INVENTION AND EVALUATION OF CAPSAICIN TRANSDERMAL NANOFIBERS PATCH FOR PAIN RELIEF: *IN VITRO* STUDY

CHUTHARAT THANCHONNANG

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การประดิษฐ์และการประเมินประสิทธิภาพแผ่นแปะเส้นใยนาโนที่มีส่วนผสมของแคปไซซินสำหรับบรรเทาอาการปวด: การศึกษาในหลอดทดลอง

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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาปรัชญาดุษฎีบัณฑิต
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มหาวิทยาลัยเทคโนโลยีสุรนารี
ปีการศึกษา 2567

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Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

Thesis Examining Committee

.....

(Dr. Natthawut Charoenphon)

Chairperson

.....

(Dr. Wiwat Nuansing)

Member (Thesis Advisor)

.....

(Assoc. Prof. Schawanya Rattanapitoon, M.D.)

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and Quality Assurance

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(Assoc. Prof. Sutham Pincharoen, M.D.)

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คำสำคัญ : แคปไซซิน, แผ่นแปะเส้นใยนาโน, อิเล็กโตรสปินนิ่ง, อาการปวด, การศึกษาในหลอดทดลอง, สารต้านการอักเสบ

แคปไซซิน (Capsaicin; 8-methyl-N-vanillyl-trans-6-nonenamide) ซึ่งเป็นสารออกฤทธิ์ชีวภาพสำคัญที่พบในพริก (*Capsicum spp.*) เป็นที่ยอมรับอย่างกว้างขวางในด้านคุณสมบัติในการบรรเทาอาการปวดและต้านการอักเสบ โดยแม้จะมีการใช้มาอย่างยาวนานในทางการแพทย์แผนโบราณและได้รับการรับรองให้ใช้ภายนอกในการรักษาอาการปวดจากระบบประสาท (neuropathic pain) หลากหลายประเภท แต่การประยุกต์ใช้ทางคลินิกยังคงมีข้อจำกัดเนื่องจากความสามารถในการละลายน้ำต่ำและความเสี่ยงในการระคายเคืองผิวหนัง แคปไซซินออกฤทธิ์โดยการกระตุ้นและทำให้เกิดการเสื่อมความไวของตัวรับ TRPV1 (transient receptor potential vanilloid 1) ซึ่งอยู่บนเส้นประสาทรับความรู้สึกชนิด ซีไฟเบอร์ ส่งผลให้การส่งสัญญาณความปวดลดลง

การศึกษานี้มีวัตถุประสงค์เพื่อพัฒนาแผ่นแปะเส้นใยนาโนที่มีส่วนผสมของแคปไซซิน โดยใช้พอลิไวนิลแอลกอฮอล์และพอลิไวนิลไพร์โรลิโดน ผ่านกระบวนการอิเล็กโตรสปินนิ่ง รวมถึงศึกษากลไกการปลดปล่อยยาและลักษณะการซึมผ่านผิวหนังของแผ่นแปะนาโนไฟเบอร์บรรจุแคปไซซิน โดยใช้ผิวหนังเทียม Strat-M™ เป็นแบบจำลองการซึมผ่านทางผิวหนัง ซึ่งแคปไซซินในความเข้มข้น 0.1 mg/mL ถูกบรรจุอยู่ในเมทริกซ์พอลิเมอร์ชนิดไฮโดรฟิลิกที่ประกอบด้วย ใช้พอลิไวนิลแอลกอฮอล์และพอลิไวนิลไพร์โรลิโดน ผลการศึกษา พบว่า แผ่นแปะเส้นใยนาโนที่ได้มีลักษณะเรียบเนียน สม่ำเสมอ มีการเรียงตัวที่สม่ำเสมอ โดยมีขนาดเส้นผ่านศูนย์กลางเฉลี่ยอยู่ที่ 667 ± 19.5 นาโนเมตร การวิเคราะห์ด้วย Fourier-transform infrared (FT-IR) แสดงให้เห็นถึงการกักเก็บแคปไซซินไว้ในพอลิเมอร์ผ่านพันธะไฮโดรเจน โดยไม่มีการเสื่อมสลายทางเคมี การประเมินความเป็นพิษต่อเซลล์โดยใช้เซลล์ผิวหนังมนุษย์ชนิดไฟโบรบลาสต์ พบว่า แผ่นแปะมีความเข้ากันได้ทางชีวภาพสูง โดยเฉพาะในช่วงความเข้มข้นต่ำ (0.001–0.01 mg/mL) และมีค่า IC_{50} ประมาณ 20 mg/mL บ่งชี้ถึงขอบเขตความปลอดภัยที่กว้าง นอกจากนี้ แผ่นแปะยังสามารถลดการแสดงออกของยีน *COX-2* ได้

ถึงประมาณ 8.1 เท่าในเซลล์ที่ถูกกระตุ้นให้เกิดการอักเสบด้วย H_2O_2 และมีผลช่วยฟื้นฟูรูปร่างของเซลล์ที่ถูกทำลายจากภาวะเครียดออกซิเดชัน

การศึกษาการซึมผ่านทางผิวหนังโดยใช้ Franz diffusion cells และผิวหนังเทียม Strat-M™ แสดงให้เห็นว่าแคปไซซินถูกปลดปล่อยอย่างต่อเนื่องเป็นระยะเวลา 12 ชั่วโมง โดยมีค่าฟลักซ์ในภาวะคงตัว (J_{ss}) เท่ากับ 63.30 µg/cm²/hr ขณะที่ค่า permeability coefficient (K_p) ลดลงตามเวลา โดยเริ่มสูงสุดที่ 0.77 cm/hr ที่ชั่วโมงแรก และเข้าสู่ค่าคงที่ในช่วงประมาณ 0.05–0.07 cm/hr หลัง 6 ชั่วโมง ซึ่งสอดคล้องกับหลักการของกฎการแพร่ของฟิก (Fick's First Law of Diffusion) ภาพจาก FTIR imaging ยังแสดงให้เห็นถึงการแพร่ของแคปไซซินไปยังชั้นลึกของเยื่อเมมเบรนได้อย่างต่อเนื่อง จากชั้นผิวหนังกำพร้าลงไปยังชั้นผิวหนังแท้ ในการเป็นระบบส่งยาทางผิวหนังแบบไม่รุกราน ที่มีความปลอดภัย ความเข้ากันได้ทางชีวภาพ และคุณสมบัติในการปลดปล่อยยาอย่างต่อเนื่อง เหมาะสมสำหรับการประยุกต์ใช้ในการบรรเทาอาการปวดเฉพาะที่ในทางคลินิก นอกจากนี้ การใช้เทคนิค FTIR mapping ยังเป็นวิธีการที่มีความน่าเชื่อถือสำหรับการประเมินประสิทธิภาพของการซึมผ่านเข้าสู่ชั้นผิวหนัง ดังนั้น ผลการศึกษาเน้นให้เห็นถึงศักยภาพของแผ่นแปะเส้นใยนาโนที่มีส่วนผสมของแคปไซซินนี้ และสามารถส่งยาไปยังตำแหน่งเป้าหมายได้อย่างเฉพาะเจาะจง ผลลัพธ์เหล่านี้ยังเน้นย้ำถึงความเป็นนวัตกรรมแผ่นแปะเส้นใยนาโน และศักยภาพอันโดดเด่นในการประยุกต์ใช้ทางคลินิกในอนาคต

สาขาเวชศาสตร์ปริวรรต
ปีการศึกษา 2567

ลายมือชื่อนักศึกษา.....
ลายมือชื่ออาจารย์ที่ปรึกษา.....
ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

CHUTHARAT THANCHONNANG: INVENTION AND EVALUATION OF THE EFFICACY OF CAPSAICIN TRANSDERMAL NANOFIBERS PATCH FOR PAIN RELIEF: *IN VITRO* STUDY THESIS ADVISOR: Dr. Wiwat Nuansing 184 PP.

Keywords: capsaicin, nanofiber patch, electrospinning, pain, *COX-2*, anti-inflammation

Capsaicin (8-methyl-N-vanillyl-trans-6-nonenamide), a prominent bioactive compound derived from chili peppers (*Capsicum spp.),* is well recognized for its potent analgesic and anti-inflammatory properties. Although extensively utilized in traditional medicine and approved for topical application in the treatment of various neuropathic pain conditions, its clinical use remains limited due to poor aqueous solubility and the potential for cutaneous irritation. Capsaicin exerts its therapeutic effects primarily through activation and subsequent desensitization of transient receptor potential vanilloid 1 (TRPV1) receptor on afferent C fibers, resulting in reduced nociceptive transmission.

This study aimed to develop a capsaicin-loaded transdermal nanofiber patch composed of polyvinyl alcohol (PVA) and polyvinylpyrrolidone (PVP) using the electrospinning technique. Additionally, it investigated the drug release mechanism and transdermal permeation behavior of the capsaicin-loaded nanofiber patch through the Strat-M™ membrane. Capsaicin was incorporated into a hydrophilic polymer matrix composed of polyvinyl alcohol (PVA) and polyvinylpyrrolidone (PVP) at a concentration of 0.1 mg/mL. The electrospun fibers exhibited smooth, uniform, and bead-free morphology, with an average diameter of 667 ± 19.5 nm. Fourier-transform infrared (FT-IR) spectroscopy confirmed successful encapsulation through hydrogen bonding interactions, without evidence of chemical degradation.

In vitro cytocompatibility assays using human dermal fibroblasts (HDFs) indicated enhanced cell viability at low concentrations (0.001–0.01 mg/mL), with an IC_{50} of approximately 20 mg/mL, signifying a favorable safety profile. The patch demonstrated marked anti-inflammatory effects, reducing cyclooxygenase-2 *(COX-2),* gene expression by approximately 8.1-fold in H_2O_2 induced inflamed HDFs.

Morphological assessments further corroborated its protective effects against oxidative stress.

Transdermal permeation studies employing Franz diffusion cells and Strat-M™ membranes revealed sustained capsaicin release over a 12-hour period, with a calculated steady-state flux (J_{ss}) of 63.30 µg/cm²/hr. The permeability coefficient (K_p) exhibited a time-dependent decline, with a peak of 0.77 cm/hr at 1 hour and stabilization to quasi-steady state levels (~0.05–0.07 cm/hr) after 6 hours, aligning with the principles of Fick's First Law of Diffusion. FTIR imaging provided visual confirmation of progressive capsaicin penetration into deeper membrane layers.

Collectively, the results underscore the potential of this capsaicin-loaded nanofiber patch as a non-invasive, biocompatible, and sustained-release transdermal system for localized pain management. The integration of FTIR mapping, cytocompatibility, and gene expression analyses offers a robust framework for evaluating formulation performance. Future *in vivo* investigations are warranted to validate clinical applicability, address sensory tolerability, and explore large-scale manufacturing potential. This delivery platform represents a promising advancement in topical analgesic therapy, offering site-specific action with reduced systemic exposure.

Translational Medicine Program
Academic Year 2024

Student's Signature.....
Advisor's Signature.....
Co-Advisor's Signature.....

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Chutharat Thanchonnang

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LIST OF ABBREVIATIONS

°C	=	Degrees Celsius
CAP	=	Capsaicin
cm	=	Centimeter
cm^2.	=	Square centimeters
cm^{-1}	=	Reciprocal centimeters
TRPV1	=	Transient receptor potential vanilloid 1
DW	=	Distilled Water
FTIR	=	Fourier transform infrared spectroscopy
H_2O_2	=	Hydrogen peroxide
HDFs	=	Human dermal fibroblasts
HPLC	=	High-performance liquid chromatography
IL-1β	=	Interleukin-1 beta, lipopolysaccharide
mL	=	milliliter
nm	=	Nanometers
mM	=	Millimolar
MAPK	=	Mitogen-activated protein kinase
NF-**κ**B	=	Nuclear factor kappa-light-chain-enhancer of activated B cells
TDDS	=	Transdermal drug delivery systems
PVA	=	Polyvinyl alcohol
PVP	=	Polyvinylpyrrolidone
SEM	=	scanning electron microscopy SEM
µL	=	Microliter
µg/mL	=	Micrograms per milliliter
w/v	=	weight/volume

CHAPTER I

INTRODUCTION

1.1 Background and Problem

Currently, chili peppers are natural plants rich in capsaicinoids, which have been widely utilized in traditional medicine. Capsaicin (8-methyl-N-vanillyl-trans-6-nonenamide), the primary active component in chili, has been approved for topical application in managing different types of neuropathic pain. This compound offers diverse therapeutic benefits, functioning as both an analgesic and anti-inflammatory agent, and is used in the treatment of gastrointestinal and cardiovascular disorders, as well as for pain relief (Anantaworasakul et al., 2020).

Capsaicin (CAP) in addition exerts its analgesic effect by activating the transient receptor potential vanilloid 1 (TRPV1) receptor located on afferent C fibers of peripheral nerves. Activation of these ligand-gated cation channels leads to membrane depolarization, initiation of action potentials, and the release of pain-related neurotransmitters, such as Substance P, to the spinal cord. Prolonged stimulation by TRPV1 agonists, like capsaicin, results in desensitization of these receptors, thereby diminishing nociceptive signaling and potentially alleviating pain (Bode & Dong, 2011). Since TRPV1-expressing peripheral nociceptors are implicated in the generation of pain and hyperalgesia at tendon sites and myofascial trigger points, targeting these receptors in the overlying skin or tendon regions presents a promising approach for managing myofascial pain. Capsaicin is a well-characterized agonist of the TRPV1receptor, a non-selective cation channel embedded within the membrane of primary sensory neurons (Fattori et al., 2016). TRPV1 is activated by thermal stimuli exceeding 43 °C, acidic environments (pH < 6), and various endogenous lipid mediators. Upon activation, the receptor facilitates the influx of calcium (Ca^{2+}) and sodium (Na^{+}) ions, leading to membrane depolarization and subsequent initiation of action potentials. These electrical signals are transmitted via predominantly unmyelinated C fibers and, to a lesser extent, Aδ fibers to the central nervous system, as a result, they are perceived

as pain or thermal sensations (Iftinca et al., 2021). The sensory response to capsaicin is commonly experienced as a sensation of heat, tingling, stinging, or burning. Notably, capsaicin induces a more sustained activation of TRPV1 compared to naturally occurring stimuli, resulting in a phenomenon known as “defunctionalization,” wherein sensory neurons exhibit diminished responsiveness to further stimulation. This prolonged activation disrupts nociceptive signaling through a combination of intracellular mechanisms, including alterations in enzymatic activity, cytoskeletal structure, osmotic balance, and mitochondrial respiration (Iftinca et al., 2021). Collectively, these effects contribute to a reversible impairment of nociceptor function, supporting capsaicin’s utility as a topical analgesic agent. Therefore, physiologically, capsaicin exerts its action by binding intracellularly to the TRPV1 receptor, thereby modulating peripheral pain pathways (Benítez-Angeles et al., 2020). CAP is important for the discovery of TRVP1 and its therapeutic effects in pain disorders. Recently, the studies have been made in understanding the mechanisms responsible for the effects of capsaicin on pain relief in patients.

Moreover, recent studies have shown that capsaicin also influences human dermal fibroblasts by modulating inflammatory mediators and promoting wound healing (Cuijpers et al., 2025; Hudita et al., 2021). Its interaction with dermal cells suggests a dual role not only in alleviating pain but also in contributing to skin regeneration and anti-inflammatory responses at the cellular level. Natural compounds with antioxidant and anti-inflammatory properties have demonstrated promising effects in protecting and repairing damaged skin. Capsaicin, the principal bioactive compound found in chili peppers, is well recognized for its therapeutic potential in various biomedical applications. Recent research has highlighted capsaicin's ability to modulate oxidative stress and inflammatory pathways, both of which are key contributors to skin damage. Thus, the present study investigates the effects of capsaicin and its major constituents on human dermal fibroblasts (HDFs), with the aim of elucidating their protective roles against oxidative and inflammatory (Hudita et al., 2021). Furthermore, evidence-based recommendations support the use of topical capsaicin as a viable therapeutic option for managing pain-related conditions (Laklouk & Baranidharan, 2016) for safety and patient tolerability of high dose capsaicin

patch is used to manage pain associated with anticancer, pain relief as well. The reported by Romero et al. (2019) that the placebo group had no hyperemia or burning at the application site, but the CAP group had 85% at 15 minutes. Symptoms vanished 24 hours after the cream was withdrawn. The capsaicin group's pain score dropped steadily until the 60th day ($p < 0.0001$). Capsaicin 8% did not create macroscopic acute or chronic skin lesions in individuals and was helpful and well tolerated. A pharmacokinetic study with 8% CAP patches showed no appreciable systemic absorption (Babbar et al., 2009) . Moreover, 24 weeks after the 8% patch was applied, there was no statistically significant change in heat or cold sensitivity thresholds (Kennedy et al., 2010). So, the capsaicin 0.1% patch is safe from local skin irritation, systemic absorption, and epidermal nerve fiber destruction (Cho et al., 2012).

One of the most common reasons people seek medical attention is because of pain (Schappert & Burt, 2006). Approximately 20% of all patients worldwide suffer from pain, and 10% of those suffer from chronic pain (Enright & Goucke, 2016). Many pain relievers have debilitating side effects, such as hepatotoxicity, depression, respiratory depression, and addiction, which are reported by more than 40% of patients treated for pain. There is an urgent need for better treatment options for chronic pain in light of the recent opioid epidemic, which is the leading cause of medication-induced overdose (Bhansali et al., 2021). Although it has been successfully applied the clinical in dermatology and pain control, but the usage of capsaicin in the treatment of myofascial pain syndrome are limited. Capsaicin's present treatment is considered. It is an effective and risk free therapy alternative as well as effective pain relief (Laklouk & Baranidharan, 2016). As a result, it's intriguing that CAP could be used to alleviate upper back muscle pain. Current evidence-based for the treatment consider topical capsaicin as a therapeutic option safety and patient tolerability of low-dose capsaicin patch is used to manage pain as well.

In fact, transdermal drug delivery systems (TDDS) represent a noninvasive approach to administering therapeutic agents through the skin and have emerged as a promising alternative to conventional methods such as oral ingestion and parenteral injection. TDDS has garnered significant attention for its ability to enhance drug delivery across various therapeutic areas, including pain management, hormone replacement

therapy, and the treatment of cardiovascular and central nervous system disorders (Murthy, 2012). Compared to oral and injectable routes, transdermal delivery offers several distinct advantages. It circumvents the gastrointestinal environment, thereby avoiding enzymatic degradation, pH variability, and fluctuations in gastric emptying time. Moreover, it bypasses hepatic first-pass metabolism, which can significantly reduce the bioavailability of orally administered drugs (Alkilani et al., 2015). In addition, these pharmacokinetic benefits, combined with patient-friendly, noninvasive application, make TDDS an attractive platform for sustained and controlled drug delivery. They also have a benefit over oral delivery in that they can be administered to patients even if they are asleep or nauseated (Leppert et al., 2018). With TDDS administration, pain, bruising, and bleeding are all minimized, which leads to an overall improvement in patient acceptance and compliance with therapy. Furthermore, they eliminate the risk of developing a disease linked with needles, as well as the risk of inadvertently harming oneself with a needle, and they reduce the generation of hazardous waste sharps associated with medical activities (Alkilani et al., 2015; Murthy, 2012). The advantages of TDDS are not limited to those based on safety; it has been established that they can significantly cut overall healthcare expenses. Furthermore, TDDS can give a sustained and regulated release of the drug, reduce the drug's peak concentration, and reduce the associated systemic toxicity (Cramer & Saks, 1994). TDDS offer an effective alternative for administering medications that exhibit limited therapeutic efficacy via oral, topical, intravenous, or intramuscular routes. Recent advancements in TDDS have focused on the incorporation of nanoparticle (NP)-based technologies to enhance transdermal absorption. As a result, nanoparticles facilitate improved drug permeation across the skin barrier and allow for controlled and sustained release profiles. Additionally, they enable the delivery of both hydrophilic and hydrophobic compounds, reduce the likelihood of systemic side effects, and support a non-invasive mode of administration. Among the various TDDS platforms under development, transdermal patches incorporating nanocarrier systems have emerged as a particularly promising innovation in targeted and patient-compliant drug delivery (Sim & Wong, 2021). Nanoparticles (NPs) are substances characterized by dimensions between 1 and 100 nanometers. In oncology, nanomaterials are typically

grouped into distinct categories to enhance their clinical applicability. However, continued investigation is necessary to advance the precision and effectiveness of targeted drug delivery strategies (Cheng et al., 2021). Nanoparticles are capable of incorporating therapeutic agents at relatively high loading capacities, and their surfaces can be readily modified to facilitate targeted drug delivery (Sim & Wong, 2021). Despite the rapid growth of nanomedicine, its application in pain management remains limited, largely due to the complex nature of pain mechanisms and the challenges of treating chronic pain. However, emerging nanotechnologies are poised to transform future analgesic therapies. Advanced nanomaterials are being developed as stimuli-responsive drug carriers capable of targeting specific tissues and cellular structures, and as nanosensors for detecting pain at the molecular level. These systems enhance therapeutic efficacy by enabling lower drug dosages, prolonged analgesic effects, and reduced side effects. With ongoing progress, nanomaterials are increasingly engineered not only for drug delivery but also to directly modulate pain pathways, offering promising solutions for chronic pain management (Palmer & DeLouise, 2016).

Polymers form the structural basis of transdermal drug delivery systems, and advancements in nanotechnology have enabled the development of highly adaptable delivery platforms using natural, synthetic, and semisynthetic materials to regulate drug diffusion across the skin. Electrospinning has emerged as a highly efficient and cost-effective technique for fabricating nanofibers, utilizing electrostatic forces to create fibers with an ultrafine morphology, high porosity, and a large surface area-to-volume ratio. Compared to conventional film-casting methods, electrospun fiber mats exhibit superior porosity and surface characteristics, which enhance drug release and diffusion from the polymer matrix (Sa'adon et al., 2019). Nanofibers may now be a great alternative for nanomedicine (Kumar et al., 2021). Various polymers, including chitosan, fibrinogen, cellulose triacetate, polyacrylic acid, polyvinyl chloride, polyurethane, polyvinyl alcohol (PVA), and polyvinyl pyrrolidone (PVP), have been utilized in electrospinning processes, enabling the development of innovative and efficient drug delivery systems (Rahmani et al., 2021). PVP is an amorphous synthetic polymer known for its strong water affinity and excellent adhesive properties. Due to its low chemical toxicity and high biocompatibility, PVP is widely utilized in biomedical

and pharmaceutical applications (Wang et al., 2015). PVP show significant promise as next-generation wound dressings, as they maintain a moist environment that prevents dehydration and scab formation. PVP-based nanofibers are frequently employed as carriers in various drug delivery systems. Prior studies have demonstrated that PVP nanofibers enable rapid release of water-soluble drugs, attributed to their high porosity, large surface area-to-volume ratio, and excellent water solubility of the polymer matrix. Electrospun PVA, alongside PVP, has been widely utilized as a drug delivery matrix for various therapeutic agents. PVA is a water-soluble, biocompatible, and biodegradable polymer with extensive biomedical applications. According to Li et al., PVA nanofiber matrices exhibit rapid drug release behavior, achieving complete release of caffeine and approximately 40% release of riboflavin within 60 seconds, highlighting its potential for burst-release formulations (Li et al., 2013). Electrospun membranes exhibit high fluid absorption and slow degradation rates, maintaining a moist environment essential for wound healing. Incorporating pharmaceuticals into these nanofibrous patches enables efficient transdermal drug delivery. Various therapeutic agents, including anti-inflammatory drugs, analgesics, and herbal extracts such as capsaicin or chili-derived compounds, have been effectively integrated into electrospun polymer matrices for use in dermal patch applications (Tanadchangsaeng et al., 2016). Strat-M™ is a synthetic membrane composed of polyethersulfone and polyolefin, designed to simulate human skin for evaluating percutaneous absorption. It serves as a reliable alternative to human or animal skin in safety assessments of dermal and cosmetic products, showing high permeability correlation with human skin (Kunita et al., 2022). Strat-M™ is gaining popularity as a skin alternative for *in vitro* permeation experiments. According to research, Strat-M™ could be used in permeation studies instead of animal or human skin. Due to these chemical and physical properties, the Strat-M™ membrane is a great alternative to a skin model for testing penetration (Haq et al., 2018; Kunita et al., 2022; Pulsoni et al., 2022). Modifications to ibuprofen (IBU) and its vehicle can enhance skin permeation and tissue accumulation (Arce et al., 2020; Klebeko et al., 2021). Transdermal hydrogels, in particular, show promise for rapid drug delivery and improved therapeutic efficacy. Additionally, Strat-M™ membranes have proven to be a suitable alternative to human skin for evaluating

parameters such as permeability coefficient, flux, and compound accumulation, confirming their relevance in transdermal permeation studies (Arce et al., 2020; Klebeko et al., 2021). While capsaicin is well known for its analgesic properties through the stimulation of transient receptor potential vanilloid 1 (TRPV1) receptor on sensory neurons, emerging studies indicate that its therapeutic effects may also extend to non-neuronal systems. Notably, its impact on cells such as human dermal fibroblasts (HDFs) remains insufficiently characterized. HDFs are not merely structural components of the skin but also play critical roles in regulating inflammation, oxidative stress, and tissue repair. Although the anti-inflammatory potential of capsaicin has been reported in several immune cell models, its molecular mechanisms particularly its ability to downregulate inflammation-associated genes like cyclooxygenase-2 (*COX-2*), a gene strongly implicated in inflammatory pain have yet to be thoroughly investigated in human dermal fibroblasts.

Previous studies have identified 0.1% capsaicin as a safe and well-tolerated therapeutic option for pain management. Electrospinning has emerged as a promising technique for fabricating nanofiber-based drug delivery systems. In transdermal research, Franz diffusion cells are widely used to evaluate skin permeation. Findings indicate that drug-loaded PVA/PVP nanofibers demonstrate superior physicochemical properties compared to individual polymers. While human skin explants are considered the gold standard for transdermal delivery evaluation, Strat-M™ membranes offer an ethical and practical alternative for *in vitro* permeation studies. Moreover, capsaicin not only alleviates pain through neuronal pathways but also modulates inflammatory mediators and promotes wound healing in human dermal fibroblasts (HDFs). The findings suggest a dual therapeutic role in both pain relief and skin regeneration. As a natural compound with strong antioxidant and anti-inflammatory properties, capsaicin shows significant potential in protecting against oxidative stress and anti-inflammatory properties. In conclusion, the objective of this study to develop the fabrication of a capsaicin transdermal nanofibers patch, the nanofibers patch of capsaicin-loaded polyvinyl alcohol and polyvinyl pyrrolidone (CAP/PVA/PVP) by the electrospinning process and to study the mechanism of the release and skin permeation characteristics of capsaicin nanofibers patch via Strat-M^{TM}

membrane. The study's findings provide a foundation for advancing new knowledge and innovation in the development of capsaicin-loaded nanofiber patches and transdermal drug delivery systems.

1.2 Research hypotheses

1. The electrospinning process successfully produced a capsaicin-loaded nanofiber patch, which effectively retained the bioactivity and therapeutic efficiency of capsaicin.

2. The capsaicin-loaded nanofiber patch demonstrates potential as an effective drug delivery system. It is suitable for evaluating in vitro drug release and skin permeation through the Strat-M™ membrane.

1.3 Research objectives

The aims of this study to:

1. To develop the invention of a capsaicin transdermal nanofibers patch, the nanofibers patch of capsaicin-loaded polyvinyl alcohol and polyvinyl pyrrolidone (CAP/PVA/PVP) by the electrospinning process.

2. To study the mechanism of the release and skin permeation characteristics of capsaicin transdermal nanofibers patch via Strat-M™ membrane.

1.4 Scope and limitations of the study

The study was conducted over a two-year period from 2022 to 2024 at the Parasitic Diseases Research Center (PDRC) and the Advanced Materials Physics (AMP) laboratory, Suranaree University of Technology, Thailand. A prototype of the capsaicin transdermal nanofibers patch (CTNP) was developed using the electrospinning technique and subsequently characterized for its physicochemical properties using scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR), and Franz diffusion cells. This research was part of an ongoing investigation into the research and development (R&D) of CTNPs at Suranaree University of Technology, Thailand.

The operational procedures of this study were divided into three main parts:

1. Development of the transdermal nanofiber patch.

2. Characterization of the nanofiber patch, focusing on morphological assessment and drug incorporation using an electrospinning machine. In addition, the stability of this formulation was assessed on the basis of its physical appearance, and morphology from the patches. The efficacy of the transdermal nanofiber patch was investigated in this study. characterization of nanofiber patch morphology. The electrospinning processes will be carried out with the aid of an electrospinning machine. This device has controllable components such as high voltage (0–30 kV). The transdermal nanofiber patches were taken using the scanning electron microscope (SEM). Fourier transform infrared spectroscopy (FT-IR) was obtained at the spectral range. The mechanical properties of electrospun webs were examined using a Franz diffusion cell testing machine. The experiment was carried out and replicated three times for each sample.

3. Synthesis and in vitro permeation testing of the CTNPs. The Franz diffusion cell system, in combination with Strat-M™ membranes, was used to assess transdermal permeation and essence retention under laboratory conditions.

1.5 Contribution

This study contributes to the advancement of transdermal drug delivery systems by introducing a novel capsaicin-loaded nanofiber patch formulation. The research provides significant insights into the design, fabrication, and evaluation of electrospun nanofibers for pharmaceutical applications. Specifically, the study offers:

1. A new formulation approach utilizing polyvinyl alcohol (PVA) and polyvinylpyrrolidone (PVP) polymers for the effective encapsulation of capsaicin.

2. Demonstration of successful nanofiber production through electrospinning, resulting in uniform, bead-free nanofibers with nanoscale diameters suitable for skin application.

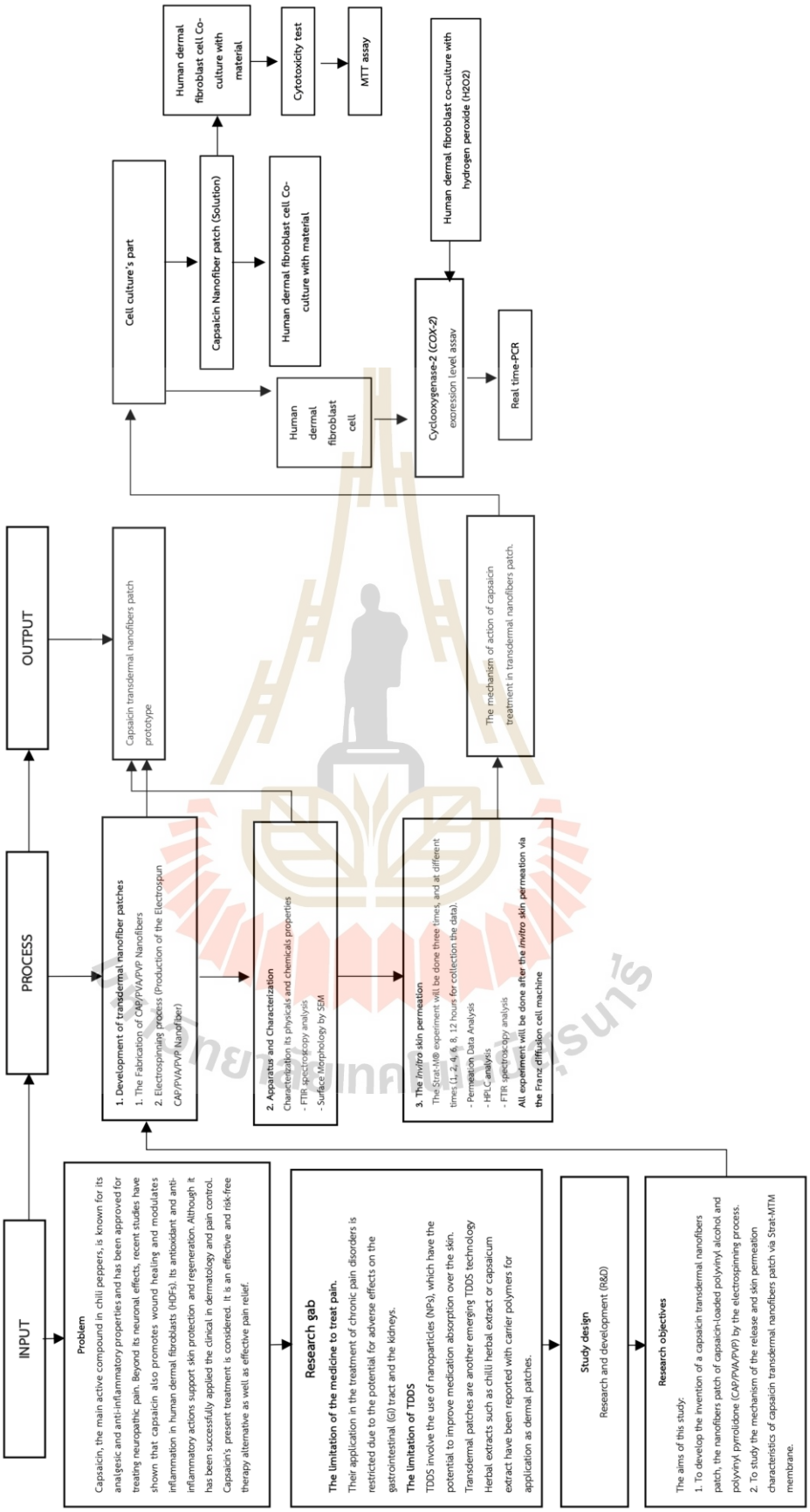
3. Validation of drug release kinetics and transdermal permeation through *in vitro* testing using Strat-M™ membranes, supporting the patch's potential for sustained capsaicin delivery.

4. Experimental evidence of anti-inflammatory and cytoprotective activities, confirming the patch's bioactivity and dermal safety.

5. A conceptual framework for future innovation in pain management therapies using biocompatible nanofiber-based delivery platforms.

These findings underscore the potential of capsaicin-loaded nanofiber patches as an innovative, non-invasive system for delivering therapeutic agents through the skin, with promising applications in pain relief and the treatment of inflammatory skin disorders.

1.6 Conceptual Framework



1.7 Expected results

The expected results of this study include the successful fabrication of a capsaicin-loaded transdermal nanofiber patch via the electrospinning technique, yielding uniform, bead-free nanofibers with optimal physicochemical characteristics for transdermal drug delivery The developed patch is expected to provide a sustained release profile of capsaicin and demonstrate efficient permeation across the Strat-M™ membrane. Furthermore, the patch is expected to exhibit biocompatibility with human dermal fibroblasts and significant anti-inflammatory activity, as evidenced by reduced *COX-2* gene expression. Overall, these outcomes will support the advancement of a novel, non-invasive transdermal delivery system for pain and inflammation management.

CHAPTER II
LITERATURE REVIEWS

2.1 Capsaicin

2.1.1 Structure of Capsaicin

Capsaicin was first identified in a partially purified crystalline form by Christian Friedrich Bucholz in 1816. Subsequently, in 1876, John Clough Thresh succeeded in isolating it in its pure crystalline form and officially named the compound capsaicin. In addition, the physiological effects of capsaicin were first documented by Rudolf Buchheim, who observed that it elicited a burning sensation upon contact with mucous membranes and also stimulated the secretion of gastric juice (Bode & Dong, 2011). Furthermore, capsaicin's chemical structure was discovered in the early twentieth century by scientists L. E. Dawson and E. K. Nelson (Idrees et al., 2020). More specifically, capsaicin (trans-8-methyl-N-vanillyl-6-nonenamide) is the primary bioactive compound found in chili peppers, which belong to the *Capsicum* genus (Mullins et al., 2022). In its pure form, capsaicin is a colorless, odorless solid with a texture ranging from crystalline to waxy, and it is insoluble in water. In terms of chemistry, it is classified as an acid amide, consisting of vanillylamine and a fatty acid moiety containing a carbon chain length between C8 and C13. As shown in Figure 2.1, capsaicin contains a 3-methoxy-4-hydroxybenzylamine (vanilloid) ring and an alkyl side chain, which are essential to its biological activity. In a similar fashion, structurally related analogs are synthesized through similar pathways but have shorter fatty acid chains, which influence their binding affinity and activation potential at the capsaicin (TRPV1) receptor. Of these, capsaicin exhibits the highest binding affinity to the vanilloid receptor, making it the most pungent and pharmacologically potent molecule within the *Capsicum genus*.

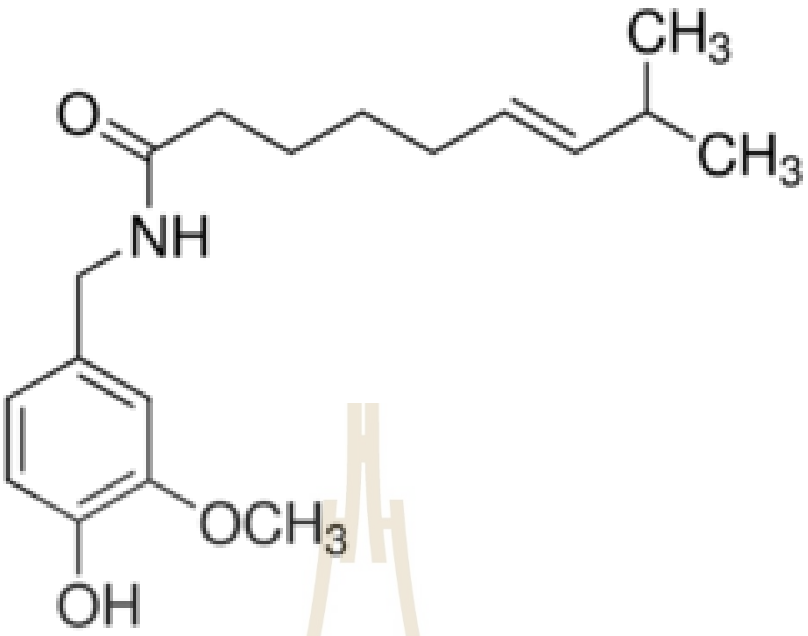


Figure 2.1 Chemical structure depiction the structure of capsaicin

The chemical structure of capsaicin comprises three key components: a vanilloid ring, specifically a 3-methoxy-4-hydroxybenzylamine moiety, and a hydrophobic alkyl side chain. These structural elements are critical to its biological activity, particularly its high-affinity interaction with the TRPV1 receptor ($C_{18}H_{27}NO_3$) (Mullins et al., 2022).

2.1.2 The molecular targets of capsaicin

Over the years, capsaicin has garnered significant attention in the scientific literature as a promising anti-cancer agent, primarily due to its antiproliferative effects. Thus, this review aims to comprehensively examine the principal mechanisms of capsaicin-mediated analgesia reported in contemporary studies and propose an integrated model of its pain-relieving actions. In particular, capsaicin exerts its effects primarily through the transient receptor potential vanilloid 1 (TRPV1) receptor, a ligand-gated, nonselective cation channel predominantly expressed in sensory neurons, particularly those involved in nociception. Currently, both agonists and antagonists targeting TRPV1 are currently undergoing clinical investigation for their analgesic potential. In this context, capsaicin itself acts as a potent TRPV1 agonist, triggering receptor activation that underlies its characteristic effects on pain modulation (Luo et

al., 2011). Additionally, according to (Bode & Dong, 2011), capsaicin exerts its physiological effects on sensory neurons primarily through intracellular binding to the transient receptor potential vanilloid 1 (TRPV1) receptor. Its role was pivotal in the identification and characterization of TRPV1, and the analgesic properties of capsaicin are largely attributed to this receptor interaction. Moreover, in recent years, significant advances have been made in elucidating the molecular mechanisms underlying capsaicin-induced pain relief. Furthermore, beyond its analgesic effects, capsaicin has been shown to modulate key signaling pathways involved in carcinogenesis and tumor progression, suggesting a potential antineoplastic role (Wang et al., 2016). Interestingly, in the majority of cases, capsaicin's effects on cancer cell metabolism appear to occur independently of TRPV1 activation, suggesting alternative molecular targets may be involved in its antineoplastic mechanisms (Rollyson et al., 2014). In line with this, our findings appear to support existing recommendations, indicating that complete. TRPV1 blockade may be a viable strategy for pain relief. Finally, it is noteworthy that capsaicin exhibits a relatively short half-life in systemic circulation. Therefore, to evaluate the oral bioavailability of capsaicin in humans, several pharmacokinetic parameters such as absorption rate, plasma concentration, and elimination half-life have been utilized.

2.1.3 The pharmacokinetics of capsaicin

Capsaicin is a naturally occurring protoalkaloid and the principal pungent compound found in chili peppers (*Capsicum annuum L.*). More specifically, chemically identified as trans-8-methyl-N-vanillyl-6-nonenamide, capsaicin is a crystalline, off-white, lipophilic solid that is both colorless and odorless. It has a melting point of 62–65 °C and is insoluble in water, though it readily dissolves in organic solvents such as ethanol, acetone, and fatty oils (Ilie et al., 2019). Furthermore, capsaicin is well recognized for its effective transdermal absorption. For instance, in a study involving 12 participants who received a 3% capsaicin formulation delivered via three different topical vehicles, capsaicin demonstrated rapid absorption and quickly reached its maximum plasma concentration following application. Additionally, the compound exhibits an approximate elimination half-life of 24 hours, supporting its suitability for sustained topical therapeutic use (Pershing et al., 2004). In another study,

a comprehensive investigation into the tissue distribution, elimination, and metabolism of capsaicin in animal models following oral administration revealed that approximately 94% of the administered dose was absorbed, with peak plasma concentrations occurring within 1 hour. Interestingly, within the same timeframe, 24.4% of the absorbed capsaicin was distributed across the blood, liver, kidneys, and intestines. Nevertheless, tissue levels declined sharply and became undetectable after four days. Moreover, *in vitro* studies using human skin demonstrated that capsaicin undergoes slow biotransformation, with the majority of the compound remaining unchanged. Only a small portion was metabolized into vanillylamine and vanillic acid. Thus, excretion of capsaicin primarily occurs via the renal and gastrointestinal pathways, with the compound eliminated through urine and feces (Reyes-Escogido et al., 2011). Additionally, capsaicin is initially found in plasma 10 minutes after capsicum consumption. Capsaicin had a maximal plasma concentration (C_{max}) of 2.47 $\pm$ 0.13 ng/ml and a T_{max} of 47.08 $\pm$ 1.99 minutes. As a result, the area under the curve (AUC_{0-t}) showed that the amount of capsaicin absorbed into the body was 103.6 $\pm$ 11.3 ng.min/mL. (Chaiyasit et al., 2009; Wang et al., 2017). *In vitro* studies have shown that capsaicin metabolism in human skin proceeds slowly, and the use of topically applied capsaicin patches with an extended elimination half-life reflects a sustained release of the compound at the application site. Collectively, these distinctive pharmacokinetics properties make topical delivery an optimal route for the therapeutic administration of capsaicin in the management of various clinical conditions.

2.1.4 The roles of capsaicin in pain relief

Capsaicin has been used extensively throughout the history of folk medicine. Specifically, this practice relies heavily on the concept of "using like to treat like," which means, for instance, treating a chemical that causes burning pain with another substance that causes burning sensation. For example, in 1850, a recommendation was made to apply an alcoholic extract of hot peppers to any parts of the body that were burning or itching (Turnbull, 1850). Moreover, capsaicin-induced analgesia's underlying processes are increasingly being explored. In particular, receptor

activity is inhibited after long or repetitive exposure to capsaicin, which is known as desensitization in the context of TRPV1.

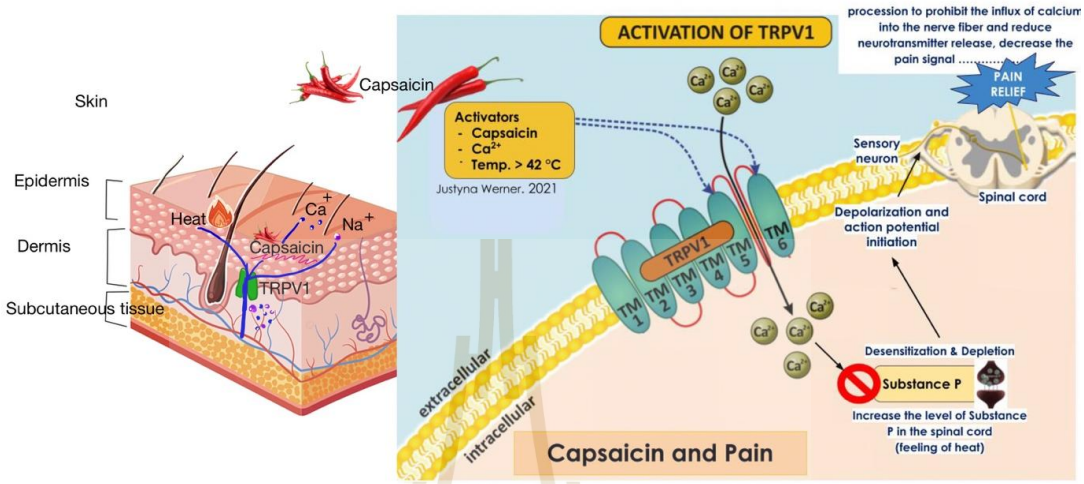


Figure 2.2 The mechanism of capsaicin and pain
(Jeszka-Skowron et al., 2021)

In particular, capsaicin-induced defunctionalization occurs through multiple interrelated mechanisms. For example, one primary pathway involves the direct pharmacological desensitization of TRPV1 receptors on the plasma membrane, as well as the inhibition of voltage-gated sodium (Na^+) channels, both of which contribute to an immediate reduction in neuronal excitability and responsiveness. Moreover, extracellular calcium (Ca^{2+}) influx through TRPV1 channels, coupled with release from intracellular stores, may exceed the cell's buffering capacity, leading to the activation of calcium-dependent proteases and subsequent cytoskeletal degradation. As a result, disruption of microtubule integrity may further impair axonal transport, contributing to functional loss. Furthermore, at supraphysiological concentrations, capsaicin can also induce mitochondrial dysfunction by inhibiting electron transport chain activity, positioning mitochondria as a critical convergence point in the cascade of defunctionalization events. In order to better understand its effects, capsaicin is an agonist following:

1. The transient receptor potential vanilloid 1 (TRPV1) receptor is a transmembrane ion channel complex that is activated by noxious heat (≥ 43 °C), acidic

conditions (pH < 6), and certain endogenous lipid mediators. Exposure to one or more of these stimuli can transiently open the channel, leading to membrane depolarization.

2. A-delta and C fibers express TRPV1 often, so depolarization causes action potentials that convey impulses to the central nervous system.

3. There are several capsaicin effects that come from these electrical impulses. Capsaicin also activates these receptors for a longer period of time than environmental agonists, causing a "defunctionalization" of sensory responses.

4. Over time, capsaicin impairs nociceptor function through disrupting enzymatic and osmotic processes, as well as cytoskeletal and osmotic structure.

5. In addition, TRPV1 activation upon application of Capsaicin, the skin ingests the components and activates the TRPV1 Receptor, which activates the C-Fiber to transmit pain signals and raises the level of Substance P in the spinal cord. This causes the sensation of heat to increase as a result of the increased activity in the pain pathway.

6. Notably, Depletion and desensitization are two related concepts. P - for substance.

Regular applications of capsaicin for 2 to 3 weeks result in desensitization of TRPV1 and Substance P and a reduction in neurotransmitter release, which reduces the pain signal or the sensation of pain.

Moreover, recent studies suggest that capsaicin-induced analgesia may be mediated not only through TRPV1 activation and subsequent desensitization but also by the inhibition of Piezo proteins—a family of mechanically activated cation-selective ion channels in mammals. Activation of TRPV1 by capsaicin triggers calcium-dependent activation of phospholipase Cδ (PLCδ), which in turn leads to the depletion of phosphoinositides. This biochemical change is associated with the suppression of Piezo channel activity, as evidenced by the inhibition of inward ionic currents during mechanical stimulation. Notably, the suppression of Piezo function is reversible upon cytosolic reintroduction of phosphoinositides, as demonstrated in excised inside-out patch clamp experiments. These findings highlight a novel mechanism by which TRPV1 activation indirectly modulates mechanosensation

through Piezo channel regulation (Borbiro et al., 2015). Overall, this study provides insight into the mechanisms underlying capsaicin-induced mechanical analgesia at the local level. Capsaicin has been widely used in the management of muscle pain, joint discomfort, and neuropathic pain, with numerous studies supporting its efficacy and safety. Topical chili-based formulations have demonstrated therapeutic benefits in the treatment of chronic pain conditions such as osteoarthritis, rheumatoid arthritis, diabetic neuropathy, cancer-related pain, postherpetic neuralgia, and psoriasis all of which significantly impair patients' quality of life. In clinical practice, for the treatment of neuropathic and musculoskeletal pain, commercially available topical products typically contain capsaicin concentrations ranging from 0.0125% to 0.075% by weight (Anantaworasakul et al., 2020). In addition to capsaicin-based treatments, drug delivery systems are designed to address limitations associated with certain pharmaceutical agents by enabling controlled release, targeted delivery, and a reduction in adverse effects. Among these, solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) represent advanced lipid-based nanocarriers optimized for topical application. These systems offer notable advantages, including sustained drug release, enhanced skin penetration, and diminished side effects associated with high concentrations of active compounds. For example, a prescription-strength capsaicin patch containing 8% capsaicin has demonstrated clinical efficacy in managing severe neuropathic pain, particularly in conditions such as diabetic peripheral neuropathy and postherpetic neuralgia (Anantaworasakul et al., 2020).

2.1.5 The advantages therapy of capsaicin

Capsaicin is known for its selective activation of nociceptive neurons and has been extensively utilized as a tool in the investigation of pain-related mechanisms. This section will explore key aspects of capsaicin's therapeutic potential in pain relief, emphasizing its contributions to the current understanding of neuronal pathways involved in nociception and pain modulation.

In particular, capsaicin has been shown to exhibit a range of pharmacological and physiological effects, most notably its analgesic and anticancer properties (Macho et al., 2003), anti-inflammation, antioxidant, and anti-obesity (Joo et

al., 2010). Moreover, capsaicin demonstrates promising therapeutic potential in pain relief, cancer prevention, and weight management. In addition, it has been shown to exert beneficial effects on the cardiovascular and gastrointestinal systems. Notably, among the various capsaicinoids, capsaicin is the most extensively investigated for its analgesic properties. For instance, studies have indicated that both oral and topical formulations of capsaicin can effectively reduce pain, particularly by attenuating inflammatory heat sensitivity and chemical-induced hyperalgesia (Luo et al., 2011). Additionally, red chili peppers exhibit notable anti-inflammatory effects, primarily attributed to capsaicinoid compounds, which have been shown to possess both anti-inflammatory activity and analgesic properties (Idrees et al., 2020). Furthermore, capsaicin possesses several distinctive properties that render it valuable for applications across the pharmaceutical, food, and agricultural (pesticide) industries. In particular, one of its most prominent characteristics is pungency, which arises from the compound's interaction with sensory receptors in mammals, specifically a family of molecules collectively known as vanilloids. The vanilloid receptor subtype 1 (VR1), now known as TRPV1, is a non-selective cation channel embedded in the plasma membrane, activated by noxious heat and capsaicin, leading to increased sodium and calcium influx. This activation underlies the perception of pungency and, under certain conditions, may contribute to neurogenic inflammation. Moreover, the pungent properties of capsaicin have also been utilized in the development of mammalian deterrents. Importantly, in the medical context, capsaicin's involvement in carcinogenic processes has received growing attention. However, this role remains controversial, as some studies have reported carcinogenic potential, while others provide evidence supporting its antitumor and chemo preventive effects (Díaz et al., 2004). Specifically, the anticarcinogenic effects of capsaicin are thought to result primarily from its capacity to induce apoptosis through the generation of reactive oxygen species (ROS), which are predominantly produced via capsaicin's action on the mitochondrial electron transport chain. Moreover, in addition to its pro-apoptotic properties, capsaicinoids have been identified as potent antioxidants, further supporting their potential therapeutic applications. For example, the use of capsaicin in managing long-term neuropathic pain among cancer patients was first established

by Ellison et al. (1997), marking an important milestone in the clinical application of this compound (Ellison et al., 1997). Looking ahead, future perspectives on capsaicin research will emphasize its therapeutic potential, particularly its clinical applicability in the treatment of pain and related pathophysiological conditions. Indeed, as a potent TRPV1 receptor agonist, capsaicin plays a critical role in modulating oxidative stress, pain perception, and inflammatory responses. Nevertheless, despite certain adverse effects, capsaicin continues to be incorporated as an active pharmaceutical ingredient in various formulations aimed at managing a range of human disorders, underscoring its enduring relevance in medical therapeutics.

2.1.6 Translating *in vitro* to *in vivo* studies into clinical trials

Capsaicin has demonstrated significant anti-proliferative effects against prostate cancer cells in both in vitro and *in vivo* experimental models, highlighting its potential as a therapeutic agent in prostate cancer treatment (Mori et al., 2006). Ellison et al. (1997) reported that capsaicin is an effective therapeutic agent for the treatment of neuropathic conditions such as postherpetic neuralgia and diabetic dysesthesia. Additionally, topical capsaicin formulations have been successfully employed in cancer patients to manage persistent neuropathic pain, particularly that arising post-surgically (Ellison et al., 1997). For instance, according to Nolano et al. (1999), a three-week course of topical capsaicin treatment at a concentration of 0.075% led to approximately 80% reduction in epidermal nerve fiber density, indicating significant epidermal denervation following prolonged exposure (Nolano et al., 1999). In another study, Malmberg et al. (2004) demonstrated that a single 60-minute application of capsaicin could result in up to 60% epidermal denervation, underscoring its potent neurophysiological impact. Specifically, topical capsaicin formulations are generally categorized into low-dose preparations (ranging from 0.025% to 0.075%) and high-dose patches containing 8% capsaicin. High-dose patches are typically applied to the most painful regions of intact skin and left in place for approximately one hour. On the other hand, while low-concentration capsaicin creams have shown moderate clinical efficacy in the treatment of peripheral neuropathic pain (PNP), high-dose formulations offer enhanced therapeutic potential in selected patient populations (Malmberg et al.,

2004). Furthermore, a single 60-minute application of the 8% capsaicin dermal patch has been shown to provide rapid onset and sustained analgesic effects in patients experiencing neuropathic pain (Blair, 2018). Despite their therapeutic potential, low-concentration capsaicin creams require multiple daily applications, and their use is often limited by poor tolerability, particularly due to the initial burning sensation. In response, to overcome these limitations, Qutenza®, a high-dose 8% capsaicin dermal patch, was developed to deliver long-lasting pain relief from a single application. Notably, the capsaicin 8% patch has been approved in the European Union (EU), either as a monotherapy or in combination with other analgesics, for the management of peripheral neuropathic pain (PNP) in adults (Bonezzi et al., 2020). Moreover, topical capsaicin has been proposed as an effective adjunctive therapy for pain management in a variety of conditions, including rheumatoid arthritis, osteoarthritis, neuralgias, and diabetic neuropathy. Additionally, it has shown therapeutic potential in the treatment of neurological dysfunction, inflammatory disorders, and painful or pruritic cutaneous conditions associated with surgical procedures, trauma, or tumor-related complications (Babbar et al., 2009). Importantly, according to evidence-based treatment guidelines, topical capsaicin 8% is recognized as a viable therapeutic option for pain management, with studies such as Laklouk and Baranidharan (2016) supporting its use. In fact, the high-dose capsaicin patch has demonstrated acceptable safety and tolerability profiles, making it particularly beneficial for managing cancer-related and other chronic pain conditions (Laklouk & Baranidharan, 2016). In a recent study, Zis et al. (2016) demonstrated that the 8% capsaicin patch effectively relieves neuropathic pain and enhances quality of life in patients with lumbosacral neuropathic pain. Their study assessed both the safety and efficacy of the capsaicin patch in the treatment of peripheral neuropathic pain (PNP), whether used as a standalone therapy or as an adjunct to existing treatment regimens (Zis et al., 2016). Furthermore, *In vitro* and *in vivo* into the clinical trial studies have explored the antitumor roles of capsaicin in various cancers, such as breast, lung, prostate, and gastric cancers and cholangiocarcinoma, and pain (Zheng et al., 2016). Despite its successes, although capsaicin has been successfully applied in clinical settings for dermatological and pain management purposes, its broader use in pain therapy remains limited. Capsaicin, a

capsaicinoid compound derived from chili peppers, is commonly used to provide temporary relief from musculoskeletal pain associated with conditions such as arthritis. Its mechanism of action involves inducing a burning sensation, which overwhelms nociceptive nerve fibers, thereby inhibiting pain transmission for an extended period.

In order to improve the efficiency of treatment, transdermal therapeutic systems (TTSs) offer a convenient and controlled route of administration. Modified silicone polymer-based matrix diffusion systems have been shown to provide cost-effective and well-regulated drug release. For instance, a study by László S. et al. (2022) evaluated the release kinetics, skin penetration, and analgesic effects of a low-dose capsaicin-loaded TTS. Drug release was assessed using both Franz diffusion cells and continuous flow-through systems, with HPLC and FTIR spectroscopy confirmed that capsaicin penetrated the epidermal and dermal layers of human skin, reaching areas where TRPV1 receptors are expressed. In an in vivo model using male Wistar rats with induced traumatic or inflammatory pain, patches were applied for 6 hours. Capsaicin administration reversed thermal hyperalgesia and increased the mechanical pain threshold in treated animals. The findings suggest that the modified silicone-polymer capsaicin TTS is a promising tool for managing both traumatic and inflammatory pain, offering sustained and targeted analgesic effects through controlled transdermal delivery (László et al., 2022).

Currently, capsaicin is available in the form of creams, gels, and dermal patches containing low doses for topical application. In line with this, previous studies have demonstrated its safety profile in the treatment of pain. Nevertheless, the therapeutic efficacy and target specificity of conventional topical formulations remain limited, primarily due to poor transdermal penetration through the epidermal barrier. This limitation may contribute to adverse effects such as skin atrophy, burning sensations, and systemic absorption, which can negatively impact patient compliance. As a solution, nanotechnology-based drug delivery systems have emerged as promising alternatives. Nano formulations enhance skin permeation, enable targeted delivery, and improve drug release kinetics, thereby offering improved therapeutic outcomes while minimizing unwanted side effects in topical drug administration.

Table 2.1 Therapeutic applications and trials of topical capsaicin for treating pain

Compound	Indicated	Application	Concentration	Route of Administration	Efficacy	References
Capsaicin Gel	Acute Back/Neck Pain	Subjects received one of four topical gels, 2 g twice daily, with 12 ± 4 h between applications: 2% diclofenac+0.075% capsaicin gel, 2% diclofenac, 0.075% capsaicin, and placebo gel	0.075% Capsaicin	Transdermal	Capsaicin, whether used alone or in combination with diclofenac, showed greater pain relief compared to placebo. However, since diclofenac alone was no more effective than placebo, combining it with capsaicin did not enhance analgesic outcomes beyond what capsaicin achieved on its own.	(Iftinca et al., 2021; Predel et al., 2020)
Capsaicin Cream	Diabetic peripheral neuropathy (DPN)	This 12-week, randomized, double-blind, parallel-group experiment compared topical clonidine with capsaicin. Visual analog scale (VAS) pain score of at least 4 treated for 3 months.	0.075% Capsaicin	Topical	In an efficacy analysis involving 69 patients treated with clonidine and 70 with capsaicin, both agents significantly reduced pain over a 12-week period ($P < 0.01$). However, no statistically significant difference in analgesic efficacy was observed between the two treatments.	(Kiani et al., 2015)
Capsaicin 8% patch (Qutenza™)	Lumbosacral Pain	All selected patients were assessed before and 2 weeks, 8 weeks, and 12 weeks in 60 minutes. VAS was used to measure pain intensity and EQ-5D to measure quality of life.	8% Capsaicin	Transdermal	Application of the 8% capsaicin patch led to notable reductions in pain intensity and enhanced quality of life among patients suffering from lumbosacral neuropathic pain, demonstrating its clinical effectiveness in this population.	(Zis et al., 2016)

Table 2.1 Therapeutic applications and trials of topical capsaicin for treating pain (Continued)

Compound	Indicated	Application	Concentration	Route of Administration	Efficacy	References
Capsaicin hydrogel patch	chronic neck pain	Participants were assigned to wear either capsaicin 0.1% (500 µg) hydrogel patches or placebo hydrogel patches (capsaicin-free) for 12 hours daily over a 4-week period. Outcome assessments were conducted at baseline, 2 weeks after treatment initiation, upon completion of the 4-week intervention, and again at 4 weeks post-treatment using standardized evaluation instruments.	0.1% Capsaicin	Transdermal	Both groups' mean VAS scores reduced at 2, 4, and 8 weeks after intervention. No difference in VAS score or other outcome indicators was seen between the two groups.	(Brodsky et al., 2012)
Capsaicin hydrogel patch	Knee osteoarthritis (OA)	All patients received capsaicin gel or placebo gel three times daily for 4 weeks, then capsaicin gel or placebo gel for another 4 weeks. A blinded examiner did weekly VAS and WOMAC assessments.	0.0125% Capsaicin	Transdermal	VAS and total WOMAC scores were significantly different between the capsaicin and placebo groups (p 0.05). Only burning was recorded. 67% of patients felt a burning sensation during capsaicin therapy, but none withdrew. 0.0125% capsaicin gel helped moderately uncomfortable OA knees.	(Kosuwon et al., 2010)
Capsaicin hydrogel patch	Myofascial Neck Pain	During the 4-week trial, all participants were advised to apply one patch to each side of the neck and shoulder girdle over the region of peak pain for 12 hours daily.	0.1% Capsaicin	Transdermal	Both groups' mean VAS, NDI, and BDI scores dropped 2 and 4 weeks following intervention. No outcome measure differed significantly between the groups.	

2.2 General Pain

2.2.1 The definition of Pain

Historically, pain was defined in 1986 by the International Association for the Study of Pain (IASP) as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage." In addition, the International Association for the Study of Pain (IASP) defines chronic pain as pain that extends beyond the expected period of tissue healing, typically characterized as persisting for more than three months. Furthermore, this standardized definition has played a pivotal role in the establishment and consolidation of pain medicine as a formal medical discipline, building upon the foundational contributions of Dr. John Bonica (Noe, 2020).

More recently, in 2018, Cohen and colleagues proposed an alternative conceptualization of pain, describing it as a shared somatic experience that conveys an individual's perceived threat to their physical or existential well-being (Cohen et al., 2018). However, in a published commentary, Treede critiqued Cohen et al.'s definition for overlooking the multidimensional aspects of pain. Moreover, he also raised concerns about the vague expansion of the concept to include threats to "bodily integrity" and questioned the implication that the recognition of pain necessitates validation by an external observer (Treede, 2018). As a result, the discourse surrounding the optimal definition of pain remains ongoing. Additionally, it is now widely acknowledged that pain including myofascial pain can manifest in the absence of identifiable tissue damage, challenging the outdated structural-pathology model that once equated pain solely with physical injury. This shift in perspective emphasizes that pain is not always a direct indicator of tissue harm (Donnelly et al., 2018). In fact, the IASP definition of pain has been widely accepted by clinicians and researchers in the field and has been officially adopted by numerous professional, governmental, and nongovernmental organizations, including the World Health Organization (WHO). Despite these changes, the accompanying glossary of pain-related terms has undergone multiple revisions over time, the core IASP definition has remained consistent.

IASP definition of pain (1979)

"Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage.

Pain is always subjective. Each individual learns the application of the word through experiences related to injury in early life. Biologists recognize that those stimuli which cause pain are liable to damage tissue. Accordingly, pain is that experience which we associate with actual or potential tissue damage. It is unquestionably a sensation in a part or parts of the body but it is also always unpleasant and therefore also an emotional experience. Experiences which resemble pain, eg, pricking, but are not unpleasant, should not be called pain. Unpleasant abnormal experiences (dysaesthesiae) may also be pain but are not necessarily so because, subjectively, they may not have the usual sensory qualities of pain. Many people report pain in the absence of tissue damage or any likely pathophysiological cause; usually this happens for psychological reasons. There is no way to distinguish their experience from that due to tissue damage if we take the subjective report. If they regard their experience as pain and if they report it in the same ways as pain caused by tissue damage, it should be accepted as pain. This definition avoids tying pain to the stimulus. Activity induced in the nociceptor and nociceptive pathways by a noxious stimulus is not pain, which is always a psychological state, even though we may well appreciate that pain most often has a proximate physical cause."

Revised IASP definition of pain (2020):

"Pain is an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage. Moreover, pain is always a personal experience that is influenced to varying degrees by biological, psychological, and social factors. Importantly, pain and nociception are different phenomena. Pain cannot be inferred solely from activity in sensory neurons. Through life experiences, individuals learn the concept of pain, and a person's report of an experience as pain should be respected. Although pain usually serves an adaptive role, it may have adverse effects on function and social and psychological well-being. Finally, verbal description is only one of several behaviors to express pain;

thus, inability to communicate does not negate the possibility that a human or a nonhuman animal experiences pain."

2.2.2 Etymology

The Declaration of Montr ´eal, a document developed during the First International Pain Summit on September 3, 2010, states that "**Access to pain management is a fundamental human right**.

The IASP's 1979 definition of pain remains influential in recognizing that pain can occur in the absence of observable tissue damage, highlighting its multidimensional and subjective nature. Its concise and accessible wording has facilitated global consensus among clinicians, researchers, and policymakers, which has significantly shaped health care, pain research, and patient understanding. Pain may be acute, chronic, intermittent, or a combination thereof, and is the most common reason individuals seek medical care.

However, despite its prevalence, pain is often undertreated, largely due to its classification as a symptom rather than a standalone condition. For example, acute pain frequently arises from injury, illness, surgery, or childbirth, and affects 30–80% of patients in clinical settings. If left inadequately managed, it can transition into chronic pain, prolong recovery, and increase both morbidity and hospital stay.

On a global scale, pain affects around 20% of adults, with approximately 10% experiencing persistent pain. Chronic pain, especially-particularly that with neuropathic components, is associated with greater severity and duration, affecting an estimated 6–10% of the population. Furthermore, it significantly diminishes quality of life, contributes to increased mortality risk, and imposes considerable socioeconomic burdens, including reduced work productivity and higher rates of absenteeism. Notably, pain is a common medical problem that is reported in developed countries as well, and it is associated with enormous personal costs and a significant burden on the health care system of the society (Dureja et al., 2017). To better understand the mechanisms and treatment approaches, Table 2.2 outlines the three primary classifications of chronic pain: nociceptive, neuropathic, and nociplastic pain.

Nociceptive pain

Nociceptive pain arises from the activation of neural pathways in response to actual or potentially harmful stimuli affecting body tissues. It is the most prevalent form of chronic pain and typically includes conditions such as arthritis and most types of spinal pain.

Neuropathic pain

According to the IASP, neuropathic pain is defined as pain resulting from injury or disease affecting the somatosensory nervous system (Finnerup et al., 2016). In contrast to nociceptive pain, neuropathic pain is frequently associated with sensory disturbances such as numbness, allodynia, and sudden bursts of intense pain. Additionally, neurological deficits may also be present, depending on the affected nerves (see Table 2.2). While nociceptive pain is often characterized as aching or throbbing, neuropathic pain is commonly described as lancinating or shooting in nature. Common clinical examples include diabetic neuropathy, postherpetic neuralgia, and radiculopathy. Whereas neuropathic pain is estimated to represent approximately 15–25% of all chronic pain cases (Cohen & Mao, 2014). While multiple validated assessment instruments exist for the classification of chronic pain, physician-based clinical evaluation continues to be regarded as the gold standard for accurate diagnosis (Liu et al., 2017). Chronic neuropathic pain, unlike many forms of nociceptive pain or acute nerve injury, is consistently linked to maladaptive behavioral responses. Although the correlation between pain intensity and functional disability is relatively weak, neuropathic pain tends to result in more pronounced impairments in quality of life compared to nociceptive pain of similar severity (Saavedra-Hernández et al., 2012; Spahr et al., 2017).

Nociplastic pain

Nociplastic pain refers to pain arising from altered nociceptive processing without clear evidence of tissue damage or identifiable pathology within the somatosensory system. Notably, previously termed functional pain syndromes, this category includes conditions such as fibromyalgia, irritable bowel syndrome, and potentially non-specific low back pain (see Table 2.2). Moreover, the underlying pathophysiology is thought to involve heightened sensory processing alongside

diminished activity in descending inhibitory pathways. As a result, patients with nociplastic pain often experience less favorable outcomes following interventional procedures—such as joint or epidural steroid injections—compared to those with nociceptive or neuropathic pain, with few exceptions to this trend (Cohen et al., 2021).

Table 2.2 The three main categories of chronic pain: nociceptive, neuro pathic, and nociplastic

	Nociceptive pain	Neuropathic pain	Nociplastic pain
Causes	Actual or potential damage to bodily tissues	Neurological dysfunction resulting from disease or injury to the nervous system	Maladaptive neurophysiological changes influencing pain processing and regulation, despite the lack of detectable tissue or nerve pathology
Descriptors	Pain descriptors indicative of somatic origin, such as rhythmic throbbing, diffuse aching, or a sensation of internal pressure	Sharp, electric shock-like, transient, or piercing sensations	Visceral pain, such as that experienced in conditions like interstitial cystitis or irritable bowel syndrome, may share similarities with neuropathic pain and is often described using terms such as diffuse, gnawing, aching, or occasionally sharp.
Sensory deficits	Rarely observed, and when present, follows a pattern that does not conform to dermatomal or peripheral nerve distributions	Commonly observed sensory disturbances, including paresthesias such as numbness, tingling, and prickling sensations	Occasionally observed, typically presenting in patterns that do not correspond to dermatomal or specific peripheral nerve distributions
Motor deficits	Weakness	Motor nerve involvement may lead to observable neurological weakness. Movement disorders such as dystonia or spasticity are commonly linked to central nervous system (CNS) lesions but can also occur in certain peripheral nerve conditions.	Generalized fatigue is frequently reported, and any observed weakness is often attributed to physical deconditioning rather than direct neurological impairment.

Table 2.2 The three main categories of chronic pain: nociceptive, neuro pathic, and nociplastic (Continued)

	Nociceptive pain	Neuropathic pain	Nociplastic pain
Hypersensitivity	Typically rare, except for localized hypersensitivity near the site of an acute injury.	Frequently reported in neuropathic pain, where pain is triggered by normally non-painful stimuli (allodynia) or an exaggerated response to painful stimuli (hyperalgesia)	Common in nociplastic pain, often presenting as diffuse pain, with heightened sensitivity to mechanical stimuli and hyperalgesia being more prevalent than allodynia.
Autonomic signs	Uncommon	Autonomic symptoms, such as alterations in skin color or temperature, swelling, and abnormal sweating (sudomotor activity), are observed in approximately one-third to one-half of affected individuals.	Increased sympathetic nervous system activity is commonly reported in patients with widespread pain syndromes, such as fibromyalgia, as well as in visceral pain conditions like irritable bowel syndrome.
Effective nonopioid pharmacological treatments	Commonly managed with NSAIDs, administered either topically or systemically, and muscle relaxants particularly effective for acute and subacute spinal pain. Additional treatments include serotonin–norepinephrine reuptake inhibitors (SNRIs), tricyclic antidepressants (TCAs), disease-modifying antirheumatic drugs (DMARDs) for inflammatory arthritis, nerve growth factor (NGF) inhibitors, and tramadol.	The typically treated with TCAs, SNRIs, and gabapentinoids. For regional neuropathic pain, high-concentration capsaicin patches and lidocaine patches may be utilized. Tramadol may also be considered.	The management includes TCAs, SNRIs, and gabapentinoids. In select cases, ketamine infusions have shown therapeutic benefit.

Table 2.2 The three main categories of chronic pain: nociceptive, neuro pathic, and nociplastic (Continued)

	Nociceptive pain	Neuropathic pain	Nociplastic pain
Precipitating or relieving factors	Flare-ups are relatively infrequent and typically triggered by physical activity.	Exacerbations occur frequently and are often unpredictable in nature.	Exacerbations are common, frequently correlating with psychosocial stressors.
Examples	Common examples include musculoskeletal and injury-related pain such as back pain, headaches, neck pain, shoulder pain, and pain resulting from burns or physical trauma.	These include pain syndromes associated with nerve damage or dysfunction, such as peripheral neuropathy, diabetic neuropathy, trigeminal neuralgia, complex regional pain syndrome (CRPS), and neuropathic pain following spinal cord injury.	Characterized by altered nociceptive processing without clear tissue or nerve injury, this group includes chronic widespread pain, fibromyalgia, chronic non-specific low back pain, chronic temporomandibular joint (TMJ) disorders, irritable bowel syndrome (IBS), chronic primary bladder pain syndrome, and chronic primary pelvic pain syndromes in both men and women (Fitzcharles et al., 2021)

2.2.3 The mechanism of pain and pain pathways

Pain arises from both physical injury and psychological responses. Specifically, nociceptive signals originate in the periphery and are transmitted to the central nervous system (CNS) for modulation. Within the spinal cord, primary afferent fibers terminate in the dorsal horn, where they synapse with second-order neurons. These neurons project through ascending pathways, including the spinothalamic and spinoreticular tracts, conveying pain signals to higher cortical centers. In addition, descending modulatory pathways—such as those involving the periaqueductal grey and nucleus raphe magnus—play a critical role in regulating pain perception. In neuropathic pain, several mechanisms have been proposed. Among these, both peripheral and central sensitization contribute to abnormal pain processing following nerve injury (Steeds, 2009). Overall, pain pathways converge to form a complex and dynamic system encompassing sensory, cognitive, and behavioral components.

Evolutionarily, this system has developed to detect and integrate harmful stimuli, coordinating protective responses essential for organismal survival (Melzack, 1999). The pain defense system ranges from basic spinal reflexes serving as primary protective mechanisms in simple organisms all the way to highly sophisticated emotional and cognitive responses in humans. Ultimately, the perception of pain results from intricate interactions between the peripheral and central nervous systems, modulated by a balance of excitatory and inhibitory neurotransmitters released in response to noxious stimuli.

The sensation of pain is composed of Figure 2.3**):**

1. **Transduction**: The nociceptors are responsible for this process, which entails the transformation of noxious stimuli into electrical impulses or action potentials. These action potentials can be triggered by a wide variety of stimuli, including those that are mechanical, thermal, or chemical in nature.

2. **Transmission**: It is a process in which the action potential that is generated at the nociceptor is propagated along the axon of the main afferent neuron. This process takes place in the primary afferent neuron.

3. **Perception**: The somatosensory cortex is primarily involved in processing the sensory-discriminative aspects of nociception, such as intensity and location, whereas the deeper limbic structures are responsible for the affective-motivational (emotional) dimensions of the pain experience.

4. **Modulation**: This refers to a neural mechanism that functions to suppress activity within the pain transmission pathways, thereby diminishing the overall perception of pain.

The process of nociception

Pain perception is mediated by multiple neural signaling pathways. When tissues are exposed to harmful thermal, mechanical, or chemical stimuli, they release various inflammatory mediators such as globulins, proteins, kinases, arachidonic acid, histamine, nerve growth factor (NGF), substance P (SP), and calcitonin gene-related peptide (CGRP). These substances activate transducer ion channels functionally similar to voltage-gated channels thereby initiating receptor potentials (**1. transduction**). These receptor potentials trigger action potentials in sensory neurons.

Peripheral sensitization refers to the heightened responsiveness of nociceptors to noxious stimuli, commonly observed in inflamed tissues. During inflammation, nociceptors located in the skin and deeper tissues exhibit increased sensitivity, thus, reducing the activation threshold. As a result, even normally non-painful or mildly painful stimuli elicit amplified responses. Moreover, this process can also activate previously silent nociceptors, which substantially intensifying the pain experience. To manage this, pharmacological agents such as non-steroidal anti-inflammatory drugs (NSAIDs), opioids, cannabinoids, and TRPV1 receptor antagonists target peripheral nociceptors to modulate pain at the site of injury (Dureja et al., 2017). One key mediator, prostaglandin E2 (PGE2), is produced from arachidonic acid via the enzyme cyclooxygenase-2 (*COX-2*). Traditional and *COX-2*-selective NSAIDs exert their analgesic effects by inhibiting this enzyme, thereby reducing the synthesis of PGE2 and mitigating pain and inflammation.

During pain transmission, afferent signals travel via sensory nerve fibers to the dorsal root ganglia and the dorsal horn of the spinal cord (**2. transmission**). Next, these signals are then relayed upward through the spinal cord to the brainstem and thalamus, where complex processing occurs (**3. modulation**). Finally, the signals reach the somatosensory cortex, enabling conscious awareness of pain (**4. Perception**). In addition, the perception of pain is further shaped by biopsychosocial factors, involving multiple brain regions:

Amygdala: Mediates the emotional and affective aspects of pain and contributes to pain modulation.

Hypothalamus: Regulates the neuroendocrine response to pain, particularly through the corticotropin-releasing pathway.

Periaqueductal gray (PAG): Serves as a central hub for descending pain modulation and is involved in aversive and defensive pain behaviors.

Basal ganglia: Contributes to the cognitive, emotional, and discriminative aspects of pain processing, including the localization and interpretation of sensory input. Stage 4 also activates a wide range of autonomic, emotional, cognitive, and behavioral responses, reflecting the integrative nature of pain perception (Dureja et al., 2017). The cerebral cortex serves as the terminal site for pain perception

and possesses the ability to initiate descending modulatory pathways that regulate nociceptive input, as illustrated in Figure 2.3. Within the spinal cord and dorsal root ganglia, endogenous opioid peptides including endorphins, dynorphins, and enkephalins play a critical role in attenuating pain transmission by modulating nociceptive afferent input. This occurs through activation of descending inhibitory pathways, particularly involving the periaqueductal gray (PAG) in the midbrain. Endogenous opioids exert their effects by binding to mu (μ), kappa (κ), and delta (δ) opioid receptors, resulting in decreased presynaptic calcium influx and reduced release of excitatory neurotransmitters such as glutamate and substance P (SP). Additionally, these mechanisms enhance potassium conductance in dorsal horn neurons, contributing to neuronal hyperpolarization and further inhibition of pain signaling. Other key neurotransmitters involved in descending modulation include norepinephrine (NE), glycine, and gamma-aminobutyric acid (GABA).

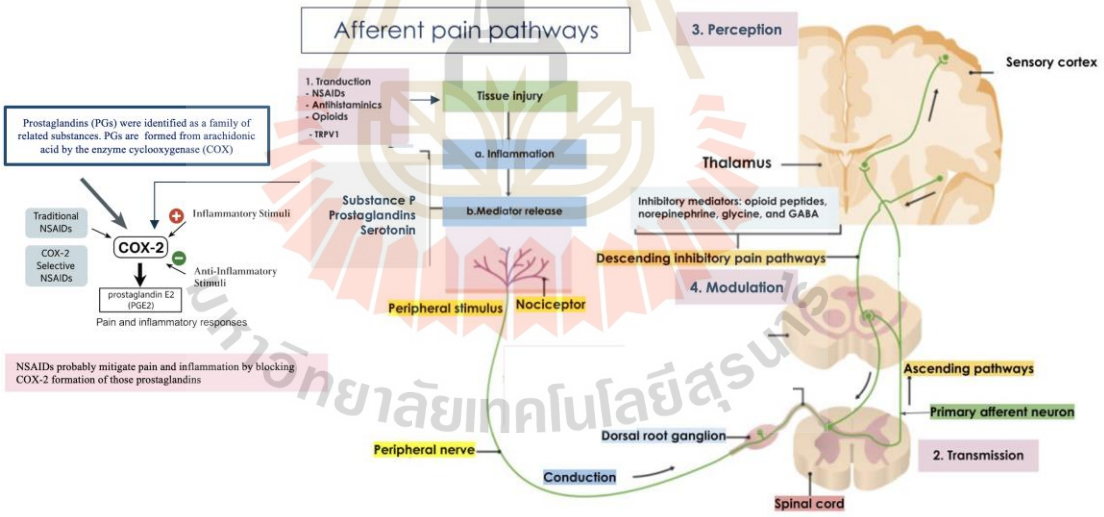


Figure 2.3 The mechanism of pain

(Modified form Lecturio and Dureja et al. (2017))

Afferent pain pathways illustrating the four primary stages of pain processing: (1) Transduction – conversion of noxious stimuli into electrical signals at the site of tissue injury involving inflammatory mediators such as prostaglandins and substance P; (2) Transmission – propagation of electrical impulses via peripheral nerves to the

spinal cord and ascending tracts; (3) Perception – conscious awareness of pain within the sensory cortex; and (4) Modulation – regulation of pain signals by descending inhibitory pathways using neurotransmitters like opioids, GABA, and norepinephrine. The diagram also highlights the role of *COX-2* in prostaglandin synthesis and the action of NSAIDs in reducing pain and inflammation.

2.2.4 Pain management

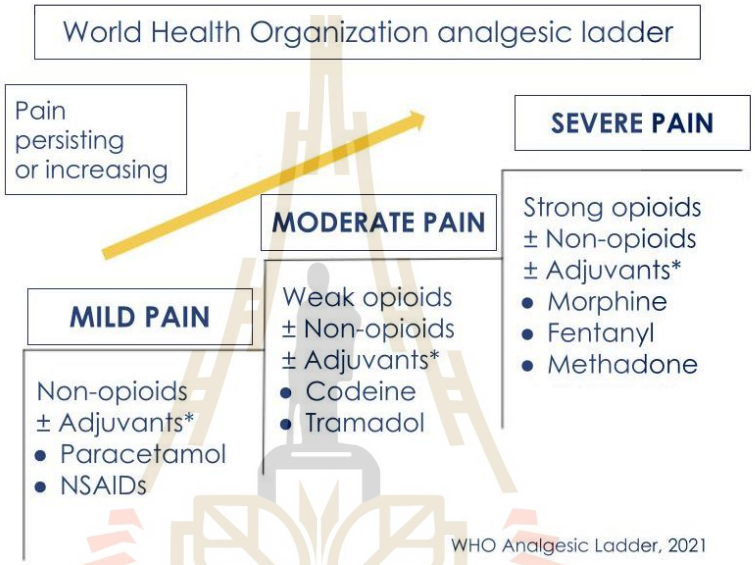


Figure 2.4 The World Health Organization (WHO) pain relief ladder (Anekar & Cascella, 2021)

The first ladder consisted mostly of three steps:

Step 1 – Mild Pain: Management begins with non-opioid analgesics such as nonsteroidal anti-inflammatory drugs (NSAIDs) or acetaminophen, which may be administered alone or in combination with adjuvant therapies.

Step 2 – Moderate Pain: For more pronounced pain, treatment involves weak opioids (e.g., hydrocodone, codeine, tramadol), either alone or in combination with non-opioid analgesics and appropriate adjuvants.

Step 3 – Severe or Chronic Pain: In cases of intense or persistent pain, strong opioids—including morphine, methadone, fentanyl, oxycodone, buprenorphine, tapentadol, hydromorphone, and oxymorphone—are prescribed, with or without concurrent use of non-opioid agents and adjuvants.

Adjuvant analgesics, or co-analgesics, encompass a broad category of medications that are primarily prescribed for non-analgesic indications, yet have demonstrated efficacy in managing various pain conditions. These include tricyclic antidepressants (e.g., amitriptyline, nortriptyline), serotonin–norepinephrine reuptake inhibitors (SNRIs) such as duloxetine and venlafaxine, anticonvulsants like gabapentin and pregabalin, as well as topical agents including lidocaine patches.

Furthermore, multimodal analgesia is widely advocated as an optimal approach for pain management due to the involvement of multiple receptor systems across both peripheral and central nervous pathways. This strategy allows for more effective pain control while minimizing adverse effects. Specifically, pharmacological agents used in multimodal regimens include: Opioids, which modulate afferent pain transmission and activate descending inhibitory pathways at both spinal and supraspinal levels, local anesthetics, which transiently block nociceptive signal conduction, antidepressants (TCAs and SNRIs), which enhance monoaminergic modulation of pain, and NSAIDs, such as ibuprofen, which reduce inflammation and peripheral sensitization. Importantly, successful pain management must also address the psychosocial dimensions of pain, including its impact on mood, anxiety, and overall physical and social functioning. A comprehensive biopsychosocial approach remains essential for achieving effective and sustainable pain relief (Webb & Steeds, 2022). In addition, distinct patterns of sensory neuronal alterations highlight the complexity and variability of pain as a dynamic physiological process. Currently, pharmacological strategies for pain management include analgesics such as acetaminophen, NSAIDs, antidepressants, and various combination therapies. Moreover, advances in molecular neuroscience have reshaped our understanding of nociception by revealing that nociceptors possess receptor-coupled ion channels capable of detecting environmental stimuli, leading to neuronal depolarization and the perception of pain. This discovery has not only deepened insight into pain mechanisms, but also facilitated the development of novel therapeutic agents—particularly in light of Mendelian mutations in these receptor proteins that are directly linked to altered pain sensitivity (Fattori et al., 2016; Wolkerstorfer et al., 2016). The foundational principle of the analgesic ladder is that effective pain management relies on thorough assessment and

understanding of the patient's pain severity to guide appropriate pharmacologic intervention. Given that many patients may require opioid therapy at some stage, it is essential to balance effective dosing with the potential for adverse effects. To address this, opioid rotation may be employed to enhance analgesic outcomes while minimizing side effects.

Additionally, patient education on the appropriate use, therapeutic benefits, and potential risks of medications is essential to prevent misuse and ensure sustained treatment efficacy. Certain chemical families exhibit a wide range of pharmacological properties, including anti-inflammatory, antioxidant, and TNF-α inhibitory effects, making them relevant in the management of conditions such as psoriasis. These compounds may also possess cardiostimulant, antioxidant, and anticancer activities. Methyl salicylate, a derivative of salicylic acid, serves as an analgesic and non-steroidal anti-inflammatory drug (NSAID), and has additionally been identified as a TRPV1 receptor activator (Ohta et al., 2009). Although significant progress has been made in elucidating pain mechanisms through emerging methodologies, experimental models remain indispensable tools in pain research. Notably, capsaicin continues to serve as a widely utilized and valuable experimental agent for investigating nociceptive pathways.

2.3 Capsaicin: Mechanisms of Pain Modulation and Cellular Responses in Human Dermal Fibroblasts

Capsaicin is a pungent vanilloid compound derived from chili peppers (*Capsicum spp.)* and is widely recognized for its potent ability to modulate various types of pain, particularly neuropathic, myofascial, and inflammatory pain. Its primary pharmacological activity is mediated through the activation of the transient receptor potential vanilloid 1 (TRPV1) channel, which is predominantly expressed on peripheral nociceptive neurons. Upon binding to TRPV1, capsaicin induces calcium (Ca^{2+}) and sodium (Na^{+}) influx, leading to neuronal depolarization, neurotransmitter release, and subsequent transmission of pain signals to the central nervous system (CNS). However, with prolonged exposure, capsaicin causes TRPV1 desensitization, which reduces

nociceptive signaling and ultimately produces analgesic effects (Bode & Dong, 2011; Caterina et al., 1997).

Recent evidence has expanded our understanding of capsaicin's mechanism of action, revealing that TRPV1 is also expressed in non-neuronal cells, including human dermal fibroblasts (HDFs). These fibroblasts play crucial roles in skin inflammation, tissue remodeling, and wound healing. Within this context, capsaicin exhibits anti-inflammatory effects by modulating intracellular signaling pathways associated with inflammation. Notably, one key mechanism is the downregulation of cyclooxygenase-2 (*COX-2*) an inducible enzyme responsible for prostaglandin E2 (PGE2) synthesis, which contributes to inflammatory pain sensitization at the tissue level. Under inflammatory stimuli, such as interleukin-1β (IL-1β) or lipopolysaccharide (LPS), *COX-2* expression is significantly upregulated in HDFs, thereby intensifying the pain cascade. Capsaicin, however, can suppress *COX-2* expression at both mRNA and protein levels by inhibiting the NF-**K**B and MAPK signaling pathways (Hudita et al., 2021)

In addition to *COX-2* suppression, capsaicin also reduces oxidative stress in dermal fibroblasts by lowering the production of reactive oxygen species (ROS) and increasing antioxidant defenses such as superoxide dismutase (SOD) and glutathione (GSH). This antioxidant activity plays a significant role in protecting skin cells from inflammation-induced damage and contributes to the attenuation of oxidative inflammatory pain (Cuijpers et al., 2025).

Moreover, capsaicin enhances fibroblast migration, proliferation, and collagen synthesis via activation of PI3K–AKT–mTOR signaling pathways, promoting tissue regeneration and supporting wound healing. These effects are crucial for reducing wound-associated pain, which often arises from chronic inflammation and delayed dermal repair (Lee et al., 2013).

Taken together, these findings highlight the dual action of capsaicin: (1) as an analgesic agent that modulates nociceptive pathways via TRPV1 and (2) *COX-2* suppression, and as a dermal modulator that reduces inflammation and oxidative stress in human dermal fibroblasts. Such properties make capsaicin a strong candidate for transdermal delivery systems targeting pain and inflammatory skin conditions.

2.3.1 Anti-Inflammatory and Effects on Human Dermal Fibroblasts (HDFs)

Human dermal fibroblasts (HDFs) are essential components of the skin's structural and functional integrity, as they play pivotal roles in wound healing, extracellular matrix (ECM) remodeling, and regulation of inflammatory responses. Therefore, understanding the molecular and cellular effects of capsaicin on HDFs is critical for evaluating its therapeutic potential, particularly in the context of transdermal drug delivery. Such insights are especially valuable for developing targeted treatments for inflammatory skin disorders and dermal pain syndromes, where fibroblast-mediated mechanisms are central to both pathophysiology and recovery.

Overview of Inflammatory Processes in HDFs

Human dermal fibroblasts (HDFs) are not merely structural components; rather, they actively participate in immune surveillance and regulation. Under pathological conditions such as tissue injury, microbial infection, or oxidative stress fibroblasts significantly upregulate the expression of several pro-inflammatory mediators, including tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and cyclooxygenase-2 (*COX-2*). These molecules not only initiate and sustain local inflammation but also facilitate the recruitment of immune cells to the site of injury, thereby amplifying the inflammatory response and contributing to pain perception. Furthermore, in dermal tissues, prolonged or dysregulated inflammation often results in adverse outcomes such as fibrosis, delayed wound healing, or the development of chronic, non-healing wounds particularly in individuals with diabetes or advanced age. Therefore, modulating the inflammatory response in fibroblasts is a key strategy for promoting effective tissue repair and managing inflammation-associated pain.

Capsaicin's Anti-Inflammatory Properties

Capsaicin exerts potent anti-inflammatory effects, primarily through the downregulation of key cytokines and enzymes involved in inflammatory signaling. Specifically in human dermal fibroblasts (HDFs), capsaicin has been shown to suppress the expression of interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and cyclooxygenase-2 (*COX-2*)—all of which play central roles in mediating pain sensitization and inflammatory cascades. By attenuating these molecular mediators, capsaicin contributes to the modulation of inflammatory responses, ultimately

supporting its therapeutic utility in conditions involving chronic inflammation and associated pain (Iftinca et al., 2021). This suppressive effect appears to be mediated through the inhibition of key intracellular signaling pathways, most notably the nuclear factor kappa B (NF-**K**B) and mitogen-activated protein kinases (MAPKs) pathways. Both pathways are known to be rapidly activated in response to various stress-related stimuli, including microbial components, pro-inflammatory cytokines, and oxidative stress. By blocking these upstream signaling cascades, capsaicin effectively downregulates the transcription of genes involved in inflammatory mediator production, thereby attenuating the cellular inflammatory response (Ilie et al., 2019).

NF-KB Pathway Inhibition

Nuclear factor kappa B (NF-**K**B) is a key transcription factor complex that, once activated, translocates to the nucleus and promotes the transcription of various pro-inflammatory genes. Capsaicin, however, has been demonstrated to inhibit the activation of I**K**B kinase (IKK), which is an upstream regulator of NF-**K**B. By blocking IKK activity, capsaicin prevents the phosphorylation and subsequent degradation of I**K**B**α**, the inhibitory protein that normally sequesters NF-**K**B in the cytoplasm. As a result, NF-**K**B remains in its inactive cytoplasmic state, thereby reducing its nuclear translocation and limiting the transcription of inflammatory mediators such as *COX-2*, IL-6, and TNF-**α**. In a study conducted by Hudita et al. (2021), HDFs treated with capsaicin showed significantly decreased expression of these pro-inflammatory markers following exposure to lipopolysaccharide (LPS), a potent inflammatory stimulus. These findings indicate that capsaicin effectively suppresses NF-**K**B-dependent inflammatory signaling in dermal fibroblasts, reinforcing its therapeutic potential for managing skin inflammation and pain (Hudita et al., 2021).

***COX-2* Expression Suppression**

Cyclooxygenase-2 (*COX-2*) is an inducible enzyme responsible for the conversion of arachidonic acid into prostaglandins, most notably prostaglandin E2 (PGE2). PGE2 plays a central role in vasodilation, edema formation, and pain sensitization during inflammation. Overexpression of *COX-2* in skin tissue has been strongly linked to chronic inflammatory conditions and hyperalgesia.

Importantly, capsaicin has been shown to downregulate *COX-2* gene expression and inhibit PGE2 production in human dermal fibroblasts (HDFs), suggesting a direct mechanism by which capsaicin attenuates peripheral sensitization and inflammatory pain. This effect is particularly relevant in pathological conditions such as myofascial pain syndrome, psoriasis, and burn wounds, where *COX-2*–mediated pathways are known to be upregulated (Chen et al., 2018).

Role in Pain Relief

Pain is not solely a neuronal phenomenon; it is also modulated by non-neuronal cells, particularly fibroblasts, which play a critical role in chronic inflammatory states and wound-related conditions. These cells actively contribute to the production of pro-inflammatory cytokines, regulation of extracellular matrix, and interactions with immune cells, thereby influencing the persistence, intensity, and resolution of pain. Consequently, targeting fibroblast-mediated pathways may offer novel therapeutic strategies in managing inflammatory and neuropathic pain syndromes (Fang et al., 2023). Fibroblasts can secrete nociceptive mediators that amplify pain and modulate nociceptor sensitivity (Pinho-Ribeiro et al., 2017). The ability of capsaicin to suppress both inflammatory mediators and oxidative stress in fibroblasts underscores its potential to alleviate not only primary nociceptive pain but also secondary inflammatory pain that originates from the dermal microenvironment. In addition, capsaicin contributes to tissue repair and regeneration, which in turn supports long-term pain relief by restoring structural integrity and mitigating chronic inflammation. Mechanistically, capsaicin stimulates fibroblast migration, proliferation, and collagen synthesis, particularly of type I and type III collagen, which are essential components for dermal remodeling and wound resolution. Furthermore, activation of PI3K/Akt/mTOR and ERK1/2 signaling pathways has been implicated in mediating these regenerative effects. Taken together, these properties highlight capsaicin's therapeutic potential not only as an anti-inflammatory and analgesic agent but also as a promising candidate for wound-healing applications (Lee et al., 2013).

Several studies have extensively investigated the anti-inflammatory and antioxidant properties of capsaicin, particularly its role in modulating pain-related molecular pathways in both neuronal and non-neuronal cells. Notably, research on

human dermal fibroblasts (HDFs) has demonstrated that capsaicin not only suppresses the expression of pro-inflammatory genes such as cyclooxygenase-2 (*COX-2*), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) but also enhances cellular antioxidant defenses.

Specifically, capsaicin has been shown to increase levels of superoxide dismutase (SOD) and glutathione (GSH) while reducing the generation of reactive oxygen species (ROS). These molecular effects collectively contribute to the attenuation of inflammation-induced oxidative stress, thereby leading to pain reduction. This dual modulation of both inflammatory and oxidative stress pathways is particularly relevant in the context of inflammatory skin disorders and dermal pain syndromes, as summarized in Table 2.3.

Table 2.3 Comparisons of Capsaicin Studies on Human Dermal Fibroblasts and Pain

Study	Cell Model / System	Key Findings	Implication for Pain Modulation
(Cuijpers et al., 2025)	HDFs under oxidative stress	Capsaicin decreased ROS and MDA, increased SOD and GSH; antioxidant protection in HDFs.	Protection from oxidative stress supports pain control in inflammatory skin conditions.
(Hudita et al., 2021)	Human Dermal Fibroblasts (HDFs)	Capsaicin reduced *COX-2*, IL-6, MMP-1 expression; anti-inflammatory effects via NF-κB inhibition.	Reduction of inflammatory gene expression may alleviate local inflammatory pain.
(Lee et al., 2013)	Fibroblasts (wound model)	Capsaicin promoted fibroblast migration and proliferation; activated Akt/mTOR signaling.	Enhanced wound healing may reduce wound-associated pain.
(Kim et al., 2004)	LPS-stimulated immune cells	Capsaicin suppressed *COX-2* and PGE2 production; reduced inflammation in macrophages.	Inflammation suppression indicates potential for systemic or local pain relief.

Although capsaicin has been widely recognized for its analgesic effects via the activation of transient receptor potential vanilloid 1 (TRPV1) receptor on sensory neurons, recent evidence suggests that its biological activity extends beyond the nervous system. In particular, its effects on non-neuronal cells, such as human dermal fibroblasts (HDFs), remain inadequately understood. HDFs are not only structural components of the skin but also key regulators of inflammation, oxidative stress, and wound healing. While the anti-inflammatory role of capsaicin has been demonstrated in various immune cell lines, few studies have explored its molecular influence on inflammation-related gene expression particularly the suppression of cyclooxygenase-2 (*COX-2*), a gene closely associated with the pathogenesis of inflammatory pain. In addition, the interaction between capsaicin and oxidative stress pathways in HDFs is poorly characterized, despite growing evidence that reactive oxygen species (ROS) contribute to chronic skin inflammation and fibroblast dysfunction.

Another research gap lies in the lack of studies evaluating capsaicin in the form of transdermal delivery systems, especially electrospun nanofiber patches. While capsaicin's use in topical creams and high-dose patches (e.g., 8%) has been approved for neuropathic pain, its potential for localized, controlled delivery via nanofibers has not been fully investigated. Moreover, studies involving in vitro skin permeation using Strat-M™ synthetic membranes are limited, despite the growing need for ethical and reproducible skin models that replace animal or human tissue. These knowledge gaps limit the understanding of capsaicin's full therapeutic potential, particularly in non-neuropathic pain types such as inflammatory or myofascial pain, which involve both neural and dermal components.

Therefore, this study was undertaken to address these gaps by developing a capsaicin-loaded nanofiber transdermal patch using biocompatible polymers (PVA/PVP) via electrospinning, and to investigate its anti-inflammatory and antioxidant effects on HDFs. Specifically, the study aims to evaluate the expression of *COX-2* and oxidative stress markers following capsaicin exposure, and to assess the release profile and skin permeation using a Strat-M™ membrane model. By elucidating the cellular and molecular mechanisms of capsaicin in human dermal fibroblasts, the

findings are expected to support the development of innovative, non-invasive therapies for pain relief.

2.4 Transdermal drug delivery systems

Oral and parenteral drug delivery remain the two most commonly employed administration routes, with the oral route being the preferred method for small-molecule therapeutics due to its convenience, portability, and ease of self-administration (Anselmo & Mitragotri, 2014; Han & Das, 2015). However, the oral route of drug administration is widely favored due to its convenience, portability, and ease of self-administration with fixed dosing regimens, making it one of the most practical delivery methods. However, oral delivery is unsuitable for many therapeutic peptides and proteins, primarily due to enzymatic degradation in the gastrointestinal tract and limited epithelial permeability for large molecules. Consequently, parenteral administration, particularly injection, is the predominant approach for delivering such macromolecules. Nonetheless, this route presents several challenges, including its invasive nature, the potential to cause pain and discomfort, and reduced patient compliance, as it often requires skilled personnel for proper administration. These limitations highlight the need to re-evaluate and innovate beyond conventional drug delivery strategies (Alkilani et al., 2015). Advanced drug delivery approaches, such as transdermal drug delivery (TDD), offer promising solutions to the limitations associated with conventional administration routes. A drug delivery system (DDS) encompasses a range of physicochemical technologies designed to regulate the transport and controlled release of pharmacologically active agents into targeted cells, tissues, or organs, thereby maximizing therapeutic efficacy and minimizing systemic side effects (Vargason et al., 2021; Vega-Vásquez et al., 2020). In essence, drug delivery systems (DDS) encompass both the formulation strategies and routes of administration designed to optimize therapeutic efficacy while minimizing potential adverse effects. Various administration modalities include oral, transdermal (through the skin), mucosal (e.g., nasal or buccal), pulmonary (inhalation via the lungs), and intravenous delivery, each offering unique advantages based on the drug's physicochemical properties and

clinical application (Jeong et al., 2021). Among these, the transdermal drug delivery system (also known as TDDS) stands out as a potentially useful method.

The transdermal drug delivery system (TDDS), a noninvasive method for administering therapeutics through the skin, has emerged as a widely researched alternative to conventional injection-based delivery. TDDS has demonstrated considerable potential in enhancing the administration of a range of pharmacological agents, with notable applications in pain management, hormonal therapies, and the treatment of cardiovascular and central nervous system (CNS) disorders (Leppert et al., 2018; Peña-Juárez et al., 2022; Roohnikan et al., 2019). One of the key advantages of TDDS is that they bypass the gastrointestinal (GI) tract, thereby avoiding interference from pH fluctuations, digestive enzymes, and intestinal microbiota that can compromise drug stability and absorption. TDDS is also characterized by its high persistence, as it enables controlled and sustained drug release in accordance with therapeutic needs. Furthermore, as a noninvasive and painless method, TDDS offers enhanced patient comfort and compliance, making it especially suitable for use in pediatric and geriatric populations (Jeong et al., 2021). However, the natural barrier properties of the skin, particularly the stratum corneum, pose a significant limitation to the full therapeutic potential of transdermal drug delivery systems. An organ with a multilayered structure, the skin serves as a barrier to protect our bodies from harmful substances such as chemicals and heat (Ali et al., 2015; Wang et al., 2021). The epidermis acts as a major barrier to transdermal drug delivery, while the dermis, containing vasculature and various cell types, presents additional challenges to effective drug penetration. Existing transdermal technologies such as patches, ointments, and creams have primarily enhanced the delivery of low molecular weight, lipophilic drugs that are effective at low doses. Although TDDS have been utilized for decades, current research efforts are focused on improving the cutaneous penetration of larger, hydrophilic molecules and macromolecular therapeutics for applications in both disease treatment and vaccination. Nanocarrier systems, composed of lipids, metals, or polymers, have shown promising results in enhancing transdermal drug penetration, enabling controlled drug release, and facilitating site-specific drug targeting within the skin. These advances hold the potential to significantly expand the

therapeutic scope of TDDS; nevertheless, further investigation is needed to fully establish the safety and biocompatibility of nanocarrier-based formulations. This study provides an overview of the current landscape of nanoparticle-mediated skin delivery systems, with an emphasis on their application in the treatment of dermatological disorders. Historically, the first FDA-approved transdermal patch, containing scopolamine for motion sickness, was introduced in 1979, marking a milestone in the field of transdermal therapeutics (Palmer & DeLouise, 2016). The TDD system has since been used to formulate other drugs, such as nicotine, fentanyl, estrogen, and testosterone. Technology is currently being used to improve transdermal drug systems.

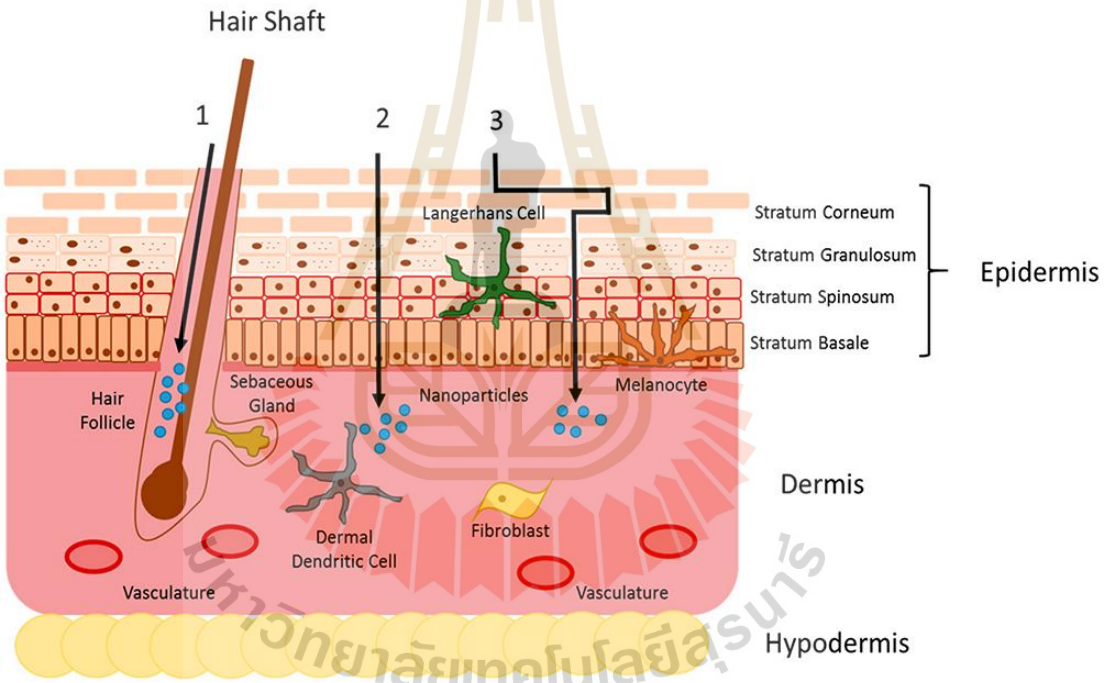


Figure 2.5 Nanoparticle skin penetration illustration

Topically applied nanoparticles can traverse the skin through three primary pathways: (1) the appendageal route, (2) the intracellular route, and (3) the intercellular route. The appendageal pathway involves nanoparticle entry through skin structures such as hair follicles, sweat glands, or cutaneous furrows, facilitating drug retention or enhanced dermal penetration. The intracellular route allows for direct translocation across the cell membranes of the epidermal layers, while the intercellular route involves diffusion through the extracellular lipid matrix between

adjacent skin cells, following a more tortuous path. The physicochemical properties of the nanoparticles namely size, surface charge, shape, and composition play a critical role in determining the preferred penetration pathway (Palmer & DeLouise, 2016).

2.4.1 A Brief Review of Skin Structure

The epidermis and the dermis are the two layers that make up the stratified structure that is the skin (Palmer & DeLouise, 2016; Wysocki, 1999). The skin, often recognized as the largest organ of the human body, comprises multiple layers, each serving distinct yet complementary roles. Among its key physiological functions are thermoregulation, ultraviolet (UV) protection, immune defense, and maintenance of water homeostasis. The epidermis, the outermost layer, plays a pivotal role in acting as a physical barrier, protecting the body from external insults such as pathogens, particulate matter, and large or hydrophilic molecules. In addition, it is critical for preventing transepidermal water loss, thereby contributing to overall skin integrity and systemic hydration (Brandner, 2009; Matsui & Amagai, 2015). The epidermis is primarily composed of keratinocytes, melanocytes, and Langerhans cells, each contributing to distinct physiological roles. Keratinocytes, which constitute the majority of epidermal cells, are central to the formation of the skin's physical barrier. These cells undergo a process of terminal differentiation, progressing from the stratum basale—the innermost layer—to the stratum corneum, the outermost layer of the epidermis. Upon reaching the stratum corneum, keratinocytes lose their nuclei and organelles, becoming corneocytes, which are essentially non-viable, flattened cells. These corneocytes are embedded within a protein-rich matrix composed of keratin, filaggrin, and loricrin, and are enveloped by a lipid envelope primarily consisting of ceramides, free fatty acids, and cholesterol, which together contribute to the skin's barrier function and water retention (van Smeden et al., 2014). The stratum corneum, along with tight junctions in the stratum granulosum, forms a highly selective barrier to water and solutes, effectively restricting the penetration of most hydrophilic drug molecules larger than 500 kDa. In addition, epidermal melanocytes produce melanin, which is transferred to keratinocytes to absorb ultraviolet (UV) radiation and protect against DNA damage, thereby contributing to the skin's defense mechanisms (Bos & Meinardi,

2000; Tolleson, 2005). Langerhans cells in the epidermis, along with macrophages and dermal dendritic cells in the dermis, serve as key components of the skin's immune surveillance system. These immune cells detect xenobiotics and pathogens and can either act locally or migrate to regional lymph nodes to initiate an adaptive immune response through B and T lymphocyte activation (Clausen & Stoitzner, 2015; Haniffa et al., 2015). Due to the skin's natural barrier—particularly the stratum corneum of the epidermis it is challenging to design drug formulations and nanocarriers capable of effective penetration. However, once drugs or nanocarriers traverse the viable epidermis, they can interact with living keratinocytes and immune cells, enabling potential transport to draining lymph nodes. Unlike the avascular epidermis, the dermis is highly vascularized, containing extensive blood and lymphatic vessels, which facilitates systemic drug absorption. The dermis consists of three layers: the papillary dermis (the most superficial), the reticular dermis, and the hypodermis. The upper dermis is rich in collagen and extracellular matrix proteins, synthesized primarily by fibroblasts, providing structural integrity and support (Amano, 2016; Lawlor & Kaur, 2015). The subcutaneous fat is located in the lowest layer of the skin, which is called the hypodermis (Lawlor & Kaur, 2015). The dermis also comprises numerous specialized structures, including sweat glands, hair follicles, nerve fibers, and vascular and lymphatic vessels. These appendages play vital roles in maintaining physiological homeostasis. Specifically, sweat glands and hair follicles, embedded within the dermal layer, are essential for thermoregulation, helping to control body temperature through sweat secretion and modulation of blood flow (Tansey & Johnson, 2015). Secondary structures in skin and the papillary dermis create furrows and invaginations in the skin. These furrows and invaginations have the potential to trap topically applied drugs or nanocarriers (German et al., 2012; Lawlor & Kaur, 2015). These structures may allow for increased drug penetration due to a decreased distance between the stratum corneum and the dermis in these areas (Gupta et al., 2012; Patzelt & Lademann, 2013). In fact, transdermal drug delivery systems (TDDS) are primarily designed to enable drugs to reach the dermis, thereby facilitating systemic absorption. The dermis, richly supplied with blood vessels and lymphatics, supports not only efficient drug uptake into the circulation but also hosts a diverse population of immune cells including

macrophages, T cells, mast cells, and dendritic cells that may interact with administered compounds. Additionally, skin appendages such as hair follicles and sweat glands may act as drug reservoirs, allowing for sustained release while simultaneously enhancing dermal penetration. An example of this systemic approach is seen in the transdermal administration of scopolamine (Graybiel et al., 1976). In transdermal drug delivery (TDD) research, significant efforts are directed toward enhancing drug penetration and retention within the skin by leveraging natural skin structures such as sweat glands, hair follicles, and skin furrows. Strategies include the use of chemical penetration enhancers and physical methods like abrasion to temporarily disrupt the stratum corneum, thereby facilitating improved nanocarrier permeability.

Furthermore, nanocarrier design is optimized based on the stratum corneum's architecture, with a preference for nanoparticles under 100 nm in size that possess surface charges or lipid coatings to promote either deeper flux or retention in the lipid-rich layers of the skin (Abdel-Mottaleb et al., 2012; Lee et al., 2013). Nanoparticle skin penetration is dependent on a number of factors, including size, charge, morphology, and material composition. These factors are discussed in the following section.

Nanocarrier Skin Penetration: In recent decades, there has been a growing incorporation of nanoparticles into various consumer products. Since the 1990s, nano-sized titanium dioxide and zinc oxide have been widely employed in sunscreens and cosmetic formulations for their ability to protect the skin against harmful ultraviolet (UV) radiation. Their nanoscale size enhances product transparency while maintaining effective UV-blocking properties, thereby improving both aesthetic and functional aspects of topical applications (Suzuki, 1987). More recently, silica nanoparticles and fullerenes have been incorporated into cosmetic formulations to enhance product functionality. Silica nanoparticles serve as desiccants, helping to absorb moisture and improve texture, while fullerenes function as free radical scavengers, offering antioxidant protection to mitigate oxidative stress on the skin. These applications highlight the expanding role of nanotechnology in enhancing the efficacy and performance of topical products (Contado, 2015; Xiao et al., 2006).

Together, these developments highlight the expanding role of nanotechnology in enhancing the functionality of topical formulations. Although most nanoparticles used in consumer products are not intended to penetrate the skin, their increasing prevalence has stimulated research into both their therapeutic potential and safety concerns, particularly in the context of transdermal drug delivery (TDD) systems. Early investigations focused on *ex vivo* and *in vivo* skin penetration models, as well as *in vitro* cytotoxicity assays in skin cells. Traditionally, it was believed that intact skin posed an impenetrable barrier to nanoparticles. However, accumulating evidence now challenges this notion. Recent studies have shown that nanoparticles can indeed penetrate the skin, and this capability is strongly influenced by several key factors, including particle size, surface charge, morphology, and chemical composition (Fernandes et al., 2015). Nanoparticle skin penetration is influenced by several physicochemical and biological factors. Beyond size, surface charge, and material composition, additional key determinants include the administered dose, morphological characteristics (such as shape and surface roughness), and biological adhesiveness, which affects their interaction with skin components. Furthermore, the *in vivo* dissociation behavior of nanoparticleshow they disassemble or release their payload also plays a critical role in modulating both penetration efficiency and biological activity within the skin. These variables collectively determine the effectiveness and safety profile of nanoparticles in transdermal drug delivery systems.

2.4.2 Drug absorption via the skin

As illustrated in Figure 2.6 (Ramadon et al., 2022), drug absorption through the stratum corneum (SC) occurs via two principal pathways: transepidermal and transappendageal routes. The transepidermal route considered the primary mechanism of absorption leverages the SC's extensive surface area to enable drug penetration. Within this route, substances may pass directly through the keratinocytes (transcellular pathway) or navigate the intercellular spaces between cells (intercellular pathway). Both modes support diffusion of therapeutic agents from transdermal systems into deeper skin layers, ultimately reaching systemic circulation (Barbero & Frasch, 2006; Haque & Talukder, 2018). The transepidermal route is subdivided into

two distinct pathways: the transcellular and intercellular routes. In the transcellular pathway, drugs penetrate directly through the corneocytes of the stratum corneum (SC), requiring traversal across multiple lipid bilayers of cell membranes. Due to the hydrophobic nature of these lipid domains, this route is generally more favorable for lipophilic (hydrophobic) compounds. Conversely, in the intercellular pathway, drugs must diffuse through the extracellular lipid matrix that surrounds the keratinocytes. This route offers a more tortuous path but serves as a primary route for many substances due to the layered lipid structure of the SC (Zhang et al., 2017). The intercellular route, the most prevalent pathway for transdermal drug absorption, facilitates the movement of hydrophilic compounds and small molecules toward the dermal vascular capillaries. Effective absorption via this route depends on the drug's amphiphilic properties, meaning it must possess both lipid and water solubility to navigate the lipid-rich extracellular matrix. The transappendageal route, the secondary pathway, involves drug transport through hair follicles and sweat glands. This route is particularly important for delivering polar, ionizable compounds and large macromolecules that face challenges in permeating the compact structure of the epidermis due to their size and solubility limitations (Ramadon et al., 2022). Despite the potential advantages of the transappendageal route, its use is limited by the small surface area it occupiesapproximately 0.1% of total skin area, compared to the more dominant transepidermal route. To overcome the barrier posed by the stratum corneum (SC) and enhance drug permeability, researchers have developed a variety of strategies aimed at modifying the SC's structure. These include chemical enhancers, physical techniques, and combinatory approaches, all designed to facilitate more efficient drug transport across the skin. The subsequent sections explore the progress in transdermal product development and detail the innovative technologies that have been employed to improve cutaneous drug absorption (Alkilani et al., 2015).

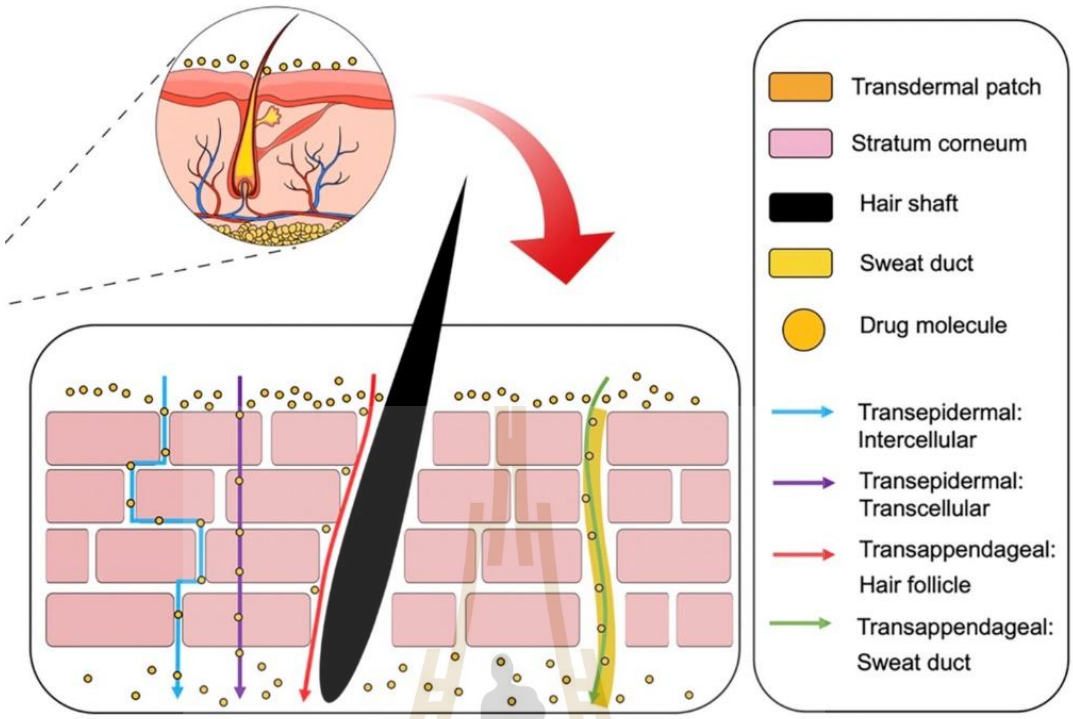


Figure 2.6 Mechanisms for transdermal medication administration

2.4.3 *In vitro* skin permeation by Strat-M™ membranes

The transdermal route is increasingly utilized for systemic drug delivery due to several advantages, including bypassing hepatic first-pass metabolism, ease of administration, prolonged drug release compared to oral routes, and improved patient adherence. However, a major limitation is the low permeability of the stratum corneum (SC), the skin's outermost layer, which serves as a formidable barrier to most compounds. To overcome this challenge, both chemical and physical enhancement techniques are employed to facilitate drug permeation. In a study conducted by Kouchak and Handali (2013), *in vitro* percutaneous absorption using Franz diffusion cells and snakeskin demonstrated that various chemical enhancers significantly increased drug permeability. Specifically, increasing the concentration of lauric acid enhanced drug diffusion, while higher levels of sodium tauroglycocholate (STGC) showed a diminishing effect on enhancement. These findings underscore the importance of optimizing both the type and concentration of penetration enhancers to maximize transdermal drug delivery efficacy (Kouchak & Handali, 2014). However, the skin acts as a highly effective physical barrier that protects the internal environment

from external stressors. This barrier function is largely attributed to the stratum corneum (SC), which presents the primary obstacle to cutaneous drug delivery. The SC's structural composition significantly limits the permeation of molecules, especially those with physicochemical properties that are not inherently favorable for skin absorption. As a result, the delivery of therapeutics through the skin remains a challenge when dealing with compounds that lack optimal solubility, size, or lipophilicity for transdermal penetration (Basto et al., 2021). Permeation enhancers temporarily modify the skin's structure to facilitate drug penetration. Ideal enhancers should be reversible, non-toxic, non-irritating, non-allergenic, and compatible with both drugs and excipients. However, many chemical enhancers carry local or systemic side effects. Consequently, safer and more effective alternatives are being explored, with natural essential oils emerging as promising candidates due to their favorable safety profiles and proven ability to enhance skin penetration in transdermal drug delivery (Nawaz et al., 2022). Transdermal patches offer a promising approach for systemic drug delivery by enabling passive diffusion of therapeutic agents through the skin. This method facilitates direct entry of the drug into systemic circulation, bypassing the gastrointestinal tract and first-pass metabolism, making it a convenient and non-invasive option for managing various systemic conditions (Sabbagh & Kim, 2022). Permeation enhancers are utilized in transdermal drug delivery systems to improve skin permeability by temporarily disrupting or modifying the structural integrity of the stratum corneum. This disruption enhances the diffusion of therapeutic agents across the skin barrier, thereby facilitating the attainment of effective plasma drug concentrations. The use of permeation enhancers is critical in optimizing drug bioavailability in transdermal applications, particularly for compounds with limited intrinsic skin permeability (Latif et al., 2022). Similarly, the study by Klebeko J. et al. (2021) evaluated how modifications to both the drug structure and formulation vehicle influence the skin permeation and accumulation of ibuprofen (IBU). Using human abdominal skin and Strat-M™ membranes in in vitro Franz diffusion experiments, the researchers compared hydrogels containing IBU and its derivatives with commercial ibuprofen gels. Quantitative analysis was conducted via HPLC. The findings demonstrated that the Celugel® formulation significantly enhanced IBU skin

penetration, delivering over three times the cumulative amount through human skin after 24 hours compared to the commercial product. These results highlight the potential of Celugel® as an effective transdermal delivery system when paired with structurally modified drugs. Furthermore, the study supports the utility of Strat-M™ membranes as a reliable substitute for human skin in evaluating transdermal drug permeation and accumulation (Klebeko et al., 2021). Transdermal drug delivery has become increasingly prominent due to its advantages over traditional oral and injectable routes, such as bypassing hepatic first-pass metabolism, protecting drugs from degradation in the gastrointestinal tract, enabling sustained drug release, and enhancing patient adherence. During pharmaceutical development, *ex vivo* permeation testing plays a crucial role in evaluating the quality and performance of transdermal systems. These studies typically utilize excised human skin from the intended application site or appropriate animal skin models to assess drug permeation characteristics under controlled conditions (Neupane et al., 2020). However, the limited availability of human skin and ethical concerns associated with animal use have reduced the appeal of these models in permeation studies. Despite this, permeation studies remain essential for demonstrating the suitability and efficacy of transdermal drug delivery systems, as they provide critical insights into the system's ability to deliver therapeutic agents across the skin barrier.

Franz diffusion cells are commonly used to assess drug permeation through the skin, and the permeation study across the dermatome of human skin explants is regarded as the gold standard for evaluating drug delivery via a transdermal system. Nevertheless, ethical and economic concerns limit the availability and use of human skin. As a result, isolated skin from inbred animals—such as porcine, primates, rodents (guinea pig, rat, and mouse), rabbit, and shed snake skin—has been routinely considered as an alternative to human skin, since it is easier to obtain, can be excised fresh prior to skin permeation studies while maintaining viability and enzymatic activity, and exhibits less variability (Kerimoğlu & Şahbaz, 2018; Todo, 2017).

2.4.4 Strat-M™ membranes

Strat-M™ is a synthetic membrane developed as an alternative to animal or human skin in permeation studies. Specifically, it replicates essential structural and chemical properties of human skin, offering a multilayer design. Its uppermost layer is tightly compacted and coated with a lipid composition that closely resembles the lipid matrix of the human stratum corneum (SC), while its underlying porous layers mimic the structural characteristics of the viable epidermis and dermis. This design enables Strat-M™ to simulate both the barrier and diffusion properties of real skin, making it a valuable tool in evaluating transdermal drug delivery systems (Haq et al., 2018). Moreover, the Strat-M™ membrane demonstrates permeability characteristics that are closely equivalent to those of human skin, particularly in drug diffusion studies. It is therefore designed to offer superior correlation with human skin compared to other biological membranes, and serves as a reliable and reproducible model for transdermal drug evaluation—without the ethical and variability concerns associated with human or animal tissue (Arce et al., 2020).

A previous study conducted in 2015 utilized infinite dose models to evaluate the permeation of various chemicals dissolved in phosphate-buffered saline (PBS, pH 7.4) through the Strat-M™ membrane. The findings revealed that there was a strong correlation between the permeation profiles observed in Strat-M™ and those seen in both rat and human skin, thus supporting the membrane's validity as a surrogate for biological tissues in transdermal drug delivery studies (Uchida et al., 2015). Furthermore, a recent investigation into nicotine permeation from formulations using binary solvents (comprising water and chemical penetration enhancers) applied at a high finite dose (200 µL/0.64 cm²) demonstrated strong correlation between Strat-M™ and human skin in terms of permeation behavior. This consistency further reinforces Strat-M™ 's relevance as a surrogate model. As a result, multiple studies have recommended the use of Strat-M™ as an alternative membrane in cosmetic development, formulation optimization, regulatory evaluations, and safety testing. To validate its suitability, it is essential to characterize and compare membrane parameters such as permeability coefficient, flux, and penetrant distribution between Strat-M™ and porcine skin, thereby establishing their equivalency for active ingredient

assessment (Arce et al., 2020). For ex *vivo* permeation testing, the use of excised human or animal skin from relevant anatomical sites is traditionally recommended. However, limitations such as the limited availability of human skin and ethical issues associated with animal use have reduced the appeal of these biological models. In response to these limitations, significant progress over the past three decades has led to the development of artificial membranes, including the Strat-M™ model, as viable alternatives. Indeed, Strat-M™ has been shown to effectively mimic human skin in terms of permeability characteristics and is now widely recognized as a suitable substitute for assessing drug permeation and accumulation in transdermal drug delivery research.

Electrospinning was employed to fabricate both drug-free and diclofenac sodium (DS)-loaded polyvinyl alcohol (PVA) patches. Scanning electron microscopy (SEM) confirmed successful integration and structural compatibility between the electrospun PVA nanofiber and the PVA cryogel matrix, with DS uniformly incorporated. Moreover, Fourier-transform infrared spectroscopy (FTIR) analysis revealed no chemical interaction between DS and PVA, as evidenced by the presence of DS-specific peaks in all medicated dual-layer patches. Higher cross-linking density and the presence of DS led to reduced swelling capacity, attributed to diminished water uptake after 24 hours in phosphate-buffered saline (PBS). *In vitro* drug release studies using Franz diffusion cells with a cellulose nitrate membrane (as a skin model) demonstrated that patches containing 2% w/v DS achieved sustained drug release for up to 24 hours (Sa'adon et al., 2021). These studies support the development of cost-effective and environmentally sustainable nanofiber patch technologies that avoid the use of toxic components. The novel capsaicin-loaded nanofiber patches show potential as transdermal drug delivery systems, offering a means to reduce the gastrointestinal side effects commonly associated with nonsteroidal anti-inflammatory drugs (NSAIDs) while meeting growing demands in pharmaceutical and biomedical fields. A 2014 study optimized a capsaicin nanoemulsion formulation for topical use, demonstrating its successful permeation through the Strat-M™ membrane in a Franz diffusion cell system. The nanoemulsions, with particle sizes between 20 and 62 nm, effectively penetrated the membrane layers, indicating their suitability for transdermal

application (Kim et al., 2014). The Strat-M™ membrane has been validated as a reliable substitute for human skin in drug permeation and accumulation studies, offering a consistent and ethical alternative for *in vitro* testing. Moreover, prior literature reviews support the effectiveness of artificial skin models in evaluating skin permeability from transdermal patches.

Therefore, these findings contribute to the advancement of knowledge and technological innovation in the development of capsaicin-loaded transdermal nanofiber patches. The study lays a foundation for improving transdermal drug delivery systems through the fabrication and optimization of capsaicin nanofiber formulations.

2.4.5 Advantages of Transdermal drug delivery system (TDDS)

Transdermal drug delivery systems (TDDS) offer a compelling alternative to oral and injectable routes because they circumvent key limitations such as first-pass hepatic metabolism, gastrointestinal degradation, as well as issues related to gastric emptying and pH variability. Unlike oral medications, TDDS can be administered to unconscious or nauseated patients, and unlike injectable routes, they avoid pain, bruising, bleeding, and needle-associated risks—such as infections, accidental injury, and sharps waste. Therefore, these attributes contribute to enhanced patient compliance and treatment safety, while also supporting cost-effectiveness in healthcare delivery. TDDS systems offer controlled, prolonged drug release, which help to reduce peak plasma concentrations, and minimize the risk of systemic toxicity. Their ease of application and removal enhances administrative flexibility, making them particularly suitable for self-administration. Furthermore, they are effective in targeting localized dermatological conditions, thereby improving therapeutic outcomes while limiting systemic side effects. However, TDDS are not without drawbacks. The most common concerns involve skin irritation and sensitization. Components such as adhesives, excipients, or the active pharmaceutical ingredient may provoke irritant contact dermatitis (ICD) or allergic contact dermatitis (ACD). ICD results from chemical or physical irritants triggering an innate immune response, characterized by erythematous, pruritic, or painful patches or plaques, without the formation of antigen-specific memory T cells. In contrast, ACD is a type IV delayed hypersensitivity reaction

mediated by T cells. It involves a two-phase immune response induction followed by elicitation which explains the delayed onset of symptoms following repeated allergen exposure. Transdermal drug delivery systems (TDDS) serve as a viable alternative to traditional routes of administration, including oral, intravenous, subcutaneous, and transmucosal methods. The advantages of TDDS include the avoidance of first-pass hepatic metabolism, improved patient adherence, reduced systemic drug interactions, the potential for on-demand dose adjustments, elimination of the need for healthcare-assisted administration, sustained drug release, and enhanced therapeutic efficacy. Transdermal patches were among the earliest TDDS technologies introduced and are based on relatively simple engineering principles. The first FDA-approved transdermal patch, Transderm-Scop®, was developed for the prevention of motion sickness using scopolamine. The success of this system led to the subsequent approval of transdermal delivery platforms for drugs such as nitroglycerin, fentanyl, estradiol, nicotine, and testosterone. These advancements paved the way for innovative therapeutic strategies, offering alternative delivery options for existing medications while potentially reducing adverse effects. For instance, estradiol patches, which are widely used by millions of patients annually, avoid the hepatic complications associated with oral estrogen therapies, thus representing a safer and more efficient method for hormone replacement therapy (Cramer & Saks, 1994). Transdermal nicotine delivery systems represent a significant advancement in medical therapy, having assisted millions of individuals in smoking cessation. Their widespread use has not only improved public health outcomes but has also likely contributed to increased life expectancy among former smokers by reducing the risks associated with long-term tobacco use (Murthy, 2012). Over the past three decades, the U.S. Food and Drug Administration (FDA) has approved more than 35 transdermal patch products across a wide range of therapeutic categories. Among these is the capsaicin 179 mg (8% w/w) cutaneous patch, commonly referred to as the capsaicin 8% patch, which delivers localized treatment for peripheral neuropathic pain. Notably, this formulation has demonstrated the ability to provide significant and sustained pain relief following a single application, making it a valuable option in the management of chronic neuropathic conditions (van Nooten et al., 2017). Topical formulations of capsaicin are

commonly utilized for pain management. According to meta-analyses of multiple studies, low-concentration capsaicin preparations are generally safe but exhibit limited efficacy, often requiring frequent daily self-application to achieve therapeutic benefits. To address these limitations, a high-concentration capsaicin 8% patch (Qutenza™) has been developed and has received regulatory approval in both the European Union and the United States for the treatment of peripheral neuropathic pain, offering a more convenient and effective alternative through single-application therapy (Anand & Bley, 2011). In recent years, innovative drug delivery systems have garnered significant research attention due to their potential to overcome limitations of conventional pharmacotherapy. These systems aim to address issues such as drug instability, high systemic toxicity, unfavorable pharmacokinetics, low cellular uptake, and the emergence of drug resistance. By enhancing the precision, efficiency, and safety of therapeutic agents, novel delivery strategies offer promising avenues for improving treatment outcomes across a range of medical conditions (Bibi et al., 2017). Nanostructure-based drug delivery systems offer significant advantages over conventional therapeutic approaches by enhancing drug safety, efficacy, and patient adherence. Their ability to control the spatial and temporal release of pharmacological agents allows for targeted and sustained delivery, thereby improving therapeutic outcomes. Owing to these benefits, the development and commercialization of such nanocarrier systems have rapidly progressed, positioning them as a promising advancement in modern drug delivery strategies.

2.4.6 Disadvantages of Transdermal drug delivery system (TDDS)

A significant limitation in transdermal drug delivery is that many drugs, especially hydrophilic compounds, may exhibit insufficient skin penetration rates, thus preventing them from reaching therapeutic concentrations. Furthermore, local adverse reactions such as erythema, pruritus, and edema may arise due to the active pharmaceutical ingredient, the adhesive, or other excipients within the patch formulation. In addition, the barrier function of the skin is not uniform, as it can vary significantly between anatomical sites, among individuals, and is also influenced by age-related factors.

1. One possible side effect of TDDS is contact dermatitis.

2. The natural constraints of drug penetration mean that transdermal patches can only be used with very effective medications.

3. Some medications, such as the scopolamine transdermal patch put behind the ear, cause discomfort to the patient.

4. Long-term patch adhesion is a problem with TDDS.

5. High manufacturing costs of transdermal patch as comparison to conventional dose form.

6. TDDS does not have the ability to administer ionic medicines via the skin.

7. TDDS is unable to achieve high blood/plasma concentrations of drugs.

8. There is no way to build TDDS for big molecules.

9. If a medicine or formulation irritates the skin, TDDS cannot develop.

2.4.7 Limitation of Transdermal drug delivery system (TDDS)

1. There must be some physicochemical qualities for medication penetration via the skin, and transdermal distribution is extremely challenging if the drug dose is greater than 10-25mg/day. Less than 5mg/day was preferred for the daily dose of medication.

2. Itching, erythema, and local edema may be brought on by the medicine itself or by the excipients used in the formulations.

3. A transdermal product's clinical requirement must also be thoroughly assessed before a choice is made.

4. The components of the system cause contact dermatitis in certain patients.

5. The skin's barrier function varies depending on the location, the individual, and the passage of time.

6. There are only a limited number of medications that may be administered in this technique because of poor skin permeability.

7. Drugs that cause tolerance or those that need to be administered at specific times (e.g. hormones) are not acceptable candidates.

TDDS is an alternative route of drug administration for medications with low efficacy when administered orally, topically, intravenously, or intramuscularly. Recent breakthroughs in TDDS involve the use of nanoparticles (NPs), which have the potential to improve medication absorption over the skin. NPs can also enable controlled release, the capacity to administer both hydrophilic and hydrophobic medications, minimize side effects, and are non-invasive when utilized in a TDDS manner. Transdermal patches are another emerging TDDS technology. TDDS using a minimally invasive technique in which micron-sized pores are formed in the epidermis to allow medication delivery to blood vessels in the dermal layer of the skin. New studies have concentrated on integrating various TDDS methodologies to overcome past limitations of drug delivery using conventional methods. Recent advances in nanotechnology have had an impact on all areas of basic and applied research. The emergence of nanostructured systems offering multiple advantages has significantly heightened scientific interest in the application of nanotechnology within transdermal drug delivery systems (TDDS). In recent decades, it has become increasingly evident that the route of administration plays a critical role in determining a drug's therapeutic efficacy by modulating its pharmacokinetics profile, biodistribution, pharmacodynamics, metabolism, and toxicity. The advent of various nanotechnologies including nanoparticles, nanofibers, nanogels, micelles, and microspheres has paralleled the advancement of innovative drug delivery strategies, establishing these nanocarriers as promising tools in the pharmaceutical and biomedical fields (Pant et al., 2019). By using passive or active targeting strategies based on the final formulation, nanocarriers can be employed to wrap and distribute medications that are too poisonous, insoluble, rapidly removed, or unstable as free molecules. Electrospinning is a cost-effective, easy, and adaptable method for producing polymer nanofibers (Luraghi et al., 2021). Electrospinning is a technique that utilizes a high-voltage electric field to generate fibers from a polymer solution extruded through a needle. The resulting electrospun fibers can be tailored in terms of porosity, morphology, and surface area by modifying both processing parameters and ambient conditions,

allowing for precise control to suit specific drug delivery applications. Drug incorporation into these fibers can be achieved either through direct blending of the drug with the polymer solution or via surface immobilization post-spinning, each offering distinct drug release profiles. A wide range of therapeutics including small molecules, proteins, and nucleic acids can be successfully encapsulated. Advanced electrospinning systems enable the co-delivery of multiple agents for synergistic effects, or allow for stimuli-responsive release, thereby enhancing the therapeutic potential of nanofiber-based drug delivery systems.

2.5. Electrospinning

Electrospinning, also referred to as electrostatic spinning, is a well-established technique for producing micro- and nanofibers from polymer solutions or melts. Although its modern application in material science is relatively recent, the underlying principle—the influence of electrical charges on liquid droplets—has been recognized for centuries. For instance, as early as the 17th century, William Gilbert observed that a droplet of water placed on a dry surface could be drawn into a conical shape when exposed to a charged object, such as a piece of rubbed amber held nearby. This foundational observation laid the groundwork for the development of electrohydrodynamic technologies like electrospinning (Asmatulu & Khan, 2018). Electrospinning is a versatile and relatively simple technique that utilizes a high-voltage electric field to draw micro- and nanofibers from polymer solutions or melts. The process enables fine control over fiber morphology by adjusting both electrospinning parameters (such as voltage, tip-to-collector distance, and flow rate) and solution properties (such as viscosity, conductivity, and surface tension). These adjustments allow for the modulation of key fiber characteristics including diameter, length, surface roughness, porosity, pore interconnectivity, and alignment, as well as the presence or absence of structural defects such as beads (Vong et al., 2021). A core element of the process is the formation of a Taylor cone at the tip of the spinneret under a high-voltage field. Once the electrostatic force overcomes surface tension, nanofibers are ejected and deposited onto a collector, forming nonwoven mats or aligned structures, depending on the setup. Electrospinning also supports the fabrication of ceramic,

composite, and functionalized fibers, thus making it a highly adaptable technique. Because of its tunability and scalability, it has gained widespread attention across diverse domains, such as biomedicine (e.g., wound dressings, transdermal drug delivery systems, scaffolds for tissue engineering), biosensing and diagnostics, cosmetics and personal care products, protective clothing and filtration materials, as well as catalysis and adsorption applications (e.g., dye removal, chromatography), and energy storage (e.g., electrodes for batteries and fuel cells). The combination of precise structural control, broad material compatibility, and functional flexibility makes electrospinning an attractive technology for next-generation material design and industrial innovation (Vong et al., 2018). Electrospinning is an innovative fiber fabrication technique that employs a high-voltage electrostatic field to generate ultrafine fibers from polymer solutions or melts. The method has garnered significant attention within the scientific community due to its ability to produce nanofibers with controlled dimensions and diverse functional properties. Electrospun fibers are characterized by a high surface area-to-volume ratio, excellent porosity, and structural flexibility, making them suitable for various biomedical, environmental, and industrial applications. The process involves dispensing a polymer solution through a syringe equipped with a metallic spinneret, where a high-voltage DC power source is applied. This causes the polymer droplet at the spinneret tip to elongate under the influence of electrostatic forces, forming a structure known as a Taylor cone. Once the electrostatic repulsion surpasses the surface tension, a fine jet is ejected from the cone and travels toward a grounded collector, solidifying into continuous nanofibers. Electrospinning offers a relatively simple, cost-effective, and scalable route for producing fibers with diameters ranging from a few nanometers to several micrometers. Its broad applicability and tunable processing parameters make it a valuable tool in advancing nanotechnology-driven innovations across scientific and industrial sectors (Asmatulu & Khan, 2018).

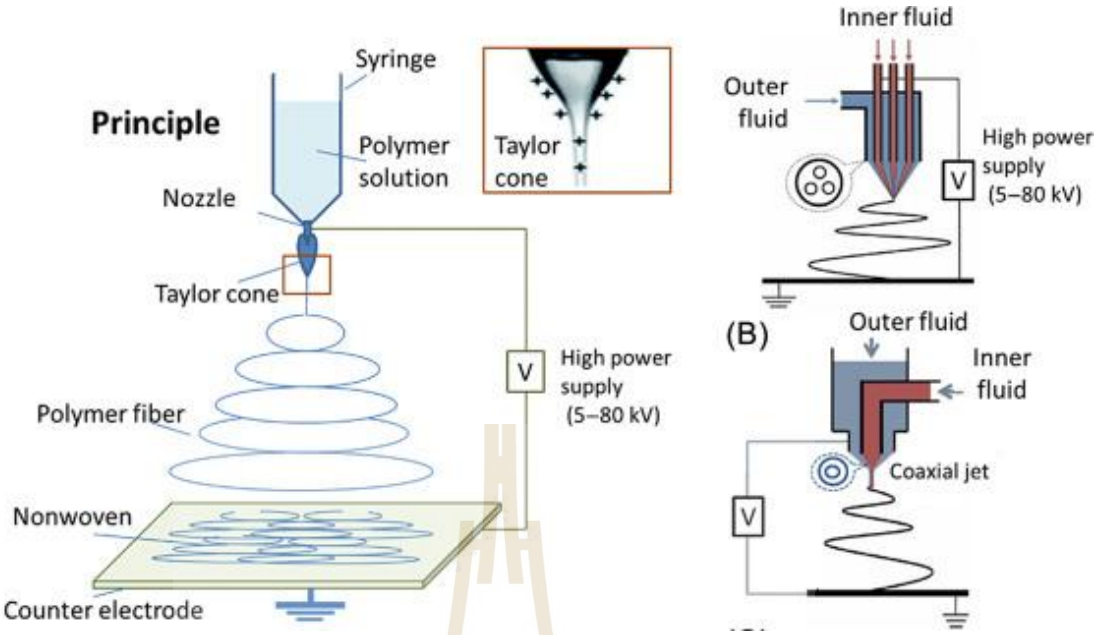


Figure 2.7 Nanofibers electrospinning process

(Zheng, 2019)

2.5.1 Nanofibers Patch

Synthetic and natural polymer-based nanoparticles represent a promising alternative for therapeutic applications owing to their favorable properties, including biocompatibility, non-immunogenicity, non-toxicity, and biodegradability. These characteristics enable their safe interaction with biological systems, thus making them ideal carriers for targeted drug delivery, controlled release, and enhanced therapeutic efficacy, while minimizing adverse effects. Moreover, their versatility in formulation also allows for the encapsulation of a wide range of therapeutic agents, thereby contributing to their growing significance in biomedical and pharmaceutical research (Crucho & Barros, 2017). The advantageous properties of polymer-based nanoparticles stem from their ability to be tailored using both synthetic and natural polymers. For example, synthetic polyester polymers such as polycaprolactone (PCL) and polylactic acid (PLA), along with their monomers, are often employed to reduce immunogenicity and toxicity. In contrast, natural polymers including chitosan, gelatin, albumin, and alginate further enhance biocompatibility and are generally more effective at minimizing toxicity, thereby improving the therapeutic efficacy of encapsulated agents compared to conventional drug delivery systems. Polymeric

nanoparticles are typically structured as matrix systems in which the active therapeutic agents are either uniformly distributed or encapsulated, depending on the nanoparticle design. When the drug is uniformly dispersed within the polymer matrix, the structure is termed a nanosphere. Conversely, when the drug is enclosed within a polymer shell, forming a core–shell configuration, it is referred to as a nano capsule. This structural versatility allows for controlled release, improved stability, and targeted delivery of various pharmaceutical agents (Letchford et al., 2009). Polymeric nanoparticles offer customizable platforms for the controlled release of therapeutic agents, thereby enabling targeted delivery with elevated drug concentrations at the desired site. Their surfaces can be readily modified or functionalized with specific recognition ligands, thus enhancing tissue-specific targeting and minimizing off-target effects. In particular, polymers such as polyacrylic acid (PAA), polyvinyl chloride (PVC), polyurethane (PU), polyvinyl alcohol (PVA), and polyvinylpyrrolidone (PVP) have demonstrated significant potential in electrospinning applications. These materials facilitate the fabrication of nanofiber-based carriers, providing promising avenues for the development of advanced drug delivery systems with improved therapeutic efficacy (Rahmani et al., 2021). Polyvinylpyrrolidone (PVP) is an amorphous synthetic polymer characterized by its high-water affinity and excellent adhesive properties. Due to its low chemical toxicity, biocompatibility, and ease of formulation, PVP has become a crucial material in biomedical and pharmaceutical applications. It is widely utilized in drug delivery systems, wound dressings, and nanofiber fabrication owing to its ability to enhance solubility, stabilize active pharmaceutical ingredients, and promote controlled drug release (Wang et al., 2015). Polyvinylpyrrolidone (PVP) films exhibit promising potential as advanced wound-dressing materials due to their ability to maintain a moist wound environment, which prevents dehydration and scab formation—crucial factors in promoting optimal healing. Electrospun PVP nanofibers are frequently employed as polymeric carriers in drug delivery systems, particularly for water-soluble drugs. Their high porosity, large surface-to-volume ratio, and excellent solubility in water enable the rapid release of encapsulated therapeutics. Similarly, electrospun polyvinyl alcohol (PVA) has gained attention as a biocompatible and biodegradable polymer with broad applications in biomedical fields. PVA nanofibers are well-suited for rapid

drug delivery, as demonstrated in Li., et al's study, where complete release of caffeine and partial release of riboflavin occurred within 60 seconds. These properties underscore the suitability of both PVP and PVA nanofibers for fast-acting transdermal and topical drug delivery applications (Li et al., 2013).

Incorporating hydrophobic polymers into polyvinyl alcohol (PVA) matrices can enhance drug release properties, particularly for hydrophobic compounds. A study investigating the impact of increasing PVA content in PVA/PVP composite nanofibers revealed that such composites could achieve sustained release profiles for ciprofloxacin hydrochloride, a commonly used antibiotic. The results demonstrated that the addition of PVP to PVA not only contributed to the controlled release of the drug but also improved the mechanical properties of the nanofiber membranes, notably increasing the ultimate yield strength. This suggests that PVA/PVP composite nanofibers offer a robust and effective platform for sustained drug delivery applications (Rahmani et al., 2021). Additionally, the high fluid absorption capacity and slow degradation rate of these membranes confirm their ability to maintain a moist wound environment, which is essential for promoting optimal healing conditions and tissue regeneration.

2.5.2 Advantages of nanotechnology

The integration of nanotechnology into medical science offers promising advancements, particularly through the use of nanoparticles, which serve as fundamental units in this field. Recent innovations have highlighted the potential of nanoparticles in therapeutic applications, especially for targeted and controlled delivery of both small and large molecules. Their versatility in size and shape, high drug-loading capacity, and ability to encapsulate both hydrophilic and hydrophobic compounds make them ideal candidates for precision medicine. Additionally, nanoparticles can establish stable interactions with ligands, enhancing tissue-specific delivery. However, despite their advantages, concerns regarding their toxicity and possible side effects persist, necessitating careful evaluation prior to clinical application. Understanding the physicochemical properties of nanoparticles and the strategies employed for their effective delivery is crucial to ensuring their safe and

efficient therapeutic use (Yetisgin et al., 2020). Advancements in nanotechnology engineering have significantly integrated multidisciplinary fields such as materials science, chemical engineering, tissue engineering, and nanomedicine. These innovations are inherently tied to the unique properties and functionalities of materials at the nanometer scale, where quantum and surface phenomena enable novel applications and enhanced performance across biomedical and pharmaceutical domains (Chauhan et al., 2020). Nanostructured materials offer distinct advantages over conventional therapeutic approaches by addressing key limitations, including poor target tissue specificity, uncontrolled drug release rates, and rapid biodegradation of bioactive agents. These materials can effectively encapsulate both hydrophilic and hydrophobic compounds, enhancing drug stability and bioavailability while minimizing systemic side effects. As versatile carriers, nanostructures contribute to more precise, sustained, and safer drug delivery (Karimi et al., 2017). Moreover, advanced strategies such as surface functionalization, passivation, and co-loading of multiple therapeutic agents within a single nanocarrier have significantly enhanced the efficacy of nanomedicines. These approaches contribute to improved pharmacokinetics, targeted delivery, and controlled release, thereby ensuring more uniform and predictable biological responses compared to conventional drug delivery systems (Charelli et al., 2022). This section provides a comprehensive overview of the unique properties of nanoparticles within biological systems, highlighting their clinical applications and therapeutic specificity. Emphasis is placed on both the types of nanoparticles currently employed in clinical practice and the targeted delivery strategies developed for various diseases, including cancer, infectious diseases, autoimmune disorders, cardiovascular conditions, neurodegenerative diseases, ocular pathologies, and pulmonary illnesses. A deeper understanding of nanoparticle biological system interactions will pave the way for the development of novel diagnostic, therapeutic, and preventive approaches, especially for diseases that remain challenging or incurable with current medical interventions.

2.5.3 Nanotechnology as therapeutic agents

The primary objective of nanomedicine is to harness nanotechnology for enhancing the efficacy and safety of pharmaceutical agents. This is often achieved by incorporating uncoated drugs into biocompatible nanocarriers, including nanoparticles, liposomes, micelles, and dendrimers. Nanoparticulate drug delivery systems (NDDSs) are engineered with adjustable parameters such as particle size, morphology, surface charge, and drug loading capacity to enable extended systemic circulation and facilitate precise targeting of tissues or even specific subcellular compartments (Almeida et al., 2011; Blanco et al., 2015). As illustrated in the accompanying figure, nanoparticulate drug delivery systems (NDDSs) can be engineered with surface modifications, such as cell-penetrating peptides or target-specific ligands, to enable drug transport across the blood–brain barrier, thereby facilitating central nervous system (CNS) delivery. By precisely controlling spatial localization, reducing required dosages, and minimizing adverse effects, NDDSs significantly enhance therapeutic efficacy in targeted medical applications (Bhansali et al., 2021). Despite the many advantages offered by nanomaterials, their limited success in alleviating chronic pain underscores the pressing need for more effective therapeutic strategies. Given the heterogeneous etiology of chronic pain, both the type of drug and the optimal dosage must be tailored to the underlying condition. One promising approach to enhancing treatment efficacy while minimizing systemic side effects is to increase drug concentration at the specific site of action. This targeted delivery can be achieved by functionalizing nanomaterials with targeting ligands, such as peptides or antibodies. Additionally, the route of administration plays a crucial role in optimizing therapeutic outcomes. For instance, topical formulations, such as anti-inflammatory creams or sprays, are suitable for cutaneous or localized neuropathic pain, whereas spinal injections are more appropriate for chronic spinal nerve pain. Other conditions involving internal organs or systemic pain may benefit from oral, intranasal, intramuscular, or intravenous administration. The following section reviews recent advancements and targeted strategies in the use of nanomaterials for chronic pain management.

In a recent study, Joshi et al. employed a nanofibrous transdermal delivery system (TDS) characterized by controlled drug release and enhanced mucoadhesive properties for the treatment of periodontitis (Joshi et al., 2015). This approach demonstrated improved therapeutic selectivity and reduced side effects. Among emerging delivery vehicles, nano-sized drug capsules are garnering increased attention due to their distinct advantages over conventional formulations. Compared to traditional capsules, nano capsules possess a substantially larger surface area, enabling accelerated drug degradation and absorption rates, even at equivalent drug masses. Additionally, electrospun nanofibers represent another advanced nanomaterial-based drug carrier, offering sustained drug release and enhanced bioavailability, particularly for compounds with poor solubility. These nanoscale carriers facilitate the gradual and efficient absorption of therapeutics into the body, thereby improving pharmacological outcomes. When constructed from biodegradable polymers, such carriers degrade into non-toxic byproducts that can be safely metabolized or excreted, further enhancing their biocompatibility. The electrospinning process used to fabricate these nanofibers involves the application of a high-voltage electric field to a polymer melt or solution, resulting in the formation of a Taylor cone at the needle tip. The solution is subsequently stretched into a liquid jet, which solidifies into nanofibers upon reaching the collector surface yielding fibrous structures with nanometric diameter and high surface-area-to-volume ratio, ideal for drug delivery applications.

Table 2.4 Electrospinning Nanofibers for transdermal drug delivery

Treatment	Materials	Drugs	Hight Voltage	Results	References
Cancer	Polyvinyl alcohol, polyethylene oxide and polyvinylpyrrolidone	Proteins (e.g., zein, gelatine, and silk) and polysaccharides (e.g., chitosan, cellulose, and sodium alginate)	10 kV–30 kV	Encapsulating drugs in coaxial electrospun nanofibers offers an effective approach for achieving controlled and prolonged drug release.	(Li et al., 2022)
Antibacterial	Polyvinyl alcohol	L-lysine/ PEO solution/ (ibuprofen (IBP)	0–30 kV	The findings indicate that PVA-Lysine (PVA Lys) electrospun membranes incorporating ibuprofen (IBP) or linalool (LO) effectively promote wound healing. Specifically, the PVA Lys LO membranes demonstrated strong antibacterial activity. These results support the potential use of PVA Lys electrospun membranes as effective wound dressing materials.	(Sequeira et al., 2019)
Fabrication, antibacterial and cytocompatibility evaluation and in vitro healing assay	Polyvinyl alcohol	chitosan	25 kv.	To validate the potential of the nanofibrous mats for wound applications.	(Adeli et al., 2019)
Gingivitis	Polyvinylpyrrolidone	ornidazole	-	The findings indicate that ornidazole-loaded electrospun fibers hold potential as an effective drug delivery system for managing gingivitis, offering localized and sustained therapeutic action at the site of inflammation.	(Tort et al., 2019)

Table 2.4 Electrospinning Nanofibers for transdermal drug delivery (Continued)

Treatment	Materials	Drugs	Hight Voltage	Results	References
Psoriasis	polymethyl vinyl ether-alt-maleic acid	salicylic acid, methyl salicylate, and capsaicin	13 kV	GC-MS analysis demonstrated that most encapsulated compounds remained stable over 15 days, with the exception of methyl salicylate. The encapsulated drugs preserved or enhanced their activation of the TRPV1 channel, which is associated with psoriasis treatment.	(Martínez-Ortega et al., 2019)
	Polyvinylpyrrolidone	Ibuprofen	15 kV	*In vitro* dissolution tests showed fiber mats dissolved in 10 s via a polymer-controlled mechanism.	(Yu et al., 2009)
Pain Management	Polyvinylpyrrolidone Polyvinyl alcohol	Buprenorphine	0–30 kV	The buprenorphine-loaded PVP/PVA nanofiber system demonstrates superior physicochemical properties compared to PVP-only formulations. The integration of PVA enhances fiber strength and stability, while cross-linking within the nanofiber matrix enables sustained drug release. This formulation strategy improves drug retention and prolongs therapeutic effects, supporting its potential application as an effective transdermal patch for pain management.	(Rahmani et al., 2021)
Comprehensive wound care	Polyvinyl alcohol	Diclofenac sodium Capsaicin Gentamicin	20 kV 15 kV 20 kV	Multilayer dressing sped rat wound healing from 21 to 7 days (including Diclofenac sodium, capsaicin and gentamicin). Histopathology showed intact epidermis in treated samples.	(Nada et al., 2020)

Table 2.4 Electrospinning Nanofibers for transdermal drug delivery (Continued)

Treatment	Materials	Drugs	Hight Voltage	Results	References
Patches Loaded with a Long-Acting Pharmacological	Polyvinylpyrrolidone Polyvinyl alcohol	Diclofenac Sodium Salt (DS), Gentamicin	20 kV	The study revealed that the release rate of Cucurbitacin (CC) from CC-loaded electrospun polyvinyl alcohol (PVA) mats was significantly higher than that from corresponding as-cast PVA films, with the rate increasing proportionally to CC concentration. These findings highlight the potential of electrospun PVA mats as effective transdermal delivery systems for medicinal applications of CC.	(Hindi et al., 2021)
Topical skin treatment	Polyvinyl alcohol	Capsaicin	15kV,17.5kV	Capsaicin derived from chili extract (CE)-loaded electrospun polyvinyl alcohol (PVA) mats demonstrated enhanced release rates and improved skin permeation compared to non-electrospun formulations. These results support the potential application of CE-loaded electrospun PVA mats as effective transdermal therapeutic delivery systems.	(Sa'adon et al., 2019)
A fast-dissolving loratadine	Polyvinylpyrrolidone	Loratadine	10 kV, 20 kV	Diameter and loratadine amount affected nanofiber drug release and disappearance time. Nanofiber solubility and release time increase with fiber diameter and medication quantity. Electrospinning can produce fast-dissolving loratadine nanofibers.	(Akhgari et al., 2016)

2.6 Related work and study summary

The effectiveness of transdermal drug delivery systems (TDDS) lies in their ability to deliver drug molecules efficiently to targeted cells, tissues, or organs at a precise therapeutic concentration for a specified duration and at a controlled rate. Additionally, the use of suitable drug carriers within TDDS facilitates sustained drug release, ensuring consistent pharmacological activity.

2.6.1 Clinical trials

Transdermal NSAIDs: Nonsteroidal anti-inflammatory drugs (NSAIDs) exert their anti-inflammatory and analgesic effects primarily by inhibiting cyclooxygenase (*COX*) enzymes, thereby reducing prostaglandin synthesis and mitigating chronic hyperalgesia. When administered topically, NSAIDs can achieve therapeutic drug concentrations directly at the site of inflammation or pain with minimal systemic absorption, potentially minimizing adverse systemic effects. The clinical efficacy of topical NSAIDs is contingent upon their ability to penetrate the skin and reach the target site. Various NSAIDs exhibit differing rates of dermal permeation and are utilized in managing conditions such as acute musculoskeletal injuries, back pain, chronic musculoskeletal disorders, and neuropathic pain. Topical formulations include ointments, gels, pastes, and transdermal patches. Among these, patches demonstrate superior skin permeation and adherence compared to gels and ointments. Nonetheless, topical NSAIDs can still lead to local and systemic adverse effects. Approximately 1–2% of users may experience dermatological reactions such as rashes, pruritus, burning sensations, or contact dermatitis, although these are generally mild and resolve upon cessation of therapy.

Transdermal opioids: In China, the most commonly utilized opioid transdermal patches are those containing fentanyl and buprenorphine. In contrast, capsaicin has gained increasing attention due to its promising preclinical, clinical, and pharmacological applications. Clinical evidence suggests that repeated topical applications (three to five times daily for two to six weeks) of low-concentration capsaicin formulations yield modest pain relief in conditions such as post-herpetic neuralgia, diabetic neuropathy, and chronic musculoskeletal pain. A high-concentration

capsaicin patch (8%) is approved in both Europe and the United States (specifically for post-herpetic neuralgia) and is also used for HIV-related neuropathy and other neuropathic pain syndromes. This formulation ensures rapid transdermal delivery of capsaicin with minimal systemic exposure. Clinical studies, including one conducted in Scotland and another across 22 countries involving 629 participants, have demonstrated that the 8% capsaicin patch has comparable efficacy to pregabalin, with no significant difference in time to therapeutic response, highlighting its potential as a reliable alternative in neuropathic pain management. The single 30- to 60-minute application under medical supervision minimizes variability in administration and enhances patient adherence while reducing environmental exposure. Oral formulations of capsaicin, often delivered in chili pepper capsules, are commercially available; however, an official therapeutic dose has not been established. The recommended daily intake ranges from 1350 to 4000 mg of capsicum containing approximately 0.25% capsaicin. Doses ranging from as low as 0.4–2 mg to as high as 135–150 mg have shown benefits in promoting thermogenesis, increasing fat oxidation, and suppressing appetite.

Additional pharmacological formulations include capsaicin-containing nasal sprays and homeopathic preparations, which have demonstrated efficacy in managing nonallergic rhinitis. One prior study reported therapeutic benefit using capsicum nasal sprays (4 g/puff) administered thrice daily over three consecutive days in patients with nonallergic, non-infectious perennial rhinitis (Fattori et al., 2016).

2.6.2 Related work and study summary

The Qutenza® capsaicin 8% dermal patch, containing synthetic capsaicin at 8% w/w, is designed for localized delivery to pain-affected areas. It has received regulatory approval in the European Union for the management of peripheral neuropathic pain (PNP) in adults, either as monotherapy or in combination with other analgesics. Clinical studies have demonstrated that a single 30-minute application can provide up to 12 weeks of sustained pain relief and improved sleep quality when compared to placebo. Extended use over 52 weeks, in conjunction with standard care, has also shown durable analgesic effects without evidence of neurological toxicity. In

individuals with non-diabetic PNP, the patch was associated with a faster onset of action and greater patient satisfaction. Similarly, patients with postherpetic neuralgia experienced both rapid and prolonged pain relief. Results in HIV-associated neuropathy were mixed, with one study showing significant benefit and another reporting no effect. The most frequently observed adverse events were transient, localized skin reactions at the site of application. Overall, the capsaicin 8% dermal patch represents an effective and well-tolerated adjunct in the treatment regimen for various forms of peripheral neuropathic pain (Blair, 2018).

In a 2022 retrospective post-authorization study, María Dolores Ausín-Crespo et al. evaluated the efficacy and tolerability of the capsaicin 8% dermal patch for the treatment of peripheral neuropathic pain within a specialized pain unit. The diagnosis of neuropathic pain was confirmed using the DN4 questionnaire, and treatment outcomes were assessed using visual analog scale (VAS) scores for pain intensity and the EQ-5D instrument for health-related quality of life. A total of 66 patients, most of whom suffered from iatrogenic neuropathic pain (47%) and reported severe baseline pain, participated in the study. Over the course of three months, the mean VAS pain score decreased significantly from 7.20 (±1.95 SD) to 6.02 (±2.77 SD), representing a mean reduction of 1.19 points (95% CI: 0.59–1.78; $p < 0.001$; Cohen's d = 0.49), indicating a moderate effect size. Additionally, the mean pain area significantly reduced from 169.5 cm^2 to 121.2 cm^2 ($p < 0.001$). Improvements were also observed across multiple EQ-5D quality-of-life dimensions, particularly in usual activities, pain/discomfort, and anxiety/depression. The capsaicin patch was well tolerated, with adverse events consistent with known application-site reactions. These findings support the use of the capsaicin 8% dermal patch as a viable treatment option for managing peripheral neuropathic pain in clinical pain management settings (Ausín-Crespo et al., 2022).

In a 2022 clinical trial, Valéria Romero et al. investigated the analgesic efficacy of an 8% capsaicin cream in patients diagnosed with myofascial pain syndrome (MPS). The study employed a double-blind, randomized design involving 40 participants, who were assigned to receive either a capsaicin 8% (CPS) or placebo (PLA) cream. Prior to the application, all participants received local anesthetic pretreatment

for 50 minutes. Subsequently, 10 grams of the test cream were applied for 30 minutes over the trigger point within a standardized 24 mm diameter area. Pain intensity was assessed using a verbal numerical scale (0–10) at multiple time points: baseline, during application, and at 1 hour, 7 days, 30 days, and 60 days post-treatment. While none of the PLA group experienced skin irritation, 85% of patients in the CPS group reported transient hyperemia and a burning sensation at the application site within 15 minutes, which resolved within 24 hours. Over time, the CPS group demonstrated a significant and sustained reduction in pain scores, with statistical significance maintained through Day 60 ($p < 0.0001$). The findings confirm that 8% capsaicin cream is effective, safe, and well-tolerated in MPS patients, with no observed short- or long-term dermatologic adverse effect (Romero et al., 2019).

Transdermal drug delivery systems (TDDSs) have become a prominent focus in pharmaceutical technology and are widely produced globally due to their potential to overcome the limitations associated with conventional administration routes such as oral or parenteral delivery. Notably, TDDSs offer several advantages, including bypassing hepatic first-pass metabolism and enabling convenient self-administration. However, the stratum corneum (SC) with its tightly packed, hydrophobic structure poses a significant barrier, rendering many drugs unsuitable for standard transdermal application. Multiple factors affect cutaneous drug absorption, with skin physiology playing a critical role. For instance, SC thickness and lipid content, which vary by anatomical location, can markedly influence the rate and extent of transdermal permeation. In recent years, nanoparticle-based drug carriers have shown considerable promise for enhancing transdermal drug delivery, offering unique advantages such as improved permeation and targeted release. Importantly, some nanoparticle formulations have advanced to the stage of clinical trials, highlighting their translational potential in therapeutic applications (Jiang et al., 2022; Mitchell et al., 2021). While nanoparticles (NPs) can be administered orally or intravenously, transdermal delivery using microneedles (MNs) has garnered significant research interest due to its potential to enhance drug bioavailability while avoiding the pain and invasiveness associated with hypodermic injections. This review highlights the types of nanoparticles currently

utilized in drug delivery and explores strategies developed to improve the transdermal transport of nanoparticle-loaded therapeutics.

A transdermal patch, or medicated skin patch, offers a controlled method for delivering active pharmaceutical ingredients into systemic circulation through the skin and is increasingly viewed as a promising alternative to oral drug administration. Capsaicin, a bioactive compound derived from chili peppers, is well known for inducing both pain and thermal sensations, which has made it a valuable tool in pain research. By selectively activating nociceptive neurons, capsaicin has been widely applied in the investigation of pain mechanisms. Clinically, its most common therapeutic use is in the management of pain, with low-concentration capsaicin formulations (0.025–0.1% w/w) having been available in many countries since the early 1980s for routine topical application (Anand & Bley, 2011). Topical analgesics are frequently self-administered, with clinical studies showing that three to five applications per day over a period of two to six weeks can yield modest therapeutic benefits in managing various pain conditions, including postherpetic neuralgia, diabetic neuropathy, and chronic musculoskeletal pain.

Recent advances in nanotechnology have highlighted the unique structural and functional properties of nanomaterials, positioning them as promising candidates for the development of innovative therapeutic platforms. In particular, nanofibers characterized by distinctive physicochemical and biological attributes—are increasingly explored in biomedical research for their potential in sustained and controlled drug delivery. These fibers can be fabricated from diverse polymeric materials using electrospinning, a scalable and versatile technique that enables the production of nanofibers with varied morphologies. Nanoparticles, defined as solid particles ranging from 0.1 to 100 nanometers in diameter, exhibit high permeability and a large surface area-to-volume ratio, making them especially suitable for transdermal drug delivery systems. Among the materials investigated for this purpose, polyvinyl alcohol (PVA) has emerged as a favorable carrier due to its non-toxic, biocompatible, and biodegradable nature, as well as its hydrogel-forming and electrospinning capabilities, which enhance drug encapsulation and skin permeation efficiency.

Previous research has demonstrated that capsaicin at a concentration of 0.1% is a promising therapeutic option for pain management, offering favorable safety and tolerability profiles. Concurrently, the electrospinning technique has gained considerable attention for fabricating nanoscale polymer-based drug delivery systems, particularly nanofibers. In this context, Franz diffusion cells are widely employed to evaluate transdermal drug permeation, and studies using Strat-M™ membranes have shown that drug-loaded PVP/PVA nanofibers possess superior physicochemical properties compared to nanofibers composed of either polymer alone. Moreover, skin permeation studies using human skin explants across the dermatome are recognized as the gold standard for assessing transdermal delivery efficacy. However, due to ethical concerns regarding the use of human and animal tissues, the Strat-M™ synthetic membrane has emerged as a reliable in vitro alternative. Accordingly, the objective of this study is to develop and optimize a capsaicin-loaded transdermal nanofiber patch, fabricated from a polyvinyl alcohol/polyvinylpyrrolidone (CAP/PVA/PVP) polymer matrix using the electrospinning process. This work further aims to investigate the drug release mechanism and transdermal permeation behavior of the nanofiber patch through the Strat-M™ membrane.

CHAPTER III

RESEARCH METHODOLOGY

This chapter gives an overview of the research design used and was conducted as a research and development (R&D) methodology to evaluate the efficacy of capsaicin transdermal nanofibers patches made from capsaicin extract for pain relief. This chapter gives a brief description of the study design and treatment, the study design, the study are, materials and methods, statistical analysis, and the methodology used to determine the setting recruitment strategy, the data collection, data analysis, ethical statement, research plan, and budgets for this study.

3.1 Study design

This study is to investigate of research and development (R&D) that will be conducted at Suranaree University of Technology, Thailand.

3.2 Study area

This study was conducted at Nakhon Ratchasima, Thailand

3.3 Materials and methods

3.3.1 Materials

1. Reagents and Materials

1) Poly (vinyl alcohol) PVA (MW = 360,000, Sigma Aldrich)

2) Polyvinylpyrrolidone average (Mw ~1,300,000, Sigma Aldrich)

3) Capsaicin analytical standard

4) Deionized water

5) Ethanol absolute ≥99.8%, AnalaR NORMAPUR® ACS, Reag. Ph. Eur. analytical reagent

2. Apparatus

1) Nipro Syringe 10 ml

2) Aluminum foil (Diamond brand)

3) Magnetic stirrer and magnetic bar (size 40x0.8 mm.)

4) Electrospinning Machine

5) Start-M membranes Transdermal diffusion Test Model (Sigma Aldrich, Merk KGaA, Darmstadt, Germany)

6) The scanning electron microscope (SEM) (JEOL JSM-6010LV InTouchScope)

7) Fourier transform infrared (FTIR)

8) Franz diffusion cells (FDC)

9) High-performance liquid chromatography (HPLC)

MTT assay

DPBS: Dulbecco's Phosphate Buffered Saline, CORNING, USA

DMSO: DIMETHYL SULPHOXIDE (CH3)2SO, Sigma-Aldrich Co. (St. Louis, MO)

MTT: 3-4,5-dimethyl-2-thiazolyl-2,5-diphenyl-2H-tetrazolium bromide, Sigma-Aldrich Co. (St. Louis, MO)

DMEM/ high glucose > Dulbecco's Modified Eagle Medium (High glucose), HyClone (Logan, UT)

Penicillin-Streptomycin Solution, HyClone (Logan, UT)

FBS: Fetal bovine serum, HyClone (Logan, UT)

Real time PCR

Trypsin: 25% Trypsin EDTA (1x), gibco, CA, USA

qPCR: 2x qPCRBIO SyGreen Mix Lo-ROX, PCR BIOSYSTEMS

RNA: NucleoSpin® RNA Plus

ReverTra Ace™ qPCR RT Master Mix with gDNA Remover, TOYOBO CO., LTD. (2-8 Dojima Hama 2-Chome Kita-ku Osaka 530-8230 JAPAN

3.3.2. Methods

1) The Fabrication of CAP/PVA/PVP Nanofibers: To prepare the polymer solutions, 20 g of polyvinyl alcohol (PVA) was dissolved in a solvent mixture consisting of 45 mL of distilled water and 45 mL of absolute ethanol, achieving a 20% w/v concentration. The solution was continuously stirred at 80 °C for 3 hours using an electromagnetic stirrer to obtain a homogeneous and transparent solution. Similarly, 20 g of polyvinylpyrrolidone (PVP) was dissolved in the same solvent mixture and stirred at 60 °C for 3 hours until a clear and uniform solution was achieved. The two polymer solutions were then combined to yield a final polymeric blend containing 10% w/v of each polymer (PVA/PVP). Subsequently, capsaicin (CAP) powder at a concentration of 0.1 mg/mL was incorporated into the PVA/PVP blend at 10% v/w. The resulting drug-polymer mixture was allowed to cool to room temperature for several hours prior to electrospinning as shown in Figure 3.1 (Hindi et al., 2021).

2) Production of the Electrospun PVA/PVP Nanofiber: The electrospinning will be performed at room temperature (Salles et al., 2015) using a 10 ml disposable syringe with a metal needle, 0.55 mm internal diameter, is a common misunderstanding. The resulting polymer solution was loaded into syringe. The solution was powered by a syringe pump with a feeding rate of 10 µL/min. The solution was electrospun under an applied voltage of 15 kV at a 17-cm tip-to-collector distance as well as a room temperature. The fibers were put on aluminum foil that was stuck to a drum that was moving cylindrical collector that represented in Figure 3.1.

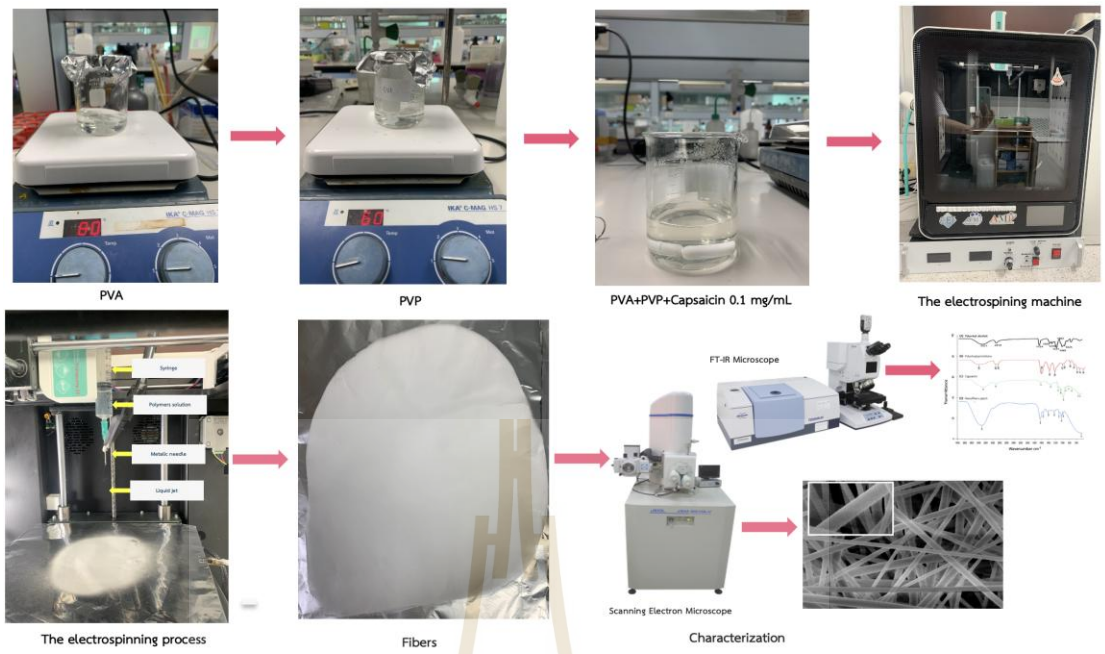


Figure 3.1 Schematic representation of the electrospinning process used to fabricate CAP/PVA/PVP nanofibers

3) Physicochemical Characterizations:

3.1) A Fourier transform infrared spectroscopy (FT-IR) was employed to analyze the chemical composition of the capsaicin-loaded nanofiber patches. The spectra were recorded over a wavelength range of 4000–600 cm^{-1} at a controlled ambient temperature of 25 °C using the Attenuated Total Reflectance (ATR) mode. Each sample was analyzed using a resolution of 4 cm^{-1}, with both the sample and background scanned 64 times to enhance signal-to-noise ratio. All measurements were performed in triplicate to ensure reproducibility. The data acquisition and spectral processing were conducted using OPUS software (Bruker Optics) as shown the workflow in Figure 3.2, enabling detailed evaluation of functional group interactions and confirmation of capsaicin encapsulation within the polymeric nanofiber matrix. (Hindi et al., 2021). The patch sample was analyzed using a Bruker VERTEX 70 FTIR spectrometer, followed by spectral analysis with OPUS software. The spectra show the characteristic transmittance peaks of polyvinyl alcohol (A), polyvinylpyrrolidone (B), capsaicin (C), and the final nanofiber patch formulation (D), confirming successful component incorporation.

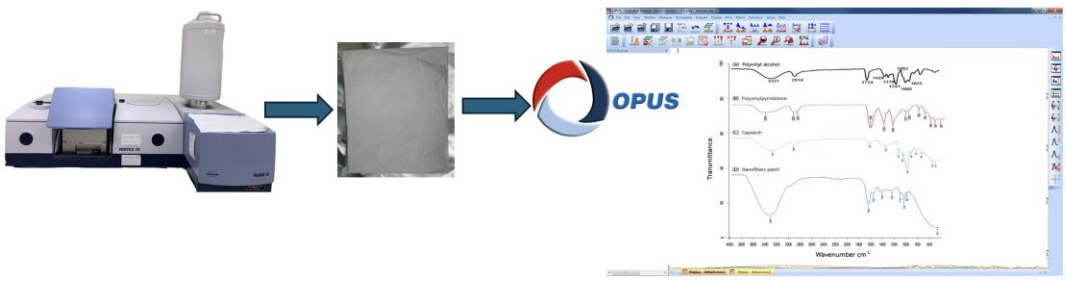


Figure 3.2 FTIR characterization workflow of capsaicin-loaded PVA/PVP nanofiber patch

3.2) Surface Morphology Characterizations (SEM) is the surface morphology of the electrospun nanofibers was examined using scanning electron microscopy (SEM). Prior to imaging, the nanofiber samples were sputter-coated with a thin layer of platinum to enhance conductivity as shown in Figure 3.3. The average fiber diameter and its distribution were quantitatively analyzed using image analysis software based on the SEM micrographs (Hindi et al., 2021).

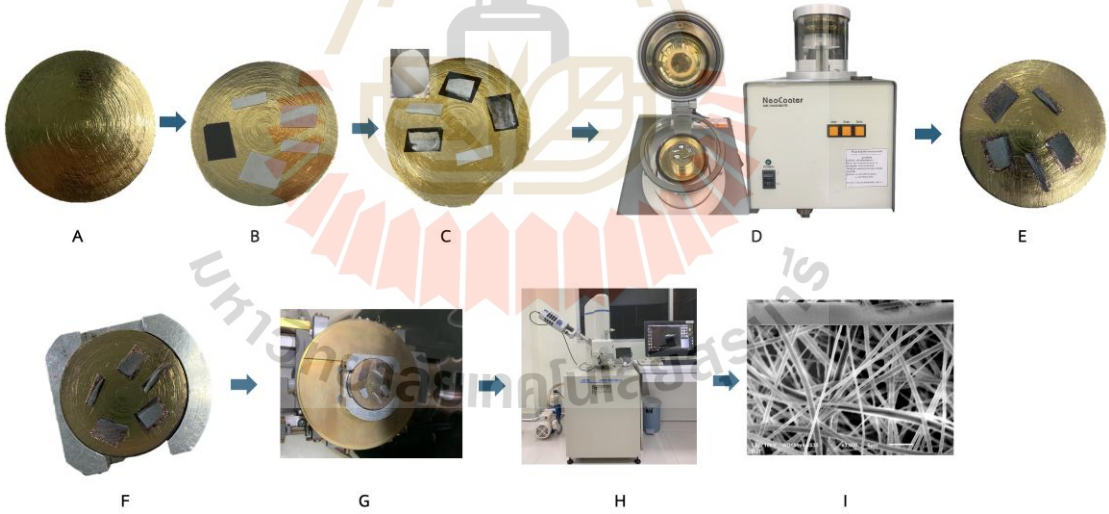


Figure 3.3 Workflow of SEM sample preparation and imaging of electrospun nanofiber patches were prepared for SEM observation by first cutting into square sections (1 × 1 cm²).

(A) Clean bare aluminum stub surface.

(B) Placement of carbon tape on the stub surface.

(C) Mounting nanofiber samples onto carbon tape.

(D) Sputter coater (Neo Coater/MP-19020NCTR) used for gold coating to enhance conductivity.

(E) Gold-coated nanofiber samples post-coating.

(F) Stub secured into the SEM holder.

(G) Mounted holder being positioned into the SEM chamber.

(H) Scanning Electron Microscope (JEOL JSM-6010LV) system setup.

(I) SEM micrograph of electrospun nanofiber mat, revealing uniform, interconnected fibrous morphology.

The samples were mounted on aluminum stubs using double-sided conductive carbon tape (Figures 3.3 (B-C). To reduce charging during imaging, a thin layer of gold was sputter-coated onto the sample surface using a Gold (Au) Sputter Coater (Neo Coater/MP-19020NCTR) (Figure 3.3 (D–E). The coated stubs were then loaded into a JEOL JSM-6010LV SEM system (Figure XG–H), and surface morphology was observed under an accelerating voltage. A representative SEM image of the capsaicin-loaded nanofiber mat is shown in Figure 3.3 (I). capsaicin nanofiber patches.

Cytotoxicity assay

1. Evaluation of Anti-inflammatory Activity on Human Dermal Fibroblasts (HDF) Using the MTT Assay

The experiment was conducted as represented in Figure 3.4 as follows:

1.1 Human dermal fibroblast (HDF) cells were seeded at a density of 6,000 cells/well in 96-well plates using DMEM medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. The cells were incubated for 24 hours in a CO_2 incubator maintained at 37°C with 5% CO_2.

1.2 The test extract was prepared at various concentrations by dilution in DMEM medium. Each concentration (0.0001, 0.001, 0.01, 0.1, 1, 10, 50, and 100 mg/mL) was added to the wells at a volume of 100 µL and incubated for 24 hours.

1.3 After 24 hours, 100 µL of MTT solution (0.5 mg/mL) was added to each well and incubated in the dark for 3 hours. Subsequently, the supernatant was removed, and the resulting formazan crystals were dissolved in 100 µL of DMSO.

1.4 The absorbance was measured at 570 nm using a microplate reader.

1.5 The percentage of cell viability was calculated using the following formula (Jaiboonma et al., 2020):

$$\% \text{ cell viability} = \frac{\text{Absorbance of treated cell}}{\text{Absorbance of control cell}} \times 100 \quad (1)$$

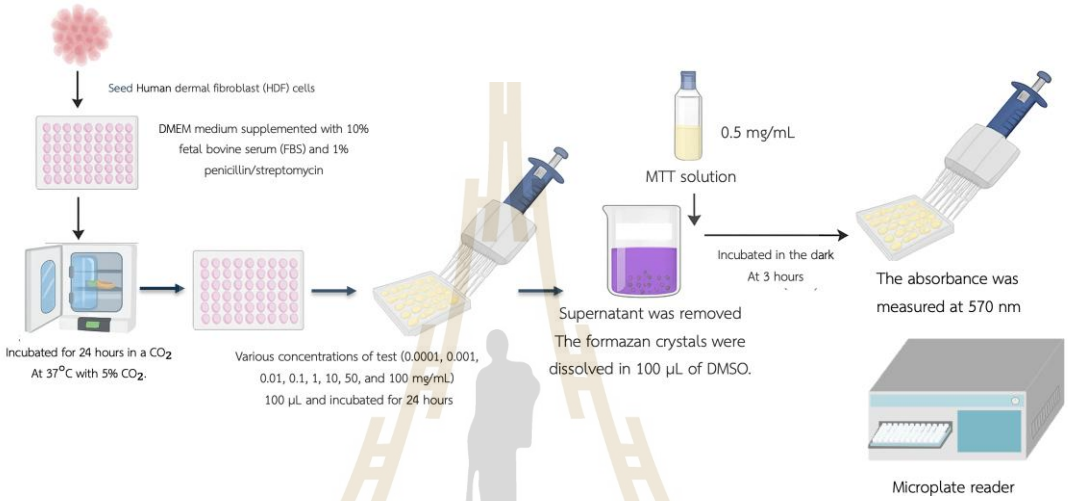


Figure 3.4 Evaluation of Anti-inflammatory Activity on Human Dermal Fibroblasts (HDFs) Using the MTT Assay

2. Investigation of Cyclooxygenase-2 (*COX-2*) Inhibitory Activity for Analgesic Properties

To assess the *COX-2* inhibitory activity of capsaicin at different concentrations, the experiment was performed in Figure 3.5 as follows:

2.1 HDF cells were cultured in 6-well plates at a density of 1×10^6 cells/well and incubated for 24 hours in a CO_2 incubator at 37°C with 5% CO_2.

2.2 The study was divided into three groups: 1) Control group (untreated), 2) Positive control group, where inflammation was induced using 1 mM H_2O_2 for 30 minutes, and 3) Treatment group, where 1 mM H_2O_2 (Kar et al., 2021) was applied for 30 minutes followed by treatment with capsaicin patch extract at 0.1 mg/mL, then incubated for 24 hours.

2.3 After 24 hours, the cells were harvested using trypsinization.

2.4 Total RNA was extracted using the NucleoSpin RNA kit (Macherey-Nagel, Dueren, Germany). RNA concentration was quantified using a microplate reader (BMG LABTECH, Ortenberg, Germany). Subsequently, 1 µg of total RNA was reverse-transcribed into complementary DNA (cDNA) using the ReverTra Ace® qPCR RT Master Mix with gDNA Remover (Toyobo Co., Ltd). The cDNA samples were stored at −20°C for subsequent *COX-2* gene expression analysis by quantitative real-time PCR (qRT-PCR). All experiments were performed in triplicate.

2.5 qRT-PCR analysis was conducted using the QuantStudio 5 Real-Time PCR System (Thermo Fisher Scientific, MA, USA). The PCR reactions were prepared using SYBR® Green Master Mix (Thermo Fisher Scientific) with primers specific for *COX-2* and GAPDH (used as an internal control). Thermal cycling conditions included initial denaturation at 95°C for 1 minute, followed by 40 cycles of 95°C for 30 seconds, 60°C for 30 seconds, and 72°C for 1 minute. Melting curve analysis was performed with the following steps: 95°C for 15 seconds, 60°C for 1 minute, and 95°C for 15 seconds.

2.6 Gene expression was analyzed using the 2^ (-delta delta C(T)) method (Rao et al., 2013), with *COX-2* expression normalized to GAPDH as the housping gene.

2.7 Data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using the Student's t-test. Statistical significance was indicated as follows: **p* < 0.05, ***p* < 0.01, ****p* < 0.001 (compared to control group); #*p* < 0.05, ##*p* < 0.01, ###*p* < 0.001 (compared to the positive control group).

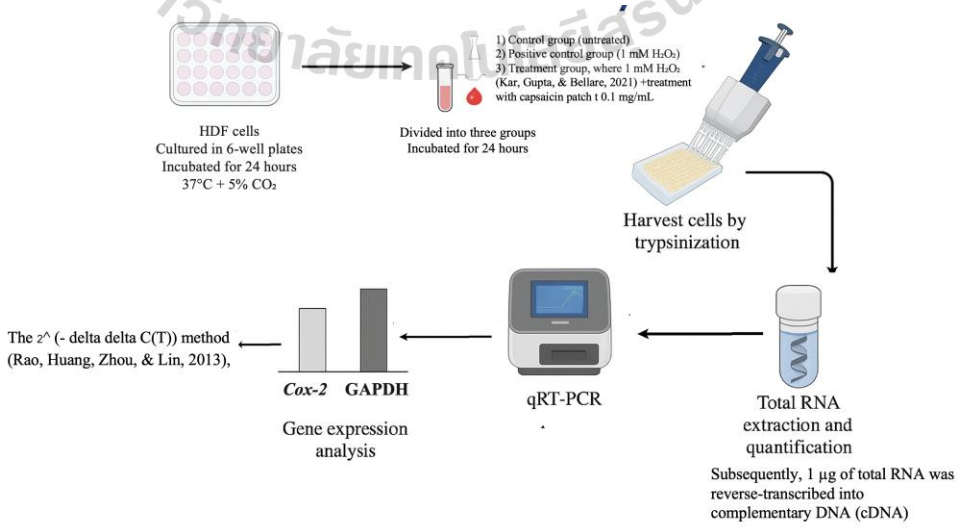


Figure 3.5 *Cox-2* Inhibition assay

The *in vitro* skin permeation

1) Franz diffusion cells: The *in vitro* permeation experiments were carried out using Franz diffusion cells with Strat-M™ Skin permeation as shown in Figure 3.6. The release medium was maintained at 37 °C and the experiment was carried out for 24 h. The Strat-M experiment was done three times, and at different times (1, 2, 4, 6, 8, 12 hours), the circulating solution in the receiver compartment was collected (Pulsoni et al., 2022). After the permeation study was completed, the skin was removed from the Franz cell, then washed with a sodium chloride 0.9%, and dried with a paper towel. (Klebeko et al., 2021).

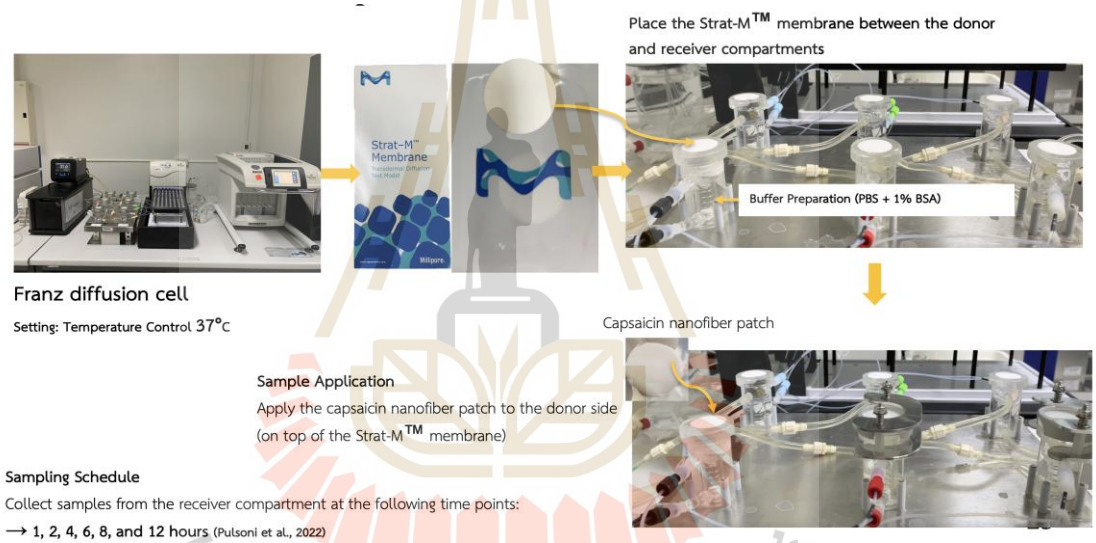


Figure 3.6 Schematic Representation of Capsaicin Permeation Study Using the Franz Diffusion Cell System

The capsaicin-loaded nanofiber patch was applied to the donor compartment of a Franz diffusion cell system, with a Strat-M™ membrane positioned between the donor and receiver chambers to simulate skin permeation. The receiver compartment was filled with PBS containing 1% BSA and maintained at 37 °C. Samples were collected at predetermined time points (1, 2, 4, 6, 8, and 12 hours) to evaluate capsaicin diffusion. This method enables in vitro assessment of transdermal drug delivery and membrane permeation.

2) High-performance liquid chromatography (HPLC) analysis: Following a 12-hour permeation study as represented in Figure 3.7, the skin surface was rinsed with deionized water and air-dried. Capsaicin levels in the stratum corneum (SC) were assessed using a tape-stripping technique with 20 pieces of 3M Scotch Magic™ tape (1 × 1 cm). To determine capsaicin retained within the deeper skin layers, the tape-stripped skin was finely minced. The tape samples and skin fragments were each extracted with a 1:1 (v/v) solution of phosphate-buffered saline (PBS, pH 7.4) and absolute ethanol, using 5 mL and 2 mL volumes, respectively. All samples were subjected to 30 minutes of sonication, followed by filtration. The resulting filtrates were analyzed for capsaicin content by HPLC using a detection wavelength of 280 nm and an injection volume of 10 µL.

High-performance liquid chromatography (HPLC) was carried out using an HP1100 system equipped with a UV detector set at 280 nm (Hewlett-Packard, Waldbronn, Germany). Separation was achieved on a Hypersil ODS column (250 × 4.0 mm i.d., 5 µm; Agilent, CA, USA) using a mobile phase consisting of acetonitrile and 1% acetic acid (1:1, v/v) at a flow rate of 1.0 mL/min. The injection volume was 10 µL. Method validation for capsaicin quantification was assessed based on accuracy, precision, and linearity. Quantification was performed using a calibration curve, which demonstrated strong linearity. All measurements were conducted in triplicate (Anantaworasakul et al., 2020).

The permeation rate of capsaicin across a Strat-M™ membrane was determined from the slope of the plot representing the cumulative amount permeated over time (hours). The lag time was identified as the x-intercept of the linear segment of the curve. The steady-state flux of capsaicin was subsequently calculated according to Equation (2) (Tatke et al., 2018).

According to Equation (3), the permeability coefficient (Kp) and enhancement ratio (Er) of capsaicin were systematically calculated to assess transdermal performance (Tatke et al., 2018).

Steady state flux (Jss) = (dQ/dt)/A **(2)**

Permeability coefficient (Kp) = Steady state flux/Donor concentration **(3)**

Where: J is the steady-state flux (μg/cm²/hr)

dQ/dt is the rate of drug permeation (μg/hr)

A is the diffusion area (cm²)

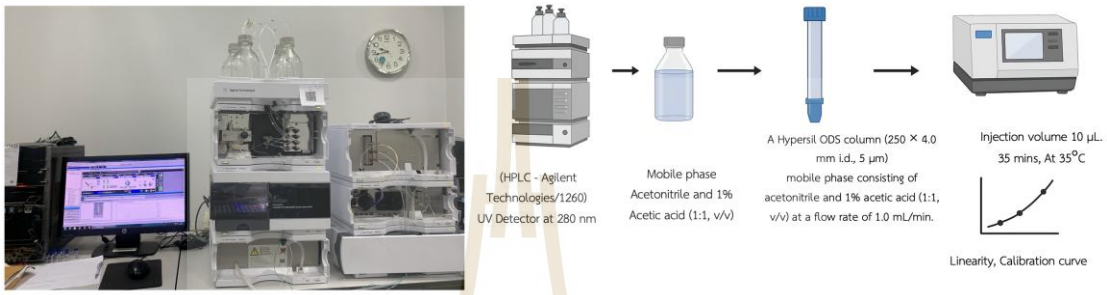


Figure 3.7 High-Performance Liquid Chromatography (HPLC) Setup and Analytical Procedure for Capsaicin Quantification

The quantification of capsaicin was conducted using an HPLC system (Agilent 1260, Agilent Technologies) with a UV detector set at 280 nm. The mobile phase consisted of acetonitrile and 1% acetic acid (1:1, v/v). Chromatographic separation was performed on a Hypersil ODS column (250 × 4.6 mm, 5 μm) at a flow rate of 1.0 mL/min and a column temperature of 35 °C. A sample injection volume of 10 μL was used, with a total run time of 35 minutes. Quantification was based on a linear calibration curve constructed from standard capsaicin solutions (Anantaworasakul et al., 2020).

3) **FTIR microspectroscopy**

Sample preparation for FTIR microspectroscopy analysis: Strat-M membrane samples were prepared by sectioning into small tissue-like pieces, each approximately 1 cm in length and 1 cm in thickness. For each test sample, three pieces were randomly excised from different regions to ensure representative distribution. The tissue fragments were then placed in an aluminum bowl and fully immersed in OCT compound (Tissue-Tek®, Electron Microscopy Sciences, Hatfield, PA, USA). The

bowl was gently placed above liquid nitrogen until the OCT medium was completely solidified. Subsequently, the frozen samples were stored at −80 °C until cryosectioning.

Tissue sections were prepared using a cryostat to obtain slices approximately 5 µm in thickness as shown in Figure 3.8. These sections were carefully mounted onto infrared-transparent (IR) slides. The mounted samples were then dehydrated in a vacuum desiccator for 48 hours to ensure complete drying prior to FTIR microspectroscopic analysis (Thumanu et al., 2017; Thumanu et al., 2015).

FTIR microspectroscopy analysis: FTIR analysis was performed using a Tensor 27 FTIR spectrometer (Bruker Optics, Germany) coupled with a Hyperion 3000 IR microscope (Bruker Optics, Germany) operating in transmission mode. Spectral acquisition and instrument control were carried out using OPUS software version 7.5 (Bruker Optics, Germany). Measurements were conducted at 36× magnification with a spectral resolution of 4 cm^{-1}. The background spectrum was collected using 64 scans. Infrared absorption spectra were acquired in the range of 4000–900 cm^{-1}. Each sample spectrum was recorded using an aperture size of 15 × 15 µm². For spatially resolved chemical imaging, spectral mapping was performed over an area of 22 × 15 µm² (Thumanu et al., 2017; Thumanu et al., 2015)

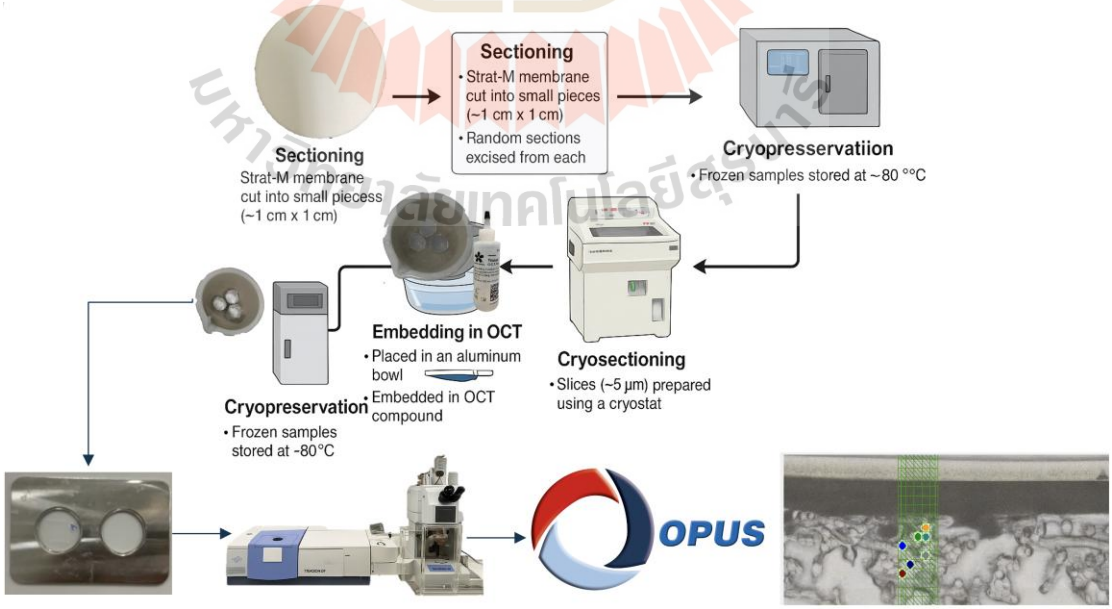


Figure 3.8 Schematic Representation of Strat-M™ Membrane Preparation and FTIR Imaging Workflow

This scheme illustrates the stepwise preparation of Strat-M™ membrane samples for FTIR imaging analysis. The membrane was cut into 1 cm × 1 cm sections and cryopreserved at −80 °C. Selected sections were embedded in optimal cutting temperature (OCT) compound using aluminum molds. The embedded samples were then cryosectioned using a cryostat to obtain thin slices (~5 µm). These slices were subjected to FTIR imaging, and spectral data were analyzed using OPUS software to investigate the spatial distribution of chemical components within the membrane.

3.4 Ethical statement

Ethical consideration: This study does not have any conflicts of interest.

Ethical approval was granted by the Human Research Ethics Committee, Health Research and Development Center of Nakhon Ratchasima Public Health Provincial Office, Nakhon Ratchasima, Thailand, via reference letter No. NRPH 004 dated 01-02-2023.

3.5 Research plan

Table 3.1 Research plan

Descriptions	Month, Year (Thesis started in semester 1/2021)											
	1	2	3	4	5	6	7	8	9	10	11	12
Starts in year 2021												
Meeting committee	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Literature review				✓	✓	✓	✓					
Year 2022												
Proposal & ethic approval											✓	✓
Year 2023												
1. Development of transdermal nanofiber patches - The Fabrication of CAP/PVA/PVP Nanofibers - Electrospinning process (Production of the Electrospun CAP/PVA/PVP Nanofiber) 2. Apparatus and Characterization Characterization its physicals and chemicals properties	✓	✓	✓	✓	✓	✓						
Data collection	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Data and statistical analysis								✓	✓	✓	✓	✓
Discussion and conclusion										✓	✓	✓
Year 2024												
Full report	✓	✓	✓	✓						✓	✓	✓
The *in vitro* skin permeation The Strat-M® experiment was done three times, and at different times for collection the data, the CAP transdermal nanofibers patch prototype	✓	✓	✓	✓	✓							
Conference and publication	✓	✓	✓	✓	✓							
Thesis defense				✓	✓	✓						
PhD completion in 2024					✓	✓						

CHAPTER IV
RESULTS AND DISCUSSION

This chapter presents the results obtained from the in vitro evaluation of the capsaicin-loaded transdermal nanofiber patch and discusses the significance of the findings in the context of its potential for pain relief applications. The study involved the formulation of nanofiber patches incorporating capsaicin, and subsequent biological assessments including cytotoxicity (MTT assay) and anti-inflammatory activity (*COX-2* inhibition) in human dermal fibroblast (HDF) cells.

Table 4.1 Experimental design and testing workflow for evaluation of capsaicin-loaded nanofiber patch

Parameter	Purpose/Design	Result/Outcome
Polyvinyl Alcohol (PVA)	Polymer matrix for nanofiber fabrication	Formed smooth nanofibers when mixed with PVP
Polyvinylpyrrolidone (PVP)	Co-polymer for nanofiber formulation	Enhanced fiber formation and drug dispersion
Capsaicin (CAP)	Active compound	Successfully encapsulated, bioactive in patch
Materials	PVA+PVP	Formed nanofibers when mixed
Materials	PVA+PVP+Capsaicin (Active compound)	20 g of PVA, 20 g of PVP+ 0.1 mg/ml of Capsaicin
Nanofibers	Diameter 500-1000 nm	667 ± 19.5 nm
Physicochemical Characterizations	FTIR SEM	- Identify and confirm the functional groups and - Distribution of nanofibers
Cytotoxicity assay	MTT assay (Nontoxic)	Nontoxic (< 10 mg/ml)

Table 4.1 Experimental design and testing workflow for evaluation of capsaicin-loaded nanofiber patch (Continued)

Parameter	Purpose/Design	Result/Outcome
Anti-Inflammatory Activity Test in Human Dermal Fibroblast (HDF) Cells	Cyclooxygenase-2 (*COX-2*) Inhibitory Activity	*COX-2* inhibition, anti-inflammatory effect
***In vitro* skin permeation**	Franz cell diffusion	The permeation of capsaicin through the Strat-M™ membrane
	FTIR, HPLC	FTIR spectral analysis revealed time-dependent variations in signal intensity, indicating progressive diffusion of capsaicin into deeper membrane layers.

4.1 Results

4.1.1 The Fabrication of CAP/PVA/PVP Nanofibers

In this study, capsaicin (CAP)-loaded nanofibers were successfully fabricated using a blend of polyvinyl alcohol (PVA) and polyvinylpyrrolidone (PVP) via electrospinning. The preparation of the polymer solution was performed by dissolving 20 g of PVA powder in a binary solvent system comprising 45 mL of distilled water and 45 mL of absolute ethanol, yielding a final concentration of 20% w/v. The solution was stirred at 80 °C for 3 hours using an electromagnetic stirrer until a clear, homogenous solution was obtained. Likewise, 20 g of PVP was dissolved under the same solvent conditions and stirred at 60 °C for 3 hours, resulting in a similarly transparent and uniform solution. The two polymer solutions were then mixed in equal volumes to achieve a final concentration of 10% w/v for both PVA and PVP, forming a stable polymeric matrix suitable for electrospinning.

Capsaicin was incorporated into the mixed polymer solution at a concentration of 0.1 mg/mL, corresponding to 10% v/w relative to the total polymer content. The final CAP/PVA/PVP solution was allowed to cool to room temperature

and rested for several hours to ensure complete homogenization before proceeding to electrospinning, as previously described by Hindi et al. (2021).

The electrospinning process was conducted at room temperature using a 10 mL disposable syringe fitted with a metal needle with an internal diameter of 0.55 mm. The polymer solution was loaded into the syringe and extruded at a flow rate of 3 mm/hr using a syringe pump. An electric voltage of 15 kV was applied across a 17-cm tip-to-collector distance. The nanofibers were collected on aluminum foil affixed to a rotating cylindrical drum collector, which ensured consistent fiber deposition and alignment.

The electrospun fibers appeared continuous, uniform, and free from visible bead formation, indicating that the process parameters solvent system, polymer concentration, applied voltage, flow rate, and distance were well optimized. The combination of PVA and PVP offered a favorable balance of viscosity, conductivity, and mechanical properties, enabling stable electrospinning and nanofiber formation, highlighting the compatibility of PVA/PVP blends in producing nanofibers with smooth morphology.

The successful fabrication of CAP-loaded nanofibers under these optimized conditions establishes a foundational step for further physicochemical and biological characterization. Subsequent studies were focus on evaluating fiber morphology using scanning electron microscopy (SEM), drug release kinetics, and biological efficacy to validate the application of this formulation as a transdermal system for pain relief.

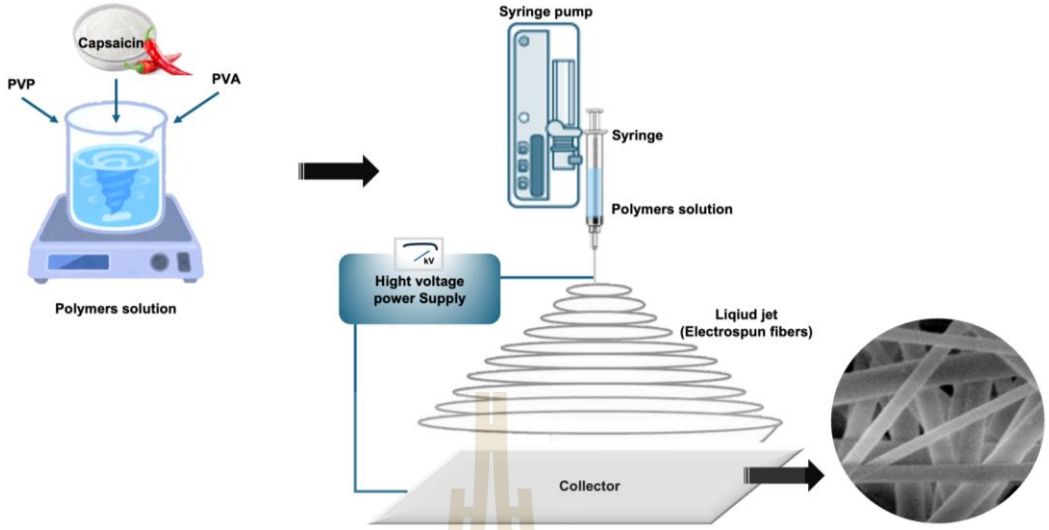


Figure 4.1 Schematic representation of the electrospinning process used to fabricate CAP/PVA/PVP nanofibers

The process involves applying a high-voltage electric field to a polymer solution loaded in a syringe, resulting in the formation of continuous nanofibers collected on a grounded rotating drum. The accompanying histograms illustrate the distribution of fiber diameters, confirming uniform morphology and consistent nanofiber formation.

4.1.2 Physicochemical Characterizations

Figure 4.2 displays the Fourier-transform infrared (FT-IR) spectra of of polyvinyl alcohol (A), polyvinylpyrrolidone (B), capsaicin (C), and the capsaicin-loaded nanofibers patch (D). These spectra were used to identify and confirm the functional groups of capsaicin and to determine whether capsaicin remained chemically stable after incorporation into the nanofiber matrix.

In the spectrum of pure capsaicin (Figure 4.2C), the broad absorption peak observed at approximately 3300 cm^{-1} corresponds to O–H stretching, indicative of phenolic hydroxyl groups. The bands at 2923 cm^{-1} and 2854 cm^{-1}represent asymmetric and symmetric C–H stretching vibrations of aliphatic $–CH_2$ and $–CH_3$ groups. A sharp peak at around 1640 cm^{-1} corresponds to C=O stretching of the amide group, while the peaks in the region of 1510–1450 cm^{-1} are attributed to aromatic C=C ring

stretching vibrations. Additional peaks near 1260 cm^{-1} and 1020 cm^{-1}can be assigned to C–N stretching and C–O stretching, respectively, confirming the characteristic structure of capsaicin.

The FT-IR spectrum of the capsaicin-loaded nanofibers patch (Figure 4.2D) shows several overlapping and shifted peaks, consistent with the successful incorporation of capsaicin into the polymer matrix. The broad O–H stretching band at 3300 cm^{-1} is retained, though broadened, indicating hydrogen bonding interactions between capsaicin and the polymeric components particularly PVA and PVP. The peaks at 2925 cm^{-1} and 2850 cm^{-1}, corresponding to aliphatic C–H stretching, were also observed, confirming the presence of capsaicin within the patch.

Notably, the carbonyl stretching band (1640 cm^{-1}) was slightly shifted and broadened in the nanofiber spectrum, suggesting possible physical interactions between the capsaicin and the hydroxyl or carbonyl groups present in the polymers. This interaction implies molecular entrapment rather than covalent bonding, which is desirable for controlled drug release without altering drug activity.

In the fingerprint region (1200–700 cm^{-1}), characteristic peaks associated with the PVP and PVA backbone such as C–O, C–C, and C–N stretching were clearly evident, confirming the structural integrity of the polymer network post-electrospinning. Importantly, no new peaks or significant changes were detected, indicating that capsaicin remained chemically stable and did not undergo degradation during the fabrication process.

These findings confirm the successful physical incorporation of capsaicin into the nanofiber matrix, while preserving its functional groups and overall chemical structure. The observed shifts in peak position and intensity further support the existence of non-covalent interactions, such as hydrogen bonding, between capsaicin and the polymer system. Such interactions are advantageous for sustained drug release and stability within the patch formulation.

Overall, FT-IR analysis validated the chemical compatibility of capsaicin with the PVA/PVP polymer blend and demonstrated that the electrospinning process did not alter the integrity of the bioactive compound.

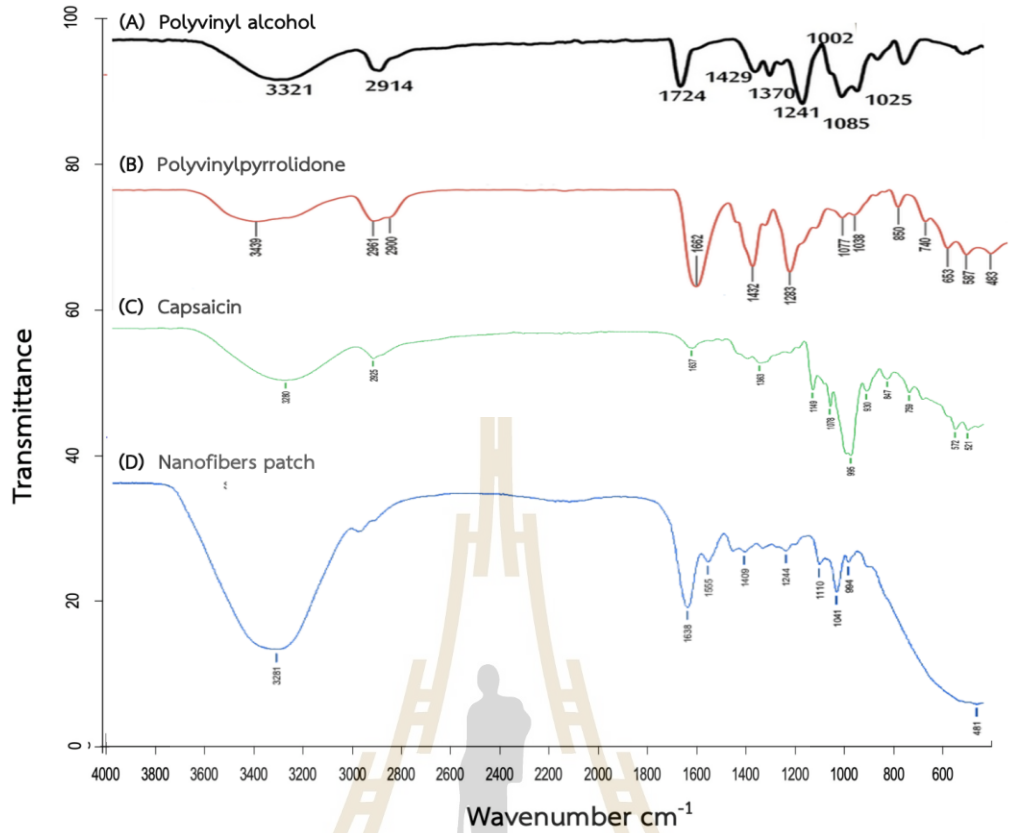


Figure 4.2 FT-IR spectrum and chemical formula of polyvinyl alcohol (A), polyvinylpyrrolidone (B), capsaicin (C), and nanofibers patch (D)

4.1.3 Surface Morphology Characterizations (SEM)

The surface morphology of nanofibers was evaluated by using the scanning electron microscope (SEM). The average diameter distribution of nanofibers was calculated by image analysis software. The nanofiber specimen was sputter coated with platinum before subjecting to the SEM imagery (Hindi et al., 2021).

The morphology of the capsaicin-loaded nanofibers was investigated using Scanning Electron Microscopy (SEM), as shown in Figure 4.4A. The image captured at 5,000× magnification revealed that the nanofibers were smooth, continuous, and bead-free, indicating a homogenous polymer solution and well-optimized electrospinning parameters as shown in Figure 4.3. The fibers appeared randomly oriented but uniformly distributed, which is desirable for consistent drug release and mechanical performance in transdermal applications. The fiber diameters were quantitatively analyzed using ImageJ software (NIH, USA), based on SEM images. A total

of 50 randomly selected fibers were measured, and the results are illustrated in the histogram in Figure 4.3B. The fiber diameters followed a unimodal, slightly right-skewed distribution. The mean fiber diameter was found to be 0.667 ± 0.195 μm, which is equivalent to 667 ± 19.5 nanometers (nm). This confirms that the fibers fall well within the nanoscale range (1–1000 nm), validating the successful formation of true nanofibers.

The relatively narrow standard deviation indicates high uniformity of fiber thickness, which is essential for ensuring consistent surface area for drug release, mechanical stability, and patch flexibility. The absence of bead formation also suggests favorable polymer–solvent interactions and appropriate electrospinning voltage and flow rate settings. In comparison with other capsaicin-loaded nanofiber systems reported in the literature, the fiber diameter obtained in this study is comparable or smaller, suggesting enhanced surface-area-to-volume ratio, which may contribute to improved drug dispersion and skin permeation performance. These morphological characteristics confirm the suitability of the CAP/PVA/PVP nanofiber formulation for transdermal delivery applications. The incorporation of capsaicin into the polymeric matrix appears homogeneously distributed throughout the fibers, indicating successful encapsulation and suggesting a potential for sustained drug release across the membrane.

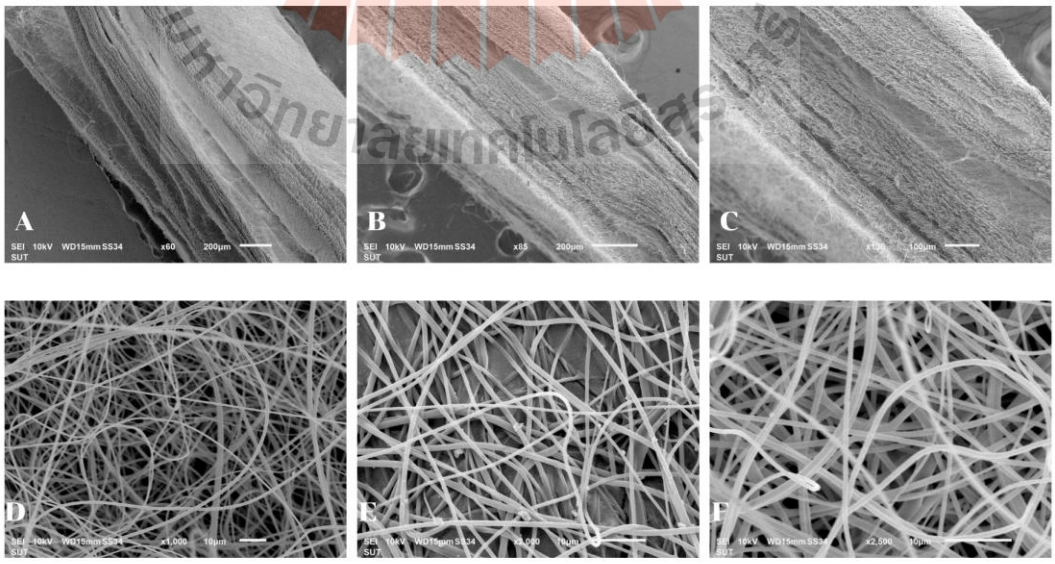


Figure 4.3 SEM micrographs of capsaicin-loaded nanofiber patch and skin interface

(A–C) Cross-sectional SEM images of skin after transdermal patch application at different magnifications (A: ×60, B: ×85, C: ×130). These images demonstrate the interaction of the fibrous layer can be seen adhering to the outer skin layer. (D–F) SEM images of the surface morphology of electrospun nanofibers at increasing magnifications (D: ×1,000, E: ×2,000, F: ×2,500). The capsaicin nanofibers incorporated show a continuous, bead-free, and smooth structure with randomly oriented networks, which is favorable for high surface area and effective drug loading and release.

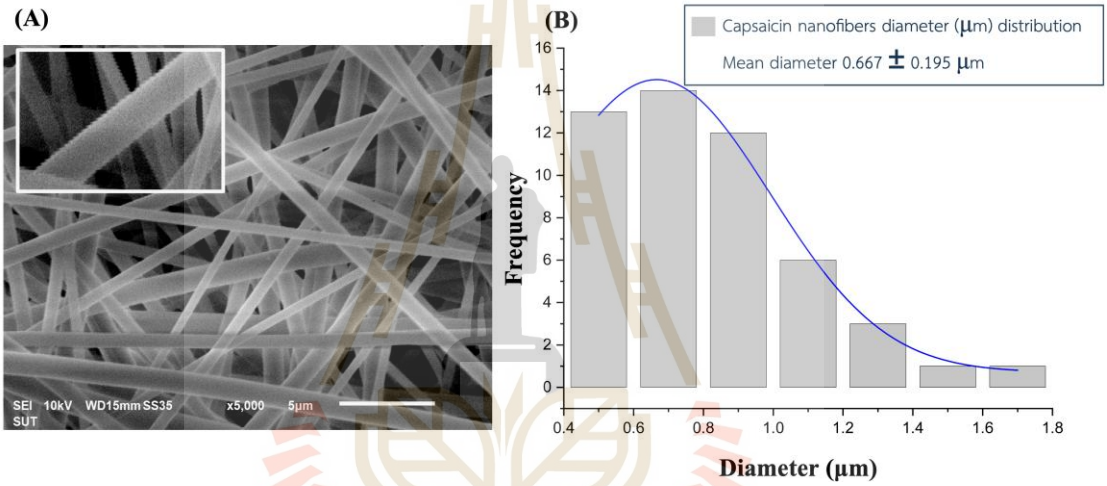


Figure 4.4 Surface morphology of nanofibers

(A) Scanning Electron Microscopy (SEM) image of capsaicin-loaded nanofibers at 5,000× magnification, showing uniform and bead-free morphology. (B) Diameter distribution histogram of capsaicin nanofibers, with a mean diameter of 0.667 ± 0.195 μm, indicating consistent fiber formation with a slight right-skewed distribution.

4.1.4 Cytotoxicity assay

Cytotoxicity Evaluation of Capsaicin-Loaded Nanofiber Patch on Human Dermal Fibroblasts (HDF)

The cytotoxicity of the capsaicin-loaded nanofiber patch was evaluated on human dermal fibroblast (HDF) cells using the MTT assay, and the results are shown in Figure 4.5. Cells were treated with varying concentrations of nanofiber extract (0.0001 to 100 mg/mL) for 24 hours.

At lower concentrations (0.0001–1 mg/mL), the nanofiber extract did not exhibit cytotoxic effects. In fact, at 0.001 and 0.01 mg/mL, a significant increase in cell viability was observed ($p < 0.001$), with viability exceeding 130% relative to the untreated control group. This suggests that low concentrations may not only be safe but also potentially promote fibroblast activity or exert mild protective effects, possibly due to the antioxidant and anti-inflammatory properties of capsaicin at sub-cytotoxic levels.

Conversely, at higher concentrations (10–100 mg/mL), a dose-dependent decrease in cell viability was observed. Cell viability dropped to approximately 75% at 50 mg/mL and further declined to ~40% at 100 mg/mL ($p < 0.001$), indicating notable cytotoxic effects at elevated doses. From the dose–response curve, the half-maximal inhibitory concentration (IC_{50}) was estimated to be approximately 20 mg/mL, which defines the concentration at which capsaicin causes a 50% reduction in cell viability under these *in vitro* conditions.

This IC_{50} value provides a useful benchmark for establishing the upper safety limit for formulation development. Notably, the concentrations that showed any cytotoxicity (≥10 mg/mL) are significantly higher than those typically used in in vivo transdermal delivery, where localized drug concentrations on the skin surface are much lower due to controlled release and limited permeation. Clinical formulations of capsaicin patches (e.g., 0.025–0.1% w/w) correspond to microgram-per-milliliter levels once applied to skin, which are well below the IC_{50} threshold observed here.

Therefore, the capsaicin nanofiber patch demonstrates a wide safety margin for dermal application, and concentrations effective in pain relief are expected to remain well within the non-cytotoxic range *in vivo*. These findings confirm the biocompatibility of the formulation and support its potential for further development as a safe transdermal therapeutic system.

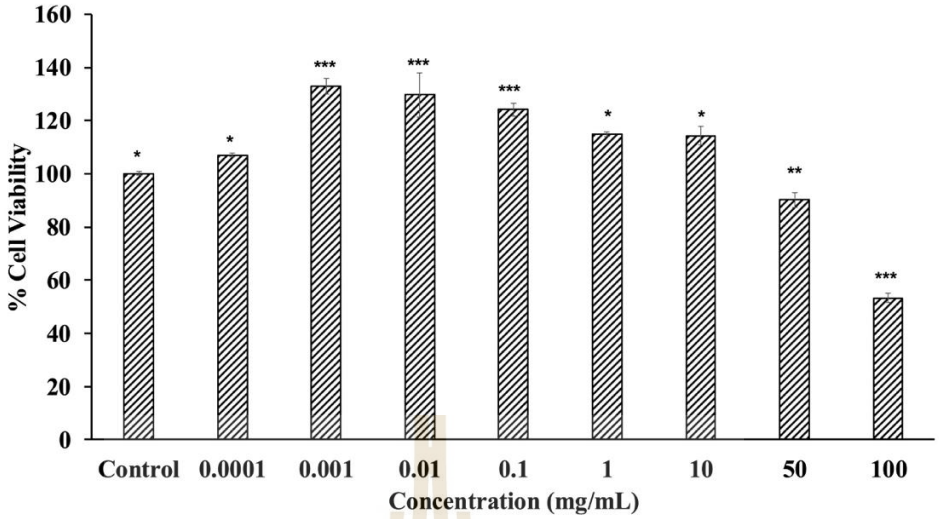


Figure 4.5 Human dermal fibroblasts (HDF) cells treatment with nanofiber patch Using the MTT Assay

The bar graph illustrates the percentage of cell viability in HDFs treated with different concentrations of capsaicin (ranging from 0.0001 to 100 mg/mL), as measured by the MTT assay. Results are expressed as a percentage relative to the untreated control group (100%).

At low concentrations (0.001–0.1 mg/mL), capsaicin significantly enhanced cell viability compared to the control, suggesting a stimulatory or protective effect on HDFs (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). However, at higher concentrations (50–100 mg/mL), a significant decrease in cell viability was observed, indicating dose-dependent cytotoxicity. Notably, the viability dropped below 80% at 50 mg/mL and further to around 60% at 100 mg/mL, showing potential toxicity at these levels. These findings support the use of low-dose capsaicin for therapeutic applications with minimal cytotoxic risk.

4.1.5 Investigation of Cyclooxygenase-2 (*COX-2*) Inhibitory Activity for Analgesic Properties

To assess the anti-inflammatory efficacy of the capsaicin-loaded nanofiber patch, gene expression analysis of cyclooxygenase-2 (*COX-2*) was performed using quantitative real-time polymerase chain reaction (qRT-PCR) in human dermal fibroblast (HDF) cells. The *GAPDH* gene was used as an internal reference for normalization.

As shown in Figure 4.6, exposure of HDF cells to hydrogen peroxide (H_2O_2, 1 mM) resulted in a marked increase in *COX-2* expression, approximately 3.5-fold higher than in the untreated control group. This significant upregulation confirmed that H_2O_2 effectively induced oxidative stress and mimicked an inflammatory microenvironment within the fibroblast population. *COX-2* is a key inducible enzyme involved in the biosynthesis of pro-inflammatory prostaglandins and is commonly used as a molecular marker for inflammatory activity.

In contrast, treatment with the capsaicin-loaded nanofiber patch at a concentration of 0.1 mg/mL following H_2O_2 stimulation led to a significant downregulation of *COX-2* mRNA expression. Quantitative analysis showed that *COX-2* levels were reduced by approximately 8.1-fold when compared to the H_2O_2-treated group. This robust suppression indicates that the patch formulation effectively mitigates inflammation at the transcriptional level. The observed reduction in *COX-2* expression is consistent with the known mechanisms of capsaicin, which include inhibition of pro-inflammatory signaling pathways such as NF-**K**B and MAPKs. Capsaicin is also known to modulate oxidative stress and reduce the expression of downstream inflammatory mediators, making it a promising candidate for topical anti-inflammatory therapy.

The successful delivery of capsaicin via the nanofiber matrix likely contributed to its enhanced bioavailability and sustained action at the cellular level. The polymer blend of PVA/PVP may also have supported the biological compatibility of the patch, allowing for efficient interaction with the cell membrane and intracellular uptake. These findings demonstrate that the capsaicin-loaded nanofiber patch possesses significant anti-inflammatory potential by downregulating *COX-2* expression in inflamed dermal cells. The patch could thus serve as an effective non-NSAID alternative for managing localized inflammation, such as in myofascial pain syndrome (MPS) or other soft tissue inflammatory conditions. Further validation through in vivo experiments is warranted to confirm therapeutic efficacy and pharmacodynamic behavior.

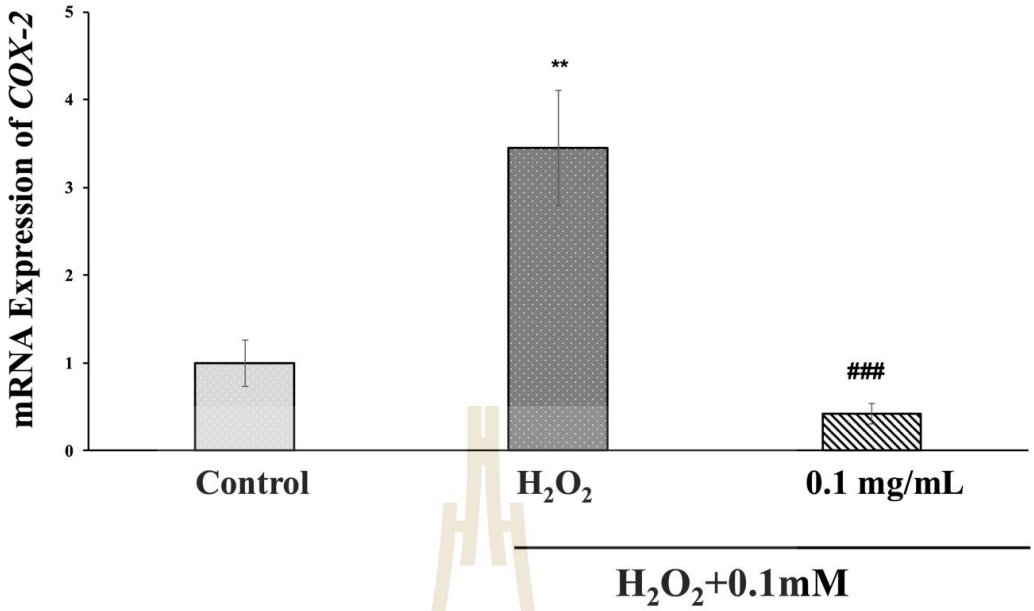


Figure 4.6 Evaluation of Anti-inflammatory Activity in HDF Cells by Real-Time PCR Targeting Two Genes (*GAPDH* and *COX-2*)

The bar graph illustrates the relative mRNA expression levels of cyclooxygenase-2 (*COX-2*) in human dermal fibroblasts (HDFs) under different treatment conditions. Exposure to 0.1 mM hydrogen peroxide (H_2O_2) significantly upregulated *COX-2* expression compared to the untreated control (**$p < 0.01$). Treatment with the capsaicin nanofiber patch (0.1 mg/mL) in combination with H_2O_2 markedly reduced *COX-2* mRNA expression (###$p < 0.001$) compared to the H_2O_2-treated group alone, indicating a strong anti-inflammatory effect. Data are presented as mean ± SD.

Effects of Capsaicin-Loaded Nanofiber Patch on Cell Morphology in HDF Cells

The protective and restorative effects of the capsaicin-loaded nanofiber patch on human dermal fibroblast (HDF) morphology were evaluated under oxidative stress conditions using phase-contrast microscopy, as illustrated in Figure 4.6. In the control group, HDF cells exhibited a typical spindle-shaped fibroblastic morphology with clear cell borders, well-defined cytoplasm, and healthy adherence to the culture surface indicating normal cell integrity. Following exposure to 1 mM

hydrogen peroxide (H_2O_2), the cells displayed significant morphological alterations, including cell shrinkage, elongation, cytoplasmic condensation, and partial detachment. These changes are consistent with oxidative stress-induced cellular damage and inflammation. However, cells treated with the capsaicin-loaded nanofiber patch at 0.1 mg/mL after H_2O_2 induction showed a noticeable recovery of normal fibroblastic morphology in Figure 4.7. The cells appeared more elongated and better attached, with fewer signs of structural damage, suggesting that the patch formulation helped mitigate the harmful effects of oxidative stress and promoted cellular protection or recovery.

These visual observations align with gene expression results from the *COX-2* qRT-PCR analysis and further support the anti-inflammatory and cytoprotective role of capsaicin delivered via the nanofiber patch system.

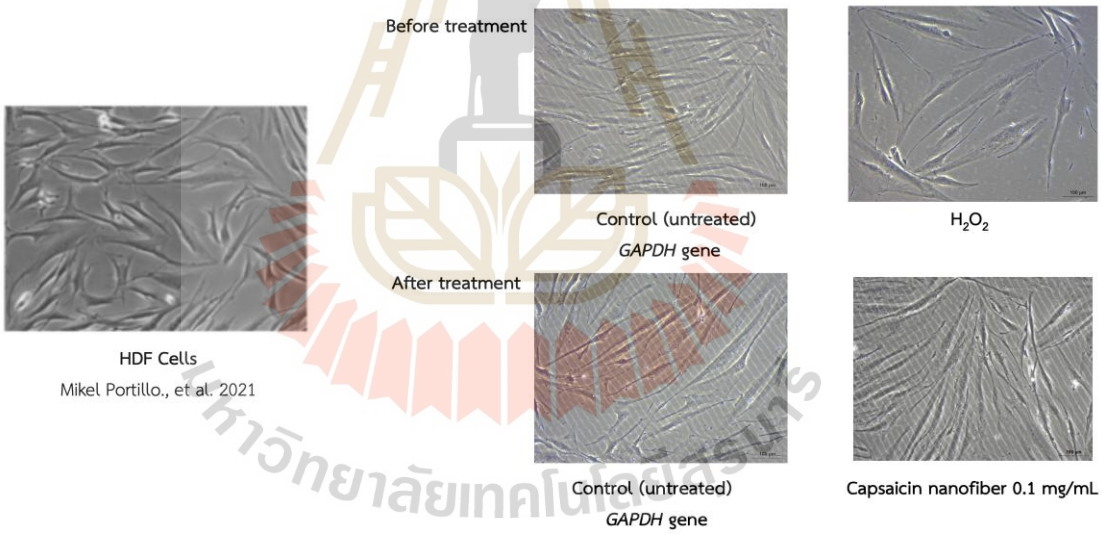


Figure 4.7 Morphological Observation of HDF Cells After Treatment with H_2O_2 and Capsaicin-Loaded Nanofiber Patch

This panel shows phase-contrast microscopy images illustrating the morphology of human dermal fibroblasts (HDFs) before and after treatment under different conditions.

When comparing the observed HDF morphology in this experimental image with the reference image from the following conclusions can be drawn:

Normal (Untreated) HDF Morphology: Both control groups (before and after treatment) and the reference image exhibit classic spindle-shaped, elongated fibroblast morphology with well-organized alignment. This confirms that the cultured HDFs maintain normal cell characteristics under non-stressed conditions, consistent with Portillo et al.'s report.

H_2O_2-Treated Cells: this study shows clear morphological deterioration cells appear rounded, detached, and fragmented, contrasting sharply with the healthy reference cells. This supports that oxidative stress induces significant cellular damage, disrupting the fibroblastic phenotype.

Capsaicin Nanofiber Treatment (0.1 mg/mL): Cells partially regain their spindle-like shape and alignment after treatment, resembling the healthy morphology seen in Portillo et al., 2021. This suggests a restorative or protective role of capsaicin nanofibers against oxidative insult.

Conclusion: Compared to the morphological standard from (Portillo et al., 2021), these findings demonstrate that capsaicin nanofibers help maintain or restore HDF morphology under oxidative stress, providing visual evidence of cytoprotective efficacy.

4.1.6 High-performance liquid chromatography (HPLC) analysis

After a 12-hour skin permeation experiment, the surface of the skin was washed with deionized water and allowed to dry. Capsaicin content in the stratum corneum (SC) was determined via the tape-stripping method using 20 pieces of 3M Scotch Magic™ tape (1 × 1 cm). To assess capsaicin retention in the underlying skin layers, the remaining tape-stripped skin was minced. Extraction of capsaicin from the tape samples and minced tissue was carried out using a 1:1 (v/v) mixture of phosphate-buffered saline (PBS, pH 7.4) and absolute ethanol, with volumes of 5 mL and 2 mL, respectively. All samples were sonicated for 30 minutes, filtered, and analyzed by high-performance liquid chromatography (HPLC).

HPLC analysis was performed using an HP1100 system with UV detection at 280 nm. Separation was conducted on a Hypersil ODS column (250 × 4.0 mm i.d., 5 μm) with a mobile phase comprising acetonitrile and 1% acetic acid (1:1,

v/v) at a flow rate of 1.0 mL/min. The injection volume was set at 10 µL. Method validation confirmed the accuracy, precision, and linearity of capsaicin quantification. Calibration curves showed strong linearity as shown in Figure 4.8, and all measurements were performed in triplicate. The graph shows the linear relationship between capsaicin concentration (µg/mL) and peak area. The regression equation is y=0.05256x, y=0.05256x, with a correlation coefficient of R^2=0.99700, indicating excellent linearity across the tested range (50–500 µg/mL)

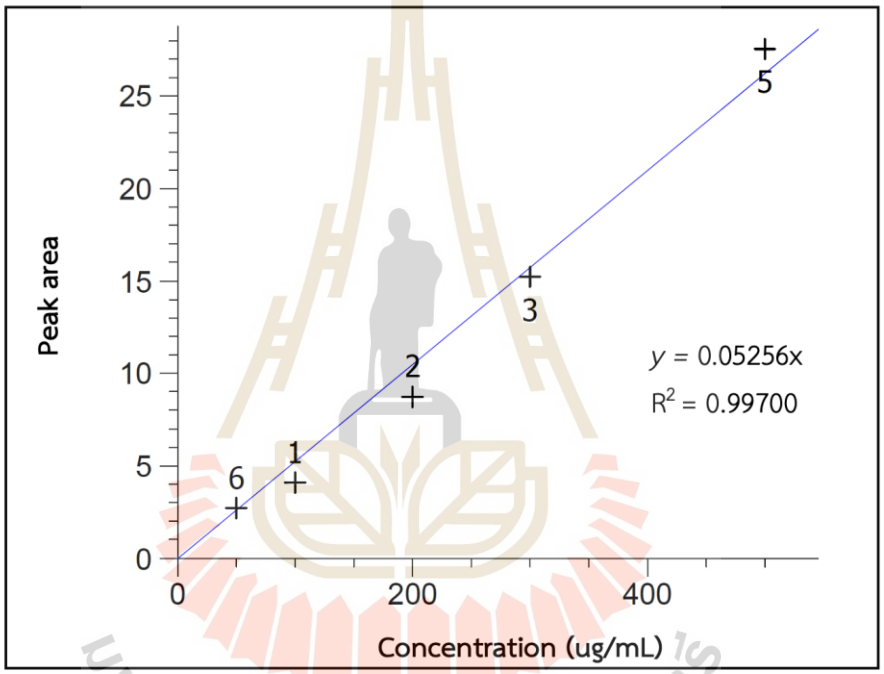


Figure 4.8 The standard calibration curve of capsaicin analyzed by HPLC

High-performance liquid chromatography (HPLC) was used to monitor capsaicin permeation through the skin at various time intervals: 1, 2, 4, 6, 8, and 12 hours. The chromatograms demonstrated consistent retention times for capsaicin, approximately between 22.3 and 22.6 minutes, across all time points, confirming the presence and stability of capsaicin in the samples. As shown in Figures 4.9–4.14, capsaicin was clearly detectable at each time point, with peak intensity correlating with permeation duration. The retention time and peak shape remained sharp and reproducible, indicating the robustness of the analytical method. The gradual increase

in peak area from 1 to 12 hours suggests a sustained release and continuous permeation of capsaicin through the skin model over time. This observation supports the potential of the tested transdermal delivery system for providing prolonged therapeutic effect. These findings are consistent with the chromatographic profile of standard capsaicin under identical HPLC conditions and confirm that capsaicin remains chemically stable throughout the 12-hour experimental period.

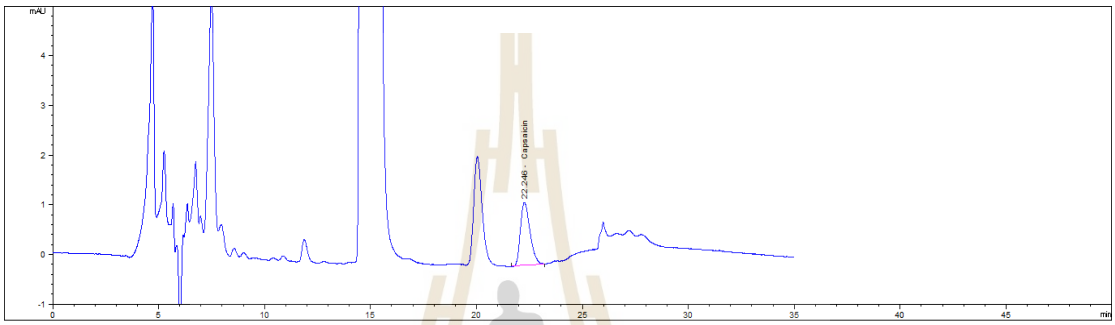


Figure 4.9 HPLC Chromatogram at 1 Hours Showing Capsaicin Retention Time

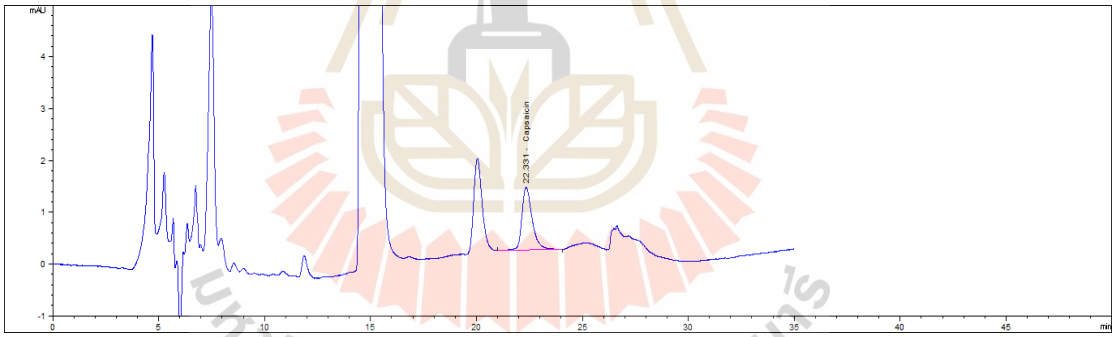


Figure 4.10 HPLC Chromatogram at 2 Hours Showing Capsaicin Retention Time

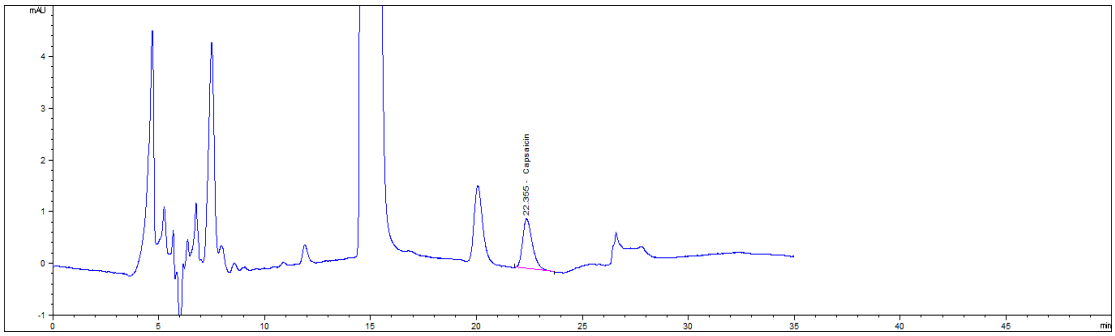


Figure 4.11 HPLC Chromatogram at 4 Hours Showing Capsaicin Retention Time

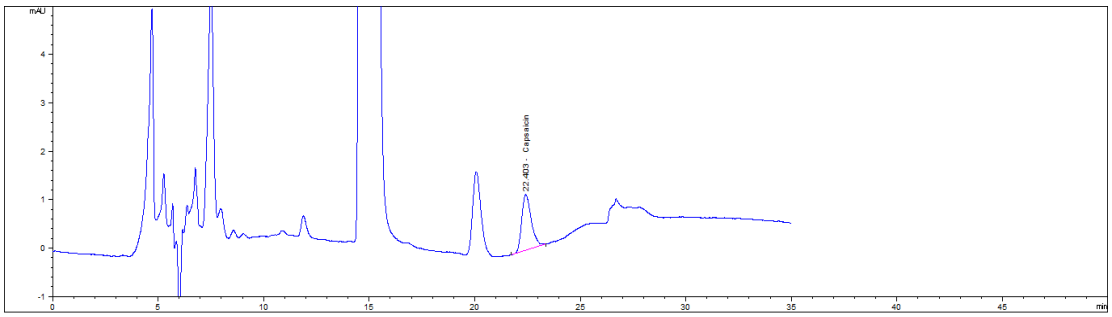


Figure 4.12 HPLC Chromatogram at 6 Hours Showing Capsaicin Retention Time

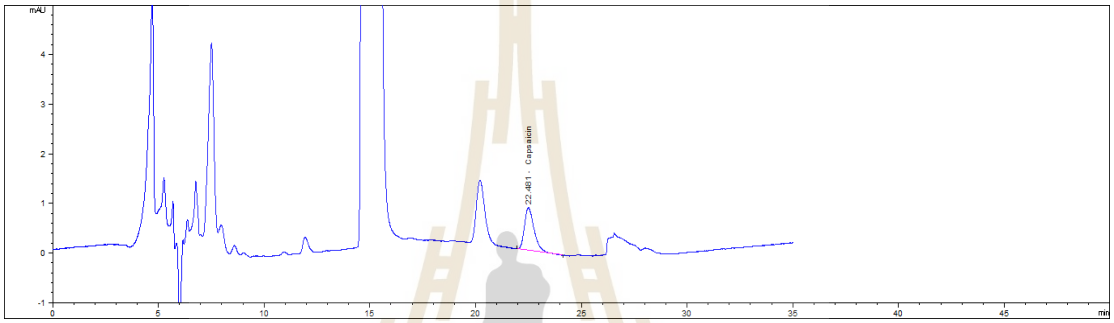


Figure 4.13 HPLC Chromatogram at 8 Hours Showing Capsaicin Retention Time

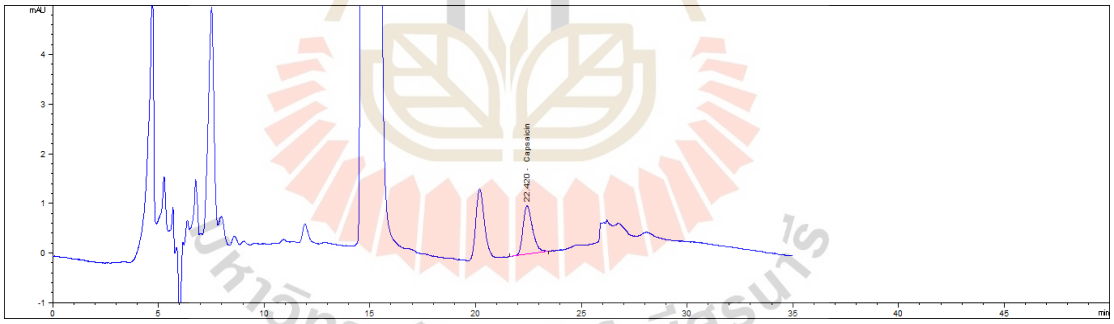


Figure 4.14 HPLC Chromatogram at 12 Hours Showing Capsaicin Retention Time

The permeation rate of capsaicin through the Strat-M™ membrane was determined by calculating the slope of the graph plotting the cumulative amount of capsaicin permeated against time (hours). The lag time corresponds to the x-intercept of the linear segment of this curve. The steady-state flux of capsaicin was then calculated using Equation (2), where steady-state flux (J_{ss}) is defined as the rate of change in cumulative permeation (dQ/dt) divided by the surface area (A) of the Strat-M™ membrane.

Steady state flux (Jss) = (dQ/dt)/A

= 63.30 µg/cm²/hr

The graph illustrates the relationship between the cumulative amount of capsaicin permeated per unit area (µg/cm²) and time (hours). The results demonstrate a consistent increase in permeation over a 12-hour period, indicating sustained drug release behavior. Notably, the time interval between 2 and 8 hours exhibits a clearly linear trend, suggesting that capsaicin permeation reached a steady-state phase during this period. Based on the slope of this linear portion, the steady-state flux (Jss) was calculated to be 63.30 µg/cm²/hr, reflecting the formulation's efficiency in delivering capsaicin across the Strat-M™ membrane via transdermal diffusion.

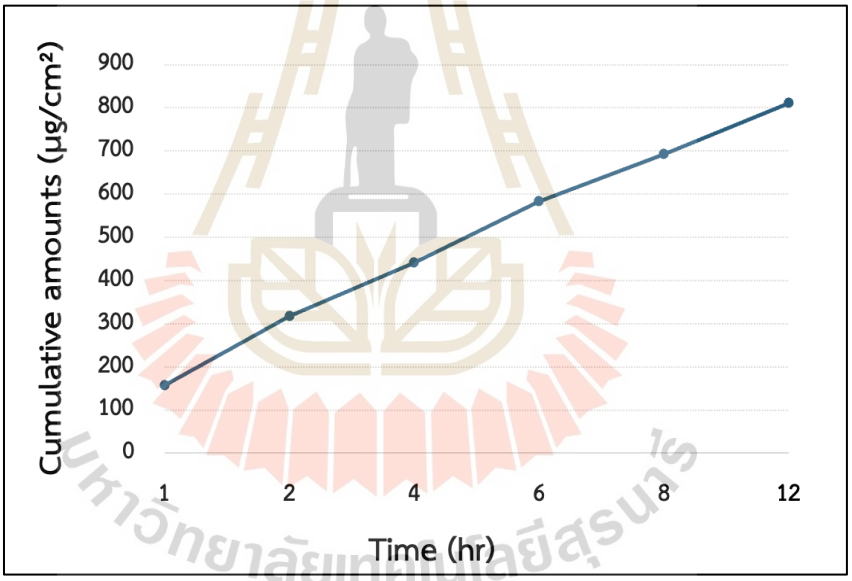


Figure 4.15 Cumulative Permeation of Capsaicin Through Strat-M™ Membrane

The graph illustrates the cumulative amount of capsaicin permeated per unit area (µg/cm²) over time, showing a sustained release profile. The linear segment between 2 and 8 hours was used to calculate the steady-state flux (J_{ss}).

The graph illustrates the permeability coefficient (K_p) of capsaicin through the Strat-M™ membrane over a 12-hour period as shown in Figure 4.16. The K_p value, which reflects the drug's ability to diffuse through the membrane relative to its donor concentration, demonstrates a clear time-dependent decline. At the 1-hour

mark, the K_p value was at its peak (~0.77 cm/hr), indicating a rapid initial permeation rate likely driven by a high concentration gradient and the availability of capsaicin on the membrane surface. During the first 2 to 4 hours, the K_p sharply decreased, suggesting a reduction in the diffusion driving force as the drug began to penetrate deeper into the membrane. Beyond 6 hours, the K_p values approached a plateau (~0.05–0.07 cm/hr), signifying the transition into a quasi-steady state of drug release. This behavior is consistent with Fick's First Law of Diffusion, where the rate of diffusion is proportional to the concentration gradient, which gradually diminishes over time as equilibrium is approached.

This trend reflects the controlled release characteristics of the capsaicin-loaded nanofiber patch, which initially releases the drug rapidly and then sustains a slower, more consistent permeation rate. Such a release profile is desirable in transdermal drug delivery systems designed for prolonged therapeutic effects, especially for conditions such as pain or inflammation.

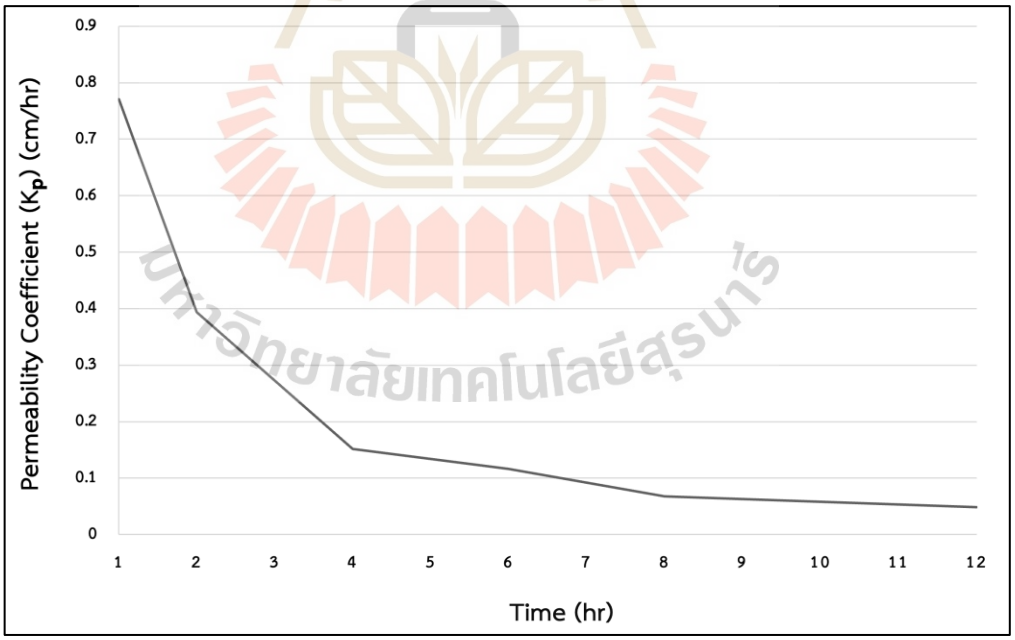


Figure 4.16 Permeability coefficient (K_p) of capsaicin from nanofiber patch over 12-hour transdermal diffusion

The graph shows the permeability coefficient (K_p) of capsaicin calculated at different time intervals (1–12 hr) based on transdermal permeation through the Strat-M™ membrane. The results illustrate an initial high K_p at 1 hour, followed by a gradual decline over time, indicating a transition from rapid to controlled drug release.

4.1.7 The *in vitro* skin permeation

The transdermal permeation of capsaicin from the nanofiber patch was evaluated using the Franz diffusion cell system, employing a Strat-M™ membrane as the skin model. Capsaicin concentrations within the stratum corneum were quantified using a tape-stripping method, while retention in the deeper dermal layers was assessed through a separate retention analysis.

FTIR microspectroscopy analysis: FTIR Imaging and Spectral Mapping of Strat-M™ Membrane Treated with Capsaicin Patch Compared to Control (1–12 hr). The FTIR imaging and spectral mapping of Strat-M™ membrane across different diffusion time points were used to monitor the permeation behavior and spatial localization of capsaicin through the membrane. The vibrational signals were analyzed and compared to the control to evaluate chemical penetration and retention over time. The cumulative amount of capsaicin permeated across the Strat-M membrane over a 12-hour period was determined and is illustrated in Figures 4.17-4.23 and APPENDIX B.

At 1 hr, strong FTIR absorption bands were observed near 2920–2850 cm^{-1} (C–H stretching) and 1650–1500 cm^{-1} (amide and aromatic groups), indicating early-stage surface adsorption of capsaicin. The chemical map shows high intensity in the upper region (epidermis-like layer), with little to no signal in deeper layers, suggesting minimal permeation.

After 2 hr, the spectral intensity in the upper layer slightly decreased, while signals extended deeper into the membrane. Increased peak intensity at 1250–1000 cm^{-1} (C–O and C–N stretching) reflected gradual permeation. The heatmap revealed more diffuse distribution compared to 1 hr.

At 4 hr, the chemical mapping indicates enhanced diffusion across the middle layer. The spectrum showed stronger intensity at 2920 cm^{-1} and 1030 cm^{-1},

which correspond to alkyl chains and ether groups from capsaicin, supporting deeper penetration.

At 6 hr, significant spectral intensity in the dermis-like layer was observed. Strong absorption at 1510 cm^{-1} (aromatic C=C) and 1100 cm^{-1}suggests active diffusion. The chemical map displays widespread distribution of capsaicin, indicating sustained release and permeation.

The FTIR at 8 hr profile reveals plateauing of permeation, with stable intensity across major peaks. Chemical imaging confirms accumulation in lower membrane layers, though peak shifts slightly suggest possible molecular interaction or binding with matrix components.

At 12 hr, capsaicin signal reached the deepest regions. The FTIR spectrum showed elevated intensity and broadened peaks, particularly around 1000–1100 cm^{-1}, indicating full-depth penetration. Compared to control (final image), the capsaicin-treated membrane demonstrates markedly higher chemical intensity and deeper spatial diffusion.

FTIR imaging and spectral analysis revealed the progressive permeation of capsaicin through the Strat-M™ membrane over a 12-hour period. At 1 hr, capsaicin signals were confined to the membrane surface (epidermis), with high intensity in C–H and amide regions. By 2–4 hrs, diffusion increased into the middle layers, with stronger C–O and C–N signals. At 6 hrs, capsaicin reached deeper (dermis-like) layers with enhanced aromatic and ether group peaks. At 8–12 hrs, full-depth permeation was observed, with broader and intensified peaks around 1000–1100 cm^{-1}, indicating maximal diffusion. Compared to the control, capsaicin-treated membranes showed significantly stronger and deeper chemical penetration.

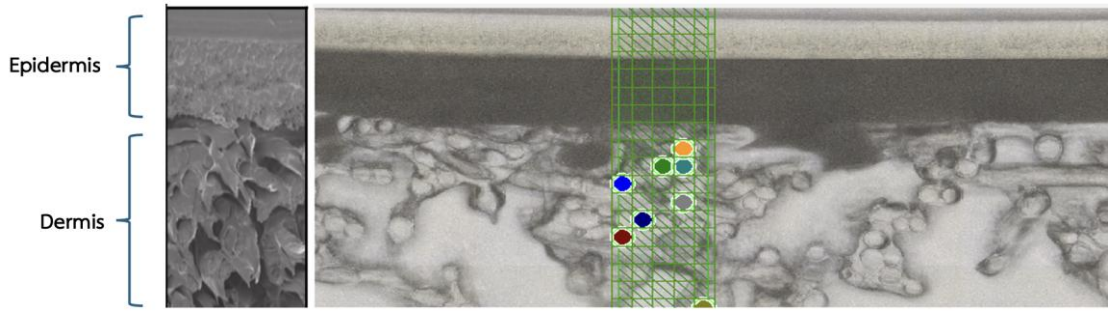


Figure 4.17 FTIR Imaging and Spectral Mapping of Strat-M™ Membrane

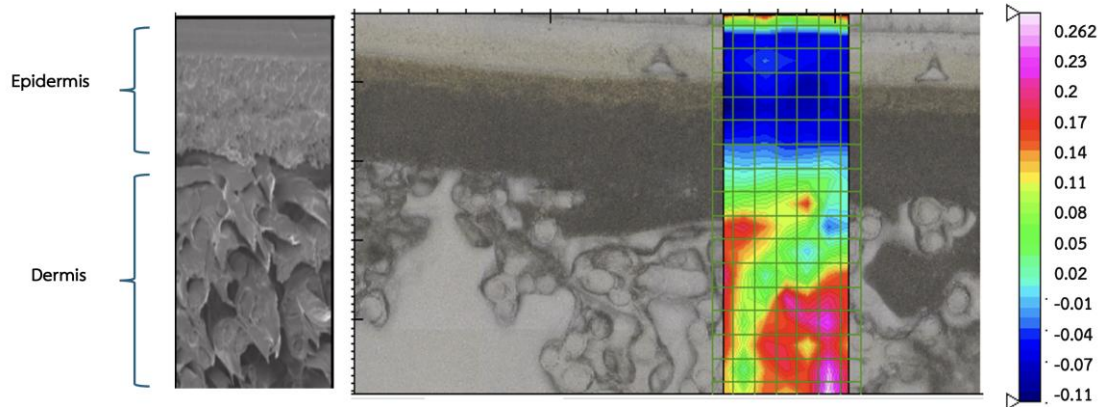


Figure 4.18 FTIR Imaging and Spectral Mapping of Strat-M™ Membrane at Various Time Points (1 hr) in Franz Diffusion Cell System

Figure 4.19 FTIR Imaging and Spectral Mapping of Strat-M™ Membrane at Various Time Points (2 hr) in Franz Diffusion Cell System

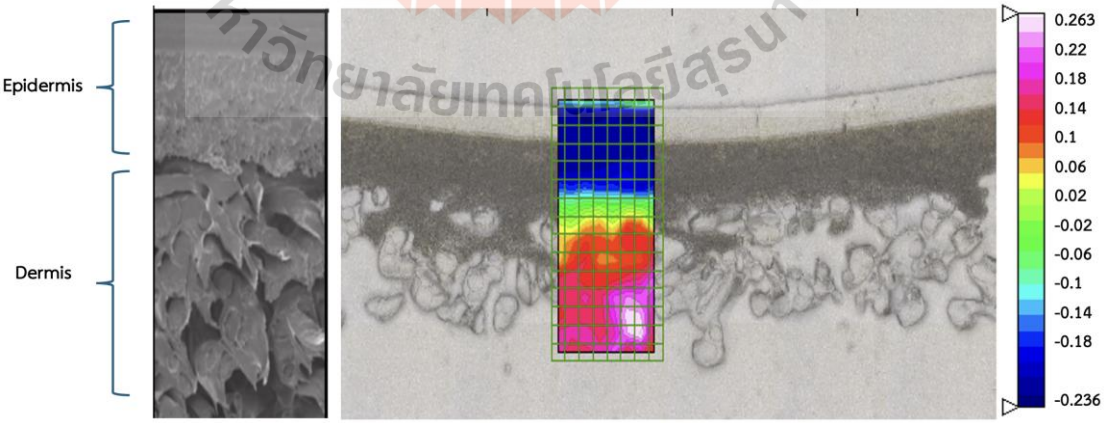


Figure 4.20 FTIR Imaging and Spectral Mapping of Strat-M™ Membrane at Various Time Points (4 hr) in Franz Diffusion Cell System

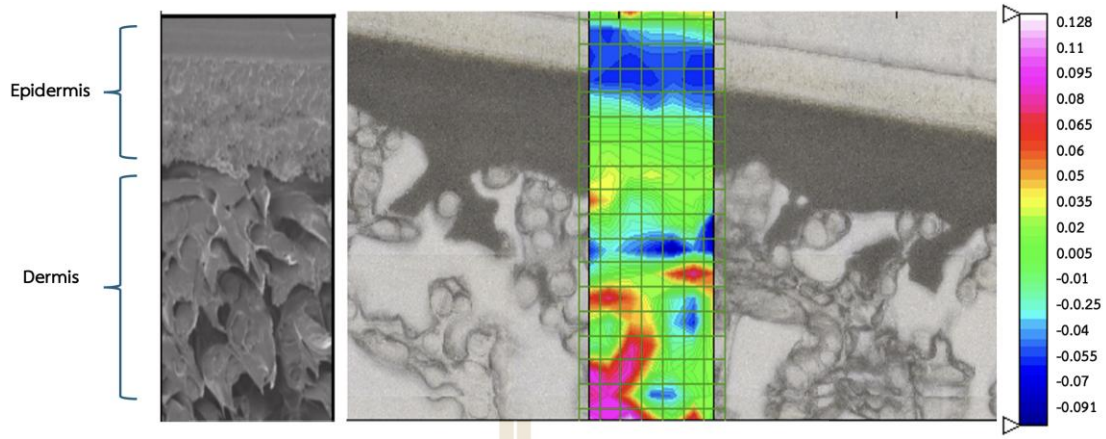


Figure 4.21 FTIR Imaging and Spectral Mapping of Strat-M™ Membrane at Various Time Points (6 hr) in Franz Diffusion Cell System

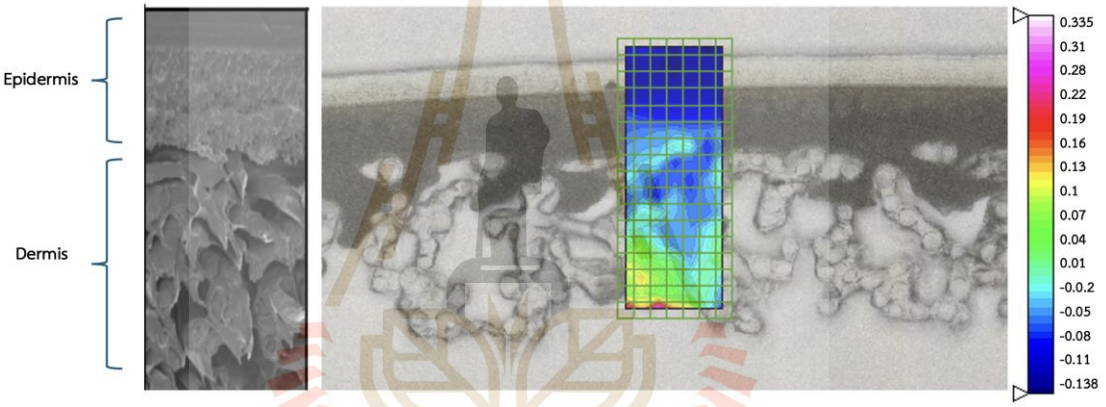


Figure 4.22 FTIR Imaging and Spectral Mapping of Strat-M™ Membrane at Various Time Points (8 hr) in Franz Diffusion Cell System

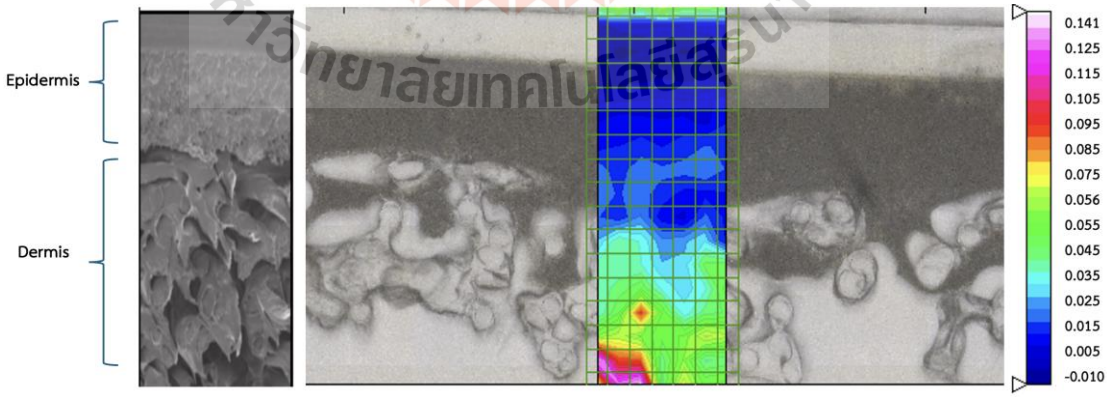


Figure 4.23 FTIR Imaging and Spectral Mapping of Strat-M™ Membrane at Various Time Points (12 hr) in Franz Diffusion Cell System

Figures 4.17-4.23 Representative FTIR images and corresponding spectral mapping at time intervals following application of the capsaicin formulation. Each panel includes the chemical intensity heatmap, selected spatial, and FTIR spectra of the mapped areas showing variations in intensity over time. The progressive diffusion of capsaicin is observed as signal penetration increases into deeper layers.

4.2 Discussion

The successful development of a capsaicin-loaded nanofiber patch using electrospinning technology presents a significant advancement in transdermal drug delivery systems. Capsaicin, a hydrophobic compound known for its analgesic and anti-inflammatory properties, poses formulation challenges due to its low water solubility and potential for skin irritation at high doses. In this study, encapsulating capsaicin in a hydrophilic polymer matrix composed of PVA and PVP proved to be an effective strategy to overcome these limitations. The blend not only improved the solubility and dispersion of capsaicin but also allowed for the formation of smooth, uniform nanofibers with desirable mechanical and morphological characteristics.

The FT-IR spectral analysis provided strong evidence for the physical entrapment of capsaicin within the nanofiber matrix. The observed shifts and broadening of O–H and C=O peaks suggest hydrogen bonding interactions between the drug and polymer, which are favorable for sustained release without compromising the drug's bioactivity. These findings are consistent with previous studies that have demonstrated the ability of PVA and PVP to form hydrogen bonds with small molecules, thus enhancing drug stability and retention within the fiber structure (Hindi et al., 2021).

The FT-IR spectrum of pure capsaicin exhibited a broad absorption peak at approximately 3300 cm^{-1}, corresponding to O–H stretching vibrations indicative of phenolic hydroxyl groups. Additional prominent bands at 2923 cm^{-1} and 2854 cm^{-1} were attributed to asymmetric and symmetric C–H stretching vibrations of aliphatic CH_2 and $–CH_3$ groups (Prietto et al., 2018), respectively. A sharp peak observed at approximately 1640 cm^{-1} was assigned to C=O stretching of the amide group, while signals in the region of 1510–1450 cm^{-1} were characteristic of aromatic C=C ring

stretching. Furthermore, peaks near 1260 cm^{-1} and 1020 cm^{-1} were assigned to C–N and C–O stretching, respectively, confirming the distinct functional groups of capsaicin, consistent with previous reports (Cortés-Estrada et al., 2020; Rezazadeh et al., 2022).

In comparison, the FT-IR spectrum of the capsaicin-loaded nanofiber patch (Figure 4.2B) demonstrated several overlapping and slightly shifted peaks, suggesting the successful incorporation of capsaicin into the polymeric matrix. The broad O–H stretching band around 3300 cm^{-1} was retained but became noticeably broader, indicating the presence of hydrogen bonding interactions between capsaicin and the polymeric components, particularly PVA and PVP (Hashmi et al., 2020). Aliphatic C–H stretching peaks at 2925 cm^{-1} and 2850 cm^{-1} were also observed, further confirming the presence of capsaicin within the nanofiber structure (Amna et al., 2019).

Importantly, the carbonyl stretching band at 1640 cm^{-1} exhibited a slight shift and broadening in the nanofiber spectrum, implying potential physical interactions between capsaicin and the hydroxyl or carbonyl groups within the polymer matrix. These interactions suggest molecular entrapment of capsaicin rather than covalent bonding, a favorable condition for maintaining the pharmacological activity of the compound and enabling a controlled drug release mechanism (Mondal et al., 2019; Tanadchangsaeng et al., 2016).

Additionally, in the fingerprint region (1200–700 cm^{-1}), characteristic peaks associated with the PVP and PVA backbone such as C–O, C–C, and C–N stretching vibrations remained clearly visible (Tahir et al., 2024). This observation confirmed the structural integrity of the polymer network following the electrospinning process. Notably, no new peaks or significant spectral changes were observed, indicating that capsaicin remained chemically stable and did not undergo degradation during fabrication.

The surface morphology of the capsaicin-loaded nanofibers, as examined by scanning electron microscopy (SEM), revealed critical insights into the structural properties of the electrospun fibers (Figure 4.3A). The SEM micrograph, captured at 5,000× magnification, demonstrated that the fibers were smooth, continuous, and free of beads, indicating a homogenous polymer solution and optimal electrospinning parameters. The random yet uniform distribution of fibers across the membrane

suggests favorable morphological conditions for consistent drug release and mechanical integrity in transdermal applications.

Quantitative analysis of fiber diameters, conducted using ImageJ software (NIH, USA), based on measurements of 50 randomly selected fibers (Mitxelena-Iribarren et al., 2023), is presented in Figure 4.4B. The diameter distribution followed a unimodal, slightly right-skewed pattern, with a mean diameter of 0.667 ± 0.195 μm (667 ± 19.5 nm), placing the fibers well within the nanoscale range of 1–1000 nm. This observation confirms the successful formation of true nanofibers, consistent with previous findings on electrospun drug-loaded systems (Hindi et al., 2021).

The relatively narrow standard deviation indicates a high degree of uniformity in fiber thickness, which is essential for maintaining consistent surface area for drug diffusion, mechanical stability, and flexibility of the transdermal patch. The absence of bead formation further suggests favorable polymer solvent interactions and the use of appropriate electrospinning voltage and flow rate settings (Martínez-Ortega et al., 2019). These features collectively support the robustness of the fabrication process and the physicochemical compatibility of the CAP/PVA/PVP system. When compared to other capsaicin-loaded nanofiber systems reported in the literature, the fiber diameters observed in this study are comparable or smaller, which may result in an enhanced surface-area-to-volume ratio (Martínez-Ortega et al., 2019).

The morphological analysis via SEM revealed that the fabricated nanofibers had a narrow diameter distribution, with an average size of approximately 667 nm. This nanoscale size is optimal for dermal applications, as it facilitates close contact with the skin, enhances drug absorption through increased surface area, and contributes to the flexibility and adherence of the patch. Similar findings have been reported, indicating that nanofibers with diameters in the nanoscale range provide a large specific surface area and highly porous structures, which are beneficial for transdermal drug delivery systems (Ahmadi Bonakdar & Rodrigue, 2024). Importantly, the absence of bead formation and the uniformity of the fibers indicate optimal electrospinning conditions, suggesting that the chosen polymer concentration, solvent system, and electrospinning parameters were well-balanced to yield high-quality nanofibers (Al-Abduljabbar & Farooq, 2023).

This characteristic is particularly beneficial for improving drug dispersion and skin permeation efficiency. Overall, the morphological attributes of the developed nanofibers affirm their suitability as a platform for transdermal drug delivery applications.

The cytotoxicity of the capsaicin-loaded nanofiber patch was assessed on human dermal fibroblast (HDF) cells using the MTT assay, and the results are presented in Figure 4.5. Cells were exposed to varying concentrations of nanofiber extract (ranging from 0.0001 to 100 mg/mL) for 24 hours to determine the concentration-dependent effects on cell viability. At lower concentrations (0.0001–1 mg/mL), the nanofiber extract did not exhibit significant cytotoxic effects. Notably, at concentrations of 0.001 and 0.01 mg/mL, a significant increase in cell viability was observed ($p < 0.001$), with viability exceeding 130% relative to the untreated control group. These results suggest that low concentrations of the nanofiber extract may not only be safe but could also enhance fibroblast activity or exert mild protective effects (Ahmady et al., 2023). This effect is likely attributed to the antioxidant and anti-inflammatory properties of capsaicin at sub-cytotoxic concentrations, which may promote cell health and tissue repair.

In contrast, at higher concentrations (10–100 mg/mL), a dose-dependent reduction in cell viability was observed. Specifically, cell viability decreased to approximately 75% at 50 mg/mL and further declined to approximately 40% at 100 mg/mL ($p < 0.001$), indicating significant cytotoxic effects at elevated doses. The dose–response curve allowed for the estimation of the half-maximal inhibitory concentration (IC_{50}), which was determined to be approximately 20 mg/mL. This value represents the concentration at which capsaicin induces a 50% reduction in cell viability under the in vitro conditions used in this study. Similar cytotoxic effects of capsaicin have been reported in previous studies. For instance, Kim et al. demonstrated that capsaicin was toxic to human skin fibroblasts, reducing survival rates to 25–50% after 24 hours of treatment (Chittasupho et al., 2020; Kim et al., 2004). Additionally, research by Szoka & Palka found that capsaicin exhibited cytotoxicity in fibroblasts, with an IC_{50} value of approximately 314.8 ± 14.1 μM at 48 hours of treatment (Szoka & Palka, 2020). These

findings underscore the importance of carefully optimizing capsaicin concentrations in therapeutic applications to balance efficacy and safety.

The IC_{50} value provides a critical reference for defining the upper safety limits in formulation development. Importantly, the concentrations exhibiting cytotoxicity (≥10 mg/mL) are much higher than those typically encountered in transdermal delivery applications. In vivo, the localized concentrations of capsaicin on the skin surface are significantly lower, due to controlled release mechanisms and limited permeation. For example, clinical formulations of capsaicin patches, such as those containing 0.025–0.1% w/w, correspond to microgram-per-milliliter concentrations once applied to the skin, which are well below the observed IC_{50} threshold.

Therefore, the capsaicin nanofiber patch demonstrates a broad safety margin for dermal applications, with effective concentrations for pain relief expected to remain within the non-cytotoxic range *in vivo*. These findings confirm the biocompatibility of the capsaicin-loaded nanofiber patch and support its potential for further development as a safe and effective transdermal therapeutic system.

The protective and restorative effects of the capsaicin-loaded nanofiber patch on human dermal fibroblast (HDF) morphology under oxidative stress conditions were evaluated using phase-contrast microscopy (Figure 4.6). In the untreated control group, HDF cells maintained a typical spindle-shaped fibroblastic morphology, with clearly defined cell borders, healthy cytoplasmic structure, and strong adherence to the culture surface features indicative of intact cellular integrity and normal physiological function.

Upon exposure to 1 mM hydrogen peroxide (H_2O_2) (de Oliveira-Marques et al., 2007), cells exhibited pronounced morphological alterations, including shrinkage, elongation, cytoplasmic condensation, and partial detachment from the culture surface. These changes are characteristic of oxidative stress-induced damage and are consistent with prior reports on H_2O_2-induced cellular inflammation and apoptotic responses in fibroblast models. Such morphological deterioration reflects the detrimental effects of reactive oxygen species (ROS) on cellular structure and function.

Remarkably, treatment with the capsaicin-loaded nanofiber patch at a concentration of 0.1 mg/mL following H_2O_2 exposure resulted in a visible restoration

of fibroblastic morphology. The treated cells appeared more elongated, retained better adhesion, and exhibited fewer structural abnormalities compared to the H_2O_2-only group. These observations suggest that the capsaicin nanofiber formulation conferred a protective or restorative effect on fibroblasts under oxidative stress. This aligns with studies indicating that capsaicin possesses antioxidant properties, reducing oxidative stress and promoting cell survival in various cell types (Zhang et al., 2024).

The morphological recovery observed in this group is in agreement with the anti-inflammatory profile indicated by *COX-2* gene expression levels, as assessed via qRT-PCR. Together, these findings support the cytoprotective and anti-inflammatory potential of capsaicin when delivered through a nanofiber-based transdermal system. The results further highlight the therapeutic relevance of this formulation in mitigating oxidative stress-related cellular damage in skin tissue models.

The anti-inflammatory efficacy of the nanofiber patch was substantiated by the significant reduction in *COX-2* expression following oxidative stress induction with hydrogen peroxide. This suppression is consistent with capsaicin's known mechanism of action, including the inhibition of *COX-2* synthesis via modulation of NF-**K**B and MAPK signaling pathways. Studies have demonstrated that capsaicin effectively inhibits the production of pro-inflammatory mediators such as *COX-2* by suppressing NF-**K**B activation in lipopolysaccharide-stimulated cells (Zheng et al., 2018). Additionally, capsaicin has been shown to affect macrophage anti-inflammatory activity through the modulation of MAPK and NF-**K**B signaling pathways (Li et al., 2021).

Notably, the sustained delivery offered by the nanofiber system may prolong capsaicin's interaction with target cells, thereby enhancing its anti-inflammatory potential compared to conventional topical creams or gels that often suffer from rapid drug clearance and poor skin penetration (Esentürk Güzel et al., 2022). Research indicates that nano-sized carriers can improve the bioavailability and absorption of capsaicinoids, thereby enhancing their anti-inflammatory and analgesic effects (Nava-Ochoa et al., 2021). Furthermore, nanofiber-based delivery systems have been shown to enhance the skin permeability and therapeutic efficacy of capsaicin, suggesting a promising approach for transdermal applications (Ghiasi et al., 2019).

Microscopic evaluation of cell morphology further demonstrated the patch's protective effects. Cells exposed to oxidative stress showed classical signs of damage, including shrinkage and detachment, whereas those treated with the capsaicin patch maintained structural integrity and normal morphology. These observations suggest not only anti-inflammatory effects, but also potential cellular recovery or protection facilitated by the formulation.

The present study investigated the transdermal permeation profile of capsaicin over a 12-hour period using HPLC analysis. Capsaicin was consistently detected across all time intervals (1–12 hours), with a steady increase in peak intensity over time, indicating continuous drug permeation. The observed retention times (approximately 22.3–22.6 minutes) and sharp chromatographic peaks suggest both the chemical stability of capsaicin and the reliability of the analytical method.

These results align with previous research findings. For instance, P. Anantaworasakul et al. (2020) demonstrated that capsaicin could successfully permeate skin layers and accumulate in both the stratum corneum and deeper tissues following topical application. Similarly, a study by Kleber et al. (2014) using Strat-M™ membranes and Franz diffusion cells showed that capsaicin-loaded nanoemulsions with particle sizes ranging from 20 to 62 nm penetrated the membrane layers effectively, supporting the suitability of such models in transdermal delivery evaluations (Kim et al., 2014).

Compared to prior research, the current study further confirms that electrospun or film-formulated capsaicin delivery systems enable sustained release across a prolonged period. In earlier studies utilizing low-concentration capsaicin creams, drug retention was often limited by formulation instability or rapid clearance. In contrast, the formulation tested in this study maintained a progressive release rate, similar to the findings reported by László et al. (2022), who observed that capsaicin-loaded silicone-based TTS formulations provided continuous drug delivery for 12 hours in animal models(Latif et al., 2022). Additionally, the HPLC results presented here corroborate findings from Qutenza™ patch studies, where the 8% capsaicin formulation achieved localized dermal uptake without significant systemic distribution, ensuring sustained therapeutic effect with minimal adverse reactions. While previous work

primarily focused on end-point measurements (e.g., skin deposition after several hours), this study contributes dynamic, time-resolved insight into the release kinetics and chemical stability of capsaicin over time. Taken together, these findings not only reinforce the potential of transdermal systems for capsaicin delivery but also highlight the compatibility of HPLC in evaluating temporal drug release and permeation behavior. Further quantitative assessment of capsaicin concentration at each time point, as well as in vivo confirmation, would strengthen these results and provide clinical relevance for pain management or anti-inflammatory applications.

The FTIR microspectroscopic analysis provided spatial and temporal insights into the diffusion behavior of capsaicin through the Strat-M™ membrane over a 12-hour period. The results clearly demonstrate progressive penetration of capsaicin, with characteristic spectral changes correlating with increased diffusion depth and chemical interaction over time.

At the early stage (1 hour), FTIR spectral signals were primarily localized in the upper membrane layers, as evidenced by strong absorption bands near 2920–2850 cm^{-1} (C–H stretching) and 1650–1500 cm^{-1} (amide and aromatic ring vibrations). These results suggest initial adsorption of capsaicin onto the surface of the membrane without significant diffusion. This surface localization is consistent with prior findings that highlight the stratum corneum-like barrier function of the upper Strat-M™ membrane. By 2 to 4 hours, spectral intensity began to shift deeper into the membrane, with notable increases in C–O and C–N stretching peaks in the 1250–1000 cm^{-1} region. These findings support earlier studies indicating that capsaicin exhibits moderate lipophilicity, allowing gradual partitioning into mid-layers mimicking the viable epidermis. This trend aligns with the permeation kinetics who observed delayed but steady penetration of capsaicin through synthetic and porcine membranes.

At 6 hours, prominent signals at 1510 cm^{-1} (aromatic C=C) and 1100 cm^{-1} (ether C–O) indicate that capsaicin reached deeper, dermis-like layers of the membrane. The associated heatmaps show widespread distribution and increased intensity, suggesting sustained release from the patch and effective translocation across membrane strata. These findings corroborate the sustained-release behavior observed in nanofiber-based capsaicin patches, as described by László et al. (2022).

Notably, the FTIR spectra at 8 and 12 hours revealed peak broadening and intensity stabilization in the 1000–1100 cm^{-1} region, indicative of molecular accumulation and possible interaction with deeper membrane components. This plateauing effect suggests that capsaicin achieved full-depth permeation, consistent with the final phase of Franz diffusion profiles. Comparatively, untreated control membranes displayed negligible chemical signals, further validating the specificity and extent of capsaicin permeation in treated samples.

Overall, the FTIR imaging technique effectively visualized the dynamic and spatial evolution of capsaicin diffusion. The observed spectral shifts and intensity distributions correspond well with capsaicin's chemical structure and known pharmacokinetics in skin models. These findings highlight the value of FTIR spectral mapping in transdermal drug delivery research and support the potential of capsaicin-loaded patches for achieving deep skin penetration and sustained release, crucial for managing localized and pain. Additionally, electrospun nanofibers allow for site-specific delivery, minimizing systemic exposure and side effects.

The calculated permeability coefficient (K_p) of capsaicin demonstrated a distinct time-dependent decline throughout the 12-hour permeation study. The initial high K_p value observed at the 1-hour time point (~0.77 cm/hr) can be attributed to a steep concentration gradient between the donor and receptor compartments, which is known to facilitate rapid transdermal diffusion. This observation is consistent with Fick's First Law of Diffusion, which states that the diffusion flux is directly proportional to the concentration gradient across the membrane (Bajaj et al., 2011; Wokovich et al., 2006).

As time progressed, K_p values declined, reaching a quasi-steady state after 6 hours. This decrease likely reflects both the depletion of surface drug content and the reduction in the driving force for diffusion, as the system approaches equilibrium. Additionally, saturation of the membrane and polymer relaxation within the nanofiber structure may contribute to the gradual attenuation of drug permeation.

Such a biphasic permeation pattern comprising an initial burst followed by sustained releaseis characteristic of electrospun nanofiber drug delivery systems, which are designed to provide both immediate and long-acting therapeutic effects (Hindi et

al., 2021; Vatankhah, 2018). This is particularly advantageous for transdermal applications targeting chronic pain or inflammation, where rapid onset followed by consistent therapeutic levels is desired.

When compared to conventional topical formulations such as gels or creams, which often suffer from poor permeation and uncontrolled release, the nanofiber patch exhibited superior performance in terms of both early-stage permeability and sustained release kinetics. Similar findings were reported by Rezazadeh et al. (2022) (Rezazadeh et al., 2022), who demonstrated enhanced transdermal delivery of capsaicin using zein-based nanofibers, albeit with lower initial K_p values (0.045–0.075 cm/hr).

In summary, the permeability profile obtained from this study supports the hypothesis that the capsaicin-loaded nanofiber patch functions as an efficient and controlled transdermal drug delivery system, capable of enhancing skin permeation and maintaining therapeutic levels of the drug over extended periods.

Despite these promising outcomes, this study also highlights areas for future research. *In vivo* evaluations are necessary to fully assess pharmacokinetics, drug permeation, and long-term therapeutic efficacy. Additionally, studies investigating the sensory effects (e.g., initial burning sensation) associated with capsaicin patches, skin irritation potential, and user acceptability will be critical for clinical translation. Furthermore, scaling up the production process while maintaining uniformity and quality will be essential for commercialization.

In conclusion, the CAP/PVA/PVP nanofiber patch developed in this study exhibits excellent physicochemical properties, safety, and biological activity, making it a strong candidate for further development as a non-invasive treatment for localized pain and inflammation. Its ability to deliver capsaicin in a sustained and biocompatible manner could address current limitations in topical analgesic therapy and open new avenues for nanofiber-based biomedical applications.

CHAPTER V

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

5.1.1. Fabrication of CAP/PVA/PVP Nanofibers

Capsaicin-loaded nanofibers were successfully fabricated using electrospinning of a polymer blend composed of polyvinyl alcohol (PVA) and polyvinylpyrrolidone (PVP). As illustrated in Figure 4.1, the schematic diagram highlights the electrospinning system and nanofiber collection process. The optimized conditions, including a polymer concentration of 10% w/v for both PVA and PVP and a capsaicin loading of 0.1 mg/mL, led to the formation of continuous and bead-free nanofibers. This confirms that the electrospinning parameters solvent system, polymer viscosity, voltage (15 kV), and flow rate (3 mm/hr) were well balanced.

5.1.2. Physicochemical Characterization (FT-IR Analysis)

Fourier-transform infrared (FT-IR) spectroscopy was used to confirm the incorporation of capsaicin and its interaction with the polymer matrix (Figure 4.2). The spectrum of pure capsaicin displayed characteristic peaks such as O–H, C–H, and C=O stretching vibrations. These peaks were retained in the nanofiber formulation with minor shifts and broadening, indicating non-covalent interactions primarily hydrogen bonding between capsaicin and the PVA/PVP matrix. The absence of new peaks suggests that capsaicin remained chemically stable during electrospinning, supporting the suitability of the process for drug encapsulation.

5.1.3. Surface Morphology (SEM Analysis)

SEM images (Figure 4.3A) confirmed that the capsaicin-loaded nanofibers were smooth, uniform, and free from bead formation. The fibers exhibited random orientation with consistent distribution. The histogram of fiber diameters (Figure 4.3B) showed a unimodal, slightly right-skewed distribution with an average

diameter of 0.667 ± 0.195 µm. This narrow distribution suggests that the electrospinning process was well-controlled and reproducible. The nanoscale fiber size is considered ideal for transdermal applications due to increased surface area and enhanced drug release properties.

5.1.4. Cytotoxicity Evaluation (MTT Assay)

The cytotoxicity of the capsaicin-loaded nanofiber patch was assessed in human dermal fibroblasts (HDF) using the MTT assay (Figure 4.5). At low concentrations (0.0001–1 mg/mL), the patch extract did not exhibit cytotoxicity and significantly enhanced cell viability, especially at 0.001 and 0.01 mg/mL ($p < 0.001$). Conversely, at higher concentrations (≥10 mg/mL), a dose-dependent reduction in cell viability was observed, with an IC_{50} of approximately 20 mg/mL. These findings confirm the biocompatibility of the patch at therapeutically relevant concentrations, which are typically far lower than those causing cytotoxicity.

5.1.5. Anti-inflammatory Activity (*COX-2* Gene Expression)

As shown in Figure 4.5, exposure to 1 mM hydrogen peroxide (H_2O_2) markedly increased *COX-2* gene expression in HDF cells, simulating an oxidative stress-induced inflammatory state. Treatment with the capsaicin-loaded nanofiber patch at 0.1 mg/mL significantly downregulated *COX-2* expression by approximately 8.1-fold compared to the H_2O_2 only group. This suggests potent anti-inflammatory activity of capsaicin, likely due to its ability to inhibit key signaling pathways such as NF-**K**B and MAPKs.

Phase-contrast microscopy (Figure 4.6) further supported the anti-inflammatory and cytoprotective effects of the patch. While HDF cells treated with H_2O_2 alone showed signs of damage including shrinkage and detachment—cells treated with the capsaicin-loaded patch recovered typical fibroblast morphology with improved attachment and cytoplasmic integrity. This visual evidence complements the qRT-PCR data and demonstrates the formulation's protective effect under oxidative stress.

This study comprehensively demonstrated the successful development, characterization, and in vitro biological evaluation of a capsaicin-loaded

transdermal nanofiber patch fabricated via electrospinning of a polyvinyl alcohol (PVA) and polyvinylpyrrolidone (PVP) polymer blend. The optimized electrospinning parameters produced smooth, bead-free nanofibers with a mean diameter of 667 ± 19.5 nm, confirming the formation of true nanoscale fibers with high uniformity. These physical characteristics are essential for consistent drug release, skin adhesion, and overall patch performance in transdermal applications. Fourier-transform infrared (FT-IR) spectroscopy confirmed the successful incorporation of capsaicin into the polymer matrix without any degradation or chemical alteration. The retention of key functional groups and the observed spectral shifts suggest favorable non-covalent interactions, particularly hydrogen bonding, between capsaicin and the PVA/PVP matrix. These interactions are critical for ensuring drug stability and controlled release over time. The nanofiber patch demonstrated excellent cytocompatibility with human dermal fibroblasts (HDFs). At lower concentrations (0.001–0.01 mg/mL), the patch even enhanced cell viability, likely due to capsaicin's known antioxidant and anti-inflammatory activities at sub-cytotoxic levels. Higher concentrations exhibited a dose-dependent reduction in viability, with an IC_{50} of approximately 20 mg/mL. This value is significantly higher than the concentrations typically used in clinical formulations, indicating a broad therapeutic safety window.

Crucially, the patch exhibited potent anti-inflammatory activity, as evidenced by the significant downregulation of *COX-2* gene expression in HDF cells pre-treated with hydrogen peroxide to induce oxidative stress. The ability of the patch to attenuate *COX-2* expression aligns with capsaicin's known pharmacological profile, suggesting that the nanofiber matrix effectively preserved and delivered the bioactivity of the drug. Furthermore, morphological analysis revealed that capsaicin-treated cells retained or regained normal fibroblast structure, reinforcing the formulation's protective and restorative potential.

Together, these findings confirm that the capsaicin-loaded nanofiber patch possesses all the key attributes of an effective transdermal drug delivery system: (1) nanoscale morphology for enhanced surface area and skin adherence, (2) chemical and mechanical stability, (3) biocompatibility, and (4) targeted therapeutic efficacy through anti-inflammatory and cytoprotective effects.

Capsaicin, a bioactive compound from chili peppers, exerts dual roles in pain modulation and inflammation control through both neuronal and non-neuronal mechanisms. Its analgesic effect primarily arises from binding to TRPV1 receptors on nociceptive neurons, causing an initial depolarization and neurotransmitter release, followed by receptor desensitization and pain attenuation. Beyond neurons, capsaicin also affects human dermal fibroblasts (HDFs), which are key mediators of skin inflammation and wound healing.

In HDFs, capsaicin exhibits significant anti-inflammatory properties by downregulating pro-inflammatory mediators such as *COX-2*, IL-6, and TNF-**α**. This occurs via inhibition of NF-**K**B and MAPK signaling pathways, reducing the transcription of inflammation-related genes. Furthermore, capsaicin suppresses oxidative stress by decreasing reactive oxygen species (ROS) and enhancing antioxidant defenses including superoxide dismutase (SOD) and glutathione (GSH).

In addition to its anti-inflammatory effects, capsaicin promotes fibroblast migration, proliferation, and collagen synthesis through activation of the PI3K/Akt/mTOR and ERK1/2 pathways, thereby supporting tissue regeneration and wound repair. These processes contribute to long-term pain relief by reducing dermal inflammation and restoring tissue integrity.

Overall, the evidence underscores capsaicin's potential as a therapeutic agent for transdermal drug delivery systems targeting both inflammatory skin conditions and pain syndromes, with mechanisms involving TRPV1 activation, *COX-2* suppression, oxidative stress reduction, and dermal remodeling

From a broader perspective, this formulation offers a promising non-invasive alternative for localized pain and inflammation management, potentially addressing limitations of conventional topical agents such as poor skin penetration, irritation, and frequent reapplication. The polymeric nanofiber matrix not only facilitates sustained drug release but also enhances capsaicin's solubility and stability two longstanding challenges in topical capsaicin therapy.

The *in vitro* evaluations revealed that the nanofiber patch exhibited excellent biocompatibility with human dermal fibroblasts, with no significant cytotoxicity at therapeutic concentrations. Moreover, the patch demonstrated anti-

inflammatory and cytoprotective effects by downregulating *COX-2* gene expression and restoring cell morphology under oxidative stress. The transdermal permeation study further confirmed the sustained release profile of capsaicin through the Strat-M™ membrane, with decreasing K_p values over time, reflecting a controlled release behavior.

The promising in vitro performance of this capsaicin patch supports its potential for further development and clinical translation. This study offers an alternative, evidence-based approach for pain relief, grounded in mechanistic findings from laboratory investigations and demonstrating the feasibility of nanofiber-based transdermal systems for treating localized inflammation and pain.

This study presents a novel approach to transdermal drug delivery by developing a capsaicin-loaded nanofiber patch fabricated via electrospinning using a biocompatible polymer blend of PVA and PVP. The innovation lies in:

The integration of capsaicin into a nanofibrous matrix that enhances solubility and minimizes skin irritation addressing key limitations of conventional capsaicin formulations.

The use of Strat-M™ synthetic membrane as a model to investigate transdermal permeation, providing a reproducible and ethical alternative to human or animal skin in early-stage studies.

The combination of multiple analytical techniques including FT-IR spectroscopy, SEM imaging, *COX-2* gene expression analysis, and FTIR spectral mapping to comprehensively characterize both physicochemical properties and biological responses. The demonstration of sustained release behavior with a clearly defined steady-state flux and permeability coefficient, supporting its application in controlled and localized pain relief

The cytoprotective and anti-inflammatory evaluation in human dermal fibroblasts under oxidative stress, which provides new mechanistic insight into its therapeutic potential at the cellular level.

Overall, the findings highlight the potential of this capsaicin-loaded nanofiber patch as a non-invasive, biocompatible, and targeted transdermal drug delivery system. These results further emphasize the formulation’s novelty and its strong promise for future clinical translation.

5.2 Future work

Based on the successful fabrication, characterization, and in vitro biological evaluation of the capsaicin-loaded nanofiber patch, several recommendations are proposed to advance this formulation toward clinical application. First, comprehensive in vivo studies are essential to validate the pharmacokinetics, dermal permeation, and therapeutic efficacy of the patch under physiological conditions. Animal models of localized inflammation or chronic pain should be employed to simulate clinical scenarios and assess drug absorption, retention time, and systemic exposure. Second, further optimization of the drug release profile is recommended. While the current formulation shows promising encapsulation and sustained delivery potential, modifying polymer ratios, introducing release modulators, or incorporating skin-permeation enhancers could enhance therapeutic outcomes and prolong capsaicin's analgesic effect.

Long-term stability testing under various storage conditions (e.g., temperature, humidity, light exposure) is also crucial to establish shelf-life and packaging requirements. Such studies would support regulatory approval and commercial translation. In addition, sensory evaluation and dermal irritation testing should be conducted in preclinical models and human volunteers to ensure safety, comfort, and compliance, particularly given capsaicin’s known potential to cause local burning sensations at higher concentrations. Human-based testing will also provide insight into tolerability during extended patch application.

Moreover, clinical trials are strongly recommended to assess the efficacy, safety, and patient acceptability of the patch in real-world settings. These trials could begin with pilot-scale evaluations for specific indications such as myofascial pain syndrome, neuropathic pain, or musculoskeletal inflammation. For broader applicability, future research could also explore the design and customization of patch formats, including adjustable dosage levels, sizes, or anatomical fits tailored to different patient needs or anatomical regions. Lastly, incorporating other synergistic compounds, such as natural anti-inflammatory agents or antioxidants, may also enhance the patch’s multimodal therapeutic potential.

References

Abdel-Mottaleb, M. M., Moulari, B., Beduneau, A., Pellequer, Y., & Lamprecht, A. (2012). Surface-charge-dependent nanoparticles accumulation in inflamed skin. **Journal of Pharmaceutical Sciences.** 101(11): 4231-4239.

Adeli, H., Khorasani, M. T., & Parvazinia, M. (2019). Wound dressing based on electrospun PVA/chitosan/starch nanofibrous mats: Fabrication, antibacterial and cytocompatibility evaluation and in vitro healing assay. **International Journal of Biological Macromolecules.** 122: 238-254.

Ahmadi Bonakdar, M., & Rodrigue, D. (2024). Electrospinning: Processes, Structures, and Materials. **Macromol.** 4(1): 58-103.

Ahmady, A. R., Solouk, A., Saber-Samandari, S., Akbari, S., Ghanbari, H., & Brycki, B. E. (2023). Capsaicin-loaded alginate nanoparticles embedded polycaprolactone-chitosan nanofibers as a controlled drug delivery nanoplatform for anticancer activity. **Journal of Colloid and Interface Science.** 638: 616-628.

Akhgari, A., Ghalambor Dezfuli, A., Rezaei, M., Kiarsi, M., & Abbaspour, M. (2016). The design and evaluation of a fast-dissolving drug delivery system for loratadine using the electrospinning method. **Jundishapur Journal of Natural Pharmaceutical Products.** 11(2; e33613).

Al-Abduljabbar, A., & Farooq, I. (2023). Electrospun Polymer Nanofibers: Processing, Properties, and Applications. **Polymers.** 15(1): 65.

Ali, S., Shabbir, M., & Nabeel Shahid, M. (2015). The Structure of Skin and Transdermal Drug Delivery System-A Review. **Research Journal of Pharmacy and Technology.** 8: 103.

Alkilani, A. Z., McCrudden, M. T., & Donnelly, R. F. (2015). Transdermal Drug Delivery: Innovative Pharmaceutical Developments Based on Disruption of the Barrier Properties of the stratum corneum. **Pharmaceutics.** 7(4): 438-470.

Almeida, J. P., Chen, A. L., Foster, A., & Drezek, R. (2011). In vivo biodistribution of nanoparticles. **Nanomedicine (Lond).** 6(5): 815-835.

References (Continued)

Amano, S. (2016). Characterization and mechanisms of photoageing-related changes in skin. Damages of basement membrane and dermal structures. **Experimental Dermatology.** 25 Suppl 3: 14-19.

Amna, T., Gharsan, F., Shang, K., Hassan, M. s., Khil, M.-S., & Hwang, I. (2019). Electrospun Twin Fibers Encumbered with Intrinsic Antioxidant Activity as Prospective Bandage. **Macromolecular Research.** 27.

Anand, P., & Bley, K. (2011). Topical capsaicin for pain management: therapeutic potential and mechanisms of action of the new high-concentration capsaicin 8% patch. **British Journal of Anaesthesia.** 107(4): 490-502.

Anantaworasakul, P., Chaiyana, W., Michniak-Kohn, B. B., Rungseevijitprapa, W., & Ampasavate, C. (2020). Enhanced Transdermal Delivery of Concentrated Capsaicin from Chili Extract-Loaded Lipid Nanoparticles with Reduced Skin Irritation. **Pharmaceutics.** 12(5).

Anekar, A. A., & Cascella, M. (2021). *WHO analgesic ladder*: StatPearls Publishing.

Anselmo, A. C., & Mitragotri, S. (2014). An overview of clinical and commercial impact of drug delivery systems. **Journal of Controlled Release.** 190: 15-28.

Arce, F. J., Asano, N., See, G. L., Itakura, S., Todo, H., & Sugibayashi, K. (2020). Usefulness of Artificial Membrane, Strat-M®, in the Assessment of Drug Permeation from Complex Vehicles in Finite Dose Conditions. *Pharmaceutics, 12*(2). doi:10.3390/pharmaceutics12020173

Asmatulu, R., & Khan, W. S. (2018). *Synthesis and applications of electrospun nanofibers*: Elsevier.

Ausín-Crespo, M. D., Martín-de Castro, E., Roldán-Cuartero, J., de la Beldad-Diez, M. L., Salcedo-Gámez, M., & Tong, H. (2022). Capsaicin 8% Dermal Patch for Neuropathic Pain in a Pain Unit. **Pain Management Nursing.** 23(4): 452-457.

Babbar, S., Marier, J. F., Mouksassi, M. S., Beliveau, M., Vanhove, G. F., Chanda, S., & Bley, K. (2009). Pharmacokinetic analysis of capsaicin after topical administration of a high-concentration capsaicin patch to patients with peripheral neuropathic pain. **Therapeutic Drug Monitoring.** 31(4): 502-510.

References (Continued)

Bajaj, S., Whiteman, A., & Brandner, B. (2011). Transdermal drug delivery in pain management. **Continuing education in anaesthesia, Critical care & pain.** 11(2): 39-43.

Barbero, A. M., & Frasch, H. F. (2006). Transcellular route of diffusion through stratum corneum: results from finite element models. **Journal of Pharmaceutical Sciences.** 95(10): 2186-2194.

Basto, R., Andrade, R., Nunes, C., Lima, S. A. C., & Reis, S. (2021). Topical Delivery of Niacinamide to Skin Using Hybrid Nanogels Enhances Photoprotection Effect. **Pharmaceutics.** 13(11).

Benítez-Angeles, M., Morales-Lázaro, S. L., Juárez-González, E., & Rosenbaum, T. (2020). TRPV1: Structure, Endogenous Agonists, and Mechanisms. **International Journal of Molecular Sciences.** 21(10).

Bhansali, D., Teng, S. L., Lee, C. S., Schmidt, B. L., Bunnett, N. W., & Leong, K. W. (2021). Nanotechnology for pain management: Current and future therapeutic interventions. **Nano Today.** 39: 101223.

Bibi, N., Ahmed, N., & Khan, G. M. (2017). Nanostructures in transdermal drug delivery systems. In *In Nanostructures for drug delivery* (pp. 639-668).

Blair, H. A. (2018). Capsaicin 8% Dermal Patch: A Review in Peripheral Neuropathic Pain. **Drugs.** 78(14): 1489-1500.

Blanco, E., Shen, H., & Ferrari, M. (2015). Principles of nanoparticle design for overcoming biological barriers to drug delivery. **Nature biotechnology.** 33(9): 941-951.

Bode, A. M., & Dong, Z. (2011). The two faces of capsaicin. **Cancer Research.** 71(8): 2809-2814.

Bonezzi, C., Costantini, A., Cruccu, G., Fornasari, D. M. M., Guardamagna, V., Palmieri, V., . . . Dickenson, A. H. (2020). Capsaicin 8% dermal patch in clinical practice: an expert opinion. **Expert Opinion on Pharmacotherapy.** 21(11): 1377-1387.

References (Continued)

Borbiro, I., Badheka, D., & Rohacs, T. (2015). Activation of TRPV1 channels inhibits mechanosensitive Piezo channel activity by depleting membrane phosphoinositides. **Science Signaling.** 8(363): ra15.

Bos, J. D., & Meinardi, M. M. (2000). The 500 Dalton rule for the skin penetration of chemical compounds and drugs. **Experimental Dermatology.** 9(3): 165-169.

Brandner, J. M. (2009). Tight junctions and tight junction proteins in mammalian epidermis. **European journal of pharmaceutics and biopharmaceutics.** 72(2): 289-294.

Brodsky, M., Cho, J., Fang, J., Kim, E., Cho, Y., & Song, M. (2012). P02.02. Efficacy of a topical 0.1% Capsaicin hydrogel patch to treat chronic neck pain: a double-blind randomized clinical trial. **BMC Complementary and Alternative Medicine.** 12(1): P58.

Caterina, M. J., Schumacher, M. A., Tominaga, M., Rosen, T. A., Levine, J. D., & Julius, D. (1997). The capsaicin receptor: a heat-activated ion channel in the pain pathway. **Nature.** 389(6653): 816-824.

Chaiyasit, K., Khovidhunkit, W., & Wittayalertpanya, S. (2009). Pharmacokinetic and the effect of capsaicin in Capsicum frutescens on decreasing plasma glucose level. **Journal of the Medical Association of Thailand.** 92(1): 108-113.

Charelli, L. E., de Mattos, G. C., de Jesus Sousa-Batista, A., Pinto, J. C., & Balbino, T. A. (2022). Polymeric nanoparticles as therapeutic agents against coronavirus disease. **Journal of Nanoparticle Research.** 24(1): 12.

Chauhan, G., Madou, M. J., Kalra, S., Chopra, V., Ghosh, D., & Martinez-Chapa, S. O. (2020). Nanotechnology for COVID-19: Therapeutics and Vaccine Research. **ACS Nano.** 14(7): 7760-7782.

Chen, J. H., Chen, H. J., Kao, C. H., Tseng, C. H., & Tsai, C. H. (2018). Is Fibromyalgia Risk Higher Among Male and Young Inflammatory Bowel Disease Patients? Evidence from a Taiwan Cohort of One Million. **Pain Physician.** 21(3): E257-e264.

References (Continued)

Cheng, Y., Chen, Z., Yang, S., Liu, T., Yin, L., Pu, Y., & Liang, G. (2021). Nanomaterials-induced toxicity on cardiac myocytes and tissues, and emerging toxicity assessment techniques. **Science of The Total Environment.** 800: 149584.

Chittasupho, C., Thongnopkoon, T., Burapapisut, S., Charoensukkho, C., Shuwisitkul, D., & Samee, W. (2020). Stability, permeation, and cytotoxicity reduction of capsicum extract nanoparticles loaded hydrogel containing wax gourd extract. **Saudi Pharmaceutical Journal.** 28(12): 1538-1547.

Cho, J.-H., Brodsky, M., Kim, E.-J., Cho, Y.-J., Kim, K.-W., Fang, J.-Y., & Song, M.-Y. (2012). Efficacy of a 0.1% Capsaicin Hydrogel Patch for Myofascial Neck Pain: A Double-Blinded Randomized Trial. **Pain Medicine.** 13(7): 965-970.

Clausen, B. E., & Stoitzner, P. (2015). Functional Specialization of Skin Dendritic Cell Subsets in Regulating T Cell Responses. **Frontiers in immunology.** 6: 534.

Cohen, M., Quintner, J., & van Rysewyk, S. (2018). Reconsidering the International Association for the Study of Pain definition of pain. **Pain reports.** 3(2): e634.

Cohen, S. P., & Mao, J. (2014). Neuropathic pain: mechanisms and their clinical implications. **Bmj.** 348: f7656.

Cohen, S. P., Vase, L., & Hooten, W. M. (2021). Chronic pain: an update on burden, best practices, and new advances. **Lancet.** 397(10289): 2082-2097.

Contado, C. (2015). Nanomaterials in consumer products: a challenging analytical problem. **Frontiers in Chemistry.** 3.

Cortés-Estrada, C. E., Gallardo-Velázquez, T., Osorio-Revilla, G., Castañeda-Pérez, E., Meza-Márquez, O. G., López-Cortez, M. d. S., & Hernández-Martínez, D. M. (2020). Prediction of total phenolics, ascorbic acid, antioxidant capacities, and total soluble solids of Capsicum annuum L. (bell pepper) juice by FT-MIR and multivariate analysis. **LWT.** 126: 109285.

Cramer, M. P., & Saks, S. R. (1994). Translating safety, efficacy and compliance into economic value for controlled release dosage forms. **Pharmacoeconomics.** 5(6): 482-504.

References (Continued)

Crucho, C. I. C., & Barros, M. T. (2017). Polymeric nanoparticles: A study on the preparation variables and characterization methods. **Materials Science and Engineering: C.** 80: 771-784.

Cuijpers, I., Sthijns, M. M. J. P. E., van den Bogart, V. A. R., Katsburg, J., Leenders, C. F. M., & Troost, F. J. (2025). Quercetin, Kaempferol and Capsaicin Counteract the TGF-β1-Induced Upregulation of αSMA and Collagen in Myoblasts. **International Journal of Molecular Sciences.** 26(11): 5151.

de Oliveira-Marques, V. n., Cyrne, L. s., Marinho, H. S., & Antunes, F. (2007). A Quantitative Study of NF-**K**B Activation by H2O2: Relevance in Inflammation and Synergy with TNF-α1. **The Journal of Immunology.** 178(6): 3893-3902.

Díaz, J., Pomar, F., Bernal, A., & Merino, F. (2004). Peroxidases and the metabolism of capsaicin in Capsicum annuum L. **Phytochemistry Reviews.** 3(1): 141-157.

Donnelly, J. M., de las Peñas, C. F., Finnegan, M., & Freeman, J. L. (2018). *Travell, Simons & Simons' Myofascial Pain and Dysfunction: The Trigger Point Manual*: Wolters Kluwer Health.

Dureja, G. P., Iyer, R. N., Das, G., Ahdal, J., & Narang, P. (2017). Evidence and consensus recommendations for the pharmacological management of pain in India. **Journal of Pain Research.** 10: 709-736.

Ellison, N., Loprinzi, C. L., Kugler, J., Hatfield, A. K., Miser, A., Sloan, J. A., . . . Cascino, T. L. (1997). Phase III placebo-controlled trial of capsaicin cream in the management of surgical neuropathic pain in cancer patients. **Journal of Clinical Oncology.** 15(8): 2974-2980.

Enright, A., & Goucke, R. (2016). The Global Burden of Pain: The Tip of the Iceberg? **Anesthesia & Analgesia.** 123(3): 529-530.

Esentürk Güzel, I., Abdo, L., Algın Yapar, E., Esentürk, E., Büyükkayhan, D., & Sindhu, R. K. (2022). An overview of nanofiber applications for development of phytopharmaceuticals. **Bezmialem Science.** 10(5): 666 - 673.

References (Continued)

Fang, X. X., Zhai, M. N., Zhu, M., He, C., Wang, H., Wang, J., & Zhang, Z. J. (2023). Inflammation in pathogenesis of chronic pain: Foe and friend. **Molecular Pain.** 19: 17448069231178176.

Fattori, V., Hohmann, M. S. N., Rossaneis, A. C., Pinho-Ribeiro, F. A., & Verri, W. A. (2016). Capsaicin: Current Understanding of Its Mechanisms and Therapy of Pain and Other Pre-Clinical and Clinical Uses. **Molecules.** 21(7).

Fernandes, R., Smyth, N. R., Muskens, O. L., Nitti, S., Heuer-Jungemann, A., Ardern-Jones, M. R., & Kanaras, A. G. (2015). Interactions of skin with gold nanoparticles of different surface charge, shape, and functionality. **Small.** 11(6): 713-721.

Finnerup, N. B., Haroutounian, S., Kamerman, P., Baron, R., Bennett, D. L. H., Bouhassira, D., . . . Jensen, T. S. (2016). Neuropathic pain: an updated grading system for research and clinical practice. **PAIN.** 157(8): 1599-1606.

Fitzcharles, M. A., Cohen, S. P., Clauw, D. J., Littlejohn, G., Usui, C., & Häuser, W. (2021). Nociplastic pain: towards an understanding of prevalent pain conditions. **Lancet.** 397(10289): 2098-2110.

German, G. K., Engl, W. C., Pashkovski, E., Banerjee, S., Xu, Y., Mertz, A. F., . . . Dufresne, E. R. (2012). Heterogeneous drying stresses in stratum corneum. **Biophysical journal.** 102(11): 2424-2432.

Ghiasi, Z., Esmaeli, F., Aghajani, M., Ghazi-Khansari, M., Faramarzi, M. A., & Amani, A. (2019). Enhancing analgesic and anti-inflammatory effects of capsaicin when loaded into olive oil nanoemulsion: An in vivo study. **International Journal of Pharmaceutics.** 559: 341-347.

Graybiel, A., Knepton, J., & Shaw, J. (1976). Prevention of experimental motion sickness by scopolamine absorbed through the skin. **Aviation, Space, and Environmental Medicine.** 47(10): 1096-1100.

Gupta, M., Agrawal, U., & Vyas, S. P. (2012). Nanocarrier-based topical drug delivery for the treatment of skin diseases. **Expert Opinion on Drug Delivery.** 9(7): 783-804.

References (Continued)

Han, T., & Das, D. B. (2015). Potential of combined ultrasound and microneedles for enhanced transdermal drug permeation: a review. **European journal of pharmaceutics and biopharmaceutics.** 89: 312-328.

Haniffa, M., Gunawan, M., & Jardine, L. (2015). Human skin dendritic cells in health and disease. **Journal of Dermatological Science.** 77(2): 85-92.

Haq, A., Goodyear, B., Ameen, D., Joshi, V., & Michniak-Kohn, B. (2018). Strat-M® synthetic membrane: Permeability comparison to human cadaver skin. **International Journal of Pharmaceutics.** 547(1): 432-437.

Haque, T., & Talukder, M. M. U. (2018). Chemical Enhancer: A Simplistic Way to Modulate Barrier Function of the Stratum Corneum. **Advanced Pharmaceutical Bulletin.** 8(2): 169-179.

Hashmi, M., Ullah, S., Ullah, A., Akmal, M., Saito, Y., Hussain, N., . . . Kim, I. S. (2020). Optimized Loading of Carboxymethyl Cellulose (CMC) in Tri-component Electrospun Nanofibers Having Uniform Morphology. **Polymers.** 12(11): 2524.

Hindi, A., Masri, M., & Hardcastle, S. (2021). Synthesis of Polymeric (Self-Disappearing) Nano Medical Patches Loaded with a Long-Acting Pharmacological Substance by Electrospinning Method. **Nanotechnology & Applications.** 4(1): 1-7.

Hudita, A., Galateanu, B., Costache, M., Negrei, C., Ion, R.-M., Iancu, L., & Ginghina, O. (2021). In vitro cytotoxic protective effect of alginate-encapsulated capsaicin might improve skin side effects associated with the topical application of capsaicin. **Molecules.** 26(5): 1455.

Idrees, S., Hanif, M. A., Ayub, M. A., Hanif, A., & Ansari, T. M. (2020). Chapter 9 - Chili Pepper. In M. A. Hanif, H. Nawaz, M. M. Khan, & H. J. Byrne (Eds.), *Medicinal Plants of South Asia* (pp. 113-124): Elsevier.

Iftinca, M., Defaye, M., & Altier, C. (2021). TRPV1-Targeted Drugs in Development for Human Pain Conditions. **Drugs.** 81(1): 7-27.

References (Continued)

Ilie, M. A., Caruntu, C., Tampa, M., Georgescu, S.-R., Matei, C., Negrei, C., . . . Boda, D. (2019). Capsaicin: Physicochemical properties, cutaneous reactions and potential applications in painful and inflammatory conditions (Review). **Experimental and Therapeutic Medicine.** 18(2): 916-925.

Jaiboonma, A., Kaokaen, P., Chaicharoenaudomrung, N., Kunhorm, P., Janebodin, K., Noisa, P., & Jitprasertwong, P. (2020). Cordycepin attenuates Salivary Hypofunction through the Prevention of Oxidative Stress in Human Submandibular Gland Cells. **International Journal of Medical Sciences.** 17(12): 1733-1743.

Jeong, W. Y., Kwon, M., Choi, H. E., & Kim, K. S. (2021). Recent advances in transdermal drug delivery systems: a review. **Biomaterials Research.** 25(1): 24.

Jeszka-Skowron, M., Zgoła-GrzeŚkowiak, A., GrzeŚkowiak, T., & Ramakrishna, A. (2021). *Analytical Methods in the Determination of Bioactive Compounds and Elements in Food*: Springer.

Jiang, X., Zhao, H., & Li, W. (2022). Microneedle-Mediated Transdermal Delivery of Drug-Carrying Nanoparticles. **Frontiers in Bioengineering and Biotechnology.** 10: 840395.

Joo, J. I., Kim, D. H., Choi, J.-W., & Yun, J. W. (2010). Proteomic Analysis for Antiobesity Potential of Capsaicin on White Adipose Tissue in Rats Fed with a High Fat Diet. **Journal of Proteome Research.** 9(6): 2977-2987.

Joshi, D., Garg, T., Goyal, A. K., & Rath, G. (2015). Development and Characterization of Novel Medicated Nanofibers Against Periodontitis. **Current Drug Delivery.** 12(5): 564-577.

Kar, N., Gupta, D., & Bellare, J. (2021). Ethanol affects fibroblast behavior differentially at low and high doses: A comprehensive, dose-response evaluation. **Toxicology Reports.** 8: 1054-1066.

Karimi, M., Zangabad, P. S., Mehdizadeh, F., Malekzad, H., Ghasemi, A., Bahrami, S., . . . Hamblin, M. R. (2017). Nanocaged platforms: modification, drug delivery and nanotoxicity. Opening synthetic cages to release the tiger. **Nanoscale.** 9(4): 1356-1392.

References (Continued)

Kennedy, W. R., Vanhove, G. F., Lu, S. P., Tobias, J., Bley, K. R., Walk, D., . . . Selim, M. M. (2010). A randomized, controlled, open-label study of the long-term effects of NGX-4010, a high-concentration capsaicin patch, on epidermal nerve fiber density and sensory function in healthy volunteers. **The Journal of Pain.** 11(6): 579-587.

Kerimoğlu, O., & Şahbaz, S. (2018). Animal Skin Models for Percutaneous Absorption Studies. **Journal of Biopharmaceutics and Therapeutic Challenges**: 1.

Kiani, J., Sajedi, F., Nasrollahi, S. A., & Esna-Ashari, F. (2015). A randomized clinical trial of efficacy and safety of the topical clonidine and capsaicin in the treatment of painful diabetic neuropathy. **Journal of Research in Medical Sciences.** 20(4): 359-363.

Kim, J. H., Ko, J. A., Kim, J. T., Cha, D. S., Cho, J. H., Park, H. J., & Shin, G. H. (2014). Preparation of a capsaicin-loaded nanoemulsion for improving skin penetration. **Journal of Agricultural and Food Chemistry.** 62(3): 725-732.

Kim, S. J., Won, Y.-H., & Kim, J.-K. (2004). Cytotoxicity of capsaicin on cultured human skin fibroblast. **Experimental Dermatology.** 13(9): 588-588.

Klebeko, J., Ossowicz-Rupniewska, P., Nowak, A., Janus, E., Duchnik, W., Adamiak-Giera, U., . . . Klimowicz, A. (2021). Permeability of Ibuprofen in the Form of Free Acid and Salts of L-Valine Alkyl Esters from a Hydrogel Formulation through Strat-M™ Membrane and Human Skin. *Materials, 14*(21). doi:10.3390/ma14216678

Kosuwon, W., Sirichatiwapee, W., Wisanuyotin, T., Jeeravipoolvarn, P., & Laupattarakasem, W. (2010). Efficacy of symptomatic control of knee osteoarthritis with 0.0125% of capsaicin versus placebo. **Medical journal of the Medical Association of Thailand.** 93(10): 1188-1195.

Kouchak, M., & Handali, S. (2014). Effects of various penetration enhancers on penetration of aminophylline through shed snake skin. **Jundishapur Journal of Natural Pharmaceutical Products.** 9(1): 24-29.

References (Continued)

Kumar, L., Verma, S., Joshi, K., Utreja, P., & Sharma, S. (2021). Nanofiber as a novel vehicle for transdermal delivery of therapeutic agents: challenges and opportunities. **Future Journal of Pharmaceutical Sciences.** 7(1): 175.

Kunita, R., Nishijima, T., Todo, H., Sugibayashi, K., & Sakaguchi, H. (2022). A Mathematical Approach Using Strat-M(®) to Predict the Percutaneous Absorption of Chemicals under Finite Dose Conditions. **Pharmaceutics.** 14(7).

Laklouk, M., & Baranidharan, G. (2016). Profile of the capsaicin 8% patch for the management of neuropathic pain associated with postherpetic neuralgia: safety, efficacy, and patient acceptability. **Patient Prefer Adherence.** 10: 1913-1918.

László, S., Bátai, I. Z., Berkó, S., Csányi, E., Dombi, Á., Pozsgai, G., . . . Pintér, E. (2022). Development of Capsaicin-Containing Analgesic Silicone-Based Transdermal Patches. **Pharmaceuticals.** 15(10): 1279.

Latif, M. S., Nawaz, A., Rashid, S. A., Akhlaq, M., Iqbal, A., Khan, M. J., . . . Alfatama, M. (2022). Formulation of Polymers-Based Methotrexate Patches and Investigation of the Effect of Various Penetration Enhancers: In Vitro, Ex Vivo and In Vivo Characterization. *Polymers, 14*(11). doi:10.3390/polym14112211

Lawlor, K. T., & Kaur, P. (2015). Dermal Contributions to Human Interfollicular Epidermal Architecture and Self-Renewal. **International Journal of Molecular Sciences.** 16(12): 28098-28107.

Lee, O., Jeong, S. H., Shin, W. U., Lee, G., Oh, C., & Son, S. W. (2013). Influence of surface charge of gold nanorods on skin penetration. **Skin Research and Technology.** 19(1): e390-396.

Leppert, W., Malec-Milewska, M., Zajaczkowska, R., & Wordliczek, J. (2018). Transdermal and Topical Drug Administration in the Treatment of Pain. **Molecules.** 23(3).

Letchford, K., Liggins, R., Wasan, K. M., & Burt, H. (2009). In vitro human plasma distribution of nanoparticulate paclitaxel is dependent on the physicochemical properties of poly (ethylene glycol)-block-poly (caprolactone) nanoparticles. **European journal of pharmaceutics and biopharmaceutics.** 71(2): 196-206.

References (Continued)

Li, J., Liu, Y., & Abdelhakim, H. E. (2022). Drug Delivery Applications of Coaxial Electrospun Nanofibres in Cancer Therapy. *Molecules, 27*(6). doi:10.3390/molecules27061803

Li, J., Wang, H., Zhang, L., An, N., Ni, W., Gao, Q., & Yu, Y. (2021). Capsaicin affects macrophage anti-inflammatory activity via the MAPK and NF-**K**B signaling pathways. **International Journal for Vitamin and Nutrition Research.** 93.

Li, X., Kanjwal, M. A., Lin, L., & Chronakis, I. S. (2013). Electrospun polyvinyl-alcohol nanofibers as oral fast-dissolving delivery system of caffeine and riboflavin. **Colloids Surf B Biointerfaces.** 103: 182-188.

Liu, R., Kurihara, C., Tsai, H. T., Silvestri, P. J., Bennett, M. I., Pasquina, P. F., & Cohen, S. P. (2017). Classification and Treatment of Chronic Neck Pain: A Longitudinal Cohort Study. **Regional Anesthesia & Pain Medicine.** 42(1): 52-61.

Luo, X.-J., Peng, J., & Li, Y.-J. (2011). Recent advances in the study on capsaicinoids and capsinoids. **European journal of pharmacology.** 650(1): 1-7.

Luraghi, A., Peri, F., & Moroni, L. (2021). Electrospinning for drug delivery applications: A review. **Journal of Controlled Release.** 334: 463-484.

Macho, A., Lucena, C., Sancho, R., Daddario, N., Minassi, A., Muñoz, E., & Appendino, G. (2003). Non-pungent capsaicinoids from sweet pepper. **European Journal of Nutrition.** 42(1): 2-9.

Malmberg, A. B., Mizisin, A. P., Calcutt, N. A., von Stein, T., Robbins, W. R., & Bley, K. R. (2004). Reduced heat sensitivity and epidermal nerve fiber immunostaining following single applications of a high-concentration capsaicin patch. **PAIN.** 111(3): 360-367.

Martínez-Ortega, L., Mira, A., Fernandez-Carvajal, A., Mateo, C. R., Mallavia, R., & Falco, A. (2019). Development of A New Delivery System Based on Drug-Loadable Electrospun Nanofibers for Psoriasis Treatment. **Pharmaceutics.** 11(1).

Matsui, T., & Amagai, M. (2015). Dissecting the formation, structure and barrier function of the stratum corneum. **International Immunology.** 27(6): 269-280.

Melzack, R. (1999). From the gate to the neuromatrix. **PAIN.** Suppl 6: S121-s126.

References (Continued)

Mitchell, M. J., Billingsley, M. M., Haley, R. M., Wechsler, M. E., Peppas, N. A., & Langer, R. (2021). Engineering precision nanoparticles for drug delivery. **Nature Reviews Drug Discovery.** 20(2): 101-124.

Mitxelena-Iribarren, O., Riera-Pons, M., Pereira, S., Calero-Castro, F. J., Castillo Tuñón, J. M., Padillo-Ruiz, J., . . . Arana, S. (2023). Drug-loaded PCL electrospun nanofibers as anti-pancreatic cancer drug delivery systems. **Polymer Bulletin.** 80(7): 7763-7778.

Mondal, R., Bobde, Y., Ghosh, B., & Giri, T. (2019). Development and Characterization of a Phospholipid Complex for Effective Delivery of Capsaicin. **Indian Journal of Pharmaceutical Sciences.** 81.

Mori, A., Lehmann, S., O'Kelly, J., Kumagai, T., Desmond, J. C., Pervan, M., . . . Koeffler, H. P. (2006). Capsaicin, a component of red peppers, inhibits the growth of androgen-independent, p53 mutant prostate cancer cells. **Cancer Research.** 66(6): 3222-3229.

Mullins, C. F., Walsh, S., Rooney, A., McCrory, C., & Das, B. (2022). A preliminary prospective observational study of the effectiveness of high-concentration capsaicin cutaneous patch in the management of chronic post-surgical neuropathic pain. **Irish Journal of Medical Science (1971 -).** 191(2): 859-864.

Murthy, N. (2012). Transdermal drug delivery: approaches and significance. **Research and Reports in Transdermal Drug Delivery**: 1.

Nada, A. A., Ali, E. A., Soliman, A. A. F., Shen, J., Abou-Zeid, N. Y., & Hudson, S. M. (2020). Multi-layer dressing made of laminated electrospun nanowebs and cellulose-based adhesive for comprehensive wound care. **International Journal of Biological Macromolecules.** 162: 629-644.

Nava-Ochoa, A. E., Antunes-Ricardo, M., & Guajardo-Flores, D. (2021). Nano-sized carriers for capsaicinoids with topic analgesic and anti-inflammatory effects. **Journal of Biotechnology.** 333: 77-85.

References (Continued)

Nawaz, A., Farid, A., Safdar, M., Latif, M. S., Ghazanfar, S., Akhtar, N., . . . Khan, M. W. (2022). Formulation Development and Ex-Vivo Permeability of Curcumin Hydrogels under the Influence of Natural Chemical Enhancers. **Gels.** 8(6): 384.

Neupane, R., Boddu, S. H. S., Renukuntla, J., Babu, R. J., & Tiwari, A. K. (2020). Alternatives to Biological Skin in Permeation Studies: Current Trends and Possibilities. **Pharmaceutics.** 12(2).

Noe, C. E. (2020). *Pain Management for Clinicians: A Guide to Assessment and Treatment*: Springer Nature.

Nolano, M., Simone, D. A., Wendelschafer-Crabb, G., Johnson, T., Hazen, E., & Kennedy, W. R. (1999). Topical capsaicin in humans: parallel loss of epidermal nerve fibers and pain sensation. **PAIN.** 81(1).

Ohta, T., Imagawa, T., & Ito, S. (2009). Involvement of transient receptor potential vanilloid subtype 1 in analgesic action of methylsalicylate. **Molecular pharmacology.** 75(2): 307-317.

Palmer, B. C., & DeLouise, L. A. (2016). Nanoparticle-Enabled Transdermal Drug Delivery Systems for Enhanced Dose Control and Tissue Targeting. **Molecules.** 21(12).

Pant, B., Park, M., & Park, S. J. (2019). Drug Delivery Applications of Core-Sheath Nanofibers Prepared by Coaxial Electrospinning: A Review. **Pharmaceutics.** 11(7).

Patzelt, A., & Lademann, J. (2013). Drug delivery to hair follicles. **Expert Opinion on Drug Delivery.** 10(6): 787-797.

Peña-Juárez, M. C., Guadarrama-Escobar, O. R., & Escobar-Chávez, J. J. (2022). Transdermal Delivery Systems for Biomolecules. **Journal of Pharmaceutical Innovation.** 17(2): 319-332.

Pershing, L. K., Reilly, C. A., Corlett, J. L., & Crouch, D. J. (2004). Effects of vehicle on the uptake and elimination kinetics of capsaicinoids in human skin in vivo. **Toxicology and applied pharmacology.** 200(1): 73-81.

References (Continued)

Pinho-Ribeiro, F. A., Verri, W. A., Jr., & Chiu, I. M. (2017). Nociceptor Sensory Neuron-Immune Interactions in Pain and Inflammation. **Trends in Immunology.** 38(1): 5-19.

Portillo, M., Mataix, M., Alonso-Juarranz, M., Lorrio, S., Villalba, M., Rodríguez-Luna, A., & González, S. (2021). The Aqueous Extract of Polypodium leucotomos (Fernblock®) Regulates Opsin 3 and Prevents Photooxidation of Melanin Precursors on Skin Cells Exposed to Blue Light Emitted from Digital Devices. **Antioxidants.** 10(3): 400.

Predel, H.-G., Ebel-Bitoun, C., Peil, B., Weiser, T. W., & Lange, R. (2020). Efficacy and Safety of Diclofenac + Capsaicin Gel in Patients with Acute Back/Neck Pain: A Multicenter Randomized Controlled Study. **Pain and Therapy.** 9(1): 279-296.

Prietto, L., Pinto, V. Z., El Halal, S. L. M., de Morais, M. G., Costa, J. A. V., Lim, L.-T., . . . Zavareze, E. d. R. (2018). Ultrafine fibers of zein and anthocyanins as natural pH indicator. **Journal of the Science of Food and Agriculture.** 98(7): 2735-2741.

Pulsoni, I., Lubda, M., Aiello, M., Fedi, A., Marzagalli, M., von Hagen, J., & Scaglione, S. (2022). Comparison Between Franz Diffusion Cell and a novel Micro-physiological System for In Vitro Penetration Assay Using Different Skin Models. **SLAS Technology.** 27(3): 161-171.

Rahmani, F., Ziyadi, H., Baghali, M., Luo, H., & Ramakrishna, S. (2021). Electrospun PVP/PVA Nanofiber Mat as a Novel Potential Transdermal Drug-Delivery System for Buprenorphine: A Solution Needed for Pain Management. *Applied Sciences, 11*(6). doi:10.3390/app11062779

Ramadon, D., McCrudden, M. T. C., Courtenay, A. J., & Donnelly, R. F. (2022). Enhancement strategies for transdermal drug delivery systems: current trends and applications. **Drug Delivery and Translational Research.** 12(4): 758-791.

Rao, X., Huang, X., Zhou, Z., & Lin, X. (2013). An improvement of the 2ˆ (–delta delta CT) method for quantitative real-time polymerase chain reaction data analysis. **Biostatistics, bioinformatics and biomathematics.** 3(3): 71.

References (Continued)

Reyes-Escogido, M. D. L., Gonzalez-Mondragon, E. G., & Vazquez-Tzompantzi, E. (2011). Chemical and Pharmacological Aspects of Capsaicin. **Molecules.** 16(2): 1253-1270.

Rezazadeh, A., Moghaddas Kia, E., Hamishehkar, H., Kafil Gazi Jahani, B., & Ghasempour, Z. (2022). Capsaicin-incorporated zein electrospun nanofibers: Characterization and release behavior. **Food Bioscience.** 49: 101843.

Rollyson, W. D., Stover, C. A., Brown, K. C., Perry, H. E., Stevenson, C. D., McNees, C. A., . . . Dasgupta, P. (2014). Bioavailability of capsaicin and its implications for drug delivery. **Journal of Controlled Release.** 196: 96-105.

Romero, V., Lara, J. R., Otero-Espinar, F., Salgado, M. H., Modolo, N. S. P., & de Barros, G. A. M. (2019). Capsaicin topical cream (8%) for the treatment of myofascial pain syndrome. **Brazilian Journal of Anesthesiology (English Edition).** 69(5): 432-438.

Roohnikan, M., Laszlo, E., Babity, S., & Brambilla, D. (2019). A Snapshot of Transdermal and Topical Drug Delivery Research in Canada. **Pharmaceutics.** 11(6).

Sa'adon, S., Ansari, M. N. M., Razak, S. I. A., Yusof, A. H. M., Faudzi, A. A. M., Sagadevan, S., . . . Amin, K. A. M. (2021). Electrospun Nanofiber and Cryogel of Polyvinyl Alcohol Transdermal Patch Containing Diclofenac Sodium: Preparation, Characterization and In Vitro Release Studies. **Pharmaceutics.** 13(11).

Sa'adon, S., Abd Razak, S. I., Ismail, A. E., & Fakhruddin, K. (2019). Drug-Loaded Poly-Vinyl Alcohol Electrospun Nanofibers for Transdermal Drug Delivery: Review on Factors Affecting the Drug Release. **Procedia Computer Science**.

Saavedra-Hernández, M., Castro-Sánchez, A. M., Cuesta-Vargas, A. I., Cleland, J. A., Fernández-de-las-Peñas, C., & Arroyo-Morales, M. (2012). The contribution of previous episodes of pain, pain intensity, physical impairment, and pain-related fear to disability in patients with chronic mechanical neck pain. **American Journal of Physical Medicine & Rehabilitation.** 91(12): 1070-1076.

Sabbagh, F., & Kim, B. S. (2022). Recent advances in polymeric transdermal drug delivery systems. **Journal of Controlled Release.** 341: 132-146.

References (Continued)

Salles, T., Lombello, C., & d'Ávila, M. (2015). Electrospinning of Gelatin/Poly (Vinyl Pyrrolidone) Blends from Water/Acetic Acid Solutions. **Materials Research.** 18: 509-518.

Schappert, S. M., & Burt, C. W. (2006). Ambulatory care visits to physician offices, hospital outpatient departments, and emergency departments: United States, 2001-02. **Vital and Health Statistics 13**(159): 1-66.

Sequeira, R. S., Miguel, S. P., Cabral, C. S. D., Moreira, A. F., Ferreira, P., & Correia, I. J. (2019). Development of a poly(vinyl alcohol)/lysine electrospun membrane-based drug delivery system for improved skin regeneration. **International Journal of Pharmaceutics.** 570: 118640.

Sim, S., & Wong, N. K. (2021). Nanotechnology and its use in imaging and drug delivery (Review). **Biomedical Reports.** 14(5): 42.

Spahr, N., Hodkinson, D., Jolly, K., Williams, S., Howard, M., & Thacker, M. (2017). Distinguishing between nociceptive and neuropathic components in chronic low back pain using behavioural evaluation and sensory examination. **Musculoskeletal Science and Practice.** 27: 40-48.

Steeds, C. E. (2009). The anatomy and physiology of pain. **Surgery (Oxford).** 27(12): 507-511.

Suzuki, M. (1987). Protective effect of fine-particle titanium dioxide on UVB-induced DNA damage in hairless mouse skin. **Photo-Dermatology.** 4(4): 209-211.

Szoka, L., & Palka, J. (2020). Capsaicin up-regulates pro-apoptotic activity of thiazolidinediones in glioblastoma cell line. **Biomedicine & Pharmacotherapy.** 132: 110741.

Tahir, M., Vicini, S., Jędrzejewski, T., Wrotek, S., & Sionkowska, A. (2024). New Composite Materials Based on PVA, PVP, CS, and PDA. **Polymers.** 16(23): 3353.

Tanadchangsaeng, N., Khanpimai, D., Kitmongkonpaisan, S., Chobchuenchom, W., Koobkokkruad, T., & Sathirapongsasuti, N. (2016). Fabrication and characterization of electrospun nanofiber films of PHA/PBAT biopolymer blend containing chilli herbal extracts. **International Journal of Food Engineering.** 2(1).

References (Continued)

Tansey, E. A., & Johnson, C. D. (2015). Recent advances in thermoregulation. **Advances in Physiology Education.** 39(3): 139-148.

Tatke, A., Dudhipala, N., Janga, K. Y., Balguri, S. P., Avula, B., Jablonski, M. M., & Majumdar, S. (2018). In situ gel of triamcinolone acetonide-loaded solid lipid nanoparticles for improved topical ocular delivery: tear kinetics and ocular disposition studies. **Nanomaterials.** 9(1): 33.

Thumanu, K., Darawadee, W., Mathukorn, S., Piyaporn, P., Toan, L. T., Weravart, N., . . . and Buensanteai, N. (2017). Synchrotron-based FTIR microspectroscopy of chili resistance induced by Bacillus subtilis strain D604 against anthracnose disease. **Journal of Plant Interactions.** 12(1): 255-263.

Thumanu, K., Mathukorn, S., Piyaporn, P., Kanokwan, N., & and Buensanteai, N. (2015). Use of infrared microspectroscopy to determine leaf biochemical composition of cassava in response to Bacillus subtilis CaSUT007. **Journal of Plant Interactions.** 10(1): 270-279.

Todo, H. (2017). Transdermal Permeation of Drugs in Various Animal Species. **Pharmaceutics.** 9(3).

Tolleson, W. H. (2005). Human melanocyte biology, toxicology, and pathology. **Journal of Environmental Science and Health, Part C.** 23(2): 105-161.

Tort, S., Yıldız, A., Tuğcu-Demiröz, F., Akça, G., Kuzukıran, Ö., & Acartürk, F. (2019). Development and characterization of rapid dissolving ornidazole loaded PVP electrospun fibers. **Pharmaceutical Development and Technology.** 24: 864 - 873.

Treede, R. D. (2018). The International Association for the Study of Pain definition of pain: as valid in 2018 as in 1979, but in need of regularly updated footnotes. **Pain reports.** 3(2): e643.

Turnbull, A. (1850). Tincture of capsaicin as a remedy for chilblains and toothache. **Dublin Free Press.** 1: 95-96.

References (Continued)

Uchida, T., Kadhum, W. R., Kanai, S., Todo, H., Oshizaka, T., & Sugibayashi, K. (2015). Prediction of skin permeation by chemical compounds using the artificial membrane, Strat-M™. **European Journal of Pharmaceutical Sciences.** 67: 113-118.

van Nooten, F., Treur, M., Pantiri, K., Stoker, M., & Charokopou, M. (2017). Capsaicin 8% Patch Versus Oral Neuropathic Pain Medications for the Treatment of Painful Diabetic Peripheral Neuropathy: A Systematic Literature Review and Network Meta-analysis. **Clinical Therapeutics.** 39(4): 787-803.e718.

van Smeden, J., Janssens, M., Gooris, G. S., & Bouwstra, J. A. (2014). The important role of stratum corneum lipids for the cutaneous barrier function. **Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids.** 1841(3): 295-313.

Vargason, A. M., Anselmo, A. C., & Mitragotri, S. (2021). The evolution of commercial drug delivery technologies. **Nature Biomedical Engineering.** 5(9): 951-967.

Vatankhah, E. (2018). Rosmarinic acid-loaded electrospun nanofibers: In vitro release kinetic study and bioactivity assessment. **Engineering in Life Sciences.** 18(10): 732-742.

Vega-Vásquez, P., Mosier, N. S., & Irudayaraj, J. (2020). Nanoscale Drug Delivery Systems: From Medicine to Agriculture. **Frontiers in Bioengineering and Biotechnology.** 8.

Vong, M., Diaz Sanchez, F. J., Keirouz, A., Nuansing, W., & Radacsi, N. (2021). Ultrafast fabrication of Nanofiber-based 3D Macrostructures by 3D electrospinning. **Materials & Design.** 208: 109916.

Vong, M., Speirs, E., Klomkliang, C., Akinwumi, I., Nuansing, W., & Radacsi, N. (2018). Controlled three-dimensional polystyrene micro- and nano-structures fabricated by three-dimensional electrospinning. **RSC Advances.** 8.

Wang, C., Ma, C., Wu, Z., Liang, H., Yan, P., Song, J., . . . Zhao, Q. (2015). Enhanced Bioavailability and Anticancer Effect of Curcumin-Loaded Electrospun Nanofiber: In Vitro and In Vivo Study. **Nanoscale Research Letters.** 10(1): 439.

References (Continued)

Wang, F., Zhao, J., Liu, D., Zhao, T., Lu, Z., Zhu, L., . . . Cai, Y. (2016). Capsaicin reactivates hMOF in gastric cancer cells and induces cell growth inhibition. **Cancer Biology & Therapy.** 17(11): 1117-1125.

Wang, M., Luo, Y., Wang, T., Wan, C., Pan, L., Pan, S., . . . Chen, X. (2021). Artificial Skin Perception. **Advanced Materials.** 33(19): e2003014.

Wang, X. R., Gao, S. Q., Niu, X. Q., Li, L. J., Ying, X. Y., Hu, Z. J., & Gao, J. Q. (2017). Capsaicin-loaded nanolipoidal carriers for topical application: design, characterization, and in vitro/in vivo evaluation. **International Journal of Nanomedicine.** 12: 3881-3898.

Webb, C. M., & Steeds, C. E. (2022). The anatomy and physiology of pain. **Clinics in Integrated Care.** 14: 100115.

Wokovich, A. M., Prodduturi, S., Doub, W. H., Hussain, A. S., & Buhse, L. F. (2006). Transdermal drug delivery system (TDDS) adhesion as a critical safety, efficacy and quality attribute. **European journal of pharmaceutics and biopharmaceutics.** 64(1): 1-8.

Wolkerstorfer, A., Handler, N., & Buschmann, H. (2016). New approaches to treating pain. **Bioorganic & Medicinal Chemistry Letters.** 26(4): 1103-1119.

Wysocki, A. B. (1999). Skin anatomy, physiology, and pathophysiology. **Nursing Clinics of North America.** 34(4): 777-797, v.

Xiao, L., Takada, H., Gan, X., & Miwa, N. (2006). The water-soluble fullerene derivative "Radical Sponge" exerts cytoprotective action against UVA irradiation but not visible-light-catalyzed cytotoxicity in human skin keratinocytes. **Bioorganic & Medicinal Chemistry Letters.** 16(6): 1590-1595.

Yetisgin, A. A., Cetinel, S., Zuvin, M., Kosar, A., & Kutlu, O. (2020). Therapeutic Nanoparticles and Their Targeted Delivery Applications. **Molecules.** 25(9).

Yu, D.-G., Zhang, X.-F., Shen, X.-X., Brandford-White, C., & Zhu, L.-M. (2009). Ultrafine ibuprofen-loaded polyvinylpyrrolidone fiber mats using electrospinning. **Polymer International.** 58(9): 1010-1013.

References (Continued)

Zhang, A., Jung, E. C., Zhu, H., Zou, Y., Hui, X., & Maibach, H. (2017). Vehicle effects on human stratum corneum absorption and skin penetration. **Toxicology and Industrial Health.** 33(5): 416-425.

Zhang, W., Zhang, Y., Fan, J., Feng, Z., & Song, X. (2024). Pharmacological activity of capsaicin: Mechanisms and controversies (Review). **Molecular Medicine Reports.** 29(3): 38.

Zheng, J., Zhou, Y., Li, Y., Xu, D. P., Li, S., & Li, H. B. (2016). Spices for Prevention and Treatment of Cancers. **Nutrients.** 8(8).

Zheng, Q., Sun, W., & Qu, M. (2018). Anti-neuro-inflammatory effects of the bioactive compound capsaicin through the NF-**K**B signaling pathway in LPS-stimulated BV2 microglial cells. **Pharmacognosy Magazine.** 14(58).

Zheng, Y. (2019). *Bioinspired design of materials surfaces*: Elsevier.

Zis, P., Bernali, N., Argira, E., Siafaka, I., & Vadalouka, A. (2016). Effectiveness and Impact of Capsaicin 8% Patch on Quality of Life in Patients with Lumbosacral Pain: An Open-label Study. **Pain Physician.** 19(7): E1049-1053.

APPENDICES

APPENDIX A

SUPPLEMENTARY DATA

Table A1 Supplementary data for Figure 4.5

Concentration					Relative Ctrl		
	1	2	3	Avg ctrl	1	2	3
100	0.113	0.112	0.115		0.531348	0.526646	0.540752
50	0.192	0.193	0.19		0.902821	0.907524	0.893417
10	0.241	0.25	0.238		1.133229	1.175549	1.119122
1	0.264	0.229	0.24		1.241379	1.076803	1.128527
0.1	0.259	0.264	0.269		1.217868	1.241379	1.26489
0.01	0.275	0.278	0.274		1.293103	1.30721	1.288401
0.001	0.29	0.282	0.275		1.363636	1.326019	1.293103
0.0001	0.234	0.222	0.227		1.100313	1.043887	1.067398
ctrl	0.209	0.213	0.216	0.212667	0.982759	1.001567	1.015674

Table A2 Supplementary data for Figure 4.5

%Percentage of Cell viability				
1	2	3	Avg	SD
53.1348	52.66458	54.07524	53.29154	0.718272
90.28213	90.75235	89.34169	90.12539	0.718272
113.3229	117.5549	111.9122	114.2633	2.936519
124.1379	107.6803	112.8527	114.8903	8.415921
121.7868	124.1379	126.489	124.1379	2.351097
129.3103	130.721	128.8401	129.6238	0.97884
136.3636	132.6019	129.3103	132.7586	3.529257
110.0313	104.3887	106.7398	107.0533	2.834348
98.27586	100.1567	101.5674	100	1.651356

Table A3 Supplementary data for Figure 4.5

Condition	AVG
Control	100
0.0001	107.0533
0.001	132.7586
0.01	129.6238
0.1	124.1379
1	114.8903
10	114.2633
50	90.12539
100	53.29154

Table A4 Supplementary data for Figure 4.6

Sample Name	*GAPDH*	*COX-2*	^CT	Ctrl Avg	^^CT	2^-(^^ct)	Avg	SD
Ctrl	26.39558	28.29672	1.901135		-0.36759	1.290199		
Ctrl2	26.03218	28.28772	2.255541		-0.01319	1.009183		
Ctrl3	25.7205	28.37001	2.649509	2.26873	0.380781	0.768022	1.022468	0.261342
H_2O_2	26.80688	27.24627	0.439383		-1.82935	3.553759		
H_2O_2	26.20566	26.95932	0.75366		-1.51507	2.858123		
H_2O_2	26.72614	26.93192	0.205784		-2.06294	4.178382	3.530088	0.660448
H_2O_2+0.1mM	23.87998	26.99248	3.112501		0.843773	0.557185		
H_2O_2+0.1mM	23.44164	27.34332	3.901688		1.632959	0.322426		
H_2O_2+0.1mM	23.42923	26.93548	3.506252		1.237524	0.4241	0.43457	0.117729

APPENDIX B

FTIR MICROSCOPY ANALYSIS: FTIR IMAGING AND SPECTRAL MAPPING

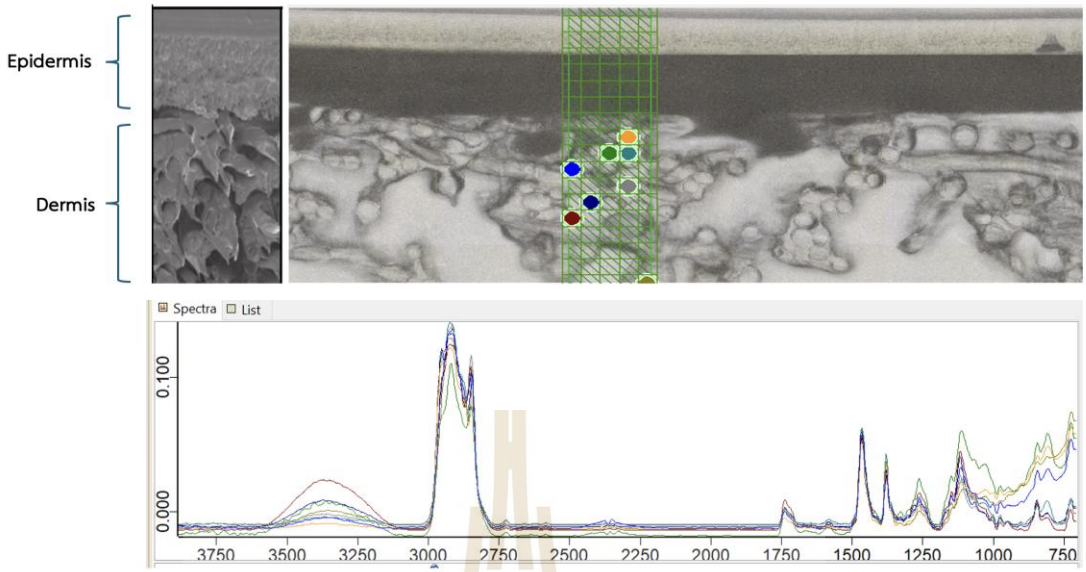


Figure B1 FTIR Microspectroscopy Analysis: FTIR Imaging and Spectral Mapping of Strat-M™ Membrane

The figure illustrates the FTIR microspectroscopy analysis of capsaicin permeation through the Strat-M™ membrane, simulating transdermal diffusion across skin layers. The upper panel shows a cross-sectional image of the membrane with distinguishable epidermis-like and dermis-like layers, visualized under FTIR imaging.

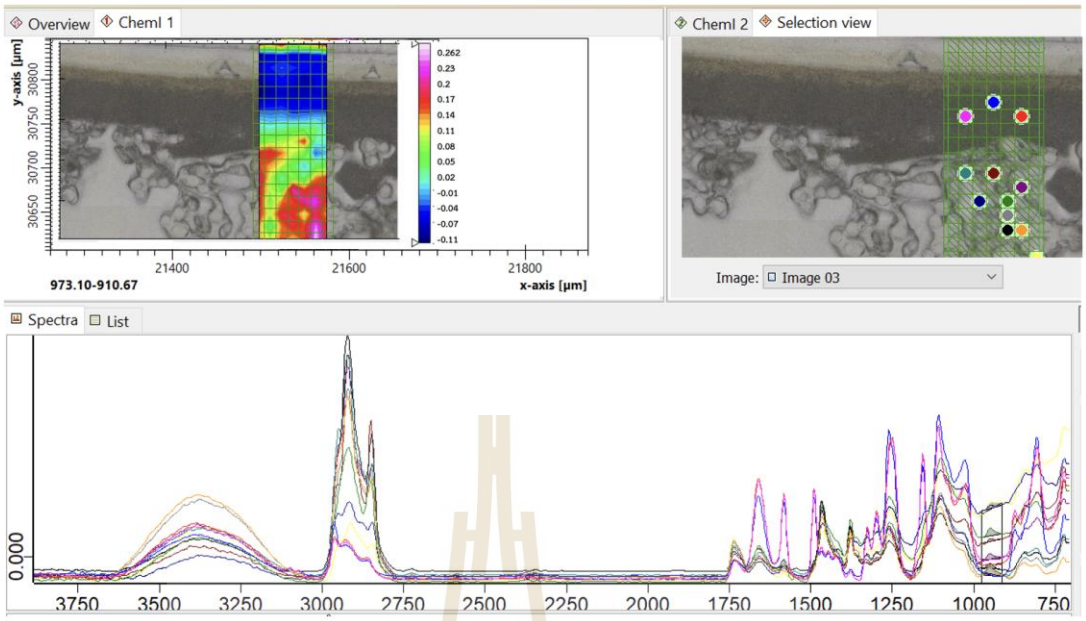


Figure B2 FTIR Microspectroscopy Mapping of Capsaicin Diffusion at 1 Hour.

This figure illustrates the FTIR microspectroscopic mapping and spectral analysis of the Strat-M™ membrane after 1 hour of contact with the capsaicin-loaded nanofiber patch. The analysis was performed to monitor the early-phase diffusion behavior and spatial localization of capsaicin across the membrane cross-section.

In the top left panel, the FTIR chemical map visualizes the intensity of capsaicin-related signals along the vertical axis of the membrane. The heatmap uses a color gradient scale, with red and white regions representing high spectral intensity and blue/purple indicating low or undetectable signal levels. At 1 hour, the signal is concentrated predominantly in the upper region, corresponding to the epidermis-like layer, while little to no signal is observed in the dermis-like layer, suggesting limited permeation at this early stage.

The top right panel presents the region of interest (ROI) used for spectral extraction. Each colored dot represents a point from which an FTIR spectrum was collected for comparison. These points are distributed along the depth of the membrane, allowing spatial correlation between signal intensity and depth.

The bottom panel shows the FTIR spectra corresponding to the selected ROIs. Notable absorption bands are observed around 2920–2850 cm^{-1}, which correspond to C–H stretching vibrations, and 1650–1500 cm^{-1}, representing amide I and aromatic C=C bonds, all of which are characteristic of capsaicin. Additional signals in the 1250–1000 cm^{-1} region (C–O and C–N stretching) further confirm the chemical presence of capsaicin. Variations in peak height and sharpness among different spectra reflect heterogeneity in compound distribution.

This result supports the hypothesis that capsaicin remained largely on the membrane surface during the initial 1-hour period, undergoing surface adsorption rather than full-layer permeation. The observed chemical localization pattern is consistent with a lag phase in drug diffusion, as expected in sustained-release transdermal delivery systems. It also emphasizes the importance of monitoring time-dependent permeation behavior to determine optimal delivery kinetics.

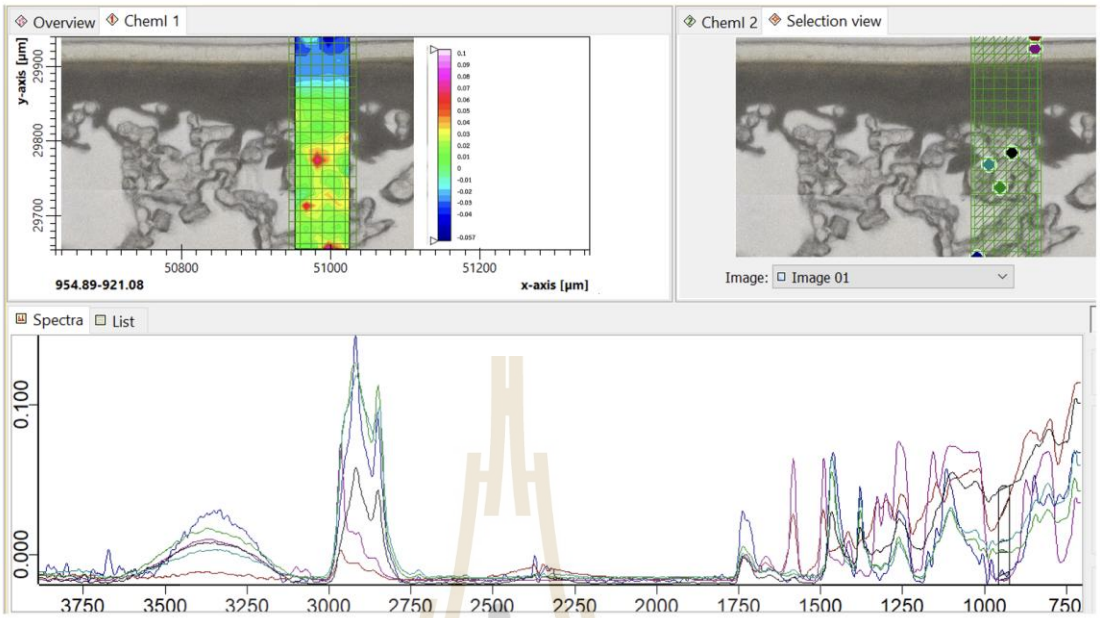


Figure B3 FTIR Microspectroscopy Mapping of Capsaicin Diffusion at 2 Hours

This figure illustrates the FTIR imaging and spectral mapping of the Strat-M™ membrane after 2 hours of exposure to the capsaicin-loaded nanofiber patch.

The top left panel shows a chemical intensity map indicating spatial distribution of capsaicin-related vibrational bands. Compared to the 1-hour time point, the signal extends further into the membrane. Higher intensities (in red/yellow) remain concentrated in the upper layers, but green-blue areas begin to appear in deeper sections, suggesting the onset of capsaicin permeation into dermis-like layers.

The top right panel identifies selected regions of interest (ROIs) where spectra were acquired for analysis. These points span from the surface through to the mid-depth of the membrane, capturing the gradient of chemical penetration.

The bottom panel displays the FTIR spectra of these regions. Peaks in the 2920–2850 cm^{-1} range (C–H stretching), 1650 cm^{-1} (C=O stretching), and 1250–1000 cm^{-1} (C–O and C–N vibrations) are clearly evident, with intensity increasing in deeper regions compared to the 1-hour mark. This indicates that diffusion of capsaicin through the membrane is actively progressing.

These results suggest that, at 2 hours, capsaicin has begun to permeate beyond the surface layer and into the interior of the Strat-M™ membrane. The increasing spectral intensity in deeper layers compared to the earlier time point supports the time-dependent diffusion behavior of the nanofiber-delivered capsaicin.

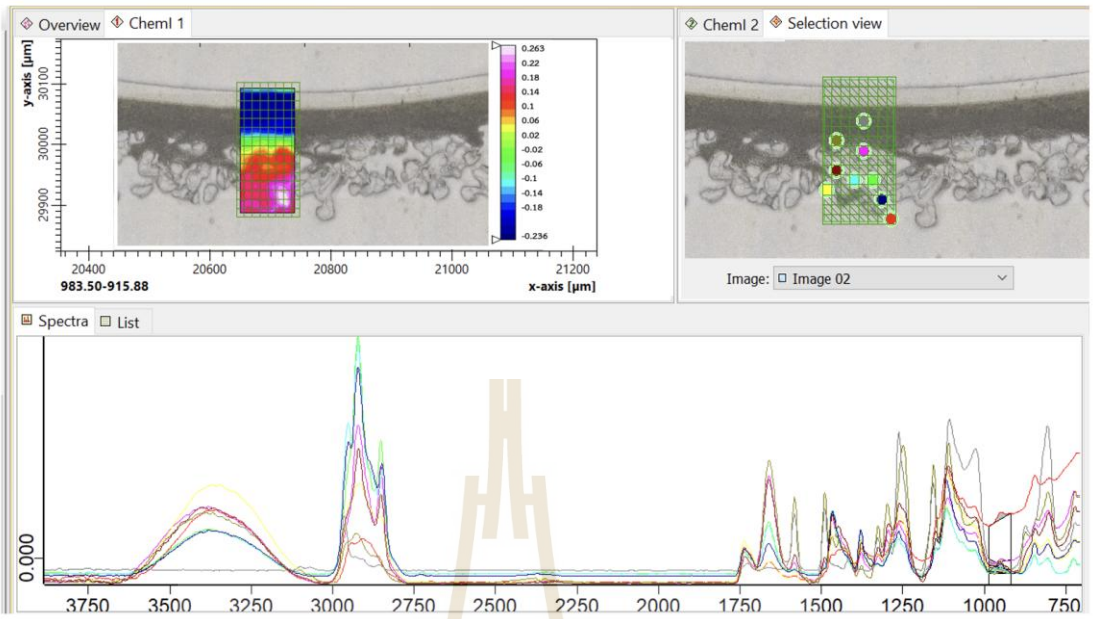


Figure B4 FTIR Microspectroscopy Mapping of Capsaicin Diffusion at 4 Hours

This figure presents FTIR microspectroscopy analysis of the Strat-M™ membrane following 4 hours of exposure to the capsaicin-loaded nanofiber patch. The data provide spatial and spectral evidence of capsaicin diffusion through the membrane structure over time.

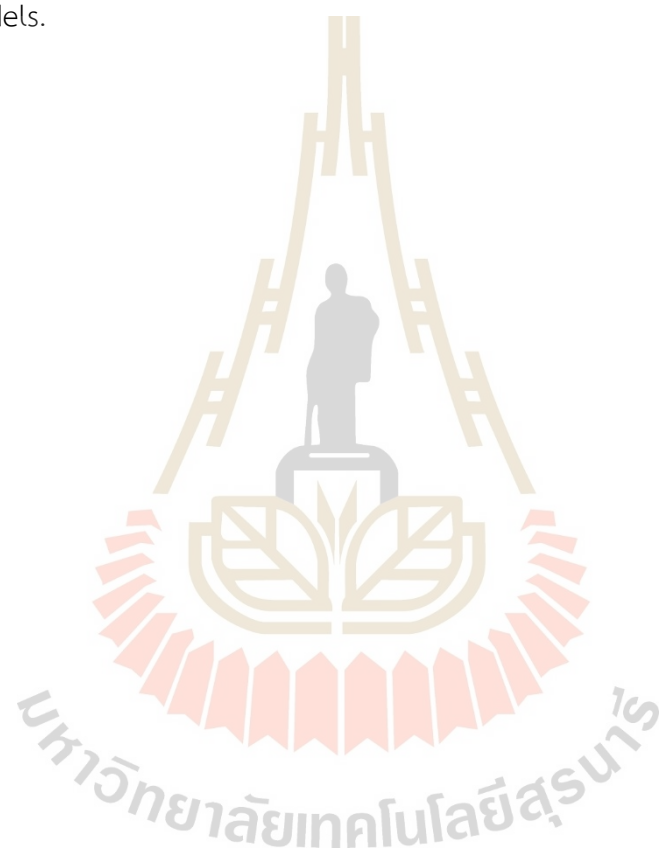
The top left panel illustrates a chemical intensity map derived from FTIR imaging. The heatmap reveals stronger capsaicin-related signals extending into deeper membrane regions compared to earlier time points. Red and magenta zones representing the highest absorbance are now seen penetrating below the upper epidermal-like layer and into the dermis-like region, suggesting significant mid-depth permeation.

The top right panel displays the selected regions of interest (ROIs) used for collecting individual FTIR spectra across different depths. These points allow for detailed evaluation of molecular signal distribution in the vertical axis of the membrane.

The bottom panel shows overlaid FTIR spectra from these ROIs. Peaks around 2920–2850 cm^{-1} (aliphatic C–H stretching), ~1650 cm^{-1} (C=O amide group), and 1000–1250 cm^{-1} (C–O and C–N stretching) are consistently present, and their intensities are

noticeably increased in deeper ROIs compared to the 2-hour profile. These spectral signatures confirm enhanced capsaicin permeation into lower layers of the membrane.

Overall, this 4-hour dataset indicates a clear progression of capsaicin permeation across the membrane. The increased chemical signal in both surface and dermal-like regions reflects sustained release behavior of the nanofiber patch, consistent with a controlled diffusion mechanism. This stage represents the transition between initial diffusion and steady-state permeation, as described in transdermal delivery models.



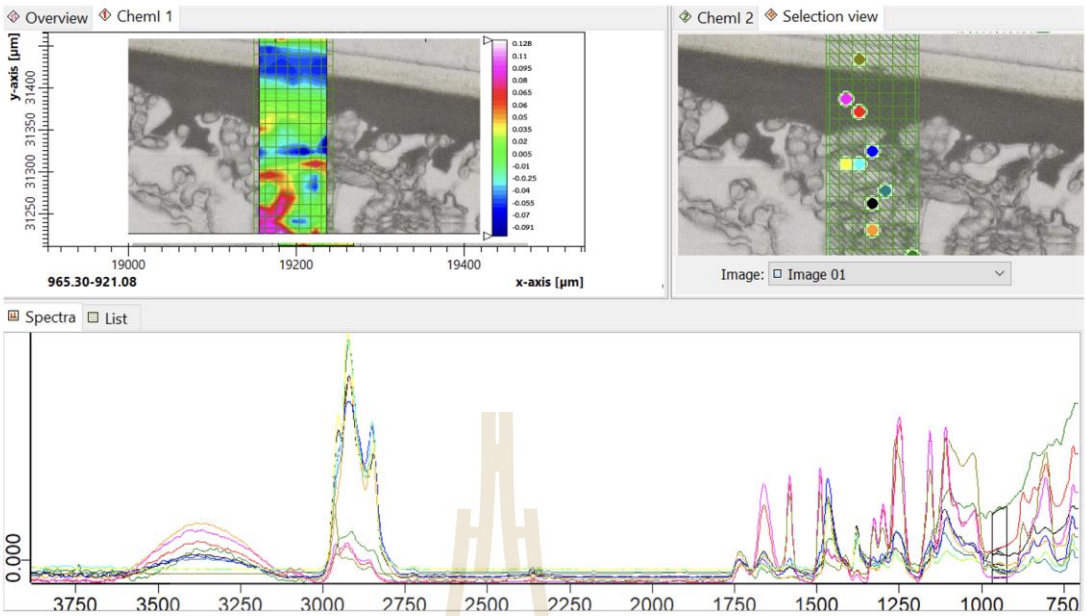


Figure B5 FTIR Microspectroscopy Mapping of Capsaicin Diffusion at 6 Hours

This figure illustrates the chemical mapping and spectral analysis of the Strat-M™ membrane after 6 hours of exposure to the capsaicin-loaded nanofiber patch, highlighting the intermediate stage of transdermal drug permeation.

The top left panel displays a color-coded FTIR chemical intensity map. The red and pink zones corresponding to high capsaicin-related absorbance are distributed throughout the membrane's vertical axis, indicating effective diffusion beyond the epidermal layer and into the dermis-like region. The presence of signal in lower membrane depths supports sustained release and cumulative permeation behavior at this stage.

The top right panel shows selected regions of interest (ROIs) from the membrane surface to its deeper layers. These ROIs provide a basis for evaluating changes in signal intensity and spectral profile across different membrane depths.

The bottom panel presents overlaid FTIR spectra from the selected ROIs. Peaks at ~2920–2850 cm^{-1} (aliphatic C–H stretching), ~1650 cm^{-1} (amide I), and 1000–1250 cm^{-1} (C–O and C–N stretching) remain prominent and well-defined across all depths. Notably, the spectral signals in deeper regions have increased, compared to earlier time points (1–4 hours), indicating stronger chemical presence and retention of capsaicin in the dermis-like layer.

At 6 hours, the data suggest that capsaicin has reached a quasi-steady state, wherein the rate of diffusion through the membrane has stabilized. This diffusion profile aligns with Fickian-controlled release, reflecting efficient and sustained transdermal delivery. These observations support the performance of the nanofiber patch as a long-acting drug delivery system.

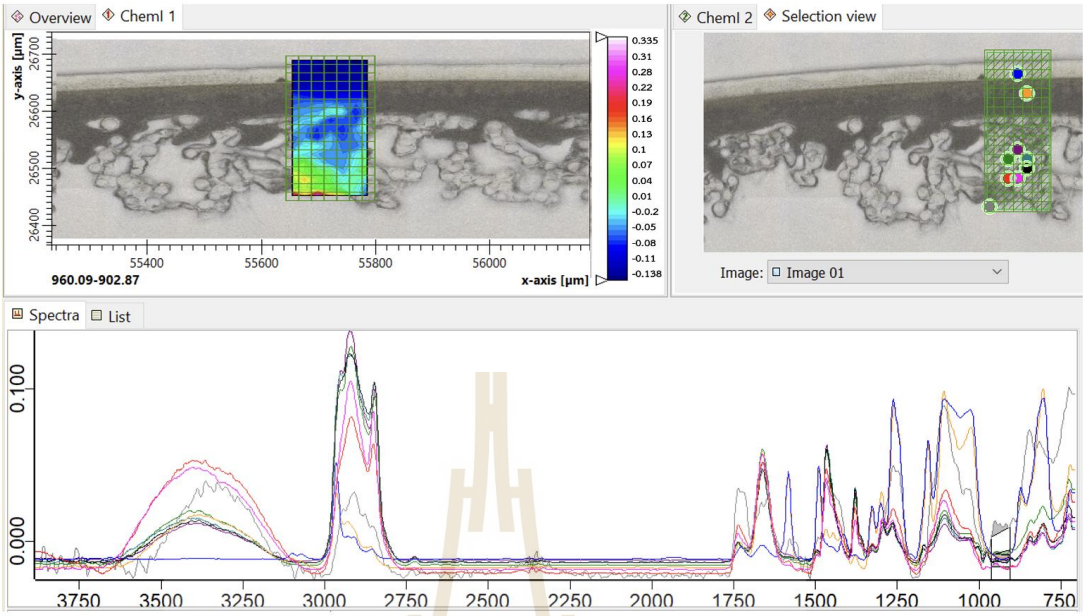


Figure B7 FTIR Microspectroscopy Mapping of Capsaicin Diffusion at 8 Hours

This figure illustrates the FTIR chemical imaging and spectral analysis of capsaicin permeation across the Strat-M™ membrane after 8 hours of contact with the capsaicin-loaded nanofiber patch.

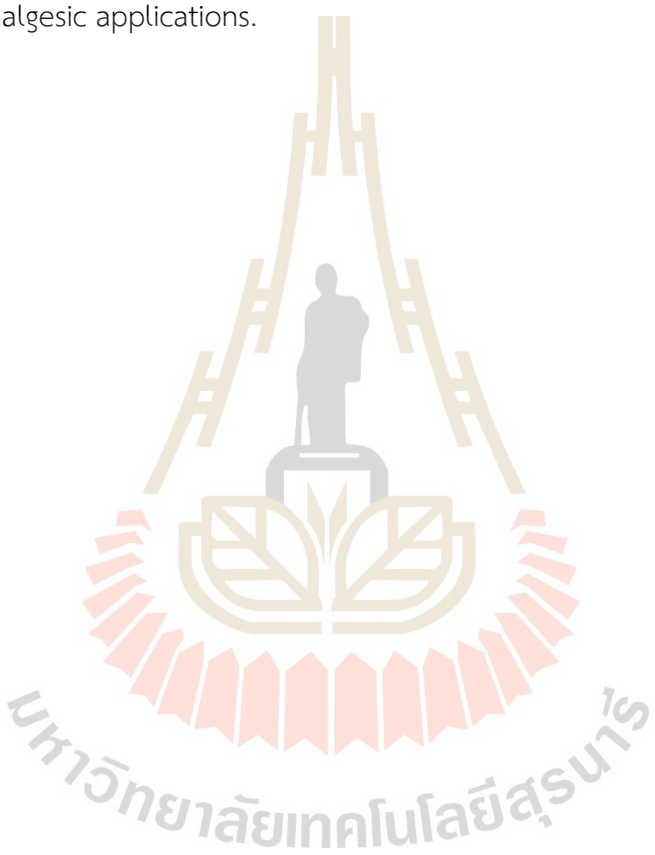
The top left panel displays a heatmap generated from FTIR microspectroscopic analysis. At this time point, high-intensity signals (yellow, green, and blue tones) extend through both the epidermal and dermal-like layers of the membrane, indicating continued diffusion and deep tissue-like accumulation. Compared to earlier time points, the signal intensity is more homogeneously distributed across the vertical membrane profile.

The top right panel shows the selected regions of interest (ROIs) used for point-specific spectral analysis. These ROIs are distributed across different depths to characterize the extent of capsaicin penetration.

The bottom panel presents the corresponding FTIR spectra extracted from each ROI. Characteristic absorption bands at ~2920–2850 cm^{-1} (C–H stretching), 1650 cm^{-1} (C=O amide), and ~1000–1250 cm^{-1} (C–N, C–O stretching) are well-resolved, with strong intensities noted especially in deeper regions. This suggests that capsaicin has

reached and remained in the dermal-like membrane layers, confirming the sustained-release performance of the patch.

The data at 8 hours demonstrate a broad and well-established diffusion pattern, with retention of capsaicin throughout the membrane depth. These findings align with the expected behavior of an electrospun nanofiber patch designed for controlled transdermal delivery and prolonged therapeutic effect. The even distribution and consistent spectral intensities support the formulation's potential for long-acting analgesic applications.



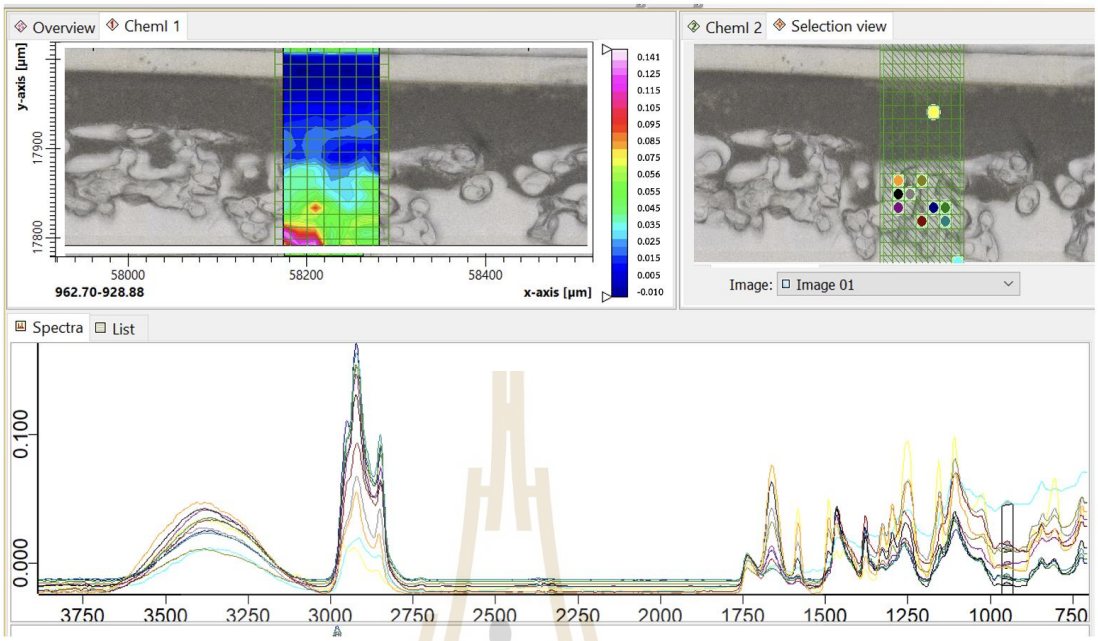


Figure A6 FTIR Microspectroscopy Mapping of Capsaicin Diffusion at 12 Hours

This figure depicts FTIR microspectroscopic imaging of the Strat-M™ membrane after 12 hours of contact with the capsaicin-loaded nanofiber patch, representing the final time point in the diffusion study.

The top left panel presents the FTIR chemical intensity map, showing a broad spatial distribution of capsaicin-associated signals (pink, red, green, and blue) that extend throughout the full thickness of the membrane. High-intensity regions are concentrated in the lower dermis-like zone, indicating deep penetration and accumulation of capsaicin in the membrane matrix.

The top right panel highlights the selected regions of interest (ROIs) used for spectral extraction. These include points from both surface and deep tissue-like regions, providing a representative profile of vertical chemical distribution.

The bottom panel displays the corresponding FTIR spectra. Strong vibrational peaks are consistently observed at ~2920–2850 cm^{-1} (aliphatic C–H stretching), ~1650 cm^{-1} (C=O amide I), and ~1000–1250 cm^{-1} (C–N and C–O stretching). Spectral intensity in the deeper layers is sustained and well-defined**,** confirming the retention of capsaicin after 12 hours**.**

At this final time point, the data suggest that capsaicin has fully permeated the membrane, with no significant additional diffusion expected beyond this duration. The intensity plateau seen across deeper ROIs indicates equilibrium-like distribution**,** consistent with the steady-state phase of transdermal drug delivery. These findings support the controlled, sustained release characteristics of the nanofiber patch and highlight its suitability for prolonged dermal therapeutic applications.

APPENDIX C

ETHICAL APPROVAL

KHE 2023 - 004

สำนักงานสาธารณสุขจังหวัดนครราชสีมา
Nakhonratchasima Provincial Public Health

สำนักงานสาธารณสุขจังหวัดนครราชสีมา

กระทรวงสาธารณสุข

255 หมู่ 11 ตำบลโคกกรวด อำเภอเมือง จังหวัดนครราชสีมา 30280 โทร. 0-4446-5101-4 ต่อ 310,311

เอกสารรับรองโครงการวิจัยแบบยกเว้น

คณะกรรมการจริยธรรมการวิจัยในมนุษย์ สำนักงานสาธารณสุขจังหวัดนครราชสีมา ดำเนินการให้การรับรองการยกเว้นพิจารณาจริยธรรมโครงการวิจัยตามแนวทางหลักจริยธรรมการวิจัยในคนที่เป็นมาตรฐานสากล ได้แก่ Declaration of Helsinki, The Belmont Report, CIOMS Guideline International Conference on Harmonization in Good Clinical Practice หรือ ICH-GCP

ชื่อโครงการวิจัย	การศึกษาผลทางคลินิกจากผลิตภัณฑ์ของสารสกัดจากพริกเคลือบอานุภาคนาโนเพื่อลดปวด
เลขที่โครงการวิจัย	NRPH 004
ชื่อหัวหน้าโครงการ	จุฑารัตน์ ถันชนนาง
หน่วยงานที่สังกัด	มหาวิทยาลัยเทคโนโลยีสุรนารี
วิธีการทบทวน	แบบยกเว้น
รายงานความก้าวหน้า	ส่งรายงานความก้าวหน้าอย่างน้อย 1 ครั้ง/ปี หรือส่งรายงานฉบับสมบูรณ์หากดำเนินโครงการเสร็จสิ้นก่อน 1 ปี
เอกสารที่รับรอง	1. แบบเสนอโครงการวิจัย 2. เอกสารชี้แจงผู้เข้าร่วมการวิจัย 3. หนังสือยินยอมตนในการทำวิจัย 4. แบบการเก็บรวบรวมข้อมูล/โปรแกรม/กิจกรรม

ลงนาม.....

(นายแพทย์วิชาญ คิดเห็น)

ประธานคณะกรรมการพิจารณาจริยธรรมการวิจัยในมนุษย์

วันที่รับรอง 1 กุมภาพันธ์ 2566 **วันหมดอายุ** 1 กุมภาพันธ์ 2567

ทั้งนี้ การรับรองนี้มีเงื่อนไขดังที่ระบุไว้ด้านหลังทุกข้อ (ดูด้านหลังของเอกสารรับรองโครงการวิจัย)

Figure C1 ETHICAL APPROVAL in February 2022

KHE 2024 - 004

สำนักงานสาธารณสุขจังหวัดนครราชสีมา
Nakhonratchasima Provincial Public Health

สำนักงานสาธารณสุขจังหวัดนครราชสีมา

กระทรวงสาธารณสุข

255 หมู่ 11 ตำบลโคกกรวด อำเภอเมือง จังหวัดนครราชสีมา 30280 โทร. 0-4446-5101-4 ต่อ 310,311

เอกสารรับรองโครงการวิจัยแบบยกเว้น

คณะกรรมการจริยธรรมการวิจัยในมนุษย์ สำนักงานสาธารณสุขจังหวัดนครราชสีมา ดำเนินการให้การรับรองการยกเว้นพิจารณาจริยธรรมโครงการวิจัยตามแนวทางหลักจริยธรรมการวิจัยในคนที่เป็นมาตรฐานสากล ได้แก่ Declaration of Helsinki, The Belmont Report, CIOMS Guideline International Conference on Harmonization in Good Clinical Practice หรือ ICH-GCP

ชื่อโครงการวิจัย	การศึกษาผลทางคลินิกจากผลิตภัณฑ์ของสารสกัดจากพริกเคลือบอานุภาคนาโนเพื่อลดปวด
เลขที่โครงการวิจัย	NRPH 004
ชื่อหัวหน้าโครงการ	จุฑารัตน์ ดันชนนาง
หน่วยงานที่สังกัด	มหาวิทยาลัยเทคโนโลยีสุรนารี
วิธีการทบทวน	แบบยกเว้น
รายงานความก้าวหน้า	ส่งรายงานความก้าวหน้าอย่างน้อย 1 ครั้ง/ปี หรือส่งรายงานฉบับสมบูรณ์หากดำเนินโครงการเสร็จสิ้นก่อน 1 ปี
เอกสารที่รับรอง	1. แบบเสนอโครงการวิจัย 2. เอกสารชี้แจงผู้เข้าร่วมการวิจัย 3. หนังสือยินยอมตนในการทำวิจัย 4. แบบการเก็บรวบรวมข้อมูล/โปรแกรม/กิจกรรม

ลงนาม.....

(นายแพทย์สมบัติ วัฒนะ)

ประธานคณะกรรมการพิจารณาจริยธรรมการวิจัยในมนุษย์

วันที่รับรอง 23 มกราคม 2567 **วันหมดอายุ** 23 มกราคม 2568

ทั้งนี้ การรับรองนี้มีเงื่อนไขดังที่ระบุไว้ด้านหลังทุกข้อ (ดูด้านหลังของเอกสารรับรองโครงการวิจัย)

Figure C2 ETHICAL APPROVAL in January 2023

APPENDIX D

INTERNATIONAL PUBLICATION

IJBM International Journal of BIOMEDICINE
ISSN: 2158-0510, e-ISSN: 2158-0529

442 5th Avenue #1196, Manhattan, NY 10018, USA Phone 1-917-740-3053 e-mail: editor@ijbm.org

Dear Assoc. Prof. Schawanya K. Rattanapitoon, MD

We are pleased to inform you that your article, "**Capsaicin Hydrogel Skin Patch: Development, Characterization, and Safety Evaluation of Cytotoxicity, Anti-Inflammatory Effects, and Pain-Relief Applications,**" has been accepted for publication in the September issue of IJBM (#3, 2025). You will receive page proofs within four weeks of receiving this acceptance letter.

Sincerely,
Prof. Marietta Eliseyeva, PhD, ScD
Editor-in-Chief
International Journal of Biomedicine

05.20.2025

Title: Capsaicin Hydrogel Skin Patch: Development, Characterization, and Safety Evaluation of Cytotoxicity, Anti-Inflammatory Effects, and Pain-Relief Applications

Authors: Chutharat Thanchonnang, Alisa Boonsuya, Phornpitcha Pechdee, Patpicha Arunsan, Nav la, Nattawut Keeratibharat, Nathkapach Kaewpitoon Rattanapitoon, Wiwat Nuansing, and Schawanya Kaewpitoon Rattanapitoon

Status: Accepted

APPENDIX E

INTERNATIONAL CONFERERENCE

The Controlled Release Society (CRS) proudly presents this
Certificate of Presentation to:

Chutharat Thanchonnang

Who presented the abstract titled:

Development of Capsaicin-Loaded Polyvinyl alcohol (PVA) and Polyvinylpyrrolidone (PVP) Nanofiber Patches for Transdermal Drug Delivery and Pain Relief: An Electrospinning Approach

at the Controlled Release Society
CRSingapore 2025: Connecting Continents in Delivery Science
February 19th-21st, 2025
NUHS, Singapore

Certificate of Presentation: Chutharat Thanchonnang presented a research abstract at the Controlled Release Society (CRS) conference, CRSingapore 2025: Connecting Continents in Delivery Science, held at NUHS, Singapore, on February 19–21, 2025.

Abstract Title: Development of Capsaicin-Loaded Polyvinyl alcohol (PVA) and Polyvinylpyrrolidone (PVP) Nanofiber Patches for Transdermal Drug Delivery and Pain Relief: An Electrospinning Approach

MAVMA CPD:
MAVMACPD-2023-059 (30 CPD)
MMA CPD:
CPDE43602 (16 points)

MALAYSIAN SOCIETY OF
PARASITOLOGY & TROPICAL MEDICINE

Certificate of Participation

presented to

Chutharat Thanchonnang

(Poster Presenter)

in recognition and appreciation of your participation in the

59th Annual Scientific Conference of the
Malaysian Society of Parasitology and Tropical Medicine

15 - 16 March 2023
The Wembley - A St Giles Hotel, Penang

DR. CHEN CHEE DHANG
President
59th Council of MSPTM (2022/2023)

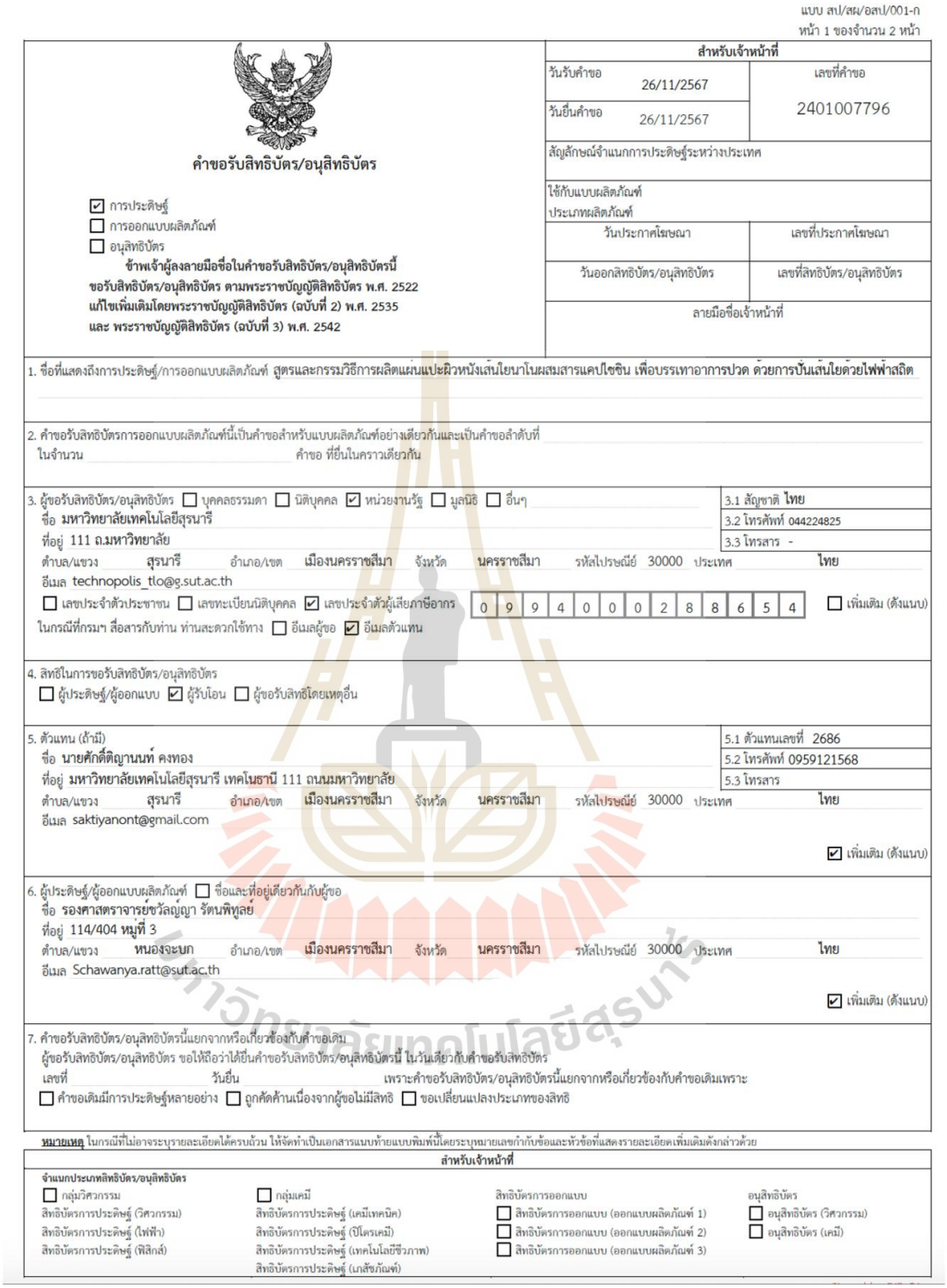
DR. STANLEY TAN TIONG KAI
Scientific Chairperson
MSPTM Annual Scientific Conference 2023

Certificate of Participation: Chutharat Thanchonnang participated as a Poster Presenter in the 59th Annual Scientific Conference of the Malaysian Society of Parasitology and Tropical Medicine (MSPTM), held on 15–16 March 2023 at The Wembley - A St Giles Hotel, Penang, Malaysia.

Title: Topical Capsaicin Hydrogel Skin Patch with Potential Pain-Relieving Effect: A One-Group Pretest and Posttest Clinical Trial Study

APPENDIX F

PATENT



แบบ สป/สผ/อสป/001-ก
หน้า 1 ของจำนวน 2 หน้า

คำขอรับสิทธิบัตร/อนุสิทธิบัตร

☑ การประดิษฐ์
☐ การออกแบบผลิตภัณฑ์
☐ อนุสิทธิบัตร

ข้าพเจ้าผู้ลงลายมือชื่อในคำขอรับสิทธิบัตร/อนุสิทธิบัตรนี้ ขอรับสิทธิบัตร/อนุสิทธิบัตร ตามพระราชบัญญัติสิทธิบัตร พ.ศ. 2522 แก้ไขเพิ่มเติมโดยพระราชบัญญัติสิทธิบัตร (ฉบับที่ 2) พ.ศ. 2535 และ พระราชบัญญัติสิทธิบัตร (ฉบับที่ 3) พ.ศ. 2542

สำหรับเจ้าหน้าที่

วันรับคำขอ 26/11/2567 | เลขที่คำขอ 2401007796
วันยื่นคำขอ 26/11/2567
สัญลักษณ์จำแนกการประดิษฐ์ระหว่างประเทศ
ใช้กับแบบผลิตภัณฑ์ประเภทผลิตภัณฑ์
วันประกาศโฆษณา | เลขที่ประกาศโฆษณา
วันออกสิทธิบัตร/อนุสิทธิบัตร | เลขที่สิทธิบัตร/อนุสิทธิบัตร
ลายมือชื่อเจ้าหน้าที่

1. ชื่อที่แสดงถึงการประดิษฐ์/การออกแบบผลิตภัณฑ์ สูตรและกรรมวิธีการผลิตแผ่นแปะผิวหนังเส้นใยนาโนผสมสารแคปไซซิน เพื่อบรรเทาอาการปวด ด้วยการปั่นเส้นใยด้วยไฟฟ้าสถิต

2. คำขอรับสิทธิบัตรการออกแบบผลิตภัณฑ์นี้เป็นคำขอสำหรับแบบผลิตภัณฑ์อย่างเดียวกันและเป็นคำขอลำดับที่
ในจำนวน คำขอ ที่ยื่นในคราวเดียวกัน

3. ผู้ขอรับสิทธิบัตร/อนุสิทธิบัตร ☐ บุคคลธรรมดา ☐ นิติบุคคล ☑ หน่วยงานรัฐ ☐ มูลนิธิ ☐ อื่นๆ
ชื่อ มหาวิทยาลัยเทคโนโลยีสุรนารี
ที่อยู่ 111 ถ.มหาวิทยาลัย
ตำบล/แขวง สุรนารี อำเภอ/เขต เมืองนครราชสีมา จังหวัด นครราชสีมา รหัสไปรษณีย์ 30000 ประเทศ ไทย
อีเมล technopolis_tlo@g.sut.ac.th
☐ เลขประจำตัวประชาชน ☐ เลขทะเบียนนิติบุคคล ☑ เลขประจำตัวผู้เสียภาษีอากร 0 9 9 4 0 0 0 2 8 8 6 5 4 ☐ เพิ่มเติม (ดังแนบ)
ในกรณีที่กรมฯ สื่อสารกับท่าน ท่านสะดวกใช้ทาง ☐ อีเมลผู้ขอ ☑ อีเมลตัวแทน

3.1 สัญชาติ ไทย
3.2 โทรศัพท์ 044224825
3.3 โทรสาร -

4. สิทธิในการขอรับสิทธิบัตร/อนุสิทธิบัตร
☐ ผู้ประดิษฐ์/ผู้ออกแบบ ☑ ผู้รับโอน ☐ ผู้ขอรับสิทธิโดยเหตุอื่น

5. ตัวแทน (ถ้ามี)
ชื่อ นายศักดิ์ติญานนท์ คงทอง
ที่อยู่ มหาวิทยาลัยเทคโนโลยีสุรนารี เทคโนธานี 111 ถนนมหาวิทยาลัย
ตำบล/แขวง สุรนารี อำเภอ/เขต เมืองนครราชสีมา จังหวัด นครราชสีมา รหัสไปรษณีย์ 30000 ประเทศ ไทย
อีเมล saktiyanont@gmail.com
☑ เพิ่มเติม (ดังแนบ)

5.1 ตัวแทนเลขที่ 2686
5.2 โทรศัพท์ 0959121568
5.3 โทรสาร

6. ผู้ประดิษฐ์/ผู้ออกแบบผลิตภัณฑ์ ☐ ชื่อและที่อยู่เดียวกันกับผู้ขอ
ชื่อ รองศาสตราจารย์ชวัลญญา รัตนพิทูลย์
ที่อยู่ 114/404 หมู่ที่ 3
ตำบล/แขวง หนองจะบก อำเภอ/เขต เมืองนครราชสีมา จังหวัด นครราชสีมา รหัสไปรษณีย์ 30000 ประเทศ ไทย
อีเมล Schawanya.ratt@sut.ac.th
☑ เพิ่มเติม (ดังแนบ)

7. คำขอรับสิทธิบัตร/อนุสิทธิบัตรนี้แยกจากหรือเกี่ยวข้องกับคำขอเดิม
ผู้ขอรับสิทธิบัตร/อนุสิทธิบัตร ขอให้ถือว่าได้ยื่นคำขอรับสิทธิบัตร/อนุสิทธิบัตรนี้ ในวันเดียวกับคำขอรับสิทธิบัตร
เลขที่ วันยื่น เพราะคำขอรับสิทธิบัตร/อนุสิทธิบัตรนี้แยกจากหรือเกี่ยวข้องกับคำขอเดิมเพราะ
☐ คำขอเดิมมีการประดิษฐ์หลายอย่าง ☐ ถูกคัดค้านเนื่องจากผู้ขอไม่มีสิทธิ ☐ ขอเปลี่ยนแปลงประเภทของสิทธิ

หมายเหตุ ในกรณีที่ไม่อาจระบุรายละเอียดได้ครบถ้วน ให้จัดทำเป็นเอกสารแนบท้ายแบบพิมพ์นี้โดยระบุหมายเลขกำกับข้อและหัวข้อที่แสดงรายละเอียดเพิ่มเติมดังกล่าวด้วย

สำหรับเจ้าหน้าที่

จำแนกประเภทสิทธิบัตร/อนุสิทธิบัตร

☐ กลุ่มวิศวกรรม
สิทธิบัตรการประดิษฐ์ (วิศวกรรม)
สิทธิบัตรการประดิษฐ์ (ไฟฟ้า)
สิทธิบัตรการประดิษฐ์ (ฟิสิกส์)

☐ กลุ่มเคมี
สิทธิบัตรการประดิษฐ์ (เคมีเทคนิค)
สิทธิบัตรการประดิษฐ์ (ปิโตรเคมี)
สิทธิบัตรการประดิษฐ์ (เทคโนโลยีชีวภาพ)
สิทธิบัตรการประดิษฐ์ (เภสัชภัณฑ์)

สิทธิบัตรการออกแบบ
☐ สิทธิบัตรการออกแบบ (ออกแบบผลิตภัณฑ์ 1)
☐ สิทธิบัตรการออกแบบ (ออกแบบผลิตภัณฑ์ 2)
☐ สิทธิบัตรการออกแบบ (ออกแบบผลิตภัณฑ์ 3)

อนุสิทธิบัตร
☐ อนุสิทธิบัตร (วิศวกรรม)
☐ อนุสิทธิบัตร (เคมี)

Title of the Invention/Product Design: Formulation of capsaicin transdermal nanofibers patch for pain relief by using electrospinning method, Patent/Utility Model Application Number: 2401007796

CURRICULUM VITAE

Name-Surname Miss Chutharat Thanchonnang
Ph.D. Candidate in Translational Medicine Program (International Program), Institute of Medicine, Suranaree University of Technology

Address 47 M. 4 Banthon, Tha Bo, Nong Khai, Thailand 43110

Tel: +6682-4809892

E-mail D6300111@g.sut.ac.th, chutharat.than@gmail.com

Education

2020 - present Doctor of Philosophy in Translational Medicine Program (International Program) Institute of Medicine, Suranaree University of Technology, (Scheme 2.2), Thailand

2018 Bachelor's degree of Public Health, Bachelor of Public Health Program (Second honor), Mahasarakham University, Thailand

Scholarship

2020 Kittibandit scholarship, Suranaree University of Technology (SUT), Thailand

2017 The scholarship: Exchange Student at University of Negeri Semarang (UNNES), Republic of Indonesia

Conference Contributions

2025 Controlled Release Society; CRS Singapore 2025: Connecting Continents in Delivery Science, February 19th-21st, 2025, NUHS, Singapore (Poster presentation)

2023 The 59th Annual Scientific Conference of the Malaysian Society of Parasitology & Tropical Medicine, MSPTM 2023, which will be held on 15-16 March 2023 at the Wembley Hotel, Penang, Malaysia (Poster presentation)

Publication

1) **Thanchonnang, C**., Boonsuya, A., Pechdee, P., Arunsan, P., La, N., Keeratibharat, N., Nuansing, W., Rattanapitoon, N. K., & Rattanapitoon, S. K. (2024). Capsaicin Hydrogel Skin Patch: Development, Characterization, and Safety Evaluation of Cytotoxicity, Anti-Inflammatory Effects, and Pain-Relief Applications. International Journal of Biomedicine 15(3) (2025) xxx-xxx (**Accepted**)

2) Pechdee, P., Boonsuya, A., Arunsan, P., **Thanchonnang, C**., La, N., Rattanapitoon, N. K., ... & Rattanapitoon, S. K. (2024). RESEARCH ARTICLE Effect of Allium sativum, Thunbergia laurifolia, and Eurycoma longifolia crude extracts on the minute intestinal fluke, *Haplorchis taichui*. Tropical Biomedicine, 41(4), 543-552.

3) Boonsuya, A., Arunsan, P., Pechdee, P., La, N., **Thanchonnang, C**., Rattanapitoon, N. K., & Rattanapitoon, S. K. (2024). RESEARCH ARTICLE Detection of the carcinogenic liver fluke, *Opisthorchis viverrini*: comparison of two coprological methods versus the automatic feces analyzer. Tropical Biomedicine, 41(3), 264-270.

4) Sangkam, W., Arunsan, P., Pechdee, P., Boonsuya, A., **Thanchonnang, C**., Chatdumrong, W., Rattanapitoon, N. K., & Rattanapitoon, S. K. (2024). Anthelmintic activity and pathophysiological effect of anthelmintic drugs against carcinogenic liver fluke, *Opisthorchis viverrini*. Tropical biomedicine, 41(2), 196–205. https://doi.org/10.47665/tb.41.2.010

5) Wisetmora, A. Wattanawong, O. Wijit, A. Phukowluan, J. Nachairan, A. Jaksuay, P. Sungpradit, S. Ekobol, N. Boonmars, T. Boonsuya, A. Pechdee, P. **Thanchonnang, C**. La, N. Rattanapitoon, N. K. Arunsan, P. Rattanapitoon, S. K. Gastrointestinal Helminthic Infection among the Population in Northern Thailand. Acta Parasitologica 2024, 69 (3), 1648-1660.

6) Pechdee, P., Boonsuya, A., Arunsan, P., **Thanchonnang, C**., La, N., Rattanapitoon, N. K., & Rattanapitoon, S. K. (2024). Anthelmintic activity and pathophysiological effect of Allium sativum crude extract against carcinogenic liver fluke, *Opisthorchis viverrini*. Tropical biomedicine, 41(4), 427-437.

Publication (Continued)

7) La, N., Leng, M., Arunsan, P., Pechdee, P., Boonsuya, A., **Thanchonnang, C**, Rattanapitoon, N.K, & Rattanapitoon, S.K. (2023). Molecular identification of *Opisthorchis viverrini* among the northeastern Cambodian population by internal transcribed spacer 2 based polymerase chain reaction. Tropical Biomedicine.

8) Chitpitaklert, P., Boonsuya, A., Pechdee, P., **Thanchonnang, C**., LA, N., Rattanapitoon, N.K., Arunsan, P., & Rattanapitoon, S.K. (2023). "Molecular detection of oral *Trichomonas tenax* in periodontal disease patients by polymerase chain reaction -based 18S rRNA gene. Tropical Biomedicine.

9) Boonsuya, A., Chitpitaklert, P., Pechdee, P., Srithongklang, W., **Thanchonnang, C**., La, N., Gordon, C.N., Rattanapitoon, N.K., Arunsan, P., & Rattanapitoon, S.K. (2023). Oral parasitic protozoan Entamoeba gingivalis in periodontal disease patients, northeastern Thailand. Tropical Biomedicine.