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Formulation of topical products with antiviral and antibacterial activity

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A Thesis

entitled

Formulation of Topical Products with Antiviral and Antibacterial Activity

by

Mei Xin Chen

Submitted to the Graduate Faculty as partial fulfillment of the requirements for the

Master of Science Degree in Pharmaceutical Sciences,

Industrial Pharmacy Option

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The University of Toledo

December 2014

An Abstract of

Formulation of Topical Products with Antiviral and Antibacterial Activity

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Antimicrobial agents are drugs, chemicals, or other substances that either kill or slow the growth of microbes [1]. Antibacterial agents which kill bacteria, antiviral agents which kill viruses, antifungal agents which kill fungi, and antiparisitic drug which kill parasites are antimicrobial agents that are in use today [1, 2]. They have been used for the last 70 years to reduced illness and death from infectious diseases [1]. The necessity of antimicrobial agents has increased due to the development of resistance to antimicrobial agents. The antibacterial and antiviral properties for zinc sulfate and copper sulfate are well-known in agriculture and pharmaceutical industries [3-6]. The present research aims at formulating various topical dosage forms including a cream and gel with antiviral and antibacterial activity. Zinc sulfate and copper sulfate were used as the model drug substances. A cream and two gels formulations (C1, G1 and G5) were successfully incorporated with the drugs. C1 was prepared with oil-in-water emulsion cream base, G1 was prepared with carrageenan-based gel and G5 was designed by using hydroxylpropylmethyl cellulose (HPMC) based gel. Their physicochemical properties including color, physical appearance, homogeneity, and texture were evaluated. All

formulated products were analyzed for spread ability, drug content, pH, viscosity and in vitro antibacterial studies against escherichia coli and staphylococcus aureus. The antibacterial activity for all formulations was determined and compared with the commercial products when a same amount of formulations were applied. The stability study of the formulations on physicochemical properties, viscosity and antibacterial activity was carried out at 4°C, 25°C and 40°C in glass and plastic containers for over a 12 week period. It was found that all of the formulations were good in appearance and homogeneity. The values of spreadability indicated that the formulated products exhibited better spreadibility than the commercial products. Further study is needed for drug content analysis due to a low percent of drug content was obtained. The pH for all formulations with drug was found to be lower than the formulations without drug. The viscosities for the formulated products at 10 rpm were found to be 44000 cps for C1, 33500 cps for G1 and 13900 cps for G5 with pseudoplastic behavior. Zinc and copper were found to have a synergistic antibacterial effect when they were used together in formulations. The desirable antibacterial activity for all formulations was observed in the concentration that were greater than 2% in both tested microorganisms, therefore, the final concentration that used in the formulations was 3% of both zinc sulfate and copper sulfate. The formulations showed significant zones of inhibition when compared with commercial products. The physicochemical properties for all three formulations were found to be stable at 4°C and 25°C for over a 12 week period in both glass and plastic containers. However, the results for viscosity and antibacterial activity were not consistent in all formulations. Therefore, further studies were suggested to provide an improved formulation.

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Chapter 1

Introduction

1.1 Topical drug delivery system

There are two categories of drug products that are topically administered through the skin. The category includes products that are applied for local action. In this case, the active ingredient(s) stay on the skin surface or penetrate through the epidermal layers and may reach the dermis, but are not absorbed into the blood circulation. This category is usually defined as topical drug delivery system. The other category is termed transdermal drug delivery or transdermal patches that are applied for their systemic effect. The active ingredients are delivered into the general blood circulation to produce a therapeutic response by traversing through the different layers of the skin. Transdermal delivery systems are usually physical devices, such as patches which are applied to the skin. They release their active ingredients via different mechanisms due to their composition and method of fabrication [7-9].

Figure 1-1: Structure of the skin [10].
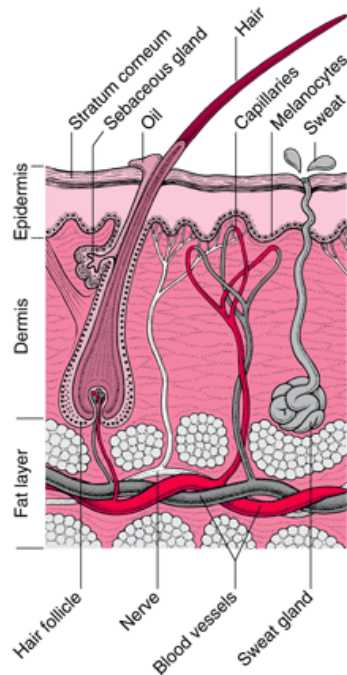
Topical drug delivery systems are administered to a particular site on the outer surface of the body. They deliver drugs locally rather than systemically to treat local dermatological conditions [8, 11, 12]. They are used when a local effect of a drug is desired. Examples of drugs delivered topically include corticosteroids, antifungals, antivirals, antibiotics, antiseptics, local anesthetics, and antineoplastics. Topical agents that act by physical action would include protective ingredients, adsorbents, emollients, and cleansing agents [8].

Conventional topical dosage forms are classified into three categories: liquid, semi-solid and solid. Liquid topical dosages include low viscosity emulsions (otherwise known as lotions), suspensions, and solutions. Collodions, foams, ointments, pastes, creams and gels are included in semi-solid topical dosage forms. Solid topical dosage forms include powders, patches, gauzes, tapes and sticks. These dosage forms vary widely according to their physical characteristics [7, 8].

There are numerous advantages for topical drug delivery over other routes of administration. The primary advantage for topical delivery is that it has minimal systemic side effects when the topical product is applied to the affected tissues [13]. Secondly, topical drug delivery systems bypass first pass metabolism by transferring the active through the skin. This avoids the metabolism of the active before it reaches the target site of action. Topical products are easy to apply and suitable for self-medication, thus can increase patient compliance. Topical drug delivery does not need complicated application procedures and, therefore, it avoids the risks and inconveniences of intravenous drug delivery. Furthermore, the skin occupies the largest surface area on the human body. It provides a relatively large area of application and has the ability to deliver drug more selectively to a specific site. It can maintain a high local concentration of drug in the surrounding tissues over an extended period, thus reducing dosing frequency. Topical administration can reduce the risk of systemic side-effects since the drugs do not reach the systemic circulation [14].

However, there are also some disadvantages. These include the following: skin irritation due to drugs or excipients; poor permeability through the skin for some medications; this route is not suitable for drugs with large particle size; the possibility of allergic reactions; and the denaturation of drugs due to the enzymes found in the epidermis [14].

A variety of semi-solid topical dosage forms were formulated in the course of this research including a cream and two gels with antiviral and antibacterial activity. The characteristics and properties of these various semi-solid topical dosage forms will be discussed in the following introduction.

1.2 Gels

Gels are semisolid systems consisting of dispersions of small or large molecules in an aqueous or hydroalcoholic liquid vehicle by the addition of a gelling agent/polymer [9, 15]. Almdal et al. have proposed that the term 'gel' should be limited to systems which fulfill the following phenomenological characteristics: (a) they consist of two or more components, one of which is a liquid, being present in substantial quantity; and (b) they are soft, solid, or solid-like materials [16]. Gels are sometimes defined as jellies. Gels can be classified as single-phase and two-phase system. Single-phase gels consist of organic macromolecules uniformly distributed throughout a liquid with no apparent boundary between the dispersed macromolecule and liquid. Two-phase systems usually refer to a gel mass consisting of floccules of small distinct particles.

A gelling agent, also called polymer, and water are the primary ingredients used to form gels. Additional ingredients can include one or more drug substances; additional solvents, such as alcohol, glycerin and propylene glycol; preservatives, such as methylparaben and propylparaben; and chelating agents, such as edetate disodium may be used to formulate gels. Certain polymers need neutralization, a process of shifting the pH of the product, to gain optimal viscosity. Such polymers include carbomer and certain types of cellulose derivatives. In such cases, pH buffers, such as triethanolamine may also be used in the formulation. Medicated topical gels can be administered through various routes, including the skin, the eye, the nose, the vagina, and the rectum [9]. Two primary properties of gels that will be discussed are swelling and syneresis. Syneresis refers to the expulsion of liquid from the gel or through a shrinkage process. Swelling, on the other hand, is the opposite of syneresis [17, 18]. The mechanism of swelling of gels can be

explained by exerting osmotic pressure on the polymer in the gel. This acts as a driving force for water to enter the gel and causes swelling [19]. Most of the polymers have the ability to adsorb water or other liquid to increase the volume of the gel. The degree of swelling in a gel depends upon the temperature of the system, pH of the solution and the individual nature of the gel [20]. The development of syneresis usually occurs in the ageing of unstable gels. There are some external factors which play an important role in inducing syneresis. The most important factors include temperature, pressure of the electrolyte, dispersion medium, state of the gel and mechanical disturbance of the gel [19, 20].

Type of gels

Gels can be further classified into organogels and hydrogels. The difference between organogels and hydrogels is based on the external solvent that is used to disperse the polymer [15, 21, 22].

1.2.1 Organogels

Organogels are thermodynamically stable, clear, viscoelastic, and biocompatible semi-solid systems, in which an apolar phase is immobilized by a three-dimensional network composed of self-assembled structures of compounds regarded as gelators [22, 23]. Some common examples of gelators include sterol, sorbitan monostearate, lecithin and cholesteryl anthraquinone derivates [24]. Organogels can be subdivided based on the nature of the gelling molecule: low molecular weight organogelators and polymeric gelators. Both of these subdivisions can be distinguished based on the nature of the

intermolecular interactions. Low molecular weight gelators self-assemble via physical intermolecular interactions composed of entangled networks of solid-fiber matrix and/or fluid-fiber matrix. Conversely, polymeric gelators solidify organic solvents based on physical intermolecular interactions to form a cross-linked matrix and chemical intermolecular interactions to form entangled-chain linked matrix [21].

1.2.2 Hydrogels

Hydrogels are three dimensional, cross-linked structures composed of hydrophilic homopolymers or copolymers, which are insoluble due to the presence of chemical crosslinks or physical crosslinks, such as entanglements or crystallites [22, 25, 26]. These hydrogels have the ability to swell in an aqueous environment due to the thermodynamic compatibility exhibited with water. Hydrogels exhibit a wide variety of applications in the medical and pharmaceutical areas. They can be formulated into numerous physical forms, including slabs, microparticles, nanoparticles, coatings, and films [27, 28]. Due to the various properties of hydrogels, including high water content and soft consistency, hydrogels can be used as contact lenses, membranes for biosensors, linings for artificial hearts, materials for artificial skin, and drug delivery devices [26].

Advantages of hydrogels [29, 30] include:

- Easy to modify.

- Highly biocompatible.

- Have good transport properties.

- Can act as protectants for cells.

- Provide a soothing effect on the skin as compared to an oily feeling caused by the application of ointments.

- Relatively deformable and can conform to the shape of the surface to which they are applied.

- Some have muco- or bioadhensive properties in immobilizing them at the site of application or in applying them on non-horizontal surfaces.

- Can increase the permeation of drugs through the keratinized epidermis.

- Can be biodegradable or bioabsorbable.

Disadvantages of hydrogels [29, 30] include:

- Expensive to produce and use.

- Non-adherent; they may need to be secured by a secondary dressing.

- Exhibit low mechanical strength.

- Can be hard to handle and difficult to load with drugs and/or nutrients.

- May be difficult to sterilize.

Classification of Hydrogels

Hydrogels can be grouped based on their origin. We can distinguish among natural polymer hydrogels, synthetic polymer hydrogels and the combinations of the two groups [29]. Examples for natural polymers include proteins, such collagen and gelatin, and polysaccharides, such as starch, alginate, and agarose. Natural polymers are usually

biocompatible and biodegradable and they support cellular activities. However, they may contain biological pathogens or induce an immune response. Other limitations of natural polymers include their low mechanical strength and high batch variation. Synthetic polymers that form hydrogels are traditionally prepared using chemical polymerization methods. They are made from monomers such as vinyl acetate, acrylamide, ethylene glycol and lactic acid. Polymers can be synthesized precisely to give a wide range of properties that result in low risk of biological pathogens and evoking an immune response. On the other hand, synthetic polymers have low biodegradability and may contain toxic substances.

Natural polymers can be subdivided into neutral and ionic (anionic polymers, cationic polymers, and amphipathic) hydrogels according to the nature of the side groups. In addition, they can also be classified as amorphous, semicrystalline, hydrogen-bonded structures, supermolecular structures, and hydrocolloidal aggregates [26, 31].

Hydrogels can be classified into two major categories: permanent (chemical gel) and reversible (physical gel). Permanent (chemical gels) are covalently cross-linked networks that always result in a strong gel. There are three main chemical gelation processes, which includes: condensation; vulcanization; and addition polymerization. An equilibrium swelling state is accomplished. This depends on the polymer-water interaction parameter and the crosslink density [22, 25]. Accordingly, reversible (physical gels) are not homogeneous, since clusters of molecular entanglements, or hydrophobically or ionically-associated domains can create non-homogeneity. The network of physical gels are held together by molecular entanglements, and/or secondary forces including ionic, H-bonding or hydrophobic forces. All of these interactions are

reversible, and can be disrupted by changes in physical conditions or application of stress [32-34].

1.2.2.1 Stimuli responsive or "smart" hydrogels

There are some hydrogel products that are sensitive to environmental conditions. They can display changes in the swelling characteristic of the network structure depending on the external environment [25]. Because of the capability of hydrogels for swelling and de-swelling reversibly in water and retaining large volume of liquid in swollen state, they can be designed to have controllable responses by changing the external environmental conditions (as shown in Figure 1-2). A variety of physical and chemical stimuli can control the shrinkage and expansion of the polymers. Physical stimuli include temperature, electric or magnetic field, light, pressure, and sound; while the chemical stimuli include pH, solvent composition, ionic strength, and molecular species [31].

Figure 1-2: Stimuli response swelling hydrogel [31].
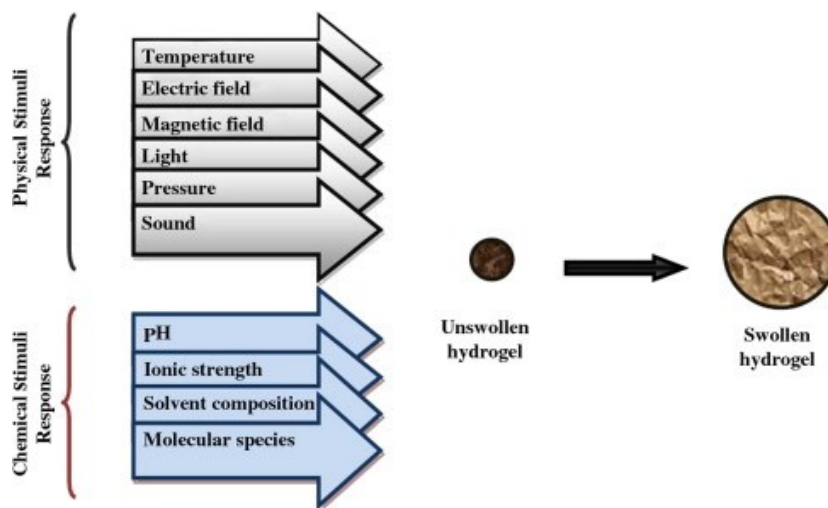
pH-sensitive hydrogels

Hydrogels, which exhibit pH-dependent swelling behavior, usually contain either acidic or basic groups, which respond to changes in environmental pH by gaining or losing protons. In aqueous media with appropriate pH and ionic strength, the pendant groups can ionize and develop fixed charges on the gel which is depicted in Figure 1-3. This process results in the uptake of solvent in the network due to electrostatic repulsions.

Figure 1-3: Expansion of an environmentally responsive hydrogel due to ionization of pendent groups at specific pH values [26].

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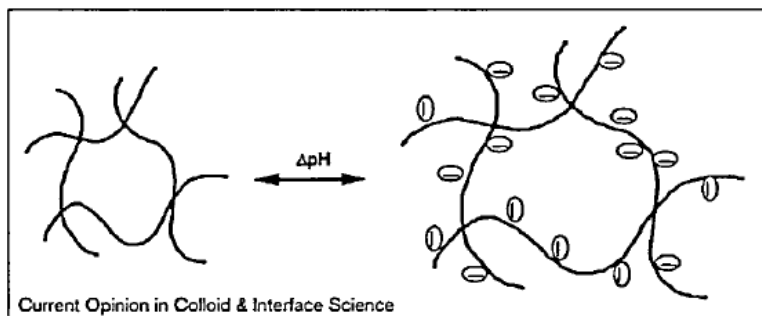
Anionic materials contain pendent groups such as carboxylic or sulfonic acid, ionize in media, which are at a pH above the pK_a of the ionizable group. Thus, an increased hydrophilicity of the network and greater swelling ratio will be obtained as the degree of ionization increases. On the contrary, ionization of cationic materials, such as amines will occur when the pH of the environment is below the pK_b of the ionizable species. Therefore, the hydrogel becomes increasingly hydrophilic and will swell to an increased level by increasing ionization and electrostatic repulsions in a low pH media. The human body presents variations in pH along the gastrointestinal tract (GIT), and also in some specific areas such as certain tissues or sub-cellular compartments. Therefore, pH-sensitive polymers play an important role in drug delivery especially along the

gastrointestinal tract [35]. Enteric coating that employs pH-sensitive polymers, prevent the degradation of drugs in the gastric fluid and the drugs are then cause gastric irritation [36]. Moreover, pH-sensitive hydrogels also play an important role in insulin delivery for diabetes therapy [37, 38]. Wound pH-responsive sustained release of therapeutics from a poly(NIPAAm-co-AAc) hydrogel was reported by Banerjee et al. They found that a pH-responsive sustained release delivery system could be beneficial for the effective treatment of wounds due to the influence of wound pH and the residence time and activity of various growth factors during wound healing [39].

Temperature-sensitive hydrogels

Polymers that are sensitive to temperature have the ability to swell or de-swell by changing the temperature of the surrounding fluid. Temperature responsive polymers exhibit a critical solution temperature at which the phase of polymer and solution is changed in accordance with their composition. They can be classified as positive or negative temperature-sensitive systems. A positive temperature-sensitive hydrogel exhibits one phase above a certain temperature and phase separation below it. Thus, possessing an upper critical solution temperature (UCST). These hydrogels contract upon cooling below the UCST. On the other hand, negative temperature-sensitive hydrogels have a lower critical solution temperature (LCST). They appear as monophasic below a specific temperature and biphasic above it. These hydrogels contract upon heating above the LCST [35, 40, 41]. The primary temperature-sensitive polymers that are used for medical and pharmaceutical purposes are poly(acryloyl-L-prolinemethylester), poly(vinylcaprolactam), poly(vinylmethyl ether), hydroxypropyl cellulose, poly(N-

isopropyl acrylamide), and polyphosphazene derivatives [41]. There are also some naturally occurring polymers that can be used to prepare thermoreversible gels, for example, gelatin and carrageenan [42]. These polysaccharide polymers adopt a random coil conformation in solution as the temperature raises. A continuous network is formed by partial helix formation upon cooling [42]. The focus of the present thesis will be on carrageenan and hydroxypropyl-methylcellulose (HPMC) in the preparation of topical formulations with antiviral and antibacterial activity.

1.2.2.1.1 Carrageenan

Carrageenan is non-toxic, cheap, biodegradable, biocompatible and natural polysaccharide that is obtained from edible red seaweeds. It is derived from a number of seaweeds of the class Rhodophyceae, which is commonly found in the Atlantic Ocean near Great Britain, Europe and North America. Carrageenan is an anionic sulfated linear polysaccharide that is widely utilized as a gelling agent, thickening agent, and emulsifying agent in experimental medicine, pharmaceutical formulations, cosmetics and personal care products, and the food industry [43]. In general, carrageenan consists of a linear galactam backbone with alternating disaccharide repeating units of 1,4-linked α-D-galactose and 1,3-linked-β-D-galactose and a variable proportion of sulfate groups on different positions [44, 45]. There are three major forms of the polysaccharides of carrageenan of commercial interests: namely, kappa (κ), iota (ι), and lambda (λ) carrageenan. These different types of carrageenans can be distinguished from each other by the number and position of the ester sulfate groups on the repeating galactose units

[46]. They have different properties due to their slightly varied chemical structures, which are shown in Figure 1-4 and Table 1.1 [44, 45, 47].

Figure 1-4: Chemical structures of repeating units of various types of carrageenan [47].
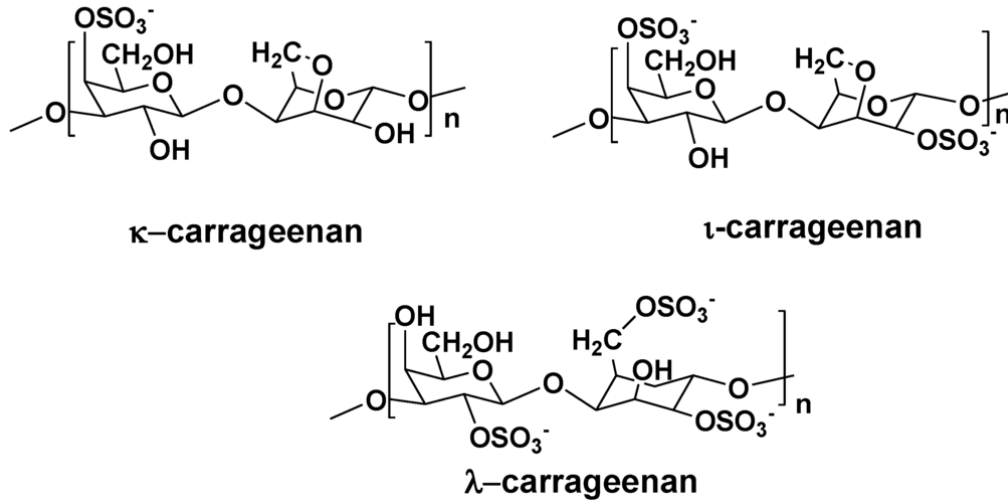
Table 1.1: An overview of the types and properties of carrageenan. Modified from [44].

Properties of carrageenan	Type of carrageenan		
	Lambda	Iota	Kappa
Solubility			
Hot water (80°C)	Soluble	Soluble	Soluble
Cold water (20°C)	All water soluble	Na^+ salt soluble, Ca^{2+} salt gives thixotropic sols	Na^+ salt soluble, limited swelling of K^+, Ca^{2+} salts
Gelation			
Effect of cations	Non-gelling	Strongest gels with Ca^{2+}	Strongest gels with K^+
Gel texture	N/A	Elastic	Brittle
Shear reversible gel	N/A	Yes	No
Syneresis	N/A	No	Yes
Salt tolerance	Good	Good	Poor
Stability in acid	Hydrolysis	Hydrolysis of solution, Gels are stable	Accelerated by heat

The mechanism of gelation for the these types of carrageenan has been adequately discussed in the literature [48]. Normally, the gel formation involves a conformational transition of the carrageenan molecule according to the following scheme, seen in Figure 1-5 [46, 49].

Figure 1-5: Models of conformational transition of κ-carrageenan and ι-carrageenan [46].
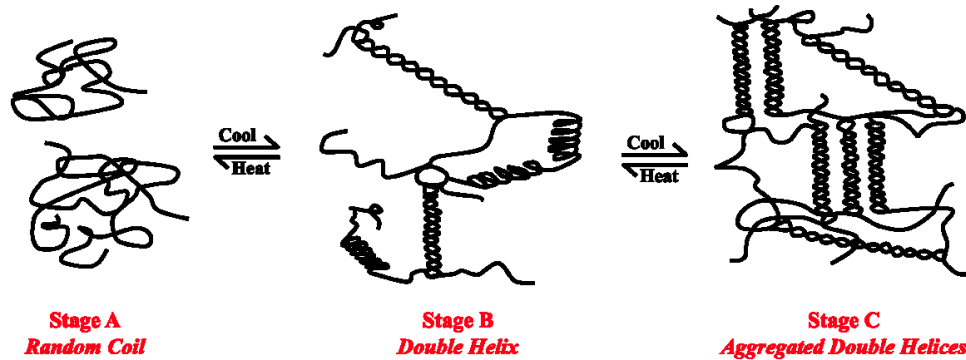
The gelling ability of a carrageenan depends on the ability of carrageenan itself to form ordered helical structures from the regular repeating disaccharides [50]. Among the three major types of carrageenan, the structure of (κ) and (ι) carrageenan allows the double helices to form a thermoreversible three-dimensional network gel, which is stabilized by hydrogen bonds between the double helices and thus, they are gel-forming carrageenans. On the other hand, (λ) carrageenan has a structure that lacks the ability to form a double helix, which then can be characterized only as a thickening agent [48, 51, 52]. The gelation of (ι) carrageenan solution is normally induced by the conformational transition of random coil to double helix (Figure 1-5) upon cooling, whereas (κ) carrageenan has a two-step gel mechanism from transition random coil to double helix and to aggregated helices (Figure 1-5), to form a brittle gel [49, 51, 53]. In additional to the gel formation of (κ) and (ι) carrageenan, is well established in that the gelling ability of carrageenan can be dependent on the presence of a suitable cation, typically potassium or calcium. The

cations associated with the sulfated groups play an important role in the mechanism of gelation by formation of intramolecular salt bridges [45, 52, 53].

1.2.2.1.2 Hydroxypropylmethyl cellulose (HPMC)

Cellulose is a natural polysaccharide that is insoluble in hot or cold water because of its strong intramolecular hydrogen bonding. However, cellulose derivatives become water soluble by adding hydrophilic moieties to the cellulose [54-56]. Hence, the advantages of modified cellulose derivatives include enhancing water retention capacity, pseudoplastic behavior, film forming properties and complexation. Moreover, their biocompatible property makes them widely used in applications in food, pharmaceutical, and cosmetic industries [56]. The most common cellulose derivatives include methyl cellulose, hydroxypropyl cellulose, carboxymethyl cellulose and HPMC [55]. The chemical structure of natural cellulose is differed from HPMC by replacing the hydroxypropyl and methyl groups from the hydroxyl groups, which is seen in Figure 1-6 (a-b).

Figure 1-6: Chemical structure of (a) natural cellulose, and (b) hydroxypropylmethyl cellulose (HPMC) [55].

15
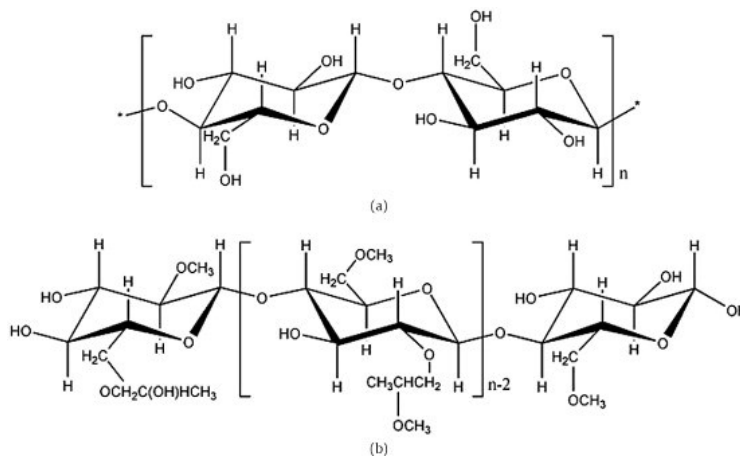
According to the United States Pharmacopeia (USP), HPMC can be classified into three types based on the chemical substitution of the ether: E (hypromellose 2910), F (hypermellose 2906), and K (hypromellose 2208) [55]. These types of HPMC can be distinguished by: (i) the methoxy group content; (ii) the hydroxypropoxyl group content; and (iii) the molecular weight [55, 57, 58]. The aqueous solution of HPMC performs temperature-dependent behavior in order to form a gel. There are some studies which show that the presences of methoxy residues on HPMC are responsible for gelation by their hydrophobic interaction, while the presences of hydroxypropyl residues play an important role in the gelation characteristics in a temperature-dependent manner. At low temperature, the polymers are hydrated and cause little polymer-polymer interaction other than simple entanglement. However, they tend to lose their water of hydration as the temperature is increased, and hence, a decline in relatively viscosity is observed [42, 54, 55, 57].

1.3 Creams and lotions

The USP defines creams as "a semisolid emulsion that contains more than 20% of aqueous phase and/or less than 50% of oleaginous phase for external application to the skin" [59, 60]. Ansel et al. proposed to define a pharmaceutical cream as follows: "semisolid preparations containing one or more medicinal agents dissolved or dispersed in either a water-in-oil (W/O) emulsion or an oil-in-water (O/W) emulsion or in another type of water-washable base." [9]. Another type of dosage form that shares similar characteristics with creams is a lotion. Lotions are low viscosity emulsions that are used for external application to the skin [7]. An emulsion is a two-phase system consisting of

at least two immiscible liquids, in which one phase is dispersed throughout a vehicle. There are two types of emulsions: O/W and W/O. The O/W emulsions are emulsions with an oleaginous internal or dispersed phase and an aqueous external or continuous phase. They are non-occlusive and used mostly as water-washable bases. These emulsions are mainly formulated for the topical application of water-soluble drugs with a local effect. They are more acceptable to consumers because they provide a pleasant skin feel and are easily washed from skin surfaces. O/W emulsions do not give a greasy texture or 'feel' since they contain the oils in the internal phase, which can increase patient compliance. On the other hand, the system designated as a W/O emulsion has an aqueous phase dispersed as the internal phase in an oleaginous external phase. They act as an emollient or occlusive by hydration of the upper layers of the stratum corneum (SC, the outer layer of the epidermis) and the inhibition of evaporation of eccrine secretions. In addition, W/O emulsions are useful for cleansing the skin of oil-soluble dirt when compared to O/W emulsions, which are less efficient as cleansers. They are more efficient in preventing the drying of the SC and giving moisture to the skin by producing a coherent, water-repellent film. [7, 60-62]. The choice of an emulsion to be applied to the skin can be determined by factors including the nature of the therapeutic agents, the desirability for an emollient or tissue-softening effect, and the condition of the skin [9]. There are several simple methods which can be performed to identify the emulsion type. The most common of these include, miscibility test with oil or water. An emulsion will only be miscible with liquids that are miscible with its continuous or external phase and as a result, an O/W emulsion is miscible with water, a W/O emulsion with an oil. Moreover, conductivity measurements can be used to distinguish between O/W and W/O

emulsions. Systems with an aqueous continuous phase will have a much higher conductivity than the systems with an oleaginous continuous phase. Furthermore, another method that can be used to determine the type of emulsion is staining or dye tests. Water-soluble and oil-soluble dyes are used, one of which will dissolve in and color the continuous phase. Therefore, it can be determined that it is O/W emulsion by observing under microscopic examination, if a water-soluble dye has been taken up by the continuous phase[60, 63, 64].

1.3.1 Emulsion Stability

Emulsion stability can be investigated by observing the properties of an emulsion that remains unchanged over a certain period of time. The longer the period of time an emulsion maintains its properties, the more stable it is. However, in fact, emulsions are thermodynamically unstable [63]. That means any emulsion will breakdown as the contact area of two phases is decreased. This can be explained from the Gibbs free energy contained in an emulsion. Gibbs free energy states;

$$\Delta G = \Delta A \, \gamma$$

where:

ΔG is the change in free energy
ΔA is the change in total surface area of dispersed particles
γ is the interfacial tension

A stable emulsion should show negative overall free energy with a large surface area by decreasing the interfacial tension, which is thermodynamically favorable [9, 65-67]. There are five main phenomena that can lead to emulsion instability: (1) creaming; (2)

coalescence; (3) flocculation (Brownian flocculation); (4) Disproportionation (Ostwald ripening); (5) phase inversion [65, 67].

Figure 1-7: Schematic representation of the various types of instability occurring in emulsions [68].
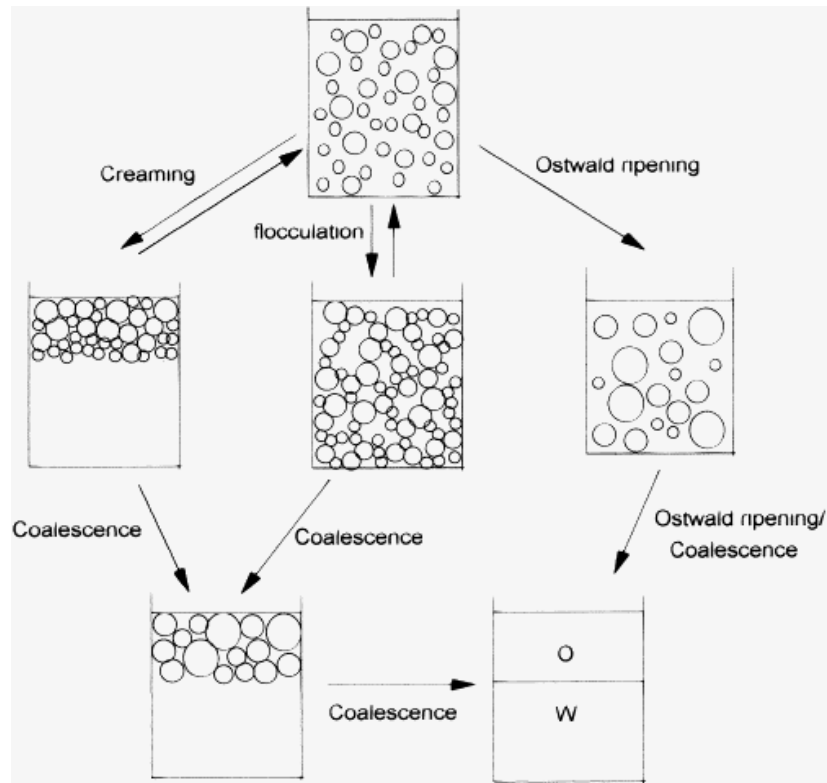
These phenomena alter the properties of emulsion, which depends on the particle size distribution and density difference between the two phases.

1.3.1.1 Creaming

The process of creaming occurs due to the influence of external forces such as gravitational or centrifugal. It is a separation of the emulsion into two phases by the relocation of the less dense phase to form a top layer of emulsion in a container [65, 67, 69]. The separation of the emulsion due to creaming usually depends on the droplet

concentration, polydispersity and inter-particle interactions in the emulsion [70]. In another words, creaming is favored when a large difference in liquid densities and a large droplet dimensions are observed, thus causing a low volume fraction [71]. This is a reversible process. Therefore, the creamed portion of an emulsion may be redistributed upon shaking or mixing [9]. Several solutions are discussed in order to obtain a stable emulsion: (1) minimize the density difference between the oil and water phase; (2) reduce the interfacial tension by reducing the droplet size; (3) increase the viscosity of continuous phase by adding a thickener or a gelling agent; (4) increase the droplet concentration to prevent the movement of droplets [9, 66].

1.3.1.2 Coalescence

This result from two or more droplets merging into a larger one is due to the disruption of the liquid film between the droplets. This irreversible process separates the emulsion into two distinct liquid phases completely [65]. A thin film of the continuous phase acts as a barrier between the droplets to prevent the contact of droplets, and thus, droplets coalescence is observed as the film barrier is disrupted. Coalescence can be prevented by reducing the contact of droplets and the rupture of the interfacial membrane or film barrier. The presence of emulsifying agents adsorbed at the oil-water interface has shown a greatly effect on preventing droplet coalescence [66].

1.3.1.3 Flocculation

Flocculation is the aggregation of the droplets into larger units without coalescence occurring [65, 69]. The van der Waals force plays an important role in the

process of flocculation. Since the van der Waals force is weak, then there is not sufficient repulsion to hold the droplets apart at a distance, and flocculation may occur. Flocculation may be reversible when the system displays weak flocculation or irreversible when the system displays strong flocculation [65, 66]. This process can be subdivided into two general categories: sedimentation flocculation and Brownian flocculation. Sedimentation flocculation can be distinguished from Brownian flocculation by the way that the droplets aggregate, in which the sedimentation of the droplets are vertically linear in sedimentation flocculation and at random Brownian movement of the droplets is resulted in Brownian flocculation [69].

1.3.1.4 Disproportionation

Disproportionation can also be called as Ostwald ripening. This phenomenon can be determined by the solubility of the disperse droplets and the particle size distribution [65]. This process can be clearly observed from the effect of an increase in the average radius of the droplets with time when the smaller droplets dissolve onto the larger droplets [68]. Previous studies have shown that Ostwald ripening can be retarded by adding a component that is soluble in the dispersed phase but essentially insoluble in the continuous phase, for example, adding a highly hydrophobic molecule to the oil phase [72].

1.3.1.5 Phase inversion

This is an instability process of an emulsion which occurs from an exchange between the dispersed phase and the medium. For example, an O/W emulsion can invert

to a W/O emulsion, and vice-versa. There are two types of phase inversion of emulsions: (1) transitional phase inversion and (2) catastrophic phase inversion. Transitional phase inversion is induced by changing factors such as temperatures, solvent and/or electrolyte concentration, whereas catastrophic phase inversion is induced by increasing the volume fraction of the disperse phase [73, 74].

1.3.2 Emulsifying Agents

As mentioned previously, emulsions are thermodynamically unstable [63]. Therefore, emulsifying agents, otherwise known as emulsifiers are usually used in the formulation of emulsions to stabilize the emulsion by reducing the interfacial tension [64]. Most emulsifying agents are surface-active agents (surfactants). The use of surfactants in emulsions reduces the interfacial tension of the two immiscible liquids by decreasing the repellent force between the liquids and diminishing each liquid's attraction for its own molecules, according to the surface tension theory of emulsification [9, 64, 75]. Qualities and characteristics of an ideal emulsifying agent include the following:

1. Surface-active and reduces surface tension to below 10 dynes/cm;

2. Adsorbs quickly around the dispersed drops as a condensed, non-adherent film which prevents coalescence;

3. Imparts to the droplets an adequate electrical potential so that mutual repulsion occurs;

4. Increases the viscosity of the emulsion;

5. Effective in a reasonably low concentration;

6. Odorless, tasteless, or colorless, non-toxic, and nonirritant;

7. Compatible with other excipients and must not interfere with the stability or efficacy of the therapeutic agent;

8. Stable and not deteriorate in the preparation [9, 63, 64].

1.3.2.1 Classification of emulsifying agents

According to Remington, emulsifying agents can be grouped according to the mechanism of action of emulsifying agents, which is the type of film formed at the interface between the two phases. Generally, there are three types of film formation with various emulsifying agents at the oil/water interface. The first type of films is a monomolecular film, which is a coherent, flexible film formed by the surfactant. This type of film promotes lower interfacial tension and results in a more stable emulsion due to a proportional reduction in the surface free energy. The examples of emulsifying agents that form monomolecular films include potassium laurate and polyoxyethylene sorbitan monooleate. Multimolecular films are another type of film which has strong, rigid films and are mostly formed by hydrocolloids which produce O/W emulsions. They are primarily formed around droplets of dispersed oil by hydrated lyophilic colloids that are adsorbed at an interface without a lowering in surface tension. As a result, their efficiency depends on the strength of the interfacial film to enhance the emulsion stability. Acacia and gelatin are examples of multimolecular film formation as

emulsifying agents. Lastly, films formed by solid particles demonstrate a stable emulsion. The solid particles, such as bentonite, graphite, and magnesium hydroxide, are small in size compared to the droplet of dispersed phase and they are wetted by both phases to some extent in order to remain at the interface [63].

There are three main categories of emulsifying agents depending on their sources: naturally occurring, synthetic surfactants and finely divided solids. The naturally occurring emulsifying agents come from natural sources, such as vegetables or animals. They are mostly carbohydrate or polysaccharide materials and include: acacia, tragacanth, agar, carrageenam, and pectin. These polysaccharide materials are added to water and form hydrophilic colloids to produce O/W emulsions. They promote good stability in pharmaceutical emulsions; however, they have a high risk to develop microbial contamination and degradation. Therefore, it is necessary to preserve emulsions that are formulated with polysaccharide materials to protect against microbial attack. Acacia is the most common emulsifying agent in the preparation of extemporaneous emulsions. This gum is frequently used in the preparation of emulsions due to its ability to provide long-term stability over a wide pH range. It stabilizes an emulsion by forming a thick film at the oil-water interface to act as a barrier to coalescence. Moreover, tragacanth and agar are generally used as a thickening agent by increasing the viscosity of an emulsion and prevent creaming. Methylcellulose and carboxymethylcellulose are examples of semi-synthetic polysaccharides. These are low-viscosity grades which act as thickening agents that are usually dissolved or dispersed in water or aqueous solvent. These materials retard particle settling and provide dispersion stability in both suspensions and emulsions. The other emulsifying agents that come from animal sources

are sterol-containing substances, such as beeswax, cholesterol, lecithin, wool fat and wool alcohols. Cholesterol is a major constituent of wool alcohols that is obtained by the saponification and fractionation of wool fat. It gives wool fat its capacity to absorb water and form a W/O emulsion. Lecithin is used as an emulsifying agent which is obtained from both plant and animal sources. It is composed of various phosphatides. Lecithin gives the best results at a pH around 8. The derivatives of wool fat or wool alcohol are widely used in the formulations of face cream in the cosmetic industry [76].

The second category of emulsifying agents that are commonly used to formulate emulsion is synthetic surfactants. Rees et al. classified synthetic surfactants based on their ionic characteristics as anionic, cationic, non-ionic and amphoteric [64].

1.3.2.2 Anionic Surfactants

According to Myers, the anionic classification group is the largest class of surface-active materials in general use today. They constitute 70-75% of the total worldwide surfactant consumption [77]. In the anionic surfactant subgroup, the surfactant ion bears a negative charge in aqueous solutions. The negatively charged anions are responsible for their emulsifying ability. They are widely used in external preparations as O/W emulsifying agents. They are only effective when they are in their ionized form and at a more alkaline pH. The anionic surfactants that are available in the pharmaceutical industry include alkali metal and ammonium soaps, soaps of divalent and trivalent metals, amine soaps, and alkyl sulphates.

Ammonium oleate is a good example of an alkali metal and an ammonium soap. Ammonium oleate, is used in the formulation of White Liniment B.P, is produced from

the reaction of ammonia and oleic acid. Alkali metal and ammonium soaps are sometimes called soft soaps. They are salts of fatty acids that have the positive ion which is univalent. Soft soaps produce stable O/W emulsions. However, a high pH is required for this type of emulsifying agents in order for it to be functional. This is due to the fact that these materials will precipitate out as the free fatty acids under acidic conditions. Additional, the free fatty acids are ineffective as an emulsifying agent and thus, emulsions formed from alkali soaps are not stable at a pH lower than 10. These emulsifying agents are incompatible with multivalent cations, high concentrations of electrolytes and high-molecular-weight cations. The main reasons are the anionic portion of the soap becomes inactive when it binds to the high-molecular-weight cations, such as benzalkonium chloride and the replacement of univalent to multivalent may interfere with the stability of the emulsion. Soaps of divalent and trivalent metals will also produce satisfactory emulsions when calcium, magnesium and aluminum salts of fatty acids are used. These salts of fatty acids have a positive ion which is divalent or trivalent. They are called hard soaps. Hard soaps form W/O emulsions and they are incompatible with high-molecular-weight cations like benzalkonium chloride. For example, the production of calcium oleate is obtained from the reaction of oleic acid with calcium hydroxide. Calcium oleate is commonly used in the formulations of Zinc Cream BP and some of the oily calamine lotions and liniments. Triethanolamine is the most widely used amine soap, which is in another class of soaps. They form more stable emulsions than the alkali soap emulsifying agents due to the cation of the amine soap is more balanced and less hydrophilic. Amine soaps have some dissimilarity with alkali soap in that they are effective as emulsifying agents around pH 8 and they are less irritating than the alkali

soaps. However, they are also incompatible with acids and high concentrations of electrolytes. For instance, triethanolamine soaps are commonly used in the formulation of O/W vanishing creams in the cosmetic and pharmaceutical industry [78].

The last class of anionic surfactants is alkyl sulphates or sulphonated compounds. These compounds are an important group of pharmaceutical surfactants. They are sometimes named as detergents. These types of soaps are very hydrophilic and are soluble in water. Sodium lauryl sulfate and dioctyl sodium sulfosuccinate are probably the most frequently used of the alklyl sulphates or sulphonated compounds to produce O/W emulsions. Sodium lauryl sulphate is used with cetostearyl alcohol to produce Emulsifying Wax, which stabilizes preparations such as Aqueous Cream and Benzoate Application. Unlike alkali soaps, this type of soap is not sensitive to high concentrations of electrolytes and thus, they are more stable to acids [9, 60, 63, 64, 79, 80].

1.3.2.3 Cationic Surfactants

In 1938, cationic surfactants were recognized to have bacteriostatic properties. Since then, they have become an important material and have been introduced into hundreds of commercial products. During World War II, numerous new applications for cationic surfactants were developed. Cationic surfactants play an important role as antiseptic agents, fungicides, germicides, fabric softeners and hair conditioners and also have additional bulk chemical applications [77]. Unlike anionic surfactants, cationic surfactants carry a positive charge. Due to their bactericidal activity, they are widely used in wound cleaning. Their aqueous solutions can be used for cleaning contaminated utensils [81]. Alkylamines are cationic surfactants that are mainly used in textile

treatment and sometimes in fabric rinse softeners. Salts of amines are frequently used in hair care applications due to their conditioning and antistatic properties. Quaternary ammonium compounds (often referred to as 'quats') and pyridinium cationic surfactants are extensively used in cosmetics formulations. The reason for their use is cationic emulsifying agents are effective at pH 4-6 which includes the normal pH of the skin, and thus, they play a significant role in topical formulations. However, cationic emulsifying agents are incompatible with anionic surfactants and polyvalent anions [60, 63, 64, 80, 82].

1.3.2.4 Nonionic Surfactants

In nonionic surfactants class, these materials are electrically neutral and do not dissociate. Some of the most important advantages include: nonionic surfactants have a significantly lower sensitivity to the presence of electrolytes in the system than the previously discussed types; they are not susceptible to pH changes; and they possess the proper balance of hydrophilic and lipophilic groups within the molecule. They differ from anionic or cationic surfactants since nonionics have a greater degree of compatibility with other materials. However, they tend to be more expensive. The most commonly used nonionic surfactants include the glyceryl esters, polyoxyethylene glycol esters and ethers, and the sorbitan fatty acid esters and their polyoxyethylene derivatives. Among a variety of nonionic surfactants, the polyoxyethylene family exhibits an inverse temperature-solubility relationship. This means that the solubility of polyoxyethylene in water decreases as the solution temperature is increased. The temperature at which components of the polyoxyethylene surfactant begin to precipitate from solution is

defined as the "cloud point" [77]. In addition, sorbitan esters are commercially available as Spans®. They are usually insoluble in water and promote W/O emulsions. These materials are produced by the esterification of one or more of the hydroxyl groups of sorbitan with either lauric, oleic, palmitic or stearic acids. Polysorbates are another class of nonionic surfactant, which are polyethylene glycol derivatives of the sorbitan esters. They are used in conjunction with the corresponding sorbitan ester to form a complex condensed film at the oil/water interface. Polysorbates are suitable for oral use and some in parenteral preparations due to their low toxicity [60, 77, 81].

1.3.2.5 Amphoteric surfactants

Amphoteric surfactants possess polar head groups which contains both positively and negatively charged groups, based on the pH of the system. The positive charge is mostly carried by an ammonium group whereas the negative charge is often carried by a carboxylate [81]. Since these types of surfactants have both cationic and anionic sites, their properties are influenced by pH. In the amphoteric class, a zwitterionic form of the surfactant, usually obtained around the isoelectric point by changing the charge of the molecule with pH, exhibits the lowest solubility. The anionic form is predominant at high pH, whereas the cationic form is predominant at low pH. The anionic form of surfactants has foam and detergency properties in alkaline condition, while the cationic form of surfactants provides surfactant substantivity. Because of the foam stabilizing effect, thickening capacity and skin-irritation reduction capacity of amphoteric surfactants, they are mainly used as secondary emulsifying agents [60, 81, 82]. Moreover, this type of materials is compatible with other classes of surfactants and possesses a synergism effect

in many formulations [77]. Some formulators had claimed that utilizing cationic surfactant, such as quaternary compounds in combinations with amphoteric surfactants provided unique properties to the hair. These properties include softness, easiness of combing with a lower chance for static buildup. However, these combinations alone are rarely used these days due to the insufficient cleansing property and "over condition" while leaving the hair limp, weighed down, and lacking in body [83]. According to Handbook of Cosmetic Science and Technology [82], amphoteric surfactants are often obtained from acyl ethylenediamines and derivatives, and N-alkyl amino acids or imno diacids. Acyl ethylenediamines and derivatives are produced by the reaction of an alkyl imidazoline with chloroacetic acid or with acrylic acid. These surfactants are widely used in the applications as fabric softeners, industrial cleaners, car cleaners and personal products. They reduce eye irritation and are incorporated into baby shampoos. Additionally, the reaction of chloroacetic acid or acrylic acid with an alkylamine to yield N-alkyl amino acids or imno diacids, which are chemical derivatives of amino acids. These molecules become a significant applicant in industry due to their excellent properties of good emulsifying effect, good foaming effect, substantive to surfaces, antistatic effects and reducing skin and eye irritation [82].

1.4 Rheology

Rheological studies are frequently described in many applications, such as plastic materials, lubricating materials, coatings, inks, adhesives, food, pharmaceuticals, cosmetics, and toiletries [84]. Rheology is one of the terms used to characterize semisolid dosage forms, for instance, gels and creams. It is used to study the deformation of solids

and the flow of liquids under the influence of external forces [85, 86]. Generally, rheological properties of a pharmaceutical system play important roles in patient acceptability, physical stability of products, and the manufacture process of products. Viscosity (η) is a term to express the resistance of a fluid to flow. It can be explained as the higher the viscosity, the greater the resistance of a fluid. There are two categories which classify the types of flow and deformation: Newtonian and non-Newtonian systems [84-87].

1.4.1 Newtonian System

In Newtonian systems, simple fluids that obey Newtonian's law of flow are known as Newtonian fluids. Newtonian fluids show a constant viscosity dependent on temperature but independent of the applied shear rate. In another words, shear stress is directly proportional to shear rate in a Newtonian fluid [85, 86].

$$Newton's\ Law: \sigma = \eta \cdot \gamma$$

$$Then: \eta = \frac{\sigma}{\gamma}$$

Where

$\boldsymbol{\sigma}$ $is\ shear\ stress, the\ force\ applied\ per\ unit\ area$
$which\ allows\ the\ material\ to\ start\ flowing.$
$\boldsymbol{\gamma}$ $is\ shear\ rate, the\ velocity\ with\ which\ the\ material\ starts$
$flowing\ upon\ applying\ a\ force.$
$\boldsymbol{\eta}$ $is\ viscosity, the\ resistance\ of\ a\ fluid\ to\ flow.$

A plot of shear stress versus shear rate with a straight line passing through the origin is obtained. This represents the flow curve or rheogram for a Newtonian system is seen in Figure 1-8a. The viscosity curve is another plot of viscosity versus shear rate which can

characterize a Newtonian system. Viscosity curves show a straight line at a constant value equal to η, which is seen in Figure 1-8b. The most common examples of Newtonian fluids are water, mineral oil, vegetable oil, whole milk, pure sucrose solutions, shampoos and liquid soap [84-86].

Figure 1-8: A rheogram (a) and a viscosity (b) curve for a Newtonian system [85].

(a)   (b)
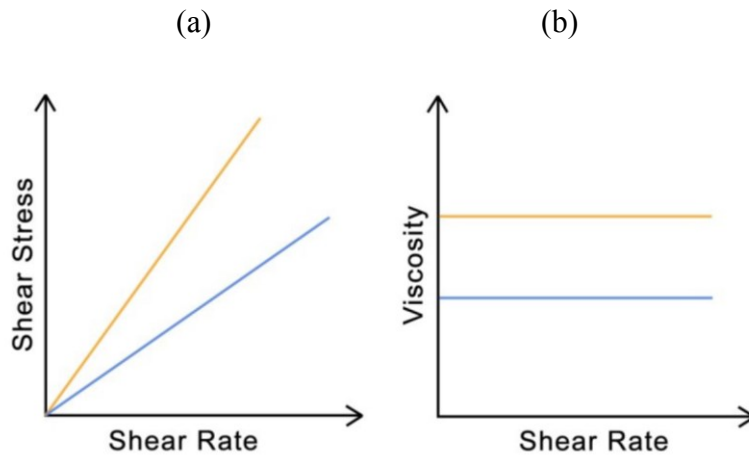
1.4.2 Non-Newtonian System

A non-Newtonian system is another category that will be introduced here. It is different from a Newtonian system due to the fact that non-Newtonian materials do not follow Newton's equation of flow [85]. Colloidal solutions, emulsions, liquid suspensions and ointments are examples of non-Newtonian materials [84]. In non-Newtonian systems, the rheogram shows a flow curve of shear stress versus shear rate, which is non-linear or does not pass through the origin. Unlike a Newtonian fluid, a non-Newtonian fluid is not constant at a given temperature and pressure but is dependent on flow conditions such as shear rate [88]. There are several types of non-Newtonian flow behavior that exhibits different fluid viscosity, which is dependent on variations of shear

rate [85]. Non-Newtonian systems which are not influenced by time are known as time-independent behavior. Materials that exhibit time-dependent behavior are subdivided into three types: shear-thinning or pseudoplastic; shear-thickening or dilatant; and plastic. In addition, there are also some non-Newtonian materials that are influenced by time, time dependent behavior systems include, thixotropic, rheopectic and anti-thixotropic [85, 86, 88, 89].

1.4.2.1 Shear-thinning or pseudoplastic flow

This type of time-independent non-Newtonian fluid behavior has the characterization of an apparent viscosity which decreases with increasing shear rate. In polymeric solution, pseudoplastic flow exhibit Newtonian behavior, where the viscosity is independent of shear rate at both very low and high shear rates [88, 89]. A rheogram and a viscosity curve of a shear-thinning material is seen in Figure 1-9a and 1-9b [85]. The rheogram exhibits a shear rate which increases, the shear stress of a pseudoplastic substance increase, whereas the viscosity curve shows that as the shear rate increases, the viscosity of a pseudoplastic substance decreases.

Figure 1-9: A rheogram (a) and a viscosity (b) curve of a shear thinning material [85].

(a)   (b)
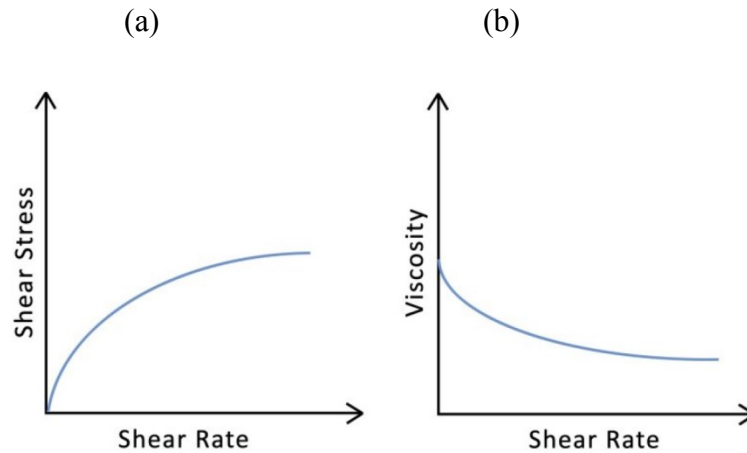
There are a large number of pharmaceutical products that display pseudoplastic flow, including liquid dispersions of tragacanth, sodium alginate, methylcellulose, and sodium carboxymethylcellulose [84]. Some typical examples in general are creams, juice concentrates, shampoo and salad dressings [86].

1.4.2.2 Shear-thickening or dilatant flow

Dilatant behavior flow shows an increase in resistance to flow or viscosity with increasing shear rates. This system is the inverse of a pseudoplastic system and does not show a yield stress, which is a specific shear stress value that must be exceeded in order to make a structured fluid/semisolid flow [85, 88, 89]. Dilatant systems are usually observed in suspensions that containing a high concentration of small and deflocculated particles [84]. This can be explained when the solvent acts as a lubricant between the particles at low shear rate, but is squeezed out at higher shear rates and results in a denser packing of the particles [85]. The common examples of shear-thickening systems are wet

sand and concentrated starch suspensions [86]. The flow properties of dilatant substances are illustrated by Figure 1-10a and 1-10b [85].

Figure 1-10: A rheogram (a) and a viscosity (b) curve of a shear thickening material [85].
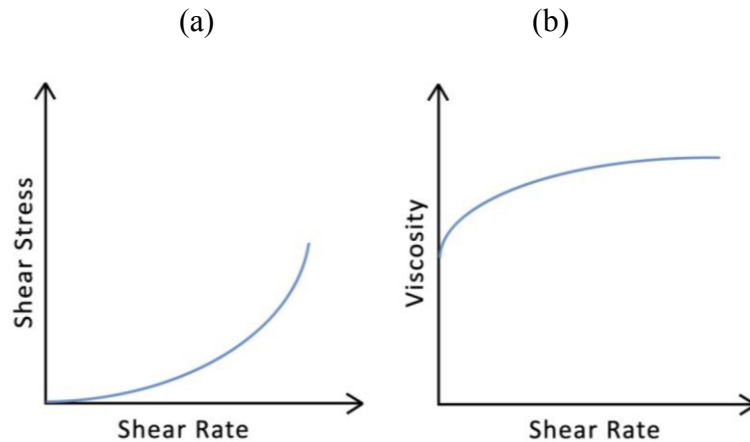
1.4.2.3 Plastic flow

Unlike pseudoplastic and dilatant behavior flow, plastic materials exhibit a yield stress. A typical example that explaine this plastic behavior is toothpaste. In order for toothpaste to flow like a liquid, a significant amount of force must be applied. If the force applied is smaller than the force corresponding to the yield stress, the material stores the deformation energy and thus, it behaves as a solid. Conversely, if the yield stress is exceeded, the fluid may exhibit Newtonian behavior that can be described as a Bingham plastic fluid or shear-thinning characteristics that can be described as a viscoplastic fluid [85, 88, 89]. In Figure 1-11a and 1-11b, the curves represent a body that exhibits plastic flow [85]. Several examples of plastic flow are tomota paste, toothpaste and hand cream [86].

Figure 1-11: A rheogram (a) and a viscosity (b) curve of a plastic material [85].

<center>(a)   (b)</center>
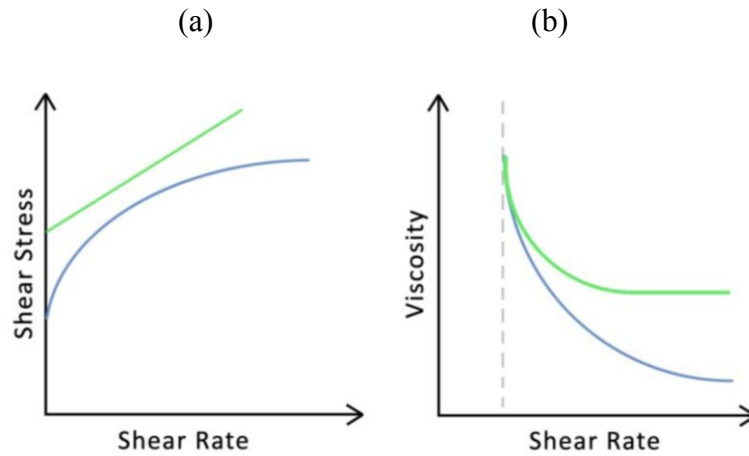
1.4.2.4 Thixotropic flow

Materials that exhibit thixotropic flow behavior has an apparent viscosity decrease with the time of shearing at a constant shear rate [88]. In time dependent behavior, the bonds between particles or molecules are broken; therefore, in order to recover its structure, the material must rest for a certain period of time [86]. This class of flow is normally performed in a loop test, which allows the material to increase the shear rates and followed by the same shear rates in decreasing order. This type of flow behavior is frequently found in all gel-forming systems. Some examples of thixotropic fluids are gels, creams, mayonnaise, ice cream and brush paint [86]. Typical rheogram and viscosity curve of thixotropic systems are shown in Figure 1-12a and 1-12b [85].

<center>36</center>

Figure 1-12: A rheogram (a) and a viscosity (b) curve of a thixotropic material [85].

(a)   (b)
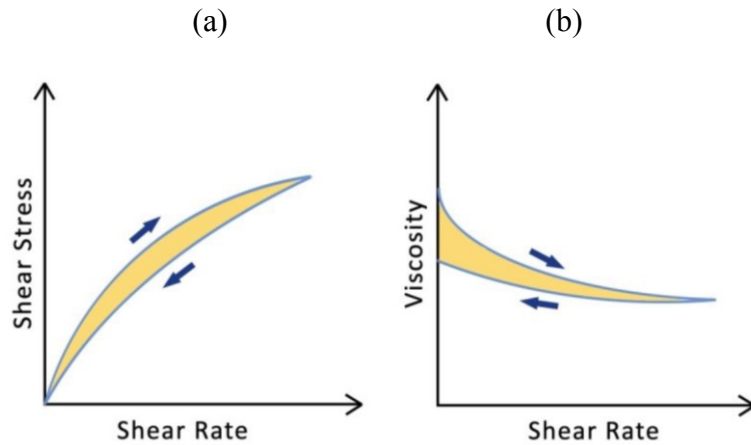
1.4.2.5 Rheopectic and Anti-thixotropic flow

Rheopectic fluids have the same behavior as a thixotropic fluid, however, in rheopectic system, the structure of the fluid will only recover completely when a small shear rate is applied. Unlike thixotropic fluid, a rheopectic fluid will not recover its structure at rest [85, 86]. The examples of rheopectic fluid are latex dispersions, casting slips and surfactant solutions [87]. Anti-thixotropic fluid shows a system that has the ability to shear-thickening. The viscosity of an anti-thixotropic substance increases with increasing shear rate, and time at a constant shear rate. Similarly with thixotropic fluid, this flow behavior is studied by a loop test as well [85, 86].

1.5 Antimicrobial agent

In the latter half of the 19th century, a variety of microorganisms were exposed to cause infectious diseases. Salvarsan® was the first antimicrobial agent in the world that was synthesized for syphilis by Ehrlich in 1910. This was followed by the discovery of penicillin in 1928. Penicillin is a fungal metabolite, which was used as an antibiotic

during World War II for wounded treatment. As a result, the development of semi-synthetic and synthetic antimicrobial agents was carried out to treat infectious diseases. These powerful and effective array compounds include sulfonamides and fluoroquinolones [90, 91]. The term antibiotic that is used previously is well-defined as "a low molecular weight substance produced by a microorganism that at low concentrations inhibits or kills other microorganisms". In opposition, an antimicrobial can be defined as "any substance of natural, synthetic or semi-synthetic origin, which kills or inhibits the growth of micro-organisms at low concentrations, but causes little or no host damage" [91]. Antimicrobial agents are a collection of antibacterials, antivirals, antifungals and antiparasitic drugs that are widely used therapeutic drugs worldwide [2]. They are appropriate treatment for acute, severe, persistent, or progressive infectious diseases by different mechanisms [92]. Bacteriostatic and bactericidal are terms that are used to describe the activity of an antibacterial. In general, an antimicrobial agent is said to be bactericidal when the minimum bactericidal concentration (MBC) is no more than four times the minimum inhibitory concentration (MIC). The MIC is the lowest concentration of an antimicrobial agent required to prevent the growth of the pathogen, whereas MBC is the lowest concentration of an antimicrobial agent required to kill the pathogen [91]. In other words, bacteriostatic is defined as an agent which prevents the growth of bacteria by keeping them in the stationary phase of growth. Bactericidal means that it kills bacteria. Most antibacterial agents that are available in the market are better described as potentially being both bactericidal and bacteriostatic [93, 94]. They are extensively applied to such areas are food, care, packaging, synthetic textiles, environmental and so on [95].

There are two categories of chemical synthesis antibacterial agent, which include organic and inorganic antibacterial agents. The organic antibacterial agents show high toxicity hazard to the human body and instability at high temperature and pressure. In contrast, inorganic antibacterial agents are heat and chemically stable, thus they have longer life compared to organic antibacterial agents [95]. In recent years, a number of metal ions have been studied as antibacterial agents in research. These metal ions include silver [96-98], copper [99, 100], zinc [101, 102], ferric [103], magnesium [104, 105], and titanium[106].

1.5.1 Zinc ions

According to the National Institutes of Health (NIH) [107], zinc is an essential element that is found in some foods and available as a dietary supplement. Zinc plays an important role in cellular metabolism in all living organisms. It is required in immune function, protein synthesis, wound healing, DNA synthesis and cell division [107]. In 1973, the first registration for pesticide products containing zinc salts was first issued in the United States [3]. Ten registered products are currently available. Out of these ten, nine contain zinc salts as their only active ingredient while the remaining one contains another active ingredient [3]. Several types of zinc salts that are included in pesticides as active ingredients include zinc chloride, zinc oxide, and zinc sulfate [3]. Zinc salts are also commonly used as herbicides to control the growth of moss on structures, walkways, patios and lawns in rainy areas [3]. It is claimed that zinc ion has the antibacterial activity against both gram-negative and gram-positive [108]. In the research of Faiz et al., they have claimed that zinc has an excellent antibacterial activity against enteric bacterial

pathogens that cause diarrhea [109]. Zinc salts were proposed as an antiviral agent in the study of Arens and Travis [6]. They found that the clinical isolates of Herpes Simplex virus (HSV) was inactivated by zinc salts in vitro. The mechanism of zinc on viruses was found to be the binding of zinc ions to the surface glycoproteins of many viruses that are required for adsorption to or penetration of the host cell [6]. The applications of zinc oxide have been studied broadly in various areas. Zinc oxide is used as an industrial preservative, incorporated into latex paints and in coatings for paper to inhibit growing of mold [110]. In previous studies, zinc oxide was determined to be bactericidal to control microbial contaminants in food by Xie et al.[111]. Zinc oxide is also a skin protectant and sunscreen (depending on the particle size), both of which are considered active ingredients and regulated by the FDA. The commercial available zinc oxide topical cream is used to treat and prevent diaper rash [112]. With the exception of zinc oxide, zinc sulfate is found to be widely used in the areas of agriculture [113], medicine [114], cosmetics [115] and environmental [116]. Because of the anti-infective properties of zinc, zinc sulfate is used in ophthalmic solution with astringent and weak antiseptic activity [117]. In the study of Surjawidjaja et al.[114], zinc sulfate was found to have an antimicrobial effect on enteric pathogens and also may contribute to the treatment of diarrhea [114]. In 1990, the mechanism of the antiviral activity of zinc sulfate was investigated. It was found that the molecular mechanism of the therapeutic effect of zinc sulfate in the treatment of herpetic lesions is due to the drastic inactivation of free virus in skin tissues, intercellular vesicles and blisters [118]. More evidences for antibacterial activity of zinc sulfate solution was found in the experiment of Boots et al. [119].

1.5.2 Copper ions

Copper is another essential trace element that is found in most living organisms [120, 121]. It is a required nutrient that helps to regulate blood pressure and heart rate. It involves in the formation of several essential enzymes such as cytochrome c oxidase, lysyl oxidase, feroxidase, and others [120, 121]. In 1956, the first copper-containing pesticide was approved for agricultural uses by the US Environmental Protection Agency (EPA) [4]. Copper is registered for use in agricultural crops, aquatic applications, antimicrobial applications and various residential applications, including home gardens and lawns [4]. In addition, there are five copper-containing alloy products registered by the EPA in February, 2008 [122]. The marketed products such as door knobs, counter tops, hand rails, and so on, have a claim that copper, "kills 99.9% of bacteria within two hours", when used in accordance with the label [122]. Some studies showed that copper surfaces have the ability to inhibit healthcare-associated pathogens, including MRSA, pseudomonas aerugonosa, and the influenza virus [120, 123, 124]. In the application of food processing, it is claimed that metallic copper surfaces act as an antibacterial agent against bacterial pathogens that cause foodborne diseases and to control escherichia coli cross-contamination [125, 126]. Copper sulfate is the best well-known and the most widely used of the copper salts which have been introduced as pesticide, algicide, germicide and fungicide for many years [127, 128]. Copper sulfate, also known as "blue stone" is primarily used as aquatic herbicide to control algae. However, if it is improperly used, it can be toxic to fish and zooplankton [129, 130]. Copper sulfate was used to prepare copper nanoparticles in the presence of a chitosan stabilizer with antimicrobial properties [131]. An in vitro study on antimicrobial evaluation of copper sulfate(II) on

strains of enterococcus faecalis was carried out and concluded that copper sulfate(II) showed antimicrobial activity at lower concentrations than chlorhexidine, a commonly used antimicrobial [132]. Several antimicrobial mechanisms of copper were proposed in recent articles, including: free radicals produced from redox cycle can damage the cell integrity; denaturation of DNA by binding of copper to protein molecules; and inactivation of enzymes and obstruction of functional groups of proteins from displacement of essential ions [120, 132, 133].

Chapter 2

Significance of the Thesis Research

The antimicrobial activities of both zinc sulfate and copper sulfate have been investigated for many years [6, 108, 114, 119, 132]. However, there is no antimicrobial formulation which incorporates these metal ions into a marketable product. The aim of the present study was to formulate various topical dosage forms including both creams and gels with antiviral and antibacterial activity. This study was inspired from a patented antimicrobial solution that composes of zinc sulfate and copper sulfate, known as SAFI Clean Water Drops [134]. It is used to treat contaminated water and surfaces in order to kill or to reduce biological contaminants, such as algae, bacteria and viruses. Therefore, zinc sulfate and copper sulfate were suggested to be used as the antiviral and antibacterial agents. In this study, it is claimed that the topical antiviral and antibacterial dosage forms can be used as a diaper rash cream and for cold sore treatment. Diaper rash or nappy rash is indicated for any skin irritation or inflammation that develops in the nappy area [135, 136]. It is estimated that the occurrence of infants to develop diaper rash is 7% to 35% between the ages of 9 and 12 months [135-137]. In the United States, a study showed that 75% of parents reported diaper rash in the preceding 2 months [136]. It is found that diaper rash is caused by the colonization of yeast, such as Candida albicans (C. albicans), and bacteria, such as Staphylococcus aureus (S. aureus), on the delicate skin with

prolonged contact with urine and faeces enzymes [136, 138, 139]. A previous study indicated that zinc and copper have the antimicrobial activity against S. aurues and C. albicans [5]. Cold sores, also known as fever blisters, are typically developed on the lips and are caused by herpes simplex virus type 1 (HSV-1) [140]. Other types of herpes simplex viruses (HSV) is HSV-2, can cause genital herpes. These types of viral infections produce an eruption of tiny blister and redness of skin around the infection area [140]. Some studies showed that zinc and copper exhibit inactivation of HSV both in vivo and in vitro [6, 141-143]. In this thesis, the characterizations of formulated creams and gels were carried out. The antimicrobial activity of the formulated creams and gels were then compared with the marketed products by comparing the zone of inhibitions against Escherichia coli (E. coli) and S. aureus. Lastly, a stability study for the topical products was performed over a 12 week period.

Chapter 3

Materials and Methods

3.1 Materials

3.1.1 Active Pharmaceutical Ingredients (API)

Copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) [134], was used as one of the API to provide antibacterial and antiviral activities in the topical formulations. The chemical structure for $CuSO_4$ is given in Figure 3-1. The $CuSO_4$ (Batch No. 12H22-U03-016184) was purchased from Fagron, Inc. (St. Paul, MN). Zinc sulfate heptahydrate USP ($ZnSO_4 \cdot 7H_2O$) [134], was another API that was used as antibacterial and antiviral agents in the topical formulations. Figure 3-2 illustrates the chemical structure for $ZnSO_4$. The $ZnSO_4$ (Lot No. 1309120189) was purchased from Letco Medical (Decatur, AL).

Figure 3-1: Chemical structure for copper sulfate pentahydrate
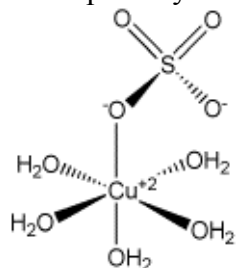
Figure 3-2: Chemical structure for zinc sulfate heptahydrate
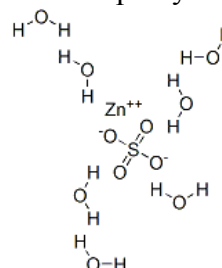
3.1.2 Polymers

Polymers were used as gelling and thickening agents in both cream and gel formulations. The (ι)-Carrageenan (Lot No. 81K1556) was purchased from Sigma-Aldrich (St. Louis, MO). Hypromellose (Benecel, K4M PHARM, also known as hydroxypropylmethyl cellulose, HPMC) (Lot No. 0001476884) was gifted from Ashland (Wilmington, DE). Kollidon® 90F (Polyvinylpyrrolidone, PVP) (Lot No. 59163897VO) was obtained from BASF (Ludwigshafen, Germany). Poloxamer 407 NF (Lot No. C144872) was purchased from PCCA (Houston, TX). FlexiThix™ (2-Pyrrolidinone, 1-ethenyl-, homopolymer) (Lot No. 274370) was a donated by ISP Technologies, Inc. (Wayne, NJ). Xanthan gum N.F. (Lot No. XV0506) and guar gum N.F. (Lot No. YV3076) were obtained from Spectrum Chemical Mfg. Corp. (Gardena, CA). Carbomer 940 (Lot No. 1301310901) was purchased from Letco Medical (Decatur, AL).

3.1.3 Emollients, Moisturizers and Stabilizer

Prolipid 141 was used as a thickener, moisturizer and stabilizer in the cream formulation. Prolipid 141 was composed of glyceryl stearate, behenyl alcohol, palmitic acid, stearic acid, lecithin, lauryl alcohol, myristyl alcohol and cetyl alcohol. Prolipid 141 (Lot No. 013318960) was donated by Ashland Chemical Company (Wilmington, DE). Refined corn oil NF was used as an emollient to provide softening or soothing effect to the skin in cream formulation. Refined corn oil NF (Lot No. 11211204) was purchased from Letco Medical (Decatur, AL). Almond oil sweet NF acted as an emollient to soften and smooth the surface of skin in the cream formulation. Almond oil sweet NF (Lot No. 1207160041) was purchased from Letco Medical (Decatur, AL). Medium chain

triglycerides (MCT) (Lot No. MKC10) was obtained from Mead Johnson & Company (Evansville, Indiana). Super refined™ Soybean USP (Lot No. SB2-478C) was donated by Croda Inc. (Edison, NJ). Florasolvs® PEG-16 Macadamia (Lot No. MPHH55) and Florasolvs® PEG-10 Sunflower (Lot No. SPGK32) were used as emollient and were obtained from FloraTech (Gilbert, Arizona). Cocoa butter (Lot No. 15509) was donated by Koster Keunen, Inc. (Watertown, CT). Cetyl alcohol (Lot No. 020781), stearic acid (Lot No. 092680), stearyl alcohol (Lot No. 041692) and isopropyl myristate (Lot No. 041592) were obtained from Sherman Research Labs (Toledo, OH). Coconut oil (Lot No. W005987) was purchased from Spectrum Organic Products, (Melville, NY).

3.1.4 Emulsifiers and Co-emulsifiers

Tefose HC was used as an O/W emulsifier that was composed of cetyl alcohol, glyceryl stearate, cereth-20 and steareth-20. Tefose HC (Batch No. 127745) was donated by Gattefossé (Saint Priest Cedex, France). Cithrol™ GMS 40 is glyceryl stearate and belongs to fatty acid esters category. It was used as a co-emulsifier to improve stability and modify viscosity. The Cithrol™ GMS 40 (Lot No. 24788) was donated by Croda Inc. (Edison, NJ). PEG-8 beeswax, also known as polyoxyethylene 8 beewax, is a non-ionic self-emulsifying wax that is obtained from the esterification of the free fatty acids of beeswax with polyethylene glycol. It was used a co-emulsifier and stabilizer in cream formulation. PEG-8 beeswax (Batch No. 5935) was donated by Koster Keunen, Inc. (Watertown, CT). Lecithin soya granular (Lot No. 06281101) was purchased from Letco Medical (Decatur, AL). Arlacel™ 165 (Lot No. 000683637) and Tween 60 (Lot No.

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0000423566) were used as an O/W emulsifier, while Span 80(Lot No. 0000694086) was used as W/O emulsifier. These emulsifiers were obtained from Croda Inc. (Edison, NJ).

3.1.5 Humectant

Glycerin USP 99% Natural was added to the cream formulation and acted as a humectant to attract water when applied to the skin. Theoretically, it improves the hydration of the stratum corneum. Glycerin USP 99% Natural (Lot No. 11100534) was purchased from Letco Medical (Decatur, AL). Urea (Lot No. 0508264X06) was purchased from Gallipot® Inc. (St. Paul, MN).

3.1.6 Solvent

Distilled deionized water was used as a solvent to disperse the polymers and other water-soluble ingredients in both cream and gels formulations. It was supplied by the University of Toledo Health Science Campus deionization system.

3.1.7 Preservatives, Antioxidant, Buffering agent and Neutralizer

Paraben concentrate was used in both cream and gel formulations to prevent the growth of mold and bacteria. Paraben concentrate was prepared by dissolving methylparaben and propylparaben powder in propylene glycol. Methylparaben (Lot No. KD388) and propylparaben (Lot No. SX0526) were obtained from Spectrum Chemical Mfg. Corp. (Gardena, CA). Propylene glycol USP (Lot No. 11090501) was purchased from Letco Medical (Decatur, AL). Butylated hydroxytoluene NF (BHT) served as an antioxidant and citric acid was used as a buffering agent to help adjust the acid/base

balance in the cream formulations. BHT (Lot No. TC2116) and citric acid monohydrate USP (Lot No. YV0659) were purchased from Spectrum Chemical Mfg. Corp. (Gardena, CA). Triethanolamine (Lot No. 24493) was used as a neutralizer and was purchased from Making Cosmetics (Snoqualmie, WA).

3.1.8 Ingredients that used for ICP-MS analysis

Nitric acid (Lot No. A200S-212) and hydrogen peroxide were supplied by Fisher Scientific (Pittsburgh, PA).

3.1.9 Ingredients that used for in-vitro antibacterial activity test

Mueller-Hinton (MH) agar was used to grow the microorganisms. Mueller-Hinton agar (Lot No. 3240477) and gentamicin 10μg standard discs (Lot No. 3172494) were purchased from Becton, Dickinson and Company (Sparks, MD).

3.1.10 Over-the-Counter Diaper Rash Cream and Cold Sore Treatment Gel and Cream

Equate® Diaper Rash Relief (Lot No. 0228118), a thick and creamy diaper rash cream, was distributed by Wal-Mart Stores, Inc. (Bentonville, AR). Nexcare™ (Lot No. 2291A), a yellowish cold sore treatment cream, was purchased from 3M (St. Paul, MN). Medicated Campho-Phenique® (Lot No. NAA1TD2), a clear cold sore treatment gel, was purchased from Bayer Health Care LLC (Morristown, NJ).

3.2 Methods

3.2.1 Formulation of topical cream

The oil phase was prepared by melting the waxes at 75°C and mixing the ingredients uniformly. In another clean beaker, the aqueous phase was prepared by dissolving the water-soluble ingredients, including polymer, in distilled water. The water-phase was warmed to 75-80°C until all the water-soluble ingredients dissolved. When the oil phase was about 75°C, the aqueous phase which has about the same temperature was slowly added into the oil phase with moderate agitation until the temperature reached 40°C. The emulsion was cooled to room temperature and formed a semi-solid cream base. A certain quantity of zinc sulfate and copper sulfate were dissolved in warmed distilled water. A small portion of the zinc and copper solution was incorporated into the cream base by stirring with an overhead stirrer (Talboys Engineering Corp, Emerson, NJ). The mixture was stirred for about 15 minutes until all portions of the zinc and copper solution were incorporated homogenously into cream base. The drug loaded cream was preserved with paraben concentrate. The exact concentration of each ingredient is given in Table 4.1.

3.2.1.1 HLB (hydrophile-lipophile balance) calculation

An HLB calculation was determined for the formulation of the cream when determining the ratio of emulsifiers to make a stable emulsion. Initially, "required HLB" value was calculated for typical ingredients to be emulsified. Once the "required HLB" value was obtained, the HLB value for an emulsifier blend was calculated. Theoretically,

in order to get a stable emulsion, the calculated HLB value for an emulsifier mixture should be the same or close to the "required HLB" value [144].

For example, in formula C15, there were 22.73% of cetyl alcohol, 22.73% of stearic acid, 27.27% of sweet almond oil, and 27.27% of soy bean oil to be emulsified in water. The required HLB for the combination of oleaginous ingredients can be calculated as follows:

Cetyl alcohol: ………22.73% × Req. HLB 15.5 = 3.523
Stearic acid: …………..22.73% × Req. HLB 15 = 3.409
Sweet almond oil: ……...27.27% × Req. HLB 7 = 1.909
Soy bean oil: ……………27.27% × Req. HLB 7 = 1.909
Estimated HLB for emulsifier system …………….10.75

The emulsifier mixture that was used in formula C15 was Span 80 and Tween 60. To calculate the ratio of emulsifiers to reach the required HLB of X, the following equation was used [144]:

$$\% \ (emulsifier \ A) = \frac{100(X - HLB_{(B)})}{HLB_{(A)} - HLB_{(B)}}$$

$$\% \ (emulsifier \ B) = 100 - \%(emulsifier \ A)$$

In this case, HLB of X was calculated to be 10.75.

$$\%Tween \ 60 = \frac{10.75 - 4.3}{14.9 - 4.3} = \frac{6.45}{10.6} = 60.85\%$$

$$\%Span \ 80 = 100 - 60.85\% = 39.15\%$$

3.2.2 Formulation of topical gels

The polymers were hydrated with distilled water before they were incorporated with the active ingredients. Polymers have different optimal conditions in order for them

to hydrate and form a gel. The exact concentration of each ingredient is given in Table 4.2.

3.2.2.1 (ɩ)-carrageenan, xanthan gum, or guar gum

The polymer powder was dispersed in 75°C warm water with stirring. When all of the polymer was dissolved, the mixture was removed from the hot plate. A certain quantity of zinc sulfate and copper sulfate were dissolved in the clear gel with intense stirring. The mixture was cooled to room temperature and preserved with paraben concentrate.

3.2.2.2 Hypromellose

A hypromellose solution was prepared by dispersing hypromellose in 75°C warm water with stirring. The hypromellose solution was stored at room temperature overnight until a clear gel was obtained. Zinc sulfate crystals were added into the gel solution and mixed intensely. Some distilled water was added into the mixture and moderate stirring was continued until the gel formed. Copper sulfate crystal was dispersed into the zinc sulfate gel with stirring until all the crystal particles dissolved. Preservative was finally added into the final gel formulation.

3.2.2.3 Poloxamer 407

Poloxamer solution was prepared using the cold method by dissolving poloxamer in cold water and then was stored under refrigerated conditions at 4°C overnight. The oil phase was prepared by mixing lecithin and isopropyl myristate in a 1:1 ratio mixture of

lecithin to isopropyl myristate. The mixture was stored at room temperature overnight for the complete dissolution of the lecithin in the isopropyl myristate. The active ingredients were added directly into the aqueous phase. The gel was prepared by mixing 1 part of oil phase (mixture of lecithin and isopropyl myristate) with 4 parts of aqueous phase (poloxamer solution) using a vortex mixer (VORTEX – T, Genie® 2).

3.2.2.4 Kollidon® 90F, FlexiThix™ or Carbomer 940

Kollidon® 90F, FlexiThix™ or carbomer 940 was directly dispersed into distilled water at room temperature with intensive agitation. Active ingredients were incorporated into the gel solution uniformly. In order for carbomer 940 solution to form a gel, triethanolamine was added to neutralize the pH to 6-6.5.

3.2.3 Evaluation of topical cream and gels

3.2.3.1 Organoleptic characters

All the formulations (blank and drug loaded) were tested for their color, texture and phase separation by visual observations. The feel on application once the preparation was applied on the skin and after two minutes of application was also performed.

3.2.3.2 Homogeneity test

Test was carried out by pressing a small quantity of the formulated cream and gel between the thumb and index finger. The consistency of the formulations and the

appearance of the coarse particles on the fingers were used to evaluate the homogeneity of the formulations.

3.2.3.3 Spreadability test

The spreadability of the formulations was determined by measuring the spreading diameter of 0.1g of sample between two horizontal glass plates (10 cm × 20 cm) after one minute. The standard weight applied on the upper plate was 25g [145].

3.2.3.4 Determination of drug content

The drug content of the gel and cream formulations was determined by using Inductively Coupled Plasma Mass Spectrometry or ICP-MS (XSERIES 2, Thermo Scientific, MA, USA) analysis. ICP-MS is an analytical technique that is used to determine elemental content. Microwave digestion was applied to the formulations before the samples were run through the ICP-MS. Samples were digested in 8 mL of nitric acid, HNO_3 and 2 mL of hydrogen peroxide, H_2O_2 using a CEM Mars microwave with program of heating from 25 to 200℃ in 15 minutes and temperature was held at 200℃ for 15 minutes. After digestion, samples were filtered to remove particulate material. The filtrate was diluted with 2% HNO_3 for analysis in ICP-MS. For quantitative analysis, standards were prepared by using the certified ICP-MS Complete Standard from Inorganic Ventures.

3.2.3.5 Determination of pH

A 1 gram portion of each formulation (blank and drug loaded) was dispersed in 25 mL of distilled water and the pH was determined using a Mettler Toledo pH meter (Mettler-Toledo Ingold Inc., Billerica, MA). The pH meter was calibrated with standard buffer solutions of pH 4, 7, 10 before each use.

3.2.3.6 Viscosity measurement

A Brookfield viscometer DV-I (Brookfield Engineering Laboratories, Middleboro, MA) was used with a concentric cylinder spindle SC4-29 to determine the viscosities of the different topical formulations. The tests were carried out at 21°C. The spindle was rotated at 5, 10, 20, 50, 100 RPM.

3.2.4 In-vitro antibacterial activity

3.2.4.1 Preparation of Mueller-Hinton (MH) agar plates

The MH agar medium was prepared according to the manufacturer's instruction and autoclaved for 20 minutes at 20 psig. After autoclaving, the agar medium was cooled to 40-45°C in a water bath. 60 mL of cooled agar medium was poured onto the sterile prepared 150 × 15 mm petri dish. The agar was allowed to cool to room temperature and stored in a refrigerator (2°C - 8°C) until used.

3.2.4.2 Preparation of inoculum

Escherichia coli (ATCC 25922) and staphylococcus aureus (ATCC 29213) were used for performing the antibacterial activity of the topical formulations containing zinc sulfate and copper sulfate. The microorganisms were sub-cultured the previous day to ensure the tested microorganisms are in their log phase of growth in order for results to be valid. One or two isolated colonies of the tested microorganisms were touched using a sterile cotton swab. The microorganisms were suspended in 2mL of sterile saline medium and vortexed well until a uniform suspension was obtained. The turbidity of the suspension was measured at 625 nm using a spectrophotometer (Thermo Scientific, Fair Lawn, USA). The turbidity of the suspension was adjusted to a 0.5 McFarland standard by adding more microorganisms if the suspension is too light or diluting with sterile saline if the suspension are too heavy. The suspension was prepared before inoculating the microorganisms on the agar plate.

3.2.4.3 Inoculation of the MH plate

To inoculate onto the MH agar plates, a sterile cotton swab was dipped into the suspension and streaked over the surface of the agar plates. This procedure was repeated for two additional times with each time the plate was rotated approximately 60˚ to ensure even distribution of the inoculum. The plates were allowed to dry at room temperature for 5 minutes before applying the drug.

3.2.4.4 Preparation of Agar Well Diffusion Assay

The dried inoculated MH agar plates that were prepared above were used to perform the Agar Well Diffusion Assay. A sterile cork borer was used to make the wells by punching the holes on the inoculated MH agar plates. Each well was 5 mm in diameter and the cut out of the agar was removed using a sterile needle. A certain amount of formulation was weighed and placed into each well on an analytical balance. Gentamicin 10 μg standard discs were used as a control to ensure the agar medium was able to support the growth of microorganism beyond the zone of inhibition. The Gentamicin standard disc was placed and pressed gently onto the same inoculated agar plate by using a sterile forceps. The inoculated agar plate was incubated in an incubator at 37^0C for 18 hours. The observed diameters of the zones of inhibition were measured using a ruler to the nearest millimeter. A study was performed to determine the synergistic effect of zinc and copper in antibacterial activity by comparing 3% of both zinc sulfate and copper sulfate, 3% of zinc sulfate, 3% of copper sulfate, 6% of zinc sulfate and 6% of copper sulfate solutions.

Another study was carried out with the formulations in a series of concentrations: 0%, 0.1%, 0.25%, 0.5%, 1%, 2%, and 3% that contain both zinc sulfate and copper sulfate. The formulations in their finalized concentrations, namely 3% of both zinc sulfate and copper sulfate were used to compare with the marketed products.

3.2.5 Stability study

All final formulations that contain 3% of both active ingredients were stored at 4^0C, 25^0C and 40^0C in both glass and plastic containers. The physicochemical properties

of all formulations were evaluated after week 12 to describe the physical stability of the formulations. The viscosities of the formulations were determined every week for over a 12 week period. The formulations were tested for antibacterial activity against Escherichia coli (ATCC 25922) by using the agar well diffusion assay, at regular time intervals of 0, 1, 3, 6, 9 and 12 weeks. The antibacterial activity of the formulations in glass container was compared with the formulations in plastic container.

3.2.6 Statistical analysis

One-way ANOVA followed by Tukey's post hoc test was used in statistical analysis to analyze the data. A difference was considered statistically significant when $p < 0.05$.

Chapter 4

Results and Discussions

4.1 Preparation and evaluation of topical creams and gels

Twenty different cream bases were formulated using various compositions of oil phase and aqueous phases. The exact amount of each ingredient composition in cream formulation is given in Table 4.1. Initially, the cream bases were incorporated with both active ingredients at a concentration of 3%. However, the properties of the bases, into which the drugs were incorporated, were shown to affect their physical stability and appearance. Formulations from C5 to C20 were obtained with the issues such as physical and appearance instability that were described previously. Therefore, they were discontinued from further characterization studies. Formulations C1 to C4 were prepared with the same ingredients but with different compositions of emulsifiers and thickeners, such as Tefore HC, Prolipid 141, PEG-beeswax and Cithrol™ GMS 40. Four of the cream formulations were observed to have similarity in consistency. No apparent change in the physical appearance was observed with these four compositions. Therefore, C1 was chosen as the final formulation to prepare the diaper rash cream for this thesis. In this study, eighteen different gels were developed using various polymers as gelling agents. However, there were only two types of polymers that can be used to incorporate the active ingredients, namely carrageenan and HPMC. Table 4.2 showed the exact amount

of the ingredient composition for the gel formulation. Formulations G1 and G2 were formulated with carrageenan gel. They incorporated the active ingredients extremely well. They have similar textures and consistency, however G2 has a lower viscosity than G1 using visual observation. Therefore, G1 was used as the final formulation. Formulations G3 to G6 were prepared with different concentrations of HPMC using the cold method. Nevertheless, G5 was the only formulation that could satisfactorily to be used in this study among the four different concentrations of HPMC. The main reason determined by this study might be the amount of water used in the formulation. Precipitation or aggregation of the polymers was observed when the active ingredients were added into the 5% HPMC solution followed by a small amount of water. This observation was found in formulations G3 and G4. Conversely, formulation G6 was found to be less viscos than formulation G5 due to presence of a lower concentration of HPMC. Clearly, this shows that the hydration of the polymers plays an important role in forming a stable gel. The properties of the polymers such as, xanthan gum, guar gum, poloxamer 407, Kollidon®90F, FlexiThix™ and carbomer 940, that the active ingredients were incorporated were shown to affect their physical stability. The final formulations for semi-solid dosage forms were cream-based (C1), carrageenan-based gel (G1) and HPMC-based gel (G5).

Table 4.1: Ingredients composition for all the cream formulations from C1 to C10

Ingredients	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10
Corn Oil	4%	4%	4%	4%	-	-	-	-	-	-
MCT	4%	4%	4%	4%	-	3%	3%	3%	5%	5%
Sweet Almond Oil	4%	4%	4%	4%	5%	4%	4%	5%	-	-
Coconut Oil	-	-	-	-	-	-	-	-	5%	5%
Cocoa Butter	-	-	-	-	-	-	-	-	-	-
Soy Bean Oil	-	-	-	-	5%	6%	6%	6%	5%	5%
PEG-16 Macadamia	-	-	-	-	-	-	-	-	-	-
PEG-10 Sunflower	-	-	-	-	-	-	-	-	-	-
Tefose HC	4%	5%	6%	6%	-	-	-	-	-	-
Prolipid 141	5%	6%	5%	7%	-	-	-	-	-	-
PEG-8 Beeswax	7%	8%	6%	6%	-	-	-	-	-	4%
GMS 40	5%	6%	6%	6%	-	-	-	-	-	-
Span 80	-	-	-	-	-	-	-	-	-	-
Tween 60	-	-	-	-	-	-	-	-	-	-
Stearyl Alcohol	-	-	-	-	-	-	-	-	-	-
Stearic acid	-	-	-	-	3%	2%	3%	4%	4%	4%
Cetyl Alcohol	-	-	-	-	5%	4%	5%	7%	6%	6%
Arlacel 165	-	-	-	-	5%	5%	5%	5%	5%	5%
Urea	-	-	-	-	3%	-	-	-	-	-
2% HPMC Solution	-	-	-	-	-	-	-	-	-	-
Xanthan Gum	-	-	-	-	-	0.5%	0.25%	0.25%	0.25%	0.25%
Carrageenan	0.35%	0.35%	0.35%	0.35%	-	-	-	-	-	-
Glycerin	5%	5%	5%	5%	5%	5%	5%	5%	5%	5%
Citric Acid	1%	1%	1%	1%	1%	1%	1%	1%	1%	1%
BHT	0.05%	0.05%	0.05%	0.05%	0.05%	0.05%	0.05%	0.05%	0.05%	0.05%
DI water	60.1%	56.6%	58.6%	56.6%	67.95%	69.45%	67.7%	63.7%	63.7%	59.7%

Table 4.1: Ingredients composition for all the cream formulations from C11 to C20

(Continued)

Ingredients	C11	C12	C13	C14	C15	C16	C17	C18	C19	C20
Corn Oil	-	-	-	-	-	-	-	-	-	-
MCT	4%	4%	-	-	-	6%	-	4%	4%	4%
Sweet Almond Oil	-	-	10%	10%	6%	-	5%	4%	4%	4%
Coconut Oil	4%	4%	-	-	-	4%	5%	4%	4%	4%
Cocoa Butter	-	-	-	-	-	-	3%	-	-	-
Soy Bean Oil	4%	4%	-	-	6%	4%	4%	-	-	-
PEG-16 Macadamia	-	-	-	-	-	4%	-	-	-	-
PEG-10 Sunflower	-	-	2%	2%	-	-	-	-	-	-
Tefose HC	-	-	-	-	-	-	-	-	-	-
Prolipid 141	-	-	-	-	-	-	-	-	-	-
PEG-8 Beeswax	6%	7%	-	-	-	-	-	7%	6%	7%
GMS 40	-	-	-	-	-	-	-	-	-	3%
Span 80	-	-	1.05%	2.25%	1.57%	1.42%	1.77%	-	-	-
Tween 60	-	-	0.95%	2.75%	2.43%	2.58%	2.23%	-	-	-
Stearyl Alcohol	-	-	-	-	-	4%	5%	-	5%	2%
Stearic acid	4%	5%	2%	2%	5%	-	-	4%	-	-
Cetyl Alcohol	6%	7%	2%	4%	5%	3%	5%	6%	6%	6%
Arlacel 165	5%	5%	-	-	-	-	-	5%	5%	5%
Urea	-	-	4%	4%	3%	3%	-	-	-	-
2% HPMC Solution	-	-	-	-	-	-	-	5%	-	-
Xanthan Gum	0.25%	0.25%	-	-	0.5%	0.5%	-	-	-	-
Carrageenan	-	-	-	-	-	-	-	-	-	-
Glycerin	5%	5%	5%	5%	5%	5%	5%	5%	5%	5%
Citric Acid	1%	1%	1%	1%	1%	1%	1%	1%	1%	1%
BHT	0.05%	0.05%	0.05%	0.05%	0.05%	0.05%	0.05%	0.05%	0.05%	0.05%
DI water	60.7%	57.7%	71.95%	66.95%	64.45%	61.45%	62.95%	54.95%	59.95%	58.95%

Table 4.2: Ingredient composition for all the gel formulations from G1 to G10

Ingredients	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10
ι-Carrageenan	2%	1%	-	-	-	-	-	-	-	-
5% HPMC Solution	-	-	q.s. 100%	q.s. 100%	50%	25%	-	-	-	-
Xanthan Gum	-	-	-	-	-	-	2%	-	-	-
Guar Gum	-	-	-	-	-	-	-	2%	-	-
Poloxamer 407	-	-	-	-	-	-	-	-	32%	24%
Lecithin	-	-	-	-	-	-	-	-	10%	10%
Isopropyl myristate	-	-	-	-	-	-	-	-	10%	10%
Kollidon®90F	-	-	-	-	-	-	-	-	-	-
FlexiThix™	-	-	-	-	-	-	-	-	-	-
Carbomer 940	-	-	-	-	-	-	-	-	-	-
Triethanolamine	-	-	-	-	-	-	-	-	-	-
BHT	-	-	-	-	-	-	-	-	0.05%	0.05%
DI water	q.s. 100%	q.s. 100%	-	25%	q.s. 100%	q.s. 100%	q.s. 100%	q.s. 100%	q.s. 100%	q.s. 100%

Table 4.2: Ingredient composition for all the gel formulations from G11 to G18

(Continued)

Ingredients	G11	G12	G13	G14	G15	G16	G17	G18
ι-Carrageenan	-	-	-	-	-	-	-	-
5% HPMC Solution	-	-	-	-	-	-	-	-
Xanthan Gum	-	-	-	-	-	-	-	-
Guar Gum	-	-	-	-	-	-	-	-
Poloxamer 407	16%	-	-	-	-	-	-	-
Lecithin	10%	-	-	-	-	-	-	-
Isopropyl myristate	10%	-	-	-	-	-	-	-
Kollidon®90F	-	30%	20%	10%	-	-	-	-
FlexiThix™	-	-	-	-	6%	4%	2%	-
Carbomer 940	-	-	-	-	-	-	-	1%
Triethanolamine	-	-	-	-	-	-	-	1.35%
BHT	0.05%	-	-	-	-	-	-	-
DI water	q.s. 100%	q.s. 100%	q.s. 100%	q.s. 100%	q.s. 100%	q.s. 100%	q.s. 100%	q.s. 100%

4.1.1 Physicochemical properties evaluations

The physicochemical properties of the topical formulations are given in Table 4.3. From the results, it is concluded that all the formulated cream and gels showed good appearance and homogeneity. The physical appearance of the cream and gel formulations was blue in nature, which was the color obtained from the natural copper sulfate. The texture of the formulations was smooth with no phase separation.

Table 4.3: Physicochemical evaluations for different topical formulations

Formulation	Color	Physical Appearance	Homogeneity	Texture	Phase Separation	Feel After Application
C1	Light blue-greenish	Opaque	Homogeneous	Smooth	No	Moisture
G1	Blue	Transparent	Homogeneous	Smooth	No	Refreshing
G5	Blue	Transparent	Homogeneous	Smooth	No	Film formed after dry

4.1.2 Spreadability test

The spreadability of the semisolid formulations plays a role in the efficacy of the topical therapy which depends on the patient spreading the drug formulation in an even layer to administer a standard dose [146]. The values in Figure 4-1 illustrated the spreading diameter after one minute. This indicated the extent of the area to which the formulation readily spreads on application to skin or the affected part by a small amount of shear [147]. According to the results, it was claimed that the formulated products: G1, G5 and C1 exhibited better spreadability than the commercial products.

Figure 4-1: Spreadability values for formulated and commercial products
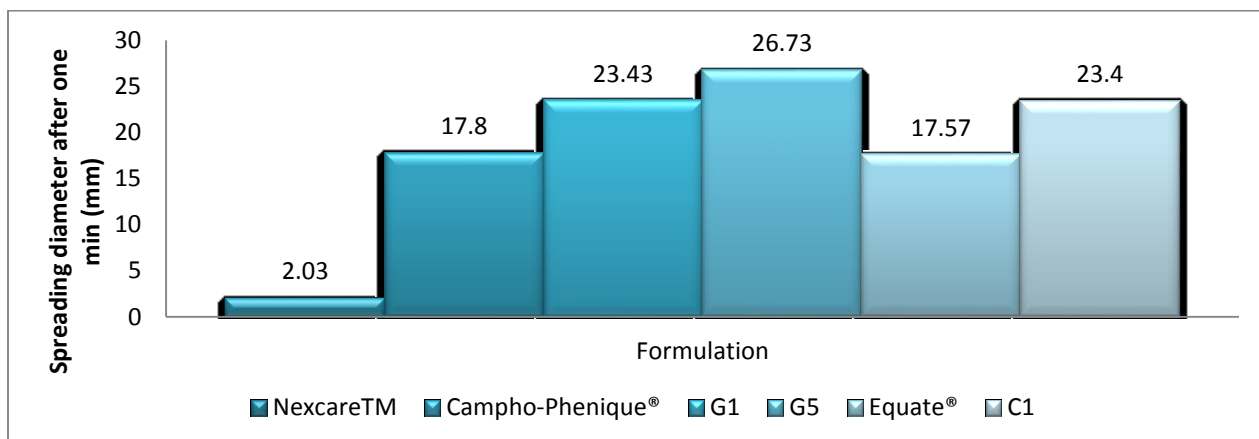
4.1.3 Determination of drug content

The determination of drug content in the various formulations was conducted using ICP-MS analysis. The percent of drug content was calculated and presented in Table 4.4. However, a relatively low percent of drug content was obtained from the ICP-MS analysis which fell between 20-24% for copper ion and 18-21% for zinc ion. The ICP-MS method is a powerful and sensitive technique for the analysis and quantification of trace elements in both solid and liquid samples [148]. One of the reasons for getting such a low percent of drug content might be due to the microwave digestion system that was used to digest the gel polymer and cream base. During the digestion, some nitric acid and hydrogen peroxide were mixed with the formulations at a high temperature of 200°C. This procedure can break down the sample matrices and leave behind a clear solution containing the analytes of interest. The digestion might interfere in the detection of the metal ion. Another possible reason might be the metal ion bound to the sulfate group on the carrageenan, therefore, only a small amount of free metal ion was detected.

Table 4.4: Percent drug content in the different formulations

Formulation	% Drug content	
	Cu^{2+}	Zn^{2+}
C1	20.80%	18.36%
G1	20.6%	18.17%
G5	24.06%	21.1%

4.1.4 Determination of pH

The pH of the cream and gel formulations without active ingredients (Table 4.5) was found to be 3.073±0.0197 for C1, 6.467±0.0943 for G1 and 6.392±0.0395 for G5. The pH was decreased to 2.85±0.0282 for C1, 4.95±0.0562 for G1, and 4.96±0.0386 for G5 when the active ingredients were incorporated into the bases. The pH of the skin is normally in the range of pH values of 4 to 6, which is acidic [149]. The cream formulation showed slightly acidic to the skin, while the gel formulations exhibited a similar pH to the skin.

Table 4.5: pH of formulations with and without active ingredients

Formulation	pH (Mean ± SD)		
	Blank	Drug (Day 1)	Drug (Week 12)
C1	3.073±0.0197	2.85±0.0282	2.90±0.0095
G1	6.467±0.0943	4.95±0.0562	4.96±0.0370
G5	6.392±0.0395	4.96±0.0386	5.05±0.0386

4.1.5 Viscosity measurement

The viscosity of the different formulations was determined at varying shear stresses using a Brookfield viscometer. The viscosity was reported in a unit of centipoise (cps). Figure 4-2 showed the apparent viscosity vs. revolutions per minute (RPM or shear stress) for the formulations. The viscosities for the formulated products at 10 rpm were

found to be 44,000 cps for C1, 33,500 cps for G1 and 13,900 cps for G5. It is claimed that a non-Newtonian behavior with pseudoplastic flow was observed in all of the formulated products. It is clearly to observe from Figure 4-2 that as the shear stress increased, the viscosity decreased in all the formulations. Also, C1 and G1 tend to have similar viscosity pattern, while G5 exhibited a lower viscosity than C1 and G1. This conclusion holds true since the resistance to flow of G5 was low when compare to C1 and G5 by pouring the formulation out of the container.

Figure 4-2: Viscosity data of various formulations including C1, G1 and G5
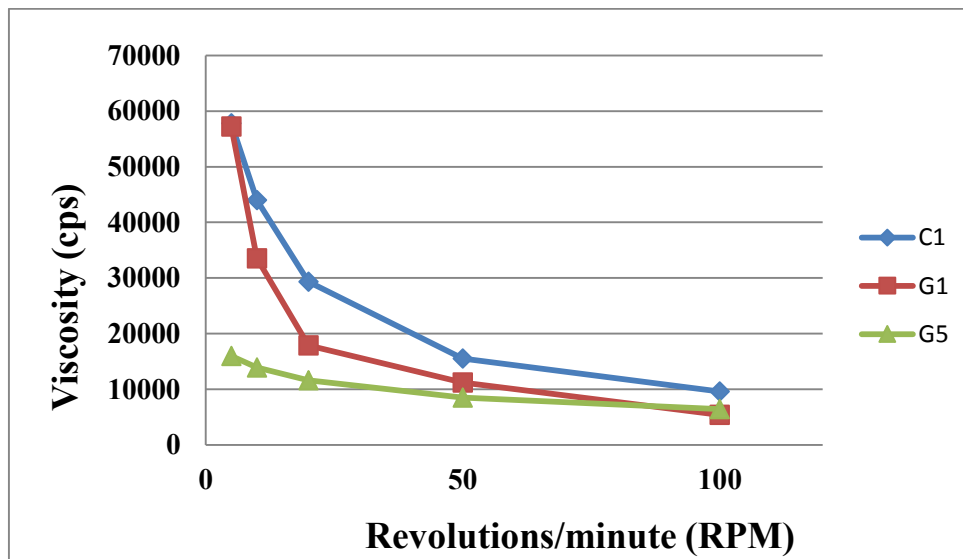
4.2 In vitro antibacterial activity

The in vitro antibacterial study was performed by measuring the diameter of the zone of inhibition on the inoculum agar plate. The zone of inhibition can be defined as the clear region around the susceptible disc or well with an antimicrobial agent on the agar surface. It is designated to test the ability of the antimicrobial agent to inhibit the

growth of microorganisms [150]. The larger the zone of inhibition is, the better the inhibition of the particular antimicrobial agent.

4.2.1 Determination of synergistic effect for zinc and copper

The synergistic effect of zinc and copper was determined in the study of the antibacterial activity against escherichia coli and staphylococcus aureus. A 20 µL portion of each solution was tested for antibacterial activity in this study. The results of this study showed that the solution with 3% of both zinc and copper has the best antibacterial activity against E. coli and S. aureus. The average zone of inhibition with different concentrations of zinc and copper is given in Figure 4-3. The results indicated that there were statistically significant differences between different formulations of the solutions ($p < 0.05$). It was suggested that the bacterial activity against S. aureus was 3% Zn & Cu = 6% Zn > 6% Cu = 3% Zn > 3% Cu while, for E. coli, it was 3% Zn & Cu > 6% Zn > 6% Cu = 3% Zn > 3% Cu. Zones of inhibition ≥15-20mm might suggestive that they have antibacterial activity against E. coli and S. aureus. As a conclusion, this study has proved that zinc and copper had a synergistic antibacterial effect when they were used together in the experimentally prepared formulations.

Figure 4-3: Comparison of antibacterial activity with varying concentrations of zinc sulfate and copper sulfate solution against Escherichia coli and Staphylococcus aureus (n=3)
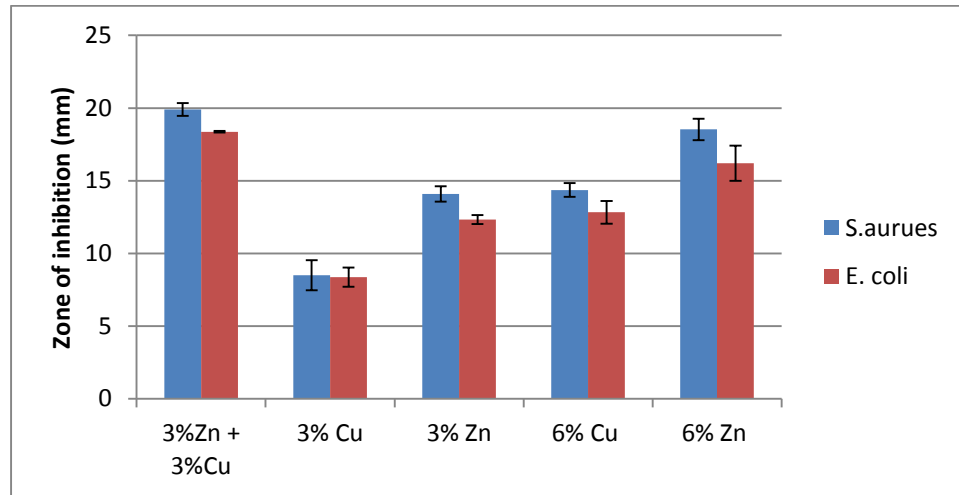

Figure 4-4: Zones of inhibition produced by (1, 2) 3% zinc and 3% copper, (3) 3% copper, (4) 3% zinc, (5) 6% copper and (6) 6% zinc against (a) E. coli and (b) S. aureus


(a)

(b)

4.2.2 Microbial studies comparison between formulations

Another study was performed to test the antibacterial activity for formulation C1, G1 and G5 with varying concentrations of zinc sulfate and copper sulfate against E. coli and S. aureus. Table 4.6 showed the antibacterial activity for formulation C1, G1 and G5 against microorganisms of E.coli and S. aureus with different concentrations of zinc and copper: blank (without drug and paraben), paraben (without drug), 0.1%, 0.25%, 0.5%, 1%, 2%, and 3% with a control, gentamicin 10 μg standard disc, by measuring the zone of inhibition in millimeters, (n=3). The mean ± standard deviation of weight for each formulation was 0.0802 ± 0.000265 (mg) in this study. There were no zones of inhibition observed on the agar plates with the blank, paraben and 0.1% in all three formulations, but the zone of inhibitions was increased as the concentration of copper and zinc increased. This also indicated that the antibacterial activity against E. coli and S. aureus increases with increasing concentrations in copper and zinc ion. In the C1 formulation, the statistical analysis showed that there was a significant difference in the concentration of 1% and above. While, in G1 and G5 formulation, there was a significant difference in the concentration of 0.25% and above. Therefore, it can be concluded that the desirable antibacterial activity was observed in the concentrations that were greater than 2% in both tested microorganisms.

Table 4.6: Antibacterial activity of different formulations with varying concentration of zinc sulfate and copper sulfate with standard gentamicin disk against Escherichia coli and Staphylococcus aureus (n=3)

Concentration of both Zn and Cu	Zone of inhibition (mm) (Mean ± SD)					
	C1		G1		G5	
	E. coli	S. aureus	E. coli	S. aureus	E. coli	S. aureus
Blank	0	0	0	0	0	0
Paraben	0	0	0	0	0	0
0.1%	0	0	0	0	0	0
0.25%	7.1±0.26	7.8±0.5	8.4±0.17	9.13±0.32	9.17±0.5	9.2±0.17
0.5%	7.8±0.1	9.2±1.15	11.37±0.06	11.93±0.49	12.3±0.64	11.3±0.42
1%	10.9±1.04	14±0.92	16.53±0.11	18.13±0.85	16.6±1.29	13.9±0.17
2%	18.77±0.61	23.3±0.57	21.93±0.55	23.07±0.15	21.8±0.15	17.6±0.44
3%	27.4±0.3	30.4±0.21	24.87±0.32	26.3±0.43	24.4±0.36	19.7±0.40
Gentamicin	24±0.36	22.2±0.56	22.43±0.153	23.53±0.15	22.7±1.77	21.3±1.16

Figure 4-5: Zones of inhibition for various concentrations of zinc and copper in (a) C1, (b) G1 and (c) G5 formulations against E. coli and (d) C1, (e) G1 and (f) G5 formulations against S. aureus

(a)

(b)

(c)

(d)

(e)

(f)
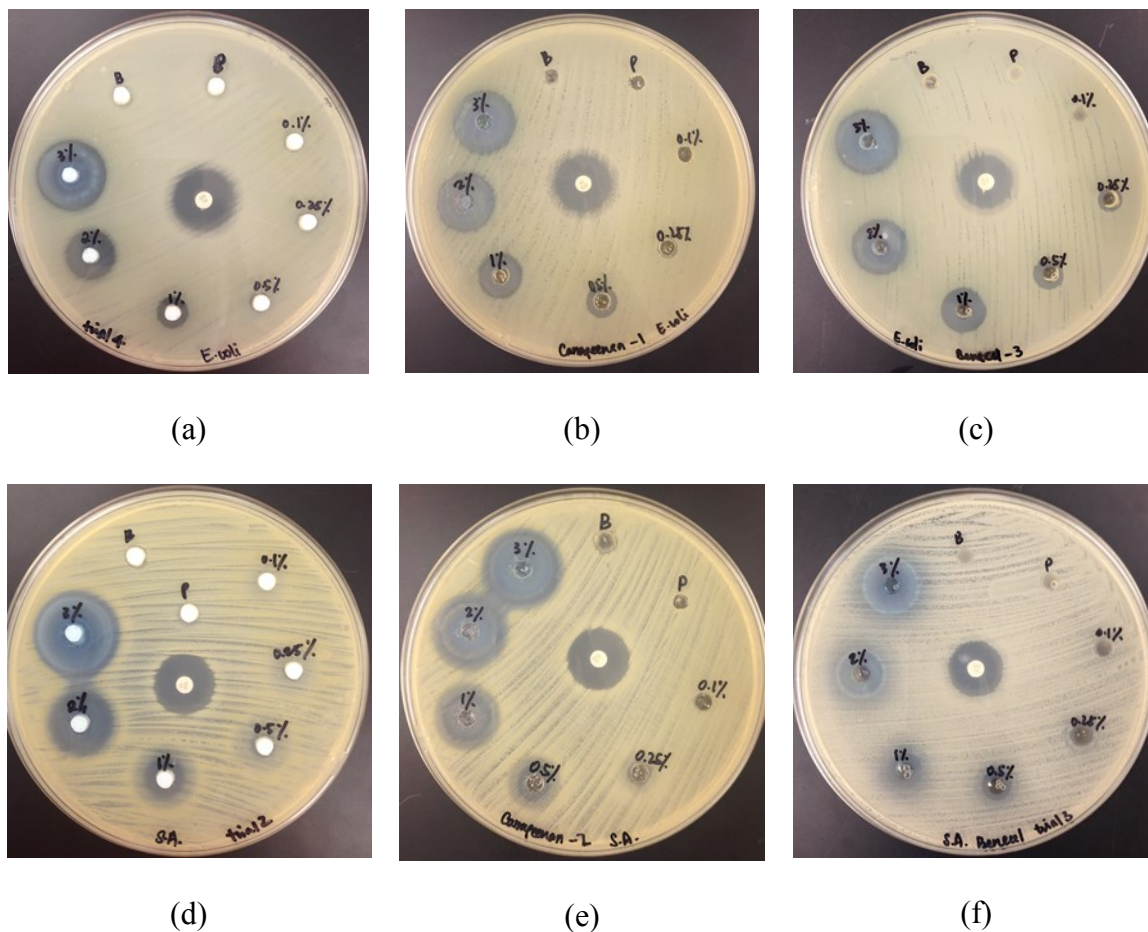
The next antibacterial study was to compare the formulated products with marketed products against E. coli and S. aureus. Table 4.7 showed the zone of inhibition for formulation C1, G1 and G5 when comparing them to the commercially available products: Nexcare™, Campho-phenique® and Equate®, with a gentamicin 10 µg standard disc, (n=3). In this study, 0.0723 ± 0.001022 (mg) of each formulation was injected into the well to test the antibacterial activity. Since there is no antimicrobial formulation

which incorporates zinc and copper into a marketable product, the formulated products were compared with the commercial available cold sore gels and diaper rash creams containing different active ingredients. The active ingredients for Nexcare™ were 5% of benzocaine as external analgesic and 1% of allantoin as skin protectant. Campho-phenique® contained 10.8% of camphor and 4.7% of phenol as pain reliever or antiseptic. Equate® diaper rash relief was 13% of zinc oxide as the skin protectant. The results indicated that there was no significant difference between G1 and G5 but there was a significant difference between the formulated gels with the formulated cream and commercial products in the same amount of formulations that applied. Therefore, commercial products did not appear to have a desirable antibacterial activity against E. coli and S. aureus in this study. According to the National Committee for Clinical Laboratory Standards (NCCLS) guidelines [151], the acceptable limits for E. coli quality control strain are 19-26 mm and for S. aureus quality control strain are 19-27 mm [151]. The results have clearly demonstrated that the zones of inhibition for the formulated products are within the range of 19-26 mm and 19-27 mm in both tested microorganism. Besides that, there was a significant difference between the formulated gels and cream when comparing their antibacterial activity. The gel formulations were found to have greater antibacterial activity than the cream formulation. This might be due to the composition of the cream formulation that was different from the gels. The cream base contains oil phases, which are insoluble in water and thus they are an obstacle for the drugs to diffuse out from the cream base.

Table 4.7: Antibacterial activity of formulated products with marketed products and standard gentamicin disk against Escherichia coli and Staphylococcus aureus (n=3)

Microorganisms	Zone of inhibition (mm) (Mean ± SD)						
	Cold sore treatment gels				Diaper rash cream		Gentamicin
	Nexcare™	Campho-Phenique®	G1	G5	Equate®	C1	
E. coli	9.63±2.82	12.2±0.98	23.73±1.41	24.1±1.85	0	20.9±1.96	22.2±2.7
S. aureus	7.5±1.32	14.8±0.29	29.1±0.58	29.1±0.29	0	26.5±0.5	24.3±0.58

Figure 4-6: Zones of inhibition produced by (N) Nexcare™, (CP) Campho-Phenique®, (ZO) Equate®, (cream) C1, (B) G5, and (C) G1 against (a) E. coli and (b) S. aureus

(a)
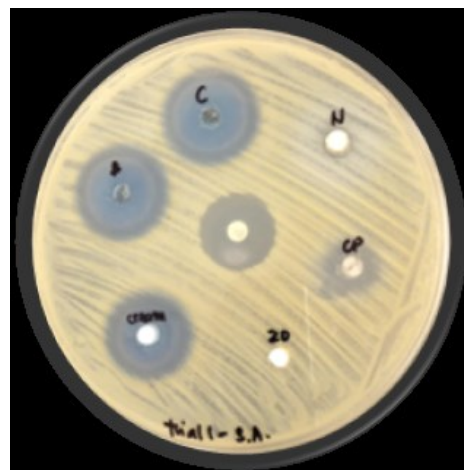
(b)

4.3 Stability study

All formulations were stored at 4°C, 25°C and 40°C in both glass and plastic containers. All formulations were analyzed for their physical appearance, phase separation, texture, homogeneity, viscosity and antibacterial activity for over a 12 week period. The physicochemical evaluation for all three topical formulations is given in Table 4.8. The color for all formulations remained the same after week 12 at all three temperatures. The physical appearance, homogeneity and texture for all formulations at 4°C and 25°C remained the same as week zero. However, phase separation was observed in all formulations at 40°C. The blue color of the liquid that came out from the cream base was observed in C1, water condensation was found on the lid and around the containers in G1, and cloudiness of the gel was observed in G5. The phenomenon seen as the physical instability of the gel formulations is known as syneresis. The syneresis effect was observed in G1 after week 3 and in G5 after week zero. ι-carrageenan in G1 usually produces an elastic gel without syneresis due to the presence of two sulfate groups per two galactose residues [152]. However, some water condensation was observed in both containers after week 3. The physical instability of G5 was observed after day 1 at 40°C which went from a clear to a cloudy gel. This indicated the presence of a thermo-sensitive HPMC polymer which caused syneresis effect in G5. A study showed that HPMC was not stable at high temperature and caused syneresis [153]. As a result, the stability study for all formulations at 40°C was terminated due to the physical instability at high temperature.

Table 4.8: Physicochemical evaluations for different topical formulations after week 12

Formulation	Temperature	Color	Physical Appearance	Homogeneity	Texture	Phase Separation
C1	4°C	Blue	Opaque	Good	Smooth	No
	25°C	Blue	Opaque	Good	Smooth	No
	40°C	Blue	-	-	-	Yes
G1	4°C	Blue	Transparent	Good	Smooth	No
	25°C	Blue	Transparent	Good	Smooth	No
	40°C	Blue	Transparent	-	-	Yes
G5	4°C	Blue	Transparent	Good	Smooth	No
	25°C	Blue	Transparent	Good	Smooth	No
	40°C	Blue	Cloudy	-	-	Yes

The viscosity of all the formulations at three different temperatures was also measured to determine the stability of the formulations. Figure 4-7 (a-d) illustrated the viscosity for C1 at 4°C (F) and 25°C (RT) in glass (G) and plastic (P) containers. It was clearly explained that the viscosity of C1 at 4°C and 25°C was found to be between 20,000 cps and 40,000 cps. No significant difference in the viscosity of C1 was observed in either glass or plastic containers. Figure 4-8 (a-d) demonstrated the viscosity for G1 at 4°C (F) and 25°C (RT) in glass (G) and plastic (P) containers. The viscosity for G1 was found to be between 20,000 cps and 40,000 cps. The viscosity increased over a period of time. This might be because the polymer was not fully hydrated or swelled from week zero. It started to increase after week 1 as the polymer swelled. Figure 4-9 (a-d) showed the viscosity for G5 at 4°C (F) and 25°C (RT) in glass (G) and plastic (P) containers. The viscosity for G5 was found to be between 10,000 cps and 35,000 cps. The results

indicated an inconsistent phenomenon of viscosity in G5 at 4°C and 25°C in both containers. The reason for this phenomenon was undetermined but there could be some reasons that cause the inconsistency of the viscosity. One of the reasons may be the degradation of the polymer in this formulation. Polymers are complexity chemical; there are many possibilities of interactions with the drug and the containers, which affect the viscosity of the formulation. Further studies should be carried out to justify the long-term storage condition for this formulation.

Figure 4-7 (a-d): Viscosity data for C1 at 4°C (F) and 25°C (RT) in glass (G) and plastic (P) container for over a 12 week period
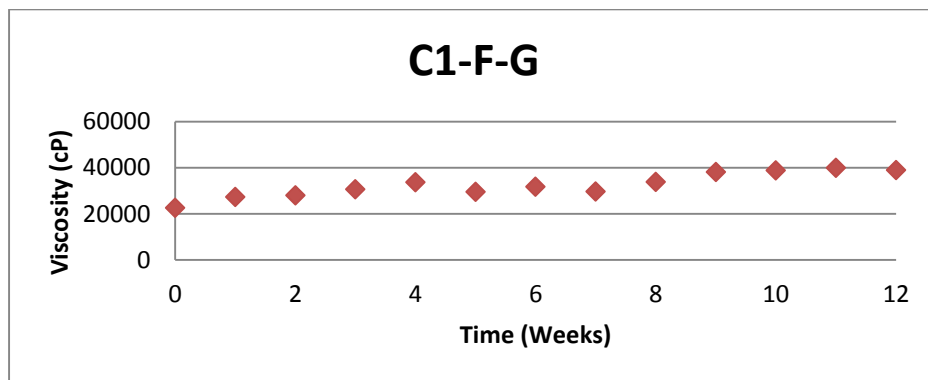
(a)
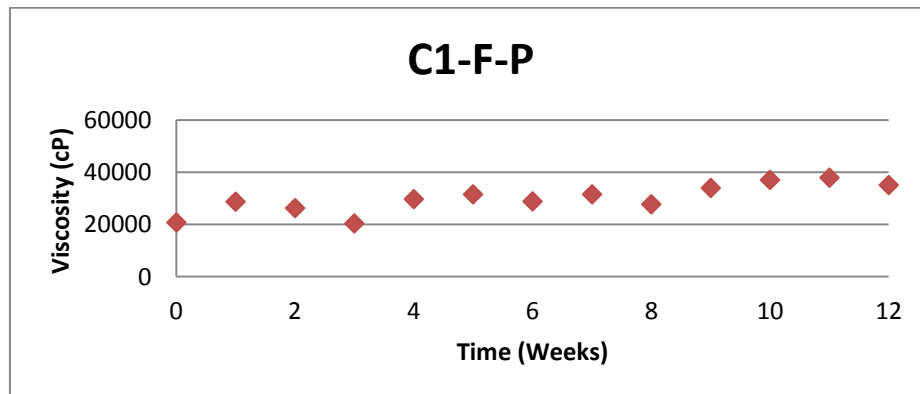
(b)

(c)

(d)
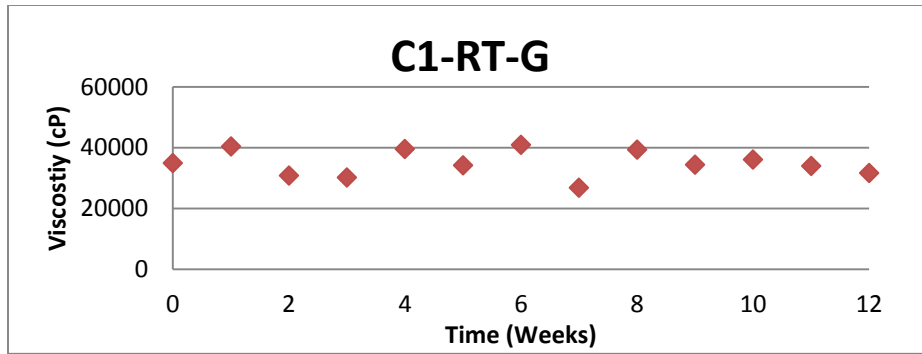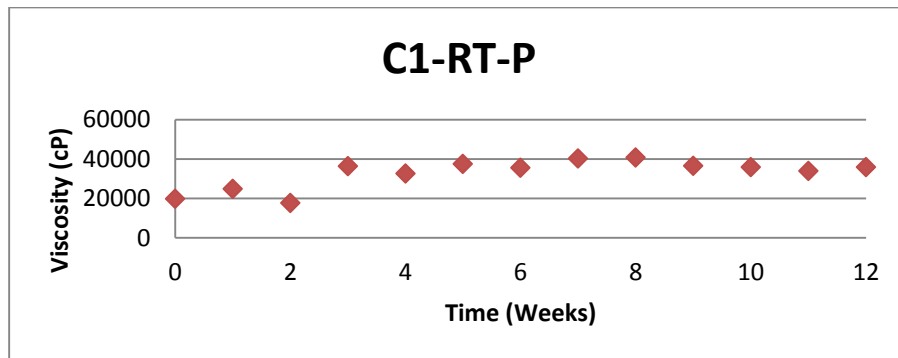
Figure 4-8 (a-d): Viscosity data for G1 at 4°C (F) and 25°C (RT) in glass (G) and

plastic (P) container for over a 12 week period

(a)

G1-F-P

(b)

G1-RT-G

(c)

G1-RT-P
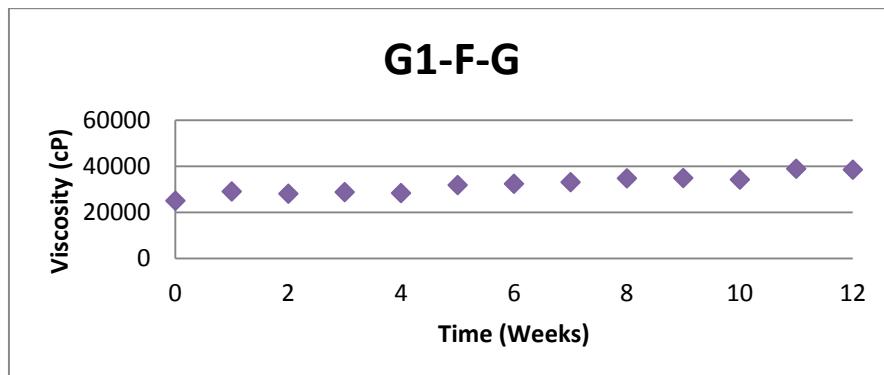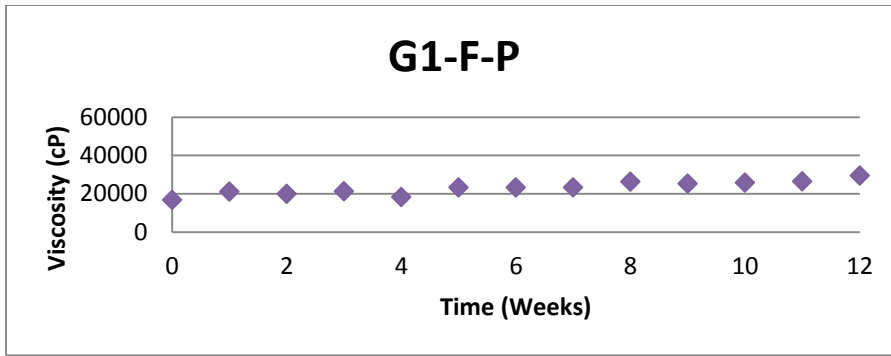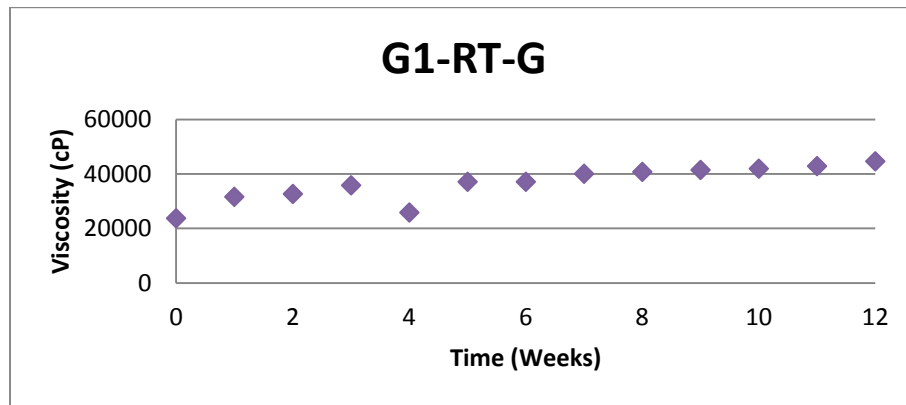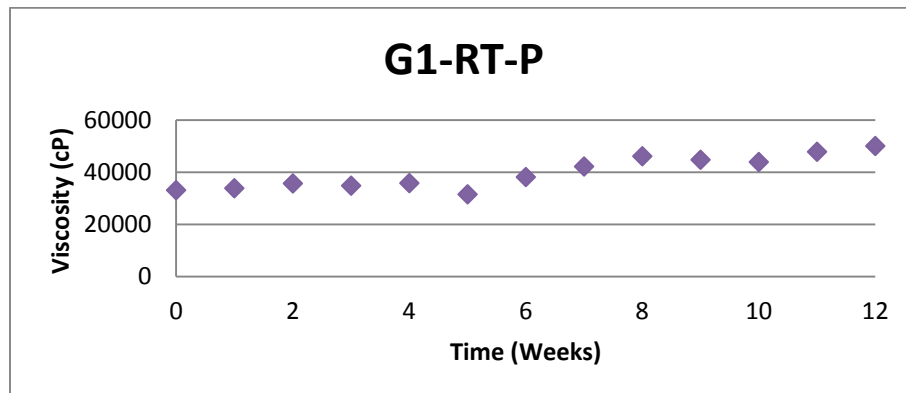
(d)

Figure 4-9 (a-d): Viscosity data for G5 at 4°C (F) and 25°C (RT) in glass (G) and

plastic (P) container for over a 12 week period
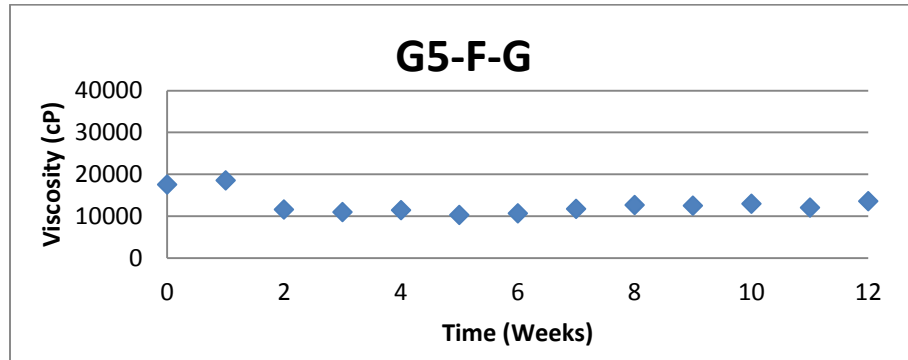
(a)
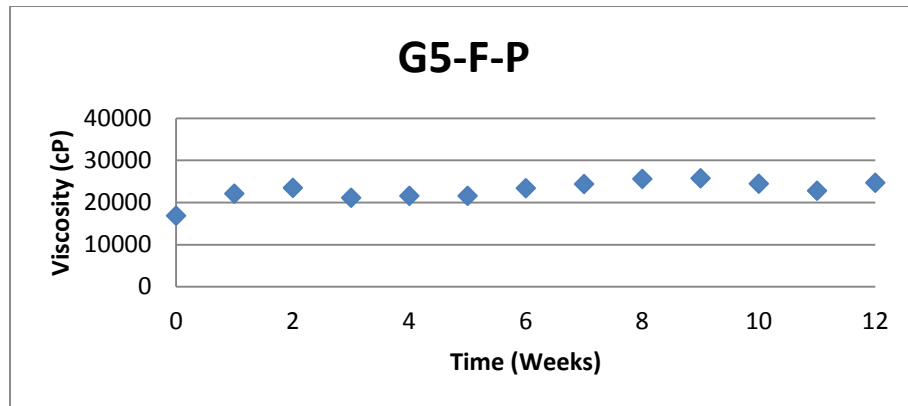
(b)
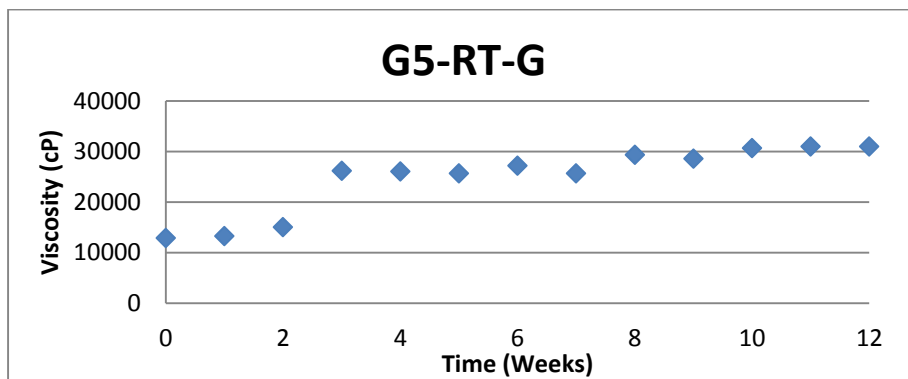
(c)

(d)
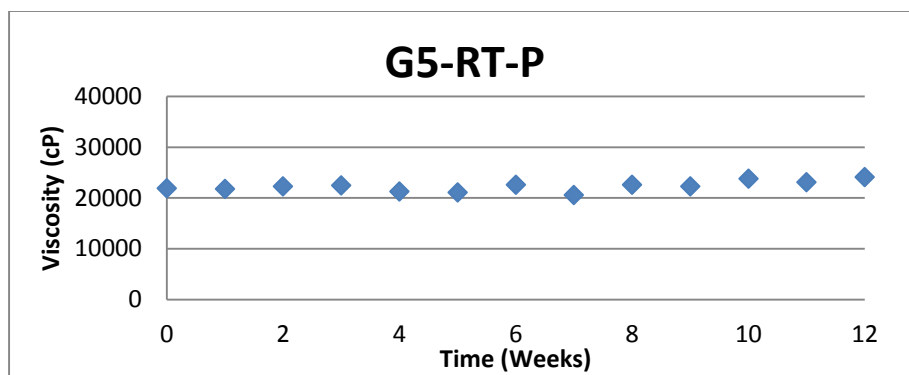
The antibacterial activity for all formulations was analyzed using the agar well diffusion assay for a 12 week period at 4°C, 25°C and 40°C in both glass and plastic containers. The average zone of inhibition for all formulations was reported in Table 4.9, Table 4.10 and Table 4.11. No significant difference was observed in zones of inhibition over time between the glass or plastic containers. It can be concluded that the material of containers did not alter the antibacterial activity of any of the formulations. There is no significant difference in the zones of inhibition between the different temperatures. However, there is a statistically significant difference in zones of inhibition over time and between temperatures over time. The temperatures did not affect the antibacterial effect of the formulations, but the temperatures did alter the physical stability of the formulations. The antibacterial activity was found to be not stable over time. Unfortunately, a decrease in the zones of inhibition was observed at both 4°C and 25°C over a 12 week period.

Table 4.9: Antibacterial activity of C1 formulation over 12 week period, (n=3)

| Type of storage container | Period | Zone of inhibition (mm) (Mean ± SD) | | | Control |
| | | Temperature | | | |
		4°C	25°C	40°C	Gentamicin (10μg)
Glass container	Day 1	17.63±0.15	19.06±0.25	20±0.88	21.4
	Week 1	24.7±0.36	24.23±0.21	-	22.9
	Week 3	21.87±0.23	23.2±0.35	-	23.6
	Week 6	22.13±0.23	21.7±0.46	-	22.9
	Week 9	22.1±0.46	21.6±0.26	-	22.1
	Week 12	21.87±0.15	21.23±0.15	-	23.8
Plastic container	Day 1	22.73±0.38	23.87±0.21	23.63±0.70	20.9
	Week 1	25.13±0.38	24.5±0.1	-	22.3
	Week 3	23.4±0.6	22.37±0.65	-	23.2
	Week 6	23.07±0.35	22.17±0.45	-	22
	Week 9	22.8±0.45	21.43±0.058	-	22.2
	Week 12	22.13±0.31	21.7±0.36	-	23.7

Table 4.10: Antibacterial activity of G1 formulation over 12 week period, (n=3)

| Type of storage container | Period | Zone of inhibition (mm) (Mean ± SD) | | | Control |
| | | Temperature | | | |
		4°C	25°C	40°C	Gentamicin (10μg)
Glass container	Day 1	25.3±0.3	24.73±0.70	24.57±0.058	21.1
	Week 1	25.97±0.47	25.83±0.42	26.4±0.61	20.9
	Week 3	25.6±0.26	25.1±0.2	26.27±0.23	23.5
	Week 6	24.1±0.26	24.2±0.17	-	21.9
	Week 9	25.23±0.31	25.73±0.15	-	23.5
	Week 12	24.4±0.2	25.1±0.36	-	22.6
Plastic container	Day 1	25.33±0.32	24.8±0.36	25.03±0.25	21.5
	Week 1	26.37±0.49	25.8±0.31	25.83±0.40	22.5
	Week 3	25.47±0.40	25.03±0.31	25.73±0.15	22.9
	Week 6	24.2±0.26	24.23±0.57	-	22.2
	Week 9	25.53±0.35	25.63±0.47	-	23.4
	Week 12	24.33±0.64	25.13±0.12	-	22.3

Table 4.11: Antibacterial activity of G5 formulation over 12 week period, (n=3)

| Type of storage container | Period | Zone of inhibition (mm) (Mean ± SD) | | | Control |
| | | Temperature | | | |
		4°C	25°C	40°C	Gentamicin (10µg)
Glass container	Day 1	25.1±0.46	25.43±0.32	25.47±0.32	20.3
	Week 1	26.3±0.17	26.03±0.40	-	22.6
	Week 3	25.3±0.26	25.5±0.26	-	22.2
	Week 6	24.43±0.23	24.73±0.38	-	22.3
	Week 9	25.13±0.058	25.03±0.35	-	21.8
	Week 12	24.87±0.47	24.9±0.2	-	22.6
Plastic container	Day 1	26.4±0.17	25.47±0.42	25.8±0.4	21.6
	Week 1	25.53±0.35	25.8±0.1	-	21.6
	Week 3	25.53±0.21	25.5±0.66	-	22.9
	Week 6	24.6±0.36	25.33±0.31	-	23
	Week 9	25.13±0.32	25.13±0.21	-	21.4
	Week 12	24.43±0.21	24.77±0.058	-	22.7

Chapter 5

Conclusion

In this study, we formulated three different antibacterial formulations of topical products with copper sulfate and zinc sulfate that act as antibacterial agents. During the formulation process, the interactions of the excipients and the drugs were found to have the ability to alter the physical stability of the formulations due to the complexity of the excipients. However, we successfully developed one cream and two gels formulations that can be used to incorporate the drugs. The final formulations were determined to be formulations C1, G1 and G5 which contained carrageenan as the gelling agent in C1 and G1, and HPMC as the gelling agent in G5. The results observed in this study clearly indicate all the formulations exhibited pseudoplastic flow and that the antibacterial activity was better than the commercial products when compared with the amount of products that were applied. Even though low percent drug content was analyzed using the ICP-MS method, the antibacterial activity for all the formulations was not altered. Therefore, a quantitative analysis which can differentiate the concentration should be found to determine the drug content in the formulations. Studies such as ex-vivo permeation of copper and zinc, skin irritation, human test studies, and stress studies with drug content should be carried out as future works.

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