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Formulation and Development of Quercetin Loaded Microsponges for Topical Sunscreen

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ABSTRACT :

The aim of this work was to formulate quercetin loaded starch microsponges incorporated in sunscreen lotion for photoprotection from the harmful UV radiations. Quercetin is a polyphenolic class of flavonoid which shows antioxidant and anti-inflammatory effects. The starch microsponges were prepared using emulsion gelation technique by optimizing independent variables were screened by fractional factorial design and optimized by three-level two-factor full factorial design. Polymer concentration and volume of dichloromethane and their response on entrapment efficiency and production yield the microsponges showed 91.16±1.24% and 95.23±2.11% respectively. The Quercetin loaded microsponges were incorporated in sunscreen lotion. Sunscreen lotion was evaluated for viscosity, Spreadability, homogeneity and grittiness, stability study, in-vitro drug release study and in-vitro SPF determination sunscreen lotion showed 5672 cps, good Spreadability, Homogeneous with no aggregates, stable, 92.35±1.22% drug release and SPF-15.95 respectively. Thus, quercetin microsponges loaded sunscreen lotion could be safe and suitable for photoprotection from harmful UV radiation.

Keywords: Microsponges, Sunscreen, Quercetin, Formulation and Development

INTRODUCTION

Every year, about one million people are diagnosed with skin cancer due to exposure to UV radiation. This causes several harmful effects on human skin mainly on the areas of the body that are most frequently exposed to the sun, such as the face, neck, head and back of the hands (Dutra *et al.*, 2004). Many molecules are used as UV-filters absorbing UVA and UVB radiation which protects skin from harmful solar radiation. However, the major problem of sunscreens products is their allergic effects and diffusion through the skin into the systemic circulation (Bhuptani and Patravale, 2019).The harmful effects of solar radiation are caused by the ultraviolet (UV) region of the electromagnetic spectrum, which can be divided into three regions: UVA from 320 to 400 nm, UVB from 290 to 320 nm and the UVC from 200 to 290 nm. The UVC rays which are filtered by the atmosphere before reaching earth. UVB rays are not completely filtered out by the ozone layer and are responsible for the damage due to sunburn. UVA rays reach the deeper layers of the epidermis and dermis and produce the premature aging of the skin. The Ultraviolet radiations can cause skin damage during any season or at any temperature. Sunscreens are mainly used to protect our skin from the harmful UVA and UVB rays and also to conserve moisture content of skin. The photoprotective action of a sunscreen formulation is measured by the sun protection factor (SPF) (Donglikar and Deore, 2017).

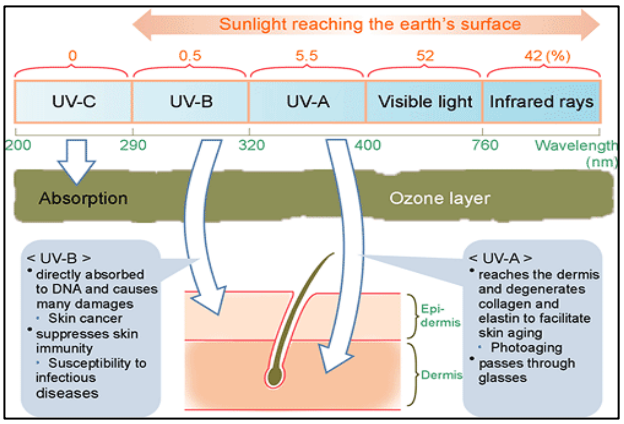

Figure 1: Types of UV rays and their influences on health (ref. Ichihashi M. et al., 2002)

To control the delivery rate of active agents to determine site in the human body has been one of the biggest challenges faced by pharmaceutical industries. Several systems have been derived for the Transdermal Drug Delivery system to improve the efficacy of drugs that may be better

administered through skin. But it is not effective whose final target is skins itself. The Microsponges technology was developed in 1987 by Won. Microsponges are porous microspheres having a particle size range of 5-150 µm. The advantage of this system is to entrap a wide range of active ingredients due to its interconnected pores on the surface and release over extended periods of time (Osmani *et al.*, 2015). There are two main methods for preparation of microsponges: Liquid-liquid suspension polymerization and Quasi-emulsion solvent diffusion method (Jyoti and Kumar, 2018).

Advantages of microsponges drug delivery system:

- It can enhance the product performance.
- It can improve the bioavailability of active ingredients.
- They have stability over pH range of 1-11 and temperature upto130^0 C.
- It is free flowing and cost effective
- It acts as a controlled drug delivery system.
- It can reduce the skin irritation problem of the drug and improve the patient compliance.
- It can improve product elegance and improve formulation flexibility.
- Microsponges are non-allergic, non-mutagenic and non-toxic also.
- It can control the release rate of the drug for an extended period of time (Kaity *et al.*, 2010).

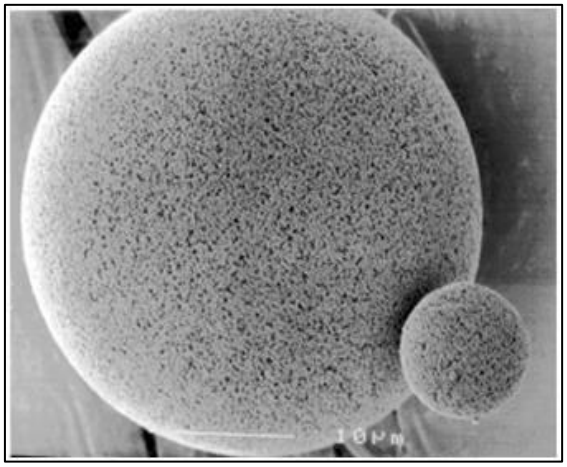

Figure 2: Structure of Microsponges

Properties of drug for loading into microsponges

- The drug must be fully immiscible in monomer or by the addition of little water it should be made miscible.
- The drug should be water immiscible or it should be slightly soluble.
- It should be inert with respect to monomers.
- To avoid cosmetic problems, the solubility of drugs in the vehicle must be checked; otherwise before the application the vehicle will be removed by the microsponges.

Release mechanism

- **Pressure:** the release of drugs from microsponges onto skin by rubbing or applying pressure.
- **Temperature change:** The flow rate of active ingredients from microsponges generally depend upon the skin temperature, increase in the skin temperature causes increase in the flow rate and hence increase in the release of active ingredient.
- **Solubility:** The water-soluble active ingredients are loaded in the microsponges in the presence of water**.** The partition coefficient of the ingredient between the microsponges and the outside system plays a major role in release which can also be activated by diffusion (Osmani *et al.*, 2015).

Application of microsponges system

- In the sunscreens, it is applicable for the long-lasting product efficacy by improving the protection from sunburn and reduced irritation and sensitization.
- Anti-acne (e.g. Benzoyl peroxide) cream, microsponges applicable for maintaining the efficacy by decreasing the irritation and sensitization of the skin.
- Anti-inflammatory (e.g. Hydrocortisone) the microsponges make the long-lasting activity by reducing the allergic reactions of skin and dermatoses.
- sustained release activity for antifungal formulation
- It can reduce unpleasant odour by lowering irritation with extended safety and efficacy of Anti- dandruffs formulations (e.g. zinc pyrithione, selenium sulfide).
- Improved activity and extended release in the antipruritic formulation.

- It is also applicable for improving stabilization against oxidation by improving efficacy and aesthetic appeal.
- It gives prolonged activity by reducing irritation, odour and greasiness e.g. Rubefacients (Kaity
- *et al.*, 2010).

DRUG AND EXCIPIENT PROFILE

A. Description

a) Name: Quercetin

b) Molecular formula: $C_{15}H_{10}O_7$

c) Structural formula:

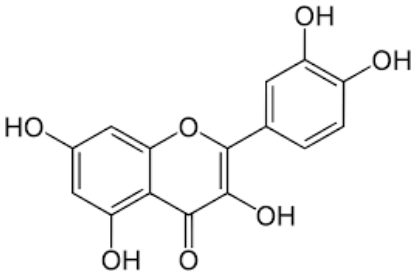

Figure: Structure of Quercetin

d) IUPAC name: 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy chromen-4-one

e) Molecular weight: 302.238 g/mol

f) Category: Antioxidant, Anti-inflammatory, Anti-cancer agent, etc.

g) BCS Class: II

h) Physical state and appearance: Yellowish crystalline powder.

i) Odour: Practically Odourless

j) Taste: Bitter

k) Colour: Yellow

l) Melting point: 316ºC

m) Solubility: Poorly soluble in water, freely soluble in ethanol, methanol and acetone

n) Storage: Store in tightly closed containers, in cool and well-ventilated places.

o) Dose: dose is 10 / 100 mg

B. Indication: Quercetin is multi-functional Phyto-constituents used in various disease conditions such as Photoprotection, cancer, inflammation.

3. MATERIALS EQUIPMENT AND METHODS

Materials

Potato starch, ethanol and dichloromethane. Quercetin (98.5% pure) was purchased from PC Chem, India. Tween 80 were received as gift samples from Mohini Organics Pvt. Ltd., India respectively. All other chemicals were of analytical grade and were used as received.

3.1 List of Equipments

Table 2: List of Equipments

Sr. no.	Equipments	Model/ make/ manufacturer
1	UV Visible Spectrophotometer	UV 1800, Shimadzu Corporation, Japan
2	FTIR	ATR, Alpha Brucker, Germany
3	Mechanical stirrer	REMI Instruments, Mumbai, India
4	Hot air oven	Dolphin, laboratory oven, India
5	Microscope	Advance microscope, India
6	Texture Analyzer	CT3, Brookfield engineering labs, Inc, US
7	Distillation assembly	Infusil India Pvt. Ltd, Bangalore, India
8	Electrical balance	Contech, Instruments, India
9	pH meter	S-20, Mettler Toledo, India
10	Thermometer	Jyoti scientific, India
11	Digital Ultra Sonicator	Citizen, India
12	Refrigerator	Godrej. EDGE. Mumbai, India
13	Diffusion cell apparatus	DBK Diffusion Cell Apparatus Mumbai, India
14	Stability chamber	Thermolab Scientific Equipments, Mumbai, India
15	Viscometer	LDVDE, Brookfield Engineering Corporation, US

Method

3.1.1 Preformulation study

3.1.1.1 Characterization of the Quercetin:

A Organoleptic properties of the quercetin

Sample of Quercetin was analysed for color, odor and physical appearance.

B Melting point:

The melting point of quercetin was measured using capillary method by using melting point apparatus.

C Determination of wavelength (λ) maxima of quercetin

Stock solution A (1000ppm) of quercetin was prepared in methanol by dissolving 10 mg of quercetin in 10 ml of distilled water and further diluted by methanol to obtain 100ppm solution. Then, the solution was scanned between 200-400 nm using ethanol as a blank solution. The UV spectrum of quercetin was compared with the reference standard spectrum at 270 nm using (double beam UV Visible Spectrophotometer, Shimadzu Corporation, Japan).

D Identification of quercetin by FT-IR spectroscopy

IR spectrum of quercetin was measured by using the FT-IR spectrophotometer. Samples were scanned over a range of 4000-400 cm^{-1} in the FTIR instrument (Alpha Brucker, Germany).

3.1.2 Drug – excipient compatibility study

The interaction of drug and excipients under experimental conditions is an important parameter before formulation. Drug- excipient compatibility study is necessary to confirm that the drug does not react with the excipients or polymers and does not affect the shelf life of the product. Thus, drug-excipient interaction study was carried out by using FTIR.

3.1.3 UV analysis of quercetin

3.1.3.1 Calibration curve of quercetin in methanol

a) Standard stock solution

Accurately weighed quercetin (10 mg) was dissolved in 10 ml with methanol to get 1000ppm solution. From the above solution 1 ml was taken into a volumetric flask (10ml) and volume was made up to 10 ml with methanol to give 100ppm.

b) Sample solution

From the above standard stock solution, (100 ppm) 0.5, 1, 1.5, 2, and 2.5 ml was taken into 10 ml volumetric flask and volume was made to 10 ml with methanol to give different drug concentrations of 5-25 ppm, respectively. The absorbance of the above solution was measured at 270 nm (ref. Sachin thesis 2019).

3.1.3.2 Calibration curve of quercetin in phosphate buffer pH 7.4

a) Standard stock solution

Accurately weighed quercetin (10mg) was transferred to 10 mL volumetric flask and volume was made up to 10 mL with phosphate buffer pH 7.4 to give 1000 ppm solution. From the above solution, 1ml

7.4 to give 100ppm.

b) Sample solution

From above standard stock solution, (100ppm) 0.5, 1, 1.5, 2 and 2.5ml was taken into 10 mL volumetric flasks and volume was made to 10 mL with phosphate buffer pH 7.4 to give different drug concentrations of 5-25 ppm, respectively. The absorbance of above solutions was measured at 270 nm.

3.2 Formulation of microsponges

3.2.1 Preparation of Starch Microsponges

Starch Microsponges were prepared by the emulsion gelation method using a high-speed mechanical stirrer. The starch microsponges were prepared with optimized parameters using different concentration of polymer and volume of dichloromethane. To form a clear aqueous suspension of starch (1000 mg starch in 15 ml water) was heated up to 100°C with continuous stirring in a beaker. Cool the clear starch solution (internal phase) at room temperature and then added into the dichloromethane (external phase) (50 ml) and stirred at 2500 rpm for 30 mins. to form w/o emulsion with 1 ml Tween 80. The emulsion was frozen in a refrigerator, after 24 h the emulsion was then subjected to solvent exchange using a different graded concentration of ethanol (40%, 60%, and 80%) every 1 h and at the last pure ethanol (100%) was used to completely remove the water. The porous starch formed was filtered and dried under a vacuum dryer to obtain free flowing Microsponges. (Bhuptani and Patravale, 2019).

3.2.2 Drug loading in starch Microsponges

Quercetin was loaded into the formed free flowing starch Microsponges powder by the immersion/solvent evaporation method. The quercetin (10-100mg) was gradually added in 10ml ethanol. Accurately weighed 100mg of Microsponges were soaked in the solution of quercetin and stirred for 4 h at room temperature then dried in a vacuum dryer at 50–60°C (Bhuptani and Patravale, 2019).

3.3 Preliminary trial batches of starch Microsponges

Selection of formulation parameters and process variables for factorial design

Preliminary trials were undertaken to establish the effect of polymer concentration on the physical characteristics of starch Microsponges. Quercetin loaded starch Microsponges were prepared by emulsion gelation method using starch by trial and error basis. Formulations were prepared by varying parameters such as, polymer concentration, volume of solvent

The levels and factors of quercetin loaded starch Microsponges formulation were selected based on preliminary experiments. The screening of variables was performed using Contour plot and 3D Surface graph using Design-Expert® software (version 11.0, Stat-Ease, UK). Four independents variables, namely, Concentration of Polymer (X_1) (800-1000 mg), Volume of Dichloromethane (X_2) (40-60 ml) were selected for Full Factorial Design (3^2). Final parameter was selected: polymer concentration 1000 mg, internal phase volume (Dichloromethane) 60 ml. Based on the result of Contour plot and 3D Surface graph, further, two of the above independent variables, which contributed with high effect (except stirring speed) were selected for further optimization study. A two-factor, three-level full factorial design was applied to dependent variable (Y1) Production yield (%) and (Y2) entrapment efficiency (EE %). The study design consists of nine runs (at zero center point) as shown in Table 3.

3.3.1 Experimental design

A three level, two factor full factorial design was used to optimize the Microsponges. Formulation batches were coded as F1 to F9. The variables selected for optimization were as follows:

Table 3: Formulation batches of Microsponges in coded form

3^2 Factorial Design			
Independent Variables	**Level**		
	Low	**Medium**	**High**
Concentration of Polymer (mg) (X_1)	800	900	1000
Volume of Dichloromethane (ml) (X_2)	40	50	60
Dependent Variable			
Production yield (%)	**(Y1)**		
Entrapment efficiency (%)	**(Y2)**		

Table 4: Formulation composition of Starch Microsponges

Batch code	Concentration of Polymer (mg) (X1)	Volume of Dichloromethane (ml) (X2)	Stirring Speed (nm)	Stirring Time (min)
F1	800	40	2500	30
F2	900	60	2500	30
F3	1000	50	2500	30
F4	900	40	2500	30
F5	1000	60	2500	30
F6	800	60	2500	30
F7	900	50	2500	30
F8	800	50	2500	30
F9	1000	40	2500	30

3.3.2 Characterization of Quercetin Microsponges

A Drug content

Accurately weighed (100mg) Microsponges were dissolved in methanol (100ml) and sonicated for 15mins. This solution was filtered using Whatman filter paper. The part of solution (1ml) was withdrawn in a 10ml volumetric flask and diluted up to the mark. The quantitative determination of Quercetin in the starch Microsponges was carried out using linear model UV absorbance detector using double beam UV spectrophotometer (1800, Shimadzu, Japan) at 285 nm against the blank (methanol) (Pandit *et al.*, 2017). Drug content was calculated by using the following formula:

$$\text{Drug content} = \frac{\text{Absorbance} - \text{Intercept}}{\text{Slope}} \times 100 \quad \ldots\ldots (1)$$

B Entrapment efficiency (EE %)

Entrapment efficiency is the total amount of drug present in the product (Pandit *et al.*, 2017). Entrapment efficiency was calculated by using the following formula:

$$\text{Drug Entrapment efficiency} = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100 \quad \ldots\ldots (2)$$

C Production yield (%)

Production yield of Microsponges was determined by calculating accurately the initial weight of raw materials and the final weight of Microsponges obtained (Pandit *et al.*, 2017). Production yield was calculated by using the following formula:

$$\text{Production yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100 \quad \ldots\ldots (3)$$

D Particle Shape

Particle shape of starch microsponges was observed under the Optical Microscope.

3.6 Formulation of Microsponges loaded sunscreen lotion

a. Dose calculation

Total amount of microsponges to be incorporated in the sunscreen lotion to make 6% w/w of sunscreen lotion of quercetin was calculated depending on the total drug content in optimized formulation batch of starch microsponges.

b. Formulation of Sunscreen lotion

The total amount of Microsponges required to be incorporated in sunscreen lotion to get 1.8% w/w of quercetin was calculated based on drug content**.** The base of Sunscreen lotions was prepared. A little quantity of water was taken in a small beaker, heating upto 85°C and Carbopol 934 was slowly added with continues stirring till it completely dispersed in water. The beaker was removed from heating and glycerin and Triethanolamine were added (water phase). In a separate beaker Cetyl alcohol, glyceryl stearate, propyl paraben and olive oil were mixed and heated at 85°C (oil phase). Then the both phases were mixed and stirred for 20mins, after cooling rose oil were added for the fragrance. Quercetin loaded Microsponges was incorporated in sunscreen base to form Microsponges loaded sunscreen lotion. The sunscreen lotion was stored in a closed container for 24h (ref. Sharma PP book 2018).

Table 5: Formulation of sunscreen lotion

Sr. no.	Ingredients	F1	Uses
1	Quercetin loaded Microsponges	6.87g	Sunscreening agent
2	Carbopol 940	2.5g	Thickener
3	Glycerin	1.5ml	Humectant
4	Triethanolamine	0.9ml	Neutralizer
5	Cetyl alcohol	1.0g	Co-emulsifier
6	Glyceryl stearate SE	0.75g	Emulsifier
7	Propylparaben	0.05g	Preservative
8	Olive oil	2.5ml	Occlusive

9	Rose oil	0.025ml	Fragrance
10	Distilled water	Upto 30g	Diluent

3.7 Evaluation of sunscreen formulation

The physicochemical properties such as color, odour, pH, viscosity, Spreadability, thermal stability and adhesiveness were evaluated.

a. Physical evaluation

The sunscreen formulation was evaluated for organoleptic characteristics, visual appearance, odour, colour, texture, homogeneity and grittiness.

b. pH measurement

The pH of the sunscreen formulation was measured using pH meter (digital instrument corporation, India), Standardized using buffer, pH 7 before use, by putting the tip of the electrode into the lotion and after 2 min the result was recorded. The measurement of pH of sunscreen lotion was done in triplicate and the mean value was recorded (Pandit *et al.*, 2017).

c. Determination of Viscosity

The Viscosity measurements were done by Brookfield viscometer (Brookfield Engineering Corporation, USA), by spindle no.64 at 25rpm. The 25g of sunscreen formulation was poured into the sample holder of the viscometer. The viscosity of formulation was measured and recorded (Pandit *et al.*, 2017).

d. Texture profile analysis

Texture profile analysis (TPA) of sunscreen formulation was performed using (CT3 Brookfield Engineering Labs.inc., USA) in compression mode by using tensile strength accessory (TA3/100). Optimized microsponges loaded sunscreen lotion was filled in the female probe, taking care to avoid air pockets into the samples. A conical analytical male probe (30 mm diameter, 60^0) was forced down into each sample at defined rate (1mm/s) and to a defined depth (10 mm). At Least two replicates analysis samples were performed. When the trigger force of 5 g was attained, the probe proceeded to pierce samples at speed of 2 mm/s depth of 25mm. When a specified distance was achieved, the probe departed from the sample at post- test speed 2mm/s. From the resulting forced-time plot, hardness and adhesiveness of sunscreen lotion was determined (Pandit *et al.*, 2017).

Figure 5: Texture profile analyzer

e. Homogeneity and grittiness

A small quantity of sunscreen lotion was pressed between the thumb and the index finger. The consistency of lotion was noticed (whether homogeneous or not), if there was any coarse particle on fingers. Also, the homogeneity can be detected when a small quantity of the sunscreen lotion is rubbed on the skin of the back of the hand. The grittiness of the prepared sunscreen lotion is also observed in the same manner (Pandit *et al.*, 2017).

f. In-vitro release study

In-vitro release of Microsponges loaded sunscreen formulation was carried out using Franz diffusion cell (DBK Diffusion cell, India) with receptor compartment (25 ml). Cellulose dialysis membrane 150 LA401-1MT (Himedia, India) on which the effective diffusion area of 3.14cm² was pre-wetted for 24h in Phosphate buffer solution (pH 7.4) with 0.5% Tween 80 before the experiment. A predetermined amount of quercetin loaded Microsponges sunscreen lotion (1g) was placed into the donor chamber. Temperature was maintained at 32±0.5°C with a water jacket by the continuous stirring of receptor media at 50 rpm. At predetermined time intervals, 2ml samples of receptor medium was withdrawn and immediately replaced with equal volume of fresh PBS. The concentration of the drug in the receptor compartment was determined by UV spectrophotometer (UV 1800, Shimadzu Corporation, Japan) at 285nm (Pandit *et al.*, 2017).

Figure 6: DBK Diffusion cell apparatus

g. In vitro SPF determination

The in-vitro SPF was determined according to the methods described by Mansur.

Procedure:

1gm of quercetin loaded Microsponges sunscreen lotion was weighed and transferred into 100 ml of volumetric flask diluted with ethanol, and then filtered through cotton, to give 10000 ppm solution. Rejecting the first 10 ml, a 5.0 ml aliquot was transferred to 50 ml volumetric flask and diluted to volume with ethanol to produce 1000ppm solution. Then a 5.0 ml aliquot was transferred to a 25 ml volumetric flask and the volume completed with ethanol (200 ppm solution). The absorption spectra of each aliquot prepared were determined from 290-400 nm, taking ethanol as a blank. The absorption data were obtained in the range of 290nm to 320nm for every 5 nm and 2 determinations were made at each point, followed by the application of Mansur equation. The in- vitro SPF of sunscreen formulation equivalent to 200ppm of drug was also determined by the same method (Dutra *et al.*, 2004).

$$\mathrm{SPF}_{in\ vitro} = \mathrm{CF} \times \sum_{290}^{320} \mathrm{EE}(\lambda) \times I(\lambda) \times \mathrm{abs}(\lambda)$$

Where,

- CF is a correction factor (= 10);
- EE (λ) – erythemal effect of radiation with wavelength λ;
- I (λ) – solar intensity spectrum; Abs (λ) - absorbance of sunscreen product.
- The values of EE (λ) x I (λ) are constants as given determined by Sayre *et al.* 1979 and are shown in the table. The obtained absorbance values Abs (λ) were multiplied with the respective EE (λ) x I (λ) values and then summation was taken and multiplied with the correction factor 10.

Table 6: Normalized product function used in the calculation of SPF (Sayre *et al.*, 1979).

Wavelength (nm)	EE x I (normalized)

290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180
Total	1

h. Stability study as per ICH guidelines

According to the International Council of Harmonization of technical requirements for pharmaceutical for human use (ICH) guidelines, the stability studies of the prepared quercetin loaded microsponges sunscreen lotion were evaluated. The prepared formulation was studied under two variations 25±2 ℃ (room temperature) and 40±2 ℃ (accelerated stability condition) for a period of one month and were evaluated for their physicochemical parameters such as color, pH, viscosity, and SPF(Bhatia and Saini, 2018).

RESULTS AND DISCUSSION

Preformulation study of quercetin

Table 7: Organoleptic properties of Quercetin

Parameter	Observed result
Color	Pale yellow
Odour	Odourless
Appearance	Yellowish white amorphous powder

Identification of drug

The drug identification was carried out by melting point, Fourier Transformed Infrared Spectroscopy & UV visible spectroscopy.

3.3.3 Determination of melting point of quercetin

Melting point of the quercetin was determined by capillary method by using melting point apparatus and it was found to be in the range of 318-320°C which was similar to the reference standard value of 317-320°C.

3.3.4 Determination of wavelength max (λmax) of quercetin

The UV spectrum of quercetin was obtained by scanning the methanolic stock solution in the range of 200 to 400 nm. The maximum absorbance occurred at the 370 nm which is quite near to previous findings.

3.3.5 Identification of quercetin by FT-IR Spectroscopy

FT-IR spectrum of quercetin was obtained by scanning the sample in the range of 400– 4000 cm^{-1}. The characteristic peaks of quercetin were observed at 3666.38, 1006, 2975 and 1663 cm^{-1} which corresponded to the functional group present in the structure of the drug. From the interpretation, the drug was identified and confirmed.

Table 8: FT-IR peaks of quercetin

Observed peaks (cm-1)	Interpretation of chemical group
3666.38	O-H stretching
1006	C-O stretching
2975	-CH3 stretching
1663	C=O stretching
1507	Aromatic C = C stretching

Drug – excipient compatibility study

3.3.6 Drug - excipients Compatibility Study by FT-IR:

Table 10: Standard calibration curve of quercetin in methanol

Concentration (µg/ml)	Absorbance (nm)
0	0 ± 0
5	0.148 ± 0.002
10	0.337 ± 0.004
15	0.482 ± 0.003
20	0.636 ± 0.002
25	0.847 ± 0.003

7.3.2 Calibration curve of quercetin in phosphate buffer pH 7.4

The λmax of quercetin solution in phosphate buffer pH 7.4 by UV spectrophotometer was found at 370 nm. At this wavelength, the standard curve was plotted to confirm the Beer-Lambert's law in the concentration range 5-25 µg/ml (Figure 16).

Table 12: Standard calibration curve of quercetin in Phosphate buffer pH 7.4

Concentration (µg/ml)	Absorbance (nm)
0	0 ± 0
5	0.048 ± 0.002
10	0.145±0.0025
15	0.219±0.003
20	0.301±0.0045
25	0.364±0.003

Figure 11: Standard calibration curve of quercetin in phosphate buffer pH 7.4

Table 13: Observations of Calibration curve in phosphate buffer pH 7.4

Parameter	Observations in PBS pH 7.4
Wavelength (max)	369.8
Beer's limit (µg/ml)	5-25
Correlation coefficient (R^2)	0.995
Slope	0.039
Intercept	0.021

Preliminary study of preparation of starch microsponges formulation

Table 14: Preliminary selection of polymer concentration

Batch code	Concentration of Polymer (mg)	Volume of Dichloromethane (ml)	Production yield (%)	Entrapment efficiency (%)
FT1	800	50	72.35±1.32	70.18±1.75
FT2	900	50	78.28±1.26	79.21±1.83
FT3	1000	50	86.21±2.41	88.26±2.35

Table 15: Preliminary selection of External phase volume

Batch code	Concentration of Polymer (mg)	Volume of Dichloromethane (ml)	Production yield (%)	Entrapment efficiency (%)
FT1	1000	40	68.42±1.35	71.25±1.64
FT2	1000	50	73.56±2.31	79.16±1.23
FT3	1000	60	87.35±1.26	89.24±1.20

Formulation of Quercetin loaded microsponges using three level, two factor (3^2) Factorial Design

3^2 Factorial Design for Quercetin loaded microsponges

Various batches of quercetin laded microsponges with starch were prepared to study the effect of different independent variables on dependent variables by using design expert 11 (stat ease). Nine formulation batches (F1 to F9) were obtained for software design expert 11 (stat ease). (Table 13)

Table 16: Formulation using 3^2 factorial design

Formulation Batches	Concentration of Polymer (mg) (X1)	Volume of Dichloromethane (ml) (X2)	Drug content (%)	Production yield (%) (Y1)	Entrapment efficiency (%) (Y2)
F1	800	40	15.66±1.45	81.21±1.75	80.24±1.83
F2	900	60	14.71±2.21	90.11±1.24	83.66±1.56
F3	1000	50	22.10±1.12	93.38±1.43	90.25±2.16
F4	900	40	20.34±1.15	86.61±1.62	79.28±1.75
F5	1000	60	26.17±1.28	95.23±2.11	91.16±1.24
F6	800	60	15.28±2.33	84.36±1.14	82.75±1.38
F7	900	50	16.37±2.19	89.13±1.32	83.22±1.52
F8	800	50	15.95±1.24	82.42±1.56	84.22±1.22
F9	1000	40	28.66±1.21	92.18±1.23	85.41±1.35

5. CONCLUSION

Quercetin loaded starch microsponges for topical sunscreen lotion were successfully formulated and developed. This study showed beneficial topical effects of quercetin of photoprotection from exposure of UV radiations. The starch microsponges were prepared by emulsion gelation method. this newly developed technique of microsponges which gives controlled release and better permeation of drugs through the skin, due to control release the frequency can be reduced and it becomes more economic and patient compliance is also improves. Overall, a safe and novel sunscreen lotion was developed to achieve more patient compliance.

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