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DESIGN AND EVALUATION OF VESCICULAR EMULSOME CONTAINING GEL FOR RHEUMATOID ARTHRHITIS

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Abstract
Rheumatoid arthritis (RA) is a chronic autoimmune disease affecting the joints, characterized by progressive symmetrical inflammation of the affected joints, leading to cartilage destruction, bone erosion and disability. Quercetin is one of the most important bioflavonoids known for its anti-inflammatory, anti-hypertensive, vasodilator, anti-obesity, anti-cholesterol and anti-atherosclerosis effects. The use of the drug is largely limited by its low hydrophilicity, so the present study improves its solubility by adding ethanol. Local use of high concentrations of ethanol is not recommended due to the risk of local irritation. Therefore, a suitable quercetin carrier for effective topical application is necessary for the treatment of rheumatoid arthritis. The use of quercetin is largely limited due to its low hydrophilicity. The aim of this study is to investigate the possible use of emulsomes to deliver quercetin in the treatment of rheumatoid arthritis.Emulsomes provide an effective topical drug delivery system due to the high retention flux and high skin retention of the drug, resulting in increased antifungal activity and reduced skin irritation. The characterization of the prepared emulsomes was performed using PDI index, zeta potential measurement, capture efficiency, etc. The pH, extrudability, dispersibility, roughness, viscosity, adhesion efficiency, etc. of the emulsome gel were checked.Emulsomes containing phosphatidylcholine (soy lecithin) cholesterol and or solid lipid were prepared and optimized for lipid ratios.. The solubility of the drug in 0.1N HCl and phosphate buffer pH 7.4 was found to be 0.0276 and 0.0094 mg/ml in different solvent respectively. The results of gel formulation having pH range 7.2 to 7.8 are desirable to skin pH, since they do not interfere with the physiology of skin.
Keywords: Emulsomes, Joints, Disease, Autoimmune, Disease

1. Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease affecting the joints. It is characterized by a progressive symmetric inflammation of affected joints resulting in cartilage destruction, bone erosion, and disability. While initially only a few joints are affected, in later stages many joints are affected and extraarticular symptoms are common. With a prevalence

ranging from 0.4% to 1.3% of the population depending on both sex (women are affected two to three times more often than men), age (frequency of new RA diagnoses peaks in the sixth decade of life), and studied patient collective (RA frequency increases from south to north and is higher in urban than rural areas), RA is one of the most prevalent chronic inflammatory diseases[24].

Clinically, the symptoms of RA significantly differ between early stage RA and insufficiently treated later stages of the disease. Early stage RA is characterized by generalized disease symptoms such as fatigue, flu-like feeling, swollen and tender joints, and morning stiffness; and is paralleled by elevated levels of C-reactive protein (CRP) and an increased erythrocyte sedimentation rate (ESR). In contrast, insufficiently treated RA displays a complex clinical picture with the occurrence of serious systemic manifestations such as pleural effusions, lung nodules and interstitial lung disease, lymphomas, vasculitis in small or medium-sized arteries, keratoconjunctivitis, atherosclerosis, hematologic abnormalities (e.g., anemia, leukopenia, neutropenia, eosinophilia, thrombocytopenia, or thrombocytosis), joint misalignment, loss of range of motion, bone erosion, cartilage destruction, and rheumatic nodules Taken together, these systemic manifestations caused by the chronic inflammatory state in RA patients result in an increased mortality[39].

1.1 Development of Rheumatoid Arthritis:

While the cause of RA is unknown, both genetic and environmental factors were shown to contribute to RA development. As it is hypothesized for other autoimmune diseases, it is likely that the initial establishment of RA requires two separate events:

(1) Genetic predisposition of the respective patient resulting in the generation of auto reactive T and B cells, and

(2) Triggering event, such as viral and bacterial infections or tissue injury, providing the activated Antigen-presenting cells (APCs) to activate the previously generated auto reactive lymphocytes, resulting in disrupted tolerance and subsequent tissue/organ destruction. Therefore, RA likely develops in genetically predisposed individuals due to a combination of genetic variation, epigenetic modification, and environmental factors initiated by a stochastic event (e.g., injury or infection). Risk factors for the development of RA were reported to include smoking, obesity, exposition to UV-light, sex hormones, drugs, changes in micro biome of the gut, mouth, and lung, periodontal disease (periodontitis), and infections. Among these factors, the link between periodontal diseases and RA development is especially interesting[12].

1.2. Emulsomes:

Emulsomes is a novel lipoidal vesicular system with an internal solid fat core surrounded by a phospholipid bilayer. Emulsome is an advance nanocarrier technology for poorly aqueous soluble drugs. It possess both emulsion and liposomes features. Emulsome represents lipid-based drug delivery systems with broad variety of therapeutic applications particularly for drugs that are poor aqueous soluble. Emulsomes consist of microscopic lipid assembly with a polar core, which contains water insoluble drugs in the solution form without requiring any surface active agent or co-solvent. Beside the other vesicular formulations, emulsomes are much stabilized and nano range vesicles. It is a new emerging delivery system and therefore could play a fundamental function in the effective treatment of life-threatening viral infections and fungal infections such as hepatitis, HIV, Epstein-Barr virus, leishmaniasis, etc. appear promising for the treatment of visceral leishmaniasis specifically and hepato-splenic candidiasis. Emulsomal formulations composed of solid lipid core material and stabilized by cholesterol and soya lecithin. The drug is loaded followed by sonication to produce emulsomes of small size. The polymer used for core material should be solid at room temperature (25°C). The high soya lecithin concentration stabilized the emulsomes in form of O/W emulsion. These fat cored lipid particles are dispersed in an aqueous phase. These systems are often prepared by melt expression or emulsion solvent diffusive extraction[7].

Emulsomes area promising approach for the formulation of drug compounds with poor aqueous solubility with varied oral bio availability. Theoral delivery of hydrophobic drugs can be made possible by emulsomes, which have been shown to substantially improve oral bioavailability. Renaissances in the use of emulsomes over the past few decades are inviting increasing attention. Recent trends are focused on the development of modified emulsomal solid or semisolid formulations as an alternative to the conventional liquid system. The development of emulsomes, however, is still largely empirical, and in vitro models that are predictive of oral bioavailability enhancement are lacking. There is aneed for in vitro methods for predicting the dynamic changes involving the drug in thegutinorder to monitor the solubilisation state of the drug *in vivo*. Attention also needsto be paid to the interactions between lipid systems and thepharmacologically active substance. The characteristics ofvarious lipid formulations also need to be understood, sothat guidelines can be established that allow identificationof suitable candidate formulations at an early stage. Futureresearch should involve human bioavailability studies aswell as more basic studies on the mechanisms of action of this fascinating and diverse group of formulations.

Quercetin is one of the important bioflavonoids present in more than twenty plants material and which is known for its anti-inflammatory, antihypertensive, vasodilator effects, ant obesity, anti-hypercholesterolemic and anti-atherosclerotic activities. Free-radical are one of the key factors for the development of the diseases such as hypertension, vascular disorders, and metabolic syndrome. Drug has been limited by its poor percutaneous permeation and skin deposition. The application of drug is greatly restricted by its low hydrophilicity, thus present investigation improve its solubility by adding ethanol. Nanoliposomes have several advantages as drug carriers, such as high solubility, better stability and enhancement of cellular uptake. The topical application of a high concentration of ethanol is not advisable because of the risk of local irritation. Therefore, an appropriate carrier of quercetin for efficient topical application is required for treatment of rheumatoid arthrhitis. RA is a progressive symmetric inflammation of affected joints resulting in cartilage destruction, bone erosion, and disability. While initially only a few joints are affected, in later stages many joints are affected and extraarticular symptoms. Rheumatoid arthritis (RA) is the rheumatism mainly manifested as disabling joint disease and mainly involves hands, wrists, feet and other small joints. Recurrent arthritis attacks, synovial cell hypertrophy and hyperplasia and bone and cartilage damages eventually lead to joint dysfunction and other complications, and there is no cure. Quercetin (QU) is a kind of natural flavonoids, with lipid lowering, anti-inflammatory and other pharmacological activities, and minor toxic side effects. Thus, we assume that QU may be an adjuvant natural drug for treatment of RA**.** Application of Qu is greatly restricted by its low hydrophilicity. Many researchers have attempted to improve its solubility by adding ethanol. Topical application of a high concentration of ethanol is not advisable because of the risk of local irritation. The present study is to investigate the potential application of emulsomes for delivery of Quercetin to treat Rheumatoid arthritis[18].

2.MATERIAL AND METHODS

2.1. Spectrophotometric methods: The drug samples (Quercetin) use for determination of absorption maxima (λmax) in various solvents i.e. pH 7.4 phosphate buffer.

2.1.1. Identification of maximum wavelength (λ max): The Quercetin drug sample was studied for determination of absorption maxima (λmax) in pH 7.4 phosphate buffer solution. UV absorption spectroscopy of drug was carried out using UV–VIS scanning spectrophotometer (Shimadzu UV-1800, Japan). A solution of Quercetin containing the concentration 10 µg/ml was prepared in pH 7.4 phosphate buffer. The UV absorption spectra were recorded using pure drugs (conc. of 10 µg/ml) and absorption peaks were determined. 10 mg of drug was dissolved in 10 ml of dissolution medium in 10 ml volumetric flask with the help of sonication in bath sonicator for 15 min to obtain 1000 µg/ml solution. Now, from the resulting solution 1 ml was kept and

diluted up to 100 ml with same solvent separately with the aid of sonication for 15 min to obtain 10 µg / ml solution. The spectrum of these solutions was run in 200 – 400 nm range in double beam UV spectrophotometer (Shimadzu, UV-1800).

2.1.2. Preparation of calibration curve

2.1.2.1. Preparation of Standard Stock Solution: 10 mg of drug was dissolved in 10 ml of phosphate buffer pH 7.4 as dissolution medium in 10 ml volumetric flask with the help of sonication in bath sonicator for 15 min to obtain 1000 µg/ml solution.

2.1.2.2. Preparation of Working Standard Solution: The prepared solution was known as Stock-1. 1ml amount was withdrawn from stock-1 and diluted up to 10 ml separate volumetric flask. From this solution 0.5, 1.0, 1.5, 2.0 and 2.5 ml solutions were transferred to 10ml volumetric flasks and make up the volume up to 10 ml with diluting medium, thus give concentration of drug solution of 5, 10, 15, 20, 25 µg/ml concentrations of drug. These solutions were analyzed at 217 nm by double beam ultraviolet spectrophotometer.

2.2. Pre-formulation studies of drug sample:

The drug samples will be studied for organoleptic properties, microscopic examination. The physical characteristics with density, particle size, flow properties, compatibility, solubility in various dissolution medias, partition coefficient and drug-excipients compatibility study done.

2.2.1. Physical appearance: The physical appearance of drug was noted by visual study for identification of color, odor, taste and microscopic characteristics.

2.2.2. Melting point: The melting point of drug was determined by capillary tube method. The drug was milled upto fine powder and it was filled in glass capillary tube. The capillary tube was previously sealed with one end. The capillary tube was placed in light paraffin oil bath and thermometer was placed in melting point apparatus, the melting temperature of drug sample was noted.

2.2.3. Loss on drying: Loss on drying was determined by accurately weighing 1.0 g of the drug and drying at 105^{o}C for three hours. It lost 0.005 gm of its initial weight.

2.2.4. Solubility determination: The solubility measurements were performed according to as drug was carried out in 0.1 N HCl (pH 1.2) and phosphate buffer pH 7.4. The saturated solutions of drug sample were prepared in screw capped tubes by adding excess amount of drug to the medium and shaking on the wrist action shaker for 48 h at 25 ± 0.5°C. The drug solution was filtered after this period, diluted and drug content was analyzed by double beam ultraviolet spectrophotometer at 217 nm to calculate the solubility of quercetin.

2.2.5. Drug-excipients compatibility studies (FT-IR Spectroscopy): The Infra-red spectroscopy of the sample was carried out to ascertain identity of the drug. The pellet is much thicker than a liquid film, hence a lower concentration in the sample is required (Beer's Law). A pellet of approximately 1.0 mm diameter of drug was prepared by compressing 3-5 mg of the drug with 100-150 mg of potassium bromide in KBr press (Model M-15, Techno Search Instruments). The pellet was mounted in IR compartment and scanned between wave number 4000 – 400 cm^{-1} using Bruker Alpha FT-IR Spectrophotometer. The compatibility of drug with excipients was assessed by FTIR spectroscopy. Drug and excipients were mixed physically and stored at 40 °C and 75% RH for one month. After this period of time IR spectra were recorded to assess the compatibility of the drugs and mixture.

2.3. Preparation of emulsomes:

Emulsomes were prepared by lipid film hydration method. By using rotary vacuum evaporator with modifications. Drug: LC: CHL ratio was altered and vesicle size and drug entrapment efficiency were studied. Briefly, a chloroform: ethanol (2:1) mixture of different ration of drug: LC: CHL evaporator under vacuum at 400±0.5°C to form a lipid film on the wall of a round bottom flask. The resulting lipid film was then hydrated with PBS (pH 7.4) for 2 hours at 37.0±0.5°C. The preparation was sonicated at 40°C in 3 cycles of 30 sec. and rest of 2 minutes between each cycle by using probe sonicator. The formulation was homogenized at 10,000 psi

pressure in 3 cycles using high-pressure homogenizer to get emulsomes.

2.4. Evaluation of emulsomes:

2.4.1. Vesicle size determination: Vesicle size was determined using the particle size analyzer (Malvern Master Sizer).

2.4.2. Entrapment efficiency: Drug was estimated in emulsomes by ultra centrifugation method. Emulsomal suspension was transferred to 10 ml centrifuge tube. This suspension was diluted with distilled water up to 5 ml and centrifuged at 2000 rpm for 20 minutes. By this we can separate undissolved drug in the formulation. Suitable volume of the protamine solution was added to the resulting supernatant and retained for 10 minutes. Emulsomes were aggregated in presence of protamine and then separated by ultra centrifugation at 15,000 rpm for 20 minutes. Supernatant and sediment were separated out. Volumes of the supernatant and sediment were measured. Sediment was diluted with distilled water up to 5 ml. The unentraped and entrapped drug contents were analyzed by estimating drug in supernatant and emulsomes (sediment) by calibration curve method using U.V. Vis. Spectroscopy at 214 nm. (Shimadzu UV-1800).

2.4.3. Transmission Electron Microscopy: Surface morphology was determined by TEM, for TEM a drop of the sample was placed on a carbon-coated copper grid and after 15 min it was negatively stained with 1% aqueous solution of phosphotungustic acid. The grid was allowed to air dry thoroughly and samples were viewed on a transmission electron microscopy (TEM Hitachi, H-7500 Tokyo, Japan).

2.5. Preparation of nano-liposomal gel:

The required quantity of drug containing emulsomes (equivalent to 100 mg), methyl paraben, glycerine and polyethylene glycol were dissolved in 30 ml of water in a beaker. All were stirred at high speed by using mechanical stirrer.Now carbopol 940 and xanthan gum were added slowly in given amount to the beaker containing liquid during continuous stirring.The triethanolamine (act as gelling agents) was added slowly during stirring to attain gel structure.The prepared gel base was finally transferred to aluminium collapsible tubes and labelled accordingly required.

2.6. Evaluation of emulsomal gel formulation:

2.6.1. Physical appearance: The physical appearance of prepared nano-liposomal was visually checked as parameters i.e. colour, appearance and feel on application.

2.6.2. pH determination: The pH of nano-liposomal gel formulations were determined by using the digital pH meter. 1 gram of liposomal gel was dissolved in 100 ml distilled water and stored for two hours. pH electrodes were completely dipped into the formulations and pH was noted. The measurement of pH of each formulation was done in triplicate manner and average values were calculated.

2.6.3. Extrudability determination: The nano-liposomal gel formulations were filled into collapsible metal tubes. The tubes were pressed with same pressure by using fingers and the extrudability of the formulations was checked. The extrudability of the formulation was determined in terms of weight in grams required to extrude a 0.5 cm ribbon of gel in 10 seconds.

2.6.4 Viscosity determination: The viscosity of the prepared nano-liposomal gel formulations was measured by Brook field viscometer. The sufficient quantity of gel base was filled in wide mouth jar separately and it should sufficiently allow dipping the spindle. The RPM of the spindle was adjusted to 2.5 RPM. The viscosities of the formulations were recorded.

2.6.5. Spreadability: Spreadability means the extent of area to which nano-liposomal gel readily spreads on application to skin or affected part of skin. The therapeutic potency of a formulation also depends upon its spreading value. Spreadability of formulation is expressed in terms of time in seconds taken by two slides to slip off from gel base, which is placed in between the slides under the direction of certain load.

Lesser the time taken for the separation of two slides, better the spreadability. It is calculated by

using the formula

S = M * L / T

where, M = Weight tied to upper slide

L = Length of glass slide

T = Time taken to separate the slides

2.6.6. Stability studies: The stability studies were carried out for all the prepared nano-liposomal gel formulations by freeze – thaw cycling. Here, by subjecting the formulations to a temperature of 4^o C, 25^o C, 40^o C for one month and syneresis was observed. After stored in given temperature, the gel is exposed to ambient room temperature and liquid exudate separating is noted[23].

2.6.7. Homogeneity: The nano-liposomal gel formulations have been set in the container; all prepared gels were tested for homogeneity by visual inspection. They were tested for their appearance and presence of any lumps, flocculates or aggregates.

2.6.8. Grittiness: The liposomal gel formulations were evaluated microscopically for the presence of any appreciable particulate matter under light microscope. The preparation should free from particles and the grittiness of any topical preparation can check.

2.6.9. Drug content studies: The nano-liposomal gel (equivalent to 100 mg) was taken and dissolves in 10 ml of phosphate buffer pH 7.4. The volumetric flasks were kept for 2 h and shaken well in a shaker to mix it properly. The solution was passed through the Whatman filter paper and filtrates were analyzed for drug content spectrophotometrically at 320 nm against corresponding gel concentration as blanks[34].

2.7.10. In Vitro Diffusion Studies: Before experiment, the cellophane membrane was washed in the running water and then soaked in distilled water for 24 h to remove glycerine present on membrane. The release of nanoliposomal gel was studied by dialysis method in pH 7.4 artificial skin pH. 2 ml samples were instilled in the dialysis bag which was screwed with two clamps at each end. The dialysis bag was dipped into the receptor compartment containing 35 ml of dissolution medium and stirred continuously at 100 rpm. The donor compartment was kept in contact with a receptor compartment and the temperature was maintained at 37±0.5°C. The receptor compartment was closed to prevent evaporation of the dissolution medium. The solution on the receptor side was stirred by externally driven teflon coated magnetic bars. At predetermined time intervals, 5 ml of solution from the receptor compartment was pipette out and immediately replaced with fresh 5 ml phosphate buffer. Samples were withdrawn at regular time intervals, and the same volume was replaced with fresh dissolution medium. The samples were spectrophotometricially measured at 320 nm to determine drug release.

Calculation of percentage drug release was done using the formula:

% drug release = (Conc. of drug (in mg) x Volume of receptor compartment) x 100 / Label claim (amount of drug in donor compartment).

Zero order release kinetics: Zero order release kinetics refers to the process of constant drug release from a drug delivery device such as nano-liposomal gel, transdermal systems, matrix tablets with low-soluble drugs and other delivery systems. In its simplest form, zero order release can be represented as:

$Q = Q0 + K0\ t$

where Q is the amount of drug released or dissolved (assuming that release occurs rapidly after the drug dissolves), Q0 is the initial amount of drug in solution (it is usually zero), and K0 is the zero order release constant. The plot made was cumulative % drug release vs time (zero order kinetic models).

First order release kinetics: The rate laws predicted by the different mechanisms of dissolution both alone and in combination, have been discussed by Higuchi.

$\text{Log } C = \text{Log } C_0 - kt / 2.303$

where, C_0 is the initial concentration of drug and K is first order constant. The equation in

resemblance to the other rate law equations, predicts a first order dependence on the concentration gradient (i.e. C_s - C_t) between the static liquid layer next to the solid surface.

3. RESULTS AND DISCUSSION

3.1 Determination of λ_{max}:

Quercetin was identified using different methods viz. melting point determination, determination of absorption maxima (λmax), loss on drying, and FTIR spectroscopy. The physical appearance of the quercetin was found as a yellow powder. Absorption maxima (λ_{max}) of quercetin was found to be at wavelength 217 nm corresponding to the values reported in literature (217 nm). A calibration curve of quercetin was prepared in phosphate buffer pH 7.4 and data was subjected to linear regression analysis. The linearity was found to be obeyed in the concentration range of 5-25 µg/ml in the media, r-values were found to be 0.998 in 0.1N and followed Beer and Lambert's law.

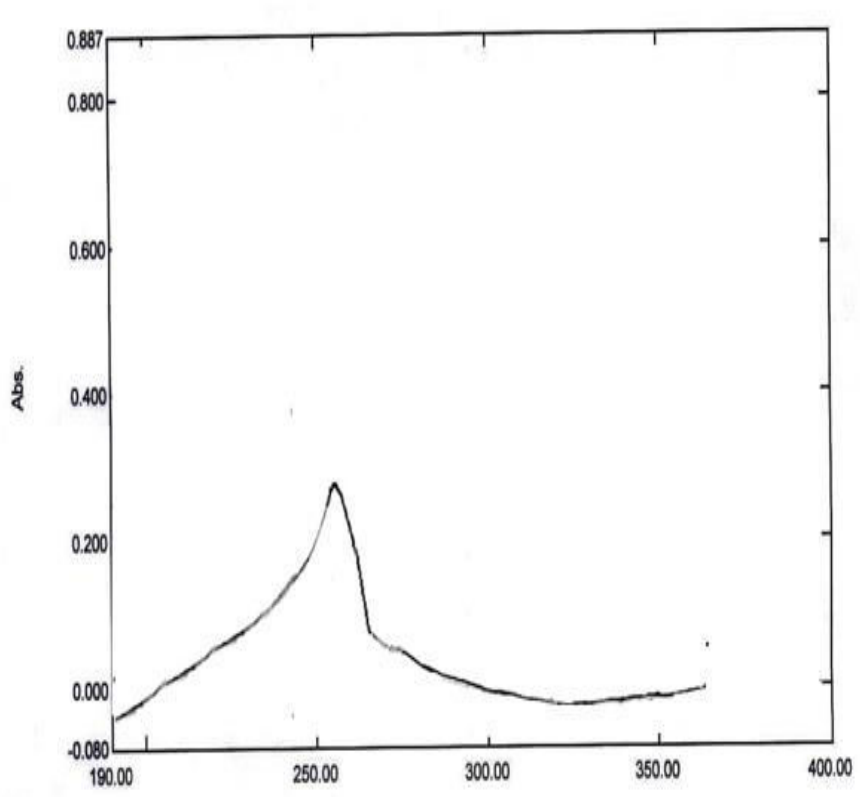

Figure 3.1: Determination of maximum wavelength λ_{max}

S. No.	Concentration (µg / ml)	Absorbance
1	0	0
2	5	0.117
3	10	0.236
4	15	0.352
5	20	0.462
6	25	0.594

Table 3.1: Calibration curve of drug in phosphate buffer pH 7.4

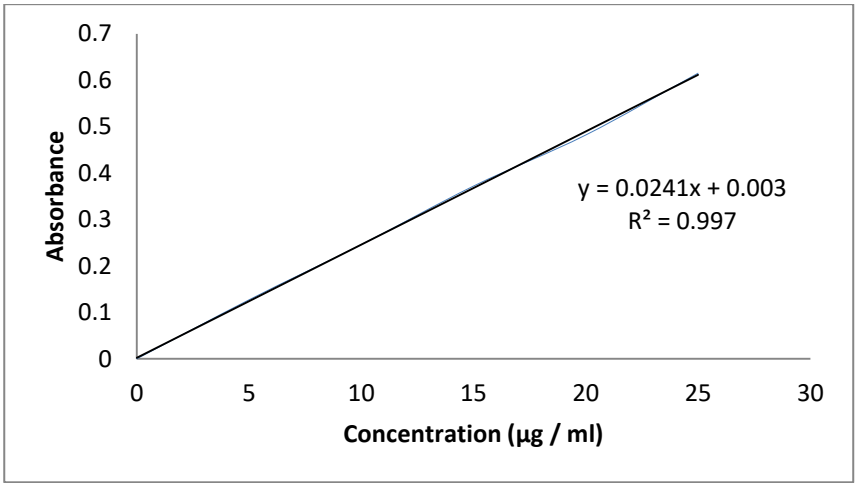

Figure 3.2: Calibration Curve of Quercetin in phosphate buffer pH 7.4

S.No.	Parameter	Quinapril hydrochloride
1	Linearity Range	5-25 µg/ml
2	Regression Equation	Y= 0.0241x+0.003
3	Correlation Cofficient	0.997

Table 3.2: Stastical Data For Linearty

3.2 Pre-formulation studies of drug sample:

3.2.1 Determination of Melting Point:

Melting point of drug was found to be 317^{0}C, which comes under the range (315-320^{0}C) as given in reference. The loss on drying for drug was found to be 0.5% (limit 1.0 %). The solubility of quercetin was determined in different media.

S. No.	Values	Physical appearance	Melting point (^{0}C)	Loss on drying (%)
1.	Observed	llow powder	7	

Table 3.3: Comparative values of different parameters used to identify the drug.

The drug was found to be freely solublein both the selected media. The solubility of the drug in 0.1N HCl and phosphate buffer pH 7.4 was found to be 0.0276 and 0.0094 mg/ml in different solvent respectively.

S. No.	Solubility (mg/ ml)	
	0.1 N HCl (pH 1.2)	Phosphate buffer pH 7.4
1	0.0276	0.0094

Table 3.4: Solubility of drugin various medium

FTIR spectra showed that the drug is compatible with all the excipients studied as no changes in the peaks were noted. FTIR spectrum of the quercetin was indicated that the characteristics peaks belonging to measure functional groups The result of FTIR drug complex study was confirmed that, their was no interference between both materials. The formulation has no interaction.

S. No.	Reported Peaks (cm^{-1})	Observed Peak (cm^{-1})	Inference
1	1745-1696	1741	-C=O (Carbonyl)
2	1680-1620	1639	C=C stretching (Alkenyl)
3	1650-1590	1618	N-H bending (Primary amine)
4	1360-1310	1345	C-N stretching (Aromatic tertiary amine)
5	1350-1260	1290	O-H in plane bending
6	680-610	640	C-H bending (Alkyne)

Table 3.5: Interpretation of FTIR spectrum of pure Quercetin

S. No.	Reported Peaks (cm^{-1})	Observed Peak (cm^{-1})	Inference
1	2900-2880	2885	C-H stretching (Methylene)
2	1680-1620	1650	C=C stretching (Alkenyl)
3	1405-1465	1445	C-H bending (Methyl)
4	895-885	890	C-H bending
5	800-700	760	C-Cl stretching(Aliphatic Chloro Compound)

Table 3.6.: Interpretation of FTIR spectrum of quercetin and excipients

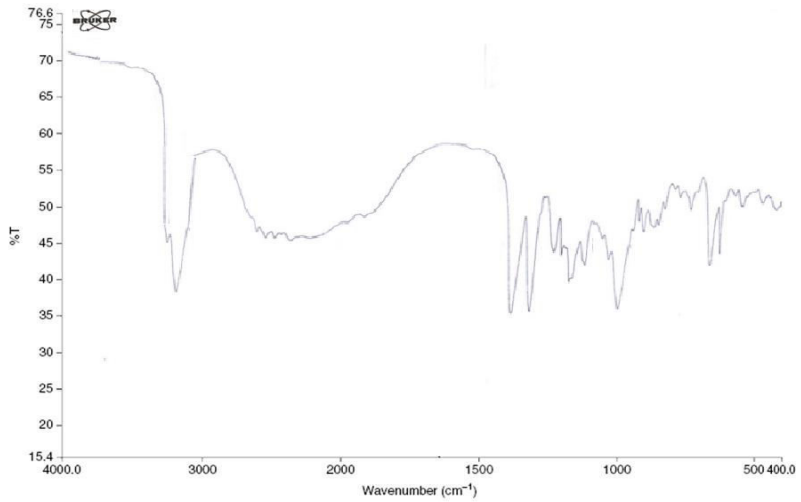

Figure 3.3: FTIR Spectrum of quercetin

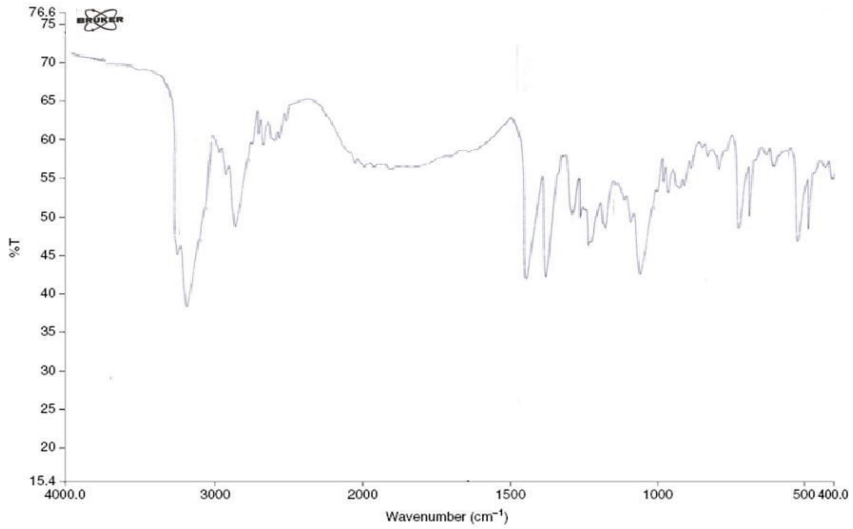

Figure 3.4: FTIR Spectra of physical mixture of Quercetinand excipients

The characterization of emulsomes of various different liposomal formulations were developed and in order to optimize factorial design was utilized wherein 2^3 design was employed for screening of significant formulation and process variables involved in the development of emulsome which is 2 level 3 factor design. Independent variable selected amount of lecithin, amount of cholesterol and rotation speed at high and low levels. Dependent variables were size, zeta potential, Entrapment efficiency and polydispersity index. The vesicle size of all emulsomal formulations prepared as per the experimental design varied between vesicle size 152 and 319 nm, Zeta potential between -27.1 to 35.8 mV and entrapment efficiency between 95.43±1.48 to 99.10±1.52 %.

The prepared emulsomes (QEM1 – QEM4) were evaluated with respect to zeta potential, entrapment efficiency, size and polydispersity index were calculated and on the basis of results the formulation QEM3 with lower level of lecithin, cholesterol and rotation speed was selected as optimized formulation since the results for the dependent variables were in close agreement to the attributes needed for the developed formulation. Lower size of the emulsomes is needed since they are to be entrapped into emulsomal gel and the lower size would also be beneficial for the localization of the active constituents into the deeper layer of the skin particularly to target the causative agent for acne. The high zeta potential is indicative of the stability of formulation and the liposomal vesicles. The entrapment efficiency of the QEM3 formulation was higher as compared to the other formulations. The polydispersity index was lower as compared to the other formulations. Hence on the basis of results obtained from the study and above discussion QEM3 was selected for transmission electron microscopy and as optimized formulation.

F. Code	**Vesicle size (nm)**
QEM1	161.8
QEM2	251.3
QEM3	152.2
QEM4	319.3

Table 3.7: Evaluations of vesicle size QuercetinEmulsomes formulations

F. Code	**Zeta Potential** (mV)
QEM1	-33.5
QEM2	27.1
QEM3	-35.8
QEM4	30.6

Table 3.8: Evaluations of zeta potential Quercetinemulsomes formulations

F. Code	**Entrapment efficiency (%)**
QEM1	96.73±0.93
QEM2	95.43±1.28
QEM3	99.10±1.11
QEM4	96.27±1.19

Table 3.9: Evaluations of entrapment efficiency Quercetinemulsomes formulations

F. Code	**Polydispersity Index (PDI±SD)**
QEM1	0.311
QEM2	0.129
QEM3	0.121
QEM4	0.539

Table 3.10: Evaluations of Polydispersity index quercetinemulsomes formulations

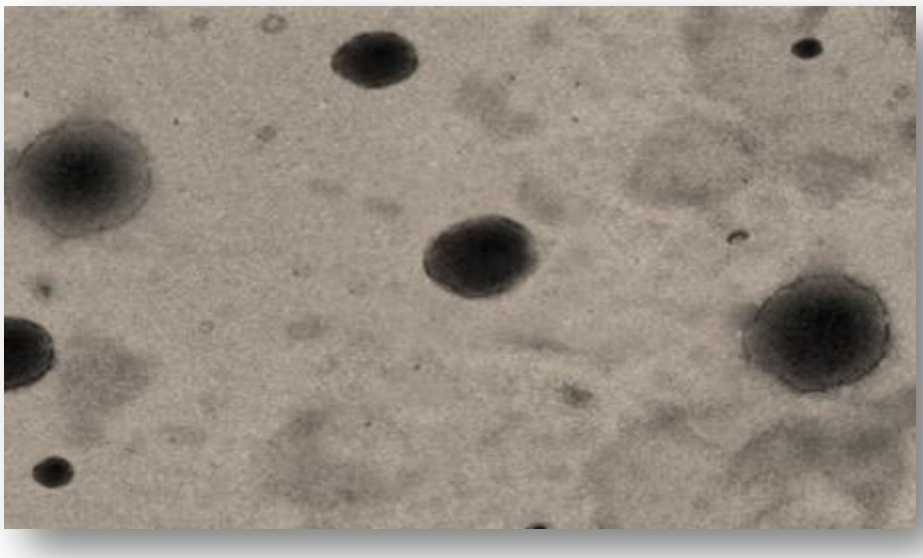

Figure 3.5: TEM image of prepared emulsomes

Characterization of emulsomal gel formulation: The main ingredient of the emulsomalemulgel formulation is the gelling agent from all required chemicals. The concentration of viscosity enhancer or gel former is of huge value as a less concentration will lead to simple solution or lotion with very low consistency. The high concentration of liposomal emulgel forming agent may lead to formation of gels with high viscosity leading to non –

uniform distribution of drug and showed problem with handling of gel.
On the basis of results obtained from the study QEM3 was selected as optimized formulation.

S. No.	Batch No.	Drug (mg)	Lecithin (mg)	Cholesterol (mg)	Stirring Speed (rpm)
1	QEM1	100	80	20	200
2	QEM2	100	60	40	200
3	QEM3	100	80	20	100
4	QEM4	100	60	40	100

Table 3.10: Optimization formulation composition of Emulsomes

Formulation	Lecithin (mg)	Cholesterol(mg)	Speed(rpm)
QEM3	80	20	200

Table 3.11:Composition of optimized Emulsomes formulation

The prepared emulsomal gels containing 0.5 % and 1 % of carbopol 940 form a very thin gel that liquefies within 4 and 5 hours of after preparation respectively. The prepared emulsomal gel containing 1.5 % carbopol 940 gelling agent to some extent better gel was obtained but the problem of liquefaction after 24 hours was observed. When the gel base formulated with 2 % of carbopol 940 showed uniform and smooth gel and does not liquefy upon keeping long time interval. The pH of the formulation was determined in order to be sure that the formulation can be used without the risk of irritancy to the skin. The pH was found to be 7.54 for G4 gel base formulation, which was very nearer to the neutral skin pH, thus the formulation G4 can be used without the risk of irritancy to the skin. The spread ability of formulations was found to decrease with increasing the concentration of gelling agent. The value of spread ability for optimized gel base was found to be 10.1 cm indicating that the gel is easily spreadable by small amount of shear stress. The result indicated that the formulation can be applied easily without being excess stress. This indicate that the formulation maintain a good wet contact time, when applied to the site of application. As the result of all evaluation parameters, 2 % of carbopol 940 was selected as the optimized concentration of gelling agent and this gel formulation is used for further gel preparations.

The physical evaluation as the colour of prepared emulsomal gels was pale yellow in colour and appearance of gel was translucent in nature and smooth on application at skin. The individual properties such as consistency of fomulations were good and texture of prepared herbal hair gel was found to be smooth. The pH value of the prepared gel formulation was observed at room temperature and valued range at 7.2 to 7.8. The literature informed that from epidermis to dermis, pH of the skin increases and attained the neutral value. The results of gel formulation having pH range 7.2 to 7.8 are desirable to skin pH, since they do not interfere with the physiology of skin.

Formulation	QFG1	QFG2	QFG3	QFG4	QFG5
Emulsomes (g) **(QEM3)**	1	1	1	1	1
Carbopol 940 (g)	1.0	1.5	2	2.5	3.0
Xanthan gum (g)	2.0	1.5	1.0	0.5	0
Propyl paraben sodium (mg)	75	75	75	75	75
Glycerine (ml)	5	5	5	5	5
Triethanolamine (ml)	5	5	5	5	5

Table 3.11: Different composition of emulsomal gel formulation

Parameters	Formulations				
	QFG1	**QFG2**	**QFG3**	**QFG4**	**QFG5**
Colours	Pale yellow colour	Pale yellow colour	Pale yellow colour	Pale yellow colour	Pale yellow colour
Appearance	Translucent	Translucent	Translucent	Translucent	Translucent
Odour	Pleasant odour	Pleasant odour	Pleasant odour	Pleasant odour	Pleasant odour
Spreadability (g.cm/sec)	10.7	10.4	10.1	10.3	10.4
Homogeneity	Good	Good	Good	Good	Good
Feel of application	Smooth	Smooth	Smooth	Smooth	Smooth
Consistency	Good	Good	Good	Good	Good
pH	7.8	7.6	7.2	7.7	7.3
Viscosity (cps)	0.97	0.99	1.15	1.09	0.96
Extrudibility	Excellent	Excellent	Excellent	Excellent	Excellent
Stability	Stable	Stable	Stable	Stable	Stable

Table 3.12: Evaluation of emulsomal gel formulation

The prepared emulsomal gel formulations were evaluated or stored to accelerated stability testing. All prepared emulsomal gels were stored at 4^0 C, 25^0 C and 45^0 C in refrigeration, room temperature and stability chamber for a period of 30 days to study effect of temperature and at different humidity condition. The physical parameters were evaluated during study period. The result of the study indicates that the preparation is physically stable at all temperatures during storage period.

Time (min)	Viscosity (cps)				
	QFG1	**QFG2**	**QFG3**	**QFG4**	**QFG5**
0	0.79	0.81	0.89	0.95	0.77
20	0.81	0.84	0.89	0.96	0.82
40	0.84	0.87	0.92	0.98	0.84
60	0.85	0.89	0.94	1.01	0.86
80	0.88	0.91	0.95	1.06	0.89
100	0.91	0.93	0.99	1.09	0.91
120	0.92	0.96	1.01	1.11	0.93
140	0.97	0.99	1.09	1.15	0.96

Table 3.13: Viscosity of emulsomal gel

S. No.	Formulation	Drug content (%)
1	QFG1	97.37
2	QFG2	98.25
3	QFG3	99.43
4	QFG4	98.12
5	QFG5	97.98

Table 3.14: Drug content in formulations

Time	QFG1	QFG2	QFG3	QFG4	QFG5
0	0	0	0	0	0
1	3.21	1.12	0.541	0.677	1.12
2	4.71	1.54	0.781	0.877	1.23
3	7.81	4.45	1.54	2.01	2.22
4	13.21	5.43	3.45	2.45	3.39
5	18.68	9.23	7.46	4.67	3.39
6	35.67	19.87	13.23	16.46	14.5
7	66.34	52.34	48.34	49.87	58.45
8	76.54	64.21	58.34	59.03	74.23
9	88.74	74.34	69.87	71.23	86.46
10	91.12	78.89	71.67	73.23	91.23
11	95.37	82.1	81.26	81.23	93.6
12	99.99	99.68	99.21	99.25	99.34

Table 3.15: in-vitro drug diffusion study emulsomal gel

Time	√Time	Log time	Cummulative % drug released	Log cummulative % drug released	Cummulative % drug retained	Log cummulative % drug retained
0	0	#NUM!	0	#NUM!	100	2
1	1	0	3.21	0.506505032	96.79	1.98583049
2	1.414214	0.30103	4.71	0.673020907	95.29	1.979047327
3	1.732051	0.477121	7.81	0.892651034	92.19	1.964683815
4	2	0.60206	13.21	1.120902818	86.79	1.938469688
5	2.236068	0.69897	18.68	1.271376872	81.32	1.91019737
6	2.44949	0.778151	35.67	1.552303109	64.33	1.808413551
7	2.645751	0.845098	66.34	1.821775467	33.66	1.527114112
8	2.828427	0.90309	76.54	1.883888458	23.46	1.370328008
9	3	0.954243	88.74	1.948119424	11.26	1.051538391
10	3.162278	1	91.12	1.959613711	8.88	0.948412966
11	3.316625	1.041393	95.37	1.979411783	4.63	0.665580991
12	3.464102	1.079181	99.99	1.999956568	0.01	-2

Table 3.16: in-vitro drug diffusion study of emulsomal gel QFG1

Time	√Time	Log time	Cummulative % drug released	Log cummulative % drug	Cummulative % drug	Log cummulative % drug

				released	**retained**	**retained**
0	0	#NUM!	0	#NUM!	100	2
1	1	0	1.12	0.049218023	98.88	1.995108458
2	1.414214	0.30103	1.54	0.187520721	98.46	1.993259831
3	1.732051	0.477121	4.45	0.648360011	95.55	1.980230691
4	2	0.60206	5.43	0.73479983	94.57	1.975753389
5	2.236068	0.69897	9.23	0.965201701	90.77	1.957942335
6	2.44949	0.778151	19.87	1.298197867	80.13	1.903795143
7	2.645751	0.845098	52.34	1.718833718	47.66	1.678154038
8	2.828427	0.90309	64.21	1.80760267	35.79	1.553761698
9	3	0.954243	74.34	1.871222557	25.66	1.409256652
10	3.162278	1	78.89	1.897021956	21.11	1.324488233
11	3.316625	1.041393	82.1	1.914343157	17.9	1.252853031
12	3.464102	1.079181	99.68	1.998608029	0.32	-0.494850022

Table 3.17: in-vitro drug diffusion study of emulsomal gel QFG2

Time	**√Time**	**Log time**	**Cummulative % drug released**	**Log cummulative % drug released**	**Cummulative % drug retained**	**Log cummulative % drug retained**
0	0	#NUM!	0	#NUM!	100	2
1	1	0	0.541	-0.266802735	99.459	1.997644088
2	1.414214	0.30103	0.781	-0.107348966	99.219	1.996594846
3	1.732051	0.477121	1.54	0.187520721	98.46	1.993259831
4	2	0.60206	3.45	0.537819095	96.55	1.984752278
5	2.236068	0.69897	7.46	0.872738827	92.54	1.966329495
6	2.44949	0.778151	13.23	1.121559844	86.77	1.938369597
7	2.645751	0.845098	48.34	1.684306646	51.66	1.713154402
8	2.828427	0.90309	58.34	1.765966425	41.66	1.619719266
9	3	0.954243	69.87	1.844290743	30.13	1.478999132
10	3.162278	1	71.67	1.855337404	28.33	1.452246575
11	3.316625	1.041393	81.26	1.909876818	18.74	1.272769587
12	3.464102	1.079181	99.21	1.99655545	0.79	-0.102372909

Table 3.18: In-vitro drug diffusion study of emulsomal gel QFG

Time	**√Time**	**Log time**	**Cummulative % drug released**	**Log cummulative % drug released**	**Cummulative % drug retained**	**Log cummulative % drug retained**
0	0	#NUM!	0	#NUM!	100	2
1	1	0	0.677	-0.169411331	99.323	1.997049829
2	1.414214	0.30103	0.877	-0.057000407	99.123	1.996174438
3	1.732051	0.477121	2.01	0.303196057	97.99	1.991181758
4	2	0.60206	2.45	0.389166084	97.55	1.989227274
5	2.236068	0.69897	4.67	0.669316881	95.33	1.979229593
6	2.44949	0.778151	16.46	1.216429831	83.54	1.921894471
7	2.645751	0.845098	49.87	1.697839368	50.13	1.700097705

8	2.828427	0.90309	59.03	1.771072783	40.97	1.612465964
9	3	0.954243	71.23	1.852662944	28.77	1.458939862
10	3.162278	1	73.23	1.864689034	26.77	1.427648371
11	3.316625	1.041393	81.23	1.909716453	18.77	1.273464273
12	3.464102	1.079181	99.25	1.996730515	0.75	-0.124938737

Table 3.19: in-vitro drug diffusion study of emulsomal gel QFG4

Time	**√Time**	**Log time**	**Cummulative % drug released**	**Log cummulative % drug released**	**Cummulative % drug retained**	**Log cummulative % drug retained**
0	0	#NUM!	0	#NUM!	100	2
1	1	0	1.12	0.049218023	98.88	1.995108458
2	1.414214	0.30103	1.23	0.089905111	98.77	1.994625054
3	1.732051	0.477121	2.22	0.346352974	97.78	1.990250033
4	2	0.60206	3.39	0.530199698	96.61	1.985022082
5	2.236068	0.69897	3.39	0.530199698	96.61	1.985022082
6	2.44949	0.778151	14.5	1.161368002	85.5	1.931966115
7	2.645751	0.845098	58.45	1.766784515	41.55	1.618571028
8	2.828427	0.90309	74.23	1.870579461	25.77	1.411114419
9	3	0.954243	86.46	1.936815231	13.54	1.131618664
10	3.162278	1	91.23	1.960137675	8.77	0.942999593
11	3.316625	1.041393	93.6	1.971275849	6.4	0.806179974
12	3.464102	1.079181	99.34	1.997124156	0.66	-0.180456064

Table 3.20: in-vitro drug diffusion study of emulsomal gel QFG5

Formulation code	**Zero-order kinetics**		**First-order kinetics**	
	r^2	**k**	**r^2**	**k**
F1	0.983	9.38	0.825	-0.148
F2	0.983	9.135	0.655	-0.116
F3	0.972	9.113	0.611	-0.111
F4	0.893	9.086	0.621	-0.112
F5	0.945	10.14	0.783	-0.144

Table 3.21: Kinetic release study data

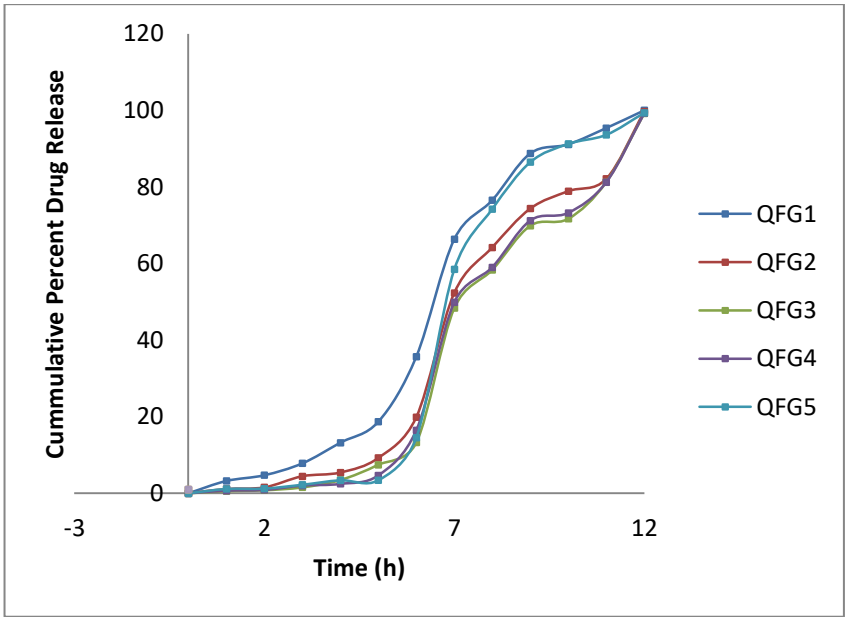

Figure 3.6: Zero - order kinetic plot (in-vitro drug diffusion study of emulsomal gel)

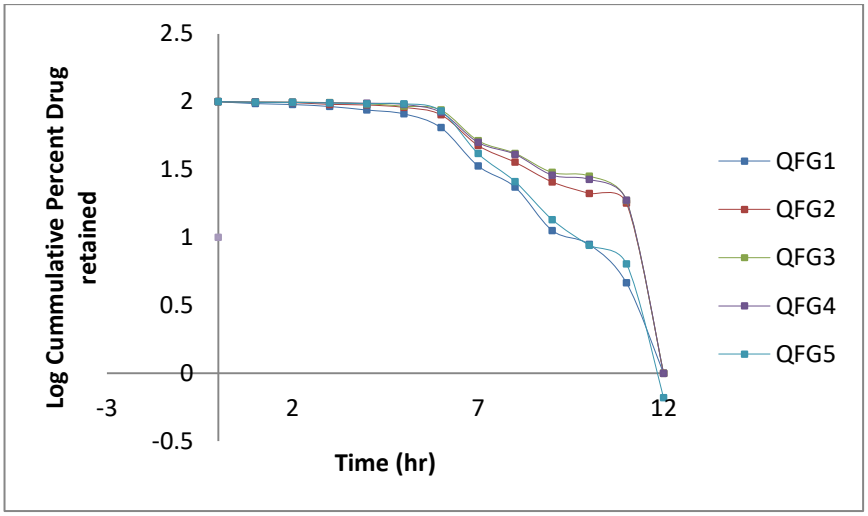

Figure 3.7: First - order kinetic plot (in-vitro drug diffusion study of emulsomal gel)

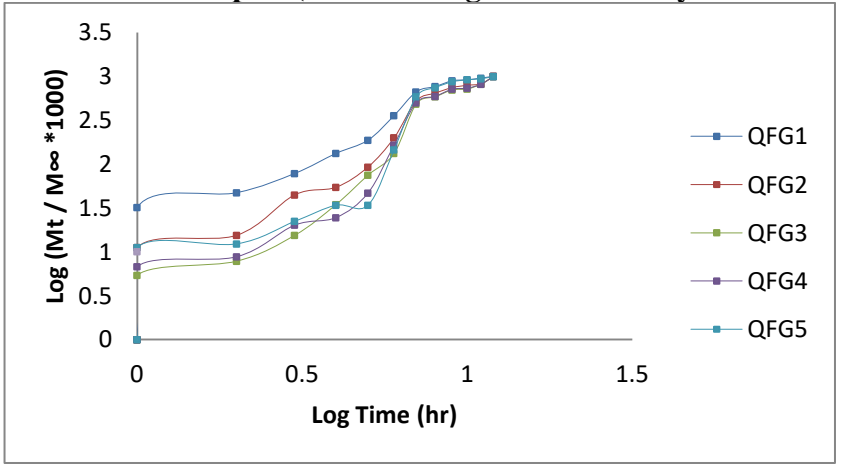

Figure 3.8: Korsmeyer-peppas kinetic plot (in-vitro drug diffusion study of emulsomal gel)

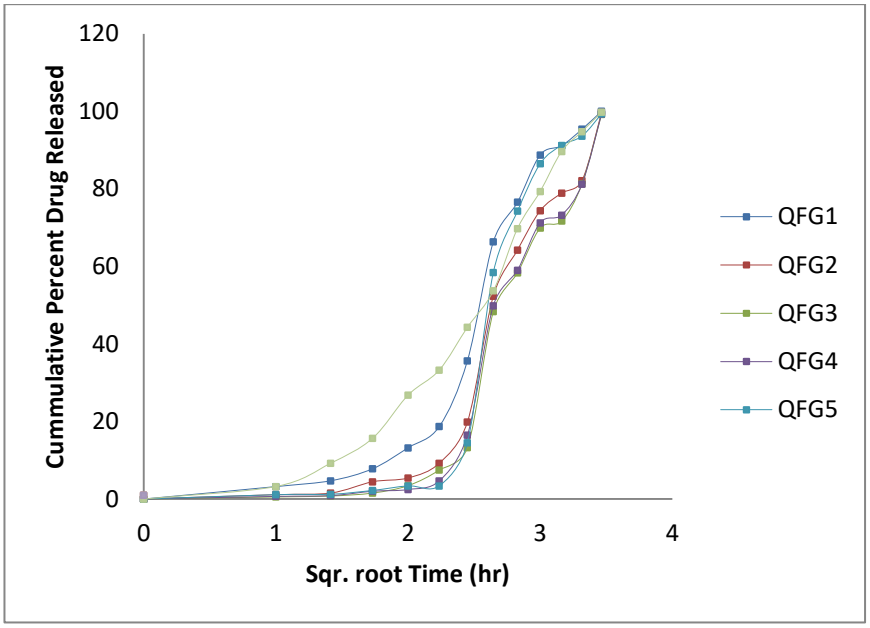

Figure 3.9: Higuchi kinetic plot (in-vitro drug diffusion study of emulsomal gel)

CONCLUSION

Emulsomes offers an effective topical drug delivery system owing to its potential of high retention flux as well as high skin retention of drug and hence enhanced antifungal activity and reduce skin irritation. Advantages of emulsomes are: protect medicament from harsh gastric environment, improve solubility and bioavailability of drug, reduce dose of drug, improve pharmacological activity, also provides sustained release of medicament by prolongs the release of medicament up to 24 hours and provide target specific drug delivery. Emulsomes containing phosphatidylcholine (soya-lecithine), cholesterol and either of the solid lipid were prepared and optimized for the lipid ratios. Hence it was desired to develop formulations which would be avoid the problems of toxicity and rapid elimination of drug.

Quercetin was identified using different methods viz. melting point determination, determination of absorption maxima (λmax), loss on drying, and FTIR spectroscopy. The physical appearance of the quercetin was found as a yellow powder. Absorption maxima (λ_{max}) of quercetin was found to be at wavelength 217 nm corresponding to the values reported in literature (217 nm). A calibration curve of quercetin was prepared in phosphate buffer pH 7.4 and data was subjected to linear regression analysis. The linearity was found to be obeyed in the concentration range of 5-25 µg/ml in the media, r-values were found to be 0.998 in 0.1N and followed Beer and Lambert's law. Melting point of drug was found to be 317^0C, which comes under the range ($315\text{-}320^0$C) as given in reference. The loss on drying for drug was found to be 0.5% (limit 1.0 %). The solubility of quercetin was determined in different media. The drug was found to be freely solublein both the selected media. The solubility of the drug in 0.1N HCl and phosphate buffer pH 7.4 was found to be 0.0276 and 0.0094 mg/ml in different solvent respectively. FTIR spectra showed that the drug is compatible with all the excipients studied as no changes in the peaks were noted. FTIR spectrum of the quercetin was indicated that the characteristics peaks belonging to measure functional groups The result of FTIR drug complex study was confirmed that, their was no interference between both materials. The formulation has no interaction. The characterization of emulsomes of various different liposomal formulations were developed and in order to optimize factorial design was utilized wherein 2^3 design was employed for screening of significant formulation and process variables involved in the development of emulsome which is 2 level 3 factor design. Independent variable selected amount of lecithin, amount of cholesterol and rotation speed at high and low levels. Dependent variables were size, zeta

potential, Entrapment efficiency and polydispersity index. The vesicle size of all emulsomal formulations prepared as per the experimental design varied between vesicle size 152 and 319 nm, Zeta potential between -27.1 to 35.8 mV and entrapment efficiency between 95.43±1.48 to 99.10±1.52 %. The prepared emulsomes (QEM1 – QEM4) were evaluated with respect to zeta potential, entrapment efficiency, size and polydispersity index were calculated and on the basis of results the formulation QEM3 with lower level of lecithin, cholesterol and rotation speed was selected as optimized formulation since the results for the dependent variables were in close agreement to the attributes needed for the developed formulation. Lower size of the emulsomes is needed since they are to be entrapped into emulsomal gel and the lower size would also be beneficial for the localization of the active constituents into the deeper layer of the skin particularly to target the causative agent for acne. The high zeta potential is indicative of the stability of formulation and the liposomal vesicles. The entrapment efficiency of the QEM3 formulation was higher as compared to the other formulations. The polydispersity index was lower as compared to the other formulations. Hence on the basis of results obtained from the study and above discussion QEM3 was selected for transmission electron microscopy and as optimized formulation. The main ingredient of the emulsomalemulgel formulation is the gelling agent from all required chemicals. The concentration of viscosity enhancer or gel former is of huge value as a less concentration will lead to simple solution or lotion with very low consistency. The high concentration of liposomal emulgel forming agent may lead to formation of gels with high viscosity leading to non – uniform distribution of drug and showed problem with handling of gel.

On the basis of results obtained from the study QEM3 was selected as optimized formulation.

The prepared emulsomal gels containing 0.5 % and 1 % of carbopol 940 form a very thin gel that liquefies within 4 and 5 hours of after preparation respectively. The prepared emulsomal gel containing 1.5 % carbopol 940 gelling agent to some extent better gel was obtained but the problem of liquefaction after 24 hours was observed. When the gel base formulated with 2 % of carbopol 940 showed uniform and smooth gel and does not liquefy upon keeping long time interval. The pH of the formulation was determined in order to be sure that the formulation can be used without the risk of irritancy to the skin. The pH was found to be 7.54 for G4 gel base formulation, which was very nearer to the neutral skin pH, thus the formulation G4 can be used without the risk of irritancy to the skin. The spreadability of formulations was found to decrease with increasing the concentration of gelling agent. The value of spreadability for optimized gel base was found to be 10.1 cm indicating that the gel is easily spreadable by small amount of shear stress. The result indicated that the formulation can be applied easily without being excess stress. This indicate that the formulation maintain a good wet contact time, when applied to the site of application. As the result of all evaluation parameters, 2 % of carbopol 940 was selected as the optimized concentration of gelling agent and this gel formulation is used for further gel preparations.

The physical evaluation as the colour of prepared emulsomal gels was pale yellow in colour and appearance of gel was translucent in nature and smooth on application at skin. The individual properties such as consistency of fomulations were good and texture of prepared herbal hair gel was found to be smooth. The pH value of the prepared gel formulation was observed at room temperature and valued range at 7.2 to 7.8. The literature informed that from epidermis to dermis, pH of the skin increases and attained the neutral value. The results of gel formulation having pH range 7.2 to 7.8 are desirable to skin pH, since they do not interfere with the physiology of skin.

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