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Research Article

Formulation and Evaluation of Bifonazole Nanoemulsion Topical Spray for Antifungal Activity

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ABSTRACT

Bifonazole is a broad-spectrum imidazole antifungal agent that is poorly water-soluble (BCS Class IV) and shows very limited percutaneous absorption from conventional topical dosage forms, restricting its local bioavailability at the site of infection. In the present study, a bifonazole-loaded nanoemulsion was developed and converted into a topical spray with the objective of improving drug solubility, enhancing skin penetration and increasing local antifungal efficacy while minimising systemic exposure. Nanoemulsions (F1–F3) were prepared by high-speed homogenization followed by ultrasonication of an oil phase (liquid paraffin, oleic acid), a surfactant/co-surfactant system (Tween 40, isopropyl alcohol) and an aqueous phase containing sodium lauryl sulphate and methyl paraben, gelled with one of three polymers – Carbopol 934, HPMC or CMC. Drug–excipient compatibility was confirmed by physical compatibility and FT-IR studies, which showed no significant interaction. The optimized spray (F1, Carbopol-based) exhibited an acceptable pH (5.5), viscosity (48 cp), spray angle (88.8°), spray-pattern ovality ratio of 1.25, 98% content uniformity and a drying time of 4 minutes, and remained stable on storage at room temperature for one month. The formulation produced a clear zone of fungal growth inhibition (2.5 cm) in an in-vitro cup-plate assay. These findings suggest that a Carbopol-based bifonazole nanoemulsion spray is a promising alternative topical dosage form for the management of cutaneous fungal infections.

INTRODUCTION

Topical drug delivery is a widely used route of administration in which a formulation is applied directly to the skin to achieve a localized therapeutic effect while minimizing systemic exposure. It is particularly suited to dermatological disorders such as fungal infections, eczema, psoriasis and acne. The stratum corneum, the outermost layer of the epidermis, acts as a highly

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efficient biological barrier that limits drug permeation, making formulation design critical to therapeutic success. Compared with the oral route, topical delivery avoids hepatic first-pass metabolism, reduces systemic adverse effects, and improves patient compliance because it is painless, non-invasive and convenient for long-term use. Conventional topical dosage forms such as creams, ointments, gels and lotions are widely used, but their efficacy is often limited by poor skin penetration and inadequate drug retention at the target site.

Nano emulsions are thermodynamically or kinetically stable, transparent to translucent dispersions of oil and water stabilized by a surfactant/co-surfactant film, with droplet sizes typically in the nanometre range. Because of their small droplet size and large interfacial surface area, nanoemulsions markedly improve the solubility of poorly water-soluble drugs, enhance permeation across the stratum corneum, and provide a stable vehicle with reduced risk of phase separation or creaming compared with conventional emulsions. Incorporating a nanoemulsion into a spray dosage form combines these advantages with the convenience of a non-contact, easy-to-apply, quick-drying delivery system that further improves patient acceptability.

Bifonazole, an imidazole derivative, is a broad-spectrum antifungal agent effective against dermatophytes, yeasts and moulds. It is, however, a BCS Class IV drug with poor aqueous solubility and shows very low absorption after topical application (about 0.6% of the applied dose, rising only to around 2.5% in lesioned skin), which limits the amount of drug retained at the site of infection when formulated conventionally. These properties make bifonazole a suitable candidate for a nanoemulsion-based topical spray, which is expected to improve its solubility, skin retention and local antifungal action while limiting the systemic side effects – such as irritation, redness and dryness – associated with the drug. The present work was therefore undertaken to formulate a bifonazole-loaded nanoemulsion, develop it into a topical spray, and evaluate the formulation for its physicochemical, spray and antifungal performance.

MATERIALS AND METHODS:

Materials

Bifonazole was obtained ex-gratis from Dhamtec Pharma and Consultants, Mumbai. Oleic acid, Tween 40, liquid paraffin, methyl paraben, isopropyl alcohol, Carbopol 934, hydroxypropyl methylcellulose (HPMC), carboxymethylcellulose (CMC) and sodium lauryl sulphate (SLS) were procured from local laboratory chemical suppliers, Melmaruvathur. All materials used were of analytical grade.

Table 1: Materials used in the formulation

Sr. No	Material	Manufacturer / Supplier
1	Bifonazole	Dhamtec Pharma and Consultants, Mumbai
2	Oleic acid	Lab chemicals, Melmaruvathur
3	Tween 40	Lab chemicals, Melmaruvathur
4	Liquid paraffin	Lab chemicals, Melmaruvathur
5	Methyl paraben	Lab chemicals, Melmaruvathur
6	Isopropyl alcohol	Lab chemicals, Melmaruvathur
7	Carbopol 934	Lab chemicals, Melmaruvathur
8	HPMC	Lab chemicals, Melmaruvathur
9	CMC	Lab chemicals, Melmaruvathur
10	Sodium lauryl sulphate	Lab chemicals, Melmaruvathur

Instruments

Table 2: Instruments used in the study

Sr. No	Instrument	Manufacturer
1	Digital electronic balance	Wesner
2	Magnetic stirrer with hot plate	REMI
3	UV-Visible spectrophotometer	Shimadzu UV-1800
4	FT-IR spectrophotometer	Shimadzu
5	Ultrasonicator	Lark
6	Melting point apparatus	Guna Enterprises, Chennai
7	pH meter	Infra Digi equipment
8	Viscometer	Brookfield DV-II+ Pro

Preformulation studies

Melting point of bifonazole was determined in triplicate using the capillary tube method. Solubility of the pure drug was tested in distilled water, methanol, DMSO and phosphate buffer pH 6.8. Physical compatibility of the drug with each excipient, and with the admixture of all excipients, was assessed by storing samples in sealed amber vials at room temperature and at 40°C ± 2°C / 75% ± 5% RH for 30 days, with visual observation at 10, 20 and 30 days. Chemical (drug–excipient) compatibility was investigated by FT-IR spectroscopy (4000–400 cm^{-1}) using a Shimadzu FT-IR spectrophotometer, comparing the spectrum of bifonazole alone with that of the drug in combination with each excipient and with the full excipient mixture.

The wavelength of maximum absorbance (λmax) of bifonazole was determined by scanning a 10 µg/mL solution in phosphate buffer pH 6.8 between 200–400 nm on a UV-visible spectrophotometer. A calibration curve was then constructed over the range 2–10 µg/mL by measuring absorbance at the λmax against a blank.

Formulation of bifonazole nanoemulsion

Bifonazole nanoemulsions were prepared by the conventional emulsification technique. The drug was dissolved in the oil phase (liquid paraffin and oleic acid), and Tween 40 was incorporated into this phase with stirring to obtain a clear, homogeneous mixture. The aqueous phase was prepared by dissolving SLS and methyl paraben in purified water, followed by dispersion and hydration of the selected polymer (Carbopol 934, HPMC or CMC). The hydrated aqueous phase was added gradually to the oil phase under continuous magnetic stirring for 20–30 minutes to obtain a coarse emulsion. The coarse emulsion was then subjected to high-speed homogenization followed by ultrasonication to reduce the droplet size into the nanometre range, yielding a stable nanoemulsion, which was allowed to cool to room temperature before further characterization.

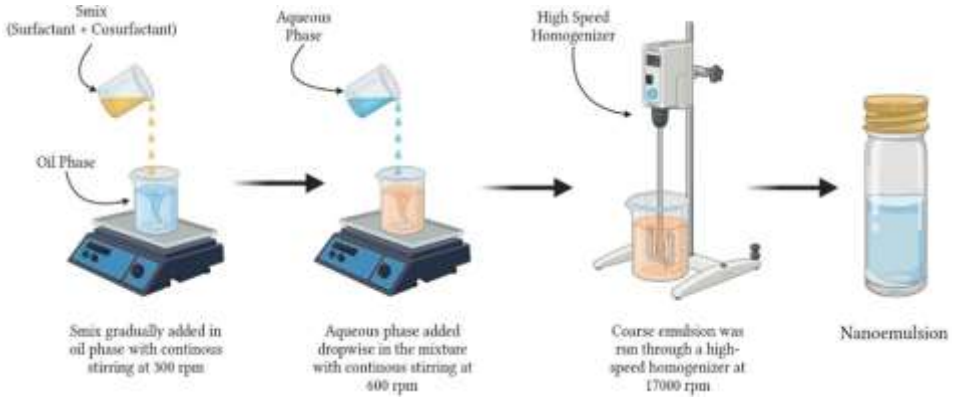

Fig. 1: Schematic representation of preparation of bifonazole nanoemulsion

Three formulations (F1–F3), differing only in the gelling polymer used (Carbopol 934, HPMC and CMC respectively), were prepared as summarised in Table 3.

Table 3: Composition of bifonazole nanoemulsion spray formulations (F1–F3)

Ingredient	F1	F2	F3
Bifonazole (g)	1	1	1
Liquid paraffin (mL)	10	10	10
Oleic acid (mL)	3	3	3
Isopropyl alcohol (mL)	30	30	30
Tween 40 (mL)	3	3	3
Methyl paraben (mg)	2	2	2
Carbopol 934 (mg)	0.5	–	–
HPMC (mg)	–	0.5	–
CMC (mg)	–	–	0.5
SLS (mg)	3	–	3
Purified water	q.s. to 100 mL	q.s. to 100 mL	q.s. to 100 mL

The optimized nanoemulsion was converted into a topical spray by adjusting the pH to a range compatible with the physiological pH of skin and filling the formulation into sterilized spray bottles fitted with a metered spray pump.

Characterization and evaluation

The nanoemulsion spray formulations were evaluated for the following parameters: (i) Viscosity – measured using a Brookfield DV-II+ Pro viscometer at 25°C using spindle no. 3, taking spindle readings at 0.5, 1, 2.5 and 5 rpm after equilibration for 5 minutes; (ii) pH – measured using a digital pH meter calibrated with pH 4, 7 and 9 buffers; (iii) Spray angle – sprays were actuated horizontally onto white paper placed 10 cm from the nozzle, and the angle was calculated from $\theta = \tan^{-1}(L/r)$, where L is the nozzle-to-paper distance and r is the average radius of the spray circle; (iv) Spray pattern – assessed by impingement of the dye-loaded (methyl red) spray onto paper and expressed as the ovality ratio (Dmax/Dmin); (v) Dilution test – performed to confirm nanoemulsion type by dilution with the continuous phase; (vi) Percentage content uniformity – determined spectrophotometrically at 254 nm after appropriate dilution with phosphate buffer pH 6.8, expressed as (test absorbance / standard absorbance) × 100; (vii) Leakage test – container-closure integrity was checked by the dye-penetration method; (viii) Drying time – the time taken for the sprayed film to dry on a glass surface at room temperature was recorded; (ix) Antifungal activity – evaluated by the cup-and-plate method against mould on starch-dextrose agar, incubated aerobically at 25°C for 24 h, with the zone of inhibition measured using a ruler; and (x) Stability studies – the optimized formulation was stored at room temperature and monitored periodically for changes in appearance, pH, viscosity, content uniformity, spray angle, spray pattern and drying time, in line with ICH guidance.

RESULTS AND DISCUSSION:

Preformulation studies

Bifonazole was obtained as a white, odourless crystalline powder. Its solubility in different media is summarised in Table 4; the drug was freely soluble in methanol and DMSO, sparingly soluble in phosphate buffer pH 6.8, and poorly soluble in distilled water, consistent with its BCS Class IV classification.

Table 4: Solubility of bifonazole in various media

Sr. No	Medium	Solubility profile
1	Methanol	Freely soluble
2	DMSO	Freely soluble
3	Phosphate buffer pH 6.8	Sparingly soluble
4	Distilled water	Poorly soluble

The melting point of bifonazole was found to be 148°C (average of three determinations: 148°C,

147°C, 148°C), which is in close agreement with the reported value, confirming the purity of the drug sample used.

The λmax of bifonazole in phosphate buffer pH 6.8 was found to be 254 nm.

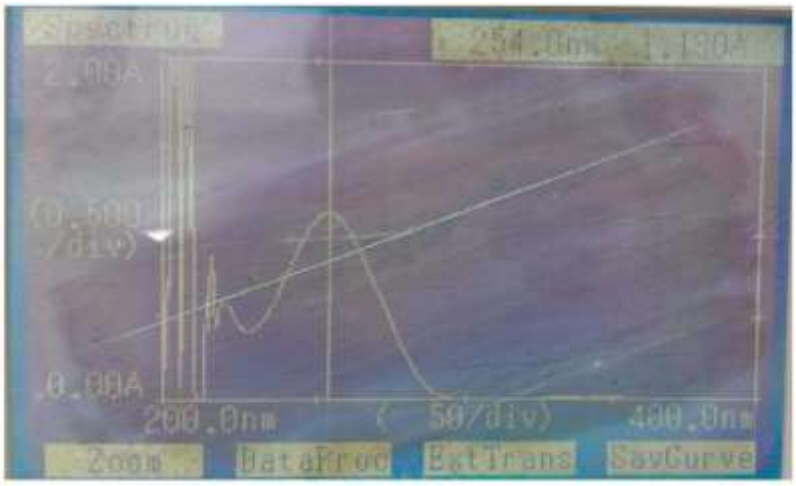

Fig. 2: UV absorption spectrum of bifonazole showing λmax at 254 nm

Calibration curve

Table 5: Calibration data of bifonazole in phosphate buffer pH 6.8

Sr. No	Concentration (µg/mL)	Absorbance (254 nm)
1	0	0
2	2	0.060
3	4	0.119
4	6	0.182
5	8	0.244
6	10	0.304

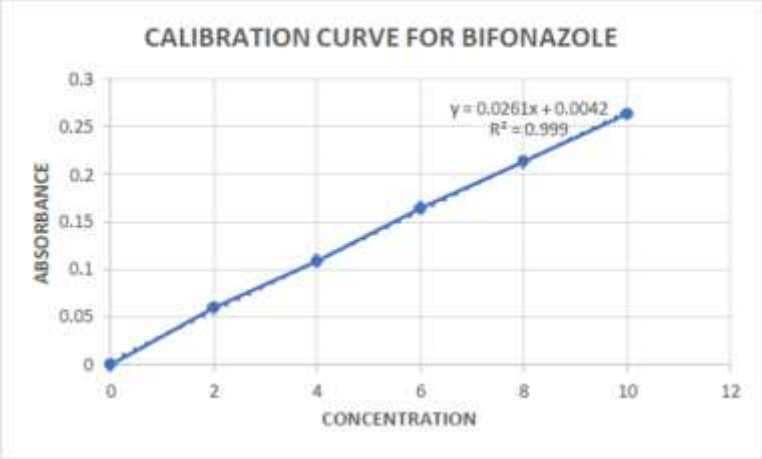

Fig. 3: Calibration curve of bifonazole (λmax 254 nm)

The drug obeyed Beer–Lambert's law over the concentration range studied, with a linear regression equation of y = 0.0261x + 0.0042 and a correlation coefficient (R^2) of 0.999, confirming good linearity of the method used for subsequent content and uniformity determinations.

Drug–excipient compatibility

On physical compatibility testing, bifonazole alone and in combination with each excipient showed no colour change or physical alteration ("No Change") at room temperature or at 40°C ± 2°C / 75% ± 5% RH over 30 days, indicating good physical compatibility of the drug with all the selected excipients.

FT-IR spectra of bifonazole alone showed characteristic peaks corresponding to C–H, C=N, C=C and C–N vibrations. These characteristic peaks were retained, without significant shift or disappearance, in the spectra of the drug combined individually with Carbopol, HPMC, methyl paraben, Tween 40, CMC, isopropyl alcohol, and the complete excipient mixture. The absence of any new peaks or loss of the drug's characteristic bands confirmed that there was no significant chemical interaction between bifonazole and the excipients used in the formulation.

Formulation and physical appearance

The three nanoemulsion spray formulations (F1–F3) were successfully prepared. F1 (Carbopol) was translucent to bluish white, F2 (HPMC) was bluish-white, and F3 (CMC) was milky white in appearance, reflecting the influence of the gelling polymer on the optical clarity of the nanoemulsion.

Content uniformity

Table 6: Content uniformity of bifonazole nanoemulsion spray formulations

Formulation code	Absorbance (254 nm)	Content uniformity (%)
F1	0.245	98
F2	0.244	97
F3	0.243	96

The percentage drug content of all three formulations was in the range of 96–98%, indicating uniform distribution of the drug within each formulation.

Evaluation of spray characteristics

Table 7: Evaluation parameters of bifonazole nanoemulsion spray formulations (F1–F3)

Parameter	F1	F2	F3
Viscosity (cp)	48	49	61
Spray pattern (ovality ratio)	1.25	1.33	1.33
Spray angle	88.8°	88.8°	88.2°
Drying time	4 min	5 min	6 min
pH	5.50	5.51	5.54
Content uniformity	98%	97%	96%

The viscosity of all formulations (48–61 cp) was within a range suitable for spray application. The pH of the formulations (5.5–5.54) was close to the physiological pH of skin, minimizing the likelihood of irritation. F1 showed the narrowest spray pattern (ovality ratio 1.25), the shortest drying time (4 min) and the highest content uniformity (98%) among the three formulations, indicating that the Carbopol-based formulation (F1) provided the most favourable overall spray and physicochemical performance.

Fig. 4: Determination of viscosity using a Brookfield viscometer

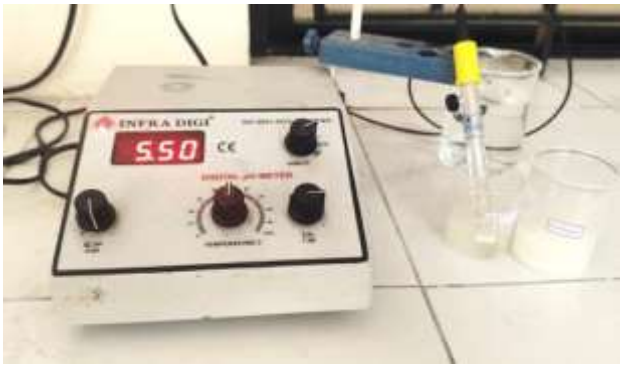

Fig. 5: Determination of pH using a digital pH meter

Stability studies

Table 8: Stability data of the optimized bifonazole nanoemulsion spray (F1)

Parameter	Initial	After 1 month (room temperature)
Visual appearance	Translucent to white	Translucent to white
pH	5.5	5.5
Viscosity	48 cp	47 cp
Content uniformity	98.5%	98%
Spray angle	88.8°	88.8°
Spray pattern	1.30	1.30
Drying time	5 min	5 min

No significant changes were observed in physical appearance, pH, viscosity, drug content, spray angle, spray pattern or drying time of the optimized formulation after one month of storage at room temperature, indicating that the bifonazole nanoemulsion spray was physically and chemically stable under the conditions tested.

Antifungal activity

The in-vitro antifungal activity of the optimized formulation (F1) was assessed against mould, isolated from bread, by the cup-and-plate method. A clear zone of fungal growth inhibition measuring 2.5 cm was observed after 24 hours of incubation, confirming that the bifonazole nanoemulsion spray retained good antifungal activity following its conversion into the nanoemulsion spray dosage form.

Fig. 6: Fungal culture plate before treatment

Fig. 7: Zone of fungal growth inhibition after treatment with bifonazole nanoemulsion spray

CONCLUSION:

A bifonazole-loaded nanoemulsion was successfully developed and formulated into a topical spray with the aim of improving the solubility, skin penetration and local antifungal efficacy of this poorly water-soluble drug. Physical and FT-IR compatibility studies confirmed the absence of any significant drug–excipient interaction, and the UV-spectrophotometric method used showed good linearity (R^2 = 0.999) for quantification of the drug. Among the three formulations prepared with different gelling polymers, the Carbopol-based formulation (F1) exhibited the most favourable overall characteristics – an acceptable pH (5.5), suitable viscosity (48 cp), narrow spray pattern, spray angle of 88.8°, 98% content uniformity, a

short drying time (4 minutes) and good physical and chemical stability on storage. The optimized formulation also produced a distinct zone of fungal growth inhibition in vitro, confirming retention of antifungal activity. These results indicate that a Carbopol-incorporated bifonazole nanoemulsion spray is a promising, patient-friendly alternative to conventional topical antifungal dosage forms, offering improved solubility, enhanced skin penetration and better local drug delivery along with emollient and hydrating properties.

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