IJPSR (2022), Volume 13, Issue 7 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 04 October 2021; received in revised form, 26 November 2021; accepted, 22 December 2021; published 01 July 2022

FORMULATION DEVELOPMENT, CHARACTERIZATION, OPTIMIZATION AND *IN-VITRO* EVALUATION OF CHITOSAN COATED FLEXIBLE LIPOSOMES OF MUPIROCIN FOR TREATMENT OF LACERATION

Shagun Gogna

Department of Pharmaceutics, Rayat Bahra Institute of Pharmacy, Hoshiarpur - 146104, Punjab, India.

Keywords:

Chitosan coated flexible liposomes, Laceration, Mupirocin, Wound healing

Correspondence to Author:
Shagun Gogna

Department of Pharmaceutics,
Rayat Bahra Institute of Pharmacy,
Hoshiarpur - 146104, Punjab, India.

E-mail: sgogna28786@gmail.com

ABSTRACT: In present era research work has the continuous focus on the formulation development of chitosan-coated flexible liposomes of Mupirocin that has persistent mitigation of laceration. Skin injuries (laceration), an important health problem that needs to be managed properly in order to avoid serious consequences. The current focus in wound therapy is on upholding the moisture balance in the wound bed and protection against pathogenic invasion. Wound dressings in a form of flexible liposomes incorporating antibiotic mupirocin, can be applied to control dermal infections and thereby ensure optimal wound healing. Drug in flexible liposome provides prolonged contact between drug and wounded area, reducing the need for frequent application of wound dressing. Our project aims to develop the chitosan-coated flexible liposomes of Mupirocin in a soya lecithin and edge activators. The drug Mupirocin was analysed through various physio-chemical and analytical parameters like pre-formulation studies, melting point, solubility, calibration curve, FTIR and drug excipient compatibility study. The chitosan-coated flexible liposomes which were prepared by Rota evaporator technique and incorporation into flexible liposomes with a dose 100mg/50 ml. The chitosan-coated flexible liposomes were evaluated by using various parameters like particle size, polydispersity index, percentage yield, encapsulation efficiency, %dug content and in vitro drug release studies. The stability study for all formulations were conducted for 2 months and found to be satisfactory as per requirement. This above novel formulation requires further *in-vivo* and clinical study then it will able to proof an excellent tool for persistent management of laceration.

INTRODUCTION: A laceration is commonly known as wound. Skin wounds are the basic tissue in which the skin is cut, punctured or torn. When injury shows up within the frame of a burn wound, it can influence a few skin layers. Wounds can be caused in a number of different ways by a variety of different objects, be it blunt, sharp or projectile. They are classified into several categories depending on the cause and resulting injury: incised wound, laceration, abrasion, puncture, avulsion, and amputation. Separate burn wounds can be a colossally difficult errand to treat in burn units as they incorporate expanded hazards of fluid loss, hypothermia, infections and impeded scarring.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.13(7).2824-43

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.13(7).2824-43

Burn injury to the skin makes a nearby immune-compromised zone, leading to possibly life-threatening microbial contaminations. Despite the progress in laceration treatment, wound diseases are still the major cause of wound-related morbidity and mortality [1]. Effective burn treatment represents a challenge regarding therapeutic

outcome, terrifying, utilitarian, and corrective results. A few promising lines in the advancement of burn treatment were proposed, among which flexible liposomes fulfill numerous criteria for perfect wound dressing. The perfect dressing ought to accomplish lasting skin recovery, have great utilitarian and tasteful characteristics, have ideal mechanical properties, be bio-adhesive and conceivably give a controlled discharge of active ingredients [2]. Chitosan may be a natural biodegradable polymer with wound healing properties on it possess. Chitosan-coated flexible liposome gives a sodden environment at wound location and shows bioadhesive properties. To guarantee the controlled release of the active ingredient, liposome bearing mupirocin was consolidated in a chitosan coat. Mupirocin was chosen as an antimicrobial drug due to its action against different microbes, commonly infecting injured regions of the skin. Its extra advantage is that it appears low activity against microorganisms within the normal skin flora. This could be seen as an advantage because the skin's typical resistance against pathogens will not be interfered with by the chosen medicate [3].

Transdermal Drug Delivery System: Transdermal drug delivery systems (TDDS) are dosage forms designed to deliver a therapeutically effective amount of drug across a patient's skin. So, as to deliver therapeutic agents through the human skin for systemic effects, the great morphological, biophysical, and physicochemical properties of the skin are to be considered. Transdermal delivery provides a number one edge over injectable and oral routes by increasing patient compliance and avoiding first-pass metabolism especially. Transdermal delivery addresses a delightful option in contrast to oral delivery of medication and is ready to supply another to hypodermic infusion as well. The primary Transdermal system, Trans-dermal-SCOP, was approved by FDA in 1979 for the prevention of nausea and vomiting related to ravel, particularly by sea. The evidence of percutaneous drug absorption could also be found through measurable blood levels of the drug, detectable excretion of the drug and its metabolites within the urine, and thru the patient's clinical response to the administered drug therapy [4]. TDDS offers pharmacological focal points over the oral course and improves patient worthiness and consistency. As such, they need been a crucial area of pharmaceutical research and development over a previous couple of decades. Today, there are 19 transdermal delivery systems for such drugs as oestradiol, fentanyl, lidocaine, and testosterone; combination patches contain one drug for contraception and hormone replacement iontophoretic and ultrasonic delivery systems for analgesia. Molecules were greater than 500 Da normally don't cross the skin. This prevents epicutaneous delivery of the high relative molecular mass therapeutics and non-invasive transcutaneous immunization. There's considerable interest within the skin as a drug application site both for local and systemic effect. Be that as it may, the skin, particularly the corneum, represents a considerable hindrance to medicate entrance in this manner, restricting effective and transdermal bioavailability.

Additionally, transdermal systems are non-invasive and may be self-administered. They will provide a release for long periods of your time (up to at least one week). They likewise improve tolerant consistency, and along these lines, the system is for the most part, modest. Perhaps the best challenge for transdermal delivery is that only a limited number of medicines are amenable to administration by this route. With Current delivery techniques, effective transdermal medications have molecular masses that are simply up to a couple of hundred Daltons. These display octanol-water partition coefficients vigorously favour lipids and need dosages of milligrams each day or less [5].

Advantages of Transdermal Drug Delivery System: The benefits of transdermal delivery over other delivery systems are as follows:

1. Evasion of first-pass metabolism of medications.
2. Diminished plasma concentration levels of medications, with diminished side effects.
3. Decrease of changes in plasma levels of medications, Utilization of medication applicants with a short half-life and low therapeutic index.
4. Simple disposal of drug delivery in the event of poisonousness.

5. Decrease of dosing recurrence and improvement of patient consistency [6].

Limitation of TDDS: Limits for a medication substance to be consolidated into a transdermal delivery system are:

1. Heavy drugs molecules (>500 Da) are normally hard to infiltrate the layer cornea.
2. Drugs with low or high partition coefficient neglect to arrive at blood circulation.
3. This course can give drugs that are highly melting because of their low dissolvability both in water and fat.
4. Many methodologies have been endeavored to convey medicament across skin obstruction and improve the adequacy[7].

Skin and Wounds:

Skin Structure: The biggest organ of the body is the skin. Human skin comprises three layers, specifically the epidermis, dermis, and hypodermis, separately. The epidermis comprises a few layers of epithelial cells. From within to the outside, these layers are the layer germinativum, layer spinosum, layer granulosum, layer lucidum, and layer corneum [8]. The deepest epidermal layer comprises of cells that are immediately isolating, in as opposed to the external layer of the epidermis, involving dead cells, level in appearance. This last layer is alluded to as the layer corneum (SC) or the horny layer. SC changes incredibly in thickness, going from 0.8 to 0.006 mm on the palm, soles and eyelids, individually. The SC might be simply 10 µm thick when it is dry, yet will grow a few overlays when in contact with water[9]. The epidermis relies on disseminating supplements from the dermis, as there is no blood supply in the epidermis. Beneath the epidermis is the dermis. This layer goes from 3-5 mm in thickness. The dermis capacities as a connective tissue layer, which contains elastin and collagen strands, giving the skin capacity to stretch and strength, individually. Veins, skin extremities, lymphatic's, and concentrated sensitive spots are bountiful in this layer. Not exclusively is the dermis giving nourishment to the epidermis; it likewise assumes a vital part in temperature control. Because of the particular sensitive spots in the dermis, all the more precisely the afferent nerve strands, dermis sees pressure, agony, temperature, and other soma-to-tangible information sources. The hair erection and release by the skin's exocrine organs are constrained by the efferent sensitive spots situated in the dermal layer. The subcutis is otherwise called the hypodermis. The hypodermis is a free layer of connective tissue that secures the fundamental tissue. Fat tissue makes up a large portion of the hypodermis [10].

Intact Skin: Human skin fills in as a defensive boundary against synthetic substances and bright light. The skin likewise fills in as a shield against pathogenic microorganisms. The combination of nutrient D in the epidermis and the capacity of fat tissue in the hypodermis are extra significant highlights of flawless skin. Sound and unblemished skin are substantial for our wellbeing and prosperity. The outside of flawless skin has pH esteem in the scope of 5.4-5.9. The protecting corrosive obstruction differs according to both endogenous and exogenous elements. Because of the acidic idea of skin, a few bacterial strains are ordinarily present, which make up the regular microbial vegetation for sound skin [11]. Section of outside particles through, across, and using human skin can be encouraged by three possible pathways **Fig. 1.**

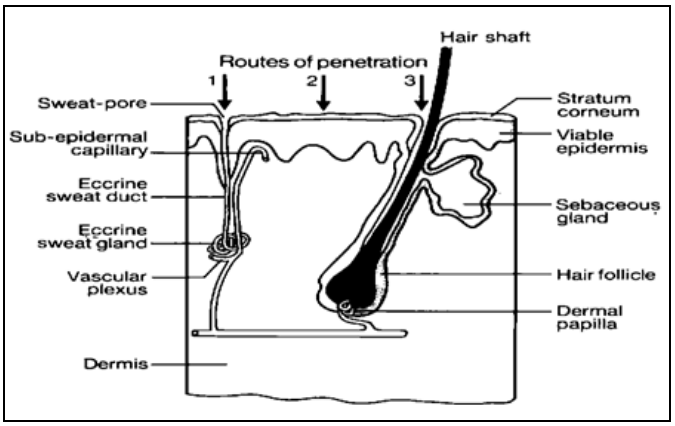

FIG. 1: ILLUSTRATING ROUTES OF PENETRATION: 1. THROUGH SWEAT GLANDS; 2. ACROSS STRATUM CORNEUM; 3. *VIA* THE HAIR FOLLICLES

They incorporate the pathways through the perspiration channels using the hair follicles and the sebaceous organs (altogether called the appendageal course) or the section straight-forwardly 5 across the SC. For the most part, it is

acknowledged that the appendageal course addresses roughly 0.1 % of the entry of medications through the skin. The SC is viewed as the rate restricting advance in transdermal infiltration of essentially all particles [12]. The construction of the SC can be viewed as an alleged block and mortar game plan where the blocks address the keratin-rich corneocytes, and the mortar addresses the intracellular lipid-rich network. Outside atoms can go through the "blocks and concrete" structure by either intracellular or Trans cell pathway as **Fig. 2** [13].

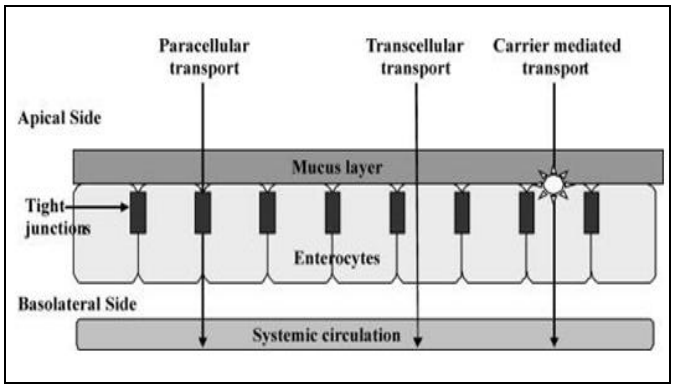

FIG. 2: ILLUSTRATION OF THE INTRACELLULAR AND THE TRANSCELLULAR ROUTE

The intracellular course is presently viewed as the preeminent course for saturation of the majority of the medications applied to the skin. The lipophilicity level and the particle's size will assume a significant part for atoms going through the lipid spaces[14].

Injured Skin and its Barrier Properties: Laceration and constant skin wounds, for example, skin ulcers, are intense medical problems. These wounds weaken personal satisfaction and take up considerable medical care assets. Improved injury treatment has become a definitive objective in injury treatment. There is agreement on injury treatment that mending the injury climate ought to be kept soggy for an ideal injury. Simultaneously, the changed skin obstruction capacity ought to be considered when creating dressings/drug conveyance frameworks for harmed skin. The lipophilic and hydrophilic properties of the skin are destined to be changed when the skin is harmed. The pH of the skin may likewise be changed. Contingent upon the seriousness and the intricacy of the harmed skin, the penetrability of skin for different medications may be exceptionally high. The defensive job of the skin in holding in body liquids goes under the genuine treat after cut off consumes wounds. Bacterial contaminations can get backbone in the helpless basic tissue without much of a stretch. Additionally, the foundational outcomes of loss of water and plasma proteins that escape from the revealed consumed outside are much more genuine. The unsettling influences in the course can be perilous [15].

Laceration (Wounds): Lacerations are wounds to the underlying tissue where the skin is cut, penetrated, or torn. Skin wounds can be partitioned into two classes depending on their appearance and capacity to recuperate individually as intense or ongoing injuries. An intense injury is a tissue injury that mends inside 8-12 weeks. The meaning of an ongoing injury is the harmed tissue that has a debilitated capacity to mend up. The underlying conditions for a persistent injury might be chemotherapy, steroid use, diseases, blood vessel inadequacy, diabetes mellitus, radiation, pressure, and venous deficiency [16]. Ongoing injuries can display complex microbiological consistency that can influence the recuperating interaction without giving any indications of underlying disease) explored ongoing injuries and their seven microbial verdure and found a high level of methicillin-resistant *S. aureus* (MRSA), *P. aeruginosa*, and *B. Streptococcus* (45, 28, and 21%, separately). The microbial investigation affirmed that injuries are inclined to diseases and that the joining of antimicrobial specialists in injury dressings is hence suggested [17]. When epithelisation is deferred past three weeks, the mended wound will leave hypertrophic scarring.

Consume wounds can be separated into a few classes relying upon the skin layers influenced by the injury.

1. Epidermal Burns: just influencing the epidermis, regularly the consequence of burns from the sun. Rankling can happen with this kind of consume; notwithstanding, it isn't regularly seen. Mending of the skin ordinarily takes five to seven days.

2. Superficial fractional thickness burns influence the epidermis and the underlying upper dermis. In this sort, rankling is

usually seen. Because of the effect on the dermal layer of the skin and the revealed shallow nerves, consumption is difficult. Recuperating of the skin generally requires fourteen days[18].

3. Deep incomplete thickness burns: these consume influence the epidermis, and more profound into the dermis. For the most part, mending of the skin takes two to about a month and is frequently identified with generous scarring.

4. Full-thickness burns: The whole type of regenerative components have been broken in these consumes. The recuperating of these consumes happens from the edges and is often connected with constriction. The way toward mending will require a little while, and the scarring will be obvious.

5. Severely charred areas: broaden farther than the skin layers, down to the subcutaneous fat, muscles and bone [19].

Etiology of Laceration: In general, factors that adversely affect wound healing can be remembered by using the mnemonic device ‘didn't HEAL,’ as follows:

- D = Diabetes: The long-term effects of diabetes impair wound healing by diminishing sensation and arterial inflow. In addition, even acute loss of diabetic control can affect wound healing by causing diminished cardiac output, poor peripheral perfusion, and impaired polymorph nuclear leukocyte phagocytosis.
- I = Infection: Infection potentiates collagen lysis. Bacterial contamination is a necessary condition but is not sufficient for wound infection. A susceptible host and wound environment are also required. Foreign bodies (including sutures) potentiate wound infection.
- D = Drugs: Steroids and antimetabolites impede the proliferation of fibroblasts and collagen synthesis.
- N = Nutritional problems: Protein-calorie malnutrition and deficiencies of vitamins A, C and zinc impair normal wound-healing mechanisms.
- T = Tissue necrosis: resulting from local or systemic ischemia or radiation injury, impairs wound healing. Wounds in characteristically well-perfused areas, such as the face, and neck, may heal surprisingly well despite unfavorable circumstances. Conversely, even a minor wound involving the foot, which has a borderline blood supply, may mark the onset of a long-term, nonhealing ulcer. Hypoxia and excessive tension on the wound edges also interfere with wound healing because of local oxygen deficits.
- H = Hypoxia: Inadequate tissue oxygenation due to local vasoconstriction resulting from sympathetic overactivity may occur because of blood volume deficit, unrelieved pain, or hypothermia, especially involving the distal extent of the extremities.
- E = Excessive tension on wound edges: This leads to local tissue ischemia and necrosis.
- A = Another wound: Competition between several healing areas for the substrates required for wound healing impairs wound healing at all sites.
- L = Low temperature: The relatively low tissue temperature in the upper and lower extremities (a reduction of 1-1.5°C [2-3°F] from normal core body temperature) is responsible for slower healing of wounds at these sites [20].

Wound Healing: Wound healing could be a complicated method consisting of 4 steps: hemostasis, inflammatory reaction, proliferation, and remodeling, all of that square measure regulated by cytokines and growth factors discharged by cells within the wounded space. The phases square measure overlapping and linear for acute wounds, whereas the chronic wounds, will be found at completely different stages of the healing method and don't heal in an orderly manner [21]. Haemostasis happens for a number of minutes when tissue is dislocated.

The disruption of blood vessels and, therefore, the ensuing leak of blood into the wound square measure followed by protoplasm activation and aggregation. This can then result in forming a protein clot that causes the hemorrhage to prevent and plugs the defect and seal off the exposed tissue. Drying the clot forms a scab that gives a short-lived protection to the broken skin and serves as a probationary matrix for cell migration and as a supply for cytokines and growth factors [22].

The inflammatory reaction takes place shortly when stopped and may last for quite seventy-two hours. This part consists of the attraction of neutrophils and monocytes from the current blood to the wounded space resulting in cleansing and elimination of germs and trash. The infiltration of immune cells could result from chemotactic signals from growth factors, epitopes of incursive microorganisms, and by-products of chemical change of protein and different matrix elements. The proliferative part of the wounded skin starts 4-5 days when injury and lasts for concerning two-three days. It consists of re-epithelization and wound contraction. Reepithelization involves the migration of keratinocytes into the wound, proliferation of keratinocytes, regeneration of the basal cells that connect the cuticle and, therefore, the derma, and reconstitution.

The latter is meted out by the formation of recent blood vessels, embryonic cell proliferation, and animate thing matrix-like scleroprotein. Wound contraction is achieved by the differentiation nine of fibroblasts to myofibroblasts that have the power to increase and retract. Therefore, the attachment of fibroblasts to scleroprotein results in the inspiration of a connective tissue [23].

Remodeling of wounds is associated with equilibrium between recent cellular animal tissue formation and its degradation by proteases. This stage, which can continue for months, is characterized by modifying the structural integrity of the tissue to restore traditional skin design. Counting on the regulation of this maturation method, the ultimate result could either be a scar that's indistinguishable from the healthy skin that is that the goal, or connective tissue that elevates on top of the encircling uninjured skin, indicating a deficient regulation of the method [24].

Transfersomes (Flexible Liposomes): The term Transfersome and also the underlying thought was introduced in 1991 by Gregor Cevc. A Transfersome may be an extremely all-mains and stress responsive, complicated mixture in the broadest sense. Its most popular kind is an associate ultra-deformable cyst possessing an associate liquid core encircled by the complicated lipoid bilayer. Reciprocity of native composition and form of the bilayer makes the cyst self-activating and self-optimizing. These permit the Transfersome to cross varied transport barriers efficiently, so act as a Drug carrier for non-invasive targeted drug delivery and sustained unharnessed therapeutic agents [24].

The idea may be an in-camera command biopharmaceutical company with headquarters in Germany. A plan develops and commercializes non-invasive, targeted medical specialty applied through the skin and/or nose. The proprietary carriers are usually applied on the skin and may be designed to attain high drug concentration at or close to the positioning of application, diminish native or general adverse aspect effects, and infrequently increase drug efficiency. The Company's Transfersome carriers are locally applied on the skin. They may be designed to attain high drug concentration at or close to the positioning of application, increasing drug efficiency and decreasing aspect [25].

Transfersomes were developed to require the advantage of phospholipids vesicles as transcutaneous drug carriers. With the immoderate versatile membrane, these self-optimized aggregates can deliver the drug reproducibly either into or through the skin, looking on the selection of administration or application, with high potency. These sac transfersomes are many orders of magnitudes, a lot more elastic than the quality liposomes and therefore compatible for skin penetration. Transfersomes overcome the skin penetration problem by compressing themselves on the intracellular protection lipoid of the stratum. There's provision for this, owing to the high cyst deformability, which allows the entry thanks to the mechanical stress of close, during a self-adapting manner. The flexibility of transfersomes membrane is achieved by mixture appropriate active parts within correct ratios.

The ensuing flexibility of transfersome membrane minimizes the danger of complete cyst rupture within the skin and permits transfersomes to follow the natural water gradient across the cuticle once applied below nonocclusive conditions. Transfersomes will penetrate the intact stratum spontaneously on 2 routes within intracellular lipid that dissent in their bilayers properties [26].

Mechanism of Penetration of Transfersomes: Transfersomes, once applied beneath appropriate conditions will transfer 0.1 mg of lipid per hour and cm^2 space across the intact skin. This price is well over that that is usually driven by the transdermic concentration gradients. The explanation for this high flux rate is, of course, occurring "transdermal diffusion gradients," *i.e.,* another way more distinguished gradient is obtainable across the skin. This diffusion gradient is developed thanks to the skin penetration barrier, prevents water loss through the skin, and maintains a water activity distinction within the viable part of the cuticle (75% water content) and nearly fully dry horny layer, just about the skin surface (15% water content). This gradient is extremely stable as a result of close air could be a good sink for the water molecule even once the transdermic water loss is unphysiologically high [27].

Consequently, all lipid vesicles made up of the polar lipid vesicles move from the rather dry location to the sites with a sufficiently high-water concentration. Thus, once lipid suspension (transfersomes) is placed on the skin surface, that's part dehydrated by the water evaporation loss, so the lipid vesicles feel this "osmotic gradient" and take a look at to flee complete drying by moving on this gradient. they'll solely accomplish this if they're sufficiently deformable to taste the slender pores within the skin, as a result of transfersomes composed of surface-active agent have additional appropriate rheological and association properties than that accountable for their larger deformability less deformable vesicles as well as customary liposomes are confined to the skin surface, wherever they dehydrate fully and fuse, so that they have less penetration power than transfersomes. Transfersomes are optimized during this respect and therefore attain the most flexibility will take full blessings of the Transepidermal diffusion gradient (water concentration gradient).

The carrier combination consists of a minimum of one amphipathic (such as phosphatidylcholine), that in liquid solvents self-assembles into a lipid bilayer that closes into a straightforward lipid sac. Lipid bilayer flexibility and porosity are greatly accumulated by the addition of at least one bilayer softening element (such as a biocompatible surface-active agent or an amphiphile drug). The ensuing flexibility and porosity optimized, Transfersome sac will so adapt its form to close simply and speedily, by adjusting the native concentration of every bilayer element to the native stress intimate by the bilayer **Fig. 3** [28].

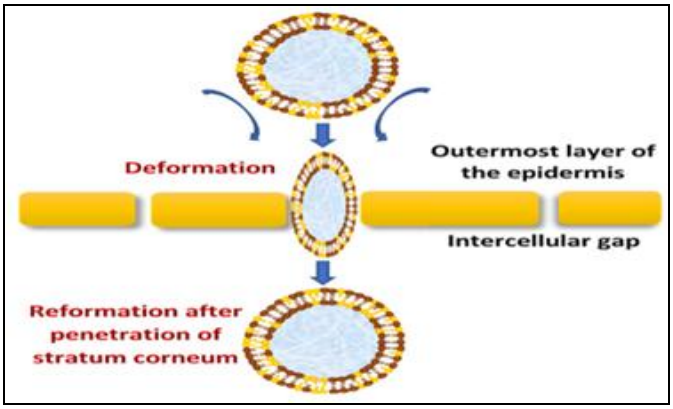

FIG. 3: MECHANISM OF TRANSFERSOMES

Vesicles will act as drug carrier systems, whereby intact vesicles enter the stratum, carrying vesicle-bound drug molecules into the skin, below the influence of present *in-vivo* connective tissue association gradient. Sac will act as penetration enhancers, whereby sac bilayer enters the stratum and then modifies its animate thing lipids, raising its thinness. Phospholipids have a high affinity for biological membranes; thus, the blending of sac phospholipids bilayer with the animate thing supermolecule layer of skin may additionally contribute to porosity sweetening of deformable vesicles [29].

Salient Features of Transfersomes: Transfersomes possess associate degree infrastructure consisting of hydrophobic and deliquescent moieties and will accommodate drug molecules with a wide selection of solubility. Transfersomes will deform and suffer slim constriction (from five to ten times but their diameter) while not measurable loss. This high deformability provides higher penetration of intact vesicles.

They will act as carriers for low moreover as high relative molecular mass medication, *e.g.,* analgesic, anaesthetic, corticosteroids, hormone, anticancer, insulin, gap junction macromolecule, and albumen. They're biocompatible and perishable as they're made of natural phospholipids, the same as liposomes. They need high defense potency, just in case of oleophilic drug almost ninetieth. They defend the encapsulated drug from metabolic degradation. They act as depot, cathartic their contents slowly and step by step. They will be used for each general moreover as topical delivery of the drug. Straightforward to proportion, as the procedure is easy, don't involve extended procedure and excess use or pharmaceutically unacceptable additives [30].

Limitations of Transfersomes:

- Transfersomes are with chemicals unstable as a result of their predisposition to aerobic degradation.
- Purity of natural phospholipids is another criterion militating against the adoption of transfersomes as drug delivery vehicles.
- Transfersomes formulations are high-priced [31].

Characterization of Transfersomes: The characterization parameters of Transfersomesinclude entrapment efficiency, vesicle diameter, number of vesicle per cubic mm , confocal scanning laser microscopy study, degree of deformability or permeability measurement, turbidity measurement, surface charge and charge density, penetration ability, drug content, *in-vitro* drug release, *in-vitro* skin permeation studies (*ex vivo* drug release study), physical stability [32].

Mupirocin: Mupirocin (MC) could be a metallic element salt of the antibiotic created by bacteria genus fluorescens. Its action mechanism is inhibiting microorganism isoleucyl tRNA synthetase, which ends in obstruction macromolecule synthesis and indirectly inhibiting ribonucleic acid synthesis [33]. Mupirocin expresses a broad activity against numerous microorganisms. Those embody *Staphylococci*, in conjunction with methicillin-resistant strains (MRSA) and *Streptococci*. *Haemophilus influenzae, Moraxella catarrhalis, bacteria, Neisseria meningitides* and *Bordetella* respiratory disease square measure a number of the gram-negative organisms that mupirocin shows activity against. The drug can bear reaction *in-vivo*, which ends in its inactivation and also the drug also will bind resiliently to body fluid (95%), thereby reducing its bioavailability. Thanks to these limitations, it's usually used as a locally applied medication. Mupirocin is additionally used nasally in infection-control programs to eradicate nasal colonization by MRSA.

It shows low activity against members of the conventional skin flora; together with *corynebacterial, micrococci,* and *Propionibacterium spp.* this will be seen as a plus thanks to traditional skin defenses against pathogens that remain unaffected by mupirocin. The potent medicine activity of MHz is any increased in an acidic atmosphere and might therefore be a plus in relevance to the acidic hydrogen ion concentration related to the skin and its surroundings [34]. Once the skin is battle-scarred or traumatized in any type, mupirocin will probably penetrate to deeper layers. This can be additionally true once occlusive dressings square measure used, leading to higher permeation. However, the skin metabolizes MHz to the major inactive matter monic acid step by step. The employment of MHz ointment has shown well acceptableness, and the connected aspect impact was reportable to be negligible. MC is slightly soluble in water, meagrely soluble in anhydrous plant products, and dichloromethane. It's a log P price at a pair of 7 (o/w) and a pka price of 4.7. Patients tormented by burn injury square measure at high risk of attracting pathogens and developing infections. Burn wounds colonisation with *S. aureus* vary to an excellent extent, similarly like the severity of the burn wound, the patient's age, the patient's own nasal and tubular cavity *S. aureus* colonization, the health care staff, and also the sort of care given by the health care skilled at the center of treatment. Burn wounds infected by *S. aureus* are related to a delay within the wound healing method, an exaggerated demand for surgery, and an extended hospital residence. Thirty administering nasal mupirocin to patients with a high risk of developing infections reduces the risk of wound colonization with *S. aureus* [35].

Chitosan: Chitosan (CS) has been widely used as an adhesive coating polymer for flexible liposomal drug delivery systems because of its adhesive properties on mucous layers. The coating mechanism or interaction of chitosan and liposomes or mucin mainly depends on electrostatic forces. Chitosan can be used to prevent or treat the wound and burn infections not only because of its intrinsic antimicrobial properties but also by virtue of its ability to deliver extrinsic antimicrobial agents to wounds and burns. It can also be used as a slow-release drug-delivery vehicle for growth factors to improve wound healing. A large number of publications in this area suggest that chitosan will continue to be an important agent in the management of wounds and burns [36].

MATERIALS AND METHODS:

Materials: Mupirocin (Neptune Life Sciences Pvt. Ltd.Baddhi, Himachal Pradesh, India) was used as a model drug Soya lecithin (Himedia Laboratories, Mumbai, India) was selected as a Phospholipid, Spans (SD Fine Chemicals, New Delhi, India) as edge activators was added to promote drug dispersion. Chitosan (Qualikems Fine Chemicals Pvt. Ltd., Vadodara, India) was used as a coating agent to control the drug release. Methanol and chloroform were selected as good solvents; distilled water was used as a poor solvent was chosen as a bridging agent due to its good wettability with regard to both the drug and polymers and its immiscibility with the poor solvent. All solvents used were of analytical grade.

Preparation of Flexible Liposomes of Mupirocin: Weighed the desired quantity of soya lecithin and sorbitan esters which were dissolved in 4ml of methanol and chloroform(2:1) in round bottom flask, and a thin layer was formed on the inner side of the round bottom flask by evaporating under vacuum using rotary evaporator for 10 min. At 40 °C. After that, the given amount of 10% w/v mupirocin was dissolved in methanol, and a 40ml volume made up with pH 7.4 phosphate buffer was added to the above layer formed with the continuous shaking for 1hr at 40 °C to anneal liposome structures.

Then the resulting solution was sonicated for 30 min in a bath sonicator. Six formulations were prepared using the different concentrations of soya lecithin and sorbitan esters **Table 1.**

TABLE 1 FORMULA FOR PREPARATION AND OPTIMIZATION OF FLEXIBLE LIPOSOMES

Ingredient	Mupirocin (mg)	Soya lecithin (mg)	Span 20 (mg)	Span 60 (mg)	Span 80 (mg)
F1	100	85	15	-	-
F2	100	90	10	-	-
F3	100	85	-	15	-
F4	100	90	-	10	-
F5	100	85	-	-	15
F6	100	90	-	-	10

Preparation of Chitosan Coated Flexible Liposomes: Chitosan solutions 0.1% and 0.3% (w/v) were prepared in 0.1% (v/v) glacial acetic acid. A volume of 25ml of chitosan solution was added drop-wise to the 25ml of flexible liposomes under magnetic stirring at room temperature for 1 hour, followed by incubation in refrigerator overnight **Table 2.**

TABLE 2: COMPOSITION OF CHITOSAN COATED FLEXIBLE LIPOSOMES

Ingredient	Mupirocin (mg)	Soya lecithin (mg)	Span 20 (mg)	Span 60 (mg)	Span 80 (mg)	Chitosan solution
F1A	100	85	15	-	-	0.1%
F1B	100	85	15	-	-	0.3%
F2A	100	90	10	-	-	0.1%
F2B	100	90	10	-	-	0.3%
F3A	100	85	-	15	-	0.1%
F3B	100	85	-	15	-	0.3%
F4A	100	90	-	10	-	0.1%
F4B	100	90	-	10	-	0.3%
F5A	100	85	-	-	15	0.1%
F5B	100	85	-	-	15	0.3%
F6A	100	90	-	-	10	0.1%
F6B	100	90	-	-	10	0.3%

RESULTS AND DISCUSSION:

Characterization for Physio-chemical Properties of Pure Drug Mupirocin:

Organoleptic Properties: Organoleptic properties of the drug were determined by observing the drug sample under a microscope for its appearance, colour and crystal morphology. The results of organoleptic properties are mentioned in **Table 3.**

TABLE 3: LIST OF SENSORY CHARACTERS

S. no.	Sensory characters	Result
1	Colour and morphology	White to off white powder
2	Odour	Odourless

Solubility Studies: The drug sample was qualitatively and quantitatively tested for solubility in various solvents. Solubility of the drug was determined by taking 1ml of various solvents such as distilled water, methanol, chloroform and phosphate buffer pH 7.4 in Eppendorf tube and frequently adding 10mg drug until the solvent gets saturated and shake for 10 min and filter the solution and determine the concentration of saturated solubility under UV spectrophotometry and calculate %solubility.

TABLE 4: SATURATED SOLUBILITY OF MUPIROCIN

S. no.	Solvent used	Observation	% Solubility
1	Distilled water	----	20%
2	Methanol	++++	98%
3	Chloroform	++++	99%
4	Phosphate buffer pH 7.4	+++-	72%

*++++= freely soluble; +++-= soluble; ----=insoluble

Melting Point: The melting point of the drug was determined by observing the drug sample under the melting point apparatus using the capillary tube method.

TABLE 5: MELTING POINT OF MUPIROCIN

S. no.	Melting point of standard drug	Melting point of sample drug	Average Melting point of sample drug
1		77-78^{o}C	
2	77-78^{o}C	76-77^{o}C	77-78^{o}C
3		77-78^{o}C	

Determination of pH (1% w/v Solution in Water): 10mg of the drug powder was taken and dissolved in 10ml of distilled water. The solution was filtered after sonication for 1 min. The pH of the solution was determined using a standard glass electrode pH meter.

TABLE 6: pH OF MUPIROCIN

S. no.	pH of solution	Average pH of the solution
1	4.15	
2	4.20	4.17±0.021
3	4.16	

Determination of Partition coefficient:

K = Amount of drug in organic layer / Amount of drug in organic layer

TABLE 7: PARTITION COEFFICIENT OF MUPIROCIN

S. no.	Medium	K
1	n-Octanol:PBS pH 7.4	2.45

Preparation of Calibration Curve of Mupirocin: From the calibration curve, the correlation co-efficient (R^2) value was found to be 0.9991, and the calibration curve shows that the drug obeys Beer's law limit within the concentration range. Spectrophotometric data for the calibration curve of Mupirocin is done in triplicate at absorbance 224nm as shown in **Table 8,** and the calibration curve graph is shown in **Fig. 4.**

TABLE 8: SPECTROPHOTOMETRIC DATA FOR CALIBRATION CURVE OF MUPIROCIN

Concentration (µg/ml)	Absorbance ± S.D.
2	0.080 ±0.0020
4	0.126±0.0015
6	0.195 ±0.0038
8	0.240 ±0.0040
10	0.299 ±0.0017
12	0.356 ±0.0055
14	0.401 ±0.0042
16	0.458 ±0.0011
18	0.511 ±0.0036
20	0.564 ±0.0082

*Each value is the average of three determinations

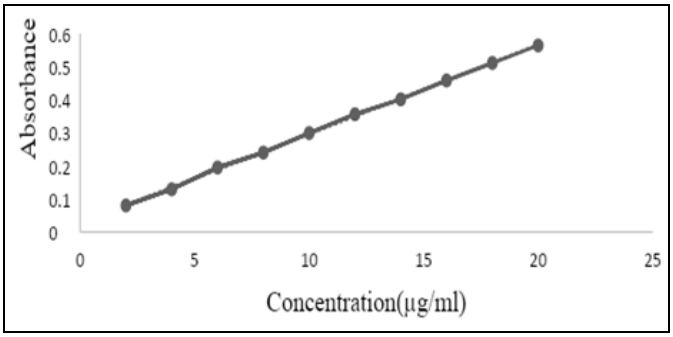

FIG. 4: CALIBRATION CURVE OF MUPIROCIN

Preformulation Studies: The Preformulation studies of the drug can be done by identifying the drug by following methods *i.e.*, Ultraviolet Spectroscopy (UV), Infrared Spectroscopy (IR), Differential Scanning Calorimetry (DSC), and melting point determination.

Identification of Drug:

Ultraviolet Spectroscopy: When the drug solution (using phosphate buffer pH 7.4 as solvent) was examined in the 200-400 nm range, the solution showed absorption maxima at about 224 nm, as shown in **Fig. 5**.

Thus, confirming the presence of the drug when compared with standard.

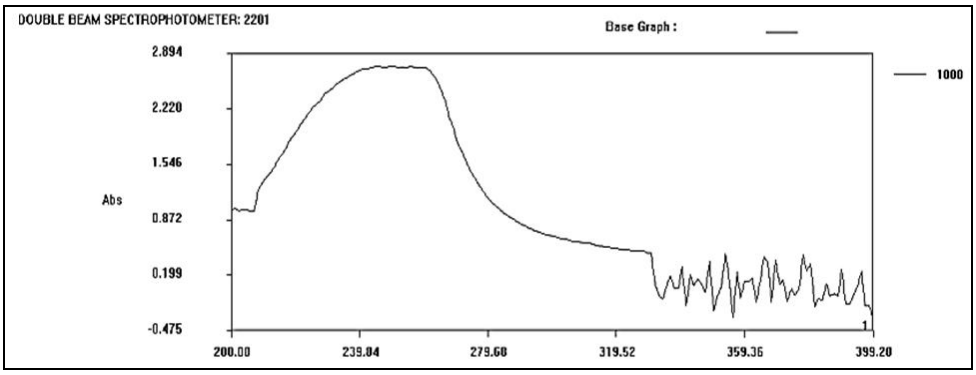

FIG. 5: UV-SPECTRUM OF MUPIROCIN

Infrared Spectroscopy: FT-IR studies are used to determine the possible interaction between the drug and excipients used. The existence of an interaction is detected by the alteration, shift or disappearance of a functional group peak of the drug. The FT-IR spectra of mupirocin and its physical mixture with polymer are shown in **Fig. 6.** There was no significant differences in the characteristic peaks position of mupirocin (1,712 cm^{-1} corresponding to C=O stretching, at 2,859 and 2,929 cm^{-1} corresponding to C−H deformation, at 1,150 cm^{-1} corresponding to the C−O−C stretching vibration, and at 1,052 cm^{-1} corresponding to the C−C skeleton) compared to its physical mixture with the polymers. Hence, mupirocin was compatible with excipients. The results of FTIR spectroscopic interpretation of pure drugs are shown in **Table 9.**

TABLE 9: FTIR SPECTROSCOPIC INTERPRETATION OF PURE DRUG (MUPIROCIN)

Wavenumber (cm^{-1})	Group confirmed
3481 and 3308 cm-1	O-H Stretch
1,712 cm^{-1}	C=O Stretch
1232 and 1222 cm-1	C-O Stretch
2,859 and 2,929 cm^{-1}	C-H Stretch
1150cm^{-1}	C-O-C Stretch
1,052 cm^{-1}	C−C Stretch

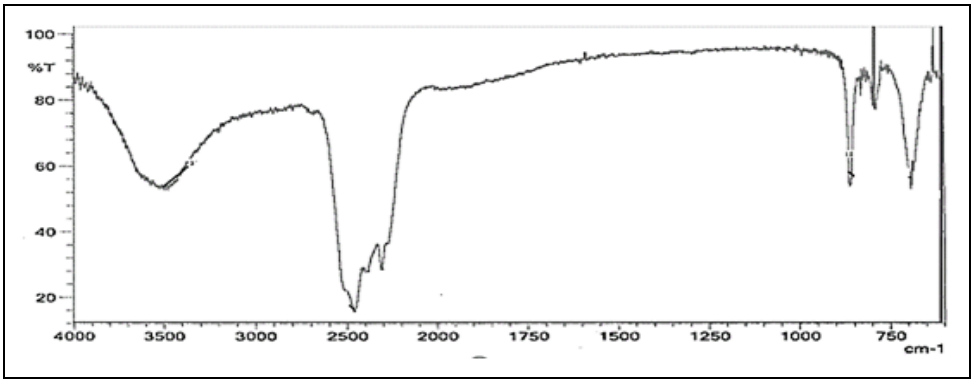

FIG. 6: FTIR SPECTRUM OF PURE DRUG (MUPIROCIN)

Differential Scanning Calorimetry (DSC): When the drug was analyzed under DSC at a heating rate of 80°C/min, the temperature range was from 30°C to 300°C. The DSC thermograms showed sharp endothermic peak corresponding to mupirocin melting point 77.31°C for mupirocin and 75.82°C of its physical mixture with polymers; it can be inferred that there is no interaction between

mupirocin and polymers selected for the study. The DSC thermograms of mupirocin and its physical mixture with polymers are given in The Merck Index, 2005. This confirmed the presence of the drug. The DSC scan of pure drug and polymer is given in **Fig. 7.**

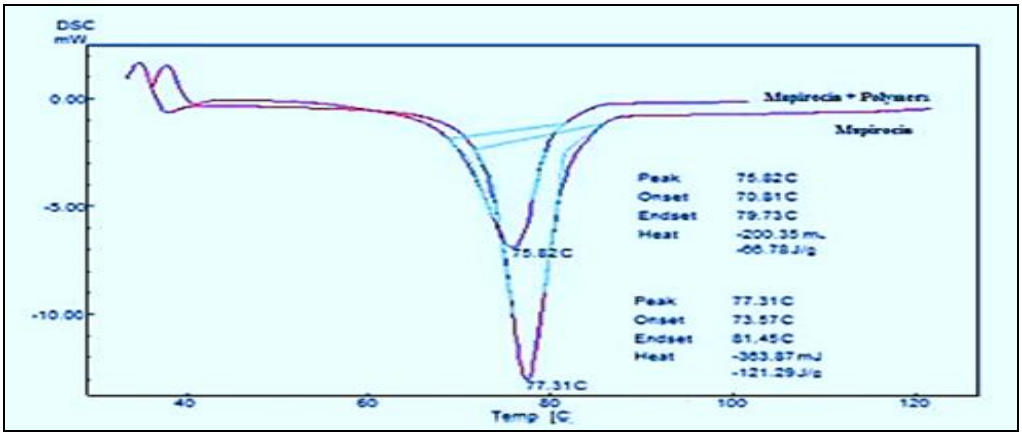

FIG. 7: DSC SCAN OF PURE DRUG (MUPIROCIN) AND PHYSICAL MIXTURE OF MUPIROCIN AND POLYMERS

Melting Point: Capillary technique was used as the standard method for melting point determination. There was the complete melting of the drug in the temperature range from 77-78°C.

Formulation Studies:

Optimization of Prepared Formulations: The **Fig. 8**. shows the images of prepared formulations. Total of 12 formulations was prepared using different concentrations of excipients and mupirocin as the main active ingredient.

FIG. 8: IMAGE OF FORMULATIONS PREPARED (50ML EACH)

Post Formulation Evaluation:

***In-vitro* Evaluation Parameters:** The various *in-vitro* evaluation parameters include percentage yield, particle size, polydispersity index, encapsulation efficiency, %drug content and *in-vitro* drug release studies.

Percentage Yield: It was observed that the percentage yield of the chitosan-coated flexible liposomal formulations of mupirocin was in the range of 68.03±0.82% - 77.11±0.54%, which was significant with respect to the ratios of polymers used. The increase in the soya lecithin ratio shows decreased percentage yield and of increase in edge activator ratio increase in percentage yield. The percentage yield of all the formulations has been shown in **Table 10,** and each value is the average of three determinations.

TABLE 10: PERCENTAGE YIELD OF CHITOSAN COATED FLEXIBLE LIPOSOMES OF MUPIROCIN

Formulation code	Percentage yield * (%)
F1A	76.46±0.56
F1B	70.67±0.77
F2A	74.14±0.61
F2B	71.21±0.98
F3A	77.11±0.54
F3B	71.79±0.44
F4A	68.28±0.68
F4B	68.03±0.82
F5A	70.83±0.48
F5B	69.27±0.14
F6A	72.53±0.36
F6B	73.46±0.74

*Standard deviation, n=3

Particle size and Polydispersity Index: The particle size of the chitosan-coated flexible liposomal formulations of mupirocin was in the range of 115.30-391.41nm. The particle size increased with the increase in chitosan and soya lecithin concentration. The polydispersity index of the flexible liposomal formulations varied from 0.210-0.470. The results suggest that all formulations were homogeneous.

TABLE 11: PARTICLE SIZE AND POLYDISPERSITY INDEX OF CHITOSAN-COATED FLEXIBLE LIPOSOMES OF MUPIROCIN

Formulation code	Particle size (nm)	Polydispersity index
F1A	165.51	0.387
F1B	264.40	0.210
F2A	237.60	0.253
F2B	265.30	0.300
F3A	149.17	0.370
F3B	291.41	0.233
F4A	205.11	0.470
F4B	314.63	0.371
F5A	115.30	0.320
F5B	365.66	0.287
F6A	237.42	0.315
F6B	391.41	0.298

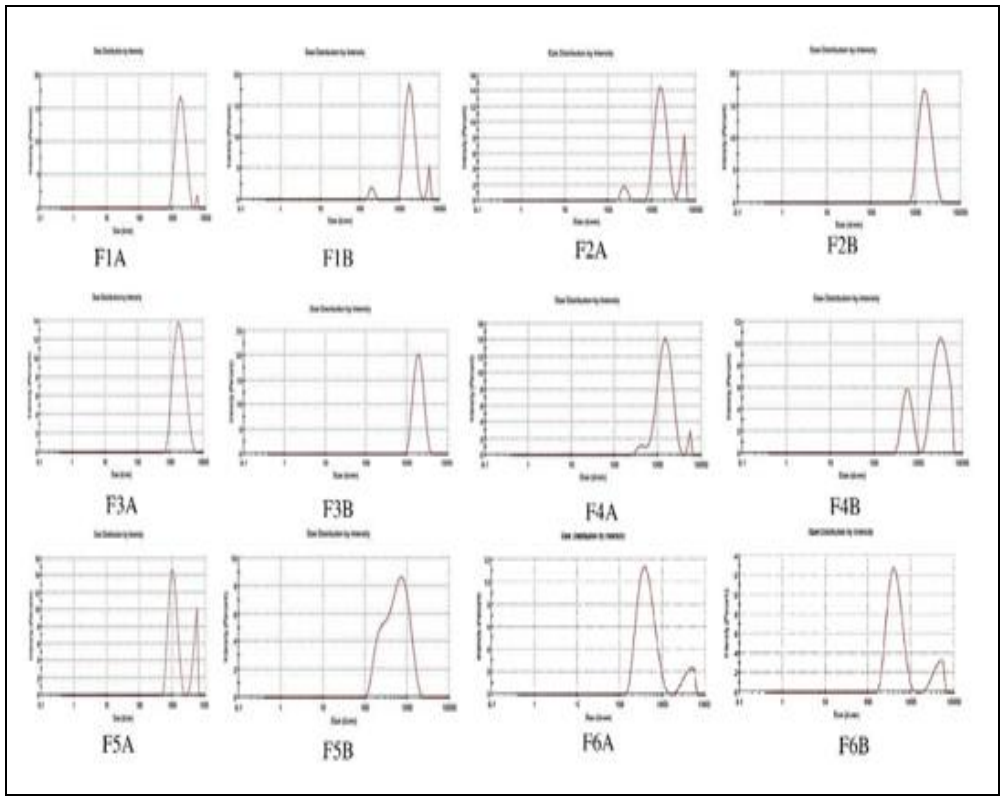

FIG. 9: PARTICLE SIZE DISTRIBUTION

Encapsulation Efficiency: It was observed that encapsulation efficiency of the formulation varied with the soya lecithin and edge activator composition and the entrapment ranged from 52.83±0.66 - 82.90±0.52%.

An increase in the amount of soya lecithin in the flexible liposomes has increased the mupirocin's encapsulation to a certain extent.

Thus the high concentration of soya lecithin produce more rigid liposomes and may reduce the bilayer permeability, enhance the stability of liposomes and result in high encapsulation efficiency; at the same time, beyond a certain concentration, soya lecithin may disrupt the regular structure of the liposomal membrane, result in lower encapsulation efficiency.

An increase in edge activator ratio increased the entrapment efficiency, and an increase in soya lecithin ratio decreased the entrapment efficiency.

This may be attributed to the saturation of lipid domains concerning the drug where a lower concentration of edge activator limits entrapment capacity.

Encapsulation efficiency amongst all the formulations are given in **Table 12** and **Fig. 10** and each value is the average of three determinations.

%Dug Content: The drug content of all the formulations was calculated as a mean of three values.

The drug content of the mupirocin-loaded chitosan coated flexible liposomal formulations was in the range of 91.3±0.28-98.9±0.13%, indicating the mupirocin was uniformly distributed in the chitosan-coated flexible liposomes.

Drug content amongst all the formulations is given in **Table 12** and **Fig. 10,** and each value is the average of three determinations.

TABLE 12: RESULTS OF ENCAPSULATION EFFICIENCY AND % DRUG CONTENT OF CHITOSAN COATED FLEXIBLE LIPOSOMES OF MUPIROCIN

Formulation Code	Encapsulation Efficiency* (%)	Drug Content* (%)
F1A	82.90±0.52	94.6±0.56
F1B	71.89±0.34	93.3±0.48
F2A	59.35±0.52	98.9±0.13
F2B	56.37±0.34	97.6±0.66
F3A	79.21±0.73	95.3±0.16
F3B	76.94±0.35	96.6±0.32
F4A	52.83±0.66	91.3±0.28
F4B	54.74±0.19	92.3±0.23
F5A	65.75±0.36	90.9±0.33
F5B	59.62±0.24	93.6±0.30
F6A	62.59±0.18	95.9±0.27
F6B	68.73±0.44	94.8±0.34

*Standard deviation, n=3

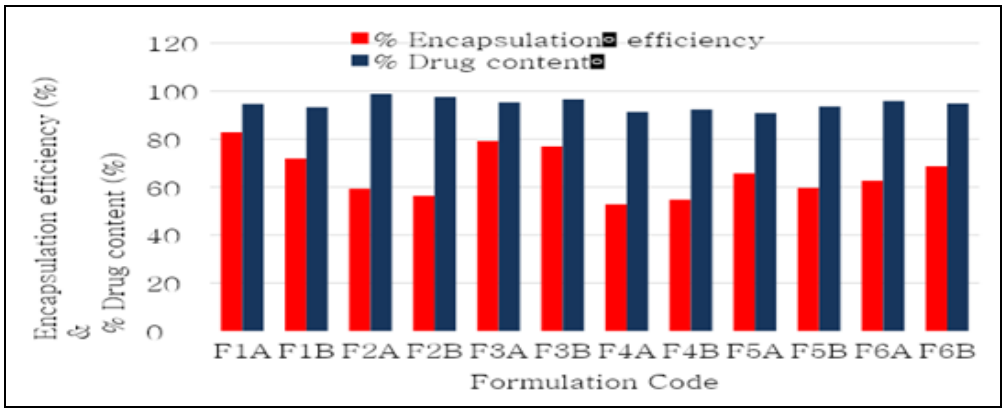

FIG. 10: ENCAPSULATION EFFICIENCY AND DRUG CONTENT OF CHITOSAN COATED FLEXIBLE LIPOSOMES OF MUPIROCIN

***In-vitro* Drug Release Studies:** The *in-vitro* release of the mupirocin from the chitosan-coated flexible liposomes was 89.53±0.15% at the end of 8 hrs **Table 13** and **Fig. 11**. It was observed that the inclusion of higher proportion of chitosan in flexible liposomal formulation results in prolonged drug retention. The delayed release of mupirocin might be attributed to its lipophilic nature held by the small fragment of the liposomal membrane. Each value is the average of three determinations.

TABLE 13: RESULTS OF *IN-VITRO* DISSOLUTION DATA OF CHITOSAN COATED FLEXIBLE LIPOSOMES OF MUPIROCIN

Time (hrs)	F1A* ± S.D.	F1B* ± S.D.	F2A* ± S.D.	F2B* ± S.D.	F3A* ± S.D.	F3B* ± S.D.
0.25	9.78±0.34	9.29±0.27	18.55±0.35	11.73±0.23	18.55±0.73	11.73±0.11
0.5	12.76±0.32	13.93±0.17	19.05±0.28	15.21±0.52	19.05±0.27	19.05±0.72
1	17.22±0.41	30.57±0.12	24.61±0.34	26.50±0.67	22.66±0.17	22.66±0.26
2	21.50±0.36	38.93±0.25	34.59±0.46	44.87±0.86	34.58±0.82	34.58±0.16
3	37.70±0.15	43.43±0.37	34.59±0.57	57.31±0.43	38.08±0.38	38.08±0.73
4	48.93±0.35	56.64±0.85	50.98±0.13	69.22±0.26	50.96±0.52	50.96±0.25
5	54.95±0.28	69.81±0.54	66.85±0.19	70.48±0.18	52.61±0.17	52.61±0.55
6	64.80±0.26	71.56±0.36	74.92±0.27	75.35±0.25	66.74±0.64	66.74±0.22
7	72.46±0.36	79.36±0.63	77.37±0.63	81.60±0.72	74.41±0.91	74.41±0.32
8	88.55±0.74	83.49±0.26	86.47±0.72	82.34±0.34	89.53±0.26	89.53±0.15

*Standard deviation, n=3

Time (hrs)	F4A* ± S.D.	F4B* ± S.D.	F5A* ± S.D.	F5B* ± S.D.	F6A* ± S.D.	F6B* ± S.D.
0.25	18.55±0.34	11.73±0.25	15.53±0.56	17.97±0.83	10.46±0.22	13.78±0.10

0.5	19.05±0.12	15.21±0.93	19.03±0.62	23.53±0.34	13.84±0.25	30.13±0.62
1	24.61±0.33	26.50±0.26	19.62±0.38	31.36±0.26	27.95±0.29	32.44±0.18
2	34.59±0.27	44.87±0.48	22.75±0.64	46.74±0.15	33.66±0.51	38.76±0.82
3	38.10±0.63	47.27±0.41	28.43±0.18	58.50±0.11	49.93±0.37	48.92±0.15
4	50.98±0.24	59.42±0.71	38.53±0.92	64.37±0.28	54.49±0.38	51.72±0.82
5	54.28±0.72	62.08±0.18	47.03±0.17	73.69±0.21	62.49±0.25	60.97±0.45
6	64.81±0.11	64.96±0.73	61.22±0.12	75.95±0.36	70.04±0.11	62.96±0.34
7	75.78±0.18	74.08±0.19	71.50±0.53	81.72±0.34	81.34±0.29	77.34±0.27
8	81.75±0.25	80.14±0.82	83.78±0.24	82.45±0.72	83.44±0.61	82.34±0.30

*Standard deviation, n=3

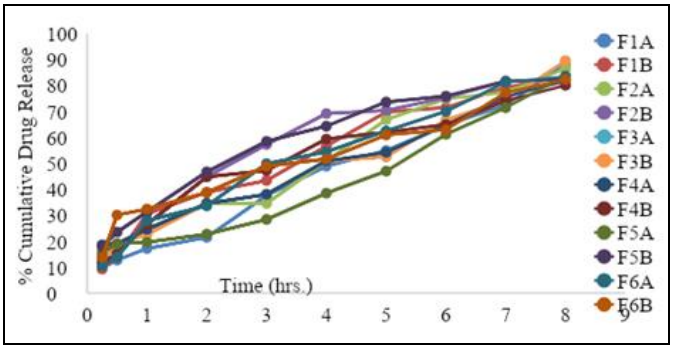

FIG. 11: *IN-VITRO* % CUMULATIVE DRUG RELEASE V/S TIME PLOT ('0TH' DAY)

***In-vitro* Drug Release Kinetics:** Data of the *in-vitro* release were fit into different equations and kinetic models *i.e.* Zero order kinetic model **Fig. 12,** First order kinetic model **Fig. 13**, Higuchi's model **Fig. 14** and Korsemeyer-Peppa's model **Fig. 15**. The better fit (highest R^2 value) for all formulations was observed in the case of Higuchi's model compared with all the three other kinetic models.

Hence, mechanism of drug release was found to be diffusion controlled. According to the Korsmeyer-Peppas model, a value of slope (n) was less than 0.5, it indicates that the release mechanism from all the optimized formulations follows Fickian diffusion.

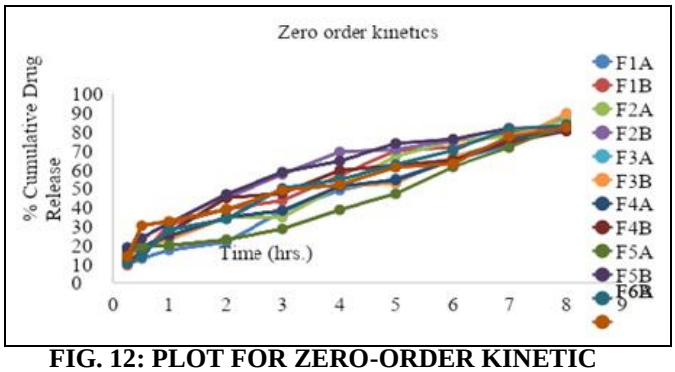

FIG. 12: PLOT FOR ZERO-ORDER KINETIC MODEL OF CHITOSAN COATED FLEXIBLE LIPOSOMES OF MUPIROCIN

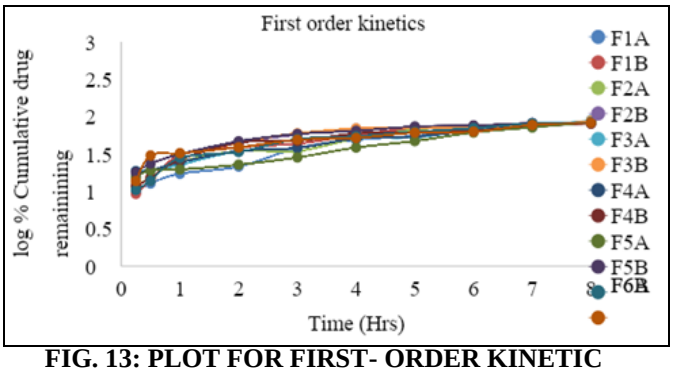

FIG. 13: PLOT FOR FIRST- ORDER KINETIC MODEL OF CHITOSAN COATED FLEXIBLE LIPOSOMES OF MUPIROCIN

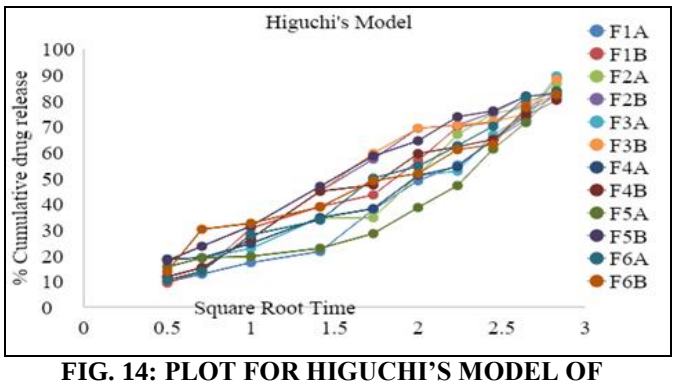

FIG. 14: PLOT FOR HIGUCHI'S MODEL OF CHITOSAN COATED FLEXIBLE LIPOSOMES OF MUPIROCIN

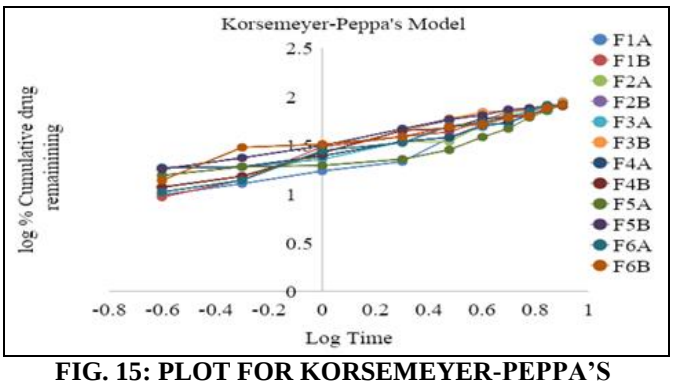

FIG. 15: PLOT FOR KORSEMEYER-PEPPA'S MODEL OF CHITOSAN COATED FLEXIBLE LIPOSOMES OF MUPIROCIN

FTIR Analysis of Optimized Formulations from Each Batch: In case of formulations, F3A formulation containing 85mg soya lecithin ,15mg Span 60 and 0.1% chitosan solution and F3B formulation containing 85mg soya lecithin, 10mg Span 60 and 0.3% chitosan solution were further subjected for FTIR analysis as shown in **Fig. 16** and **Fig. 17** respectively.

All the two formulations showed the same FTIR wave number peaks as that of pure mupirocin. Thus confirmed that there was not any significant change in principal peaks of pure drug in optimized formulations and all ingredients are chemically compatible with each other in optimized formulations. The results of FTIR data interpretation of selected formulations are given in **Table 14.**

TABLE 14: RESULTS OF FTIR DATA INTERPRETATION FOR TWO SELECTED FORMULATIONS

Functional group	Pure Drug	F3A	F3B
C=O Stretch	1712.46	1735.69	1735.79
C-O Stretch	1232.19	1231.59	1232.66
C-H Stretch (Alkane)	2859.75	2854.89	2850.32
C-H Stretch (Aromatic)	2929.55	2928.81	2929.81
C-O-C Stretch (Aromatic)	1150.46	1153.15	1157.37
C-C Stretch (Aromatic)	1052.45	1051.46	1051.43

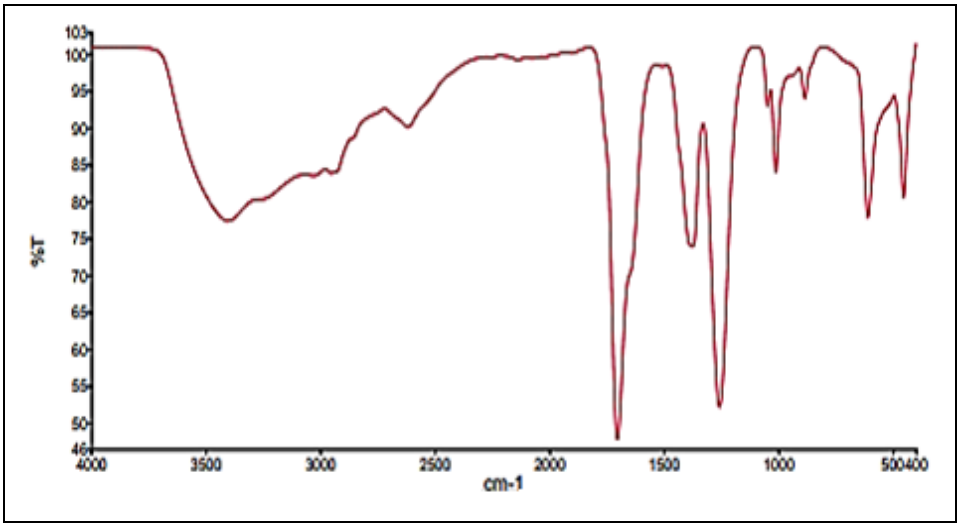

FIG. 16: FTIR SPECTRUM OF FORMULATION F3A

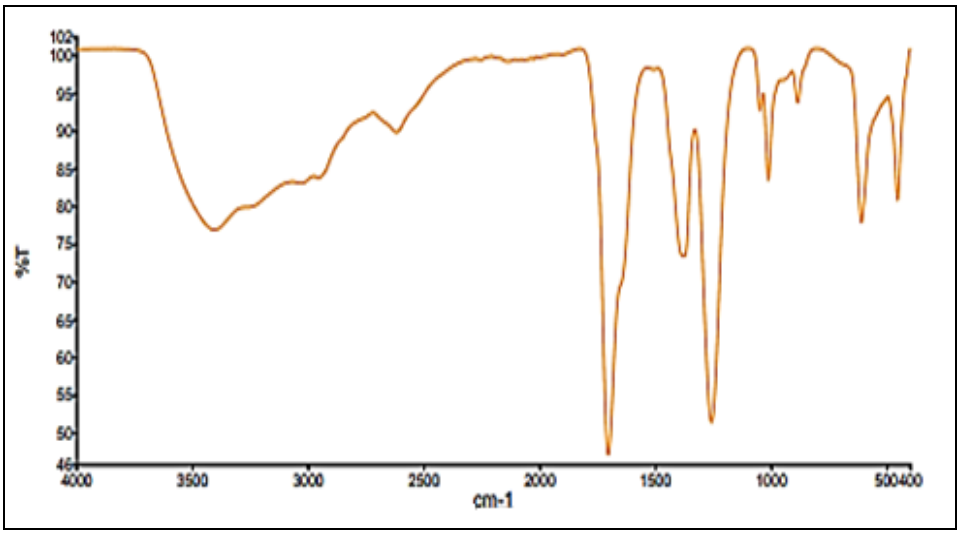

FIG. 17: FTIR SPECTRUM OF FORMULATION F3B

Characterization of Final Optimized Formulation:

Shape of Liposomes: The shapes of most of the chitosan-coated flexible mupirocin-loaded liposomes were spherical in shape, as shown in **Fig. 18.** The shape of flexible liposomes was observed under Photomicrograph under 45X10 magnification.

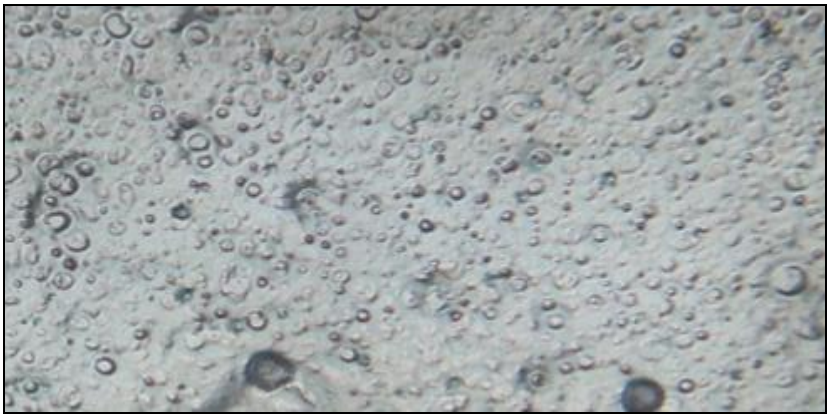

FIG. 18: PHOTOMICROGRAPH OF OPTIMIZED MUPIROCIN LIPOSOMES (OLM) UNDER 45X 10 MAGNIFICATIONS

SEM Analysis: Chitosan-coated flexible liposomes of mupirocin surface morphology and shape were investigated by SEM analysis, as shown in **Fig. 19**. The mupirocin-loaded liposomes have a vesicular structure and are spherical in shape.

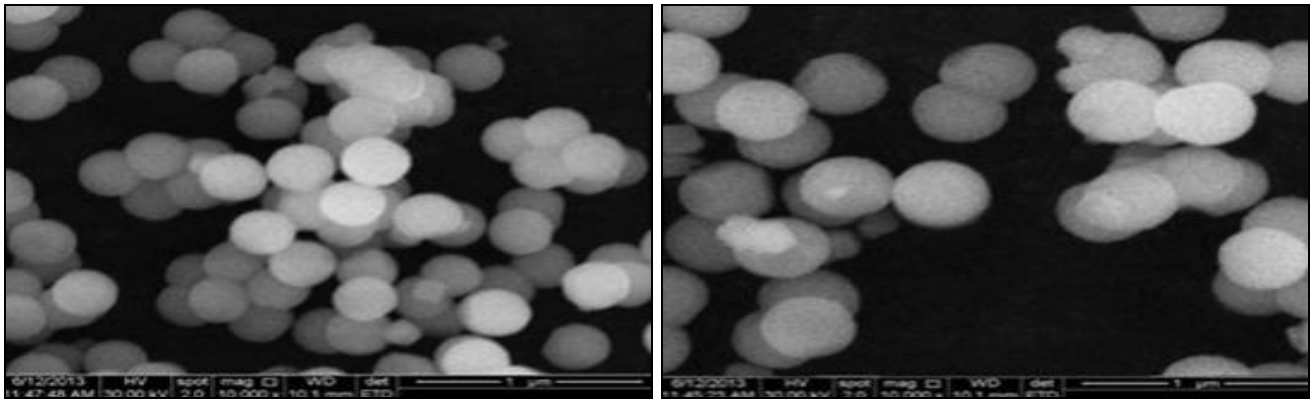

FIG. 19: SEM IMAGES CHITOSAN COATED FLEXIBLE LIPOSOMES OF MUPIROCIN

Accelerated Stability Study: The accelerated stability studies were conducted for all the formulations. The formulations were exposed to the environmental condition of 25±2 °C temperature and 60 ±5% R.H. The various evaluation parameters such as colour change **Table 15**, % drug content **Table 16**, encapsulation efficiency **Table 16** and *in-vitro* drug release were evaluated after zero-day, thirty day **Table 17** and sixty-day **Table 18** respectively and each value is the average of three determinations. There was not any significant deviation in the results of these parameters from zero-day to two months of the stability study period, as given in the tables below. Thus, the final optimized formulation was stable after a two-month accelerated stability study**.**

TABLE 15: RESULTS OF TWO MONTHS ACCELERATED STABILITY STUDY

Evaluation parameter	At 25±2^{0}C and 60±5% R.H.		
	At 0th day	At 30th day	At 60th day
Colour Change	No	No	No

TABLE 16: RESULTS OF ACCELERATED STABILITY STUDY OF ENCAPSULATION EFFICIENCY AND % DRUG CONTENT OF CHITOSAN-COATED FLEXIBLE LIPOSOMES OF MUPIROCIN

Formulation code	Encapsulation Efficiency* (%)		Drug Content* (%)	
	At 30th day	At 60th day	At 30th day	At 60th day
F1A	80.42±0.32	78.90±0.52	92.6±0.37	90.4±0.63
F1B	69.89±0.62	68.34±0.34	91.3±0.28	89.3±0.26
F2A	57.35±0.27	54.32±0.52	97.9±0.12	95.2±0.72
F2B	53.37±0.73	52.24±0.34	95.6±0.18	94.4±0.38
F3A	77.21±0.23	74.32±0.73	92.3±0.22	90.5±0.82
F3B	74.94±0.81	72.54±0.35	95.6±0.83	92.3±0.72
F4A	49.83±0.16	47.26±0.66	89.3±0.92	87.2±0.53

F4B	51.74±0.22	50.23±0.19	90.3±0.17	88.6±0.27
F5A	63.75±0.81	61.64±0.36	87.9±0.92	86.3±0.44
F5B	56.62±0.28	54.24±0.24	91.6±0.16	90.5±0.26
F6A	60.59±0.18	59.64±0.17	93.9±0.91	91.3±0.15
F6B	64.73±0.44	61.26±0.64	92.8±0.36	90.5±0.34

*Standard deviation, n=3

TABLE 17: RESULTS OF '30TH' DAY ACCELERATED STABILITY STUDY OF *IN-VITRO* DISSOLUTION DATA OF CHITOSAN COATED FLEXIBLE LIPOSOMES OF MUPIROCIN

Time (hrs)	F1A* ± S.D.	F1B* ± S.D.	F2A* ± S.D.	F2B* ± S.D.	F3A* ± S.D.	F3B* ± S.D.
0.25	13.78±0.32	12.51±0.72	11.63±0.26	12.61±0.16	17.38±0.32	19.33±0.23
0.5	30.13±0.13	27.20±0.17	24.37±0.17	22.43±0.32	28.79±0.24	30.94±0.13
1	32.44±0.12	31.35±0.35	31.13±0.45	23.62±0.82	33.82±0.72	33.84±0.43
2	38.76±0.35	33.86±0.38	32.47±0.62	34.57±0.17	42.29±0.17	38.51±0.35
3	48.92±0.82	43.50±0.73	42.79±0.27	41.39±0.27	46.62±0.36	42.72±0.82
4	51.72±0.52	51.44±0.82	49.17±0.37	45.81±0.54	51.55±0.32	50.27±0.58
5	60.97±0.82	58.15±074	54.89±0.32	56.88±092	53.01±0.78	52.98±0.54
6	62.96±0.26	61.79±0.73	61.43±0.26	68.01±0.30	65.09±0.51	71.39±0.38
7	77.34±0.37	72.94±0.12	71.80±0.82	72.86±0.39	77.33±0.17	78.51±0.32
8	86.92±0.81	82.01±0.16	83.01±0.17	81.74±0.31	88.28±0.37	86.05±0.20

*Standard deviation, n=3

Time (hrs)	F4A* ± S.D.	F4B* ± S.D.	F5A* ± S.D.	F5B* ± S.D.	F6A* ± S.D.	F6B* ± S.D.
0.25	18.07±0.34	14.85±0.28	16.02±0.26	12.61±0.25	14.85±0.36	13.58±0.32
0.5	22.46±0.17	22.63±0.21	23.71±0.32	21.45±0.32	24.68±0.31	22.63±0.28
1	27.06±0.18	29.09±0.32	26.96±0.18	25.66±0.28	29.11±0.32	26.94±0.38
2	37.35±0.23	32.57±0.33	35.79±0.38	32.53±0.17	37.94±0.43	33.63±0.27
3	42.53±0.72	43.47±0.17	39.20±0.18	35.93±0.48	45.65±0.32	43.75±0.49
4	43.23±0.38	51.41±0.29	48.09±0.29	48.50±0.32	53.90±0.14	57.44±0.32
5	55.67±0.18	54.71±0.16	59.17±0.30	53.06±0.31	62.48±0.22	61.56±0.10
6	70.11±0.21	64.37±0.27	70.12±0.16	71.18±0.10	65.16±0.42	69.30±0.37
7	77.22±0.36	71.83±0.81	75.08±0.12	76.93±0.28	80.52±0.45	78.16±0.01
8	80.85±0.53	78.45±0.38	81.82±0.47	80.76±0.17	82.13±0.13	81.90±0.13

*Standard deviation, n=3

TABLE 18: RESULTS OF '60TH' DAY ACCELERATED STABILITY STUDY OF *IN-VITRO* DISSOLUTION DATA OF CHITOSAN COATED FLEXIBLE LIPOSOMES OF MUPIROCIN

Time (hrs)	F1A* ± S.D.	F1B* ± S.D.	F2A* ± S.D.	F2B* ± S.D.	F3A* ± S.D.	F3B* ± S.D.
0.25	13.58±0.10	12.61±028.	12.41±0.63	13.78±0.41	15.73±0.11	12.71±0.24
0.5	22.43±0.69	25.74±0.25	24.47±0.15	22.71±0.27	22.44±0.61	21.26±0.53
1	32.40±0.20	32.41±0.36	31.14±0.23	28.89±0.38	26.47±0.18	28.00±0.24
2	36.87±0.84	35.52±0.64	38.81±0.43	37.44±0.45	38.99±0.31	39.37±0.76
3	46.83±0.59	46.82±0.14	47.90±0.54	44.46±0.61	49.83±0.25	47.38±0.35
4	51.66±0.75	51.66±0.52	53.42±0.24	51.24±0.17	57.41±0.35	53.01±0.47
5	60.91±0.17	58.18±0.52	55.86±0.67	53.86±0.82	71.08±0.24	66.45±0.25
6	75.66±0.35	71.37±0.63	69.32±0.35	68.68±0.17	79.07±0.46	75.78±0.36
7	81.73±0.27	77.11±0.13	78.08±0.25	71.88±0.18	81.84±0.24	80.58±0.47
8	85.19±0.52	81.92±0.34	82.89±0.92	80.16±0.02	84.43±0.47	84.23±0.25

*Standard deviation, n=3

Time (hrs)	F4A* ± S.D.	F4B* ± S.D.	F5A* ±S.D.	F5B* ±S.D.	F6A* ±S.D.	F6B* ±S.D.
0.25	11.73±0.62	11.82±0.16	12.61±0.15	13.26±0.14	11.09±0.16	12.61±0.24
0.5	18.03±0.32	17.06±0.17	13.85±0.18	22.43±0.26	23.15±0.01	21.26±0.64
1	27.98±0.14	22.71±0.37	25.81±0.32	28.01±0.47	27.15±0.28	23.62±0.52
2	36.61±0.41	36.78±0.24	38.72±0.43	38.99±0.68	33.96±0.82	34.62±0.35
3	48.90±0.35	49.75±0.26	46.74±0.28	42.61±0.34	40.10±0.16	45.57±0.42
4	54.63±0.41	57.43±0.32	52.45±0.43	51.23±0.32	43.05±0.82	52.45±0.57
5	62.53±0.52	60.95±0.54	58.10±0.65	53.95±0.45	60.05±0.16	59.17±0.43
6	68.31±0.47	66.09±0.76	62.90±0.32	66.91±0.76	71.29±0.71	63.10±0.67
7	71.92±0.25	71.60±0.32	72.99±0.46	71.95±0.44	78.40±0.52	74.17±0.22
8	79.14±0.74	77.44±0.11	78.45±0.23	79.07±0.74	80.88±0.73	79.83±0.53

*Standard deviation, n=3

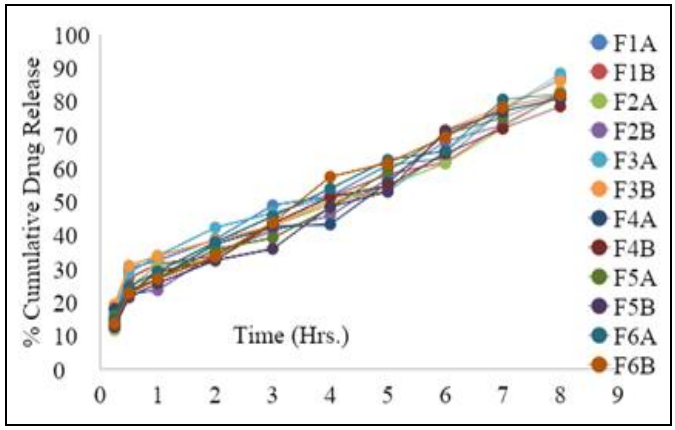

FIG. 20: *IN-VITRO* % CUMULATIVE DRUG RELEASE V/S TIME PLOT ('30TH' DAY)

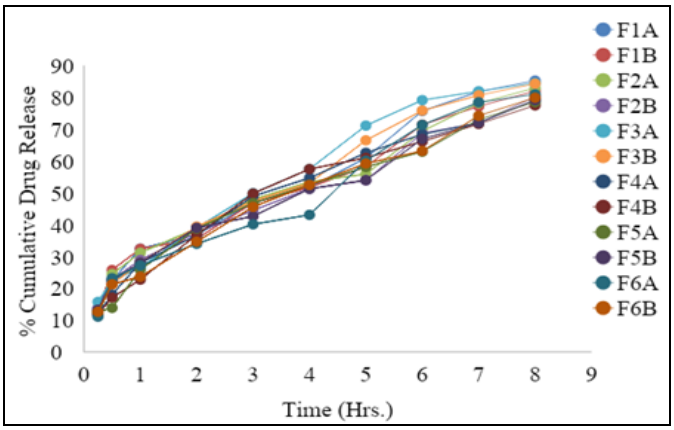

FIG. 21: *IN-VITRO* % CUMULATIVE DRUG RELEASE V/S TIME PLOT ('60TH' DAY)

CONCLUSION: The research aimed to formulate and evaluate chitosan-coated flexible liposomes for laceration treatment. The main objective of the present research work was to deliver the drug in a controlled manner to improve the therapeutic efficacy and patient compliance of the dosage form at the site of application. The pure drug was successfully identified using various analytical techniques *i.e.,* UV spectroscopy, FTIR spectroscopy, DSC analysis, and melting point determination. The drug excipient compatibility was performed by using FTIR spectroscopy, DSC and it was confirmed that there was no interaction between drug and excipients used in the formulation. The chitosan-coated liposomes were prepared by rota evaporator technique (handshaking method) using chloroform: methanol as solvent, using different concentrations of span 20, span 60, span 80 as edge activators, soya lecithin as phospholipid, and chitosan as a coating agent. The prepared flexible liposomal were evaluated for percentage yield, particle size, polydispersity index, encapsulation efficiency, %drug content, and *in-vitro* drug release studies. Satisfactory results were obtained in all prepared formulations. The % *in-vitro* drug release data of all formulations were subjected to various *in vitro* release kinetic models such as zero order, first order, Higuchi model and Korsmeyer Peppa's model to find out the exact mechanism of drug release from the prepared formulations. The *in-vitro* drug release kinetics showed that all the formulations were best fitted to Higuchi's model. Hence mechanism of drug release from all the formulations was found to be diffusion controlled. According to Korsmeyers-Peppa's model, a slope (n) value was less than 0.5, indicating that the release mechanism from all the formulations follows Fickian diffusion. Based on the highest encapsulation efficiency, particle size, and % *in-vitro* drug release, the F3A, and F3B formulation was selected as the final formulation. The final formulation was further characterized by using SEM and FTIR. The surface images of flexible liposomes taken by SEM showed that the drug was uniformly distributed in the selected formulation. The final formulation was further subjected to two months of accelerated stability studies. The accelerated stability studies confirmed the better stability of all the formulations. From the results observed from the post formulation evaluation parameters, it was concluded that the prepared chitosan-coated flexible liposomes had achieved the set objectives to deliver the drug in a controlled manner to improve the therapeutic efficacy at the site of application.

ACKNOWLEDGEMENT: The author is very grateful to Honorable Chairman S. Gurvinder Singh Bahra, Rayat Bahra Group, Punjab, India, for their constant encouragement and support in preparing this article. The authors hereby declare no "conflict of interest.

CONFLICTS OF INTEREST: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

REFERENCES:

1. Ling H, Luoma JT and Hilleman D: A review of currentlyavailable fenofibrate and fenofibric acid formulations. Cardiology Research 2013; 4: 47-55.

2. Akbarzadeh A, Samiei M and Davaran S: Magneticnanoparticles: preparation, physical properties, andapplications in biomedicine. Nanoscale Res Lett 2012; 7(1): 144.
3. Murdande SB, Shah DA and Dave RH: A review: Pharmaceutical and pharmacokinetic aspect of nano-crystalline suspensions. J of Pharm Sci 2016; 105: 10-12.
4. Mozafari MR, Danaei M, Javanmard R, Raji M and Maherani B: Liposomes: A review of manufacturing techniques and targeting strategies. Current Nanoscience 2011; 7: 436-52.
5. Marripati S, Umasankar K and Reddy PJ: A Review onliposomes. International Journal of Research in Pharmaceutical and Nano Sciences 2014; 3: 159-69.
6. Sercombe L, Veerati T, Moheimani F, Wu SY, Sood AK and Hua S: Advances and challenges of liposome assisted drug delivery. Frontiers in Pharmacology 2015; 6: 286.
7. Al-Ahmady ZS, Chaloin O and Kostarelos K: Monoclonal antibody-targeted, temperature-sensitive liposomes: *in-vivo* tumor chemotherapeutics in combination with mild hyperthermia. J of Con Release Official Journal of the Control R Society 2014; 28: 196-32.
8. Han HD, Jeon YW, Kwon HJ, Jeon HN, Byeon Y, LeeCO, Cho SH and Shin BC: Therapeutic efficacy of doxorubicin delivery by a CO2 generating liposomal platform in breast carcinoma. Acta Biomater 2015; 24: 279-85.
9. Sawant R and Torchilin V: Challenges in development of targeted liposomal therapeutics. American Association of Pharmaceutical Scientists Journal 2012; 14(2): 303-15.
10. Yuan DF, Zong TL, Gao HL and He Q: Cell penetrating peptide TAT and brain tumor targeting peptide T7 dual modified liposome preparation and *in-vitro* targeting evaluation. Yao Xue Xue Bao 2015; 50: 104-10.
11. Shantil V: An Imperative Note on Novel Drug Delivery Systems. J Nanomedic Nanotechnol 2011; 2: 125.
12. Agrawal P: Significance of Polymers in Drug Delivery System. J Pharmacovigil Edition 2015; 3: 127.
13. Bhagwat, Vaidhya: Novel drug delivery systems: an overview. International Journal of Pharmaceutical Sciences and Research 2013; 4(3): 970-82.
14. Akshay, Das S and Samant A: Design, formulation and evaluation of liposome containing isoniazid. International Journal of Applied Pharmaceutics 2018; 10: 2.
15. United States Pharmacopoeia 2011; 1: 964-65.
16. Kaur, Kaur P and Khan: Liposome as a Drug Carrier – A Review. IJRPC 2013; 3(1).
17. Chinnala KM and Panigrahy R: Formulation and Evaluation of Acyclovir Liposomes. International Research J of Pharmaceutical and Biosciences 2017; 4(1).
18. Raheem, Ahmad, Abbas and Dakhil: Effect of method of preparation on physical properties of ciprofloxacin hydrochloride elastic liposomes intended to be utilized in the treatment of Acne Vulgaris. Int J Res Ayuveda Pharm2013; 4(5); 742-46.
19. Lankalapall S, Tenneti VK and Adama R: Preparation andevaluation of liposome formulations for poorly soluble drug itraconazole by complexation. Der Pharmacia Lettre 2015; 7(8): 1-17.
20. Chandran SMP: Formulation and evaluation of Glimepiride loaded liposomes. International Journal of Research in Pharmaceutical Sciences 2015; 6(4): 333-38.
21. Suraj, Faizi and Abhisek: Formulation and development of liposomal gel for topical drug delivery system. Int J Pharm Sci Res 3(11): 4461-74.
22. Moghimipour E, Salami A and Mahsa M: Formulation and Evaluation of Liposomes for Transdermal Delivery of Celecoxib. Jundishapur J of Nat Pharm Prod 2015; 10(1).
23. Visser CC, Stevanovic S and Voorwinden LH: Targetingliposomes with protein drugs to the blood-brain barrier *in-vitro*. J of Pharma Sciences 2005; 25: 299-305.
24. Sadhukhan B, Mondal NK and Chattoraj S: Optimisation using central composite design and the desirability function for sorption of methylene blue from aqueous solution onto Lemna major. Karbala International Journal of Modern Science 2016; 2: 145-155.
25. Yang LP and Keating GM: Fenofibric acid: in combination therapy in the treatment of mixed dyslipidemia. American Journal of Cardiovasc Drugs 2009; 9: 401-9.
26. Shah DA: Investigation of thermal and kinetic properties of nanocrystals of the poorly soluble pharmaceuticals, Doctoral dissertation. Proquest LLC 2015.
27. Bangham AD, Standish MM and Watkins JC: Diffusion of univalent ions across the lamellae of swollen phospholipids. J of Molecular Biology 1965; 13: 238-52.
28. Papahadjopoulos D and Bangham AD: Biophysical properties of phospholipids. Imperme ability of phosphatidylserine liquid crystals to univalent ions. Biochimica et Biophysica Acta 1966; 126: 185-8.
29. Korang-Yeboah M, Rahman Z, Shah D, Mohammad A, Wu S, Siddiqui A and Khan MA: Impact of formulation and process variables on solid-state stability of theophylline in controlled release formulations. International Journal of Pharmaceutics 2016; 499: 20-28.
30. Murdande SB, Shah DA and Dave RH: Impact of nanosizing on solubility and dissolution rate of poorly soluble pharmaceuticals. Journal of Pharmaceutical Sciences 2015; 104: 2094-2102.
31. Patil SD and Burgess DJ: Liposomes: design and manufacturing, in injectable dispersed systems: formulation, processing and performance. D. J. Burgess Editor 2005.
32. Maxwell K, Ziyaur R, Shah D and Khan M: Spectroscopic-based chemometric models for quantifying low levels of solid-state transitions in extended release theophylline formulations. Journal of pharmaceutical Sciences 2015; 105: 97-105
33. Sawant R and Torchilin V: Challenges in development of targeted liposomal therapeutics. American Association of Pharmaceutical Scientists Journal 2012; 14(2): 303-315.
34. Kern A, Golbraikh A and Sedykh A: Quantitative structure property relationship modeling of remote liposome loading of drugs. Journal of controlled release: Official Journal of the Control Release Society 2012; 160: 147-57.
35. Slingerland M, Guchelaar HJ and Gelderblom H: Liposomal drug formulations in cancer therapy: 15 years along the road. Drug Discovery Today 2012; 17: 160-6.

How to cite this article:
Gogna S: Formulation development, characterization, optimization and *in-vitro* evaluation of chitosan coated flexible liposomes of mupirocin for treatment of laceration. Int J Pharm Sci & Res 2022; 13(7):2824-43. doi: 10.13040/IJPSR.0975-8232.13(7).2824-43.