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# Fabrication of topical film forming polymeric solution for pain management

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# FABRICATION OF TOPICAL FILM FORMING POLYMERIC SOLUTION FOR PAIN MANAGEMENT

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by

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## ABSTRACT

Polymeric film-forming solutions (FFSs) are novel and emerging drug delivery systems for topical application to the skin. In their simplest composition, they constitute an active drug substance, film-forming polymer, and a volatile skin-tolerant solvent. When applied to the skin, FFSs form a thin and transparent polymeric film shortly after solvent evaporation. Owing to their unique composition and formation mechanism, these systems offer many superior advantages to the more conventional topical dosage forms. Thereby, this work aimed to develop and characterize film forming solutions for the skin delivery of two of the most commonly used topical non-steroidal anti-inflammatory drugs (NSAIDS); ketoprofen and diclofenac sodium.

FFS were developed by varying the type and content of the film forming polymer. The resulting formulations were evaluated according to favorable film characteristics, *in vitro* and *ex vivo* drug release profiles. Eudragit E100 was identified as a suitable release matrix for ketoprofen. In the case of diclofenac Na; however, the ex vivo permeation study results failed to show a characteristic release profile for either the test formulation or the marketed formulation VOLTAREN® Gel.

Nevertheless, the presented work provided a rationalized way for the development and evaluation of FFS and investigated their potential as delivery systems for ketoprofen and diclofenac sodium.

# DEDICATION

I dedicate my thesis to my beloved family; may God bless their souls.

ACKNOWLEDGMENTS

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# CHAPTER I

## INTRODUCTION

In situ film forming polymeric solution is a novel and emerging approach for dermal and transdermal drug delivery. These solutions in their simplest composition comprise a drug substance, a film-forming polymer, and a skin-tolerant volatile solvent[1]. They are applied on the skin as liquids forming a very thin polymeric film after the rapid evaporation of the solvent. The formed films provide superior advantages over the more conventional topical dosage forms; they are flexible, fast-drying, less greasy and do not carry the risk of being wiped off the skin compared to semisolid formulations. Above all, the most crucial attribute of in situ film forming solutions is the complete skin contact over the entire application period without causing any skin fixation or irritation as in the case of topical patches. This potential advantage is especially essential for the management of chronic skin diseases where the repetitive application is a major cause of poor patient compliance and satisfaction as well as poor therapeutic outcomes.

Based on this rational, two of the most prescribed topical non-steroidal anti-inflammatory drugs (NSAIDS); ketoprofen and diclofenac Na were formulated as polymeric film-forming solutions for skin delivery. Table 1 represents some of the physical and chemical properties of diclofenac Na and ketoprofen[2,3].

**Table 1: Some of ketoprofen and diclofenac Na physical and chemical properties**

| **Property** | **Ketoprofen** | **Diclofenac Na** |
|---|---|---|
| Molecular weight | 254.28 g/mol | 296.14 g/mol |
| Melting point | 94° C | 283° C |
| Octanol/water partioning coefficient | 3.12 | 4.51 |
| Dissociation coefficient | 4.45 | 4.15 |
| Aqueous solubility | 51 mg/L | 2.37 mg/L |

Accordingly, the presented work aimed to develop FFS formulations of ketoprofen and diclofenac Na using different types and concentrations of film-forming polymers and to characterize and optimize the resulting formulations according to favorable film characteristics, *in vitro* and *ex vivo* drug release profiles.

# CHAPTER II

## OBJECTIVE OF THE STUDY

The objective of the present work was to develop, characterize and optimize FFSs for the topical delivery of ketoprofen and diclofenac Na.

**Research Strategy**: The study was divided into two parts; the former was focused on the formulation of different polymeric FFS formulations by varying the type and content of the polymer. A selection of 13 polymers from different chemical groups, all described by their manufacturer or in the literature as being film formers, were tested and evaluated for their film characteristic and drug loading capacities. In the second part, we determined the *in vitro,* and *ex vivo* release profiles of the formulations that passed the first stage of testing. All formulations were made in two batches and were subjected to stability conditions for three months. Formulations were kept at two conditions, 25$^{o}$C/60% RH and 40$^{o}$C/75% RH respectively.

# CHAPTER III

# MATERIALS AND METHODS

## Materials

Ketoprofen was purchased from Tokyo Chemical Industry Co., Ltd. Diclofenac sodium salt, Chitosan and Poly( acrylic acid) were purchased from Sigma -Aldrich. Eudragit E100® (dimethylaminoethyl methacrylate, butyl methacrylate, and methyl methacrylate), Eudragit EPO® (dimethylaminoethyl methacrylate, butyl methacrylate, and methyl methacrylate), Eudragit RLPO® (Methacrylic acid methylacrylate copolymer), and Eudragit RS100® were purchased from Evonik Industries. Kollidon® 30 (Polyvinylpyrrolidone), Kollidon 90F®, Kollidon SR, Soluplus® ( polyethylene glycol, polyvinyl acetate, and polyvinylcaprolactame-based graft copolymer) and Luterol® (Plyoxyl propylene-polyoxyethylene block copolymer) were purchased from BASF. Carbopol was purchased from Lubrizol Corporation. Menthol was purchased from Ward's Natural Science. High-Performance Liquid Chromatography (HPLC)-grade solvents like methanol, ethanol were purchased from Fisher Chemicals, USA. Porcine skin was obtained from Pontotoc Slaughterhouse, Pontotoc, MS, USA. Dermatomed human cadaver skin was purchased from New York Firefighters Skin Bank.

## Methods

### Polymer screening

A selection of 13 polymers from different chemical classes were evaluated for their solubility in 95% ethanol and the resulting film characteristics. The screened polymers herein were all described by their manufacturer or in the literature as being film formers. Table 2 represents all the polymers that were used in this experiment.

**Table 2: Polymers used in the screening experiment**

| Trade name | Polymer |
|---|---|
| Eudragit E 100 | Poly(butyl methacrylate, (2-dimethylaminoethyl)methacrylate, methyl methacrylate) 1:2:1 |
| Eudragit RLPO | Ammonio methacrylate copolymer type A |
| Eudragit EPO | Butyl methylacrylate-(2-Dimethylaminoethyl) methacrylate-Methyl methacrylate-copolymer |
| Kollidon 30 | Polyvinylpyrrolidone |
| Kollidon 90F | Polyvinylpyrrolidone higher molecular weight |
| Kollidon SR | A blend of polyvinyl acetate and povidone (K 30) in the ratio 8:2 |
| PVA 7200 | Polyvinyl alcohol |

| | |
|---|---|
| Poly(acrylic acid) | Polyacrylic acid |
| Luterol® | Plyoxyl propylene-polyoxyethylene block copolymer |
| Eudragit RS 100 | Ammonio Methacrylate copolymer type B |
| Carbopol | Acrylic acid and C10-C30 alkyl acrylate crosslinked with allyl pentaerythritol |
| Soluplus | Polyethylene glycol, polyvinyl acetate and polyvinylcaprolactame-based graft copolymer |
| Chitosan | Polysaccharide composed of randomly distributed β--linked D-glucosamine and N-acetyl-D-glucosamine |

**Preparation of polymeric film forming solutions**

The polymer was dissolved in 95% ethanol kept on stirring overnight until a clear solution was obtained. The volume was made up to compensate the solvent lost due to evaporation. Subsequently, the formulations were kept in glass vials sealed tightly with Parafilm®. Three different concentrations were prepared for each of the screened polymers; 1%, 2.5% and 5% w/w.

**In vitro evaluation of the polymeric film forming solutions**

The prepared polymeric solutions were initially evaluated according to the following characteristics: solution appearance, solution viscosity, film drying time, outward stickiness and cosmetic attractiveness of the produced films.

The appearance of the solutions was evaluated visually and described as clear or opaque with or without precipitation of the polymer4. Likewise, the viscosity of the polymeric formulations was visually assessed and rated as low (water-like), medium (glycerol-like) or high (syrup-like)[5].

For the evaluation of film drying time, the films were formed in small weighing boats. After five minutes a glass slide was placed gently on the surface of the film. If no liquid droplets are visible on the glass after removing it, the film was considered to be dry. If liquid droplets were still visible on the slide, the test was repeated at seven minutes6.

Additionally, the outward stickiness of the films was estimated by pressing cotton wool on the dry film with minimum pressure. Stickiness was rated high if heavy amount of fibers were retained on the surface of the film, medium if a thin layer of fibers was formed on the film and low if no adherence of fibers was noted5.

Moreover, the cosmetic appearance of the films was assessed. Complete, uniform and transparent films were rated high in cosmetic attractiveness. While incomplete, non-uniform and/or visible films were considered to be less attractive.

Formulations were considered successful when solutions were clear and of low viscosity. And when the formed films had a drying time of $\leq 7$ minutes, rated low on outward stickiness and high in cosmetic attractiveness.

## Preparation of drug-loaded formulations

Fourteen FFS formulations passed the in vitro evaluation experiments and were loaded with ketoprofen and diclofenac sodium. Table 3 shows the content of such formulations.

**Table 3: Composition of drug loaded FFSs.**

| | **Drug levels (w/w%)** | | | | |
|---|---|---|---|---|---|
| **Polymer (w/w%)** | **1 %** | **1.5%** | **3%** | **5%** | **10%** |
| | **Amount of the drug added (mg)** | | | | |
| **Kollidon 30 (2.5%)** | 41 | 62 | 125.5 | 213.5 | 451 |
| **Kollidon 30 (5%)** | 42.5 | 64 | 129 | 220 | 463 |
| **Kollidon 90F (2.5%)** | 41 | 62 | 125.5 | 213.5 | 451 |
| **Kollidon 90F (5%)** | 42.5 | 64 | 129 | 220 | 463 |
| **Kollidon SR (2.5%)** | 41 | 62 | 125.5 | 213.5 | 451 |
| **Kollidon SR (5%)** | 42.5 | 64 | 129 | 220 | 463 |
| **Eudragit E100 (2.5%)** | 41 | 62 | 125.5 | 213.5 | 451 |

| | | | | | |
|---|---|---|---|---|---|
| **Eudragit E100 (5%)** | 42.5 | 64 | 129 | 220 | 463 |
| **Eudragit RLPO (2.5%)** | 41 | 62 | 125.5 | 213.5 | 451 |
| **Eudragit RLPO (5%)** | 42.5 | 64 | 129 | 220 | 463 |
| **Eudragit EPO (2.5%)** | 41 | 62 | 125.5 | 213.5 | 451 |
| **Eudragit EPO (5%)** | 42.5 | 64 | 129 | 220 | 463 |
| **Soluplus (2.5%)** | 41 | 62 | 125.5 | 213.5 | 451 |
| **Soluplus (5%)** | 42.5 | 64 | 129 | 220 | 463 |

For the preparation of drug loaded FFS formulations, the polymers were first added to 95% ethanol kept on stirrer overnight until completely dissolved. To the obtained clear solutions, different amounts of ketoprofen and diclofenac sodium were added as shown in table 4. The formulations were kept in glass vials sealed tightly with parafilm.

## Compound light microscopy

Crystallization of the drugs from the polymeric film forming solutions was evaluated with compound light microscopy equipped with 10x, 20x, and 40x objectives. 100 µl of each of the formulations was casted on a glass slide and allowed to dry at room temperature. The presence or absence of drug's crystals and their distribution was investigated at four different time points; 15 minutes, 1 hour, 2 hours and one day.

## Film evaluation on pig skin

The formulations with the higher drug concentration that didn't show any signs of drug's crystallization at any time point were considered successful were further assessed using pig skin. Fresh porcine skin was brought from a local slaughterhouse. The abdominal skin regions were taken and shaved using an electric shaver. The hairless skin was cut into small pieces. The skin was mounted on a solid surface and used for film forming solution evaluation.

The drying time, outward stickiness and cosmetic attractiveness were evaluated as mentioned above. Film flexibility was assessed by stretching the skin in 2-3 directions. The film was rated flexible if no signs of cracking or skin fixation were observed or non-flexible if cracking or skin fixation occurred1.

Formulations that showed short drying time, low outward stickiness, high cosmetical attractiveness, and excellent flexibility were considered successful and were evaluated in the in vitro drug release study.

## In vitro drug release study

Determination of the transport of the drug across cellulose dialysis membrane was performed using vertical Franz diffusion cells. The cells had receiver volume and diffusional

surface area of 5 ml and 0.64 $cm^2$ respectively. Dialysis membranes were cut into small pieces to fit and were mounted on each diffusion cell. The donor and receiver chambers were clamped and sealed with Parafilm®. The receiver fluid, Phosphate buffer saline (PBS) pH 7.4 was added and any air bubbles trapped next to the membrane were removed initially and after each sampling point. Small magnetic stirbars were added to the receiver chamber, and the temperature was maintained at 32 °C by a circulating water jacket. To evaluate drug transport, 10 µl/$cm^2$ of each formulation was added to the donor chamber.

Table 4 shows the composition of the formulations that were tested at this stage. Samples of 200 µl of receiver fluid were removed at 0, 1, 2, 3, 4, 5, 6, 12 and 24 h. Following removal of each sample, the same volume of fresh PBS was added to the receiver compartment to maintain sink conditions. Samples were analyzed by HPLC.

**Table 4: Composition of FFSs used in the *In vitro* release study**

| **Formulation Code** | **A** | **B** | **C** | **D** |
|---|---|---|---|---|
| **Polymer% (w/w)** | Kollidon 30 5% | Kollidon 90F 5% | Eudragit E100 5% | Kollidon 90F 2.5% |
| **Drug % w/w** | Diclofenac Na 3% | Diclofenac Na 3% | Ketoprofen 3% | Ketoprofen 3% |
| **95% ethanol %w/w** | 92% | 92% | 92% | 94.5% |

### *Ex vivo* permeation study

Dermatomed human cadaver skin was obtained from New York Firefighters skin Bank (525 E. 68th St. New York, NY 100065 USA). The skin was kept at -20 °C and slowly thawed before use. The skin was rinsed with water and cut into pieces sufficient enough to cover the 0.64 cm2 diffusion area of the Franz cells. The skin was then fixed between the absorption and the diffusion compartments of the cells, with the epidermis facing the receiver compartment. 10-15 µl/cm2 of the formulations were applied on the skin. After applying the formulations, 5 ml PBS was immediately added to diffusion cells. Incubation temperature was maintained at 32 C, and magnetic stirring rate was 600 rpm. Samples were withdrawn at 1, 2, 3, 4, 5, 6, 12 and 24 h, filtered and replaced with 5 ml pre-heated fresh PBS at each time point. Drug concentration was determined by HPLC.

### Quantification of the drug within skin layers

Skin samples were removed carefully from the diffusion apparatus, and the exact diffusion area was identified and punched out using a biopsy punch. Each skin disc then was rinsed using 5ml of a rinsing solvent (1ml at a time), and the resulting solutions were collected in glass vials. The dried pre-weighted skin discs were immersed in NaOH (1M); the mixture was placed on a stirrer for 24h. Subsequently, the extraction mixture was then centrifugated at 1300 rpm for 15min; the supernatant fluid was then collected and analyzed by HPLC.

### Stability Studies

Film forming solutions that passed the pig skin testing was kept for stability studies. The samples were kept at conditions of 25°C/60% RH and 40°C/75% RH for three months in stability chambers. The samples were withdrawn and analyzed for initial and three months. The

formulations were evaluated visually for any physical changes in solution appearance and color. And drug content in the FFS was analyzed using UV-vis spectrophotometry. Absorbances of diclofenac sodium samples were read spectrophotometrically at 275 nm, and for ketoprofen samples at 254 nm taking ethanol as blank for both. All measurements were carried out at ambient temperature, in a quartz cuvette of 1.00 cm optical length.

**HPLC Analysis**

The samples from in vitro release study and the ex vivo permeation study were analyzed for their drug content by HPLC. An isocratic HPLC method was developed for the quantification of ketoprofen and diclofenac sodium. The experiment was performed using a Waters HPLC system (Water 600 Controller, USA) equipped with a 600-pump unit, a 717 plus autosampler with an injection valve with a sample loop of 50 µl, and a 2487 dual absorbance UV detector.

Diclofenac sodium method: reversed phase Luna® 100° A C18 column (100x4.6 mm, 3µm, Phenomenex Inc, CA the USA) was utilized at ambient temperature. The mobile phase was acetonitrile/water (3:1) adjusted to pH 3 with glacial acetic acid. The flow rate was set at 0.65 ml/min. Diclofenac sodium was detected at a wavelength of 275 nm with a retention time of 3.5. 20 µl of the injection was eluted in the column. The calibration curve was prepared using different concentrations of diclofenac sodium in the range of 1µg to 10ng using methanol as a solvent. LOD, LOQ were determined.

Ketoprofen method: A method stated in USP-NF was used. Phenomenex Luna® C18 reverse phase column (100 Å, 250 × 4.6 mm, 5 µm) was used as the solid phase. The mobile phase was water, acetonitrile, and glacial acetic acid in the following ratio (90:110:1). The flow rate was set at 1.2 mL/min. Ketoprofen was detected at 256 nm (Waters 2489 UV/detector) with a retention time of approximately 4.7. Twenty microliters was injected from each sample. A ten point

calibration curve was plotted and found to be linear in the concentration range of 2 µg/mL to 100 µg/mL with a correlation coefficient (R2) of 0.999. The limit of detection and limit of quantification values for the method were found to be 0.2 and 0.7 µg/mL, respectively.

# CHAPTER IV

# RESULTS AND DISCUSSION

## Polymer screening and *in vitro* evaluation of polymeric FSSs

A selection of 13 polymers from different chemical classes, all described by their suppliers or in literature as film formers were evaluated. Each polymer was tested at three different concentrations, 1%, 2.5% and 5% w/w.

The evaluation criteria employed was based on critical features for practical, accurate and patient-friendly application of this novel dosage form. The viscosity of the film forming solution is required to be low to enable an application of the dosage form as a spray, which would ensure accurate and flexible dosing. As a result, only solutions with low viscosity were considered successful and were chosen for the next experiment.

Drying time is a very important characteristic of the formed films. Conveniently, the films should have a drying time $\leq 7$ minutes so as to avoid long waiting times for the patient.

Likewise, the prepared films are required to be non-sticky to avoid adhesion to clothes or any other surfaces. The cosmetic attribute of films is another essential feature important for patients. Patients prefer films that are transparent and flexible

Table 5 represents the successful formulations that were clear, with low viscosity and produced films that were fast drying, with low stickiness and high cosmetic attractiveness.

**Table 5: Composition of the positively evaluated formulations**

| Polymer | w/w % | Appearance | Viscosity | Drying time (min) | Stickiness | Film formation | Cosmetic attractiveness |
|---|---|---|---|---|---|---|---|
| **Kollidon® 30** | 2.5% | Clear | Low | ≤5 | Low | Complete | Transparent |
| | 5% | | Low | 7 | | | |
| **Kollidon® 90F** | 2.5% | Clear | Low | ≤5 | Low | Complete | Transparent |
| | 5% | | Low | 5 | | | |
| **Kollidon® SR** | 2.5% | Clear | Low | 7 | Low | Complete | Transparent |
| | 5% | | Low | 7 | | | |
| **Eudragit RLPO** | 2.5% | Clear | Low | ≤5 | Low | Complete | Transparent |
| | 5% | | Low | 7 | | | |
| **Eudragit E100** | 2.5% | Clear | Low | 5 | Low | Complete | Transparent |
| | 5% | | Low | 5 | | | |
| **Eudragit EPO** | 2.5% | Clear | Low | 5 | Low | Complete | Transparent |
| | 5% | | Low | 7 | | | |
| **Soluplus** | 2.5% | Clear | Low | 5 | Low | Complete | Transparent |

As can be deducted from the results, both the nature and the content of the polymer have a vital impact on the properties of the formed films. The choice of the polymer is important

because the polymer has first to be soluble in a volatile skin-tolerant solvent. Polymers that aren't sufficiently soluble in volatile solvents will have the problem of prolonged drying time or lacking the ability to give clear solutions and subsequently homogenous clear films.

Equally important parameter is the polymer content. While increasing the polymer amount increases the drug loading capacity of the formulation, this has an inverse impact on the viscosity of the formulation as well as the thickness and cosmetic attributes of the produced film. More viscous solutions are difficult to dispense and produce films that are thicker, less invisible and less flexible. For these reasons the type and the amount of the film forming polymer have to be determined carefully when formulating polymeric film-forming solutions.

**Compound light microscopy**

Five different concentrations of each drug in each of the selected polymeric solutions were prepared (1%, 1.5%, 3%, 5% and 10% w/w). The results varied with polymer type and concentration. Solutions with higher polymer concentrations were able to prevent and stabilize the drugs against crystallization. However, the anti-nucleating capacity of the drug was limited by the drug solubility in the polymeric matrix. Accordingly, the solutions that passed the microscopic evaluation were the ones with the higher polymer concentration and medium drug loading capacity.

Table 6 shows the composition of the formulations that didn't show any signs of drug precipitation and or crystallization at all time points.

**Table 6: Composition of drug loaded FFSs that passed the microscopic evaluation.**

| Formulation Code | Composition |
|---|---|
| Formulation A | 5% kollidon 30+ 3% Diclofenac Na |
| Formulation B | 5% kollidon 90F+ 3% Diclofenac Na |
| Formulation C | 5% Soluplus + 3% Diclofenac Na |
| Formulation D | 2.5% kollidon 90F+ 3% ketoprofen |
| Formulation E | 5% kollidon 90F+ 3% ketoprofen |
| Formulation F | 5% Eudragit E100 + 3% Ketoprofen |
| Formulation G | 2.5% Eudragit E100+ 3% ketoprofen |
| Formulation H | 2.5% Eudragit EPO+ 3% ketoprofen |

## Formulation evaluation on pig skin

After loading the formulations with drugs, it's crucial at this stage to make sure that the drugs' incorporation hasn't led to any changes in the desirable film characteristics. Evaluation using pig skin provides a better assessment of the films as they are formed on a surface that most closely resembles the actual wearing conditions. Consequently, full thickness pig skin was used to evaluate the films drying time, stickiness, cosmetic attractiveness and flexibility.

Film flexibility is a key feature of in situ films produced by FFS. As the solutions are expected to be used on considerably large surface areas of the skin, it's very important for the films to be of sufficiently flexible and elastic. This is required to prevent any fissures or cracks disrupting the film upon movements of the patient.

Table 7 represents the composition of the formulations that gave positive results in all the testing criteria.

**Table 7: Composition of FFS that passed the pig skin evaluation experiment.**

| Formulation Code | Drug (w/w%) | Polymer (w/w%) |
|---|---|---|
| A | Diclofenac Na (3%) | Kollidon 30 (5%) |
| B | Diclofenac Na (3%) | Kollidon 90F (5%) |
| C | Ketoprofen (3%) | Kollidon 90F (2.5%) |
| D | Ketoprofen (3%) | Eudragit E100 (5%) |

### *In vitro* drug release study

As the principal goal of this work was to formulate FFS capable of producing films that prolong topical delivery of NSAIDS. An in vitro experiment method was designed using an artificial membrane. The artificial membrane chosen was Cellulose dialysis membrane, which has been extensively used to investigate drug release from topical formulations. The membrane-formulation interactions were assessed in preliminary work, to make sure that the membrane acts only as an inert holding not a barrier for drug diffusion once it's released from the polymeric matrix. Data, not showing here, from the 24 h soaking of the membrane in formulations with different drug concentrations showed that the membrane didn't cause any changes in the amount of the drug indicating the inertness of the membrane.

Similarly, solubility testing of the ketoprofen and diclofenac Na in PBS revealed that the solubilities of both drugs are at least 20x the expected amounted of the drugs to permeate. The solubility of ketoprofen was found to be at least 5 mg/ml and that for diclofenac Na 9 mg/ml.

The formulations that adequately satisfied the criteria about drying time, outward stickiness, cosmetic acceptability and film flexibility in pig skin testing were evaluated for their diffusion ability and release characteristics.

Diclofenac release form kollidon 90F and kollidon 30 was investigated. The cumulated drug amount released (µg) per unit surface area was plotted as a function of time. Although both films sustained diclofenac release for up to 24 h, the release of diclofenac was higher from kollidon 90F corresponding to 105 µg/cm² (almost 50% of drug loading) reaching a maximum release rate at three h with a steady state release rate reached after 6 h. The differences in the release profiles between the two polymers are due to differences in the polymer-drug interactions. Such interactions determine the diffusivity of the drug in the polymeric matrix, the ability of the polymer to prevent crystallization of the drug and the extent to which the polymer is able to support the supersaturated state after solvent evaporation.

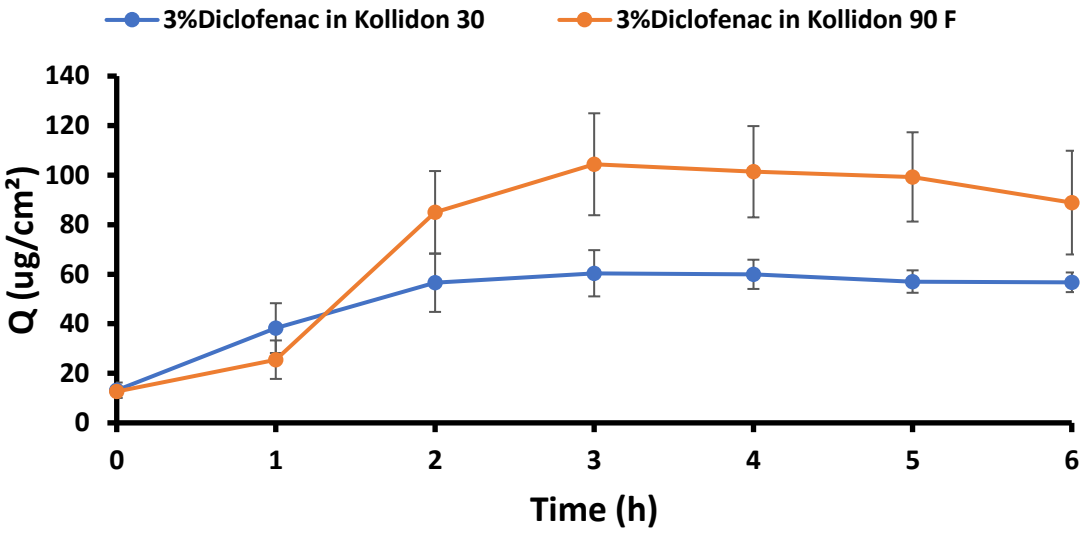


**Figure 1: In vitro release profile of Diclofenac Na from polymeric film-forming solutions. (mean of n=3± S.D)**

The drug transport data across cellulose dialysis membrane of 3% ketoprofen in kollidon 90F and 3% ketoprofen in Eudragit are shown in figure 2. As evident from the figure, the release of ketoprofen form Eudragit E100 was higher than kollidon 90F. The total amount of ketoprofen released was 70 µg/cm² (almost 35% of the loading dose) reaching a maximum release at 2 h after which a relatively constant drug release was maintained.

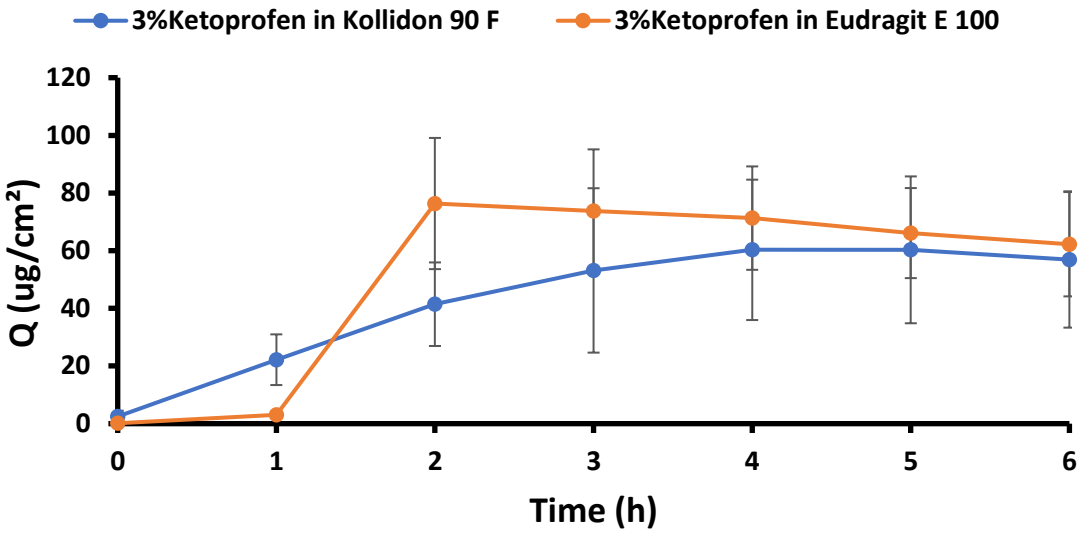


**Figure 2: In vitro release profile of Ketoprofen from polymeric film-forming solutions. (mean ± standard deviation; n=3)**

Based on the presented results 3% Diclofenac in kollidon 90F and 3% Ketoprofen in Eudragit E100 were chosen for further optimization and permeation testing using dermatomed human cadaver skin.

### *Ex vivo* permeation study

To further optimize the performance of the two selected formulations, menthol was added at a concentration of 0.08% w/w. Incorporation of penetration enhancer is one of the most common methodologies used to improve drug permeation and partitioning into the skin. Key features that play an important role when selecting a penetration enhancer are; safety and performance. Penetration enhancers shouldn't cause any irritation or allergizing effect to the skin; also, they should have a quick, predictable and reversible effect on the *stratum corneum* (SC).

Menthol a monocyclic terpenoid alcohol has as a long history of use in topical products, either for its cooling and refreshing sensation or to enhance the diffusivity and partitioning of both hydrophilic and lipophilic drugs [7-10]. Menthol acts on the intercellular lipids impeded within the stratum corneum corneocyte cells. It exerts its effect via disrupting the highly ordered structure of the lipid bilayer, by increasing the fluidity of the SC lipids[11]. Additionally, menthol is included in the list of generally recognized as safe agent list established by the US Food and Drug Administration. Therefore, menthol was included in the formulations that gave better results in the in vitro release testing.

**Table 8: Final composition of the optimized formulation (w/w%)**

| **Formulation** | **Diclofenac in Kollidon 90F** | **Ketoprofen in Eudragit E100** |
|---|---|---|
| **Drug %** | 3 | 3 |
| **Menthol %** | 0.08 | 0.085 |
| **Polymer %** | 5 | 5 |
| **Ethanol %** | 91.92 | 91.92 |

The permeation profile of ketoprofen from Eudragit E100 and 2% ethanolic solution were investigated. Statistically, the difference in the steady-state flux of ketoprofen from the test formulation and the control is nonsignificant. ($p \geq 0.05$). The fact that the test formulation gave comparable results to the saturated ethanolic solution indicates that the polymer has a major enhancing effect on the drug flux.

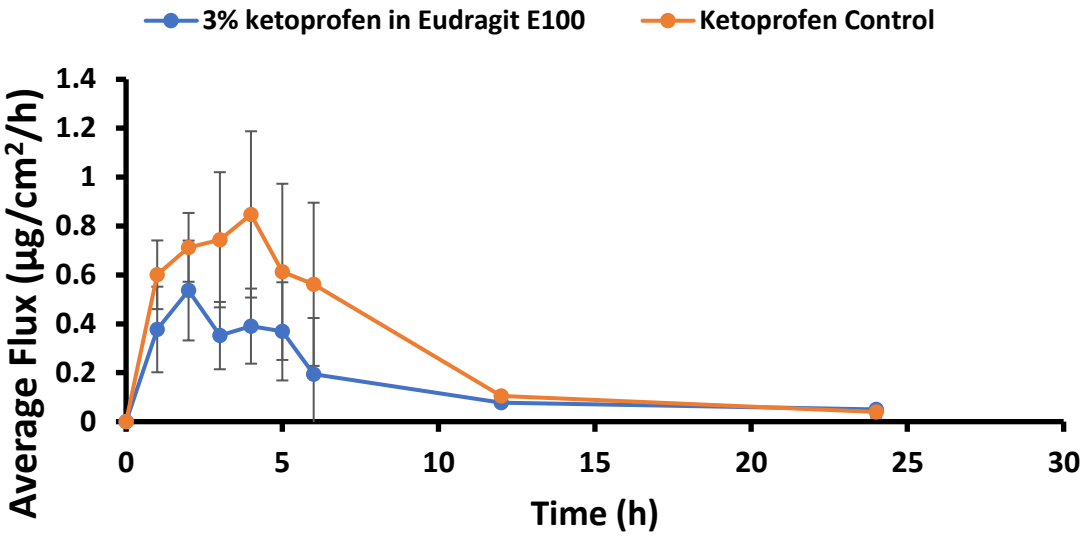


**Figure 3: Ex vivo permeation profile of Ketoprofen from FFS and polymer-free saturated ethanolic solution (mean of n=6± S.D)**

As for diclofenac sodium permeation study, the results of the permeation study for both test formulation and marketed gel Voltaren® didn't result in a good flux profile similar to what we saw with ketoprofen. Accordingly, we are only showing the cumulative amount of diclofenac sodium retained in the skin.

**Drugs quantification within skin layers**

Figure 4 and 5 show the recovered amount of ketoprofen and diclofenac sodium respectively, represented in µg/mg weight of dermatomed skin. In the case of ketoprofen, the 2% w/w ethanolic solution of ketoprofen showed a higher amount of retained drug than the polymeric FFS, an observation that requires more investigation.

Even though permeation studies of diclofenac sodium didn't result in a good flux profile, we were able to quantify the amount of drug retained within the skin at the end of the permeation

study. Voltaren® gel resulted in a higher amount of the active retained within the skin compared to diclofenac sodium FFS.

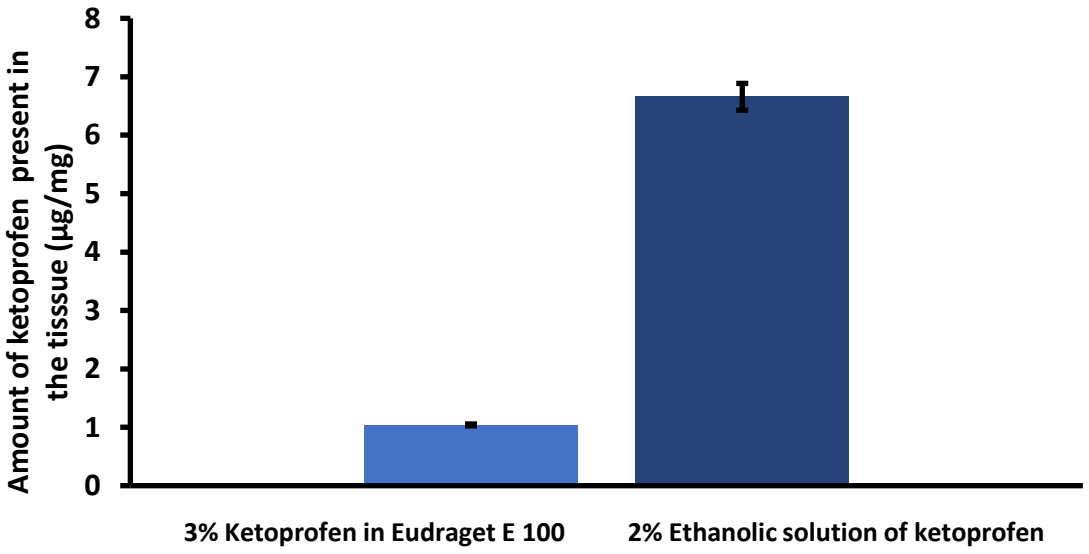


**Figure 4: Recovered amount of ketoprofen in µg/mg of dermatomed skin from ketoprofen FFS and a control solution. (mean of n=6 ± SD)**

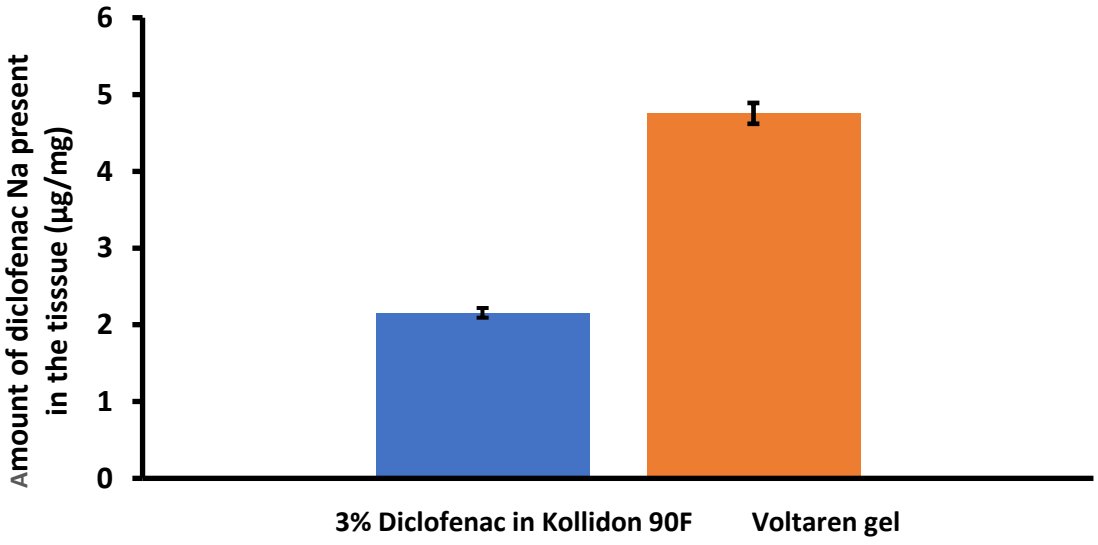


**Figure 5: The Recovered amount of diclofenac sodium in µg/mg of dermatomed skin from diclofenac sodium FFS and Voltaren® marketed gel. (mean of n=6 ± SD)**

**Stability study**

Results from these studies are an essential part of drug development process and mandatory requirement by regulatory authorities. Table 10 shows the drug assay results for the four formulations that were kept for stability testing at 25°C /40% RH and 40°C /75% RH for 3 months.

According to the obtained results, all formulations passed stability testing at 3 months, they all gave results within the acceptable limits.

**Table 9: 3-month stability testing drug Assay% results (n=3, mean ± SD)**

| **Formulation** | **Fresh solutions** | **25°C /40% RH** | **40°C /75% RH** |
|---|---|---|---|
| **3% ketoprofen in Eudragit E100** | 101.5 ± 0.65 | 101.16 ± 0.47 | 101.90 ± 0.18 |
| **3% ketoprofen in Kollidon 90F** | 102.35 ± 0.34 | 97.20 ± 0.51 | 97.37 ± 0.68 |
| **3% diclofenac Na in Kollidon 30** | 102.21 ± 0.62 | 97.48 ± 0.60 | 102.29 ± 0.42 |
| **3% diclofenac Na in Kollidon 90F** | 103.08 ± 0.05 | 99.59 ± 0.70 | 99.20 ± 0.44 |

## HPLC Analysis

**Ketoprofen:** table 11 shows the HPLC results of ketoprofen standards in methanol. The limit of quantification (LOQ) was found to be 0.0625 μg/mL. Lower concentrations than 0.0625 μg/mL of ketoprofen in methanol were detectable but not quantified precisely. Figure 6 shows ketoprofen calibration curve that was found to be linear in the range 0.0625 μg/mL to 100 μg/mL with a correlation coefficient ($R^2$) of 1.

**Table 10: HPLC calculated areas for different ketoprofen standards in methanol**

| **Concentration (μg/ml)** | **Area** |
|---|---|
| 100 | 3281097 |
| 50 | 1636094 |
| 10 | 344777 |
| 5 | 166785 |
| 2.5 | 83156 |
| 1 | 32974 |
| 0.5 | 15877 |
| 0.25 | 7826 |
| 0.125 | 4169 |
| 0.0625 | 1549 |

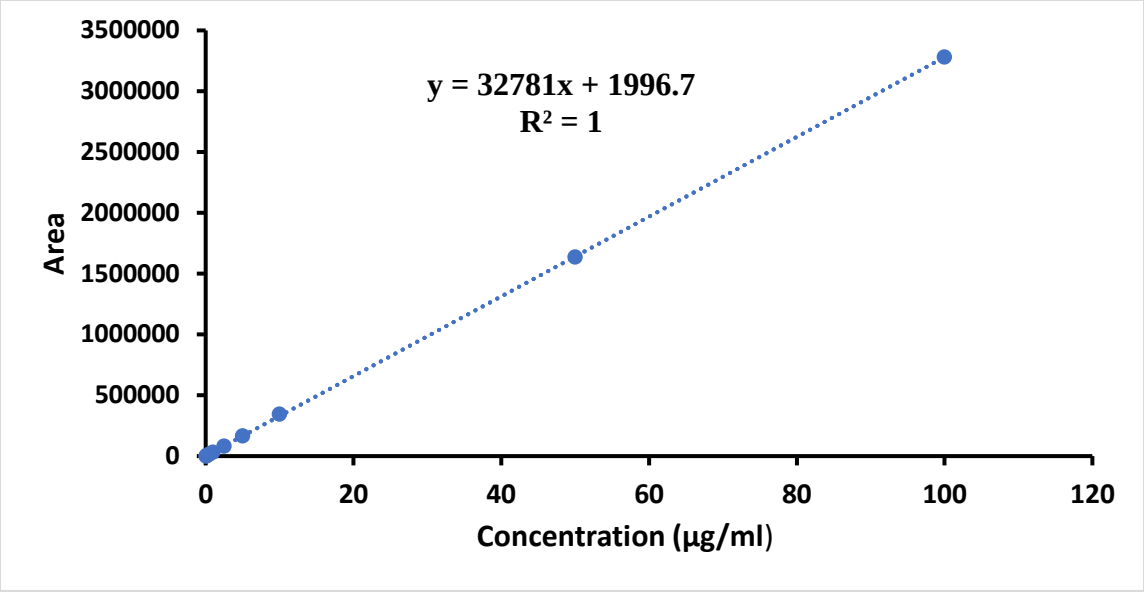


**Figure 6: Ketoprofen standard calibration curve.**

**Diclofenac sodium:** table 12 shows the HPLC results of diclofenac sodium standards in methanol. The limit of quantification (LOQ) was found to be 0.125 µg/mL. Lower concentrations than 0.125 µg/mL of ketoprofen in methanol were detectable but not quantified precisely. Figure 7 shows diclofenac sodium calibration curve that was found to be linear in the range 0.125 µg/mL to 50 µg/mL with a correlation coefficient ($R^2$) of 0.999.

**Table 11: HPLC calculated areas for different ketoprofen standards in methanol**

| Concentration (µg/ml) | Area |
|---|---|
| 50 | 810039 |
| 10 | 162273 |
| 5 | 79609 |
| 2.5 | 37629 |
| 1 | 14757 |
| 0.5 | 15863 |
| 0.25 | 3459 |
| 0.125 | 2977 |

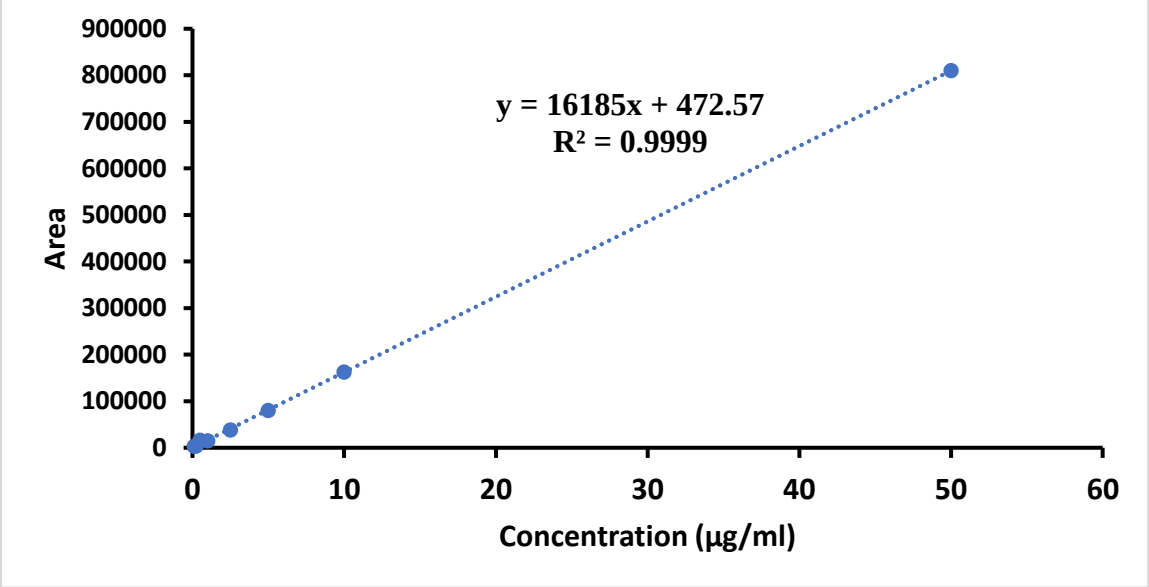


**Figure 7: Diclofenac sodium standard calibration curve**

# CHAPTER V

# CONCLUSION

Film forming solutions were formulated with polymers from different chemical groups such as acrylates (Eudragit_ RL PO, Eudragit_ E100, Eudragit_ EPO) Kollidon ( 30, 90F, SR), Soluplus, Carbopol, and PVA. These formulations contained one of the polymers, a volatile solvent, and the drug substance. The developed rating system, even though based on qualitative test methods, provided a good basis for the evaluation of the developed formulations comprising key features for patients that would ensure higher patient satisfaction and compliance. The positively evaluated preparations resulting from the formulation experiments provided the basis for the development of film-forming polymeric solutions for ketoprofen and diclofenac Na as a novel dosage form for topical delivery of non-steroidal anti-inflammatory drugs (NSAIDS).

The focus of this work was to develop and investigate the release potential of FFS of diclofenac Na and ketoprofen. In the case of ketoprofen, Eudragit E100 was identified as a potential matrix; producing high-quality films and showing promising release profile comparable to a saturated ethanolic solution. For diclofenac Na, due to the poor permeability nature of the drug, we didn't manage to get a flux profile for neither the

marketed or our formulation. However, detectable amounts of the drug were found retained in the skin after applying the formulations for up to 24 hours.

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# VITA

Maha Alkurdi is a graduate student at the department of pharmaceutics and drug delivery, University of Mississippi. Her project involved the investigation and formulation of in-situ film-forming solution for the topical delivery of non-steroidal ant-inflammatory drugs. She has completed her BSC in Pharmacy from the University of Jordan, Amman-Jordan. She will receive her master's degree in May 2019 and plans to enroll in the industry soon after her graduation.