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FORMULATION AND EVALUATION OF ANTIMICROBIAL ENROFLOXACIN PRONIOSOMAL GEL FOR VETERINARY DRUG DELIVERY SYSTEM; RESEARCH

[1] **Smital Suresh Kokate**

[1]Student. M.V.P. Samaj"s College Of Pharmacy, Gangapur, Road, Nashik.

[1]Department of Quality Assurance

Abstract : The purpose of this research is to design proniosomal gel drug delivery system of Enrofloxacin for veterinary drug delivery system. This can be done by the use of vesicular drug delivery system. Encapsulation of a drug in the vesicular structure of the phospholipids plays role to prolong the existence of the drug in the systemic circulation and thus enhance penetration into target tissue and reduce toxicity. The potential of proniosomes as a transdermal drug delivery system of Enrofloxacin wasinvestigated by encapsulating the drug in various formulations of proniosomal gel composed of various ratios of cholesterol, prepared by coacervation-phase separation method. The formulated systems were characterized in vitro for size, vesicle count, drug content; drug release .The method of proniosome loading resulted in an encapsulation yield of 70.00 % to 88.04 %. In-vitro studies showed prolonged release of entrapped Enrofloxacin. This study results in a promising prolonged delivery system for Enrofloxacin and has good stability characteristics.

IndexTerms - Enrofloxacin, Proniosomes, Veterinary, Phospholipid, Transdermal.

Materials &Method:

Materials: The following chemicals and instruments were used in the experimental studies.

Chemicals: - The following chemicals were used for the study.

Table 2: List of Chemicals

Sr. No.	Chemicals	Manufacturer
1	Enrofloxacin	Aarti chemicals, Mumbai
2	Lecithin	Padmaja Chemicals
3	Cholesterol	Modern science lab
4	Span 20	Modern science lab
5	Span 60	Modern science lab
6	Ethanol	Modern science lab
7	Distilled water	NDMVP's college of pharmacy
8	0.1% Glycerol solution	Modern science lab

Table 3: List of Instruments

Instruments	Company
Bath Sonicator	Leelasonic
Weighing Balance	Shimadzu AUX 220
Sonicator	Citizen lab CD 4820
Brookfield Viscometer	DV-E viscometer, USA
Digital Balance Dona Balance	Wensar Nabco 200 DS, Varanasi
Homogenizer	Remi Motors Ltd., Mumbai
Magnetic Stirrer with hot plate	Remi Motors Ltd., Mumbai
Melting point apparatus	DBK Programmable Apparatus
Research Centrifuge UV-Visible Spectrophotometer	Shimadzu AUX 220
SEM	JEOL JSM – 5600
FTIR	Shimadzu 84005
pH meter	Hanna Instrument

1. INTRODUCTION:

1.1 Topical drug delivery System

Topical drug administration is a localized drug delivery system anywhere in the body through ophthalmic, rectal, vaginal and skin as topical routes. Skin is one of the most readily accessible organs on human body for topical administration and is main route of topical drug delivery system. Topical delivery can be defined as the application of the drug containing formulation to skin to directly treat cutaneous disorders (psoriasis, acne etc.) with the intentof containing the pharmacological or the effect of the drug to surface of skin or within. Topical application of several moieties widely used in several diseased treatments is asubstituted route to overcome the adverse effect of oral and rectal route of administration. There are two basic types of drug delivery products, external topical and internal topical. Theexternal topical are applied to mucous membrane orally, vaginally or on the rectal tissues for local activity (Bhowmik D. et al, 2012, Ashara K. et al, 2014).[1]

1.2Advantages of topical drug delivery systems

- Avoidance of first pass metabolism
- Convenient and easy to apply
- Avoids fluctuations in drug levels, inter and intra patient variations
- Avoidance of gastro-Intestinal incompatibility
- Ability to deliver drug more selectively to a specific site
- Achievement of efficacy with lower total daily dosage of drug by continuous drug input
- A relatively large area of application in comparison with buccal or nasal Cavity

Avoidance of the risks and inconveniences of intravenous therapy and of the variedconditions of absorption, like pH changes, presence of enzymes, gastric emptying time etc.

- Ability to easily terminate the medications when needed.
- Improving physiological and pharmacological response.
- Improved patient compliance.

- Provide suitability for self-medication.[1]
-

1.3 Disadvantages of topical drug delivery system

- Drugs of larger particle size are not easy to absorb through the skin
- Poor permeability f some drugs through the skin.
- Skin irritation or contact dermatitis may occur due to the drug and excipients. Possibility of allergic reactions.
- Can be used only for drugs which required very small plasma concentration for action
- Enzymes in epidermis may denature the drugs (Hyma P.et al, 2014, Singla V.et al, 2012).

2. Skin as a site for trans-dermal drug administration:

The skin is the one of the most extensive and readily accessible organs of human body covers a surface area of approx. 2 cm and receives about the third of the blood circulating through the body. Skin is the largest organ in the body account for more than 10% of body mass. Skinis the complex organ and allows the passage of various chemicals into and across the skin. Skin serves as the point of administration for systematically circulation and then transported to target tissues. The potential of using intact skin as the site of administration for dermatological preparations to elicit pharmacological action in the skin tissue has beenrecognized for several years. Skin mainly consist of three layers epidermis, dermis and subcutaneous tissues as shown in Fig. 1 with respect drug delivery, interest in these structureshas centered upon the possibility that they may provide “shunt” pathway across the skin, circumventing the need to cross the full stratum corneum. Skin is structurally complex and thick membrane. Molecules moving from the environment must penetrate the stratum corneum and any material of endogenous or exogenous origin of its surface. They must then penetrate the viable epidermis, the papillary dermis and the capillary walls into the blood stream or lymph channels, where upon they are removed from the skin by flow of blood.[3]

2.1 Drug transport -through the Skin:

2.1.1 Diffusion process

In passive diffusion, matter moves from one region of a system to another following random molecular motion. The basic hypothesis underlying the mathematical theory for isotropic materials (which have identical structural and diffusional properties in all direction) is that the rate of transfer of diffusing substance per unit area of a section is proportional to the concentration gradient measured normal to the section. This is expressed as Fick's first law ofdiffusion

$J= -D\ \delta C/ \delta x$.................................. (1)

Where,

J is the rate of transfer per unit area of surface (the flux), C is the concentration of diffusing substance, x is the space coordinate measured normal to the section

D is the diffusion coefficient.

The negative sign indicate that flux is in direction of decreasing concentration i.e. down tothe concentration gradient, Fick's second law is expressed as,

$\delta C / \delta t = D (\delta C2/ \delta x2)$................................. (2)

By performing multiple experimental design a membrane separating two compartments, with a concentration gradient performing during sink condition. If we measured the cumulative mass of diffusant m which passes per unit area through the membrane as a function time. At long times the plot approaches a straight line and from it we obtain steady flux, dm/dt. We obtain plot as follows,

$dm/dt = DC0K/h$ .... (3)

Where,

Co=Constant concentration of drug in the donor solution,

K = Portion coefficient of the solute between the membrane and bathing solution, h = Thickness of the membrane (Aulton M.,2007).

Ideal Molecular Properties for Drug Penetration A low molecular mass, preferably less than 600 Dalton. An adequate solubility in oil and water, so the concentration gradient in the membrane is high. A balanced partition coefficient and A low melting point, because it correlates good solubility (Aulton, M. 2007).[1]

2.2 Factors affecting absorption of drug through skin:

2.2.1 Skin condition

Intact, healthy skin is a tough barrier, but many agents can damage it. Many solvents that are used in formulations open complex dense structure of horny layer. Mixture of non- polar and polar solvents such as chloroform and methanol remove lipid fraction, forming artificial shunts through which molecules pass more easily. Diseased skin alters skin condition so percutaneous absorption through diseased skin increases.

2.2.2 Skin age

Skin of young and elderly people are more permeable than adults. Children are more susceptible to toxic effects of drugs and chemicals because of greater surface area/ unit body weight thus; potent drugs produce severe side effects.

2.2.3 Blood flow

Changes in peripheral circulation affect trans-dermal absorption, an increase in blood flow reduce amount of time a penetrating agent remains in dermis and raises concentration gradient across the skin. In clinically hyperemic skin, increase in absorption always arises because the disease damages skin barrier. Potent vasoconstriction agents such as topical steroids reduce their own clearance rate or that of another drug.

2.2.4 Regional skin sites

Variation in cutaneous permeability is due to different thickness and nature of SC and skin appendages. There is different in rate of absorption for a specific substance passing through identical skin site in different healthy volunteers. E.[4]

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Skin metabolism Skin metabolizes steroid hormones, chemicals, etc. Such metabolism may determine therapeutic efficacy of topically applied compounds. It is estimated that skin can metabolize almost 5% of candidate topical drugs.

2.4 Physicochemical factors

2.4.1 Skin hydration

When water saturates the skin, tissue swells and softens and it get wrinkles therefore, the permeability increases. Hydration of stratum corneum is one of the most important factors in increasing penetration rate.

2.4.2 Temperature and pH

Penetration of material through human skin can change tenfold for a large temperature variation as diffusion coefficient decreases as temperature falls. According to simple form of pH partition hypothesis only unionized molecules pass readily across lipid membranes.

Weak acids and bases dissociate to different degrees depending on pH and their pKa or pKb values. Stratum Corneum remarkably resistant to alteration in pH, tolerating a range of 3 to 9.

2.4.3 Diffusion coefficient

Diffusion speed depends on state of matter of medium.

2.4.4 Drug concentration

Flux of solute is proportional to concentration gradient across the entire barrier phase. Drug permeation follows Fick's law. For maximal flux in a thermodynamically stable Situation the donor solution should be saturated.

2.4.5 Partition coefficient

Partition coefficient is referred as 'K'. Molecule should have optimal K value. Below K value molecules are too water soluble to partition well into the horny layer. At higher K values compound have more lipid soluble that do not readily pass from stratum corneum into water rich viable tissue. For a drug series this behaviour produces a parabolic or bilinear relation between pharmacological activity and partition coefficient.

2.4.6 Molecular size

Absorption is inversely related to molecular weight (Aulton M., 2007),[4]

3. Inflammation:

3.1 Definition:

Inflammation is an important nonspecific defence reaction to tissue injury, caused by pathogen or wound. Acute Inflammation is the immediate responce of the body to injury or cell death. The gross features were described over 2,000 years ago and still considered as cardinal signs (Willey M. et al, 2008). Inflammation is the result of concerted participation ofa large number of

vasoactive, chemo active and proliferative factors at different stage (Tripathi K. et al, 2003).

Inflammation is local protective response of the body tissue injury. In last few decades research demonstrate that inflammation is regulated by many pro and antiinflammatorychemical mediators like histamine, prostaglandins (PEG2 and prostacyclins), leukotriens (LTBA), serotonin bradikinin, cytokines (IL-I, IL-6, IL-8, IL-11, TNF-a), reactive oxygen species, growth factors, liposomal enzymes of neutrophills. The extent of involvement of these chemical mediators varies depending upon the nature of Inflammation (Khatib N. et al, 2010).[6]

Types of inflammation

1. Acute inflammation
2. Chronic inflammation

3.2 Mechanism of inflammation

The release of inflammatory mediators from injured tissue cells which cause development of the signs of the inflammation. Mediator's increases acidity in surrounding fluid result into activation of enzyme kallikrein, it releases bradykinin which binds to receptor on capillary wall. Simultaneously bradikinin binds to mast cells and activates mast cells by causing influx of calcium ions leads to the release of histamine. Histamine causes move out of leukocytes and bradykinin which causing swelling or oedema. Bradykinin then binds to the capillary wall and stimulates production of prostaglandins to promote tissue swelling in the infected area and it also binds to the free nerve endings and stars a pain impulse (Willey M. et al, 2008). 1.10 Various topical drug delivery systems

3.2.1 Gels

Gels are defined as semi rigid system in which the movement of dispersing medium is restricted by an interlacing three-dimensional network of particles of solvated macromolecules of the dispersed phase. The USP defines gels as a semisolid system consisting of either suspension made up of small inorganic particles or large organic

molecules interpenetrated by a liquid. The favourable properties of dermatological gel are thixotropic, have good spreadability, greaseless, easily removable, emollient, demulcent and non-staining (Tahsildar A. et al, 2013).

3.2.2 Ointments

The preparations intended for external application to the skin or mucous membrane is referred as ointment. These are greasy, semisolid preparations which are often anhydrous and contain medicament either dissolved or dispersed in the vehicles. It contains bases like hydrocarbon fats and fixed oils, absorption base, emulsifying bases and water soluble bases.

3.2.3 Creams

Creams are semisolid preparations containing one or more medicinal agents dissolved or dispersed in either water-in- oil emulsion or an oil-in-water emulsion or in other type ofwater washable base. Creams find important applications in topical skin products. Many patients and physicians prefer creams because they are easier to spread and remove.

3.2.4 Liquid Preparations

These are preparations meant for external application to the skin includes simple bath,liniments, lotions, paints, varnishes and tinctures. A simple soak or bath provides an active, ingredients in an aqueous solution or suspension. Bath additives such as oil and emollient deposit a layer of liquid paraffin on the skin in an attempt to maintain the moisture content ofthe stratum corneum.

3.2.5 Powders

The main purpose of formulating dusting powders for application to skin by mixing together several finely divided in soluble powders. It functions like drying, protective and lubricating agent. For e.g. talc, zinc oxide, starch, and kaolin. Dusting powder should no longer consistof boric acid because abraded skin may absorb it into toxic amounts.

3.2.6 Aerosols

Aerosols may function as drug delivery system for solutions, suspensions, powders, semisolids and emulsions. Solution forms of aerosols are simple products consisting of an active ingredient dissolved in propellants or a mixture of propellants and miscible solvents (Ansel C., 2011, Jain N. 2010).

Gels The term 'gel represents a physical state with properties intermediate between those of solids and liquids. However, it is often wrongly used to describe any fluid system that exhibits some degree of rigidity.

A gel consists of a polymer which swells in the presence of fluid and perhaps it within its structure. The rigidity of the gel is determined by the amount of fluid it entraps. These gels are wet and soft and look like a solid material. These are capable of undergoing large deformation in their physical state i.e. from solid to liquid.[7]

4 Definitions

Gels are semisolid systems in which a liquid phase is constrained within a three dimensional polymeric matrix of natural or synthetic gums in which a high degree of physical or chemicalcross linking has been established. The term 'gel' is broad encompassing semisolids of a widerange of characteristics from gaily rigid gelatine slabs to suspensions of colloidal clays to certain greases. A gel can be looked upon as being composed of two interpenetrating phases (the gelling agent and a fluid component).

The United State Pharmacopoeia (USP) defines gels as semisolids, being either suspensions of small inorganic particles or large organic molecules interpenetrating with liquid. In the first case, the inorganic particles, such as bentonite form a three dimensional house of cards structure throughout the gel. This is true two phase system, as the inorganic particles are not soluble merely being dispersed through the continuous phase.

It is the interaction between the units of the colloidal phase, inorganic or organic which sets up the structural viscosity immobilizing the liquid continuous phase. Thus, gels exhibitcharacteristics intermediate to solids and liquids (Bhardwaj S. et al, 2012).

4.1 Classification of gels

According to USP, gels are classified as

Single-Phase Gels

Two-Phase Gels

4.1.1 Single phase gels

Single Phase Gels consist of organic macromolecules uniformly distributed through a liquid in such manner that no apparent boundaries exist between the dispersed macromolecules and the liquid. Single phase gel may be made from synthetic macromolecules or from natural

gums. Although these gels are commonly aqueous, alcohols and oils can be used as the continuous phase,

4.1.2Two phase gels

Two Phase Gels containing bentonite may be used as a base for topical preparations such as plaster and ointment. Aluminium hydroxide gel, USP is an example of a two phase gel. The USP states that "Aluminium hydroxide gel is a suspension of amorphous aluminium hydroxide in which there is a partial substitution of carbonate for hydroxide." The gel is usually prepared by the interaction of soluble aluminium salt, such as chloride or sulphate with ammonia solution, sodium carbonate or bicarbonate (Singh M. et al, 2013).[8]

4.2 Based on Nature of Solvent

A) Hydro gels (water based)

Here they contain water as their continuous liquid phase e.g. bentonite magma, gelatine cellulose derivatives, carbopol, and poloxamer gel.

B) Organic gels (with non aqueous solvent)

These contain a non aqueous solvent on their continuous phase e.g. plastibase (low molecular wt. Polyethylene dissolved in mineral oil)

C) Xerogels

Solid gels with low solvent concentration are known gels Xerogels. These are produced by evaporation of solvent or freeze drying leaving the gel framework behind, on contact with the fresh fluid they swells and can be reconstituted e.g. Tragacanth ribbons, acacia tear, B- cyclodextrin, dry cellulose and polysterene.

4.3 Based on Rheological Properties

Usually gels exhibit non-Newtonian flow properties. They are classified as

a) Plastic gels

b) Pseudo plastic gels

c) Thixotropic gels

A) Plastic gels

e.g. Bingham bodies, flocculated suspension of aluminium hydroxide exhibit a plastic flow and the plot of rheogram gives the yield value of gels above which the elastic gels distort andbegins to flow.

Pseudo-plastic gels

e.g. Liquid dispersion of tragacanth, sodium alginate, Na CMC etc, exhibits pseudoplastic flows. The viscosity of these gels decreases with increasing the rate of share with no yield value. As the shearing stress increased the disarranged molecules begin to align their long axis in the direction of flow with release of solvent from gel matrix.

4.3.1 Thixotropic gels

The bonds between the particles in these gels are very weak and can be broken down by shaking. The resulting solution will revert back to gel due to the particle colliding and linking together again (the reversible isothermal gel-so-gel transformation).This occurred in colloidal systems with non spherical particle to build up a scaffold like structure e.g. kaolin, bentonite and agar.[5]

4.4 Based On Physical Nature

A) Elastic gels

Gels of agar, alginates and guar gum exhibit an elastic behaviour. The fibrous molecules are being linked at the point of junction by relatively weak bonds such as hydrogen bonds and dipole attraction. If the molecule possesses free -COOH group then additional bonding take place by salt bridge of type COO-X-COO between two adjacent strand networks e.g. alginateand carbopol.

B) Rigid gels

This can be formed from macromolecule in which the framework linked by primary valance bond. e.g. in silica gel, silicic acid molecules are held by SiO-Si-O bond to give a polymer structure possessing a network of pores (Kaur L. et al, 2013).

4.5 Properties of Gels

Gels should posses following properties

1.It should be inert, compatible with other additives and non-toxic

2.It should be stable at storage condition.

3.It should be free from microbial contamination.

4.It should maintain all rheological properties of gel.

5.Economical.

6.It should be washable with water and free from staining nature,

7.It should not affect biological nature of drug.

8.It should be convenient in handling and its application.

9.It should posses properties such as thixotropic, greaseless, emollient, non-staining etc (Singh M. et al, 2013).

4.6 Proniosomal gel

Proniosomes

Colloidal particulate carriers such as liposomes or niosomes have been widely employed in drug delivery systems and producing them from proniosomes provides them a distinctive advantage. These carriers can act as drug reservoirs and the rate of drug release can be controlled by modification of their composition. These lipid vesicles can carry both hydrophilic drugs (by encapsulation) and hydrophobic drugs (in lipid domain). Due of their capability to carry a variety of drugs, these lipid vesicles have been extensively used in various drug delivery systems like drugtargeting controlled release and permeation enhancement of drugs but there remains certain draw backs to be addressed and can be avoided if they are prepared in dry form. Proniosomes, prepared in dry form and hydrated by agitation in hot water to form niosomes provide an alternative with prospective for drug delivery via the transdermal route.[9]

4.7 Niosomes

Niosomes are non ionic surfactant vesicles that can entrap a solute in a manner analogous to liposomes. They are osmotically active, and are stable on their own, while also increasing thestability of the entrapped drugs. Handling and storage of surfactants required no special conditions. Niosomes posses an infrastructure consisting of hydrophilic and hydrophobic moieties together, and as a result, can accommodate drug molecules with a wide range of solubilities. Although niosomes as drug carriers have shown advantages such as being cheap and chemically stable, they are associated with problems related to physical stability such as fusion, aggregation, sedimentation and leakage on storage. All methods traditionally used for preparation of niosomes are time consuming and many involve specialised equipments. Most of these methods allows only for a predetermined lot size so material is often wasted if smaller quantities are required for particular dose application. The size of niosomes ismicroscopic and lies in non metric scale. The particle size ranges from 10nm-100nm.[9]

4.7.1 Disadvantages of niosomes

1. Physical instability
2. Aggregation
3. Fusion
4. Leaking of entrapped drug
5. Hydrolysis of encapsulated drugs which limiting the shelf life of the dispersion To overcome these Disadvantages, proniosomes are prepared and reconstituted into niosomes.

4.6.1 Proniosomes as a carrier:

Hu and Rhodes et al reported that Proniosomes are dry formulations of surfactantcoated carrier, which can be measured out as needed and rehydrated by brief agitation in hot water. These “Proniosomes” minimizes the problems of niosomes physical stability such as aggregation, fusion and leaking and provided additional convenience in transportation, distribution, storage and dosing. Proniosome-derived niosomes are superior to conventional niosomes in convenience of storage, transport, and dosing. Stability of dry proniosomes is expected to be more stable than a pre-manufactured niosomal formulation. In release studies proniosomes appears as to be equivalent to conventional niosomes. Size distributions of proniosome-derived niosomes are somewhat better that those of conventional niosomes sothe release performance in more critical cases turns out to be superior. Proniosomes are dry powder, which makes further processing and packaging possible. The powder form provides optimal flexibility, unit dosing, in which the proniosome powder is provided in capsule couldbe beneficial. A proniosome formulation based on maltodextrin was recently developed that has potential applications in deliver of hydrophobic or ampiphilic drugs. The better of these formulations used a hollow particle with expectionally high surface area. The principleadvantage with these formulations was the amount of carrier required to support the surfactant could be easily adjusted and proniosomes with very high mass ratios of surfactant to carrier could be prepared.[9]

Because of the ease of production of proniosomes using the maltodextrin by slurry method, hydration of surfactants from proniosomes of a wide range of compositions can be studied.

4.7.1 Advantages of proniosomes over the niosomes

1. Avoiding problem of physical stability like aggregation, fusion, leaking,
2. Avoiding hydrolysis of encapsulated drugs which limiting the shelf life of the dispersion.

4.7.2 Formation of niosomes from proniosomes

The niosomes can be prepared from the proniosomes by adding the aqueous phase with the drug to the proniosomes with brief agitation at a temperature of the surfactant.

>Tm

Where,

T = Temperature

Tm = mean phase transition temperature Blazek- walsh A.I. et al has reported the formulation of niosomes from maltodextrin based proniosomes. This provides rapid reconstitution of niosomes with minimal residual carrier. Slurry of maltodextrin and surfactant was dried to form a free flowing powder, which could be rehydrated by addition of warm water. (Sudhamani T. et al 2010).

4.6.3 Preparation of proniosomal gel

Coacervation phase separation method is widely adopted for the preparation of proniosomal gel. Proniosomal gel is basically mixture of many phases of liquid crystal, viz. Lamellar, hexagonal and cubic phase liquid crystals which on hydration tend to form unilamellar to multivesicular, multilamellar and spherical shaped structures.

Cholesterol + Surfactant + Phosphatidyl Choline + Drug + Suitable Alcohol (minimum amount of suitable alcohol is added so that micelle formation not takes

place)

Mixing is done and after that it is covered with lid to prevent the loss of solvent(Warmed at 60-70°C on water bath)

Mixing is done and after that it is covered with lid to prevent the loss of solventDiluted glycerol solution isotonic buffer solution (phosphate buffer or saline solution)

Limited aqueous solution is then added.

(Only limited amount of aq. Phase added so that gel formation takes place and not thedispersion)

Liquid gel formed

Warmed again at 60-70°C on water bath due to formation of range of less viscouscomposition

(Overnight cooling in dark)

Proniosomal gel formed

Addition of water leads to swelling of bi-layer (Due to interaction between water and the polar groups of the surfactants). Uni-lamellar to Multi-vesicular, Multi-lamellar and sphericalshaped structures.

5. Formulation aspects of proniosomes: [1,5,10]

Proniosomal gel comprises of variety of ingredients such as: Cow Ghee, , Nonionic surfactants (Sorbitan, polysorbatan), Cholesterol, Alcohol (Butanol, Ethanol, Isopropanol, Propanol), Aqueous phase (Water, 0.1% Glycerol, Phosphate buffer pH 7.4), Miscellaneous (Dicetyl Phosphate, Solutan C24 etc.)

A. Surfactants

A wide range of surfactant is available (Table 1) and therefore selection of surfactant should be done on the basis of the following

a. Hydrophilic Lipophilic Balance (HLB value)

Surfactants having HLB number in between 4 and 8 are good candidate for vesicle formationas when Hydrophilic surfactants are taken into account to attain a concentrated systems and it inhibits the free hydrated units to exist aggregates and coalesced to form lamellar structure. High HLB value results in reduction of surface free energy which allows forming vesicles of larger size. High HLB value of Span 40 and 60, results reduction in surface free energywhich allows forming vesicles of larger size hence large area exposed to the dissolution medium and skin. High aqueous solubility on hydration do not allow

b. Phase transition temperature

Phase Transition Temperature plays a vital role in the degree of entrapment as the transition temperature of surfactants increase it leads to increase in the entrapment efficiency and decrease in the permeability, Spans with highest phase transition temperature provided the highest entrapment for the drug and vice versa. The drug leaching from the vesicles can be reduced due to high phase transition temperature and low permeability.

c. Critical packing parameter (CPP)

CPP can be defined as the relationship between the structure of the surfactant including size of hydrophilic lead group, the structure of the surfactant including size of hydrophilic lead group, and length of hydrophobic alkyl chain in the ability to form vesicles is described as CPP = v/lca Where, v=hydrophobic group volume, lc = the critical hydrophobic group lengtht o corder a = the area of the hydrophilic head group As we know the entrapment efficiency and particle size are inversely proportional to each other therefore CPP value holds an important place in the formulation. Interpretation of relation related to CPP.

a. CPP of between 0.5 and 1 indicates that the surfactant is likely to form vesicles.
b. A CPP of below 0.5 is said to give spherical micelles due to largehydrophilic head group area

c. CPP of above 1 because of large contribution from the hydrophobic group volume should produce inverted micelles, the latter presumably only in an oil phase, or precipitation would occur. Spans are most widely used in the preparation of provesicles. Fundamentally all spans have the same head group but can be differentiate on the basis of their alkyl chain. Literature suggests that entrapment efficiency increases as the alkyl chain length increases and it followed the trend Span 60 (C18)>Span 40 (C16) >Span 20(C12)>Span 80(C18). Spans 60 and 80 have the same head group, but difference lies in the alkyl chain of Span 80 that is unsaturated. Introduction of a double bond into the paraffin chain of Span 80 causes a marketenhancement in the permeability. This might be the reason for the lower entrapment efficiency of the Span 80 systems.

B. Cholesterol

El-Laithy et al, reported that as the cholesterol content increase there is a significant increase in entrapment efficiency (%) but after certain limit further Cholesterol increase results in significant decrease in entrapment efficiency. Reason revealed for this type of behaviour is Cholesterol molecules accommodate itself as "vesicular cement" in the molecular cavities formed when surfactant monomers are assembled into bi-layers to form niosomal membranes and this space filling action results in the increased rigidity, decreased permeability of cholesterol-containing membranes compared to cholesterol-free membranes and the improved entrapment efficiency. On further increase of cholesterolbeyond certain concentration it completes with the drug for the space within the bi-layers, hence excluding the drug and can disrupt the regular linear structure of vesicular membranes.

C. Solvent

Selection of alcohol is another important aspect as it is having huge impact on the vesicle size and the drug permeation rate. Literature revealed that as the solubility of alcohol in waterincreases the size increases and they follow the order Ethanol > Propanol > Butanol > Isopropanol It also affect the spontaneity of formation of niosomes the formulation containing Isopropanol and Butanol were formed more spontaneously than niosomes containing Propanol and Ethanol due to faster phase separation of Isopropanol and Butanol due to their lower solubility in water.

D. Aqueous phase

Phosphate buffer pH 7.4, 0.1% glycerol, hot water is generally used as Aqueous phase in preparation of proniosomal gel. Mokhtara et al. found that pH of the hydrating medium also play a very important role in the entrapment efficiency as the fraction of flurbiprofen encapsulated was increased to about 1.5 times as the pH decreased from pH 8 to 5.5. The increase in the % entrapment efficiency of flurbiprofen by decreasing the pH may be attributed due to the presence of the ionisable carboxylic group in its chemical structure. Decrease in the pH leads to proportions increase in the unionised species of flurbiprofen, which have higher partitioning to the bilayer lipid phase compared to the ionized species. Reason suggested by Ammara et al. For such type of behaviour is that the type of aqueous medium might influence the tactness of proniosomes, thus affecting their entrapment efficiencies.

E. Miscellaneous

a. Dicetyl Phosphate (DCP) And Stearyl Amine (SA)

Dicetyl phosphate and stearyl amine are charged lipid used to impart charges on niosomal vesicles. Proniosomal formulation having DCP shows slightly greater amount of drug as compare to formulation containing surfactant and cholesterol only but on other hand much less than those formulation containing egg/soya lecithin. It is also reported that due to the charge present in the curvature and decrease vesicle size results in the maximum drug releasefor the formulation containing DCP. It is reported that SA also decreases the entrapment efficiencies.

b. Solutan C24 (poly-24 ox ethylene cholesteryl ether)

6 Applications of proniosomes derived niosomes

6.1 Targeting of bioactive agents

To Reticulo-Endothelial System (RES) are the cells of RES preferentially taken up the vesicles. The uptake of niosomes by the cells is also by circulating cerum factors known as oposins, which mark them for clearance. Such localized drug accumulation has, however, been exploited in treatment of animal tumours known to metastasize to the liver and spleen and in parasitic infestation of liver to organs other than RES. It has been suggested that carrier system can be directed to specific sites in the body by use of antibodies. Immunoglobulin seems to bind quite readily to the lipid surface, thus offering a convenient means for targeting of drug carrier. Many cells posses the intrinsic ability to recognize and bind particular carbohydrate determinants and this can be exploited to direct carriers system to particular cells. Delivery of peptide drugs investigated oral delivery of 9-deglycinamide, 8-arginine vasopressin entrapped in niosomes in an in-vitro intestinal loop model and reported that stability of peptide increased significantly. Neoplacia Doxorubicin, the anthracyclic antibiotic with broad spectrum antitumour activity, shows a dose dependent irreversible cardio toxic effect. Niosomal delivery of this drug to mice bearing S-180 tumour increased their life span and decreased the rate of proliferation of sarcoma.

6.2 Niosomes as carrier for haemoglobin

Niosomes can be used as a carrier for haemoglobin. Niosomal suspension shows a visible spectrum super imposable onto that of free haemoglobin. Vesicles are permeable to oxygen and haemoglobin dissociation curve can be modified similarly to non-encapsulated haemoglobin.

6.3 Transdermal delivery of drugs by niosomes

Slow penetration of drug through skin is the major drawback of transdermal route of delivery. An increase in the penetration rate has been achieved by trans-dermal delivery of drug incorporated in niosomes. It has studied that topical delivery of erythromycin from various formulations including niosomes or hairless mouse, From the studies, and confocal microscopy, it was seen that nonionic vesicles could be formulated to target pilocebaceous glands.

6.4 Delivery of peptide drugs

It has investigated that oral delivery of 9-desglycinamide, 8-arginine vasopressin entrappedin niosomes in an in-vitro intestinal loop model and reported that stability of peptide increased significantly. Immunological application of niosomes; Niosomes have been used for studying the nature of the immune response provoked by antigens. It has reported that niosomes as potent adjuvant in terms of immunological selectivity, low toxicity and stability.

6.5 Neoplacia

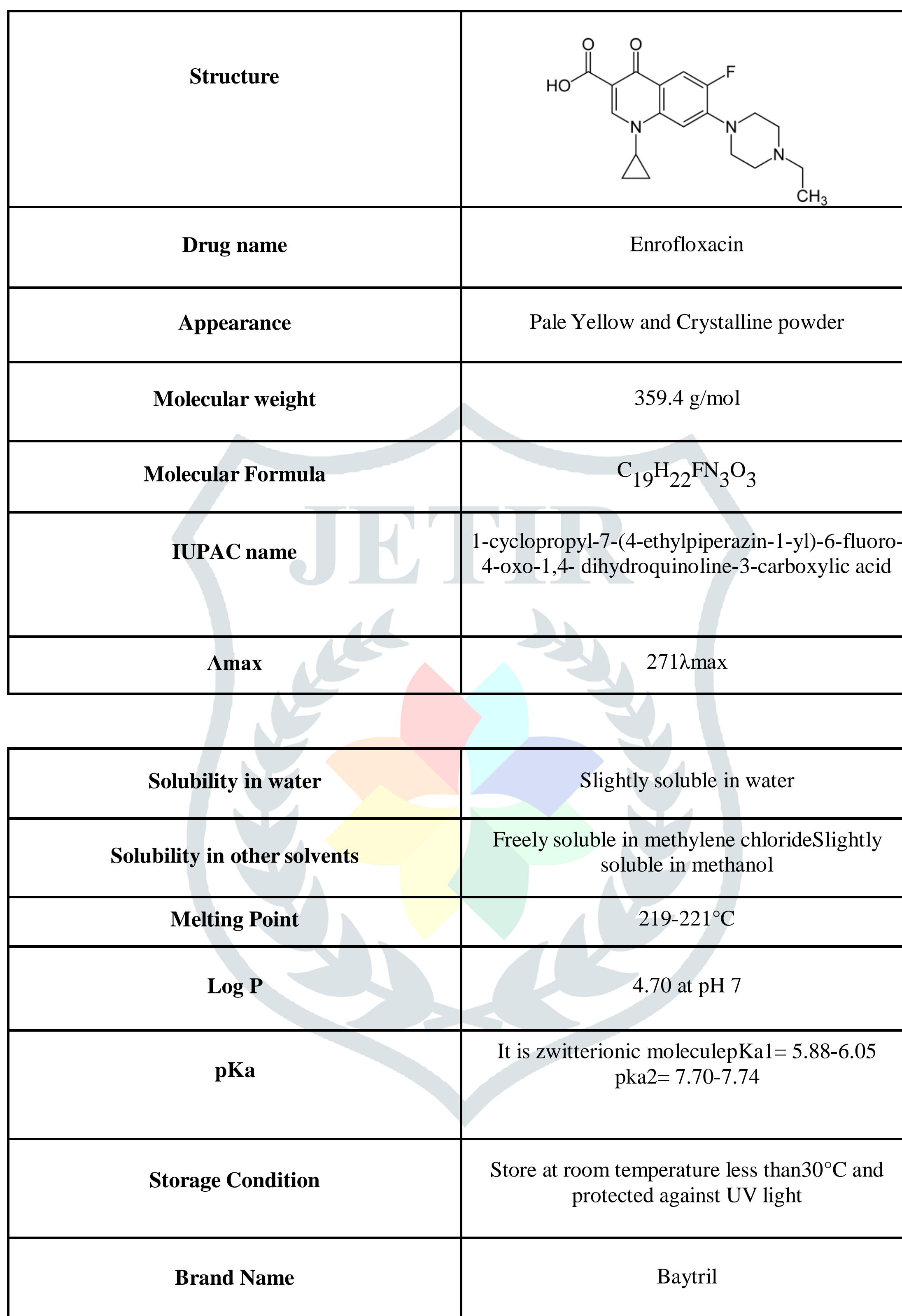
Doxorubicin, the anthracyclic antibiotic with broad spectrum anti-tumour activity, shows a dose dependent irreversible cardio toxic effect. Niosomal delivery of this drug to micebearing S-180 tumour increased their life span and decreased the rate of proliferation of sarcoma. Niosomal entrapment increased the half-life of the drug, prolonged its circulation and altered its metabolism. Intravenous administration of methotrexate entrapped in niosomes to S-180 tumour bearing mice resulted in total regression of tumour and also higherplasma level and slower elimination.

6.6 Leishmaniasis

Niosomes can be used for targeting of drug in the treatment of diseases in which the infectingorganism resides in the organ of reticulo-endothelial system. Leishmaniasis is such a disease in which parasite invades cells of liver and spleen. The commonly prescribed drugs are antimonial, which are related to arsenic, and at high concentration they damage the heart, liver and kidney. The study of antimony distribution in mice, performed by researcher showed high liver level after intravenous administration of the carrier's forms of the drug. It has reported that increased sodium stibogluconate efficacy of niosomal formulation and that the effect of two doses given on successive days was additive.

7. Drug Profile

Table 1: Drug Profile



Structure	
Drug name	Enrofloxacin
Appearance	Pale Yellow and Crystalline powder
Molecular weight	359.4 g/mol
Molecular Formula	$C_{19}H_{22}FN_3O_3$
IUPAC name	1-cyclopropyl-7-(4-ethylpiperazin-1-yl)-6-fluoro-4-oxo-1,4- dihydroquinoline-3-carboxylic acid
Λmax	271λmax
Solubility in water	Slightly soluble in water
Solubility in other solvents	Freely soluble in methylene chlorideSlightly soluble in methanol
Melting Point	219-221°C
Log P	4.70 at pH 7
pKa	It is zwitterionic moleculepKa1= 5.88-6.05 pka2= 7.70-7.74
Storage Condition	Store at room temperature less than30°C and protected against UV light
Brand Name	Baytril

8.0 EXPERIMENTAL WORK

8.1 Preformulation studies:

8.1.1 Physical characters

The drug powder was evaluated for colour and odour

8.1.2 Melting Point

The melting point was determined by introducing a minute amount of drug into a small thin-walled capillary melting point tube sealed at one end by inserting the tip into a Bunsen flame. The drug was transferred from the open end to the bottom of the capillary tube, the bottom was tapped gently. A packed capillary was attached to a normal mercury thermometer then capillary was inserted into melting point apparatus and the temperature was observed at which melting begins and is complete.

8.1.3 Solubility Determination

The solubility of enrofloxacin was determined in distilled water and in different solvents. For this an excess amount of drug was added to volumetric flasks containing solvent and shaken vigorously for 12 hours with frequent intervals.

8.2 Characterization: [11]

UV-visible Spectroscopy

Determination of λ max: The UV absorption spectrum of Enrofloxacin was obtained using a UV- visible Spectrophotometer. The spectrum was scanned from 200 nm to 400 nm.

Preparation of Standard Stock Solution: Standard stock solution of Enrofloxacin were prepared by dissolving 10 mg of drug separately in 10 ml of 0.1 KOH solution and sonicated for 15 minutes and filtered through whatman filterpaper in order to get dilution of 1 mg/1 ml i.e.1000 µg/ml.

Determination of Absorption Maxima: By appropriate dilution of standard stock solutions of drug with

0.1 KOH, solution containing and 10 µg/ml of drug was scanned separately in the range of 200-400nm. Wavelength of absorption maxima was determined for drug. Drug showed absorption maxima at 271 nm.

FTIR Spectroscopy

The IR spectrum of drug & excipients was obtained in a KBR pellet using FT-IR spectrophotometer. FT-IR spectra were recorded in the region of 400–4,000 cm-1 . Assign the major absorption bands.

Formulation

Preparation of Proniosomal gel Coacervation phase separation Method:

Proniosomal formulae were prepared using different types of nonionic surfactants, lecithin, and cholesterol.

Appropriate amounts of proniosomal components mixed together with the drug were mixed with 2.5 ml of absolute ethanol in a clean and dry, wide-mouth tube.

After mixing all the ingredients, the open end of the glass tube was covered with a lid to prevent loss ofsolvent from it and warmed in a water bath at 65 ± 3°C for ~5 min, until the surfactants were dissolved completely.

Then, 1.6 ml of pH 7.4 phosphate buffer was added, and warming was continued on the water bath for ~2 min till a clear solution was formed which was converted into Proniosomal gel on cooling.

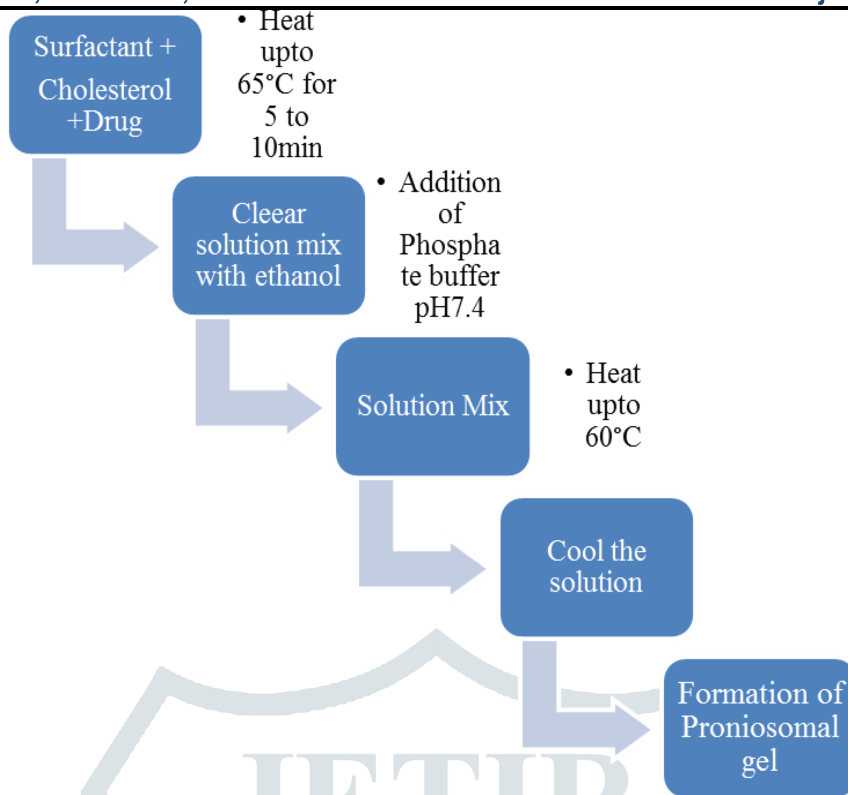
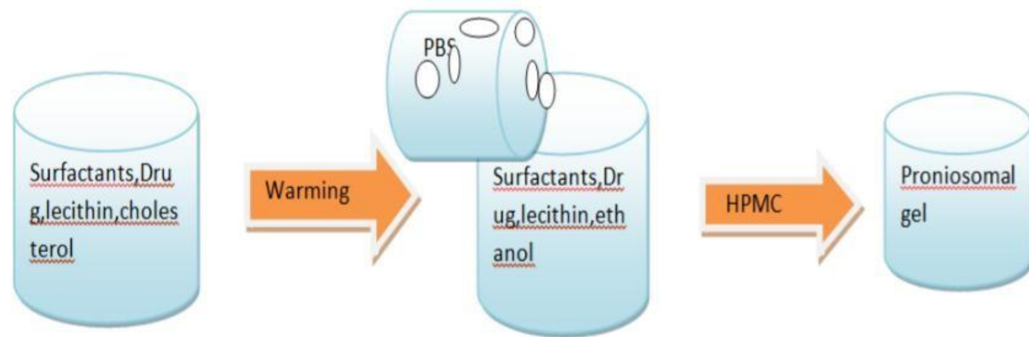


Fig 1.Preparation of Enrofloxacin Proniosomal gel

Table 4: Optimization of batch

Sr.no	Components	B1 (%)	B2 (%)	B3 (%)	B4 (%)
1	Enrofloxacin	5	5	5	5
2	Cholesterol	1.5	1.5	1.5	1.5
3	Lecithin	12.5	12.5	12.5	12.5
4	Span 60	-	-	5	2.5
5	Span 80	2.5	5	-	-
6	Distilled water	27.5	27.75	27.75	27.75
7	Ethanol	20	20	20	20
8	0.1% Glycerol Solution	31.25	31.25	31.25	31.25



3. EVALUATION STUDIES: [12,13,14]

Optical Microscopic Examination

Proniosomal suspension was diluted with phosphate buffer pH 7.4. A drop was placed on glass slideand the shape of vesicles was measured using optical microscopic camera.

Surface Morphology (SEM)

The sizes of the vesicles were measured by scanning electron microscopy. Small amount of proniosomal suspension was placed on the specimen stub, coated with carbon and then with gold vapor using Hitachi vacuum evaporator. The samples were examined under scanning electron microscope,and then photographed

Determination of pH

The pH measurements are performed using digital pH meter. Before measurements pH meter was calibrated and readings were taken by dipping the glass electrode into the gel formulations

Drug Content

1 ml of formulation was taken in 10 ml columetric flask and diluted with water and volume adjusted to 10ml. 1 ml from this solution was withdrawn and diluted with 10m ml of distilled water. Finally the absorbance of prepared solution was measured at 271 nm by using UV spectrophotometer.

Drug release

The releases of drug from proniosomal formulation (with different solvents and surfactants) were determined by using membrane diffusion technique. The proniosomal formulation equivalent to 5 mg ofdrug was placed in a glass tube of 2.5 cm diameter with 8 cm effective length which was tied with previously soaked dialysis membrane, which acts as a donor compartment. The glass tube was placed ina beaker containing 100 ml of phosphate buffer (pH 7.4), acting as a receptor compartment

The temperature of receptor medium was maintained at 37 ± 05°C and was agitated at the speed of 100 rpm using magnetic stirrer. The whole assembly was fixed in such a way that the lower end of tube containing suspension was just touching (1-2 mm depth) the surface of diffusion medium. Aliquots of 3ml sample were withdrawn and after each withdrawal same volume of medium was replaced. The collected sample was analyzed by UV spectrophotometer using phosphate buffer (pH 7.4) as blank Antimicrobial study

Agar plate technique was used. It is a technique used for evaluation of microbial stability of formulation and mainly applied for semisolid formulations. Previously prepared Sabouraud's agar dried plates were used. Three grams of the optimized proniosomal gel was placed in a bore of plate. Freshly prepared culture loops were streaked across the agar at a right angle from the ditch to the edge of the plate. After incubation for 18 to 24 hours at 25°C, the microbial growth was observed visually.

Spreadibility

0.5g gel was placed within a circle of 1 cm diameter premarked on a glass plate over which a second glass plate was placed. A weight of 10 g was allowed to rest on the upper glass plate. The increase inthe diameter due to spreading of the gels was determined.

$S = m \times L/t$

Where, S: Spreadability,m: weight of load,

L: length travel by upper slide, t: time.

Viscosity

The measurement of viscosity of the prepared proniosomal gel was done with Brookfield Viscometer (DV-E). Ten g of gel was taken in a beaker and the spindle was dipped into the gel formulation, viscosity of the gel formulation was measured by rotating the spindle 06 at 10 rpm because gel comes under the HA (high viscosity) category.[15]

Zeta potential

The zeta potential of the selected batch (B3) of proniosomal formulation was determined at 25ºC using Zetasizer (Malvern Instruments). Proniosomal suspension was diluted 100 times with double-distilled

9. Results and Discussiom

Preformulation Studies:

Physical Characters

Parameter	Observation
Colour	Pale Yellow
Odour	Odourless

Fig 3: Picture of Formulated Proniosomal gel

Melting point:

Reported	Observed
221°C	220°C
	219°C
	221°C

Solubility:

Chemical	Solubility
Water	Slightly soluble
Methanol	Slightly soluble
Methylene Chloride	Slightly soluble

Characterization

UV-visible Spectroscopy

Determination of λ max: After the analysis of Enrofloxacin through range of UV spectrophotometer, λ max 271nm was found to be maxima.

Table 5: Concentration & absorbance

Sr. No.	Concentration (µg/mL)	Absorbance
1	10	0.0504
2	50	0.1427
3	100	0.2343
4	150	0.3311
5	200	0.4323
6	300	0.6282

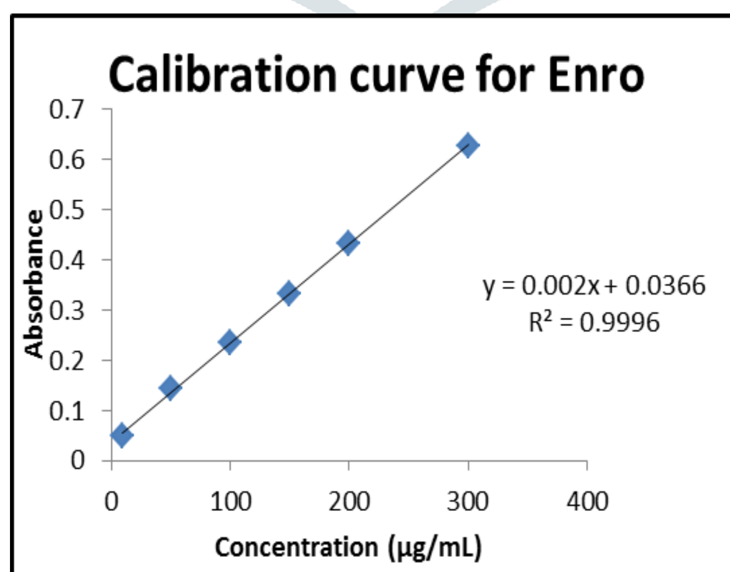


Fig 4: Calibration curve for Enrofloxacin

FTIR Spectroscopy

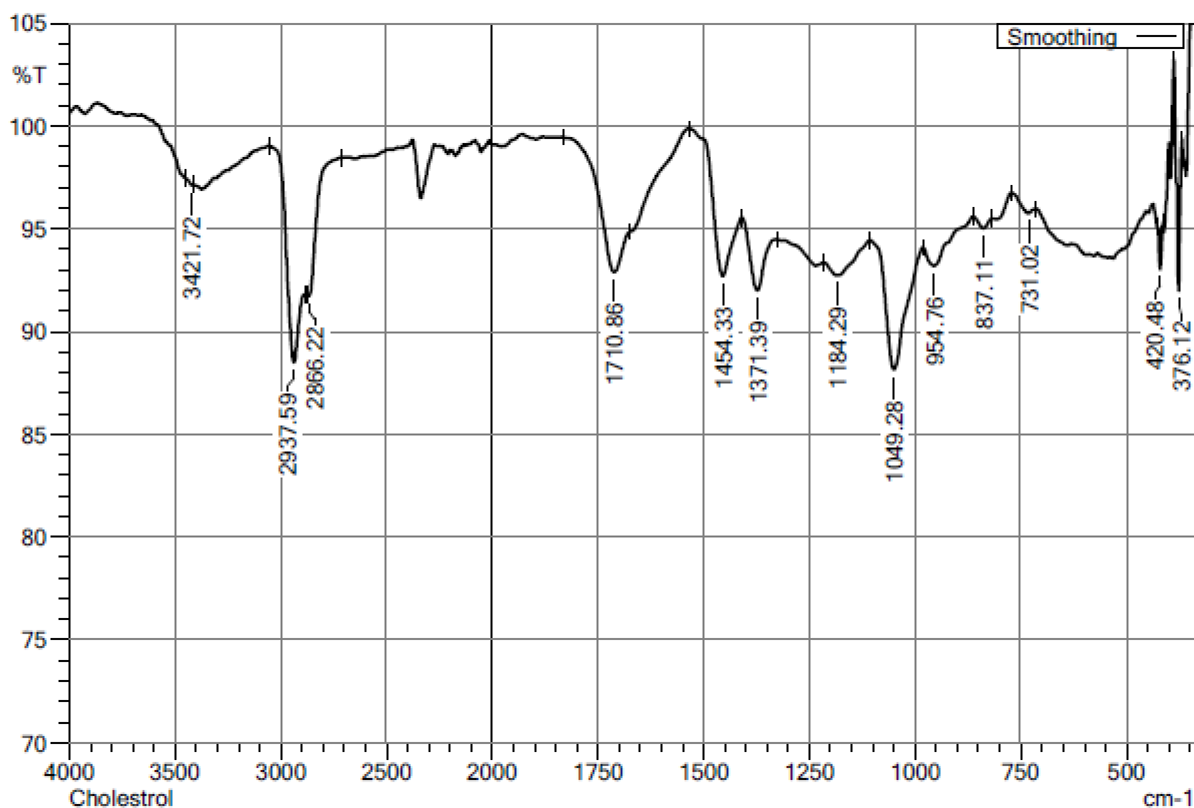


Fig 5: FTIR spectra of Cholesterol

Table 6: Interpretation of FTIR Spectra of Cholesterol

Functional Group	Peak cm-1 Reported	Peak cm-1 Observed
O-H (stretching)	3550-3200	3421.72
C=C (Stretching)	1629	1371.39
C-H (Bending)	1460	1454.33
C=O (Stretching)	1730-1750	1710.86

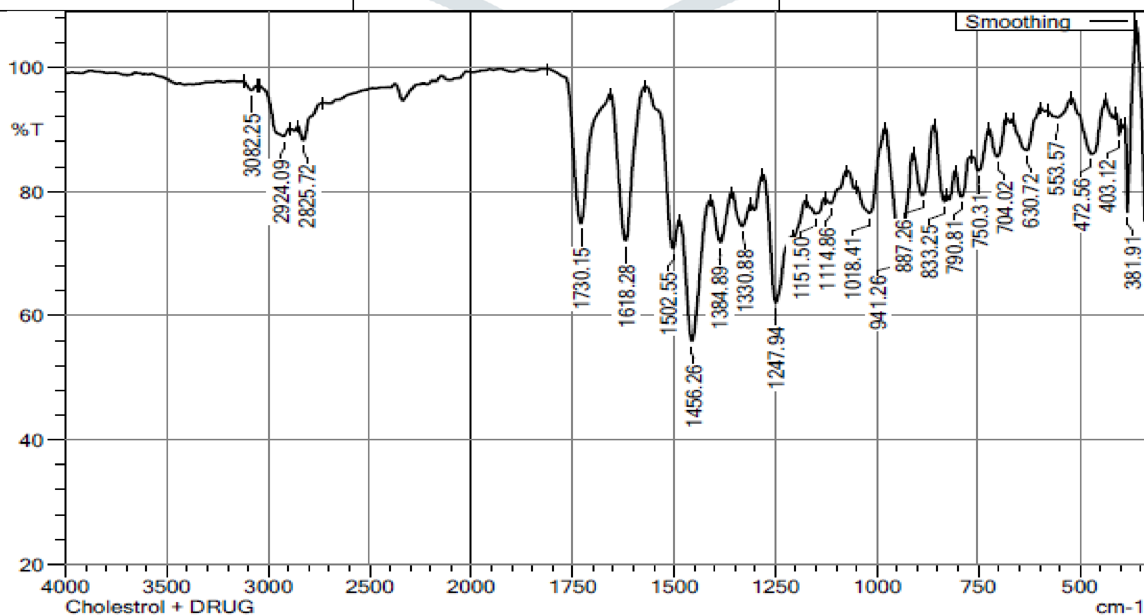


Fig 6: FTIR spectra of Cholesterol + Drug (Enrofloxacin)

Table 7: Interpretation of FTIR Spectra of Cholesterol + Drug (Enrofloxacin)

Functional Group	Peak cm-1 Reported	Peak cm-1 Observed
O-H (stretching)	3550-3200	3082.25
C=C (Stretching)	1629	1618.28
C=O (Stretching)	1730-1750	1730.15
C-F	1400-1000	1247.94

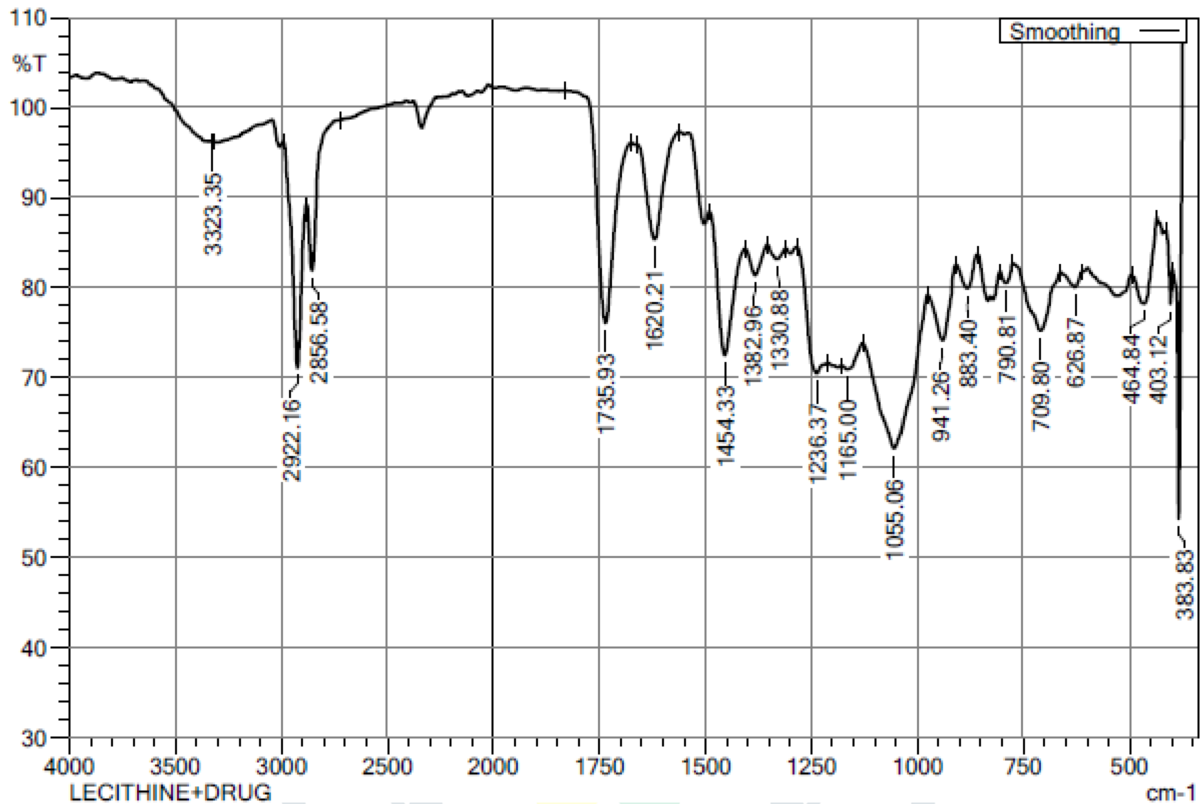


Fig 7: FTIR spectra of Lecithin + Drug (Enrofloxacin)

Table 8: Interpretation of FTIR Spectra of Lecithin + Drug (Enrofloxacin)

Functional Group	Peak cm-1 Reported	Peak cm-1 Observed
O-H (stretching)	3550-3200	3323.35
N-H (stretching)	3000-2800	2922.16
C=O (Stretching)	1730-1750	1735.93
C-N (stretching)	1250-1020	1055.06

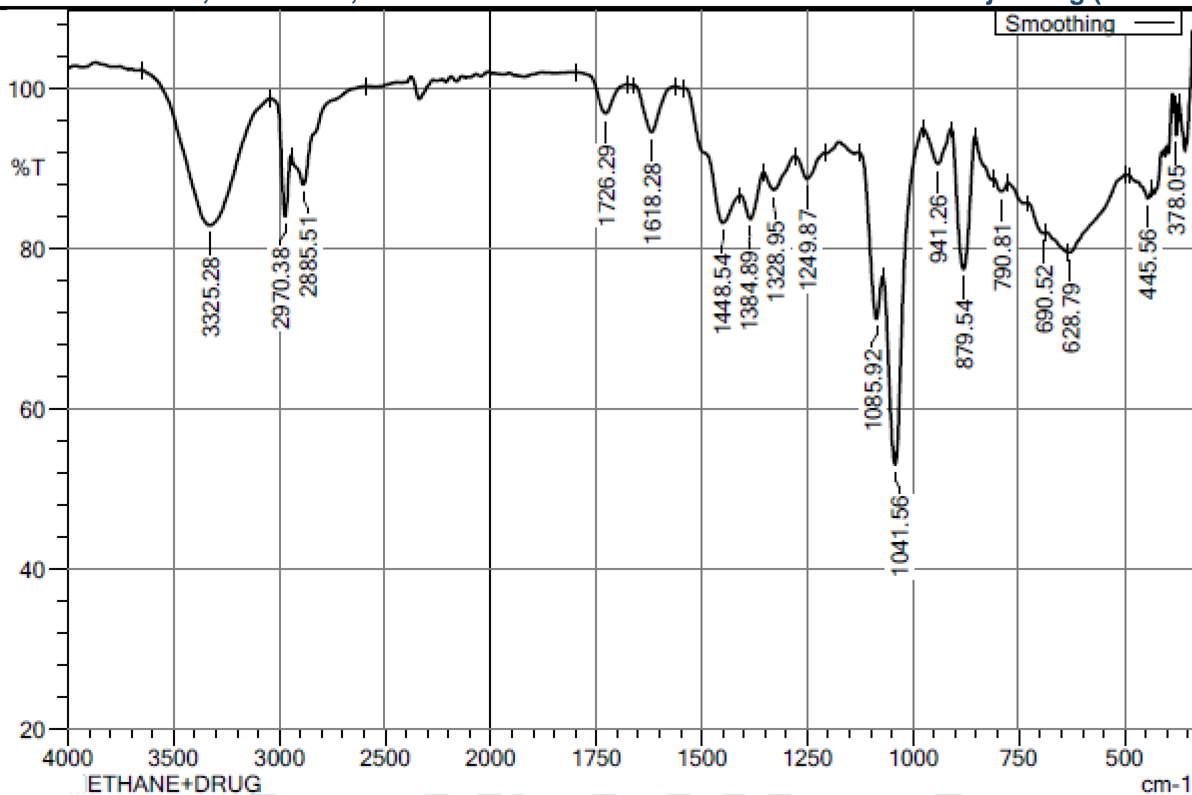


Fig 8: FTIR Spectra of Ethanol+ Drug (Enrofloxacin)

Table 9: Interpretation of FTIR Spectra of Ethanol+ Drug (Enrofloxacin)

Functional Group	Peak cm-1 Reported	Peak cm-1 Observed
O-H (stretching)	3550-3200	3325.28
N-H (stretching)	3000-2800	2970.38
C=O (Stretching)	1730-1750	1726.29
C-N (stretching)	1250-1020	1249.87

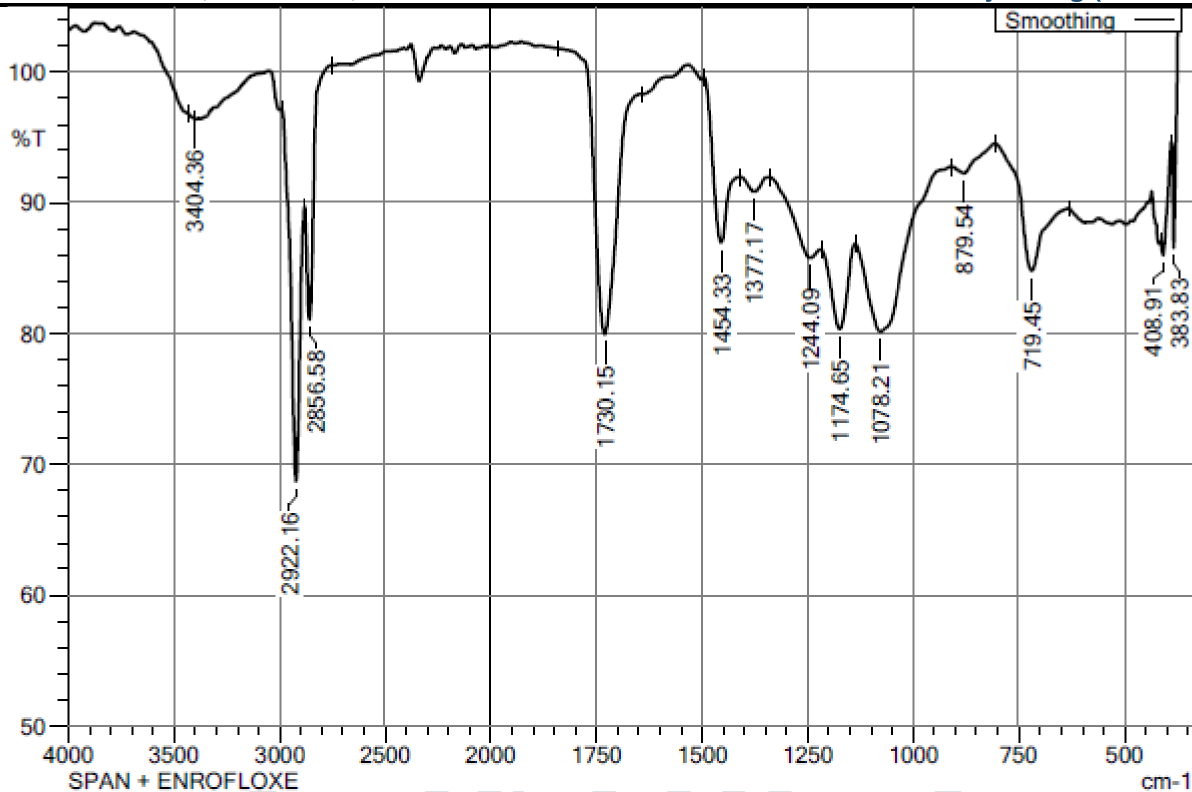


Fig 9: FTIR spectra of span+ Enrofloxacin

Table 10: Interpretation of FTIR Spectra of span+ Enrofloxacin

Functional Group	Peak cm-1 Reported	Peak cm-1 Observed
O-H (stretching)	3550-3200	3404.36
N-H (stretching)	3000-2800	2922.16
C=O (Stretching)	1730-1750	1730.15
C-F	1400-1000	1377.17

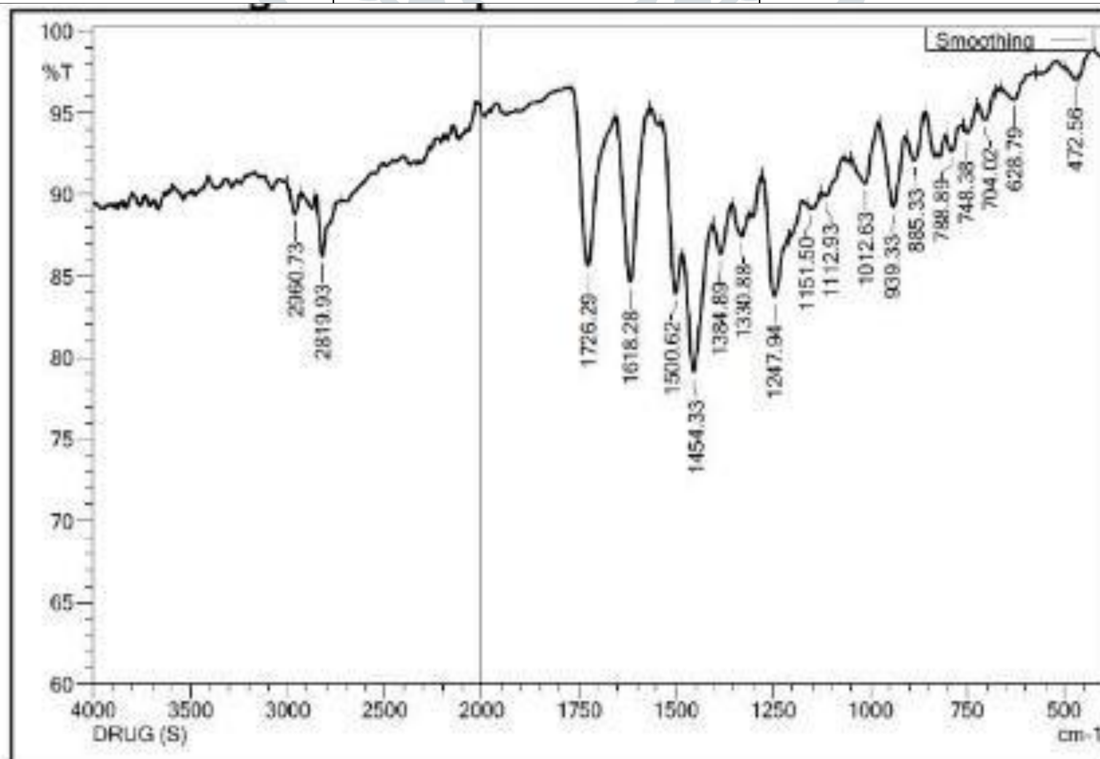


Fig 10: FTIR spectra of Enrofloxacin

Table 11: Interpretation of FTIR Spectra of Enrofloxacin

Functional Group	Peak cm-1 Reported	Peak cm-1 Observed
C=O (Stretching)	1730-1750	1726.29
C=C (Stretching)	1629	1618.26
C-H (Bending)	1460	1454.33
C-F	1400-1000	1247

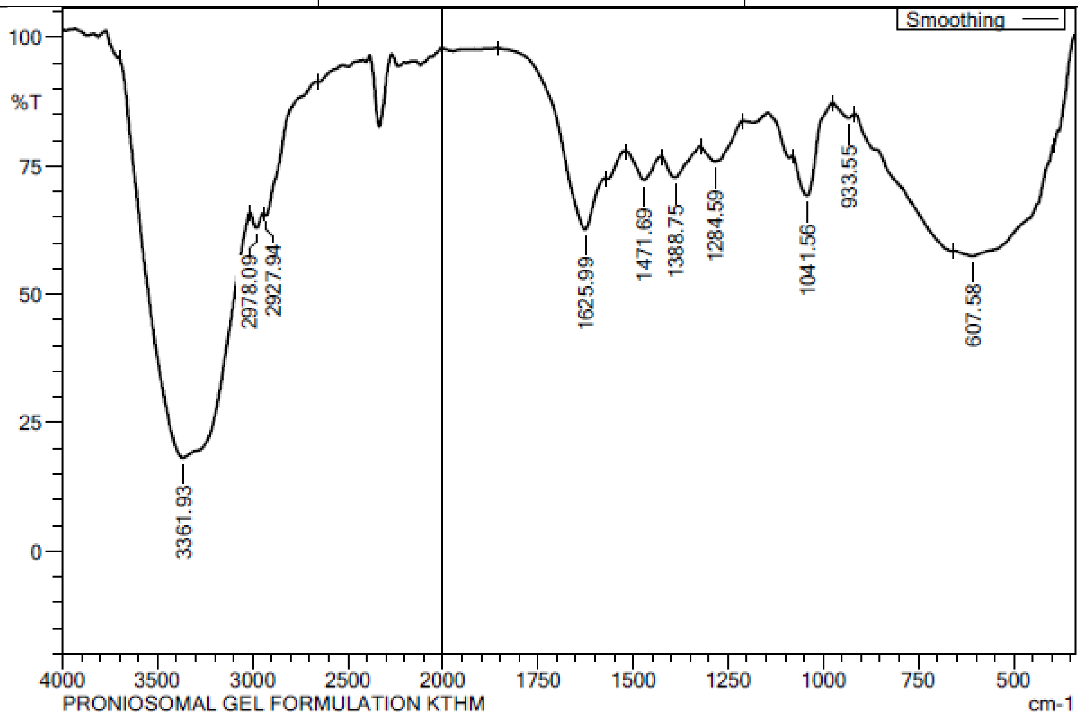


Fig 11: FTIR spectra of Proniosomal gel formulation

Table 12: Interpretation of FTIR Spectra of Proniosomal gel formulation

Functional Group	Peak cm-1 Reported	Peak cm-1 Observed
N-H (Stretching)	3400-3300	3361.93
O-H (Stretching)	3200-2700	2978.09, 2927.94
C=C (Stretching)	1629	1625.99
C-H (Bending)	1475	1471.69
C-F	1400-1000	1388.75

EVALUATION STUDIES

Optical Microscopic Examination

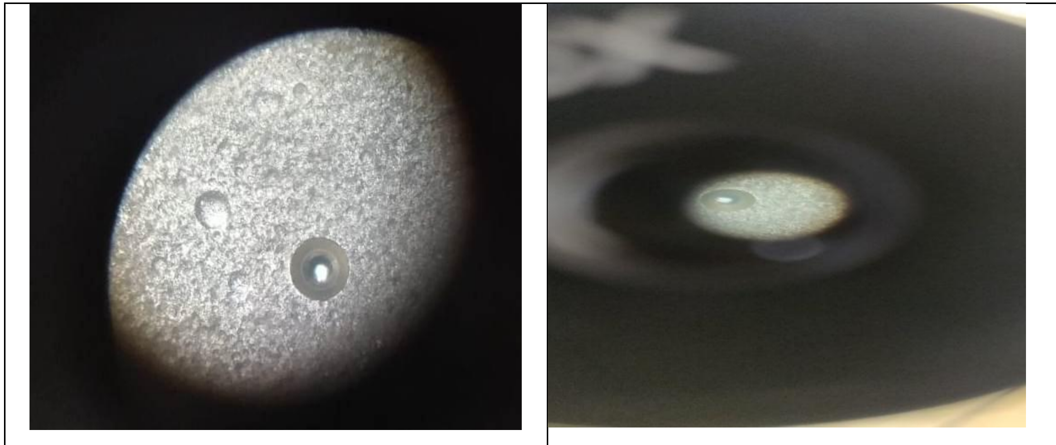


Fig 12: Microscopic Evaluation of Enrofloxacin Pro-niosomal gel

From the Optical Microscopic examination of Pronisomal gel of Enrofloxacin it was found themost of the vesicles has size less than 200 nm which confirms the formation of niosomes of Enrofloxacin which is entrapped in the vesicles.

Surface Morphology (SEM)

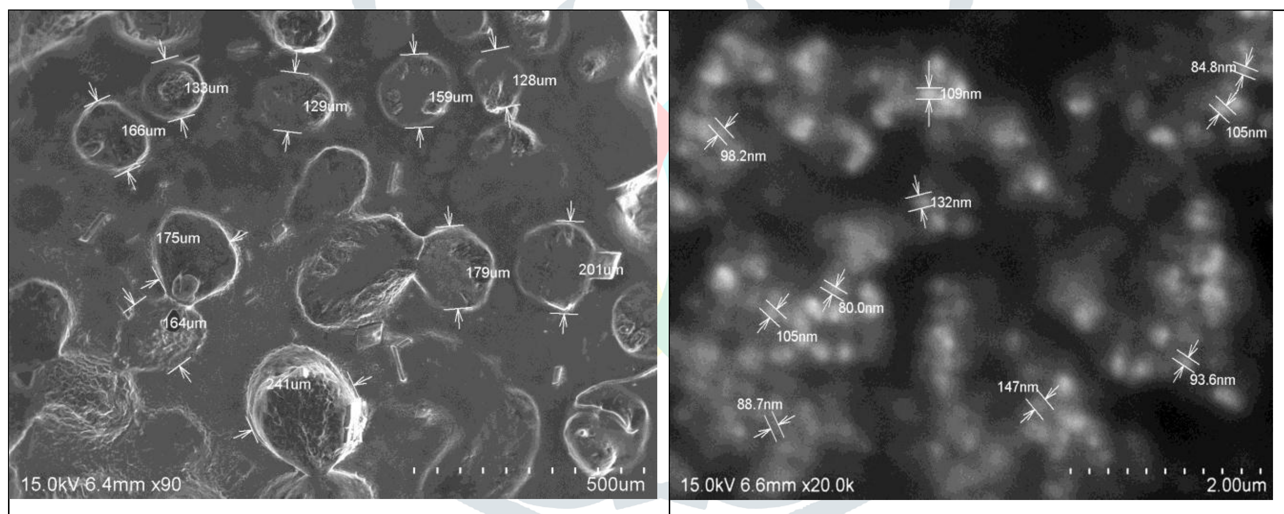


Fig 13: Surface morphology of Enrofloxacin Pro-niosomal gel

From the Scanning Electron Microscopy it was observed that, the F3 batch has maximum concentration of lecithin with glycerol solution which improves the surface characteristics of the proniosomal gel of Enrofloxacin.

9.4.3 Determination of pH

Table 13: Determination of pH of formulations

Formulation	pH
B1	6.71 ±0.93
B2	6.7 ± 0.70
B3	6.10 ± 0.5
B4	6.86 ± 0.13

All Formulation batches were examined for their pH measurement, the pH found to be in therange of 6.1 to 6.8 which is slightly acidic in nature.

9.5.4 Drug Content

Table 14: Determination of drug content of formulations

Formulation	Drug content
B1	98.12 ± 0.3 %
B2	96.10 ± 0.2 %
B3	97.30 ± 0.2%
B4	98.21 ± 0.2 %

The drug content of pro-niosomal gel was found to be in the range of 96.10 ± 0.2 % to 98.21±0.2 %.

9.5.6 Drug release

Table 15: % Cumulative release of Enrofloxacin

Sr. No.	Batch code	% Cumulative release
1	B1	80.31 ± 0.3
2	B2	70.67 ± 0.5
3	B3	88.04 ± 0.3
4	B4	70.00 ± 0.2

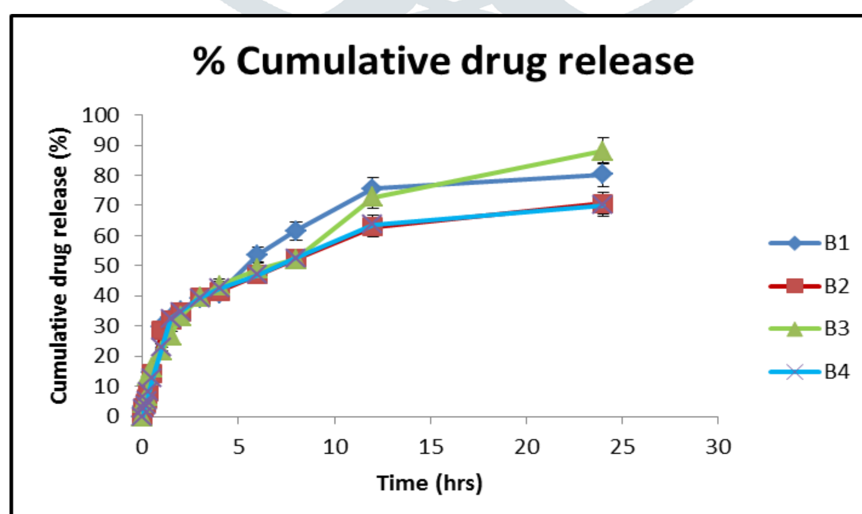
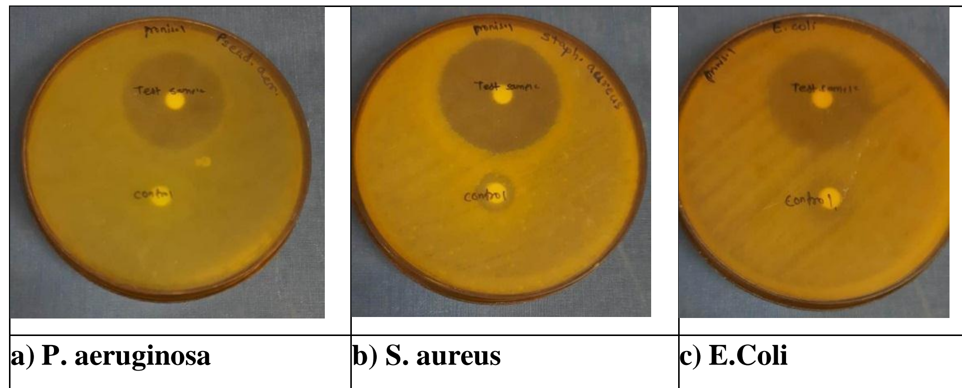


Fig 14: Percent cumulative release of batch B1-B4

Drug release from vesicles and drug dissolution ensured sustained reproducibility of rate and duration of drug release. From results it was found that that batch B3 showed highest drug release (88.04%) and the lowest release was from batch B4 (70%) This may be due

to its higher lecithin and span 60 content. The Span 60 surfactant was found to be prominent which helps to increase the solubility hence enhance dissolution of Enrofloxacin.

Antimicrobial study



a) P. aeruginosa	b) S. aureus	c) E.Coli

Fig 15: Antimicrobial study of Proniosomal gel

Table 16: Antimicrobial study of Proniosomal gel

Sr.	Sample	P. aeruginosa	S. sureus	E. coli
1	Proniosomal Control sample	No Zone	No Zone	No Zone
2	Proniosomal Test sample	31 mm	32mm	39mm

The antimicrobial study of the Proniosomal gel batch 3 was performed by using the Agar diffusion method on three different strains of bacteria. There is no zone of inhibition is observed in a control sample and a Zone of inhibition is observed for the test sample. The zone of inhibition in agar plate containing E - Coli shows 39 mm of the zone of inhibition, for Pseudomonas aeruginosa, it is 31 mm, and for Staphylococcus aureus it is 32 mm.

The study shows that the drug is able to diffuse in agar media from the proniosomal gel without affecting its ability to inhibit the growth of bacteria.

Spreadability test

All the proniosomal gel formulations showed good spreadability i.e. gel is easily spreadable.Spreadability of proniosomal gel was in the range of 3.6-2.3 g.cm/sec

Table17: Details of Spreadability test of proniosomal gel

Sr. No.	Batch code	Spreadability
1	B1	3.2 ± 0.021
2	B2	2.3 ± 0.014
3	B3	3.4 ± 0.014
4	B4	2.4 ± 0.016

9.6.8 Viscosity

Viscosity was found in the range of 8213 cps to 10477 cps

Table 18: Details of viscosity of proniosomal gel

Sr. No.	Batch code	Viscosity
1	B1	10477±0.12
2	B2	9541± 0.24
3	B3	8213±0.34
4	B4	9365±0.25

Zeta potential

The formulation B3 which was subjected to Zeta potential Analysis had a zeta value of -20.0 mV, which is a measure of net charge of proniosomal gel. High surface charge provides sufficient electrostatic repulsion between the vesicles which made them stable, by preventing aggregation. Negative charge leads to rapid blood clearance.

			Mean (mV):	Area (%)	Width (mV):
Zeta Potential (mV):	-26.1	Peak 1:	-26.1	100.0	9.71
Zeta SD (mV):	9.71	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm):	0.794	Peak 3:	0.00	0.0	0.00

Zeta potential out of range

Conductivity is out of range - check cell or sample

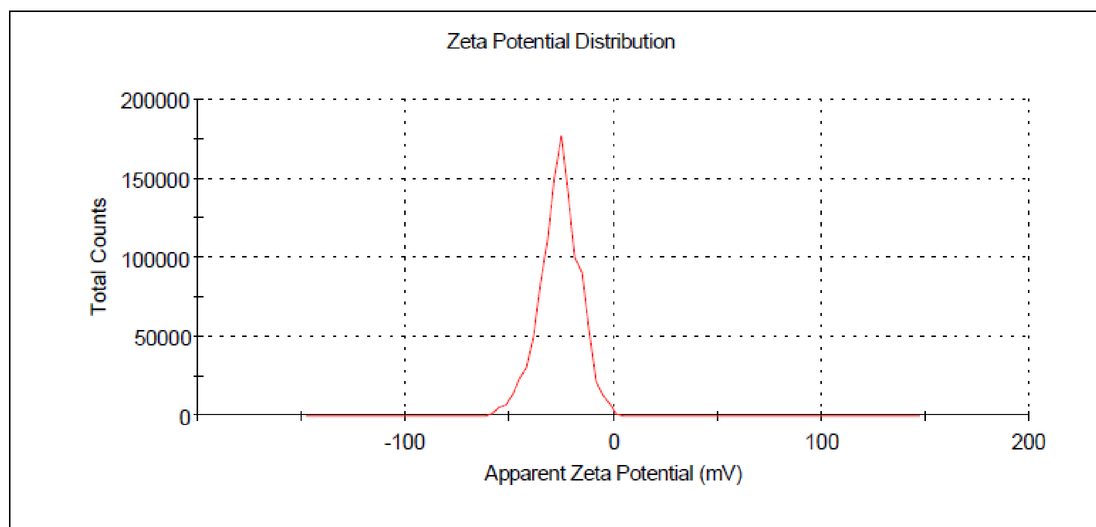


Fig 16: Zeta potential determination of Enrofloxacin proniosomal gel

			Size (d.nm):	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	126.1	Peak 1:	176.4	98.1	111.3
PdI:	0.274	Peak 2:	3569	1.9	1187
Intercept:	0.921	Peak 3:	0.000	0.0	0.000
Result quality :	Good				

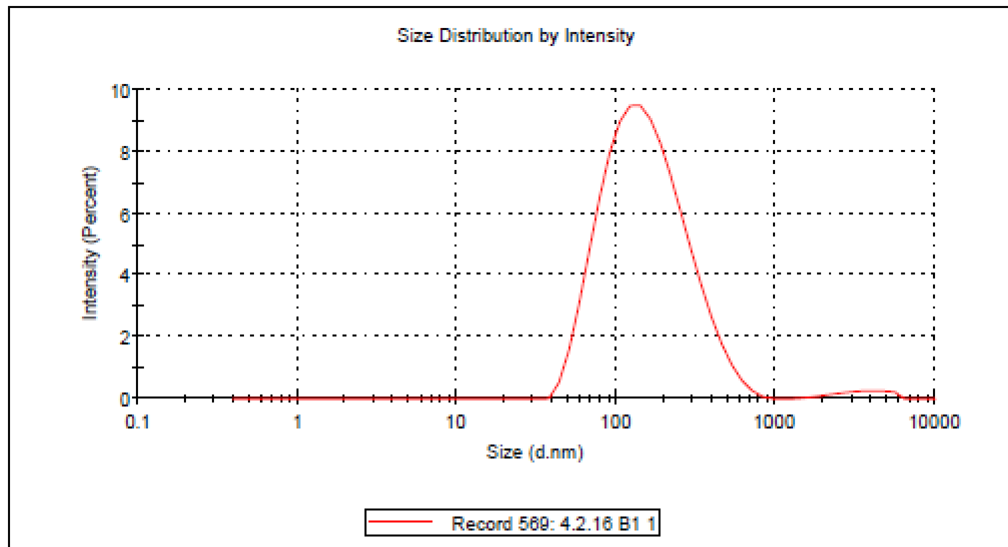


Fig 17: Particle size determination of Enrofloxacin proniosomal gel

Stability Study

In the present study, sta bility studies were performed on the B3 formulation. It was stored at 4°C ± 1°C for 6 months and observed for the change in %EE, average PS of the vesicles, and ZP. It was observed that there was no change in the physical appearance as there was neither change in the formulation consistency nor aggregation of vesicles. Furthermore, there was no significant change at P > 0.05 in EE%, PS, and ZP of fluconazole proniosomal gel after the storage for 6 months. This result showed the high stability and suitability of Enrofloxacin proniosomal gel.

Table 19: Stability data of Enrofloxacin Proniosomal gel formulation

Parameter	**% Entrapment**			**Particle Size**			**Zeta potential**		
Period	Initial	2 Months	6 Months	Initial	2 Months	6 Months	Initial	2 Months	6 Months
B3	97.30 ± 0.2%	96.86 ± 0.2%	95.23 ± 0.2%	126.1± 0.34	125.5±0.26	125 ± 0.21	-26.1	-26.1	-25.20

10.0 Conclusion:

The prepared proniosomes gel was evaluated for vesicle shape and drug content, in vitro drug release study and stability. The presence of cholesterol made the proniosomes more stable with high drug entrapment efficiency and retention properties. Formulation B3 showed highest drug release of 88.04 % after 24 hrs. Proniosomal gel was evaluated for physical appearance, clarity, pH, spreadability, viscosity, drug content and in occlusion studies it was observed that proniosomal gel reduces more water loss.

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