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FORMULATION AND EVALUATION OF BENZOYL PEROXIDE LOADED LIPOSOMES FOR IMPROVED STABILITY

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ABSTRACT

The objective of this investigation was to formulate benzoyl peroxide (BPO) loaded liposomes. The liposomes were prepared using lecithin and cholesterol as the lipid forming agents using solvent evaporation method and sonication at 65 W power at 1 min pulses. Six formulations were prepared and characterized for particles size, drug entrapment and release study. The particle size of the liposome ranged from 2.22 to 8.01 µm. The entrapment efficiency was found to be in the range of 2.143 to 53.77%. Sustained release of the encapsulated BPO was obtained, with less than 70% of the drug released in 24 h. The formulation was stable for a period of 12 weeks in accelerated conditions suggesting an improved stability of the drug.

Keywords: Acne, benzoyl peroxide, liposome, stability, encapsulation, release

INTRODUCTION

Acne vulgaris is a most common skin condition and the biggest distress of a teenager's life. It is a chronic inflammatory disease of the pilosebaceous follicle and is more frequent and severe in males, whereas more persistent in women **[1]**. The presently used effective strategies of management of acne are aimed to intervene at one or more of the four main stages of pathogenesis of the disease and include topical and systemic antibiotics and retinoids, benzoyl peroxide, azelaic acid, salicylic acid and oral antiandrogens, depending on the severity of the disease.

Benzoyl Peroxide (BPO), a therapeutic agent for acne, is known to induce concentration-related skin irritation. BPO is the most commonly used treatment prescribed to patients with acne and is available in a variety of strengths (2.5-10%)

and formulations (cream, gel, wash, foam, aqueous gel, leave-on, and wash-off) **[2]**. The use of BPO is limited by concentration-dependent irritation, staining and bleaching of fabric, and uncommon contact allergy **[3]**. Liposomes are colloidal particles in which phospholipid bilayers encapsulate a portion of the medium into their interior. They are formed spontaneously by the self-assembly of phospholipid molecules in an aqueous medium **[4]**. Liposomal formulations are good for topical application because they can spread excellently to form depots of active ingredients in the horny layer of the skin, which allows for the transport of dermatological and cosmetic agents of different types. Also liposomes are known to improve the stability of the encapsulated drug and reduce its toxicity **[5]**. Literature evidence suggests that the topical administration of BPO encapsulated in liposomes may be advantageous in reducing the irritation and itching associated with current BPO formulations **[6-9]**. The literature revealed that liposome encapsulated benzoyl peroxide formulations have been a topic of wide interest among the researchers and helps in improving the antibacterial efficacy and storage stability of the drug **[10-13]**. The method of preparation of the liposomes influences the physicochemical characteristics of and hence the release kinetics and clinical efficacy of the drug. The objective of this work was to test the hypothesis that drug incorporated into liposomes possess higher stability. Hence we developed, and characterized benzoyl peroxide loaded liposomes and to determine its antibacterial efficacy in storage stability.

MATERIAL AND METHODS

High purity Soy Lecithin and cholesterol were procured from Merck Life Sciences, Mumbai. Benzoyl peroxide (BPO), methanol, acetonitrile, ortho phosphoric acid, and chloroform were purchased from Oxford Fine Chemicals, Mumbai.

Preformulation and compatibility study of drug and excipients [14]

The procured BPO was studied for identification characteristics whereas the FTIR spectrum of benzoylperoxide and a physical mixture of drug with lecithin and cholesterol were obtained to study the interaction (if any) between drug and the excipients.

Formulation of liposomes

The liposomal vesicles were prepared using physical dispersion method from a lipid mixture of lecithin: cholesterol in varying ratios **[15]**. The quantities of lipids was accurately weighed **(Table 1)** and dissolved in 100 ml of a methanol:chloroform (2:1, v/v) solution in a round bottomed flask. BPO (200 mg) dissolved in 5 ml of chloroform was added to the lipid mixture. The organic solvent was evaporated to dryness in a rotary evaporator that was rotated at 180 rpm in a

40ºC water bath. When a thin film of lipid was deposited on the inner wall of the flask, phosphate buffered saline (PBS, pH 7.4, 5 ml) was added and the preparation was rotated for a further 30 min until a white homogenous dispersion of liposomes was obtained. The dispersion was then incubated in a shaker bath for 2 h at 37ºC to complete the swelling process. Smaller MLVS were produced from the larger MLVs by probe sonication. Sonication was performed intermittently, with each cycle comprising of sonication at 65 W for 1 min followed by 1 min of rest, and 10 - 100 of such cycles were applied to a batch of liposomes. The alternating cycle was to ensure that the liposomes were maintained at the ambient temperature of film hydration. The liposomes were incubated for another 2 h at 37ºC to allow for the completion of the annealing process.

Evaluation of liposomes [16]

Encapsulation efficiency of BPO

The efficiency of drug encapsulation (EE) is defined as the percentage of encapsulated BPO in a liposome dispersion, was determined as previously reported. 5ml samples of liposomes were subjected to high-speed centrifugation at 86,000 g for 4 h at 4ºC. The supernatant containing the free drug was isolated, and the pellet containing the BPO-loaded liposomes was reconstituted in 5 ml of PBS. The BPO contents in the pre-centrifuged liposome sample, the supernatant and reconstituted pellet were determined by HPLC using C18 column (30 x 4.6 mm, 5 µm pore size). The mobile phase used was 70% v/v acetonitrile, 28% v/v water, and 2% v/v phosphoric acid at flow rate of 1 ml/min and detection wavelength of 254 nm using UV detector.

BPO encapsulation efficiency of the liposomal samples was calculated as follows:

$$\% \text{ Encapsulation} = \frac{\text{Amt of drug encapsulated}}{\text{Amt of BPO in formulation}} \times 100\%$$

In vitro drug release

The *in vitro* BPO release study was carried out over 24 h. Dispersions of liposomes (5ml) were placed in dialysis sacs immersed in glass bottles each containing 100 ml of stirred PBS (pH 7.4). At designated time periods (0.5 h, 1 h, 3 h, 5 h, 8 h, 24 h), 1-ml aliquots were removed from the receptor compartments and replaced with an equal volume of PBS. Drug concentration in the receptor compartment was determined by HPLC. The *in vitro* BPO release profile was constructed by plotting the mean cumulative percent drug release (calculated based on the actual BPO content in the liposomes) against time.

Size

The particle size of the liposomes was determined by using a Magnus microscope, employing the calibrated eye piece and stage micrometer method. Size of liposomal

vesicles was measured at different location on slide by taking a small drop of liposomal dispersion on it and average size of liposomal vesicles was determined.

Stability of Liposomes

The stability of the liposomal preparations was evaluated as a function of storage time. In the preliminary experiments, liposomal samples were stored in a refrigerator at 4ºC for 3 months immediately after preparation. Once every 2 weeks, the size of the samples were determined using ocular micrometer.

RESULTS AND DISCUSSION

The BPO procured was white solid with faint odor and melting point of 105-107°C. It exhibited a log P value of 3.5. The HPLC chromatogram exhibited retention of 5.3 min using the followed chromatographic conditions.

The FTIR spectra of BPO exhibited stretching and bending vibrations in the region of 600 to 2000 cm^{-1}. The vibrations of BPO and the excipients were present in the physical mixture with slight variation in frequencies suggesting no chemical interaction between the drug and the excipients **(Figure 2, 3)**.

Evaluation of liposomes

The process and formulation and parameters strongly affect the properties of drug-loaded liposomes. As with all drug delivery systems, a precise characterization of the physicochemical parameters of the BPO-loaded liposomes is important in order to develop drug products of high quality. The parameters used to characterize the liposomes in the preliminary experiments included particle size, encapsulation efficiency and the drug release profile. Stability studies using particle size as an indicator of stability were also conducted over a 3-month period.

Particle size

As the concentration of lecithin increased, the particle size increased. It was also found that increasing the amount of cholesterol in the formulation also caused a significant increase in the particle size of the liposomes **(Table 2)**. The sonication time on the other hand also affected the particles size. Drug loaded liposomes in the size range of 1.8 – 3.2 µm, were obtained over 30 min of repeated sonication and cooling. Ideally smaller particle size is desirable but the increased process time and lower stability might present problems. Also it has been earlier stated that the maximum amount of drug incorporated into liposomes is dependent on the quantity of membrane component.

Drug entrapment in liposomes

The result of drug entrapment efficiency of liposomes **(Table 2)** indicates that drug entrapment efficiency of liposomes decreases with decreasing concentration of lecithin which might be due to the saturation of lipid bilayer with reference to the drug where low lecithin content provides limited

entrapment capacity. The encapsulation efficiency of liposomes is governed by the ability of formulation to retain drug molecules in the aqueous core or in the bilayer membrane of the vesicles. Chlolesterol seems to help in keeping the drug entrapped in the core and hence increasing cholesterol improves entrapment marginally. Cholesterol improves the fluidity of the bilayer membrane and improves the stability of bilayer membrane in the presence of biological fluids such as blood/plasma. Annealing was performed on the prepared liposomes to prevent physical degradation and drug loss. This process involved incubating the liposome dispersion for 2 h at 35ºC, a temperature higher than the phase transition temperatures of the lipids, to allow for the equilibrium of opposite sides of the lipid bilayer by transmembrane flip-flop.

From the results of particle size and entrapment efficiency, formulation **F4** containing soy lecithin-cholesterol in the ratio 9:2 was considered the most optimum formulation with particle size of 6.05 µm and 53.77 % drug entrapment post 5 minutes of sonication.

In vitro release of BPO from liposome

The *in vitro* release of BPO from the most optimum liposomal formulation (**F4**) was studied for a 24 h duration. It was observed that a maximum of 61.7% of BPO was released from the formulation over a period of 24 h while around 23% of BPO was released in the first 30 min of the study. In order to establish the mechanism of drug release, different kinetic models are used **(Figure 4)**. The drug release data were subjected to various mathematical kinetic model including zero order release kinetics (plot of cumulative percent release vs time), Highuchi's equation (plot of cumulative percentage of drug release vs log time) and Korsmeyer-Peppas equation (log cumulative percentage release vs log time). The Korsmeyer model is widely used when the release mechanism is not well known or when more than one type of release phenomena could be involved. The interpretation of data was based on the value of the resulting regression coefficients. For the formulation, the values of R^2 of zero order, Higuchi and Peppas model were calculated. It was clearly observed that for most of the formulations, the value of resulting regression coefficient is highest for Higuchi model which shows that all the formulations predominantly followed the Higuchi square root kinetics. The corresponding n values of maximum formulations were below 0.5 which indicates that the formulations released drug though Fickian diffusion mechanism.

Stability of liposomes

The change in particle size over a period of three months was considered to ascertain the stability of the liposomal formulation

(Table 3). The optimized liposomal formulation was stored in six different batches in refrigerator to monitor the stability. The particle size was observed every two weeks for changes.

Table 1: Composition of liposome formulations

Formulation code	Lecithin	Cholesterol	Total weight of lipids (mg)
	Ratio		
F1	9	1	800
F2	8	1	800
F3	7	1	800
F4	9	2	800
F5	8	2	800
F6	7	2	800

Table 2: Particle size of liposomes

Formulation Code	Sonication time (min)	Particle Size (µm)		% BPO encapsulated
		Blank liposome	BPO loaded liposome	
F1	5	3.21	5.62	48.92
	15	2.24	4.16	43.51
	30	1.89	2.22	30.51
F2	5	3.82	7.01	47.23
	15	2.68	4.83	40.62
	30	2.02	3.01	25.28
F3	5	4.38	7.69	44.71
	15	3.24	6.23	26.13
	30	2.96	4.98	20.64
F4	5	3.59	6.05	53.77
	15	2.91	5.54	46.42
	30	2.46	3.19	33.69
F5	5	4.11	7.43	49.16
	15	3.35	5.29	42.58
	30	2.76	3.62	29.84
F6	5	4.97	8.01	45.73
	15	3.91	6.82	29.67
	30	3.17	5.22	21.43

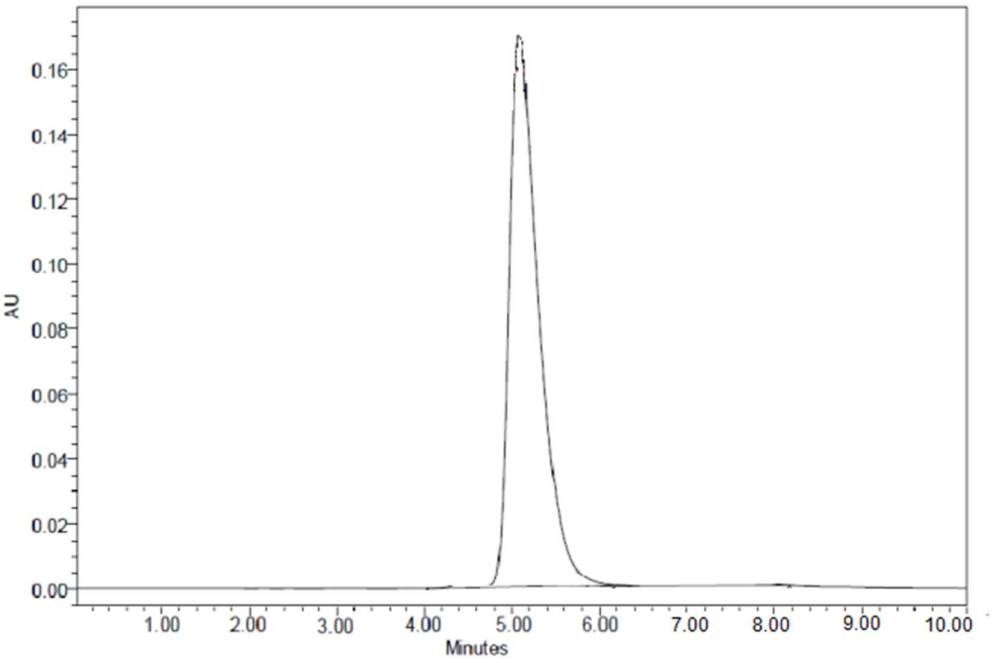

Figure 1: HPLC chromatogram of BPO

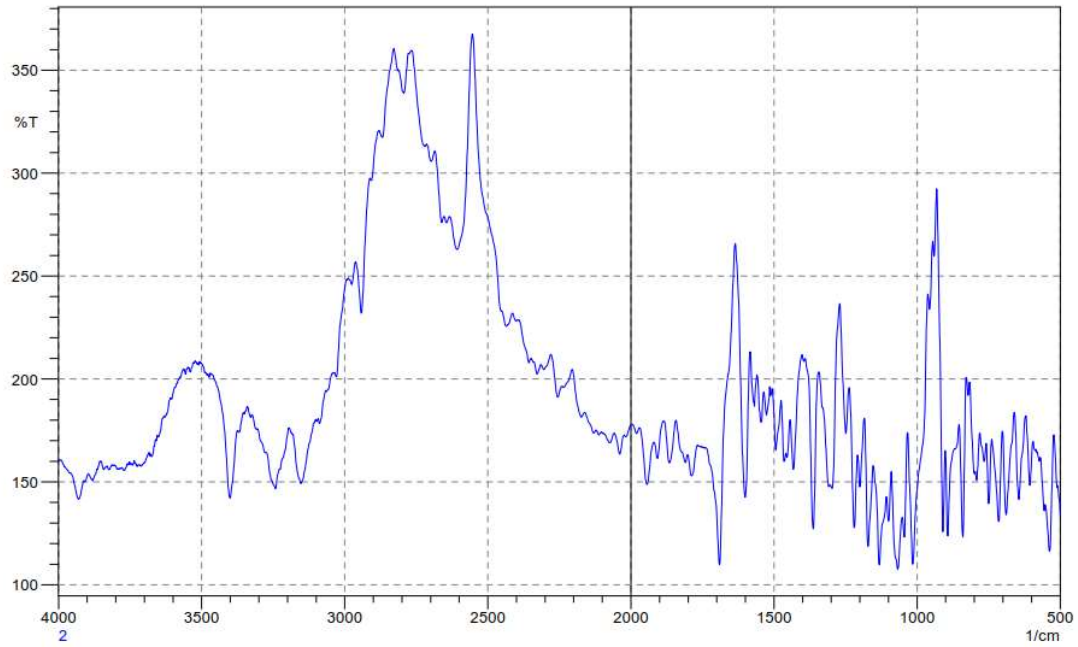

Figure 2: FTIR Spectra of benzoyl peroxide

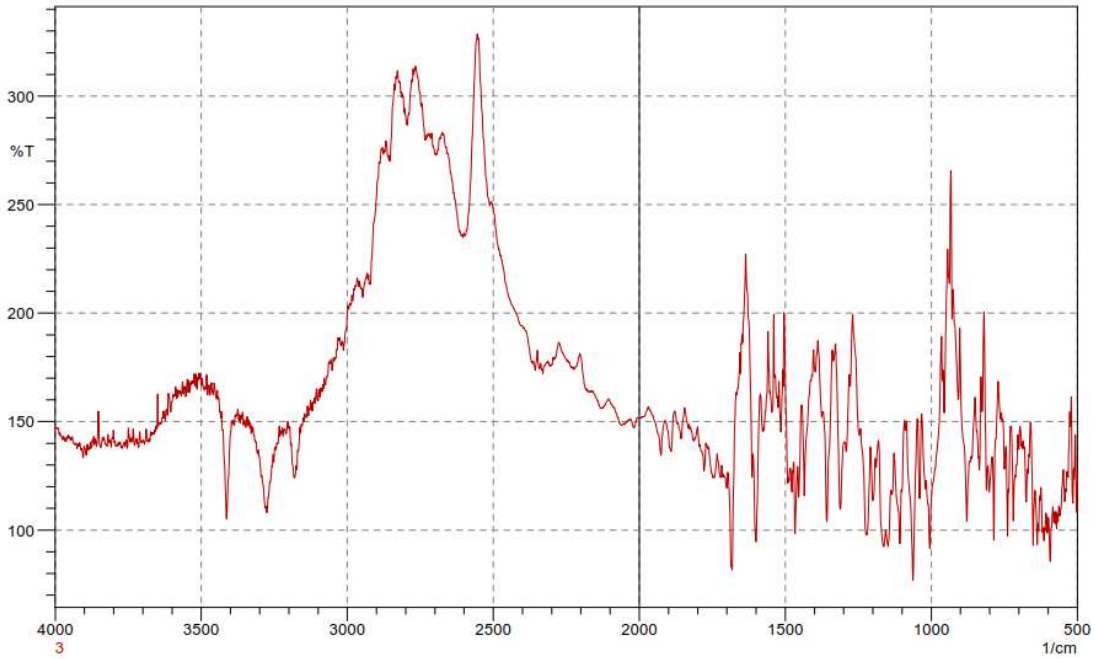

Figure 3: FTIR Spectra of physical mixture of BPO, lecithin & cholesterol

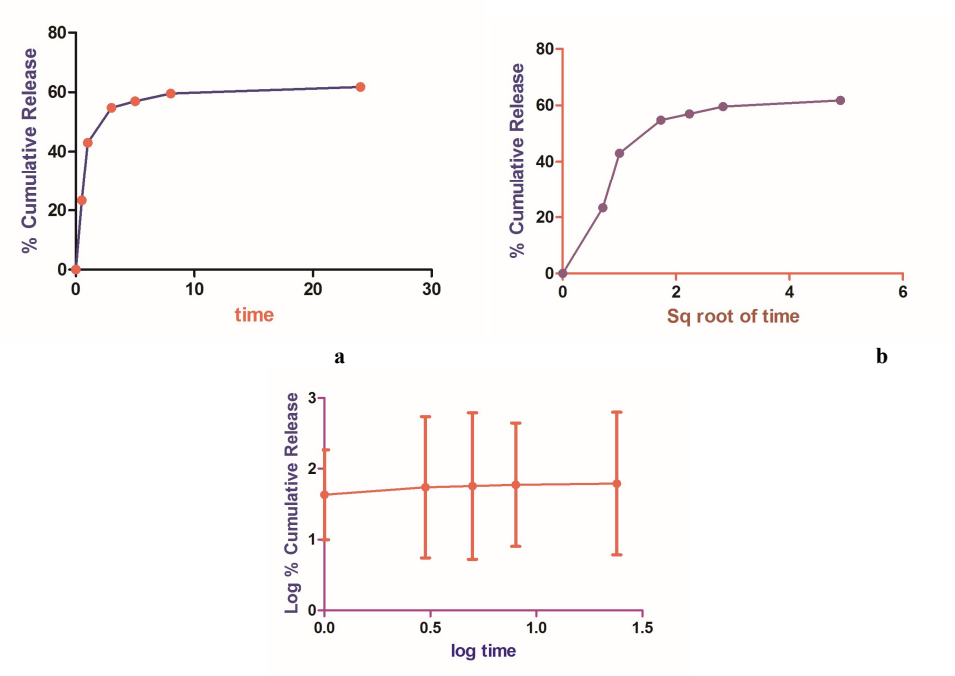

Figure 4: Release of BPO from liposome a) zero order b) Higuchi c) Peppas

CONCLUSION

Solvent evaporation method was successfully applied for preparation of liposomes loaded with Benzoyl Peroxide for improving the stability of the drug molecule. The use of soy lecithin and cholesterol in varying proportions was studied and the effect of sonication time on the size of the liposomes was also studied. The formulation could control the release of the drug for more than 24h. This formulation would be helpful in formulating topical preparations of benzoyl peroxide which would be applied to acne and would be helpful in the treatment of acne.

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