989	-14.630519	20.286260
		215	-2.3113664	5.9445536	.995	-19.769756	15.147023

Homogeneous Subsets

release10

Tukey HSD[a]

group	N	Subset for alpha = .05
		1
214	6	65.285276
20	6	71.271426
212	6	73.323111
215	6	76.473459
2151	6	77.527883
Sig.		.094

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

release20

Tukey HSD[a]

group	N	Subset for alpha = .05	
		1	2
214	6	75.576175	
20	6	80.520024	80.520024
215	6	88.555322	88.555322
212	6		89.122200
2151	6		91.558902
Sig.		.057	.135

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

release30

Tukey HSD[a]

group	N	Subset for alpha = .05
		1
214	6	83.070851
20	6	86.256046
212	6	94.583429
215	6	95.000966
2151	6	96.024215
Sig.		.083

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

release45

Tukey HSD[a]

group	N	Subset for alpha = .05
		1
214	6	88.760984
20	6	90.362334
2151	6	97.173361
212	6	99.123298
215	6	99.127523
Sig.		.301

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

release60

Tukey HSD[a]

group	N	Subset for alpha = .05
		1
214	6	92.050640
20	6	92.521150
2151	6	97.655187
215	6	99.219544
212	6	100.7591
Sig.		.519

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

release90

Tukey HSD[a]

group	N	Subset for alpha = .05
		1
20	6	94.293788
214	6	95.297413
2151	6	98.125283
215	6	100.4366
212	6	101.9107
Sig.		.705

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

8.3 The percent of drug release at 120, 180, 240 and 300 min

Oneway

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
release120	20	6	96.326179	14.2446585	5.8153575	81.377327	111.275031	80.3020	111.9973
	212	6	102.6869	6.9531476	2.8386106	95.390051	109.983813	94.6209	111.0890
	214	6	96.599646	10.3476174	4.2243971	85.740488	107.458805	82.3267	109.9592
	215	6	100.7053	10.9419504	4.4670325	89.222380	112.188126	87.1673	117.7990
	2151	6	97.948408	6.6188518	2.7021349	91.002349	104.894467	91.5138	107.3085
	Total	30	98.853284	9.8116567	1.7913552	95.189551	102.517017	80.3020	117.7990
release180	20	6	97.653315	15.4637430	6.3130467	81.425112	113.881518	80.5823	113.5966
	212	6	103.2957	7.4430013	3.0385926	95.484794	111.106696	94.0036	112.8978
	214	6	97.526097	10.7034389	4.3696606	86.293527	108.758667	82.7134	111.3254
	215	6	102.9193	11.2283051	4.5839364	91.135888	114.702655	87.6655	119.5390
	2151	6	98.291225	6.4281414	2.6242777	91.545304	105.037146	91.8609	106.9578
	Total	30	99.937131	10.3155281	1.8833491	96.085249	103.789012	80.5823	119.5390
release240	20	6	98.810046	15.8262645	6.4610454	82.201400	115.418692	81.4725	114.7299
	212	6	103.8765	6.9090960	2.8206266	96.625858	111.127161	95.8363	112.4954
	214	6	98.503070	10.9353244	4.4643275	87.027151	109.978989	83.7711	112.0567
	215	6	103.6641	11.0825406	4.5244282	92.033719	115.294545	88.0760	120.3349
	2151	6	98.512468	6.4622911	2.6382193	91.730709	105.294226	92.2492	107.5065
	Total	30	100.6732	10.3460738	1.8889260	96.809958	104.536532	81.4725	120.3349
release300	20	6	99.148742	16.1488519	6.5927412	82.201561	116.095922	82.7795	115.4238
	212	6	103.0477	7.1524557	2.9199778	95.541637	110.553721	95.2875	111.9766
	214	6	97.469467	10.7951952	4.4071200	86.140604	108.798329	82.6984	111.3671
	215	6	102.1747	10.8253924	4.4194479	90.814167	113.535272	86.4675	116.9236
	2151	6	98.431938	6.4162327	2.6194160	91.698515	105.165362	92.2187	107.0499
	Total	30	100.0545	10.2985133	1.8802427	96.208981	103.900037	82.6984	116.9236

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
release120	3.173	4	25	.031
release180	3.928	4	25	.013
release240	4.142	4	25	.010
release300	5.320	4	25	.003

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
release120	Between Groups	182.464	4	45.616	.437	.781
	Within Groups	2609.326	25	104.373		
	Total	2791.790	29			
release180	Between Groups	203.468	4	50.867	.441	.778
	Within Groups	2882.425	25	115.297		
	Total	3085.893	29			
release240	Between Groups	192.339	4	48.085	.413	.798
	Within Groups	2911.857	25	116.474		
	Total	3104.196	29			
release300	Between Groups	141.540	4	35.385	.301	.874
	Within Groups	2934.182	25	117.367		
	Total	3075.722	29			

Post Hoc Tests

Multiple Comparisons

Dunnett C

Dependent Variable	(I) group	(J) group	Mean Difference (I-J)	Std. Error	95% Confidence Interval	
					Lower Bound	Upper Bound
release120	20	212	-6.3607528	6.4711740	-32.319898	19.598392
		214	-.2734671	7.1877614	-29.107206	28.560272
		215	-4.3790741	7.3329914	-33.795404	25.037256
		2151	-1.6222293	6.4124813	-27.345928	24.101470
	212	20	6.3607528	6.4711740	-19.598392	32.319898
		214	6.0872857	5.0895227	-14.329359	26.503930
		215	1.9816788	5.2926449	-19.249791	23.213149
		2151	4.7385235	3.9190871	-10.982913	20.459960
	214	20	.2734671	7.1877614	-28.560272	29.107206
		212	-6.0872857	5.0895227	-26.503930	14.329359
		215	-4.1056069	6.1481632	-28.768993	20.557779
		2151	-1.3487622	5.0146849	-21.465194	18.767670
	215	20	4.3790741	7.3329914	-25.037256	33.795404
		212	-1.9816788	5.2926449	-23.213149	19.249791
		214	4.1056069	6.1481632	-20.557779	28.768993
		2151	2.7568447	5.2207196	-18.186097	23.699786
	2151	20	1.6222293	6.4124813	-24.101470	27.345928
		212	-4.7385235	3.9190871	-20.459960	10.982913
		214	1.3487622	5.0146849	-18.767670	21.465194
		215	-2.7568447	5.2207196	-23.699786	18.186097
release180	20	212	-5.6424304	7.0062545	-33.748054	22.463193
		214	.1272178	7.6777921	-30.672282	30.926718
		215	-5.2659570	7.8017325	-36.562644	26.030730
		2151	-.6379101	6.8367676	-28.063636	26.787816
	212	20	5.6424304	7.0062545	-22.463193	33.748054
		214	5.7696483	5.3223095	-15.580822	27.120118
		215	.3764735	5.4995925	-21.685168	22.438115
		2151	5.0045203	4.0149568	-11.101498	21.110539
	214	20	-.1272178	7.6777921	-30.926718	30.672282
		212	-5.7696483	5.3223095	-27.120118	15.580822
		215	-5.3931748	6.3329619	-30.797882	20.011532
		2151	-.7651280	5.0971333	-21.212302	19.682046
	215	20	5.2659570	7.8017325	-26.030730	36.562644
		212	-.3764735	5.4995925	-22.438115	21.685168
		214	5.3931748	6.3329619	-20.011532	30.797882
		2151	4.6280468	5.2819794	-16.560639	25.816732
	2151	20	.6379101	6.8367676	-26.787816	28.063636
		212	-5.0045203	4.0149568	-21.110539	11.101498
		214	.7651280	5.0971333	-19.682046	21.212302
		215	-4.6280468	5.2819794	-25.816732	16.560639

Multiple Comparisons

Dunnett C

Dependent Variable	(I) group	(J) group	Mean Difference (I-J)	Std. Error	95% Confidence Interval	
					Lower Bound	Upper Bound
release240	20	212	-5.0664629	7.0498966	-33.347157	23.214231
		214	.3069760	7.8533641	-31.196832	31.810783
		215	-4.8540858	7.8876840	-36.495568	26.787396
		2151	.2975785	6.9789189	-27.698388	28.293545
	212	20	5.0664629	7.0498966	-23.214231	33.347157
		214	5.3734389	5.2807343	-15.810252	26.557130
		215	.2123771	5.3316400	-21.175522	21.600276
		2151	5.3640414	3.8621413	-10.128957	20.857040
	214	20	-.3069760	7.8533641	-31.810783	31.196832
		212	-5.3734389	5.2807343	-26.557130	15.810252
		215	-5.1610618	6.3561522	-30.658797	20.336673
		2151	-.0093975	5.1855974	-20.811446	20.792651
	215	20	4.8540858	7.8876840	-26.787396	36.495568
		212	-.2123771	5.3316400	-21.600276	21.175522
		214	5.1610618	6.3561522	-20.336673	30.658797
		2151	5.1516643	5.2374280	-15.858303	26.161632
	2151	20	-.2975785	6.9789189	-28.293545	27.698388
		212	-5.3640414	3.8621413	-20.857040	10.128957
		214	.0093975	5.1855974	-20.792651	20.811446
		215	-5.1516643	5.2374280	-26.161632	15.858303
release300	20	212	-3.8989376	7.2104443	-32.823669	25.025794
		214	1.6792747	7.9301288	-30.132475	33.491024
		215	-3.0259783	7.9369866	-34.865238	28.813281
		2151	.7168032	7.0940522	-27.741021	29.174627
	212	20	3.8989376	7.2104443	-25.025794	32.823669
		214	5.5782123	5.2866792	-15.629326	26.785751
		215	.8729593	5.2969605	-20.375823	22.121741
		2151	4.6157408	3.9227045	-11.120207	20.351689
	214	20	-1.6792747	7.9301288	-33.491024	30.132475
		212	-5.5782123	5.2866792	-26.785751	15.629326
		215	-4.7052530	6.2413321	-29.742386	20.331880
		2151	-.9624715	5.1267969	-21.528642	19.603699
	215	20	3.0259783	7.9369866	-28.813281	34.865238
		212	-.8729593	5.2969605	-22.121741	20.375823
		214	4.7052530	6.2413321	-20.331880	29.742386
		2151	3.7427815	5.1373982	-16.865916	24.351479
	2151	20	-.7168032	7.0940522	-29.174627	27.741021
		212	-4.6157408	3.9227045	-20.351689	11.120207
		214	.9624715	5.1267969	-19.603699	21.528642
		215	-3.7427815	5.1373982	-24.351479	16.865916

8.4 The residual patch

Oneway

Descriptives

repatch

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
20	6	.756393	.1373307	.0560650	.612273	.900512	.6341	.9620
212	6	1.086212	.1629388	.0665195	.915219	1.257206	.8947	1.3434
214	6	1.376798	.2427700	.0991104	1.122027	1.631570	1.0179	1.6299
215	6	1.388461	.2083862	.0850733	1.169773	1.607148	1.0142	1.6548
2151	6	1.818231	.2028387	.0828086	1.605365	2.031097	1.6408	2.1974
Total	30	1.285219	.4016784	.0733361	1.135230	1.435208	.6341	2.1974

Test of Homogeneity of Variances

repatch

Levene Statistic	df1	df2	Sig.
.501	4	25	.735

ANOVA

repatch

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.734	4	.934	24.710	.000
Within Groups	.945	25	.038		
Total	4.679	29			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: repatch

Tukey HSD

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
20	212	-.3298198*	.1122243	.050	-.659408	-.000231
	214	-.6204054*	.1122243	.000	-.949994	-.290817
	215	-.6320680*	.1122243	.000	-.961656	-.302480
	2151	-1.0618381*	.1122243	.000	-1.391426	-.732250
212	20	.3298198*	.1122243	.050	.000231	.659408
	214	-.2905856	.1122243	.103	-.620174	.039003
	215	-.3022482	.1122243	.084	-.631837	.027340
	2151	-.7320183*	.1122243	.000	-1.061607	-.402430
214	20	.6204054*	.1122243	.000	.290817	.949994
	212	.2905856	.1122243	.103	-.039003	.620174
	215	-.0116626	.1122243	1.000	-.341251	.317926
	2151	-.4414327*	.1122243	.005	-.771021	-.111844
215	20	.6320680*	.1122243	.000	.302480	.961656
	212	.3022482	.1122243	.084	-.027340	.631837
	214	.0116626	.1122243	1.000	-.317926	.341251
	2151	-.4297701*	.1122243	.006	-.759358	-.100182
2151	20	1.0618381*	.1122243	.000	.732250	1.391426
	212	.7320183*	.1122243	.000	.402430	1.061607
	214	.4414327*	.1122243	.005	.111844	.771021
	215	.4297701*	.1122243	.006	.100182	.759358

*. The mean difference is significant at the .05 level.

Homogeneous Subsets

repatch

Tukey HSD[a]

group	N	Subset for alpha = .05		
		1	2	3
20	6	.756393		
212	6		1.086212	
214	6		1.376798	
215	6		1.388461	
2151	6			1.818231
Sig.		1.000	.084	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

9 *In vitro* penetration of verapamil hydrochloride-chitin whiskers-crab chitosan buccal patch

9.1 The percent of penetrated drug at 1.5, 2, 3 and 4 hr

Oneway

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
penetrate1.5	20	6	.516888	.2923816	.1193643	.210053	.823724	.2779	1.0282
	212	6	1.791930	.6685516	.2729351	1.090328	2.493532	.9983	2.6214
	214	6	2.168249	1.5003490	.6125149	.593730	3.742769	.5494	4.8479
	215	6	.693440	.3742520	.1527877	.300687	1.086194	.3621	1.3158
	220	6	.384242	.1692283	.0690872	.206648	.561836	.1599	.6422
	2151	6	1.066713	.2368877	.0967090	.818115	1.315311	.8325	1.4775
	Total	36	1.103577	.9391945	.1565324	.785799	1.421355	.1599	4.8479
penetrate2	20	6	1.046669	.4943527	.2018186	.527878	1.565460	.6016	1.9643
	212	6	3.034046	1.4275570	.5827977	1.535917	4.532176	1.1426	4.8734
	214	6	3.614829	2.3494678	.9591662	1.149213	6.080444	1.3638	7.5162
	215	6	1.362390	.5805831	.2370220	.753105	1.971674	.9107	2.3266
	220	6	.876900	.4249895	.1735012	.430901	1.322899	.3836	1.5073
	2151	6	1.672188	.5410178	.2208696	1.104425	2.239952	1.1501	2.5917
	Total	36	1.934504	1.5219892	.2536649	1.419537	2.449471	.3836	7.5162
penetrate3	20	6	2.119067	1.2341677	.5038469	.823888	3.414247	1.0988	4.3546
	212	6	5.109389	2.8418564	1.1601830	2.127043	8.091734	2.9015	9.6174
	214	6	6.192568	3.0298315	1.2369235	3.012955	9.372181	3.2413	10.0352
	215	6	2.678656	1.2997088	.5306039	1.314695	4.042616	1.0605	4.5005
	220	6	2.009072	1.7685803	.7220199	.153061	3.865084	.4119	5.3156
	2151	6	3.095349	1.2226685	.4991523	1.812237	4.378461	1.8858	5.3220
	Total	36	3.534017	2.4742328	.4123721	2.696857	4.371177	.4119	10.0352
penetrate4	20	6	3.352379	1.9302085	.7880043	1.326749	5.378009	1.8246	6.9437
	212	6	8.124283	4.1391343	1.6897945	3.780528	12.468038	4.2114	13.4790
	214	6	8.833033	3.5584785	1.4527428	5.098639	12.567427	4.5512	12.6695
	215	6	4.236493	1.8112957	.7394584	2.335655	6.137332	1.6958	7.1908
	220	6	4.526627	1.2613525	.5149450	3.202919	5.850335	2.2681	5.8204
	2151	6	4.698794	1.2469508	.5090655	3.390199	6.007389	3.7088	7.1386
	Total	36	5.628601	3.1798790	.5299798	4.552685	6.704518	1.6958	13.4790

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
penetrate1.5	2.605	5	30	.045
penetrate2	5.292	5	30	.001
penetrate3	5.237	5	30	.001
penetrate4	6.479	5	30	.000

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
penetrate1.5	Between Groups	15.831	5	3.166	6.315	.000
	Within Groups	15.042	30	.501		
	Total	30.873	35			
penetrate2	Between Groups	38.012	5	7.602	5.296	.001
	Within Groups	43.063	30	1.435		
	Total	81.076	35			
penetrate3	Between Groups	88.808	5	17.762	4.247	.005
	Within Groups	125.456	30	4.182		
	Total	214.264	35			
penetrate4	Between Groups	154.169	5	30.834	4.631	.003
	Within Groups	199.738	30	6.658		
	Total	353.907	35			

Post Hoc Tests

Multiple Comparisons

Dunnett C

Dependent Variable	(I) group	(J) group	Mean Difference (I-J)	Std. Error	95% Confidence Interval	
					Lower Bound	Upper Bound
penetrate1.5	20	212	-1.2750419*	.2978949	-2.545834	-.004250
		214	-1.6513612	.6240371	-4.313445	1.010723
		215	-.1765522	.1938864	-1.003653	.650549
		220	.1326462	.1379162	-.455691	.720984
		2151	-.5498249	.1536244	-1.205172	.105522
	212	20	1.2750419*	.2978949	.004250	2.545834
		214	-.3763194	.6705729	-3.236921	2.484282
		215	1.0984897	.3127901	-.235844	2.432823
		220	1.4076880*	.2815432	.206651	2.608725
		2151	.7252169	.2895620	-.510028	1.960461
	214	20	1.6513612	.6240371	-1.010723	4.313445
		212	.3763194	.6705729	-2.484282	3.236921
		215	1.4748090	.6312833	-1.218186	4.167805
		220	1.7840074	.6163989	-.845492	4.413507
		2151	1.1015363	.6201025	-1.543763	3.746836
	215	20	.1765522	.1938864	-.650549	1.003653
		212	-1.0984897	.3127901	-2.432823	.235844
		214	-1.4748090	.6312833	-4.167805	1.218186
		220	.3091983	.1676816	-.406116	1.024513
		2151	-.3732728	.1808223	-1.144644	.398098
	220	20	-.1326462	.1379162	-.720984	.455691
		212	-1.4076880*	.2815432	-2.608725	-.206651
		214	-1.7840074	.6163989	-4.413507	.845492
		215	-.3091983	.1676816	-1.024513	.406116
		2151	-.6824711*	.1188514	-1.189480	-.175462
	2151	20	.5498249	.1536244	-.105522	1.205172
		212	-.7252169	.2895620	-1.960461	.510028
		214	-1.1015363	.6201025	-3.746836	1.543763
		215	.3732728	.1808223	-.398098	1.144644
		220	.6824711*	.1188514	.175462	1.189480
penetrate2	20	212	-1.9873773	.6167527	-4.618387	.643632
		214	-2.5681595	.9801687	-6.749467	1.613148
		215	-.3157209	.3113040	-1.643715	1.012273
		220	.1697689	.2661455	-.965583	1.305121
		2151	-.6255194	.2991891	-1.901832	.650793
	212	20	1.9873773	.6167527	-.643632	4.618387
		214	-.5807822	1.1223427	-5.368591	4.207027
		215	1.6716564	.6291523	-1.012248	4.355561
		220	2.1571463	.6080755	-.436847	4.751140
		2151	1.3618579	.6232468	-1.296855	4.020570
	214	20	2.5681595	.9801687	-1.613148	6.749467
		212	.5807822	1.1223427	-4.207027	5.368591
		215	2.2524386	.9880179	-1.962353	6.467230
		220	2.7379285	.9747320	-1.420187	6.896044
		2151	1.9426401	.9842679	-2.256155	6.141435
	215	20	.3157209	.3113040	-1.012273	1.643715
		212	-1.6716564	.6291523	-4.355561	1.012248
		214	-2.2524386	.9880179	-6.467230	1.962353
		220	.4854899	.2937382	-.767570	1.738549
		2151	-.3097985	.3239797	-1.691865	1.072269
	220	20	-.1697689	.2661455	-1.305121	.965583
		212	-2.1571463	.6080755	-4.751140	.436847
		214	-2.7379285	.9747320	-6.896044	1.420187
		215	-.4854899	.2937382	-1.738549	.767570
		2151	-.7952883	.2808666	-1.993439	.402862
	2151	20	.6255194	.2991891	-.650793	1.901832
		212	-1.3618579	.6232468	-4.020570	1.296855
		214	-1.9426401	.9842679	-6.141435	2.256155
		215	.3097985	.3239797	-1.072269	1.691865
		220	.7952883	.2808666	-.402862	1.993439

*. The mean difference is significant at the .05 level.

Multiple Comparisons

Dunnett C

Dependent Variable	(I) group	(J) group	Mean Difference (I-J)	Std. Error	95% Confidence Interval	
					Lower Bound	Upper Bound
penetrate3	20	212	-2.9903213	1.2648661	-8.386122	2.405479
		214	-4.0735011	1.3356053	-9.771068	1.624066
		215	-.5595883	.7317118	-3.681002	2.561826
		220	.1099948	.8804399	-3.645879	3.865869
		2151	-.9762816	.7092353	-4.001813	2.049250
	212	20	2.9903213	1.2648661	-2.405479	8.386122
		214	-1.0831797	1.6958786	-8.317639	6.151280
		215	2.4307330	1.2757606	-3.011542	7.873008
		220	3.1003161	1.3665055	-2.729069	8.929701
		2151	2.0140398	1.2630034	-3.373815	7.401894
	214	20	4.0735011	1.3356053	-1.624066	9.771068
		212	1.0831797	1.6958786	-6.151280	8.317639
		215	3.5139127	1.3459273	-2.227687	9.255513
		220	4.1834958	1.4322334	-1.926278	10.293269
		2151	3.0972195	1.3338414	-2.592823	8.787262
	215	20	.5595883	.7317118	-2.561826	3.681002
		212	-2.4307330	1.2757606	-7.873008	3.011542
		214	-3.5139127	1.3459273	-9.255513	2.227687
		220	.6695831	.8960208	-3.152758	4.491924
		2151	-.4166933	.7284871	-3.524351	2.690965
	220	20	-.1099948	.8804399	-3.865869	3.645879
		212	-3.1003161	1.3665055	-8.929701	2.729069
		214	-4.1834958	1.4322334	-10.293269	1.926278
		215	-.6695831	.8960208	-4.491924	3.152758
		2151	-1.0862764	.8777618	-4.830726	2.658173
	2151	20	.9762816	.7092353	-2.049250	4.001813
		212	-2.0140398	1.2630034	-7.401894	3.373815
		214	-3.0972195	1.3338414	-8.787262	2.592823
		215	.4166933	.7284871	-2.690965	3.524351
		220	1.0862764	.8777618	-2.658173	4.830726
penetrate4	20	212	-4.7719036	1.8644989	-12.725682	3.181874
		214	-5.4806538	1.6526985	-12.530911	1.569603
		215	-.8841144	1.0806246	-5.493958	3.725729
		220	-1.1742480	.9413390	-5.189912	2.841416
		2151	-1.3464151	.9381357	-5.348414	2.655584
	212	20	4.7719036	1.8644989	-3.181874	12.725682
		214	-.7087502	2.2284225	-10.214992	8.797492
		215	3.8877893	1.8445065	-3.980703	11.756281
		220	3.5976556	1.7665146	-3.938130	11.133442
		2151	3.4254885	1.7648097	-4.103024	10.954001
	214	20	5.4806538	1.6526985	-1.569603	12.530911
		212	.7087502	2.2284225	-8.797492	10.214992
		215	4.5965394	1.6301105	-2.357359	11.550438
		220	4.3064058	1.5413079	-2.268669	10.881481
		2151	4.1342387	1.5393535	-2.432499	10.700977
	215	20	.8841144	1.0806246	-3.725729	5.493958
		212	-3.8877893	1.8445065	-11.756281	3.980703
		214	-4.5965394	1.6301105	-11.550438	2.357359
		220	-.2901336	.9010921	-4.134108	3.553841
		2151	-.4623007	.8977452	-4.291998	3.367396
	220	20	1.1742480	.9413390	-2.841416	5.189912
		212	-3.5976556	1.7665146	-11.133442	3.938130
		214	-4.3064058	1.5413079	-10.881481	2.268669
		215	.2901336	.9010921	-3.553841	4.134108
		2151	-.1721671	.7240967	-3.261096	2.916762
	2151	20	1.3464151	.9381357	-2.655584	5.348414
		212	-3.4254885	1.7648097	-10.954001	4.103024
		214	-4.1342387	1.5393535	-10.700977	2.432499
		215	.4623007	.8977452	-3.367396	4.291998
		220	.1721671	.7240967	-2.916762	3.261096

9.2 The percent of penetrated drug at 5, 6 and 9 hr

Oneway

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
penetrate5	20	6	4.368723	2.1853237	.8921547	2.075367	6.662080	2.3200	8.3597
	212	6	10.620680	5.5085762	2.2488668	4.839784	16.401576	3.6809	17.5269
	214	6	11.562643	4.3931832	1.7935095	6.952280	16.173006	5.9848	16.1569
	215	6	6.548163	2.7163009	1.1089252	3.697580	9.398746	3.9112	9.9175
	220	6	7.209114	3.1088605	1.2691870	3.946565	10.471663	3.3443	12.8535
	2151	6	7.042093	2.8403973	1.1595874	4.061279	10.022907	4.5615	12.5570
	Total	36	7.891903	4.1939430	.6989905	6.472877	9.310929	2.3200	17.5269
penetrate6	20	6	5.998249	2.9241186	1.1937664	2.929574	9.066923	3.2541	10.7661
	212	6	13.037344	5.3005142	2.1639258	7.474796	18.599893	6.1505	20.1413
	214	6	14.124014	5.0748510	2.0717992	8.798284	19.449743	6.8168	19.4825
	215	6	8.541281	3.3072864	1.3501940	5.070497	12.012065	5.2565	12.8582
	220	6	10.380325	4.1485678	1.6936457	6.026670	14.733980	5.1907	17.3205
	2151	6	8.859509	3.3869500	1.3827166	5.305123	12.413895	6.2569	15.3797
	Total	36	10.156787	4.7332782	.7888797	8.555276	11.758298	3.2541	20.1413
penetrate9	20	6	10.631846	5.9324199	2.4219003	4.406153	16.857539	4.9550	19.7859
	212	6	19.443525	7.1845523	2.9330812	11.903800	26.983250	9.1822	29.5174
	214	6	20.743864	7.8308434	3.1969284	12.525898	28.961830	9.6745	33.1343
	215	6	15.284075	3.1962771	1.3048747	11.929788	18.638362	11.8008	20.6797
	220	6	20.763731	5.7183903	2.3345231	14.762648	26.764813	12.7476	29.6246
	2151	6	15.622374	5.3429547	2.1812521	10.015287	21.229461	11.6479	26.2629
	Total	36	17.081569	6.7082818	1.1180470	14.811813	19.351325	4.9550	33.1343

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
penetrate5	2.229	5	30	.077
penetrate6	.899	5	30	.495
penetrate9	.620	5	30	.685

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
penetrate5	Between Groups	217.964	5	43.593	3.289	.017
	Within Groups	397.656	30	13.255		
	Total	615.621	35			
penetrate6	Between Groups	274.036	5	54.807	3.223	.019
	Within Groups	510.101	30	17.003		
	Total	784.137	35			
penetrate9	Between Groups	477.052	5	95.410	2.607	.045
	Within Groups	1097.984	30	36.599		
	Total	1575.037	35			

Post Hoc Tests

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
penetrate5	20	212	-6.2519564	2.1020000	.058	-12.645387	.141475
		214	-7.1939194*	2.1020000	.020	-13.587351	-.800488
		215	-2.1794399	2.1020000	.902	-8.572871	4.213991
		220	-2.8403909	2.1020000	.755	-9.233822	3.553040
		2151	-2.6733698	2.1020000	.798	-9.066801	3.720061
	212	20	6.2519564	2.1020000	.058	-.141475	12.645387
		214	-.9419631	2.1020000	.997	-7.335394	5.451468
		215	4.0725165	2.1020000	.400	-2.320915	10.465948
		220	3.4115655	2.1020000	.590	-2.981866	9.804997
		2151	3.5785866	2.1020000	.541	-2.814845	9.972018
	214	20	7.1939194*	2.1020000	.020	.800488	13.587351
		212	.9419631	2.1020000	.997	-5.451468	7.335394
		215	5.0144795	2.1020000	.193	-1.378952	11.407911
		220	4.3535285	2.1020000	.329	-2.039903	10.746960
		2151	4.5205496	2.1020000	.290	-1.872881	10.913981
	215	20	2.1794399	2.1020000	.902	-4.213991	8.572871
		212	-4.0725165	2.1020000	.400	-10.465948	2.320915
		214	-5.0144795	2.1020000	.193	-11.407911	1.378952
		220	-.6609510	2.1020000	1.000	-7.054382	5.732480
		2151	-.4939299	2.1020000	1.000	-6.887361	5.899501
	220	20	2.8403909	2.1020000	.755	-3.553040	9.233822
		212	-3.4115655	2.1020000	.590	-9.804997	2.981866
		214	-4.3535285	2.1020000	.329	-10.746960	2.039903
		215	.6609510	2.1020000	1.000	-5.732480	7.054382
		2151	.1670211	2.1020000	1.000	-6.226410	6.560452
	2151	20	2.6733698	2.1020000	.798	-3.720061	9.066801
		212	-3.5785866	2.1020000	.541	-9.972018	2.814845
		214	-4.5205496	2.1020000	.290	-10.913981	1.872881
		215	.4939299	2.1020000	1.000	-5.899501	6.887361
		220	-.1670211	2.1020000	1.000	-6.560452	6.226410
penetrate6	20	212	-7.0390956	2.3807121	.060	-14.280256	.202065
		214	-8.1257651*	2.3807121	.021	-15.366925	-.884605
		215	-2.5430323	2.3807121	.890	-9.784193	4.698128
		220	-4.3820765	2.3807121	.456	-11.623237	2.859084
		2151	-2.8612603	2.3807121	.832	-10.102421	4.379900
	212	20	7.0390956	2.3807121	.060	-.202065	14.280256
		214	-1.0866695	2.3807121	.997	-8.327830	6.154491
		215	4.4960632	2.3807121	.428	-2.745097	11.737223
		220	2.6570191	2.3807121	.871	-4.584141	9.898179
		2151	4.1778352	2.3807121	.508	-3.063325	11.418995
	214	20	8.1257651*	2.3807121	.021	.884605	15.366925
		212	1.0866695	2.3807121	.997	-6.154491	8.327830
		215	5.5827327	2.3807121	.208	-1.658427	12.823893
		220	3.7436886	2.3807121	.622	-3.497472	10.984849
		2151	5.2645048	2.3807121	.262	-1.976655	12.505665
	215	20	2.5430323	2.3807121	.890	-4.698128	9.784193
		212	-4.4960632	2.3807121	.428	-11.737223	2.745097
		214	-5.5827327	2.3807121	.208	-12.823893	1.658427
		220	-1.8390441	2.3807121	.970	-9.080204	5.402116
		2151	-.3182280	2.3807121	1.000	-7.559388	6.922932
	220	20	4.3820765	2.3807121	.456	-2.859084	11.623237
		212	-2.6570191	2.3807121	.871	-9.898179	4.584141
		214	-3.7436886	2.3807121	.622	-10.984849	3.497472
		215	1.8390441	2.3807121	.970	-5.402116	9.080204
		2151	1.5208161	2.3807121	.987	-5.720344	8.761976
	2151	20	2.8612603	2.3807121	.832	-4.379900	10.102421
		212	-4.1778352	2.3807121	.508	-11.418995	3.063325
		214	-5.2645048	2.3807121	.262	-12.505665	1.976655
		215	.3182280	2.3807121	1.000	-6.922932	7.559388
		220	-1.5208161	2.3807121	.987	-8.761976	5.720344

*. The mean difference is significant at the .05 level.

Multiple Comparisons

Dependent Variable: penetrate9
Tukey HSD

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
20	212	-8.8116796	3.4928248	.149	-19.435435	1.812076
	214	-10.112019	3.4928248	.069	-20.735774	.511737
	215	-4.6522298	3.4928248	.765	-15.275986	5.971526
	220	-10.131885	3.4928248	.068	-20.755641	.491871
	2151	-4.9905282	3.4928248	.710	-15.614284	5.633228
212	20	8.8116796	3.4928248	.149	-1.812076	19.435435
	214	-1.3003391	3.4928248	.999	-11.924095	9.323417
	215	4.1594498	3.4928248	.838	-6.464306	14.783206
	220	-1.3202054	3.4928248	.999	-11.943961	9.303550
	2151	3.8211514	3.4928248	.880	-6.802604	14.444907
214	20	10.1120186	3.4928248	.069	-.511737	20.735774
	212	1.3003391	3.4928248	.999	-9.323417	11.924095
	215	5.4597888	3.4928248	.628	-5.163967	16.083545
	220	-.0198663	3.4928248	1.000	-10.643622	10.603889
	2151	5.1214904	3.4928248	.687	-5.502265	15.745246
215	20	4.6522298	3.4928248	.765	-5.971526	15.275986
	212	-4.1594498	3.4928248	.838	-14.783206	6.464306
	214	-5.4597888	3.4928248	.628	-16.083545	5.163967
	220	-5.4796551	3.4928248	.624	-16.103411	5.144101
	2151	-.3382984	3.4928248	1.000	-10.962054	10.285457
220	20	10.1318849	3.4928248	.068	-.491871	20.755641
	212	1.3202054	3.4928248	.999	-9.303550	11.943961
	214	.0198663	3.4928248	1.000	-10.603889	10.643622
	215	5.4796551	3.4928248	.624	-5.144101	16.103411
	2151	5.1413567	3.4928248	.684	-5.482399	15.765112
2151	20	4.9905282	3.4928248	.710	-5.633228	15.614284
	212	-3.8211514	3.4928248	.880	-14.444907	6.802604
	214	-5.1214904	3.4928248	.687	-15.745246	5.502265
	215	.3382984	3.4928248	1.000	-10.285457	10.962054
	220	-5.1413567	3.4928248	.684	-15.765112	5.482399

Homogeneous Subsets

penetrate5

Tukey HSD[a]

group	N	Subset for alpha = .05	
		1	2
20	6	4.368723	
215	6	6.548163	6.548163
2151	6	7.042093	7.042093
220	6	7.209114	7.209114
212	6	10.620680	10.620680
214	6		11.562643
Sig.		.058	.193

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

penetrate6

Tukey HSD[a]

group	N	Subset for alpha = .05	
		1	2
20	6	5.998249	
215	6	8.541281	8.541281
2151	6	8.859509	8.859509
220	6	10.380325	10.380325
212	6	13.037344	13.037344
214	6		14.124014
Sig.		.060	.208

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

penetrate9

Tukey HSD[a]

group	N	Subset for alpha = .05
		1
20	6	10.631846
215	6	15.284075
2151	6	15.622374
212	6	19.443525
214	6	20.743864
220	6	20.763731
Sig.		.068

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

9.3 The slope of a plot of percent of drug penetration versus time (hr)

Oneway

Descriptives

slope

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
20	6	1.338245	.7311779	.2985021	.570921	2.105569	.6316	2.4523
212	6	2.345859	.9562230	.3903764	1.342364	3.349353	.9760	3.6347
214	6	2.516648	.9362807	.3822350	1.534081	3.499214	1.1986	3.9578
215	6	1.954114	.4333569	.1769172	1.499334	2.408894	1.4770	2.6790
220	6	2.740105	.7811779	.3189146	1.920309	3.559901	1.6327	3.9575
2151	6	1.890142	.6769648	.2763697	1.179711	2.600573	1.4322	3.2370
Total	36	2.130852	.8554292	.1425715	1.841416	2.420288	.6316	3.9578

Test of Homogeneity of Variances

slope

Levene Statistic	df1	df2	Sig.
.632	5	30	.677

ANOVA

slope

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7.702	5	1.540	2.580	.047
Within Groups	17.910	30	.597		
Total	25.612	35			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: slope

Tukey HSD

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
20	212	-1.0076136	.4460894	.242	-2.364437	.349209
	214	-1.1784027	.4460894	.118	-2.535226	.178420
	215	-.6158690	.4460894	.738	-1.972692	.740954
	220	-1.4018596*	.4460894	.040	-2.758683	-.045037
	2151	-.5518972	.4460894	.815	-1.908720	.804926
212	20	1.0076136	.4460894	.242	-.349209	2.364437
	214	-.1707891	.4460894	.999	-1.527612	1.186034
	215	.3917446	.4460894	.949	-.965078	1.748568
	220	-.3942460	.4460894	.948	-1.751069	.962577
	2151	.4557164	.4460894	.907	-.901107	1.812539
214	20	1.1784027	.4460894	.118	-.178420	2.535226
	212	.1707891	.4460894	.999	-1.186034	1.527612
	215	.5625336	.4460894	.803	-.794289	1.919357
	220	-.2234569	.4460894	.996	-1.580280	1.133366
	2151	.6265054	.4460894	.724	-.730318	1.983328
215	20	.6158690	.4460894	.738	-.740954	1.972692
	212	-.3917446	.4460894	.949	-1.748568	.965078
	214	-.5625336	.4460894	.803	-1.919357	.794289
	220	-.7859906	.4460894	.504	-2.142814	.570832
	2151	.0639718	.4460894	1.000	-1.292851	1.420795
220	20	1.4018596*	.4460894	.040	.045037	2.758683
	212	.3942460	.4460894	.948	-.962577	1.751069
	214	.2234569	.4460894	.996	-1.133366	1.580280
	215	.7859906	.4460894	.504	-.570832	2.142814
	2151	.8499624	.4460894	.419	-.506861	2.206785
2151	20	.5518972	.4460894	.815	-.804926	1.908720
	212	-.4557164	.4460894	.907	-1.812539	.901107
	214	-.6265054	.4460894	.724	-1.983328	.730318
	215	-.0639718	.4460894	1.000	-1.420795	1.292851
	220	-.8499624	.4460894	.419	-2.206785	.506861

*. The mean difference is significant at the .05 level.

Homogeneous Subsets

slope

Tukey HSD[a]

group	N	Subset for alpha = .05	
		1	2
20	6	1.338245	
2151	6	1.890142	1.890142
215	6	1.954114	1.954114
212	6	2.345859	2.345859
214	6	2.516648	2.516648
220	6		2.740105
Sig.		.118	.419

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

9.4 The residual drug in donor compartment

Oneway

Descriptives

redonor

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
20	6	10.460345	2.9577215	1.2074847	7.356406	13.564283	7.5008	15.0725
212	6	14.267715	3.9456134	1.6107899	10.127048	18.408383	9.5399	18.1337
214	6	20.351210	6.4416814	2.6298054	13.591080	27.111340	13.8727	28.3643
215	6	11.478028	4.0536921	1.6549129	7.223939	15.732117	7.5954	18.4022
220	6	39.365356	7.2253255	2.9497268	31.782842	46.947870	32.2027	50.3125
2151	6	20.783128	2.5204793	1.0289814	18.138047	23.428209	17.7184	23.3524
Total	36	19.450964	10.8583185	1.8097198	15.777037	23.124890	7.5008	50.3125

Test of Homogeneity of Variances

redonor

Levene Statistic	df1	df2	Sig.
2.548	5	30	.049

ANOVA

redonor

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3422.599	5	684.520	29.170	.000
Within Groups	704.009	30	23.467		
Total	4126.608	35			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: redonor

Dunnett C

(I) group	(J) group	Mean Difference (I-J)	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
20	212	-3.8073707	2.0131228	-12.395164	4.780423
	214	-9.8908650	2.8937685	-22.235411	2.453680
	215	-1.0176832	2.0485985	-9.756813	7.721446
	220	-28.905012*	3.1873041	-42.501753	-15.308271
	2151	-10.322783*	1.5864495	-17.090428	-3.555138
212	20	3.8073707	2.0131228	-4.780423	12.395164
	214	-6.0834943	3.0839132	-19.239180	7.072191
	215	2.7896876	2.3094114	-7.062045	12.641420
	220	-25.097641*	3.3608827	-39.434852	-10.760430
	2151	-6.5154124	1.9113992	-14.669262	1.638438
214	20	9.8908650	2.8937685	-2.453680	22.235411
	212	6.0834943	3.0839132	-7.072191	19.239180
	215	8.8731819	3.1071873	-4.381789	22.128152
	220	-19.014147*	3.9518052	-35.872178	-2.156115
	2151	-.4319181	2.8239475	-12.478614	11.614778
215	20	1.0176832	2.0485985	-7.721446	9.756813
	212	-2.7896876	2.3094114	-12.641420	7.062045
	214	-8.8731819	3.1071873	-22.128152	4.381789
	220	-27.887328*	3.3822514	-42.315697	-13.458960
	2151	-9.3051000*	1.9487276	-17.618190	-.992010
220	20	28.9050116*	3.1873041	15.308271	42.501753
	212	25.0976409*	3.3608827	10.760430	39.434852
	214	19.0141466*	3.9518052	2.156115	35.872178
	215	27.8873285*	3.3822514	13.458960	42.315697
	2151	18.5822285*	3.1240504	5.255322	31.909135
2151	20	10.3227831*	1.5864495	3.555138	17.090428
	212	6.5154124	1.9113992	-1.638438	14.669262
	214	.4319181	2.8239475	-11.614778	12.478614
	215	9.3051000*	1.9487276	.992010	17.618190
	220	-18.582229*	3.1240504	-31.909135	-5.255322

*. The mean difference is significant at the .05 level.

9.5 The residual patch

Oneway

Descriptives

repatch

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
20	6	11.946445	2.3607616	.9637769	9.468977	14.423912	9.2290	14.9665
212	6	8.307781	2.3641029	.9651410	5.826807	10.788755	5.2921	10.6558
214	6	9.478059	1.2441013	.5079022	8.172455	10.783664	7.9226	10.6711
215	6	10.905502	.6993764	.2855192	10.171552	11.639453	10.0561	12.1088
2151	6	5.463741	.3366282	.1374279	5.110471	5.817011	5.1963	6.1105
Total	30	9.220306	2.7433801	.5008704	8.195911	10.244701	5.1963	14.9665

Test of Homogeneity of Variances

repatch

Levene Statistic	df1	df2	Sig.
10.101	4	25	.000

ANOVA

repatch

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	151.696	4	37.924	14.244	.000
Within Groups	66.562	25	2.662		
Total	218.258	29			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: repatch

Dunnett C

(I) group	(J) group	Mean Difference (I-J)	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
20	212	3.6386636	1.3639512	-1.832833	9.110160
	214	2.4683855	1.0894175	-1.901818	6.838589
	215	1.0409424	1.0051801	-2.991342	5.073227
	2151	6.4827039*	.9735257	2.577401	10.388007
212	20	-3.6386636	1.3639512	-9.110160	1.832833
	214	-1.1702780	1.0906245	-5.545323	3.204767
	215	-2.5977212	1.0064881	-6.635253	1.439811
	2151	2.8440403	.9748762	-1.066680	6.754761
214	20	-2.4683855	1.0894175	-6.838589	1.901818
	212	1.1702780	1.0906245	-3.204767	5.545323
	215	-1.4274431	.5826542	-3.764763	.909877
	2151	4.0143183*	.5261664	1.903599	6.125037
215	20	-1.0409424	1.0051801	-5.073227	2.991342
	212	2.5977212	1.0064881	-1.439811	6.635253
	214	1.4274431	.5826542	-.909877	3.764763
	2151	5.4417615*	.3168717	4.170629	6.712894
2151	20	-6.4827039*	.9735257	-10.388007	-2.577401
	212	-2.8440403	.9748762	-6.754761	1.066680
	214	-4.0143183*	.5261664	-6.125037	-1.903599
	215	-5.4417615*	.3168717	-6.712894	-4.170629

*. The mean difference is significant at the .05 level.

BIOGRAPHY

NAME	Miss Wicharanee Tongsima
DATE OF BIRTH	18 July 1976
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INSTITUTIONS ATTENDED	Mahidol University, 1999: Bachelor of Science in Pharmacy Mahidol University, 2005: Master of Science in Pharmacy Programme in Pharmaceutics
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