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Formulation and Evaluation of Linalool loaded Microsponges for topical delivery

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ABSTRACT
The aim of the present study was to formulate and evaluate topical microsponges based delivery system containing Linalool. This formulation showed controlled release of the drug for proficient treatment of fungal infection. Linalool loaded microsponges were prepared by quasi emulsion solvent diffusion method using Eudragit RS100 polymer with active constituent of Linalool. The prepared microsponges were characterized by SEM, FTIR, % drug loading and % entrapment efficiency, particle size, zeta potential. Compatibility studies using UV and FTIR indicate that there is no chemical interaction between drug and polymers. SEM studies revealed that the prepared microsponges were spherical and porous in nature. The formulation was subjected to in-vitro diffusion studies for 9hrs which showed controlled release. Microsponges were further incorporated into cream formulation for topical delivery. Prepared cream formulation was evaluated for physical parameters like pH, spredability and in-vitro drug diffusion study. Cream loaded with Linalool microsponges showed desirable physical parameters and in-vitro drug diffusion i.e. 92% in 9hrs. Antifungal activity of prepared formulation cream was done against Candida albicans, which showed zone of inhibition i.e. 1.7 cm.
Keywords: *Microsponge, Topical formulation, Oral administration, Controlled release, Particle size.*

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INTRODUCTION

The microsponges technology was developed by Won in 1987 and the original patents were assigned to advanced polymer system, Inc. This company developed a large number of variations of the technique and applied to the cosmetic as well as over the counter (OTC) and prescription pharmaceutical product. At present, this technology has been licensed to Cardinal Health, Inc, for use in topical products [1].

The microsponges Delivery system (Microsponge drug delivery system) is a patented polymeric system consisting of porous microspheres. They are tiny sponge like spherical particles that consist of a myriad of interconnecting voids within a noncollapsible structure with a large porous surface through which active ingredient are released in a controlled manner. The size of the microsponge's ranges from 5-300µm in diameter and a typical 25µm sphere can have up to 250000 pores and an internal pore structure equivalent to 10 feet in length, providing a total pore volume of about 1ml/g for extensive drug retention [2].

The Microsponge Drug Delivery System has advantages over other technologies like microencapsulation and liposomes. Microcapsules cannot usually control the release rate of actives. Once the wall is ruptured the actives contained within microcapsules will be released. Liposome suffers from lower payload, difficult formulation, limited chemical stability and microbial instability [3].

A Microsponge Delivery System is patented, highly cross-linked, porous, polymeric microspheres that can entrap wide range of actives and then release them with desired rate. This system is useful for the improvement of performance of topically applied drug. It is a unique technology for the controlled release of topical agents and consists of micro porous beads, typically 10-25 microns in diameter, loaded with

active agent. Their high degree of cross-linking results in particles that are insoluble, inert and of sufficient strength to stand up to the high shear commonly used in manufacturing of creams, gels, lotions, and powder. Their characteristics feature is the load a high degree of active materials into the particle and on to its surface [4]. Moreover they may enhance stability, reduce side effect and modify drug release favorably. Microsponge technology has many favorable characteristics, which make it a versatile drug delivery vehicle. Microsponge Drug Delivery System can provide increased efficacy for topically actives agents with enhanced safety, extended product stability and improved aesthetic properties in an efficient manner [5, 6, 7].

Linalool was found to be a potent inhibitor of *Candida albicans*, which act as a human pathogen and has been shown to be resistant against a variety of antifungal agents. Linalool may be beneficial in dental care products, as it displays strong antimicrobial activity against cariogenic bacteria. Linalool further exhibits a variety of effects on central nervous system such as antinociceptive and sedative activities [8].

The present work has aimed at formulating microsponges for Linalool, which can release the drug in a controllable manner, thus reducing the side effects. The drug loaded microsponges are converted to cream for better applicability and patient compliance.

MATERIAL AND METHODS

MATERIALS:

Linalool was gift sample from S.D fine Chem. Limited, Mumbai. All other chemical used were of analytical grade and purchased from authentic suppliers.

METHODS:

Drug loaded microsponges were prepared by Quasi- emulsion solvent diffusion method. The inner phase was prepared by dissolving eudragit RS 100 in a suitable solvent i.e. dichloromethane. Then drug was added to solution and dissolved under ultrasonication at 35^0C. The inner phase was then added into outer phase containing polyvinyl alcohol and stirs this mixture at 800-900 rpm for 2 h to get desired rigid microsponges due to the evaporation of organic solvent. The rigid microsponges were filtered through the filter paper, washed with distilled water and dried at 40^0C for 24 h. Process dependent variable like Eudragit RS 100 and Polyvinyl alcohol were optimized by 3^2 factorial design techniques. The design and composition of various microsponges is outline in Table 1 and 2 [9-11].

EVALUATION OF MICROSPONGES:

Production yield:

The production yield of microsponges was determined by using formula mentioned below

$$\text{Production Yield (PY)} = \frac{\text{Practical Mass of Microsponges}}{\text{Theoretical Mass Theoretical mass (Polymer+drug).}^{12}} \times 100 \quad (1)$$

Drug content and Entrapment efficiency:

Exactly weighed amount of (100 mg) of microsponges containing drug was kept in 100 ml of ethanolic phosphate buffer (60:40) at pH 6.8 for 12 h with constant stirring. Filtered samples (using 0.45 lm membrane filter) were analyzed at 583 nm next to blank using UV spectrophotometer (Pharmaspec 1700, Shimadzu, Japan). Evaluation of drug content and entrapment efficiency for all batches were completed using following expressions.

Drug content (%) = $Mact/ Mms \times 100$ (2)

Entrapment efficiency = $Mact / Mthe$ (3)

Where *Mact* = actual drug content in weighed quantity of microsponges,

Mms = weighed quantity of microsponges and *Mthe* = theoretical drug in microsponges [12]

Particle size analysis:

Particle size and size distribution of microsponge particles was determined using optical microsponge. A minute quantity of microsponges was spread on a clean glass slide and average size of 100 microsponges was determined in each batch. The values were given in table 5 for the formulations in form of mean particle size [13].

Scanning electron microscopy:

For evaluating morphology and surface topography, the formulated microsponges were examined under scanning electron microscope (LE0 440i, UK) operating at 5 kV. Using double adhesive tape, sample was mounted on a metal stub and coating with platinum / palladium alloy under vacuum was done [14].

Zeta potential:

Zeta potential of optimized microsponges was measured by using zeta sizer at 25^0C (Particulate system nano plus) [15].

Procedure for cream formulation:

- Cream containing drug loaded microsponge was prepared by (70-75^{0}C) at the oily ingredients to their increasing melting point and mixed them.
- Water soluble ingredients were dissolved separately into water warm until it attains temperature up to oily phase.
- Aqueous phase was then added to the oily phase and mixed by using mortar and pestle
- Glycerin was then added and mixed until homogenous mixture of cream was prepared
- The mixture was cooled at room temperature and then transferred the cream into a suitable container.[16]

EVALUATION OF CREAM [17-20]

Physical examination:

The prepared topical cream was inspected visually for their colour, homogeneity, consistency and appearance.

Colour: The colour of the formulation was checked against white and black background.

Consistency: The consistency was checked by applying on skin.

Determination of viscosity:

Viscosity measurement was carried out by using Brookfield viscometer. Sufficient sample was placed in a cylindrical tube and measured the reading at 37^{0}C ± 2^{0}C and rotating the spindle no. 63 at 1.5 rpm.

Extrudability:

The prepared cream formulation was filled in clear, collapsible aluminum tube. Afterward, the extrudability of cream formulations was estimated in weights in gram required to extrude out through tip of the tube.

Spreadability:

Spreadability of gel formulations was determined by using horizontal plate method. A glass plate was stationed on the surface and an excess of gel (1g) were allocated in the slide. The gel was sandwiched between the slide and another glass slide having the fixed dimension was placed over it. A 125 g weight was positioned over the upper slide for specific time (5 min.) to expel and to provide a uniform film of the gel between the slides.

The spreadability was then calculated using the following formula

$$S = M \times L / T$$

Where, S= spreadability, M= weight placed on the upper slide, L= length moved by the glass slide, T= time

In vitro release studies of linalool loaded microsponge cream:

In vitro release studies were performed by artificial dialysis membrane. For this testing, a vertical Franz diffusion cell with a surface area of 2.54 cm^2 and a reservoir capacity of 20 ml was used. The artificial membrane was firmly positioned between the two halves of the diffusion cell. (pH6.8), and its temperature maintained at 37± 0.5 ^{0}C and stirred constantly by magnetic stirrer. A predetermined amount of formulated cream (1 mg) of linalool was placed on the donor side. A total of 2 ml of the sample was withdrawn from the receptor compartment at definite time intervals and replaced with an equal volume of fresh receptor fluid. The aliquots were correctly diluted with the receptor medium and analyzed by an UV spectrophotometer. Measurements were performed in triplicate and their means were reported.

***In vitro* antifungal study of linalool loaded microsponge cream:**

In vitro antifungal test was determined by sabouraud dextrose agar disk diffusion method employing the 'cup plate technique' using sterilized Petri dish. Cream containing microsponges (1 mg/ml), Cream without microsponges (1 mg/ml), and standard Luliconazole cream (1 mg/ml) prepared in DMSO and was placed into cups of size 8 mm, then into wells of a sabouraud dextrose plate previously seeded with the test organism of Candida albicans. After allowing diffusion of the solution for 2 h, the plates were incubated at 27^{0}C for 48 h. The zone of inhibition measured around each cup was compared with that of the standard. The results of triplicates measurements and their means were reported.

Accelerated stability studies:

The prepared Linalool microspongic cream were packed in aluminium collapsible tube (5 g) and subjected to stability studies at 5^{0}C/60 % RH, and 40^{0}C/ 75 % RH for a period of 3 month. Samples were withdrawn at 15- day time intervals and evaluated for physical appearance, pH, rheological properties, drug content, and drug release profiles.

RESULTS AND DISCUSSION:

Evaluation of Linalool loaded microsponges:

Linalool microsponges were prepared by quasi-emulsion solvent diffusion method. The method was simple, reproducible and raid. The product was observed to be of pale white colour with good flow

properties than as compare with pure drug. Particle size, drug content, Percentage yield of different formulation was calculated and percentage entrapment efficiency were also determined for formulation and given in Table 4.

Scanning electron microscopy:

The determination of shape and surface morphology was done by scanning electron microscope HITACHISU1500,Japan.ThecapturedSEM image is shown in Fig. 1. SEM results indicated that the microsponges of Linalool with dichloromethane were highly porous, spherical discrete particles. The surface topography reveals that the microsponges were porous and the pores may be induced by the rapid escape of the volatile solvents from the surface during formulation (Fig. 1).

Zeta potential:

Highly negative or highly positive zeta value indicates good physical stability of the formulation. The optimized microsponge formulation of Linalool was measured by using zeta sizer at 25^0C, it was found to be -4.15 (mV) and it indicated the formulation to be stable.

EVALUATION OF LINALOOL LOADED MICROSPONGES CREAM:

Preparation of microsponge cream:

Linalool loaded microsponge cream was prepared as per formulation given in **Table 3.** Microsponges were inspected visually for their color, texture and appearance. The formulation was white, smooth texture and of good homogeneity without any lumps and syneresis.

Physical characterization of cream:

Physical parameters of cream formulation were shown in Table 5. Cream was evaluated for their pH value of the cream was around 5.92±o.o148 which is supposed to be suitable for skin without causing any irritation. The value of spreadability 18.5±0.656 indicated that the cream is easily spreadable by small amount of shear. The extrudability of Linalool microsponge cream was found to be 78.7±0.014 which indicates the better extrudability. Viscosity of prepared formulation was found to be 1745.3±o.309

***In vitro* release studies of linalool loaded microsponge cream:**

In vitro drug release study of cream was carried out using ethanolic phosphate buffer pH 6.8 using Franz diffusion cell. From the result it could be concluded that % CADD of cream formulation gave better release. At the end of 9 h the total amount of drug release from the formulation was found to be 92.1±0.54 %

***In vitro* antifungal study of linalool loaded microsponge cream:**

The result of antifungal activity of cream was investigated against *Candida albicans* using agar well diffusion assay. Diameter of zone of inhibition of sample, positive control and negative control are shown in Table no 7

Accelerated stability studies:

The developed Linalool loaded microsponge formulation was found to be stable upon storage for 3 month. No major changes were noticed in their physical properties and drug release profile.

Table 1: Dependent variables considered in formulation

Dependent Variable	Code	Level		
		-1	0	+1
Eudragit RS 100	X_1	0.5	1	1.5
Polyvinyl alcohol	X2	0.25	0.5	0.75

Table 2: Composition for drug loaded microsponges

Batch no.	F1	F2	F3	F4	F5	F6	F7	F8	F9
Linalool (ml)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Eudragit RS 100 (g)	0.5	1	1.5	0.5	1	1.5	0.5	1	1.5
Dichloromethane (ml)	20	20	20	20	20	20	20	20	20
Polyvinyl alcohol (g)	0.25	0.25	0.25	0.50	0.50	0.50	0.75	0.75	0.75
Distilled water (ml)	100	100	100	100	100	100	100	100	100

Table 3: Preparation of cream containing linalool loaded microsponges

Sr. No	Ingredients	Quantity
1	Microsponges containing Linalool equivalent to	0.5 gm
2	Bees Wax	8 gm
3	Borax	1 gm
4	Liquid paraffin	6 ml
5	Glyceryl monostearate	2 gm
6	Cetyl alcohol	2.5 gm
7	Glycerine	1 ml
8	Propyl paraben	0.2 gm
9	Methyl paraben	0.1 gm
10	Distilled Water	q. s

Table no 4: Production yield, entrapment efficiency, particle size, and drug content

Formulation code	% Production yield (AM±SD)*	% Entrapment efficiency (AM±SD)*	Particle size µm (AM±SD)**	Drug content (AM±SD)*
1	67.65±1.9	42±0.41	83.2±0.08	56.07±0.21
2	54.58±0.83	46.±0.48	79.3±0.93	40.23±0.34
3	48.07±1.14	54.±0.07	65.2±0.75	38.4±0.04
4	65.5±0.35	61.±1.21	61.6±0.92	37.2±0.09
5	54.6±0.48	59.13±0.78	81.25±3.89	29.7±0.05
6	60.48±0.73	69.12±0.82	89.87±2.99	22.5±0.04
7	71.5±1.63	67±1.33	75.81±6.78	24.7±0.11
8	69.35±0.94	73±0.81	97.54±4.54	21.12±0.4
9	75.66±0.68	74±0.21	116.98±0.98	19.23±0.9

*Average of three determinations, **Average of 100 particles

Table no: 5 Physical parameters of prepared cream

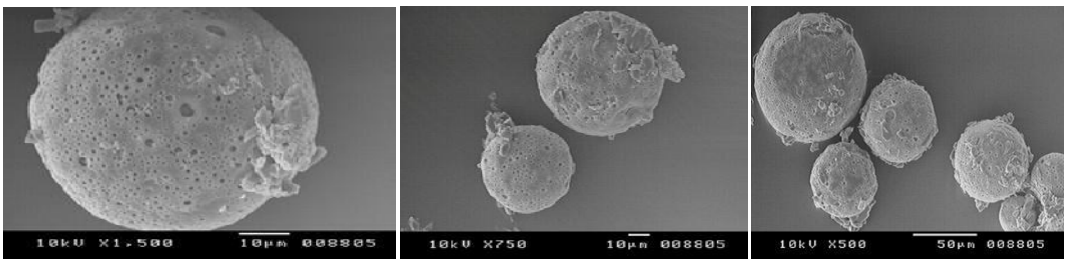
Sr. no	Test	Std. deviation
1	pH	5.92±o.o148
2	Spreadability (gm-cm/sec)	18.5±0.656
3	Extrudability (%)	78.7±0.014
4	Viscosity (cps)	1745.3±o.309

Table: 6 Pharmacokinetic modeling of optimized batch

Drug release kinetics model	Regression coefficient
Zero order	0.9948
First order	0.9428
Higuchi model	0.9335
Korsemeyer-Peppas model	0.9384

Table: 7 Antifungal activities

Microorganism used	Test sample	Zone of inhibition(CM)
Candida albicans	Cream formulation (Test sample)	1.7
	Positive control (Standard)	1.9
	Negative control (Cream without microsponge)	0.8



A B C

Fig. 1: SEM of linalool loaded k2microsponge formulation a) 500x, b) 750x, c) 1500x

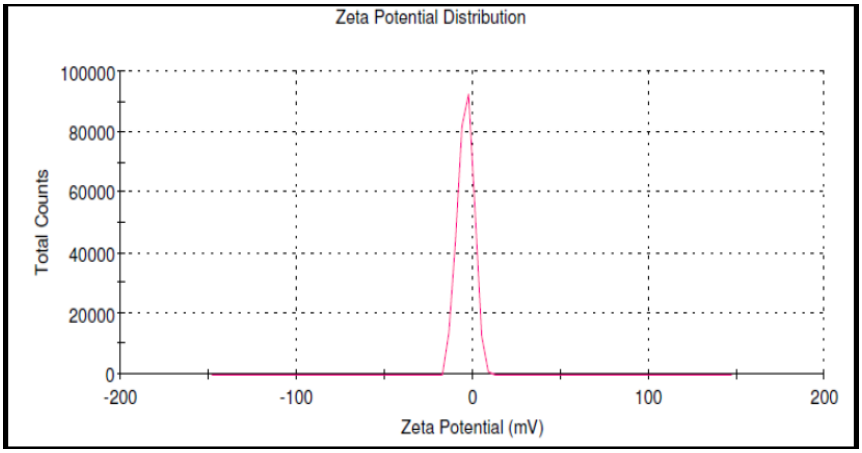


Fig. 2 Zeta potential of drug loaded microsponge

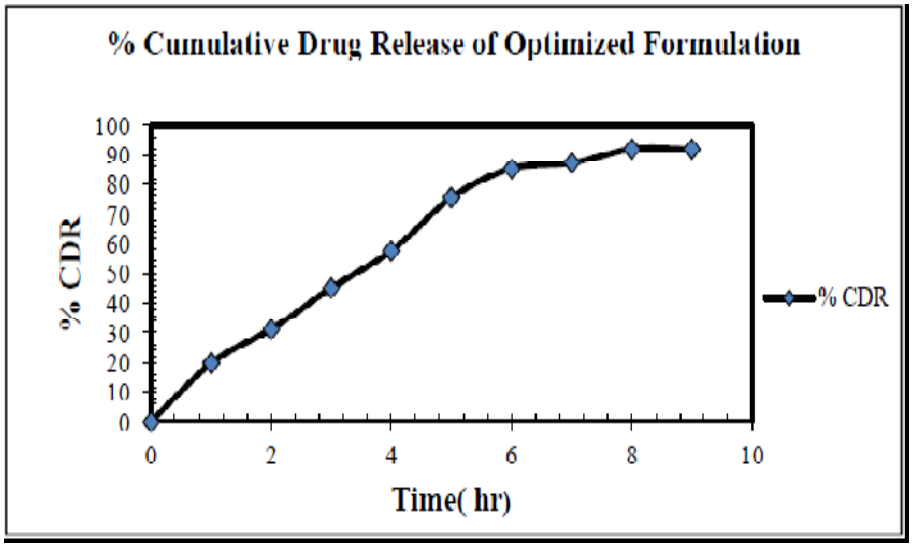


Fig 3: % Cumulative drug release of optimized formulation

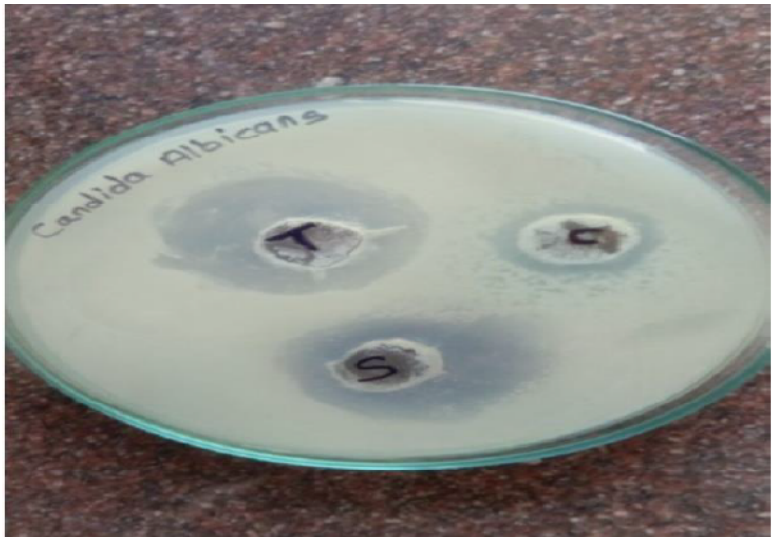


Fig 4: In-vitro antifungal activity against *candida albicans*

CONCLUSION

The present study concluded successfully formulation of Linalool loaded microsponge by using quasi-emulsion solvent diffusion method. The implemented method was found to be easy, reproducible and rapid. Formulation microsponges were spherical in shape, have porous and regular surface. The in-vitro drug release study of formulation Linalool microspobge loaded cream followed Korsmeyer Peppas model, first order, zero order, and Higuchi model on their regression (r^2) values. The in-vitro antifungal study of cream showed effective result against *Candida albicans.* Thus, it found to have potential management of fungal infection for better patient compliances.

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