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^{TM} 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.

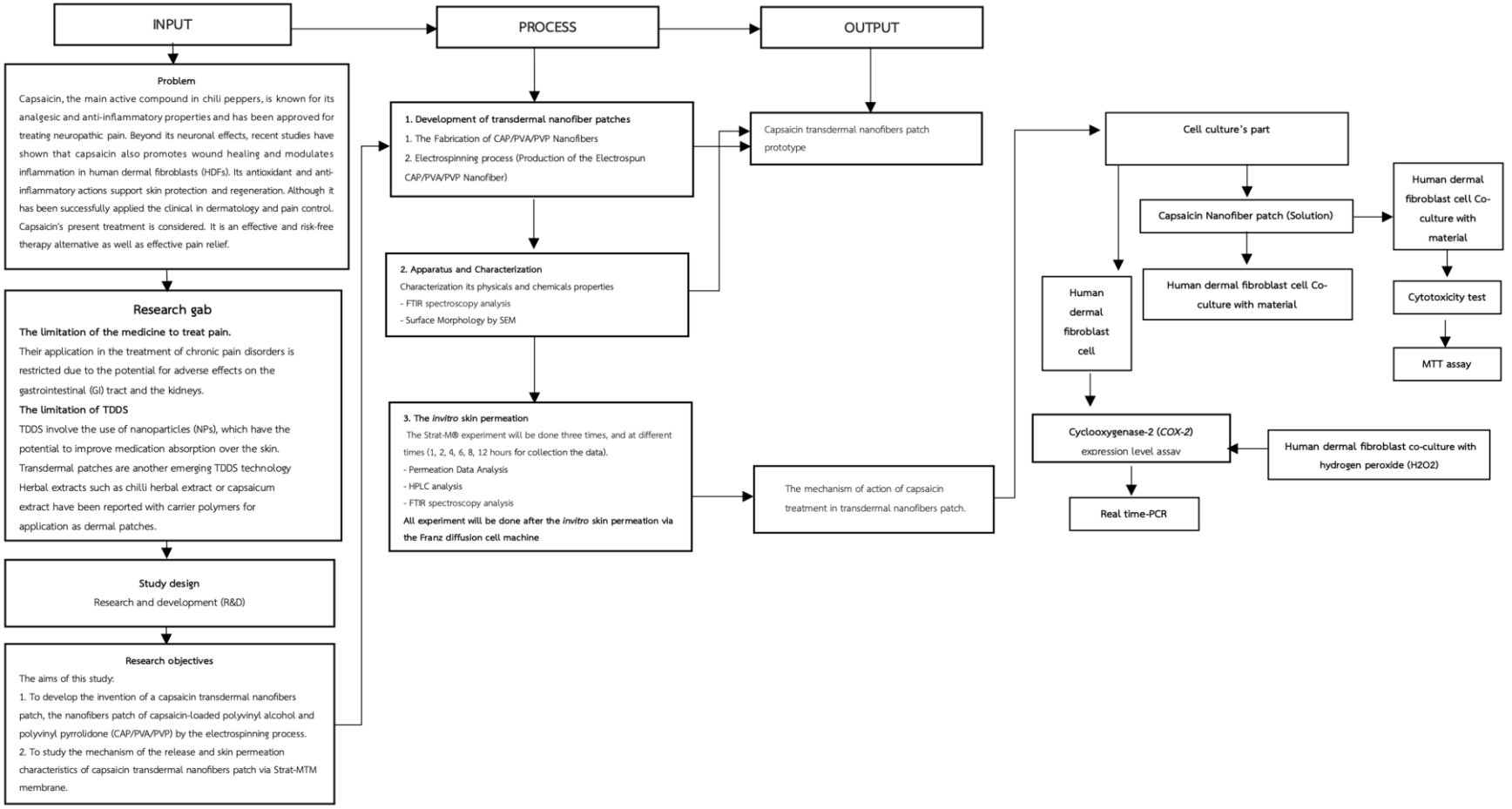
3. Validation of drug release kinetics and transdermal permeation through *in vitro* testing using Strat-M™ membranes, supporting the patch's potential for sustained capsaicin delivery.

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.