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Original Research Article

Formulation, Optimization and Evaluation of Benzophenone-3 Loaded Ethyl Cellulose Microspheres Incorporated Sunscreen Cream for Enhanced Photoprotection

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Abstract:

The present study aimed to develop and evaluate a controlled-release microsphere-based sunscreen formulation incorporating Benzophenone-3 (Oxybenzone) as an organic UV filter. Microspheres were prepared using the solvent evaporation technique with ethyl cellulose as the polymer. A 3^2 full factorial design was employed to investigate the influence of stirring speed and polymer concentration on particle size, entrapment efficiency, percentage yield, and in-vitro drug release. Preformulation studies confirmed the purity and identity of the drug through melting point determination, FTIR spectroscopy, UV spectral analysis, and solubility studies. The prepared microspheres exhibited high entrapment efficiency (72.00–86.07%), particle size ranging from 107–375 µm, and satisfactory percentage yield (32.18–87.89%). SEM analysis revealed spherical morphology with smooth surfaces. In-vitro drug release demonstrated sustained release up to 120 minutes with 41.56% cumulative release, indicating diffusion-controlled behavior. Sunscreen creams incorporating microspheres showed enhanced UV protection compared to formulations containing free drug. The combination of microspheres with inorganic UV filters such as Zinc oxide and Titanium dioxide exhibited superior photoprotective efficacy. Ex-vivo permeation studies using Franz diffusion cells demonstrated minimal systemic permeation (0.7%) and predominant drug retention (>90%) within the stratum corneum, confirming surface-retentive behavior. The developed microsphere-based sunscreen formulation provides improved photostability, controlled release, enhanced efficacy, and reduced systemic exposure, making it a promising strategy for advanced topical photoprotection.

Keywords: Benzophenone-3; Oxybenzone; Microspheres; Ethyl cellulose; Sunscreen; Controlled release; Photoprotection; Solvent evaporation; Ex-vivo permeation; Inorganic UV filters.

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Introduction

Ultraviolet (UV) radiation is one of the primary environmental factors responsible for skin damage, premature aging, immunosuppression, and photocarcinogenesis. Excessive exposure to UV-A (320–400 nm) and UV-B (280–320 nm) radiation can lead to erythema, hyperpigmentation, oxidative stress, and long-term skin malignancies. Sunscreen formulations play a critical role in minimizing these harmful effects by absorbing, reflecting, or scattering UV radiation.

Among organic UV filters, Benzophenone-3 (Oxybenzone) is widely used due to its ability to absorb both UV-A and UV-B radiation. It functions by absorbing UV energy and converting it into less harmful heat energy. However, conventional formulations containing free Benzophenone-3 present certain limitations, including photodegradation, potential skin irritation, and systemic absorption concerns. Recent regulatory discussions and safety evaluations have emphasized the need for formulation strategies that limit systemic exposure while maintaining surface efficacy. [1] Encapsulation of active ingredients into polymeric microspheres has emerged as an effective strategy to enhance photostability, control release, and reduce skin penetration. Microspheres are spherical particulate systems that can modulate drug release through diffusion or matrix-controlled mechanisms. Ethyl cellulose is commonly used as a hydrophobic polymer for sustained-release systems due to its excellent film-forming properties, chemical stability, and biocompatibility. [2-3]

The solvent evaporation technique is widely employed for preparing polymeric microspheres

due to its simplicity, reproducibility, and suitability for hydrophobic drugs. Process variables such as stirring speed and polymer concentration significantly influence microsphere characteristics including particle size, entrapment efficiency, and drug release profile. Application of factorial design allows systematic optimization and understanding of formulation variables and their interactions.

In addition to organic UV filters, inorganic agents such as Zinc oxide and Titanium dioxide provide broad-spectrum protection by reflecting and scattering UV radiation. Combination formulations containing both organic and inorganic filters may provide synergistic photoprotection. Furthermore, herbal additives such as Aloe vera gel contribute soothing and antioxidant properties, improving skin compatibility. [3-4]

Ex-vivo skin permeation studies using Franz diffusion cells provide essential information regarding dermal retention and systemic exposure. For sunscreen products, optimal performance requires maximum retention in the stratum corneum with minimal transdermal permeation. [5]

Therefore, the present study was designed to develop Benzophenone-3 loaded ethyl cellulose microspheres using solvent evaporation technique, optimize formulation variables using a 3^2 factorial design, incorporate the optimized microspheres into sunscreen cream, and evaluate photoprotective efficacy and skin permeation behaviour. The study aims to provide a safer and more effective sunscreen system with enhanced stability and controlled release properties.

Materials and Methods

The Materials and Methods are mentioned as below:

Materials: Benzophenone-3 (Oxybenzone) was used as the active pharmaceutical ingredient (API). Ethyl cellulose (EC) was used as the polymer for microsphere preparation. Sodium carboxymethyl cellulose (CMC) and Tween 80 served as stabilizer and emulsifying agent, respectively.

Polyethylene glycol (PEG) was used as a co-solvent. Chloroform and methanol were used as analytical grade solvents. Zinc oxide and titanium dioxide were used as inorganic UV filters. Aloe vera gel was used as herbal additive. Phosphate buffer (pH 7.4) was prepared according to standard procedures. All chemicals and reagents used were of analytical grade.

Preformulation Studies: Preformulation studies were performed to evaluate the physicochemical properties of Benzophenone-3. The drug was examined visually for colour, odour, texture, and physical state under daylight conditions. The melting point was determined by the capillary method using a digital melting point apparatus, and the experiment was performed in triplicate. Fourier Transform Infrared (FTIR) spectroscopy was conducted using the KBr pellet method by mixing the drug with potassium bromide (1:100), compressing into a pellet, and scanning in the range of 4000–400 cm^{-1} to confirm characteristic functional groups. For UV spectrophotometric analysis, a stock solution (100 µg/mL) of the drug was prepared in methanol and scanned between 200–400 nm to determine the λmax. A calibration curve was constructed by preparing serial dilutions (10–50 µg/mL) and measuring absorbance at 286 nm. Solubility studies were conducted in various solvents including water, methanol, ethanol, chloroform, and phosphate buffer pH 7.4 at 25 ± 2°C by visual inspection. [6]

Preparation of Benzophenone-3 Loaded Microspheres: Benzophenone-3 loaded microspheres were prepared using the solvent evaporation technique. The organic phase was prepared by dissolving 500 mg of Benzophenone-3 in 2 mL PEG and incorporating it into ethyl cellulose dissolved in 50 mL chloroform under continuous stirring to obtain a homogeneous solution. The aqueous phase consisted of 0.05% w/v CMC and 1% w/v Tween 80 dissolved in distilled water. The organic phase was added dropwise into the aqueous phase under mechanical stirring at speeds ranging from 1000 to 2000 rpm, and stirring was continued for 2–3 h at room temperature to allow complete evaporation of chloroform and formation of solid microspheres. The formed microspheres were collected by decantation, washed three times with distilled water, air-dried for 24 h, and stored in a desiccator until further evaluation. [7-8]

Experimental Design: A 3^2 full factorial design was employed to investigate the influence of formulation variables.

Stirring speed (1000, 1500, and 2000 rpm) and polymer concentration (5, 7, and 9 g) were selected as independent variables, while particle size, entrapment efficiency, percentage yield, and in-vitro drug release were evaluated as dependent responses. Data were analyzed using a quadratic polynomial equation:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2$$

Evaluation of Microspheres [9-10]

The Evaluation of Microspheres of Benzophenone-3 are mentioned below:

Particle Size Analysis: Particle size was determined using optical microscopy by measuring approximately 200 particles per batch to calculate average size of microspheres.

Entrapment Efficiency: Microspheres were dissolvetd in methanol under sonication. The solution was filtered and analyzed at 286 nm. Entrapment efficiency was calculated as:

$$\%EE = \frac{\text{Actual Drug Content}}{\text{Theoretical Drug Content}} \times 100$$

Percentage Yield

$$\%Yield = \frac{\text{Weight of Dried Microspheres}}{\text{Total Weight of Drug + Polymer}} \times 100$$

Surface Morphology: Surface morphology was examined using Scanning Electron Microscopy. Samples were sputter-coated with gold and observed under appropriate magnifications.

In-Vitro Drug Release Study: Drug release was studied using the dialysis bag diffusion technique in phosphate buffer pH 7.4 at 37 ± 0.5°C with continuous stirring. Samples were withdrawn at predetermined intervals up to 2 h and analyzed at 286 nm. Cumulative percentage drug release was calculated. [10]

Preparation of Sunscreen Cream Formulations: Sunscreen creams were prepared using a pre-formulated cream base.

- Organic formulations contained Benzophe-none-3 or microspheres.
- Inorganic formulations contained zinc oxide and titanium dioxide.
- Aloe vera gel was incorporated where applica-ble.

All components were mixed under stirring (500–700 rpm) followed by homogenization for 5–10 min to obtain uniform creams.

Evaluation of Sunscreen Cream: The In-vitro and in-vivo Evaluation of Sunscreen Cream of Benzophenone – 3 are mentioned below:

In-Vitro Sunscreen Efficacy: Sunscreen efficacy was evaluated using sodium nitroprusside photodegradation method. Sodium nitroprusside is broken into Nitric oxide (NO) and Prussian blue when exposed to sunlight. Formulations were spread over cellophane membrane placed on sodium nitroprusside solution and exposed to sunlight for 2 h. Absorbance was measured at 394 nm. Higher absorbance indicated better UV protection.

Ex-Vivo Skin Penetration Study: Ex-vivo skin penetration studies were carried out using Franz diffusion cells. Porcine flank skin was excised, cleaned, and mounted between the donor and receptor compartments with the stratum corneum facing the donor side. The receptor compartment was filled with phosphate buffer pH 7.4 and maintained at 37 ± 0.5°C under continuous stirring. The formulation (5 mg/cm²) was applied to the donor compartment, and samples were withdrawn at predetermined intervals up to 120 min and analyzed at 286 nm. After completion of the diffusion study, tape stripping was performed using adhesive tapes to remove the stratum corneum. The drug was extracted from the tape strips using methanol and quantified spectrophotometrically. The remaining skin tissue was cut into small pieces, sonicated in methanol, and analyzed to determine drug retention in the viable epidermis and dermis. [11]

Results and Discussion

Drug Evaluation: The Drug Evaluation of Benzophenone – 3 is mentioned below:

Physical Appearance: Benzophenone-3 was observed as a yellow, crystalline, odourless powder with a uniform texture and absence of visible impurities.

Melting Point: The melting point of Benzophenone-3 was found to be 63 °C, which is in close agreement with the reported literature range (62–68 °C).

FTIR Spectroscopy: The FTIR spectrum of Benzophenone-3 exhibited characteristic absorption peaks corresponding to its functional groups. A broad peak in the region of 3200–3500 cm^{-1} confirmed the presence of phenolic –OH stretching vibrations. A sharp and prominent peak at 1629 cm^{-1} corresponded to the carbonyl (C=O) stretching vibration of the benzophenone moiety. Aromatic C=C stretching vibrations were observed between 1590–1500 cm^{-1}, confirming the aromatic structure. Peaks between 1300–1200 cm^{-1} were attributed to C–O stretching vibrations, while bands in the 900–700 cm^{-1} region corresponded to aromatic C–H out-of-plane bending vibrations. The presence of all characteristic peaks without significant shifts confirms the structural integrity and purity of Benzophenone-3.

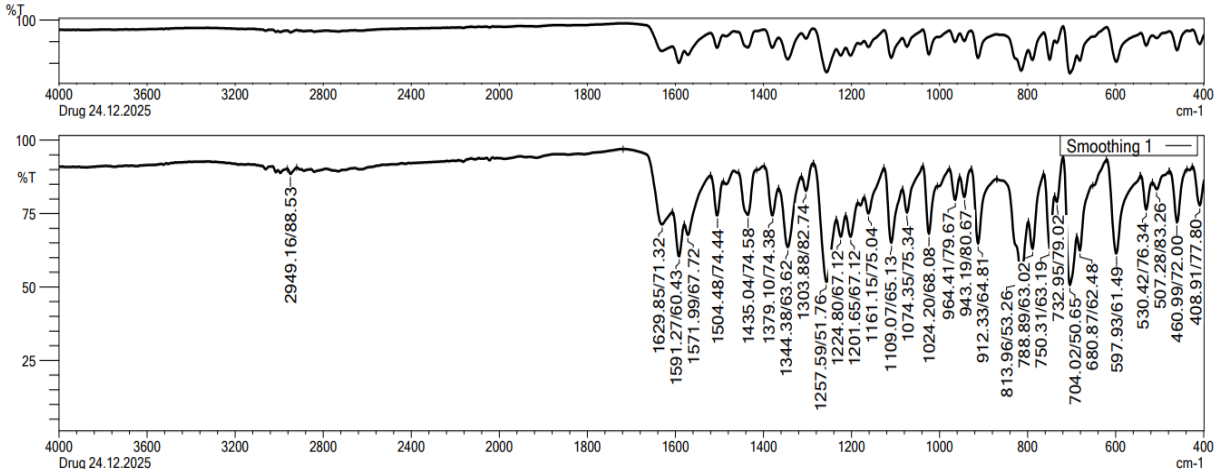

Figure 1: FTIR Spectrum of Benzophenone-3

UV Spectral Analysis and Calibration Curve: The UV spectrum of Benzophenone-3 in methanol showed a maximum absorption (λmax) at 286 nm, which is consistent with reported data for aromatic ketone derivatives. The calibration curve constructed over the concentration range of 1–5 µg/mL demonstrated a linear relationship between absorbance and concentration, indicating adherence to Beer–Lambert's law. The linearity confirms the suitability of the UV spectrophotometric method for quantitative estimation of the drug in further studies.

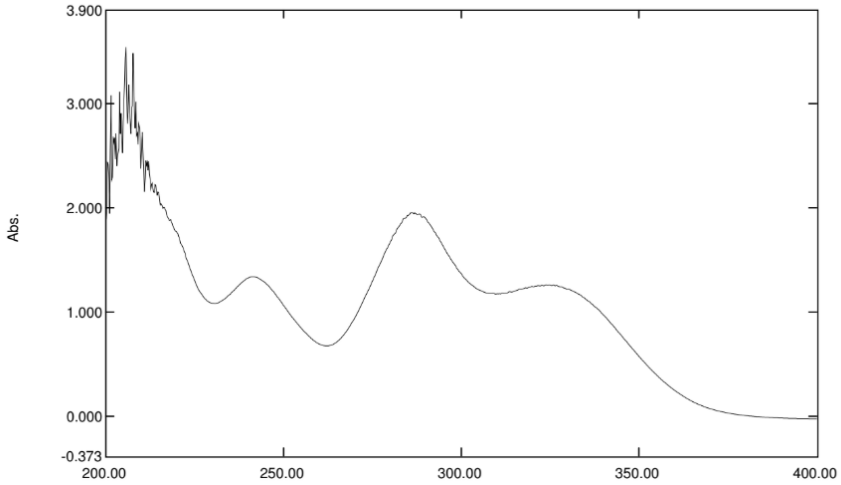

Figure 2: UV Spectra of Benzophenone-3

Solubility Studies: Benzophenone-3 exhibited poor solubility in aqueous media, including distilled water and phosphate buffer pH 7.4, confirming its hydrophobic nature. In contrast, high solubility was observed in organic solvents such as methanol, ethanol, chloroform, acetone, and isopropyl alcohol. The highest solubility was observed in chloroform, reflecting its lipophilic character. This solubility profile aligns with the chemical structure of Benzophenone-3 and explains its widespread use in oil-based and alcohol-based topical formulations. The poor aqueous solubility highlights the necessity for formulation strategies such as encapsulation in polymeric microspheres to improve dispersion and stability in topical systems.

Evaluation of Benzophenone-3 Loaded Microspheres: The Evaluation of Benzophenone-3 Loaded Microspheres are mentioned below:

Entrapment Efficiency (% EE): The entrapment efficiency of the prepared microspheres ranged from 72.00% to 86.07%, indicating successful encapsulation of Benzophenone-3 within the ethyl cellulose matrix. Both stirring speed and polymer concentration significantly influenced %EE. At higher stirring speeds (2000 rpm), efficient droplet dispersion and rapid solvent evaporation enhanced drug entrapment, with batch B3 (5 g polymer) showing the highest %EE (86.07%). At moderate stirring speed (1500 rpm), an optimal polymer concentration (7 g, batch B5) yielded improved entrapment, suggesting the importance of an

appropriate polymer-to-drug ratio. Lower stirring speeds (1000 rpm) produced relatively consistent %EE values, likely due to reduced shear-induced drug diffusion into the aqueous phase. Overall, the high entrapment efficiencies confirm the suitability of the solvent evaporation technique for encapsulating hydrophobic drugs such as Benzophenone-3.

Table 1: Entrapment Efficiency

Batch	Stirring Speed (rpm)	Polymer (g)	%EE
B1	2000	9	83.82
B2	2000	7	76.48
B3	2000	5	86.07
B4	1500	9	72.00
B5	1500	7	82.00
B6	1500	5	79.69
B7	1000	9	81.44
B8	1000	7	80.51
B9	1000	5	70.80

Particle Size Analysis: Particle size ranged from 107 µm to 375 µm and was significantly influenced by stirring speed and polymer concentration. Higher stirring speeds (2000 rpm) produced smaller microspheres due to increased shear forces, which reduced droplet size during emulsification. Conversely, lower stirring speeds resulted in larger particles. At intermediate stirring speed (1500 rpm), insufficient polymer concentration led to droplet coalescence, producing larger particles (e.g., B6: 375 µm).

Smaller and more uniform microspheres were achieved with higher polymer concentration and higher stirring speed, which is advantageous for achieving uniform topical distribution and controlled drug release.

Table 2: Particle Size Range

Batch	Stirring Speed (rpm)	Polymer (g)	Size Range (µm)
B1	2000	9	200
B2	2000	7	165
B3	2000	5	107
B4	1500	9	180
B5	1500	7	252
B6	1500	5	375
B7	1000	9	351
B8	1000	7	275
B9	1000	5	185

Percentage Yield: The percentage yield varied between 32.18% and 87.89%. Higher polymer concentrations resulted in greater yields due to improved droplet stability and reduced processing losses. Batches containing 9 g polymer showed yields above 73%, whereas 5 g batches exhibited significantly lower yields. Moderate stirring speeds favoured higher recovery by minimizing splashing and mechanical loss.

These findings indicate that polymer concentration plays a dominant role in optimizing microsphere yield.

Table 3: Percentage Yield

Batch	Stirring Speed (rpm)	Polymer (g)	% Yield
B1	2000	9	86.31
B2	2000	7	80.00
B3	2000	5	37.63
B4	1500	9	73.78
B5	1500	7	57.33
B6	1500	5	42.18
B7	1000	9	87.89
B8	1000	7	81.06
B9	1000	5	32.18

Surface Morphology (SEM): SEM analysis revealed predominantly spherical microspheres with well-defined boundaries and minimal aggregation, confirming successful preparation via solvent evaporation. Higher polymer concentrations resulted in smoother and more uniform surfaces, while lower concentrations occasionally produced slightly porous or rough surfaces. Minor surface porosity observed in some batches may facilitate controlled drug diffusion. The absence of cracks or structural collapse indicates good mechanical stability of the microspheres. Overall, SEM findings corroborate the successful encapsulation and structural integrity of the microspheres.

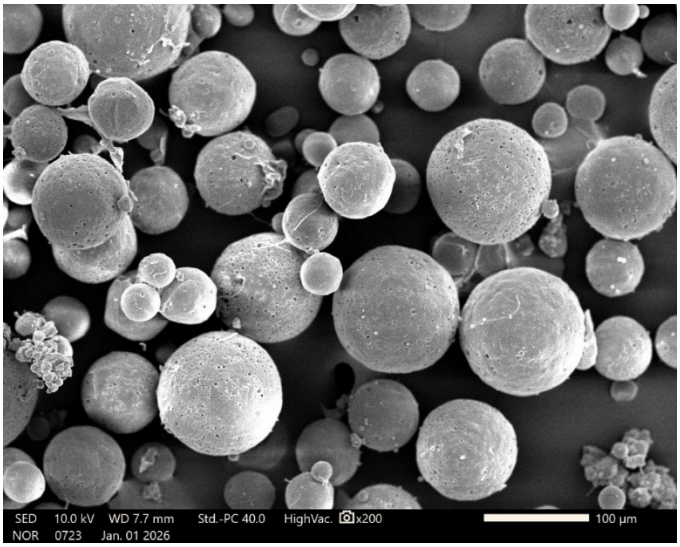

Figure 3: Scanning Electron Microscopy of Benzophenone-3 loaded microspheres

In-Vitro Drug Release: The in-vitro release study demonstrated a sustained release pattern over 120 minutes, with cumulative drug release reaching 41.56%. An initial gradual release phase was observed, followed by a controlled and progressive increase in release. The absence of burst release indicates effective encapsulation of the drug within the polymer matrix. The sustained release behavior can be attributed to the diffusion-controlled mechanism of drug release through the ethyl cellulose matrix.

Such controlled release is beneficial for sunscreen applications, as it may prolong photoprotective action and enhance photostability.

Table 4: Final In-Vitro Drug Release Profile of Benzophenone-3 Microspheres

S. No.	Time	Cumulative Drug Release (%)
1	10 min	6.42
2	20 min	8.63
3	30 min	10.02
4	40 min	13.28
5	50 min	15.77
6	60 min	18.64
7	70 min	23.47
8	80 min	28.53
9	90 min	32.92
10	100 min	35.33
11	110 min	38.78
12	120 min	41.56

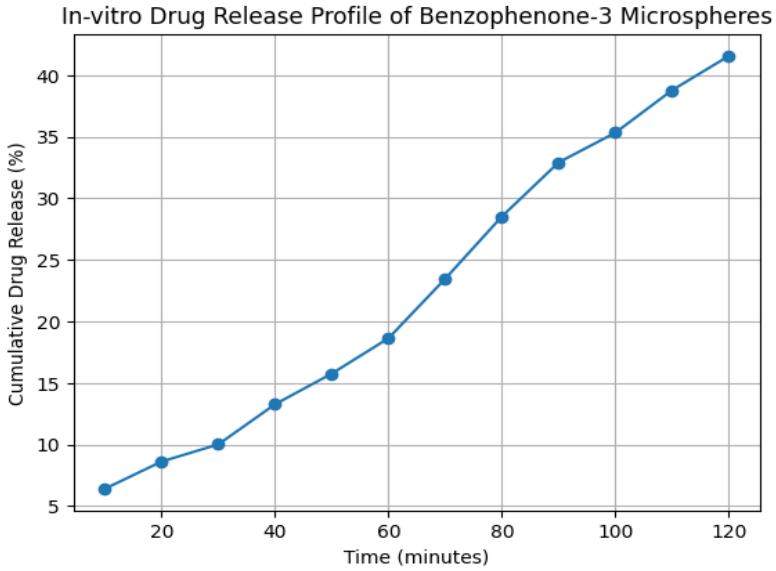

Figure 4: In-vitro drug release profile of Benzophenone-3 loaded microspheres

Evaluation of Sunscreen Cream Formulations: The Evaluation of Sunscreen Cream Formulations are mentioned below:

In-Vitro Sunscreen Efficacy: The sunscreen efficacy results demonstrated clear differences among formulations. The unprotected control showed minimal absorbance (0.229), indicating maximum photodegradation. The formulation containing free Benzophenone-3 exhibited significant protection (1.000), confirming its UV-absorbing capability. Encapsulation of the drug into microspheres further enhanced protection (1.076), likely due to improved photostability and controlled release.

Table 5: Sunscreen Efficacy of Different Cream Formulations

S. No.	Formulation	Absorbance at 394 nm
1	Unprotected (Control)	0.229
2	Cream base + Benzophenone-3	1.000
3	Cream base + Benzophenone-3 microspheres	1.076
4	Cream base + Inorganic agents (ZnO + TiO_2 + Aloe vera gel)	0.729
5	Cream base + Microspheres + Inorganic agents	1.435

Higher absorbance value indicates greater sunscreen protection.

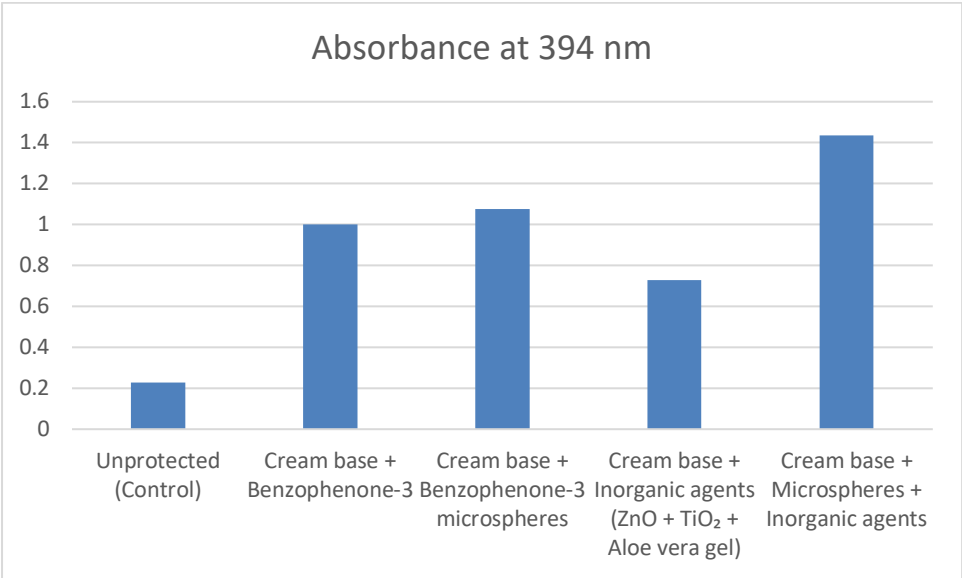

Figure 5: Bar Graph of Sunscreen Efficacy

The inorganic formulation containing zinc oxide and titanium dioxide demonstrated moderate protection (0.729), attributable to UV reflection and scattering mechanisms.

The combination formulation (microspheres + inorganic agents) exhibited the highest absorbance (1.435), indicating superior and synergistic photoprotection.

The combination of chemical absorption (organic filter) and physical reflection (inorganic filters) provided broad-spectrum UV protection. These findings highlight the advantage of integrating polymeric microspheres with inorganic UV filters for enhanced sunscreen efficacy.

Ex-Vivo Skin Permeation and Drug Distribution: Franz diffusion studies demonstrated negligible transdermal permeation of Benzophenone-3 over 120 minutes, with only 0.7% detected in the receptor compartment. The majority of the drug (>90%) remained localized within the stratum corneum, as confirmed by tape stripping studies. Approximately 6% was retained in the viable epidermis and dermis, indicating limited penetration beyond the outer skin layers.

Table 6: Cumulative Amount of Benzophenone-3 Permeated Through Porcine Skin Using Franz Diffusion Cell

Time (min)	Cumulative Amount Permeated (µg/cm²)	% Cumulative Permeation
5	ND	0.00
10	ND	0.00
15	0.02 ± 0.01	0.01 ± 0.00
30	0.04 ± 0.02	0.02 ± 0.01
45	0.06 ± 0.01	0.03 ± 0.01
60	0.08 ± 0.02	0.04 ± 0.01
75	0.09 ± 0.02	0.05 ± 0.01
90	0.11 ± 0.03	0.06 ± 0.02
105	0.12 ± 0.03	0.06 ± 0.02
120	0.13 ± 0.04	0.07 ± 0.02

ND – Not detected (below LOQ), Values expressed as mean ± SD (n = 3)

Table 7: Distribution of Benzophenone-3 after 120 min Permeation Study

Skin Compartment	Amount Recovered (µg/cm²)	% Drug Recovered
Stratum corneum (Tape stripping)	78.6 ± 4.2	92.4 ± 3.1
Viable epidermis + dermis	5.1 ± 0.8	6.0 ± 1.0
Receptor compartment	0.13 ± 0.04	0.7 ± 0.2
Total recovered	**83.8 ± 5.1**	**~99.1**

The minimal systemic permeation observed confirms the surface-retentive behavior of the formulation, which is highly desirable for sunscreen applications. Effective confinement of the drug within the stratum corneum reduces the risk of systemic absorption and potential adverse effects while maintaining adequate surface protection.

Overall Discussion

The results demonstrate that Benzophenone-3 was successfully encapsulated into ethyl cellulose microspheres using the solvent evaporation technique. The microspheres exhibited high entrapment efficiency, controlled particle size, satisfactory yield, spherical morphology, and sustained drug release behaviour. Incorporation of these microspheres into sunscreen cream significantly enhanced photoprotective efficacy, especially when combined with inorganic UV filters.

Ex-vivo permeation studies confirmed minimal systemic exposure and predominant retention within the stratum corneum, supporting the safety and suitability of the developed formulation for topical photoprotection. Overall, the optimized microsphere-based sunscreen formulation offers improved stability, controlled release, enhanced efficacy, and reduced systemic absorption, making it a promising candidate for advanced topical sunscreen applications.

Conclusion

The present study successfully developed and optimized microsphere-based sunscreen formulations containing Benzophenone-3 using the solvent evaporation technique. Preformulation studies confirmed the identity and purity of the drug. Application of a 3^2 factorial design enabled systematic evaluation of the influence of stirring speed and polymer concentration on microsphere characteristics.

The prepared microspheres exhibited high entrapment efficiency, controlled particle size distribution, satisfactory yield, and spherical morphology. In-vitro release studies confirmed sustained and diffusion-controlled drug release without significant burst effect.

Incorporation of microspheres into cream formulations significantly enhanced sunscreen efficacy compared to free drug formulations. The combination of microspheres with inorganic UV filters such as Zinc oxide and Titanium dioxide demonstrated superior photoprotection due to synergistic absorption and reflection mechanisms. Ex-vivo permeation studies revealed predominant retention of the drug within the stratum corneum and negligible systemic permeation, indicating improved safety profile. Overall, the developed microsphere-based sunscreen system offers enhanced photostability, controlled release, improved efficacy, and reduced systemic exposure. This approach represents a promising advancement in the development of safer and more effective topical photoprotective formulations.

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