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-**κ**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.