FORMULATION AND COMPARATIVE EVALUATION OF PRONIOSOME HYDROCORTISONE GEL WITH MARKETED FORMULATION

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CERTIFICATE

This is to certify that the dissertation work entitled "**FORMULATION AND COMPARATIVE EVALUATION OF PRONIOSOME HYDROCORTISONE GEL WITH MARKETED FORMULATION**" submitted by **Mr. C. Praveen,** is a bonafide work carried out by the candidate under the guidance of **Mr. V. SANKAR M. Pharm.,** and submitted to the Tamil Nadu Dr. M.G.R. Medical University, Chennai, in partial fulfillment for the Degree of **Master of Pharmacy** in **Pharmaceutics** at the Department of Pharmaceutics, PSG College of Pharmacy, Coimbatore, during the academic year 2008-2009.

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DECLARATION

I do hereby declare that the dissertation work entitled **"FORMULATION AND COMPARATIVE EVALUATION OF PRONIOSOME HYDROCORTISONE GEL WITH MARKETED FORMULATION"** submitted to the Tamil Nadu Dr. M.G.R. Medical University, Chennai, in partial fulfillment for the Degree of **Master of Pharmacy** in **Pharmaceutics,** was done by me under the guidance of **Mr. V. SANKAR, M. Pharm.,** at the Department of Pharmaceutics, PSG College of Pharmacy, Coimbatore, during the academic year 2008-2009.

C. PRAVEEN

EVALUATION CERTIFICATE

This is to certify that the dissertation work entitled **"FORMULATION AND COMPARATIVE EVALUATION OF PRONIOSOME HYDROCORTISONE GEL WITH MARKETED FORMULATION"** submitted by **Mr. C. Praveen,** University Reg. No.26074658 to the Tamil Nadu Dr. M.G.R. Medical University, Chennai in partial fulfillment for the Degree of **Master of Pharmacy** in **Pharmaceutics** is a bonafide work carried out by the candidate at the Department of Pharmaceutics, PSG College of Pharmacy, Coimbatore and was evaluated by us during the academic year 2008-2009.

Examination Center: PSG College of Pharmacy, Coimbatore.

Date:

Internal Examiner **External Examiner**

Convener of Examinations

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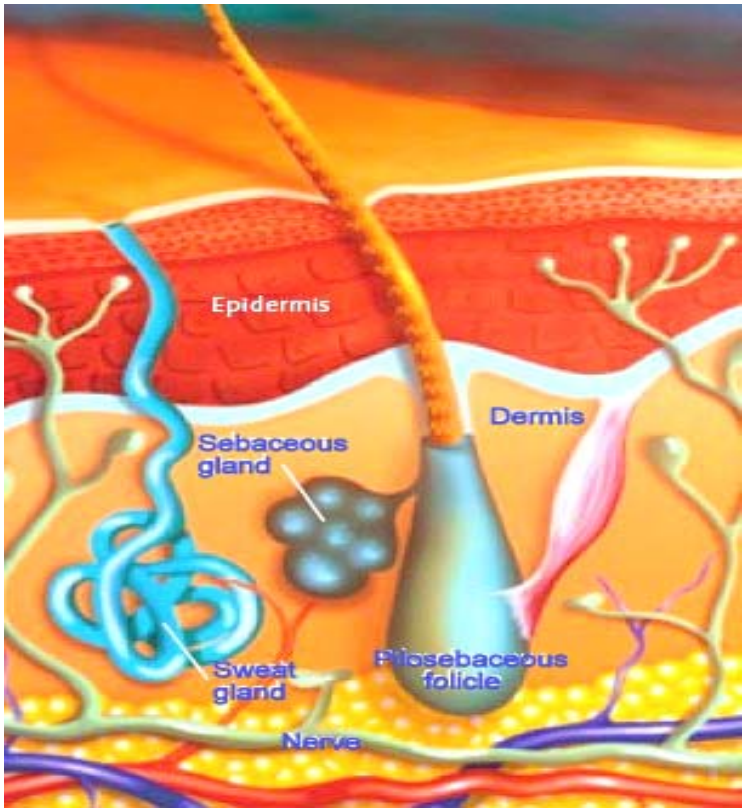
INTRODUCTION

Dermal and transdermal delivery

The skin covers 1,4,5,9,13a total surface area of approximately 1.8m2 and provides the contact between the human skin and its external environment. This large and outermost layer of the human body is easily accessible and hence attractive as a non-invasive delivery route for selected drug compounds. Dermal and Transdermal drug delivery can have many advantages as compared to other routes of drug administration. (22)

Figure 1: **Skin Structure**



Dermal drug delivery is the topical application of drugs to the skin in the treatment of localized skin diseases. The advantage of dermal drug delivery is that high concentration of drugs can be localized at the site of action, reducing the systemic drug levels and therefore also reducing the systemic side effects (22). Transdermal drug delivery, on the other hand uses the skin as an alternative route for the delivery of systemically acting drugs. This drug delivery route for systemic therapy can have several advantages as compared to conventional oral drug administration. First of all, it circumvents the variables that could influence gastrointestinal absorption. Such as pH, food intake and gastrointestinal motility. Secondly it circumvents the first pass hepatic metabolism and is therefore suitable for drugs with a low bio-availability. Thirdly transdermal drug delivery can give a constant, controlled drug input. This would reduce the need for frequent drug intake, especially of drugs with a short biological half-life. Furthermore, variations in drug plasma levels can be avoided, reducing the side effects in particular of drugs with a narrow therapeutic window. Finally, transdermal drug delivery is easy and painless, which in turn will increase patient compliance.

Despite these advantages of the skin as a site of drug delivery, only less than 10 drugs are currently available in the market as a transdermal delivery system. These transdermal delivery systems contain drugs including fentanyl, nitro-glycerine, scopolamine, clonidine, nicotine, estradiol, and testosterone. By far the most important reason for such few transdermal delivery systems is the fact that human skin is highly impermeable for most drug compounds. Previously, many attempts have been made and many methods have been employed in order to improve drug delivery across the intact human skin (22).

Routes of drug penetration:

Drugs applied to the skin surface can serve two purposes. Dermal delivery is aimed at treating localized skin diseases. In this case, it is required that the drug penetrates the outer skin layers it reach its site of action within the skin, with little or no systemic uptake. On the other hand, transdermal delivery systems are designed to obtain therapeutic systemic blood levels. Hence, it is required that the drug reaches the dermal or transdermal drug delivery, the drug has to cross the outer layer of the skin, the stratum corneum. Since this layer is the main barrier of the skin, transport across the stratum corneum is the rate-limiting step in both dermal and transdermal; drug delivery.

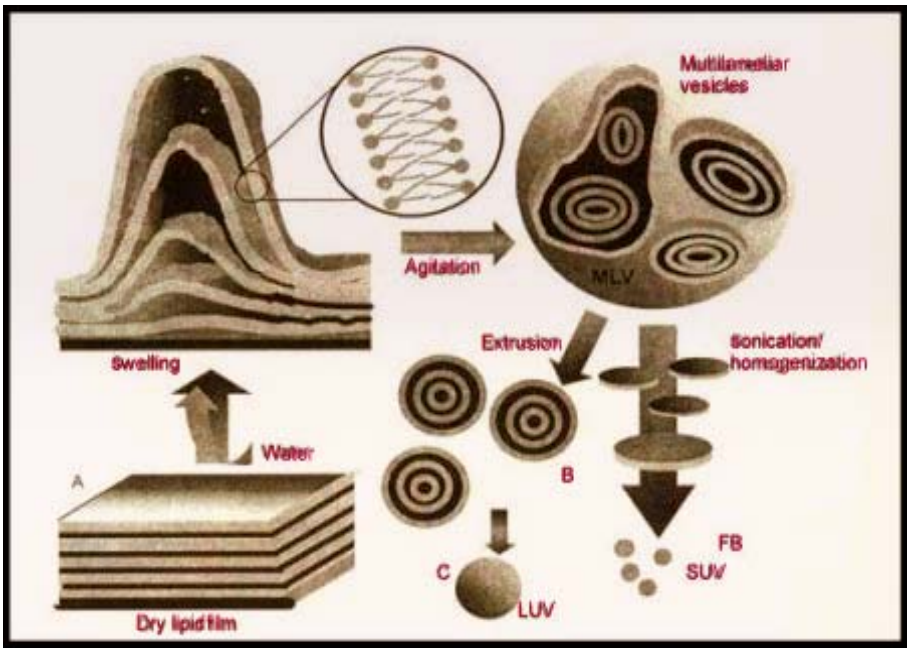
There are two potential pathways for a molecule to across the stratum corneum: (a) the transappendagel route and (b) the transepidermal route (22). The transappendagel route involves transport of drugs via the sweat glands and the pilosebaceous units. This route bypasses the intact stratum corneum and is therefore also known as a “shunt”

Route. The transapendageal route, however, is not considered to be very significant, as the appendages only contribute 0.1% to the total surface area of the skin. Hence, transport of most of the drug compounds occurs via the transepidermal route, which involves transport across the intact contagious stratum corneum. Two path ways through the intact stratum corneum can be distinguished. (a) The intercellular route, crossing through the corneocytes and the intercytes and (b) The intercellular route has been thought to be the route of preference for most drug molecules. Hence, many techniques have been aimed to disrupt and weaken the highly organized intercellular lipid lamellae in an attempt to enhance drug transport across the intact skin.

Vesicle as skin delivery systems:

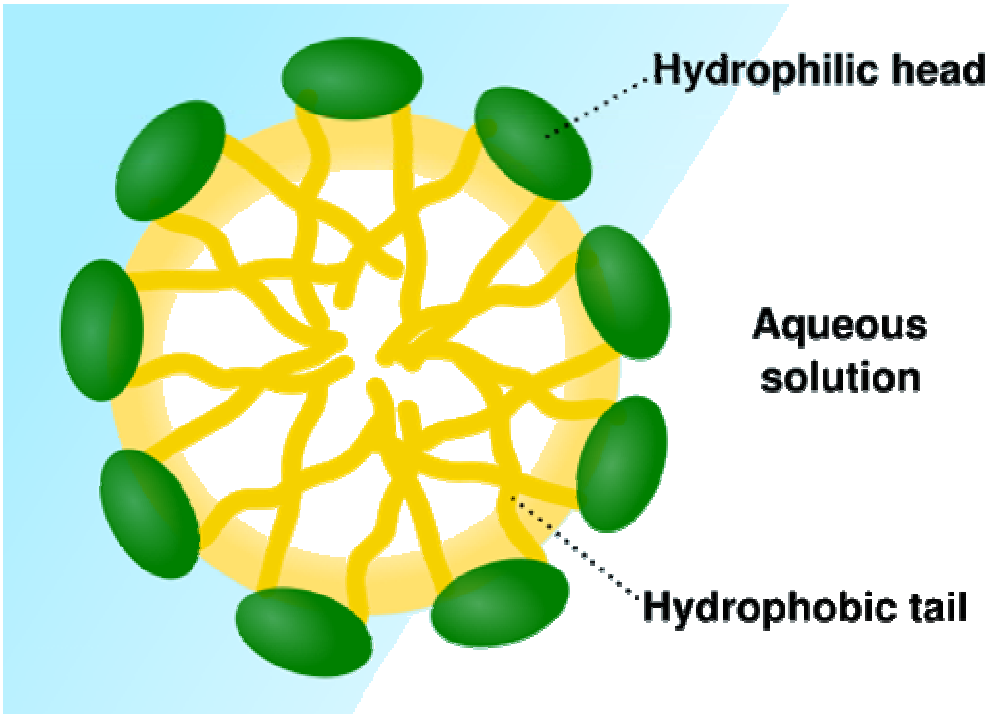
Vesicular Drug Delivery System is a novel approach having small spherical vesicles in which one or more aqueous compartments are completely enclosed by molecules that have hydrophilic and hydrophobic functionality such as phospholipids and cholesterol. These are varying in properties like composition, size, surface charge and method of preparation. They can be formed as single lipid bilayer or in multiple bilayer.These vesicular novel drug delivery systems are as a tool for the Dermal and Transdermal drug delivery.

Figure 2: **Vesicle Formation**



One of the methods to enhance drug transport across the skin is the use of vesicles. Vesicles are hollow colloidal particles, consisting of amphilic molecules. These amphiphilic molecules consist of a polar hydrophilic head group and a polar hydrophobic tail. Due to their amphiphilic properties, these molecules can form in the presence of excess of water one (uni lamellar vesicles/0 or more (multilamellar vesicles) concentric bilayer that surround an equal number of aqueous compartments. Both water-soluble and water-insoluble drugs can be entrapped into the vesicles. Hydrophilic drugs can be entrapped into the internal aqueous compartment. While lipophillic drugs can be entrapped in the vesicle bilayer or partition between the bilayer and the aqueous phase (22).

Figure 3**:** **Vesicle structure**



A wide variation of lipids and surfactants can be used to prepare vesicles. Most commonly, the vesicles are composed of phospholipids or non-ionic surfactants. These are referred to as liposomes and niosomes or non-ionic surfactants vesicles. The composition of the vesicles influences their physicochemical characteristics such as size, charge, phase state, lamellarity, and bilayer elasticity. These physicochemical characteristics in turn have a significant effect on the behaviour of the vesicles and hence also on their effectiveness as a drug delivery system

The rationale for using vesicles in dermal and transdermal drug delivery is manifold:

- Vesicles might act as drug carriers to deliver entrapped drug molecules into or across the skin.
- Vesicles might act as penetration enhancers owing to the penetration of the individual lipid components into the stratum corneum and subsequently the alteration of the intercellular lipid lamellae within the skin layer.
- Vesicles might serve as a depot for sustained release of dermal active compounds.
- Vesicles might serve as a rate-limiting membrane barrier for the modulation of systemic absorption, hence providing a controlled transdermal delivery system.
- The individual components of vesicles might have additional useful properties.
- Vesicles biodegradable, minimally toxic, and relatively nonimmunogenic.

To pursue optimal drug action, functional molecules could be transported by a carrier to the site of action and released to perform their task. Non-ionic surfactant vesicles known as niosomes are microscopic lamellar structures formed on admixture of a non-ionic surfactant, cholesterol and dicetyl phosphate with subsequent hydration in aqueous media. Niosomes are unilamellar or multilamellar vesicles of entrapping hydrophilic and hydrophobic solutes. These Niosomes can entrap solutes are quite stable, and require no special conditions and lack of many disadvantages associated with liposomes.

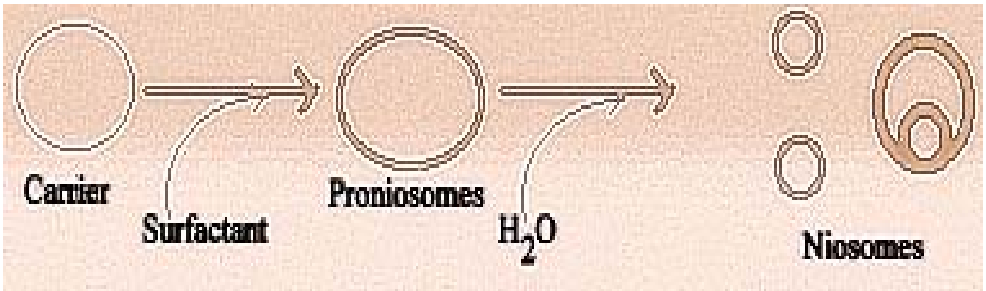
These Niosomes exhibit good chemical stability during storage, but there may be problems of physical instability in niosome dispersions, and thus limiting the shelf life of the dispersion.

PRONIOSOMES AS A VESICULAR DRUG DELIVERY SYSTEM

A novel approach to minimize the physical instability of niosomes is **Proniosomes** - derived niosomes for the delivery of poorly soluble drugs. This is based on liposome production method. These Proniosomes consist of maltodextrin powder coated with surfactant or a surfactant/drug mixture to yield a dry powder. Upon addition of hot water and brief agitation, the maltodextrin dissolves and the surfactant forms a suspension of multilamellar vesicles (niosomes) containing the poorly soluble drug. The niosomes slowly release drug in to solution (3). The proniosome powder can also be mixed with hydrogel powder. Adding hot water to the mixed powders allow formation of a hydrogel in which niosomes spontaneously form. The niosome-containing hydrogel can be formed as a gel that will degrade and release intact niosomes or as a stable gel, which slowly releases the drug from niosomes that remain in side the gel matrix.

Proniosomes offer a versatile vesicle drug delivery concept with potential for delivery of drugs via transdermal route. This would be possible if Proniosomes form niosomes upon hydration with water from skin following topical application under occlusive conditions (1).

Figure 4: **Proniosomes Methodology**



Proniosomes minimizes problems of niosomes physical stability such as aggregation, fusion and leaking and provide additional convenience in transportation, storage and dosing. Transdermal therapeutic system has generated an interest as this system provides the considerable advantage of a non-invasive parenteral route for drug therapy, avoidance of first pass gut and hepatic metabolism, decreased side effects and relative ease of drug input termination in problematic cases (2).

Proniosomes are mostly used and interested in topical formulations. While using topical formulations (creams, gel) less potent drugs are used for the prevention of atrophy (skin thin and fragile).

Dermatitis:

Dermatitis is a blanket term meaning any "inflammation of the skin" (e.g. rashes, etc.) there are several different types of dermatitis. The different kinds usually have in common an allergic reaction to specific allergens. The term may be used to refer to eczema, which is also known as dermatitis eczema or eczematous dermatitis. A diagnosis of eczema often implies childhood or dermatitis, but without proper context, it means nothing more than a "rash"

Types of Dermatitis

Spongiotic dermatitis:

This pattern of skin reaction includes many other subtypes - irritant dermatitis, seborrheic dermatitis, atopic dermatitis, allergic contact dermatitis, thermal induced dermatitis, and drug induced dermatitis.

Childhood eczema:

Also known as atopic dermatitis or atopic eczema. This can be immunologic mediated dermatitis. In dogs, it is frequently associated with airborne allergen or food allergen. In human, it can be associated with food allergy. However, most cases of human atopic dermatitis do not have an associated allergy. It is believed that the human skin is lacking in a protective lipid agent, making the skin itchy and prone to scratching.

Seborrhoeic dermatitis:

Seborrhoeic dermatitis is also known as dandruff. A rash of the scalp, face, and occasionally chest and groin. It is associated with a common yeast, Pityrosporum. It is treated with either an antiinflammatory or an antifungal agent, or both.

Psoriasis:

Psoriasis or psoriatic dermatitis is a pattern of dermatitis with distinct relationship to a defined entity, psoriasis. It can be familial, and is associated with arthritis.

Dyshidrotic dermatitis:

Dyshidrotic dermatitis is also known as Pompholyx. It is a pattern of spongiotic dermatitis presenting as small fluid filled or pus filled bumps on the hands and feet. The cause is unknown, but it has been highly associated with contact dermatitis (see Allergic Contact Dermatitis). Some cases are due to a food intolerance to nickel.

Urticaria:

Urticaria is also known as hives and is a pattern of allergic dermatitis characterized by transient wheals or welts. The definition requires that the lesions shifts, moves, or changes within 24 hours. They should not remain static, or the diagnosis of urticaria can not be rendered.

Vesicular or bullous dermatitis:

This can be caused by drug reaction, or auto immune diseases. Examples includes Steven Johnson Syndrome, bullous erythema multiforme, bullous pemphigoid, and pemphigus vulgaris. Athlete foot fungus can also cause bullous dermatitis of the foot.

Papular urticaria:

A pattern of dermatitis often presenting after insect bite reactions. Flea bite dermatitis are often grouped around the ankles in a walking adult. In a crawling infant, it can be anywhere on the body.

Eczema is a form of dermatitis, or inflammation of the epidermis. The term *eczema* is broadly applied to a range of persistent skin conditions. These include dryness and recurring skin rashes which are characterized by one or more of these symptoms: redness, skin edema (swelling), itching and dryness, crusting, flaking, blistering, cracking, oozing, or bleeding. Areas of temporary skin discoloration may appear and are sometimes due to healed lesions, although scarring is rare. In contrast to psoriasis, eczema is often likely to be found on the flexor aspect of joints.

Eczema

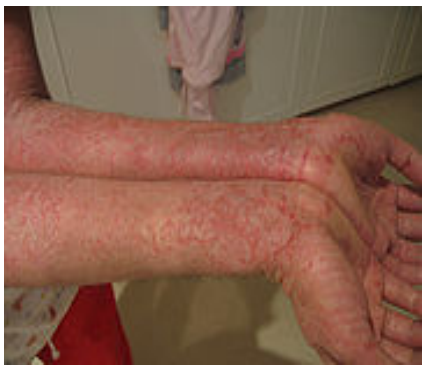


Figure - 5

Medications:

Dermatitis is often treated by glucocorticoid (a corticosteroid) ointments, creams or lotions. They do not cure eczema, but are highly effective in controlling or suppressing symptoms in most cases. Dermatitis is often treated by glucocorticoid (a corticosteroid) ointments, creams or lotions. They do not cure eczema, but are highly effective in controlling or suppressing symptoms in most cases.

For mild-moderate eczema a weak steroid may be used (e.g. hydrocortisone or desonide), whilst more severe cases require a higher-potency steroid (e.g. clobetasol propionate, fluocinonide). Medium-potency corticosteroids such as clobetasone butyrate (Eumovate), Betamethasone Valerate (Betnovate) or triamcinolone are also available. Generally medical practitioners will prescribe the less potent ones first before trying the more potent ones.

Prolonged use of topical corticosteroids is thought to increase the risk of possible side effects, the most common of which is the skin becoming thin and fragile (atrophy).[6] Because of this, if used on the face or other delicate skin, only a low-strength steroid should be used.

LITERATURE REVIEW

- **Yi-Hung Tsai a., et al**., investigated the estradiol skin permeation from various proniosome gel formulations across excised rat skin in in-vitro studies. The encapsulation efficiency and size of niosomal vesicles formed from Proniosomes upon hydration were also characterized. The encapsulation (%) of Proniosomes with Span surfactants showed a very high value of ¥100%. Proniosomes with Span 40 and Span 60 increased the permeation of estradiol across skin. Both penetration enhancer effect of non-ionic surfactant and vesicle-skin interaction may contribute to the mechanisms for Proniosomes to enhance estradiol permeation. Niosome suspension (diluted Proniosomal formulations) and proniosome gel showed different behavior in modulating transdermal delivery of estradiol across skin. Presence or absence of cholesterol in the lipid bilayers of vesicles did not reveal difference in encapsulation and permeation of the associated estradiol. The types and contents of non-ionic surfactant in Proniosomes are important factors affecting the efficiency of transdermal estradiol delivery.
- **Ankur Gupta., et al.,** studied on development of a proniosomal carrier system for captopril for the treatment of hypertension that is capable of efficiently delivering entrapped drug over an extendedperiod of time. The potential of proniosomes as a transdermal drug delivery system for captopril was investigated by encapsulating the drug in various formulations of proniosomal gel composed of various ratios of sorbitan fatty acid esters, cholesterol, lecithin prepared by coacervation-phase

separation method. The formulated systems were characterized in vitro for size, vesicle count, drug entrapment, drug release profiles and vesicular stability at different storage conditions. Stability studies for proniosomal gel were carried out for 4 weeks. The method of proniosome loading resulted in an encapsulation yield of 66.7 - 78.7%. Proniosomes were characterised by transmission electron microscopy. In vitro studies showed prolonged release of entrapped captopril. At refrigerated conditions, higher drug retention was observed. It is evident from this study that proniosomes are a promising prolonged delivery system for captopril and have reasonably good stability characteristics.

- **Almira I. Blazek-Welsh and David G. Rhodes., et al.,** investigated on moaltodextrin Proniosomes. The Niosomes are nonionic surfactant Vesicles that have potential applications in the delivery of hydrophobic or amphiphilic drugs. Our lab developed Proniosomes, a dry formulation using a sorbitol carrier coated with nonionic surfactant, which can be used to produce niosomes within minutes by the addition of hot water followed by agitation. The sorbitol carrier in the original proniosomes was soluble in the solvent used to deposit surfactant, so preparation was tedious and the dissolved sorbitol interfered with the encapsulation of one model drug. A novel method is reported here for rapid preparation of proniosomes with a wide range of surfactant loading. A slurry method has been developed to produce proniosomes using maltodextrin as the carrier. The time required to produce proniosomes by this simple method is independent of the ratio of surfactant solution to carrier material and appears to be scalable. The flexibility of the proniosome preparation method would allow for the

optimization of drug encapsulation in the final formulation based on the type and amount of maltodextrin. This formulation of proniosomes is a practical and simple method of producing niosomes at the point of use for drug delivery.

- **Jain. N.K., et al.,** investigated on development of proniosome based transdermal drug delivery system of levonorgestrel (LN) and extensively characterized both in vitro and in vivo. The proniosomal structure was liquid crystalline-compact niosomes hybrid which could be converted into niosomes upon hydration. The system was evaluated in vitro for drug loading, rate of hydration (spontaneity), vesicle size, polydispersity, entrapment efficiency and drug diffusion across rat skin. The effect of composition of formulation, amount of drug, type of Spans, alcohols and sonication time on transdermal permeation profile was observed. The stability studies were performed at 48C and at room temperature. The biological assay for progestational activity included endometrial assay and inhibition with the formation of corpora lutea. The study demonstrated the utility of proniosomal transdermal patch bearing levonorgestrel for effective contraception.
- **Ibrahim A. Alsarra., et al.,** studied whether Niosomes are nonionic surfactant vesicles that have potential applications in the delivery of hydrophobic and hydrophilic drugs. Permeation of a potent nonsteroidal anti-inflammatory, ketorolac, across excised rabbit skin from various proniosome gel formulations was investigated using Franz diffusion cells. Each of the prepared proniosomes significantly improved drug permeation and reduced the lag time (P! 0.05). Proniosomes prepared with Span 60

provided a higher ketorolac flux across the skin than did those prepared with Tween 20 (7- and 4-fold the control, respectively). A change in the cholesterol content did not affect the efficiency of the proniosomes, and the reduction in the lecithin content did not significantly decrease the flux (PO0.05). The encapsulation efficiency and size of niosomal vesicles formed by proniosome hydration were also characterized by specific high performance liquid chromatography method and scanning electron microscopy. Each of the prepared niosomes achieved about 99% drug encapsulation. Vesicle size was markedly dependent on the composition of the proniosomal formulations. Proniosomes may be a promising carrier for ketorolac and other drugs, especially due to their simple production and facile up

- **Ajay B. Solanki., et al.,** investigate the combined influence of 3 independent variables in the preparation of piroxicam proniosomes by the slurry method. A 3-factor, 3-level Box-Behnken design was used to derive a secondorder polynomial equation and construct contour plots to predict responses. The independent variables selected were molar ratio of Span 60: cholesterol (X1), surfactant loading (X2), and amount of drug (X3). Fifteen batches were prepared by the slurry method and evaluated for percentage drug entrapment (PDE) and vesicle size. The transformed values of the independent variables and the PDE (dependent variable) were subjected to multiple regressions to establish a full-model second-order polynomial equation. F was calculated to confirm the omission of insignificant terms from the full-model equation to derive a reduced-model polynomial equation to predict the PDE of proniosome-derived niosomes.

Contour plots were constructed to show the effects of X1, X2 and X3 on the PDE. A model was validated for accurate prediction of the PDE by performing checkpoint analysis. The computer optimization process and contour plots predicted the levels of independent variables X1, X2, and X3 (0, -0.158 and –0.158 respectively), for maximized response of PDE with constraints on vesicle size. The Box-Behnken design demonstrated the role of the derived equation and contour plots in predicting the values of dependent variables for the preparation and optimization of piroxicam proniosomes.

- **El-laithy. H.M., et al.,** investigated on development of Novel approach for the preparation of controlled release proniosome-derived niosomes, using sucrose stearate as non-ionic biocompatible surfactants for the nebulisable delivery of cromolyn sodium. Conventional niosomes were prepared by a reverse phase evaporation method followed by the preparation of proniosomes by spraying the optimized surfactant–lipid mixture of sucrose stearate, cholesterol and stearylamine in 7:3:0.3 molar ratios onto the surface of spray dried lactose powder. Proniosome-derived niosomes were obtained by hydrating proniosomes with 0.9% saline at 50 ◦C and mixing for approximately 2 min. All vesicles were evaluated for their particle size, morphological characteristics, entrapment efficiency, *in vitro* drug release, nebulisation efficiency and physical stability at 2–8 ◦C. In addition, coating carrier surface with the surfactant–lipid mixture, during preparation of proniosomes, resulted in smaller, free flowing, homogenous and smooth vesicles with high drug entrapment efficiency. Compared to a standard drug solution, a successful retardation of the drug release rate was

achieved with the proniosome-derived niosomes, where the *t*50% value of the release profile was 18.1 h compared to 1.8 h. Moreover, high nebulisation efficiency percentage and good physical stability were also achieved. The results are very encouraging and offer an alternative approach to minimize the problems associated with conventional niosomes like degradation, sedimentation, aggregation and fusion

- **Vyas. S.P., et al.,** studied on topical drug delivery through vesicular systems. DNA vaccines are capable of eliciting both humoral as well as cellular immune responses. Liposomes have been widely employed for DNA delivery through topical route; however, they suffer from certain drawbacks like higher cost and instability. In present study, non-ionic surfactant based vesicles (niosomes) for topical DNA deliveries have been developed. DNA encoding hepatitisB surface antigen (HBsAg) was encapsulated in niosomes. Niosomes composed of span 85 and cholesterol as constitutive lipids were prepared by reverse phase evaporation method. Prepared niosomes were characterized for their size, shape and entrapment efficiency. The immune stimulating activity was studied by measuring serum anti-HBsAg titer and cyokines level (IL-2 and IFN-_) following topical application of niosomes in Balb/c mice and results were compared with naked DNA and liposomes encapsulated DNAapplied topically as well as naked DNA and pure recombinant HBsAgadministered intramuscularly. It was observed that topical niosomes elicited a comparable serum antibody titer and endogenous cytokines levels as compared to intramuscular recombinant HBsAg and topical liposomes. The study signifies the potential of niosomes as DNA vaccine carriers for

effective topical immunization. The proposed system is simple, stable and cost effective compared to liposomes.

- **Suresh P. Vyasa, et al.,** study results shows that, Non-invasive vaccine delivery is a top priority for public health agencies because conventional immunization practices are unsafe and associated with numerous limitations. Recently, the skin has emerged as a potential alternative route for non-invasive delivery of vaccine. Topical immunization (TI), introduction of antigen through topical application onto the intact skin, has many practical merits compared to injectable routes of administration. One of the possibilities for increasing the penetration of bioactives through the skin is the use of vesicular systems. Specially designed lipid vesicles are attracting intense attention and can be used for non-invasive antigen delivery. In the present study, elastic vesicle transfersomes, non-ionic surfactant vesicles (niosomes) and liposomes were used to study their relative potential in non-invasive delivery of tetanus toxoid (TT). Transfersomes, niosomes and liposomes were prepared and characterized for shape, size and entrapment efficiency. These vesicles were extruded through polycarbonate filter (50-nm pore size) to assess the elasticity of the vesicles. The immune stimulating activity of transfersomes, niosomes and liposomes were studied by measuring the serum anti-TT IgG titre following topical immunization. The immune response elicited by topical immunization was compared with that elicited by same dose of alum-adsorbed tetanus toxoid (AATT) given intramuscularly. The results indicate that optimal formulations of transfersomes, niosomes and liposomes could entrap 72.7±3.4, 42.5±2.4 and 41.3±2.2% of antigen and

their elasticity values were 124.4±4.2, 29.3±2.4 and 21.7±1.9, respectively. In vivo study revealed that topically given TT containing transfersomes, after secondary immunization, could elicit immune response (anti-TT-IgG) that was equivalent to one that produced following intramuscularly alum-adsorbed TT-based immunization. In comparison to transfersomes, niosomes and liposomes elicited weaker immune response. Thus transfersomeshold promise for effective non-invasive topical delivery of antigen(s).

- **Chang-Koo Shim., et al**., investigated the feasibility of proliposomes as a sustained transdermal dosage form was examined. Proliposomes containing varying amount of nicotine were prepared by a standard method using sorbitol and lecithin. The porous structure of sorbitol in the proliposomes was maintained, indicating that the majority of lecithin and nicotine is deposited within their porous matrix of the sorbitol particles. As a consequence, the flow property of the proliposome particles was comparable to that of original sorbitol particles. Microscopic observation revealed that proliposomes are converted to liposomes almost completely within minutes following contact with water. It indicates that proliposomes may form liposomes by the sweat when they are applied on the skin under occlusive conditions in vivo. The size distribution of the reconstituted liposomes and nicotine release to pH 7.4 phosphate buffer from them were not significantly affected by the content of nicotine. The release pattern was apparently identical to the ExodusÒ patch, a commercially available transdermal nicotine formulation. We also studied in vitro permeation of nicotine across rat skin from proliposomes in a modified Keshary–Chien

diffusion cell where the experimental set up simulates in vivo application of the proliposomes under an occlusive condition. The nicotine flux from proliposomes was initially retarded compared with that of nicotine powder. The flux from proliposomes appeared to remain constant throughout the experimental period compared with that of nicotine powder, indicating that nicotine may be delivered across the skin in a sustained manner at a constant rate from proliposomes. These results, therefore, indicate that sustained transdermal delivery of nicotine is feasible using proliposomal formulations if the formulations are topically applied under occlusive conditions.

- **Katare. O.P., et al.,** investigated the Dithranol is one of the mainstays in the topical treatment of psoriasis. However, the use of dithranol in psoriatic condition is inconvenient and troublesome, as it has irritating, burning, staining and necrotizing effect on the normal as well as the diseased skin. The entrapment of drug in vesicles is viewed to help in the localized delivery of the drug and an improved availability of the drug at the site will reduce the dose and in turn, the dose-dependent side effects like irritation and staining. The investigations deal with critical parameters controlling the formulation and stabilization of dithranol loaded liposomes and niosomes. The entrapment efficiency of dithranol in liposomes was optimized by altering the proportion of phosphatidyl choline and cholesterol, and in case of niosomes it was between Span 60 and cholesterol. Hydration and permeation mediums were also established keeping in view the poor solubility and stability of dithranol. The mean liposome and niosomes sizes were 4_1.25 and 5_1.5 _m, respectively. The

drug-leakage study carried out at different temperatures of 4–8, 25_2 and 37 °C for a period of two months affirms that the drug leakage increased at a higher temperature. The in vitro permeation study using mouse abdominal skin shows significantly enhanced permeation with vesicles as indicated by flux of dithranol from liposomes (23.13 _g/cm2/h) and niosomes (7.78 _g/cm2/h) as compared with the cream base (4.10 _g/cm2/h).

- **Barry. B.W., et al.,** studied on optimization of drug delivery through human skin is important in modern therapy. This review considers drug–vehicle interactions (drug or prodrug selection, chemical potential control, ion pairs, coacervates and eutectic systems) and the role of vesicles and particles (liposomes, transfersomes, ethosomes, niosomes).We can modify the stratum corneum by hydration and chemical enhancers, or bypass or remove this tissue via microneedles, ablation and follicular delivery. Electrically assisted methods (ultrasound, iontophoresis, electroporation, magnetophoresis and photomechanical waves) show considerable promise. Of particular interest is the synergy between chemical enhancers, ultrasound, iontophoresis and electroporation.
- **Jia-You Fang., et al.,** investigate the skin permeation and partitioning of a fluorinated quinolone antibacterial agent, enoxacin, in liposomes and niosomes, after topical application, were elucidated in the present study. In vitro percutaneous absorption experiments were performed on nude mouse skin with Franz diffusion cells. The influence of vesicles on the physicochemical property and stability of the formulations were measured. The enhanced delivery across the skin of liposome and niosome

encapsulated enoxacin had been observed after selecting the appropriate formulations. The optimized formulations could also reserve a large amount of enoxacin in the skin. A significant relationship between skin permeation and the cumulative amount of enoxacin in the skin was observed. Both permeation enhancer effect and direct vesicle fusion with stratum corneum may contribute to the permeation of enoxacin across skin. Formulation with niosomes demonstrated a higher stability after 48 h incubation compared to liposomes. The inclusion of cholesterol improved the stability of enoxacin liposomes according to the results from encapsulation and turbidity. However, adding negative charges reduced the stability of niosomes. The ability of liposomes and niosomes to modulate drug delivery without significant toxicity makes the two vesicles useful to formulate topical enoxacin.

- **David G. Rhodes., et al.,** study described a procedure for producing a dry product which may be hydrated immediately before use to yield aqueous niosome dispersions similar to those produced by more cumbersome conventional methods. These 'proniosomes' minimize problems of niosome physical stability such as aggregation, fusion and leaking, and provide additional convenience in transportation, distribution, storage, and dosing. This report describes the preparation of dispersions of proniosome-derived niosomes, comparison of these niosomes to conventional niosomes, and optimization of proniosome formulations. In addition, conventional and proniosome-derived niosomes are compared in terms of their morphology, particle size, particle size distribution, and drug release performance in synthetic gastric or intestinal fluid. In all comparisons,

proniosome-derived niosomes are as good as or better than conventional niosomes.

- **J. Kristla., et al.,** studied the influence of liposome size on the transport of hydrophilic substance. The relative contribution of the liposome size, lamellarity, composition and charge to transport drug into the skin, which was applied entrapped in liposomes, is a subject of some controversy. For this purpose liposomes composed of dipalmitoyl-phosphatidylcholine (DPPC), or non-hydrogenated soya lecithin (NSL) or hydrogenated soya lecithin (HSL), all in combination with 30% cholesterol, as well as of two types of niosomes: from glyceryl distearate or PEG stearate in combination with 45% of cholesterol and 10% of lipoaminosalt were prepared and their physical characteristics (size, polydispersity index, zeta potential, entrapped volume) were determined. Their size was varied by extrusion and by sonication. The transport of the entrapped spin labeled hydrophilic compounds into the skin was measured by electron paramagnetic resonance imaging methods. No significant transport into the deeper skin layers (more than 100 mm deep) was observed for NSL liposomes, irrespective of vesicle size. For all other vesicular systems some transport into the deeper skin layers was observed, which did not depend on vesicle size, significantly until the vesicle diameter of approximately 200 nm was reached. However, for small vesicles (with diameter less than 200 nm) the transport is significantly decreased.We have proven that small vesicles are not stable and disintegrate immediately in contact with other surfaces. As a consequence, they lose an important influence on the topical delivery of the entrapped hydrophilic substances.

- **Işık Sarıgüllü Özgüney, Hatice Yeşim Karasulu., et al**. Study was to evaluate and compare the in vitro and in vivo transdermal potential of w/o microemulsion (M) and gel (G) bases for diclofenac sodium (DS). The effect of dimethyl sulfoxide (DMSO) as a penetration enhancer was also examined when it was added to the M formulation. To study the in vitro potential of these formulations, permeation studies were performed with Franz diffusion cells using excised dorsal rat skin. To investigate their in vivo performance, a carrageenan-induced rat paw edema model was used. The commercial formulation of DS (C) was used as a reference formulation. The results of the in vitro permeation studies and the paw edema tests were analyzed by repeated-measures analysis of variance. The in vitro permeation studies found that M was superior to G and C and that adding DMSO to M increased the permeation rate. The permeability coefficients (Kp) of DS from M and M+DMSO were higher (Kp = 4.9 × 10−3 ± 3.6 × 10−4 cm/h and 5.3 × 10−3 ± 1.2 × 10−3 cm/h, respectively) than the Kp of DS from C (Kp = 2.7 × 10−3 ± 7.3 × 10−4 cm/h) and G (Kp = 4.5 × 10−3 ± 4.5 × 10−5 cm/h). In the paw edema test, M showed the best permeation and effectiveness, andM+DMSO had nearly the same effect as M. The in vitro and in vivo studies showed that M could be a new, alternative dosage form for effective therapy.
- **Robert C. Scott, Paul H. Dugard., et al.,** investigated the absorption of undiluted phthalate diesters (dimethyl phthalate (DMP), diethyl phthalate (DEP), dibutyl phthalate (DBP) and di-(-2-ethylhexyl) phthalate (DEHP)] has been measured in vitro through human and rat epidermal membranes. Epidermal membranes were set up in glass diffusion cells and their

permeability to tritiated water measured to establish the integrity of the skin before the phthalate esters were applied to the epidermal surface. Absorption rates for each phthalate ester were determined and a second tritiated water permability assessment made to quantify any irreversible alterations in barrier function due to contact with the esters. Rat skin was consistently more permeable to phthalate esters than the human skin. As the estersbecame more lipophillic and less hydrophilic, the rate of absorption was reduced. Contact with the esters caused little change in the barrier properties of human skin, but caused marked increases in the permeability to water of rat skin. Although differences were noted between species, the absolute rates of absorption measured indicate that the phthalate esters are slowly absorbed through both human and rat skin.

- **Sushama talegaonkar., et al**., investigated on development of novel drug delivery system. Novel drug delivery system aims to deliver the drug at a rate directed by the needs of the body during the period of treatment, and channel the active entity to the site of action. At present, no available drug delivery system behaves ideally achieving all the lofty goals, but sincere attempts have been made to achieve them through novel approaches in drug delivery. A number of novel drug delivery systems have emerged encompassing various routes of administration, to achieve controlled and targeted drug delivery. Encapsulation of the drug in vesicular structures is one such system, which can be predicted to prolong the existence of the drug in systemic circulation, and reduce the toxicity, if selective uptake can be achieved. Consequently a number of vesicular drug delivery systems such as liposomes, niosomes, transfersomes, and pharmacosomes

were developed. Advances have since been made in the area of vesicular drug delivery, leading to the development of systems that allow drug targeting, and the sustained or controlled release of conventional medicines. The focus of this review is to bring out the application, advantages, and drawbacks of vesicular systems.

- **Hamidreza Moghimi., et al.,** studied the Nicotine transdermal systems are being used as an aid to smoking cessation programs. As the kinetics of nicotine delivery is important in success of a smoking cessation program, rapid and high input of nicotine is required, which is not possible by passive methods and requires enhancement strategies such as iontophoresis. Iontophoretic permeation, of nicotine looks promising, based on published data on human skin. However, to optimize this method, permeation pathways should be known and further parameters have to studied, which are the subject of the present investigation. In this study iontophoretic permeation of nicotine through rat skin was performed and the effects of different variables on this phenomenon were studied. Anodic iontophoresis of nicotine from a solution at pH 2.8, using a 0.5 mA/cm^2 current density resulted in a considerable enhancement (about 3-fold) of nicotine absorption through rat skin. Nicotine concentration and current density showed a directly increasing effect on permeation of the drug, but the effect of concentration was not linear. Pulsatile current delivery was more effective in permeation of nicotine than the continuous method. Anodic iontophoresis was around 2-fold more effective than the cathodic method in increasing the flux. Post iontophoretic permeation studies

showed good reversibility of the membrane barrier properties. Results were in good agreement with the reported human data and might be considered as an evidence of the ability of rat skin to model human skin and also the importance of intercellular pathway of the stratum corneum in iontophoretic delivery of nicotine and possibly other drugs. Donor's pH showed no effect on permeation of nicotine under the studied conditions, pH values of <3. Results also showed that the electr-osmotic flow could occur at pH values lower than 4. Finally, this study show that by controlling the effective parameters of iontophoretic delivery, a more effective nicotine transdermal delivery method would achievable**.**

PROFILE OF THE DRUG USED IN THIS STUDY

HYDROCORTISONE

Hydrocortisone Short-acting glucocorticoid that depresses formation, release, and activity of endogenous mediators of inflammation including prostaglandins, kinins, histamine, liposomal enzymes, and complement system. Also modifies body's immune response (24)

Hydrocortisone is a topical corticosteroids constitute of primarily synthetic steroids used as anti-inflammatory and anti-pruritic agents

SYNONYM: Pregn-4-ene-3, 20-dione, 11, 17, 21-trihydroxy-, (11ß)-)

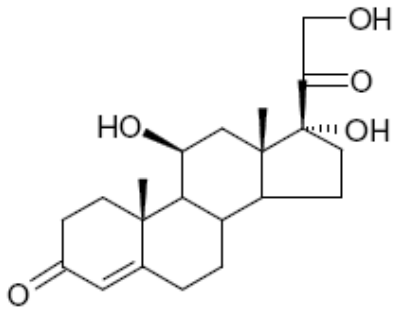
Molecular formula: $C_{21}H_{30}O_5$

Molecular weight : 362.46

Potency : 0.004-0.005 µg/ml

Melting Point : 211-214 °C (lit.)

Chemical Structure:



CLINICAL PHARMACOLOGY:

Topical corticosteroids share anti-inflammatory, antipruritic and vasoconstrictive actions.

The mechanism of anti-inflammatory activity of the topical corticosteroids is unclear. Various laboratory methods, including vasoconstrictor assays, are used to compare and to predict potencies and/or clinical efficacies of the topical corticosteroids. There is some evidence to suggest that a recognizable correlation exists between vasoconstrictor potency and therapeutic efficacy in man (25).

Pharmacokinetics:

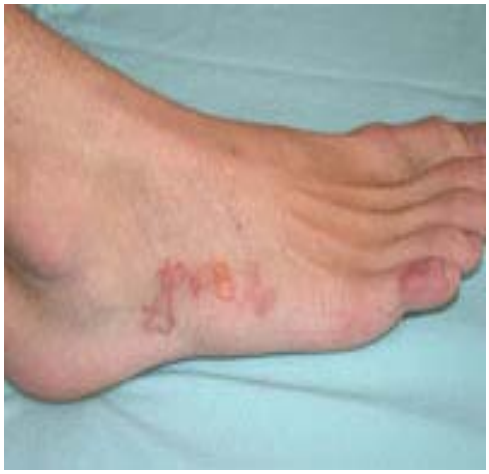
The extent of percutaneous absorption of topical corticosteroids is determined by many factors including the vehicle, the integrity of the epidermal barrier, and the use of occlusive dressings. Topical corticosteroids can be absorbed from normal intact skin. Inflammation and/or other disease processes in the skin increase percutaneous absorption. Occlusive dressings substantially increase the percutaneous absorption of topical corticosteroids.

Thus, occlusive dressings may be a valuable therapeutic adjunct for treatment of resistant dermatoses. Once absorbed through the skin, topical corticosteroids are handled through pharmacokinetic pathways similar to systemically administered corticosteroids. Corticosteroids are bound to plasma proteins in varying degrees. Corticosteroids are metabolized primarily in the liver and are then excreted by the kidneys. Some of the topical corticosteroids and their metabolites are also excreted into the bile (25).

INDICATIONS AND USAGE

Topical corticosteroids are indicated for the relief of the inflammatory and pruritic manifestations of corticosteroid-responsive dermatoses.

DIFFERENT DERMATITIS



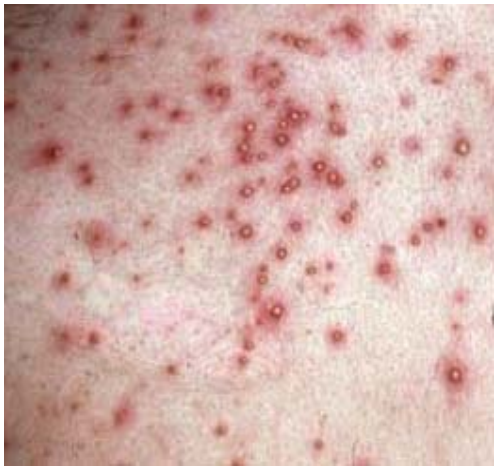


Figure 6

CONTRAINDICATIONS

Topical corticosteroids are contraindicated in those patients with a history of hypersensitivity to any of the components of the preparation.

ADVERSE REACTIONS

The following local adverse reactions are reported infrequently with topical corticosteroids, but may occur more frequently with the use of occlusive dressings.

These reactions are listed in approximate decreasing order of occurrence:

Burning, itching, irritation, dryness, folliculitis, hypertrichosis, acneiform eruptions, hypo pigmentation, perioral dermatitis, allergic contact dermatitis, maceration of the skin, secondary infection, skin atrophy, striae and miliaria (25).

OVERDOSAGE

Topically applied corticosteroids can be absorbed in sufficient amounts to produce systemic effects.

DOSAGE AND ADMINISTRATION

Topical corticosteroids are generally applied to the affected area as a thin film from 2 to 4 times daily depending on the severity of the condition.

Occlusive dressings may be used for the management of psoriasis or recalcitrant conditions. If an infection develops, the use of occlusive dressings should be discontinued and appropriate antimicrobial therapy instituted.

Polymer profile

Lecithin biochemistry also called Phosphatidyl Choline:

Any of a group of phospholipids (phosphoglycerides) that is important in cell structure and metabolism. Lecithins are composed of phosphoric acid, cholines, esters of glycerol, and two fatty acids; the chain length, position, and degree of unsaturation of these fatty acids vary, and this variation results in different lecithin with different biological functions.

Pure lecithin is white and waxy and darkens when exposed to air. Commercial lecithin is brown to light yellow, and its consistency varies from plastic to liquid.

The term lecithin is also used for a mixture of phosphoglycerides containing principally lecithin, cephalin (specifically phosphatidyl ethanolamine), and phosphatidyl inositol.

Commercial lecithin, most of which comes from soybean oil, contains this mixture and, commonly, about 35 percent neutral oil. It is widely used as a wetting and emulsifying agent and for other purposes. Among the products in which it is used are animal feeds, baking products and mixes, chocolate, cosmetics and soaps, dyes, insecticides, paints, and plastics.

Scientific names: 1,2-diacyl-sn-glycero-3-phosphatidylcholine

Lecithin is usually used as synonym for phosphatidylcholine, a phospholipid which is the major component of a phosphatide fraction which may be isolated from either egg yolk or soy beans. It is commercially available in high purity as a food supplement and for medical uses.

Lecithin is regarded as a well tolerated and non-toxic emulsifier. It is approved by the United States Food and Drug Administration for human consumption with the status "Generally Recognized As Safe". Lecithin is an integral part of cell membranes, and can be totally metabolised, so it is virtually non-toxic to humans. Other emulsifiers can only be excreted via the kidneys.

Lecithin is used commercially for anything requiring a natural emulsifier and/or lubricant, from pharmaceuticals to protective coverings. For example, lecithin is the emulsifier that keeps chocolate and cocoa butter in a candy bar from separating.

Sorbitan Esters (Sorbitan Fatty Acid Esters)

Functional Category

Emulsifying agent, non-ionic surfactant; solubilizing agent, wetting and dispersing/suspending agent (26).

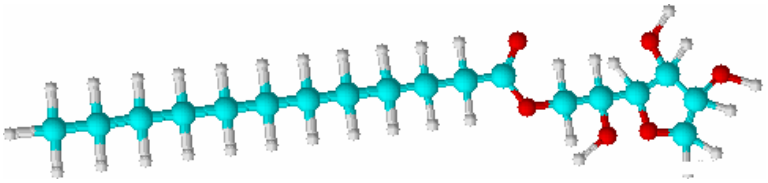
Span 20

Non-proprietary Name**:** Sorbitan monolaurate

Synonym: Arlacel 20; Armotan ML; Crill 1; Dehymuls SML; E493; Glycomul L; Hodag SML; Liposorb L; Montane 20; Protachem SML; Sorbester P12; Sorbirol L; sorbitan laurate; Span 20; Tego SML.

Chemical name**:** Sorbitan monododecanoate

Structure:



Empirical formula**:** $C_{18}H_{34}O_6$

Molecular weight**:** 346

Colour and form**:** Yellow viscous liquid

Safety**:** LD50 (rat, oral): 33.6 g/kg.

Experimental neoplastigen.

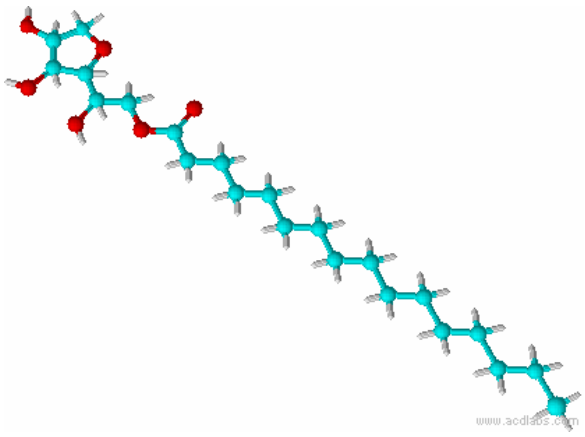
Span 40

Non-proprietary Names: Sorbitan monopalmitate

Synonym: 1,4-Anhydro-D-glucitol, 6-hexadecanoate; Ablunol S-40; Arlacel 40; Armotan MP; Crill 2; Dehymuls SMP; E495; Glycomul P; Hodag SMP; Lamesorb SMP; Liposorb P; Montane 40; Nikkol SP-10; Nissan Nonion PP-40R; Protachem SMP; Proto-sorb SMP; Sorbester P16; Sorbirol P; sorbitan palmitate; Span 40.

Chemical name: Sorbitan monohexadecanoate

Structure:



Empirical formula: $C_{22}H_{42}O_6$

Molecular weight: 403

Colour and form: Cream solid

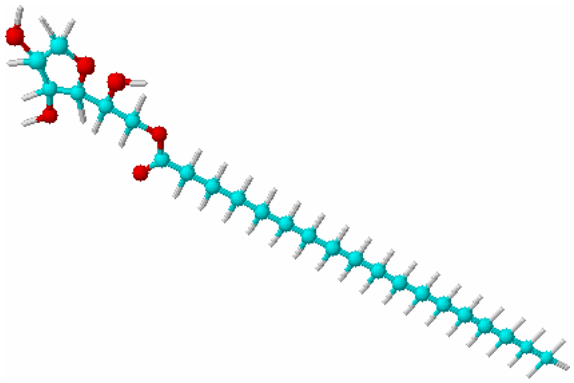
Span 60

Non-proprietary Name: Sorbitan monostearate

Synonym: Ablunol S-60; Alkamuls SMS; 1,4-Anhydro-D-glucitol, 6-octadecanoate; anhydrosorbitol monostearate; Arlacel 60; Armotan MS; Atlas 110K; Capmul S; Crill 3; Dehymuls SMS; Drewmulse SMS; Drewsorb 60K; Durtan 6O; Durtan 60K; E491; Famodan MS Kosher; Glycomul S FG; Glycomul S KFG; Hodag SMS; Lamesorb SMS; Liposorb S; Liposorb SC; Liposorb S-K; Montane 60; Nissan Nonion SP-60R; Norfox Sorbo S- 60FG; Polycon S60K; Protachem SMS; Prote-sorb SMS; S-Maz 60K; SMaz 60KHS; Sorbester P18; Sorbirol S; sorbitan stearate; Sorgen 50; Span 60; Span 60K; Span 60 VS; Tego SMS.

.Chemical name: Sorbitan mono-octadecanoate

Structure:



Empirical formula: $C_{24}H_{46}O_6$

Molecular weight: 431

Colour and form: Cream solid

Safety: LD_{50} (rat, oral): 31 g/kg.

Very mildly toxic by ingestion. Experimental reproductive effects.

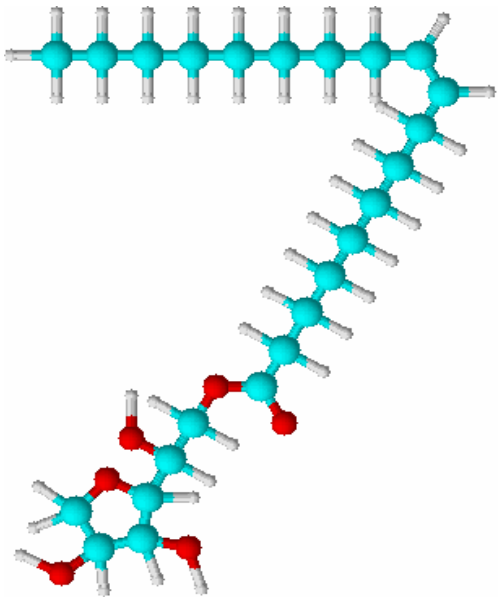
Span 80

Non-proprietary Name: Sorbitan monooleate

Synonym: Ablunol S-80; Arlacel 80; Armotan MO; Capmul O; Crill 4; Crill 50; Dehymuls SMO; Drewmulse SMO; Drewsorb 80K; E494; Glycomul O; Hodag SMO; Lamesorb SMO; Liposorb O; Montane 80; Nikkol SO-10; Nissan Nonion OP-80R; Norfox Sorbo S-80; Polycon S80 K; Proto-sorb SMO; Protachem SMO; S-Maz 80K; Sorbester P17; Sorbirol O; sorbitan oleate; Sorgen 40; Sorgon S-40-H; Span 80; Tego SMO.

Chemical name: (*Z*)-Sorbitan mono-9-octadecenoate

Structure:



Empirical formula: $C_{24}H_{44}O_6$

Molecular weight: 429

Colour and form: Yellow viscous liquid

Polyoxyethylene Sorbitan Fatty Acid Esters

Functional Category

Emulsifying agent, non-ionic surfactant, solubilizing agent, wetting, dispersing / suspending agent (26).

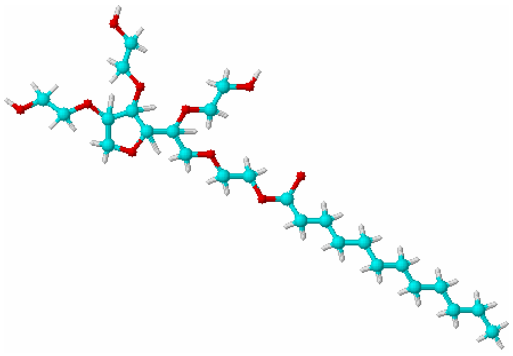
Tween 40

Non-proprietary Names**:** Polysorbate 40

Synonym**:** Crillet 2; E434; Eumulgin SMP; Glycosperse S-20; Hodag PSMP-20; Lamesorb SMP-20; Liposorb P-20; Lonzest SMP-20; Montanox 40; poly(oxy- 1,2-ethanediyl) derivatives; Protasorb P-20; Ritabate 40; sorbitan monohexadecanoate; Sorbax PMP-20; Tween 40.

Chemical name: Polyoxyethylene 20 sorbitan monopalmitate

Structure



Empirical formula**:** $C_{62}H_{122}O_{26}$

Molecular weight**:** 1284

Colour and form**:** Yellow oily liquid

Solubility: Soluble in water and ethanol. Insoluble in mineral oils

Safety**:** LD50 (rat, IV): 1.58 g/kg. Moderately toxic by IV route.

Tween 60

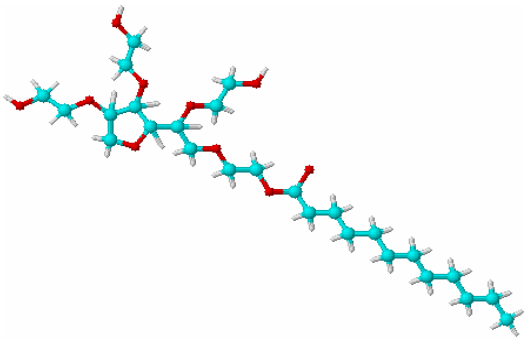
Non-proprietary Names**:** Polysorbate 60

Synonym

Atlas 70K; Atlas Armotan PMS 20; Capmul POE-S; Cremophor PS 60; Crillet3; Drewpone 60K; Durfax 60; Durfax 60K; E435; Emrite 6125; Eumulgin SMS; Glycosperse S-20; Glycosperse S-20FG; Glycosperse S-20FKG; Hodag PSMS-20; Hodag SVS-18; Lamsorb SMS-20; Liposorb S-20; Liposorb S-20K; Lonzest SMS-20; Nikkol TS-10; Norfox SorboT-60 Montanox 60; Polycon T 60 K; polyoxyethylene 20 stearate; Ritabate 60; Protasorb S-20; Sorbax PMS-20; sorbitan monooctadecanoate poly(oxy-1,2-ethanediyl) derivatives; T-Maz 60; T-Max 60KHS; Tween 60; Tween 60K; Tween 60 VS (26).

Chemical name**:** Polyoxyethylene 20 sorbitan monostearate

Structure:



Emprical formula**:** $C_{64}H_{126}O_{26}$

Molecular weight**:** 1312

Colour and form**:** Yellow oily liquid

Solubility**:** Soluble in water and ethanol. Insoluble in mineral oils

Safety: LD50 (rat, IV): 1.22 g/kg. Moderately toxic by IV route.

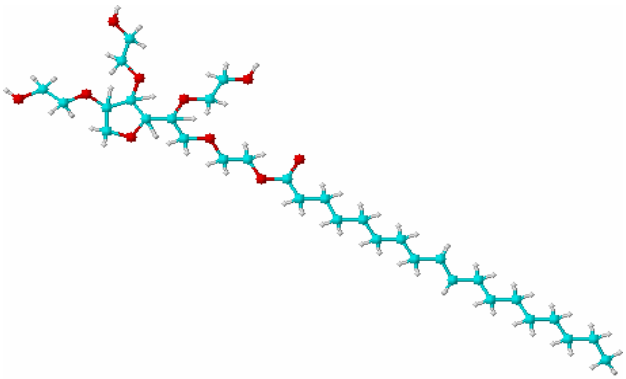
Tween 80

Non-proprietary Names: Polysorbate 80

Synonym: Atlas E; Armotan PMO 20; Capmul POE-O; Cremophor PS 80; Crillet 4; Crillet 50; Drewmulse POE-SMO; Drewpone 80K; Durfax 80; Durfax 80K; E433; Emrite 6120; Eumulgin SMO; Glycosperse O-20; Hodag PSMO-20; Liposorb O-20; Liposorb O-20K; Montanox 80; polyoxyethylene 20 oleate; Protasorb O-20; Ritabate 80; (Z)-sorbitan mono-9-octadecenoate poly(oxy1,2- ethanediyl) derivatives; Tego SMO 80; Tego SMO 80V; Tween 80(26).

Chemical name: Polyoxyethylene 20 sorbitan monooleate

Structure:



Emprical formula: $C_{64}H_{124}O_{26}$

Molecular weight: 1310

Colour and form: Yellow oily liquid

Solubility: Soluble in water and ethanol. Insoluble in mineral oils

Safety: moderately toxic by IV route. Mildly toxic by ingestion. Eye irritation. Experimental tumorigen, reproductive effects.

Mutogenic data. · LD50 (mouse, IP): 7.6 g/kg

· LD50 (mouse, IV): 4.5 g/kg

Cholesterol

Non-proprietary Name: Cholesterol

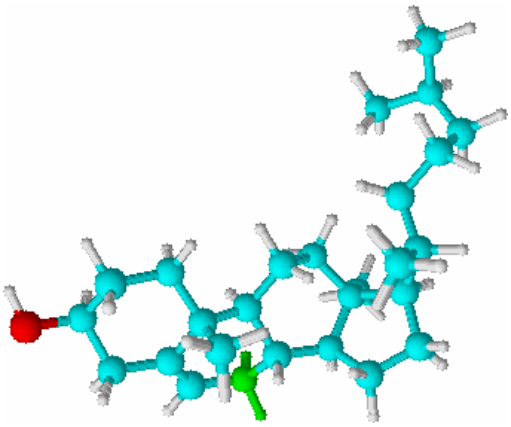
Synonyms: Cholesterin; cholesterolum.

Chemical Name: Cholest-5-en-β-ol [57-88-5]

Empirical Formula: $C_{27}H_{46}O$

Molecular Weight: 386.67

Structure



Functional Category

Emollient; emulsifying agent.

Applications in Pharmaceutical Formulation or Technology

Cholesterol is used in cosmetics and topical pharmaceutical formulations at concentrations of 0.3–5.0% w/w as an emulsifying agent. It imparts water-absorbing power to an ointment and has emollient activity. Cholesterol also has a physiological role. It is the major sterol of the higher animals, and it is found in all body tissues, especially in the brain and spinal cord. It is also the main constituent of gallstones.

Description

Cholesterol occurs as white or faintly yellow, almost odourless, pearly leaflets, needles, powder, or granules. On prolonged exposure to light and air, cholesterol acquires a yellow to tan colour.

Solubility: Chloroform1 in 4.5

Acetone	Soluble
Ethanol (95%)	1 in 78 (slowly)
	1 in 3.6 at 80°C
Ether	1 in 2.8
Methanol	1 in 294 at 0°C

Stability and Storage Conditions

Cholesterol is stable and should be stored in a well-closed container, protected from light.

Safety

Cholesterol is generally regarded as an essentially nontoxic and nonirritant material at the levels employed as an excipients. It has, however, exhibited experimental teratogenic and reproductive effects, and mutation data have been reported. Cholesterol is often derived from animal sources and this must be done in accordance with the regulations for human consumption. The risk of bovine spongiform encephalopathy (BSE) contamination has caused some concern over the use of animal-derived cholesterol in pharmaceutical products. However, synthetic methods of cholesterol manufacture have been developed (26)

Objective of the study

Hydrocortisone is given by topical application for its anti-inflammatory effect in allergic rashes, eczema and certain other inflammatory conditions.

Hydrocortisone is synthetic carticosteriod and it is also a proved anti-inflammatory drug. Hydrocortisone is available in different dosage forms for topical treatment.

Hydrocortisone is available with salt formations like hydrocortisone acetate, hydrocortisone butyrate,hydrocortisone sodium sucinate and other salt formations.

The available in market having trade names like

1. CUTISOFT cream 1 % w/w *INNOVA* (IPCA)
2. ENTOFORM cream *CIPLA*
3. HYDROCORT cream *PFISCAR*
4. TENDRONE cream *YASH PHARMA*
5. WYCORT ointment 2.5 % WYETH

Hydrocortisone is found to have 10% absorption through topical route. It is also affected by pharmacokinetic parameters like plasma half life, plasma protein binding. To improve the absorption of caroticosteroid thruough skin. Proniosome approach was tried using a less potent hydrocartisone.

- The objective of the study is to explore proniosomes for the delivery of hydrocortisone through transdermal route.
- To enhance the transport / permeation of drug through skin without side effects.
- To increase sustain pharmacodynamic activityof drug.

PLAN OF WORK

1. Construction of Standard Curve
2. Formulation of proniosome hydrocortisone gel in 1 % and 2.5 % by coacervation phase separation.
3. Determination of Vesicle size by optical microscopy.
4. Encapsulation efficiency by ultra centrifugation.
5. Drug content uniformity.
6. *In-vitro* drug release using sigma dialysis membrane.
7. Ex In-vivo studies using rat skin.
8. Anti inflammatory activity in mice Paw
9. Drug Permeation study in Human Skin by Prick test

Materials and Methods

- Hydrocortisone USP from SAMARTH LABS
- Soya lecithin (phosphatidyl choline) from Hi-Media laboratories
- Cholesterol from LOBA CHEMIE
- Span 20,40,60,80 from LOBA CHEMIE
- Tween 40, 60, 80 from LOBA CHEMIE
- Dialysis Membrane was purchased from Hi-Media Laboratories (Mumbai, India).
- Ethyl alcohol 99.9%

Instruments

- Magnetic stirrer 2MLH by REMI EQUIPMENTS
- Rotary evaporator
- Dialysis membrane 50 Himedia (Molecular weight cut-off ranges 12000 – 14000)
- UV spectrophotometer 1650 PC Shimadzu
- Eppendroff Centrifuge 5415
- pH Meter ELCO, LI 120
- Electronic balance Shimadzu ELB 300
- Eppendrop Tubes and other glass wares

Experimental Work

Preparation of Standard Stock Solution

Accurately, weighed 10 mg of Hydrocortisone was dissolved in 5 ml of absolute alcohol to get a drug concentration (2 mg/ml). From this stock solution 1 ml solution was taken and transferred to 100 ml volumetric flask and made up the volume up to the mark with PBS pH 7.4 to obtain a standard stock solution of a drug concentration, 20 µg /ml.

Selection of Analytical Wavelength for Hydrocortisone:

The λ_{max} of Hydrocortisone is determined by appropriate dilution of the standard stock solution with PBS pH 7.4, the solution was scanned using the double beam UV visible spectrophotometer (Model: UV- 1650 PC, SHIMADZU) in the spectrum mode between the wavelength range of 400 nm to 200 nm. The λ_{max} of Hydrocortisone is found to 248 nm as the wavelength for further analysis.

Figure 7: Spectrum report for Hydrocortisone

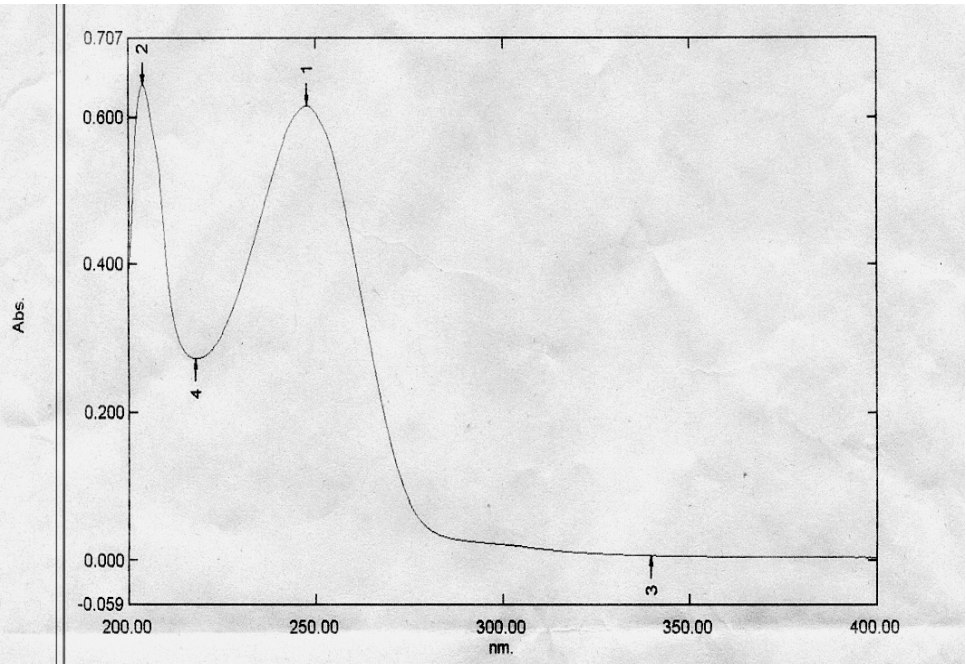


Table 1: Wavelength of spectrum

No.	P/V	Wavelength	Abs.	Description
1		247.80	0.615	
2		203.80	0.643	
3		339.80	0.007	
4		218.00	0.273	

Standard Plot of Hydrocortisone:

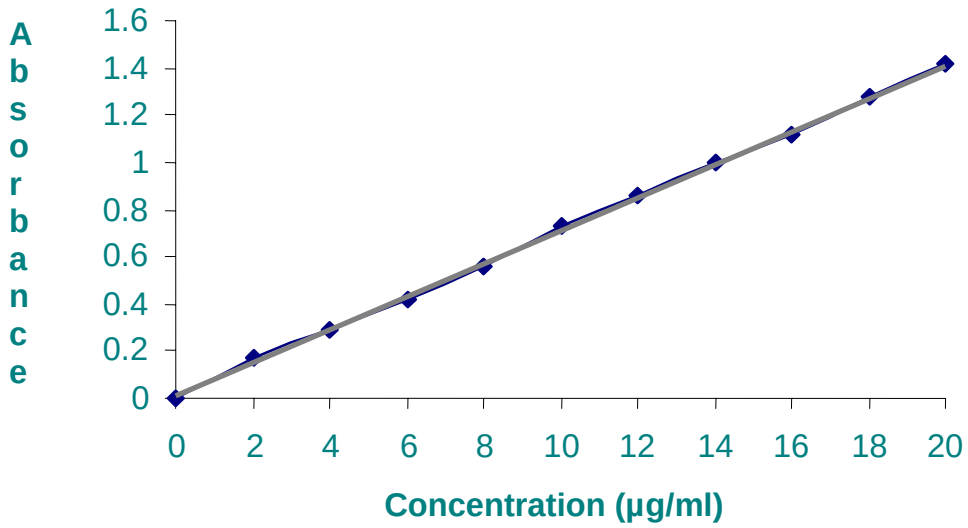
Standard stock solution was further diluted to get the different concentrations like 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 µg/ml to determine the linearity range. Linearity was obtained in the above concentration at 248 nm using UV Spectrophotometer was shown in Table 1 & fig. 2.

Table 2**:** **Standard Curve for Hydrocortisone**

S.No.	Concentration of Drug (µg / ml)	Absorbance at 248nm
1	0	0
2	2	0.1749
3	4	0.289
4	6	0.424
5	8	0.555
6	10	0.726
7	12	0.854
8	14	1.000
9	16	1.116
10	18	1.273
11	20	1.414

Figure 8:

Standard Graph for hydrocortisone



Straight line equation Y = 0.06759 x + 0.01742

Correlation co-efficient r^2 = 0.99917

Pre Formulation study:

Proniosomal gel was prepared by a coacervation-phase separation method .Precisely weighed amounts of surfactant, lecithin, cholesterol were taken in a clean and dry wide mouthed glass vial of 5.0 ml capacity and ethyl alcohol and water was added to it. After warming, all the ingredients were mixed well with a glass rod; the open end of the glass bottle was covered with a lid to prevent the loss of solvent from it and warmed over water bath at 60-70°C for about 5 min until the surfactant mixture was dissolved completely. Then the aqueous phase (0.1% glycerol solution) was added and warmed on a water bath till a clear solution was formed which was converted into Proniosomal gel on cooling (2).

Table 3:

Formulation using different ratios of Non ionic surfactant:

S.No.	Surfactant Type	Ratio	Soya lecithin (mg)	Cholesterol (mg)	Ethanol (ml)	Water (ml)
1	S20:S40	1:9 1:1 9:1	100	100	2	0.5
2	S20:S60	1:9 1:1 9:1	100	100	2	0.5
3	S20:S80	1:9 1:1 9:1	100	100	2	0.5

Formulation procedure:

The proniosome hydrocortisone gel was prepared with 1 % and 2.5 % drug concentration with the same procedure described above using appropriate ratio of surfactants. The proniosome hydrocortisone gel formulation compositions are given in Table 4. (2)

Table 4: **Composition of hydrocortisone gel formulation**

S.No.	Formulation Type	Drug conc.	Surfactant Type	Ratio	Lecithin (mg)	Cholesterol (mg)	Ethanol (ml)	Water (ml)
1	PHG 1	1%	S20:S40	1:9	100	100	2	0.5
2	PHG 2	1%	S20:S60	1:9	100	100	2	0.5
3	PHG 3	1%	S20:S80	1:9	100	100	2	0.5
4	PHG 4	1%	S20:T40	1:9	100	100	2	0.5
5	PHG 5	1%	S20:T60	1:9	100	100	2	0.5
6	PHG 6	1%	S20:T80	1:9	100	100	2	0.5
7	PHG 7	2.5%	S20:S40	1:9	100	100	2	0.5
8	PHG 8	2.5%	S20:S60	1:9	100	100	2	0.5
9	PHG 9	2.5%	S20:S80	1:9	100	100	2	0.5
10	PHG10	2.5%	S20:T40	1:9	100	100	2	0.5
11	PHG 11	2.5%	S20:T60	1:9	100	100	2	0.5
12	PHG 12	2.5%	S20:T80	1:9	100	100	2	0.5

Characterization of Proniosomal Gel

Vesicle Size Analysis:

Proniosomal hydrocortisone gel (100 mg) was hydrated in saline solution (0.9% solution) in a small glass vial with occasional shaking for 10 min. The dispersion was observed under optical microscope. The size of 50 vesicles was measured using a calibrated ocular and stage micrometer fitted in the optical microscope. Vesicle size is calculated using Equation 1. (2)

$$\text{Size of each division} = \frac{\text{Number of divisions of stage micrometer}}{\text{Number of divisions of eye piece micrometer}} \times 10 \rightarrow (1)$$

Table 5: Vesicle Size Determination by Optical Microscope

S.No	Formulation Type	Surfactant Type	Drug conc.	Vesicle size Range (μm)	Average size (μm)
1	PHG 1	S20:S40	1%	2 – 6	3
2	PHG 2	S20:S60	1%	2 – 8	4
3	PHG 3	S20:S80	1%	2 – 12	4.5
4	PHG 4	S20:T40	1%	2 – 12	4
5	PHG 5	S20:T60	1%	2 – 10	3.5
6	PHG 6	S20:T80	1%	2 – 8	3
7	PHG 7	S20:S40	2.5%	2 – 8	4
8	PHG 8	S20:S60	2.5%	2 – 14	5
9	PHG 9	S20:S80	2.5%	4 – 12	6
10	PHG 10	S20:T40	2.5%	2 – 16	6.5
11	PHG 11	S20:T60	2.5%	2 – 20	7
12	PHG12	S20:T80	2.5%	4 – 10	6

Proniosome vesicle pictures for 12 different formulations are shown below.

Figure 9

PHG 1 (S20: 40 1%) in 40X

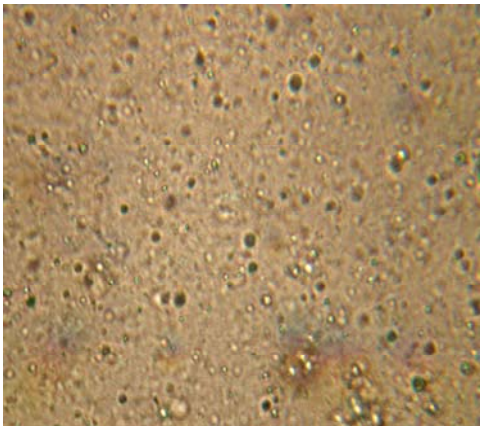


Figure 10

PHG 2 (S20:60 1 %) in 40 X

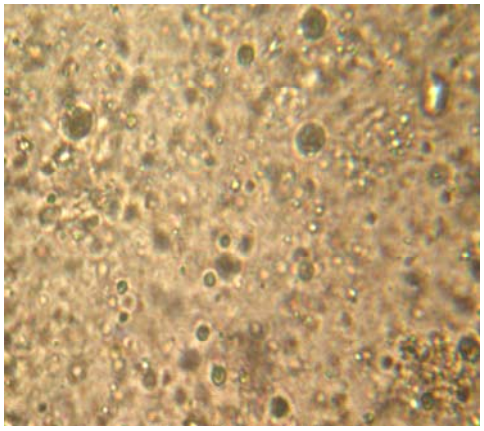


Figure 11

PHG 3 (S20:80 1%) in 40X

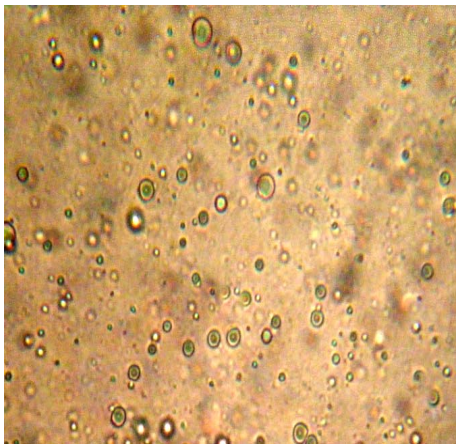


Figure 12

PHG 3 (S20:80 1%) in 10X

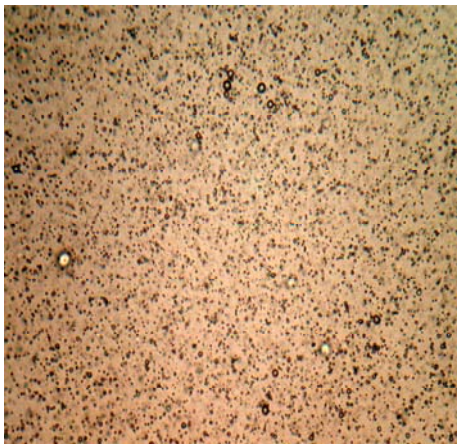


Figure 13

PHG 4 (S20:T40 1 %) in 40X

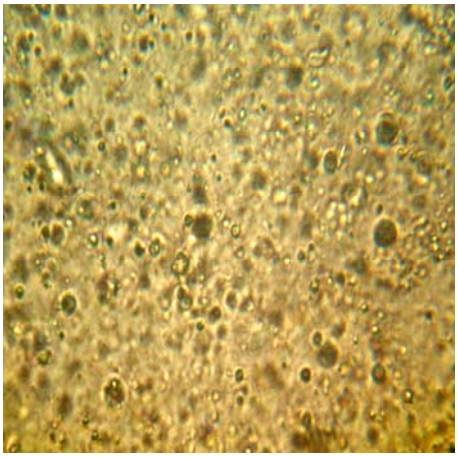


Figure 14

PHG 4 (S20:T40 1 %) in 10X

Figure 15

PHG 5 (S20:T60 1%) in 40x

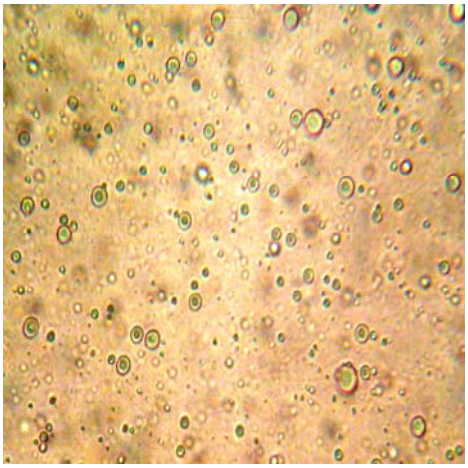


Figure 16

PHG 6 (S20:T80 1%) in 40X

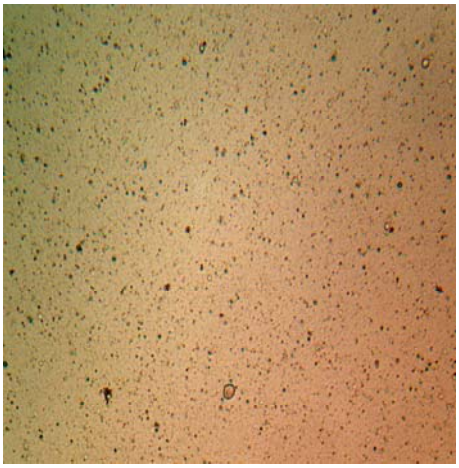


Figure 17

PHG 7 (S20:40 2.5 %) in 40X

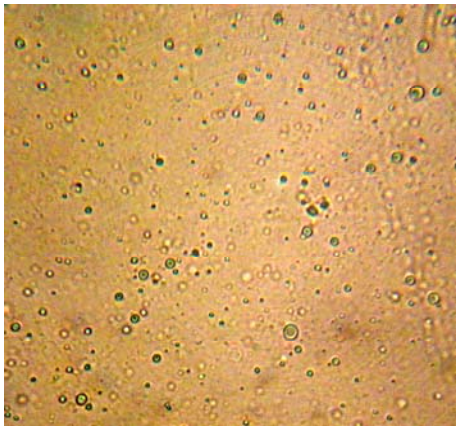


Figure 18

PHG 8 (S20:60 2.5 %) in 40X

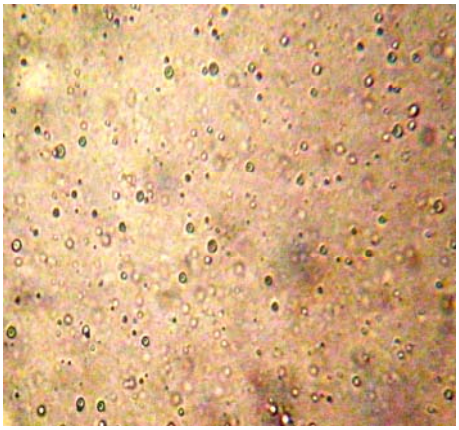


Figure 19

PHG 9 (S20:80 2.5 %) in 40X

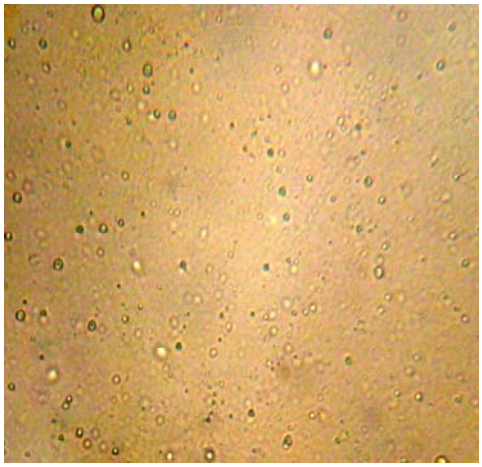


Figure 20

PHG 10 (S20:T40 2.5 %) in 40X

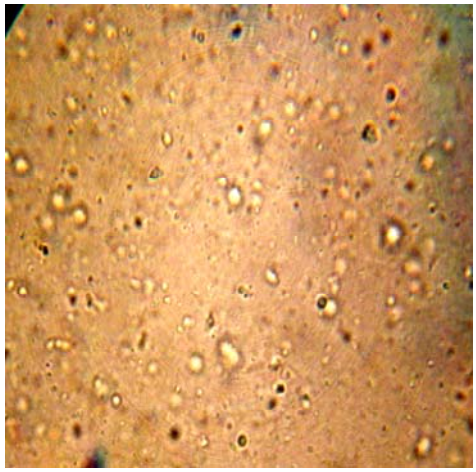


Figure 21

PHG 11 (S20:T60 2.5 %) in 40X

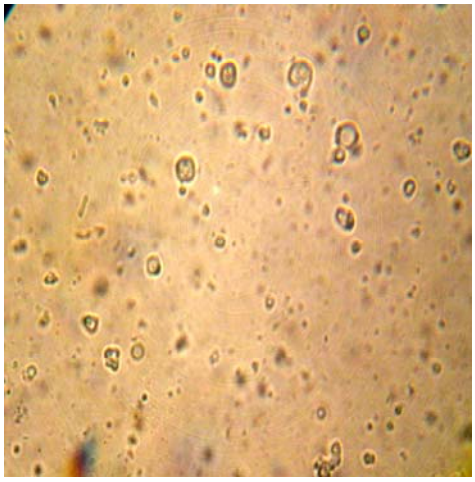
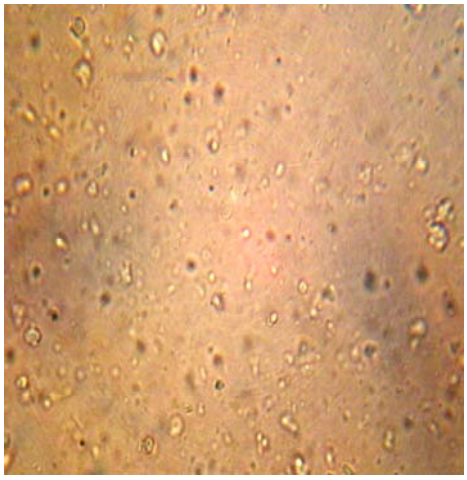


Figure 22

PHG 12 (S20:T80 2.5 %) in 40X



Encapsulation Efficiency:

Encapsulation of hydrocortisone drug in Proniosomal gel was evaluated by dispersing the Proniosomal hydrocortisone gel (100 mg) in distilled water and the dispersion was warmed gently for the formation of niosomes. Then the dispersion was centrifuged at 13000 rpm for 1hr at 5^0C. The supernatant was taken for the determination of free drug at 248 nm spectrophotometrically. (2)

The percentage encapsulation efficiency was calculated from Equation 2.

% Encapsulation Efficiency = $[(C_t - C_r)/C_t] \text{ X } 100$ → (2)

Where,

C_t – Concentration of total Hydrocortisone.

C_r – Concentration of free drug in supernatant solution.

Table 6: **Entrapment efficiency of Hydrocortisone**

S.No	Surfactant Type	% Entrapment
1	PHG 1 (S20, S40 1%)	89.67%
2	PHG 2 (S20, S60 1%)	79.66%
3	PHG 3 (S20, S80 1%)	70.66%
4	PHG 4 (S20, T40 1%)	58.88%
5	PHG 5 (S20, T60 1%)	65.15%
6	PHG 6 (S20, T80 1%)	27.20%
7	PHG 7 (S20, S40 2.5%)	83.55%
8	PHG 8 (S20, S60 2.5%)	80.00%
9	PHG 9 (S20, S80 2.5%)	76.42%
10	PHG 10(S20, T40 2.5%)	76.12%
11	PHG 11(S20, T60 2.5%)	77.02%
12	PHG 12(S20, T80 2.5%)	57.34%

Drug content uniformity:

Formulated Proniosomal gel was mixed well and 100 mg of gel was weighed and transferred into vial. The gel was dissolved in 25 ml of phosphate buffer saline (pH 7.4) with vigorous shaking, and the solutions were assayed for hydrocortisone content at 248 nm. Amount Drug content present in 100 mg gel was calculated by Equation 3. (27).

Amount of drug = [(concentration) x (1) x (100) / 1000] → (3)

Table 7: **Drug content uniformity of proniosome hydrocortisone gel**

S.No.	Formulation Type	Surfactant Type	Absorbance	Concentration (mg)	Amt. of Drug (25ml)	% of drug
1	PHG 1	S20, 40 (1 %)	3.9133	0.055984	1.399594	55.9837
2	PHG 2	S20, 60 (1 %	3.91333	0.05598	1.399594	55.9837
3	PHG 3	S20, 80 (1 %)	3.90134	0.055811	1.395287	55.8114
4	PHG 4	S20, T40 (1 %)	3.91333	0.055984	1.399594	55.9837
5	PHG 5	S20, T60 (1 %)	3.89917	0.05578	1.394507	55.7802
6	PHG 6	S20, T80 (1 %)	3.91333	0.055984	1.399594	55.9837
7	PHG 7	S20, 40 (2.5 %)	4.2137	0.0603	1.507501	24.3145
8	PHG 8	S20, 60 (2.5 %)	4.00725	0.057333	1.433335	23.1183
9	PHG 9	S20, 80 (2.5 %)	3.99988	0.057227	1.430687	23.0755
10	PHG 10	S20,T40(2.5 %)	3.99481	0.057155	1.428865	23.0462
11	PHG 11	S20,T60(2.5 %)	3.99988	0.057227	1.430687	23.0755
12	PHG 12	S20,T80(2.5 %)	3.91333	0.055984	1.399594	22.5741

In vitro release studies:

Release from proniosome hydrocortisone gel was carried out using Himedia dialysis membranes 50 with the molecular weight cut-off range from 12000 - 14000. A weighed amount of (100 mg) Proniosomal gel formulation was dispersed in the dialysis membrane and the open ends of the membrane were covered with membrane closure clips. The membrane containing gel formulation was allowed to dip in 50 ml of receptor medium pH 7.4 phosphate buffer saline. The receptor medium was stirred using magnetic bead fitted to a magnetic stirrer at 60 rpm. Samples were withdrawn and replaced by equal volumes of fresh receptor medium at each sampling intervals to maintain sink condition. Samples withdrawn were analyzed by using spectrophotometer at 248 nm.

Table 8: In-vitro Release for S20:40 1 %

S.No	TIME in min	Absorbance	Concentration (µg/ml)	Concentration (mg)	Amt. release 50 ml	Cumulative % release
1	30	0.10266	1.224889	0.001225	0.061244	4.71111
2	60	0.22437	2.973847	0.002974	0.148692	11.43787
3	90	0.3417	4.659865	0.00466	0.232993	17.92256
4	120	0.37063	5.075586	0.005076	0.253779	19.52148
5	150	0.39651	5.447478	0.005447	0.272374	20.95184
6	180	0.45712	6.318437	0.006318	0.315922	24.30168
7	210	0.50566	7.015951	0.007016	0.350798	26.98443
8	240	0.59443	8.291565	0.008292	0.414578	31.89063
9	270	0.62297	8.701681	0.008702	0.435084	33.468
10	300	0.68922	9.653686	0.009654	0.482684	37.12956
11	330	0.70133	9.827705	0.009828	0.491385	37.79887
12	360	0.77735	10.9201	0.01092	0.546005	42.0004
13	420	0.78043	10.96436	0.010964	0.548218	42.17063
14	480	0.66244	9.26886	0.009269	0.463443	35.64946

Table 9: In-vitro Release for S20:60 1 %

S.No	TIME in min	Absorbance	Concentration (µg/ml)	Concentration (mg)	Amt. release 50 ml	Cumulative % release
1	30	0.03979	0.321454	0.000321	0.016073	1.236362
2	60	0.16846	2.170427	0.00217	0.108521	8.347795
3	90	0.24219	3.229918	0.00323	0.161496	12.42276
4	120	0.32214	4.37879	0.004379	0.21894	16.8415
5	150	0.35669	4.875269	0.004875	0.243763	18.75104
6	180	0.37268	5.105044	0.005105	0.255252	19.63478
7	210	0.44147	6.093548	0.006094	0.304677	23.43672
8	240	0.47771	6.614312	0.006614	0.330716	25.43966
9	270	0.51907	7.208651	0.007209	0.360433	27.72558
10	300	0.52664	7.317431	0.007317	0.365872	28.14396
11	330	0.54043	7.515591	0.007516	0.37578	28.90612
12	360	0.58508	8.157206	0.008157	0.40786	31.37387
13	420	0.62047	8.665757	0.008666	0.433288	33.32983
14	480	0.65182	9.116252	0.009116	0.455813	35.06251

Table 10: In-vitro Release for S20:80 1 %

S.No	TIME in min	Absorbance	Concentration (µg/ml)	Concentration (mg)	Amt. release 50 ml	Cumulative % release
1	30	0.10977	1.327058	0.001327	0.066353	5.104± (0.135)
2	60	0.23584	3.138669	0.003139	0.156933	12.071± (0.563)
3	90	0.34723	4.73933	0.004739	0.236967	18.228± (0.453)
4	120	0.41711	5.743498	0.005743	0.287175	22.09± (1.453)
5	150	0.44153	6.09441	0.006094	0.304721	23.440± (0.717)
6	180	0.46606	6.446903	0.006447	0.322345	24.76 ± (1.313)
7	210	0.53442	7.429228	0.007429	0.371461	28.573± (0.329)
8	240	0.60013	8.373473	0.008373	0.418674	32.20 ± (0.190)
9	270	0.69901	9.794367	0.009794	0.489718	37.670± (0.152)
10	300	0.76355	10.7218	0.010722	0.53609	41.237± (0.77)
11	330	0.82434	11.59534	0.011595	0.579767	44.59± (0.612)
12	360	0.9013	12.70125	0.012701	0.635063	48.85 ± (0.136)
13	420	0.95372	13.45452	0.013455	0.672726	51.748 ± (0.389)
14	480	1.07216	15.15649	0.015156	0.757824	58.294 ± (0.627)

Table 11: In-vitro Release for S20:T40 1 %

S.No	TIME in min	Absorbance	Concentration (µg/ml)	Concentration (mg)	Amt. release 50v ml	Cumulative % release
1	30	0.06445	0.675815	0.000676	0.033791	2.448607
2	60	0.4519	6.243426	0.006243	0.312171	22.62111
3	90	0.52234	7.25564	0.007256	0.362782	26.28855
4	120	0.5835	8.134502	0.008135	0.406725	29.47283
5	150	0.62476	8.727403	0.008727	0.43637	31.62103
6	180	0.62708	8.760741	0.008761	0.438037	31.74182
7	210	0.63208	8.832591	0.008833	0.44163	32.00214
8	240	0.64136	8.965943	0.008966	0.448297	32.4853
9	270	0.68799	9.636011	0.009636	0.481801	34.91308
10	300	0.69666	9.760598	0.009761	0.48803	35.36448
11	330	0.72241	10.13062	0.010131	0.506531	36.70515
12	360	0.75633	10.61805	0.010618	0.530902	38.47119
13	420	0.78423	11.01897	0.011019	0.550948	39.9238
14	480	0.84415	11.88001	0.01188	0.594001	43.04352

Table 12: In-vitro Release for S20:T60 1 %

S.No	TIME in min	Absorbance	Concentration (µg/ml)	Concentration (mg)	Amt. release 50 ml	Cumulative % release
1	30	0.09998	1.186377	0.001186	0.059319	4.329844
2	60	0.42407	5.843512	0.005844	0.292176	21.32669
3	90	0.55322	7.699382	0.007699	0.384969	28.09993
4	120	0.67822	9.495617	0.009496	0.474781	34.65554
5	150	0.73352	10.29027	0.01029	0.514514	37.55574
6	180	0.74182	10.40954	0.01041	0.520477	37.99103
7	210	0.74475	10.45165	0.010452	0.522582	38.14469
8	240	0.7572	10.63055	0.010631	0.531528	38.79763
9	270	0.79407	11.16037	0.01116	0.558018	40.73127
10	300	0.82007	11.53398	0.011534	0.576699	42.09483
11	330	0.82117	11.54979	0.01155	0.57749	42.15252
12	360	0.8245	11.59764	0.011598	0.579882	42.32717
13	420	0.82753	11.64118	0.011641	0.582059	42.48607
14	480	0.83127	11.69493	0.011695	0.584746	42.68222

Table 13: In-vitro Release for S20:T80 1 %

S.No	TIME in min	Absorbance	Concentration (µg/ml)	Concentration (mg)	Amt. release 50 ml	Cumulative % release
1	30	0.07598	0.8415	0.000842	0.042075	3.048914
2	60	0.32407	4.406524	0.004407	0.220326	15.96567
3	90	0.45332	6.263831	0.006264	0.313192	22.69504
4	120	0.57712	8.042822	0.008043	0.402141	29.14066
5	150	0.63152	8.824544	0.008825	0.441227	31.97298
6	180	0.69418	9.72496	0.009725	0.486248	35.23536
7	210	0.72475	10.16425	0.010164	0.508212	36.82698
8	240	0.75572	10.60928	0.010609	0.530464	38.43943
9	270	0.78407	11.01667	0.011017	0.550833	39.91547
10	300	0.782007	10.98702	0.010987	0.549351	39.80806
11	330	0.83177	11.70211	0.011702	0.585106	42.39896
12	360	0.83425	11.73775	0.011738	0.586887	42.52808
13	420	0.83527	11.75241	0.011752	0.58762	42.58118
14	480	0.82127	11.55123	0.011551	0.577561	41.85228

Table 14: In-vitro Release for S20:40 2.5 %

S.No	TIME in min	Absorbance	Concentration (µg/ml)	Concentration (mg)	Amt. release 50 ml	Cumulative % release
1	30	0.06812	0.728553	0.000729	0.036428	2.464658
2	60	0.37146	5.087513	0.005088	0.254376	17.2108
3	90	0.41748	5.748814	0.005749	0.287441	19.44795
4	120	0.47278	6.543469	0.006543	0.327173	22.13623
5	150	0.56909	7.927432	0.007927	0.396372	26.81811
6	180	0.70898	9.937635	0.009938	0.496882	33.61852
7	210	0.71558	10.03248	0.010032	0.501624	33.93936
8	240	0.81384	11.44446	0.011444	0.572223	38.71604
9	270	0.83484	11.74623	0.011746	0.587311	39.7369
10	300	0.9198	12.96709	0.012967	0.648355	43.86703
11	330	0.91772	12.9372	0.012937	0.64686	43.76591
12	360	0.93054	13.12143	0.013121	0.656071	44.38913
13	420	1.07031	15.1299	0.01513	0.756495	51.18371
14	480	1.12439	15.90703	0.015907	0.795351	53.81268

Table 15: In-vitro Release for S20:60 2.5 %

S.No	TIME in min	Absorbance	Concentration (µg/ml)	Concentration (mg)	Amt. release 50 ml	Cumulative % release
1	30	0.09131	1.06179	0.001062	0.05309	3.604177
2	60	0.39355	5.404943	0.005405	0.270247	18.34672
3	90	0.48633	6.738181	0.006738	0.336909	22.8723
4	120	0.55054	7.660871	0.007661	0.383044	26.00431
5	150	0.59229	8.260813	0.008261	0.413041	28.04078
6	180	0.5813	8.102888	0.008103	0.405144	27.50471
7	210	0.59229	8.260813	0.008261	0.413041	28.04078
8	240	0.72974	10.23595	0.010236	0.511798	34.74526
9	270	0.84424	11.8813	0.011881	0.594065	40.33029
10	300	0.85242	11.99885	0.011999	0.599943	40.7293
11	330	0.94543	13.33539	0.013335	0.66677	45.2661
12	360	1.07349	15.1756	0.015176	0.75878	51.51256
13	420	0.95447	13.4653	0.013465	0.673265	45.70705
14	480	0.8551	12.03736	0.012037	0.601868	40.86002

Table 16: In-vitro Release for S20:80 2.5 %

S.No	TIME in min	Absorbance	Concentration (µg/ml)	Concentration (mg)	Amt. release 50 ml	Cumulative % release
1	30	0.05258	0.505245	0.000505	0.025262	1.766591
2	60	0.16797	2.163386	0.002163	0.108169	7.564285
3	90	0.27319	3.675384	0.003675	0.183769	12.85099
4	120	0.30664	4.156057	0.004156	0.207803	14.53167
5	150	0.34985	4.776979	0.004777	0.238849	16.70273
6	180	0.38867	5.334818	0.005335	0.266741	18.65321
7	210	0.45972	6.355798	0.006356	0.31779	22.22307
8	240	0.48877	6.773243	0.006773	0.338662	23.68267
9	270	0.51465	7.145136	0.007145	0.357257	24.98299
10	300	0.52112	7.238109	0.007238	0.361905	25.30807
11	330	0.5249	7.292427	0.007292	0.364621	25.498
12	360	0.52844	7.343296	0.007343	0.367165	25.67586
13	420	0.53362	7.417732	0.007418	0.370887	25.93613
14	480	0.59563	8.308809	0.008309	0.41544	29.05178

Table 17: In-vitro Release for S20:T40 2.5 %

S.No	TIME in min	Absorbance	Concentration (µg/ml)	Concentration (mg)	Amt. release 50 ml	Cumulative % release
1	30	0.09778	1.154764	0.001155	0.057738	4.037635
2	60	0.41675	5.738324	0.005738	0.286916	20.06407
3	90	0.63184	8.829142	0.008829	0.441457	30.87113
4	120	0.63184	8.829142	0.008829	0.441457	30.87113
5	150	0.76648	10.7639	0.010764	0.538195	37.63602
6	180	0.86035	12.1128	0.012113	0.60564	42.35246
7	210	0.92505	13.04253	0.013043	0.652127	45.60327
8	240	0.96753	13.65297	0.013653	0.682648	47.73765
9	300	1.1145	15.76491	0.015765	0.788245	55.12206
10	360	1.15198	16.30349	0.016303	0.815175	57.00522
11	420	1.13733	16.09297	0.016093	0.804649	56.26914
12	480	1.10632	15.64736	0.015647	0.782368	54.71106

Table 18: In-vitro release for S20:T60 2.5 %

S.No	TIME in min	Absorbance	Concentration (µg/ml)	Concentration (mg)	Amt. release 50 ml	Cumulative % release
1	30	0.0387	0.305791	0.000306	0.01529	1.0692
2	60	0.18958	2.473919	0.002474	0.123696	8.650065
3	90	0.25867	3.466734	0.003467	0.173337	12.12145
4	120	0.36536	4.999856	0.005	0.249993	17.48202
5	150	0.40076	5.50855	0.005509	0.275428	19.26066
6	180	0.43848	6.050582	0.006051	0.302529	21.15588
7	210	0.47278	6.543469	0.006543	0.327173	22.87926
8	240	0.49939	6.925851	0.006926	0.346293	24.21626
9	300	0.51819	7.196005	0.007196	0.3598	25.16086
10	360	0.59155	8.25018	0.00825	0.412509	28.84678
11	420	0.62183	8.6853	0.008685	0.434265	30.36818
12	480	0.63918	8.934617	0.008935	0.446731	31.23992

Table 19: In-vitro Release for S20:T80 2.5 %

S.No	TIME in min	Absorbance	Concentration (µg/ml)	Concentration (mg)	Amt. release 50 ml	Cumulative % release
1	30	0.04968	0.463572	0.000464	0.023179	1.67961
2	60	0.39453	5.419026	0.005419	0.270951	19.63415
3	90	0.48633	6.738181	0.006738	0.336909	24.4137
4	120	0.55054	7.660871	0.007661	0.383044	27.75678
5	150	0.6283	8.778273	0.008778	0.438914	31.80534
6	180	0.69861	9.788619	0.009789	0.489431	35.46601
7	210	0.75134	10.54634	0.010546	0.527317	38.21139
8	240	0.75867	10.65167	0.010652	0.532584	38.59302
9	300	0.77502	10.88662	0.010887	0.544331	39.44428
10	360	0.81006	11.39014	0.01139	0.569507	41.26863
11	420	0.81421	11.44978	0.01145	0.572489	41.4847
12	480	0.81531	11.46558	0.011466	0.573279	41.54197

Table 20: In-vitro Release for marketed 1 % Hydrocortisone cream

S.No	TIME in min	Absorbance	Concentration (µg/ml)	Concentration (mg)	Amt. release 50 ml	Cumulative % release
1	30	0.05273	0.5074	0.000507	0.02537	2.537002
2	60	0.53845	7.487139	0.007487	0.374357	37.43569
3	90	0.53308	7.409973	0.00741	0.370499	37.04986
4	120	0.5332	7.411697	0.007412	0.370585	37.05849
5	150	0.49219	6.822388	0.006822	0.341119	34.11194
6	180	0.44702	6.173301	0.006173	0.308665	30.8665
7	210	0.46899	6.489007	0.006489	0.32445	32.44504
8	240	0.33618	4.580543	0.004581	0.229027	22.90272
9	300	0.31897	4.333238	0.004333	0.216662	21.66619
10	360	0.29272	3.956028	0.003956	0.197801	19.78014
11	420	0.36462	4.989223	0.004989	0.249461	24.94611
12	480	0.34656	4.729703	0.00473	0.236485	23.64851

Table 21:

Release comparison of 1 % PHG preparations & Marketed Hydrocortisone 1 %

S.No.	Time in min	Cumulative % release of 1 % proniosome formulation						Hydrocortisone CREAM 1%
		S20:S40	S20:S60	S20:S80	S20:T40	S20:T60	S20:T80	
1	30	4.71111	1.23636	5.10407	2.44860	4.32984	3.04891	2.537002
2	60	11.4379	8.34779	12.0718	22.6211	21.3266	15.9656	37.43569
3	90	17.9226	12.4227	18.2281	26.2885	28.0999	22.6950	37.04986
4	120	19.5215	16.8415	22.0903	29.4728	34.6555	29.1406	37.05849
5	150	20.9518	18.7510	23.4400	31.6210	37.5557	31.9729	34.11194
6	180	24.3017	19.6347	24.7957	31.7418	37.9910	35.2353	30.8665
7	210	26.9844	23.4367	28.5739	32.0021	38.1446	36.8269	32.44504
8	240	31.8906	25.4396	32.2056	32.4853	38.7976	38.4394	22.90272
9	270	33.468	27.7255	37.6706	34.9130	40.7312	39.9154	21.66619
10	300	37.1296	28.1439	41.2376	35.3644	42.0948	39.8080	19.78014
11	330	37.7989	28.9061	44.5974	36.7051	42.1525	42.3989	24.94611
12	360	42.0004	31.3738	48.8509	38.4711	42.3271	42.5280	23.64851
13	420	42.1706	33.3298	51.7481	39.9238	42.4860	42.5811	_____
14	480	35.6495	35.0625	58.2941	43.0435	42.6822	41.8522	_____

Figure23:

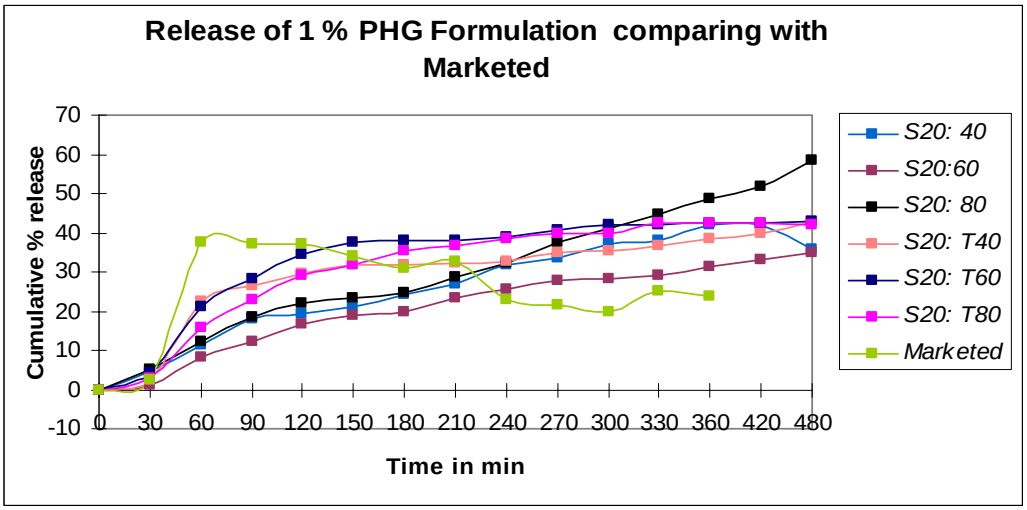
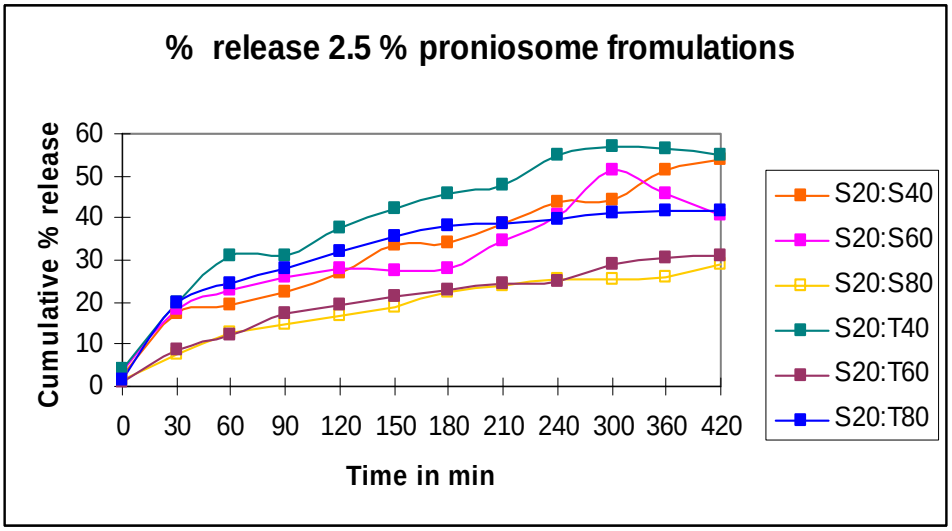


Table 22: Release comparison of 2.5 % PHG preparations

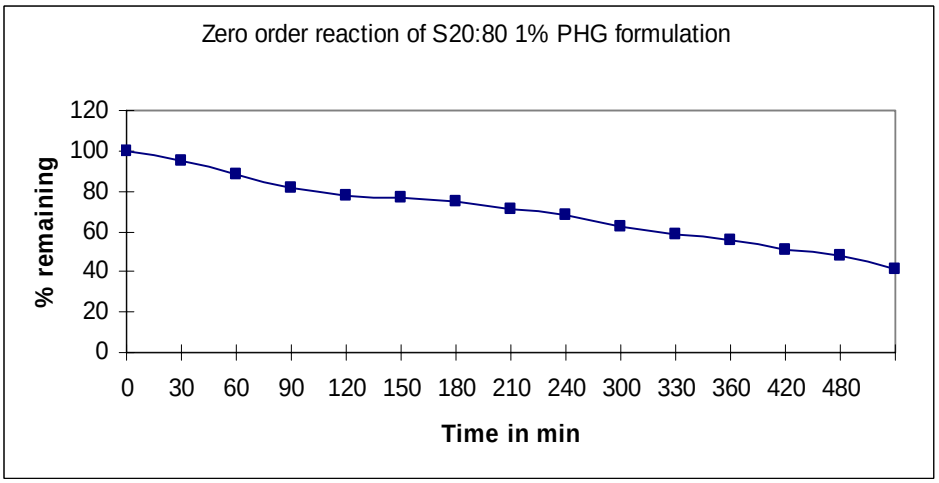
S.No	Time in mins	Cumulative % release of 1 % proniosome formulations					
		S20:S40	S20:S60	S20:S80	S20:T40	S20:T60	S20:T80
1	30	2.464658	3.604177	1.766591	4.037635	1.0692	1.67961
2	60	17.2108	18.34672	7.564285	20.06407	8.650065	19.63415
3	90	19.44795	22.8723	12.85099	30.87113	12.12145	24.4137
4	120	22.13623	26.00431	14.53167	30.87113	17.48202	27.75678
5	150	26.81811	28.04078	16.70273	37.63602	19.26066	31.80534
6	180	33.61852	27.50471	18.65321	42.35246	21.15588	35.46601
7	210	33.93936	28.04078	22.22307	45.60327	22.87926	38.21139
8	240	38.71604	34.74526	23.68267	47.73765	24.21626	38.59302
9	300	43.86703	40.7293	25.30807	55.12206	25.16086	39.44428
10	360	44.38913	51.51256	25.67586	57.00522	28.84678	41.26863
11	420	51.18371	45.70705	25.93613	56.26914	30.36818	41.4847
12	480	53.81268	40.86002	29.05178	54.71106	31.23992	41.54197

Figure 24:



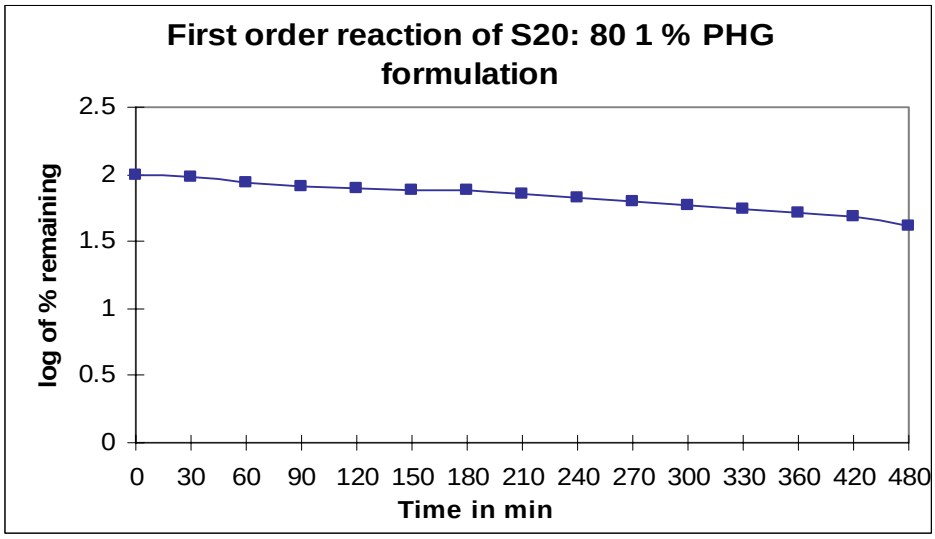
Kinetics for S20: 80 1 % PHG formulation:

Figure 25:



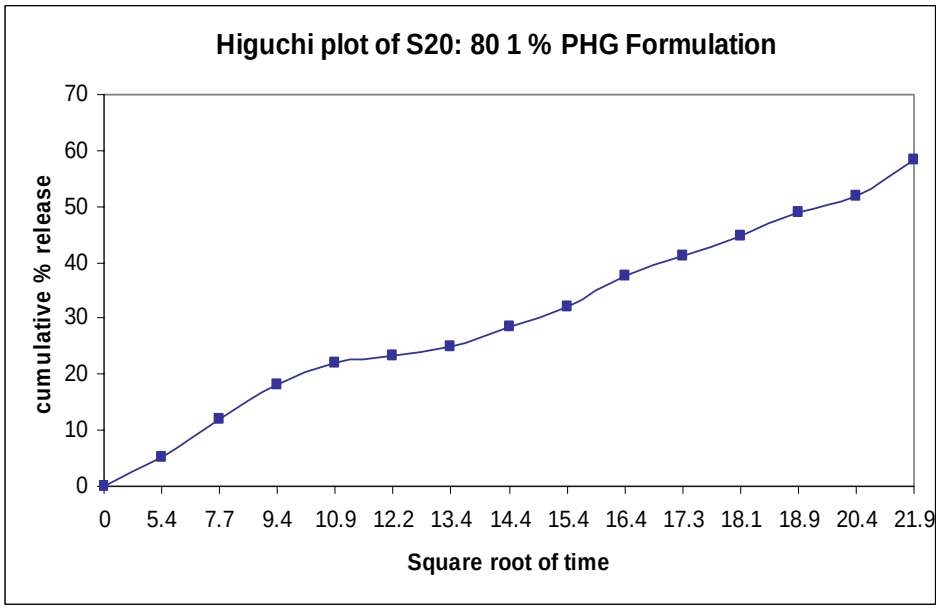
Regression coefficient r^2 = 0.998334541

Figure 26:



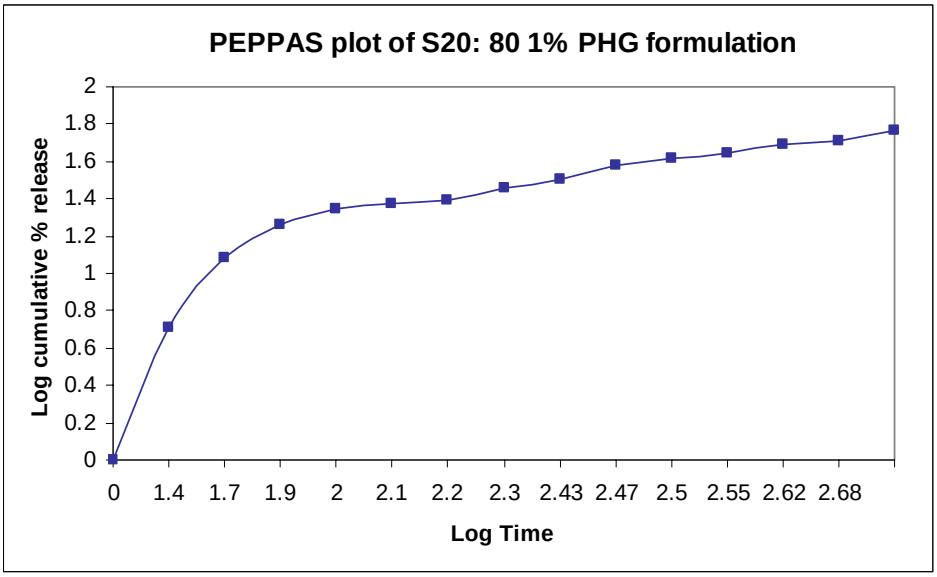
Regression coefficient r^2 = 0.9631

Figure 27:



Regression coefficient r^2 = 0.998953

Figure 28:



Regression coefficient $r^2 = 0.994415$

Slope = 0.893036

Ex In-vivo studies:

Institutional animal ethics committee (PSG institute of medical sciences and research) has grant approval for animal usage Reg No: 158 / 1999 / CPCSEA on 5th January, 2009.

The permeation of Proniosomal hydrocortisone gel was determined by Franz (vertical) diffusion cell using excised Rat skin as membrane mounted on the receptor compartment with the stratum corneum side facing upwards into the donor compartment. A weighed amount of proniosome hydrocortisone gel was applied on the stratum corneum facing upwards in to the donor compartment. The receptor compartment was filled with pH 7.4 phosphate buffer saline 15 ml medium to maintain a sink condition. Sampling was regularly done at predetermined time. The available diffusion area of the cell was 1.5 cm^2. The receptor medium was stirred by a magnetic bead. Samples withdrawn were analyzed using spectrophotometer at 248 nm. (2) (1).

Table 23:

Release comparison of 1 % PHG formulations using diffusion cell in rat skin

S.No.	TIME in MINS	Cumulative % release of 1 % proniosome formulations					
		S20:40	S20:60	S20:80	S20:T40	S20:T60	S20:T80
1	30	0.327633	0.063457	0.382986	0.107913	0.480116	0.194063
2	60	0.799167	0.196063	0.544992	0.32732	0.977059	0.506851
3	90	0.734416	0.419974	1.151976	0.358749	1.175676	0.402032
4	120	0.755971	0.711137	1.304584	0.398208	1.323362	0.570925
5	150	0.866504	0.913062	1.675758	0.543355	1.304275	0.278961
6	180	0.972812	1.072137	2.051329	0.543355	1.368349	0.455085
7	210	1.101193	1.747837	1.956747	0.557331	1.260123	0.30653
8	240	1.586693	2.054864	1.801293	0.58577	1.185863	0.253478
9	270	2.895675	2.045725	1.805087	0.663854	1.407808	0.267071
10	300	3.634488	1.81414	1.722144	1.217256	1.467223	0.199174
11	330	3.934445	2.404052	1.585314	1.161666	1.291099	0.130824
12	360	4.576692	2.70901	1.722144	1.112855	1.123041	0.107913
13	420	6.197356	3.185199	3.420319	1.667544	2.093634	0.197053
14	480	6.696738	3.29168	4.524788	1.639105	2.589951	0.114275
15	540	7.093174	3.70467	5.336887	1.690454	2.923911	0.068454

Figure29:

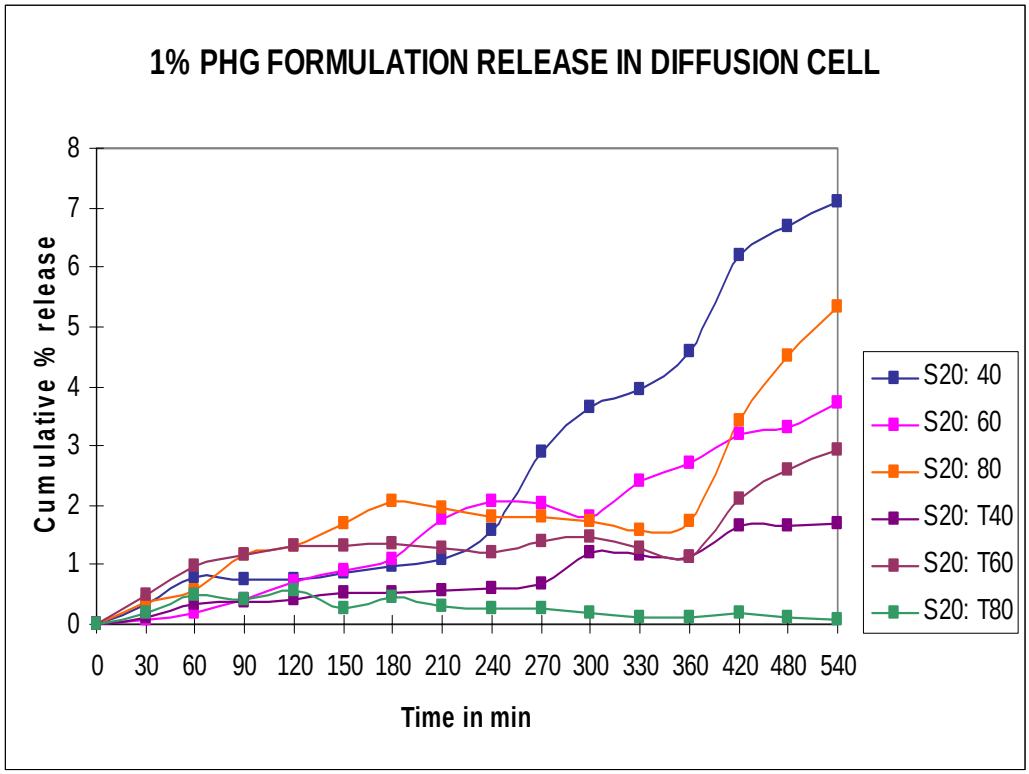
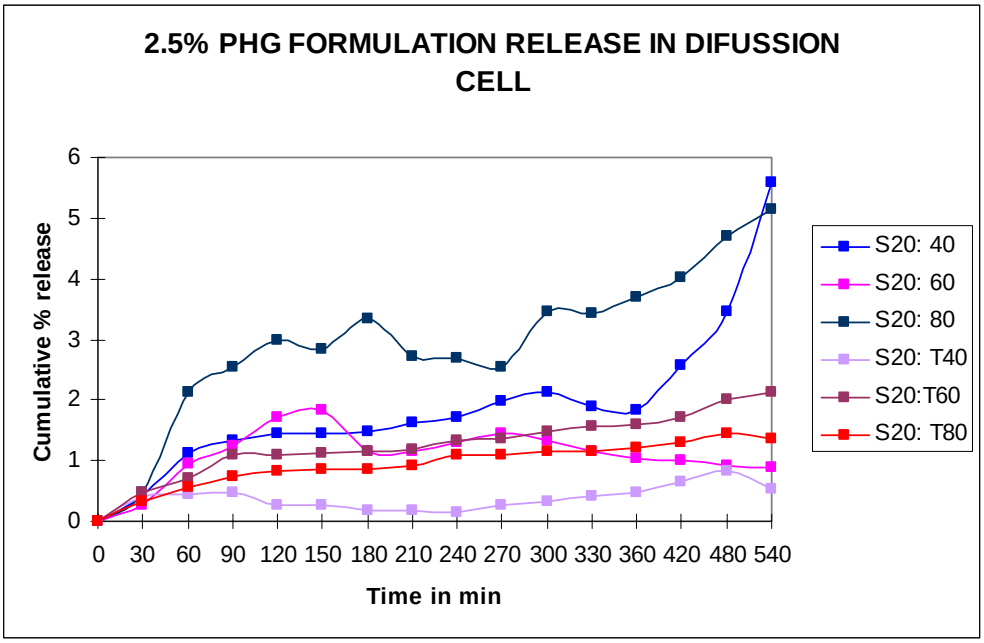

1% PHG FORMULATION RELEASE IN DIFFUSION CELL
Cumulative % release
0
1
2
3
4
5
6
7
8
0 30 60 90 120 150 180 210 240 270 300 330 360 420 480 540
Time in min
S20: 40
S20: 60
S20: 80
S20: T40
S20: T60
S20: T80

Table 24:

Release comparison of 2.5 % PHG formulations using diffusion cell in rat skin

S.No.	TIME in MINS	Cumulative % release of proniosome formulations					
		S20:40	S20:60	S20:80	S20:T40	S20:T60	S20:T80
1	30	0.399195	0.264521	0.461273	0.409229	0.482654	0.338376
2	60	1.13387	0.932806	2.114787	0.431305	0.710961	0.5493
3	90	1.339072	1.254835	2.527346	0.481368	1.095889	0.741103
4	120	1.450639	1.721109	2.988274	0.277258	1.105658	0.816232
5	150	1.462193	1.825348	2.823078	0.270443	1.125614	0.842967
6	180	1.482196	1.152752	3.349274	0.171987	1.154886	0.842967
7	210	1.612732	1.165426	2.709441	0.171569	1.176094	0.919765
8	240	1.716885	1.314844	2.696767	0.156307	1.334417	1.099296
9	270	1.970542	1.459089	2.544159	0.271313	1.373459	1.092934
10	300	2.12634	1.321138	3.467222	0.330693	1.478313	1.159962
11	330	1.90217	1.158011	3.434545	0.407803	1.577952	1.152349
12	360	1.826383	1.04334	3.693462	0.470834	1.582541	1.216804
13	420	2.564162	0.999023	4.009455	0.649043	1.713886	1.299999
14	480	3.467395	0.915994	4.696537	0.83	2.012629	1.43875
15	540	5.571921	0.886507	5.129013	0.543008	2.11651	1.366645

Figure 30:



Statistical analysis:

Comparison between the invitro and invivo results in S20:80 1 % proniosome hydrocortisone gel formulation was performed by analysis of variance (one way ANOVA with turkeys multiple camparision post test) with graph pad prism (version 3.0) software.

Anti inflammatory action:

Acute and chronic inflammation models were used to evaluate the anti-inflammatory activity, the study was carried out after the approval of animal ethical committee. Weighed mice range from 35 -49 g were used. In acute model carrageenan were used to induce inflammation in mice hind paw. 0.1ml of 1% carrageenan in normal saline was injected in to mice paw. In this three groups of animals were used. (18).

Figure 31: Injecting carrageenan to induce inflammation in mice paw

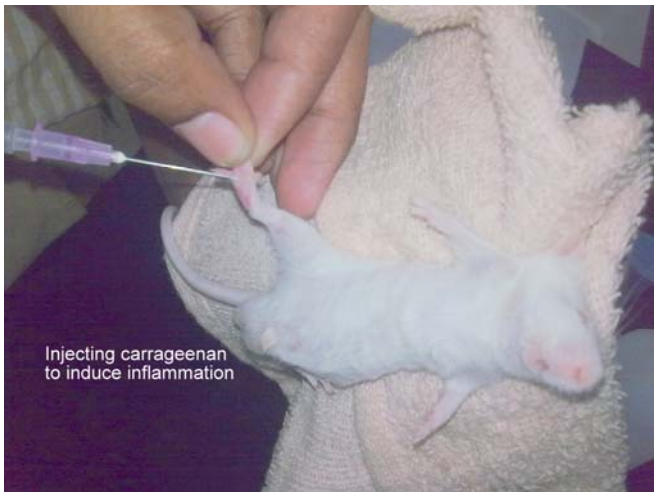


Table 25: **Animal Group Type**

S.No	Group Type	No. of Animals
1	I	1 mice
2	II	3 mice
3	III	3 mice

Group I is used as control (0.1ml 1% carrageenan in normal saline). Group II received the marketed hydrocortisone cream 1% (100 mg). Group III received the proniosome hydrocortisone gel 1% (100 mg). The Anti inflammatory effect of marketed and Proniosomal formulations were determined by Screw gauge. The paw volume was measured initially and then at 1, 2, 3 and 4hr after the carrageenan injection.

Table 26: **Comparative Anti-inflammatory activity in Mice**

S.No.	Group Type	Animals in group	Paw size before inflammation(mm)	0 hr(mm)	1hr (mm)	2hr (mm)	3hr (mm)	4hr (mm)
1	I	1	2.65	3.5	4.7	5.0	5.1	4.0
2	II	1	3.05	3.90	3.64	3.6	3.45	3.4
3		2	2.75	3.55	3.67	3.46	3.3	3.25
4		3	2.80	3.60	3.38	3.2	3.1	3.1
5	III	1	3.25	4	3.72	3.29	3.1	3.05
6		2	2.90	3.75	3.84	3.6	3.05	2.8
7		3	2.64	3.49	3.55	3.28	3.09	2.75

Drug permeation testing in human volunteers:

Prick test: Anti inflammatory activity were evaluated in human volunteers using skin prick test. It was done for Control, Marketed formulation and Proniosomal formulation.

Skin prick testing is most commonly performed on the forearm. The pronisome hydrocortisone gel 1% and hydrocortisone cream 1% marketed formulation was applied twice a day in occlusion conditions at marked area. After 24 hr. occlusion dressing was removed and the arm was cleaned, then a drop of commercially-produced allergen (histamine) is placed onto a marked area of skin. Using a sterile lancet, a small prick through the drop is made. This allows a small amount of allergen to enter the skin.

The histamine wheal suppression was measured using scale. The ability of histamine wheal suppression of proniosome hydrocortisone gel 1% was compared with marketed hydrocortisone cream 1% and control. (26)

Table 27: **Histamine Wheal Size using prick test in Human Volunteers**

S.No.	Formulation Type	Histamine wheal size			
		Sample I (mm)	Sample II (mm)	Sample III (mm)	Average mean (mm)
1	Control	4	5	4	4.6
2	Marketed hydrocortisone 1%	4.5	6	4.5	5
3	PHG (S20:80 1 %)	4	6	5	5

RESULTS AND DISCUSSION:

Hydrocortisone is a synthetic corticosteroid drug which may be given by injection or by topical application. It is given by topical application for its anti-inflammatory effect in allergic rashes, eczema and certain other inflammatory conditions. Hydrocortisone is available in creams, lotions for topical application but it is affected by number of pharmacokinetic parameters like drug absorption ,plasma half life ,plasma protein binding.(22)

Standard curve:

Spectrum of hydrocortisone was determined by double beam UV visible spectrophotometer in the spectrum mode range from 400 nm to 200nm with appropriate dilution of standard stock solution.

Standard plot for hydrocortisone was carried out in spectrophotometry at 248 nm by different concentrations of stock solution like 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 µg/ml.

Pre formulaton:

Proniosomes a novel drug delivery system for topical application was investigated in this study. Proniosome hydrocortisone gel formulations with non ionic surfactants combination were prepared by coacervation-phase separation method after optimizing the non-ionic surfactant ratio for vesicle formation in different combination of surfactants in Table 4.(2)

Formulation:

After Pre formulation studies non-ionic surfactant ratio 1:9 shows better vesicle formation than the 1:1and 9:1. Hence Proniosome Hydrocortisone gel with different non ionic surfactants in 1:9 ratio containing 1% and 2.5% Hydrocortisone with Soya lecithin was formulated.

Determination of vesicle size:

Vesicle size of proniosome hydrocortisone gel formulations was determined using optical microscope. Vesicle formation was good in S20:80 1 % (PHG 3) combination showed in (fig.11) and poor in S20: T80 2.5 % (PHG) combination showed in fig. 22. (2).

Vesicle formation and size was good in S20:80 (fig 11) and poor in S20:T80 (fig 16) in 1 % PHG formulations.

Vesicle formation and size was good in S20: 40 (fig 17) and poor in S20:T80 (fig 22) when comparing in 2.5 % PHG formulations.

Vesicles obtained from Span combination formulations was found in the size range 3 – 6 μm and Span, Tween combination formulations 4 – 7 μm.

Encapsulation efficiency:

Encapsulation of Proniosomal hydrocortisone gel prepared using different surfactant combinations was determined by ultra centrifugation.

The percentage entrapment of hydrocortisone gel varies from 27 – 89 %.

Entrapment was high in S20: 40 (89.67 %) and poor in S20:T80 (27.2 %) in 1 % proniosome hydrocortisone gel. And in 2.5 % proniosome hydrocortisone gel entrapment was found high in S20:40 (83.55 %) and poor in S20: T80 (57.34 %). This shows the entrapment of S20:40 are high and S20: T80 are poor in both 1 % and 2.5 % formulations (Table 6).

Among the 12 PHG formulations entrapment efficiency was high in S20:40 1 % (89.67 %) and poor in S20:T80 1 % (27.20 %). (Table 6)**.**

High entrapment in Span combinations may be due to hydrophobic nature of surfactants and drug. (2). (5)

Drug content uniformity:

Drug content of proniosome hydrocortisone gel was calculated (equation 3) and percentage drug content each formulation was given in Table 7.

Drug content of proniosome hydrocortisone gel shows 1 % PHG formulations having uniform distribution than in 2.5 % PHG formulations, but drug concentration is more in 2.5 % PHG formulations.

In Vitro release:

Drug release from proniosome gel was determined using dialysis membrane. Proniosome hydrocortisone gel formulation was compared with marketed 1 % hydrocortisone cream. The initial hour release from marketed formulation was found to be high when compared with PHG 1 %. But the cumulative percent release from 1 % hydrocortisone cream was not proper and linear with respect to time when compared to 1 % PHG formulation.

Maximum 34.7 % of drug was found to be release from marketed 1 % cream through dialysis membrane in 2 hr. where as 58 % of drug was found to be release from (S20: 80 1 %) PHG formulation showed extend release up to 8 hr. Cumulative release comparison for 1 % PHG formulation with marketed 1 % hydrocortisone cream was given in Table 21 and fig 23.

The cumulative release from 2.5 % PHG formulation shows sustained release similarly as 1 % PHG. But here good release was shown from S20:40 (53.81 %) and poor from S20: 80 (29.05 %). Table 22 and fig. 24

In 1 % PHG formulation S20: 80 (58.29%) shows good release and S20: 60 (35.06 %) shows poor release. Comparative release was shown in table 21 and fig. 23

Cumulative release in Span combinations S20:80 1 % (58.29 %) shows high and S20: 80 2.5 % (29.05 %) was poor. And from Span, Tween combinations S20: T40 2.5 % was high and S20: T60 2.5 % (31.23 %) was poor.

In vitro results show S20:80 combinations with 1 % drug concentration 58.29 ± 0.626 having good release than other formulations.

Release Kinetics:

Release kinetic parameters of proniosome hydrocortisone gel 1 % (S20:80) was carried out using zero order, first order, Higuchi and Peppas kinetics.

Regression value of PHG 1 % (S20:80) was 0.99833 for zero order and 0.9631 in first order. Results show the formulation obeys mixed order kinetics.

The Higuchi plot value for proniosome hydrocortisone gel 1 % (S20:80) was more than 0.998. Hence it follows diffusion release mechanism. The slope value of Peppas plot was 0.8930 which confirms non fickian diffusion type shown in fig. 25, 26, 27, and 28.

Ex *in vivo* studies:

Ex invivo release study was carried out in vertical Franz diffusion cell. The releases from all the formulations were found to be low through rat skin. But it shows linearity in release with respect to time.

The cumulative percent release of 1 % PHG formulations through rat skin was given in Table 23 and fig 29.

The cumulative percent release of 2.5 % PHG formulations through rat skin is given in Table 24 and fig 30. This shows S20: 40 1 % (7.09 %) is high and S20: T80 2.5 % is poor. (1). (5)

The lower amount of drug release through rat skin may be due to lower adsorption and fusion of proniosome onto surface of skin and the lipid bilayers of proniosomes does not show rate-limiting membrane barrier in this study.

Anti inflammatory action:

Anti inflammatory action of proniosome hydrocortisone gel 1% PHG 3 (S20: 80 1 %) was compared with the marketed topical 1% hydrocortisone cream formulation and control in mice. Measurements of inflammated mice paw before treatment and after treatment with respect to time intervals up to 4 hr. Table 6.

Anti inflammatory study results shows, the marketed formulation was good in initial hours later the activity got reduces their. But in proniosome hydrocortisone gel formulation the activity was less in initial hours but later it shows promising anti inflammatory action than in marketed after 4 hr.

When comparing with control, marketed 1 % hydrocortisone and proniosome hydrocortisone gel 1 % (S20: 80) for anti inflammatory activity significance was $P < 0.001$. When comparing with marketed hydrocortisone 1 % and proniosome hydrocortisone gel 1 % (S20: 80) the significance was $P < 0.05$.

DRUG PERMEATION STUDIES IN HUMAN VOLUNTEERS:

Skin prick test:

The formulation which has showing promising results in invivo study was taken for prick test in human volunteers.

Drug permeation and anti-inflammatory action of PHG 3 (S20:80 1 %) formulation was carried out in human volunteers. To check the drug permeability and histamine wheal suppression activity skin test was carried out in 3 volunteers as a mock trail. Comparison between marketed hydrocortisone cream 1 % with PHG (S20: 80 1 %) formulation and alcohol as control was carried out. The wheal suppression was high with alcohol followed by proniosome formulation when compared with marketed formulation. (26)

This study can extend in more no. of volunteers after getting clearance from human ethical committee and after incorporating penetration enhancers in the formation.

Conclusion:

The proniosome hydrocortisone gel 1% and 2.5% was prepared by using various surfactant combinations by coacervation phase separation method. The *in vitro* permeation of different formulations containing mixture of non-ionic surfactants have been studied and evaluated. The cumulative release from (S20: 80 1 %) PHG was 58.92 ± 0.627.

Proniosome hydrocortisone gel shows diffusion release type which was confirmed by Higuchi and Peppas plot. Comparison of Proniosome formulation with marketed 1 % hydrocortisone cream, proniosome formulation shows better cumulative release and anti inflammatory activity than in marketed formulation. Phospholipids and non-ionic surfactants in an optimum ratio in the Proniosomes may act as penetration enhancers, which are useful for increasing the permeation of hydrocortisone through skin.

Reference:

1. Yi-Hung Tsai., Yaw-Bin Huang., (2001) In vitro skin permeation of estradiol from various proniosome formulations. *International Journal of Pharmaceutics 215 91–99*
2. Ankur Gupta., Sunil Kumar Prajapati., (June 2007) Design and Development of a Proniosomal Transdermal Drug Delivery System for Captopril. *Tropical Journal of Pharmaceutical Research 6 (2): 687-693*
3. Almira. I. Blazek-Welsh., avid G. Rhodes., (2001) Maltodextrin-Based Proniosomes *AAPS Pharmsci; 3 (1) article 1*
4. Bhavana Vora, Ajay J. Khopade, N.K. Jain., (1998) Proniosome based transdermal delivery of levonorgestrel for effective contraception *Journal of Controlled Release 54 149–165*
5. Ibrahim A. Alsarra., A.A. Bosela., (2005) Proniosomes as a drug carrier for transdermal delivery of ketorolac *European Journal of Pharmaceutics and Biopharmaceutics 59: 485–490*
6. Ajay B. Solanki., Jolly R. Parikh., 2007 Formulation and Optimization of Piroxicam Proniosomes by 3-Factor, 3-Level Box-Behnken Design. *AAPS PharmSciTech; 8 (4) Article 86*
7. H.M. El-laithy., M.I. Tadros., (2008) Sucrose stearate-based proniosome-derived niosomes for the nebulisable delivery of cromolyn sodium *International Journal of Pharmaceutics xxx (2008) xxx–xxx*

8. S.P. Vyas., R.P. Singh, Sanyog Jain., Vivek Mishra., (2005) Non-ionic surfactant based vesicles (niosomes) for non-invasive topical genetic immunization against hepatitis B. *International Journal of Pharmaceutics 296 80–86*

9. Prem N. Gupta., Vivek Mishra., Amit Rawat., Praveen Dubey., Sunil Mahora, Sanyog Jain., D.P. Chatterji., Suresh P. Vyasa., (2005), Non-invasive vaccine delivery in transfersomes, niosomes and liposomes: a comparative study. *International Journal of Pharmaceutics 293: 73–82*

10. Suk-Jae Chung., Min-Hwa Lee., Chang-Koo Shim., (1997). In vitro skin permeation of nicotine from proliposomes. *Journal of Controlled Release 49: 177–184*

11. Choi M.J., H.I. Maibach., (2005) Liposomes and Niosomes as Topical Drug Delivery Systems. *Skin Pharmacol Physiol;18:209–219*

12. Agarwal. R., Katare. O.P., Vyas. S.P., (2001) Preparation and in vitro evaluation of liposomal/niosomal delivery systems for antipsoriatic drug dithranol. *International Journal of Pharmaceutics 228: 43–52*

13. B.W. Barry., (2001) Novel mechanisms and devices to enable successful transdermal drugv delivery (review)European Journal of Pharmaceutical Sciences 14: 101–114

14. Jia-You Fang.,Chi-Tzong Hong., (2001) Effect of liposomes and niosomes on skin permeation of enoxacin. *International Journal of Pharmaceutics 219: 61–72*

15. Chengjiu Hu., David G. Rhodes., (1999) Proniosomes: A Novel Drug Carrier Preparation. *International Journal of Pharmaceutics 185: 23–35*

16. entjurcb M. S., K. Vrhovnika, J. Kristla., (1999) Liposomes as a topical delivery system: the role of size on transport studied by the EPR imaging method. *Journal of Controlled Release 59: 87–97*

17. Işık Sarıgüllü Özgüney., Hatice Yeşim Karasulu., (2006) Transdermal Delivery of Diclofenac Sodium Through Rat Skin From Various Formulations *AAPS PharmSciTech; 7 (4) Article 88*

18. M Gupta., UK Mazumder., V Thamil Selvan., (2006) Anti-inflammatory evaluation of leaves of Plumeria acuminate. *BMC Complementary and Alternative Medicine* , 6:36doi:10

19. Robert c. Scott., Paul h. dugard., john D. Ramsey., Christopher Rhodes., (1987) In- vitro absorption of some o-phthalate diesters through human and rat skin. *Environmtntal health perspectives vol. 7., 223-227*

20. BIJU. S. S., SUSHAMA TALEGAONKAR., (January 29, (2009)) Vesicular systems: An overview [Downloaded free from http://www.ijpsonline.com on Thursday, January 29, 2009]

21. Hamidreza Moghimi, Abdollah Momajjad., An In-Vitro Iontophoretic Permeation Study of Nicotine through Rat Skin. *: hrmoghimi@yahoo.com*

22. Micro encapsulation methods and industrial applications volume 158, second edition, 563-585

23. Therapeutic drugs by Collin Dollery second edition

24. U.S. Pharmacopeia Asian edition 2005

25. Www. Wikepedia.com

26. Hand book pharmaceutical Excipients

27. Pandit JK., Bharathi D., (2007) Long acting ophthalmic formulation of indomethacin: evaluation of alginate gel systems, *Indian journal of Pharmaceutical Sciences,* volume 60 , 37- 40