RESEARCH ARTICLE

Design, Development and *Ex-vivo* Evaluation of Nanosponges Loaded Topical Gel for Rejuvenation of Skin

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ABSTRACT

The study aimed the development of nanosponges was the choice due to the numerous advantages of this novel technology over the others. Rutin was chosen as it has excellent antioxidant potential. The compatibility study was performed using Fourier transform infrared (FTIR) and there was no interaction found. The optimization study was brought off by Design expert software (version.13). Nanosponges were prepared by emulsion solvent diffusion method. The prepared sponges were brimmed in a night gel formulation for their suitable delivery to the skin. The prepared nanosponges were evaluated for various parameters such as particle size analysis, entrapment efficiency and zeta potential which were found in the acceptable limit. *In-vitro* permeation study was carried out using Franz-diffusion cell and the cumulative percent drug permeation was found to be 69 to 84.5% in 30 hours. A gel formulation was prepared using rutin-loaded nanosponges and studied for various parameters such as pH, viscosity, and extrudability for various combinations using NS-4. *Ex-vivo* drug diffusion study reveals that the night gel took 300 minutes for 78.26 to 94.16% permeation. The IC_{50} values of the flavonoid combination, NS-4 was found to be 54.70, 52.76, and 51.65 µg/mL in the different *in-vitro* models viz. DPPH, when compared to that of the standard ascorbic acid. Conclusively, the objective of delivery of rutin using nanosponges as carriers for the target delivery was achieved by NS-4 formulation and successfully delivered as night gel for skin rejuvenation.

Keywords: Nanosponges, Rutin, Design of experiments, Night gel, Antioxidant, Franz-Diffusion Cell.

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INTRODUCTION

Scope of Proposed Investigation

The study was helpful in understanding the delivery of the nanoparticulate system as a topical formulation, also called as cosmeceuticals. The objective of this research was the successful delivery of rutin as nanosponges loaded in night gel as a cosmeceutical preparation for skin rejuvenation. Active formulation is the choice in order to reduce the side effects of the cosmetic formulation and imparting novel technic will provide a wonderful solution for skin issues like skin damage due to lightening, laser treatments, sun exposure, free radical deposition and excess chemical treatments.[1] Moreover, the development of sponges is the choice due to the numerous advantages of this novel technology over the others. The entrapment of the drug molecule and its release is the criteria of selection that serves superior to other dosage forms.[2]

Nanoparticles in Cosmeceuticals

The most convenient way of providing the delivery through topical route for skin ailments by creams, gels, and ointments to name a few. But in order to achieve the most prominent delivery via this route, we stepped towards the novel methods.[3,4] It has been observed that novel carriers such as vesicular system, microparticulate system and nanoparticulate systems are magnificent discoveries for more effective treatment. In the vesicle systems, we have carriers such as liposomes, niosomes, transferosomes, ethosomes, etc. as they serve a suitable release pattern. Formation of a depot in the skin is the working manner and some find it efficacious in topical delivery.[5-7] These systems in such small sizes easily cross the barriers of the skin and move deeper for better results.[8] For more specific delivery we choose the nano particulate systems which have the size ranges from 100 to 1000 nm. These are way smaller particles than we thought and work immensely great to achieve any kind of target delivery.[9] Nanosponges have caught the attention of pharmaceutical researchers for drug delivery because they have the capacity to carry hydrophilic and lipophilic fragments.[10]

Topical gels: These are semi-solid substances with a confined liquid phase inside a three-dimensional gum (natural or synthetic) polymeric matrix that has a high degree of established chemical or physical cross-linking.[11] Gel formulations perform superior as compared to other topical drug deliveries. Gels are rich in liquid but do not flow in the steady state and comprise of substantially dilute cross-linked,

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and two-component semi-solid system. Gels become more popular drug delivery system because of the molecular stability of the drug, network structure, and biocompatibility.

MATERIALS AND METHODS

Rutin was purchased from Carbanio, Hyderabad, ethyl cellulose was purchased from LobaChemie, Mumbai, HPLC methanol was collected from HiMedia, polyvinyl alcohol was obtained from Sigma Aldrich. The materials are listed in Table 1.

Emulsion Solvent Diffusion Method

The organic phase containing drug and polymer was added to the external phase with an emulsifier. Then this suspension was stirred (at 1000 rpm for 3 hours). The supernatant was then separated by transferring into another beaker and the obtained powder was dried. Then the solid crystals of nanosponges are obtained by solvent evaporation.[12] The preparation method is shown in Figure 1.

Preparation of Night Gel

By adjusting carbomer-940 (0.5, 1, 1.5%), several gel formulations have been developed.[13] The polymer had been immersed in water for a duration of two hours. After stirring, triethanolamine(TEA) was incorporated to neutralize it. The optimized sponges (NS-4) was dissolved into a known amount of propylene glycol (PG) after it had been pre-weighed.[14] After that, it was put in a Carbopol mix and stirred for a further 20 minutes. The dispersion was left to hydrate and swell for 60 minutes (Table 2).

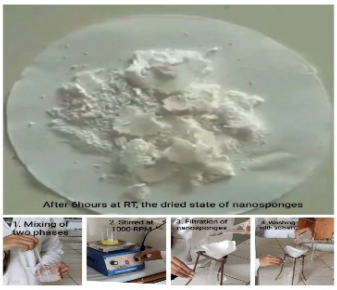

Figure 1: Preparation of nanosponges

Table 1: Optimized formula (Design Expert software version.13)

Formulation code	*Rutin (mg)*	*Ethyl cellulose(mg)*	*PVA (mg)*	*Dichloromethane (mL)*
NS-1	247.344	586.635	936.694	36.391
NS-2	250.000	500.000	850.000	30.000
NS-3	250.000	750.000	700.000	50.000
NS-4	280.875	544.008	755.009	43.729
NS-5	255.115	816.615	907.728	49.678
NS-6	244.536	664.442	755.829	47.653

Table 2: Formulation of night gel

Ingredients	*Formulations*			
	NS G1(F1)	*NS G2(F2)*	*NS G3(F3)*	*NS G4(F4)*
Carbomer-940 (%)	0.5	1.0	1.5	2.0
Propylene glycol (%)	0.5	0.5	0.5	0.5
Propylparaben (%)	0.02	0.02	0.02	0.02
Triethanolamine (mL)	0.1	0.1	0.1	0.1

(Note: Ns G – Nanosponge Gel)

RESULTS

Compatibility Studies

IR analysis and DSC analysis: FTIR spectra of the rutin, ethyl cellulose and drug in combination with polymer was performed to identify the structure and interactions. The FTIR results are shown in Figures 2-4. DSC analysis is predicted in Figure 5.

Optimization Study (Design Expert Software Version.13)[13,15]

Optimization results are predicted in Figures 6-8.

Particle size determination

Particle size analysis was done using Melvin analyzer,[16] which found all the nanosponges within the range 228 to 262 nm. The results are shown in Table 3.

Determination of entrapment efficiency

The loading efficiency of the drug was calculated as:

$$Loading\ efficiency = \frac{Actual\ drug\ content\ in\ nanosponge}{Theoretical\ drug\ content} \times 100$$

Zeta potential

Zeta potential depends on the surface charge of the particle is a very important parameter for the stability of the nanoparticle.[17] The results are shown in Table 3.

In-vitro drug release

In-vitro release kinetics experiments were carried out using a franz diffusion cell. It is important to find out the drug release

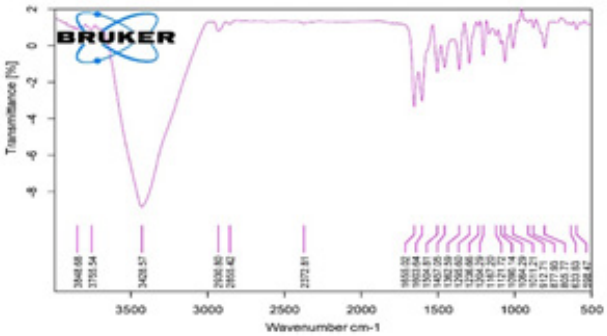

Figure 2: FTIR spectra of rutin

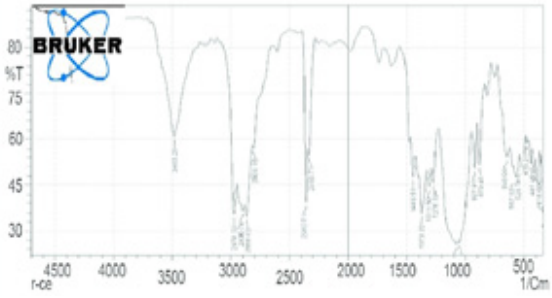

Figure 3: FTIR spectra of ethyl cellulose

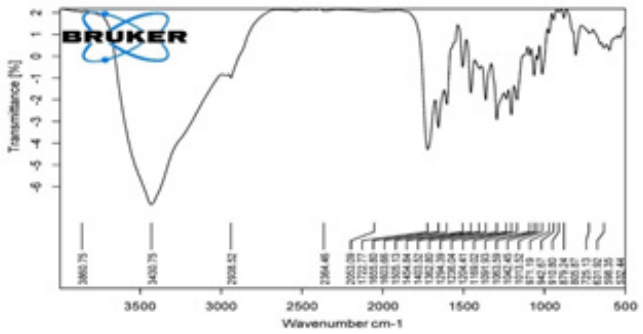

Figure 4: FTIR spectra of drug + polymer

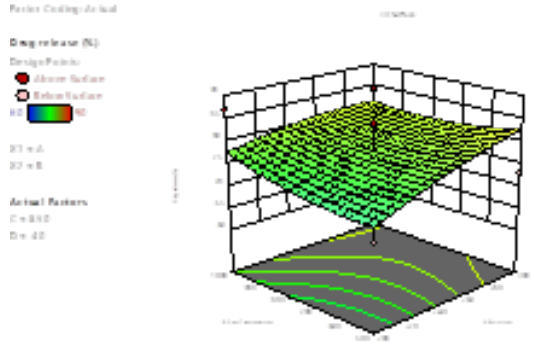

Figure 8: 3-D response plot exert influence on drug release

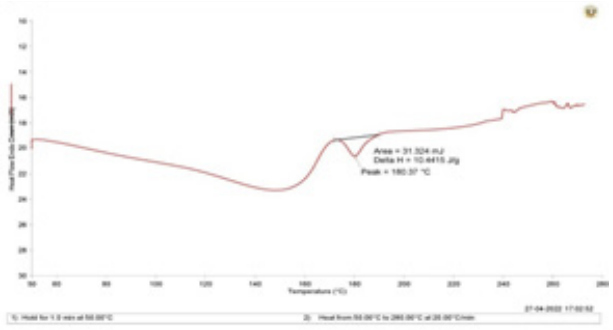

Figure 5: DSC of rutin

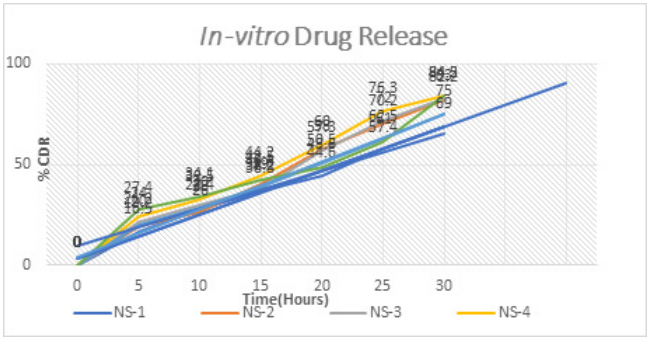

Figure 9: *In-vitro* %drug release of nanosponges

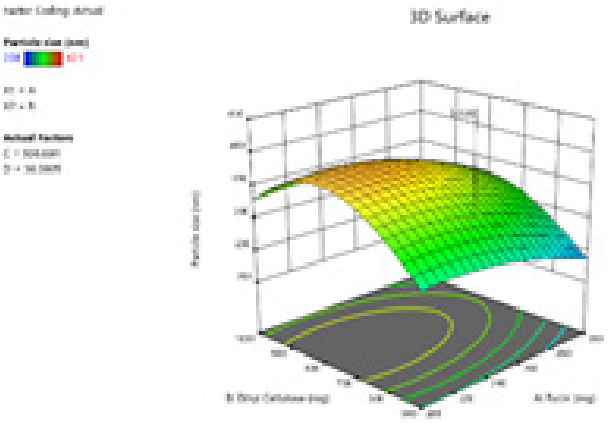

Figure 6: 3-D response plot exert influence on particle size,

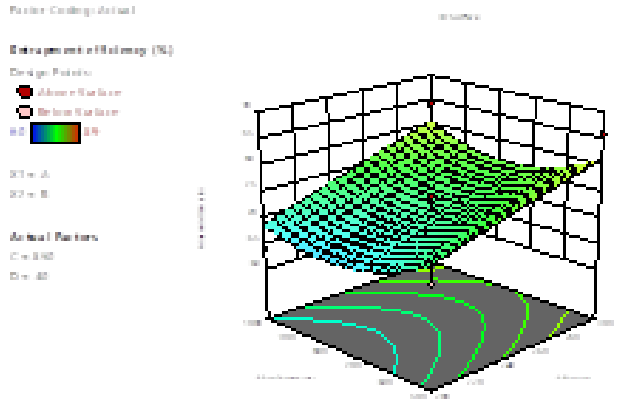

Figure 7: 3-D response plot exert influence on entrapment efficiency of nanopsonges,

pattern from the prepared nanoparticulate system.[18,19] The cumulative percent (%) drug release was observed to be 69 to 84.5% in 30 hours. The results are shown in Table 4 and a graph was predicted in Figure 9.

Microscopic study (SEM analysis)

The surface morphology was examined by SEM. It confirmed the spherical shape and particle's porousness. The results are shown in Figure 10.

In-vitro antioxidant study of flavonoid

The various concentrations ranging from 20 to 80 µg/mL of the different flavonoid combinations were tested for their free radical scavenging activity. It was found that at 80 µg/mL, among all the three combinations screened, NS-4 was found to exhibit good antioxidant activity when compared to that of the standard ascorbic acid. The IC_{50} values of the flavonoid combination, NS-4 was found to be 54.70, 52.76 and 51.65 µg/mL in the different in vitro models viz., DPPH, total anti-oxidant assay and lipid peroxidation assay respectively

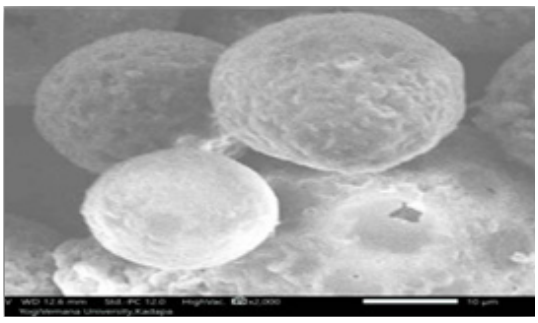

Figure 10: SEM of NS-4 formulation

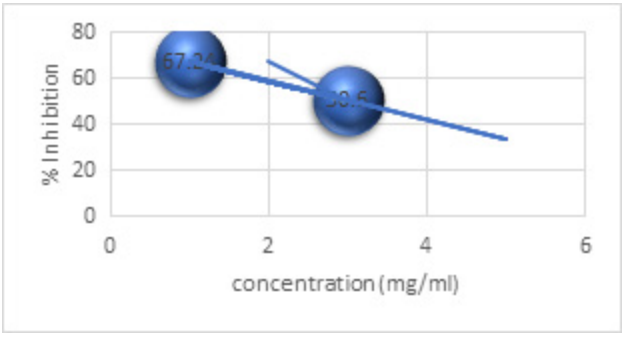

Figure 11: Antioxidant potential of NS-4 with standard

Table 3: Particle size analysis, entrapment efficiency and zeta potential

Formulation code	*Particle size (nm)*	*Entrapment efficiency (%)*	*Zeta potential (mV)*
NS-1	328 ± 1.15	77.5	-23.8
NS-2	338 ± 1.18	72.1	-22.8
NS-3	362 ± 1.15	69.5	-22.4
NS-4	334 ± 1.15	85.2	-21.2
NS-5	354 ± 1.08	74.3	-27.6
NS-6	410 ± 1.12	82.2	-25.8

Table 4: *In-vitro* %drug release

Time (hours)	*Formulations*					
	NS-1	*NS-2*	*NS-3*	*NS-4*	*NS-5*	*NS-6*
0	0	0	0	0	0	0
5	16.5	19.2	21.2	24	20	27.4
10	28	26	30	32.5	28.4	34.1
15	36.8	40.4	38.2	44.2	39	42.5
20	44.6	58	57.3	60	50.5	48.6
25	57.4	70.2	72	76.3	62.5	61
30	69	82.2	83	84.5	75	84.2

Table 5: Antioxidant potential of the flavonoid combinations

S. No.	*Formulation*	*IC_{50} values in µg/mL*	
		DPPH scavenging assay	*Ascorbic acid*
1	NS-4	67.24	50.6

when compared to that of the standard Ascorbic acid (50.6, 49.54 and 49.25 µg/mL, respectively),[20] The results indicate the antioxidant potential of the effective combination for further development as a topical night gel. The results are shown in Figure 11. Antioxidant potential of the flavonoid combinations is represented in Table 5.

In-vitro antioxidant activity (DPPH assay) and IC_{50} value

Evaluation Parameters of night gel

- *pH Determination*

Three sample readings were taken and averaged in order to determine the pH values. As result shown in Table 6, every formulation fell within the skin's standard pH range.

- *Rheological studies*

The viscous nature of the prepared night gel combinations was estimated with a Brooke field viscometer at 30 rpm and spindle number 64. The results are specified in Table 6.

- *Extrudability test*

A generous amount of force is applied to the gel formulation until it flows through the outlet of the test cell. The results are shown in Table 6.

- *Drug content determination*

The UV spectrophotometer functioning within the 200–400 nm range was utilized to estimate the drug content of various night gel formulations.

- *Ex-vivo Diffusion Study*

A prepared gel formulation's diffusion study (Figure 12) had been carried out using only recently isolated goat abdominal skin. The results are shown in Table 7 and Figure 13.

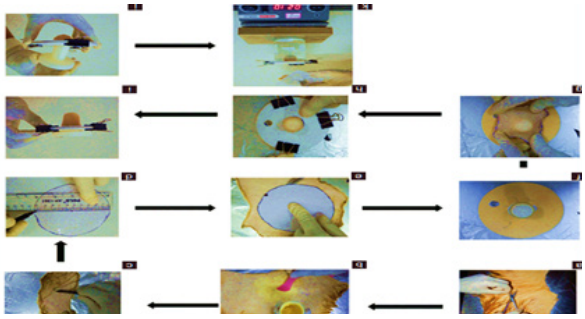

Figure 12: (a): Hair remover used to clean the hairs (b): Scalpel used for eliminating fat material (c): Diffusion assembly fabricated and semipermeable membrane measured of the size of donor section (d): Goat skin measured equal to membrane (c): Arrangement for scale up of skin at donor section (f-j): Finally the gel applied to donor section

Table 6: Estimation of pH, viscosity, extrudability and %drug content of night gel

Formulation	*pH*	*Viscosity(cp)*	*Extrudability*	*%Drug content*
NS G1(F1)	6.22	14374	19.56	96.84
NS G2 (F2)	6.86	15241	20.14	97.63
NS G3 (F3)	6.48	14353	25.12	97.42
NS G4 (F4)	6.50	14252	22.32	95.10

Table 7: Data for *ex-vivo* cumulative %drug permeation data

Time(min)	*NS G1(F1)*	*NS G2(F2)*	*NS G3(F3)*	*NS G4(F4)*
0	0.00	0.00	0.00	0.00
5	11.01	09.80	11.82	14.55
10	14.27	13.17	26.54	20.76
15	22.43	21.45	35.40	27.35
20	31.08	31.63	41.07	38.71
30	42.87	38.10	51.14	40.89
60	53.95	47.46	62.57	55.78
120	65.86	63.56	76.49	69.09
240	78.62	73.95	91.14	84.48
300	80.05	78.26	94.16	87.42

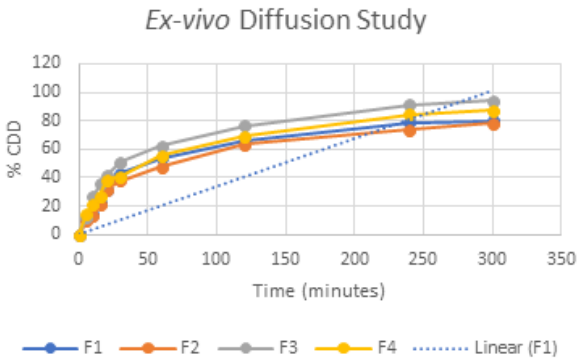

Figure 13: *Ex-vivo* diffusion study

DISCUSSION

Development of cosmeceuticals using active ingredients for rejuvenation of skin by efficacious delivery of an active ingredient by controlled release by a novel carrier. The optimization study was performed using Design Expert software (version.13) to formulate the nanosponges with less or no error. The possibility of getting actual results close to the predicted values, there is factorial designing using the model of variance. The nanosponges of rutin were successfully prepared by emulsion solvent diffusion technique and evaluated for various parameters such as particle size analysis, entrapment efficiency and zeta potential which were found in the acceptable limit. *In-vitro* permeation study was accomplished using Franz-diffusion cell and the cumulative percent drug permeation was found to be 69 to 84.5% in 30 hours. Nanosponges(NS-4) were loaded in gel for topical delivery of the drug. Gel formulations was also studied for various parameters such as pH, viscosity, and extrudability. *Ex-vivo* drug diffusion study reveals that the night gel took 300 minutes for 78.26 to 94.16% permeation. The IC_{50} values of the flavonoid combination, NS-4 were found to be 54.70, 52.76 and 51.65 µg/mL in the different *in-vitro* models viz. DPPH, when collated with standard ascorbic acid.

CONCLUSION

The objective of the preparation of a novel cosmeceutical night gel formulation was successfully done and the target delivery was achieved by loading rutin in the novel delivery vehicle known as nanosponges which is specifically used for successful topical delivery of the drug. All the parameters were resulted in the required range which provides a successful delivery of rutin nanosponges up to 30 hours and the aim of using rutin for its very prominent antioxidant properties had been utilized for skin rejuvenation. The results indicated the antioxidant potential of the effective combination for further development as a topical night gel. NS G3 formulation delivered best results as topical night gel formulation on the basis of ex-vivo permeation rate.

ACKNOWLEDGEMENT

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