

Topical Application Using Non-ionic Surfactant for Formulation and Evaluation of Flavonoid Proniosomes

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ABSTRACT

It is the goal of this research to examine the topical use of non-ionic surfactant for the Formulation and Evaluation of Flavonoid Proniosomes developed for the transdermal delivery of Rutin. The Proniosomal topical gel was prepared using the slurry technique involved with a non-ionic surfactant, Maltodextrin, Cholesterol and Glycerin. A robust design of experiments was used to optimize the various formulation variables. The optimized PG4 formulation was evaluated for entrapment efficiency, SEM, ATR -FTIR, Viscosity, Spreadability, Drug content, *in-vitro* diffusion study, and stability studies. PG4 formulation showed the highest entrapment efficiency and the higher percent of drug content. Evaluated for viscosity and spreadability which indicated the ratio of span 80: cholesterol demonstrated presenting some fluctuation. The SEM exhibited spherical vesicles and the optimal particle size for proniosome. *In vitro* drug release was better drug release throughout the process. After 3 months of storage at refrigerator temperatures, the stability in the presence of the percentage drug content of Rutin proniosomal gel formulation 4. There are several benefits to using Span 80 as the nonionic surfactant in these Proniosomal gel formulations, including increased drug accumulation in the different skin layers and increased carrier potential for the topical administration of rutin for the treatment of rheumatoid arthritis.

Keywords: Proniosome, Maltodextrin, Cholesterol, Glycerin Drug content, *In-vitro* diffusion study.

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INTRODUCTION

Pro-Niosome^[1]

Pro-niosomes are dried substances which can be converted into niosomes when hydrated with water. It's one of the vesicular drug delivery systems. Since the early 1980s, proniosomes are used as drug-targeting agents and drug carriers to obtain various advantages while avoiding the disadvantages associated with

traditional dosage forms.^[2,3] Pro-niosomes are also low in toxicity due to their non-ionic nature, lack of particular precautions, and formulation and production circumstances.

Proniosomes come in two varieties, depending on how they are Proniosomes are based on sorbitol and Proniosomes are based on maltodextrin.

Rutin is a quercetin rhamno-glucoside, also known as rutoside, which may be found in a variety of plants, including citrus, apples, vegetables, and buckwheat.^[7] and fits the profile of a prospective antioxidant and anti-inflammatory agent.

Nonionic surfactants are the most commonly used form of the surface-active agent in vesicle preparation because of the superior benefits they provide in terms

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of stability, compatibility, and toxicity especially Span 60 and Span 80.^[2]

Experimental

Materials

Rutin was purchased from (Tokyo, Japan). Cholesterol and Maltodextrin were purchased from Global calcium (Hosur, Tamilnadu). Span 80 and Glycerin were purchased from Central Drug House (Mumbai, India). Carbopol 934 gel bases were purchased from Chennai city, Tamilnadu.

Preformulation Studies

Characteristics of organoleptics^[3-4]

The organoleptic properties of rutin were observed for colour, taste, odour and solubility of the drug was observed using (Approximately 1gm) in Ethanol, Methanol and Distilled water was studied in a test tube.

Formulation of Proniosomal Gel by Slurry Method^[5]

The proniosomes were produced in multiple batches by varying the ratios of surfactants and organic solvents. The Slurry method (rotatory flask method) was used to make proniosomal suspensions by dissolving cholesterol and surfactant in alcohol. Along the margins of the flask wall, a thin coating developed. The drug was diluted in 10 mL of acetate buffered saline (ABS) solution pH 5.5 and applied to a thin film for hydration, followed by 5 min of sonication. The proniosomal suspension was stored at 4°C. in the refrigerator. Thin-film hydration was used to create a proniosomal solution. In an optimal formulation of proniosomal suspension (2ml), carbopol 934 was added as a gelling agent, along with glycerin, methylparaben, and dimethyl sulphoxide as a penetration enhancer, and the pH of the gel was adjusted with the addition of triethanolamine. Finally, Proniosomal gel was formed. The preparation procedure is shown in the Table 1.

Table 1: Formulation table of proniosomal topical gel.

Ingredients	PG ₁	PG ₂	PG ₃	PG ₄	PG ₅	PG ₆	PG ₇
Rutin (mg)	150	150	150	150	150	150	150
Cholesterol(mg)	50	100	150	200	50	50	50
Span 80(ml)	50	50	50	50	100	150	200
Maltodextrin(mg)	1000	1000	1000	1000	1000	1000	1000
Ethanol(ml)	10	10	10	10	10	10	10
Carbopol 934(mg)	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Glycerin(ml)	4.5	4.5	4.5	4.5	4.5	4.5	4.5

Characterization of Proniosomal Topical Gel pH Measurement^[6]

A digital pH metre was used to determine the pH of the proniosomal dispersion. In cleansed water, and appropriately weighed amount of gel was spread. The pH of the proniosomes should then be calibrated before using a standard buffer solution (Acetate buffer pH 5.5) to determine the pH of the gel.

Compatibility studies of Proniosomal Topical Gel Formulation^[7]

Infrared radiation in the 4000 to 400 cm⁻¹ range is delivered by the ATR-FTIR system, with some of the radiation being absorbed and some being passed through a medium. The radiation received by the sample molecules is converted into rotational and/or vibrational energy by the molecules themselves. Ftir analysis is a great tool for chemical identification and compatibility investigations since each molecule or chemical structure has its unique fingerprint. The preparation procedure is shown in the Figure 1-3.

ATR-FTIR Spectroscopy of Proniosomal Topical Gel Formulation

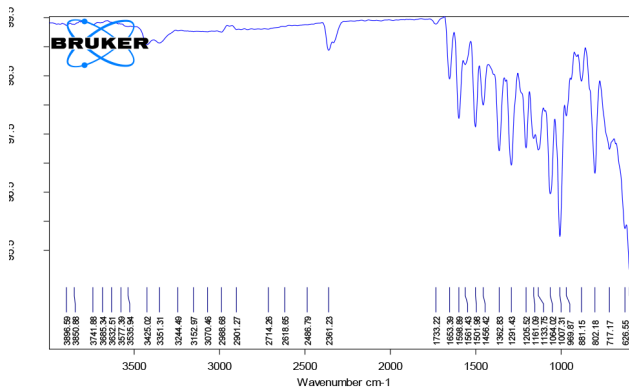


Figure 1: ATR-FTIR Spectrum of Pure Drug.

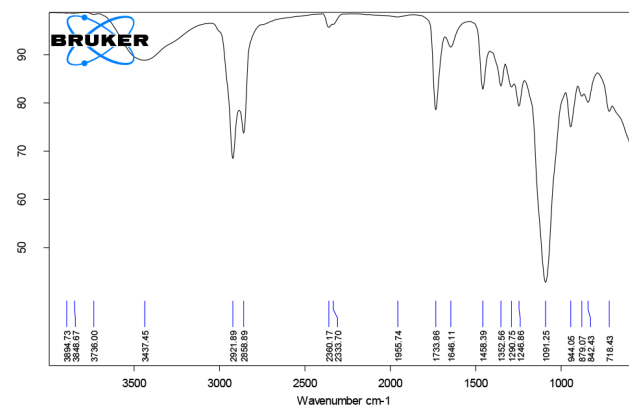


Figure 2: ATR-FTIR Spectrum of physical mixture of Rutin, Maltodextrin, Cholesterol, Span 80.

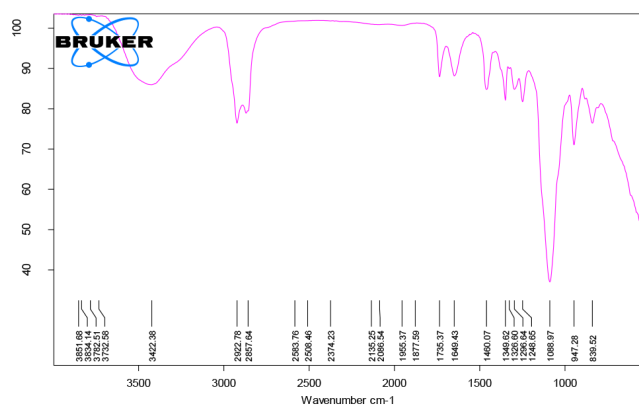


Figure 3: ATR-FTIR Spectrum of the optimized formulation.

Determination of Differential scanning calorimetry

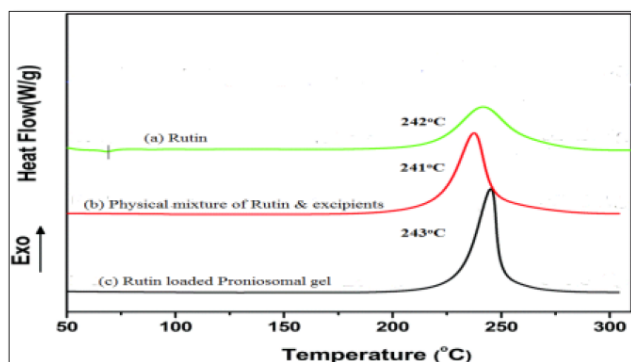


Figure 4: Differential scanning calorimetry curves of Rutin, Physical mixture and Proniosomal formulation.

Thermal Behaviour Studies of Rutin Loaded Proniosomal Topical Gel

The Perkin Elmer STA 6000 Thermal Analyzer should be used for Differential Scanning Calorimetry. The instrument has been calibrated using an indium standard. Samples are accurately weighed (ranging from 3 mg to 25 mg) and deposited in open-style ceramic sample pans. Using a steady heating rate of 100°C per minute, thermograms are produced. A dry Argon gas purge (60 ml/min) is used for all runs. Samples were heated between 37°C and 400°C for this experiment. The preparation procedure is shown in Figure 4.

Morphology Analysis of Rutin Loaded Proniosomal Topical Gel^[8]

Scanning electron microscopy may be used to examine particle size distribution and surface morphology (smoothness, roundness, and aggregate formation) (SEM). Optical microscopy can demonstrate the generation of vesicles by the specific approach. The Proniosome suspension must be applied on a glass

Evaluation Parameters of Proniosomal Gel

Scanning Electron Microscopy of Rutin Loaded Proniosome

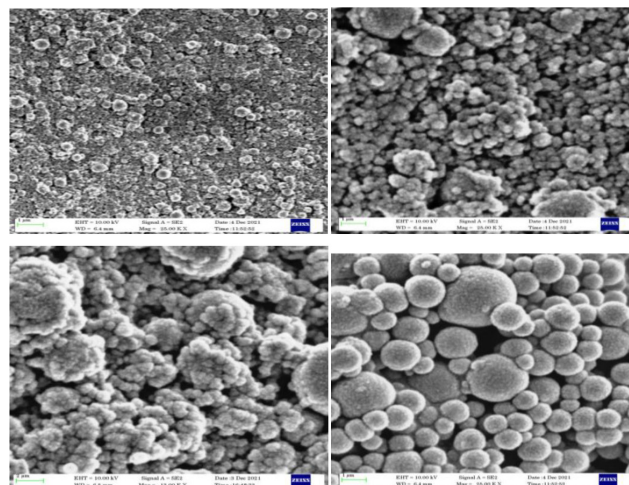


Figure 5: Scanning electron microscopy image of rutin loaded from proniosome.

slide and dried at room temperature; the resulting dry thin film of niosome suspension must be examined for vesicle formation. The preparation procedure is shown in Figure 5.

Drug Content^[9]

Ethanol is used to test the formulation's drug content after the application of a proniosomal gel. Pipetting out 50ml of proniosomes prepared from a 100ml standard flask. To ensure that the vesicles are thoroughly dissolved, an adequate quantity of Ethanol is added to the mixture and well stirred. Acetate buffered saline pH 5.5 may be made up to 100ml using this buffer. The preparation procedure is shown in Table 2.

Using empty proniosomes as a blank, the absorbance is measured at 271nm using a UV-Visible Spectrophotometer (Shimadzu UV-1700 Pharma Spec Japan).

The following formula is used to compute the drug content from the standard curve:

$$\text{Drug content} = \frac{\text{Sample Absorbance}}{\text{Standard Absorbance}} \times 100$$

Viscosity^[10]

The viscosity of the proniosomal gel was measured using a Brookfield viscometer. The viscometer was attached to spindle number 7 and dipped into a flask containing 50 g of proniosomal gel; settings such as rpm and spindle number were adjusted at (20 rpm, spindle

Table 2: Comparison of Drug Content, Viscosity, Spreadability, pH.

Sl. no	Formulation	Drug content	Viscosity (cps)	Spreadability gm.cm ²	pH
1	PG ₁	92	1896	11.06	4.5
2	PG ₂	94	1954	10.57	4.7
3	PG ₃	91	1928	10.72	4.9
4	PG ₄	96	1997	11.96	5.5
5	PG ₅	95	1985	11.88	4.8
6	PG ₆	92	1898	10.84	5.2
7	PG ₇	93	1986	11.10	5.6

no.7), and viscosity was measured. The preparation procedure is shown in the Table 2.

Spreadability^[11]

0.5 g of the produced proniosomal gel was spread onto a premarked 2 cm diameter circle on a glass plate, and the spreadability was then tested using a second glass plate. For five minutes, a half-kilogram weight was allowed to lie on the top glass surface. After spreading the gel, the diameter of the circle was measured. The diameter of the gels grew as a result of their diffusion. The spreadability formula was then used: The preparation procedure is shown in Table 2.

$$S = M.L/T$$

Where,

S stands for spreadability.

M denotes a weight that is attached to the top slide.

L is the length of the glass slide.

T is the amount of time it takes to entirely separate the slides from one another

Percentage of Entrapment Efficiency^[12]

It was decided to take approximately 20 min to dissolve 50 mg of proniosomal gel in 100 ml of volumetric ethanol, and after that, the volume was brought up to the mark with acetate buffer pH 5.5. The drug's absorbance at 271nm was then measured with a UV-Spectrophotometer using 10 ml from the above solution and the mark. The preparation procedure is shown in the Table 3.

The percentage of drug encapsulation was calculated by equation.

$$\% \text{ E.E} = 1 - \frac{(\text{Unentrapped drug})}{(\text{Total drug})} \times 100$$

Table 3: Percentage of Entrapment Efficiency.

Sl. No.	Sonication Time (minutes)	Entrapment Efficiency*
1	0	39.23
2	1	45.96
3	2	48.45
4	3	53.10
5	4	42.28
6	5	40.34
7	6	41.45

Table 4: In vitro Studies list of Percentage drug release of Cumulative.

Time	Percentage drug release of Cumulative						
	PG ₁	PG ₂	PG ₃	PG ₄	PG ₅	PG ₆	PG ₇
0	0	0	0	0	0	0	0
30min	3.58	2.51	3.69	4.15	3.74	3.21	3.95
1hr	6.25	5.98	6.95	8.65	7.14	6.53	7.71
2hr	15.31	15.19	15.81	16.58	15.86	15.62	16.11
3hr	28.59	28.72	28.35	29.65	28.69	27.26	28.81
4hr	34.76	34.43	35.24	35.73	35.19	34.69	35.27
5hr	42.65	43.19	43.14	43.87	42.85	42.95	43.42
6hr	50.59	50.21	50.72	51.16	50.14	50.61	51.02
7hr	51.65	53.54	55.12	59.81	56.59	52.98	57.18
8hr	59.51	60.41	62.98	65.69	61.74	58.21	61.95
9hr	67.25	62.98	67.95	71.48	68.14	64.53	66.71
10hr	72.31	69.19	72.81	79.54	73.86	69.62	71.01
12hr	79.76	77.43	80.24	92.41	81.69	78.29	79.11

In vitro Studies of Proniosomal Topical Gel

Drug release studies using semi-permeable membranes

Drug diffusion through egg membrane^[13]

One end of the diffusion cell was connected with an egg membrane for in vitro research that had a 20 ml capacity. This proniosomal gel had been created, and the diffusion cell's compartment had been preserved across the membrane. A magnetic stirrer kept everything all together. At 32°C, the magnetic bead was used to keep the pH 5.5 acetate buffer solution in the receptor compartment constantly stirred at 50 rpm. UV spectrophotometer at 271nm was used to measure the percentage of drug release from the proniosome in the samples collected over a period of up to 12 hr. The preparation procedure are shown in Table 4 and Figure 6.

Stability Studies^[14]

Proniosomal gel were tested for stability under accelerated conditions in accordance with ICH

guidelines. The drug's ability to maintain protection against microbial contamination. An optimal medicinal product must be well specified in terms of its physical, chemical, and microbiological properties from the start of the research and throughout the period of the shelf life intended. For three months, the microsp sponge formulations were stored at $40^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and 75% RH. Proniosomal gel was examined for physical appearance, pH, Vesicle size determination, Spreadability and *in vitro* drug release studies. The preparation procedure is shown in Table 5.

RESULTS AND DISCUSSION

Preformulation Studies

Organoleptic characteristics

The Color of Rutin was Slightly greenish-yellow in appearance with hygroscopicity, odourless and tasteless. The Rutin was freely soluble in anhydrous ethanol, slightly insoluble in Methanol and very slightly soluble in water which shows it is lipophilic.

DISCUSSION

Organoleptic Characteristics^[15]

The Preformulation studies were carried out to find out the solubility of rutin. The sample received for its

organoleptic properties such as greenish-yellow colour, odourless and appearance in powder shows results which comply with reported literature standards. Solubility test gave an idea that Rutin is water slightly soluble but soluble in solvents like Ethanol and phosphate buffer pH 7.4

ATR-FTIR Spectroscopy of Proniosomal Topical Gel Formulation

ATR-FTIR spectroscopic analyses were used to conduct drug-polymer interaction investigations, which verified that there was no compatibility between the drug and selected excipients. Finally, the resulting dry thin film of proniosomal gel must be examined for no interaction with the optimized formulation (PG4).

Determination of Differential Scanning Claorimetry

DSC studies of Rutin loaded proniosomal gel are used to conduct drug-polymer interaction on the thermal investigations, which verified that there were no interactions between the drug and polymers. The DSC studies revealed that the nature of the material should not be changed and shifted from crystalline to amorphous.

Evaluation Parameters of Proniosomal Gel

Scanning Electron Microscopy of Rutin Loaded Proniosome

The slurry method was used to create the Rutin proniosomal gel. The prepared rutin-loaded proniosomal gel in the formulation of PG4 containing different ratios of Span 80 and cholesterol (1:4) was morphologically analysed using SEM (Scanning Electron Microscopy) with the magnification power of X5 K, X10 K, and X50 K and X60 K, it observed the internal morphology. It was shown that vesicles were spheres and smooth on the surface. Finally, the resulting dry thin film of proniosome gel must be examined for vesicle formation.

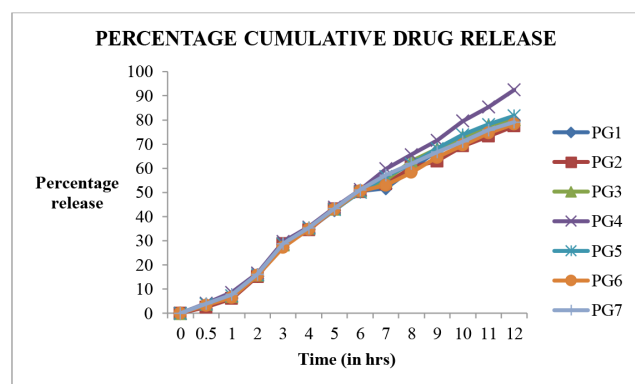


Figure 6: Percentage of cumulative Drug Release.

Table 5: List of Stability studies at different temperatures for 1 to 3 months.

Parameters	After 1 month		After 2 month		After 3 month	
	4°C	30°C	4°C	30°C	4°C	30°C
Appearance	Pale greenish-yellow	Pale greenish-yellow	Pale greenish-yellow	Pale greenish-yellow	Pale greenish-yellow	Pale greenish-yellow
pH measurement	5.5	5.5	5.3	5.3	5.1	5.1
Vesicle size determination	30nm	30nm	30nm	30nm	30nm	30nm
Spreadability	10.5	10	10.6	10.4	10.2	10.4
<i>In vitro</i> drug release studies	91.74	91.54	90.98	90.65	90.54	90.35

Drug Content, Viscosity, Spreadability and pH

The percentage of drug content was determined and the results varied from 91 to 96 respectively. The drug content of the formulation was uniformly distributed in the gel. The percentage of viscosity was calculated and the findings of the viscosity were 1896 to 1997cps respectively. These values were sufficient to obtain a good viscosity. The percentage of Spreadability varies from 10.57 to 11.96 gm.cm² depending on the calculation. To ensure that the gel is applied evenly to the skin, PG4 have high spreadability and meets the optimal quality requirements for sunscreen application. The pH measurement results revealed that all proniosomal gel formulations developed had pH values ranging from 4.5 to 5.5 respectively which was considered to be acceptable to avoid the risk of irritation upon application to the skin.

Percentage of Entrapment Efficiency

Entrapment efficiency has determined PG1 to PG6 range from 39.23% to 53.10%, Rutin loaded proniosomal gel formulation (PG4) was shown the good entrapment efficiency (53.10%) respectively.

In vitro Studies of Proniosomal Topical Gel^[16]

The *in-vitro* release of prepared Rutin loaded Proniosomal gel showed that the formulation prepared with (1:4) ratio of Span 80 and Cholesterol comprised formulation PG4 was considered as optimized formulation of 92.41%. It showed better drug release throughout the time intervals of 12 hrs in a controlled and long-duration manner when compared with the other formulation.

Stability Studies

Three months of stability testing was carried out on the optimised PG4 Proniosome gel formulation. The findings of the study revealed that there were no significant alterations. The pH appeared to be 7 at 6.9, the spreadability was 10.5, and *in vitro* kinetic tests, 86.38 percent accelerated stability studies were shown to be significantly different from room temperature. As a result was revealed to be the optimized formulation PG4 and all of the reports are within the specification ranges in refrigerator stability studies.

SUMMARY AND CONCLUSION

Sorbitanmonooleate, cholesterol, and maltodextrin were used as biocompatible excipients in the present study to generate a proniosomal gel that might serve as a drug carrier and vehicle for topical delivery of rutin. In terms of viscosity, spreadability, drug encapsulation,

pH, and *in vitro* diffusion experiments, the proniosomal formulations were successfully done by the proniosomal gel for delivery of the drug into topical region for 12 hr. The penetration depends upon the drug solubility and bioavailability which was enhanced by the concentration of cholesterol and non-ionic surfactant. The optimized formulation (PG4) showed moderate viscosity along with sufficient gel strength. 92.41% drug release achieved at the end of 12 hr. Drug release and viscosity could be adjusted by varying concentrations of cholesterol and non-ionic surfactant. The results concluded that the developed proniosomal gel could perform better for leading to improve solubility, bioavailability, efficacy and better patient compliance.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ATR-FTIR: Attenuated Total Reflectance- Fourier Transform Infrared; **DSC:** Differential Scanning Calorimetry; **Gm:** Gram; **Mins:** minutes; **PG1:** Proniosome Gel 1.

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