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Development and Evaluation of Novel DDS for Topical Delivery of Quercetin for Treatment of Rheumatoid Arthritis

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Abstract:- Rheumatoid arthritis (RA) is a chronic autoimmune disease that affects the joints, leading to progressive symmetric inflammation, cartilage destruction, bone erosion, and disability. While the initial impact may be limited to a few joints, later stages of the disease commonly involve multiple joints and exhibit extraarticular symptoms. The prevalence of RA varies between 0.4% and 1.3% depending on factors such as gender (with women being affected more frequently than men), age (with the highest frequency of new RA diagnoses occurring in the sixth decade of life), and the characteristics of the patient population studied (RA frequency increasing from south to north and being higher in urban compared to rural areas). Quercetin, an important bioflavonoid found in over twenty plant materials, is renowned for its various beneficial effects, including anti-inflammatory, antihypertensive, vasodilator, antiobesity, antihypercholesterolemic, and antiatherosclerotic activities. Free radicals play a significant role in the development of diseases such as hypertension, vascular disorders, and metabolic syndrome. However, the effectiveness of quercetin as a drug is limited by its poor permeation through the skin and its low deposition. To address these challenges, the solubility of quercetin can be improved by incorporating ethanol. Nanoliposomes serve as excellent carriers for drugs due to their high solubility, improved stability, and enhanced cellular uptake. It is important to note that caution should be exercised when topically applying high concentrations of ethanol to avoid the risk of local irritation. Therefore, the development of an appropriate carrier, such as nanoliposomes, for efficient topical application of quercetin is necessary for the treatment of rheumatoid arthritis.

Keywords:- *Tablet, Immediate release layer, Sustained release layer, Pre-formulation characteristics, UV absorbance.*

I. INTRODUCTION

Rheumatoid arthritis (RA) is one of the most prevalent chronic inflammatory diseases. The clinical presentation of RA varies significantly between early-stage RA and inadequately treated later stages of the disease. Early-stage RA is characterized by general disease symptoms, including fatigue, flu-like feelings, swollen and tender joints, morning stiffness, and elevated levels of C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR). When it comes to topical drug delivery systems, the oral route is commonly preferred for its convenience, although it is susceptible to hepatic first-pass metabolism, requiring higher drug doses. Additionally, the presence of surfactants in lipid-based formulations can cause gastric irritation, and systemic drug distribution can result in unwanted side effects. Hence, non-invasive, non-painful, and non-irritating delivery methods are desirable. The development of rheumatoid arthritis (RA) is influenced by various factors, including environmental and genetic factors. Environmental factors such as smoking, obesity, and infections with pathogens like Porphyromonas gingivalis, along with genetic factors like epigenetic modifications and genetic polymorphisms affecting antigen presentation (e.g., HLA genes HLA DRB1*01/04), T- and B-cell function, cytokine production, and signal transduction following immune cell activation, contribute to RA development. Additionally, synovial injury and hyperplasia of synovial fibroblasts can also contribute to the onset of RA by triggering inflammatory conditions. Overall, these processes lead to the modification of autoantigens, primarily through citrullination, resulting in the generation of neoepitopes with altered surface charge and increased susceptibility to proteolytic degradation. Genetic and environmental risk factors play significant roles in the development of rheumatoid arthritis.

RA patients and the possible treatments for RA can be categorized into four main strategies. The first strategy involves non-pharmacological treatments, which include a combination of physical therapy, patient counseling on lifestyle factors, and surgical procedures to remove and/or replace affected joints and bone areas. The second strategy revolves around the use of non-steroidal anti-inflammatory drugs (NSAIDs), primarily for symptomatic relief and until the RA diagnosis is confirmed. While NSAIDs can reduce pain and stiffness in affected patients, they do not have an impact on disease progression. The third strategy focuses on non-specific

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immune system suppression through the application of glucocorticoids. Glucocorticoids exhibit rapid disease-modifying effects; however, their long-term usage is limited due to severe side effects. The fourth and final strategy involves disease-modifying anti-rheumatic drugs (DMARDs), which are used to target inflammation, prevent further joint damage, and slow down disease progression. In terms of topical drug delivery systems, utilizing formulations applied to the skin offers several advantages. These include targeted delivery to the specific site of action, reduced systemic toxicity, avoidance of first-pass metabolism and gastric irritation, improved release rate for better percutaneous absorption, and increased bioavailability with sustained release profiles. Factors to consider when selecting a topical preparation include the effect of the vehicle (such as occlusive vehicles that enhance active ingredient penetration and efficacy), matching the type of preparation to the site and type of lesions, and considering the potential for irritation or sensitization.

Nanoliposomes, which are nanometric versions of liposomes, serve as widely utilized encapsulation and controlled release systems. Liposomes, composed of lipid and/or phospholipid bilayers, are ideal models of cells and biomembranes. They find applications in various industries, including food, cosmetics, agriculture, and pharmaceuticals, for protecting and delivering different materials, including drugs, nutraceuticals, pesticides, and genetic material. Nanoliposomes provide increased surface area compared to liposomes, leading to improved solubility, enhanced bioavailability, better-controlled release, and more precise targeting. The term "nanoliposome" specifically refers to nanoscale bilayer lipid vesicles. Research interest in nanoliposomes has been growing, as indicated by the increasing number of articles and patents published on the topic since 2002.

II. MATERIALS AND METHODS

A. Analytical methods:

The drug samples (Quercetin) use for determination of absorption maxima (λmax) in various solvents i.e. 0.1N HCl solution. The analytical method will be evaluated with preparation of calibration curve.

➢ *Determination of λ max in 0.1 N HCl:*

In order to determine the absorption maxima (λmax) of Quercetin, a drug sample solution was prepared in pH 7.4 phosphate buffer to simulate gastric conditions. UV absorption spectroscopy of the drug was conducted using a UV-VIS scanning spectrophotometer (Shimadzu UV-1800, Japan). A solution of Quercetin with a concentration of 10 µg/ml was prepared in pH 7.4 phosphate buffer. The UV absorption spectra were recorded using pure drugs at a concentration of 10 µg/ml, and the absorption peaks were identified. To prepare the drug solution for the dissolution study, 10 mg of Quercetin was dissolved in 10 ml of the dissolution medium using sonication in a bath sonicator for 15 minutes, resulting in a 1000 µg/ml solution. From this solution, 1 ml was taken and further diluted with the same solvent to a total volume of 100 ml, again using sonication for 15 minutes, yielding a 10 µg/ml solution. The UV spectra of these solutions were recorded in the range of 200 to 400 nm using a double beam UV spectrophotometer (Shimadzu, UV-1800).

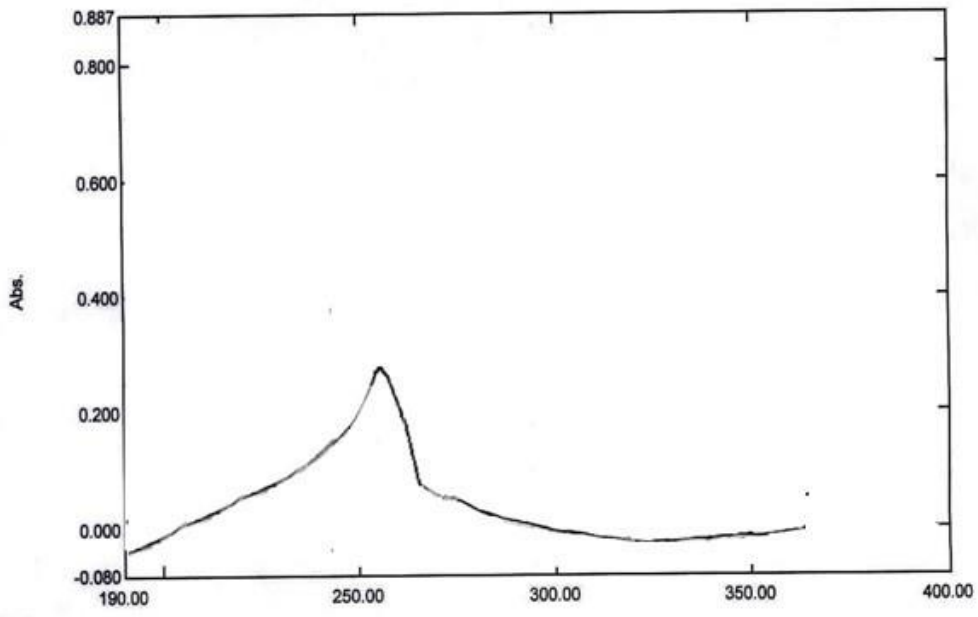

Fig. 1: Determination of maximum wavelength λmax

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➢ *Construction of calibration curve*

- **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.
- **Preparation of Working Standard Solution:** The solution mentioned above was referred to as Stock-1. From Stock-1, 1 ml was withdrawn and separately diluted up to a volume of 10 ml in a volumetric flask. Subsequently, 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml, and 2.5 ml of these solutions were transferred to 10 ml volumetric flasks and the volume was adjusted to 10 ml using a diluting medium. This resulted in Quercetin drug solutions with concentrations of 5 µg/ml, 10 µg/ml, 15 µg/ml, 20 µg/ml, and 25 µg/ml, respectively. The analysis of these solutions was performed at a wavelength of 214 nm using a double beam ultraviolet spectrophotometer.

Table 1: Calibration curve of Quercetin in phosphate buffer pH 7.4

S. No.	Concentration (µg /ml)	Absorbance
1	0	0
2	5	0.127
3	10	0.246
4	15	0.372
5	20	0.482
6	25	0.615

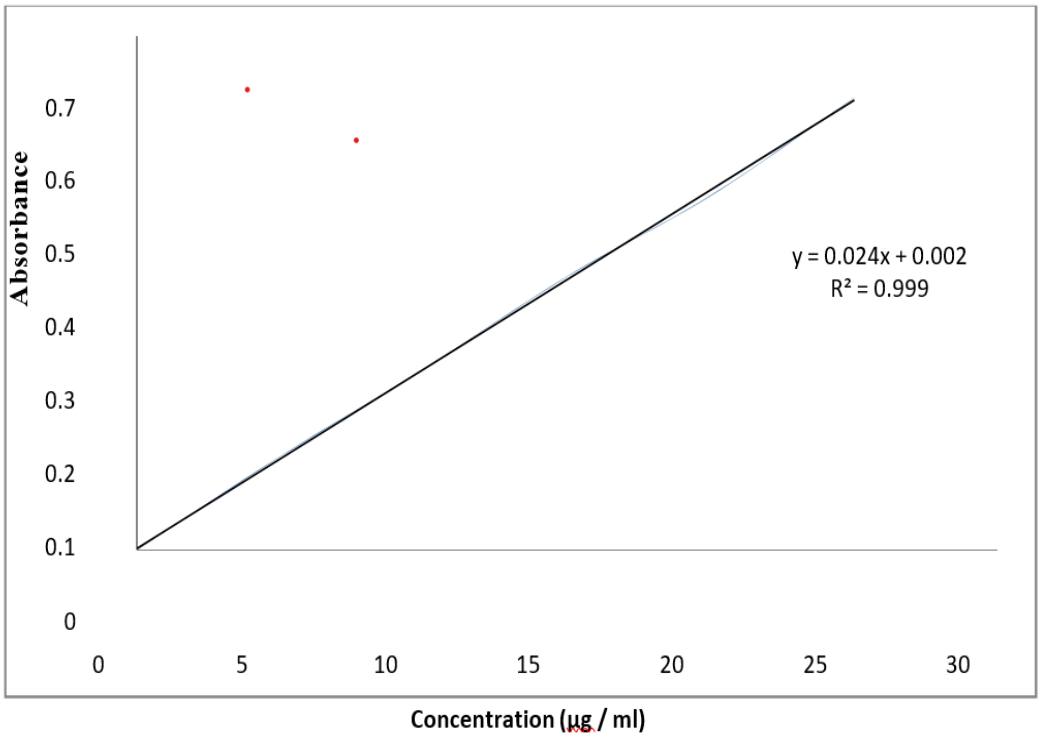

Fig. 2: Calibration Curve of Quercetin in phosphate buffer pH 7.4

Table 2: Stastical Data For Linearty

S.No.	Parameter	Quinapril hydrochloride
1.	Linearty Range	5-25 µg/ml
2.	Regression Equation	Y= 0.024x+0.002
3.	Correlation Cofficient	0.999

B. Preformulation 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 will be done.

- **Physical appearance:** The physical appearance of drug Quercetin was noted by visual study for identification of color, odor, taste and microscopic characteristics.
- **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.
- **Loss on drying:** Loss on drying was determined by accurately weighing 1.0 g of the Quercetin and drying at 105°C for three hours. It lost 0.005 gm of its initial weight.

Table 3: Comparative values of different parameters used to identify the drug.

S. No.	Values	Physical appearance	Melting point (^{0}C)	λ_{max} (nm)	Loss on drying (%)
1.	Observed	yellow powder	317	256	0.5

- **Solubility determination:** The solubility measurements were performed according to as Quercetin were 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 214nm to calculate the solubility of Quercetin.

Table 4: Solubility of Quinapril hydrochloride in 0.1 N HCl and phosphate buffer pH 7.4

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

- **Drug-excipients compatibility studies (FT-IR Spectroscopy**): The sample was subjected to infrared spectroscopy to determine the identity of the drug. In order to achieve a thicker pellet, which requires a lower concentration in the sample (according to Beer's Law), a pellet of approximately 1.0 mm diameter containing Quercetin was prepared. This was done by compressing 3-5 mg of the drug with 100-150 mg of potassium bromide in a KBr press (Model M-15, Techno Search Instruments). The resulting pellet was then mounted in the infrared (IR) compartment and scanned within the wave number range of 4000 – 400 cm^-1 using a Bruker Alpha FT-IR Spectrophotometer. To assess the compatibility of the drug with excipients, a physical mixture of the drug and excipients was prepared and stored at 40 °C and 75% relative humidity for one month. After this duration, infrared spectra were recorded to evaluate the compatibility of the drug and the mixture.

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Table 5: Interpretation of FTIR spectrum of pure Quercetin

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 (Primaryamine)
4	1360-1310	1345	C-N stretching (Aromatic tertiaryamine)
5	1350-1260	1290	O-H in plane bending
6	680-610	640	C-H bending (Alkyne)

Table 6: Interpretation of FTIR spectrum of Quercetin and All polymers

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 (AliphaticChloro Compound)

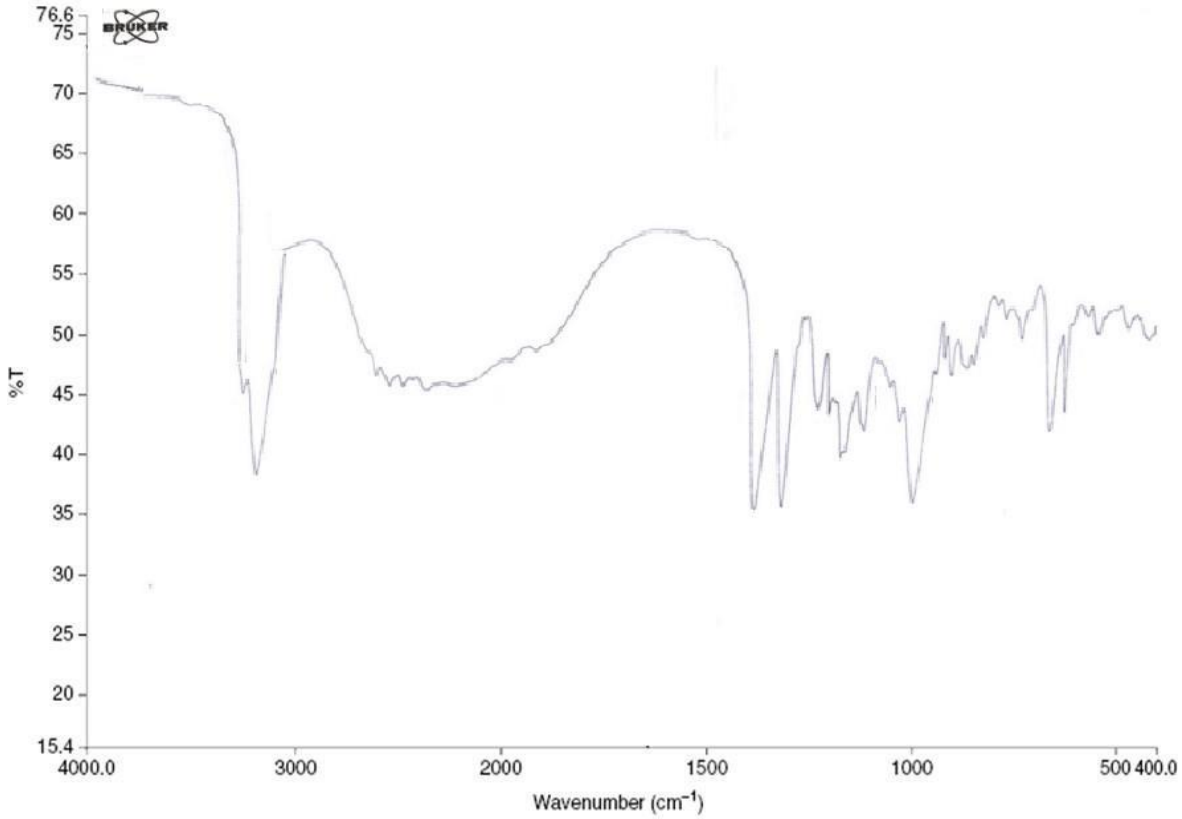

Fig. 3: FTIR Spectrum of quercetin

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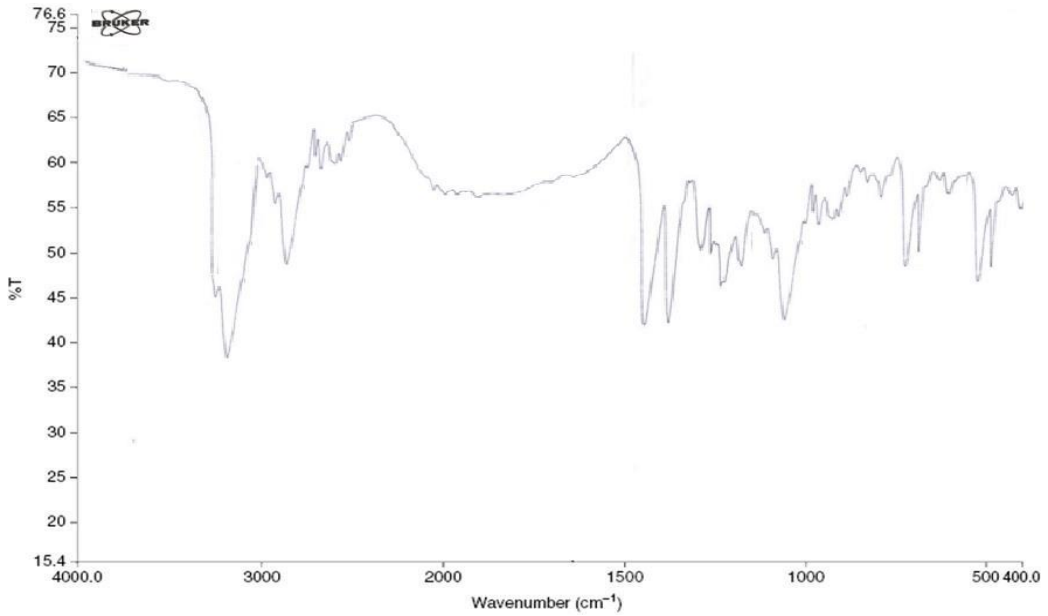

Fig. 4: FTIR Spectra of physical mixture of quercetin and All polymers

C. Preparation of nano-liposome:

Nano-liposomes were prepared using the lipid film hydration method with modifications, employing a rotary vacuum evaporator. The ratio of drug to SPC to CHOL was adjusted, and the vesicle size and drug entrapment efficiency were investigated. Initially, a mixture of chloroform and methanol (2:1) containing different ratios of drug to SPC to CHOL was evaporated under vacuum at a temperature of 400±0.5°C, resulting in the formation of a lipid film on the inner wall of a round bottom flask. Subsequently, the lipid film was hydrated with pH 7.4 PBS for a duration of 2 hours at 370±0.5°C. The preparation was then sonicated at 40°C in 3 cycles, with each cycle consisting of 30 seconds of sonication followed by a 2-minute rest period using a probe Sonicator. Finally, the formulation was homogenized using a high-pressure homogenizer in 3 cycles at a pressure of 15,000 psi to obtain nano-liposomes.

Table 7: Optimization formulation composition of nano-iposomes

S. No.	BatchNo.	Drug(mg)	Lecithin(mg)	Cholesterol (mg)	Stirring Speed(rpm)
1	QNL1	100	100	20	200
2	QNL2	100	100	40	200
3	QNL3	100	100	20	100
4	QNL4	100	100	40	100

D. Evaluation of nano-liposomes:

- **Vesicle size determination:** Vesicle size was determined using the particle size analyzer (Malvern Master Sizer).
- **Entrapment efficiency:** The entrapment efficiency of quercetin in liposomes was determined using the ultra-centrifugation method. A nano-liposomal suspension was transferred to a 10 ml centrifuge tube and diluted with distilled water to a final volume of 5 ml. The suspension was then centrifuged at 2000 rpm for 20 minutes to separate the undissolved drug in the formulation. A suitable volume of protamine solution was added to the resulting supernatant and left for 10 minutes. The presence of protamine caused aggregation of the nano-liposomes, which were then separated by ultra-centrifugation at 15,000 rpm for 20 minutes. The supernatant and sediment were separated, and their volumes were measured. The sediment was diluted with distilled water to a final volume of 5 ml. The unentrapped and entrapped drug contents were analyzed by estimating the drug in the supernatant and liposomes (sediment) using the calibration curve method with UV-Vis spectroscopy at 214 nm (Shimadzu UV-1800).
- **Transmission Electron Microscopy:** For the determination of surface morphology, transmission electron microscopy (TEM) was employed. A drop of the sample was placed on a carbon-coated copper grid and allowed to sit for 15 minutes. It was then negatively stained with a 1% aqueous solution of phosphotungstic acid. After thorough air drying, the samples were viewed using a transmission electron microscope (TEM Hitachi, H-7500 Tokyo, Japan).

Table 8: Evaluations of nano-iposomas Formulations

F. Code	Vesicle size (nm)	Zeta Potential (mV)	Entrapment efficiency (%)	Polydispersity Index(PDI±SD)
IL1	161.8	-33.5	96.73±0.93	0.311
IL2	251.3	27.1	95.43±1.28	0.129
IL3	152.2	-35.8	99.10±1.11	0.121
IL4	319.3	30.6	96.27±1.19	0.539

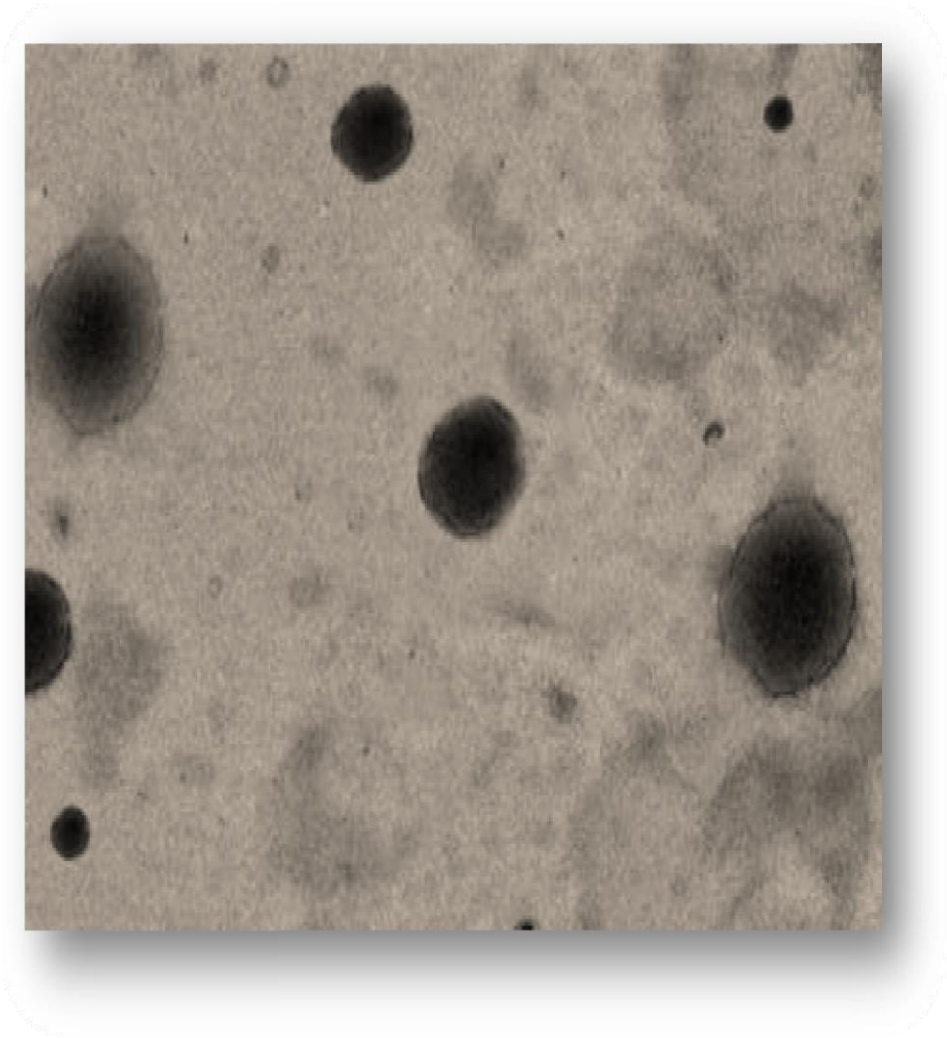

Fig. 5: TEM image of prepared Nano-liposomes as optimized

On the basis of results obtained from the study QNL3 was selected formulation.

Table 9: Composition of optimized nano-liposomes formulation

Formulation	Lecithin (mg)	Cholesterol (mg)	Speed (rpm)
QNL3	100	20	100

- **Preparation of nano-liposomal gel:** The necessary amount of drug-containing nano-liposomes (equivalent to 100 mg), methyl paraben, glycerineand polyethylene glycol were dissolved in 30 ml of water in a beaker. All the ingredients were mixed vigorously using a mechanical stirrer. Then, Carbopol 934 and PVP were gradually added to the liquid in the beaker while stirring continuously. Triethanolamine, acting as a gelling agent, was slowly added during the stirring process to achieve a gel-like consistency. Finally, the prepared gel base was transferred to aluminum collapsible tubes and appropriately labeled as required. The composition of the various gel formulations is detailed in Table 2.10

Table 10: Different composition of nano-liposomal gel formulation

Formulation	F1	F2	F3	F4	F5
Nano-lipsomes (g) **(QNL3)**	1	1	1	1	1
Carbopol 934 (g)	1	1.5	2	2.5	3
PVP (mg)	50	50	50	50	50
Methyl paraben sodium (mg)	75	75	75	75	75
Glycerine (ml)	5	5	5	5	5
PEG (ml)	5	5	5	5	5
Triethanolamine (ml)	1	1	1	1	1

E. Evaluation of nano-liposomal gel formulation:

- **Physical appearance:** The physical characteristics of the prepared nano-liposomal formulation were visually examined, including parameters such as color, appearance, and texture upon application.
- **pH determination:** The pH values of the nano-liposomal gel formulations were determined using a digital pH meter. A sample weighing 1 gram of liposomal gel was dissolved in 100 ml of distilled water and allowed to sit for two hours. The pH electrodes were fully immersed in the formulations, and the pH values were recorded. The pH measurements were performed in triplicate, and the average values were calculated.
- **Extrudability determination:** The nano-liposomal gel formulations were filled into collapsible metal tubes. The tubes were pressed with uniform pressure using fingers, and the extrudability of the formulations was evaluated. The extrudability was assessed by measuring the weight in grams required to extrude a 0.5 cm ribbon of gel within a 10-second interval.
- **Viscosity determination:** The viscosity of the prepared nano-liposomal gel formulations was measured using a Brookfield viscometer. An adequate amount of gel base was placed in a wide-mouth jar, allowing sufficient dipping of the spindle. The rotational speed of the spindle was set to 2.5 RPM, and the viscosities of the formulations were recorded
- **Spread ability:** Spread ability refers to the capacity of the nano-liposomal gel to smoothly spread over the skin or the affected area. The effectiveness of a formulation in terms of therapy is also impacted by its spreadability. To quantify the spreadability of the gel, the time in seconds it takes for two slides to separate after applying the gel between them under a specific load is measured. A shorter separation time indicates superior spreadability. The equation used to calculate spreadability is as follows: $S = (M * L) / T$ Where: M represents the weight tied to the upper slide. L denotes the length of the glass slide. T signifies the time taken for the slides to separate.
- **Stability studies:** The stability of all the prepared nano-liposomal gel formulations was assessed through freeze-thaw cycling. The formulations were subjected to temperatures of 4°C, 25°C, and 40°C for one month, and syneresis was observed. After being stored at these temperatures, the gels were exposed to ambient room temperature, and any liquid exudate separation was noted.
- **Homogeneity:** The nano-liposomal gel formulations were placed in containers, and visual inspection was conducted to evaluate their homogeneity. The formulations were examined for their appearance and the presence of any lumps, flocculates, or aggregates.
- **Grittiness:** The liposomal gel formulations were examined under a light microscope to detect the presence of any noticeable particulate matter. The preparations should be free from particles, and the presence of grittiness in any topical formulation was assessed.

The preparation should free from particles and the grittiness of any topical preparation can check.

- **Drug content studies**: A sample of nano-liposomal gel equivalent to 100 mg was taken and dissolved in 10 ml of pH 7.4 phosphate buffer. The volumetric flasks containing the solution were shaken well for 2 hours on a shaker to ensure proper mixing. The solution was then filtered through Whatman filter paper, and the filtrates were analyzed for drug content using spectrophotometry at 320 nm against corresponding gel concentration as blanks.

Table 11: Evaluation of nano-liposomal gel formulation

Parameters	Formulations				
	F1	F2	F3	F4	F5
Colours	Pale yellow colour	Pale yellow colour	Pale yellow colour	Pale yellow colour	Pale yellow colour
Appearance	Translucent	Translucent	Translucent	Translucent	Translucent
Odour	Pleasantodour	Pleasantodour	Pleasantodour	Pleasantodour	Pleasantodour
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 12: Viscosity of nano-liposomal gel

Time (min)	Viscosity (cps)				
	F1	F2	F3	F4	F5
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 13: Drug content in formulations by UV spectrophotometer

S. No.	Formulation	Drug content (%)
1	F1	97.37
2	F2	98.25
3	F3	99.43
4	F4	98.12
5	F5	97.98

- **In Vitro Diffusion Studies:** Prior to the experiment, the cellophane membrane underwent a thorough washing in running water, followed by immersion in distilled water for 24 hours to eliminate any traces of glycerine present on the membrane. The release of nano liposomal gel was examined using the dialysis method in pH 7.4 artificial skin conditions. Two milliliter samples were introduced into a dialysis bag, which was securely fastened with clamps on both ends. Subsequently, the dialysis bag was submerged in the receptor compartment containing 35 milliliters of dissolution medium and continuously stirred at 100 revolutions per minute. The donor compartment was in contact with the receptor compartment, while the temperature was carefully maintained at 37±0.5°C. To prevent evaporation of the dissolution medium, the receptor compartment was sealed shut. Stirring of the solution on the receptor side was achieved using externally driven Teflon-coated magnetic bars. At predetermined time intervals, 5 milliliters of solution from the receptor compartment was extracted using a pipette and immediately replaced with fresh 5 milliliters of phosphate buffer. Samples were withdrawn periodically, with an equal volume being substituted with fresh dissolution medium. The spectrophotometric measurement of the samples was conducted at 320 nm to determine the extent of 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 pertains to the continuous and constant release of medication from various drug delivery mechanisms, such as nano-liposomal gel, transdermal systems, matrix tablets containing poorly soluble drugs, and other delivery systems. At its core, zero-order release can be depicted as:

$Q = Q0 + K0\ t$ Eq. 1

In the context of drug release or dissolution, the variable Q represents the quantity of drug released or dissolved, assuming rapid release following dissolution. Q0 denotes the initial amount of drug present in the solution, typically zero, while K0 represents the zero-order release constant. The plot generated illustrates the cumulative percentage of drug release over time, following a zero-order kinetic model.

- **First-order release kinetics:** Higuchi has extensively discussed the rate laws predicted by various dissolution mechanisms, both independently and in combination. The equation for this scenario can be expressed as: Log C = Log C0 - kt / 2.303 (Eq. 2) Here, C0 refers to the initial drug concentration, and K represents the first-order constant. This equation, similar to other rate law equations, indicates a first-order dependence on the concentration gradient (i.e., Cs - Ct) between the static liquid layer adjacent to the solid surface and the bulk liquid.

Table 14: In-vitro drug diffusion study of nanoliposomal gel

Time	**F1**	**F2**	**F3**	**F4**	**F5**
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 15:

Time	√Time	Logtime	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

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Table 16: in-vitro drug diffusion study of nano-liposomal gel F2

Time	√Time	Logtime	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	1.54	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 17: in-vitro drug diffusion study of nano-liposomal gel F3

Time	√Time	Logtime	Cummulative % drug released	Log cummulative % drug released	Cummulat ive % drugretained	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 18: in-vitro drug diffusion study of nano-liposomal gel F4

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

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Time	√Time	Logtime	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 19: in-vitro drug diffusion study of nano-liposomal gel F5

Table 20: Kinetic release study data

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

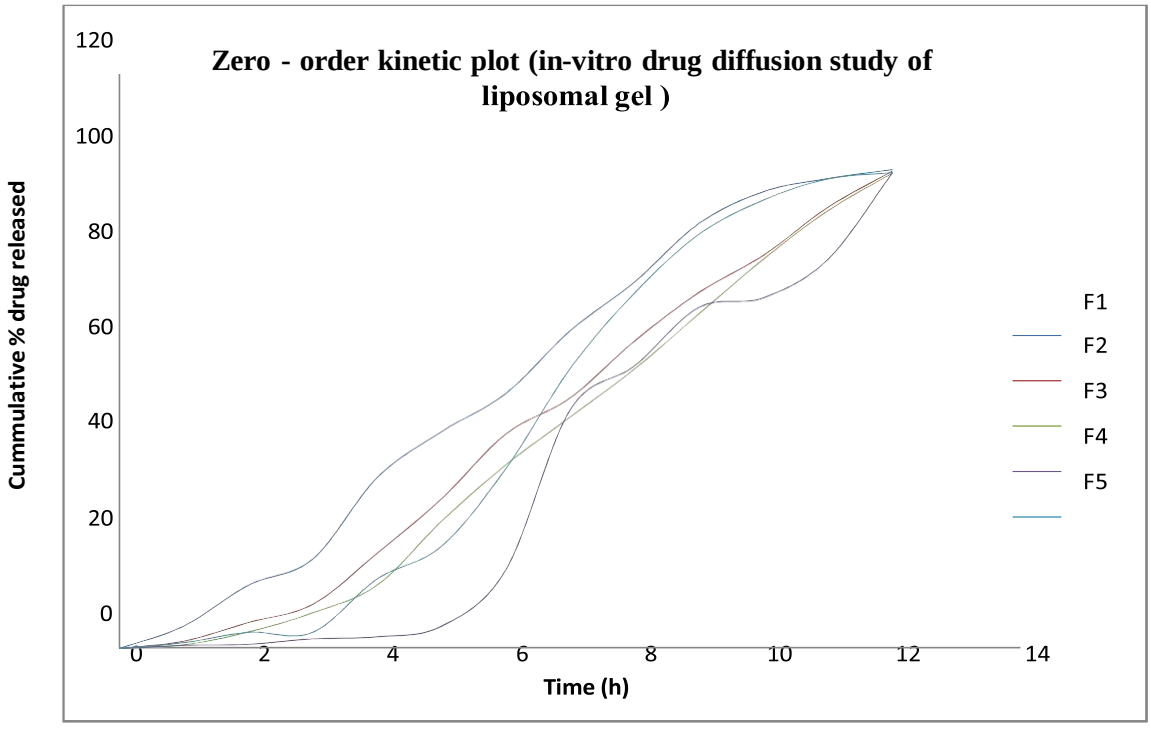

Fig. 6: Zero - order kinetic plot (in-vitro drug diffusion study of nano-liposomalgel)

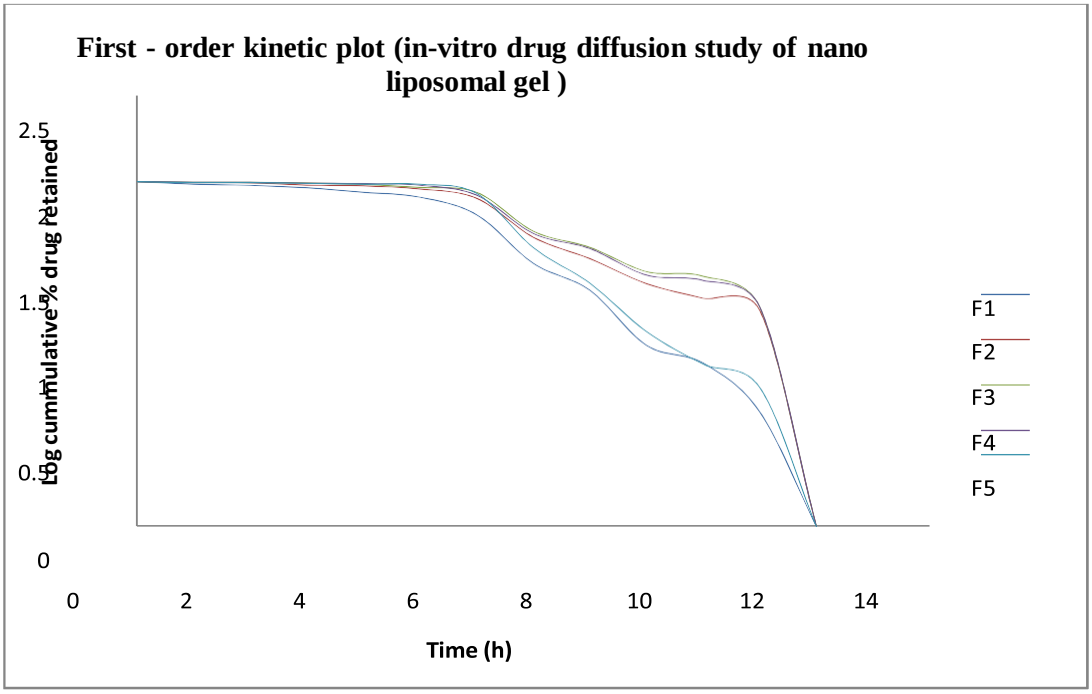

Fig. 7: First - order kinetic plot (in-vitro drug diffusion study of nano-liposomalgel

III. RESULTS AND DISCUSSION

Quercetin was identified through several methods, including determination of melting point, absorption maxima (λmax), loss on drying, and FTIR spectroscopy. The physical appearance of quercetin was observed as a yellow powder. The absorption maxima (λmax) of quercetin was measured at a wavelength of 256 nm, which corresponds to values reported in the literature (214 nm). A calibration curve for quercetin was created in 0.1N HCl at pH 1.2, and the data was subjected to linear regression analysis. The linearity was found to be within the concentration range of 5-25 µg/ml in the media, with r-values of 0.998 in 0.1N, following Beer and Lambert's law. The melting point of the drug was determined to be 317°C, falling within the range of 315-320°C as referenced. The loss on drying for the drug was found to be 0.5% (below the 1.0% limit). The solubility of quercetin was assessed in different media, and it was found to be freely soluble in both selected media. The solubility of the drug in 0.1N HCl and water was measured as 0.0276 mg/ml and 0.0094 mg/ml, respectively, in different solvents. FTIR spectra analysis revealed compatibility between the drug and excipients studied, as no changes in the peaks were observed. The FTIR spectrum of quercetin indicated the presence of characteristic peaks belonging to measured functional groups. The results of the FTIR drug complex study confirmed no interference between the materials, indicating no interaction within the formulation.

For the characterization of nano-liposomes in various liposomal formulations, a factorial design utilizing a 2-level, 3-factor design (23 design) was employed to optimize the development of nanoliposomes. The independent variables selected were the amount of lecithin, the amount of cholesterol, and the rotation speed at high and low levels. The dependent variables included size, zeta potential, entrapment efficiency, and polydispersity index. The vesicle size of all liposomal formulations prepared according to the experimental design ranged from 152 to 319 nm, with zeta potential values ranging from -27.1 to 35.8 mV, and entrapment efficiency ranging from 95.43±1.48% to 99.10±1.52%.The prepared nano-liposomes (QNL1 – QNL4) were evaluated with respect to zeta potential, entrapment efficiency, size and polydispersity index were calculated and on the basis of results the formulation QNL3 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.

A smaller size of liposomes is necessary for their entrapment in the emulgel and for effective localization of active constituents in the deeper layers of the skin, particularly targeting the causative agent of acne. The high zeta potential indicates the stability of the formulation and liposomal vesicles. Among the formulations tested, QNL3 exhibited higher entrapment efficiency and lower polydispersity index. Based on these results and the preceding discussion, QNL3 was chosen as the optimized formulation for further analysis, including transmission electron microscopy.

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A. Characterization of nano-liposomal gel formulation:

The key component of the liposomal emulgel formulation is the gelling agent, which is essential for achieving the desired consistency. The concentration of the viscosity enhancer or gel former is crucial, as too low a concentration results in a simple solution or lotion with very low consistency. On the other hand, a high concentration of the liposomal emulgel-forming agent can lead to gels with high viscosity, causing non-uniform distribution of the drug and handling issues. The prepared nanoliposomal emulgels containing 0.5% and 1% Carbopol 934 formed very thin gels that liquefied within 4 and 5 hours, respectively, after preparation. The gel formulated with 1.5% Carbopol 934 exhibited a somewhat better gel, but liquefaction was observed after 24 hours. However, the gel base formulated with 2% Carbopol 934 resulted in a uniform and smooth gel that did not liquefy over an extended period of time. The pH of the formulation was measured to ensure it could be safely used on the skin without causing irritation. The pH of the G4 gel base formulation was determined to be 7.54, which closely matches the neutral pH of the skin, indicating that the G4 formulation can be used without risk of skin irritation. The spreadability of the formulations decreased with increasing concentration of the gelling agent. The optimized gel base exhibited a spreadability value of 10.1 cm, indicating that it can be easily spread with a small amount of shear stress. This suggests that the formulation maintains good wet contact time when applied to the application site. Based on the evaluation parameters, 2% Carbopol 934 was selected as the optimized concentration of the gelling agent for further gel preparations.

The physical characteristics of the prepared nanoliposomal gels were examined. The gels were observed to be pale yellow in color, translucent in appearance, and smooth upon application to the skin. The individual properties, such as consistency and texture, of the formulations were deemed satisfactory, with the herbal hair gel demonstrating a smooth texture. The pH value of the prepared gel formulation was determined at room temperature and ranged from 7.2 to 7.8. According to literature, the pH of the skin increases from the epidermis to the dermis, eventually reaching a neutral value. The observed pH range of 7.2 to 7.8 in the gel formulation aligns with the desirable pH for the skin, as it does not disrupt the skin's physiology. To assess the stability of the prepared nanoliposomal gel formulations, accelerated stability testing was conducted. The gels were stored at different temperatures (40°C, 25°C, and 45°C) under refrigeration, room temperature, and stability chamber conditions for a duration of 30 days. The physical parameters of the gels were evaluated throughout the study period. The results indicate that the preparations remained physically stable at all temperatures during the storage period.

IV. SUMMARY AND CONCLUSION

Nano-liposomes, which are lipid vesicles, are considered highly suitable for drug delivery systems as they enable targeted delivery to the desired organ while minimizing drug distribution to non-target tissues. The intimate contact between nano-liposomes and adjacent surfaces enhances drug absorption. The nano-liposomal gel was formulated using the lipid film hydration method with necessary modifications after optimizing the formulation variables. Cholesterol was added to enhance the characteristics of the bilayers in the nano-liposomes, increasing their micro viscosity, stabilizing the membranes, and increasing vesicle rigidity. Encapsulation of quercetin Hcl in nano-liposomes was found to prolong the skin residence time, leading to faster healing of external lesions and reducing side effects and treatment duration. The vesicles can adsorb onto the stratum corneum surface, allowing direct transfer of the drug from the vesicles to the skin, or they can fuse and mix with the lipid matrix of the stratum corneum, enhancing drug penetration into the skin. Nano-liposomal gels have been reported to penetrate the skin intact and reach a depth where they can be absorbed into the systemic circulation. The formulated quercetin nanoliposomal gel formulations were prepared and evaluated. The physical evaluation revealed that the color of the prepared nanoliposomal gels was pale yellow, with a translucent appearance and smooth application on the skin. The individual properties, such as formulation consistency, were satisfactory, and smooth texture was found. The pH of the gel formulation at room temperature and ranged from 7.2 to 7.8. According to literature, the pH of the skin increases from the epidermis to the dermis, eventually reaching a neutral value. The gel formulation with a pH range of 7.2 to 7.8 aligns with the desirable pH for the skin, ensuring compatibility with the skin's physiology. To assess stability, the prepared nano liposomal gel formulations underwent accelerated stability testing. The gels were stored at various temperatures (40°C, 25°C, and 45°C) under refrigeration, room temperature, and stability chamber conditions for a period of 30 days, while also being subjected to different humidity conditions. Physical parameters were evaluated throughout the study period. The results indicate that the preparation remained physically stable at all temperature.

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