FABRICATION AND EVALUATION OF DISSOLVING MICRONEEDLES FOR TRANSDERMAL DRUG DELIVERY

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DECLARATION

I hereby declare that the thesis entitled "**FABRICATION AND EVALUATION OF DISSOLVING MICRONEEDLES FOR TRANSDERMAL DRUG DELIVERY**" submitted by me, for the award of the degree of MTech, in Clinical Engineering to Sree Chitra Tirunal Institute for Medical Science and Technology, is a record of bonafide research work carried out by me under the supervision of **Dr. Shiny Velayudhan** (SCTIMST).

I further declare that the work reported in this thesis has not been submitted and will not be submitted, either in part or full, for the award of any other degree or diploma in this institute or of any other institute or university.

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To whomsoever it may concern

This is to certify that **Ms. Gautam Suresh Moon** has fulfilled the requirements prescribed for the MPhil degree of the Sree Chitra Tirunal Institute for Medical Sciences and Technology, Thiruvananthapuram. The dissertation entitled, **"FABRICATION AND EVALUATION OF DISSOLVING MICRONEEDLES FOR TRANSDERMAL DRUG DELIVERY"** was carried out under my direct supervision. No part of the thesis was submitted for the award of any degree or diploma prior to this date.

Thiruvananthapuram Dr. Shiny Velayudhan

Date:

The dissertation entitled

FABRICATION AND EVALUATION OF DISSOLVING MICRONEEDLES FOR TRANSDERMAL DRUG DELIVERY

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Master of Technology (Clinical Engineering)

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ABSTRACT

Transdermal Drug Delivery is a preferable route of drug administration due to myriad of advantages. However, the barrier properties of skin pose a challenge in transporting drug molecules across it. Dissolving microneedles is a promising drug delivery platform that not only aids in overcoming the barrier limitation but also enhances the drug delivery through the dermis. This study reports the fabrication and evaluation of rapidly dissolving microneedles as a tool to deliver a model lipophilic analgesic (Meloxicam).

Various polymer blend solutions were formulated using Polyvinyl alcohol and polyvinyl pyrrolidone for designing dissolving microneedle patch. The optimum formulations were arrived at based on the rheological characteristics and fabrication efficiency of the polymer blend solutions. The microneedle patches were evaluated for their morphology, mechanical sturdiness, dissolvability and skin penetration efficiency. The optimized polymer blend solution was then mixed with various concentrations of drug, in presence of a surfactant, Pluronic. The drug loaded microneedle patches were evaluated for their morphology, mechanical sturdiness, dissolvability, skin penetration efficiency and drug release profile.

The study showed that dissolving drug loaded microneedle patches could be successfully. The microneedle patches had pointed pyramidal needles, which could easily penetrate through the model skin used in this study. The patches dissolved rapidly when in contact with water and released the embedded drug.

CHAPTER 1

INTRODUCTION

Drug Delivery is a vast and upcoming field. Various drug delivery methods are being explored to better manage the pain and ease of incorporation into the patient. Among all the challenges posed by the traditional methods, the major one would be pain while delivering the drug and waste disposal after using any method. The current best and quickest method for delivering any drug is to give it via a syringe, which is invasive and quite painful for the patient. This method also generates a lot of waste, which needs to be disposed of after use, and the requirement of trained staff to use them is also a challenge. To deliver a drug without any trained staff and without leaving any harmful waste for the environment to be disposed of would be a revolutionary technology in terms of drug delivery. Microneedles are one such technology that can work so that the drug can be delivered painlessly without any incorporation of trained staff. Microneedles which have been studied previously, include solid, coated, hollow, and dissolving types. Dissolving microneedles made from polymers that are biodegradable and able to deliver drugs can be the solution to the problem. Microneedles are invasive, but they do not provide pain to the skin as they do not go deep enough to give a neuronal response. This allows to reduce the reduction of pain while incorporating drugs. The whole patch will be made with biodegradable polymers so the waste patches can be thrown away quickly without any worry of reuse. The microneedles could be made simpler with the help of a device that would stamp them on the skin, removing the requirement of any trained staff. All these advantages pose significant merit in developing dissolving microneedles for drug delivery.

Chapter 2, of the thesis details the literature survey on the various aspects of drug delivery and delivery systems. It begins with a brief description on human skin anatomy, and

touches on topics such as transdermal drug delivery, concept of microneedles, various microneedle fabrication processes, classification of microneedles, various materials used in the microneedle fabrication, applications of microneedles, various strategies employed in and delivery of analgesics. The GAP area in the delivery a analgesics is also presented and the objectives arrived at.

Chapter 3, details the materials and methods employed in this study. It covers the fabrication and various techniques employed for the characterization of microneedles. The characterization techniques include morphological characterization, mechanical property analysis, rheological analysis, of polymer solution, in vitro skin insertion studies and drug release studies. which were used in our work, and how the dissolving polymeric microneedles were fabricated.

Chapter 4 describes the results of the studies and discussions. The chapter details on the results and discussion of the present investigation. The results of the microneedle fabrication, morphology of both virgin and drug loaded microneedles, mechanical strength of microneedles, in vitro skin insertion and drug released from the microneedles are presented and discussed.

Chapter 5 summarizes the conclusions and presents the future prospectus.

The study signifies the use of microneedles for drug delivery for the painless administration of a hydrophobic drug using water soluble microneedles. Dissolving microneedles based on polymers is expected to perform better in cost, pain management, and waste generation. The microneedles fabricated by dissolving polymers have shown a potential to be used for drug delivery. The approaches and the results obtained in this study have the potential to be used as a base and eventually developed to solve the unmet clinical need of providing a painless method of drug delivery.

CHAPTER 2

LITERATURE SURVEY

2.1. SKIN ANATOMY

Organs play a vital role in the overall function of our body. They work in unison and as individuals to keep the body in proper shape. Amongst them is skin, which serves the most crucial role of keeping the body intact together and acts as a barrier to the surrounding. Skin covers the whole body. It consists of layers, including the epidermis, dermis, and subcutis (Trommer & Neubert, 2006). These layers are built of other layer components. The primary components of the dermis are collagen, elastin, blood vessels, nerves, and sweat glands. Collagen gives the skin its tensile strength, while elastin provides elasticity. Blood vessels supply nutrients to the skin, and nerves play a role in skin sensation. Sweat glands regulate temperature and protect against harmful bacteria. Overall, these components work together to maintain skin health and function (Losquadro, 2017). The epidermis comprises a Hydrolipid film, Stratum corneum, Stratum granulosum, Stratum spinosum, and Stratum basale. Stratum corneum serves the function of the barrier due to its physicochemical composition (Jiao *et al.*, 2022). Keratins are fibrous proteins that are the main structural component of keratinocytes, which are the cells that make up the stratum corneum layer of the skin. There are many types of keratins, divided into type I and type II based on their charge. Keratins are expressed differently and play important roles in maintaining the skin barrier functions of reducing water loss, maintaining permeability barrier, and resisting physical and mechanical damage(Jiao *et al.*, 2022). Schematic of the structure of skin, adapted from (Gaur *et al.*, 2017) is given in Figure 2.1.

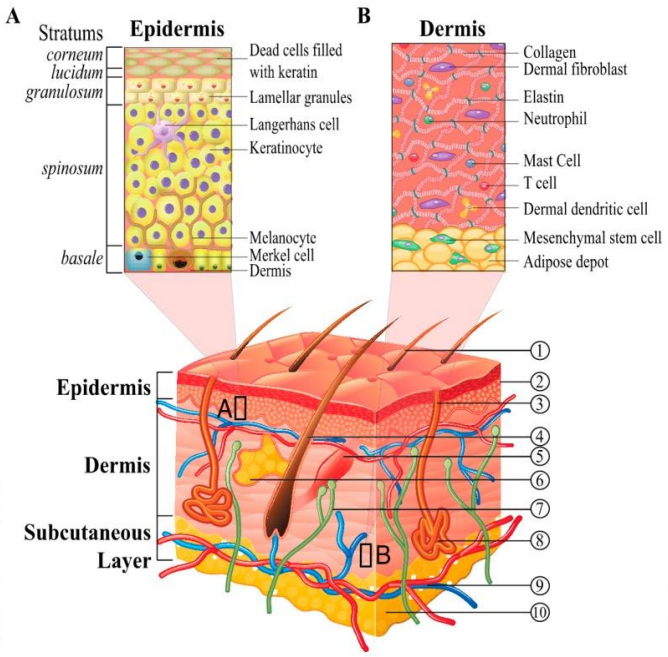

Figure 2.1 Schematics of structure of the skin

2.2. TRANSDERMAL DRUG DELIVERY

Drug delivery includes the physiochemical technologies that control the delivery and release of pharmacologically active substances to the body, i.e., to cells, tissues, and organs, so that these drugs can have the appropriate effect desired from them. Efficient drug delivery systems should have proper routes of administration along with the drug formulations, which would not have any side effects but will have maximum therapeutic efficacy (Figure 2.2). There are various ways of delivering these drugs, such as lung inhalation, oral and mucosal administration, transdermal administration, and intravenous injection (Jeong *et al.*, 2021). The most convenient way of delivering drugs through the skin is to have direct administration using needle-based injections, which are invasive. Transdermal drug delivery systems (TDDS) are being touted to allow minimal invasive drug delivery into the skin and, eventually, the body. TDDS has improved upon the delivery of therapeutic agents in pain management. TDDS, when done through appropriate methods, could involve minimal pain and little waste generation compared to needles. Since TDDS is a direct method applied to a specific part of the body, it does not need to pass through the gastrointestinal tract, and thus, there is no interference from enzymes or microorganisms in the intestine (Akhtar *et al.*, 2020).

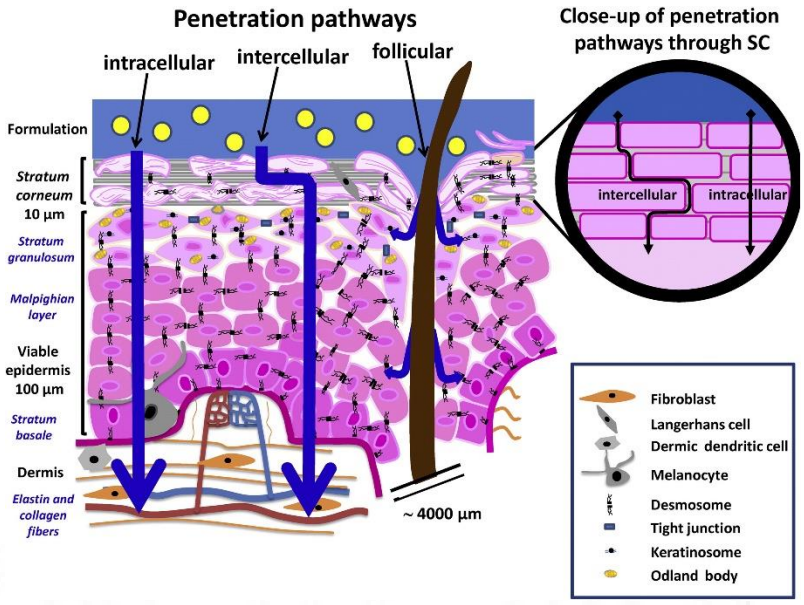

Figure 2.2 Sketch of the three penetration pathways: intracellular, intercellular and follicular. The upper right inset is a stratum corneum close-up showing the intracellular and tortuous intercellular pathways **(**Bolzinger *et al.*, 2012)

2.3. CONCEPT OF MICRONEEDLES

The most common way to deliver drugs is using a transdermal delivery of the drug, i.e., a syringe which is certainly not the best experience for them. The pain and swelling which subsides also pose a big issue in using these syringes. Not only children but people of various ages and in situations where the unavailability of syringes is a problem needs to be looked at as an opportunity to develop a new painless way to deliver drugs transdermally (Amorij *et al.*, 2010). Microneedles (MN) offer a solution to all these problems.

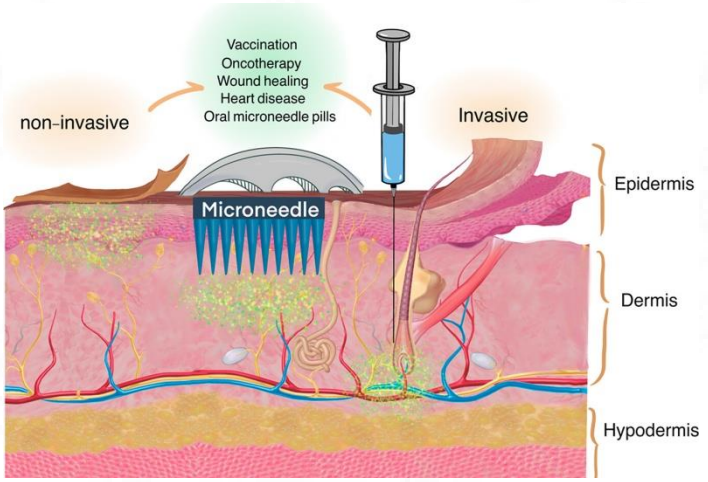

Figure 2.3 Working concept of microneedles (Zhou et al., 2023)

Microneedles pierce the topmost dead layer of the skin, i.e., stratum corneum and thus enabling them to release the drug/substances in the bloodstream (Figure 2.3). The length of the MN can range from 50µm to 900µm, which will not activate the sensory neurons and transfer the pain sensation to the brain (Donnelly *et al.*, 2010). After the application of MN, they would degrade and clear off from the body, thus making them biocompatible and relatively safe to use. Various types MN are available for application, such as solid, hollow, coated, hydrogel forming and biodegradable, and they release drugs in different ways. Mainly the interest would be in biodegradable, rapidly dissolving MN containing drugs but making them will require a specific polymer or a polymer composition. This composition should be able to encapsulate a drug that can be mixed homogenously with the polymer solution, make needles that could puncture through the upper layer of the skin and rapidly degrade over time and have the instant release of the drugs. Microneedles offer a painless, noninvasive, non-waste generative and possibly staff less way of delivery to the patient. Mostly lipophilic drugs with molecular weight less than 400-500 kDa can be delivered transdermally which are thus targeted drugs for delivery through MN array.

2.4. MICRONEEDLES FABRICATIONS PROCESSES

2.4.1. Micro-molding

One of the easiest ways of forming microneedles is via micro-molding (Figure 2.4). This method uses ready-made molds of silicon, PDMS, or metal molds to create microneedles.

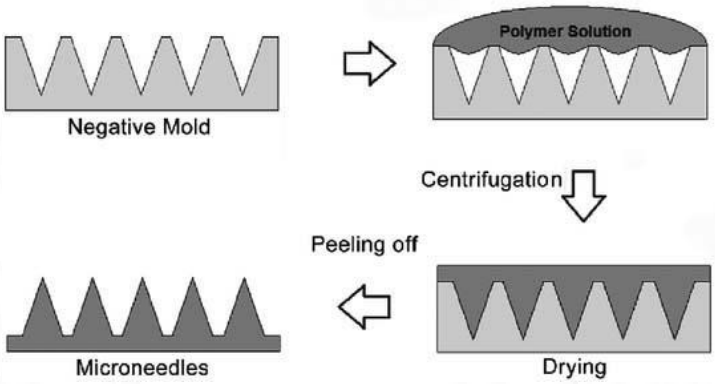

Figure 2.4 Schematics of micro-moulding (Bolzinger et al., 2012)

They have dedicated holes of different shapes and sizes, like pyramidal or circular, and of different depths, which will become the heights of the MNs. These molds play a crucial part in forming needles. The melt solutions of various materials, like silicon,

polymers, etc., are filled into these molds in vacuum or temperature-controlled conditions to fill and dry them appropriately. The schematically depicts the micro molding process.

2.4.2. Lithography

Drawing lithography is an alternative method for developing 3D polymer structures directly from a 2D film surface. In comparison to other fabrication techniques, drawing lithography claims superior speed and cost-efficiency, allowing for rapid prototyping of MNs for advanced biomedical applications. In this method, the micro-pillars drew a spin-coated SU-8 substrate and cured it to establish the polymeric bridge. The drawing speed was then raised to 700 m/s to create the MNs. In order to metalize the surface of the drawn MNs, nickel electroplating was done. After that, the hollow metallic MN array was liberated by removing the drawn SU-8 MNs using SU-8 remover. (K. Lee *et al.*, 2010)

2.4.3. Injection Moulding

Injection moulding is another process used to fabricate MN arrays. In this method, the polymer pellets are fed into an injection molding machine, which injects the molten polymer into the MN moulds (Sachan *et al.*, 2021). Schematics representation of injection moulding of MNs is given in Figure 2.5.

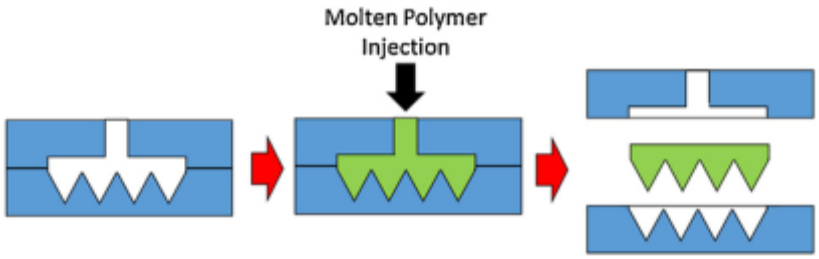

Figure 2.5 Schematics of micro-injection moulding (Bolzinger et al., 2012)

2.4.4. Additive Manufacturing

Additive manufacturing, called 3D printing, has been studied for MN formation. With the help of additive manufacturing, we can have precise control over MN design which is essential for skin piercing. Six types of 3D-printing processes can be used for the fabrication of MNs - stereolithography (SLA), selective laser sintering (SLS), digital light processing (DLP), fused deposition modeling (FDM), continuous liquid interface production (CLIP), and two-photon polymerization (2PP) (Olowe *et al.*, 2022). Most of the 3D-printing technologies available can fabricate the MN in a reproducible manner with a high resolution and quality.

The choice of material and application type will govern the type of technique which will be used for fabrication.

2.5. CLASSIFICATION OF MICRONEEDLES

Based on the type of use in delivering drug we classify the MN. They include (1) Solid MN; (2) Coated MN; (3) Hollow MN and (4) Dissolving MN. A schematic of different kinds of MNs are given in Figure 2.6.

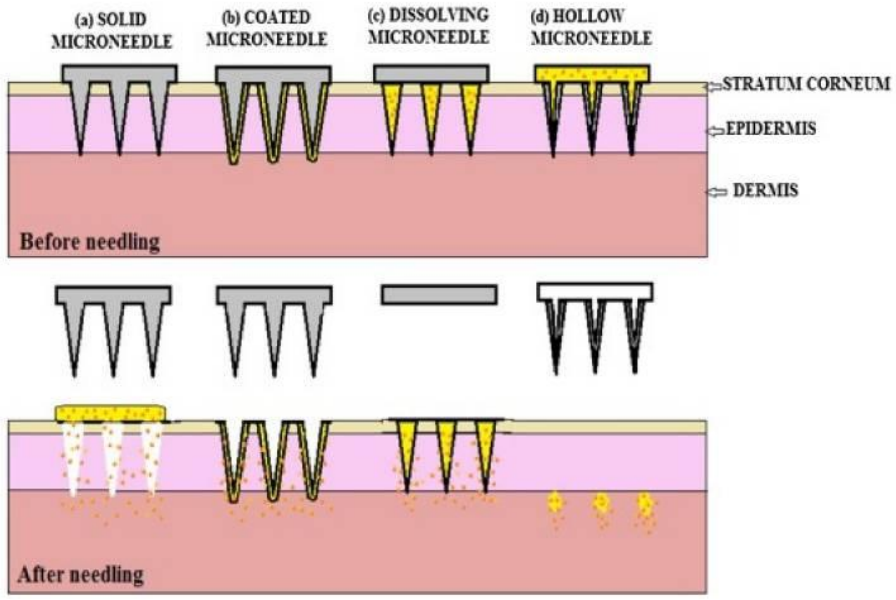

Figure 2.6 Schematics of different kinds of microneedles (Aldawood et al., 2021)

2.5.1. Solid Microneedles

This type of MN structure is designed to promote drug administration to the dermis by penetrating the stratum corneum and to increase the bioavailability and kinetic transport across the skin. This type of MNs creates channels in the skin to allow drugs reach the lower skin layer. They have adequate mechanical strength, have sharper tips, allow more drugs to pass into the skin and are easy to manufacture. The materials which have been used for fabricating solid MNs include metals (Martanto *et al.*, 2004; Matriano *et al.*, 2002), ceramics (Bystrova & Luttge, 2011) and polymers (Sadeqi et al., 2022).

2.5.2. Coated Microneedles

The coated microneedle is a solid-type MN coated with a drug solution (Gill & Prausnitz, 2007a). The drug solution can be coated by dipping MNs into solution once or multiple times depending upon dosage and sticking capacity (Kim *et al.*, 2010; Gill &

Prausnitz, 2007a),or making a roller of MNs and surface coating them each time before use (Ameri *et al.,* 2010). Multiple layer-onto-layer methods have been used to coat DNA or protein by alternatively dipping into a positively charged solution since DNA is negatively charged in nature to form a sort of multilayer (Saurer *et al.,* 2010). The coating formulations govern the method we can employ to coat the MNs. Surfactants and stabilizers can be added to coating solutions to facilitate the formulation of coating substances. Various small molecules like vitamin B (Gill & Prausnitz, 2007a) and lidocaine (Zhang *et al.,* 2012), macromolecules like insulin (Gill & Prausnitz, 2007b) and desmopressin (Cormier *et al.,* 2004), Vaccines like hepatitis B surface antigen (Andrianov *et al.,* 2009) and inactivated influenza virus (Kim *et al.,* 2010). The disadvantage of this method is that the amount that can be coated on the tip and around the MNs is minimal and hence not very feasible in the long term. The capacity of the MNs remains an issue that can be solved by making the MNs hollow from the inside to store more therapeutic substance and deliver it successfully.

2.5.3. Hollow Microneedles

Hollow MNs serve the purpose of a MN system in which the delivery of drugs and dosage can be precisely controlled. This control is achieved via the delivery system, which is usually a pressure-driven flow system of any formulation. This system helps prevent the infusion rate as a rapid, bolus, or slow. Micro-electromechanical systems (MEMS) techniques are used for the formation of hollow MN by using an appropriate material substrate, including laser micromachining. (Davis *et al.,* 2005), an integrated lithographic moulding technique (Luttge *et al.,* 2007) and wet chemical etching and micro-fabrication (Ma *et al.,* 2006). Hollow silicon MNs were fabricated using techniques like lithographic processes, wet etching, and micromachining. While those made of glass by micropipette technique drawn glass technique (Wang *et al.,* 2006), by polymer via drilling and making a bore hole, also creating the beveled tip shape and metal as they are made from hypodermic needles (Ma *et al.,* 2006). Its flow should be derived to inject the therapeutic through the hollow MN. This flow can be generated using an actuator system or syringe for better control.

2.5.4. Dissolving Microneedles

The dissolving MNs are distinct from all the other forms of MNs discussed before; the needles will dissolve during usage. To have this particular trait of dissolving, the material usually used is polymers. There are various polymers, both natural and synthetic which FDA has approved for biocompatibility for human use. The easy availability of polymers and the

way their properties can be changed by combining different kinds of polymers offers them a significant advantage. The concern for polymeric MNs arises from the scope of penetration and efficiency with which they can be produced. These dissolving MNs are usually administered using an applicator that can apply adequate pressure and hold time for the drug-loaded polymeric MNs to release and dissolve MN on the skin. The usual method of forming these dissolving polymeric MN is using solvent casting by filling the mold with the polymer melt solution mixed with the drug and letting it dry and solidify in the mold. Various polymers like Polyvinyl alcohol (PVA) (Chu *et al.,* 2010), Polyvinyl pyrrolidone (PVP) (Sullivan *et al.,* 2008) have been used, and the MN is made in a vacuum environment to get the solution into the molds to have sharper tips. The dissolving time and the sharpness of the MNs determine the time it will take for the delivery of therapeutic substances. After administration, they would give out very little waste; also, they would be patient and convenient to use. Thus, they offer the best solution among MN options available.

2.6. MATERIALS USED FOR FABRICATION OF DISSOLVING MICRONEEDLES

Dissolving MNs are primarily fabricated using polymers. These polymers are natural polymers like sugars and or synthetic polymers. The combination of various polymers has been extensively studied and performed to deliver various therapeutics. The matrix of the MN, which is made from these polymers, has a combination depending upon the drug they are to encapsulate and dissolve. Since all the therapeutics is not water soluble, it poses a challenge for dissolving polymeric MNs fabrication.

Hyaluronic Acid (HA) and PVP-K90 are used as the matrix materials for constructing MNs to deliver Propranolol hydrochloride. The use of HA and PVP-K90 provided adequate mechanical strength for skin piercing and could quickly dissolve as they came in contact with interstitial fluid when they were inserted (He *et al.,* 2021). Carboxymethyl Cellulose (CMC) was used to deliver vaccine as it could incorporate such a sensitive biomolecule because its fabrication was at 22 °C and a pH of ~6.89 and dried at near room temperature conditions. These attributes are crucial for the integrity of most vaccines (Raphael *et al.,* 2010). Chondroitin sulfate and dextran was the base polymer to construct dissolving MN to deliver protein/peptides, Recombinant human growth hormone (rhGH), and Desmopressin acetate (DDAVP). This system had 500μm length of MNs and 300μm diameters of their basements,

and feasibility studies were performed using rats for skin penetration and dissolving of MN (Fukushima *et al.*, 2011).

Sugar MNs were fabricated from sugar combinations using dehydrated sugars of formulations trehalose anhydrous (TRA), trehalose dihydrate (TRD), sucrose (SUC), maltose (MAL), mannitol (MAN), and xylitol (XYL). A model enzyme/protein was used to form these MNs, and they possessed suitable structural rigidity to puncture human skin effectively and dissolve entirely in human skin (Martin *et al.*, 2012). Fibroin MNs were made using silk fibroin harvested B. mori cocoons and served as a protein matrix to incorporate different methylene blue (MB) concentrations while maintaining the drug activity. The fabricated fibroin had dimensions of MNs as 500 μm length, base diameter as 200 μm, and radius of the tip as 5 μm. The fabricated MNs were able to penetrate the porcine skin, and the fibroin matrix dissolved within minutes to release the drug (You *et al.*, 2011). Maltose was used as a substrate for fabricating biodegradable MNs, which will dissolve in 10 minutes. These MNs could easily be disposed of after application by dissolving in the (boiled) water. Ascorbate, sodium salicylate, and calcein were used as therapeutics with a limit of 5–10 weight percentages for maintaining the strength of the MNs. They also performed a clinical study with ten patients to check the pain index according to The International Contact Dermatitis Research Group (ICDRG) criteria. All patients reported no pain score. (Miyano *et al.*, 2005)

Polylacticglycolic acid (PLGA) MN matrix was used to make MNs that used CMC or Polylactic acid (PLA) microparticles to encapsulate drugs. They determined that less than 10% of drug loadings are essential for effective microneedle design to maintain needle mechanical strength (Park *et al.*, 2006). The combination of PVA as a solubilizer and PVP for delivering Meloxicam with Phosphate Buffer Saline (PBS) as a buffer was used to form MNs. They achieved high axial needle fracture force enough to pierce the skin, and the patch could dissolve and deliver the entire amount of the drug in under 60 minutes (Amodwala *et al.*, 2017).

2.7. APPLICATIONS OF DISSOLVING POLYMERIC MICRONEEDLES

Dissolving polymeric MNs has been studied extensively for the delivery of therapeutics. These are small molecules, macromolecules, proteins, enzymes, antibodies, DNA, vaccines, etc. The critical part of this delivery is to encapsulate these therapeutics so that they are still biologically active while being delivered. However, the mechanical strength of the individual needle and the whole patch remains viable enough to puncture the human

skin. The small molecule drug delivery is most desirable in the case of MNs. The properties of less storage and, thus, lesser concentrations make a case for suitability for small drugs and vaccines

2.7.1. Vaccine Delivery

Dissolving MNs can be easily used; they lack any pain during the application, and their self-dissolving nature and no facility for reuse with ease of transport presents an attractive option for the delivery of vaccines. Dissolving MN only targets the uppermost skin layer; thus, they directly target the skin antigen-presenting cells thus, facilitating improved vaccine efficacy. The use of dissolving MN also will not require any special staff training compared to the other hypodermic needles or syringe delivery methods. Vaccines are given at a very young age to babies to protect them from various infectious diseases. Nevertheless, administration and pain in infants are complex and result in a painful procedure, leaving certain kinds of swelling and inflammation around the skin where needle administration occurs. DMNs would quickly subside in this case as well. Sullivan group in 2010 showed the first successful vaccination with dissolving MN. In the study, dissolving MN contained 3 µg of lyophilized inactivated influenza virus vaccine and was fabricated using polyvinyl pyrrolidone, with needles in a height of 650 µm. The dissolving MNs were tested on the mouse skin and had a dissolving time of a few minutes by insertion with hand. The study also compared that the immune responses elicited from the dissolving MNs were greater than the Intramuscular vaccination with the same dosage (Sullivan *et al.,* 2010). A dissolving MN was also developed for HIV, and it was designed to rapidly dissolve and deliver a stable trimeric recombinant HIV-1 CN54 clade C gp140 envelope protein. The material used in this case was a combination of Gantrez® AN-139 with PBS at pH 7.4 and Polysorbate 80. It was determined in the study that initial priming with DMNs and then the boost with Intranasal vaccination would elicit an immune response similar to the subcutaneous injection system (Pattani *et al.,* 2012). The vaccine delivery, when done on a large scale with dissolving MN, could be beneficial. Mass vaccinations could be combined with intranasal or oral administration reducing the cost, time, and (Lee *et al.,* 2020) waste management required by transdermal or subcutaneous administration.

2.7.2. Drug Delivery

Delivery of drugs, in general, is achieved via oral administration. After oral administration, it goes through the gastrointestinal tract before it gets absorbed in the body

and thus loses some of its effects. Many drugs are needed to be delivered to a particular area or spot. This cannot be achieved via oral administration. Only a transdermal syringe could achieve this effect, which is quite painful as many drugs are lipophilic. Thus, an effective way of delivering these lipophilic drugs to the body is via dissolving MNs. Various people have researched drugs that could reduce pain and inflammation, which are very specific to parts of the body. In this way, the dissolving MNs could fulfill the exact purpose of delivering drugs at a specific location.

Lidocaine is known for its local anesthetic activity in superficial and invasive procedures. Lee *et al.,* 2020 prepared to dissolve MNs composed of CMC which contained lidocaine prepared using centrifugal lithography. Lidocaine dissolving MNs were fast, and the anesthetic efficacy was higher than commercially available topical anesthetic agents. Insulin is among the most commonly administered drugs among patients with type 1 and 2 diabetes. Kim *et al.,* 2020 and his team have developed dissolving MN composed of a CMC shell with insulin powder inside its hollow core. Loading drug in such a way allowed them to load twice the amount of insulin than directly mixing CMC and insulin. The bioavailability of insulin in *in vivo* conditions remained similar for both cases. The following studies show us how dissolving MNs have recently improved in different areas as researchers are developing newer technologies and ideas for the delivery system to be more efficient and working.

2.8. STRATEGIES EMPLOYED FOR ANALGESICS ADMINISTRATION

Analgesics are medications used in the management and treatment of pain. They are broadly divided into Nonopioid analgesic agents (acetaminophen, nonsteroidal anti-inflammatory drugs, antidepressants, antiepileptics, local anaesthetics) and opioid analgesic agents (Vardanyan & Hruby, 2006).

2.8.1. Conventional Strategies for Analgesic Administration and their Limitations

Presently, most of the analgesics are administered orally. Oral administration of analgesic is simple and noninvasive and has shown good efficacy in most cases, with high patient acceptability. Vomiting or delayed gastric emptying are a contraindication to oral administration. Indeed, if the oral route is used postoperatively before the return of normal gastric motility, the “dumping effect” can occur, i.e., accumulated doses may enter the intestine at the same time once emptying resumes, resulting in a sudden large systemic uptake of analgesics with an increased risk of adverse events. The type of analgesic formulation (suspension or tablet) and the type of preparation (immediate or slow release) can affect

intestinal absorption and bioavailability, which in turn depends on the effects of first-pass hepatic metabolism of the different drugs. Intravenous (IV) route is another effective way of a analgesic administration. The IV route is the fastest route of administration of analgesics; however, the costs of intravenous opioids, paracetamol, or NSAIDs are higher, with no benefit in terms of pain control. Therefore, the IV route of administration should be used only if the oral route is not possible.

Transdermal (TD) route another strategy of administration and is used to describe drugs that, when applied to the skin, are systematically absorbed and have predominantly central effects. However, the onset and offset times of this preparation are slow, and this makes them less effective. Highly fat-soluble drugs can be administered through a transmucosal route, bypassing first-pass hepatic metabolism and rapidly entering the systemic circulation. Transmucosal routes include sublingual, buccal, intranasal, and rectal. Epidural analgesia has become a globally adopted choice for acute pain treatment in surgical settings, above all for labor of childbirth. Epidural analgesia is more efficacious than IV opioid analgesia

2.8.2. Proposed Strategy for Analgesic Administration

This thesis attempts to explore using MN technology as a delivery platform for the administration of a model analgesic namely Meloxicam. Meloxicam (MX), a non-steroidal anti-inflammatory drug (NSAID), exhibits efficacy for reducing pain and inflammatory symptoms and has definite activity in treating osteoarthritis, rheumatoid arthritis, and ankylosing spondylitis. Although MX has relatively low toxicity, oral administration is also related to frequent gastrointestinal side effects, such as gastrointestinal perforation and ulceration. Therefore, an alternative route of administration for MX is needed to avoid the disadvantages related to the existing oral medications. Drug delivery through the skin may be a good choice, which provides an advantage of delivering drugs directly to the disease site, resulting in maximal local effects with minimum systemic activity. Most topical NSAIDs have been shown to present more safety and tolerability than their oral formulations. However, few transdermal preparations of MX are available in the market. Meloxicam is known for its low permeability through the skin. Additionally, administration of MX through skin is extremely painful for the patients due to the high lipophilicity of the drug. In this scenario, we propose to explore the possibility of developing a rapidly dissolving MN patch that can not only incorporate the highly lipophilic drug MX, but also deliver it to the blood

rapidly. It is expected that the novel MX loaded MN patch will address the low MX skin permeability and improve its bioavailability.

2.9. OBJECTIVES

Following were the objectives of the present study:

1. Formulate a novel polymer blend solution for fabrication of dissolving microneedles
2. Fabrication of microneedle patches with optimized polymer blend solution
3. Characterization of microneedle patches fabricated from optimized polymer blend solution

CHAPTER 3

MATERIALS AND METHODS

3.1. MATERIALS

Polyvinyl pyrrolidone (PVP) (Mw) 10 kDa), Polyvinyl alcohol (Mw) 85-124 kDa) (PVAH), Polyvinyl alcohol (Mw 31-50 kDa) (PVAL), Pluronic® F-127 (Mw 12.5 kDa), were obtained from Sigma-Aldrich (St. Louis, MO, USA). The PVA grades used were 99% hydrolyzed. Melonex Injection - Rx Meloxicam Injection IP 5 mg/mL was obtained for use as drug solution. All the solutions for this study were prepared in distilled water.

Microneedles were fabricated using Pyramidal MN silicone templates. A master mould consisting of 100 (10X10) pyramidal needles was purchased from Micropoint Technologies Pvt. Ltd, Singapore. Another master mould consisting 225 (15X15) pyramidal needles was purchased from Central Manufacturing Technology Institute, Bangalore.

3.2. FABRICATION OF POLYMERIC MICRONEEDLES

3.2.1. Fabrication of Blank Microneedles

Prior to fabrication of MN array patch, polymer solutions were prepared by weighing appropriate amounts of polymers into a conical flask and dissolving in distilled water (80 – 90°C) to obtain final desired polymer concentrations of PVAH (20% w/V), PVAL (20% w/V), PVP (15% w/V) and Pluronic (1% w/V, 2% w/V). These solutions were blended in various ratios to generate a range of formulations. The viscous polymer blends (~ 200 µl) thus formed was cast on MN silicone template, and vacuumed to fill in MN template cavities. The filled in silicone templates were air dried and detached from the silicone

template and stored in a dessicator until use. Prior to micro-moulding, the viscous polymer solutions were vacuumed so as to remove the entrapped bubbles if any.

Table 3.1 Composition of polymer blends

Sample code	Blend ratio	Final Polymer concentration (% w/V)
PVP	1:0	15
PVAH/PVAL	1:1	10
PVAH/PVP_1	1:10	22
PVAH/PVP_2	1:1	20

3.2.2. Fabrication of Drug Loaded Microneedles

Appropriate amounts of drug were mixed to PVAH/PVP_2 either directly or in the presence of a surfactant Pluronic F127. Polymer MNs were formed from the drug loaded solutions following the same procedure mentioned in Sec 3.2.1. The drug loaded MN patches were air dried at room temperature and stored in desiccator until use. For the sake of convenience, the PVAH/PVP_2 blend will be hereafter abbreviated as PB. The compositions of the drug loaded polymer blends are given in Table 3.2.

Table 3.2 Composition of drug loaded polymer blends

Sample code	**Pluronic (% w/V)**	**Meloxicam (% w/V)**
PB/Pl/MX_1	0.4	0.08
PB/Pl/MX_2	2	0.08
PB/Pl/MX_3	0.8	0.04
PB/Pl/MX_4	0.8	0.06
PB/Pl/MX_5	0.8	0.08
PB/Pl/MX_6	0.8	0.12

3.2.3. Rheological Analysis of Polymer Solutions

A rheometer (Modular Compact Rheometer 302 Anton Paar Germany GmbH, Germany) was used to analyze the rheological properties PB solution. An oscillatory amplitude sweep (angular frequency 10 rad/s and strain 10 –1000%), oscillatory frequency sweep (shear strain γ 10% and angular frequency 0.1–100 rad/s) were carried out at 25°C.

3.3. CHARACTERIZATION MICRONEEDLES

3.3.1. Morphology of Microneedles

The MN patches were inspected using a Leica DVM6 Digital Microscope (Leica, Wetzlar, Germany). They were mounted on a platform angled at 45º made of steel such that the visualization of the MN patch was clear and distinctly seen.

3.3.2. Mechanical Properties of Microneedles

Axial compression force was applied onto the MN patches to determine the strength of the needles. Known loads were applied onto the microneedle arrays in axial compression mode press and hold of TA.XT-Plus Texture Analyser (Stable Micro Systems, Surrey, UK). Microneedle patch was attached to the probe of the Texture analyser using a double-sided tape. The MN array was pressed against a platform at a rate of 0.5mm/sec until a maximum force of 50N was applied. Multiples samples in triplicates of different formulations were performed for mechanical test. Soon after compression, the patch was examined under the microscope Leica DVM6 Digital Microscope.

3.3.3. *In vitro* Skin Insertion Tests

Parafilm M was used as an skin alternative for MN insertion studies (Larrañeta *et al.,* 2014). Microneedle arrays were inserted into a sheet assembled in 8 layers of Parafilm using Texture Analyser. A force of 40N was applied and held for 30s as shown in Figure 3.1. After insertion of the MN array Parafilm sheet unfolded and the impressions on the sheet were examined under Leica DVM6 Digital Microscope.

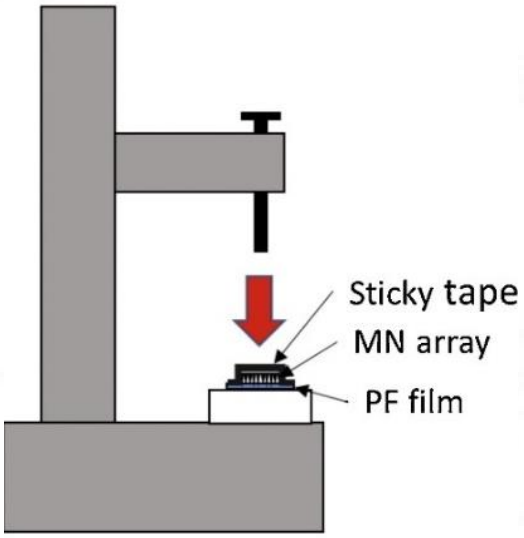

Figure 3.1 Schematics of experimental setup of microneedle array during the skin insertion test (Larrañeta *et al.,* 2014)

3.3.4. *In vitro* Dissolution Tests

Microneedles patches were gently placed on a thin layer of water taken in a petri-dish, so that they floated on the water. The MN patches were kept in contact with water for various

time intervals (2, 10, and 20 min). The images of the MN patches at the end of each time intervals were captured using Leica DVM6 Digital Microscope.

3.3.5. Drug Release from Microneedles

The dissolution test was performed by immersing the microneedle patch in a petri dish filled with distilled water (Figure 3.2). 3 mL of distilled water was put in the petri dish of size 35 mm diameter at top x 17.3 mm deep. The patch was placed on the water surface in such a way that only the needle portion is immersed into the water. After 10 minutes interval the patches were removed from the petri dish (Champeau *et al.*, 2020). They were observed and imaged using the Leica DVM6 Digital Microscope. The water solution was used for spectroscopy analysis. The concentration of drug from the water solution samples was determined by using UV-spectrophotometer at a λmax of 363 nmm.

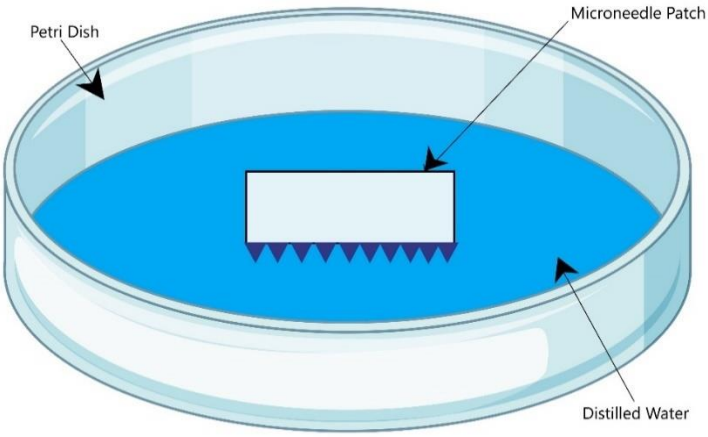

Figure 3.2 Schematics of dissolution test with microneedle patch immersed in distilled water

CHAPTER 4

RESULTS AND DISCUSSION

4.1. FABRICATION OF POLYMERIC MICRONEEDLES

The focus of this study was to develop a delivery platform based on MNs for the delivery of analgesics. Analgesics are medications that relieve pain. So they need to be available in the blood immediately after application so that the patient feels the comfort. The selection and design of the polymers and polymer blends used in this study was based on this logic. Different grades of PVA and PVP were used to create various formulations of polymer solutions. It has been reported that PVA and PVP share a high degree of miscibility owing to their ability to interact through hydrogen bonding between hydroxyl groups of PVA and carbonyl groups of PVP and reported to be miscible at 25°C and 37°C. (Teodorescu *et al.,* 2018).

Four compositions of polymer solutions, namely PVP, PVAH/PVAL, PVAH/PVP_1, and PVAH/PVP_2 (referred to as PB) were used for the fabrication of MN. A lower molecular weight (10 kDa) PVP was selected based on the literature Lee *et al.,* 2015, so that rapidly dissolving MN patches could be produced. These authors prepared MN from various combinations of low molecular weight PVP for the transdermal delivery of insulin. However, our studies showed that MNs could not be fabricated with this material even at concentrations as high as 20% w/V. The patches were rather too sticky and did not come out from the micro-moulds even after lengthy drying procedures.

Various polymer solution blends of PVP and PVA were created and MN patches were fabricated. Images of MN patches fabricated using PVAH/PVAL, PVAH/PVP_1 polymer blend solution are shown in Figure 4.1. It can be seen from the figure that the MNs formed were either fragile or deformed. The MNs formed from the PVA blend (PVAH/PVAL)

seemed stronger when compared to those formed from the PVAH/PVP_1. This is expected as PVAH used in this composition has a higher molecular weight and hence imparts better mechanical stability to the needles.

The MNs fabricated from PVAH/PVP_1 seems really fragile. This is expected as the entity imparting stability (PVAH) forms only one part of the polymer composition, hence the effect of higher molecular weight on the stability of MN is diminutive.

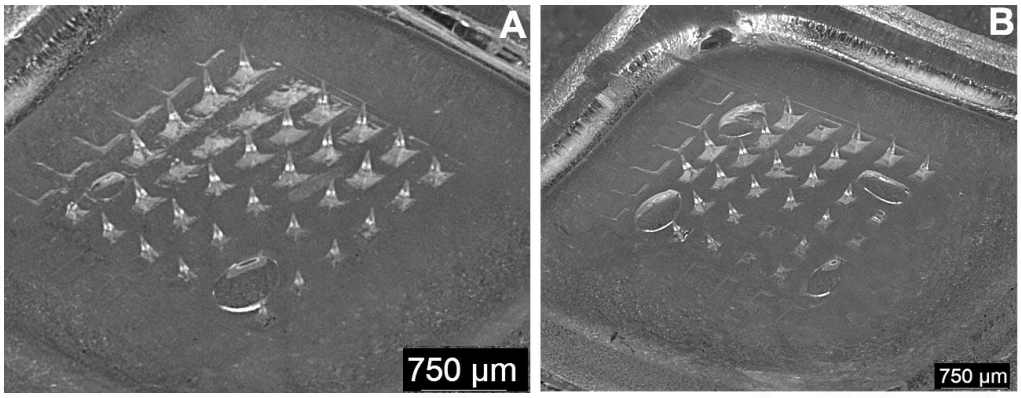

Figure 4.1 Morphology of MN fabricated from (A) PVAH/PVAL and (B) PVAH/PVP_1

Figure 4.2 shows the morphology of MN fabricated from PB. It can be seen that the MNs and base substrates were extremely smooth and transparent. For all polymeric materials tested, the needle tips were uniformly sharp for each needle in the array. The MNs fabricated from PB polymer blend solutions consisted of either polymer in equal ratios. The higher molecular weight of PVAH resulted in a viscous polymer solution. It has been documented in literature that MNs fabricated from polymers with higher molecular weights displayed better appearance and mechanical properties.

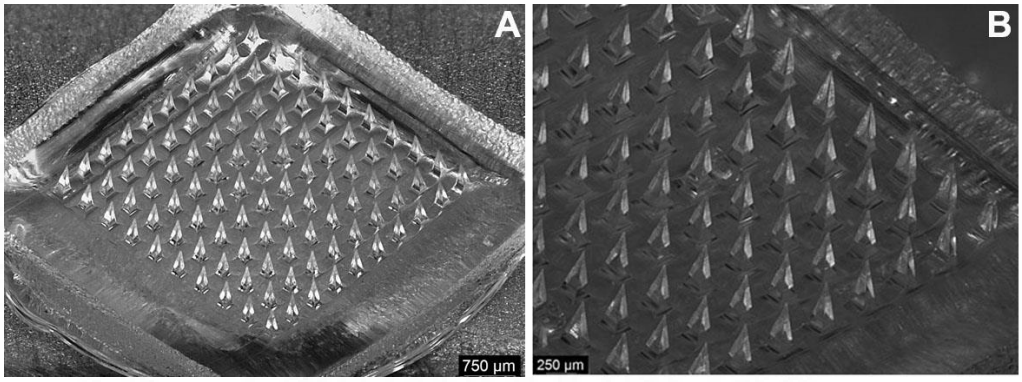

Figure 4.2 Morphology of MN fabricated from (A) PVAH/PVP_2; (B) PVAH/PVP_2@higher magnification

Fabrication of MN patches with larger sizes is a challenge. This study attempted to fabricate larger MN patches by casting the polymer blend solutions (PB) on a silicone MN

template containing an array of 225 MNs (15X15). The morphology of larger array of MN (Figure 4.3) confirmed that PB solution could be used to fabricate MN arrays with more number of needles. Both the MNs and the base substrate were smooth and clear. The MNs formed appeared very sturdy.

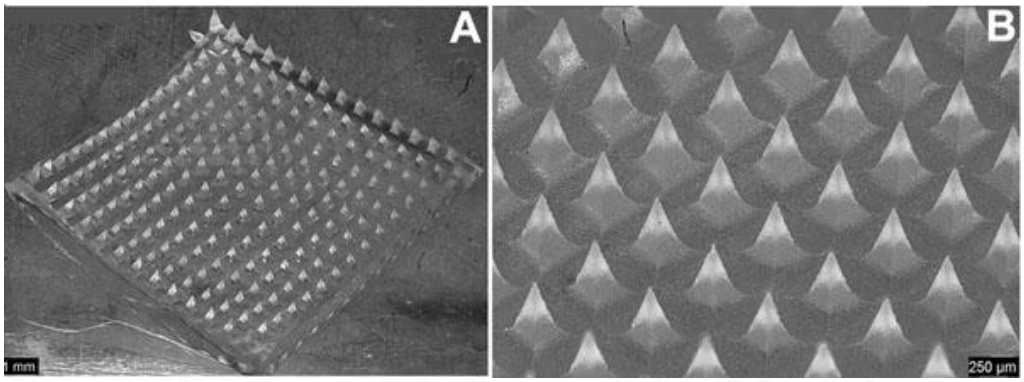

Figure 4.3 Morphology of an array of 225 MN fabricated from (A) PVAH/PVP_2; (B) PVAH/PVP_2@higher magnification

These observations confirmed that PB possessed the optimum polymer concentration which enabled the formation of sturdy and pointed MN arrays, irrespective of the size of the patch and the processing parameters employed. This polymer blend was thus subjected to further detailed analysis.

4.2. FABRICATION OF DRUG LOADED MICRONEEDLES

Drug loaded polymeric MNs were fabricated using PB solutions. Meloxicam, an analgesic used to relieve pain, tenderness, swelling, and stiffness caused by osteoarthritis (arthritis caused by a breakdown of the lining of the joints) and rheumatoid arthritis (arthritis caused by swelling of the lining of the joints) was used as the model drug. Meloxicam is a hydrophobic (lipophilic) drug; hence incorporation of MX into a hydrophilic matrix (PB) was challenging. Fabrication of MX loaded MNs have been reported in the literature (Amodwala *et al.*, 2017). The literature reported addition of MX directly to the polymer solution, which was then subjected to centrifugal casting. The adaptation of this protocol did not yield any MNs on the patch (Figure 4.4).

In order to enhance the solubility of MX in PB solution, Dimethylsulfoxide (DMSO), which is reported as an excellent solvent to MX was added to PB solution. Addition of 50µl of DMSO resulted in clear PB/MX solutions. The solutions were cast on 10X10 array mould following optimized procedures. This attempt also did not yield an MN array (Figure 4.4b).

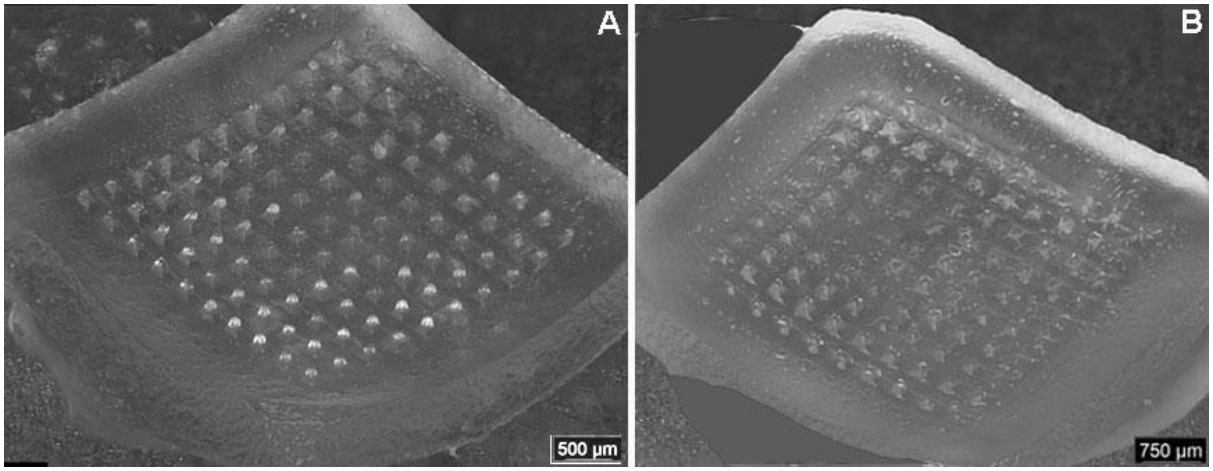

Figure 4.4 Morphology of an array of MN fabricated from (A) PB/MX; (B) PB/MX_DMSO

Another strategy employed was use of surfactant to improve the dissolution of MX in hydrophilic matrix. Basak and Bandyopadhyay reported use Pluronic F127 for the encapsulation of hydrophobic drugs to improve the solubility (Basak & Bandyopadhyay, 2013). Another researcher Rarokar *et al.*,2021 used Pluronic F127 to encapsulate MX to increase the solubility of the drug in the solution. In this study six different formulations of polymer solution containing drug (PB/Pl/MX_1, PB/Pl/MX_2, PB/Pl/MX_3, PB/Pl/MX_4, PB/Pl/MX_5 and PB/Pl/MX_6) were prepared and MN patches fabricated. The morphologies of the fabricated MN patches are given in Figure 4.5. It can be seen from the figure that all the formulations with various concentrations of drug and Pl resulted in the formation of MNs.

Formulation PB/Pl/MX_1 contained the lowest amount of Pl (0.4 % w/V) and PB/Pl/MX_2 had the highest of Pl concentration (2 % w/V); for a MX concentration of 0.08 % w/V. The MN patch fabricated from PB/Pl/MX_1 resulted in the formation of MN with sharper tips (Figure 4.5a), while fabrication using PB/Pl/MX_2 resulted in MNs with blunt needles. Among the many factors, viscosity of polymer solutions plays a vital role in the formation of MNs. Generally, a viscous solution results in the formation of sturdy MNs. However, if the viscosity increases several folds chances of formation of mechanical strong and morphologically smooth MNs low; unless specialized fabrication techniques are applied. It has been reported that in the presence of a surfactant, the viscosity of the polymer solution may be increased several times by micelles if weak attraction between a polymer segment and a surfactant exists (Wang *et al.,* 2004). For a Pl/PVA polymer blend, at certain temperature and composition of polymer mixtures they could behave either as viscous fluids and they exhibit solid-like properties (elastic hydrogels) (Bercea & Morariu, 2017).

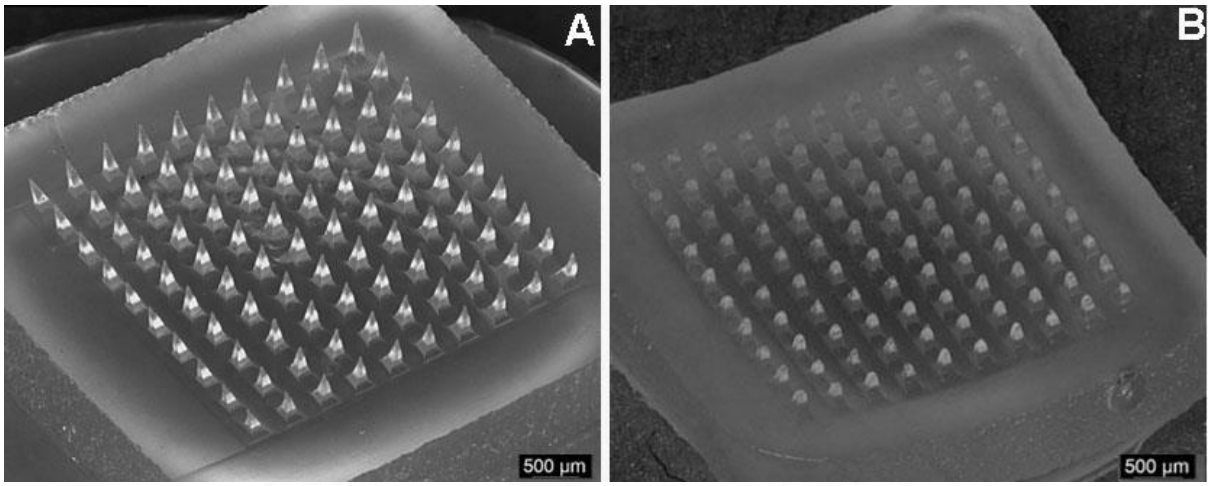

Figure 4.5 Morphology of an array of MN fabricated from (A) PB/Pl/MX_1; (B) PB/Pl/MX_2

This aspect, however, needs further investigation, wherein parameters such as polymer-polymer interactions, transition from the sol state to soft or hard hydrogel and the thermodynamics of the solution should be looked into. Polymer compositions PB/Pl/MX_3, PB/Pl/MX_4, PB/Pl/MX_5 and PB/Pl/MX_6 also yielded MN arrays with sharper needles. Formulation PB/Pl/MX_6 had the highest concentration of MX; hence a MN patch containing an array of 225 MNs (15X15) was also fabricated (Figure 4.6). It could be seen that this composition also resulted in patches with sharper MN tips.

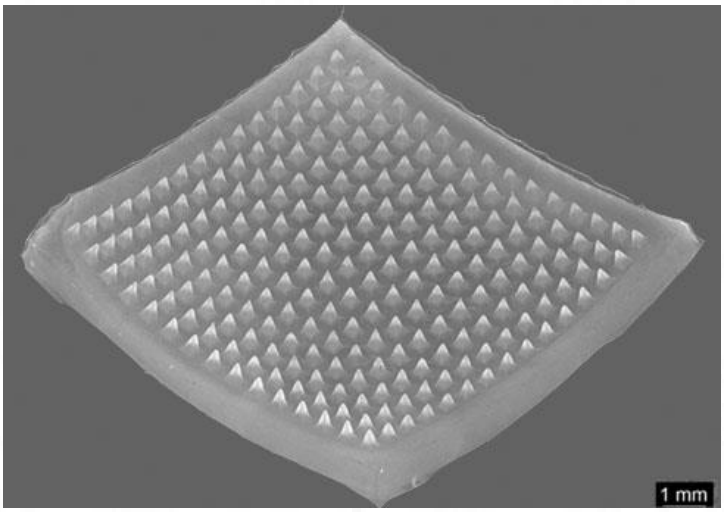

Figure 4.6 Morphology of an array of MN fabricated from (PB/Pl/MX_6)

4.3. RHEOLOGICAL PROPERTIES OF POLYMER SOLUTIONS

The rheological properties of PVAH/PVP_2 solution were studied to evaluate the viscosity and physical characteristics (Figure 4.7). The variation of storage and loss modulus against shear strain is shown in Figure 4.7a. It could be seen that as the strain increased, storage modulus decreased. The storage modulus G' represents the elastic portion of the viscoelastic behaviour, which quasi describes the solid-state behaviour of the sample. As the shear strain increases, the polymer chains get aligned to the direction of shear, which eventually leads to a decrease in elastic portion.

The loss modulus G" characterizes the viscous portion of the viscoelastic behaviour, which can be seen as the liquid-state behaviour of the sample. In this study, the values of loss modulus are higher than that of the storage modulus. The PVAH/PVP_2 was dominated by viscous portion, indicating that it the MN patches fabricated from this composition would be easily soluble in a solvent (water). The viscosity of this solution was sufficient enough to fill in the moulds well.

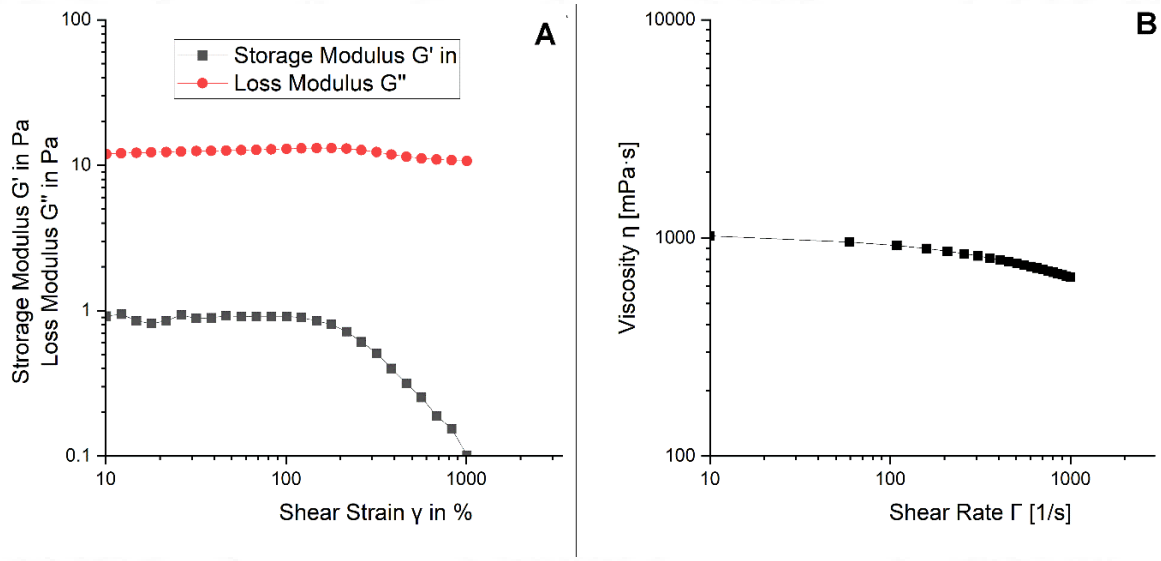

Figure 4.7 Rheological characteristics of PVAH/PVP_2 solution

4.4. MORPHOLOGY OF MICRONEEDLES

The morphology of MNs is shown in Figure 4.8. The MN were pyramidal shape with sharp tips at the end. It had a slant height of 488µm, vertical height of 388µm and a base of 180µm.

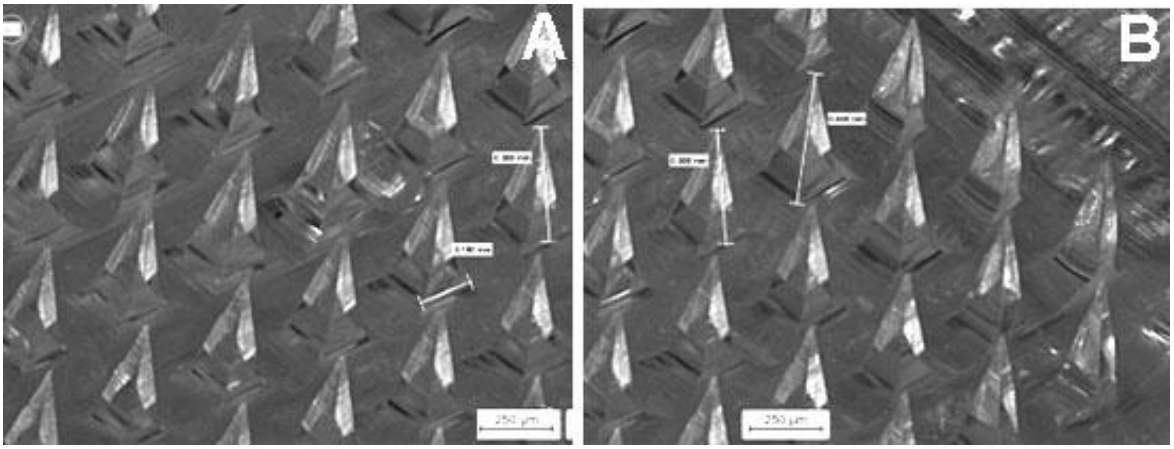

Figure 4.8 Dimensions of the MNs showing (A) base length and vertical height; (B) slant height and vertical height

The morphology and dimensions of MN of the 15×15 array (Figure 4.9) had dimensions of slant height of around 500µm, vertical height 600 µm and a base length of 345µm.

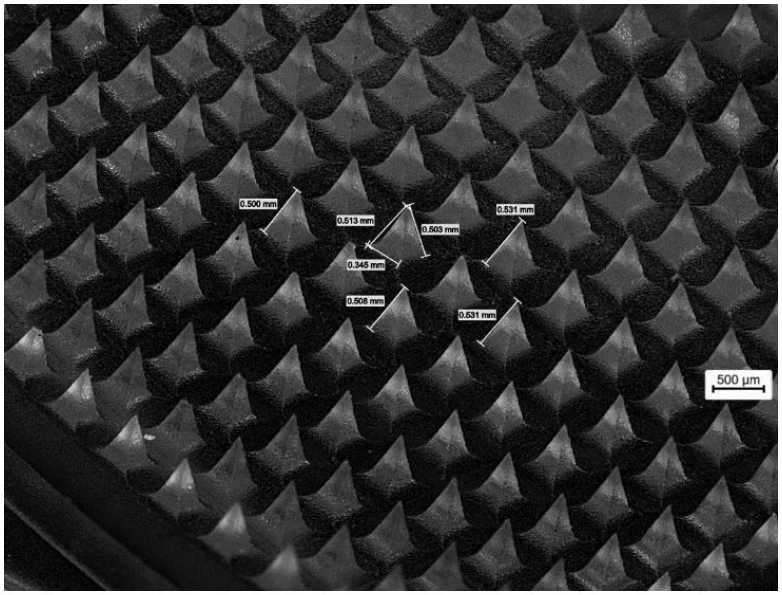

Figure 4.9 Dimensions of the MNs showing the base length and slant height

4.5. MECHANICAL STRENGTH OF MICRONEEDLES

In the practical use of MN, one of the most critical problems is the MN tip fracture during application. The mechanical properties of the MN were determined to assess if the fabricated MNs were strong enough to penetrate the skin. Several factors such as the material composition, geometry and aspect ratio are found to affect the mechanical strength of MN (Lee *et al.,* 2015).This study uses MNs with pyramidal geometry as they are considered to mechanically robust owing to their larger cross-sectional area at the same base/diameter in comparison to MNs with conical geometries. It has been reported by Larrañeta *et al.,* 2014 that the force applied by the volunteers using their thumbs ranged between 10-50N and 32N, being the maximum average force exerted by the volunteers for microneedle application. In line with these reports, in this study a force of 40N was employed continuously for 30s (Figure 4.10a), which is the time recommended for microneedle application (Donnelly *et al.,* 2014). The MN patches were imaged soon after they were subjected to compression. It was seen that the MN patches did not fracture and retained their original morphology and dimensions. They also did not undergo any permanent deformation (Figure 4.10b), confirming the mechanical stability of the MNs. A mechanically robust MN patch is easy to handle and apply.

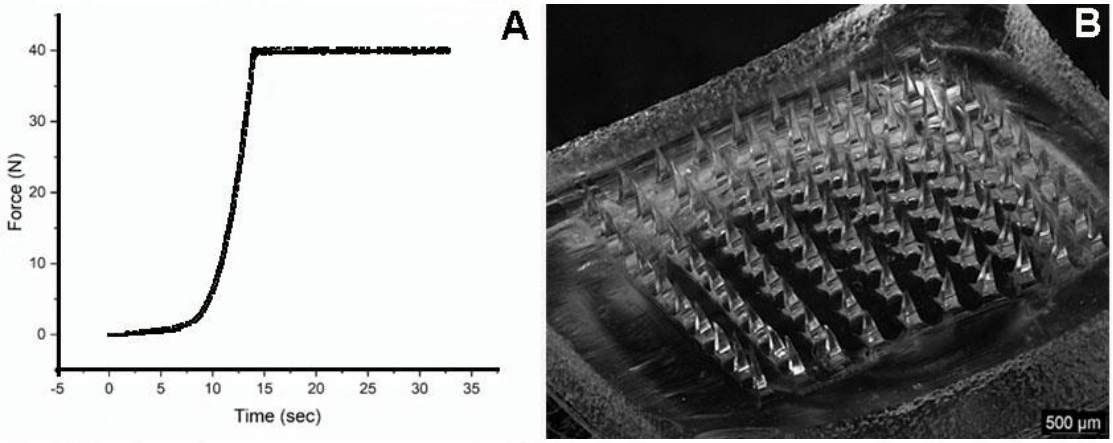

Figure 4.10 (A) The variation of force with time when the MN patch was subjected to compression; (B) Image of the patch after compression for 30s

4.6. *IN VITRO* SKIN INSERTION TEST

In order to examine if the fabricated MNs were sturdy enough to penetrate the skin, the MN patch was inserted into a Parafilm (skin model). Microneedle patches fabricated from PVAH/PVP_2 was pressed on to the Parafilm with a force of 40N. It was seen that the MNs penetrated the skin model reliably and left penetration marks (Figure 4.11). The penetration marks were square in shape confirming the pyramidal shapes of the MNs. The marks appeared deeper towards the edges than the center. This could be due to the non-uniform distribution of the applied force. The experiment was conducted on patches that were slightly warped. This would have resulted in the forces being distributed non-uniformly.

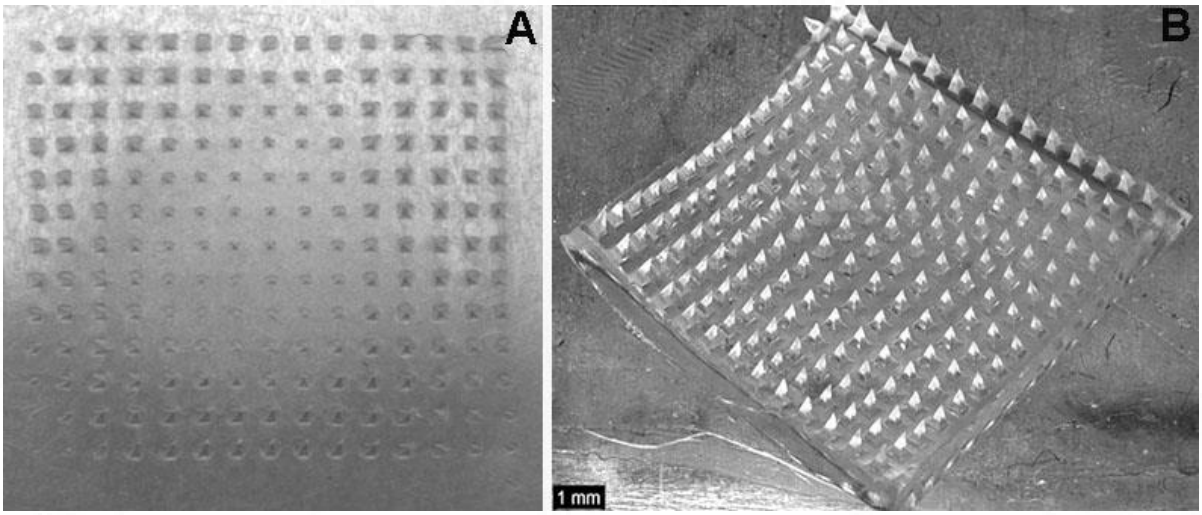

Figure 4.11 Micrographs of (A) the impression formed by MNs on Parafilm; and (B) MN patch after insertion into Parafilm

4.7. IN VITRO DISSOLUTION STUDIES AND DRUG RELEASE FROM MICRONEEDLES

One of the challenges of employing MN patches for drug delivery is to achieve the dosage accuracy comparable to that of hypodermal needles. Hence, when using drug embedded MN patches for therapeutic purposes, immense attention has to be paid so as to improve desired drug effectiveness and therapeutic safety.

The PB/MX patches with various loading of drug (meloxicam) was gently place on the surface of water in such a way that the MNs were completely immersed in water. The image of PB/MX_1 patch following immersion after 10 min is given in Figure 4.12.

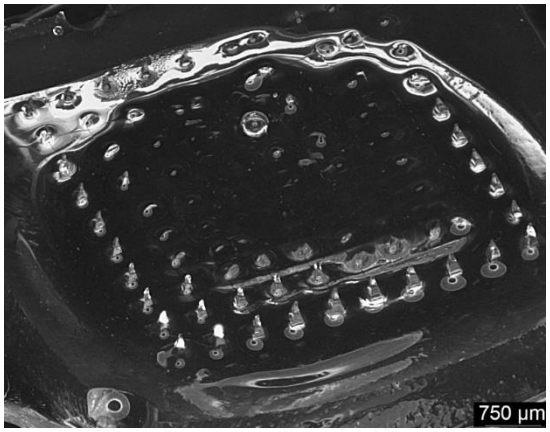

Figure 4.12 Micrographs of PB/MX_1 following immersion in water

It was observed that majority of the MNs disappeared following. It seemed that the MNs located towards the center of the patch underwent rapid dissolution that the MNs located towards the edges. This could be because; the patches when kept freely floating on a film of water had a tendency to warp towards the surface facing air. This makes the MNs in the center of the patch to be better immersed in the solvent than the MNs towards are edges. This experimental set up may not be the exact representation of the MN patches when inserted into the skin. In actual application, the patches would be inserted into the skin using an applicator and due to the inherent elasticity of the skin and the chemistry of the patches, they would remain adhered to the skin. This study however gives an indication that the MNs can readily dissolve when in contact with a fluid and thus release the embedded drug immediately.

The drugs released from the PB/MX patches with various concentration of the drugs was evaluated by sampling the drug solutions from the aforementioned experiment. For the quantification of drugs in the samples, a standard graph was plotted for various concentration MX and the concentrations of the drugs was determined by interpolation. It could be seen that amounts of drugs released from the various patches ranged from 2 – 15 µg per patch. These values may seem rather small but are significant given the various limitations of experimental parameters employed. The drug chosen MX is highly lipophilic and hence it was difficult to get them into a hydrophilic matrix. Secondly, when the MN patches were placed freely on a film of water, the patches warped outwardly resulting in non-uniform immersion of MNs. The MNs located in the centre of the patch dissolved as they completely immersed in the solvent, whereas those situated towards the edges dissolved only partially. Lastly some amount of drug was also lost in the backing plate. The researchers will further focus these aspects of the study and improve the outcoming with a better experimental model.

CHAPTER 5

SUMMARY, CONCLUSIONS AND FUTURE OUTLOOK

5.1. SUMMARY AND CONCLUSIONS

In this study, we tried to develop a formulation for rapidly dissolving MNs. These microneedles will be used for transdermal drug delivery. The drug delivery system of the transdermal route is vital for giving a direct dose without encountering any other system for drug administration. The work focused on how these microneedles can be formed in shape, size, and mechanical strength to deliver the drug to the desired location. This required the experimental formulation of various polymers chosen due to a detailed literature survey on dissolving microneedles. The polymers PVA and PVP were chosen as the base polymers for making and perfecting the protocol for the fabrication of microneedles. The fabrication method was prepared and completed after trial and error with different combinations of PVA and PVP. These combinations resulted in different kinds of needles with varying mechanical and physical properties, which were analyzed by performing the rheology of the polymer solutions. The rheology results were able to deduce the difference between the polymer combinations and give us an appropriate combination for fabrication. Fabrication of the microneedles required a specific method to accomplish the goals set for shape, size, and strength. This method, accompanied by the combinations of polymers, was crucial in forming microneedles. Size and shape were verified using a stereomicroscope.

The mechanical strength of the needles was found to be adequate to puncture human skin and deliver the drug. Meloxicam was incorporated as a drug in the formulation with direct dissolution. The solubility of Meloxicam is very low; thus, it leads to failure, and no microneedles were formed. A unique polymer Pluronic F127 belonging to the category of poloxamers was incorporated to increase the solubility of the drug in the formulation leading to the formation of needles. Combinations of F127 and Meloxicam, along with the polymer combination of PVA and PVP, were tested for the formation of needles. These combinations with varying concentrations lead to the change in properties of the strength of the microneedles. Thus, tests for mechanical strength to check for the ability to puncture the skin were performed on the Parafilm tape. The needles had dissolved and created holes in the parafilm tape when inserted. The dissolution of these microneedles was tested by dissolving them in distilled water as a solvent, and the concentration of the released drug was obtained from the microneedles.

5.2. FUTURE OUTLOOK

Microneedles have been considered an efficient but difficult-to-master device for drug delivery. They have been extensively researched to reduce the waste generated from traditional syringe needles in the past few years. The incorporation of drugs has been a challenge in the success of microneedles over the years. Thus, finding a way to incorporate any drug in the MNs would be a breakthrough for this technology. For our work, the animal studies about the drug release and eventual incorporation into the animal system would lead to the verification of MNs. Further studies may include detailed analysis on increasing the drug load of microneedles with the help of Pluronic F127 and determining the maximum drug load which can be incorporated into a patch of microneedles. This will make the product cost-efficient and deliver therapeutic doses to the patient. The kinetics for drug elution needs to be studied with an animal skin model. These tests with porcine skin will be part of future work. As the MNs will dissolve in the animal model in vivo tests, we can determine the time to deliver the drug, which will cause a therapeutic effect.

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