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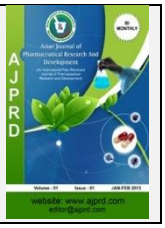

Open Access **Research Article**

Formulation and Evaluation of Phytosome for the Topical Drug Delivery

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A B S T R A C T

The aim of the present study was formulation and evaluation of phytosome for the topical drug delivery. Quercetin loaded phytosome were prepared by ether injection method. The prepared phytosome were further characterized for particle size, zeta potential, entrapment efficiency, drug content and in-vitro diffusion study.On the basis of criteria (the drug diffused at 4 Hr. not more than 20%, not less than 15%) the F2 batch is optimized and the optimized batch is incorporated in gel for further evaluation such as pH determination, drug content, viscosity and in-vitro diffusion study. From the in-vitro diffusion study we observed that the quercetin release from phytosomal gel by zero order kinetics which show controlled release of quercetin from phytosomal gel.

Keywords: Quercetin, Phytosome, Soya-lecithin, Psoriasis, Phytosomal gel, Topical delivery.

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INTRODUCTION

In recent years, topical drug delivery has garnered considerable interest due to its non-invasive nature, high bioavailability, and ability to deliver drugs directly to the target site, thereby avoiding hepatic first-pass metabolism and reducing gastrointestinal complications. This route is particularly advantageous for localized treatment of dermatological conditions, especially when systemic administration routes (oral, sublingual, rectal, or parenteral) are unsuitable. A broad spectrum of plant-derived herbal extracts has demonstrated therapeutic efficacy in managing skin disorders owing to their antimicrobial, anti-inflammatory, hemostatic, wound-healing, and burn-relieving properties. Common conditions treated include eczema, acne, urticaria, pruritus, psoriasis, and various infections. Topical formulations are generally categorized as either externally applied (to the skin surface) or internally applied (to mucosal membranes). Key benefits of this route include enhanced site-specific targeting, improved patient compliance, reduced systemic side effects, and suitability for drugs with short half-lives or narrow therapeutic windows. [1,2]

In recent years, there has been a growing global reliance on herbal treatments. Herbal medications are increasingly recommended due to their potential to reduce adverse effects. The utilization of herbal medicines and phytoconstituents has emerged as a promising approach for the management and treatment of various health conditions.[3] Herbal remedies possess a complex composition comprising numerous active constituents, which act synergistically to enhance their overall therapeutic efficacy.[4]The majority of biologically active constituents in plants are polar or water-soluble; however, their limited absorption hinders their effective utilization, ultimately reducing their bioavailability. To enhance bioavailability, herbal formulations must maintain an appropriate balance between hydrophilic properties, which facilitate absorption in gastrointestinal fluids, and lipophilic characteristics, which enable permeation across lipid biological membranes.[5]

Significant progress has been made in the past year in developing novel drug delivery systems (NDDS) for plant extracts and their active constituents. Targeted delivery systems enable site-specific and sustained drug release, enhancing therapeutic efficacy at lower doses.[6] Advancements in formulation technologies have enhanced drug delivery; however, effective administration is often hindered by the drug's physicochemical properties and

biological barriers such as the skin and organ membrane linings.[7] Novel herbal drug delivery systems employ various advanced formulations, including liposomes, phytosomes, pharmacosomes, niosomes, nanoparticles, microspheres, transferosomes, ethosomes, transdermal systems, and proniosomes.[8]

The term "phyto" denotes plant origin, while "some" implies a cell-like structure. Phytosomes, also known as herbosomes, are vesicular drug delivery systems designed to improve the absorption and bioavailability of poorly soluble compounds.[9]Phytosomes, structurally similar to liposomes, are advanced lipid-based carriers designed to enhance the absorption and bioavailability of polyphenolic phytoconstituents, particularly flavonoids, which exhibit poor oral bioavailability.[10] These vesicles form through hydrogen bonding between the polyphenolic components of herbal extracts and the phosphate groups of phospholipids in non-polar solvents.[11] The hydrophilic phytoconstituents bind to the polar head of phospholipids, while the lipophilic tails facilitate membrane permeation. This structure improves drug entrapment, reduces dosage requirements, and significantly enhances bioavailability. Phytosomes also improve skin penetration and offer better pharmacokinetic and pharmacodynamic profiles compared to conventional herbal formulations. Phosphatidylcholine, a key component, not only forms the vesicular structure but also offers therapeutic benefits such as hepatoprotection.[12]

Typically, phytosomes are prepared by combining active phytoconstituents with phospholipids like phosphatidylcholine (PC), phosphatidylserine (PS), or phosphatidylethanolamine (PE) in defined ratios under specific conditions. This nanotechnology holds great promise for improving the delivery and efficacy of herbal polyphenols in various therapeutic applications.[13]

Psoriasis is a chronic, immune-mediated dermatological condition affecting approximately 3% of the U.S. population and around 125 million individuals globally. It is characterized by accelerated skin cell proliferation, leading to the formation of red, scaly plaques, commonly on the scalp, lower back, elbows, and knees. Plaque psoriasis, the most prevalent form, accounts for over 80% of cases and presents as erythematous, scaly lesions primarily on extensor surfaces, but may also involve intertriginous areas, palms, soles, and nails.[14]

Quercetin is a polyphenolic flavonoid widely present in various edible plants and herbs. Major dietary sources include citrus fruits, apple skins, onions, garlic, parsley, sage, tea, grains, red wine, grapes, olive oil, dark cherries, and berries. It exhibits diverse biological activities, including chemopreventive, antioxidant, antiproliferative, anti-inflammatory, and antiallergic effects. Recently, quercetin has become commercially available as both a therapeutic agent and a dietary supplement.Quercetin exhibits potent anti-inflammatory and antiallergic effects by suppressing cytokine release, inhibiting mast cell and basophil degranulation, and reducing neutrophil and monocyte lysosomal secretion, leukotriene synthesis, and lipid peroxidation. It down regulates key enzymes in the eicosanoid pathway, including phospholipase A2, cyclooxygenase, and lipoxygenase, leading to decreased production of proinflammatory leukotrienes. Additionally, quercetin reduces T-helper 17 (Th17) cell production by modulating the MAPK–TLR4 signaling pathway, resulting in lowered levels of proinflammatory cytokines such as interleukin-17 (IL-17).[15]

MATERIALS AND METHOD

Materials

Quercetin was purchased from Yarrow-Chem. Mumbai, India. Soya lecithin was purchased from Shiva Biochem. Nandgaon, Khandeshwar. Diethyl-ether was obtained from Research-Lab Fine Chem. Industries, Mumbai. Other chemicals such as Ethanol, Triethanolamine, Propylene glycol and Carbopol 940 were supplied by S.D. Fine Chemicals, Mumbai.

UV Spectroscopy Study (Determination of ƛ max)

In order to ascertain the wavelength of maximum absorption of the drug, 10mg of quercetin was accurately weighed and transfer to 10ml volumetric flask. The drug was dissolved in phosphate buffer (pH 7.4) and the volume was made up to 10ml to obtain 1000ug/ml. From above solution take 1ml and dilute up to 10ml to obtain primary stock solution which is 100ug/ml. The resulting solution was scanned between 400-200nm against buffer solution as a blank. From solution having concentration 100 ug/ml aliquots of 0.5, 1.0, 1.5, 2.0 and 2.5ml were pipette out into 10ml volumetric flask. The volume was made up to the mark with buffer solution to get the final concentration of 5, 10, 15, 20 and 25 ug/ml. A graph of absorbance versus concentration was plotted. It show straight line meaning the calibration curve obeys Beers law.[16]

Method of Preparation for Quercetin Phytosome

The quercetin phytosome were prepared by using ether injection method. The quercetin phytosome were made by combining quercetin and soya-lecithin in organic solvent in 1:0.5, 1:1..and 1:2.5 molar ratios respectively. The drug were dissolved in ethanol and soya-lecithin dissolved in diethyl ether, the resultant solution are mixed and slowly inject drop by drop in aqueous phase with constant stirring. After the removal of organic solvent the tiny cell like sac are produce and phytosomal suspension was obtained.[17]

Table 1: Formulation table of quercetin phytosome

Sr. No.	Composition	F1	F2	F3	F4	F5
1	Drug	100mg	100mg	100mg	100mg	100mg
2	Soya-lecithin	125mg	250mg	375mg	500mg	625mg
3	Diethyl-ether	18ml	18ml	18ml	18ml	18ml
4	Ethanol	6ml	6ml	6ml	6ml	6ml
5	Phosphate Buffer	30ml	30ml	30ml	30ml	30ml

Characterization of the Prepared Quercetin Phytosome

Entrapment Efficiency

The phytosome suspension (equivalent to 10mg) was ultra-centrifuged at 2000 Rpm for duration of 30 min. The purpose of this process was to isolate the phytosome from the unentrapped extract. The concentration of the unbound drug in the supernatant was assessed through the utilization of a UV-Visible spectrophotometer, measuring the absorbance at a wavelength of 364nm.[18]

The Entrapment efficiency was calculated using following formula:

$$\% EE = \frac{\text{Total amount of drug} - \text{amount of free drug}}{\text{Total amount of drug}} \times 100$$

Drug Content

A precise amount of phytosomes containing 10 mg of quercetin was weighed and transferred into a volumetric flask with a capacity of 10 ml. The flask's contents were dissolved in a small quantity of phosphate buffer and subjected to sonication for a duration of 30 minutes. the drug content was quantified through spectrophotometric analysis utilizing a UV spectrophotometer following suitable dilution.[19]

The drug content was calculated using following formula:

Drug Content = Practical value / Theoretical value x 100

Particle Size

The particle size of optimized batch were estimated by using Malvernzetasizer.

Zeta Potential

The charge of thedrugloadedvesiclessurface of phytosome wasdeterminedusingMalvern.[20]

In-Vitro Diffusion Study

A diffusion study of phytosomal suspension was carried out using franz diffusion cell through dialysis membrane. Dialysis membrane was soaked in distilled water for 24 hours. The receptor compartment was filled with phosphate buffer (pH 7.4) and donor compartment contain 3ml of phytosomal suspension (equivalent to 10mg) on dialysis membrane and whole assembly was kept on magnetic stirrer at 600rpm for a period of 10 hours and samples were withdrawn at specified time interval of 1 hr and replaced with equal volume of buffer. Samples were appropriately diluted with buffer and analyzed using (Shimadzu Corporation, Japan)UV spectrophotometer at 364nm. Steady state Flux (Jss) was calculated from the slope of the linear part of the cumulative amount of drugpermeated per unit area (µg/cm2) against a time (h) plot. Permeability coefficient (Kp) =Jss / Co, (Co = initial drug concentration).[21]

Formulation of Phytosomal Gel of Quercetin

The preparation of gel was achieved by gradual addition of gelling agent 1% Carbopol 940 by mechanical mixing at 600 rpm for 30 min. A 9 ml phytosome suspension equivalent to 30mg drug was subsequently introduced gradually into the polymer gel. The ultimate amount was adjust to 100g utilizing distilled water. The gel that were produced were subsequently stored at ambient temperature in order to facilitate subsequent investigation.[22]

Table 2: Formulation plan of Phytosomal Gel

Sr.no.	Ingredients	Quantity
1	Carbopol 940	1g
2	Propylene glycol	0.5ml
3	Methyl paraben	0.2g
4	Triethanolamine	q. s.
5	Phytosome suspension	9ml
6	Distilled water	100ml

Evaluation of Prepared Phytosomal Gel

Determination of pH

1 g of gel was dispersed in 20 ml of distilled water, and a digital pH meter was used to determine the pH value. The measurement was perform three times and mean +- SD was calculated.[23]

Spread-ability

Two glass slides of 20 cm × 20 cm were selected. A small amount of gels was sandwiched between the two glass slides. A 50 g weight was placed on the upper slide so that the gel between the two slides was pressed uniformly to form a thin layer. The weight was removed and then fixed to a stand without slightest disturbance in such a way that the upper slide slides off freely, to the force of weight tied to it. The time taken for the upper slide to separate away from the lower one was noted using a stop clock.[24]

The following equation was used for this purpose:

$S = m \times L/T$

Drug Content

The drug content was performed by using 1 g of gel dissolved in 10 ml of phosphate buffer the solution was then filtered through Whatman filter paper and filtrate was analyzed using UV- spectrophotometer.[25]

Viscosity

The measurement of viscosity of the sample was done using Brookfield Viscometer (DV-E Model).The required quantity of gel was placed in small volume holder and the spindle used was LV4-64 at room temperature and at 10, 20, 50, 100

Rpm. The corresponding viscosity value in Cp (centipoises) was noted.[26]

Washability

The weighed quantity of gel was taken and spread on the hand and wash under running water for 1 min.

In-Vitro Drug Diffused

A diffusion study of Phytosomal gel was carried out using Franz diffusion cell through dialysis membrane. Dialysis membrane was soaked in distilled water for 24 hours. The receptor compartment was filled with phosphate buffer (pH 7.4) and donor compartment contain 1 g of phytosomal gel (equivalent to 0.3mg) on dialysis membrane and whole assembly was kept on magnetic stirrer at 600rpm for a period of 10 hours and samples were withdrawn at specified time interval of 1 hr and replaced with equal volume of buffer. Samples were appropriately diluted with buffer and analyzed using UV spectrophotometer at 364nm.[21]

In vitro diffusion has been recognized as an important element in drug development. To analysis the mechanism for the release and release rate kinetics of the formulated dosage form, the data obtained from conducted studies was fitted into Zero order, First order, Higuchi matrix, and KorsmeyerPeppasmodel. In this by comparing the r-values obtained, the best-fit model was selected.[27]

RESULT AND DISCUSSION

Determination of wavelength maxima (λ max) and Calibration Curve of drug

The UV spectra of the drug were acquired through the scanning of drug solutions with a concentration of 10µg/ml, revealing a peak absorption at 364nm. A calibration curve was generated using phosphate buffer pH 7.4 at 364nm concentration ranging from 5-25µg/ml. the resulting data was subjected to linear regression analysis. The calibration curve show strong linear correlation by interpreting the r^2 value so the drug was obey's Beer-Lambert law. The result are mention in Table No. 3 and Fig. No. 1.

Table 3: Standard calibration curve of quercetin in phosphate buffer

Sr. no.	Concentration ug/ml	Absorbance
1	0	0
2	5	0.15
3	10	0.27
4	15	0.40
5	20	0.54
6	25	0.68

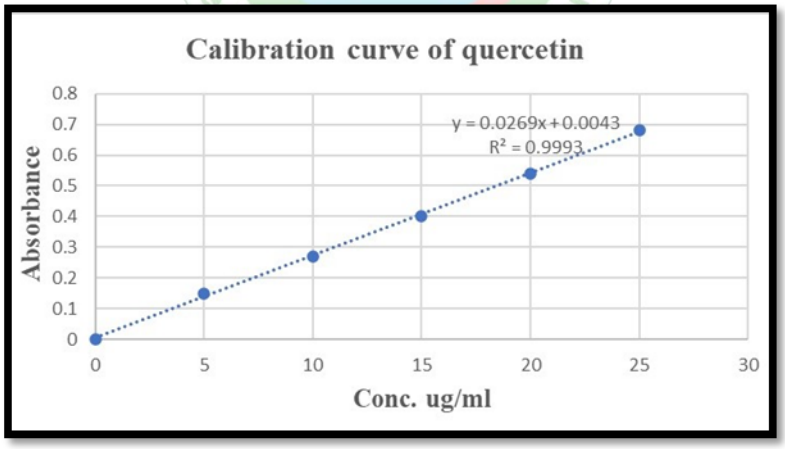

Figure: 1 Standard calibration curve of quercetin

Evaluation of Prepared Phytosome

Quercetin loaded phytosome were prepared by ether injection method. The absorbance readings obtained from the supernatant solution were used to determine the entrapment efficiency. The entrapment efficiency of F1 batch show highest entrapment efficiency which is 96.14% than other batches the reason behind that as the lipid concentration increases the entrapment of quercetin is decreases. The drug content of quercetin phytosome were observed within the range of 95.05-80.18 % , Which suggest that the formulations possess a satisfactory quantity of drug.The F3 batch show the 98.77% drug content due to the strong H- bond between the soya-Lecithin and Quercetin.

Table 4: Result of various evaluation parameters of phytosome

Batches	% Entrapment Efficiency	% Drug Content
F1	96.14	95.03
F2	95.14	91.33
F3	96.03	98.77
F4	93.35	87.62
F5	90.34	80.18

Particle Size and Zeta Potential

Particle size was measured using Malvern zetasizer. The x-axisrepresents the size of particles, typically ranging from nanometers to micrometers,while the y-axis shows the frequency or proportion of particles within each size range.TheaverageparticlesizeofoptimizeformulationF2 wasfoundtobe145.6nm, and the zeta potential was measured to be -10.9 mV (Fig. No. 2 & 3).

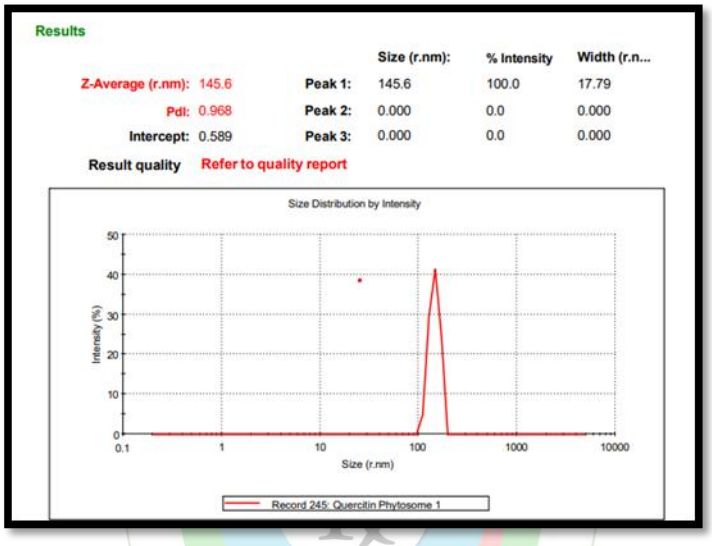

Figure 2: Particle size of phytosome

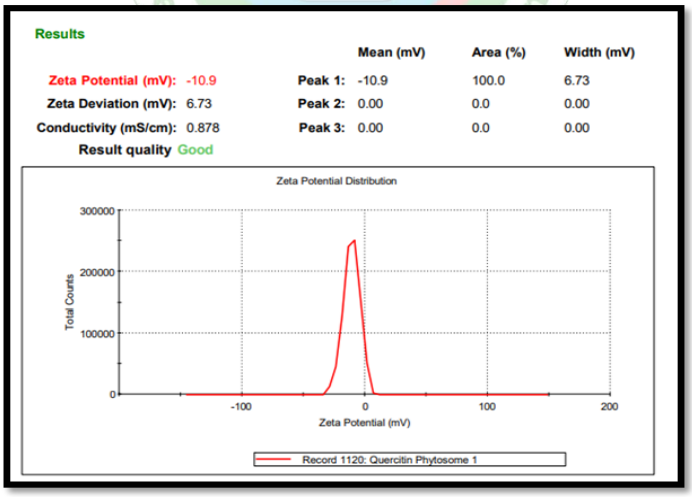

Figure 3: Zeta-potential of phytosome

In-vitro drug diffusion study

From the In-vitro drug diffusion study of quercetin after 6 Hr. study we observed that the drug diffused at various time interval ranging from 2.65- 13.34 % (fig. No. 4) and the permeability of quercetin per unit area to the diffusion membrane, which is 0.013mg/ cm^2. The drug released from phytosome suspension at various time interval ranging from 0.42 – 29.54 % and the permeability of phytosome formulation of all batches ranging from 0.021- 0.073mg/cm^2. As compared to the pure drug diffused study we set the criteria, which is at the 4 Hr. study of all batches diffused drug not more than 20% , not less than 15% on the basis of above criteria the formulation F2 (1:1) has show 17.75% drug diffused at 4 Hr. (Fig. No. 5) and the permeability coefficient of F2 batch show 0.073mg/cm^2 so we optimized the F2 batch (Table No. 5 and 6).

Table 5: In-vitro drug diffused of quercetin and phytosome formulation

	% Cumulative Amount of Drug Diffused						
Sr. no.	**Time (Hr.)**	**Pure drug (Quercetin)**	**F1**	**F2**	**F3**	**F4**	**F5**
1	1	2.65	0.42	4.14	7.1	1.16	5.62
2	2	7.99	5.64	18.88	10.3	7.17	14.08
3	3	10.61	8.16	13.44	15.3	10.50	20.72
4	4	13.34	11.52	17.75	19	13.98	24.68
5	5	6.38	16.52	20.75	22.8	19.09	29.54
6	6	5.27	21.75	26.17	28.3	23.68	7.20

Table 6: Result of permeability coefficient of quercetin and phytosome formulation

Sr.no.	**Batches**	**pKa Value (mg/cm^2)**
1	Pure drug (Quercetin)	0.013
2	F1 (phytosome Formulations)	0.021
3	F2	0.073
4	F3	0.021
5	F4	0.024
6	F5	0.021

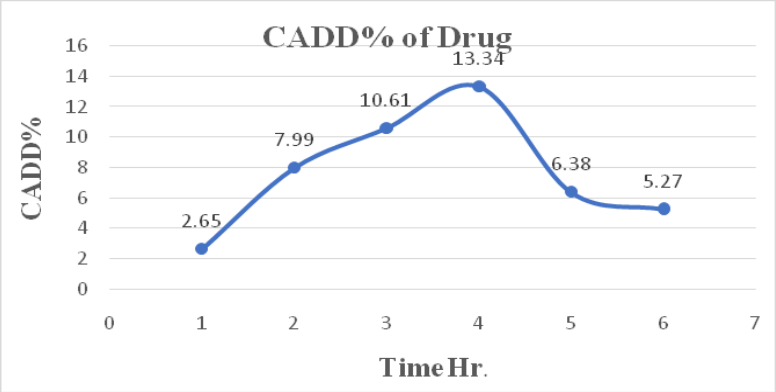

Figure 4: % Cumulative amount of drug diffused of quercetin

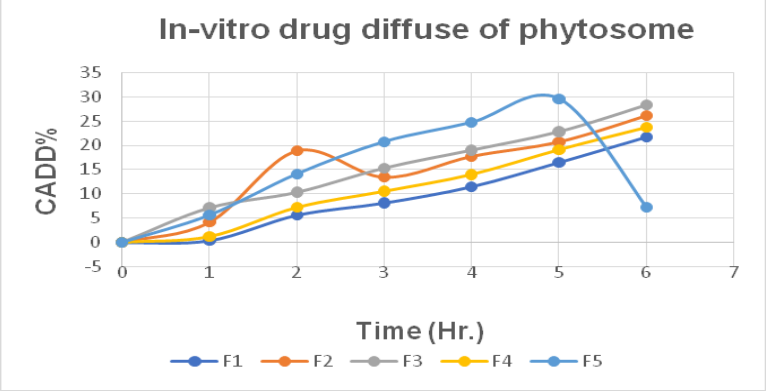

Figure 5: % Cumulative amount of drug diffused of phytosome

Evaluation of Prepared Phytosomal Gel

The gel are prepared using Carbopol 940 and the optimized phytosome suspension (F2) are incorporated. The pH of prepared phytosomal gel is measured three times and we get mean of pH value of prepared phytosomal gel is 5.7 which is compatible with the pH of skin. The spreadability value was 17.5g.cm/s, which indicates that the gel can be spread easily. The drug content of phytosomal gel were found to be 98.76%

which reveled that the drug aresatisfactory present in gel (Table No. 7). The viscosity of prepared phytosomal gel indicates that as the Rpm increases the viscosity decreases and as the Rpm decreases the viscosity increases. As we plot shear stress Vs. shear strain we observed that the gel show relatively pseudoplastic flow which is shear thinning, desirable for topical application (Table No. 8 and Fig. No. 5). The washability of prepared phytosomal gel after being subjected to running water after 1 min. we get 81% washability of gel, which show good retention and washable time of gel (Table No. 9).

Table 7: Result of prepared phytosomal gel

Sr. no.	pH	Spread ability	Drug Content
1	5.7	17.5g.cm/s	98.76%

Table 8: Result of Viscosity

Sr.no.	Temperature	RPM	Viscosity cps	RPM	Viscosity cps
1	37.9°C	10	22688	100	4150.3
2	37.9°C	20	16009	50	7223.8
3	37.9°C	50	7768.1	20	15197
4	37.9°C	100	3966.8	10	27007

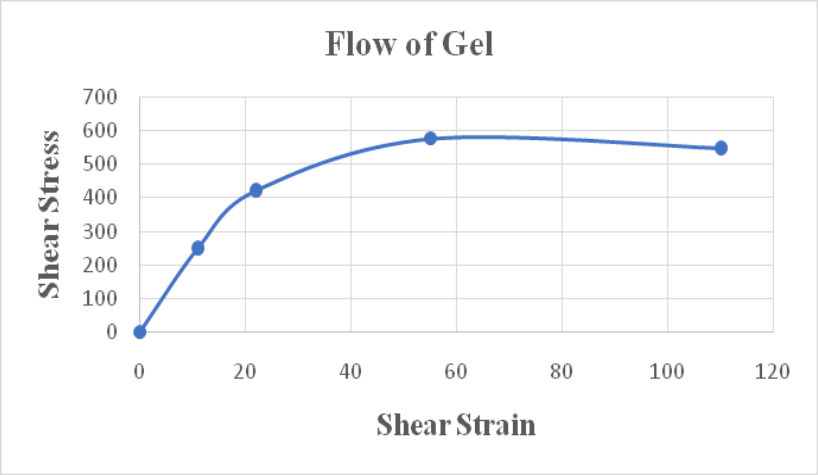

Figure 6: Flow of Gel

Table 9: Result of Washability

Sr.no.	Time (min.)	Wt. of gloves + gel	Wt. of gloves after washing and drying	Wash ability %
1	1	8.37g +1g=9.37g	8.56g	81%

In-Vitro Diffusion Study

From the in-vitro diffusion study of phytosomal gel, the cumulative amount of drug released from the phytosomal gel over a period of 6 Hr. is 69.45% but as we set criteria of 4 Hr. study for optimization we observed that the drug diffused 47.21% at 4 Hr. and the permeability coefficient of phytosomal gel to the membrane is 0.08mg/cm^2. From the R^2 value of release kinetics we observed that the drug released from gel by zero order kinetics followed by Peppas model as shown in Table No. 10.

Table 10: Regression coefficient of phytosomal gel

Sr. No.	Phytosomal gel	Zero order kinetic	First order kinetic	Higuchi model	Korsmeyer-Peppas
1	F2 (1:1)	0.9893	0.963	0.9607	0.9839

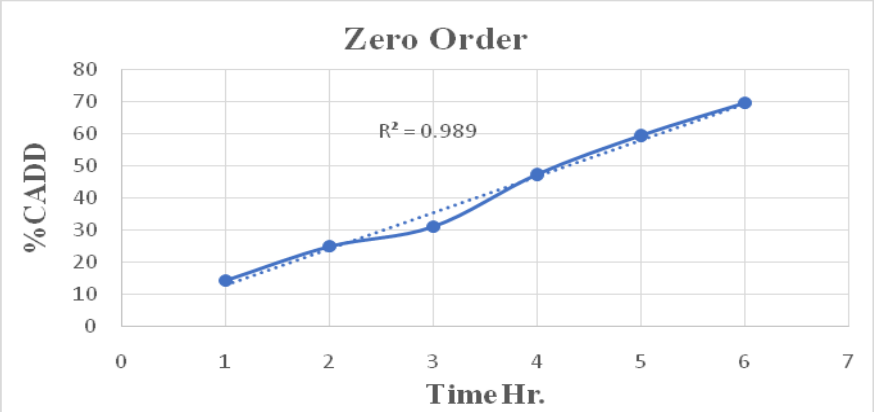

Figure 7: Plot of % Cum. Drug released Vs. Time (Zero order kinetic)

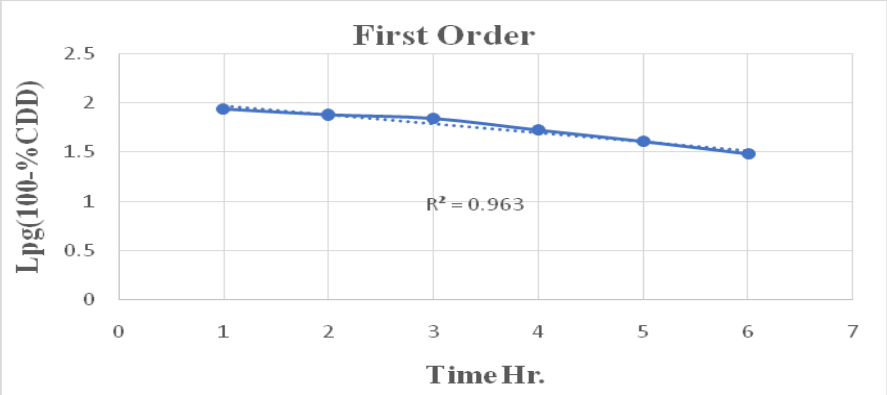

Figure 8: Plot of log % Cum. Drug retained Vs. Time (First order kinetic)

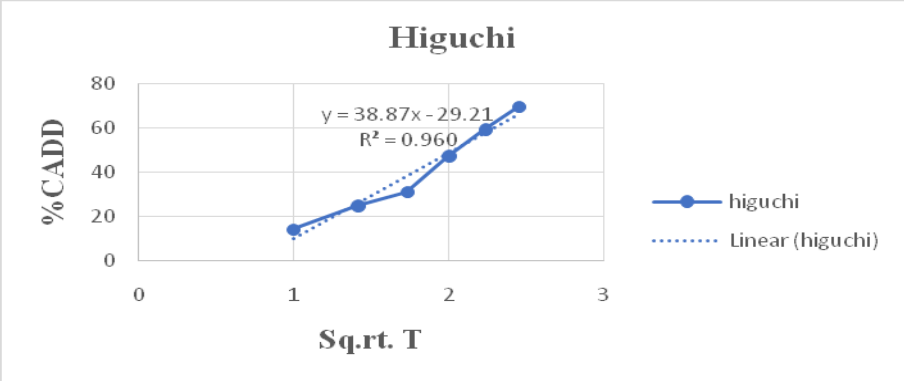

Figure 9: Plot of % Cum. Drug released Vs. square root of time (Higuchi model)

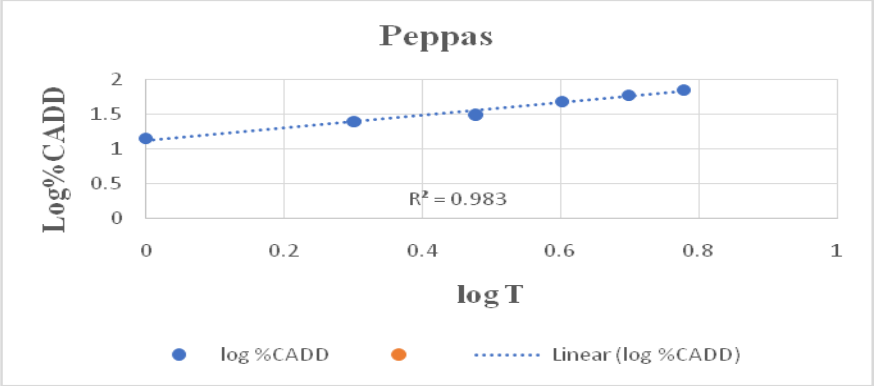

Figure 10: Plot of log % Cum. Drug released Vs. log of time (Korsmeyer-Peppas model)

CONCLUSION

The quercetin loaded phytosome were prepared by ether injection method. The formulated phytosome suspension are optimized by conducting various evaluation parameters such as particle size, zeta potential, entrapment efficiency, drug content and in-vitro diffusion study,from the set of criteria we optimized the F2 batch. The particle size of optimized batch is 145.6nm and zeta potential is -10.9 mV and the entrapment efficiency is 95.14% and drug content is 91.33%.The F2 batch is further incorporated in gel to carry out the quercetin release from the prepare phytosomal gel. By conducting release kinetics study such as zero order, first order, Higuchi and Peppas study, the quercetin release from phytosomal gel showed zero order kinetics followed by Peppa's model by comparing r^2 value, which show controlled release of quercetin. The study verified that the phytosomal gel formulation of Quercetin demonstrated a more favorable diffusion study, so we conclude that the phytosomal formulation are desirable carrier for the topical delivery of quercetin to treat various skin disorder such as psoriasis and various in-vivo study required for safety and efficacy of quercetin phytosome for topical delivery.

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