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Faculty of Graduate Studies

Formulation and Stability Evaluation of Ciprofloxacin Topical Preparation

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Dedication

To my beloved mother the most affectionate person in the world, who is encouraging, helping and strengthen me to fight hard to makes my dreams come true even her illness. Mom I will always wish god give you good health.

To my lovely father, the source of power and support who raised me until I became what I am today.

To the other half of my heart my beloved husband Dr. Ribhi Mansour your support, encouragement, enthusiasm, love and understanding is the secret of my success, and you are the greatest gift of my life.

To my soul my daughter Dima, you will always be my hope, pureness and all of the beautiful things in this world.

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To my husband family for their everlasting support and help.

I dedicate my thesis with big love

Abeer

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Thanks to my family with all my love, which doesn’t have any words to express my love, thanks for being there always for me and stood beside me throughout my study and provided me with psychological support and encouragement.

الاقرار

أنا الموقع أدناه موقع الرسالة التي تحمل العنوان:

Formulation and Stability Evaluation of Ciprofloxacin Topical Preparation

أقر بأن ما اشتملت عليه الرسالة هو نتاج جهدي الخاص، باستثناء ما تمت الإشارة إليه حيثما ورد، وأن هذه الرسالة ككل، أو أي جزء منها لم يقدم من قبل لنيل أي درجة أو لقب علمي أو بحثي لدى أي مؤسسة تعليمية أو بحثية أخرى.

Declaration

The work provide in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

Student's name: **اسم الطالب**:

Signature: **التوقيع:**

Date: **التاريخ**:

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List of Abbreviations

Symbol	Abbreviation
^{O}C	Celsius
CFU	Colony forming unit
CIP	Ciprofloxacin
DNA	Deoxyribonucleic acid
DDs	Drug delivery systems
EDTA	Ethylene diamine tetra acetic acid
EPR	Electron paramagnetic resonance
FTIR	Fourier transform infra-red
GUV	Giant unilamellar vesicle
HCl	Hydrochloride
H	Hours
H_2O	Water
Min	Minutes
MRSA	Methicillin-resistant Staphylococcus aureus
MIC	Minimum inhibitory concentration
SDS	Sodium lauryl sulfate
S. aureus	Staphylococcus aureus
SEM	Scanning electron microscope
TEM	Transmission electron microscope
TEA	Triethanolamine
UV-Vis	Ultraviolet-Visible
P. aeruginosa	Pseudomonas aeruginosa
PB	Phosphate buffer
PBS	Phosphate buffer saline
pH	Power of hydrogen
λ_{max}	Lambda max

Formulation and Stability Evaluation of Ciprofloxacin Topical Preparation

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Abstract

With increment the number of infections resistance to antibiotics and life threatening causes many researchers work on developing alternative approaches and manufacturing new drug delivery systems to traverse the disadvantages of the conventional antibiotics. The aim of this study was to encapsulate the ciprofloxacin HCl in three different surfactants such as anionic surfactant (Sodium Lauryl Sulfate), cationic surfactant (Cetrimide) and nonionic surfactant (Tween 20). The encapsulation may in one hand help improving penetration behavior of the ciprofloxacin and on the other hand reduce the used doses. The other vital role of this research was the formulation of three stable encapsulated topical dosage forms (ciprofloxacin HCl gels) in order to treat topical infections. The encapsulation of ciprofloxacin HCl lead to successful conspicuous increasing in ciprofloxacin loading with potentially more soluble pattern. Fortunately, the *in vitro* release results revealed that about almost 90% of the loaded ciprofloxacin HCl was released at pH 5.5 in the first 2 hours. The prepared three gels differentiated as stable, clear and homogeneous formulation with good rheology characteristics. Moreover, improvement in the results of the antibacterial activity manifest that the newly encapsulated

ciprofloxacin had better antibacterial activity against *S. aureus* and *P. aeruginosa* than ciprofloxacin alone.

Chapter one
Introduction

1.1 Infectious diseases

Infectious diseases are disorders caused by germ's invasion and may pose danger to human's lives, "germs" are small disease causing agents such as bacteria, viruses, protozoa and fungus [1, 2].

Bacterial infections are diseases caused by multiplications of harmful bacteria inside the body [3].

Bacteria illness caused due to various mechanisms such as production of toxins leading to cell damage or multiplication in numerous numbers that the body cannot work regularly or it may cause tissue damage directly [4, 5].

There are two kinds of bacteria sorted into two groups the gram positive and negative bacteria, the severity of infections based on the type of bacteria involved, it ranges from mild to severe and it is cured by antibiotic drugs [6].

Antibiotics are classified based on their mechanism of action on the micro-organisms such as, inhibition of the cell wall synthesis such as penicillin, cephalosporins and vancomycin, beta-lactamase Inhibitors such as carbapenems, aztreonam, polymycin, bacitracin [2].

Inhibition of protein synthesis by binding to 30S ribosome such as aminoglycosides and tetracycline, interfere with DNA function such as

sulfonamide and rifampicin, interfere with intermediary metabolism by damaging DNA such as trimethoprim and metronidazole, inhibition of DNA gyrase and leakage of cell content by damage bacteria cell membrane such as fluoroquinolones [2, 7].

One of the most used antibacterial agents is fluoroquinolone antibiotics.

Fluoroquinolones are series of large group of antibiotics that contain a fluorine atom in their chemical structure, they featured in guidelines treatments as broad spectrum against gram positive and negative infections by inhibition their DNA synthesis which act by inhibiting DNA gyrase and topoisomerase [8].

Following the first quinolone marketing was the introduction of several generation analogues that marketed after a while.

Quinolones are classified into four different generations with unique properties such as 1st generation consist of nalidixic acid and cinoxacin [9], the 2nd generation consists of norfloxacin, lomefloxacin, enoxacin, ofloxacin and ciprofloxacin [10, 11], the 3rd generation levofloxacin, sparfloxacin, gatifloxacin and moxifloxacin [12, 13], and the 4th generation such as, trovafloxacin [14-16].

One of the fluoroquinolones with overwhelming use is ciprofloxacin, the ciprofloxacin was first introduced by Bayer A.G. in 1983, following the approving of the United States Food and Drug Administration (FDA) in 1987 [17].

1.2 Ciprofloxacin

Ciprofloxacin is a broad spectrum 2nd generation fluoroquinolones antibiotic that covers gram positive and negative bacteria, it acts by inhibition of DNA gyrase (topoisomerase type 2) [18, 19].

Although it has an excellent sensitivity to gram negative bacteria such as (escherichia coli, haemophilus influenzae, klebsiella pneumoniae and pseudomonas aeruginosa from the gram negative category), it act against gram positive bacteria such as (methicillin-sensitive, streptococcus pneumoniae, staphylococcus epidermidis and enterococcus faecalis) [20, 21]. Therefore, Ciprofloxacin is used in the treatment of various diseases [22].

Ciprofloxacin is 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid, it has a chemical formula C17H18FN3O3 with a 2 hydrogen bond donor and 7 hydrogen bond accepter as shown in figure1.1 [23].

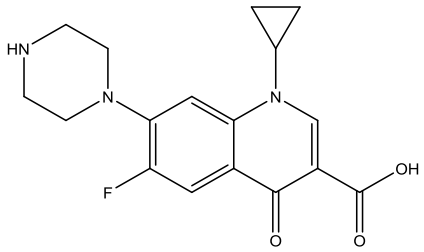

Figure 1.1: Chemical structure of ciprofloxacin [23].

Ciprofloxacin is yellowish to light yellow crystals with a molecular weight 331.35 g/mol, melting point 255-257℃, it has a zwitterionic structure which accounts for its good solubility in acidic and basic solvents, its solubility in DMSO (<1 mg/ml at 25℃), methanol, 0.1N hydrochloric acid (25 mg/ml), and ethanol (<1 mg/ml at 25℃) but insoluble in water [24].

Ciprofloxacin found in the market as formulation of tablet for different companies in strength of 750, 500 and 250 mg also as parenteral route, eye drops, eardrops, topical and oral suspension. However, the ciprofloxacin is classified as class 4, according to Biopharmaceutical Classification Systems (BCS), this means that ciprofloxacin has a low water solubility and low permeability due to the small, flat and rigid molecule of ciprofloxacin has a crystal lattice that lowers its aqueous solubility, this is due to the strong intermolecular bonds (van der Waals and hydrogen bonding interactions), these interactions prevent the water to reach inside the molecule and then prevent solubilizing process [25].

However many bacteria had developed resistance to the antibiotics as a result of major widespread prescribed use, in addition to the wrong unmonitored use of these antibiotics especially the narrow spectrum antibiotics.

The mechanism of resistance for ciprofloxacin emerge rapidly by either one or more mutations in the quinolone binding region of the target enzyme DNA gyrase and DNA topoisomerase IV or the other resistance change in

the permeability of the organism due to the effect of efflux pump that localized in the bacterial membrane [26-30].

Various attractive studies have been made to investigate and improve either the solubility or the antimicrobial activity of ciprofloxacin unfortunately these studies were not reach satisfactory results. Therefore, this study aims to improve the solubility, spectral properties and antibacterial activity through its encapsulation in various three types of surfactants such as Sodium Lauryl Sulfate (SDS), Tween 20 and Cetrimide. Encapsulation of the ciprofloxacin inside the surfactant leads to a formation of micelle with increasing in its solubility profile.

The 2nd goal is the formation of hydrogel for topical application with suitable criteria of gel and topical dosage form.

1.3 surfactants and micelles

Also called surface active agents are organic compounds that adsorbed to the surface or interfaces leading to a decrease in the surface tension [31].

Surfactants which play vital role in pharmaceutical applications are characterized by amphiphilic structure which means that they composed two regions in their structure, the hydrophobic moiety known as "tails" such as hydrocarbon chain and the hydrophilic group known as "head" with good affinity to water [32-34].

Surfactants act in three different steps, the first step is "**roll up**" which means decreasing the interfacial tension between the two layers especially

oil/solution system, the second step called "**emulsifying**" which gives emulsifying of oil easily by lowering oil/solution interfacial tension [35-38],

"**solubilizing**" is the third step by which the surfactant has good interaction with the micelle in a water or other solvents leading to easy dissolving resulting in clear and stable solution [39-41] as shown in figure 1.2.

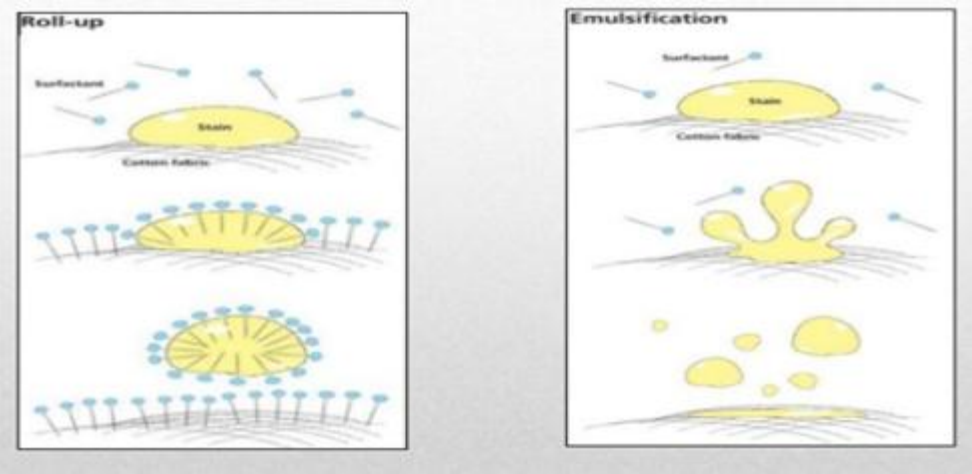

Figure 1.2: Steps of surfactant formation [21].

Surfactants self-assembled themselves in the interfacial between the two phases, until saturation occur in the surface then starting the formation of spherical globules in the aqueous medium which is called "**micelle**" [42-46] as shown in figure 1.3.

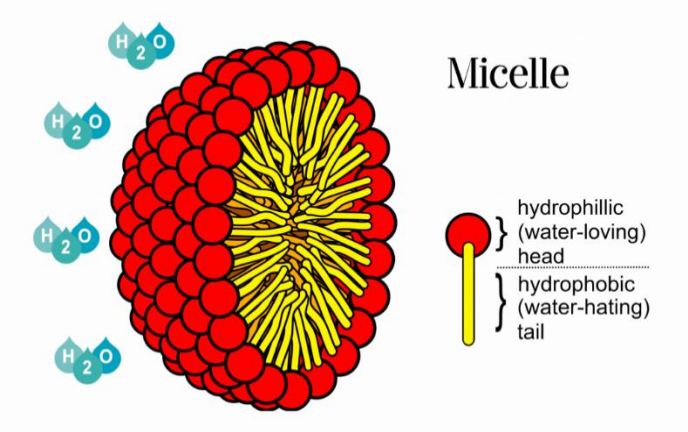

Figure 1.3: Micelle structure [2].

Micelles are reservoirs of soluble surfactants that accumulates spontaneously and reversibly formed from amphiphilic molecules [46].

It has unique structure with two parts the hydrophobic hydrocarbon chains are towards the inner core of the micelles and the hydrophilic group that still always in contact with the surrounding aqueous medium [47, 48].

Micelle is a soluble accumulate of surfactant molecules in a liquid medium formed when the concentration of surfactant exceed the critical micelle concentration (CMC) [49-52].

To achieve basic conditions for micelle formation the procedure should takes place in suitable solvent medium for the hydrophilic hydrocarbon chain.

1.4 Classifications of surfactants

The classifications based according to the charge on the polar head of the surfactant. They grouped into cationic, anionic, zwitterionic and nonionic

groups with distinctive properties and characterization for each group as shown in figure 1.4.

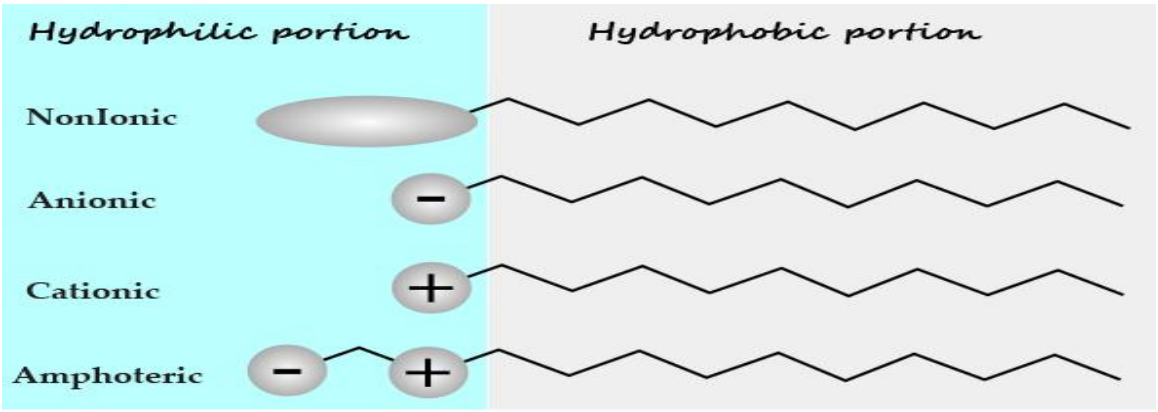

Figure 1.4: Classification of surfactants [31].

1.4.1 Cationic surfactants

They are positively charged molecules especially on the polar head, they dissociated in water into an amphiphilic cation and an anion, most often of the halogen type [53].

They are quaternary ammonium compound derived from nitrogen compounds with aryl and alkyl groups [54-56].

They have important use in pharmaceutical applications and commercial products due to their bactericidal activity against a wide range of gram positive and negative bacteria [57]. As being positively charged molecules, they adsorbed on negatively charged substances to produce antistatic effect.

Trimethylalkylammonium chloride, and the chlorides or bromides of benzalkonium and alkylpyridimium ions are examples of cationic

surfactants but the most commonly used cationic surfactants is Cetrimide [19].

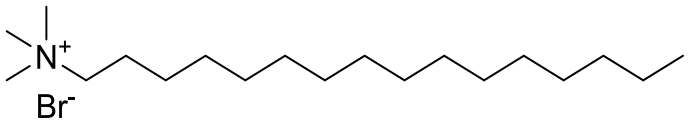

Figure 1.5: Cetrimide structure [58].

Cetrimide is a low molecular cationic surfaces active agent that lowers the surface tension between liquid and solid or two liquids, it also contains mixture of cetrimonium bromide (CTAB), cetyltrimethylammonium bromide, hexadecyltrimethylammonium bromide [31].

That's considered as quaternary ammonium surfactant with antiseptic acting against bacteria and fungi [59].

As with most surfactants, CTAB forms micelles in aqueous solutions leading to increase the solubility of many substances, the CMC value of Cetrimide is 3.88 mM at room temperature [60, 61].

1.4.2 Anionic surfactants

Are organic substances which ionized when added in solution giving negatively charge on the head region of it, leading to creation of anions [62].

They contain anionic functional group on their head such as sulfate, phosphate, sulfonate and carboxylates [63].

Noticeable alkyl sulfates include ammonium lauryl sulfate, sodium lauryl sulfate (sodium dodecyl sulfate), and sodium myreth sulfate [18] as shown in figure 1.6.

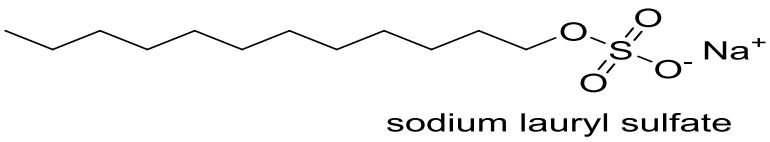

Figure 1.6: Sodium lauryl sulfate structure [58].

It has amphiphilic properties giving them excellent micelle formation leading to good detergent acting [64]. The CMC of the SDS is 8.2 mM at 25 °C [19].

1.4.3 Zwitterionic surfactant

Also called amphoteric surfactants, their head groups carries both positive and negative charges, in other words they are molecules exhibit both cationic and anionic centers [24, 31, 65].

The negatively charged group can be carboxylate such as -CO_2-, sulfate such as -OSO_3- or sulfonate such as -SO_3- [59].

The cationic part based on primary, secondary, or tertiary amines or quaternary ammonium cations.

The most prevalent biological amphoteric surfactants have a phosphate anion with an amine or ammonium, such as the phospholipids phosphatidylserine, phosphatidylethanolamine as shown in figure 1.7, phosphatidylcholine and sphingomyelins [60, 66].

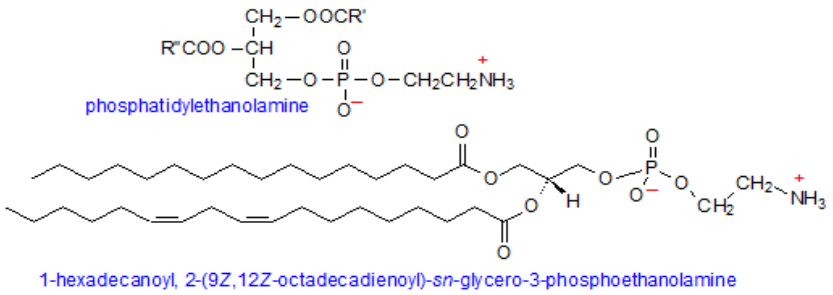

Figure 1.7: Phosphatidylethanolamine structure [67].

zwitterionic surfactants pose unique properties, they are not sensitive to pH changes although there are some of them that alter their behavior with pH changes [68].

They still being important field due to their high biological compatibility and low toxicity but they are quite expensive limiting application especially in cosmetics [69].

1.4.4 Nonionic surfactants:

They have hydrophilic group with no charge but they derive their water solubility from polar group such as hydroxyl or polyoxyethylene.

They consist of two parts, the oxygen containing hydrophilic group in the head and the hydrophobic tail [70, 71].

Strong, stable, covalent H- bonding created due to interaction between the oxygen and the water molecules [72].

Many factors affecting this H-bond interaction for instance, the elevation of temperature lead to obvious diminishing in H-bond chances, which directly affect inversely the solubility of nonionic surfactants.

Examples of nonionic surfactants such as Tween 20 as shown in figure 1.8.

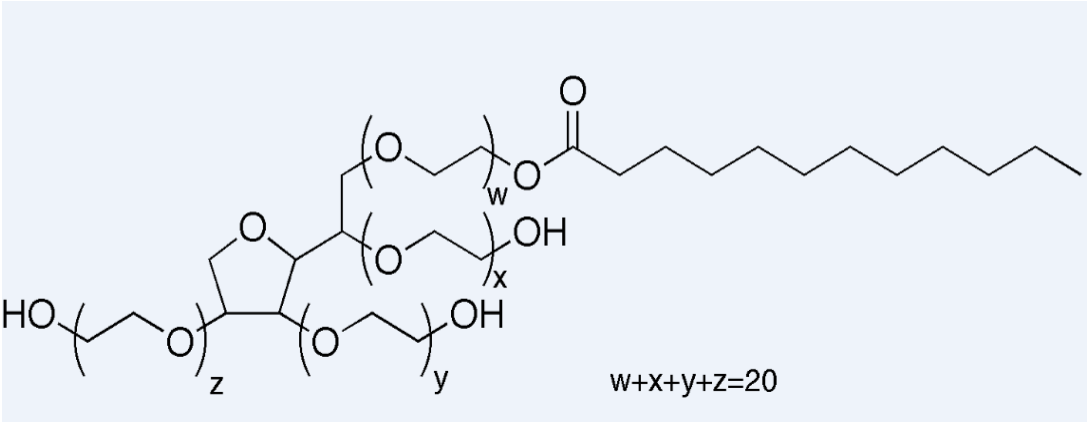

Figure 1.8: Tween 20 structure [24].

Polysorbate 20 with commercial brand name Tween 20 is amphoteric surfactant with CMC value of 8.04×10^{-2} mM at 21 °C [24].

Formed by chemical reactions of ethylene oxide called ethoxylation of sorbitan before the addition of lauric acid.

It has a lot of applications such as wetting agent in food applications or it may act in many biotechnical and pharmaceutical application as excipients (stabilizers) [73, 74].

One of our aims in this research is to develop gel as good topical dosage form to reach our expected goals.

1.5 Topical dosage form

It's a drug delivery system which applied to a particular on or in the body which mostly applied to body surfaces such as mucus membrane or skin to treat various types of illness anywhere in the body such as skin, rectal

routes, vaginal and ophthalmic route [75, 76]. It may be antiseptic, antifungal, analgesic, anesthetic, protectant and anti- inflammatory.

Topical dosage forms are classified into groups according to its physicochemical nature some are solids, the other may be semisolid until liquid dosage form.

The topical drugs are preferred due to the local therapeutic activity when applied to the mucus membranes or the skin [77, 78].

The overall goal of the topical drugs is serving local action and formulated to provide prolonged and continuous local contact with minimal systemic drug absorption. One of the most commonly used topical preparations is the gel dosage form. Many factors may affect the absorption of topical dosage forms such as physiological factors of the skin or the physicochemical factors of the drug.

There are a lot of advantages of using this delivery system characterized by avoidance of the first pass metabolism in addition to the exhibition of the gastrointestinal incompatibility also it can be considered as suitable self-medication with good site selectivity and many other advantages but unfortunately it has some disadvantages such as skin irritations or allergies.

1.5.1 Pathways of transdermal permeation

Many topical dosage forms had systematic effect in addition to the local effect that they made on the place of applications. That occurs due to the

permeation into the skin by diffusion via intercellular permeations, transdermal permeation or trans permeation [79-82] as shown in figure 1.9.

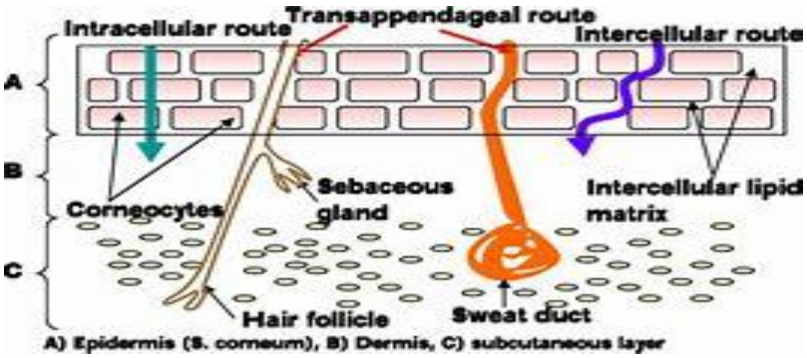

Figure1.9: Transdermal drug penetration pathways through the skin [24].

1.5.2 Gel dosage form

Gel is semisolid dosage form in which they are section of topical route of administration [83]. Gels are defined as stable, clear, transparent, homogeneous pharmaceutical system consisting of liquid phase that interpenetrated within three dimensional cross linked network, forming high level of cross linking caused by chemical or physical interactions [84] as shown in figure 1.10.

They are used externally and comes in various fields of cosmetics, food and pharmaceutical therapeutic functions [85].

They may consist of one or more active ingredients that dispense homogeneously and uniformly in the gel in addition to the inactive ingredients that the gel contains. There are many kinds of gels classified into categories such as hydrogels, organogels, xerogels and nanocomposite hydrogels [24, 86].

Topical route of administration provides some advantages for example it provides the largest surface area which means large spreading and absorbing, also it avoids first-pass effects for people having gastrointestinal irritation in addition it avoids metabolic degradation associated with oral administration.

Figure 1.10: Hydrogel [67].

1.6 Literature review

The abundance of research attempts were carried out to amend the safety and efficacy while overcoming the disadvantages of the current antibiotics by using different approaches such as nanoparticles, polymeric nanoparticles, liposomes, ciprofloxacin reactions such as esterification also encapsulation inside cyclodextrin or in unilamellar vesicle.

Another study conducted in 2011 by German A. Islan *et al*., which investigate the ciprofloxacin encapsulation on alginate/pectin model. It prepared by ionotropic gelation under acidic conditions using calcium as

crosslinker. Many approaches studied such as SEM images, release studies, FTIR to observe the component interactions [87].

In 2013, Nora Kaszas *et al.* study the encapsulation capacity of incorporated ciprofloxacin hydrochloride (CPFX) into giant unilamellar vesicle by measuring zeta potential and electron paramagnetic resonance (EPR). There was increasing in zeta potential at pH 5.4 with increasing fluidity results of the EPR observations that is due to CPFX binding to GUVs.

The results of the dialysis model showed that there was different permeation of CPFX through the membrane of GUV, interestingly; these results enhance the concept of this encapsulation design.

The encapsulated ciprofloxacin release about 42.72% of the initial CIP at the stimulated gastric pH in 2 h whereas, the commercial CIP had low release 30% leading to many sides effect. This research highlights the improvement of the release at stimulated gastric conditions, decreasing toxicity, increasing bioavailability and biodisponibility [88].

In 2016 Tigani Isa and Zuki Abu Bakar Zakaria examining the antibacterial activity against Salmonella Typhimurium of the ciprofloxacin-encapsulated cockle shells calcium carbonate nanoparticles and it's biocompatibility in macrophage. They observe good biocompatible pattern with 99.5% encapsulation capacity and 5.9% loading content with regard to the mean diameter of inhibition zone they got 18.6-+ 0.5 mm while comparing it with the 11.7-+ 0.9 mm of the ciprofloxacin alone means that

the encapsulation pattern enhance the antibacterial activity and may act as good delivery system for the ciprofloxacin [89].

However, there are no previous studies that used three different surfactants for encapsulation the ciprofloxacin drug, and prepare final product as gel topical preparation. So, the main aim of our study is to develop three formulations of ciprofloxacin based on SDS, Tween 20 and cetrimide as surfactants.

1.7 Objectives of the Research

The present study was conducted in various stages with the following objectives.

1- To encapsulate Ciprofloxacin in various surfactants in order to enhance aqueous solubility.

2- To prepare the encapsulated Ciprofloxacin in gel formulations with a good consistence.

3- To study the rheology behavior, *in vitro* release, dynamic light scattering particle size, zeta potential and morphology (TEM) of the developed Ciprofloxacin-surfactants gels.

4- To study the stability of the formulated gel dosage form.

5- To conduct the antimicrobial activity of the developed Ciprofloxacin-surfactants gels.

Chapter Two
Methodology

2.1 Materials and equipment

Ciprofloxacin HCl was purchased from (Pharmacare Ramallah), SDS (catalog # 2057881) was purchased from (Sigma-Aldrich, USA). Tween 20 (catalog # E0088) and cetrimide (catalog # 202283 were purchased from sun-pharm drug store (Nablus). Disodium hydrogen phosphate and potassium dihydrogen phosphate were purchased from (C.S. Company, Israel). EDTA was purchased from Alfa Aesar Company (England). Triethanolamine and Carbapol 940 purchased from Al-Zahra factory, Nablus.

While the instruments used in this research were as follows:

- **Transmission Electron Microscopy (TEM)** images were taken by using Morgagni 286 transmission microscope (FEI Company, Eindhoven, Netherlands) at 60 kV.

- **Ultraviolet-Visible (UV-Vis)** spectra were recorded with (7315 Spectrophotometer, Jenway, UK), using 10-mm quartz cuvettes for measuring concentration of solution and final gels at wavelength λ 280 nm.

- **Centrifuge (UNIVERSAL 320, Hettich Zentrifugen, Germany)** used in the dispersion of the encapsulated ciprofloxacin.

- **Water bath** and **Sonicator (Elmasonic S 70 H, Elma®, Germany)** used in preparation of the final three encapsulated gel.
- **Water bath shaker (memmert Gmbh, Germany)** for In vitro release test.
- **Rheometer (Brookfield viscometer, USA)** instrument for determination and characterization of gel dosage form.
- **Zeta potential analyzer model Zeta PALS (Brookhaven Instruments Co. NY, USA)** for particle size determination.
- **Accumax Variable micropipette**, UK used for pipetting.
- **(Stat Fax® 2100-Microplate reader, Awareness Technology INC, FL, USA)** for Scanning each plate at λ_{max} 630 nm for all strains.

2.2 Encapsulation of Ciprofloxacin HCl in the surfactants

Preparation of Ciprofloxacin-surfactant solutions were started with preparing three stock solutions of the surfactants (Tween 20, SDS and Cetrimide), which used to encapsulate the Ciprofloxacin HCl powder.

2.2.1 Preparation of stock solution 0.1% w/v of surfactants

0.1% of the surfactant solution was prepared by accurately weighed 1 g of the surfactant (Tween 20, SDS and Cetrimide) transferred to 1-liter volumetric flask; the volume completed with milli-Q water, the mixture sonicated for 10 minutes.

2.2.2 Ciprofloxacin surfactants formation

Accurately weighed 50 mg of Ciprofloxacin HCl powder was dissolved in measured 50 ml of 0.1% surfactant (Tween 20, SDS and Cetrimide) which was prepared previously, thereafter sonication performed for 25 minutes, centrifuge for a maximum 5000 rpm for 30 minutes three times to remove the excess of Ciprofloxacin.

After centrifugation, the supernatant taken and the amount of the encapsulated Ciprofloxacin quantified through spectrophotometric analysis.

2.2.3 Calibration curve of Ciprofloxacin HCl

The preparation of standard solution acquired by weighted 5 mg of Ciprofloxacin HCl transferred to 100 ml volumetric flask completed with milli-Q water up to 100 ml thereafter, the mixture sonicated for 25 minutes.

In order to conduct a calibration curve prepare a stock solution of 5 mg/100 ml then dilute it to twelve different concentrations levels of (2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 µg/ml) were obtained of each standard solution, conveniently diluted with milli-Q water.

All samples analyzed by spectrophotometry at λ_{max} 280 nm. The absorbance values plotted against concentration of the ciprofloxacin HCl.

The concentration of the encapsulated Ciprofloxacin in the surfactants quantified against calibration curves. The encapsulation efficiency (EE) of was calculated from the following equation:

EE (%) = (weight of Ciprofloxacin in surfactant)/ (weight of Ciprofloxacin initially used) x 100.

All samples were prepared in triplicates.

2.2.4 Ciprofloxacin surfactant final mixture

Preparation of Cetrimide Ciprofloxacin and Tween 20 Ciprofloxacin mixtures done by weighing 250 mg of Ciprofloxacin HCl power which dissolved in 250 ml of 0.1% of each surfactant (Cetrimide, Tween 20) the final mixture sonicated for 30 mins and storage at room temperature.

In contrast, the SDS mixture was prepared by weighing 100 mg of ciprofloxacin HCl in 500 ml 0.1% SDS solution, sonication for 30 mins also storage at room temperature.

2.2.5 Formation of Ciprofloxacin-surfactant gel

2.2.5.1 Preparation of Carbopol gel

Carbopol 940 solution 4% (w/w) was prepared by weighting 4 g of Carbopol and mixed with 96 ml of distilled water then stirred continuously for 24 hrs. Then, the pH of the prepared hydrogel was adjusted at pH 5.6 by triethanolamine (TEA) which considered as neutralizer.

2.2.5.2 Preparation of Ciprofloxacin-surfactant gel

The drug triturated to the gel bases, the incorporation of the drug done by using a spatula and slab. All the ingredients used took on weight/ weight basis.

For 0.5% gel preparation, a 15 g of the Ciprofloxacin-surfactant solution was added to 8.25 g of Carbopol gel then adding water up to 25 g (1.7 g) by gentle mixing having final gel of 25 g.

In addition to 0.5% gel formation, there were another 0.8%, 1%, 1.2% and 4% concentration of gel were prepared.

2.2.5.3 Preparation of 1% of Ciprofloxacin gel

To obtain 1% concentration of Ciprofloxacin gel, 1 g from each Carbopol Ciprofloxacin gel of 0.5%, 0.8%, 1%, 1.2% and 4% transferred to 100 ml volumetric flask completed with milliQ water up to 100 ml then sonicated for 1 hour, then serial dilution were done until reach the 1% concentration of Ciprofloxacin which were analyzed spectrophotometry at λ_{max} (280 nm).

2.2.6 Rheology test

The rheological behaviors of the prepared gels carried out by weighing 25 g of Ciprofloxacin gel of each concentration and added in 50 ml beaker then run on the Brookfield viscometer device using spindle number 7 in at various shear rate ranged from 0-100 rpm at room temperature.

2.2.7 Zeta potential

Zeta potential of the three encapsulated Ciprofloxacin gel was determined by zeta potential analyzer model Zeta PALS (Brookhaven Instruments Co. NY, USA) with a scattering angle of 90 degrees at 25 ℃.

2.2.8 Stability study

This study defines the period of time at which the drug maintains its characteristics and properties though the life cycle of the drug, such as chemical, physical, purity, quality, therapeutic, microbiological and toxicological specifications.

The stability study was conducted on the best final three gels of Ciprofloxacin with the best rheological, antibacterial and release results.

2.2.8.1 Determination the concentration of Ciprofloxacin on the gel over the storage time

For storage time examining, the stored Ciprofloxacin gels tested every 15, 30, 45 and 60 days. This done by dissolving 1 g of the final gel in 100 ml milliQ water and stirred for 15 minutes, then the solution was filtered and analyzed at spectrophotometry at λ_{max} 280 nm.

The absorbance results of the Ciprofloxacin gel compared with the initial standard solution of ciprofloxacin.

2.2.8.2 Determination the pH of the three encapsulated Ciprofloxacin gel over the storage time

By using pH meter the gel checked at 1st day, 15, 30, 45 and 60 days.

2.2.8.3 Physical properties

Observing the original Physical properties over the 60 days storage period including appearance palatability, odor, uniformity and color suspendability retained.

2.2.9 *In vitro* test

Ciprofloxacin In vitro release test carried out using dialysis membrane in order to test the kinetic behavior of drug release. This elucidated by using dialysis bag, which is soaked phosphate buffer.

2.2.9.1 Preparation of dialysis membrane

Dialysis bag (spectra/Por^{t}® 4) soaked in 1.0 L of 2% sodium bicarbonate/1.0 mM EDTA in a 2.0 L glass beaker, boiled for 10 minutes, then rinsed and boiled for 10 minutes thoroughly with distilled water. Finally, it submerged completely in 50% Ethanol/1.0 mM EDTA to remove the preservative and stored at 4 °C. It rinsed thoroughly with phosphate buffered saline before used in the experiments.

2.2.9.2 Phosphate buffer solution pH 5.5 preparation

To prepare PBS with a pH 5.5, two solutions were prepared as follow:

For solution 1 preparation, dissolve accurate 13.61 g of potassium dihydrogen phosphate in distilled water and diluted to 1 L with the same solvent.

For solution 2 preparation, dissolve accurate 35.81 g of disodium hydrogen phosphate in distilled water and diluted to 1 L with the same solvent.

Finally, mix 96.4 ml of solution 1 and 3.6 ml of solution 2 and adjust the pH to 5.5.

2.2.9.3 *In vitro release* Ciprofloxacin HCl test

A small membrane was filled with 3 grams of each gel and secured from both ends with a rubber band, and then the dialysis membrane immersed totally in 20 ml of the receiving medium.

The beaker immersed in a large water bath instrument at 37℃ temperature and gently shake for 24 h.

Five milliliters sample withdrawn every 30 minutes from the receiving medium and replaced with the same equal volume of new fresh phosphate buffer of pH 5.5 at 37 ℃ to mimic the sink condition then all samples measured at λ_{max} 280 nm.

2.2.10 Antibacterial activity

2.2.10.1 Bacterial strains

The antibacterial activity of the three developed Ciprofloxacin gels were studied against *S. aureus* (ATCC 25923) [90] and *P. aeruginosa* (ATCC 27853) [58] strains and compared to the activity of surfactants and Ciprofloxacin alone.

2.2.10.2 McFarland preparation

Alternately, the 0.5 McFarland may be prepared by adding 0.5 ml of 0.048 mol/L barium chloride dihydrate (1.175% w/v $BaCl_2 \bullet 2H_2O$) to 99.5 mL of 0.18 mol/L (1% v/v) sulfuric acid then mixed well to ensure homogeneous solution [91]. Thereafter, checking the optimal density by spectrophotometer at wavelength λ 630 nm. In order to get turbidity of 0.5 McFarland standard, the absorbance at a wavelength of 625 nm should be 0.08 to 0.13 that reflect bacterial concentration of about 1.5×10^8 CFU/ml. The McFarland solution sealed to prevent any contamination and covered with aluminum foil to protect it from light.

Visually with good illumination compare the turbidity of the test suspension to the 0.5 McFarland standard and make dilution if needed to reach comparable turbidity of McFarland standard.

2.2.10.3 Mueller Hinton broth preparation

Add 22 gm of Mueller Hinton broth powder to 1 liter of distilled water and shaking with heating on Bunsen burner. Mueller Hinton broth sterilized by autoclave at 121℃ for 15 min. Sterilization done by the control blanks and the utilized of the sterilization indicator tapes. The sterilized Mueller Hinton broth required in case of making the dilutions and preparing the bacterial cultures [84].

2.2.10.4 Mueller Hinton agar preparation

Adding 38 gm of Mueller Hinton broth powder to 1liter distilled water and shaking with heating on Bunsen burner. Then boiled for 1 min. Mueller Hinton agar sterilized in autoclave at 121℃ for 15 min. Sterilization confirmed by the control blanks and the usage of the sterilization indicator tapes [84].

2.2.10.5 Broth microdilution method

This is the basic vital technique in this study to obtain and investigate the minimum inhibitory concentration (MIC) for each encapsulated Ciprofloxacin gel [92]. The used protocol was according to that of CLSI (Clinical and Laboratory Standards Institute)[24, 93].

Briefly, Ciprofloxacin dissolved in milliQ water to achieve a concentration of (2.777 µg/mL) for the gram positive the *S. aureus* and the gram-negative *P. aeruginosa*. While for SDS Ciprofloxacin gel, Tween 20 Ciprofloxacin gel and Cetrimide Ciprofloxacin gel, they dissolved in milliQ water to

obtain a concentration of (0.002 µg/mL), (0.016 µg/mL) and (0.008 µg/mL) respectively for *S. aureus* and *P. aeruginosa*. These solutions were serially diluted two-fold in 11 wells with Muller Hinton broth. Well number 11 considered as a negative control and well number 12 presented as a positive control. In order to test the antibacterial activity of the surfactants in broth microdilution technique, the three kinds of surfactants (SDS, Tween 20 and cetrimide) serially diluted two-fold with Muller Hinton broth to reach concentrations form 0.098% to 50%. Then, the stored overnight grown bacterial supplement to all wells except Well number 11 that considered as negative control. After inoculation of bacteria, the prepared plates stored in the incubator for 18 hour at 35°C. Minimal inhibitory concentration (MIC) considered lowest concentration of the drug that inhibit the present of any visible growth in the test media. MIC can be read manually or by (Stat Fax® 2100-Microplate reader, Awareness Technology INC, FL, USA) which analyze the absorbance of each plate at λ_{max} 630 nm.

Chapter Three
Results and Discussion

Ciprofloxacin is a 2nd generation fluoroquinolone antibacterial agent that has broad-spectrum activity against gram negative and gram-positive bacteria. Ciprofloxacin inhibit the bacterial enzyme DNA gyrase leading to breakage the double stranded DNA [91]. Ciprofloxacin considered as one of the important drugs in the guideline treatment for many diseases and infection, unfortunately many bacteria had developed resistance to this kind of antibiotic due to widespread and the wrong prescribed use [67, 86].

The mechanism of resistance for Ciprofloxacin emerge rapidly by either one or more mutations in the quinolone binding region of the target enzyme DNA gyrase and DNA topoisomerase IV or the other resistance change in the permeability of the organism due to the effect of efflux pump that localized in the bacterial membrane.[88, 92]

Many researchers investigated ways to improve the antimicrobial activity of ciprofloxacin or to overcome the resistance to this drug unfortunately these studies did not reach satisfactory results. Therefore, in this work we aimed to improve its spectral properties and antibacterial activity.

The second vital aim of this research is to prepare a new topical formulation dosage form that will treat the topical infections, which regrettably had limited drug that target it.

Encapsulation of ciprofloxacin HCl in three types of surfactants, the anionic surfactant such as sodium lauryl sulfate (SDS), the nonionic surfactant such as Tween 20 and the cationic surfactant such as Cetrimide ending in micelle formation leading to solubility and antibacterial activity improvements.

Before encapsulating ciprofloxacin in the surfactants, we have studied the stability and the preparation of the gel formulations. As we have three different surfactants, we have prepared various concentrations of the surfactants (1%, 0.8%, 0.5% and 0.1%). These various concentrations added to the gel base component, which was 0.5% Carbapol. We have noticed instability of the gels at all concentrations except for the 0.1% of the surfactant. Therefore, this concentration used for the rest of the experiments. On the other hand, it noticed that this concentration is still above the CMC of all used surfactants.

In order to study the encapsulation behavior of Ciprofloxacin in three different surfactants, a calibration curve of Ciprofloxacin HCl constructed as shown in figure 3.1. The calibration curve was built at λ_{max} = 280 nm obtaining a linearity with a regression factor of 0.9903.

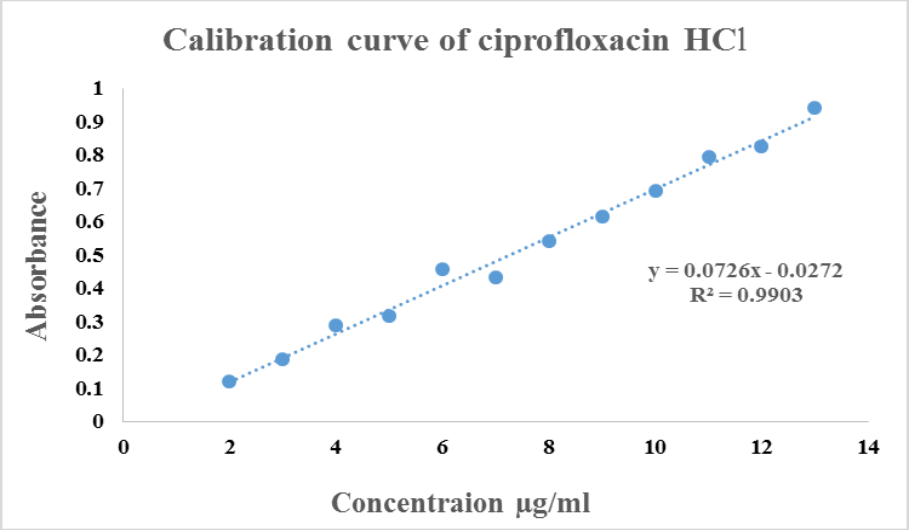

Figure 3.1: Calibration curve of Ciprofloxacin HCl.

Once the calibration curve was constructed, the encapsulation efficacy was calculated for the three different surfactants. We obtained 100% encapsulation for the Tween 20 and Cetrimide, but in the case of SDS was 20%.

3.2 Preparation of ciprofloxacin gel

In order to obtain the optimum conditions for the gel formulation, various concentrations of gel base were prepared. In this part of the study, we prepared 0.5%, 0.8%, 1.2% and 4% of Carbapol 940 base and Ciprofloxacin-surfactants added to obtain the final gel formulation. In these preparations, we aim to obtain the maximum encapsulation efficacy of Ciprofloxacin-surfactant in the final gel formula. The maximum loading achieved in the case of 0.5% of Carbapol base. Moreover, we have obtained a loading efficiency 0.5% of Ciprofloxacin in the case of

Cetramide and Tween 20 and 0.25% in the case of SDS in the 0.5% of Carbapol formulation.

The 0.5% Carbopol gel showed good stability on standings. In addition to the good appearance with compatible pH 5.5, which is similar, to skin pH so it is the dosage form of the choice, which may not make irritation to the skin. Moreover, it has perfect physical properties such as consistency, viscosity, drug content and homogeneity.

3.3 Rheology results

Generally viscosity essentially decrease as the shear stress increase which lead to change in the arrangement of the molecules to align their long axes in direction of flow orientation result in reduction of the internal resistance of the material, All of the prepared gels showed similar rheological behavior (pseudo-plastic) i.e., the viscosity decreases with an increase in the shear rate.

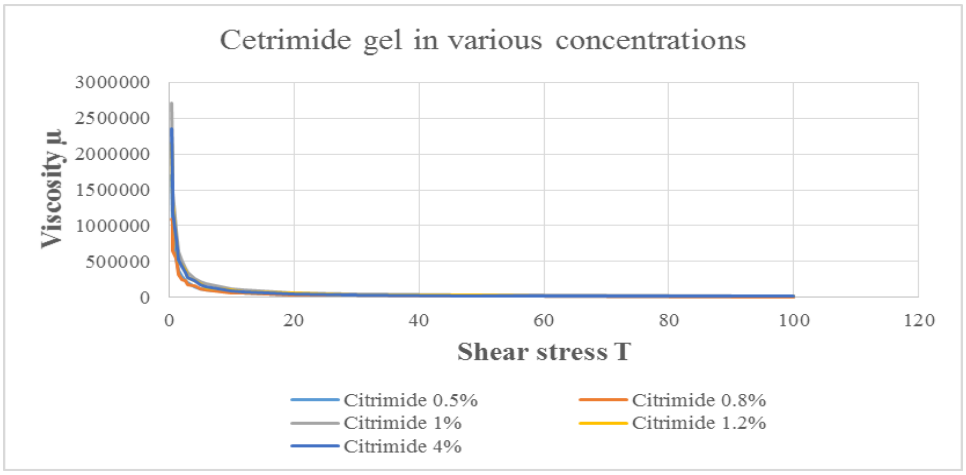

Figure 3.2: Comparison between the encapsulated Ciprofloxacin Cetrimide gels of various Carbopol concentrations (0.5%, 0.8%, 1%, 1.2% and 4%).

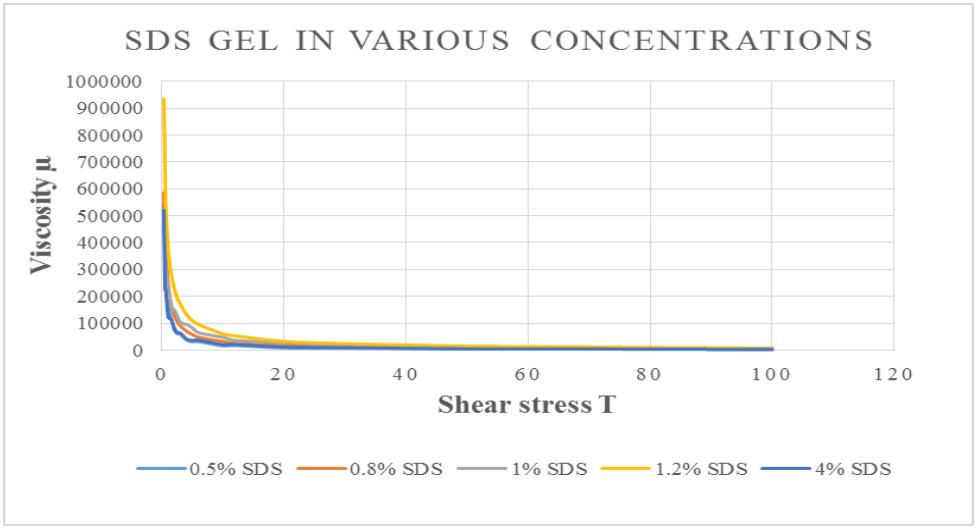

Figure 3.3: Comparison between the encapsulated Ciprofloxacin SDS gels of various Carbopol concentrations (0.5%, 0.8%, 1%, 1.2%, and 4%).

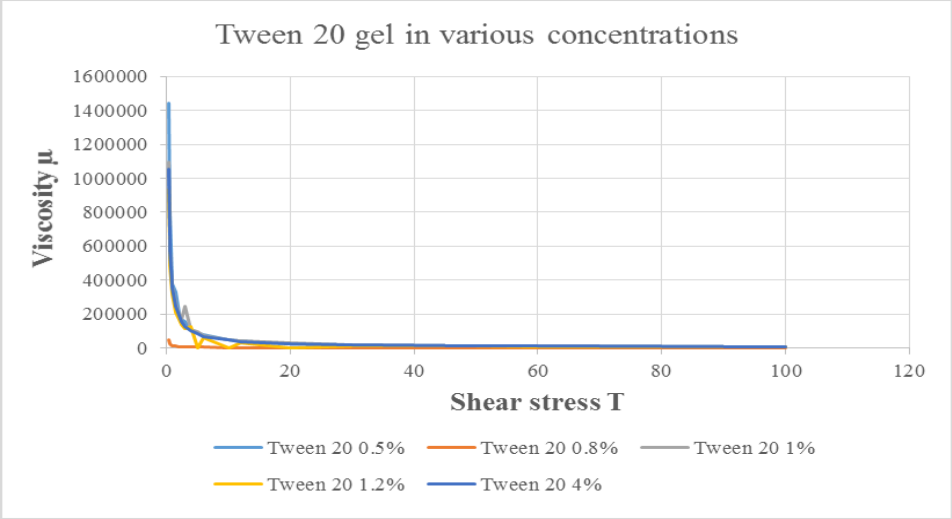

Figure 3.4: Comparison between the encapsulated Ciprofloxacin Tween20 gels of various Carbopol concentrations (0.5%, 0.8%, 1%, 1.2%, and 4%).

Statistically, the completely prepared gel formulas showed significant correspond results, which means that all of them had excellent acceptable rheological properties. According to these results, all of the prepared formulas had the necessary properties to act as excellent gel dosage form with good viscosity that is suitable for skin applications and meets the requirements of good flow. The inspection of rheological results in scheme 3.2 until 3.4 highlights the similar behavior of all gels in the various concentrations at the same temperature 25^{o}C; the results obtained enhancement the hypothesis of no great change in viscosity substantially with increasing the concentration of the Ciprofloxacin gel with each kind of the three surfactants. Due to these results, 0.5% concentration was chosen to be used as an optimum Carbopol 940 concentration, that's because it has similar rheological behavior compared to the rest of the prepared gels as shown in figures above also for the vital reason of its suitability and compatibility of the Ciprofloxacin mixture, the other gel concentrations

showed low dosage form characteristics when it mixed with the final encapsulated Ciprofloxacin mixture, on the contrary some of the prepared gels results in gel containing granules or gel with some sedimentations the others results in separating into two layers solution and not gel.

3.4 Morphology of the Ciprofloxacin-gels

The morphology of the Ciprofloxacin encapsulated gels in the three different surfactants investigated by transmission electron microscopy (TEM). As shown in figure 3.5, the three different formed gels showed the formation of the fiber like structure of the gels. The images confirm the formation of the gel with its consistent morphology.

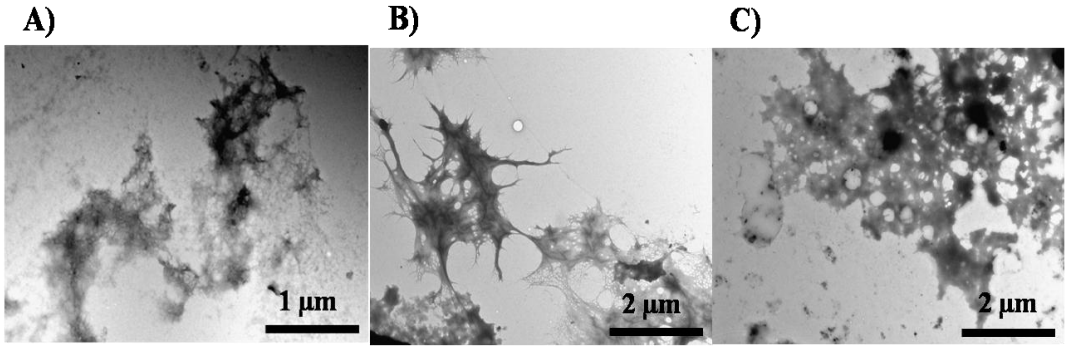

Figure 3.5: TEM images of Ciprofloxacin encapsulated gel of: A) Cetrimide gel; B) SDS gel; C) Tween 20 gel.

3.4 Zeta potential results

Zeta potential is used to determine the surface charge and the stability of the final formulation studies as increasing the zeta potential value increasing the electrostatic repulsion between the particles hence there is no tendency to fluctuate and no precipitation. In our study, as we have three different surfactants, therefore we will obtain positive and negative values

of zeta potential depending on the formulation. Good stability of the formula achieved once the zeta potential value be more than ±30 mV.

Table 3.1: and figures 3.6 until 3.8 show the obtained zeta potential results of our three-ciprofloxacin gel formulations.

Table 3.1: Summary of zeta potential value results.

Formulation type	Surfactant type	Zeta potential (mV)
Surfactant solution	SDS solution	-58 mV
	Tween 20 solution	-32.2 mV
	Cetrimide solution	+59.11 mV
Encapsulation Ciprofloxacin gel	SDS Ciprofloxacin gel	-30.5 mV
	Tween 20 Ciprofloxacin gel	-41.6 mV
	Cetrimide ciprofloxacin gel	+45.8 mV

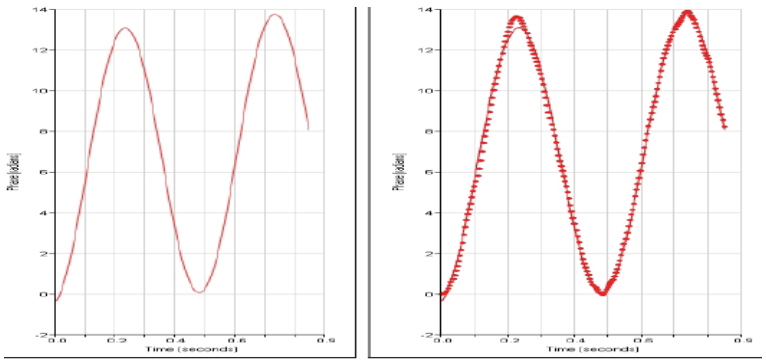

Figure 3.6: Zeta potential of Cetrimide Ciprofloxacin gel.

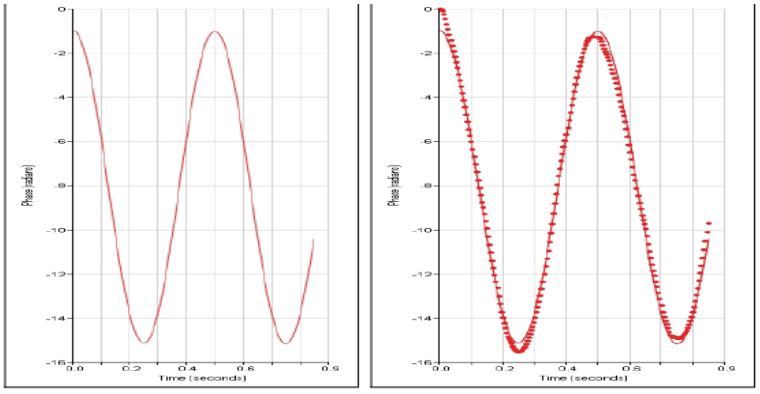

Figure 3.7: Zeta potential of SDS Ciprofloxacin gel**.**

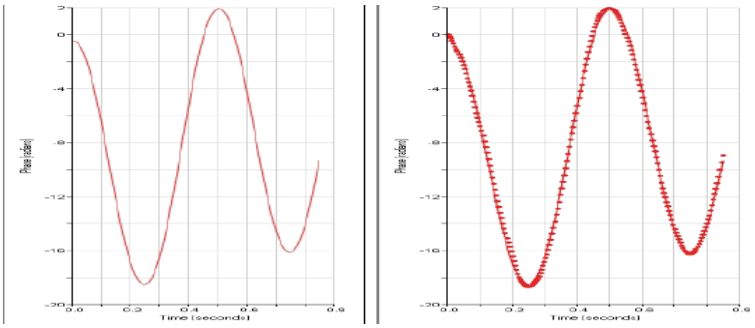

Figure 3.8: Zeta potential of Tween 20 Ciprofloxacin gel.

As expected, we obtained -30.5 mV and -41.6 mV in the case of SDS and Tween 20 gel formulations respectively and +59.11 mV in the case of Cetrimide gel formulation.

The negative charge indicate evidence towards the nature of the surface charge that is negative charge in nonionic and anionic case and in the case of cationic surfactant is positive due to the positive surface of the cationic surfactants, zeta potential value be more than ±30 mV indicates the stability of the solution as well as the gel formulation.

According to these results, the encapsulated Ciprofloxacin gels had good stability and reach the requirements to act as good gel with no sedimentation phenomena.

3.5 Stability results

3.5.1 Effect of the storage time on the concentration of the encapsulated Ciprofloxacin gels

The stability study was carried out using the three Ciprofloxacin gels that provided the best release with excellent rheological characteristics, the study was conducted at 25^{o}C for 60 days.

Samples of the prepared gels were taken at each 15 days intervals and was studied for their drug content by analysis in spectrophotometry the results compared to the initial standard gel and it showed that the gel continue to save the at the same concentration of the initial prepared gels regardless of the time which means that the Ciprofloxacin gel is stable for at least 60 days.

3.5.2 Effect of the storage time on the pH of the encapsulated Ciprofloxacin gels

There were no significant changes observed on the pH of the three stored gels over 60 days.

The pH stay around 5.5 which indicate that the storage time had no effect on the pH of the gels**.**

3.5.3 Effect of the storage time on the physical properties of the encapsulated Ciprofloxacin gels

In collapsible tube, there were no obvious change in the color, odor, uniformity and appearance after 60 days of the storage, which elucidate the physical stability of the three gels at room temperature.

3.6 Release results

It mentioned in the introduction that the pH of the skin is 5.5. Therefore, to investigate this behavior, we have studied the release profiles of our gels at pH 5.5 with gentle agitation at 37°C. As shown in figure 3.8, we can observe a time dependent cumulative release profiles of the three encapsulated Ciprofloxacin gels achieved.

The test conducted to study the effect of gel on the release of Ciprofloxacin drug in addition, to investigate the effect of each surfactant on the release profile of the drug.

Figure 3.8 showed that the three Ciprofloxacin gels display about 90% of the loaded Ciprofloxacin released in the first 2 hours at pH 5.5, these satisfactory results enhance the idea that the three prepared gels can be consider as good topical dosage form, which easily applied and can be release in selective manner through the skin with high and rapid release. A detailed analysis of drug release profile then revealed more than 30 % release in 45 min as shown in figure 3.8. In addition, the results showed that the gels continue the release over 17 hours which means that the good

application of the three ciprofloxacin gels is in the first hours so it will be good and selective formulation for topical infection diseases that need quick treatment and in case of multiple drug use per day.

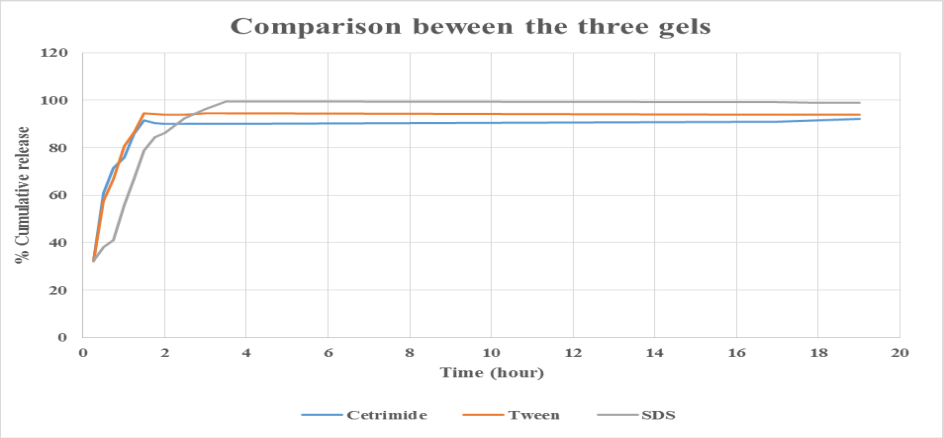

Figure 3.9: in vitro release comparison between the three kinds of the encapsulated Ciprofloxacin gels at 37°C.

3.7 Antibacterial results

As one of our main objectives of this study is to improve the antibacterial activity of Ciprofloxacin. We have studied the antibacterial activity on two bacterial species the gram-positive bacteria such as *S. aureus* and gram negative such as *P. aeruginosa*. In this study, we have determined the minimum inhibitory concentration (MIC) of each sample (surfactant alone, ciprofloxacin, ciprofloxacin gel) which was determined by broth microdilution method as described in the method section.

The antibacterial activity of SDS Ciprofloxacin gel was higher against S. aureus than of Ciprofloxacin by 390 folds and by 620 folds for and P. aeruginosa at the same concentration as shown in table 3.2.

Whereas the antibacterial activity of Tween 20 Ciprofloxacin gel higher against S. aureus than of ciprofloxacin by 390 folds and by 2480 folds for and P. aeruginosa at the same concentration as shown in table 3.2.

Finally the expected improvement in the antibacterial of the Cetrimide Ciprofloxacin gel higher against *S. aureus* than of ciprofloxacin by 781 folds and by 2480 folds for and *P. aeruginosa* at the same concentration as shown in table 3.2.

Table 3.2: Minimum inhibitory concentration (MIC) of SDS Ciprofloxacin gel, Tween 20 Ciprofloxacin gel and Cetrimide Ciprofloxacin gel compared to that of ciprofloxacin.

Sample	***S. aureus***	***P. aeruginosa***
Ciprofloxacin (2.777µ/mL)	0.390625	0.31
SDS solution	-	-
Ciprofloxacin SDS gel (0.002 µ/mL)	0.001	0.0005
Tween 20 solution	-	-
Ciprofloxacin Tween 20 gel (0.016µ/mL)	0.001	0.000125
Cetrimide solution	3.9	0.25
Ciprofloxacin Cetrimide gel (0.008µ/mL)	0.0005	0.000125

In conclusion, it noticed that the surfactants alone almost did not show any significant antibacterial activity in comparison to the Ciprofloxacin or the developed gels. The three encapsulated gels improved the antibacterial activity against *S. aureus, P. aeruginosa* with excellent improvement that could be due to the aggregation of bacteria with the gels, and as a result, the exposing of bacteria to Ciprofloxacin increased and consequently the concentration of Ciprofloxacin that entered to bacteria increased.

Moreover, the increasing in the antibacterial activity may refer to the good encapsulation of micelle to the ciprofloxacin which hence maximizing its concentration and subsequently lead to high exposure against the bacteria.

Due to these results, I found that the best gel was the cetrimide ciprofloxacin gel that’s due to its good antibacterial activity with the presence of cetrimide as antiseptic which work as synergism with the encapsulation in order to increase the antibacterial activity also it had good topical dosage form characteristics.

Conclusion

The desired new Ciprofloxacin gels successfully formulated by encapsulation Ciprofloxacin HCl inside three kinds of surfactants the SDS, Tween 20 and Cetrimide. The three encapsulated Ciprofloxacin gels seem to improve the solubility of the Ciprofloxacin with taken into consideration saving the properties of good gel dosage form with suitable excellent release of the Ciprofloxacin. The modified ciprofloxacin gels showed a good solubility in water and they characterized by the required characterization techniques. There was a huge improvement in the antibacterial activity of the three modified ciprofloxacin gels against the two tested strains of bacteria in comparison with the ciprofloxacin and surfactants alone. The improvement was marvelous against *S.aureus* and *P.aeruginosa*.

Limitation and Suggestions for future work

Future work may include:

1. Studying the antibacterial activity of the three modified ciprofloxacin gels against other different strains of bacteria both (resistance and sensitive).

2. In vivo experiments on animals to examine the effectiveness of the three modified ciprofloxacin gels.

3. Extending this work using other surfactants.

4. Comparison between the three gels in order to achieve the best gel with the maximum loading of ciprofloxacin and the best antibacterial activity.

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جامعة النجاح الوطنية

كلية الدراسات العليا

صياغة وتقييم ثبات المستحضرات الموضعية للسيبروفلوكساسين

إعداد

عبير فاروق منصور

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قدمت هذه الأطروحة استكمالاً لمتطلبات الحصول على درجة الماجستير في العلوم الصيدلانية، بكلية الدراسات العليا، في جامعة النجاح الوطنية، نابلس- فلسطين.

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الملخص

مع ازدياد عدد الإصابات بالأمراض المعدية، مقاومة المضادات الحيوية والأسباب التي تهدد الحياة، يعمل العديد من الباحثين على تطوير مناهج بديلة وتصنيع أنظمة جديدة لتوصيل الدواء لاجتياز عيوب المضادات الحيوية التقليدية. الهدف من هذه الدراسة هو تغليف الحامض الهيدروكلوري للسبروفلوكسين في ثلاثة انواع مختلفة من منشطات السطح مثل الفاعل بالسطح الأنيوني (سلفونات اللورلي الصوديوم)، الفاعل بالسطح الكاتيوني (سيترامايد) والفاعل بالسطح غير ايوني (توين 20). قد يساعد التغليف من ناحية على تحسين سلوك الاختراق للسيبروفلوكساسين ومن ناحية أخرى يقلل من الجرعات المستخدمة. الهدف الآخر لهذا البحث هو صياغة ثلاثة اشكال مغلفة ومستقرة كشكل صيدلاني موضعي (جل الحامض الهيدروكلوري للسبروفلوكسين) من أجل معالجة العدوى الموضعية. لقد ادى تغليف السيبروفلوكساسين إلى زيادة واضحة في تحميل السيبروفلوكساسين مع ازدياد قابلية الذوبان. لحسن الحظ، أظهرت دراسة نمط خروج الدواء ان حوالي 90 ٪ من حمض السيبروفلوكساسين المحمل خرج عند درجة حموضة 5.5 في الساعات الثلاث الأولى. تميزت كل من الثلاثة أنواع من الهلامات المحضرة على أنها مستقرة وواضحة ومتجانسة مع خصائص الريولوجيا الجيدة. علاوة على ذلك، فإن التحسن المزدهر في نتائج النشاط المضاد للبكتيريا يدل على أن السيبروفلوكساسين المغلف حديثًا كان لديه نشاط مضاد للبكتيريا أفضل ضد بكتريا المكورات العنقودية الذهبية وبكتريا الزائفة الزنجارية مقارنة بالسيبروفلوكساسين وحده.