DESIGN AND SYNTHESIS OF A NANOCAPSULE SYSTEM FOR TRANSDERMAL DELIVERY OF LIGNOCAINE ACROSS THE SKIN

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A dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, in fulfilment of the requirements for the degree of Master of Science in Medicine in the field of Pharmaceutical Affairs.

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DECLARATION

I, Lerato Mathabelo Makha, declare that this dissertation is my own work. It is being submitted for the degree of Master of Science in Medicine in the field of Pharmaceutical Affairs in the Faculty of Health Sciences as a research component at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

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Signature

This …….15th.....day of May 2024

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ABSTRACT

Pain is a major health problem that is caused by an emotional and sensory experience due to injury of tissues and can be differentiated as acute or chronic. Pain protects humans by assisting in making us aware that there is body injury or potentially damaging situations. If acute pain is not treated correctly, it can lead to chronic pain. Pain can be treated by several classes of medications including analgesics, Non-steroidal Anti-inflammatory drugs (NSAIDs), opioids, anti-depressants, and local anaesthetics. These medicines are administered orally. Although this is the preferred route of administration, it has many inherent limitations that can negatively impact the drug's desired pharmacological effects.

Since the skin is the most convenient and accessible region for medication administration, innovative delivery techniques like Transdermal Drug Delivery Systems (TDDS) have been developed to circumvent the drawbacks of traditional drug delivery routes. This method has displayed fewer side effects experienced with conventional oral medications like gastric irritation and first-pass hepatic metabolism causing low blood concentration of the drug leading to low therapeutic effect. Nonetheless, the stratum corneum (SC), the skin's outermost layer, functions as a strong barrier to prevent most medications from penetrating the skin. Much attention has been paid to the use of nanocarriers as a transdermal delivery system of different accessible pharmaceuticals through the SC with the potential to have local or systemic effects to treat various disorders (Alexander et al., 2012).

The purpose of this report was to synthesize Lignocaine (LIG)-loaded nanocapsules to evaluate their potential to transport LIG across the skin for increased effectiveness and fewer side effects. Lignocaine-loaded nanocapsules (LIG-PLGA-PC) were prepared by employing the solvent displacement technique. Aqueous phase consisted of 200 mg of Tween® 80 in 30 ml of purified water. The mixture was ultrasonicated for few minutes then put under electronic magnetic agitation. Organic phase was prepared by dissolving 125 mg of PLGA, 250 mg of PC and 30 mg of LIG in 5 ml of chloroform. Under probe sonication, organic phase was added drop wise to the aqueous phase and was allowed to stir overnight to remove the organic solvent. The formulated nanosystem was then frozen and lyophilized (FreeZone® 2,5, Labconco®, Kansas City, MS, USA) at -80 °C for 48 hours to yield a powder and analyzed using

different analytical techniques. Four samples were prepared with three samples having a drug loaded in the organic phase. The fourth sample was blank, without the drug in the organic phase. The synthesised nanoparticles were characterized using Scanning Electron Microscopy (SEM), zetasizer, Ultraviolet (UV) spectroscopy, Fourier transform infrared (FTIR) spectroscopy and Thermogravimetric Analysis (TGA). SEM clarified the configuration of the surface and shape of the nanocapsules by beaming electrons that interacted with the sample's atoms to produce three-dimensional surface topography. Using the zeta-sizer, the size of nanoparticles was found to be around 139.8 nm and a polydispersity index (PDI) of 0.182 a while the zeta potential was -48,2 mV. A drug entrapment efficacy (DEE) percentage of 72 % was achieved. LIG release studies showed a sustained release over a 24-hour period. The FTIR spectral evaluation of drug loaded sample showed a vibration at 3000–2800 cm−1 indicating a N-H stretch, and 1750-1735 cm−1 presented with a C=O stretching group. At 1250–1020 cm−1 there is a C-N stretching confirming the presence of an amine group.

Thermogravimetric Analysis (TGA) thermal studies indicated that the LIG nanoparticulate structure increased the thermal stability of LIG. To investigate the biocompatibility of the nanoformulation, immortalized human keratinocytes (HaCaT) were treated with LIG-PLGA-PC copolymeric nanocapsules and an MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide) assay was conducted to evaluate the cytotoxicity of the treatments. Absorbance values were measured at 570 nm employing a Thermo Lab systems Multiskan MK3 microplate reader. From the cytotoxicity study conducted, at 48-hour, LIG-PLGA-PC nano formulation showed increased cell viability of 102, 103, and 110 % for 6,25 µg/ml, 3,125 µg/ml, 1,5625 µg/ml concentrations respectively. The blank nanocapsule showed a cell viability of 99, 98 and 100 % at concentrations of 6,25 µg/ml, 3,125 µg/ml, and 1,5625 µg/ml. The HaCaT cell micrographs showed cell proliferation on the drug loaded as well as plain drug figures. This can be confirmed by the MTT assay that the nanoformulation was non-cytotoxic with predicted biocompatibility.

The combined experiments and findings of the LIG-PLGA-PC nanocapsule synthesis demonstrated that further studies and investigations are required to show that these nanoparticles can be used as a possible formulation for TDDS medication delivery of LIG for pain treatment.

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LIST OF EQUATIONS

LIST OF ABBREVIATION

COX-1	Cyclooxygenase enzymes 1
COX-2	Cyclooxygenase enzymes 2
CXB	Celecoxib
DDS	Drug Delivery
DEE	Drug Entrapment Efficiency
DMEM	Dulbecco's Modified Eagle Medium
DNA	Deoxyribonucleic acid
DSC	Differential Scanning Calorimetry
FBS	Foetal bovine serum
FDA	Food and Drug Administration
5FU	5 Fluorouracil
FTIR	Fourier Transformation Infrared Spectroscopy
HaCaT	Immortalised Human Keratinocyte
LA	Local Anaesthetic
LDC	Lidocaine
LIG	Lignocaine
LIG-PLGA-PC	Lignocaine loaded nanocapsule
MTT	3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide dye
NC	Nanocapsule
NS	Nanosphere
NLC	Nanostructured lipid nanocarriers
NSAIDS	Non-steroidal Anti-inflammatory drugs
PAMAM	Polyamido amine
PBS	Phosphate Buffered solution
PC	Phosphatidylcholine
PDI	Poly dispersed index
PGA	Poly Glycolic Acid (PGA)
PLA	Poly Lactic Acid
PLC	Prilocaine
PLGA	Poly (lactic-co-glycolic acid)
RNA	Ribonucleic acid

SASA	The South African Society of Anaesthesiologists
SEM	Scanning Electron Microscopy
s1R	sigma-1 receptor
SLN	Solid Lipid nanoparticle
SC	Stratum corneum
SFPP	S-flurbiprofen plaster
T	Transmission
TGA	Thermogravimetric Analysis
TDD	Transdermal Drug Delivery
TDDS	Transdermal Drug Delivery System
UV	Ultraviolet
WHO	World Health Organization

CHAPTER 1
INTRODUCTION

1.1. INTRODUCTION:

Pain is a major public health concern that affects individuals with all severe diseases, as well as the larger community and economy (Hua and Cabot, 2013). Overcoming pain has been the major global health challenge since the dawn of humanity. The International Association for the study of pain defines pain as an "unpleasant sensory and emotional experience connected with actual or potential tissue damage" which can be categorized into physiological and pathological or clinical. and an increase in hospitalization. Indirect cost can be measured by disability-related emotional distress, and financial costs (Negash et al., 2022).

Pain management has been declared a basic human right by healthcare professionals and organizations such as the International Association of the Study of Pain, the World Health Organization (WHO), and the United Nations, and it is the responsibility of any clinician to minimize pain development and manage existing pain as best as possible (Wrona et al., 2022).

WHO released a fundamental step based starting point called analgesic ladder for pharmaceutical management of pain (Yang et al., 2020).

WHO step 1, simple analgesics: Paracetamol is a widely used first-line basic analgesic and antipyretic. It is frequently administered orally and is used to treat moderate to severe pain. Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used analgesics and anti-inflammatory medications that inhibit the cyclooxygenase enzymes COX-1 and COX-2, thereby suppressing prostaglandin (thromboxane A2 and PGE2) formation.

WHO step 2, weak opioids: For many years, the mainstay of treatment for acute pain has been opioid analgesics, which are still in use today. Accessibility of medicines is growing in numbers, each with a different potency, onset time, duration of action, administration method, and affinity of opioids to receptors.

WHO Step 3, strong opioids: A wide range of morphine formulations can be created and administered in various ways. Diamorphine is an inactive synthetic prodrug that is deacetylated to morphine and 6-monoacetylmorphine. The metabolism that is produced is like that of morphine. Fentanyl is a derivative of phenylperidine that is synthetically manufactured and is 100 times more powerful than morphine. Oxycodone, a synthetic opioid, is available as an immediate and delayed release oral forms.

Analgesic adjunct medications: A variety of pharmaceuticals are routinely utilized as opioid adjuncts in multimodal analgesic techniques. Anaesthetists frequently utilize them intraoperatively. Gabapentinoids, gabapentin, and pregabalin are the most widely prescribed adjuncts that can be used as part of a multi-modal therapy (Hay and Nesbitt, 2019). There has been continuous innovative research focusing on creating new and the most effective alternative therapeutic strategies to overcome pain (Peptu et al., 2015).

Pain is commonly managed by administration of oral and parenteral dosage forms. These methods of administration have side effects and limitations associated with them. Oral dosage forms may cause gastrointestinal side effects. The drugs may undergo hepatic first pass metabolism causing degradation of drugs that may cause low bioavailability. This can yield low therapeutic effects of a drug. The side effects associated with parenteral dosage forms are pain at the site of administration and are not convenient to administer. These disadvantages associated with oral and parenteral medication administration led to the creation of the transdermal drug delivery system (TDDS) (Jeong et al., 2021). TDDS emerged as the most effective technique of delivering drugs across the skin for either local or systemic therapeutic effects. There are several benefits associated with this route of delivery including the fact that they are comfortable, convenient to use, not harmful to body tissues, and helps with patient's compliance. TDDS lowers the concentration of a drug imbalance, maintains stable blood levels, and reduces the possibility of overdosing (Zhou et al., 2018; Sala et al., 2018).

Conventional therapies have been used in TDDS to reduce first-pass drug degradation effects and drug-related side effects (Elmowafy et al., 2017). The use of undamaged

skin as a route of administration of medication into the human body system has been under investigated for several years (Uchechi et al., 2014). Though TDDS uses the skin as the route of administration, there are limitations associated with the drug formulation. The skin, which is composed of the stratum corneum (SC), the epidermis, and the dermis, is the biggest organ. SC prevent the entrance on drugs through the skin (Alexander et al., 2012). Figure 1.1 shows the structure of the skin illustrating the SC the epidermis and the dermis.

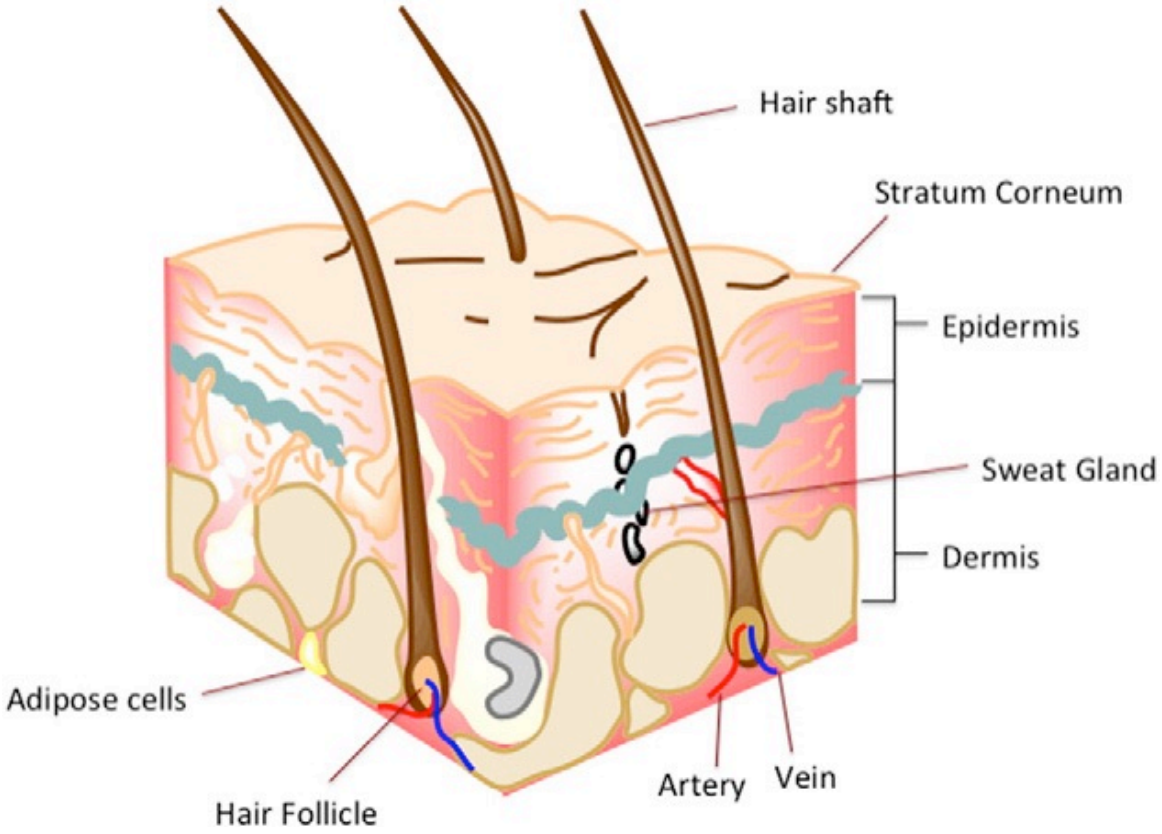


Figure 1.1 (Goyal et al., 2016) schematic of the structure of the skin illustrating the stratum corneum, the epidermis and the dermis

Dermal and transdermal delivery techniques have shown to have certain disadvantages despite their advantages over conventional topical formulations. Certain drugs are not suitable for transdermal delivery; those that need high blood levels cannot be given in this way; not all skin types can adhere to the adhesive used in patches; some drugs or drug formulations may result in skin irritation; wearing patches may be uncomfortable; the formulation may be more expensive because it requires specialized equipment; and it may not be suitable for drugs with a rapid onset

of action (Uchechi et al., 2014). As a result of these difficulties, nanotechnologically based drug dosage forms have been developed. One of the drug delivery systems likely to achieve good penetration via the skin is the use of nanoparticles or nanocarriers. Polymeric nanoparticles, lipid nanoparticles, liposomes, and other systems are some of the examples (Elmowafy et al., 2017)

Application of nanoparticle-loaded topical preparations for medical purposes has been investigated in depth in the last decade (Pitorre et al., 2017). Gels as nanocarriers provide several advantages in medicine, including drug protection, increased stability, targeting, stealthiness, decreased toxicity, controlled release, and prolonged release of the loaded-drug molecule (Pitorre et al., 2017). The use of gels as nanocarriers have been useful in the treatment of diseases like cancer and cutaneous disease (Valenzuela and Simon, 2012; Pitorre et al., 2017). Numerous delivery methods, including transdermal, buccal, intra-articular, and intravesical, were developed due to local anesthetics' systemic toxicity (Shipton, 2012). As a result, local anesthetic medication delivery has evolved (Shipton, 2012). Clinically, local anesthetics have been used in the management of different types of pain (Rogobete et al., 2016; Onuh et al., 2022).

Anesthetics have recently been delivered by innovative methods such as transdermal, oral transmucosal, sublingual, and many more (Zhai et al., 2014). The most common dosage forms for anesthetics such prilocaine, lignocaine, benzocaine, and ropivacaine include gels, ointments, and creams (Yue et al., 2018). Novel ropivacaine-loaded lipid nanocapsules were developed in which a lipid-based colloid system was used and proven to be useful for transdermal distribution in the investigation. In the study it was found that lipid-based colloid systems could be used as a transdermal delivery vehicle for ropivacaine to improve penetration (Zhai et al., 2014). Because the skin offers a convenient and wide surface area for drug delivery, scientists made enormous advancements in TDDS sphere. Aims are being made to create effective TDDS that promote longer-lasting, sustained drug release at the intended site within the suggested therapeutic window without causing adverse effects.

One of the most promising synthetic polymers was granted permission by the health regulatory authority in the United States of America, Food and Drug Administration

(FDA). The polymers were approved to be used in drug delivery systems for various drug administration routes, like transdermal drug delivery. One of the polymers was poly (lactic-co-glycolic acid) (PLGA) (Naves et al., 2017a). PLGA has been recognized as a potential synthetic polymer for many of biologicals, including the creation of effective drug delivery systems. Many different types of drugs, including hydrophilic and hydrophobic, ranging in size from tiny molecules to macromolecules, can be encapsulated in PLGA. The formulation containing the polymer can then be delivered to different locations via various administration routes such as intravenous, pulmonary, and TDDS (Naves et al., 2017; Peltonen et al., 2020).

Lignocaine (LIG) is a local anesthetic that prevents nerve conduction by blocking the stimulation of neuronal cell membranes and preventing entry of sodium ions across sodium channels (Peptu et al., 2015); Li et al., 2018). It is a safe medication that is used in various emergency medicine settings, such as topical anesthesia, local and regional anesthesia, post-stroke pain syndrome, neuropathic pain, and visceral/central pain among others (Golzari et al., 2014). LIG is chosen over other LAs because it, is considered the gold standard in local anesthesia and is the most often used (Da Silva et al., 2024). Lidocaine is the most often used local anesthetic in the amide category. It is a relatively safe medicine that can be taken in small dosages without causing significant safety issues. It has fewer adverse effects, is inexpensive and easily accessible (Golzari et al., 2014).
LIG is used in multimodal analgesic regimen in preventing opioid abuse and its associated side effects when used alone. It is therefore crucial to combine various medications with different mechanisms of action in pain management to reduce adverse effects while attaining maximum pain relief (Barker et al., 2020).

Clinically, local anesthetics are used extensively for cancer treatments, acute and chronic pain management, and postoperative analgesia. Nevertheless, conventional local anesthetic drugs and standard drug delivery methods have several drawbacks, including neurotoxicity, short half-lives, and non-sustained release, which restricts their use in clinical practice. For each local anesthetic medication, a successful characterization of the drug delivery system can help achieve more effective drug release, a longer duration of action, and less systemic toxicity (Ma et al., 2023).

A study was conducted to evaluate how intravenous lignocaine affected the acute rehabilitation program following a laparoscopic nephrectomy and pain management. After the procedure was performed, lignocaine helped with both early and late post-operative pain management and rehabilitation (Tauzin-Fin et al., 2014).

FDA approved lignocaine (5 %) patch has been shown to be effective in small randomized or open-label trials in conditions like diabetic peripheral neuralgia and peripheral neuropathic pains; it is used as an adjunctive therapy with minimal side effects profile (Shipton, 2012).

In this study, a transdermal LIG-PLGA-PC nanocapsule was designed and synthesized for controlled released formulation for penetration across the skin. The formulation was aimed at providing a sustained release of lignocaine over 24 hours, thus providing patients with improved therapeutic efficacy.

Polymeric nanoparticles are biodegradable and non-toxic colloids with a size ranging from 1-1000 nm. They are classified as polymeric nanospheres (NS) or nanocapsules. In this study, the focus was going to be on nanocapsule delivery systems. A polymeric nanocapsule was a colloidal system made up of a core that was enveloped with a polymeric membrane. Nanocapsules have gained popularity in drug delivery systems in recent years due to their increased drug-loading efficiency (Deng et al., 2020).

1.2. Rationale and Motivation of the Study

Drug administration by the oral route is the commonly used method of administration and is the most convenient and economically efficient. However, compared to transdermal delivery system, it has disadvantages of drug and food interactions, drug degradation in the gastric intestinal tract, first-pass metabolism and other problems (Alexander et al., 2012). The use of skin as a route of administration can prevent these problems associated with oral route and other routes such as intravenous. Because of its vast surface area, the skin enables drug absorption and helps in the treatment of both systemic and topical conditions (Rai et al., 2018). It is also a pain free application, which can be used in long term management of therapeutic drug application (Rai et al., 2018).

The rational of this research was to design and synthesize a nanotechnological based drug formulation that will provide a suitable therapeutic strategy for pain management through the transdermal route. A biocompatible and biodegradable polymer matrix will be used to formulate a delivery vehicle for lignocaine. The matrix will be prepared using Poly D, L-lactide-co-glycolide (PLGA), Phosphatidylcholine (PC) and Tween® 80 polymers. When a drug is loaded into a polymeric system, it will diffuse across the matrix, creating a mechanism for regulated and sustained drug administration (Kearney et al., 2016). To improve patient compliance and the other disadvantages mentioned above, Lignocaine will be combined with colloidal carriers such as) PLGA in this investigation.

PLGA is a synthetic, robust, highly biocompatible and biodegradable polymer and has since received FDA approval. It has been thoroughly researched to be used as a delivery system for medicines, proteins, and other macromolecules like DNA, RNA, and peptides. It has potential for extended drug administration, good degradation characteristics, and a lengthy clinical history (Makadia and Siegel, 2011). Because natural polymers vary greatly in purity, synthetic polymeric nanoparticles are the most used in the design of TDDS (Naves et al., 2017b).

This study developed a transdermal nanocapsule loaded with a local anaesthetic to deliver the medicine to the site of action in a controlled manner. The formulation was designed to induce a rapid and sustained onset of action following application thus producing a prolonged drug effect and reduced toxicity locally (Peptu et al., 2015; Beloqui et al., 2016). The morphology of the formulation was examined using a scanning electron microscope (SEM) after an effective nanocapsule design. The Zeta potential and particle size distribution was also be measured.

Following synthesis of Lignocaine loaded nanocapsules, physicochemical properties and evaluation of various chemical bond shifts, responsible for particle solubility and drug entrapment efficacy were analysed using Fourier Transformation Infrared Spectroscopy (FTIR). The zeta potential was used to calculate the degree of attraction between particles in a liquid suspension. The average particle size was ascertained by means of particle size analysis using the intensity of the scattered light and the polydispersity index (PDI). The nanocapsules was evaluated for cytotoxicity on HaCaT cells utilizing MTT assay. The designed polymeric nanocapsule should thus possess

the potential of delivering lignocaine with a lower drug-loaded dose, releasing drug in a continuous manner, with higher proposed affinity to cross the skin.

The FTIR spectral evaluation of drug loaded sample showed a vibration at 3000–2800 cm^{-1} indicating a N-H stretch, and 1750-1735 cm^{-1} presented with a C=O stretching group. At 1250–1020 cm^{-1} there is a C-N stretching confirming the presence of an amine group.

LIG-loaded PLGA-PC nanocapsule displayed a good particle size of 139.8 nm ± 81.46 with PDI of 0.182. As seen in Figures 3.5 a(i) and 3.5 b(i), the mean particle size of drug-loaded nanocapsules was equivalent to that of placebo nanocapsules, irrespective of LIG drug loading. Particle size with a mean average of 250 nm - 500 nm are generally obtained with nanocapsules (Mora-Huertas et al., 2010).

The LIG-PLGA-PC nanocapsule demonstrated a one-step degradation with weight loss of 67 % when subjected to elevated temperatures between 330 – 400°C.

The highest cell viability in 48 hours and 72 hours graphs were 110 % at 1.5625 µg/ml and 104,9 % at 12.5 µg/ml respectively.

Even though the high concentration was seen with drug-loaded formulation compared to drug alone, generally it was seen that at this concentration of both the graphs, there was a high cell viability. The treatments were meant to decrease cell viability as local anaesthetics are cytotoxic (Kang et al., 2016a).

1.3. Aim and Objectives

The purpose of this research is to design a transdermal lignocaine-loaded nanocapsule as a potential delivery system for pain management. The following objectives will be performed to achieve the above aim.

Objective 1: To design a biodegradable lignocaine-loaded nanocapsule delivery system, using Poly D, L-lactide-co-glycolide (PLGA), Phosphatidylcholine (PC) and Tween® 80 polymers.

Objective 2: To evaluate the physicochemical properties of the nanocapsule, employing Fourier Transformation Infrared (FTIR) spectroscopy, to determine molecular state composition.

Objective 3: To evaluate size and charge of nanoparticles, to determine the uniformity and surface charge distribution of the nanoparticles.

Objective 4: To determine drug entrapment efficiency and the release profile of lignocaine *in vitro*, using a dialysis method.

Objective 5: To undertake thermal and morphological evaluation, employing Scanning Electron Microscope (SEM), evaluating particle conformation properties of the nanocapsule delivery system, respectively.

Objective 6: To determine cytotoxicity and cell viability of HaCaT cells using MTT assay.

1.4 Overview of this dissertation

Chapter 1: Defines pain as a health problem that affects most of the population. Pain is mostly treated by oral and parenteral dosage forms. Even though these formulations are widely used, they have some challenges. Oral formulations are subjected to hepatic metabolism that degrades the product and reduces bioavailability. Low bioavailability results in low efficacy and can lead to unpredictable responses to a drug. Parenteral formulations are expensive which can result in low patient compliance. This study demonstrates the potential benefits of TDDS over other dosage forms. They avoid the liver's first-pass effect as well as systemic side effects such as gastrointestinal tract disorders. Drugs with a molecular weight that are too big, are not able to pass through the skin. In this study, TDDS was formulated using a nanotechnology approach to overcome the challenges that are associated with skin penetration.

Chapter 2: Describes in detail, a literature review on pain as a health condition, as well as the various forms and treatment methods available on the market. It describes skin anatomy and physiology, and its functions in a human being. It addressed how the skin acts as a barrier to prevent medications used in pain management from penetrating the body and treating the condition. Several types of nano formulations, with their advantages and disadvantages are also mentioned. In addition, the reason for the choice of nanotechnology formulation in the development of nanocapsules are highlighted.

Chapter 3: Describes the Lignocaine-loaded nanocapsules design, materials used, and techniques of synthesize. It illustrates the characterization of the nanocapsules, performed utilizing the FTIR, SEM, Zeta sizer, and Thermal analysis. Drug release profiles were determined by *in vitro* release study and UV Spectroscopy. Cell viability studies were also conducted utilizing MTT assay for proof of concept and confirmation of non-toxicity to physiological tissue. The nanocapsules were found to be less than 200 nm in size, with favourable poly-dispersity index. The morphological studies and the zeta sizer confirmed the synthesis of nanocapsules, with FTIR data exhibiting the formation of polymeric nanocapsules with incorporation of Lignocaine.

The size of nanoparticles was measured using zeta sizer and was found to be around 139.8 nm with a polydispersity index (PDI) of 0.182 and the zeta potential of -48,2 mV. A drug entrapment efficacy (DEE) percentage of 72 % was achieved. LIG release studies showed a sustained release over a 24-hour period. The FTIR spectral evaluation of drug loaded sample showed a vibration at 3000–2800 cm−1 indicating an N-H stretch, and 1750-1735 cm−1 presented with a C=O stretching group and at1250–1020 cm−1 there was a C-N stretching confirming the presence of an amine group. Using TGA technique, LIG nanoparticulate structure increased the thermal stability of LIG. Biocompatibility of the LIG-PLGA-PC nanocapsules was determined using HaCaT cells. MTT assay was conducted to evaluate the cytotoxicity of the treatments. At 48-hour, LIG-PLGA-PC nano formulation showed increased cell viability of 102, 103, and 110 % for 6,25 µg/ml, 3,125 µg/ml, 1,5625 µg/ml concentrations respectively. The HaCaT cell micrographs showed cell proliferation on the drug loaded as well as drug alone figures. In a drug release study, 56 % of the drug was released from LIG-PLGA-PC nanocapsule within 4 hours. After 48 hours,

about 59 % of the drug was released from LIG-PLGA-PC nanocapsule indicating a sustained release profile.

Chapter 4: Provides a conclusion and future recommendations in which the study can be further improved. Efficacious lignocaine-loaded nanocapsules were formulated, with significantly positive results from the physicochemical characterization, and *in vitro* release study. Cellular viability studies showed no toxicity to physiological tissue. Further studies should be conducted on the combination of these polymers, their behaviour *in vivo* and stability over time.

CHAPTER 2
DESIGN AND SYNTHESIS OF A NANOCPSULE SYSTEM FOR TRANSDERMAL DELIVERY OFLIGNOCAINE ACROSS THE SKIN

2.1 Pain

2.1.1 Definition of pain

The International Association for the Study of Pain (IASP) describes pain as an unpleasant sensory and emotional experience associated with actual or potential tissue damage (Everson et al., 2020). Untreated pain can lead to physical and psychosocial effects that can result in a decline in one's well-being, depression, anxiety, insomnia, and lack of appetite (Kwon et al., 2020). Insufficient pain management can also result in more complicated conditions, such as further injury of tissues, infections, hormonal imbalance, and a prolonged stay in the hospital (Cho and Hong, 2021). In patients who are unable to use their judgment to convey pain intensity, they are frequently underdiagnosed and undertreated, partly due to a lack of appropriate assessment tools to detect the condition (Wennberg et al., 2020).

Regardless of the efforts made to study pain and develop novel treatment options, pain management, particularly post-surgery, remains a major healthcare concern for physicians and patients worldwide (Negash et al., 2022). Over 300 million people undergo surgery each year, worldwide (Yoo et al., 2019). However, not all patients receive the appropriate pain relief medication after surgery, with approximately 74 % of patients experiencing moderate to extreme pain after discharge (Yoo et al., 2019). In addition, if acute pain is poorly managed, this can lead to chronic pain and some serious medical conditions such as deep vein thrombosis, pneumonia, and infections (Yoo et al., 2019). The section below discusses different classes of pain together with their treatment methods used in healthcare settings.

2.1.2 Classification of pain

The understanding of different types of pain enables healthcare professionals to choose the appropriate drug for use in pain management (Russo and Sundaramurthi, 2019). Pain is pathophysiologically classified into 2 main categories namely nociceptive and non-nociceptive pain (Abed et al., 2022). Nociceptive pain is mainly due to mechanical, thermal, or chemical injury which occurs via transduction,

transmission, perception, and modulation (Russo and Sundaramurthi, 2019). Somatic and visceral pains are two types of nociceptive pain. Somatic pain can be caused by damage to skin, tissue, bones, and joints causing a sharp pain confined to one area, while visceral pain is a dull and crampy pain of smooth muscle origin. Neuropathic and inflammatory pain are two types of non-nociceptive pain. Pins and needles, paraesthesia, burning, hyperalgesia, or allodynia are symptoms of neuropathic pain, whereas mediators that sensitize the nociceptive pain pathway are symptoms of inflammatory pain (Everson et al., 2020).

The characteristics of pain include its anatomical location, physiological nature, its duration, severity, and causation (Andreu and Arruebo, 2018). The onset of pain is crucial in establishing whether the pain is acute or persistent. Acute pain is experienced as severe or self-limiting pain that has rapid onset and can result from a specific injury or pathology. Chronic pain normally occurs because of neuropathic conditions and can persist for more than 3 months (Hay and Nesbitt, 2019). The most used method of classifying pain is based on their pathophysiology. Figure 2.1 summarizes the classification and types of physiological pain (nociceptive, neuropathic, and inflammatory).

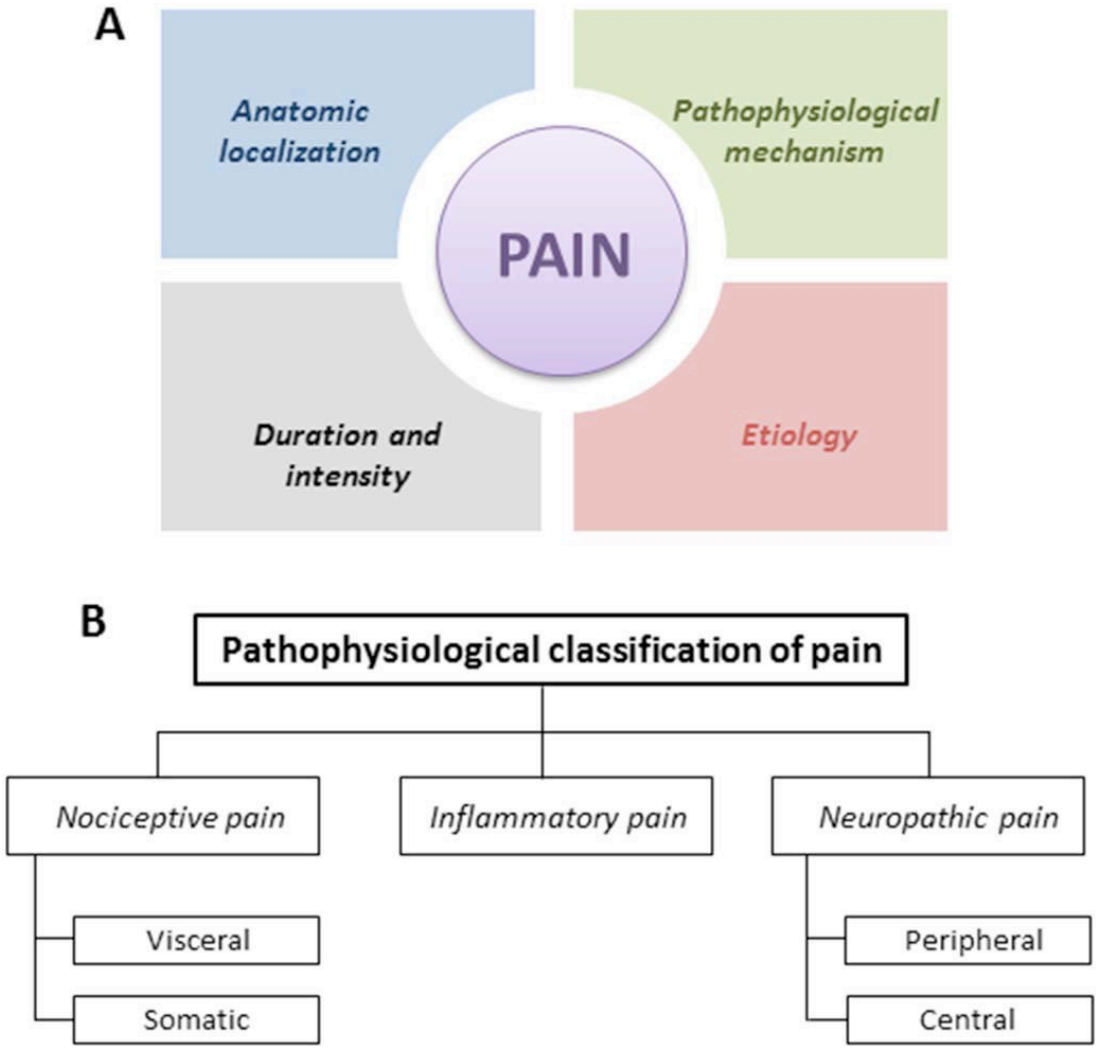


Figure 2.1 (A) Pain classification and (B) Pathophysiological classification of pain (Andreu and Arruebo, 2018)

2.1.3 Pain management

Paracetamol, Nonsteroidal anti-inflammatory medicines (NSAIDs), Selective Cyclooxygenase (COX-2) inhibitors, antiepileptic drugs, muscle relaxants, antidepressants, local anaesthetics, and opioids are all pharmaceuticals that can be used to treat or control pain (Andreu and Arruebo, 2018). These pharmaceutical products can be used individually or in combination to achieve the desired therapeutic outcome (Andreu and Arruebo, 2018). Table 2.1 below summarises various classes of medicines used in the management of pain, route of administration, conditions, mode of action and their side effects.

Table 2.1 Summary of drugs used in the management of pain.

Drug	Classification	Delivery route	Conditions	Mechanism of action	Side effects	Reference
Paracetamol	Antipyretic	Oral	Analgesia and fever	Decreases tissue concentrations of prostaglandins and proinflammatory mediators.	Gastrointestinal, liver, renal, and vascular side effects	(Freo et al., 2021)
Ibuprofen	NSAIDS	Oral	Pain and Inflammation	Nonselective (COX 1) and (COX 2) inhibitors	Gastric ulceration Renal disorders Asthma attack Platelet disorders	(Hay and Nesbitt, 2019)
Celecoxib	Selective COX-2 Inhibitor	Oral	Pain and Inflammation	Selective COX 2 inhibitors	Cardiovascular Renal adverse effects	(Sinatra, 2002)
Lignocaine	Local anaesthetics	Topical	Post-herpetic neuralgia	Through sodium channel blocking, it obstructs the propagation of action potentials	Local skin irritation	(Bajaj et al., 2011a)
Amitriptyline	Tricyclic antidepressants	Oral	Fibromyalgia, migraine, and tension headaches, diabetic polyneuropathy and traumatic mononeuropathy	Blocks the reuptake of central neurotransmitters such as serotonin and norepinephrine	Dryness of the mouth, Sedation, low blood pressure, exacerbation of glaucoma, and bladder obstruction	(Fitzgerald et al., 1998)
Fentanyl	Opioids	Intranasal/ Intravenous	Severe renal colic pain	Bind to opioid receptors.	Nausea, Dizziness, Pruritis, respiratory depression, bad taste Pharyngeal irritation	(Nazemian et al., 2020) and (Hatten et al., 2020)
Clonidine	Alpha 2 receptor agonists	Spinal/ Epidural	Intractable cancer pain Neuropathic pain	Stimulation of alpha receptors	Hypotension Bradycardia	(Eisenach, 1994) and (Van Elstraete et al., 2000)

Drug	Classification	Delivery route	Conditions	Mechanism of action	Side effects	Reference
			Reflex sympathetic dystrophy Postoperative and obstetric labour pain			
Gabapentin, pregabalin	Gabapentenoids/ Antiepileptics	Oral	Adjunctive postoperative analgesia for major surgery Neuropathic pain	Signal transmission: utilizes alpha-2 receptors to decrease neurotransmitter release	Sedation Dizziness Nausea	(Everson et al., 2020) and (Hay and Nesbitt, 2019)

2.1.4 Mechanism of action of drugs used for pain management.

Paracetamol (acetaminophen) is without a doubt one of the most often used medications in the world (Freo et al., 2021). As an over-the-counter medicine, paracetamol is the standard and first-line treatment for fever and acute pain (Ayoub, 2021).

The NSAIDs are used to treat inflammation and relieve discomfort. They work by blocking cyclooxygenase enzymes, which impede the production of prostaglandins and thromboxane from arachidonic acid (Stachowicz, 2021). Prostaglandins are responsible in the development of pain by amplifying the response of sensory neurons to painful stimuli (Langford and Mehta, 2006). Ibuprofen is an example of NSAIDs that is available as an oral formulation and is used for pain, fever, and inflammation (AL Falahi et al., 2021). However, oral formulations are associated with side effects including gastric ulceration (Hay and Nesbitt, 2019).

Selective Cyclooxygenase-2 (COX-2) inhibitors act by blocking the COX-2 enzyme, which causes pain, fever, and inflammation. Unlike NSAIDS, which are COX-1 and COX2 inhibitors, they protect the gastrointestinal mucosa while having no effect on platelets. They are commonly utilized in orthopaedic postoperative analgesia (Sinatra, 2002).

Tricyclic antidepressants are used in the treatment of pain. They function by inhibiting the reuptake of serotonin and norepinephrine neurotransmitters at central adrenergic neurons (Fitzgerald et al., 1998). Tricyclic antidepressants, such as amitriptyline, are prescribed for the treatment of migraine, tension headaches, diabetic polyneuropathy, and traumatic mononeuropathy (Fitzgerald et al., 1998).

Opioids are synthetic or natural substances that produce euphoria and analgesia by binding to μ opioid receptors. Hypotension, sedation, nausea, constipation, falls, and rapid tolerance with physical dependency are all side effects linked with this class of medicines (Hatten et al., 2020). A study was conducted where it was mentioned that the development of a safer and new class of drugs that work on the sigma-1 receptor (s1R), was urgently needed in minimising the side effects (Zhuang et al., 2021).

Fentanyl is an example of an opioid drug used intravenously or intranasally in the management of severe pain (Nazemian et al., 2020).

Alpha-2 adrenergic receptor agonists also produce analgesia. These medicines provide analgesia primarily through activities in the spinal cord. Because systemic administration provides inadequate analgesia, it has been discovered that medicines operate best when injected near the cord (spinal or epidural administration). Alpha-2 adrenergic receptor agonists have several advantages over opioids in the treatment of severe pain, including the absence of opioid-like adverse effects (Eisenach, 1994). Clonidine has an additional analgesic effect when combined with local anaesthetics in epidural anaesthesia. It also prolongs the surgical analgesic with local anaesthetics in caudal block in children (Van Elstraete et al., 2000).

Gabapentenoids are antiepileptics that work by inhibiting neurotransmitter release and are used as adjunctives in the management of pain (Everson et al., 2020). Local anaesthetics (LA) are another class of drugs used to alleviate pain. They are weak bases having an aromatic ring that is lipophilic in nature (Perrin et al., 2020). They work by prohibiting the entry of sodium in peripheral nerves and inhibiting the propagation of action potential, and reversibly inhibiting the process of excitation and conduction (Columb et al., 2017). Lignocaine is the typical example of a local anaesthetic with such inhibition properties. Currently, a range of pain disorders are treated using topical lignocaine. Health authorities in several countries have approved topical lignocaine for the treatment of post-herpetic neuralgia; research has also demonstrated that it is a safe and efficacious treatment for pain following surgery. For the best pain management and multimodal analgesia, topical lignocaine may be a better solution, either alone or in conjunction with systemic medications and non-pharmacological methods (Voute et al., 2021). An investigation was conducted to provide an overview of the effectiveness and safety of a medicated plaster containing 5 % Lignocaine in the treatment of different neuropathic pain syndromes. The plaster was found to be beneficial in treating neuropathic pain symptoms associated with past herpes zoster infection (Mick and Correa-Illanes, 2012).

2.2 TRANSDERMAL DRUG DELIVERY SYSTEMS

2.2.1 Transdermal drug delivery and its benefits

The application of pharmacologically active substances to the skin for local action or appropriate systemic therapeutic blood levels is known as transdermal drug delivery, or TDD (Valenzuela and Simon, 2012). This method of administration provides a lot of advantages over the standard oral and intravenous routes. It avoids the gastrointestinal degradation and first-pass hepatic metabolism that comes with the oral route, resulting in higher bioavailability (Shukla et al., 2018). It enhances therapeutic efficiency and maintains steady-state plasma concentrations. In addition, it is simple and easy to use with minimal pain on application, convenient and non-invasiveness resulting in the improvement of patient compliance (Valenzuela and Simon, 2012; Zhou et al., 2018).

TDD is a painless method of applying a drug formulation to healthy, undamaged skin to systemically distribute medication. The delivery method allows medication to enter the body across the SC, epidermis, and dermis without accumulating in the dermal layer. The medication can be absorbed systemically by the dermal microcirculation once it reaches the dermal layer (Alkilani et al., 2015). The skin structure and its benefits are elaborated below.

2.3 THE HUMAN SKIN

2.3.1 Structure of the human skin

The skin is the body's biggest organ that is made up of three layers. The skin's outermost layer is called the epidermis. The dermis is the intermediate layer, which contains blood vessels and connective tissues. The hypodermis is the body's deepest layer of insulation (Uchechi et al., 2014).

The basal layer, stratum lucidum, granular layer, spinous layer, stratum granulosum, and cornified layer, often known as SC, are the layers that make up the epidermis (Peptu et al., 2015). The dermis of human skin is divided into two parts. First, there is the top dermis, also called the papillary dermis, which has loose connective tissue and a high cell density. The second component is the deeper layers of the reticular dermis, which is tightly packed with collagen and other connective tissue proteins while having a low cell density (Korosec et al., 2019). Hair follicles, sweat glands, and sebaceous glands are examples of appendages found in the reticular dermis (Peptu et al., 2015).

2.3.2 General functions of the skin

The skin functions primarily to protect the body from external harsh environmental conditions. It protects against a wide range of damage, including thermal, chemical, ultraviolet, and bacterial invasion. It also functions as a barrier to water and salt loss, controls body temperature, and synthesise Vitamin D which assists in the metabolic processes (Goyal et al., 2016; Abdo et al., 2020). Additionally, the skin regulates sensation, secretion and excretion (X. Chen et al., 2020).

2.3.3 Pathways for drug permeation through the skin

The medication’s concentration, the age of the skin, the application site, the duration of the drug's interaction with the skin, and the skin's integrity are some factors that influence drug transport via the skin (Alexander et al., 2012). Figure 2.2 below is a diagrammatic representation of different pathways of penetration across the skin namely, intracellular, intercellular, and follicular penetration pathways (Alexander et al., 2012). The intercellular lipid route involves the passage of drugs between the cells of the skin. This pathway can transport lipophilic or molecules without charge or polarity across the skin (Alkilani et al., 2015). Drugs enter the corneocytes through the transcellular pathway. The follicular pathway—which includes sweat ducts, sebaceous glands, and hair follicles—allows drugs to enter the skin (Carter et al., 2019). The

intercellular pathway is the most predominant route for drug absorption and is largely reliant on the balance of the drug to be adequately of lipid and aqueous nature (Ramadon et al., 2022).

To potentially overcome some of drawbacks associated with oral and parenteral drug administration routes, the transdermal route has been investigated as another potential method for improving drug delivery (Ramadon et al., 2022). TDDS medications are available in different patch dosage forms. When applied topically, the active molecules are dispersed throughout the skin and enter the bloodstream at a regulated rate over an extended period, improving therapeutic efficacy and reducing side effects (Stanekzai et al., 2019).

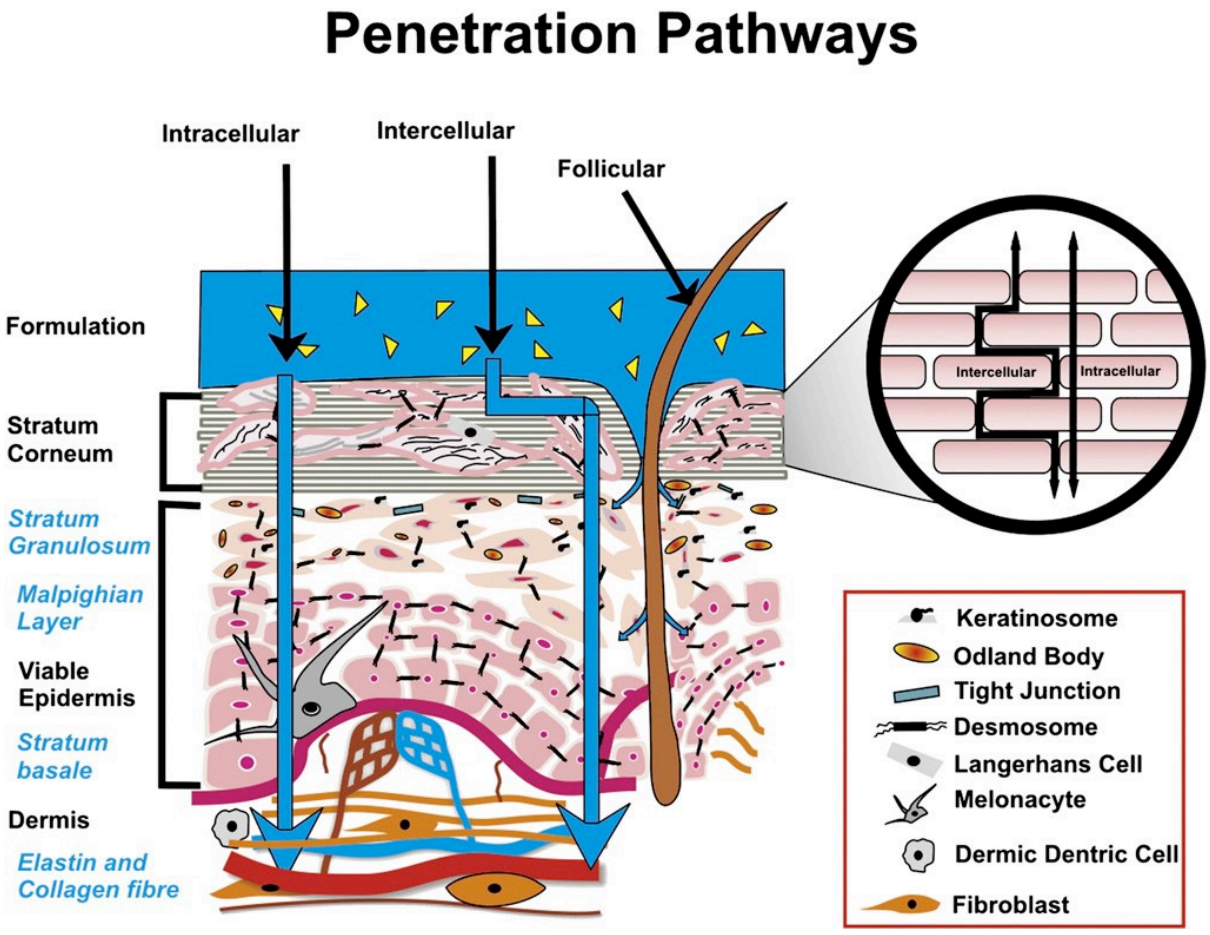


Figure 2.2 Diagrammatic representation of intracellular, intercellular, and follicular penetration pathways across the human skin (Alexander et al., 2012)

2.4 TRANSDERMAL PATCHES

Transdermal patches can be used to manage acute and chronic pain. The available patches on the market are NSAIDs patches, opioid patches, and local anaesthetic patches (Bajaj et al., 2011a). Flurbiprofen is an NSAID that has analgesic, antipyretic, and anti-inflammatory, properties that is used to manage different types of arthritis, and alkylating spondylitis (Yang and Li, 2021). However, when the drug is administered orally, it presents complications including gastrointestinal discomfort. Flurbiprofen has a low solubility, which leads to poor absorption, as well as a short half-life, which necessitates multiple dosage throughout the day, resulting in poor patient compliance (Yang and Li, 2021). In a study conducted by Yataba et al., (2017), an innovative topical NSAID patch formulation called S-flurbiprofen plaster (SFPP) was formulated. To establish the appropriate dosage for patients suffering from osteoarthritis, a multicentre, randomised, double-blind, parallel-group trial evaluated the safety and efficacy of SFPP against placebo. Pain relief was observed, especially at 40 mg, and it was determined that this dosage was the most efficient to use (Yataba et al., 2017). These oral dosage form difficulties point to the need for innovative medicine formulations for topical delivery, such as patches (Yang and Li, 2021). Fentanyl patches also known as Duragesic are an opioid used in cancer for the management of pain. It can maintain blood concentrations of up to 72 hours with minimum and maximum concentrations being 12 and 24 hours respectively (Bajaj et al., 2011a).

Buprenorphine is an agent that is used in the management of opioid dependency (Hatten et al., 2020). It is available as a sublingual dosage form and Injectable formulations. Compared to other drugs such as methadone which are used for the treatment of opioid dependency, it has been shown to have fewer side effects like less euphoria and reduced drug dependency (Dhawan et al., 2019). The sublingual formulation has complications including diversion to the use of illicit drugs. Parenteral buprenorphine causes phlebitis and is not easy to administer. To reduce these side effects associated with sublingual and injectable formulations, a transdermal patch was developed with reduced side effects. There is less diversion to illicit drug use and abuse, ease of use of a patch and increased compliance, constant blood concentrations (Dhawan et al., 2019).

Emla patch or cream is a transdermal formulation that is made up of LIG and prilocaine local anaesthetics. It was found that Emla patch lowers pain associated with a

tuberculosis test significantly (Dubus et al., 2006). Versatis (5 %), a lidocaine patch is also available to manage neuropathic pain associated with post-herpetic neuralgia (Bajaj et al., 2011a).

2.5 LOCAL ANAESTHETICS

Anaesthetic agents are an important group of drugs for the treatment of pain. They have advantages of relaxing muscles, reduction of anxiety and unwanted reflexes (Wang et al., 2019). Anaesthetics are classified into pre-anaesthetic, neuromuscular blockers, general and local anaesthetics (Wang et al., 2019). Based on the chemistry of local anaesthetics, they are characterized with lipophilic aromatic ring, a link, and a hydrophilic amino group (Hay and Nesbitt, 2019). The amines are mostly tertiary as three alkyl or aryl groups attached to the nitrogen atom. Local anaesthetics can be classified into amides and esters depending on nature of their linkage. Examples of amides are lignocaine, prilocaine, bupivacaine and ropivacaine. Examples of the ester group include cocaine, procaine, chloroprocaine, benzocaine and amethocaine (Columb et al., 2017).

Even though local anaesthetics are used in the treatment of pain, they have challenges including fluctuating plasma drug concentrations. They may need frequent dosing leading to poor patient compliance (Wang et al., 2019). To address the challenges, TDDS nano formulations loaded with a local anaesthetic has been developed to minimize or overcome challenges of the convention dosage forms. In these systems, the drug diffused out of the matrix to produce a controlled and prolonged effective drug plasma concentration (Wang et al., 2019). The most studied local anaesthetics in Drug Delivery Systems (DDS) are LIG and bupivacaine to provide safe and effective treatment options in perioperative pain management (Rogobete et al., 2016).

Local anaesthetics cause Local anaesthetic Systemic Toxicity (LAST) which is a potentially fatal adverse event that can develop after administering local anaesthetic medications via a variety of methods. Increased usage of local anaesthetic medications has made it necessary for healthcare providers to understand the adverse event (El-Boghdadly et al., 2018).

Prompt detection and treatment of LAST are critical for lowering the dangers associated with regional anaesthesia. However, LAST is a relatively uncommon

occurrence, and the diagnosis and opportunity for appropriate treatment can be easily missed (Macfarlane et al., 2021).

Local anaesthetics vary in their potential to cause LAST. Short acting drugs including lignocaine have less toxicity than longer acting ones like bupivacaine. Use of a single LA rather than multiple agents in one patient should be advocated to reduce toxicity. Healthcare practitioners are encouraged to use the lowest effective dose of LAs (Yaddanapudi, 2011).

Lignocaine as a topical anaesthetic preparation exhibits a minimal systemic absorption and increased analgesic effects because of blocking of sodium channels in afferent nerves (Oliveira et al., 2020). Due to its low molecular weight and limited water solubility, lignocaine, can be employed as a model molecule for hydrophobic drug binding. It is the most often used local anaesthetic due to its effectiveness, quick regulated release, low duration of effects, and topical anaesthetic action (Li et al., 2021). The only intravenous anaesthetic that has received FDA approval is lignocaine hydrochloride. LIG is mostly delivered intramuscularly or transdermally using mucilages; however, these delivery methods have several drawbacks when used in therapeutic settings. For instance, multiple injections are frequently needed for anaesthesia during procedures involving superficial skin, such as cosmetic surgery, tattooing, birthmark removal, scar dissolving, and skin transplantation. This causes pain and discomfort and creates a considerable number of sharp contaminants. DDS containing local anaesthetic medicines for continuous release have been created to minimize or reduce these side effects, ensuring that drug release is significantly slower, and analgesia is maintained over a longer period while generating less neurotoxicity (Ma et al., 2023). Due to the lower systemic toxicity and improved efficacy of non-conventional local anaesthetics, there has been substantial improvement in nanotechnology-based delivery systems during the last ten years. The current study looked at developing a Lignocaine Loaded Nanocapsule for topical delivery system across the skin.

2.5.1 Management of pain by Local Anaesthetics

Mechanism of action of local anaesthetics involves inhibition of excitation conduction process in the peripheral nerves by causing blockage of neural transmission (Liu and Zhao, 2019). They exist in equilibrium as unionized and as ionised. The ionized form

of the molecule binds to the inside of sodium channel, blocking its function. The unionized part of the drug prevents sodium influx and nerve transmission through blockage of sodium channels thereby inhibiting a threshold potential from being reached and temporarily disabling nerve conduction (Perrin et al., 2020). Though topical dosage forms have been used to deliver local anaesthetics, the structure of the skin makes it difficult for the drug to pass through and reach the site of action.

The use of nanotechnology to develop a TDDS formulation for local anaesthetics are beneficial in increasing penetration of drug molecules, the increasing onset of action and reducing toxic side effect (Liu and Zhao, 2019).

2.6 TRANSDERMAL DELIVERY OF NANOPARTICLES FOR PAIN MANAGEMENT

Nanoparticles have particle sizes of less than 100 nm in at least one dimension (Khan et al., 2019). Alternative therapeutic strategies have been introduced through an integrated approach in a search for effective therapeutic ways using the current existing medication. The application of nanotechnology to enhance healthcare is known as nanomedicine, and it involves the use of nanoscale materials for disease detection, tracking, treatment, and prevention (Tinkle et al., 2014). Nanomedicine has shown therapeutic advantages in several diseases including cancer (Gao et al., 2014). They are associated with enhanced pharmacological properties like prolonged systemic drug circulation and high drug loading yield which result in good pharmacokinetic properties (Pitorre et al., 2017).

Penetration of drugs across the skin depend on their physicochemical factors. This can be bypassed by the development of advanced TDDS like nanocapsules (Marto et al., 2016). TDDS have been used as analgesics for management of acute pain. Local anaesthetics are formulated as TDDS in the prevention of pain, including in venesection and vaccination (Bajaj et al., 2011a). TDDS clinical application has been limited to lipophilic drugs and those with a molecular weight of < 500 Da (M. Chen et al., 2020). Table 2.2 shows different classes of drugs used in the management of pain, their yielded results, and references (Bajaj et al., 2011a).

2.7 Classification of Nanoparticles for transdermal drug delivery

Nanoparticles are categorized as organic, inorganic, and carbon-based. The size, shape, and structure of the nanoparticles vary. It may be flat, hollow cored, conical, spherical, cylindrical, tubular, or spiral. Different methods of synthesis are used to create or improve the formulation of nanoparticles and increase optical, mechanical, physical, and chemical characteristics (Anu Mary Ealia and Saravanakumar, 2017). Nanoparticles are classified into vesicles (liposomes, transferosomes, ethosomes, niosomes and invasomes) lipid nanocarriers, polymeric nanoparticles, nano-emulsions, dendrimers, metallic nanoparticles and polymeric nanoparticles (Goyal et al., 2016; Zhou et al., 2018). Figure 2.3 shows different types of nanoformulations used for TDDS (Carter et al., 2019). Figure 2.4 depicts several nanocarriers that can be utilized to improve medication penetration across the skin (Dragicevic and Maibach, 2018). Nanoparticles can be made of lipids or polymers. Solid lipid nanoparticles (SLN) are the initial class of lipid-based nanoparticles. They consist only of solid lipids, which can be waxes, glycerides, or mixtures of the two, and have a nearly perfect solid lipid matrix. Following that, an enhanced second class of nanoparticles, nanostructured lipid nanocarriers (NLC), were developed.

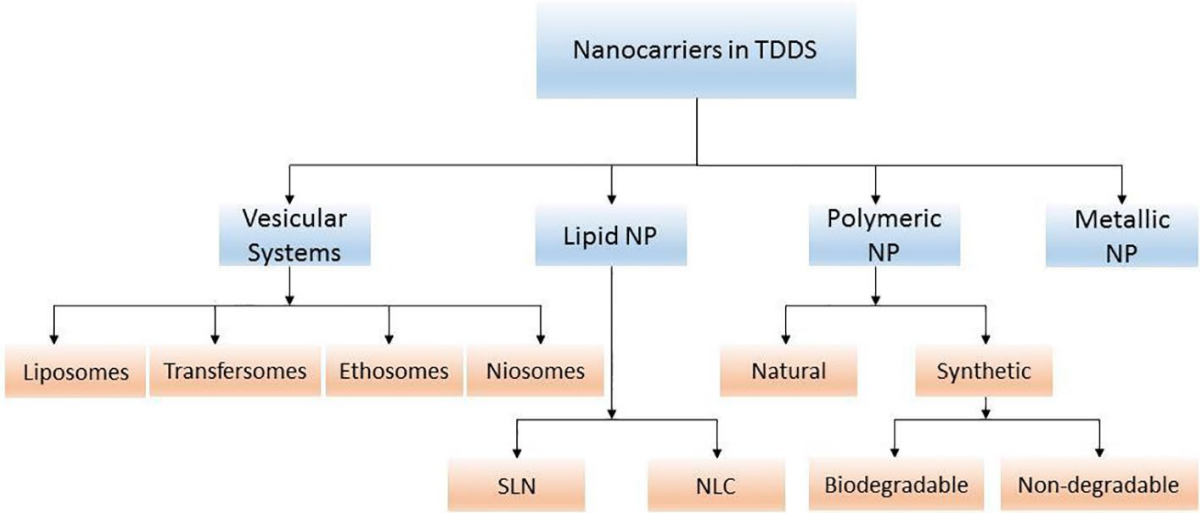


Figure 2.3 Flowchart showing various types of nanocarriers used for TDDS (Carter et al., 2019)

2.7.1 Dendrimers

Dendrimers are monodispersed, tree-like structures with a size range between 1 – 100 um and are architecturally made up of an inner core, interior branching, internal cavities in the periphery, and the terminal groups (Mignani et al., 2021). Dendrimers, also known as cascades and arborols, used in the delivery of a range of bioactive molecules including vaccines and enzymes (Narmani et al., 2019). Manikkath and colleagues examined the use of polyamido amine (PAMAM) dendrimer, one of the most common dendrimers, to create a low-frequency ultrasound on the passage of ketoprofen through across the skin (Manikkath et al., 2017). Ketoprofen is an NSAID that is used in management of pain that originates in the muscles and skeletal system. Since ketoprofen is accessible in an oral dose form, its limitation can be linked to gastrointestinal adverse effects. In the study, it was concluded that plasma drug levels obtained with a PAMAM dendrimer application combined with ultrasound were comparable to oral ketoprofen treatment. This work reveals the potential of combining sonophoresis and PAMAM dendrimers to increase the epidermal delivery of bioactive substances (Manikkath et al., 2017).

2.7.2 Vesicles

Vesicles are multilayered particles made up of amphiphilic biomaterials and lipids. When exposed to a large amount of water, these amphiphilic materials reorganize into unilamellar or multilamellar vesicle layers. Vesicles can be employed to deliver both hydrophilic and lipophilic medicines using TDDS. Liposomes, niosomes, transfersomes, ethosomes, and invasomes are examples of diverse types of vesicles (Zhou et al., 2018).

2.7.2.1 Liposomes

Liposomes is another type of nano delivery system with some applications in transdermal drug delivery and are defined as spherical vesicles (Uchechi et al., 2014). They consist of a bilayer of phospholipids that can encapsulate pharmaceuticals that are hydrophilic or hydrophobic within the bilayer or between the bilayer, respectively (Garg et al., 2020). Kim et al., 2019, conducted a study where a thin film hydration method was used where baicalein loaded liposome nano formulation was developed using novel pseudo ceramic, PO3C, PO6C and PO9C. Baicalein is a weakly soluble flavonoid found in the root of the Scutellaria baicalensis plant that has anti-

inflammatory activities amongst other properties. In the study, it was found that the pseudo ceramic liposome formulation was able to deliver the baicalein across the skin effectively (Kim et al., 2019).

2.7.2.2 Niosomes

Niosomes, are microscopic transporters, made up of cholesterol and non-ionic surfactants. They are assumed to be less successful in transdermal administration due to their inability to permeate deeper skin tissues due to trapping in the SC. They frequently fail to provide the desired transdermal penetration (Tijani et al., 2021). They are less expensive to produce than traditional liposomes (Carter et al., 2019). Niosomes nanoformulations as vehicles are delivered as parenteral, oral, ocular, nose to the brain, pulmonary, anticancer, and transdermally (Masjedi and Montahaei, 2021). In TDDS, niosomes formulations have been used in the treatment of acne, mycoses, and psoriasis (Carter et al., 2019).

Manosroi et al., 2008 conducted a study where they investigated the anti-inflammatory effect of a topical gel using niosomes as a delivery system across the skin. The drug of choice used was diclofenac diethylammonium (DCFD), an NSAID. The formulation technique used was the thin-film method with sonication. The study's findings demonstrated that the gel showed high fluxes through rat skin and strong anti-inflammatory effect (Manosroi et al., 2008). Auda et al., 2016 undertook another investigation with the goal of designing and synthesizing Celecoxib (CXB). CXB niosomal hydrogels for TDD was formulated to mitigate its oral side effects. CXB niosomal gel formulations for transdermal medication administration were produced *in vitro* and *in vivo*. All niosomal gel formulations were shown to be highly anti-inflammatory (Auda et al., 2016).

2.7.2.3 Transferosomes

Transferosomes are bilayer nanovesicles composed of phospholipids, edge activators (surfactants), and water. Due to their elasticity, they emerged as one of the most effective drug-delivery methods for topical treatment when compared to conventional topical systems (Opatha et al., 2020).

Omar et al., 2019 conducted a study to produce a topical gel containing transferosomal lidocaine as a substitute to uncomfortable local anaesthetic injections. Film hydration

was used to create the transferosomes, which contained soybean phosphatidylcholine and cholesterol. The incorporation of transferosomal lidocaine into gel enhanced drug permeability. Furthermore, embedding transferosomal vesicles in gel dosage form improved their stability. It was also determined that specific skin permeation enhancers can be utilized in conjunction with transferosomal lidocaine gel to promote medicine penetration. This approach has the potential to be used to deliver multiple topical medications without affecting the skin's structure (Omar et al., 2019).

2.7.2.4 Ethosomes

Ethosomes are a form of vesicular nanocarrier that is made up of phosphatidylcholine, cholesterol, ethanol (20-50 %), and water. They have many medical uses including their capacity to transport both hydrophilic and hydrophobic drugs (Carter et al., 2019).

In contrast to conventional liposomes, ethosomes have a substantially higher transdermal flow and can pass through the SC barrier (Uchechi et al., 2014). In a study conducted by Sakdiset et al., 2019, development of Indomethacin-loaded ethosomes was discussed. Different phospholipid concentrations, ethanolic content, the dispersion medium, cholesterol, and agents that affect surface charge were used to design the formulation for transdermal medication delivery. The thin-film hydration process was used to make the formulation. Physical appearance, particle size analysis, content of the drug, entrapment efficiency determination, and surface morphology were used to characterize the preparation. When compared to commercial and ethanolic indomethacin solutions, the formulation improved skin permeability and decreased skin disposition.

These findings supported the use of ethosomes as a carrier for indomethacin transdermal administration (Sakdiset et al., 2019).

2.7.2.5 Invasomes

Invasomes are vesicles with phospholipids and a terpene or a combination of terpenes as part of their composition. They have been demonstrated to increase medication penetration through the skin (Jain et al., 2021). Research was conducted by Duangjit et al., 2016 where Capsicum, a spice that comes from peppers was designed and developed for TDD. The combination penetrated the skin better than a regular

liposome and a commercially available ethanolic solution containing 0.15 percent capsaicin (Duangjit et al., 2016).

2.7.3 Polymeric micelles

Amphiphilic nanosystems made up of copolymers with a hydrophilic shell and a hydrophobic core are referred to as polymeric micelles (Zhang et al., 2007). Individual micelles range in size from 10 - 200 nm, encapsulating hydrophobic or poorly soluble pharmaceuticals in polymeric micelles improves medication solubility, stability, and bioavailability, as well as therapeutic results (Sezgin-bayindir et al., 2016). A study was performed by Román-Vargas et al., where the aim was to investigate the *in vivo* analgesic activity of a cannabis extract rich in Cannabidiol. It was discovered that the formulation maintained the analgesic effect and had improved efficacy which could be used in the management of chronic pain (Román-Vargas et al., 2023).

2.7.4 Nanogels

Nanogels are biocompatible and biodegradable nanoparticles that are made up of cross-linked hydrophilic polymers with a size range of 20 – 200 μm. Their materials are soft and small in size allowing increased drug penetration. They have been developed for oral, topical, vaginal, and ocular drug delivery systems (Garg et al., 2020).

Over the past ten years, nanogels have been used a lot to treat systemic diseases like autoimmune diseases, skin diseases, cancer, and wound management. This is because nanogels have superior mechanical and rheological properties than traditional semisolid dosage forms like gels, pastes, creams, and ointments (Siafaka et al., 2023). A study was conducted with the aim of creating and testing a Diclofenac sodium nanogel to deliver extended release, increase drug residence time on the skin, and to improve bioavailability. A nanogel formulation containing Diclofenac sodium was successfully developed and demonstrated to be an effective and superior carrier for transdermal/topical preparation (Talele et al., 2017).

2.7.5 Nanoemulsions

Nanoemulsions are nanoformulations with a transparent oil droplets size of about 500 μm. They have unique properties of small droplet size, large surface area and with increased stability (Kumar et al., 2021). They also have the advantage of requiring

fewer emulsifying agents in their formulations. Nanoemulsions are used mostly in food technology, petroleum, chemical, and pharmaceutical (Liu et al., 2021). An investigation conducted by Shakeel et al., 2009 was set out to look at the viability of using nanoemulsions to deliver celecoxib topically. The created nanoemulsions were examined for shape, viscosity, droplet size, and refractive index using several dispersion stability tests. Celecoxib gel and nanoemulsion gel were contrasted with optimized formulation's in vitro skin permeability profile. In nanoemulsion formulations, a significant increase in permeability metrics was seen (p 0.05). These findings imply that nanoemulsions could serve as better transdermal delivery systems for celecoxib (Shakeel et al., 2009).

2.7.6 Solid Lipid nanoparticles

Solid Lipid nanoparticles (SLN) comprises of solid lipid internal phase, aqueous external phase, and a surfactant as a stabilizer (Mohammadi-Samani et al., 2020). A study was conducted by Mohammadi-Samani et al., 2020 where oral and parenteral SLN-loaded sumatriptan for the management of migraine headaches were developed. Side effects associated with oral formulations are first-pass drug degradation effects resulting in low bioavailability. The inconvenience shown with the use of parenteral formulations is difficult with self-administration leading to low patient compliance. All these inconveniences led to the development of TDDS (Mohammadi-Samani et al., 2020).

A study was conducted to develop and evaluate a formulation of sumatriptan loaded SLN to increase the drug's rate of percutaneous penetration and minimize any potential negative effects. The solvent diffusion/evaporation approach was used to create SLNs loaded with sumatriptan. Sumatriptan skin permeation analyses, in vitro release studies, particle size, and entrapment efficiency were all used to describe the generated SLNs. To compare the percutaneous penetration of sumatriptan to that of the drug's usual gel formulation, sumatriptan-loaded SLNs were added to a topical delivery system of gel. The findings showed that compared to sumatriptan traditional gel formulation, the drug's skin penetration was increased in the sumatriptan loaded SLN formulation (Mohammadi-Samani et al., 2020).

2.7.7 Nanostructured lipid carriers (NLCs)

Following the discovery of solid lipid nanoparticles, the second group of lipid nanoparticles, nanostructured lipid carriers (NLC), was discovered. NLCs are more advantageous over SLN due to high drug encapsulation capacity, have low water content, and enhance encapsulation of a drug with minimized leakage during storage (Elmowafy and Al-Sanea, 2021). A study was conducted by Pandey et al., 2020 where an oral anti-diabetic drug, repaglinide, with low bioavailability and high first-pass hepatic metabolism was formulated as an NLC and administered using transdermal route. The repaglinide-loaded NLC showed an increase in bioavailability using the transdermal route. This study concluded that class II drugs with low solubility can be reformulated using NLC to improve patient compliance (Pandey et al., 2020).

Otarola et al., 2020, also performed research where NLC loaded with piroxicam was formulated. Piroxicam is an NSAID that is used to treat inflammation and pain. The study's goal was to create and characterize a piroxicam-loaded Nanostructured lipid carriers (NLCs) gel formulation for topical medication delivery. A stable gel was formulated. The gel when applied to the pigskin, did not show any skin irritation (Otarola et al., 2020).

2.7.8 Nanoparticles

Polymeric nanoparticles are solids produced from polymers, either natural or manmade. One of the most significant biomedical uses of polymeric nanoparticles is drug delivery. Researchers have investigated the potential of polymeric nanoparticles to carry a range of medications via different modes of administration, such as peptides, biological macromolecules, vaccines, and tiny hydrophilic and hydrophobic medicines (Peltonen et al., 2020). Polymeric nanoparticles can be categorised according to two major types namely NC (reservoir systems) and NS (matrix systems) (El-Say and El-Sawy, 2017).

Nanocapsules (NC) are nanoparticles with an oily or aqueous core enclosed in a polymeric shell and sometimes combined with a lipophilic and hydrophilic surfactant blend, whereas NS are matrix systems consisting only of polymers. The increased drug loading of NC compared to NS is one of its main benefits (Elmowafy et al., 2017).

Nanoparticles can further be classified as natural or synthetic. The commonly used natural material for the development of nanoparticles for topical medical administration

includes chitosan, alginic acid, and albumin (Goyal et al., 2016). Due of the nature of these nanoparticles, batch to batch variance is inevitable making it difficult to have reproducible results. When compared to natural polymeric nanoparticles, synthetic-based nanoparticles can give consistent outcomes (Goyal et al., 2016).

PLGA incorporated nanoparticles have shown better success in delivering drugs across the skin. PLGA is a synthetic biocompatible, biodegradable, and non-toxic polymer (Masood, 2016). PLGA was incorporated with glucose amine to treat osteoarthritis. Glucosamine experienced several side effects when taken orally, such as gastrointestinal distress and decreased bioavailability because to first-pass hepatic metabolism. To circumvent the pitfalls associated with oral glucose amine, glucosamide was incorporated with PLGA and delivered transdermally to treat osteoarthritis (Rahimi et al., 2021). Local anaesthetic agents prilocaine (PLC) and lidocaine (LDC) were employed in a study conducted by Cordeiro Lima Fernandes et al., 2021. PLC and LDC were encapsulated in a lipid nanocapsule using phase inversion method and optimised using a factorial design. Rheological studies of the gel were performed. The formulation had a low particle size and a high surface area, according to the study's findings. The work demonstrates that lipid nanocapsules are effective PLC and LDC carriers, prolonging and enhancing topical anaesthetic. The skin was not irritated by the prepared gel (Cordeiro Lima Fernandes et al., 2021).

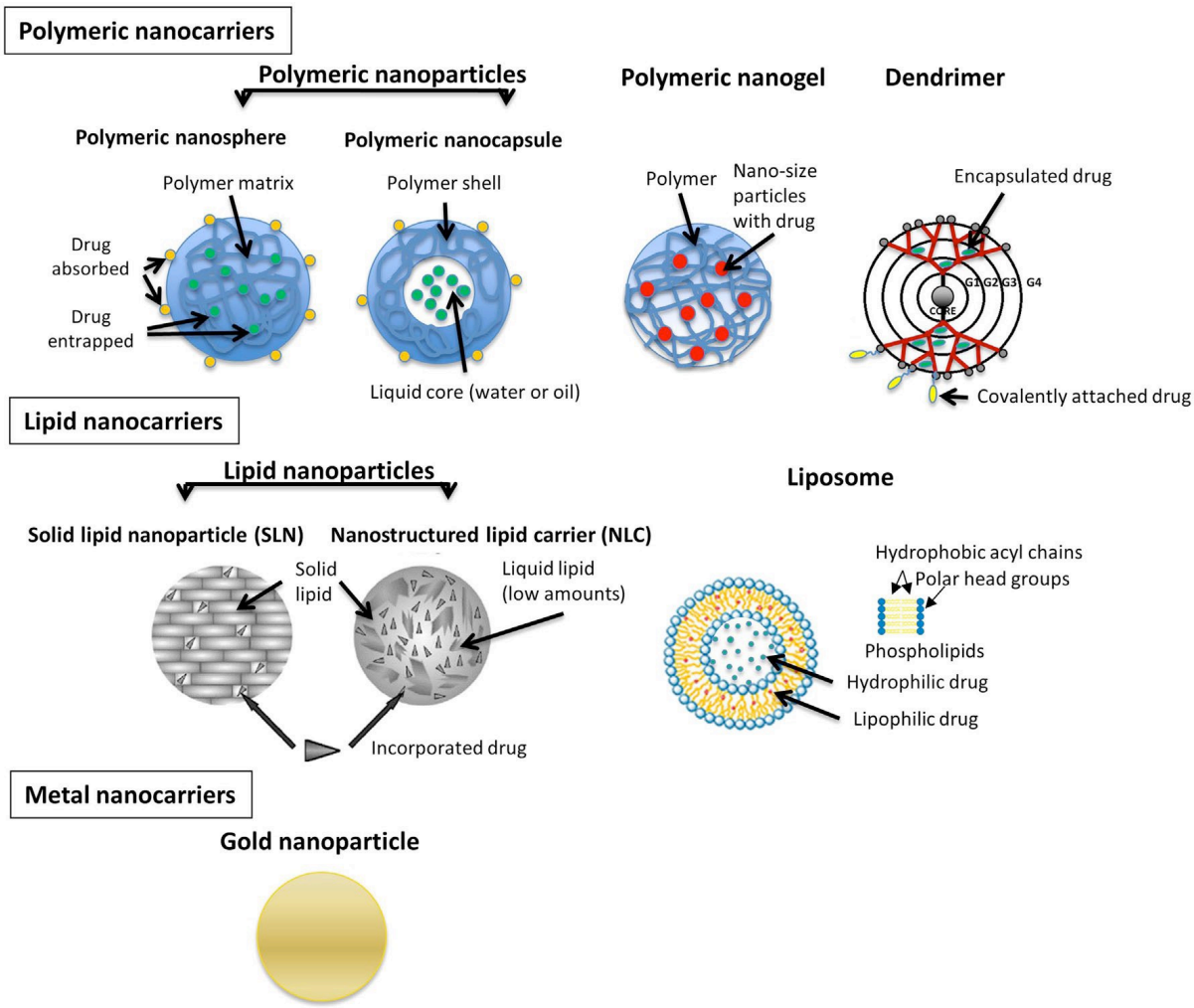


Figure 2.4 Schematic representation of various nanocarriers that can used to enhance penetration of drugs across the skin (Dragicevic and Maibach, 2018)

Table 2.2 A table showing different classes of drugs used in the management of pain, their yielded results, and references.

Drug	Nanotechnology	Pain condition targeted	Results yielded	References
Baicalein	Liposome	Anti-inflammatory activities	Increased skin penetration	(Kim et al., 2019)
diclofenac diethylammonium	Niosome	Anti-inflammatory activities	Increased physical and chemical stability. Tween 60 also showed high penetration through rat skin and increased inflammatory response on rat ear oedema assay	(Manosroi et al., 2008)
lignocaine	Transferosomes	Local anaesthetic	Shortening of onset of action while increasing the duration of action of the local anaesthetic	(Y. Liu et al., 2021)
indomethacin	Ethosomes	NSAIDs	Enhanced skin permeation compared to commercial indomethacin solutions	(Sakdiset et al., 2019)
Capsaicin	Invasome	Anti-inflammatory and pain killer	Skin penetration was higher than in commercial capsaicin in ethanol and conventional liposome	(Duangjit et al., 2016)
Sumatriptan	Solid Lipid nanoparticles (SLNs)	Migraine	Increased skin permeation of the SLN containing Sumatriptan compared to the plain gel	(Mohammadi-Samani et al., 2020)
Piroxicam	Nanostructured lipid carriers (NLC)	Anti-Inflammatory	A stable gel shown by low PDI of (0.2), elevated zeta potential, entrapment efficiency of 100 % and particles size of less than 200 nm was obtained. No skin structure alteration	(Otarola et al., 2020)
cinnamon essential oil	Nanoemulsions	Anti-Inflammatory	Increased stability and solubility of the product	(X. Liu et al., 2021)
ketoprofen	Dendrimers	Anti-Inflammatory	Increased drug permeation of Ketoprofen through the skin	(Manikkath et al., 2017)
ketoprofen	Carbon nanotubes	Anti-Inflammatory	high voltages caused an increase in drug release.	(Im et al., 2010)
Naproxen	Nanogels	Pain killer	Increased bioavailability of naproxen in the blood as shown by downregulation of COX-2	(Yurdasiper et al., 2018)
Indomethacin	Polymeric micelles	NSAIDS	Improved plasma concentration in rats	(Zang et al., 2007)
Prilocaine and lidocaine	Lipid nanocapsule	Local anaesthetics	Low particle size and high surface area. No skin irritation.	(Cordeiro Lima Fernandes et al., 2021)

2.8 Transdermal drug delivery systems (TDDS) and its limitations

The skin is the body's biggest organ. It contains an outermost layer called the SC that inhibits chemicals from passing through it and entering the system. TDDS was created to allow medicinal substances to flow through the skin. TDDS has used physical and chemical technology for penetration of macromolecules. Physical technologies such as microneedles, microjets, electroporation, sonophoresis and iontophoresis have been used. Chemical technology used to enhance drug penetration include penetration enhancers and biological peptides (Münch et al., 2017).

TDDS has been restricted to lipophilic medicines with a molecular weight of less than 500 Da (M. Chen et al., 2020). In a research study conducted, polymeric microneedles were employed as TDDS for the management of diseases like diabetes, infectious disease, cancer, and dermatological disease therapies (M. Chen et al., 2020). In the study, nanoparticles were encapsulated with polymeric microneedles. It was found that this type of TDDS has advantages in the treatment of numerous diseases. Both hydrophilic and hydrophobic medicines can be delivered through the skin with the use of TDDS (M. Chen et al., 2020). Opioids have successfully been administered using TDDS for management of pain. This class of medicines was found to be beneficial including prevention of blood peaks, caused consistent and uninterrupted drug distribution, and lessened bothersome side effects. A noticeable increase in patient compliance resulted from the fewer daily treatments to 72 hourly or weekly. Fentanyl and buprenorphine patches are frequently used to treat cancer patients' chronic pain (Mastrangelo et al., 2021).

Even though TDDS have advantages over oral and parenteral drug administration they do have limitations associated with their use. The choice of polymer matrix, the drug choice and penetration enhancers used in TDDS must be carefully considered before a formulation can be synthesised to deliver a drug across the skin and become successful. Polymers chosen in TDDS must be biocompatible, cheap and must not have interactions with the drug and other excipients (M. Chen et al., 2020). Drugs chosen for delivery through the skin must be suitable for administration in low doses, have low molecular weight, be lipophilic, have a log P value of less than 5, and a melting point of 200°C (M. Chen et al., 2020). The application of a dosage form may cause local skin irritation or sensitivity at the site of administration. Not all medications

are appropriate for transdermal administration. When compared to other delivery routes, the transdermal approach is comparatively more expensive (Bajaj et al., 2011b).

2.9 Conclusion

Pain is a healthcare problem that affects the social and economic aspects of patients and the society at large. Current treatment of pain may include non-pharmacological and pharmacological treatment options. The use of different medications is used to manage pain pharmacologically, including NSAIDS, local anaesthetics and opioids. Even though these medications are mainly administered by oral route and parenteral routes, the formulations are associated with side effects. The current research options are used to formulate dosage forms that will have less side effects (Andreu and Arruebo, 2018).

The skin is used in TDDS to allow drug penetration across the SC. Transdermal pain management plays a key role in patients because the formulations are less expensive than other dose forms, and patient compliance is higher due to the simplicity of application. Even though the route has advantages, there are few challenges that they are associated with. These may include inconsistent drug release and spike in drug release and toxicity problems. Over the last decade the use of nanotechnology was employed in drug delivery systems to enhance targeted treatments with fewer side effects (Alexander et al., 2012). It is used to overcome challenges associated with conventional transdermal drug delivery systems by allowing transportation of drugs across the skin.

To boost drug delivery to the systemic circulation and enable the administration of a variety of drugs, especially those that are challenging to deliver via chemical penetration augmentation approaches, physical enhancement tactics must be employed (Alkilani et al., 2015).

CHAPTER 3
DESIGN AND SYNTHESIS OF A NANOCAPSULE SYSTEM FOR TRANSDERMAL DELIVERY OF LIGNOCAINE ACROSS THE SKIN

3.1 INTRODUCTION

Pain is an unpleasant, uncomfortable feeling and emotional experience that is linked to tissue injury. Poor management of pain can lead to further tissue damage, infections, hormone imbalance, the lengthy stays in hospital, agitation, and delirium (Cho and Hong, 2021). There are several classes of medicines that can be used either individually or in combination with one another to treat pain. These classes include nonsteroidal anti-inflammatory drugs (NSAIDs), local anaesthetics, antiepileptic drugs, antidepressants, skeletal muscle relaxants, and opioids for the management of pain (Andreu and Arruebo, 2018).

Local anaesthetics are used in pain management to numb the skin. They are used mostly as dermal applications with examples being LIG topical gel, patch, or sprays (Wrona et al., 2022). Topical or local anaesthesia when used in conjunction with NSAID analgesia, has been shown in numerous trials to relieve pain effectively and affordably during and after a variety of procedures (Windsor, 2022).
In this study Lignocaine was used as a local anaesthetic for the treatment of pain. A class Ib antiarrhythmic and amide-type local anaesthetic, lignocaine has been utilized successfully in a wide range of conditions thanks to its biopharmaceutical qualities. LIG was designed and synthesized as a nanocapsule for transdermal delivery system across the skin.

Nanocapsules are incredibly small particles ranging with the size between 10 – 1000 nm that are comprised of a liquid and solid core with a cavity in which a medicine is deposited that is surrounded by a unique polymer membrane made of organic or synthetic polymers. They have gotten a lot of attention because of the protective coating, which is frequently pyrophoric, quickly oxidized, and delays the release of active chemicals (Kothamasu et al., 2012). Drugs are inserted into the core cavity of nanocapsules, which protects them against rapid deterioration (Pathak et al., 2019).

Scientists have made great advancements in the development of drug delivery systems (DDS), mostly because the skin offers a suitable and vast surface region for drug delivery, over the past few decades. Currently under development is the creation of efficient transdermal DDS that provide prolonged drug release at the targeted location for a longer period within the recommended therapeutic window without causing adverse effects. With FDA approval, poly (lacticcoglycolic acid, or PLGA) is among the most promising synthetic polymers for creating flexible drug administration vehicles for different drug administration routes, such as transdermal medication delivery (Naves et al., 2017).

Because of its advantageous mechanical strength, biocompatibility, and biodegradable qualities, PLGA is widely utilized and thoroughly defined for controlled drug delivery applications. This allows for its usage via a variety of routes with minimal side effects (Pardeshi et al., 2021). Local anaesthetics loaded with these polymer carriers are slowly released at the nerve periphery, resulting in a longer nerve block and a lower overall number of local anaesthetics entering the human circulation per unit time. The release of local anaesthetics from polymeric nanocarriers allows for a longer nerve block while reducing the possibility of the medication entering the bloodstream (Zhang et al., 2023). The copolymer PLGA, which is made up of Poly Lactic Acid (PLA) and Polyglycolic acid (PGA), is presently one of the most well-defined nanomaterials for drug administration. Because of these remarkable qualities, PLGA has been one of the most appealing polymeric alternatives, and its adoption as a drug delivery carrier has shown enormous potential (Phạm and Kim, 2020). In this study, a nanocapsule containing LIG loaded with PLGA is designed and synthesised for transdermal drug delivery system across the skin.

3.2 MATERIALS AND METHODS

3.2.1 Materials

Poly D, L-lactide-co-glycolide (PLGA), Tween® 80, Phosphatidylcholine (PC), chloroform, Lignocaine (LIG)., Dulbecco's Modified Eagle Medium (DMEM), Snakeskin Dialysis bag 22 mm, Foetal bovine serum (FBS), 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide dye (MTT), 0,25 % $^{w}/_{v}$ trypsin were purchased from Sigma (Sigma-Aldrich, Missouri, USA). Immortalised Human Keratinocyte, HaCaT cells (CRL# 1573, passage 21, Batch # 203970) were purchased from the

American Type Culture Collection (ATCC) (Rockville, MD, USA). All reagents and chemicals of high analytical grade were used. Pipettes, beakers, standard volumetric flasks and measuring cylinders, Syringe, polytops, filters (0,45 micron), centrifuging tubes.

3.2.2 Synthesis of lignocaine-loaded nanocapsule

The LIG-PLGA-PC nanocapsules were prepared using solvent displacement technique as shown in Figure 3.1 (Fessi et al., 1989). The aqueous phase consisted of 200 mg of Tween® 80 in 30 ml of purified distilled water. The mixture was ultrasonicated for 2 minutes then put under electronic magnetic agitation. To make the organic phase, 125 mg of PLGA, 250 mg of PC and 30 mg of LIG were dissolved in 5 ml of chloroform. The organic phase was introduced dropwise to the aqueous phase using probe sonication and stirred overnight to eliminate the organic solvent. The formulated nanosytem was then frozen and lyophilized (FreeZone® 2,5, Labconco®, Kansas City, MS, USA) at -80 ˚C for 48 hours to produce a powder and was analyzed using different analytical techniques as depicted in the figure 3.1 below. Four samples were prepared with three samples having a drug loaded in the organic phase. The fourth sample was blank, without the drug in the organic phase.

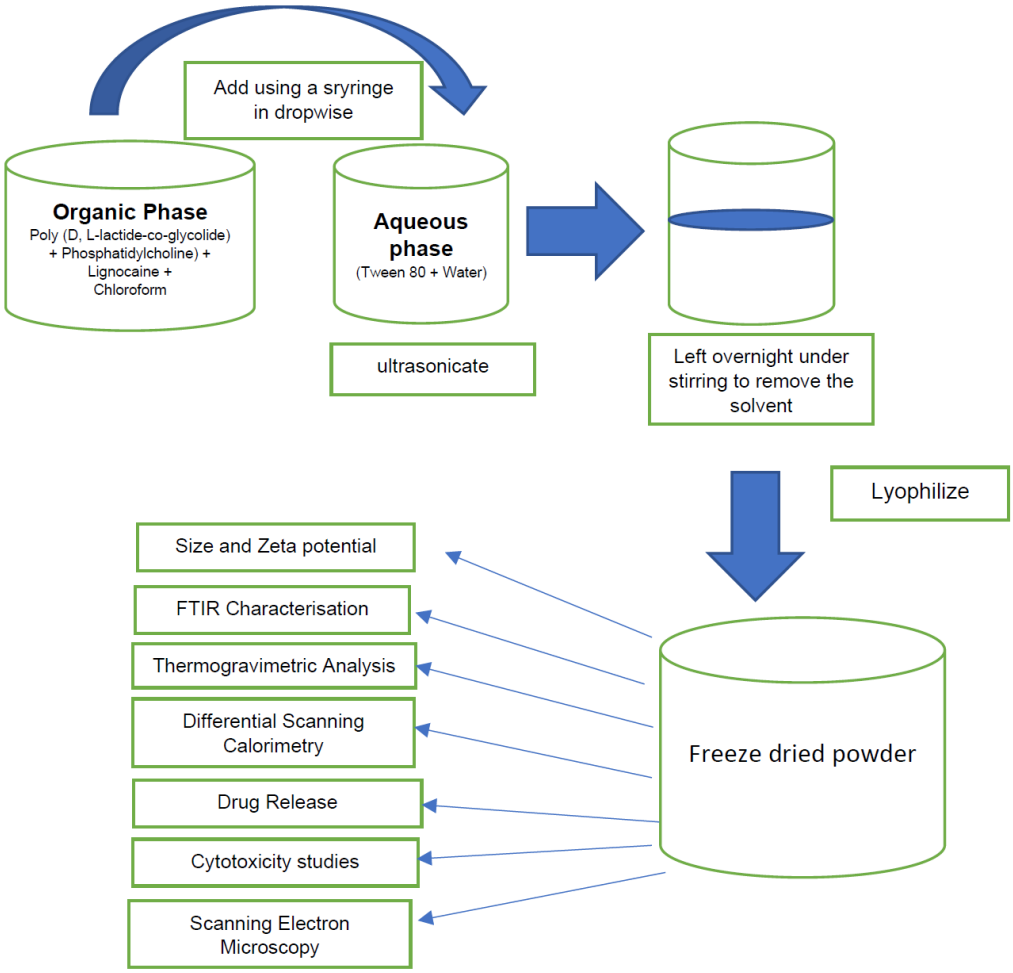


Figure 3.1 Schematic representation of LIG-PLGA-PC nanocapsule system using solvent displacement technique and physicochemical characterization of the formulation.

3.2.3 Physicochemical analysis using Fourier Transformation Infrared (FTIR) spectroscopy.

To examine the chemistry and composition of the blank nanocapsule, the polymers and drug loaded nanocapsule samples were studied using Attenuated Total Reflectance Fourier Transformation Infrared (ATR-FTIR) analysis (Nandiyanto et al., 2019). Characteristic peaks were examined to establish the molecular vibrations of the polymers in the formulations. A single-reflection diamond MIRTGS detector (Perkin Elmer® Spectrum 100, Llantrisant, Wales, UK) was used with the Perkin Elmer® Spectrum 2000 FTIR Spectrometer. Using a universal ATR polarization accessory, samples were analyzed at a resolution of 4 cm^{1}. Samples of each polymer, LIG, and

manufactured nanocapsules were placed on a diamond crystal and each was run at a scan rate of 100 to reduce signal to noise ratio, in the wavelength range of 4000 – 600 cm^1 at a constant pressure of 120 psi (Kondiah et al., 2013).

3.2.4 Determining Particle size and zeta potential of copolymeric nanoparticles.

The zeta potential of nanocapsules and particle size distribution were measured using a Zetasizer NanoZS analyser (Malvern Instruments Ltd. UK), which works by dynamic light scattering. A 90 ° light scattering angle and a temperature of 25 °C were used. 0.2 ml of each sample was drawn and diluted with 0.8 ml of distilled water. The blended sample was filtered through a 0.45-micron filter and particle size and zeta potential were determined. At a temperature of 25 °C, all measurements were made in triplicate. Calculations were made for particle size, polydispersity index, zeta potential, and organic solvent content to screen formulations and choose the most beneficial preparation parameters.

3.2.5 SEM Morphological of lignocaine-loaded nanocapsules

The LIG-PLGA-PC nanocapsule was examined with the JEOL 840 SEM (JEOL, Japan) at various magnifications at 20 keV. The lyophilized dry formulation was placed onto studs and sputter-coated with gold-palladium shadowing. The surface shape and texture of the synthesized nanocapsules were determined using micrographs of the formulation.

3.2.6 Thermal Analysis using Differential Scanning Calorimetry and Thermo Gravimetric Analysis

Thermogravimetric analysis (TGA) was performed on the LIG; 10 mg of material was heated at 10 °C/min in an open aluminium pan with nitrogen as a purge gas (flow rate of 25 ml/min). The percentage copolymer mass loss was calculated under temperature heating settings (Nasef et al., 2017).

3.2.7 Entrapment efficiency of Lignocaine-loaded nanocapsule

The formulation was centrifuged for 30 minutes at 4750 rpm. The supernatant was removed, while that of the sample with drugs was kept. 25 ml of clean purified water was added, the tubes were sonicated to mix the particles at the bottom. The tubes

were placed back in the centrifuging machine at 4750 rpm for 30 minutes and the amount of entrapped LIG was detected using UV Spectrophotometer. Drug entrapment efficiency (DEE) was calculated at 265,1 nm using the calibration curve (Figure 3.2) and the computed linear equations (1 & 2), where r^2 = 0,9999 was found. Using the created standard calibration curve that links absorbance to concentration using the Beer Lamberts Law of linearity (Equation 1), the specific absorptivity can be utilized to determine the drug concentration released. To determine the Drug Entrapment Efficiency, utilize Equation 3 (Weng et al., 2020).

Equation 1: $y = mx + C$ (1)

Where:

Y: dependent variable

M: gradient of the line

X: independent variable (concentration)

C: y -intercept

Equation 2: Lignocaine Concentration

$$Concentration\ of\ Lignocaine = \frac{y}{m} - b \quad (2)$$

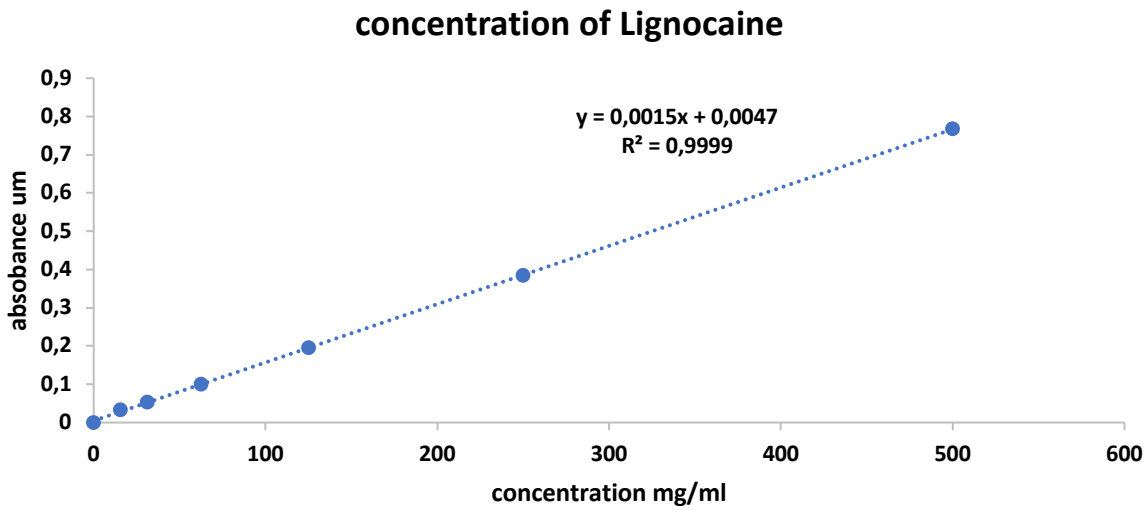


Figure 3.2 Calibration curve of Lignocaine in distilled water using Cecil 3021 scanning spectrophotometer at 200 – 400 nm (All instances: n = 3 and SD < 0,025)

The LIG-loading capacity (%$^{w}/_{w}$) was calculated using Equation 4. All measurements were conducted in triplicate.

Equation 3: Drug Entrapment Efficiency

$$DEE(\%) = \frac{\text{total amount of lignocaine} - \text{amount of free lignocaine}}{total\ amount\ of\ lignocaine} \times 100$$

(3)

Equation 4: Lignocaine Loading Capacity

$$Lignocaine\ Loading\ (\%\frac{w}{v}) = \frac{\text{Mass of lignocaine in nanocapsules}}{Mass\ of\ Nanocapsules} \times 100 \quad (4)$$

3.2.8 In vitro drug release analysis of the copolymeric nanoparticles containing Lignocaine.

Using LIG-PLGA-PC nanocapsule samples that were synthesised as per section 3.2.3, *in vitro* release tests of LIG were carried out. 3 x 2 ml of the drug-loaded samples were placed in the dialysis bags. The sample without the medication was the fourth one. The Phosphate buffer solution (PBS) release media (80 ml, 37 °C) was submerged in the sealed bags, and they were shaken orbitally at 100 rpm. 3 ml of samples were taken and replaced with brand-new PBS stored at the same temperature every 30 minutes, 1 hour, 2 hours, 4 hours, 8 hours, and 24 hours. Using a UV spectrophotometer, the absorbance at a wavelength of 265,1 nm was measured to determine the content of LIG in the samples (Raj and Prabha, 2016). The calibration curve obtained is displayed in Figure 3.2, and the release curve in Figure 3.7 shows the concentration of LIG at various time Intervals, with each point presented as mean ± SD (n=3). A comparable protocol and set of experimental parameters were utilized to ascertain the release profile of bare lignocaine.

3.2.8.1 Determination of ג max

The LIG stock solution was made by dissolving 50 mg of the medication in 50 ml of purified water to achieve a concentration of 1 mg/ml. The standard stock solution of LIG was utilized to calculate the ג max. The absorbance was measured using a UV spectrophotometer in the 200 - 400 nm range. The greatest absorbance on the curve was 265,1 nm.

3.2.8.2 Linearity profile

From the stock solution, different concentrations were prepared including 0,5, 0,25, 0,125, 0,0625, 0,003125 and 0,0015625 mg/ml. Absorbance values of these solutions were measured at ג max of 265,1 nm as shown in Figure 3.3. Table 3.1 shows the Linearity profile of lignocaine to determine the wavelength. Equation 2 was used to determine Lignocaine Concentration.

3.2.8.3 Calibration curve

The calibration curve was plotted between the concentration of LIG and respective measured absorbances. The LIG concentration at various time intervals was displayed on the release curve, with each point represented as mean ± SD (n = 3).

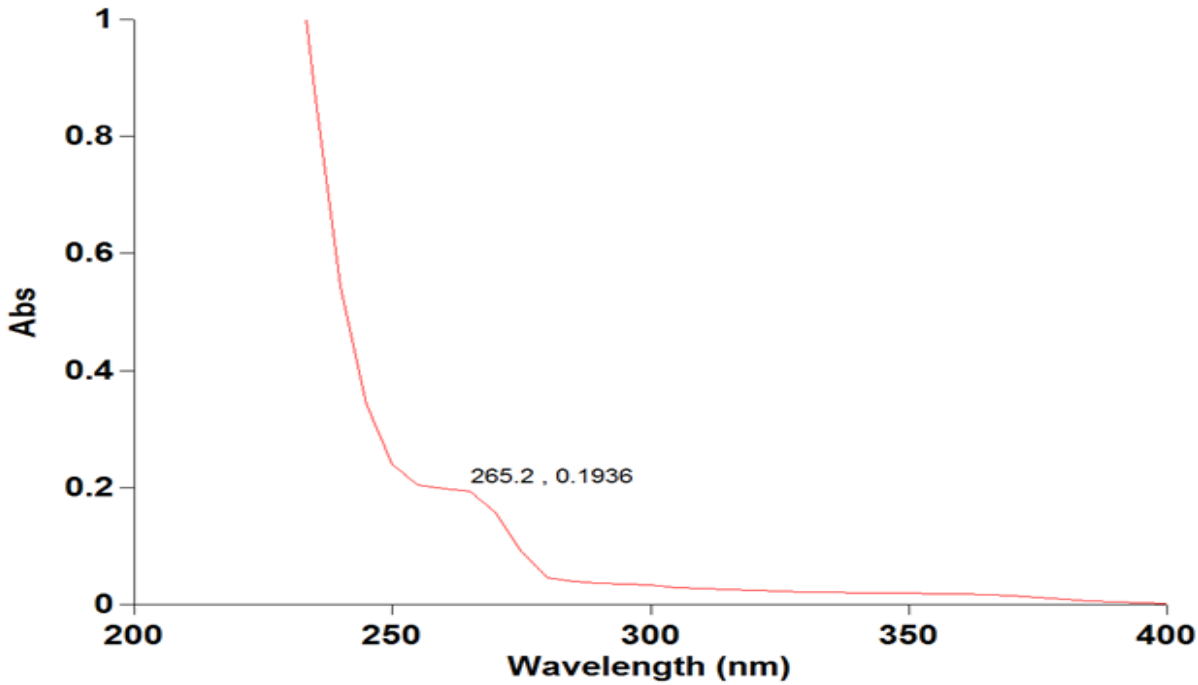


Figure 3.3 UV spectrum of Lignocaine ג max 265,1 um

Table 3.1 A table showing Linearity profile of lignocaine to determine the wavelength.

Statistical parameters	Results
Wavelength	265,1 nm
Slope	1,4653
Regression coefficient	Y=1,4653x + 0,0254
Correlation coefficient r	0,9971
Wavelength	265,1 nm

3.2.9 *In vitro* cytotoxicity studies of the lignocaine-loaded nanocapsules utilizing HaCaT seeding of cells.

The cell line Immortalised Human Keratinocyte, HaCaT, was used in the investigation. HaCaT cells were grown in an incubator at 37 °C and 5 % CO_2 (Thermo Scientific, Shanghai, China). The culture media, Dulbecco's Modified Eagle Medium (DMEM) (10 % FBS, 1 % Penicillin, and streptokinase), was aspirated from the flask and discarded. 1.5 ml of PBS was used to wash the cells and remaining solution was discarded. 2 ml of Trypsin solution was added to the flask to cover and detach the cells. The flask was incubated for 3-5 mins to resuspend the cells. 2 ml of DMEM medium was added to stop trypsination. For 5 minutes, the cells were centrifuged at 1500 rpm. To create a homogenous cell suspension, the cell pellet was resuspended in 1 ml of DMEM. The suspension was diluted x10 and a volume of 10 µL was added to 10 µL of trypan blue for counting.

3.2.9.1 Counting cells using a haemocytometer.

The chamber was cleaned, and cover slipped with alcohol. The coverslip was dried and fixed in position. Ten microlitres of the cell suspension were added to the haemocytometer. The chamber was placed in the inverted microscope under a 4X objective. The cells were counted in the large, central gridded square (1 mm^2). The average counted number of cells per square was multiplied by the dilution factor (24) and 10^4 to estimate the number of cells per ml.

3.2.9.2 Microscopy

Phase contrast images were captured after 48 hours and 72 hours of treatment using the 20X objective of inverted light microscope (Olympus CKX53, Tokyo, Japan).

3.2.9.3 Cell proliferation (MTT)

The MTT assay measures cellular metabolic activity to determine cell viability, proliferation, and cytotoxicity. The MTT test was run after 72 hours of treatment. Using a multichannel pipette, 10 µl of the MTT solution (at a final concentration of 5 mg/ml) was added to each well after the treatment period of 72 hours. The 96-well plates were then incubated for 4 hours at 37 ºC and 5 % CO_2 in a humid environment after adding MTT. After the intracellular formation of formazan crystals, 100 µl of the solubilizing agent was added, and the mixture was completely dissolved by overnight incubation at 37 ºC and 5 % CO_2. Triplicate wells containing only the growth medium and solubilization reagent were used as the blank. A multimode microplate reader (Victor X3, Perkin Elmer, Massachusetts, USA) was used to read the absorbance values at 570 nm using a reference wavelength of 620 nm. After calculating the mean values of the corrected absorbance measurements of each triplicate treatment in respect to the mean values of the PBS control (set at 100 % viability), the data was displayed in a grouped bar chart. The plates were timed for 48 and 72 hours (Colombo et al., 2017)

Percentage cell viability was calculated using quantity of cells counted over the count of quadrants, shown in Equation 5 & 6. DF represents dilution factor that was used and 10^4 is the constant.

Equation 5: Calculation of viable cell count…… (5)

$$Viable\ cells\ count = \frac{Number\ of\ cells\ counted}{Number\ of\ Quandrants\ counted}\ x\ DF\ x\ 10^4\ cells/ml$$

Equation 6: Calculation of percentage cell viability…… (6)

$$\%\ cell\ viability = \frac{\text{Absorbance of test} - \text{Absorbance of blank}}{\text{Absorbance of negative control} - \text{Absorbance of blank}} x\ 100$$

Where:

Absorbance of test: Various treatments used in this study.

3.2.10 Statistical Analysis

Origin software (version 8.5.0 SR1, Origin Lab Corporation, Northampton, MA, USA) was used to process and analyze data for nanoparticle synthesis and characterization, as well as drug release investigations. The mean standard error of three experiments was used to represent all results.

3.3 Results and Discussion

3.3.1 Characterization of the synthesised nanoparticulate formulation utilizing FTIR spectroscopy

Characterization of active pharmaceutical ingredients (APIs) using FTIR technique increases the quality characteristics of all raw materials utilized during the manufacturing process of pharmaceuticals, as well as those in the finished products (Anacleto et al., 2018).

The molecular composition and characteristics of the Lignocaine (LIG) loaded Poly D, L-lactide-co-glycolide (PLGA), and Phosphatidylcholine (PC), (LIG-PLGA-PC) nanocapsule were evaluated for their chemical characteristics. FTIR spectra of drug-loaded, Blank nanocapsules, and starting materials LIG, PC, PLGA, and Tween® 80 were illustrated in Figure 3.4.

The FTIR spectral evaluation of drug loaded sample showed a vibration at 3000–2800 cm^{-1} indicating a N-H stretch, and 1750-1735 cm^{-1} presented with a C=O stretching group. At 1250–1020 cm^{-1} there is a C-N stretching confirming the presence of an amine group. The FTIR spectral evaluation of blank nanocapsule showed a vibration at 3000–2800 cm^{-1} indicating a N-H stretch, and 1750-1735 cm^{-1} presented with a C=O stretching group. At 1250–1020 cm^{-1} the there is a C-N stretching confirming the presence of an amine group. The spectra resemble that of the drug loaded nanocapsule. The FTIR Spectra of Lignocaine showed that at 1680 cm^{-1} there is a C=O stretching indicating that lignocaine is a tertiary amine. At 1342-1266 cm^{-1}, there is a vibration with a C-N stretch showing it is an aromatic amine (Anacleto et al., 2018).

PLGA

A peak is located at 1750 cm^{-1}. The drug-loaded nanocapsule exhibits this similar peak. It can be seen from the figure 3.4 that all the samples have more than 5 absorption bands in the IR spectra, meaning that they are complex molecules.

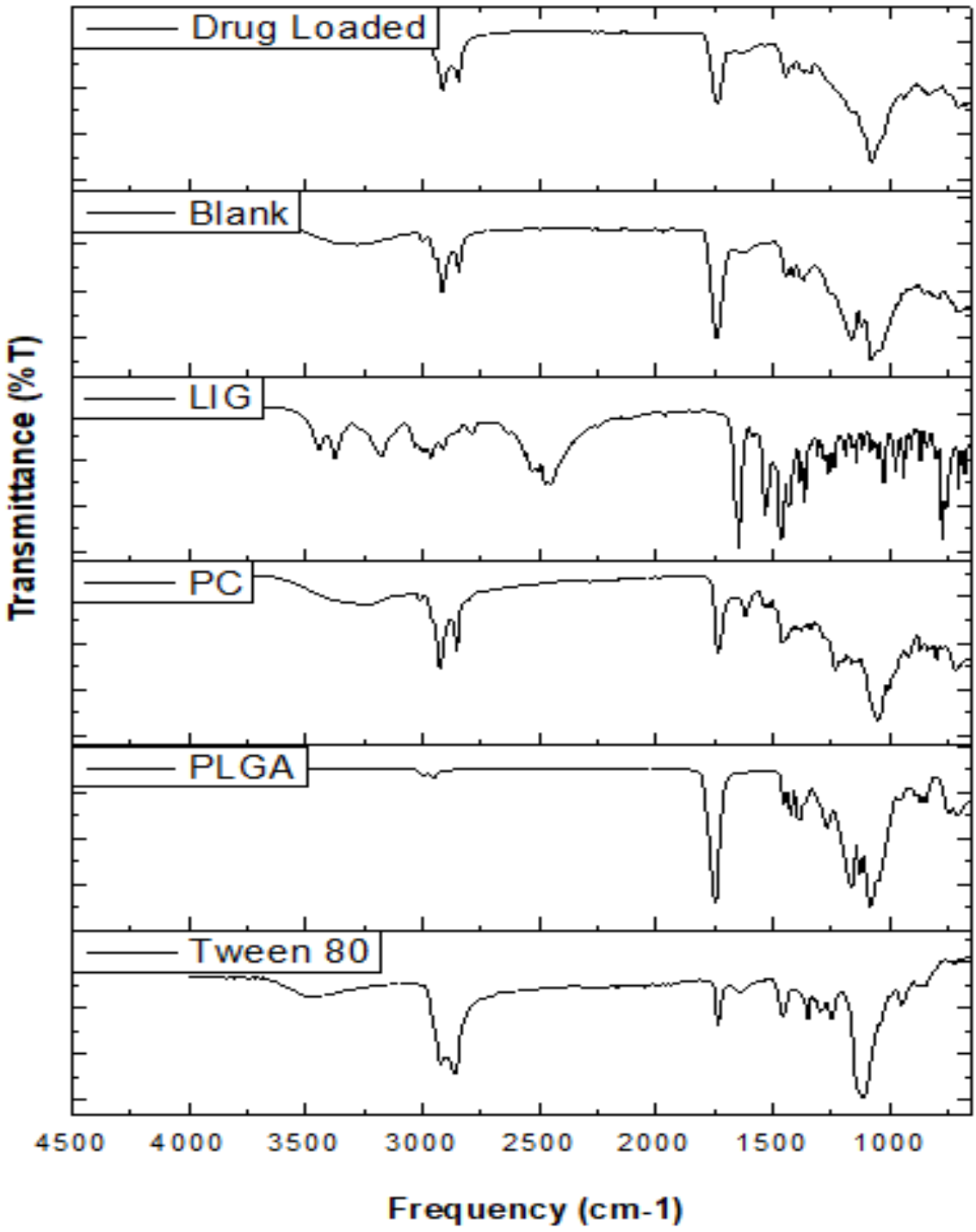
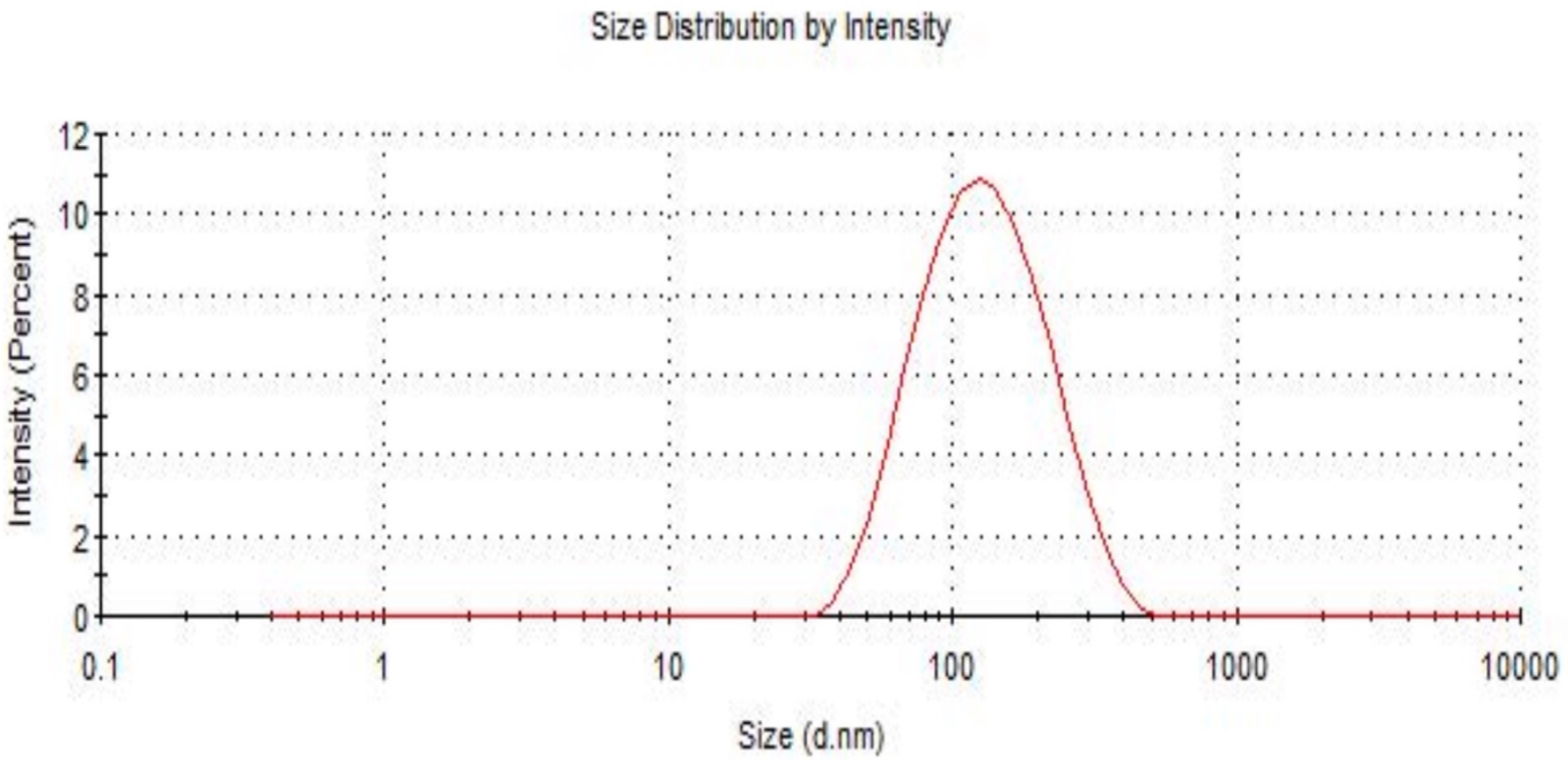


Figure 3.4 Fourier Transformation Infrared Spectra of synthesised LIG-PLGA-PC nanocapsule (Drug-loaded), blank nanocapsule (Blank), Lignocaine (LIG), PC, PLGA and Tween® 80

3.3.2 Lignocaine loaded nanoparticles size, zeta potential and SEM morphological evaluation

The Zetasizer NanoZS analyzer was used to measure the size and zeta potential of both blank and LIG-loaded PLGA-PC nanocapsules (Malvern Instruments Ltd. UK). A particle size of blank nanoparticles was 109 nm ± 72.21 with a polydispersity index (PDI) of 0.227± 0.0 PDI less than 0.5 indicating a good homogeneity of the sample with respect to the particle size distribution. In contrast, LIG-loaded PLGA-PC nanocapsule displayed a good particle size of 139.8 nm ± 81.46 with a PDI of 0.182. As seen in Figures 3.5 a(i) and 3.5 b(i), the mean particle size of drug-loaded nanocapsules was equivalent to that of placebo nanocapsules, irrespective of LIG drug loading. Particle size with a mean average of 250 - 500 nm is generally obtained with nanocapsules (Mora-Huertas et al., 2010). The blank and LIG loaded PLGA-PC nanocapsule showed a high level of nanoparticle stability in solution with a zeta potential of -49.7 mV ± 5.87 and -48.2 mV ± 4.92 respectively as shown in figure 3.6 a(ii) and 3.6 b(ii). The zeta potential values of the prepared nanocapsule can be attributed to chemical nature of both the polymer and the stabilizing agent used (Mora-Huertas et al., 2010).

Z-Average (d. nm): 109.0 ……a(i)
PdI: 0.227



Z-Average (d. nm): 139.8 …… b(i)
PdI: 0.182

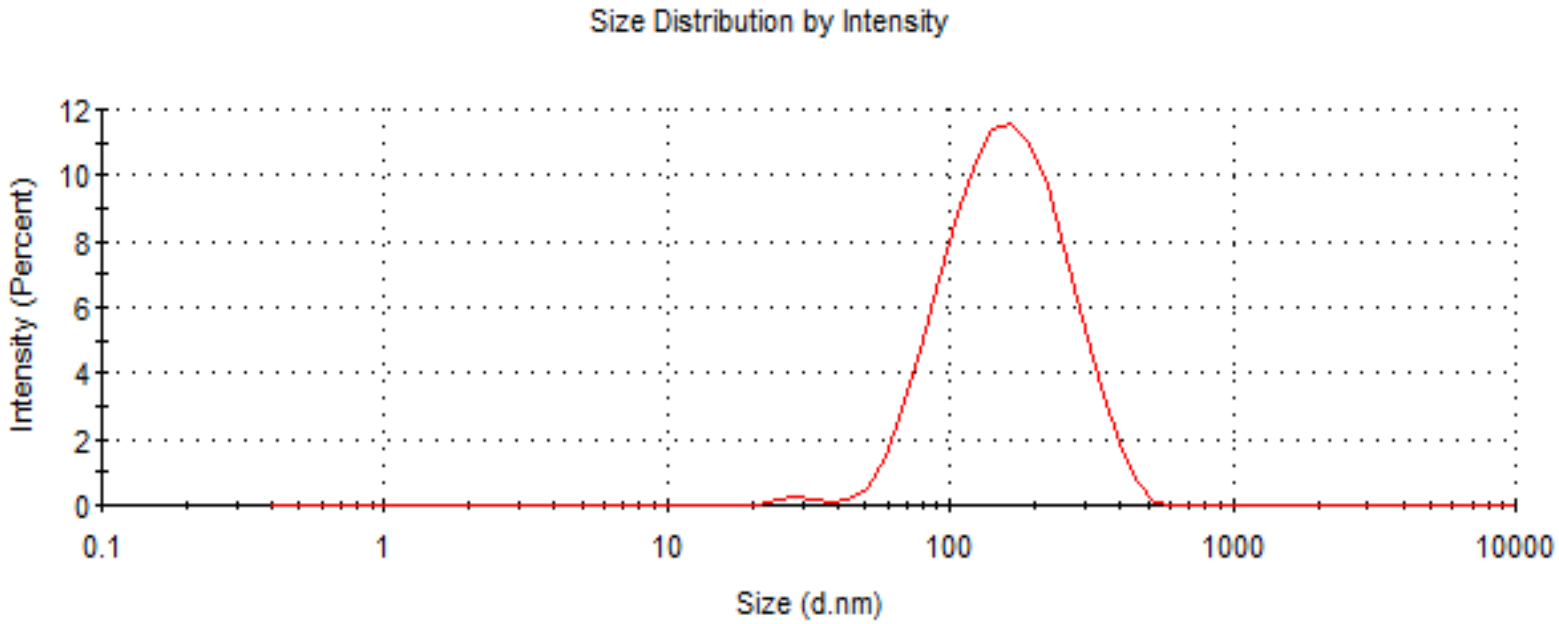
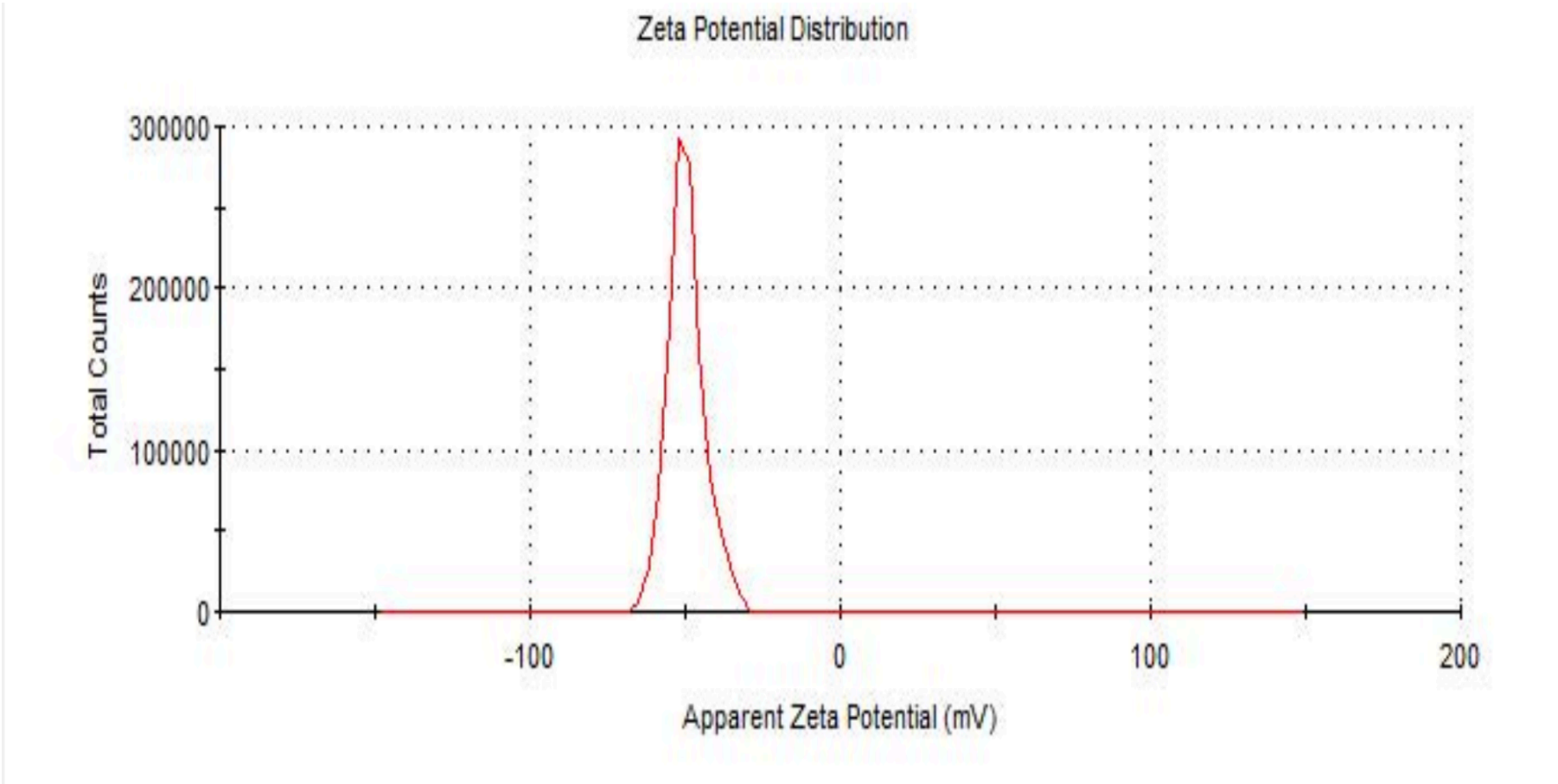


Figure 3.5 Particle size distribution graph of a(i) Placebo nanocapsules and b(i) Lignocaine loaded nanocapsules.

Zeta Potential (mV: -49.7 …… a(ii)



Zeta Potential (mV: -48.2) ..... b(ii)

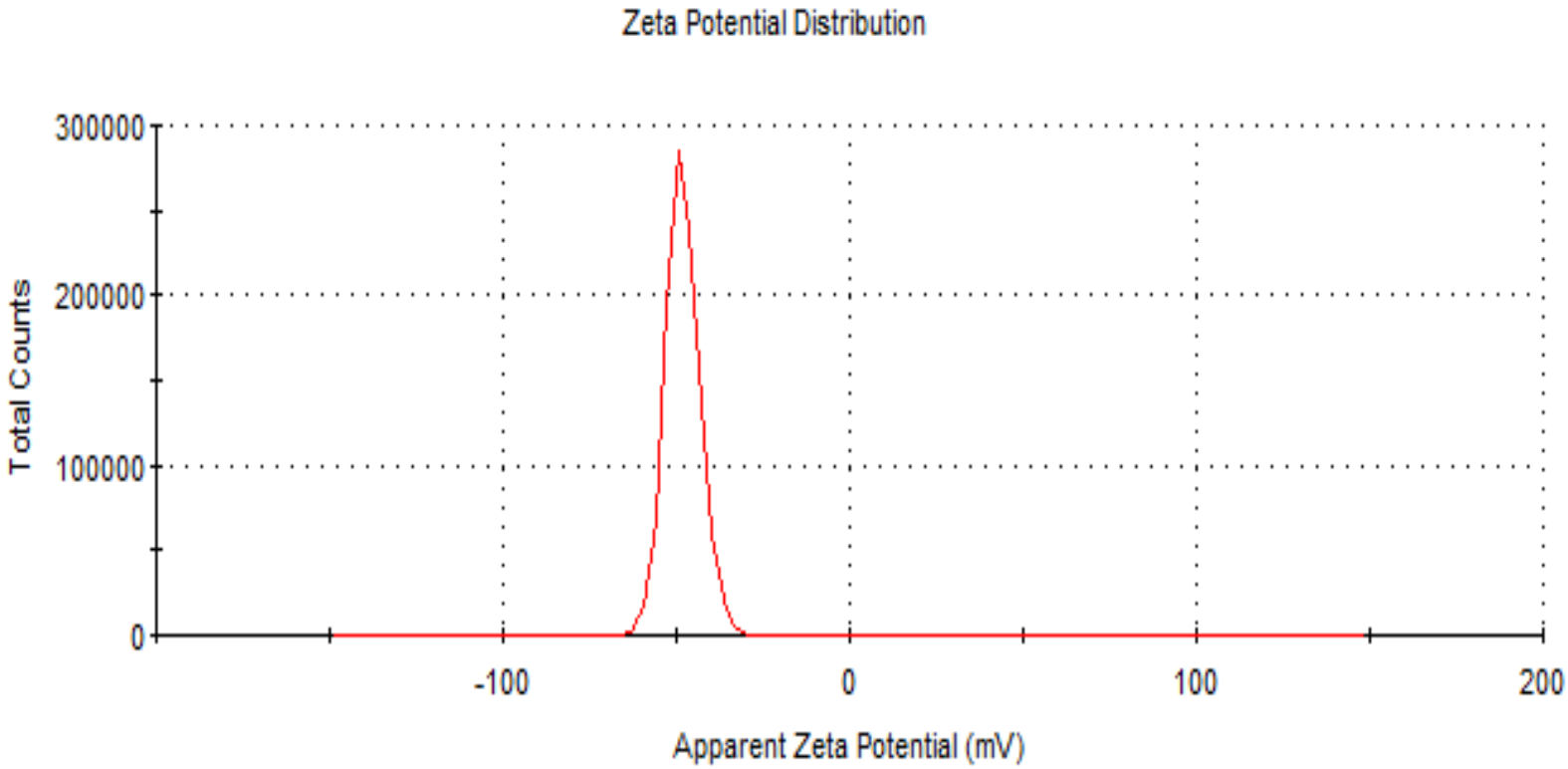


Figure 3.6 Zeta potential graph of a(ii) Placebo nanoparticles and b(ii) Lignocaine loaded nanocapsules

Surface Morphology of the formulated nanocapsules was examined and characterized utilizing scanning electron micrograph (SEM). When compared to SEM images (Figure 3.7), the particle size indicates core shaped particles with a limited size distribution and particle size of roughly 579 nm. The figure depicts the structure of a nanocapsule with a core region surrounding the encapsulated drug. This size falls within the range of the nanocapsule particulate dispersion, which is between 10 and 1000 um (Lima et al., 2022).

Polymeric nanoparticles (NPs) generated from various techniques typically have mean diameters ranging from 100 to 300 nm. The large particle size seen on the figure 3.7 may be because of many factors, such as the quali-quantitative composition, can affect the size of polymeric nanoparticles. The amount of drug present may also have an impact on the average diameter of the nanoparticles, perhaps resulting in larger particles with a wider size distribution. The zeta potential of more than 30 mV can be seen and that is crucial for the colloidal suspension's physicochemical stability since strong repulsive forces often inhibit aggregation (Zielińska et al., 2020).

The aggregation seen in the figure 3.7 below could be as a result of the process of solvent removal used in the synthesis of the nanocapsule (Shrestha et al., 2020). The aggregation of particles could have been as a result of drying process to increase stability of the nanocapsules (Deng et al., 2020).

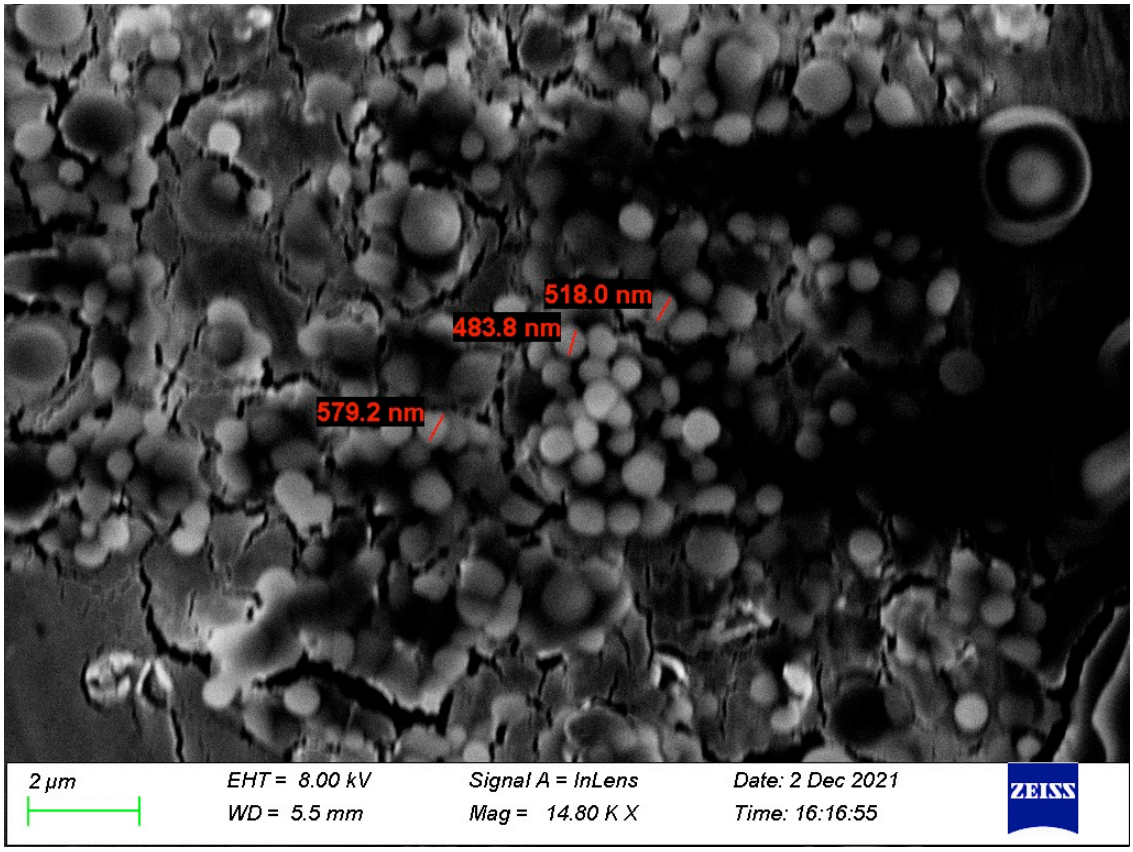
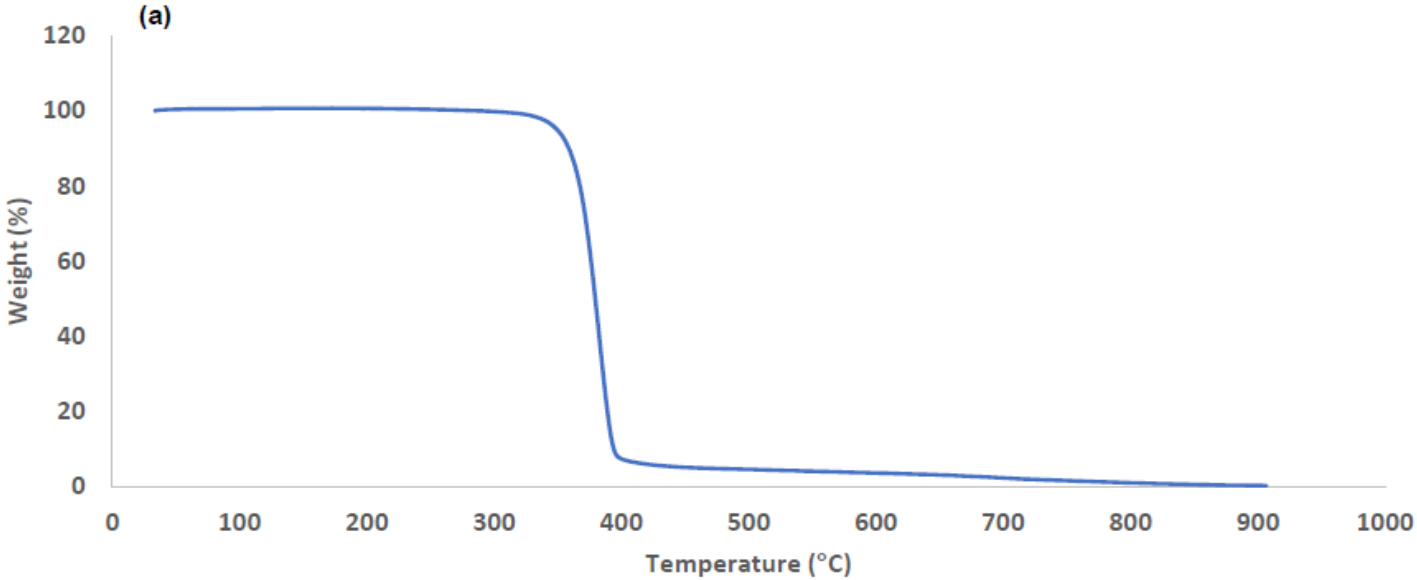


Figure 3.7 Illustration of scanning electron micrograph (SEM) image of self-assembled LIG-PLGA-PC nanocapsule formulation in dry state.

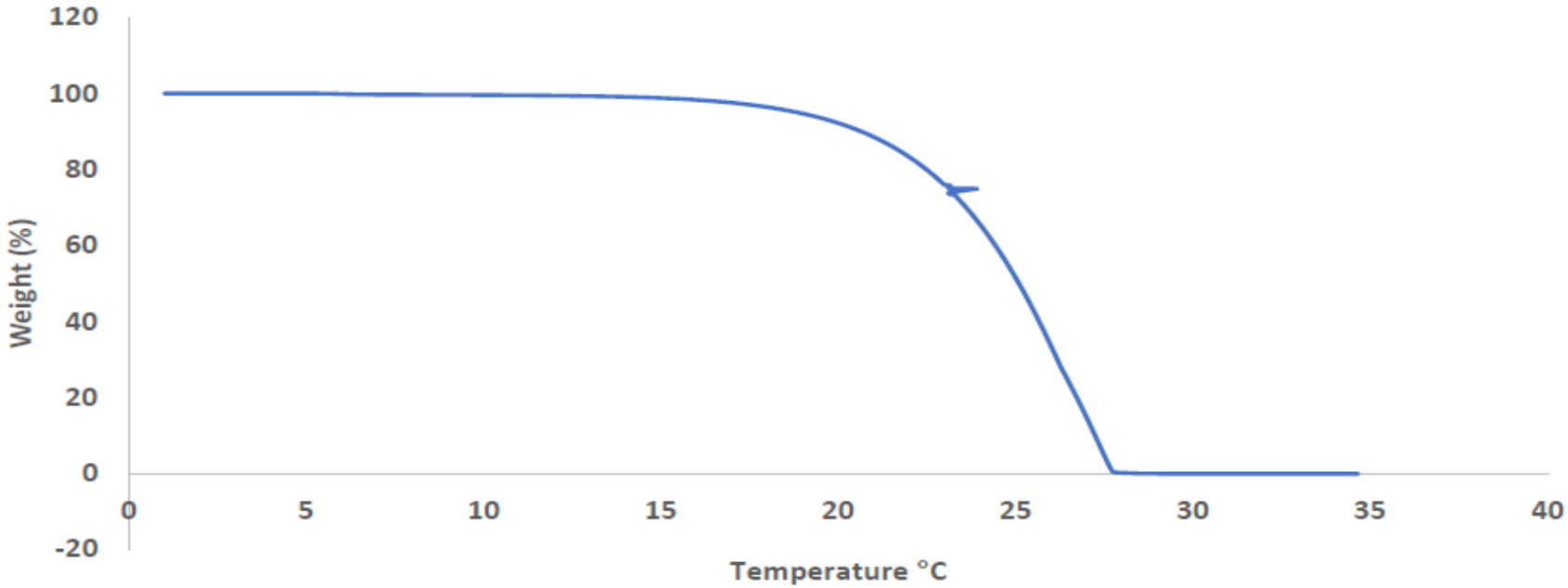
3.3.3 Thermogravimetric analysis

Thermal stability experiments were carried out to evaluate the integrity and variations in the mass of the polymeric nano-formulation under thermal stress. LIG is known to be thermally unstable; analysis was conducted to examine the magnitude of change in thermal stability properties of LIG incorporated in the nanocapsule. Utilizing the TGA, the thermal degradation profile of LIG-PLGA-PC nanocapsule was generated as shown in Figure 3.8. The LIG-PLGA-PC nanocapsule demonstrated a one-step degradation with weight loss of 67 % when subjected to elevated temperatures between 330 – 400°C. These points correspond to the points of inflection on the nano-formulation TGA thermogram in Figure 3.8 (a). The thermograph for the model drug LIG showed a weight loss, at 20 °C as shown in Figure 3.8 (b). In Figure 3.8 (c), the blank nanoparticles showed a weight loss and degradation at a temperature range of 300 – 400 °C with a total loss of 94 % of weight, which is attributed to the copolymeric structure degradation. The inclusion of the drug in the formulation results in a one-step

degradation difference between LIG-PLGA-PC nanocapsule and blank nano-formulation at 330 – 400 °C.


(a)
120
100
80
60
40
20
0
Weight (%)
0
100
200
300
400
500
600
700
800
900
1000
Temperature (°C)

(b)


120
100
80
60
40
20
0
-20
Weight (%)
0
5
10
15
20
25
30
35
40
Temperature °C

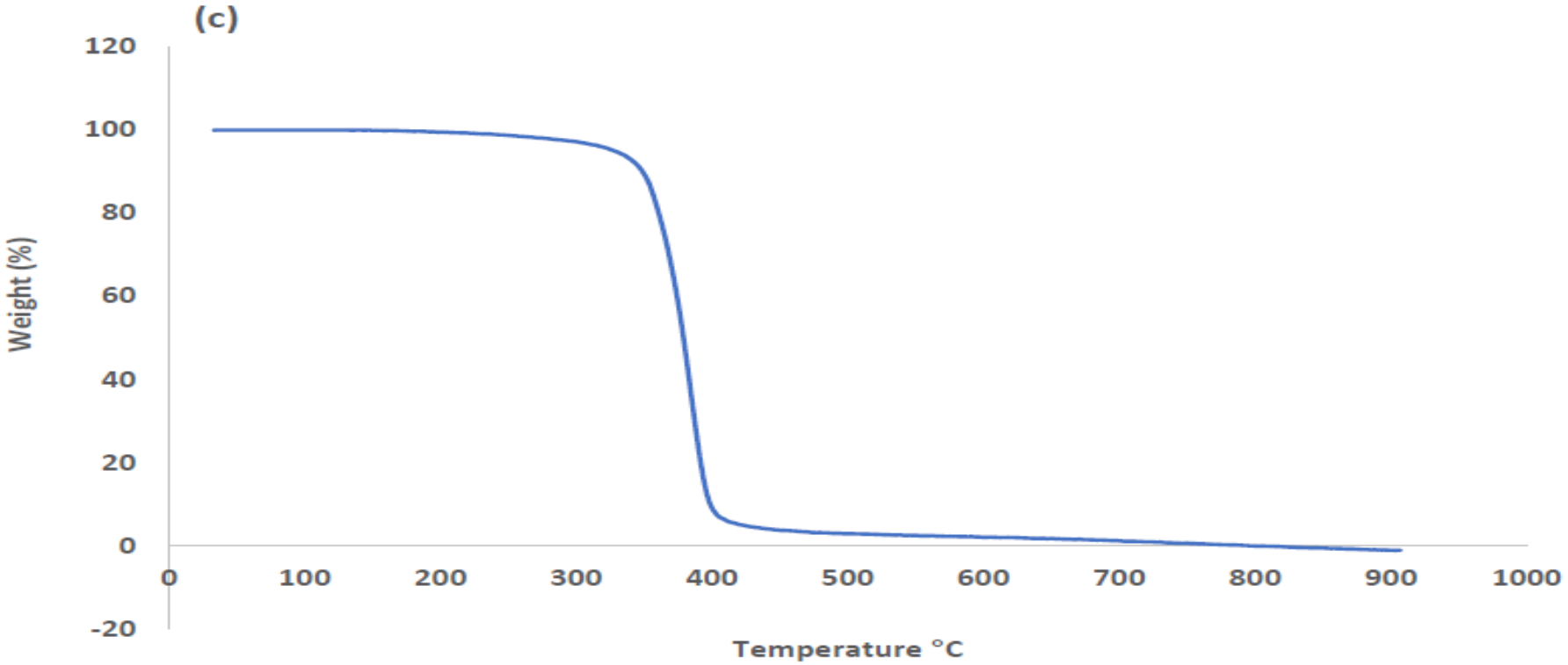


Figure 3.8 Thermogravimetric curves (TGA) depicting the stability and thermal degradation of (a) LIG-PLGA-PC nanocapsule, (b) Plain LIG drug (c) Blank nanocapsules

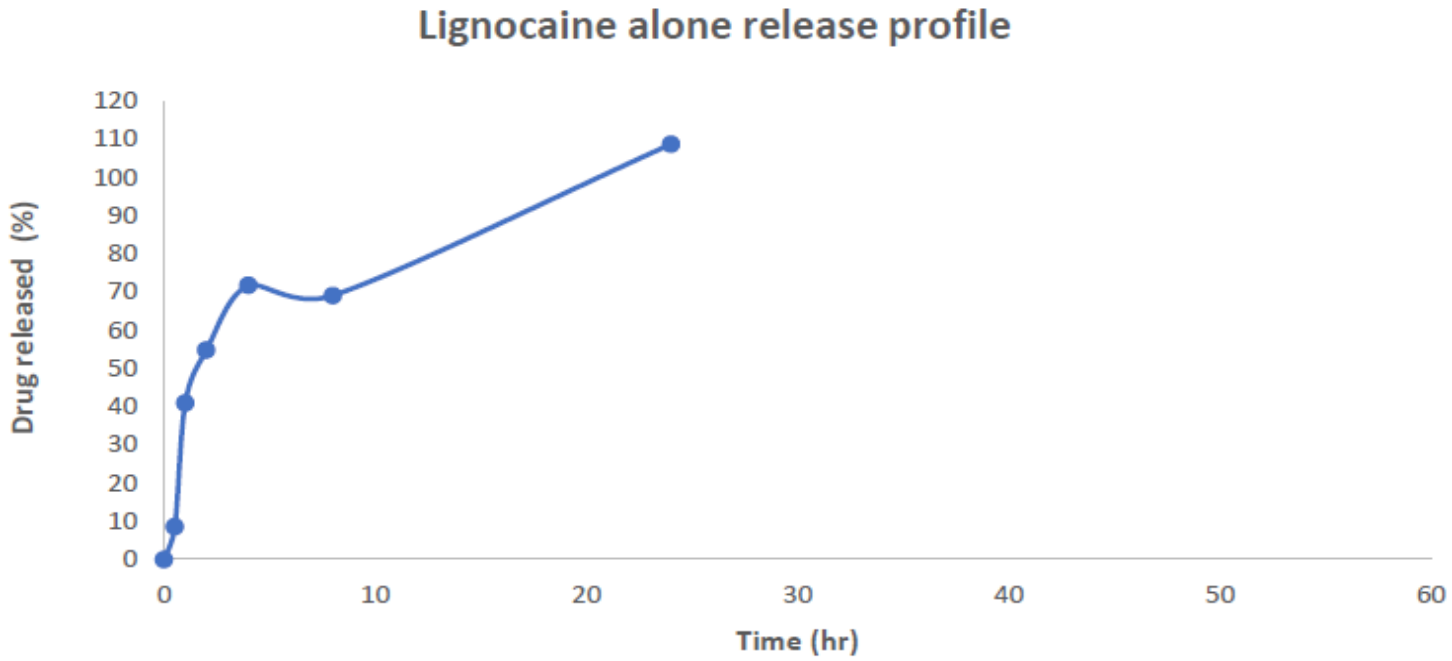
3.3.4 Entrapment efficiency of Lignocaine loaded nanocapsule

Drug entrapment efficiency (DEE) was calculated at 265,1 nm. A computed linear curve of r^2 = 0,9999 was found. Using the created standard calibration curve that linked absorbance to concentration, the specific absorptivity was utilized to determine the drug concentration. A drug entrapment efficacy (DEE) percentage of 72 % was achieved (Weng et al., 2020).

3.3.5 Lignocaine-loaded Nanocapsule Drug Release Studies

The nanocapsules were formulated and suspended in PBS at the pH of 7,4. at which the body maintains the pH of blood. The study was conducted using LIG-PLGA-PC nanocapsule and free LIG drug over 24 hours. Predetermined time intervals (0,5, 1, 2, 4, 8, and 24 hours) were used as sampling period to determine amount of the drug release at each point. The *in vitro* release profile of bare dug and of LIG-PLGA-PC nanocapsule is presented in Figure 3.9 (a) and in Figure 3.9 (b) respectively. The figures showed an increase in release of the drug by about 70 % within 4 hours and approximately 100 % of the drug was release after 24 hours of the study. On other hand 56 % of the drug was released from LIG-PLGA-PC nanocapsule within 4 hours. After 48 hours, about 59 % of the drug release from LIG-PLGA-PC nanocapsule indicating a sustained release profile. In a study conducted by (Wang et al., 2019), LIG loaded to PLGA system demonstrated the same release profile, suggesting a sustained release behaviour of LIG from PLGA nanoparticle when compared to drug alone.

(b)


Lignocaine alone release profile
Drug released (%)
120
110
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
60
Time (hr)

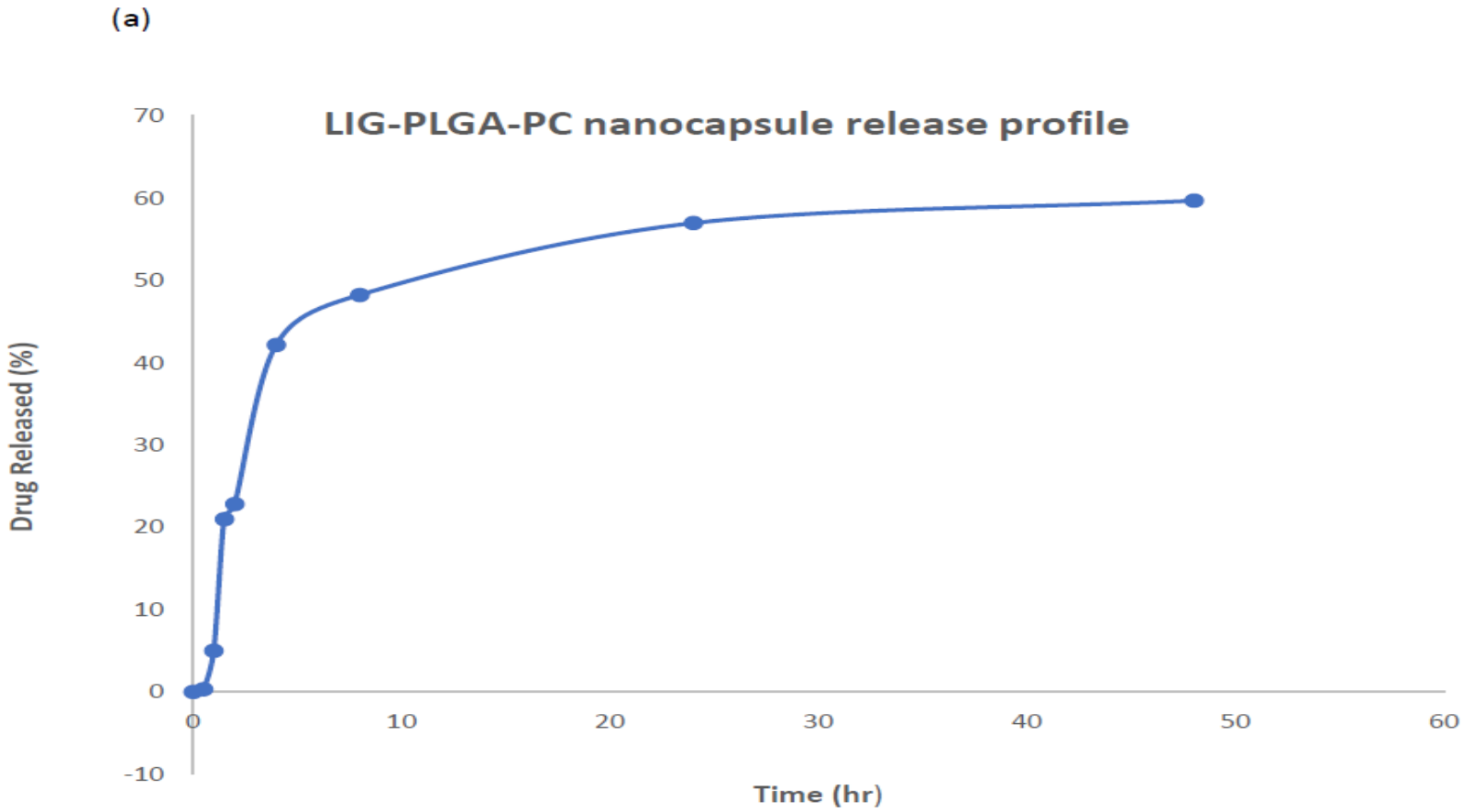


Figure 3.9 Drug release profile of (a) Lignocaine from lignocaine loaded nanocapsule and (b) Lignocaine alone in PBS. Each individual point represents mean ± SD (n=3)

3.3.5 Evaluation of Cytotoxicity of Lignocaine loaded nanocapsule utilizing HaCaT cells

A cytotoxicity assay was conducted to determine the effect of the LIG-PLGA-PC nanocapsules on the viability of HaCaT cells. HaCaT cells provided an excellent platform for investigating skin-related processes and assessing potential therapies. It closely resembled the skin epidermis. Their endurance and capacity to differentiate make them a dependable candidate for in vitro studies. The cells were treated with samples of drug alone, LIG-PLGA-PC and blank nanocapsules in the following concentration: 200, 100, 50, 25, 12,5, 6,25, 3,125 and 1,5625 µg/ml. After 48 and 72 hours the cells were visualised using brightfield micrographs (at 10X) and an MTT assay was conducted as described in Section 3.2.11 (Kang et al., 2016a).

Figure 3.10 (a) and (b) depict the graph for MTT assays for plain LIG, LIG-PLGA-PC nanocapsule and blank nanocapsules at 48 hours and 72 hours, respectively. Figure 3.11 displays light microscopic images of the HaCaT cells treated with cells with a) 5FU, b) Blank c) Drug-loaded d) Plain drug e) 1X PBS f).

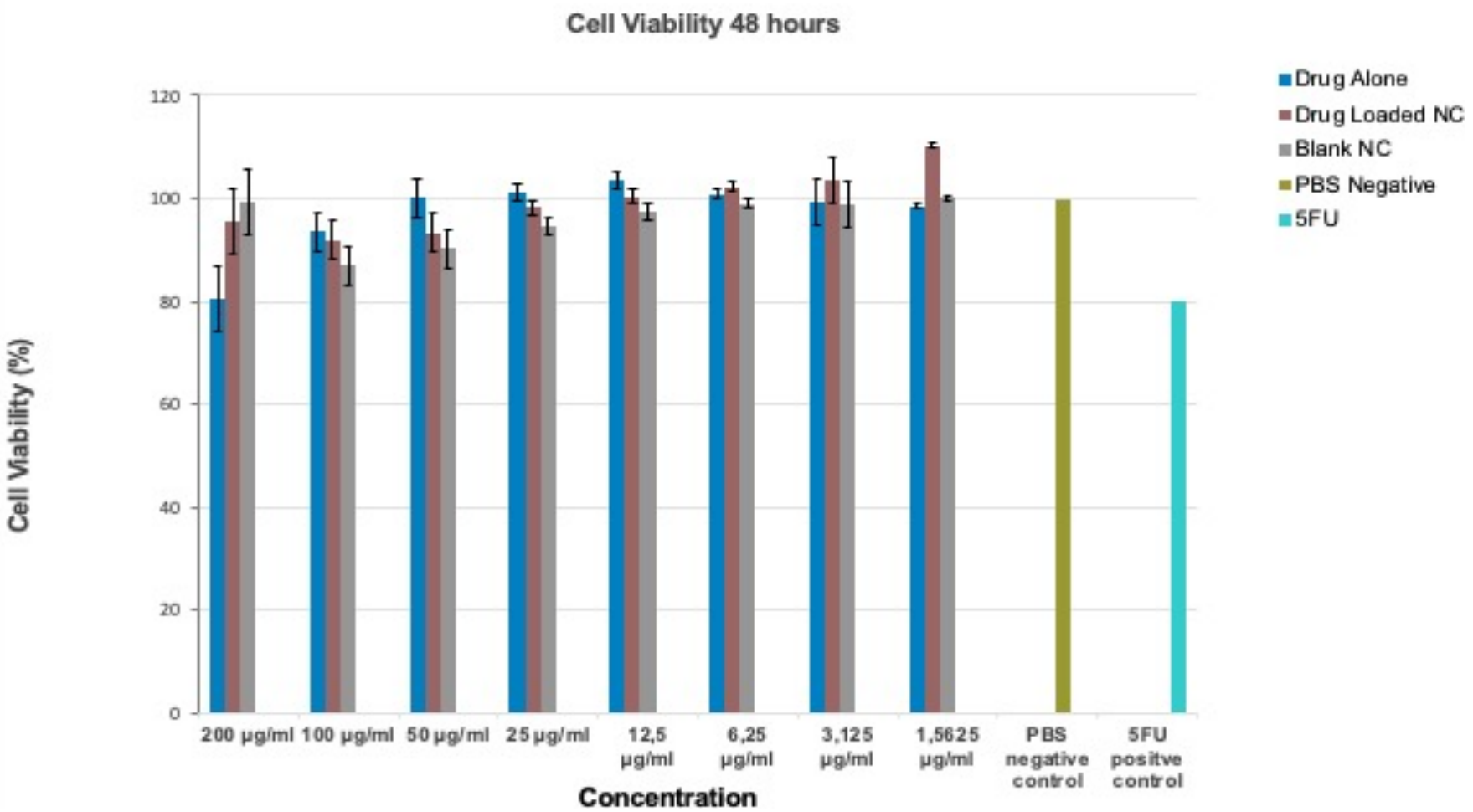
On the 48-hour graph, the Drug alone showed cell viability of 103 % at 12.5 µg/ml, 101 % at 25 µg/ml and 100 % at both 6.25 and 50 µg/ml. LIG-PLGA-PC nanocapsule showed cell viability of 110 % at 1,5625 µg/ml, 103,5 at 3,125 µg/ml, and 100 % at 12,5 µg/ml. The blank nanocapsule showed a cell viability of 110 % at 1.5625 µg/ml, 99 % at 6.25 µg/ml and 200 µg/ml. LIG-PLGA-PC nanocapsule had cell viability of over 100 % at lower concentrations between 12.5 and 1.5625 µg/ml. The highest concentration of 200 µg/ml used in the study showed cell viability of 95 %. This could mean that at high sample dose the drug loaded nanocapsule showed less cell viability when compared to a negative control of PBS of 100 %.

In the drug alone sample, at the same concentration of 200 µg/ml, there was less cell viability of 80 % as confirmed by the positive control cells treated with 5-Fluorouracil (5FU) display which showed a high cytotoxicity as expected.

In the 72-hour cell viability investigation, Drug alone graph has 101 % at 12.5 µg/ml. The drug-loaded nanocapsule formulation showed higher cell viability of 104 % at both 12.5 µg/ml and 3.125 µg/ml, and 102 % at 6.25 and 25 µg/ml. The blank nanocapsule had cell viability of 104 % at 12,5 µg/ml, 103 % at 6.25 and 100.9 % at 3.125 µg/ml.

Cell viability was greater than 100 % in LIG-PLGA-PC nanocapsules at lower doses ranging from 25 to 3.125 µg/ml. The maximum concentration utilized in the study, 200 µg/ml, resulted in cell viability of 97.8 %. This could imply that the drug-loaded nanocapsule exhibited less cell at high doses when compared to a 100 % negative control of PBS. As expected, positive control cells treated with 100 µg/ml 5FU exhibited substantial cytotoxicity. From the study conducted, it can be seen that the samples used had cell viability as confirmed by Zielińska et al., 2022 that Cell viability more than 70 % is commonly regarded as confirmation of minimal cytotoxicity of the evaluated nanoparticle composition (Zielińska et al., 2022).

The highest cell viability in 48 hours and 72 hours graphs were 110 % at 1.5625 µg/ml and 104,9 % at 12.5 µg/ml respectively.
Even though the high concentration was seen with drug-loaded formulation compared to drug alone, generally it is seen that at this concentration of both the graphs, there is a high cell viability. The treatments were meant to decrease cell viability as local anaesthetics are cytotoxic (Kang et al., 2016a). A study was conducted by Fiat et al., 2023, where the aim of the study was to investigate the cytotoxic effect of lignocaine on A-375 cutaneous melanoma cells, as well as the possible safety of LIG on HaCaT immortalized healthy human keratinocytes. The obtained data revealed that LIG had no cytotoxicity on HaCaT cells (Fiat, 2023). More *invitro* studies as well as clinical studies will need to be carried out to agree with the findings in the study.
As shown in Figure 3.11 there are little or no morphological changes associated with cytotoxicity on the drug loaded nanocapsule when administered to skin keratinocytes (HaCaT) at concentrations of up to 200 µg/ml. Nevertheless, morphological characteristics associated with cytotoxicity are clearly observed in the positive control cells treated with 100 ug/ml of 5FU (Fig 3.11 a). Morphological features associated with good cell viability of HaCaT epithelial cells and demonstrated that LIG nanocapsules exhibited reduced cytotoxicity. However there seems to be cell proliferation on the Figure 3.11 d). Cell proliferation was observed in all the samples containing Drug alone, Drug loaded NC and blank NC.


Cell Viability 48 hours
Cell Viability (%)
120
100
80
60
40
20
0
200 µg/ml
100 µg/ml
50 µg/ml
25 µg/ml
12,5 µg/ml
6,25 µg/ml
3,125 µg/ml
1,5625 µg/ml
PBS negative control
5FU positve control
Concentration
Drug Alone
Drug Loaded NC
Blank NC
PBS Negative
5FU

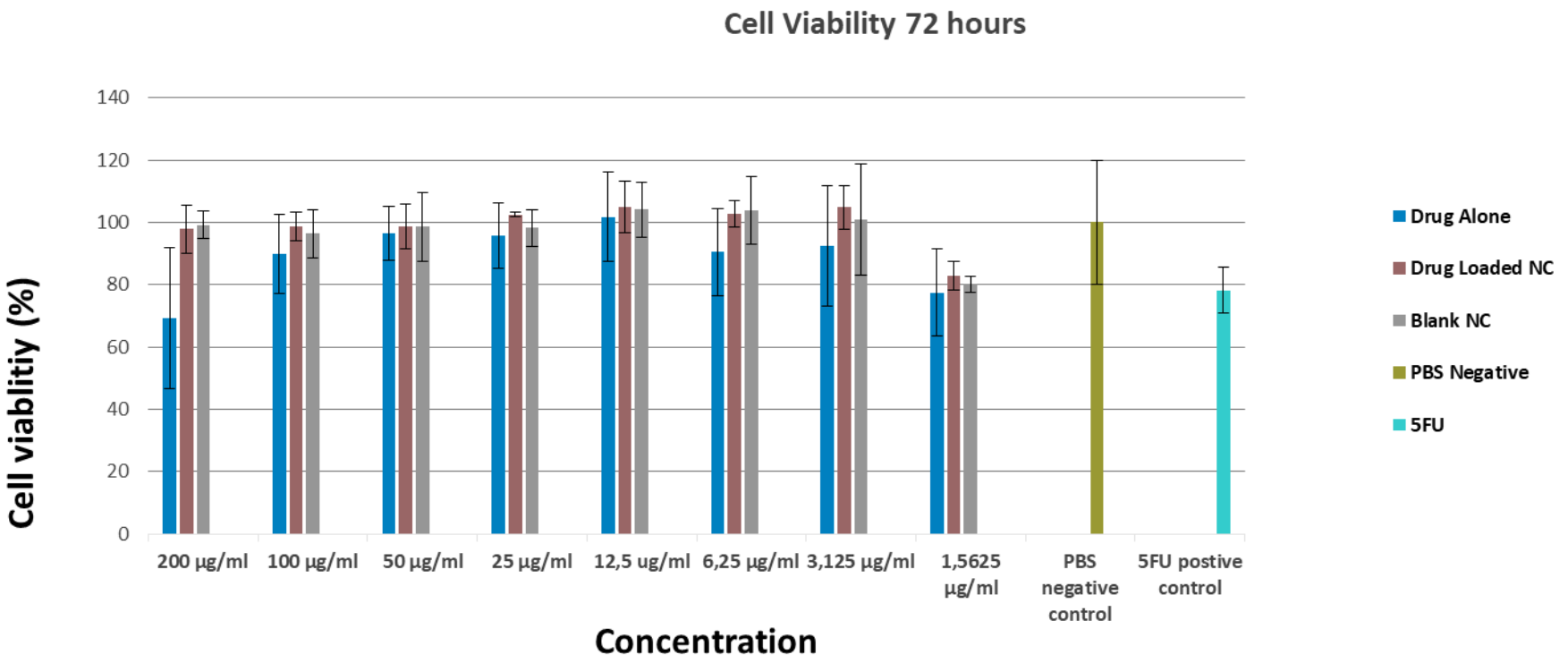


Figure 3.10 MTT assay for evaluation of the outcome of lignocaine-loaded nanocapsule, blank nanoparticles and lignocaine plain drug on the percentage viability of HaCaT cells. The cells treated with various formulations were incubated for 48 hours (a) and 72 hours (b), prior to evaluation of cell viability. Each percentage cell viability point represents average +- SD (n=3). Controls

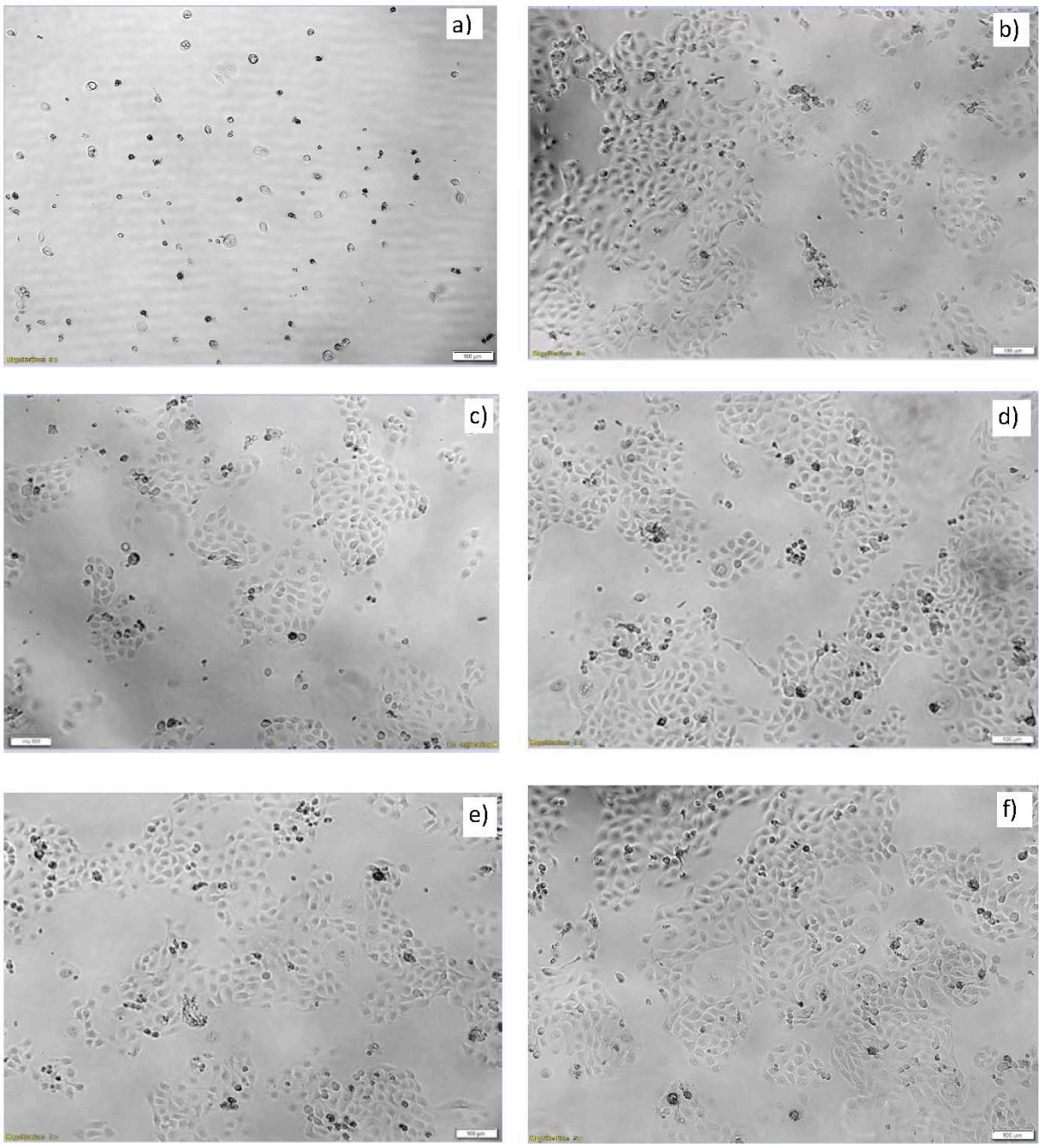


Figure 3.11 Micrographs of HaCaT cells taken with a 10X objective of an inverted microscope showing growth after treatment with a) cells with 100 ug/ml 5FU, b) Blank nanoparticles, c) Drug-Loaded, d) Plain drug, e) 1X PBS, f) Untreated.

CHAPTER 4
CONCLUSIONS AND RECOMMENDATIONS

4.1 Conclusions

Pain is an uncomfortable sensation in the body that affects majority of people worldwide. Depending on the type of pain whether chronic or acute, there several classes of therapeutic agent available for pain management. In June 2019, World Health Organisation (WHO) announced a revised guideline on pain management. The drugs included in the guideline are analgesics, Non-Steroidal Anti-Inflammatory agents, weak and strong opioids. Adjunct medications like gabapentin, pregabalins and local anaesthetics are also used in the management of pain (Hay and Nesbitt, 2019). These medications are mainly available in oral and parenteral formulations and their therapeutic classes have challenges including first pass hepatic metabolism seen in oral medications and pain at the site of administration for parenteral dosage forms. The need for a drug formulation to improve management of pain is important. To lessen the pain, topical local anaesthetics have been applied. (Bajaj et al., 2011b).
Transdermal drug delivery system (TDDS) is a method of drug administration across the skin. When compared to other routes of administration, this route is associated with numerous pharmacological benefits such as less invasiveness, less pain, ease of administration, and minimal side effects. However, the layers that make up the skin create a barrier for drugs to pass through, which has a negative impact on the therapeutic outcome.

In this study a local anaesthetic, Lignocaine, was used as a model drug using Nanotechnology to penetrate the active molecule through the skin to the site of action. Lately, polymeric nanoparticle research has gained a lot of interest. The purpose of this work was to design and synthesize polymeric LIG-PLGA-PC nanocapsules for use as a drug delivery mechanism of lignocaine for management of pain Using the interfacial deposition method, the nanocapsule was synthesized. The properties of lignocaine and its intended usage were the primary determinants of the polymer selection and formulation technique employed.

4.2 Limitations and future perspectives

Analgesic medications can be applied topically to the skin using topical patches for local effects or transdermal patches for regulated and extended release of the active substance. Transdermal drugs are among the most advanced pharmaceutical products available worldwide, and transdermal drug delivery has grown in prominence as a field of study for pharmaceutical technology. By using these technologies, the drawbacks of other distribution methods, such oral and parenteral, may be mitigated. Nano synthesis of pain medications administered by Transdermal drug delivery system is likely to increase due to the impact that pain has on the health system, globally. There are improvements and new discoveries done to enhance safety, quality, and efficacy profiles of pain medications. TDDS provides a formulation that can benefit the health system due to its low toxicity, increased patient compliance, and increased bioavailability. In this report, through various review of different studies, it has been seen that Nanotechnology increase drug releases and act as carriers of lipophilic and hydrophobic drugs across the skin.

The synthesized nanocapsule formulation was found to have high cell viability when applied to human skin cells concluding that they are not toxic. Even though the active ingredient was synthesized by being incorporated into a polymer, there was drug release from LIG-PLGA-PC nanocapsule indicating a sustained release profile.

The synthetic formulation must be redesigned to find an exact dose form that can be employed in the future to transport the medicine over the skin. The underperformance of the formulation could be attributed to several reasons, including the wrong choice of the drug or the suitability of the drug with the correct polymers. To overcome these challenges, going forward, different concentrations of the drug and polymers should be used. Different method of syntheses can also be investigated. Further tests like DSC must be incorporated to assess the behaviour of the formulation.

In the study it is seen that there is an extremely high percentage of cell proliferation. This could be attributed to several reasons including the fact that only one cell line was used in the study therefore it cannot be entirely concluded that lignocaine is not cytotoxic.

The regulatory framework for topically applied nanomedicine is still developing, and there have been fewer requests for marketing authorization. Additional research is required to demonstrate the mechanisms of penetration and define the quality profile.

Furthermore, because of the greater costs associated with their research and manufacturing, the rationale for a nanomedicine product is mainly based on establishing clinical superiority over existing therapeutic options on the market.

The strong potential of nanotechnology provides an outstanding opportunity to resolve several challenges in pain treatment. Multidisciplinary teamwork is required to make the most significant achievements in this area. As a result, efforts should be made simultaneously to expand research into the impact of nanoparticles on multiple pain pathways.

REFERENCES

Abdo, J.M., Sopko, N.A., Milner, S.M., 2020. The applied anatomy of human skin: A model for regeneration. Wound Med. 28, 100179. https://doi.org/10.1016/j.wndm.2020.100179

Abed, D.Z., Sadeghian, R., Mohammadi, S., Akram, M., 2022. Thymus persicus (Ronniger ex Rech. f.) Jalas alleviates nociceptive and neuropathic pain behavior in mice: Multiple mechanisms of action. J. Ethnopharmacol. 283, 114695. https://doi.org/10.1016/j.jep.2021.114695

AL Falahi, O.A., Abdullah, S.R.S., Hasan, H.A., Othman, A.R., Ewadh, H.M., Al-Baldawi, I.A., Kurniawan, S.B., Imron, M.F., Ismail, N. 'Izzati, 2021. Simultaneous removal of ibuprofen, organic material, and nutrients from domestic wastewater through a pilot-scale vertical sub-surface flow constructed wetland with aeration system. J. Water Process Eng. 43, 102214. https://doi.org/10.1016/j.jwpe.2021.102214

Alexander, A., Dwivedi, S., Ajazuddin, Giri, T.K., Saraf, Swarnlata, Saraf, Shailendra, Tripathi, D.K., 2012. Approaches for breaking the barriers of drug permeation through transdermal drug delivery. J. Controlled Release 164, 26–40. https://doi.org/10.1016/j.jconrel.2012.09.017

Alkilani, A., McCrudden, M.T., Donnelly, R., 2015. Transdermal Drug Delivery: Innovative Pharmaceutical Developments Based on Disruption of the Barrier Properties of the Stratum Corneum. Pharmaceutics 7, 438–470. doi:10.3390/pharmaceutics7040438

Anacleto, S.D.S., Borges, M.M.C., De Oliveira, H.L., Vicente, A.R., De Figueiredo, E.C., De Oliveira, M.A.L., Borges, B.J.P., De Oliveira, M.A., Borges, W.D.S., Borges, K.B., 2018. Evaluation of physicochemical properties as supporting information on quality control of raw materials and veterinary pharmaceutical formulations. J. Pharm. Anal. 8, 168–175. https://doi.org/10.1016/j.jpha.2018.01.001

Andreu, V., Arruebo, M., 2018. Current progress and challenges of nanoparticle-based therapeutics in pain management. J. Controlled Release 269, 189–213. https://doi.org/10.1016/j.jconrel.2017.11.018

Anu Mary Ealia, S., Saravanakumar, M.P., 2017. A review on the classification, characterisation, synthesis of nanoparticles and their application. IOP Conf.

Ser. Mater. Sci. Eng. 263, 032019. https://doi.org/10.1088/1757-899X/263/3/032019

Auda, S.H., Fathalla, D., Fetih, G., El-Badry, M., Shakeel, F., 2016. Niosomes as transdermal drug delivery system for celecoxib: in vitro and in vivo studies. Polym. Bull. 73, 1229–1245. https://doi.org/10.1007/s00289-015-1544-8

Ayoub, S.S., 2021. Paracetamol (acetaminophen): A familiar drug with an unexplained mechanism of action. Temperature 8, 351–371. https://doi.org/10.1080/23328940.2021.1886392

Bajaj, S., Whiteman, A., Brandner, B., 2011a. Transdermal drug delivery in pain management. Contin. Educ. Anaesth. Crit. Care Pain 11, 39–43. https://doi.org/10.1093/bjaceaccp/mkq054

Bashford, G., Tan, S.X., McGree, J., Murdoch, V., Nikles, J., 2021. Comparing pregabalin and gabapentin for persistent neuropathic pain: A protocol for a pilot N-of-1 trial series. Contemp. Clin. Trials Commun. 24, 100852. https://doi.org/10.1016/j.conctc.2021.100852

Barker, J.C., Joshi, G.P., Janis, J.E., 2020. Basics and Best Practices of Multimodal Pain Management for the Plastic Surgeon. Plastic and Reconstructive. Surgery. - Global. Open 8, e2833. https://doi.org/10.1097/GOX.0000000000002833

Beloqui, A., Solinís, M.Á., Rodríguez-Gascón, A., Almeida, A.J., Préat, V., 2016. Nanostructured lipid carriers: Promising drug delivery systems for future clinics. Nanomedicine Nanotechnol. Biol. Med. 12, 143–161. https://doi.org/10.1016/j.nano.2015.09.004

Carter, P., Narasimhan, B., Wang, Q., 2019. Biocompatible nanoparticles and vesicular systems in transdermal drug delivery for various skin diseases. Int. J. Pharm. 555, 49–62. https://doi.org/10.1016/j.ijpharm.2018.11.032

Chen, M., Quan, G., Sun, Y., Yang, D., Pan, X., Wu, C., 2020. Nanoparticles-encapsulated polymeric microneedles for transdermal drug delivery. J. Controlled Release 325, 163–175. https://doi.org/10.1016/j.jconrel.2020.06.039

Chen, X., Zhu, L., Li, R., Pang, L., Zhu, S., Ma, J., Du, L., Jin, Y., 2020. Electroporation-enhanced transdermal drug delivery: Effects of logP, pKa, solubility and penetration time. Eur. J. Pharm. Sci. 151, 105410. https://doi.org/10.1016/j.ejps.2020.105410

Cho, Y., Hong, G.-R.S., 2021. Behavioral and Physiological Pain Responses in Brain-Injured Patients Who Are Unable to Communicate in the Intensive Care Unit. Pain Manag. Nurs. 22, 80–85. https://doi.org/10.1016/j.pmn.2020.08.006

Colombo, I.,Sangiovanni, E., Maggio, R., 2017. HaCaT Cells as a Reliable In Vitro Differentiation Model to Dissect the Inflammatory/Repair Response of Human Keratinocytes. Hindawi, 7435621, 12. doi: 10.1155/2017/7435621

Columb, M.O., Cegielski, D., Haley, D., 2017. Local anaesthetic agents. Anaesth. Intensive Care Med. 18, 150–154. https://doi.org/10.1016/j.mpaic.2016.11.012

Cordeiro Lima Fernandes, P., David de Moura, L., Freitas de Lima, F., Henrique Rodrigues da Silva, G., Isaias Carvalho Souza, R., de Paula, E., 2021. Lipid nanocapsules loaded with prilocaine and lidocaine and incorporated in gel for topical application. Int. J. Pharm. 602, 120675. https://doi.org/10.1016/j.ijpharm.2021.120675

Da Silva, C.B., Dos Santos, C.P., Serpe, L., Sanchez, J.B., Ferreira, L.E.N., De Melo, N.F.S., Groppo, F.C., Fraceto, L.F., Volpato, M.C., Franz-Montan, M., 2024. Polymeric Nanocapsules Loaded with Lidocaine: A Promising Formulation for Topical Dental Anesthesia. Pharmaceuticals 17, 485. https://doi.org/10.3390/ph17040485

Deng, S., Gigliobianco, M.R., Censi, R., Di Martino, P., 2020. Polymeric Nanocapsules as Nanotechnological Alternative for Drug Delivery System: Current Status, Challenges and Opportunities. Nanomaterials 10, 847. https://doi.org/10.3390/nano10050847

Dhawan, A., Modak, T., Sarkar, S., 2019. Transdermal buprenorphine patch: Potential for role in management of opioid dependence. Asian J. Psychiatry 40, 88–91. https://doi.org/10.1016/j.ajp.2019.02.002

Dragicevic, N., Maibach, H., 2018. Combined use of nanocarriers and physical methods for percutaneous penetration enhancement. Adv. Drug Deliv. Rev. 127, 58–84. https://doi.org/10.1016/j.addr.2018.02.003

Duangjit, S., Nimcharoenwan, T., Chomya, N., Locharoenrat, N., Ngawhirunpat, T., 2016. Design and development of optimal invasomes for transdermal drug delivery using computer program. Asian J. Pharm. Sci. 11, 52–53. https://doi.org/10.1016/j.ajps.2015.10.039

Dubus, J.-C., Mely, L., Lanteaume, A., 2006. Use of lidocaine-prilocaine patch for the mantoux test: Influence on pain and reading. Int. J. Pharm. 327, 78–80. https://doi.org/10.1016/j.ijpharm.2006.07.037

Eisenach, J.C., 1994. Alpha-2 agonists and analgesia. Expert Opin. Investig. Drugs 3, 1005–1010. https://doi.org/10.1517/13543784.3.10.1005

El-Boghdadly, K., Pawa, A., Chin, K.J., 2018. Local anesthetic systemic toxicity: current perspectives. Local Reg. Anesth. Volume 11, 35–44. https://doi.org/10.2147/LRA.S154512

El-Say, K.M., El-Sawy, H.S., 2017. Polymeric nanoparticles: Promising platform for drug delivery. Int. J. Pharm. 528, 675–691. https://doi.org/10.1016/j.ijpharm.2017.06.052

Elmowafy, M., Al-Sanea, M.M., 2021. Nanostructured lipid carriers (NLCs) as drug delivery platform: Advances in formulation and delivery strategies. Saudi Pharm. J. 29, 999–1012. https://doi.org/10.1016/j.jsps.2021.07.015

Elmowafy, M., Samy, A., Abdelaziz, A.E., Shalaby, K., Salama, A., Raslan, M.A., Abdelgawad, M.A., 2017. Polymeric nanoparticles based topical gel of poorly soluble drug: Formulation, ex-vivo and in vivo evaluation. Beni-Suef Univ. J. Basic Appl. Sci. 6, 184–191. https://doi.org/10.1016/j.bjbas.2017.03.004

Everson, M., McLain, N., Collins, M.J., Rayborn, M., 2020. Perioperative Pain Management Strategies in the Age of an Opioid Epidemic. J. Perianesth. Nurs. 35, 347–352. https://doi.org/10.1016/j.jopan.2020.01.001

Fessi, H., Puisieux, F., Devissaguet, J.Ph., Ammoury, N., Benita, S., 1989. Nanocapsule formation by interfacial polymer deposition following solvent displacement. Int. J. Pharm. 55, R1–R4. https://doi.org/10.1016/0378-5173(89)90281-0

Fiat, F., 2023. *In vitro* characterization of the cytotoxic profile of lidocaine on a-375 cutaneous melanoma cells and hacat keratinocytes. farmacia 71, 856–864. https://doi.org/10.31925/farmacia.2023.4.23

Fitzgerald, J.F., Romero, R., Saberski, L.R., 1998. Complications of antidepressants, anticonvulsants, and antiarrhythmics for chronic pain management. Tech. Reg. Anesth. Pain Manag. 2, 119–129. https://doi.org/10.1016/S1084-208X(98)80021-9

Freo, U., Ruocco, C., Valerio, A., Scagnol, I., Nisoli, E., 2021. Paracetamol: A Review of Guideline Recommendations. J. Clin. Med. 10, 3420. https://doi.org/10.3390/jcm10153420

Gao, Y., Xie, J., Chen, H., Gu, S., Zhao, R., Shao, J., Jia, L., 2014. Nanotechnology-based intelligent drug design for cancer metastasis treatment. Biotechnol. Adv. 32, 761–777. https://doi.org/10.1016/j.biotechadv.2013.10.013

Garg, A., Sharma, G.S., Goyal, A.K., Ghosh, G., Si, S.C., Rath, G., 2020. Recent advances in topical carriers of anti-fungal agents. Heliyon 6, e04663. https://doi.org/10.1016/j.heliyon.2020.e04663

Gehdoo, R., Singh Bajwa, S., Jain, D., Dogra, N., 2022. Postoperative pain management: Stepping towards newer frontiers. Indian J. Anaesth. 66, 481. https://doi.org/10.4103/ija.ija_605_22

Golzari, S.E., Soleimanpour, H., Mahmoodpoor, A., Safari, S., Ala, A., 2014. Lidocaine and Pain Management in the Emergency Department: A Review Article. Anesthesiol. Pain Med. 3. https://doi.org/10.5812/aapm.15444

Goyal, R., Macri, L.K., Kaplan, H.M., Kohn, J., 2016. Nanoparticles and nanofibers for topical drug delivery. J. Controlled Release 240, 77–92. https://doi.org/10.1016/j.jconrel.2015.10.049

Hatten, B.W., Cantrill, S.V., Dubin, J.S., Ketcham, E.M., Runde, D.P., Wall, S.P., Wolf, S.J., Wolf, S.J., Byyny, R., Carpenter, C.R., Diercks, D.B., Gemme, S.R., Gerardo, C.J., Godwin, S.A., Hahn, S.A., Hatten, B.W., Haukoos, J.S., Hickey, S.M., Kaji, A., Kwok, H., Lo, B.M., Mace, S.E., Nazarian, D.J., Promes, S.B., Shah, K.H., Shih, R.D., Silvers, S.M., Smith, M.D., Thiessen, M.E.W., Tomaszewski, C.A., Valente, J.H., Wall, S.P., Winger, J., Cantrill, S.V., Hirshon, J.M., Mims, M., Schulz, T., 2020. Clinical Policy: Critical Issues Related to Opioids in Adult Patients Presenting to the Emergency Department. Ann. Emerg. Med. 76, e13–e39. https://doi.org/10.1016/j.annemergmed.2020.06.049

Hay, D., Nesbitt, V., 2019. Management of acute pain. Surg. Oxf. 37, 460–466. https://doi.org/10.1016/j.mpsur.2019.05.004

Hua, S., Cabot, P.J., 2013. Targeted nanoparticles that mimic immune cells in pain control inducing analgesic and anti-inflammatory actions: a potential novel treatment of acute and chronic pain condition. Pain Physician 16, E199-216. https://doi.org/10.3389/fphar.2014.00211

Im, J.S., Bai, B.Ch., Lee, Y.-S., 2010. The effect of carbon nanotubes on drug delivery in an electro-sensitive transdermal drug delivery system. Biomaterials 31, 1414–1419. https://doi.org/10.1016/j.biomaterials.2009.11.004

Jain, S., Tripathi, S., Tripathi, P.K., 2021. Invasomes: Potential vesicular systems for transdermal delivery of drug molecules. J. Drug Deliv. Sci. Technol. 61, 102166. https://doi.org/10.1016/j.jddst.2020.102166

Jeong, W.Y., Kwon, M., Choi, H.E., Kim, K.S., 2021. Recent advances in transdermal drug delivery systems: a review. Biomater. Res. 25, 24. https://doi.org/10.1186/s40824-021-00226-6

Kang, D.-K., Zhao, L.-Y., Wang, H.-L., 2016a. Cytotoxic effects of local anesthesia through lidocaine/ropivacaine on human melanoma cell lines. Braz. J. Anesthesiol. Engl. Ed. 66, 594–602. https://doi.org/10.1016/j.bjane.2015.04.002

Kearney, L., Whelan, D., O'Donnell, B.D., Clover, A.J.P., 2016. Novel methods of local anesthetic delivery in the perioperative and postoperative setting—potential for fibrin hydrogel delivery. J. Clin. Anesth. 35, 246–252. https://doi.org/10.1016/j.jclinane.2016.07.033

Khan, Ibrahim, Saeed, K., Khan, Idrees, 2019. Nanoparticles: Properties, applications and toxicities. Arab. J. Chem. 12, 908–931. https://doi.org/10.1016/j.arabjc.2017.05.011

Kim, A.R., Lee, N.H., Park, Y.M., Park, S.N., 2019. Preparation and characterization of novel pseudo ceramide liposomes for the transdermal delivery of baicalein. J. Drug Deliv. Sci. Technol. 52, 150–156. https://doi.org/10.1016/j.jddst.2019.04.009

Kondiah, P.P.D., Tomar, L.K., Tyagi, C., Choonara, Y.E., Modi, G., du Toit, L.C., Kumar, P., Pillay, V., 2013. A novel pH-sensitive interferon-β (INF-β) oral delivery system for application in multiple sclerosis. Int. J. Pharm. 456, 459–472. https://doi.org/10.1016/j.ijpharm.2013.08.038

Korosec, A., Frech, S., Gesslbauer, B., Vierhapper, M., Radtke, C., Petzelbauer, P., Lichtenberger, B.M., 2019. Lineage Identity and Location within the Dermis Determine the Function of Papillary and Reticular Fibroblasts in Human Skin. J. Invest. Dermatol. 139, 342–351. https://doi.org/10.1016/j.jid.2018.07.033

Kothamasu, P., Kanumur, H., Ravur, N., Maddu, C., Parasuramrajam, R., Thangavel, S., 2012. Nanocapsules: The Weapons for Novel Drug Delivery Systems. BioImpacts ISSN 2228-5660. https://doi.org/10.5681/BI.2012.011

Kumar, N., Verma, A., Mandal, A., 2021. Formation, characteristics and oil industry applications of nanoemulsions: A review. J. Pet. Sci. Eng. 206, 109042. https://doi.org/10.1016/j.petrol.2021.109042

Kwon, S.-H., Kim, H., Park, S., Jeon, W., 2020. Development of Knowledge and Attitudes Survey on Pain Management for Korean Long-term Care Professionals. Asian Nurs. Res. 14, 105–113. https://doi.org/10.1016/j.anr.2020.04.002

Langford, R.M., Mehta, V., 2006. Selective cyclooxygenase inhibition: its role in pain and anaesthesia. Biomed. Pharmacother. 60, 323–328. https://doi.org/10.1016/j.biopha.2006.06.017

Li, Y., Zhao, E., Li, L., Bai, L., Zhang, W., 2021. Physicochemical and pharmacological investigations of polyvinylpyrrolidone - tetrahydroxyborate hydrogel containing the local anesthetic lidocaine. J. Mol. Liq. 335, 116526. https://doi.org/10.1016/j.molliq.2021.116526

Lima, A.L., Gratieri, T., Cunha-Filho, M., Gelfuso, G.M., 2022. Polymeric nanocapsules: A review on design and production methods for pharmaceutical purpose. Methods 199, 54–66. https://doi.org/10.1016/j.ymeth.2021.07.009

Liu, X., Chen, L., Kang, Y., He, D., Yang, B., Wu, K., 2021. Cinnamon essential oil nanoemulsions by high-pressure homogenization: Formulation, stability, and antimicrobial activity. LWT 147, 111660. https://doi.org/10.1016/j.lwt.2021.111660

Liu, X., Zhao, Q., 2019. Long-term anesthetic analgesic effects: Comparison of tetracaine loaded polymeric nanoparticles, solid lipid nanoparticles, and nanostructured lipid carriers in vitro and in vivo. Biomed. Pharmacother. 117, 109057. https://doi.org/10.1016/j.biopha.2019.109057

Liu, Y., Cheng, M., Zhao, J., Zhang, X., Huang, Z., Zang, Y., Ding, Y., Zhang, J., Ding, Z., 2021. Transdermal Delivery of Lidocaine-Loaded Elastic Nano-Liposomes with Microneedle Array Pretreatment. Biomedicines 9, 592. https://doi.org/10.3390/biomedicines9060592

Ma, H., Pan, Z., Lai, B., Zan, C., Liu, H., 2023. Recent Research Advances in Nano-Based Drug Delivery Systems for Local Anesthetics. Drug Des. Devel. Ther. Volume 17, 2639–2655. https://doi.org/10.2147/DDDT.S417051

Makadia, H.K., Siegel, S.J., 2011. Poly Lactic-co-Glycolic Acid (PLGA) as Biodegradable Controlled Drug Delivery Carrier. Polymers 3, 1377–1397. https://doi.org/10.3390/polym3031377

Manikkath, J., Manikkath, A., Shavi, G.V., Bhat, K., Mutalik, S., 2017. Low frequency ultrasound and PAMAM dendrimer facilitated transdermal delivery of ketoprofen. J. Drug Deliv. Sci. Technol. 41, 334–343. https://doi.org/10.1016/j.jddst.2017.07.021

Manosroi, A., Jantrawut, P., Manosroi, J., 2008. Anti-inflammatory activity of gel containing novel elastic niosomes entrapped with diclofenac diethylammonium. Int. J. Pharm. 360, 156–163. https://doi.org/10.1016/j.ijpharm.2008.04.033

Marto, J., Gouveia, L.F., Gonçalves, L.M., Gaspar, D.P., Pinto, P., Carvalho, F.A., Oliveira, E., Ribeiro, H.M., Almeida, A.J., 2016. A Quality by design (QbD) approach on starch-based nanocapsules: A promising platform for topical drug delivery. Colloids Surf. B Biointerfaces 143, 177–185. https://doi.org/10.1016/j.colsurfb.2016.03.039

Masjedi, M., Montahaei, T., 2021. An illustrated review on nonionic surfactant vesicles (niosomes) as an approach in modern drug delivery: Fabrication, characterization, pharmaceutical, and cosmetic applications. J. Drug Deliv. Sci. Technol. 61, 102234. https://doi.org/10.1016/j.jddst.2020.102234

Masood, F., 2016. Polymeric nanoparticles for targeted drug delivery system for cancer therapy. Mater. Sci. Eng. C 60, 569–578. https://doi.org/10.1016/j.msec.2015.11.067

Mastrangelo, S., Capozza, M.A., Triarico, S., Attinà, G., Maurizi, P., Romano, A., Ruggiero, A., 2021. Opioid transdermal delivery system: a useful method for pain management in children. Ann. Transl. Med. 9, 185–185. https://doi.org/10.21037/atm-20-2619

Mick, G., Correa-Illanes, G., 2012. Topical pain management with the 5% lidocaine medicated plaster – a review. Curr. Med. Res. Opin. 28, 937–951. https://doi.org/10.1185/03007995.2012.690339

Mignani, S., Shi, X., Guidolin, K., Zheng, G., Karpus, A., Majoral, J.-P., 2021. Clinical diagonal translation of nanoparticles: Case studies in dendrimer nanomedicine. J. Controlled Release 337, 356–370. https://doi.org/10.1016/j.jconrel.2021.07.036

Mohammadi-Samani, S., Salehi, H., Entezar-Almahdi, E., Masjedi, M., 2020. Preparation and characterization of sumatriptan loaded solid lipid nanoparticles for transdermal delivery. J. Drug Deliv. Sci. Technol. 57, 101719. https://doi.org/10.1016/j.jddst.2020.101719

Mora-Huertas, C.E., Fessi, H., Elaissari, A., 2010. Polymer-based nanocapsules for drug delivery. Int. J. Pharm. 385, 113–142. https://doi.org/10.1016/j.ijpharm.2009.10.018

Münch, S., Wohlrab, J., Neubert, R.H.H., 2017. Dermal and transdermal delivery of pharmaceutically relevant macromolecules. Eur. J. Pharm. Biopharm. 119, 235–242. https://doi.org/10.1016/j.ejpb.2017.06.019

Nandiyanto, A.B.D., Oktiani, R., Ragadhita, R., 2019. How to Read and Interpret FTIR Spectroscope of Organic Material. Indones. J. Sci. Technol. 4, 97. https://doi.org/10.17509/ijost.v4i1.15806

Narmani, A., Mohammadnejad, J., Yavari, K., 2019. Synthesis and evaluation of polyethylene glycol- and folic acid-conjugated polyamidoamine G4 dendrimer as nanocarrier. J. Drug Deliv. Sci. Technol. 50, 278–286. https://doi.org/10.1016/j.jddst.2019.01.037

Nasef, A.M., Gardouh, A.R., Ghorab, M.M., 2017. Formulation and in-vitro evaluation of pantoprazole loaded pH-sensitive polymeric nanoparticles. Future J. Pharm. Sci. 3, 103–117. https://doi.org/10.1016/j.fjps.2017.04.004

Naves, L., Dhand, C., Almeida, L., Rajamani, L., Ramakrishna, S., Soares, G., 2017a. Poly(lactic-co-glycolic) acid drug delivery systems through transdermal pathway: an overview. Prog. Biomater. 6, 1–11. https://doi.org/10.1007/s40204-017-0063-0

Nazemian, N., Torabi, M., Mirzaee, M., 2020. Atomized intranasal vs intravenous fentanyl in severe renal colic pain management: A randomized single-blinded clinical trial. Am. J. Emerg. Med. 38, 1635–1640. https://doi.org/10.1016/j.ajem.2019.158483

Negash, T.T., Belete, K.G., Tlilaye, W., Ayele, T.T., Oumer, K.E., 2022. Knowledge, attitudes and practices of health professionals towards postoperative pain

management at a referral hospital in Ethiopia. Ann. Med. Surg. 73, 103167. https://doi.org/10.1016/j.amsu.2021.103167

Oliveira, D.M.L., Rezende, P.S., Barbosa, T.C., Andrade, L.N., Bani, C., Tavares, D.S., da Silva, C.F., Chaud, M.V., Padilha, F., Cano, A., de Albuquerque Júnior, R.L.C., Souto, E.B., Severino, P., 2020. Double membrane based on lidocaine-coated polymyxin-alginate nanoparticles for wound healing: In vitro characterization and in vivo tissue repair. Int. J. Pharm. 591, 120001. https://doi.org/10.1016/j.ijpharm.2020.120001

Omar, M.M., Hasan, O.A., El Sisi, A.M., 2019. Preparation and optimization of lidocaine transferosomal gel containing permeation enhancers: a promising approach for enhancement of skin permeation. Int. J. Nanomedicine Volume 14, 1551–1562. https://doi.org/10.2147/IJN.S201356

Onuh, O., Boehm, K., Hao, Y., Nan Huang, K., Brydges, H., Chiu, D., 2022. Effectiveness of Local Anesthetics for Postoperative Pain Management in Elective Hand Surgery: A Systematic Review. Plast. Reconstr. Surg. - Glob. Open 10, 74–74. https://doi.org/10.1097/01.GOX.0000898696.54788.87

Opatha, S.A.T., Titapiwatanakun, V., Chutoprapat, R., 2020. Transfersomes: A Promising Nanoencapsulation Technique for Transdermal Drug Delivery. Pharmaceutics 12, 855. https://doi.org/10.3390/pharmaceutics12090855

Otarola, J.J., Cobo Solis, A.K., Farias, M.E., Garrido, M., Mariano Correa, N., Molina, P.G., 2020. Piroxicam-loaded nanostructured lipid carriers gel: Design and characterization by square wave voltammetry. Colloids Surf. Physicochem. Eng. Asp. 606, 125396. https://doi.org/10.1016/j.colsurfa.2020.125396

Pandey, S.S., Patel, M.A., Desai, D.T., Patel, H.P., Gupta, A.R., Joshi, S.V., Shah, D.O., Maulvi, F.A., 2020. Bioavailability enhancement of repaglinide from transdermally applied nanostructured lipid carrier gel: Optimization, in vitro and in vivo studies. J. Drug Deliv. Sci. Technol. 57, 101731. https://doi.org/10.1016/j.jddst.2020.101731

Pardeshi, S.R., Nikam, A., Chandak, P., Mandale, V., Naik, J.B., Giram, P.S., 2021. Recent advances in PLGA based nanocarriers for drug delivery system: a state of the art review. Int. J. Polym. Mater. Polym. Biomater. 1–30. https://doi.org/10.1080/00914037.2021.1985495

Pathak, C., Vaidya, F.U., Pandey, S.M., 2019. Mechanism for Development of Nanobased Drug Delivery System, in: Applications of Targeted Nano Drugs

and Delivery Systems. Elsevier, pp. 35–67. https://doi.org/10.1016/B978-0-12-814029-1.00003-X

Peltonen, L., Singhal, M., Hirvonen, J., 2020. Principles of nanosized drug delivery systems, in: Nanoengineered Biomaterials for Advanced Drug Delivery. Elsevier, pp. 3–25. https://doi.org/10.1016/B978-0-08-102985-5.00001-2

Peptu, Cristian, Rotaru, R., Ignat, L., Humelnicu, A., Harabagiu, V., Peptu, Catalina, Leon, M.-M., Mitu, F., Cojocaru, E., Boca, A., Tamba, B., 2015. Nanotechnology approaches for pain therapy through transdermal drug delivery. Curr. Pharm. Des. 21, 6125–6139. https://doi.org/10.2174/1381612821666151027152752

Perrin, S.L., Bull, C., Black, S., 2020. Local anaesthetic drugs. Anaesth. Intensive Care Med. 21, 113–117. https://doi.org/10.1016/j.mpaic.2019.12.003

Pitorre, M., Gondé, H., Haury, C., Messous, M., Poilane, J., Boudaud, D., Kanber, E., Rossemond Ndombina, G.A., Benoit, J.-P., Bastiat, G., 2017. Recent advances in nanocarrier-loaded gels: Which drug delivery technologies against which diseases? J. Controlled Release 266, 140–155. https://doi.org/10.1016/j.jconrel.2017.09.031

Rahimi, M., Charmi, G., Matyjaszewski, K., Banquy, X., Pietrasik, J., 2021. Recent developments in natural and synthetic polymeric drug delivery systems used for the treatment of osteoarthritis. Acta Biomater. 123, 31–50. https://doi.org/10.1016/j.actbio.2021.01.003

Rai, V.K., Mishra, N., Yadav, K.S., Yadav, N.P., 2018. Nanoemulsion as pharmaceutical carrier for dermal and transdermal drug delivery: Formulation development, stability issues, basic considerations and applications. J. Controlled Release 270, 203–225. https://doi.org/10.1016/j.jconrel.2017.11.049

Raj, V., Prabha, G., 2016. Synthesis, characterization and in vitro drug release of cisplatin loaded Cassava starch acetate–PEG/gelatin nanocomposites. J. Assoc. Arab Univ. Basic Appl. Sci. 21, 10–16. https://doi.org/10.1016/j.jaubas.2015.08.001

Raja, S.N., Carr, D.B., Cohen, M., Finnerup, N.B., Flor, H., Gibson, S., Keefe, F.J., Mogil, J.S., Ringkamp, M., Sluka, K.A., Song, X.-J., Stevens, B., Sullivan, M.D., Tutelman, P.R., Ushida, T., Vader, K., 2020. The revised International Association for the Study of Pain definition of pain: concepts, challenges, and

compromises. Pain 161, 1976–1982. https://doi.org/10.1097/j.pain.0000000000001939

Ramadon, D., McCrudden, M.T.C., Courtenay, A.J., Donnelly, R.F., 2022. Enhancement strategies for transdermal drug delivery systems: current trends and applications. Drug Deliv. Transl. Res. 12, 758–791. https://doi.org/10.1007/s13346-021-00909-6

Rogobete, A.F., Dragomirescu, M., Bedreag, O.H., Sandesc, D., Cradigati, C.A., Sarandan, M., Papurica, M., Popovici, S.E., Vernic, C., Preda, G., 2016. New aspects of controlled release systems for local anaesthetics: A review. Trends Anaesth. Crit. Care 9, 27–34. https://doi.org/10.1016/j.tacc.2016.06.004

Román-Vargas, Y., Porras-Arguello, J.D., Blandón-Naranjo, L., Pérez-Pérez, L.D., Benjumea, D.M., 2023. Evaluation of the Analgesic Effect of High-Cannabidiol-Content Cannabis Extracts in Different Pain Models by Using Polymeric Micelles as Vehicles. Molecules 28, 4299. https://doi.org/10.3390/molecules28114299

Russo, M.M., Sundaramurthi, T., 2019. An Overview of Cancer Pain: Epidemiology and Pathophysiology. Semin. Oncol. Nurs. 35, 223–228. https://doi.org/10.1016/j.soncn.2019.04.002

Sakdiset, P., Amnuaikit, T., Pichayakorn, W., Pinsuwan, S., 2019. Formulation development of ethosomes containing indomethacin for transdermal delivery. J. Drug Deliv. Sci. Technol. 52, 760–768. https://doi.org/10.1016/j.jddst.2019.05.048

Sala, M., Diab, R., Elaissari, A., Fessi, H., 2018. Lipid nanocarriers as skin drug delivery systems: Properties, mechanisms of skin interactions and medical applications. Int. J. Pharm. 535, 1–17. https://doi.org/10.1016/j.ijpharm.2017.10.046

Sezgin-bayindir, Z., Ergin, A.D., Parmaksiz, M., Elcin, A.E., Elcin, Y.M., Yuksel, N., 2016. Evaluation of various block copolymers for micelle formation and brain drug delivery: In vitro characterization and cellular uptake studies. J. Drug Deliv. Sci. Technol. 36, 120–129. https://doi.org/10.1016/j.jddst.2016.10.003

Shakeel, F., Baboota, S., Ahuja, A., Ali, J., Shafiq, S., 2009. Celecoxib Nanoemulsion for Transdermal Drug Delivery: Characterization and In Vitro Evaluation. J. Dispers. Sci. Technol. 30, 834–842. https://doi.org/10.1080/01932690802644012

Shipton, E.A., 2012. Advances in delivery systems and routes for local anaesthetics. Trends Anaesth. Crit. Care 2, 228–233. https://doi.org/10.1016/j.tacc.2012.07.004Shrestha, S., Wang, B., Dutta, P., 2020. Nanoparticle processing: Understanding and controlling aggregation. Adv. Colloid Interface Sci. 279, 102162. https://doi.org/10.1016/j.cis.2020.102162

Shukla, T., Upmanyu, N., Agrawal, M., Saraf, Swarnlata, Saraf, Shailendra, Alexander, A., 2018. Biomedical applications of microemulsion through dermal and transdermal route. Biomed. Pharmacother. 108, 1477–1494. https://doi.org/10.1016/j.biopha.2018.10.021

Siafaka, P.I., Özcan Bülbül, E., Okur, M.E., Karantas, I.D., Üstündağ Okur, N., 2023. The Application of Nanogels as Efficient Drug Delivery Platforms for Dermal/Transdermal Delivery. Gels 9, 753. https://doi.org/10.3390/gels9090753

Sinatra, R., 2002. Role of COX-2 Inhibitors in the Evolution of Acute Pain Management. J. Pain Symptom Manage. 24, S18–S27. https://doi.org/10.1016/S0885-3924(02)00410-4

Stachowicz, K., 2021. Deciphering the mechanisms of regulation of an excitatory synapse via cyclooxygenase-2. A review. Biochem. Pharmacol. 192, 114729. https://doi.org/10.1016/j.bcp.2021.114729

Stanekzai, A., Sudhakar, C., Zhakfar, Am., Karan, Vikrant.S., 2019. Recent Approaches in Transdermal Drug Delivery System. Res. J. Pharm. Technol. 12, 4550. https://doi.org/10.5958/0974-360X.2019.00783.2

Talele, S., Nikam, P., Ghosh, B., Deore, C., Jaybhave, A., Jadhav, A., 2017. A Research Article on Nanogel as Topical Promising Drug Delivery for Diclofenac sodium. IndianJ. Pharm. Educ. Res. 51, s580–s587. https://doi.org/10.5530/ijper.51.4s.86

Tauzin-Fin, P., Bernard, O., Sesay, M., Biais, M., Richebe, P., Quinart, A., Revel, P., Sztark, F., 2014. Benefits of intravenous lidocaine on post-operative pain and acute rehabilitation after laparoscopic nephrectomy. J. Anaesthesiol. Clin. Pharmacol. 30, 366. https://doi.org/10.4103/0970-9185.137269

Tijani, A.O., Thakur, D., Mishra, D., Frempong, D., Chukwunyere, U.I., Puri, A., 2021. Delivering therapeutic cannabinoids via skin: Current state and future

perspectives. J. Controlled Release 334, 427–451. https://doi.org/10.1016/j.jconrel.2021.05.005

Tinkle, S., McNeil, S.E., Mühlebach, S., Bawa, R., Borchard, G., Barenholz, Y.C., Tamarkin, L., Desai, N., 2014. Nanomedicines: addressing the scientific and regulatory gap. Ann. N. Y. Acad. Sci. 1313, 35–56. https://doi.org/10.1111/nyas.12403

Uchechi, O., Ogbonna, J.D.N., Attama, A.A., 2014. Nanoparticles for Dermal and Transdermal Drug Delivery, in: Sezer, A.D. (Ed.), Application of Nanotechnology in Drug Delivery. InTech. https://doi.org/10.5772/58672

Valenzuela, P., Simon, J.A., 2012. Nanoparticle delivery for transdermal HRT. Maturitas 73, 74–80. https://doi.org/10.1016/j.maturitas.2011.12.019

Van Elstraete, A.C., Pastureau, F., Lebrun, T., Mehdaoui, H., 2000. Caudal clonidine for postoperative analgesia in adults. Br. J. Anaesth. 84, 401–402. https://doi.org/10.1093/oxfordjournals.bja.a013448

Voute, M., Morel, V., Pickering, G., 2021. Topical Lidocaine for Chronic Pain Treatment. Drug Des. Devel. Ther. Volume 15, 4091–4103. https://doi.org/10.2147/DDDT.S328228

Wang, B., Wang, S., Zhang, Q., Deng, Y., Li, X., Peng, L., Zuo, X., Piao, M., Kuang, X., Sheng, S., Yu, Y., 2019. Recent advances in polymer-based drug delivery systems for local anesthetics. Acta Biomater. 96, 55–67. https://doi.org/10.1016/j.actbio.2019.05.044

Weng, J., Tong, H.H.Y., Chow, S.F., 2020. In Vitro Release Study of the Polymeric Drug Nanoparticles: Development and Validation of a Novel Method. Pharmaceutics 12, 732. https://doi.org/10.3390/pharmaceutics12080732

Wennberg, P., Möller, M., Sarenmalm, E.K., Herlitz, J., 2020. Evaluation of the intensity and management of pain before arrival in hospital among patients with suspected hip fractures. Int. Emerg. Nurs. 49, 100825.

Windsor, P.A., 2022. Role of Topical Anaesthesia in Pain Management of Farm Animals, a Changing Paradigm. Animals 12, 2459. https://doi.org/10.3390/ani12182459

Wrona, S.K., Quinlan-Colwell, A., Brown, L., Jannuzzi, R.G.E., 2022. Procedural Pain Management: Clinical Practice Recommendations American Society for Pain Management Nursing. Pain Manag. Nurs. 23, 254–258. https://doi.org/10.1016/j.pmn.2021.11.008

Yaddanapudi, S., 2011. Prevention of local anesthetic systemic toxicity. J. Anaesthesiol. Clin. Pharmacol. 27, 438. https://doi.org/10.4103/0970-9185.86566

Yang, C., Li, T., 2021. Transdermal delivery of flurbiprofen from polyoxypropylene-polyoxyethylene block copolymer stabilized reduced graphene oxide to manage pain in spondylitis: In vitro and in vivo studies. Eur. J. Pharm. Sci. 165, 105929. https://doi.org/10.1016/j.ejps.2021.105929

Yang, J., Bauer, B.A., Wahner-Roedler, D.L., Chon, T.Y., Xiao, L., 2020. The Modified WHO Analgesic Ladder: Is It Appropriate for Chronic Non-Cancer Pain? J. Pain Res. Volume 13, 411–417. https://doi.org/10.2147/JPR.S244173

Yataba, I., Otsuka, N., Matsushita, I., Matsumoto, H., Hoshino, Y., 2017. The efficacy and safety of S-flurbiprofen plaster in the treatment of knee osteoarthritis: a phase II, randomized, double-blind, placebo-controlled, dose-finding study. J. Pain Res. Volume 10, 867–880. https://doi.org/10.2147/JPR.S131779

Yoo, J., De Gagne, J.C., Kim, H.J., Oh, J., 2019. Development and evaluation of a web-based acute pain management education program for Korean registered nurses: A randomized controlled trial. Nurse Educ. Pract. 38, 7–13. https://doi.org/10.1016/j.nepr.2019.05.013

Yue, Y., Zhao, D., Yin, Q., 2018. Hyaluronic acid modified nanostructured lipid carriers for transdermal bupivacaine delivery: In vitro and in vivo anesthesia evaluation. Biomed. Pharmacother. 98, 813–820. https://doi.org/10.1016/j.biopha.2017.12.103

Yurdasiper, A., Ertan, G., Heard, C.M., 2018. Enhanced delivery of naproxen to the viable epidermis from an activated poly N-isopropylacrylamide (PNIPAM) Nanogel: Skin penetration, modulation of COX-2 expression and rat paw oedema. Nanomedicine Nanotechnol. Biol. Med. 14, 2051–2059. https://doi.org/10.1016/j.nano.2018.05.017

Zhai, Y., Yang, X., Zhao, L., Wang, Z., Zhai, G., 2014. Lipid nanocapsules for transdermal delivery of ropivacaine: in vitro and in vivo evaluation. Int. J. Pharm. 471, 103–111. https://doi.org/10.1016/j.ijpharm.2014.05.035

Zhang, J.X., Yan, M.Q., Li, X.H., Qiu, L.Y., Li, X.D., Li, X.J., Jin, Y., Zhu, K.J., 2007. Local Delivery of Indomethacin to Arthritis-Bearing Rats through Polymeric

Micelles Based on Amphiphilic Polyphosphazenes. Pharm. Res. 24, 1944–1953. https://doi.org/10.1007/s11095-007-9322-4

Zhang, Shuo, Wang, Y., Zhang, Shuai, Huang, C., Ding, Q., Xia, J., Wu, D., Gao, W., 2023. Emerging Anesthetic Nanomedicines: Current State and Challenges. Int. J. Nanomedicine Volume 18, 3913–3935. https://doi.org/10.2147/IJN.S417855

Zhou, X., Hao, Y., Yuan, L., Pradhan, S., Shrestha, K., Pradhan, O., Liu, H., Li, W., 2018. Nano-formulations for transdermal drug delivery: A review. Chin. Chem. Lett. 29, 1713–1724. https://doi.org/10.1016/j.cclet.2018.10.037

Zhuang, T., Xiong, J., Hao, S., Du, W., Liu, Z., Liu, B., Zhang, G., Chen, Y., 2021. Bifunctional μ opioid and σ1 receptor ligands as novel analgesics with reduced side effects. Eur. J. Med. Chem. 223, 113658. https://doi.org/10.1016/j.ejmech.2021.113658

Zielińska, A., Carreiró, F., Oliveira, A.M., Neves, A., Pires, B., Venkatesh, D.N., Durazzo, A., Lucarini, M., Eder, P., Silva, A.M., Santini, A., Souto, E.B., 2020. Polymeric Nanoparticles: Production, Characterization, Toxicology and Ecotoxicology. Molecules 25, 3731. https://doi.org/10.3390/molecules25163731