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Drug encapsulation in chitosan nanoparticles for dermal patch formulations

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The aim of this thesis was to develop drug encapsulation in chitosan nanoparticles for dermal patch formulations. The thesis project included the optimization of preparation protocols of drug capsulated nanoparticles by using a model drug and a variety of synthetic routes. Subsequently, the model drug was replaced by the local anesthetic drug, lidocaine. In addition, one part of the project was to develop a thin polymer film which incorporates lidocaine-encapsulated chitosan nanoparticles. Ph.D. Didem Sen Karaman from Åbo Akademi University acted as a supervisor and an expert during this thesis project. The thesis was commissioned by the Pharmaceutical Sciences Laboratory at Åbo Akademi University.

Nanotechnology refers to technology that utilizes nanosized materials with a diameter of 1-100 nanometers of materials. Nanoparticles have specific properties, based on their large surface area / mass ratio and specific physicochemical properties. When the material is processed to nanoscale, the chemical, physical and biological properties are either significantly better or completely different as compared to conventional materials.

Chitosan is a natural biopolymer which can be used in drug delivery due to its many good properties. For example, it is non-toxic, biodegradable and has bactericidal and growth-inhibiting effects. Lidocaine is a widely used drug for local anesthesia. Its effect is based on the fact that it is nonionic, which means it can easily pass through the cell membrane. Lidocaine has the ability to close sodium channels of nerve cells and thus prevent the transmission of nerve impulses.

Thin polymer films are being developed for use in drug delivery. They have a number of advantages, including the possibility of designing different drug dosing on the natural polymer matrix. In addition, a thin polymer film which incorporates drug-encapsulated nanoparticles modify the release rate of the drug, reduce toxicity and enhance the therapeutic efficiency.

The obtained results revealed that encapsulation of lidocaine into chitosan nanoparticles improves the release of drug compared to free lidocaine in the patch formulation. In addition, the flux of drug from the film is higher when the formulation contains nanoparticles. However, the results showed that the nanoparticles' incorporation route in the patches must be improved.

Keywords	nanotechnology, nanoparticle, chitosan, dermal drug delivery, lidocaine, local anesthetic, polymeric film, Franz cell diffusion

Tekijä Otsikko Sivumäärä Päivämäärä	Aino Oisalo Lääkkeellä kapseloitujen kitosaaninanopartikkelien muotoilu paikallispuudutukseen käytettyyn iholaastariin 33 sivua 10.3.2017
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Ohjaajat	Professori Jessica Rosenholm Tohtori Didem Sen Karaman Lehtori Hannu Turunen

Opinnäytetyön tavoitteena oli kehitellä paikallispuudutelaastari kitosaaninanopartikkeleja apuna käyttäen. Työhön kuului lääkeainetta sisältävien nanopartikkelien valmistamiseen liittyvien olosuhteiden optimointi käyttäen erilaisia synteesireittejä, minkä jälkeen malliaine korvattiin varsinaisella lääkeaineella. Lisäksi yksi osa tutkimusta oli kehitellä polymeerikalvo, johon lidokaiinilla kapseloitu kitosaaninanopartikkeli voidaan yhdistää. Opinnäytetyössä ohjaajana sekä asiantuntija-apuna toimi tohtori Didem Sen Karaman Åbo Akademista. Opinnäytetyön toimeksiantajana toimi Åbo Akademi, farmasian tutkimuslaitos.

Nanoteknologialla tarkoitetaan teknologiaa, jossa hyödynnetään nanokokoisia, halkaisijaltaan 1 - 100 nanometriä olevia materiaaleja. Nanopartikkeleilla on erityisiä ominaisuuksia, jotka perustuvat niiden suureen pinta-alan ja massan suhteeseen ja erityisiin fysikaaliskemiallisiin ominaisuuksiin. Kun materiaali pilkotaan nanomittakaavaan, sen kemialliset, fysikaaliset ja biologiset ominaisuudet ovat joko huomattavasti parempia tai täysin erilaisia verrattuna tavanomaisiin materiaaleihin.

Kitosaani on luonnollinen biopolymeeri, jota voidaan käyttää apuna muun muassa lääkeaineiden annostelussa lukuisien hyvien ominaisuuksien ansiosta. Se on muun muassa myrkytön, biohajoava ja sillä on bakteereja sekä tappava että kasvua estäviä vaikutuksia. Lidokaiini on laajalti käytetty lääkeaine paikallispuudutuksessa. Sen vaikutus perustuu siihen, että se on ionisoitumaton eli se läpäisee helposti solukalvon. Lidokaiini pystyy sulkemaan hermosolujen natriumkanavat ja näin ollen estää hermoimpulssien välittymisen. Ohuita polymeerikalvoja kehitellään käytettäväksi lääkeannostelussa. Niillä on useita etuja, kuten mahdollisuus ohjelmoituun lääkeaineen annosteluun luonnollisesta polymeerimatriisista. Lisäksi ohuet polymeerikalvot, joihin on yhdistetty lääkekapseloituja nanopartikkeleja, muokkaavat lääkkeiden vapautumisnopeutta, vähentävät myrkyllisyyttä sekä parantavat terapeuttista tehokkuutta.

Saadut tulokset osoittivat, että laastarit, jotka sisälsivät kitosaaninanopartikkeleja vapauttavat kolme kertaa enemmän lääkeainetta verrattuna vain lidokaiinia sisältäviin laastareihin. Lisäksi lidokaiinin virtaus kalvosta on suurempi, kun formulaatio sisältää nanohiukkasia. Kuitenkin tulokset osoittivat, että polymeerikalvojen valmistusmenetelmää on kehitettävä.

Avainsanat	Nanoteknologia, nanopartikkeli, kitosaani, dermaallinen lääkeannostelu, lidokaiini, paikallispuudutus, polymeerikalvo

Content

List of Abbreviations

CH-NP	Chitosan nanoparticle
CHT	Chitosan
DCM	Dichloromethane
DiI	Dialkylcarbocyanine (fluorescent dye)
DLS	Dynamic light scattering
EM	Electron microscope
HCL	Hydrochloride
HPC	Hydroxypropyl cellulose
HPMC	Hydroxypropyl methyl cellulose
MCC	Microcrystalline cellulose
PNPs	Polymeric nanoparticles
SEM	Scanning Electron Microscopy
TEM	Transmission electron microscopy
TPP	Sodium Tripolyphosphate
UV-Vis	Ultraviolet–visible spectroscopy
ZP	Zeta potential

1 Introduction

Nanotechnology is a generic term for very small-scale technology (scale from 1 to 100 nm). It offers possible keys to many current problems by means of smaller, lighter, faster and better-performing materials, components and systems. In drug delivery, nanotechnology is now an exponentially growing focus of research and development and it is being applied to improve drug delivery in several ways.

Generally, drugs have problems such as poor stability, water insolubility, low selectivity, high toxicity, side effects, poor bioavailability and lack of selectivity. One of the biggest problems in therapeutic treatment is drug delivery. For example, in cancer treatments chemotherapy also kills normal, healthy cells along the way. The promise of nanotechnology in drug delivery is to deliver a drug selectively to the target site for enhanced efficacy with reduced side effects.

Nanoparticles are widely used as drug carriers in pharmaceutical applications. There are different types of nanoparticles such as inorganic, polymeric, solid lipid, liposome, nanocrystal, nanotube and dendrimer. Several materials as metal, carbon nanotubes, polymers or other materials can be used for preparation of nanoparticles in a variety of medical applications.

Chitosan (CHT) is one of the most commonly used natural biomaterials, and it is made by treating the chitin shells of shrimp and other crustaceans. Chitosan nanoparticles as drug carriers have the advantage of providing controlled drug release, they can also aid to improve drug solubility and stability, enhance efficacy and reduce toxicity. In addition, chitosan is highly biocompatible.

Lidocaine is one of the widely used drug for local anesthesia. Skin, the biggest organ in the body, has one of the functions to protect against toxic compounds as chemicals and new drug carrier techniques are needed. Nowadays, the markets have pharmaceutical applications (gels, patches, ointments) that include lidocaine hydrochloride (Xylocaine®) or a eutectic mixture of lidocaine hydrochloride and procaine hydrochloride (EMLA®). The development of local anesthetics applications for topical drug delivery using liposomes and nanoparticles have been explored. One of the growing technique is incorporation of drugs into thin polymeric films by using, for example, nanoparticles. The thin

polymeric films have several benefits such as possibility of controlled delivery of drug by regulation of the nature of polymeric matrix. In addition, a thin polymer film which incorporates drug-encapsulated nanoparticles modifies the release rate of drugs, increasing bioadhesive properties and reducing toxicity as well as resulting in an improved therapeutic efficacy.

The aim of this thesis project was to develop a thin polymeric film containing lidocaine-encapsulated chitosan nanoparticles as a local anesthetic formulation. This thesis is part of the research run by Professor Jessica Rosenholm in the BioNanoMaterials group at the Pharmaceutical Sciences Laboratory, which is part of the Faculty of Science and Engineering at Åbo Akademi University. The BioNanoMaterials research group has three different main objectives. One of them is to develop functional nanoparticles for detection, tracking, diagnostic and therapeutic biomedical applications by smart design. They also synthesize composite nanostructures for improved bio-applicability and theranostic activity. In addition, they apply the developed nanomaterials for *in vitro* and *in vivo* drug targeting and biomedical imagining together with their collaborators.

2 Theoretical background

2.1 Nanotechnology

Nanotechnology takes part in several technology fields from electrics to cosmetics. According to the National Nanotechnology Initiative (NNI), nanoparticles have a diameter ranging from 1 to 100 nm. In this size range, it is possible to achieve control of matter at atomic and molecular scale. Nanotechnology offers possible solutions to many current problems by means of smaller, lighter, faster and better-performing materials, components and systems compared to the bigger scale. (1) (2)

However, nanotechnology refers to technologies in which the conscious use of nanoscale structures to create new properties. Material properties change completely when the material consists of nanosized particles. Then the particle surface area relative to volume and mass is very large and causes physical, chemical and biological properties of a radical change in the substance. (2)

2.2 Nanotechnology in drug delivery

Nowadays nanotechnology has been applied to improve drug delivery in many ways, and the nanosystems provide several advantages. The reason is that nanostructured materials have shown promise as drug delivery systems because of their controlled- and sustained-release properties, subcellular size, and biocompatibility with tissue and cells. Especially, nanotechnology is adapted for non-soluble and hydrophobic drugs for improving solubility. (3) (4) Various nanostructured materials were produced and applied to drug delivery such as nanoparticles, nanocapsules, nanotubes, micelles, nano- and micro emulsions and liposomes. (5) In this thesis, the focus was on nanoparticles.

2.2.1 Nanoparticles

Nanoparticles are one type of nanomaterials. Nanoparticles have been used as a physical approach to alter and improve the pharmacokinetic and pharmacodynamic properties of various types of drug molecules. Several materials such as metal (copper, zinc, gold, silver), carbon nanotubes, polymers (chitosan) or other materials can be used for preparation of nanoparticles in a variety of medical applications. Recent advancement in

nanotechnology has proven that nanoparticles can be used as drug carriers. Small size of particles (ranging from 10 nm to 100 nm) has several benefits. Various advantages of nanosizing are decreased fed/fasted variability and patient-to-patient variability, increased oral bioavailability, rate of dissolution and surface area. In addition, nanosize applications offer less amount of dose required, enhanced solubility and more rapid onset of therapeutic action. (6) A variety of nanoparticles can be designed by considering the aim of the application. Figure 1 shows different types of nanoparticles.

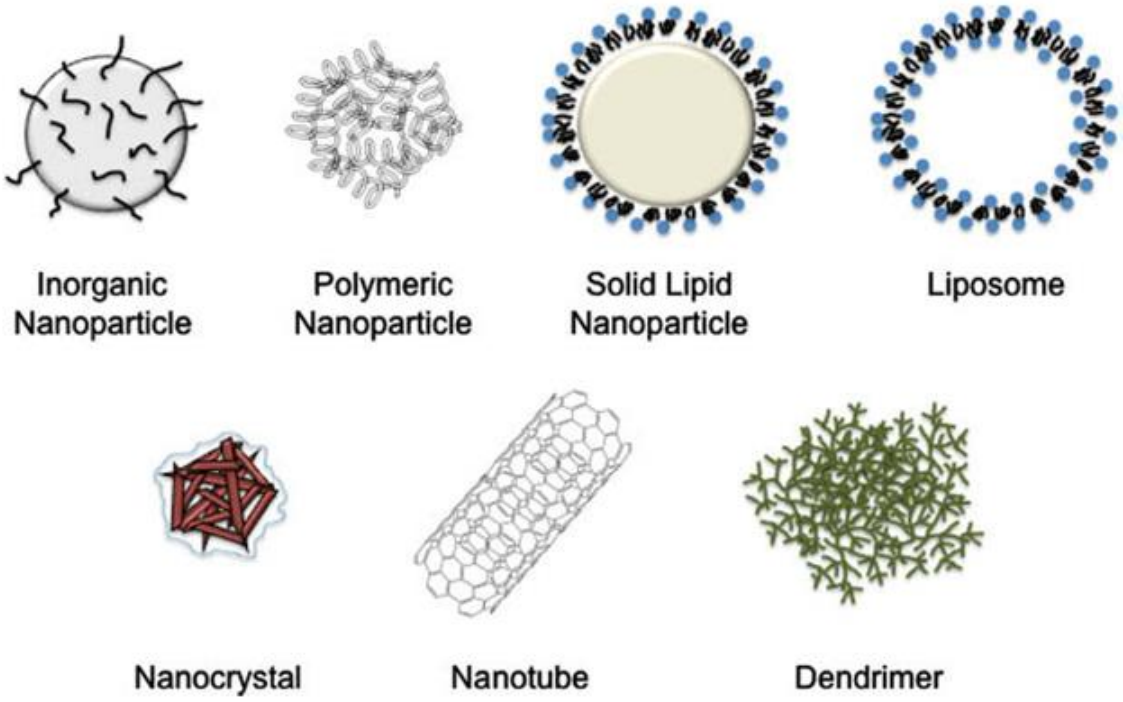

Figure 1. Some types of nanoparticles: inorganic, polymeric, solid lipid, liposome, nanocrystal, nanotube and dendrimer. (7)

In this thesis, polymeric nanoparticles (PNPs) is the type that was worked with. Most polymeric nanoparticles are biodegraded, biocompatible, non-toxic and degrade to produce readily cleared degradation by-products. They have been adopted as a preferred method for nanomaterial drug delivery. (7) PNPs have been extensively studied as particulate carriers in the pharmaceutical and medical fields. (8) Encapsulation strategies include polymers with absorbed drugs, dendritic molecules or coordination compounds with drugs bonded covalently or weakly attached, and artificial or natural micelles or liposome vesicles containing nanodoses of insoluble or toxic drugs which can be selectively released on targets. (3) It is possible to prepare polymeric nanoparticles with different polymers, and in this thesis chitosan polymer was used for the preparation of nanoparticles.

2.3 Chitosan

Chitin is the second most important natural polymer after cellulose in the world, and chitosan (CHT) is the most important derivative of chitin. When the degree of deacetylation of chitin reaches about 50%, it becomes soluble in aqueous acidic media and is called chitosan. (9) Figure 2 presents the structure of chitin and chitosan. Chitosan, N-acetyl-d-glucosamine and β-(1,4)-linked d-glucosamine, is one of the most commonly used natural biomaterials. It is a hydrophilic polymer and a natural linear biopolyaminosaccharide. Chitosan is largely used in different applications as solutions, gels, or films and fibers, because it is being soluble in aqueous solutions. This polymer presents excellent biocompatibility, biodegradability and antimicrobial activity. (9) (10) (11)

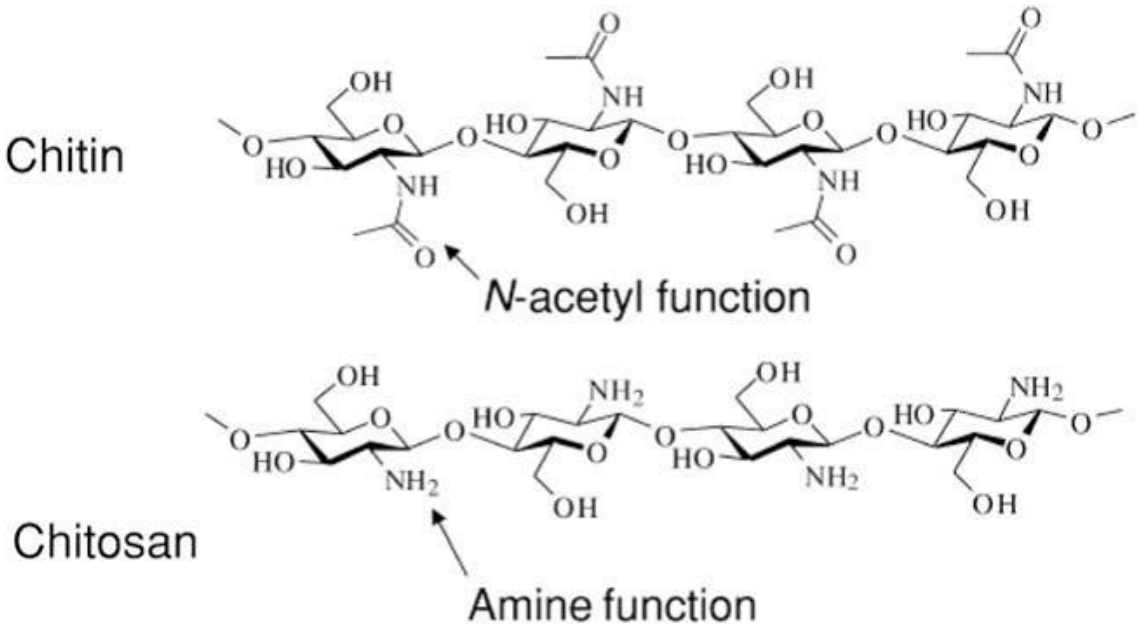

Figure 2. Structure of chitin and chitosan. Chitosan is delivered by deacetylation of chitin. (12)

Generally, drugs have problems such as poor stability, water insolubility, low selectivity, high toxicity, unsensitiveness side effects, poor biodistribution and lack of selectivity. Drug carriers play a significant role in resolving these problems. In this thesis, chitosan nanoparticles were prepared and employed as drug carrier. Chitosan nanoparticles as drug carriers have the advantage of providing controlled drug release, they can also aid to improve drug solubility and stability, enhance efficacy and reduce toxicity. (13)

Properties of chitosan are shown in Figure 3. Chitosan nanoparticles are capable of passing through biological barriers *in vivo* because of their small size. In addition, chitosan nanoparticles can deliver drugs to the lesion site to enhance efficacy, and modified nanoparticles also have other properties such as improved drug targeting. Being a natural product and having other good features such as low immunogenicity and low toxicity, chitosan is a renewable pharmaceutic adjuvant with good biocompatibility. (13) (14)

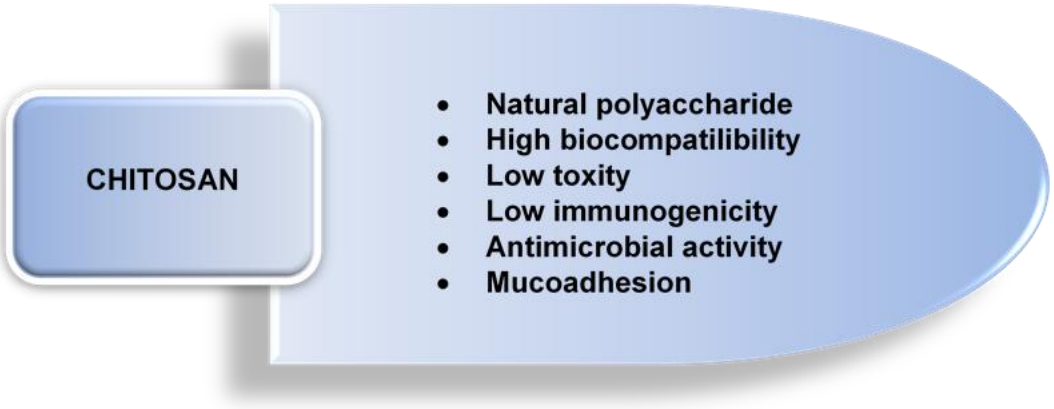

Figure 3. Properties of chitosan.

The properties of chitosan allow it to be used in local anesthetic drug delivery in the skin, because chitosan is mucoadhesive in nature, reactive (it can be produced in many different forms), and most importantly, it has a positive charge under acidic conditions. Earlier studies show that chitosan hydrogels are non-cytotoxic and potential for extended drug release, making them promising local anesthetic delivery vehicles. (12) (15)

2.4 Ionic gelation method

Several methods for preparation of polymeric nanoparticles have been developed and one of them is ionic gelation method. It is also the one that is used in this work. The method is simple and mild, and involves the mixture of two aqueous phases at room temperature.

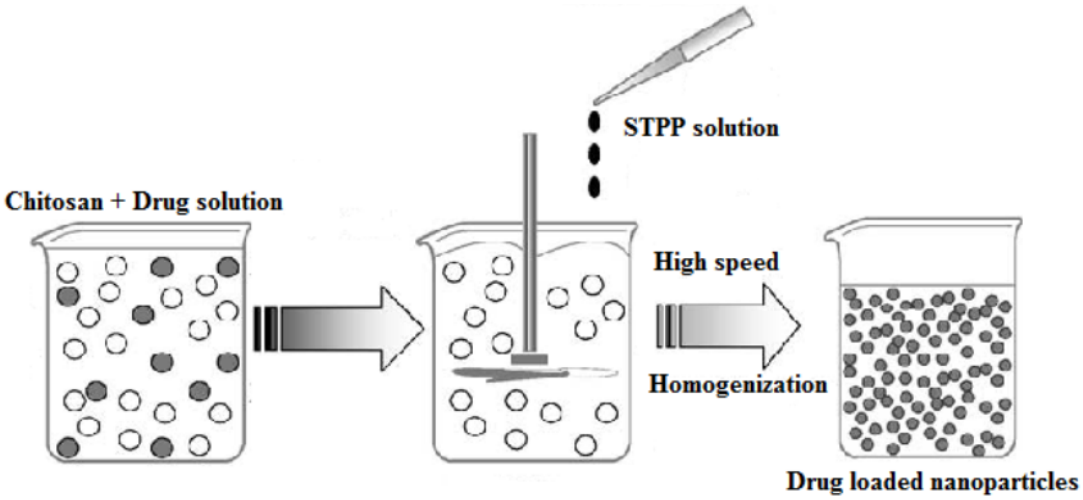

Figure 4. Schematic representation of the ionic gelation method. Tripolyphosphate is added to chitosan-drug solution dropwise with high speed mixing for making drug-loaded nanoparticles. TPP work as cross linker between chitosan and drug. (8)

In ionic gelation method (Figure 4), based on the formation of complexation between the positively charged amine group of chitosan and negatively charged polyanion, such as tripolyphosphate (TPP) to form coacervates with a size in the range of nanometer. TPP can be used for preparing chitosan nanoparticles because it is nontoxic, multivalent and able to form gels through ionic interactions. (8) (16)

Nanoemulsions are nanosized emulsions, which are manufactured for improving the delivery of active pharmaceutical ingredients. A typical nanoemulsion contains oil, water and an emulsifier. Nanoemulsions have various advantages; for example, it can improve the bioavailability of the drug, it is non-toxic and non-irritant in nature as well has improved physical stability. The most important benefit is that it helps to solubilize lipophilic drugs, and lidocaine (the drug that is used in this thesis) is a lipophilic molecule. (17)

Due to unique properties of nanoemulsions, it is an attractive candidate for applications in the food, cosmetic, and pharmaceutical industries and in drug delivery applications. In this chapter, the focus will be on applications of nanoemulsions as building blocks for complex material synthesis to produce, for example, compartmentalized nanoparticles. One of the best-known application in polymer synthesis is emulsion polymerization. It means synthesis, where hydrophobic monomers contained in droplets are polymerized to create polymeric particles. Nanoemulsions have been utilized extensively in polymer synthesis. (17) (18)

Dichloromethane (DCM) is used for making nanoemulsions in nanotechnology. DCM is colorless, organic, water immiscible and highly polar oil compound that is usually used as a solvent. Chemical formula of DCM is CH_2Cl_2 and molar mass 84.93 g/mol. In this thesis project chitosan is dissolved in dichloromethane and the drug compound is dissolved in the polymer solution. It means that the mixture is emulsified in an aqueous solution containing surfactant and producing an oil-in-water emulsion. The advantages of this method are that it is simple, fast and economical. (19) (20)

2.5 Local Anesthetic and Lidocaine Hydrochloride

Local anesthesia includes numbing an area of the body using a type of medication called a local anesthetic. These medications can be used to treat painful conditions, prevent pain during a procedure or operation, or relieve pain after surgery. However, it is temporary pain killer without damaging nerves. (21)

Skin is the biggest organ of the body. Skin performs functions such as protection against penetration of toxic exogenous compounds (e.g. chemicals, microbes), storage and synthesis for water and lipids. In addition, skin acts as a water resistance barrier. Due to all properties of skin, skin has limited possibility of clinical applications. New drug carrier techniques are needed for dermal drug delivery. A number of carrier systems, for example micro emulsions, liposomes, and nanoparticles have been investigated for dermal delivery of drugs. (15) (21)

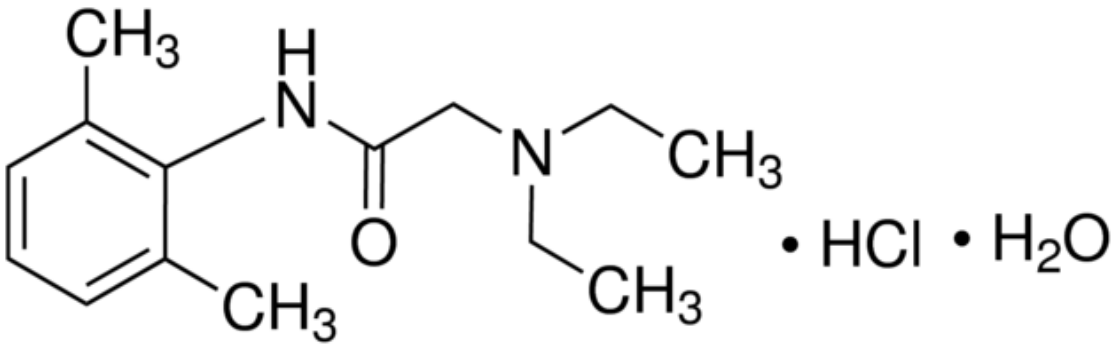

Figure 5. Structure of lidocaine hydrochloride monohydrate.

Lidocaine is one of the oldest and widely used medicines that are used as local anesthetic agents after surgery, trauma, or medical procedures. It was discovered in the

1940s. Lidocaine reversibly closes the sodium channels of nerve cells and prevents the transmission of nerve impulses. It is a synthetic amide used chiefly in its hydrochloride salt form ($C_{14}H_{22}N_2O \cdot HCl \cdot H2O$), in which from it was also used in this thesis project. The molecular weight of the hydrochloride from of lidocaine is 288.82 g/mol, and the structure of lidocaine hydrochloride is shown in Figure 5.

The development of local anesthetics applications for dermal drug delivery using liposomes and lipid nanoparticles have been explored. The idea for new nanosystems are modifying the release rate of drugs, increasing bioadhesive properties and reducing toxicity, resulting in an improved therapeutic efficacy. Nowadays, there are available commercial formulations such as lidocaine and prilocaine cream (EMLA®, Astra Zeneca), lidocaine tape (Penles®, Wyeth) or lidocaine gel path (Lidoderm®, Endo Pharmaceuticals). (15) (22)

2.6 Thin polymeric film based patches for drug delivery

Films that are made from natural, artificial, and synthetic polymers are called a polymeric film. The thickness of the film must be up to 0.2–0.3 mm so that it can be referred to as film; thicker polymeric materials are called sheets. Nowadays there is a growing interest in formulating drugs into thin polymeric films for various numbers of pharmaceutical applications such as oral strips, medical implants and for wound healing applications. (23) In many cases, the films can be of complex composition with different types of polymers with other components such as nanoparticles. The films are easy to handle, mechanically robust and should be non-toxic, biocompatible and biodegradable, so that it can be used as a platform for drug delivery. (24)

The thin polymeric films have several benefits for being used efficiently as a drug release platform. The main advantage is possibility of programmed delivery of drug by regulation of the nature of polymeric matrix. Earlier studies have shown that thin films reduce the dose frequency and enhance the drug efficacy as well as the capabilities to improve the onset of drug action. For dermal drug delivery, complete skin contact is essential and flexible film applications can offer it. In addition, polymeric films are thin and flexible to be less obtrusive and more acceptable by the patient. (23) (24)

For local anesthesia, the drug should penetrate the stratum corneum and desensitize the underlying pain receptors within the skin. Since drug delivery to the skin presents both unique opportunities as well as problems owing to the skin structure, physiology, and barrier properties, thin polymeric film incorporated lidocaine-loaded nanoparticles could solve of the problems. Nanoparticles are used for targeting of topically applied drugs, increasing bioadhesive properties and reducing toxicity, resulting in an improved therapeutic efficacy. In addition, nanoparticles are smaller and lighter than original drug molecules, which make drug release from the film easier. (15)

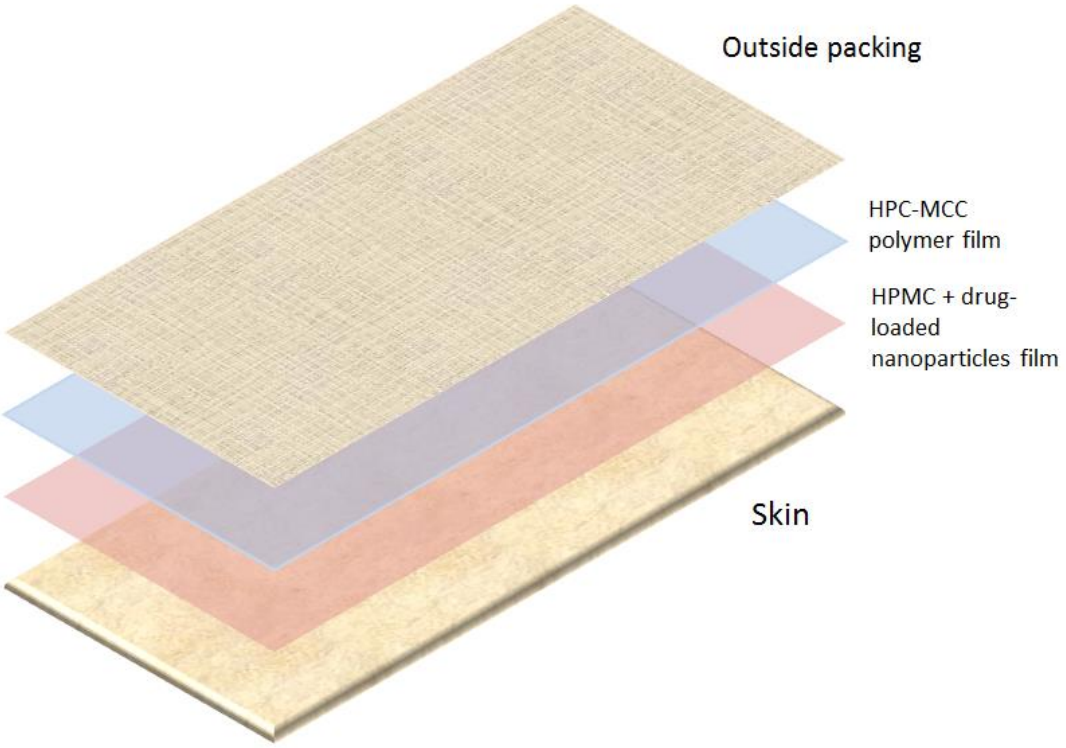

Figure 6. Structure of bi-layered patch.

There is exist various numbers of polymers that can be used to manufacture thin polymeric films. In this study, hydroxypropyl methyl cellulose (HPMC) and hydroxypropyl cellulose (HPC) was used because earlier studies have shown desirable properties, such as thickness and elastics for the film. (25) Figure 6 presents the structure of the bi-layered patch that is used and produced in this thesis project. The backing membrane must be flexible and provide a good bond to the drug reservoir. In addition, it prevents drug from leaving the dosage from through the top.

3 Materials and Methods

3.1 Materials

Lidocaine (LID) hydrochloride monohydrate, chitosan (CHT) and tripolyphosphate (TPP) and dichloromethane (DCM) were purchased from Sigma Aldrich (St. Louis, MO). Hydroxypropyl methylcellulose polymer (HPMC), hydroxypropyl cellulose (HPC) and microcrystalline cellulose (MCC) were provided by Shin-Etsu Chemical (Chiyoda-ku, Tokyo, Japan).

3.2 Dynamic Light Scattering and Zeta Potential

Dynamic light scattering (DLS) is one of the most important tool in characterization of nanoparticles. The range of particle size that is allowed is 0.3nm – 10µm diameter. The basic principle of DLS is simple. It finds the particles and molecules that are in constant random thermal motion and diffuse at a speed related to their size. Generally, smaller particles diffuse faster than larger particles.

Zeta potential (ZP) of particles is determined by measuring their velocity while they are moving due to electrophoresis. In addition, zeta potential depends of the speed that particles are moving. The speed is proportional to the field strength and their zeta potential. It is important to know the particles' zeta potential because then it is possible to make reasonable choices about the chemistry of a formulation in order to select the most appropriate materials to provide stability and to improve shelf life.

3.3 Electron Microscopy

A microscope that uses a beam of accelerated electrons to imagine a specimen is called an electron microscope (EM). It utilizes the same basic principles as light microscope. Instead of glass lenses, condenser lenses are used in the electron microscope because of glass does not pass electrons. The topology, morphology, composition and crystallographic information of the substances can be obtained.

3.3.1 Transmission Electron Microscopy

Transmission Electron Microscopy (TEM) is the original form of electron microscopy and a great tool for the characterization of nanoparticle size, size distribution and morphology. The principle of the TEM is almost the same as that of the light microscope, but the difference is that it uses electrons instead of light. It means that electrons of much lower wavelength make it possible to get a resolution a thousand times better than with a light microscope. (26) In this study, TEM was used to find a structure of chitosan nanoparticles.

3.3.2 Scanning Electron Microscopy

Scanning Electron Microscopy (SEM) is one of the three types of electron microscopes and used in many fields, including medical and materials research. The principle of SEM is very similar to that of TEM. The significant difference between those electron microscopy techniques is that TEM electrons transmit though the specimen, while the SEM electron beams scan across the specimen. In this thesis project, SEM was used for getting an idea about the distribution of chitosan nanoparticles on the polymeric film.

3.4 Ultraviolet-Visible Spectroscopy

Ultraviolet-Visible Spectroscopy (UV-Vis) is one of the spectroscopic methods, and it has been in general use for the last 35 years. During those years, it has become the most important analytical instrument in the laboratory. UV-Visible spectrometry has many benefits such as its simplicity, versatility, speed, accuracy and cost-effectiveness. (27)

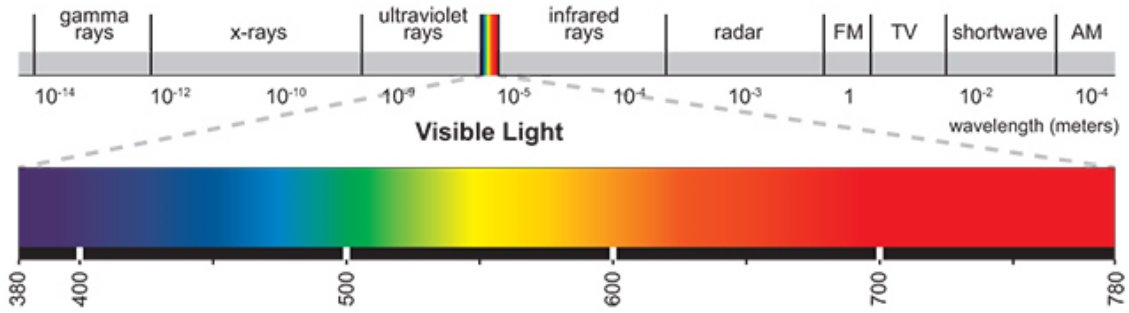

Figure 7. Light spectrum chart. UV radiation is called radiation that occurs in range of 100-400 nm and visible light appearing 400-800 nm radiation.

Ultraviolet (UV) radiation is electromagnetic radiation. UV radiation is called radiation that occurs in the range of 100-400 nm and visible light is radiation appearing at 400-800 nm (Figure 7). The molecules may absorb visible or UV light at specific wavelengths, consequently the electrons of the molecule become excited. Excitation is possible only at specific wavelengths, which correspond to the energy that is needed to displacement. When a molecule absorbs energy (which is corresponding to the energy that is needed to displacement), an absorption band is detected in the absorption spectrum. This method utilizes the phenomenon known as UV-spectroscopy or UV-Vis spectroscopy, when the visible light region is involved in the measurements. A UV spectrum is obtained by recording the absorbed radiation intensity as a function of the wave number. (28)

In this thesis project, UV-Vis was used for the detection of drug content on nanoparticles and thin polymeric films as well as to obtain drug release curve by using NanoDrop 2000c Spectrophotometer, which has a wavelength range from 190 to 840 nm.

In this thesis project, a standard curve was prepared on different concertation of lidocaine hydrochloride with the absorption of spectra by using UV-Vis spectrophotometer. Calibration curves were performed for the standards three times at five different concentrations. The method was linear in the lidocaine hydrochloride concentration range from 31.25 to 500 µg/ml (R^2 = 0.99). All analyses for encapsulation and drug release studies were according to standard curve.

3.5 Optimization process of drug encapsulated chitosan nanoparticles formulations

One of the aims of this thesis was to find the most efficient encapsulation degree of chitosan nanoparticles. At the beginning of the project, three different synthesis routes for the drug encapsulated chitosan nanoparticles were tested. Dialkylcarbocyanine (DiI) is a lipophilic carbocyanine dye that was used as model drug for optimizing the formula before using lidocaine hydrochloride. For all three different drug loaded chitosan nanoparticle preparations, the same ratio of chitosan and tripolyphosphate anion (5:1) were used.

3.5.1 Preparation of CHT-DiI-TPP nanoparticles

In the first synthesis protocol, 200 µl of CHT (5 mg) was added to up to 2 ml of 10 mM acetate buffer, pH 5.0. DiI was added in different weight percent (1, 5, 10, 25, 50 and 100 w %) to chitosan solution by simultaneous mixing. The chitosan-DiI solution was stirred at 900 rpm with a MIX 15 eco magnetic stirrer approximately 10 min. 322 µl of TPP solution (3 mg/ml) was added dropwise to each tube, mixing simultaneously. The CHT-DiI-TPP solutions were stirred at 900 rpm with a MIX 15 eco magnetic stirrer for 4 hours.

3.5.2 Preparation of CHT-TPP-DiI nanoparticles

In the second preparation route, 200 µl of CHT (5 mg) was added to up to 2 ml of acetate buffer (10 mM @ pH 5.0). 322 µl of TPP solution (3 mg/ml) was added dropwise to each tube under fast stirring. The solution was stirred at 900 rpm with a MIX 15 eco magnetic stirrer for 20 min before DiI was added in different weight percent (1, 5, 10, 25, 50 and 100 w %) to CHT-TPP solution under mixing. The CHT-DiI-TPP solutions were stirred at 900 rpm with a MIX 15 eco magnetic stirrer for 4 hours.

3.5.3 Emulsion based encapsulation of DiI into chitosan nanoparticles

In accordance with the findings of H. Liu e. al (19), the last experiment was made by encapsulating DiI containing dichloromethane (DCM) with the aid of the emulsifying property of chitosan in the DCM water emulsion system. In this process, first DiI was added in different weight percent (0.5, 2.5, 5.0, 12.5, 25.0, 50.0, 75.0 and 100.0 w %) to 1 ml of DCM while sonicating. Acetate buffer (10 mM @ pH 5.0) was added up to 2 ml and sonicate approximately 10 min. 200 µl of chitosan (5 mg) was added to solution while sonicating and sonicate again 30 min. While stirring, 332 µl of TPP (3 mg/ml) was added to solution and sonicate and vortex. Vials were left in the stirrer (900 rpm) for 2 hours. After 2 hours, DCM was evaporated by heating the sample (36°C) while stirring.

3.6 Collecting the particles

Particles were collected by using the same protocol for each batch. One drop of 1 M sodium hydroxide (NaOH) was added per milliliter of CS-NPs solution, and the solution

was centrifuged with a ScanSpeed Mini microcentrifuge (LaboGene, Denmark) at 12 000 rpm for 10 min. The pellet was washed twice with 2 ml of Milli-Q water and centrifuged at 12 000 rpm for 10 min between washes. However, if weight percent of DiI was 50 w% or more, the solution was centrifuged for 15 min instead of 10 min. After washes, 2 ml of acetate buffer (10 mM @ pH 5.0) was added on the nanoparticle pellet and sonicated for 30 min.

3.7 Hydrodynamic size

Hydrodynamic size values of chitosan nanoparticles were investigated by using Dynamic Light Scattering (DSL, Malvern, Zetasizer Nano ZS). For the measurement, 150 µl from the chitosan nanoparticle stock (9 mg/ml of particles) was added to 1.35 ml of acetate buffer (10mM @ pH 5.0) and sonicated 30 min before measurement.

3.8 Determination of drug encapsulation degree for differently prepared particles

For the drug loading degree determination, a predetermined amount of ethanol was added to nanoparticle pellet and sonicated 30 min. Tubes were left on Vortex Mixer VX-200 (Labnet, Edison, USA) shaker for 2 hours. After 2 hours, tubes were centrifuged (12 000 rpm, 7 min) and supernatant was collected. Ethanol was added to the pellet, tubes were sonicated and vortexed regularly for 30 min and left on the shaker overnight. Supernatant was collected again by using the same protocol as earlier. If the pellet was still pink, ethanol was added and left in the shaker for 4 hours. Supernatant was collected. Each supernatant was measured by using Nanodrop 2000c UV-Vis spectrophotometer (Thermo Scientific, USA) λ= 260 nm.

3.9 Encapsulation of lidocaine into chitosan nanoparticles

After optimization of the drug encapsulation for the formulation the model drug, DiI, was replaced with the actual drug molecule, lidocaine hydrochloride. See sections 3.5-3.8 (excluding 3.5.3). All preparation process was made in different weight percent of lidocaine hydrochloride (1, 5, 10, 25 and 50 w %). Lidocaine hydrochloride in different weight percent (1, 5, 10, 25 and 50 w %) was used in the all preparation processes.

On the basis of the preliminary tests, the lidocaine hydrochloride incorporation work was performed using protocol synthesis that was slightly modified from the protocol that is explained in section 3.5.3. The protocol was as follows: 5 mg of lidocaine hydrochloride was dissolved in 2 ml of DCM and sonicated 30 min. 400 µl of chitosan (25 mg/ml) was dissolved in 936 µl of acetate buffer (10 mM @ pH 5) and added to the DCM-lidocaine solution while sonicating. After 30 min sonication and 3 min homogenization, 664 µl TPP (3mg/ml) was added to the solution while stirring. CHT-DCM-lidocaine-TPP solution was stirred at 500 rpm with a MIX 15 eco magnetic stirrer for approximately 1 hour. Afterwards, the content of DCM in the lidocaine hydrochloride encapsulated chitosan-TPP particles was evaporated by heating the sample (36°C). The obtained sample was lyophilized.

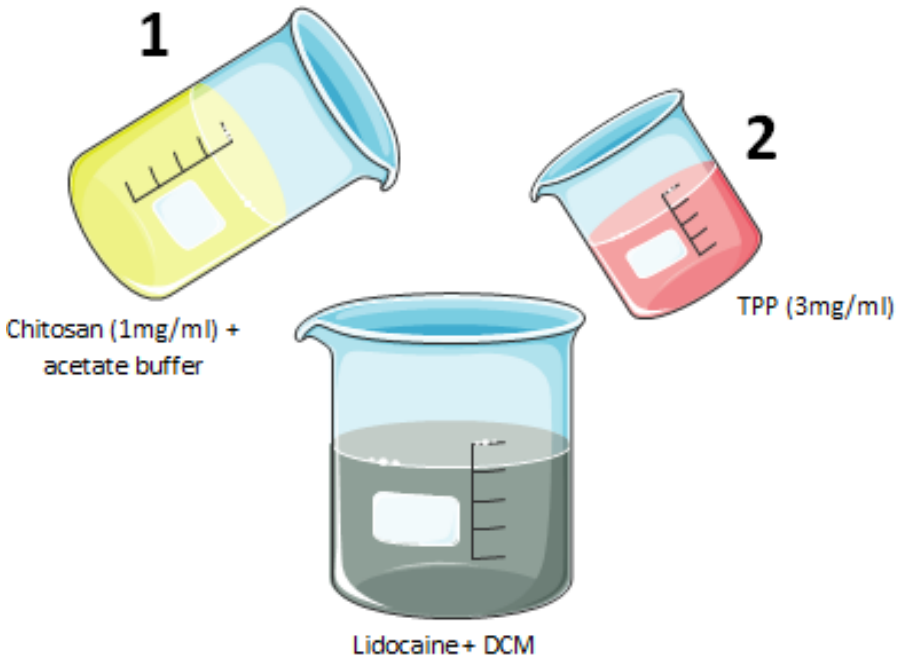

Figure 8. 50w% of lidocaine hydrochloride was dissolved in DCM (dichloromethane) and chitosan (25 mg/ml) was dissolved in acetate buffer solution (10 mM @ pH 5) separately. Afterwards, the chitosan solution was added to the DCM-lidocaine solution. After the homogenization of the obtained emulsion, TPP (3mg/ml) was added under stirring.

Figure 8 shows the synthesis of lidocaine hydrochloride encapsulated chitosan nanoparticles. In this method, DCM is used for emulsified chitosan.

3.10 Preparation of bi-layered polymeric film based patches

Earlier studies have shown that the use of polymers hydroxypropyl methyl cellulose (HPMC) and hydroxypropyl cellulose (HPC) to manufacture thin polymeric films give desirable properties to the film such as thickness and elastics. (25)

In this thesis, two different binders, ethanol and isopropanol alcohol, were tested in accordance to the findings of M. Preis et. al. (25) The solvent starts to slightly dissolve the film to obtain stickiness for adhering it to the surface of the other film. In addition, different combinations of selected polymers were tested to be as a base and an upper layer. Table1 gives the different combinations of polymers and solvent. Microcrystalline cellulose (MCC) was used to manufacture a rough surface on base layer because film attached more easily to the rough surface than to the smooth surface.

Table 1. Bi-layer polymeric films manufacturing by paste method (+/+ both layers swelling; -/+ only upper layer swelling; +/- only base layer swelling).

Base layer	Upper layer	Spray	Result
HPMC + MCC	HPMC	Ethanol	+/+
HPMC + MCC	HPC	Ethanol	+/+
HPMC + MCC	HPC	Isopropanol	-/+
HPC + MCC	HPMC	Ethanol	+/+
HPC + MCC	HPMC	Isopropanol	+/-

When the base layer was made by using HPMC-MCC polymer and the upper layer was made from the same polymer (HPMC), the spray solution had to be ethanol because of HPMC does not swell with isopropanol. However, when the solvent was ethanol, it was hard to paste it on the base layer because both the films started to swell with ethanol. In addition, dry film curled up. Preferable result was gotten when isopropanol was used as a binder. The film was easier handle because HPC was the only polymer that could swell in isopropanol. Therefore, HPMC films could easily be applied onto the isopropanol-wetted base layer.

3.10.1 Hydroxypropyl cellulose (HPC) film preparation as supporting layer of bi-layered film

The thin polymeric film was made from 15 % hydroxypropyl cellulose (HPC), and 5% microcrystal cellulose (MCC) was used as a filling material (table 2). Milli-Q water was

used as a solvent. Transparent copier film (Folex imaging, X-10.0) was used as a liner, and the film was casted by using film casting knife Film Applicator MULTICATOR 411 (Erichsen, Germany). The thickness of the wet film was 500 micrometers (casting height). This HPC-MCC film was used as a base layer.

3.10.2 Preparation of hydroxypropyl methyl cellulose (HPMC) films as an upper layer of bi-layered film

An upper layer was made from 10 % hydroxypropyl methyl cellulose (HPMC), and 2 % glycerol was used as a plasticizer (Table 2). Milli-Q water was used as a solvent. Transparent copier film (Folex imaging, X-10.0) was used as a liner, and the film was casted by using film casting knife Film Applicator MULTICATOR 411 (Erichsen, Germany). The thickness of the wet film was 200 micrometers (casting height).

Table 2. Film formulations used for layers of bi-layered films manufactures – amounts in %.

LAYER	UPPER	SUPPORTING
Film former	HPMC	HPC
Polymer	10	15
Glycerol	2	-
MCC	-	5
Particles	48	-
Water	40	80

In order to incorporate lidocaine-encapsulated CH-NPs into HPMC films, the particles were simply mixed with the polymer solution before casting. It was decided to use 50 w% of lidocaine hydrochloride loading for film preparation because it gave the best loading efficiency in the preliminary tests. Predetermined amount of particles (5.86 mg) which corresponds to 50 w% of lidocaine hydrochloride on films were mixed with 2.4 g of acetate buffer (10 mM @ pH 5) and sonicated 30 min. First, 1 ml of film solution was mixed well with the particle suspension and then added to the rest of the film solution. Particle-film solution was stirred at 120 rpm with a MIX 15 eco magnetic stirrer for approximately 1.5 hour before casting. Furthermore, free lidocaine hydrochloride film (not containing CH-NPs) was prepared by using the same protocol. Pure lidocaine hydrochloride was mixed with HPMC polymer and added to the solvent.

3.10.3 Paste method for manufacturing bilayer polymeric film

M. Preis et al studied different manufacturing of bi-layered films. (25) On the basis of this study, it was decided to use the paste method in this project. Films were prepared as described in sections 3.11.1 and 3.11.2.

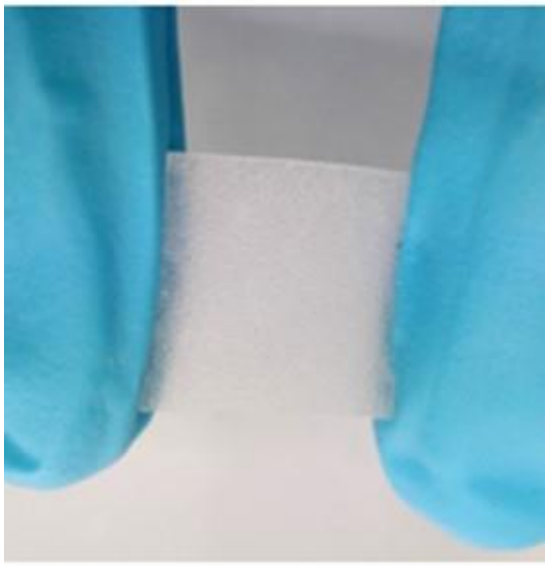

Figure 9. Bi-layered thin polymeric film. Base layer has rough surface because of MCC and transparent drug-loaded HPMC film layer is completely bound to the base film.

As the second layer's film sheets were slightly smaller (3.2 cm x 3.2 cm) than the base film (3.5 cm x 3.5 cm), potential drug load of the second layer was completely shielded from one side. HPMC film pieces were applied onto the isopropanol-wetted HPC-MCC film. As shown in Figure 8, the smooth drug-loaded layer was completely bound to the rough film surface.

3.11 Drug release studies from polymeric film

First films were cut in the squares with the dimensions of 3.2 cm x 3.2 cm (10 cm^2). The pieces were soaked to the dissolution medium that consisted of 2 ml of 10 mM acetate buffer, pH 5.0. The release was performed at room temperature, with a rotation speed of 120 rpm with a MIX 15 eco magnetic stirrer. After 35 min, the solution was analyzed by using Nanodrop 2000c UV-Vis spectrophotometer (Thermo Scientific, USA), λ= 260 nm. All the determinations were made in triplicate. Films that included lidocaine hydrochloride loaded nanoparticles were compared with free lidocaine hydrochloride films.

3.12 Franz Cell Diffusion

The release of lidocaine hydrochloride from lidocaine-encapsulated CH-NPs was determined by using Franz cell diffusion with a diffusion area of 6.25 cm^2. Prepared films were cut in the squares with the dimensions of 2.5 cm x 2.5 cm. Cellulose Acetate membrane filters which have a dimension of 0.45 µm and a pore size of 1.2 µm (Whatman, GE Healthcare Life Sciences) was used as membrane. First, the membrane was wetted by soaking in the receptor media (10 mM acetate buffer, pH 5) for 30 min. After 30 min, the membrane was applied to the dosage wafer. The prepared film was placed onto the membrane. The plastic ring was placed on top of the dosage wafer. The receptor compartment (capacity 4 ml) was filled with 10 mM acetate buffer (pH 5), and the hydrodynamics in the receptor compartment was maintained by stirring with a magnetic stirrer. The donor formulation is shown in Figure 10. Carefully the dosage wafer with membrane and the film was moved on top of the glass disk and the clamp was applied for removing pressure.

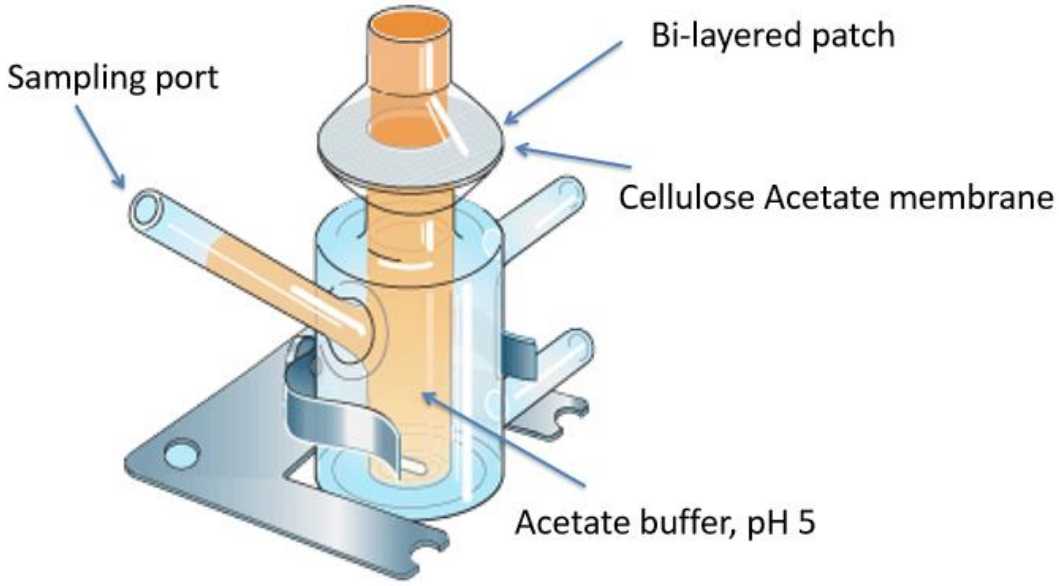

Figure 10.Franz Cell Diffusion donor formulation top of the magnetic stirrer.

Approximately 2 ml of the solution from the receiver medium was removed at regular intervals, 30, 60, 120, 180 and 240 min. Drug concentration (µg/ml) was measured by using UV-Vis, at 260 nm. All the determinations were made in triplicate for each film. Flux

from the film that included lidocaine-encapsulated CH-NPs was compared with flux from the free lidocaine hydrochloride polymeric film.

Flux (J), which means the amount of permeant crossing the membrane in time, was calculated by using the formulation:

$$J = \frac{c}{A}$$

where c is the drug content on receiver medium and A is the area of the patch. The unit of flux is µg/cm^2.

4 Results and Discussion

4.1 The encapsulation capacity of CHT-NP prepared with different routes chitosan nanoparticles

4.1.1 The encapsulation capacity of CHT-DiI-TPP nanoparticles and CHT-TPP-DiI nanoparticles prepared by ionic gelation method

In this thesis, different synthesis routes were tried for the encapsulation of the lidocaine in the CHT-NP in order to find the route with the highest drug encapsulation degree. As a starting hydrophobic dye molecule (DiI) was used as model drug. The obtained DII loading degrees are presented in Table 3.

Table 3. Loading weight percent and yield of CHT-DiI-TPP nanoparticles and CHT-TPP-DiI nanoparticles. Lines on color orange present CHT-DiI-TPP nanoparticles and white lines present CHT-TPP-DiI nanoparticles.

Starting DiI w/w%		Loaded DiI w/w%	Yield %
1	CHT-DiI-TPP	0.69	69.25
	CHT-TPP-DiI	0.64	64.02
5	CHT-DiI-TPP	4.68	93.67
	CHT-TPP-DiI	4.22	84.49
10	CHT-DiI-TPP	5.89	58.90
	CHT-TPP-DiI	9.25	92.55
25	CHT-DiI-TPP	23.00	92.00
	CHT-TPP-DiI	22.59	90.34
50	CHT-DiI-TPP	43.35	86.69
	CHT-TPP-DiI	41.46	82.93
100	CHT-DiI-TPP	100.92	100.92
	CHT-TPP-DiI	85.17	85.17

Drug loading degree and yield in CHT-NPs did not result in significant differences when they were prepared with the routes given in section 3.5.1 and 3.5.2 (table 3). For all starting weight percent of DiI, yield of DiI were approximately the same with both syntheses. The DiI preparations even resulted in 100 w/w% loading as the highest loading degree for the preparation route of explained in section 3.5.1. Most of the loading degrees also resulted in almost 80-90 % loading efficacy relative to the starting drug amount.

4.1.2 The encapsulation capacity of emulsion based chitosan nanoparticles for DiI

In this experiment, DiI was attempted to be dissolved in DCM before adding in 10 mM acetate buffer. Afterwards chitosan and TPP was added, and the protocol was followed as given in section 3.5.3. Table 4 shows low values for the drug loading amount and yield for the loading process. The obtained low values mean that DiI cannot be successfully incorporated into CHT-NP particles via the synthesis route of emulsion based CHT-NP preparation. It could be due to the low solubility of DiI in DCM, which also may lead to an unsuccessful emulsification and encapsulation process.

Table 4. Emulsion based encapsulation of DiI into chitosan nanoparticles. Both values (loading w% and yield) were quite low with this synthesis.

Starting DiI w/w%	Loaded DiI w/w%	Yield %
0.5	0.22	43.65
2.5	0.92	36.59
5.0	3.68	73.60
12.5	3.50	28.01
25.0	3.62	14.50
50.0	1.48	2.94
75.0	1.74	2.31
100.0	1.88	1.88

Compared all values of different synthesis, it shows that emulsion based encapsulation of DiI into chitosan nanoparticles results with the lowest loading weight percent and yield with the model DiI. The best synthesis protocol was found to be the preparation route described in 3.5.1, in which DiI was mixed with chitosan solution after the TPP was added for ionic gelation process.

4.1.3 The encapsulation capacity of CHT-lidocaine-TPP nanoparticles and CHT-TPP-lidocaine nanoparticles

After optimization of the drug encapsulation for the formulation DiI molecule, DiI was replaced with the actual drug lidocaine hydrochloride. Table 5 shows loading weight percent and yield of CHT-lidocaine-TPP nanoparticles and CHT-TPP-lidocaine nanoparticles.

Table 5. Loading weight percent and yield of CHT-lidocaine-TPP nanoparticles and CHT-TPP-lidocaine nanoparticles. Orange rows present CHT-lidocaine-TPP nanoparticles and white rows present CHT-TPP-lidocaine nanoparticles.

Starting lidocaine w/w%		Loaded lidocaine w/w%	Yield %
1	CHT-Lidocaine-TPP	n/a	n/a
	CHT-TPP-Lidocaine	2.00	200.00
5	CHT-Lidocaine-TPP	1.56	31.11
	CHT-TPP-Lidocaine	8.26	165.14
10	CHT-Lidocaine-TPP	2.27	22.67
	CHT-TPP-Lidocaine	16.74	167.41
25	CHT-Lidocaine-TPP	4.00	16.00
	CHT-TPP-Lidocaine	41.26	165.05
50	CHT-Lidocaine-TPP	16.67	33.33
	CHT-TPP-Lidocaine	82.88	165.77

The preparation process in which TPP was added to chitosan solution before adding the lidocaine hydrochloride (as described in section 3.5.2) resulted in higher drug amount compared to the actual starting lidocaine amount. The obtained high values may be a result of the structural changes in lidocaine molecule at the detection WL of 260 nm with the UV-VIS spectrophotometer for this preparation route of drug incorporation. Whereas, preparation process in which lidocaine was added to chitosan solution before TPP addition resulted in lower yield and loading weight percent of lidocaine compared to the obtained efficiency with the model molecule DiI.

4.1.4 The encapsulation capacity of emulsion based encapsulation of lidocaine into chitosan nanoparticles

In this preparation process of lidocaine encapsulated chitosan nanoparticles, lidocaine was dissolved in DCM, and chitosan was dissolved in acetate buffer solution separately; in other words, the protocol described in section 3.5.3 was followed. Table 6 shows loading yield and loading degree of lidocaine on CH-NPs.

Table 6. Loading efficiency weight percent and loading degree of lidocaine hydrochloride on CH-NPs.

Starting lidocaine w/w %	Yield %	Loaded Lidocaine w/w% on Particles
1	n/a	n/a
5	42,67	2,13
10	66,67	6,67
25	19,33	4,83
50	42,67	21,33

In this experiment, loading yield was between 40-70 %, but the loading degree on CH-NPs was changing. However, the highest loading degree was obtained with the starting 50 w/w % lidocaine loading. Therefore, this loading route was used in the thin polymeric films.

4.2 Characterization of lidocaine-encapsulated CH-NPs

4.2.1 Dynamic Light Scattering

Dynamic Light Scattering were used to investigate the hydrodynamic size of the particles. Chitosan stock solution was not filtered before adding to DCM-lidocaine solution, which might be the reason why DSL measurement failed with lidocaine-encapsulated CH-NPs. In all measurements, the Polydispersity Index dimensionless (PDI) was greater than 0.7 indicate, which means that the sample has a very broad size distribution and is probably not suitable for the DLS technique. Good PDI value is between 0.1-0.4. However, this does not mean that the nanoparticles do not exist. Lidocaine-encapsulated CH-NPs solution might have free chitosan polymers; because they are not filtered, they are too large (~120 kDA), which might be the reason of obtained high PDI values. Although DLS is one of the most important tools in the characterization of nanoparticles, it is quite sensitive, and it has limitations in such cases.

4.2.2 Electron Microscopy

The observation from transmission electron microscope (TEM) gave information of the structure of lidocaine-encapsulated CH-NPs (Figure 11), whereas the analysis with a scanning electron microscope (SEM) showed the general appearance of chitosan nanoparticles on the polymeric films. (Figure 12).

TEM images show that the encapsulation of lidocaine-encapsulated CH-NPs are in clusters. In addition, they were nearly spherical in shape and the size range of clusters was about 150-500 nm.

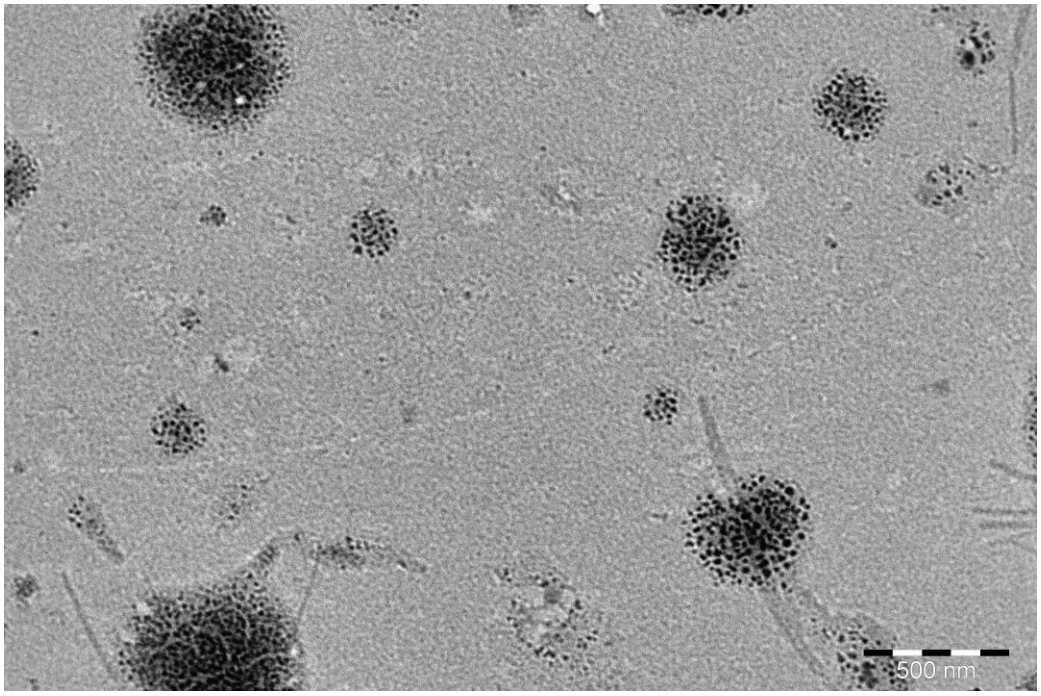

Figure 11. TEM image of lidocaine-encapsulated CH-NPs with 500 nm scale bar.

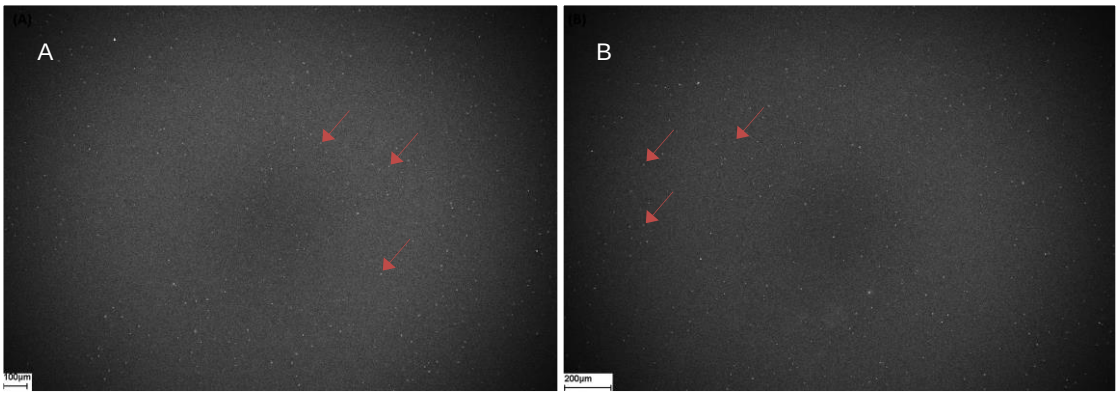

Figure 12. SEM image of center of the polymeric film incorporated CH-NPs with 100 µm scale bar (A) and SEM image of side of the polymeric film incorporated CH-NPs with 200 µm scale bar (B). White dots, some of them are pointed with red arrows, present nanoparticles.

White dots on SEM images present nanoparticles. Images from the center and side of the film indicate that the nanoparticles were evenly distributed on the film. However, according to the result of Franz Cell Diffusion experiment, white dots might also be air bubbles on film.

4.3 Determination of incorporated lidocaine hydrochloride amount on bi-layered polymeric film patches

For drug release experiment films were cut in 10 cm^2 pieces. The hypothesis was that 10-cm^2 piece of the film would have approximately 62.5 µg of lidocaine hydrochloride. Table 7 shows that release out of lidocaine hydrochloride was approximately 206 µg of lidocaine hydrochloride in 10-cm^2 film, whereas release out of the film without CH-NPs was approximately 79 µg in the 10-cm^2 film.

Table 7. Incorporated lidocaine hydrochloride amount on bilayer polymeric film patches. Piece of the films were soaked in acetate buffer (10 mM @ pH 5.0) and complete release of incorporated lidocaine hydrochloride was measured after 35 min.

	Lidocaine Release	
	1 cm^2 of film (µg)	10 cm^2 of film (µg)
With CH-NPs	20,56	205,56
Free lidocaine	7,87	78,67

Figure 13 displays a graph of lidocaine hydrochloride release from drug-loaded nanoparticle films and free lidocaine incorporated films. Three parallel experiments gave the same result that the release of lidocaine hydrochloride was three times higher when incorporated within CH-NPs than without them.

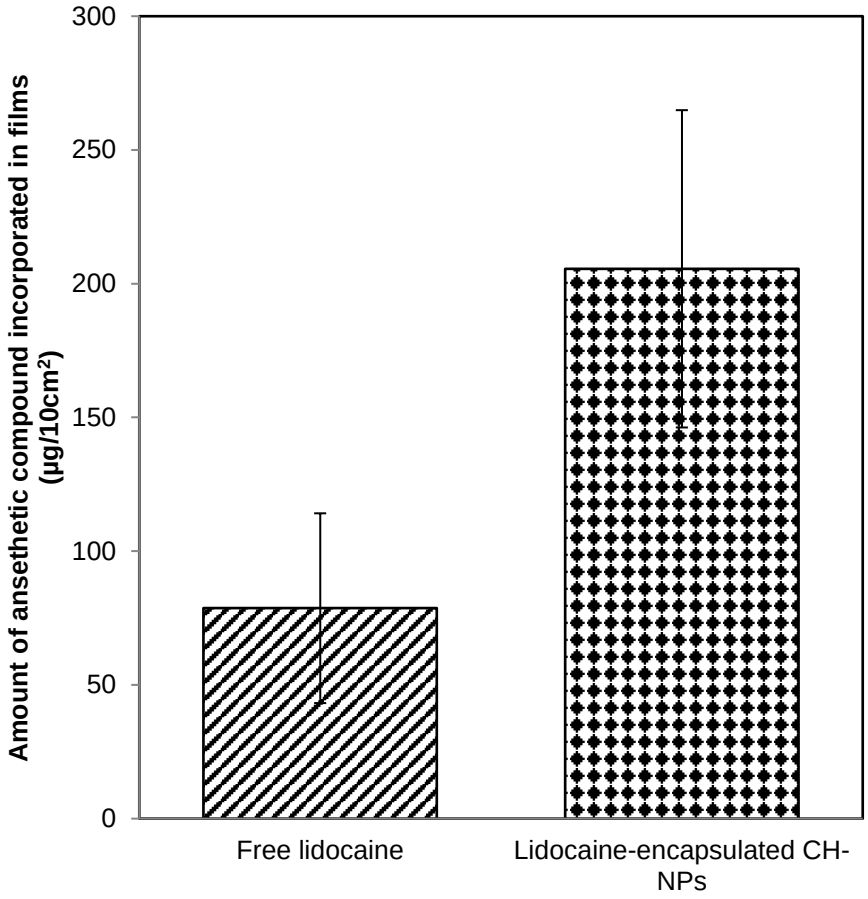

Figure 13. Amount of lidocaine hydrochloride in film compared to the film with chitosan nanoparticles between the films without CH-NPs. Films were soaked in 10 mM acetate buffer (pH 5.0) for 35 min before measurement.

Standard deviation was high in both experiments. This mean that lidocaine hydrochloride was not evenly distributed and the lidocaine hydrochloride content depends on which part of film is used.

4.4 Determination of lidocaine release profile from bi-layered polymeric film patches by Franz-diffusion cell method

The lidocaine hydrochloride flux profiles of free lidocaine hydrochloride films and lidocaine-loaded chitosan nanoparticles films are illustrated in Figure 14.

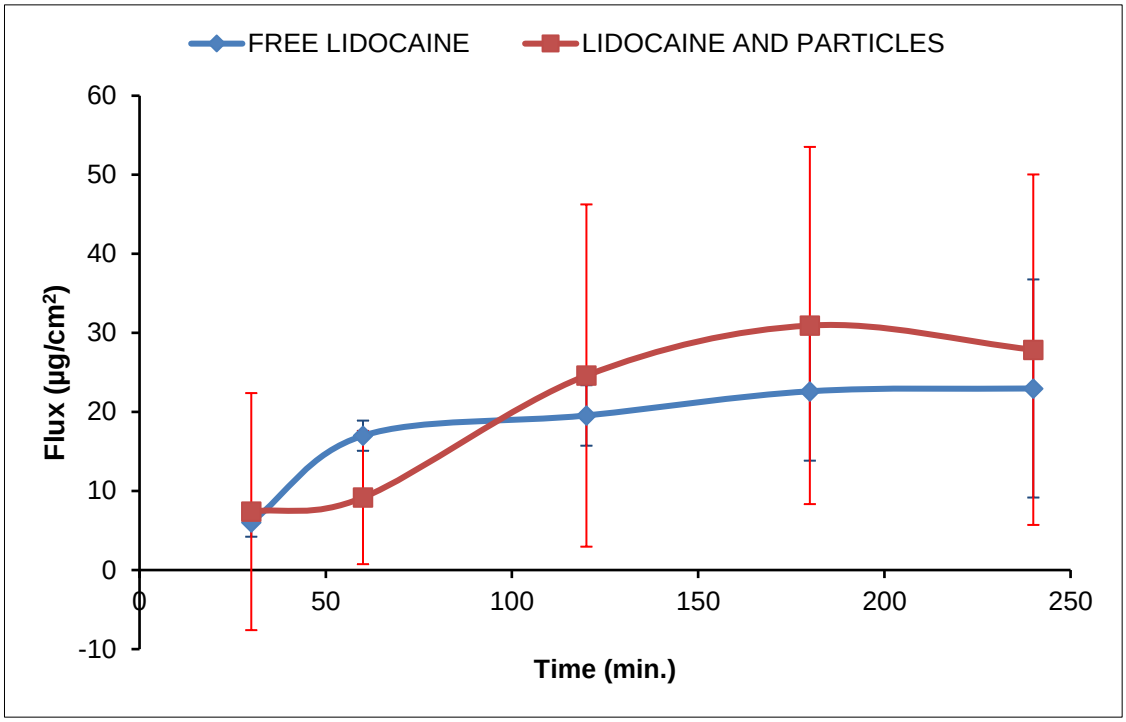

Figure 14. Flux of lidocaine measured by using Franz cell diffusion experiment. The red line is the film with lidocaine-loaded chitosan nanoparticles, and the blue line is the film without chitosan nanoparticles.

Results showed that the flux of lidocaine was higher when the formulation contained nanoparticles. When the film contained free lidocaine hydrochloride, flux increase first faster than with CH-NPS, but after one hour, the flux was approximately constant. Therefore, the flux was increasing until 180 min when films contained lidocaine-loaded chitosan nanoparticles. This means that drug release from the thin polymeric film is improved because of the nanoparticles.

However, standard deviation was high when the formulation contained lidocaine-loaded chitosan nanoparticles. This means that the lidocaine hydrochloride content depends of which part of the film is used.

5 Conclusion

This study shows that drug encapsulated chitosan nanoparticle preparation with dye, DiI, works and the protocol could be used for future studies with drugs that have similar solubility properties as DiI. The best synthesis process is when DiI was mixed with chitosan solution before TPP was added.

As to the lidocaine hydrochloride incorporation into CHT-NPs, the preparation route of encapsulating the drug with the emulsification process works more effectively compared to the other two encapsulation processes. When the lidocaine incorporation into patches is carried out by using the CHT-NPs, nanoparticle-incorporated patches seem to provide three times more drug incorporation compared to only lidocaine hydrochloride incorporated patch formulation. The nanoparticle-incorporated patch formulation seems to provide a sustained release profile. In addition, flux of lidocaine hydrochloride from films was compared using Franz cell diffusion; the results suggest that the flux is higher when the formulation contains nanoparticles.

Film casting with casting knife does not give a homogenous film. One reason might be that the casting process is manual and the speed is changing each time. In addition, inhomogeneous film might depend of how much film solution is being added on the liner. It is, therefore, recommended that next investigation could try to make films by using an inkjet printer. Polymer-particle suspension solution could act as an ink, and any size of patch could be printed.

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References

1. **Schulenburg, Mathias.** *Nanoteknologia, Innovaatioita huomisen hyväksi.* Köln : European Commissio, Research and Innovation, 2007. ISBN 92-79-00877-3.

2. **Ketul, Popat.** *Nanotechnology in Tissue Engineering and Refenerative Medicine.* Boca Raton : Taylor and Francis Group, 2011. ISBN 92-79-00877-3.

3. **Tibbals, Harry F.** *Medical Nanotachnology and Nanomedicine.* Boca Raton : Taylor and Francis Group, 2011. ISBN 978-1-4398-0874-0.

4. **Kristiina Järvinen, Vesa-Pekka Lehto, Jorma Joutsensaari, Jarno Salonen and Karl-Heinz Herzig.** *Nanoteknologia lääkehoidon työkaluna.* s.l. : Duodecim, 2008.

5. **Libo Wu, Jian Zhang and Wiwik Watanabe.** *Physical and chemical stability of drug nanoparticles.* Mountain View : Advanced Drug Delivery Reviews, 2010. doi:10.1016/j.addr.2011.02.001.

6. **Bhatia, Saurabh.** *Nanoparticles Types, Classification, Characterization, Fabrication Methods and Drug Delivery Applications.* s.l. : Springer International Publishing, 2016. DOI: 10.1007/978-3-319-41129-3.

7. **Amir H. Faraji, Peter Wipf.** *Nanoparticles in cellular drug delivery.* Pittsburgh : Bioorganic & Medicinal Chemistry, 2009. doi: 10.1016/j.bmc.2009.02.043.

8. **Nagavarma B V N, Hemant K.S.Yadav, Ayaz A, Vasudha L.S and Shivakumar H.G.** *Different Techniques for Preparation of Polymeric Nanoparticles - A Review.* Mysore : Asian Journal of Pharmaceutical and Clinical Research, 2012. ISSN: 0974-2441.

9. **Rinaudo, Marguerite.** *Chitin and chitosan: Properties and applications.* Grenoble : Progress in Polymer Science, 2006. doi:10.1016/j.progpolymsci.2006.06.001.

10. **Lifeng Qi, Zirong Xu, Xia Jiang, Caihong Hu and Xiangfei Zou.** *Preparation and antibacterial activity of chitosan nanoparticles.* Hangzhou : Elsevier Ltd, 2004. doi:10.1016/j.carres.2004.09.007.

11. **Hao Liu, Chaoyang Wang, Shengwen Zou, Zengjiang Wei and Zhen Tong.** *Simple, Reversible Emulsion System Switched by pH on the Basis of Chitosan without Any Hydrophobic Modification.* Guangzhou : American Chemical Society, 2012. dx.doi.org/10.1021/la3021113.

12. **Julie Nilsen-Nygaard, Sabina P. Strand, Kjell M. Vårum, Kurt I. Draget and Catherine T. Nordgård.** *Chitosan: Gels and Interfacial Properties .* Trondheim : Polymers, 2015. doi:10.3390/polym7030552.

13. **Wang JJ, Zeng ZW, Xiao RZ, Xie T, Zhou GL, Zhan XR, and Wang SL.** *Recent advances of chitosan nanoparticles as drug carriers.* Hangzhou : International Journal of Nanomedicine, 2011. DOI: 10.2147/IJN.S17296.

14. **Bhushan, Bharat.** *Encyclopedia of Nanotechnology.* s.l. : Springer Netherlands, 2012. ISBN 978-90-481-9751-4.

15. **Jianguo Wang, Laizhu Zhang, Huimin Chi & Shilei Wang.** An alternative choice of lidocaine-loaded liposomes: lidocaine-loaded lipid–polymer hybrid nanoparticles for local anesthetic therapy. *Drug Delivery.* s.l. : Taylor & Francis Group, 2016, ss. 1254-1260.

16. **P. Calvo, C. Remuñán-López, J. L. Vila-Jato and M. J. Alonso.** *Novel hydrophilic chitosan-polyethylene oxide nanoparticles as protein carriers.* Santiago de Compostela : Journal of Applied Polymer Sciences, 1997. CCC 0021-8995/97/010125-08.

17. **Bhatia, S.** Nanoparticles Types, Classification, Characterization, Fabrication Methods and Drug Delivery Applications. *Natural Polymer Drug Delivery Systems.* s.l. : Springer International Publishing Switzerland, 2016.

18. **Manjit Jaiswal, Rupesh Dudhe and P. K. Sharma.** *Nanoemulsion: an advanced mode of drug delivery system.* Nagar : 3 Biotech, 2014. doi: 10.1007/s13205-014-0214-0.

19. **Hao Liu, Chaoyang Wang, Shengwen Zou, Zengjiang Wei, and Zhen Tong.** *Simple, Reversible Emulsion System Switched by pH on the Basis of Chitosan without Any Hydrophobic Modification.* Guangzhou : Langmuir, 2012. ss. 11017–11024.

20. **Serveh Ghaderi, Saeed Ghanbarzadeh and Hamed Hamishehkar.** *Evaluation of Different methods for Preparing Nanoparticle Containing Gammaoryzanol for Potential Use in Food Fortification.* Tabriz : Pharmaceutical Scineces, 2015.

21. **Benjamin Balzus, Fitsum Feleke Sahle, Stefan Hönzke, Christian Gerecke, Fabian Schumacher, Sarah Hedtrich, Burkhard Kleuser and Roland Bodmeier.** *Formulation and ex vivo evaluation of polymeris nanoparticles for controller delivery od corticosteroids to the skin and the corneal epithelium.* Berlin : European Journal of Pharmaceutics and Biopharmaceutics, 2017. doi.org/10.1016/j.ejpb.2017.02.001.

22. **Nagarsenker, Pankaj Pathak and Mangal.** *Formulation and Evaluation of Lidocaine Lipid Nanosystems for Dermal Delivery.* Kalina : American Association of Pharmaceutical Scientists, 2009. DOI: 10.1208/s12249-009-9287-1.

23. **Sandeep Karki, Hyeongmin Kim, Seon-Jeong Na, Dohyun Shin, Kanghee Jo and Jaehwi Lee.** *Thin films as an emerging platform for drug delivery .* s.l. : Asian Journal of Pharmaceutical Sciences, 2016. doi: 10.1016/j.ajps.2016.05.004..

24. **Kit Frederiksen, Richard H. Guy and Karsten Petersson.** *Formulation considerations in the design of topical, polymeric film-forming systems for sustained drug delivery to the skin .* s.l. : European Journal of Pharmaceutics and Biopharmaceutics, 2015. doi: 10.1016/j.ejpb.2015.01.002.

25. **Preis M, Woertz C, Schneider K, Kukawka J, Broscheit J, Roewer N and Breitkreutz J.** *Design and evaluation of bilayered buccal film preparations for local administration of lidocaine hydrochloride.* Düsseldorf : European Journal of Pharmaceutics and Biopharmaceutics, 2013. doi: 10.1016/j.ejpb.2013.12.019.

26. **Transmission Electron Microscopy Analysis of Nanoparticles. [Online] September 2012. [Viitattu: 12. January 2017.] http://50.87.149.212/sites/default/files/nanoComposix%20Guidelines%20for%20TEM%20Analysis.pdf.**

27. Thermo Fisher Scientific. Basic UV-Vis Theory, Concepts and Applications. [Online] Thermo Fisher Scientific. [Viitattu: 20. February 2017.] http://www.uni-salzburg.at/fileadmin/oracle_file_imports/359201.PDF.

28. Overview of spectroscopy at <www.chemwiki.ucdavis.edu> accessed 20 January 2017.