CHITOSAN BLENDING WITH WATER-SOLUBLE POLYMERS AS DISSOLVING MICRONEEDLES FOR CONTROLLED DRUG RELEASE

Mr. Fuad Abdullah Farhan Saleh

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ของผสมไคโตซานกับพอลิเมอร์ชนิดละลายน้ำได้เพื่อเป็นไมโครนีดเดิ้ล ชนิดละลายได้สำหรับการปลดปล่อยยาแบบควบคุม

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Thesis Title	
By	Mr. Fuad Abdullah Farhan Saleh
Field of Study	Chemistry
Thesis Advisor	Professor SUPASON WANICHWECHARUANG, Ph.D.

Accepted by the FACULTY OF SCIENCE, Chulalongkorn University in Partial Fulfillment of the Requirement for the Master of Science

..... Dean of the FACULTY OF SCIENCE
(Professor POLKIT SANGVANICH, Ph.D.)

THESIS COMMITTEE

..... Chairman
(Professor MONGKOL SUKWATTANASINITT, Ph.D.)

..... Thesis Advisor
(Professor SUPASON WANICHWECHARUANG, Ph.D.)

..... Examiner
(CHANAT AONBANGKHEN, Ph.D.)

..... External Examiner
(Thapakorn Tree-udom, Ph.D.)

ฟาด อับดุลลาห์ ฟาร์ฮาน ซาเล : ของผสมไคโตซานกับพอลิเมอร์ชนิดละลายน้ำได้เพื่อเป็นไมโครนีดเดิ้ล ชนิดละลายได้สำหรับการปลดปล่อยยาแบบควบคุม. (CHITOSAN BLENDING WITH WATER-SOLUBLE POLYMERS AS DISSOLVING MICRONEEDLES FOR CONTROLLED DRUG RELEASE) อ.ที่ปรึกษาหลัก : ศุภศร วนิชเวชารุ่งเรือง

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Chitosan has gained significant interest due to its ability to film-forming, biodegradability, and biocompatibility, making it suitable for topical and transdermal drug delivery such as fabricating microneedles. In this study, chitosan microneedles were prepared by incorporating polyvinylpyrrolidone (PVP) and polyvinyl alcohol (PVA) in three different ratios (1:1, 2:1, and 3:1 chitosan: polymers) to control drug release. The microneedles were fabricated using a molding method, their mechanical properties were tested using the universal testing machine (UTM), and their skin penetration ability was tested using pig skin (*ex vivo*). The results indicated that the microneedles exhibited sufficient strength to penetrate the outer layer of the porcine skin effectively. The study included incorporating two drugs: curcumin as a model of hydrophobic drugs, and butterfly pea extract as a model of hydrophilic drugs. Chitosan blended with PVP demonstrated the ability to finely tune the release profiles with approximately 90% of hydrophilic and 60% of hydrophobic loaded drugs being released from the microneedles over four days. In contrast, chitosan blended with PVA did not exhibit a significant difference from the blank microneedles, releasing only 49% of the loaded drugs over two days. These findings suggest that chitosan microneedles incorporating PVP are a promising candidate for controlled transdermal drug delivery, offering versatility in releasing both hydrophobic and hydrophilic

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my field of study. This acknowledgment is a small token of my appreciation for the many hands and hearts that have played a part in this journey.

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Fuad Abdullah Farhan Saleh

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1 CHAPTER I INTRODUCTION

1.1 INTRODUCTION

Drug administration through the skin has been utilized to target the epidermis, dermis, and deeper tissues and for systemic distribution. The stratum corneum is the principal barrier to drug transport through the skin, with most transport happening through the intercellular layer [1]. Microneedle technology is promising for the transdermal administration of therapeutic proteins because it allows macromolecules to penetrate through the stratum corneum [2]. Microneedles differ from the more typical subcutaneous injection procedure in that they are painless, do not generate medical waste, may be self-administered, and do not require expert application [3]. Microneedles made of biodegradable or dissolving polymers have attracted much curiosity in recent years [4-6]. Drugs can be effectively delivered into the skin using microneedles consisting of hyaluronic acid (HA) [7], carboxymethyl cellulose (CMC) [8], polyvinyl alcohol (PVA) [9], polyvinyl pyrrolidone (PVP) [10], and maltose [11]. Nevertheless, these microneedles disintegrate fast when they come into touch with moisture in the skin, which causes speedy drug distribution [12]. Extended drug release may be possible using biodegradable microneedles composed of poly-L-lactic acid (PLA) [13], or copolymer poly(lactic-co-glycolic acid) (PLGA) [14].

Chitosan-based delivery methods have benefits over other hydrophobic polymers such as PLGA for the encapsulation of biopharmaceuticals since it does not require organic solvents. It degrades between weeks to months [12, 15]. Chitosan β-

(1⟶4)-linked D-glucosamine and N-acetyl-D-glucosamine is a family of molecules with variations in their composition, size, and distribution of monomers rather than a single polymer with a well-defined structure [16]. Chitosan's biocompatibility, degradability, and nontoxicity have made it a popular drug delivery material. Chitosan matrix swelling and disintegration can release drugs contained in chitosan carriers, producing sustained-release action [12, 17]. Its safety as dietary supplements or medication carriers has been proven in animal and human models [12, 18]. Chitosan is a biocompatible polymer that is often utilized in delivery methods to help the formulation's wound-healing benefits [19]. Creating an aqueous chitosan solution under mildly acidic conditions is simple by protonating its amino groups, which will give it positive charges [17].

It is still difficult to degrade insoluble natural polysaccharides with highly organized structures, such as cellulose and chitin. It has been observed that chitosan can be hydrolyzed enzymatically by amylases, hemicellulases, pectinase, and hyaluronidase. Although these enzymes have been proposed for the production of low-molecular-weight chitosan LMWC and chitooligosaccharides COS from chitosan, little is known about their mechanism of action [20]. Chitosan and hyaluronic acid have similar structure therefore hyaluronidase mediates the enzymatic degradation of chitosan. The breakdown of chitosan in tissue by hyaluronidase has been investigated. This enzyme has been utilized to break down hyaluronic acid in tissue. It cleaves the glycoside bond [21].

Chitosan microneedles are a type of microneedle that is made from chitosan, a biodegradable and non-cytotoxic material[2]. Chitosan microneedles have been shown to be effective for transdermal delivery of drugs[22]. They have also been shown to be effective for sustained release of drugs[2].

According to a study published in the Journal of Controlled Release, chitosan was selected as the microneedle material because of its non-cytotoxic and biodegradable properties and its immune-stimulating activity that can enhance humoral and cellular responses[2]. Another study published in the Journal of Pharmaceutical Sciences found that chitosan microneedles have the sufficient mechanical strength to be inserted in vitro into porcine skin at approximately 250 μm in depth and in vivo into rat skin at approximately 200 μm in depth[12].

Here, we detail the creation of two novel polymer blended systems chitosan-PVP and chitosan-PVA that allow for straightforward modulation of drug release. The blends were also used to demonstrate microneedle fabrication and the mechanical properties of the resulting microneedles. The work covers the specifics of modifying the chitosan: PVP and chitosan: PVA ratios to achieve varying drug release rates.

1.2 Literature Review

1.2.1 Drug Delivery

Drug delivery is an extensive field of research that focuses on the development of the carrier systems for effective therapeutic administration of drugs [23]. Since the development of medical application systems, a variety of drugs are being administered through a variety of conventional drug delivery dosage forms to treat a variety of diseases. These dosage forms include solutions, lotions, mixtures, creams, pastes, ointments, solutions, powders, suppositories, injections, suspensions, pills, immediate-release capsules, tablets, etc. [24].

Some of the traditional dosage forms are currently utilized as major drug delivery products. However, they may not always provide the optimal therapeutic responses. To overcome this limitation and enhance safety, efficacy, and patient compliance, pharmaceutical companies are actively exploring novel drug delivery systems. By incorporating these innovative approaches alongside existing medicines, the aim is to significantly improve treatment outcomes while minimizing side effects. Some examples of these advanced drug delivery systems with remarkable therapeutic potential include oral controlled release systems, fast dispersing dosage forms, liposomes, taste-masking systems, transdermal patches, aerosols, site-specific delivery systems, and etc. [25].

1.2.2 Transdermal and Intradermal Drug Delivery (TDD and IDD)

TDD is a painless method of systemic drug delivery that involves applying a drug formulation to healthy, undamaged skin. The medication first goes through the stratum corneum and then deeper layers of the epidermis and dermis. The drug becomes available for systemic absorption when it enters the dermal layer through dermal microcirculation [26]. Despite the numerous advantages, transdermal delivery of drugs is restricted to a few compounds with specific physicochemical features. the drug should ideally have a molecular weight of less than 500 Da and a log P less than 2-3 [27]. The main barrier to transdermal penetration is the stratum corneum. As a result, several technologies have been developed to penetrate the stratum corneum and increase skin permeability. Iontophoresis, sonophoresis, magnetophoresis, electroporation, and laser-microporation are among the types of the penetration technologies [28-33]. These approaches have significant applications and economic constraints. To address the issues associated with transdermal delivery, traditional drug delivery techniques such as intradermal injections are now being used. However, intradermal injections have restrictions such as needle injuries, fear, and the necessity for highly trained personnel, which raises the cost of delivery.

IDD is another type of drug delivery. IDD entails injecting a material straight into the dermis. This technique is frequently applied to immunizations or certain drugs that the skin may absorb. IDD delivery can be challenging to learn as it needs specialized training and might not target the skin properly, causing leakage or delivery

to subcutaneous tissue instead of the skin. **Intradermal drug delivery targets the dermis whereas transdermal drug delivery targets systemic drug circulation via dermis.** Microneedles are used for both transdermal and intradermal drug administration[74].

Microneedle drug delivery addresses the constraints of the two traditional dose forms [34]. The method has been developed to deliver not only small molecules [35] but also various macromolecules [36], cosmeceuticals [37], and micro/nano-particles [38].

1.2.3 Microneedles

Microneedle arrays are micron-sized needles that go through the stratum corneum, the skin's basic barrier, to administer treatment via the skin. Microneedles range in height from 50 to 900 microns (Figure 1) and are made from a variety of metals, silicon, and polymers The insertion of microneedle patches into the skin creates minute aqueous pores that allow medications to diffuse to the epidermal layer of the skin [39]. The idea of microneedles was developed decades earlier, but it was not researched widely until the mid-1990s. Microneedles, as opposed to hypodermic needles, are more patient-friendly because they are painless and can be delivered by the patient. Microneedles are so small that they can administer practically any drug or small particle composition but are not lengthy enough to induce discomfort during administration [40].

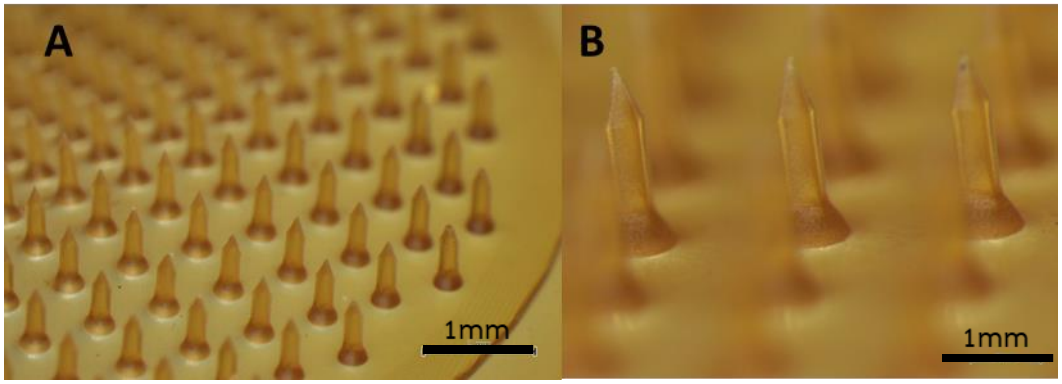


Figure 1. (A & B) Stereomicroscopic image of a dissolving microneedle array patch

1.2.3.1 Microneedle Classification

Microneedles can be generally categorized based on their delivery profile or the substance utilized in their production.

1.2.3.1.1 Based on Variations in Drug Delivery

Microneedles are characterized as solid, hollow, dissolving, or coated (Figure 2).

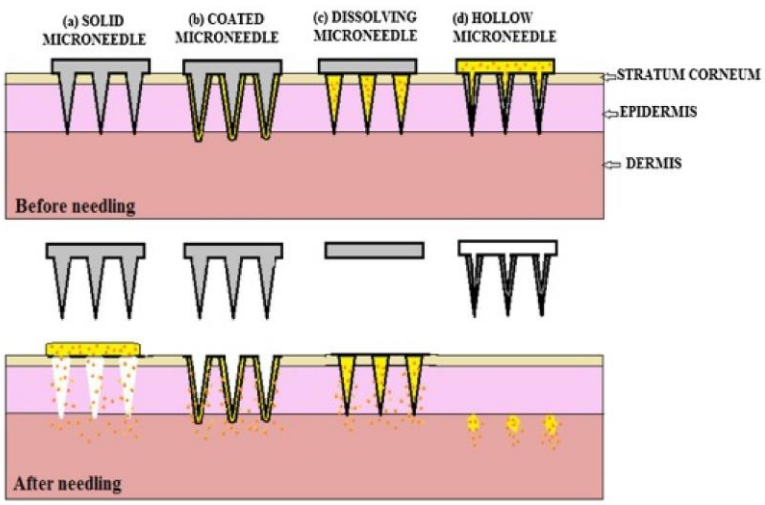


Figure 2. Types of microneedles [41].

1.2.3.1.1.1 Solid Microneedles

This microneedle construction is intended to penetrate the stratum corneum to promote medication administration to the dermis and kinetic transport across the skin [42]. Solid microneedles, which are inserted and removed to generate micron-scaled pores on the skin's surface, can be utilized as a skin pretreatment. As microchannels are formed, they operate on a 'poke and patch' basis. These microchannels improve medication permeability by allowing formulations to diffuse directly into the skin layer. Micropores created by microneedles persisted on rat skin for at least 72 hours following treatment when held under occlusive conditions, such as occlusive tape [42, 43]. The micropores closed quickly after microneedle administration in the absence of obstruction. The microchannels formed by solid MN healed quickly within 2 hours, ensuring the lack of subsequent infection [42]. The ‘scrape and patch' method is a variation on the traditional solid microneedle procedure in which microneedles, microprojections, or microblade are scraped across the skin to create micro-abrasions. On these microprojections, drug solutions contained within a patch are subsequently placed [44]. Another option is to use a roller with solid microneedles that pierce the stratum corneum many times as it spins on the skin. Based on this premise, commercially marketed Derma-rollers are utilized for skin pore opening therapy [34]. Ita et al. (2015) investigated the use of cylindrical surface Microneedle Systems to deliver medications for high blood

pressure. Transdermal flux values were found to rise 5 to 8 times after microneedle roller treatment of porcine skin [45].

1.2.3.1.1.2 Hollow Microneedles

Hollow MNs have been produced using ceramics, metal, silicon, and glass, with an empty cavity inside each needle and a bore on the needle tip. As a result, medication solutions in microvolumes can be injected into the skin [46]. The most significant advantages of this type of MN are that it has a greater drug delivery capability than solid, coated, and dissolving MN arrays [39], where chemicals, proteins, vaccines, and oligonucleotides can be administered through the skin [47, 48]. In terms of restrictions, it has been noted that blocking the bore in needle tips with skin tissue during insertion. This problem, however, can be overcome by retracting the MN array and/or positioning the bore on the side of MN. Furthermore, precise manufacturing processes such as lithography, etching, microelectromechanical system (MEMS), and 3D printing are required for the fabrication of hollow MN arrays (Figure 4) [49].

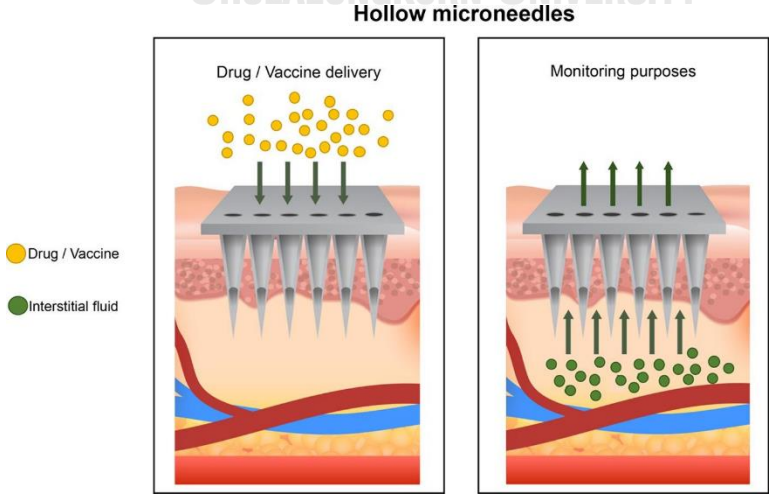


Figure 3. Hollow microneedles [49].

1.2.3.1.1.3 Coated Microneedles

A coated microneedle is made up of a sharp, solid-core microneedle structure with a solid film coating that contains the active ingredient and water-soluble inactive excipients [50]. The water-soluble excipients not only support with the microneedle coating process, but they also catalyze the film's removal from the microneedle surface. When a drug-coated microneedle gets inserted into the skin, the coating comes into contact with the interstitial fluid. Contact with these aqueous medium aids in the dissolution of the water-soluble excipients in the microneedle coating, causing the coating to separate from the microneedle surface. It is only crucial that the coating separate from the microneedle surface before the microneedles are withdrawn from the skin; the substance left behind can dissolve completely over time. Coated microneedles can be utilized to deliver coated payloads to tissues along with skin (Figure 4) [51]. Ma et al. (2018) coated solid MN with dispersed lidocaine in a polyethylene glycol matrix and found that it delivered significantly more than a commercial product [52].

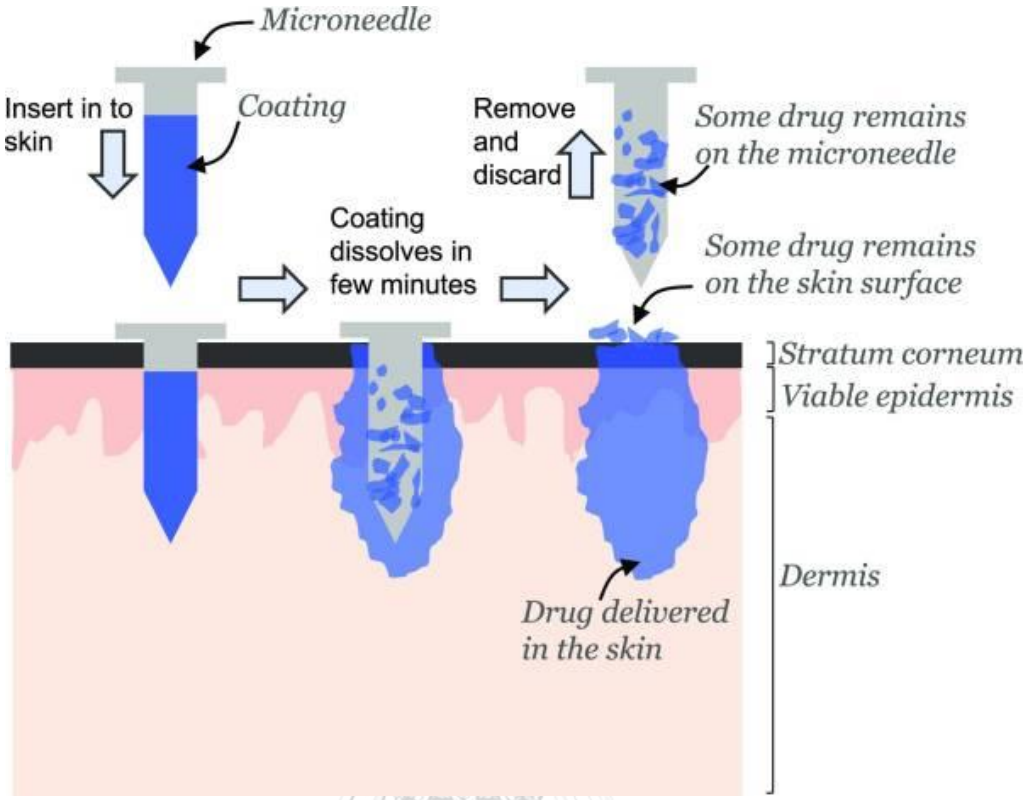


Figure 4. Schematic of coated microneedles principal [51].

1.2.3.1.1.4 Dissolving Microneedles DMs

Dissolving microneedles have recently been tested for noninvasive transdermal immunization, patient monitoring, and diagnostic applications [53, 54]. Dissolving microneedles are made by encapsulating the medication in biodegradable polymers. After piercing the stratum corneum, the polymer that makes up the needle shape degrades, releasing the incorporated medication (Figure 5). Due to the nature of their method of action, dissolving microneedles can solve a number of the problems associated with solid microneedles since they require no more manipulation after insertion. The advantage of making microneedles dissolve beneath the skin is that it essentially lowers the possibility of injuries from needle

sticks after application [55]. Maltose, polyvinylpyrrolidone, chondroitin sulfate, dextran, hyaluronic acid, and albumin are examples of water-soluble materials used to make DMs, which can be applied to the skin with a finger to transport medication molecules [54]. The materials that are utilized to make dissolving microneedles are inexpensive, readily accessible, and don't require difficult processing conditions like high temperatures [56]. They dissolve fully in the skin and don't leave any biohazardous sharps tips behind after use because they are created from biocompatible and water-soluble components like sugars and cellulose derivatives [57]. When DMs penetrate tissues in the body, they typically become softer and dissolve, avoiding injury from the mechanical forces of application [58]. In comparison to silicon and metal needles, dissolving microneedles are more beneficial. *In vivo* breakage of silicon and metal microneedles is possible [59]. DMs are also effective because they are created to deliver a variety of medications, are simple to use, and are inexpensive [60]. Detachable dissolvable microneedles (DDMNs), which provide fast (1-2 min) detachment of needles from the base by fabrication of DMNs with water penetrable layer, have also been developed and produced by our lab [61].

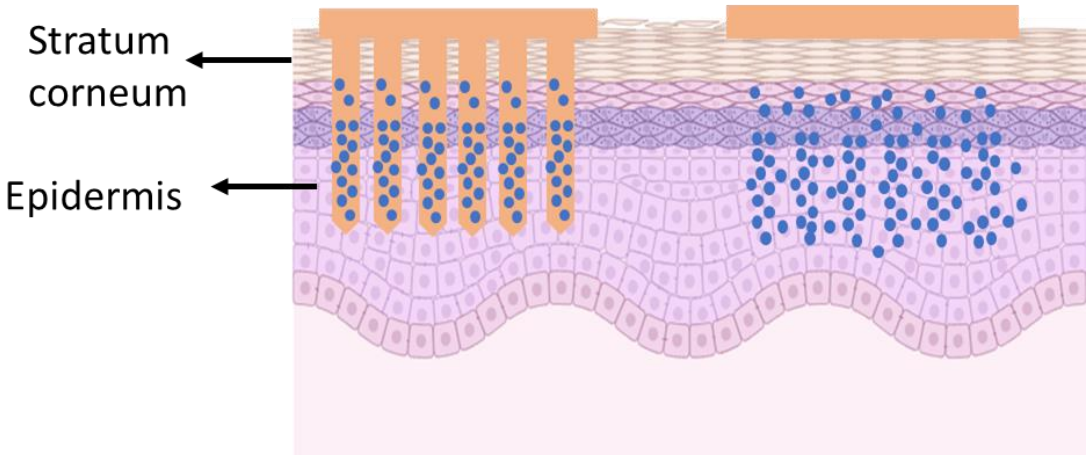


Figure 5. Schematic of dissolvable microneedles principal

1.2.4 Chitosan CS

Chitosan is a linear polysaccharide composed of randomly distributed β-(1⟶4)-linked D-glucosamine (Figure 6). Chitosan is insoluble in water at neutral and basic pH but it is soluble in acidic conditions. Acetic acid (AA) and trifluoroacetic acid (TFA) have been used to dissolve chitosan [15, 22, 62, 63]. In another method, Xie, H. et al. dissolved chitosan by using ionic liquid 1-butyl-3-methyl-imidazolium chloride ([Bmim]Cl) as a solvent. They found that the ionic liquid cannot entirely break down the crystalline domains of chitosan and can only produce a partly dissolved solution, this does not affect its usage [64]. It is still difficult to degrade insoluble natural polysaccharides with highly organized structures, such as cellulose and chitin. It has been observed that chitosan can be hydrolyzed enzymatically by amylases, hemicellulases, pectinase, and hyaluronidase. Although these enzymes have been proposed to produce low-molecular-weight chitosan LMWC and chitooligosaccharides COS from chitosan, little is known about their mechanism of action [20]. E.I. Kulish at.al. studied the degradation of chitosan in tissue by hyaluronidase. This enzyme has been used to degrade hyaluronic acid in tissue. It

cleavages the glycoside bond. Chitosan and hyaluronic acid have similar structure therefore hyaluronidase mediates the enzymatic degradation of chitosan [21].

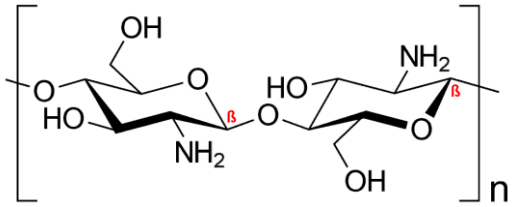


Figure 6. Chemical structure of chitosan [65]

Numerous enzymatic degradation investigations have discovered evidence that the order of glucosamine and N-acetyl glucosamine units, or the block or random distribution of polymeric units, affects lysozyme breakdown (Figure 7) [66].

Figure 7. Reaction mechanism of lysozyme breaking $\beta(1\rightarrow4)$ *glycosidic bonds in polymeric chitosan.*

Chitosan's degradation is influenced by its molecular weight and level of deacetylation. N-acetyl-D-glucosaminidase first breaks down low- and high-molecular-weight chitosan down to monomers, which is followed by renal clearance. While the high molecular weight chitosan is broken down into tiny pieces by proteases, it also travels through renal clearance. According to in vivo research, chitosan breaks down into straightforward, non-toxic components like oligosaccharides that are simple to get rid of (Figure 8) [22].

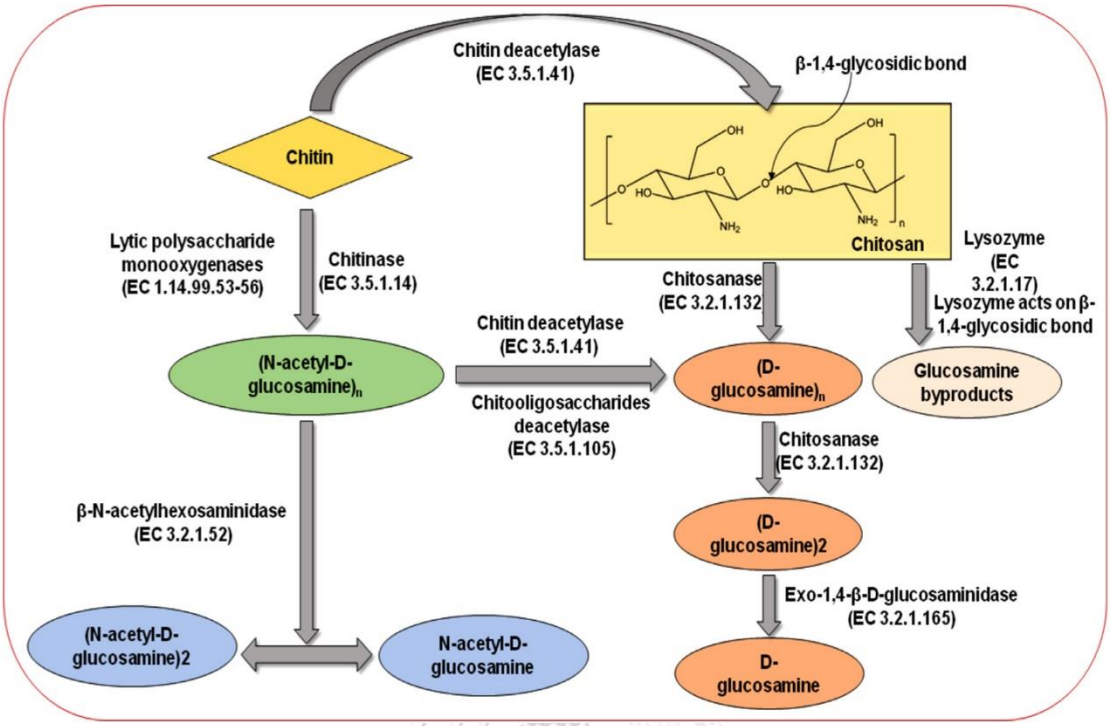


Figure 8. Enzymatic degradation pathway for chitosan: chitosan is obtained from chitin deacetylation by the enzyme chitin deacetylase. Chitin is also degraded by the enzymes chitinase and lytic polysaccharide monooxygenases to units of N-acetyl-D-glucosamine, which are further degraded by chitin deacetylase and chitooligosaccharides deacetylase to D-glucosamine units. Majority of the chitosan is degraded by lysozyme to glucosamine byproducts. In a parallel pathway, chitosan is also degraded by chitosanase and exo-1,4-β-D-glucosaminidase to glucosamine byproducts [22].

1.2.4.1 Chitosan Microneedles Literature Review

D.A. Castilla-Casadiego, et al. fabricated chitosan microneedles to deliver meloxicam. Chitosan was dissolved in an acidic medium using acetic acid at different concentrations of 90, 50, and 10% (v/v). 1 gram of chitosan has been dissolved with the previous concentration of acetic acid. Then chitosan has been mixed with meloxicam to fabricate microneedle. They found that One patch released a drug

concentration of 3.57 × 10^{-5} Mol/M^3 in the skin per week, which represents 26.2% of what is needed for pain management in cattle, established as 1.43 × 10−4 mol/m^3 [67].

In their paper in 2022 C. Ryall et al. fabricated chitosan-PVA hydrogel microneedles for dermal delivery of Centella Asiatica. Two types of microneedles have been fabricated: type I consists of (12% w/w PVA and 0.4% w/w chitosan) and type II consists of (2% w/w chitosan and 11% w/w PVP). From the optimization of the microneedles, CS/PVP patches showed the most desirable characteristics. They found that the mean percentage of drugs released by Type I microneedles was 51.12%; in contrast, the mean percentage of drugs released by Type II microneedles was 25.60%. Both had favorable release characteristics, with an initial rapid release followed by a longer-lasting release over a period of 48h. Rapid release may quickly reach therapeutically efficacious levels for a topical administration device like microneedles [63].

In another paper, Yu-Hsiu Chiu et.al. fabricated a dissolvable microneedle consisting of hyaluronic acid HA and chitosan to deliver single-dose vaccination. They prepared two layers of dissolving microneedles. The first layer consists of HA and the second layer consists of chitosan. Results revealed that OVA produced from the HA tips disappeared from the insertion site as the FITC-OVA signal rapidly decreased and became undetectable within a week. Because HA is hydrophilic, it can easily dissolve in the skin and perhaps generate a thick HA gel where it is inserted. The Texas Red OVA's fluorescence intensity gradually diminished over the course of 28 days but

remained detectable, showing that the chitosan base can keep the antigen at the delivery site for up to 4 weeks of sustained antigen exposure. Fig.9. [15]. In their paper in 2019, Chandrasekharan, A. et.al. fabricated chitosan microneedles. The water-soluble chitosan was prepared by acid hydrolysis with trifluoroacetic acid (TFA). Overall, these results indicate that the chitosan MN demonstrated appropriate mechanical characteristics for skin insertion while also exhibiting delayed dissolving behavior in wet conditions. Chitosan MN patches loaded with rhodamine B, a model hydrophilic medication, had sustained release kinetics for more than 72 hours and were proven to be biocompatible [68].

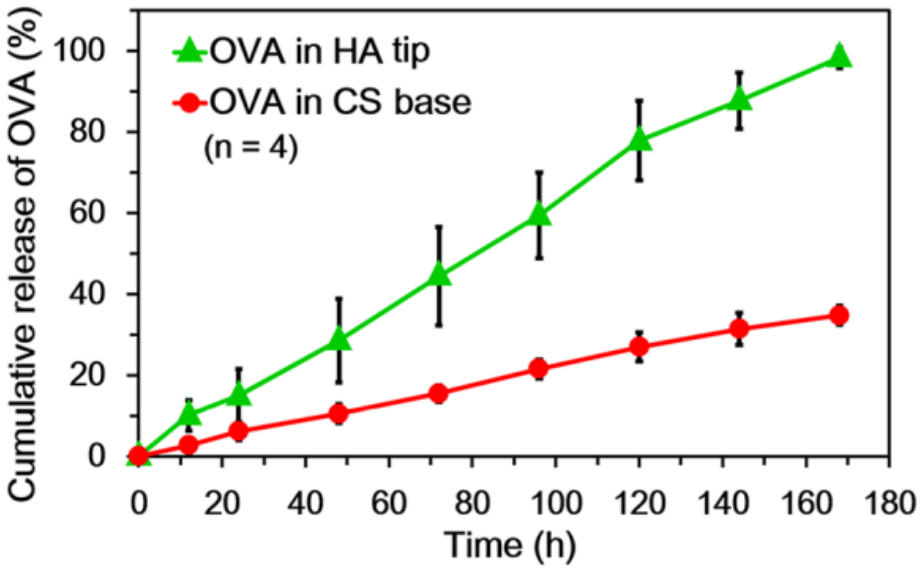


Figure 9. In vitro OVA release from the HA tip and the chitosan (CS) base [15]

1.3 Objective

This research focused on developing chitosan blended with PVP and PVA to fabricate microneedle patches for transdermal and intradermal drug delivery. Mechanical strength, and penetration ability (using *ex vivo* porcine ear skin) have been studied. Loading of hydrophobic and hydrophilic drug in the obtained microneedle and *In vitro* drug release also demonstrated.

2 CHAPTER II EXPERMINTAL

2.1 Materials and Chemicals

Chitosan (deacetylated 85%), MW 30,000 was purchased from Seafresh Chitosan (lab) Co. Ltd. Thailand; Poly (vinyl alcohol) (PVA), MW 95,000; Polyvinylpyrrolidone (PVP) MW 40,000 were purchased from Sigma Aldrich. Glacial Acetic Acid (A.A) 99% and Sodium Hydroxide of analytical reagent grade were purchased from Emsure Co. Curcumin MW 368.38 was purchased from ACROS Co. Butterfly pea powder HQ was purchased from Tipco, Thailand Biotech Co. Ethanol was purchased from Sigma Aldrich. Polydimethylsiloxane molds (PDMS): (1.6 cm in diameter, containing 277 needles arranged with a tip-to-tip distance of 1150 μm; each needle is nail-shaped with 350×350×360 μm^3 (W×L×H) square column and 500 μm high of the square pyramid on the top) Figure 10. The mechanical properties were measured by universal testing machine UTM, Shimadzu EZ-S, Shimadzu Corporation, Tokyo, Japan. The penetration ability was measured by using commercial porcine ear skin.

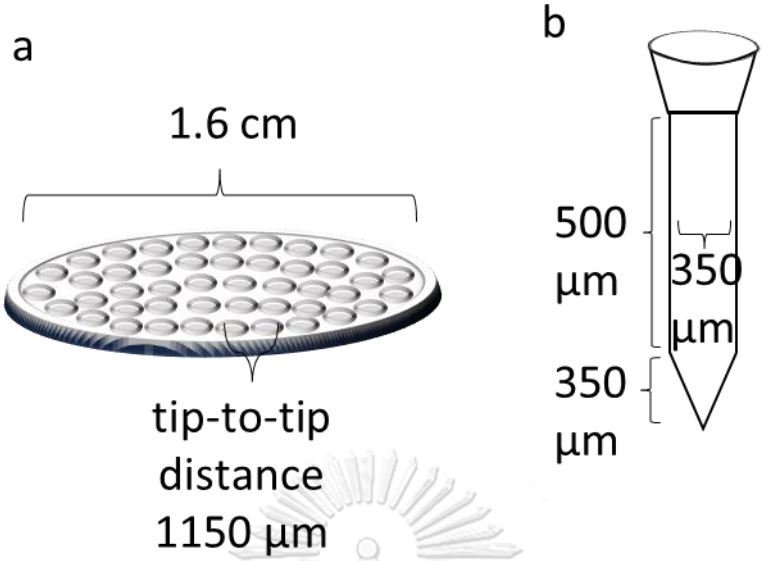


Figure 10. schematic diagram a) the dimension of the mold, b) dimension of the needle

2.2 Preparing the Microneedle Matrix

For the microneedle matrix, chitosan powder was dissolved in a 0.2 M aqueous solution of acetic acid to obtain a 3% (w/v) chitosan solution. The obtained viscous chitosan solution was then neutralized by adding drops of 0.5 M NaOH after that dialyzed at room temperature against deionized (DI) water with several water exchanges to remove excess acetic acid and salts (final pH approximately 5).

2.3 Fabrication of Chitosan- PAA, HA, PVA, PVP, Trehalose and Sucrose

6% (w/v) of chitosan solution mixed with 9% (w/v) of polymers and sugars. The mixing of chitosan solution with polymers and sugars is shown in Table 1.

Table 1: the amount of materials for the preparation of chitosan/ polymers and sugars solution

Formations	CS: polymers & sugars	mg of materials	
		CS	Polymers and sugars
1	1:1	45	45
2	2:1	60	30
3	3:1	67.5	22.5

The fabricated needles touched by fingers to feel the needles and assess the initial strength then observed by stereomicroscope to see the physical appearance.

2.4 Studying Mechanical Property

Mechanical compression tests were performed using a UTM. A microneedle array was placed on a stainless-steel base plate's flat inflexible surface. An axial force was applied by a moving sensor mount, perpendicular to the axis of the microneedle array, at a constant speed of 66 mm/min [69]. The initial distance from the tips of the microneedle arrays to the mount was set at 1 cm. The force was measured when the moving sensor touched the uppermost point of the microneedle array. The testing machine subsequently recorded the force required to move the mount as a function of microneedle displacement.

2.5 Drug-loading into Chitosan Microneedles

Curcumin was dissolved with ethanol to make 3% w/v as a stock solution and mixed with CS: PVP and CS: PVA formulations to make 13% w/w as a maximum

capacity of loading. Butterfly pea was dissolved with water to make 15% w/v as a stock solution mixed with CS: PVP and CS: PVA formulations to make 33% w/w as a maximum capacity of loading.

2.6 Skin Penetration (*ex vivo*) and Penetration Efficiency

The microneedle patches with drugs and without drugs were applied onto the porcine skin. The porcine skin is sliced, and the depth of the needles observed under the stereomicroscope. The height of the needles in the patch and the depth of the needles in porcine skin have been measured to evaluate the penetration efficiency. 102 patches contain 2040 needles have been studied for this experiment. The results were analyzed with Excel and GraphPad Prism program. The penetration efficiency was approved by measuring the p-value with ANOVA: single factor.

2.7 Drug Release *in vitro*

Briefly, needles with drugs were immersed in 100 ml PBS (pH 7.4) and a water jacket was used to control temperature at 37 $^{\circ}$C. The drug solution released was acquired at predetermined time intervals (0, 0.25, 0.5, 01, 02, 04, 08, 12, 24, and 48 hours) through the sampling port and then measured using a UV machine where curcumin absorb at 425 nm and butterfly pea 575 nm

2.8 Statistical Analysis

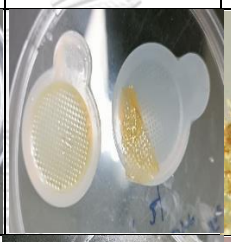
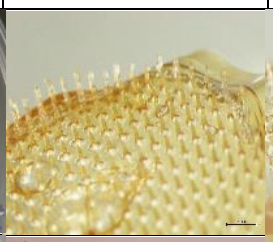
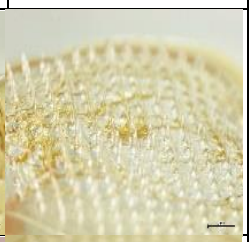
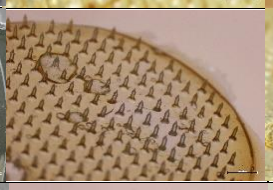
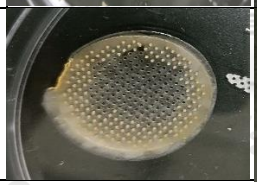
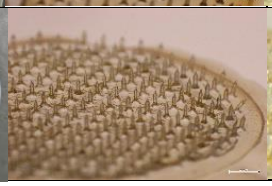
A comparison between the two groups was performed using ANOVA: single factor using statistical software (Graph Pad Prism 9). Data presented as mean ± SD. A difference of $P < 0.05$ was considered statistically significant.

3 CHAPTER III RESULTS and DISCUSSION

3.1 Fabrication of Chitosan: PAA, HA, PVA, PVP, Trehalose and Sucrose

1- Chitosan with trehalose, sucrose, PAA, and HA

Table 2. the needles images of mixing chitosan with trehalose, sucrose, PAA, and HA

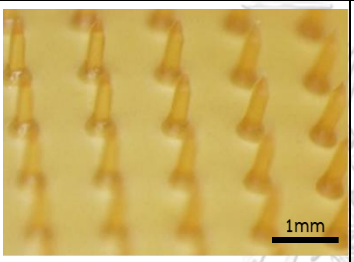
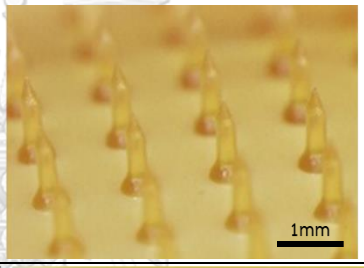
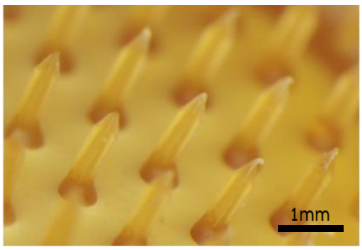
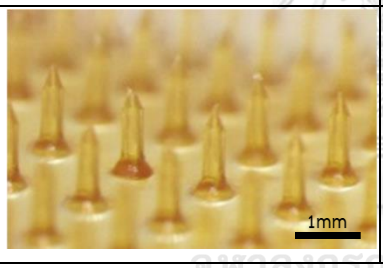
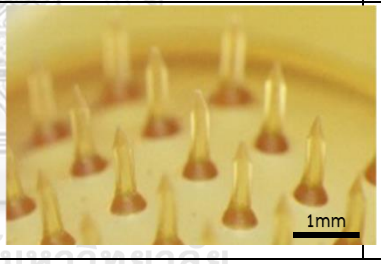
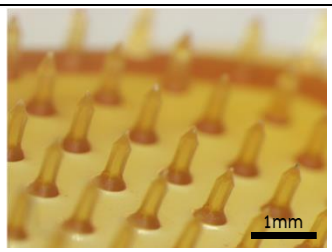
Formation	CS: polymers & sugars	Trehalose	Sucrose	PAA	HA
1	1:2				
2	1:1				
3	2:1				

Trehalose and sucrose have been mixed with chitosan individually. The microneedle arrays showed weak, unpeeled, and high moisture needles Table 2. That was attributable to the hygroscopic nature of sugars. HA and PAA blended with chitosan showed a precipitate due to the difference of pka value at pH 5.0.

2- Chitosan with PVA and PVP

PVP, PVA was mixed with chitosan in different ratios CS: PVA 1:1, 2:1, and 3:1 (mentioned in Table 3). Under the stereomicroscope, the arrays showed that the needles were un-hollow, fulfilled and met the desired criteria. PVA and PVP with chitosan are chosen for further investigation.

Table 3. stereomicroscopic images of mixing chitosan with PVP and PVA

Formulations	1:1	2:1	3:1
CS: PVP			
CS: PVA			

In the case of sugars, the results showed brittle needles when the chitosan was the major component and sticky when the sugars were the major component. The increased brittleness of microneedles, when more chitosan is added, suggests that chitosan has a significant impact on the mechanical properties of the microneedles. Chitosan is a natural polymer derived from chitin and is known for its rigidity and strength. Therefore, an increase in chitosan content can make the microneedles more brittle. The observation that microneedles became sticky when

more sugars (sucrose and trehalose) were added suggests that sugars have a plasticizing effect on the chitosan matrix. Sugar molecules are known to increase polymers' flexibility and water absorption capacity. This can lead to increased stickiness, especially in humid environments. The stickiness of microneedles could impact their handling and storage. It might also affect their ability to pierce the skin effectively if they adhere to the skin surface.

Blending chitosan with PVA and PVP showed good results. The increased presence of chitosan in microneedle formulation likely played a significant role in the transition from hollow to solid microneedles. Chitosan is known for its structural integrity and rigidity. This transition may have occurred due to chitosan's ability to form a solid matrix when it interacts with the other polymers (PVA and PVP) in the formulations. Pure PVP microneedles have been fabricated as a control condition. However, a notable issue was encountered during the fabrication process. The microneedles were prone to breaking easily. Additionally, the microneedles faced difficulties in detaching from the mold, possibly due to their brittle nature.

3.2 Drug-loading and Skin Penetration (*ex vivo)*

Two different drug models have been uploaded into the chitosan DMNs and the penetration to the porcine skin has been studied to evaluate the penetration efficiency and to study the effect of drug added on the penetration efficiency (Table 4)

3.2.1 Chitosan Microneedles with Curcumin

Curcumin was loaded as a hydrophobic drug model. It loaded to the formulation of chitosan: PVP with a maximum loading capacity of 13% w/w Figure. 11a. On the other hand, the formulation of chitosan: PVA didn't encapsulate curcumin. Fig. 11b. Pure chitosan encapsulates curcumin Fig. 11c.

The results showed that the chitosan: PVP formulation can encapsulate curcumin successfully. This formulation's maximum loading capacity achieved for curcumin is 13% w/w, as shown in Fig. 11a. This suggests that the chitosan: PVP combination is suitable for the encapsulation of hydrophobic drugs like curcumin. However, chitosan: PVA formulation did not successfully encapsulate curcumin. This means that the combination of chitosan and PVA may not be suitable for encapsulating hydrophobic drugs like curcumin under the conditions or methods used in this study regarding to the polarity of blended polymers and curcumin. Interestingly, the results also reveal that pure chitosan, without the addition of PVP or PVA, was able to encapsulate curcumin successfully. This finding suggests that chitosan alone can encapsulate hydrophobic drugs like curcumin, which could be valuable information for drug delivery applications.

Table 4. the effect of penetration efficiency after loaded drug models to the needles

Formulations Wt. CS: wt. polymers	Drug loading and penetration efficiency	
	Curcumin w/w 13 %	Butterfly pea w/w 32%
CS	Curcumin enhanced the penetration	Good penetration
1 CS: 1 PVP	Curcumin enhanced the penetration	Good penetration
2 CS: 1 PVP	Curcumin enhanced the penetration	Good penetration
3 CS: 1 PVP	Curcumin enhanced the penetration	Good penetration
1 CS: 1 PVA	-	Good penetration
2 CS: 1 PVA	-	Good penetration
3 CS: 1 PVA	-	Good penetration

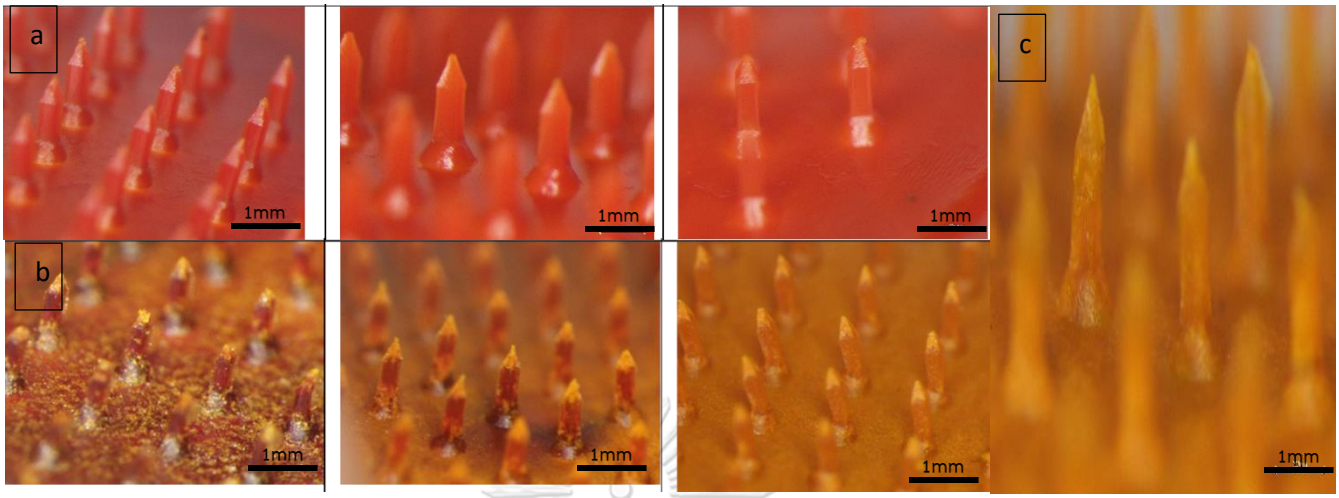


Figure 11. Stereomicroscopic images a) curcumin loaded to chitosan: PVP formulations from the left (ratios 1:1 CS: PVP, 2:1 CS: PVP, 3:1 CS: PVP), b) curcumin loaded to chitosan: PVA formulations from the left (ratios 1:1 CS: PVA, 2:1 CS: PVA, 3:1 CS: PVA), c) curcumin loaded to pure chitosan formulation.

3.2.2 Chitosan Microneedles with Butterfly pea extract

Butterfly pea extract was loaded as a hydrophilic drug model. It loaded to the formulation of chitosan: PVP, chitosan: PVA, and pure chitosan with a maximum loading capacity of 32% w/w Fig. 12. The results demonstrate that the chitosan: PVP formulation is capable of encapsulating butterfly pea extract effectively. The maximum loading capacity achieved is 32% w/w. Similar to the chitosan: PVP formulation, the results indicated that the chitosan: PVA formulation also successfully encapsulated butterfly pea extract with a maximum loading capacity of 32% w/w. This suggests that both chitosan-based formulations can effectively encapsulate hydrophilic drugs. Interestingly, the results reveal that pure chitosan,

without the addition of PVP or PVA, was able to encapsulate butterfly pea extract effectively with a maximum loading capacity of 32% w/w. This suggests that chitosan alone is a suitable carrier for hydrophilic drug models like butterfly pea extract.

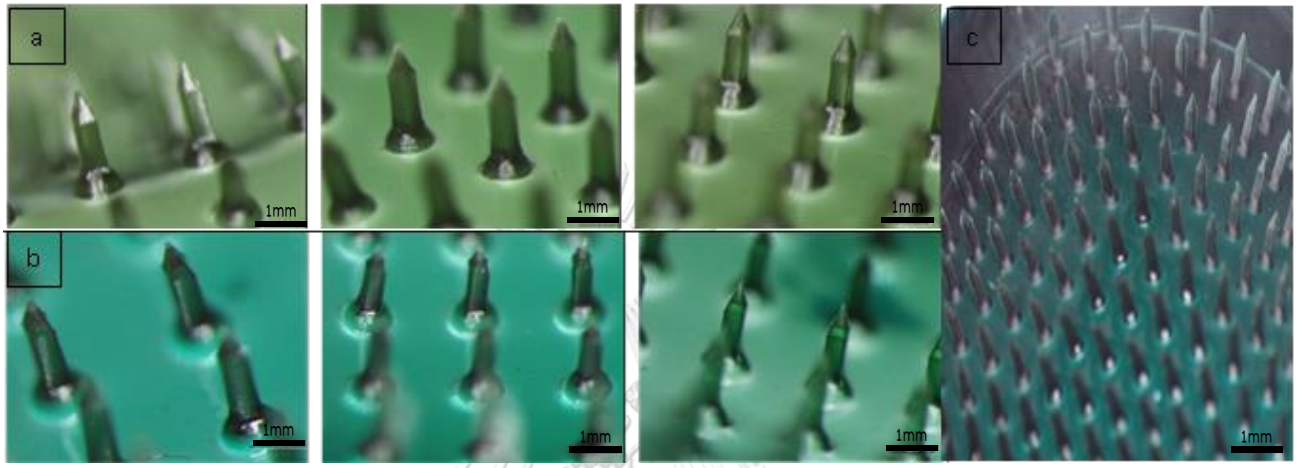
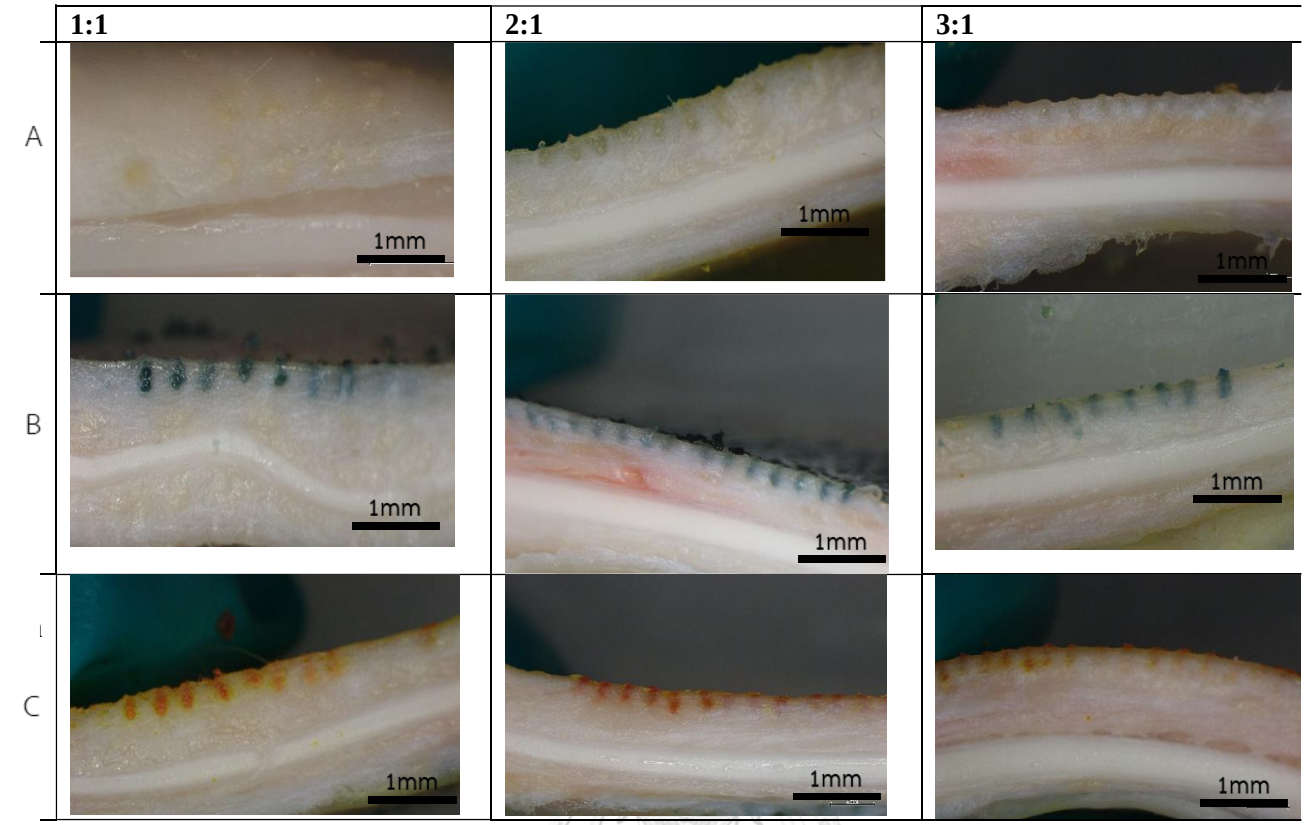


Figure 12. Stereomicroscopic images a) butterfly pea loaded to chitosan: PVP formulations from the left (ratios 1:1 CS: PVP, 2:1 CS: PVP, 3:1 CS: PVP), b) butterfly pea loaded to chitosan: PVA formulations from the left(ratios 1:1 CS: PVA, 2:1 CS: PVA, 3:1 CS: PVA) , c) butterfly pea loaded to pure chitosan formulation.

3.2.3 Skin Penetration (*ex vivo*) and Penetration Efficiency

Besides mechanical property tests, we tested the insertion ability of the DMNs in porcine skin *ex vivo*. The needles successfully penetrate the porcine skin (Figure. 13) with different depths due to the different formulas and ratios except pure PVP formulation; the needles dissolve on the pig surface skin (Figure.14) The experiment successfully demonstrated that the needles penetrated the porcine skin to different depths. This variability in penetration depth suggests that the composition of the needles, as well as the ratios of the components in the patches, have an impact on

how deeply the needles can penetrate the skin. The results indicated that the depth of the whole needles in all the ratios is more than 600 μm which is good enough to penetrate the stratum corneum reaching the epidermis layer[70, 71]. The statistical analysis (Figure. 15) approved that adding butterfly pea extract as a hydrophilic drug model didn't significantly affect the depth of the needles compared with adding curcumin. Interestingly, pure chitosan showed no significant differences in depth with either butterfly pea or curcumin. It's important to note that the length of needles on the patches was found to be equal across all formulations. This consistency in needle length suggests that the fabrication process was successful and that any variations in drug delivery depth can be attributed to the choice of polymers and drug models rather than the physical characteristics of the needles themselves.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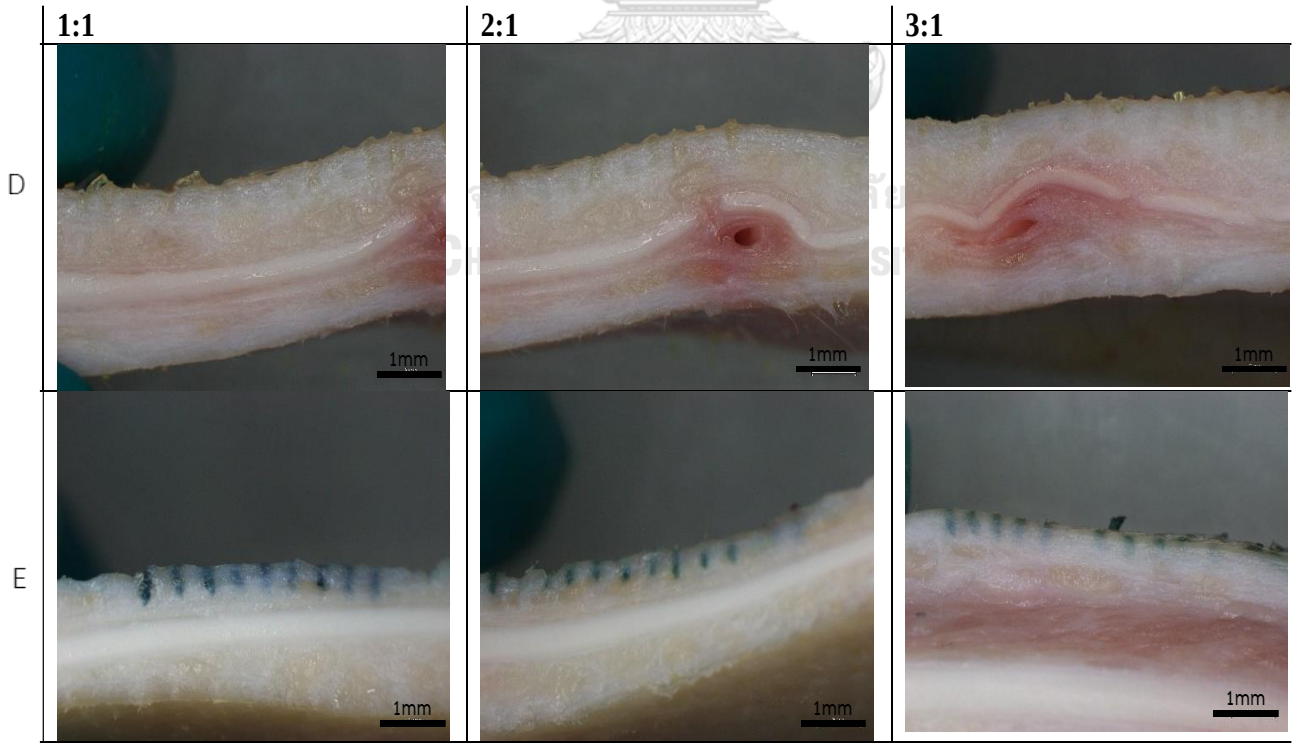
1:1
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E
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F

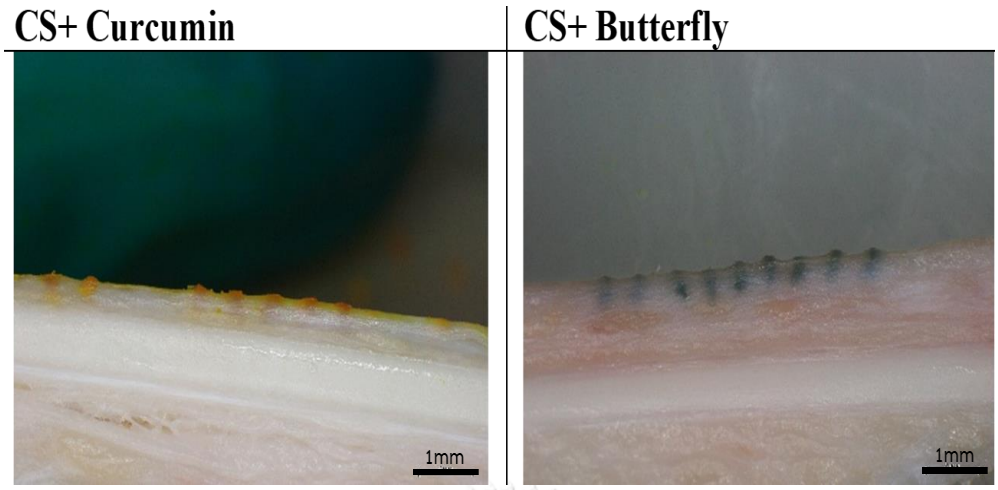

Figure 13. Ex-vivo microneedle insertion tests on ear porcine skin; cross-section images of the porcine skin after curcumin and butterfly staining. A) chitosan- PVP pure (no drug model), B) chitosan- PVP- butterfly, C) chitosan- PVP-curcumin (ratios 1:1 CS: PVP, 2:1 CS: PVP, 3:1 CS: PVP), D) chitosan- PVA pure (no drug model), E) chitosan- PVA- butterfly (ratios 1:1 CS: PVA, 2:1 CS: PVA, 3:1 CS: PVA) , and F) pure chitosan- curcumin and pure chitosan- butterfly

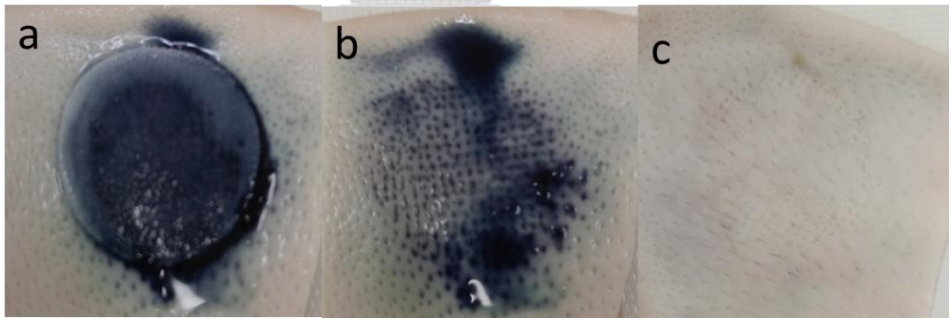

Figure 14. Ex-vivo microneedle insertion tests on ear porcine skin images of pure PVP formulation a) during the applicating the patch on the ear porcine skin, b) after detaching the patch, c) after swapping the color from the ear porcine skin surface.

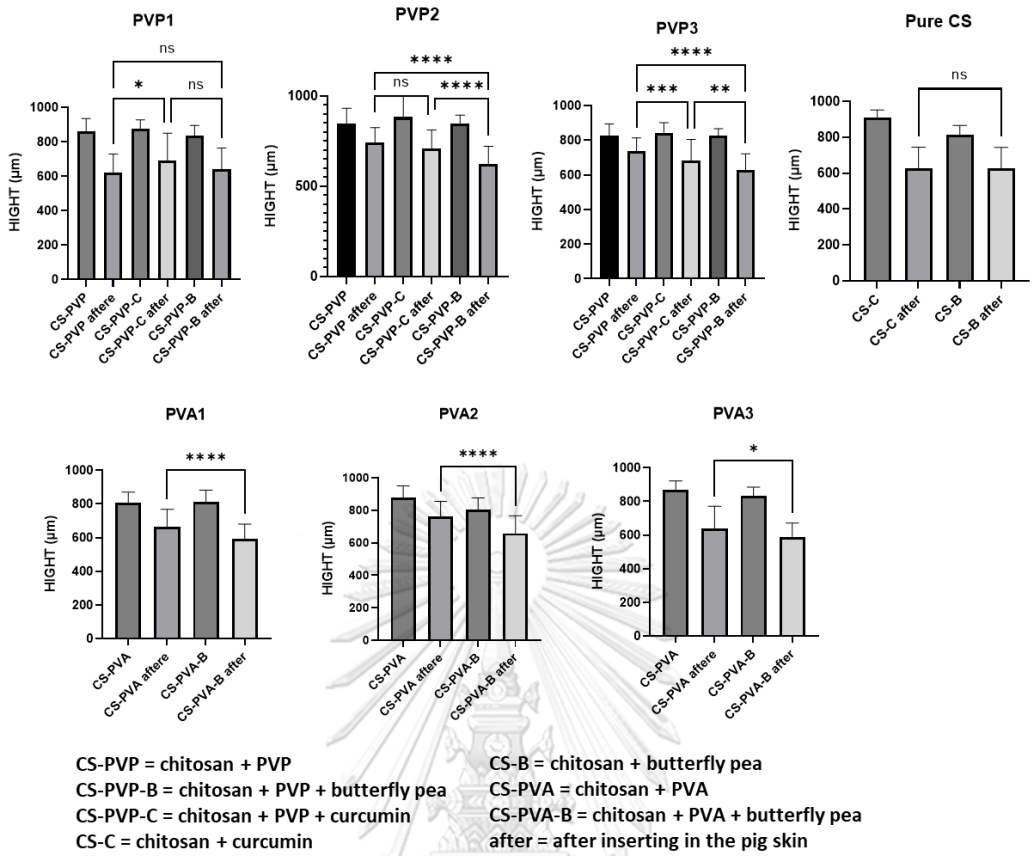

Figure 15. Statistical analysis of the needles' height in the patches and the needles' depth in porcine skin (PVP1 1:1 CS: PVP, PVP2 2:1 CS: PVP, PVP3 3:1 CS: PVP), (PVA1 1:1 CS: PVA, PVA2 2:1 CS: PVA, PVA3 3:1 CS: PVA), and pure CS (only chitosan with drugs, no polymers blended with chitosan). p-value control using ANOVA-single factor data analysis, <0.05 is considered statistically significant.

Chitosan with PVP showed fewer significant differences compared to chitosan with PVA. This could indicate that the combination of chitosan and PVP has properties that allow for more consistent needle penetration depths. Curcumin needles penetrated deeper into the porcine skin compared to butterfly pea needles. This suggests that the choice of the drug model can influence the penetration depth, which may be due to differences in the properties of these drugs, such as their

solubility or interaction with the skin. Both hydrophobic (curcumin) and hydrophilic (butterfly pea) drug models exhibited differences in needle penetration depth. However, the differences with butterfly pea needles were less significant compared to curcumin needles. Curcumin needles penetrated deeper into the porcine skin compared to butterfly pea needles. This suggests that the choice of the drug model can influence the penetration depth, which may be due to differences in the properties of these drugs, such as their solubility or interaction with the skin. When using pure chitosan, regardless of the drug model, there were no significant differences compared to the chitosan with polymers. However, the depth of penetration was less than that of chitosan with polymers. This suggests that the presence of polymers in the formulation may enhance the penetration of the needles.

3.3 Mechanical Strength

To overcome the barrier of the skin and deliver the drug efficiently into the skin, the mechanical property and the insertion ability are critical for DMNs. In brief, the DMN sample fixed on the platform was pressed slowly by the vertical sensor. The varying force and displacement were recorded during the test to obtain the mechanical curves. As shown in Figure. 16, all microneedles exhibited almost the same mechanical strength. It was clear that all of the formulations show reduction and deformation in the vertical direction but no bending or fracture after compression with a maximum force (150 N/needle). This result contributes to the

overall understanding of microneedle technology and its potential for various medical and pharmaceutical applications.

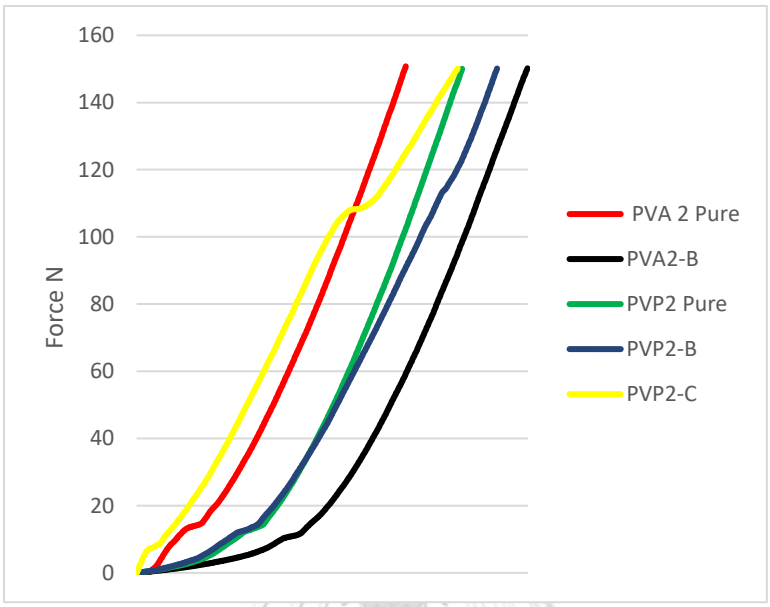

Figure 16. Mechanical characteristics of PVA 2 pure (2:1 chitosan: PVA), PVA2-B (2:1 chitosan: PVA with butterfly pea), PVP2 pure (2:1 chitosan: PVP), PVP2-B (2:1 chitosan: PVP with butterfly pea), and PVP2-C (2:1 chitosan: PVP with curcumin) microneedle patches.

3.4 Drug Release *in vitro*

Chitosan sustains the release of butterfly (hydrophilic drug model) where around 38 % w/v has been released after 2 days from pure chitosan. Chitosan blended with PVA showed faster drug release with increasing concentrations of PVA in the needles around 49%, 47%, and 46% w/v 1:1. 2:1, 3:1 CS: PVA respectively released after 2 days. the drug release decreased with the concentration of PVA. PVP blended with chitosan showed the fastest drug release 69%, 67%, and 48% w/v,

from the ratio CS: PVP 1:1, 2:1, 3:1 respectively where the high concentration of PVP. CS: PVP showed faster drug release than CS: PVA. Figure. 17.

The initial observation that pure chitosan sustained drug release aligns with the known properties of chitosan as a biocompatible and hydrophilic polymer. It offers a controlled release environment for hydrophilic drugs [17]. PVA has a manageable effect on the controlled release behavior of chitosan. The trend of decreasing drug release with decreasing PVA concentration may be due to PVA's hydrophilic nature, which can enhance drug diffusion and release from the microneedles [9]. PVP is known for its solubilizing properties and ability to enhance drug release. The high drug release observed with CS: PVP blends, especially at the 1:1 ratio, indicates a strong influence of PVP on accelerating drug release [72]. CS: PVP blends outperform CS: PVA blends in terms of drug release rate. This could be attributed to the distinct properties of PVP, which promotes rapid drug dissolution and diffusion [73].

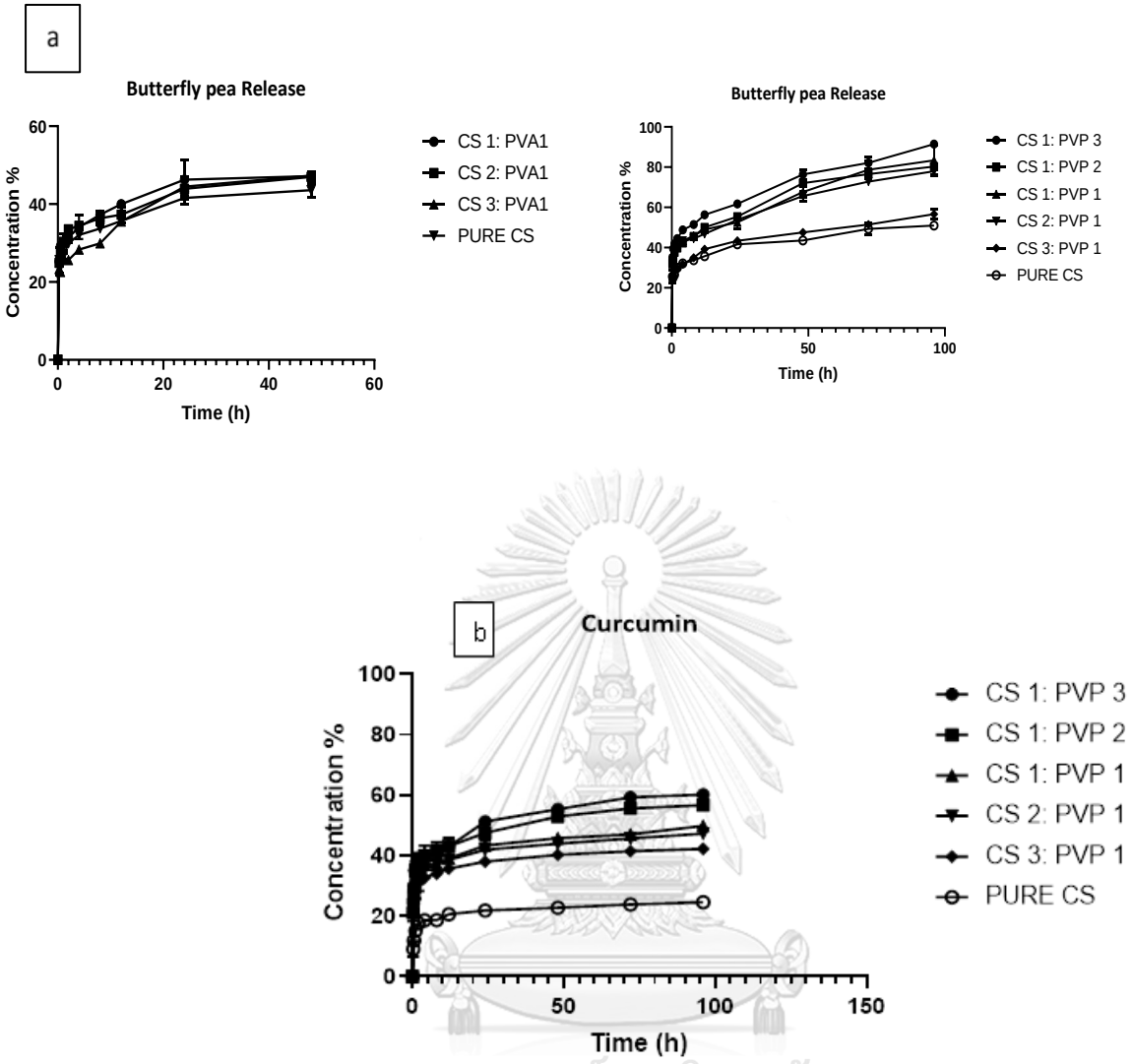

Figure 17. In vitro release profile of a) butterfly pea extract in CS: PVA (PVA1 1:1, PVA2 2:1, PVA3 3:1) and CS: PVP (PVP1 1:1, PVP2 2:1, PVP3 3:1), b) curcumin in CS: PVP (PVP1 1:1, PVP2 2:1, PVP3 3:1) patches

4 CHAPTER IV CONCLUSION

The incorporation of chitosan into blends with PVA and PVP in varying ratios has proven to be a promising approach for tunable drug release systems. The formulations showed outstanding mechanical properties. Furthermore, the penetration of these formulations through porcine ear skin demonstrates their potential for intradermal and transdermal drug delivery. The noteworthy finding in this study is the variation in drug release kinetics observed between chitosan-PVA and chitosan-PVP blends. After four days of testing, chitosan blended with PVP exhibited a notably faster drug release rate compared to chitosan-PVA blends. This suggests that the choice of polymer partner plays a crucial role in fine-tuning drug release profiles. These results underscore the importance of carefully selecting and optimizing the polymer blend composition to achieve the desired drug release characteristics. The versatility of chitosan as a biomaterial, when combined with appropriate polymers, opens exciting possibilities for tailoring drug delivery systems to meet specific therapeutic requirements. Further research in this field could explore the potential applications of these formulations in controlled drug release for various medical scenarios.

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REFERENCES

VITA

NAME	FUAD ABDULLAH FARHAN SALEH
DATE OF BIRTH	31 December 1993
PLACE OF BIRTH	Taiz, Yemen
INSTITUTIONS ATTENDED	Bachelor's Degree of Applied Science in Industrial Chemistry, Taiz University, 2018

REFERENCES

VITA

NAME	FUAD ABDULLAH FARHAN SALEH
DATE OF BIRTH	31 December 1993
PLACE OF BIRTH	Taiz, Yemen
INSTITUTIONS ATTENDED	Bachelor's Degree of Applied Science in Industrial Chemistry, Taiz University, 2018