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Formulation and evaluation of benzoyl peroxide incorporated invasomal vesicles

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ABSTRACT

The objective of the current investigation was to develop invasomes loaded with benzoyl peroxide to improve the stability of benzoyl peroxide as well as to control the release of the same from the formulation for a longer duration. The invasomes were prepared by thin layer hydration method using 1:10 ratio of drug to lecithin, employing varying concentrations of eucalyptus oil and peppermint oil (terpenes). The entrapment efficiency, particle size and percent drug release from the invasomes was evaluated. The average particle size of the formulations ranged from 153.4 to 315.4 nm. The size was found to decrease with the increasing concentration of the terpene. The zeta potential of the invasomal formulations -27 mV to -49 mV. The drug entrapment was found to be between 64.15 to 86.35 %. All the formulations could release benzoyl peroxide for upto 12 hours in the in vitro experiments. The formulation F3 was considered the most efficient formulation with smallest particle size (153.4 nm), a zeta potential of -31.6 mV, entrapment efficiency of 82.24 % and a drug release of 99.88 % at the end of the 12th hour.

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Introduction

Benzoyl peroxide (Figure 1) is an organic compound in the peroxide family. It consists of two benzoyl groups bridged by a peroxide link. It is one of the most important organic peroxides in terms of applications. Benzoyl peroxide is indicated for the treatment of mild to moderate acne vulgaris and rosacea. Combined with other agents, it may be used in the treatment of more severe acne.[1] Benzoyl peroxide formulations are available as lotions, creams, gels and solutions, at concentrations of 2.5, 5 or 10% w/w. These formulations may contain water, propylene glycol, isopropyl myristate, acetone, or alcohol as vehicle, and are often associated with side effects that include erythema, itching, burning, scaling and irritation. It has been postulated that the side effects of benzoyl peroxide can be circumvented by formulating benzoyl peroxide into nano-sized lipid vesicles.[2]

Invasomes are composed of unsaturated soybean lecithin (with high % PC), small amount of ethanol, and small amount of a mixture of terpenes (cineole, citral, and d-limonene).[3] They are the soft liposomal vesicles embodying trivial quantities of ethanol and terpene or terpene assortments, which deed as potential transporters with amplified skin penetration.[4] Several studies have linked invasomes to improved transdermal penetration of the incorporated drug.[5-10]

In the present investigation it was aimed to develop and characterize invasomal preparations of benzoyl peroxide using phospholipids as membrane components and terpenes like limonene as penetration enhancers hypothesizing that the incorporation of benzoyl peroxide in the vesicles would be able to reduce the skin irritation associated with benzoyl peroxide and also improve the skin permeation of benzoyl peroxide.

Material and Methods

Benzoyl peroxide was purchased from Oxford Fine Chemicals, Soy lecithin was procured from Himedia. All other reagents and chemicals were of analytical grade and purchased from various sources.

Preformulation Studies[11]

The pure drug was observed for color, odor and other physical characteristics and its identification was carried out by FTIR spectrophotometry. The melting point of the drug was determined using open capillary method and is uncorrected. The calibration curve of benzoyl peroxide was prepared using HPLC in concentration range of 2-10 µg/ml utilizing 70% v/v acetonitrile, 28% v/v water, and 2% v/v phosphoric acid as the mobile phase at detection wavelength of 254 nm using a C18 column. The solubility of benzoyl peroxide was qualitatively determined in various solvents.

Formulation of Invasomes[12]

Different benzoyl peroxide-loaded invasomes were prepared using the conventional thin layer hydration technique. Accurately weighted amount of soy lecithin (200 mg) was dissolved in 10-mL mixture of methanol/ chloroform (2:1, v/v) until a clear solution was obtained. benzoyl peroxide (20 mg) and different concentrations of terpene (eucalyptus oil and perppermint oil) were added and placed in a clean, dry, 1000-mL round bottom flask and mixed well. The organic solvent was evaporated by rotatory evaporator at 120 rpm at 60°C for 15 min to obtain a clear film on the walls of the flask.

The deposited lipid film was then hydrated with 10-mL ethanolic–water mixture (3% v/v) by rotation at 120 rpm for 1 h at 60°C. The resulting nanodispersion was filtered through a filter paper to remove any drug crystals and then stored at 4°C until use.

Evaluation of benzoyl peroxide-loaded invasomes

Entrapment Efficiency[13]

To determine the amount of drug entrapped in the invasomes, 1-mL of benzoyl peroxide-loaded invasome dispersion was centrifuged at 13,000 rpm at for 60 min. The clear supernatant was siphoned off carefully to separate the unentrapped benzoyl peroxide and the supernatant was analyzed by HPLC. Sediment was treated with 1 ml of 0.1% Triton X 100 to lyse the vesicles and then diluted to 100 ml with methanol and benzoyl peroxide was analyzed by HPLC method. Amount of benzoyl peroxide in supernatant and sediment gave a total amount of benzoyl peroxide in 1 ml dispersion. The percent entrapment was calculated using the formula:

% entrapment= amount of benzoyl peroxide in sediment/amount of benzoyl peroxide added ×100

Particle Size and Zeta Potential

The particle size of the prepared invasomes was measured using the dynamic light-scattering by Malvern Zetasizer at temperature of 25 ± 2°C. The zeta potential of a selected benzoyl peroxide -loaded invasome was also performed using 90° scattering angle at temperature of 25 ± 2°C.

In vitro release study

The in vitro release of benzoyl peroxide from different benzoyl peroxide-loaded invasomes was evaluated using the dialysis bag diffusion technique.[14] Amount of benzoyl peroxide-loaded invasomes (equivalent to 2 mg benzoyl peroxide) was separated by centrifugation at 13,000 rpm for 1 h, then re-dispersed in 1-mL 3% (v/v) ethanolic aqueous solution and placed in a cellulose membrane bag. The dialysis bag was tightly closed at both ends and immersed in a stoppered bottle containing 100-mL phosphate buffer saline (PBS; pH = 7.4) representing the receptor compartment. The bottle was placed in a water bath shaker, stirred at 100 rpm, and maintained at 37 ± 0.5°C. At pre-determined time intervals, 3-mL sample of the receptor compartment was withdrawn and replaced with an equal volume of fresh medium to keep constant volume. The samples were properly diluted and analyzed for the amount of drug released was determined by HPLC.

Stability study

The most efficient invasomal formulation was selected for stability study. Formulation was stored at 40 ± 2°, 8° and at room temperature. Percent drug entrapment was determined at different time intervals.

Results and Discussion

The IR spectrum of benzoyl peroxide exhibited stretching vibrations due to C-O, C=O and aromatic C-C (Figure 2).

The procured sample of benzoyl peroxide was white in color with a faint odor, melting at 105-107°C, slightly soluble in ethanol and insoluble in water. The retention time in HPLC analysis was found to be 5.183 min (Figure 2). The calibration curve is presented in Figure 3.

Evaluation of Invasomes

The invasomes were evaluated for shape and size, entrapment efficiency and *in vitro* drug release. The results of the study are presented

in the following sections.

Particle size and zeta potential

The particle size of the invasomes was determined by Malvern zeta sizer and the average particle size of the formulations ranged from 153.4 to 315.4 nm (Table 2). The size was found to decrease with the increasing concentration of the terpene. The zeta potential of the invasomal formulations -27 mV to -49 mV. A similar effect of increasing the concentration of terpene was observed in a study of invasomes of adapalene by Targhotra and Gupta.[8] In their study, a 1.0 - 1.5% of terpene was found to increase the particle size and zeta potential of the invasomes.

Entrapment efficiency

The entrapment efficiency was determined by ultrafiltration method using centrifugal tubes. The entrapment of the benzoyl peroxide in the invasomes was found to enhance with the increased concentration of the terpene. The drug entrapment was found to be between 64.15 to 86.35 % (Table 2). The higher entrapment of benzoyl peroxide in formulations with peppermint oil could be attributed to the higher lipophilicity of peppermint oil which enhances the tendency to form lipoidal interactions with the drug.[15]

In vitro drug release

The release of benzoyl peroxide from invasomes was studied for a period of 24 hours and the invasomes were able to sustain the release of benzoyl peroxide for duration of 12 hours. The results of release study are presented in table 3, figure 6.

Stability of invasomes

The most efficient formulation (F3) was selected for stability study of vesicles at different temperatures. The formulation was stored in amber glass container at different temperature. The drug content after treatment with triton X100 and % residue of benzoyl peroxide was calculated as given in Table 4. It was observed that only about 2% degradation occurred at room temperature and all formulations were almost stable at 8° and 40° with only 1.3% degradation of benzoyl peroxide thereby proving the stability of the developed system.

Conclusion

Recently, invasomes have been studied by many researchers as a choice of topical or transdermal drug delivery system to provide better oral bioavailability consideration, high penetration property of the invasome encapsulated agents through biological membrane and their stability. The present formulation study on benzoyl peroxide is an attempt to prepare invasomal drug delivery system and evaluate its in vitro performance. The formulations were prepared with different ratios of terpene. The objective of the work was accomplished as the formulations exhibited stability as well as sustained release of benzoyl peroxide.

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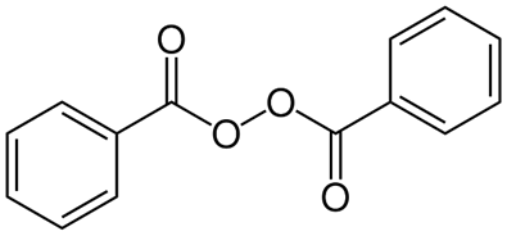

Figure 1 Chemical structure of benzoyl peroxide

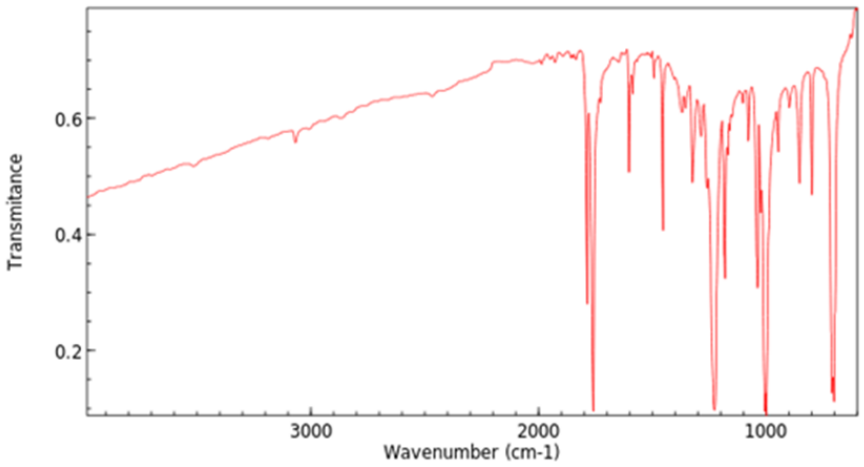

Figure 2 FTIR spectrum of benzoyl peroxide

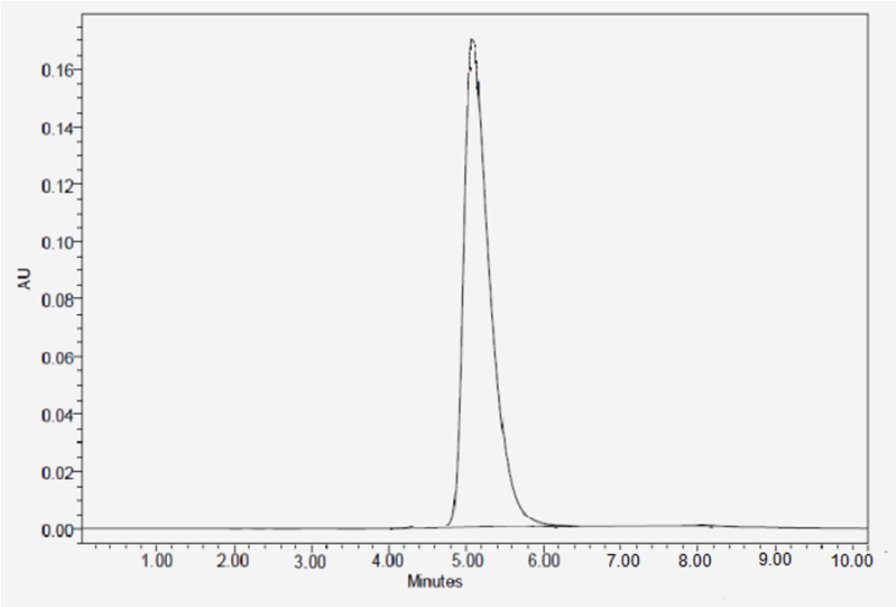

Figure 3 HPLC chromatogram of benzoyl peroxide

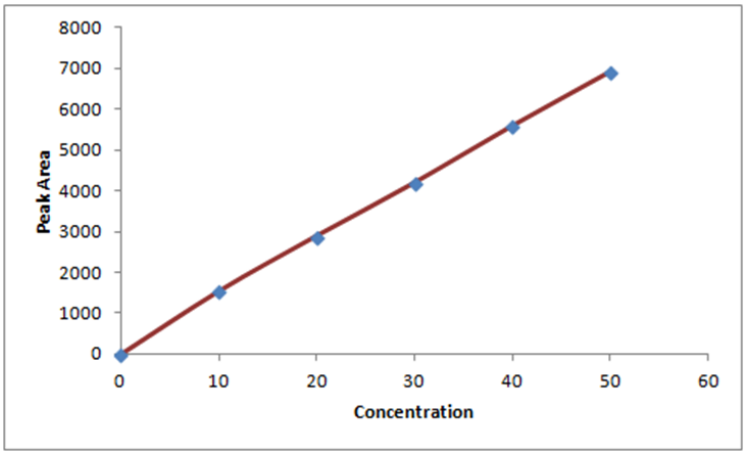

Figure 4 Calibration curve of benzoyl peroxide

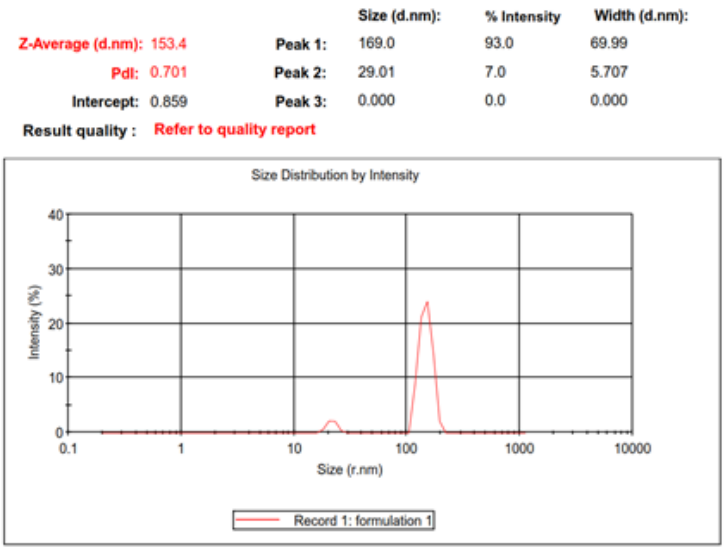

Figure 5 Particle size of F3

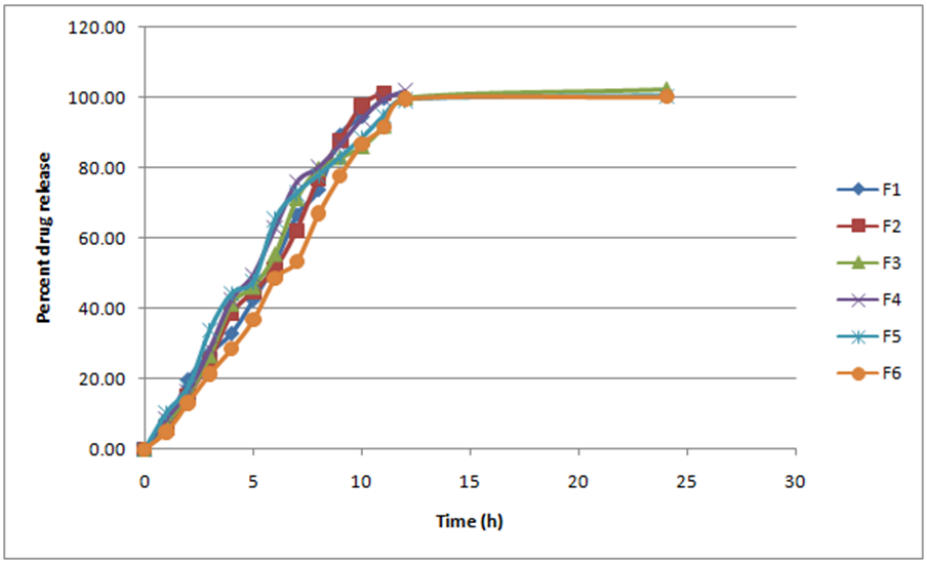

Figure 6 In vitro release of benzoyl peroxide from invasomes

Table 1 Formulation design for invasomes

S.No.	Ingredient	F1	F2	F3	F4	F5	F6
1	Benzoyl peroxide (mg)	20	20	20	20	20	20
2	Soy Lecithin (mg)	200	200	200	200	200	200
3	Methanol-chloroform (2:1 v/v) (mL)	10	10	10	10	10	10
4	Eucalyptus oil (% w/v)	0.5	1.0	1.5	-	-	-
5	Peppermint oil (% w/v)	-	-	-	0.5	1.0	1.5

Table 2 Particle size, zeta potential and entrapment efficiency of invasomes

Formulation	Particle size	Zeta Potential	% EE
F1	247.9 nm	-27.1 mV	64.15
F2	215.1 nm	-29.8 mV	78.85
F3	153.4 nm	-31.6 mV	82.24
F4	315.4 nm	-29.6 mV	70.53
F5	256.7 nm	-37.2 mV	79.64
F6	210.3 nm	-49.2 mV	86.35

Table 3 In vitro release of benzoyl peroxide from invasomes

Time (h)	% Cumulative Release					
	F1	F2	F3	F4	F5	F6
0	0.00	0.00	0.00	0.00	0.00	0.00
1	6.68	5.90	7.30	8.60	10.4	4.90
2	19.78	15.20	15.50	16.40	17.7	13.20
3	27.31	25.30	26.60	28.50	34.02	21.45
4	32.93	38.50	41.30	42.50	44.3	28.50
5	42.46	44.70	46.20	49.50	47.6	36.70
6	53.03	51.2	55.4	62.70	65.4	48.5
7	66.6	62.2	71.3	75.8	72.9	53.4
8	73.8	76.9	79.7	80.04	77.9	66.9
9	89.3	87.7	82.9	86.4	83.1	77.7
10	94.6	97.6	85.96	93.8	88.5	86.7
11	99.8	101.2	91.87	99.3	94.7	91.5
12	100.3	101.2	99.88	101.8	99.4	99.6
24	100.3	101.2	102.3	101.8	100.4	100.1

Table 4 Stability of the invasome (F3) on storage

Time (d)	Drug entrapment (%)		
	40°	8°	Room Temperature
1	82.23	82.06	82.07
15	81.91	81.46	81.75
30	81.86	81.17	81.41
45	81.76	81.89	81.02