Tailoring of Genistein loaded Transniosome for the management of photoaging: Statistical optimization with Box Behnken Design of Quality by Design (QbD), in vitro evaluation

Manjusha Jharaniya, Sarika Shrivastava, Jitendra Banweer

SIRT-Pharmacy, Sanjeev Agrawal Global Education University, Bhopal, M.P.

Abstract:

The present study aims to develop and optimize a Genistein-loaded transniosomal formulation for the effective management of photoaging using a Quality by Design (QbD) approach. Genistein, a natural isoflavone with potent antioxidant and anti-photoaging properties, exhibits limited dermal bioavailability due to poor solubility and permeability. To overcome these limitations, transniosomes were designed as a novel nanocarrier system offering enhanced skin penetration and sustained release. The formulation was statistically optimized employing a Box–Behnken Design (BBD) by evaluating the influence of surfactant concentration, cholesterol content, and hydration volume on key responses such as vesicle size, entrapment efficiency, and drug release. The optimized formulation demonstrated nanosized vesicles with uniform distribution, high entrapment efficiency, and controlled release kinetics. In vitro studies revealed improved permeation across the skin membrane and enhanced antioxidant potential compared to pure Genistein. The developed transniosomal system thus provides a promising platform for efficient dermal delivery of Genistein, potentially preventing UV-induced oxidative damage and mitigating the visible signs of photoaging. This QbD-based optimization not only ensures formulation robustness but also supports a systematic pathway toward the development of effective anti-photoaging therapeutics.

Key words: Genistein, transniosomes, Photoaging, Quality by Design (QbD), Box–Behnken Design, Dermal drug delivery

1.Introduction

Skin aging is a complex biological phenomenon driven by intrinsic and extrinsic factors. Intrinsic aging is genetically programmed, while extrinsic aging—commonly referred to as photoaging—is primarily induced by chronic exposure to ultraviolet (UV) radiation [1]. UV exposure leads to the generation of reactive oxygen species (ROS) and inflammatory cytokines that degrade the extracellular matrix (ECM), particularly collagen and elastin fibers, resulting in wrinkle formation, loss of elasticity, and pigmentation changes [2,3]. Genistein, a naturally occurring isoflavone derived from soy, has gained attention for its anti-photoaging potential

due to its antioxidant, anti-inflammatory, and tyrosine kinase inhibitory activities [4,5]. It has been demonstrated to scavenge free radicals, inhibit matrix metalloproteinases (MMPs), and reduce UV-induced erythema and skin damage [6]. Despite these promising pharmacological properties, genistein suffers from poor aqueous solubility and low skin permeability, which significantly limit its topical bioavailability and therapeutic efficiency [7]. To overcome these limitations, novel drug delivery systems such as transniosomes have been developed. Transniosomes are ultra-deformable, non-ionic surfactant-based vesicular systems that incorporate edge activators to enhance flexibility and penetration through the stratum corneum [8]. These vesicles offer several advantages, including enhanced drug entrapment, prolonged drug release, improved skin retention, and better physical stability compared to conventional formulations [9,10]. Recent studies have indicated that transniosomal systems can significantly enhance dermal and transdermal delivery of poorly soluble compounds, making them ideal carriers for delivering phytoactives like genistein [11]. The present research focuses on the formulation, optimization, and evaluation of genistein-loaded transniosomes to improve its topical delivery and therapeutic efficacy against photoaging.

2. Materials and Method

Genistein, different grades of surfactant (Pluronic L64, Pluronic F127, Pluronic P85), and Co-surfactant (Span60) were purchased from Yucca Entreprises (Wadala E.) Mumbai. Cholesterol and chloroform were used from lab. All the chemicals and solvents used in tests were analytical grade and HPLC grade water was used throughout the procedure.

2.1. Preparation of transniosomes by thin film Hydration Method:

Genistein loaded transniosomes were prepared by thin film hydration technique by using span 60, different grade Pluronic co-polymeric surfactants (L64, P85 and F127), and cholesterol ratios (1 :1:1, 1:2:1 and 1: 3:1). Accurately weighted quantities of drug, surfactants, co-polymeric surfactants and cholesterol were taken to give the desired ratio and were dissolved in 10 ml of chloroform in a round bottom flask and 5 mg of Dicetyl phosphate was added to the above mixture. The solvent mixture was evaporated in a rotary flash evaporator at rotate 100 rpm until a smooth, dry lipid film was obtained followed by introducing it under high vacuum through vacuum pump for at least three hours for removal of residual content of chloroform. Further flask was kept in vacuum desiccators overnight for complete removal of chloroform. Then dry lipid film was hydrated with 5 ml of phosphate buffer saline pH 7.4 at room temperature for a period of 15 min hour until the formation of transniosomes. The obtained transniosome dispersions were stored in a refrigerator at 4 ± 3°C.

Table 1: Composition of genestein loaded transniosomes

Ingredients (mg)	GF1	GF2	GF3	GF4	GF5	GF6	GF7	GF8	GF9
Genestein	10	10	10	10	10	10	10	10	10
Span 60	50	50	50	50	50	50	50	50	50
Pluronic L64	50	100	150	-	-	-	-	-	-
Pluronic F127	-	-	-	50	100	150	-	-	-
Pluronic L85	-	-	-	-	-	-	50	100	150
Cholesterol	50	50	50	50	50	50	50	50	50
Dicetyl phosphate	5	5	5	5	5	5	5	5	5
Chloroform	10	10	10	10	10	10	10	10	10

3. Evaluation parameters of formulated genestein loaded transniosomes

3.1. Estimation of Percentage of Entrapment efficiency

Entrapment efficiency of the transniosomes were done by separating the unentrapped drug by dialysis method and the drug remained entrapped in transniosomes was determined by complete vesicle disruption using 0.1%w/v Triton X- 100 and analyzed UV spectrophotometrically for the drug content after suitable dilution with phosphate buffer saline pH 7.4 and filtered through whatmann filter paper. The filtrate was measured spectrophotometrically using phosphate buffer saline pH 7.4 and triton X-100 mixture as blank. The percentage of drug encapsulation efficiency was calculated by the following equation:

% Entrapment efficiency = Amount of entrapped drug/Total amount of drug ×100%

3.2. Estimation of percentage of Drug content

The percentage of drug content in the formulation was determined by taking transniosomal dispersion equivalent to 5mg in a 10 ml of volumetric flask and made the volume up to required volume using phosphate buffer pH 7.4. After that 1ml of the solution was withdrawn and diluted to 10ml using phosphate buffer saline pH7.4, the absorbance of the solutions was measured in the UV-Visible Spectrophotometer the percentage of drug content was calculated. The drug content is calculated following formula,

% of Drug content = Sample Absorbance/ Standard Absorbance× 100

3.3. Vesicle size distribution measurements and surface charge

The vesicle size and surface charge of the transniosomes was determined by measuring the electrophoretic mobility of the transniosomal particles using a zeta sizer (Malvern Instruments ltd, UK) equipped with a 5 mW helium neon laser with a wavelength output. Glassware was cleaned of dust by washing with detergent and rinsing twice with water for injections. Measurements of size analysis were made at 25˚C at an angle of 90˚. Data were analyzed using the "contin" method. Polydispersity index (PI) was determined as measures of homogeneity. Values were obtained from the printed report of Malvern zeta sizer which includes the present intensity in terms of size distribution of transniosomes and their respective sizes. Small values of PI indicate a homogeneous population while high values indicate heterogeneity. Vesicle dispersions were characterized by photo microscopy for vesicle formation and morphology. The size and shape of vesicles in transniosomes formulations were also observed by optical microscopy using a calibrated eyepiece micrometer, and photographs were taken at × 400 magnifications with a digital camera (Olympus, 8.1 megapixel, Japan).

3.4. Scanning electron microscope observation of transniosomes (SEM)

The scanning electron microscopy (SEM) is one of the most important techniques used for analysis of surface morphology such as spherical shape; smoothness and formation of aggregates and size distribution of transniosomes were studied by (Hitachi, Japan). Transniosomes were sprinkled onto the double- sided tape and coated with gold film of thickness of 200 nm under reduced pressure of 0.001 mmHg that was affixed on aluminum stubs. The aluminum stub was placed in the vacuum chamber of a scanning electron microscope. Photographs were taken at suitable magnification.

3.5. In-vitro release studies of the formulations

The *in-vitro* release of genestein transniosomes was carried out using open end cylinder method. One end of the tube is tightly covered with a Himedia dialysis membrane (MW-12,000-14,000 Da). The transniosomal suspension (5 ml) was placed over the membrane in the donar chamber. The donar chamber is then lowered to the vessels of the glass beaker containing 100 ml of phosphate buffer saline pH 7.4, which act as a receptor compartment so that the dissolution medium outside and the vesicles preparation inside were adjusted at the same level. The release study was carried out at 37±0.5^{0}C, and the stirring shafts were rotated at a speed of 50 rpm. 5ml of samples were withdrawn periodically at predetermined time intervals. Every withdrawal was followed by replacement with fresh medium to maintain the sink condition. The withdrawn samples were diluted and analyzed for the drug content using UV spectrophotometer. Phosphate buffer saline was used as blank. The release pattern was observed for 24hrs. The *in- vitro* release studies were also carried out for the marketed formulation by the same method.

4.Result and Discussion

4.1. Estimation of entrapment efficiency of transniosomal formulations (GF1-GF9)

The entrapment efficiency (% EE) of transniosomes in GF1- GF9 formulations varied between 45.64 ± 0.32% and 90.03 ± 0.31%. From the results, it was observed that entrapment efficiency of drug loaded transniosomes formulation was found to be increased on equal ratio of the span 60, co-polymeric surfactants and cholesterol (1:1:1), whereas entrapment efficiency decreases on further increase in co-polymeric surfactant ratio from 1:2:1 and 1:3:1.

4.2. Estimation of Percentage of Drug content of formulations (CF1-CF9)

The percentage of drug content of genestein loaded transniosomes in GF1- GF9 formulation was found to be in the range of 97.99±0.68% to 99.01±0.60%. The results were indicated that the uniform distribution of drug in prepared transniosomes formulations. Among the nine formulations, GF7 showed maximal drug content as 99.01±0.60%.

Table 2: Formulation Table of genestein loaded transniosomes

Formulation Code	Span 60: Co-polymeric surfactant: Choleterol ratio	% of Entrapment efficiency	% of Drug content
GF1	1:1:1	61.98 ± 0.54	97.99 ± 0.68
GF2	1:2:1	60.90 ± 0.37	98.32 ± 0.21
GF3	1:3:1	45.64 ± 0.32	98.87 ± 0.62
GF4	1:1:1	75.52 ± 0.63	98.53 ± 0.32
GF5	1:2:1	71.68 ± 0.72	98.37 ± 0.13
GF6	1:3:1	68.78 ± 0.48	98.67 ± 0.41
GF7	1:1:1	90.03 ± 0.31	99.01 ± 0.60
GF8	1:2:1	78.90 ± 0.80	98.12 ± 0.53
GF9	1:3:1	70.54 ± 0.43	98.51 ± 0.36

4.3. Vesicle size and size distribution of transniosomal formulations (GF1 to GF9)

The vesicle size and surface charge of the transniosome was done by using a Malvern zeta sizer. The size was varied between 240.5±2.13 nm and 318.4±2.32 nm. Results shown that as the amount of co-polymeric surfactant increased from 1:1 to 1:3, the vesicle size also increased by the same ratio.

4.4. Surface charge of transniosomal formulations (GF1 to GF9)

As the zeta potential increases, the charged particles repel one another, and this stabilizes the system against agglomeration and prevents faster settling. Systems with the zeta potential value

of ≥-30 mV or ≥+30 mV are considered to be stable. The zeta potential of the transniosomes was changed within a range of -27.6±0.7 mV and -42.01±0.5 Mv. The zeta potential of the formulations GF7 was found to -42.01±0.5 mV this is due to higher repulsion, formulation GF7 would yield better stable formulations.

Table 3: Vesicle size and Zeta potential analysis of formulations (GF1-GF9)

Formulation Code	Vesicle Size (nm)	PDI	Zeta Potential (mV)
GF1	280.4 ± 1.74	0.22±0.01	-29.2±1.8
GF2	295±1.50	0.23±0.02	-27.6±0.7
GF3	306±2.21	0.18±0.01	-32.5±1.5
GF4	259.0±1.94	0.19±0.02	-27.8±0.4
GF5	280.0±1.18	0.20±0.01	-30.1±0.5
GF6	318.4±2.32	0.21±0.02	-28.5±1.2
GF7	240.5±2.13	0.24±0.02	-42.01±0.5
GF8	269.0±1.94	0.27±0.05	-39.2±0.8
GF9	290.4±3.92	0.23±0.03	-32.5±0.4

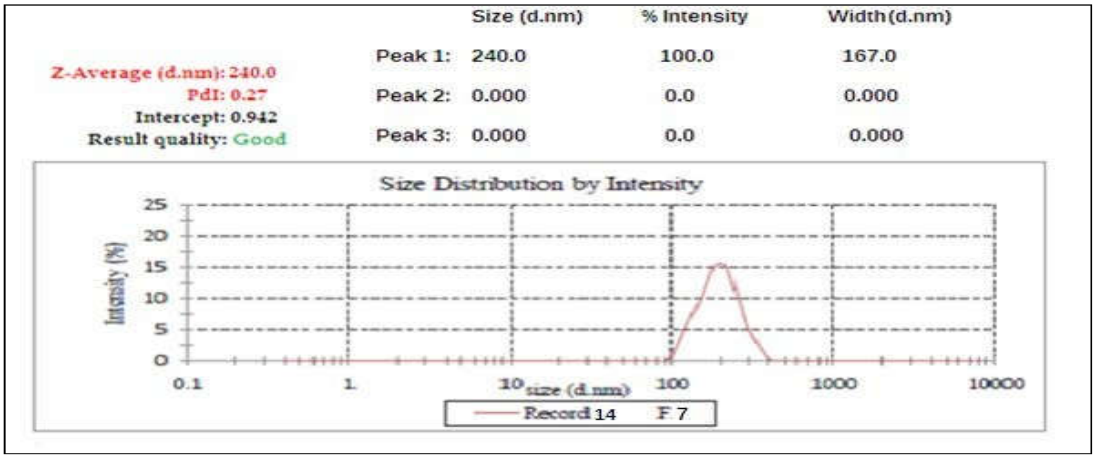

Figure 1: Size distribution by intensity of Optimized formulation (GF7)

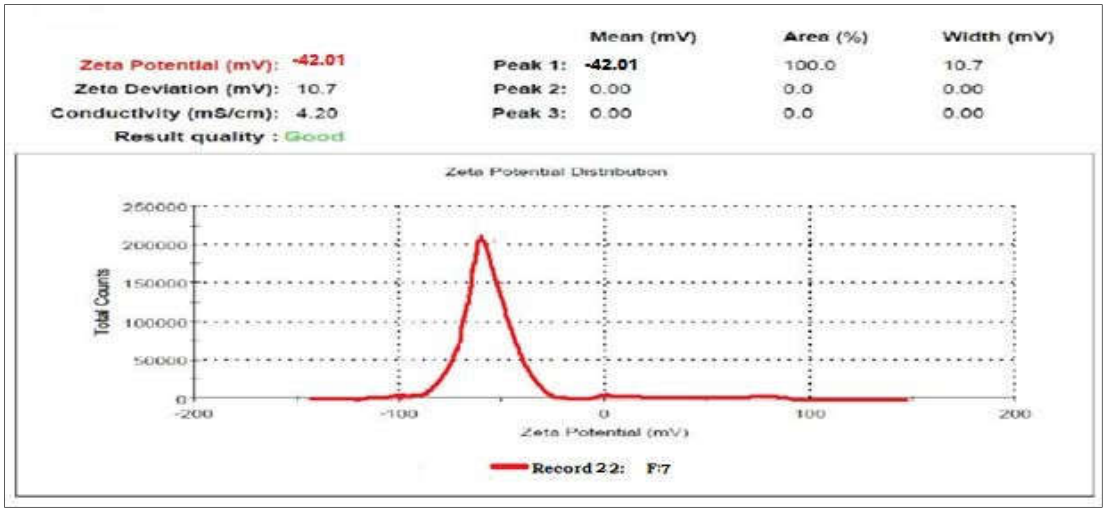

Figure 2: Zeta potential of Optimized formulation (GF7)

4.5. Scanning electron microscope observation of optimized formulation GF7

The SEM micrographs of optimized formulation GF7 are given in Figures. The SEM images confirmed the formation of transniosomes. The results showed the prepared vesicles are spherical in shapes with smooth surface and the vesicles were discrete and separate with no aggregation. The vesicle size distribution histogram revealed the uniform size distribution of drug within the transniosomes.

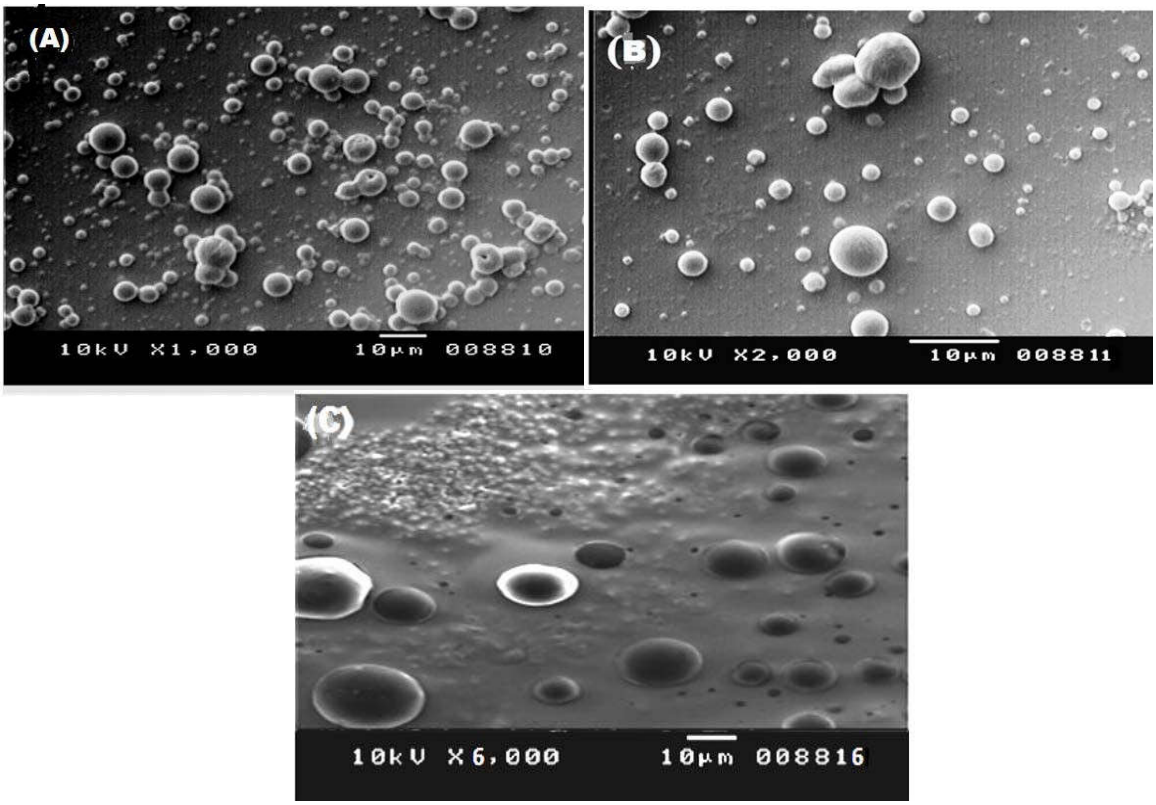

Figure 3: SEM image of Optimized formulation GF7 (X 1000 magnification)

4. 6. *In-vitro* release studies of formulations GF1 to GF9

The *in-vitro* release profile of drug from transniosomes clearly indicates that the concentration of co-polymers slows the release of drug. At the end of 24 hrs, *in-vitro* drug relased from formulations GF1 to GF9 was found to be 82.27 ± 0.94% to 99.85 ± 1.04%.in phosphate buffer saline pH7.4. The 25 to 34% of drug release was observed upto 4hrs, followed by slowing down and reaching a constant slow drug release observed after 4hrs. The formulation containing both Span 60 and Pluronic P85 ratio of 1:1, 1:2 and 1:3 showed maximum *in-vitro* release of 99.85 ± 1.04 %, 94.47 ± 0.54 % and 92.11 ± 0.57 % for 24 hrs. The cumulative percentage release at the end of 24 hrs was below 100% for all the dosage forms, this may be due to the relatively slow erosion of the transniosomes based on the Pluronic co-polymeric surfactant concentration. Among various formulations, GF7 was found to have a good release pattern and controlled release up to 24 hrs it could be suggested that the developed Pluronic P85 modified formulation could act as constant released carrier it was selected as the optimized formulation and used for the further studies.

Time (hrs)	GF1	GF2	GF3	GF4	GF5	GF6	GF7	GF8	GF9
0	0	0	0	0	0	0	0	0	0
0.5	12.65±1.10	10.09±0.55	8.95±0.33	14.45±0.11	11.30±0.08	9.30±0.46	13.34±0.16	15.34±0.63	11.14±1.04
1	16.82±0.84	14.11±1.14	11.43±0.85	19.81±0.26	14.81±0.47	1358±0.72	17.72±0.50	20.65±0.38	15.47±0.01
2	21.53±0.26	19.71±0.72	17.54±0.22	26.46±0.78	19.62±0.35	18.29±0.61	25.20±1.04	26.70±0.49	22.62±0.60
3	25.12±0.60	23.14±1.13	21.80±1.26	30.22±0.33	24.57±0.66	22.74±1.33	29.88±0.29	30.35±0.02	26.79±0.73
4	30.33±0.85	28.35±0.67	25.04±1.39	34.36±1.41	29.43±0.34	26.90±0.93	32.49±0.38	33.87±0.93	30.10±0.50
6	36.10±0.62	36.13±0.75	30.48±0.86	40.21±1.09	36.20±0.73	34.50±0.22	40.62±0.71	40.92±0.72	35.16±0.38
8	41.61±0.61	39.64±1.26	35.62±0.73	45.55±0.58	41.51±1.06	39.39±0.70	47.37±0.37	46.45±0.53	42.06±0.90
10	49.37±0.30	46.66±1.24	41.92±0.65	52.03±0.11	47.92±1.73	45.91±0.27	54.91±0.64	53.10±0.32	49.28±0.82
12	55.82±0.38	55.87±0.08	47.71±1.13	58.16±0.43	53.48±0.63	51.92±1.21	62.27±0.42	60.37±1.41	55.69±1.03
18	73.55±0.24	71.76±0.12	65.03±0.37	75.49±0.51	71.94±0.31	71.08±0.35	82.53±0.46	79.28±0.37	75.52±0.02
24	89.18±1.2	87.91±0.62	82.27±0.94	91.29±0.18	89.82±0.22	86.75±0.65	99.85±1.04	94.47±0.54	92.11±0.57

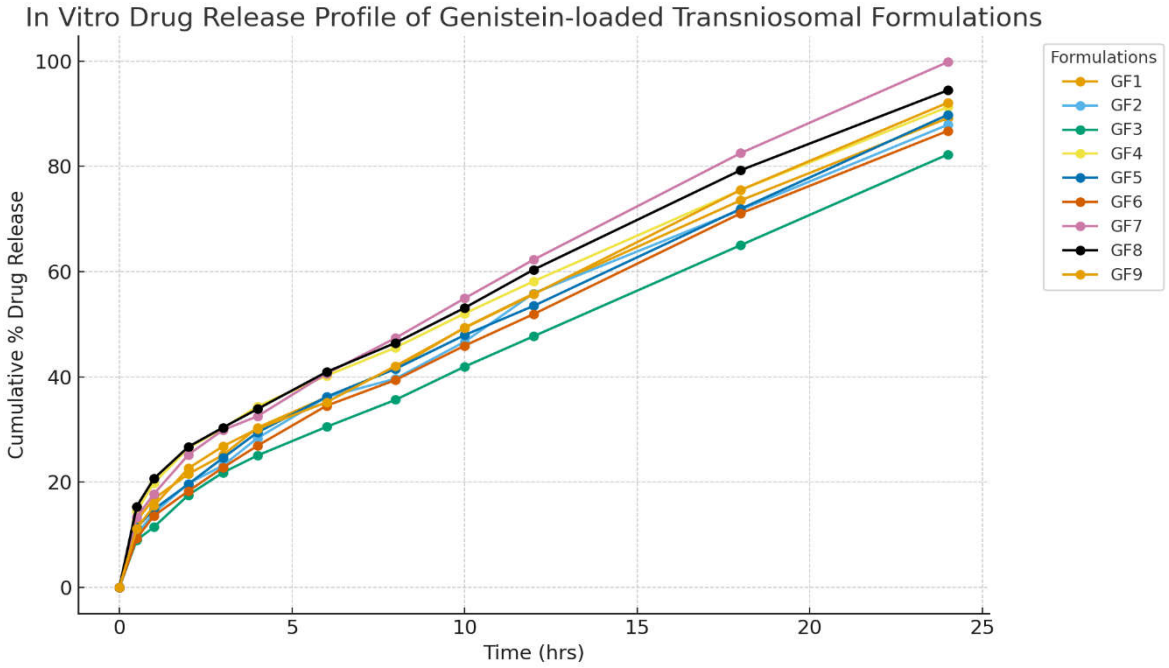

Figure 4: *In-vitro* cumulative drug release profile for all nine Genistein-loaded transniosomal formulations (GF1–GF9). Each curve shows the gradual release of the drug over 24 hours, helping visualize and compare their release kinetics.

The *in vitro* release profiles of Genistein-loaded transniosomal formulations (GF1–GF9) were evaluated over a period of 24 hours, and the results are graphically represented in Figure 1. All formulations exhibited a biphasic release pattern, characterized by an initial burst phase followed by a sustained drug release phase. The initial burst release, observed within the first 2–4 hours, may be attributed to the desorption of Genistein adsorbed on the surface of transniosomal vesicles and the release of drug molecules located near the vesicle periphery. Among the tested formulations, GF7 demonstrated the highest cumulative drug release (≈99.85% at 24 hours), indicating efficient diffusion and sustained delivery of Genistein. This superior release could be associated with the optimized ratio of surfactant and cholesterol, which promotes the formation of flexible and highly deformable vesicles with greater permeability through the dialysis membrane. GF4 and GF8 also showed comparatively higher release values (≈91.29% and 94.47%, respectively), suggesting that these formulations possess favorable bilayer fluidity and effective drug encapsulation efficiency.

Formulations such as GF2, GF3, and GF6 exhibited slower drug release rates, reaching around 82–88% at 24 hours. The relatively reduced release from these formulations might result from higher cholesterol content, which increases membrane rigidity and decreases the permeability of vesicular bilayers. Additionally, smaller vesicle size and higher surfactant content in GF7 and GF8 likely facilitated greater surface area for diffusion and better release kinetics. Overall, the data confirm that modulation of formulation variables—such as surfactant concentration, cholesterol ratio, and hydration volume—significantly affects the release characteristics of transniosomal vesicles. The observed sustained release behavior supports the hypothesis that transniosomes can act as effective carriers for topical and transdermal delivery of Genistein,

maintaining therapeutic levels for an extended period while minimizing frequent dosing. Thus, GF7 was identified as the optimized formulation, showing controlled and prolonged release up to 24 hours, which is desirable for anti-photoaging therapy. The sustained release pattern suggests potential for enhanced skin retention, improved antioxidant activity, and prolonged protection against UV-induced oxidative stress in dermal tissues.

Conclusion While microemulsions and nanogels have successfully enhanced genistein delivery and photoprotective efficacy, transniosomes with ultra-deformable vesicle properties offer a potentially superior platform but remain under-investigated in this context. The present investigation successfully demonstrated the formulation and optimization of Genistein-loaded transniosomes using the Quality by Design framework and Box–Behnken Design. The systematic approach enabled identification of critical formulation parameters influencing vesicle size, entrapment efficiency, and release behavior. The optimized transniosomal formulation exhibited nanoscale vesicles with excellent stability, high drug retention, and controlled release, ensuring effective delivery of Genistein to the deeper skin layers. In vitro studies confirmed enhanced permeation and antioxidant efficacy, suggesting superior photoprotective performance compared to the free drug. Overall, the developed transniosomal system provides a promising and scientifically robust strategy for targeted dermal delivery of Genistein in the management of photoaging, offering potential advancement toward safer and more effective anti-aging therapeutics.

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