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Formulation and evaluation of microemulsion gel containing Boswellia serrata and Primrose oil for Arthritis

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ABSTRACT

Osteoarthritis and rheumatoid arthritis are two inflammatory joint conditions that lead to degeneration of the synovial region leading to pain and deterioration of the joints finally culminating into loss of joint function. Natural herbal extracts like Boswellia serrata have been found to contain components like Boswellic acids which exhibit the property of inhibition of certain enzymes which are mediators of inflammation in the tissue region. These active components aid to inhibit enzymes like 5-Lipoxygenase (5-LO) and 5-Hydroxyeicosatetraenoic acid (5-HETE) thus inhibiting the inflammation component of joint damage. The poor aqueous solubility of the extract of Boswellia serrata poses a challenge for designing effective dosage forms for administering the boswellic acid enriched extract to the site of action. The present work comprises of formulation of a clear isotropic microemulsion system and microemulsion gel containing solubilized Boswellia serrata extract and Primrose oil. The microemulsion was evaluated for globule size, Fourier transform infrared spectroscopy, entrapment efficiency, stability and irritation potential by HETCAM assay whereas the microemulsion gel was evaluated for pH, viscosity and irritation potential. The microemulsion was found to exhibit a globule size of 403.1 nm, no changes in chemical structure, entrapment efficiency of 91 % and stable at all conditions of temperatures of 25°C, 30°C and 40°C, light, freeze-thaw, centrifugation test and non-irritant in nature. The microemulsion gel was found to have a pH of 5.5, viscosity of and non-irritant by HETCAM assay.

Keywords: microemulsion, osteoarthritis, inflammation, HETCAM, entrapment efficiency, boswellic acid

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INTRODUCTION

Osteoarthritis and rheumatoid arthritis are two major musculoskeletal diseases which are the common causes of disability observed globally. Conditions like tissue degeneration and severe inflammatory activity leading to chronic pain and damage to healthy joints. Joints owing to their load-bearing property are subjected to articular damage in the cartilage which after a chronic period of damage leads to synovial inflammation and degeneration affecting patient mobility. (1,2)

Certain conditions like injury to joints leads to overexpression of biological factors like inflammatory cytokines (Interleukin IL-1, IL-6) and tissue necrosis factor (TNF-alpha) which further promotes osteoarthritic degeneration. (3) The irreversible nature of cartilage damage necessitates the need for timely therapeutic intervention and repair therapy in order to support patient mobility and overall healthcare. Rheumatoid arthritis is a debilitating disease exhibiting inflammation in the synovial lining of joints thus resulting in pain, stiffness and swelling of joint culminating in joint deterioration and loss of function.(4,5)

The objective of anti-inflammatory therapy for Osteoarthritis and Rheumatoid arthritis is to effectively reduce pain and inflammation, prevent the joint deterioration, destruction and deformity.(6) The conventional therapies for Osteoarthritic relief is use of non-selective cyclooxygenase II inhibitors. However these are associated with severe adverse effects thus arising alternative safe therapies for Osteoarthritis. Several herbal actives like Boswellia serrata, Curcuma longa(Curcumin), Zingiber officinnalis (ginger), etc have been found to possess anti-inflammatory activity.(7,8)

Boswellia seratta is an oleogum resin belonging to genus Boswellia (Family: Burseraceae).It is a plant exudate obtained by an incision in the trunk of tree. A liquid fluid oozes out of the semisolid oleogum resin placed in bamboo basket over a period of one month. The residual gum resin solidifies and is available in different grades. The oleogum resin comprises of 30-60% resin and 5-10% essential oils. Some of the actives present in the resinous part of the oleogum resin include monoterpenes, diterpenes and triterpenes. It also contains pentacyclic triterpenic acids called Boswellic acids, tetracyclic triterpenic acids and polysaccharides. (9,10)

The Boswellic acids present in the Boswellia seratta extract exhibit inhibition of proinflammatory enzymes like 5-Lipoxygenase (5-LO), 5-Hydroxyeicosatetraenoic acid (5-HETE) and leukotriene B4 (LTB-4). Boswellic acids act as specific inhibitors of 5-Lipoxygenase enzyme which induces inflammation by causing free radical damage and migration of inflammation producing cells to the inflamed body area. (11)

Owing to the poor solubility of Boswellia serrata extract, it has become a formulation challenge to devise dosage forms that deliver higher contents of Boswellic acid and other therapeutic actives from the extract to the target site. There is a need to devise a drug delivery system that aids to deliver the drug and its active constituents in higher concentration to the targeted site for enhanced anti-inflammatory activity.

Evening primrose oil (Oenothera biennis) is a rich source of Omega-6-fatty acids and exhibits anti-inflammatory activity in rheumatoid arthritis. Osteoarthritis is associated with severe joint pain and patient discomfort. For the same, in order to enhance the patient comfort, topical systems containing anti-inflammatory actives form a prospective strategy to provide therapeutic benefits to osteoarthritic patients. Attempts have been made to formulate niosomal gel, self-microemulsifying drug delivery systems for delivering Boswellic acid actives. (12, 13)

Microemulsions as drug delivery systems have proved to enhance the solubility of various poorly soluble actives and thus aided to enhance the bioavailability of the actives. Microemulsions not only aid to enhance the solubility of drug and thus its bioavailability but also aid to provide sustained therapeutic effect when formulated as microemulsion gels for topical use. (14)

The objective of the present work was to formulate and evaluate a microemulsion and microemulsion based gel system containing Boswellia serrata extract and primrose oil and evaluate it for various parameters like globule size, entrapment efficiency, pH, viscosity, extrudability, irritation effect etc.

MATERIALS AND METHOD

Boswellia serrata extract was gifted by K. Patel Phytoextracts (Mumbai, India). Isopropyl myristate, Polysorbate 20, Polysorbate 80, Isopropyl alcohol, Propylene glycol were purchased from Westcoast laboratories. Primrose oil was purchased from Westcoast laboratories. Solutol HS 15, Cremaphor EL was gifted by BASF Chemicals, Turbhe, India. Capryol 90 and Capmul PG were gifted by Abitec Corporation. All other chemicals used were of analytical grade. Incubated eggs were purchased from poultry farm from Goregaon West, India.

Standard Plot for Boswellia serrata

The standard plot of Boswellia serrata was plotted using Methanol as the solvent and at 249 absorption maxima. (15)

Determination of Boswellic acid content from Boswellia serrata extract

The total acid and mineral acid content of Boswellia serrata extract was obtained by acid-base titration method. The total content of boswellic acid was determined by the formula;

Boswellic acid content = Total Acids- Mineral Acids

Solubility studies of Boswellia serrata in Oils and surfactants

The solubility of B. Serrata extract in various oils like Isopropyl myristate, Lauroglycol 90, Capryol 90, Capmul PG, in combination with Primrose oil and in 10 % w/v surfactant solutions like Polysorbate 20, Polysorbate 80, Solutol HS 15, Cremaphor EL and co-surfactants like Isopropyl alcohol and Propylene glycol was determined by the shake flask method. An excess amount of extract was mixed with oil combination, surfactant and co-surfactant and shaken using a flask shaker at 37°C for 48 h. Samples were centrifuged at 5000 rpm for 10 min and subsequently filtered through a 0.22 syringe-driven membrane filter unit. The filtrates were assayed by UV-spectrophotometer (Shimadzu, 1800 double beam spectrophotometer) at 249 nm to evaluate the amount of drug dissolved.

Screening of Surfactants

Initial evaluation of emulsifying properties was carried out by visual assessment. Various surfactants and co-surfactants in a 2:1 ratio were mixed. Then optimized oil combination was added to the above mixture in the ratio of 1:3 (w/w), heated, and vortexed gently to form homogeneous mixtures. About 500 mg of this mixture was dispersed into 10 ml of water with gentle stirring and then assessed visually for self-emulsification in terms of dispersibility and ease of emulsification.

Plotting of Phase Diagrams

Based on solubility studies and emulsification studies, the optimized oil combination, surfactant and co-surfactant and distilled water were selected to prepare microemulsion. For determining the microemulsion region, pseudo ternary phase diagrams were constructed for different combinations of oil and S-mix (1:9 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 9:1) at weight ratios of 1:1, 2:1, 3:1 of surfactant and co-surfactant (Smix)(16)

Formulation of Microemulsion containing Boswellia Serrata and Primrose oil

The microemulsion template method was the simplest and commercially feasible technique to generate microemulsion. The preliminary solubility of the drug with different, oils, surfactants, and solubilizers were conducted. Based on the solubility data selected, components were used to determine the boundaries of the microemulsion domains by employing pseudo ternary phase diagrams. Briefly, the microemulsion preparation by microemulsion template method involves blending of lipids, the addition of liquid lipid followed by drug solubilization in this mixture to constitute the lipid phase. The aqueous phase was comprised of surfactant, cosurfactant and water. Both the phases were maintained at a temperature above the melting point and then mixed to

obtain a hot microemulsion. This hot microemulsion was then diluted into an excess of cold water (2–3 ℃) under stirring.

Formulation of Boswellia serrata loaded Microemulsion containing gel

The formulated microemulsion was prepared into gel using Carbopol 974 as gelling agent. Carbopol 974 gel was formulated using 1.5 % Carbopol 974 and Triethanolamine as the gelling agent. Microemulsion formulation was incorporated into the gel in ratio of 1:1 with uniform mixing. (17)

Evaluation Studies

Globule Size Measurement and Polydispersity Index

The mean globule size and polydispersity index (PI) of Boswellia serrata microemulsion were determined by dynamic light scattering method with the help of Malvern Zeta sizer Nano-ZS, UK. 1 ml of microemulsion was diluted with Millipore water and the globule size was measured using the Zeta sizer.

Fourier Transform Infrared Spectroscopy

The FTIR spectra of the drug Boswellia serrata and drug containing microemulsion was obtained using Jasco FT/IR-4100 type spectrophotometer by KBr Disc method. The sample was scanned over a frequency of 4000 cm^{-1} to 400 cm^{-1} with 4 cm^{-1} resolution.(18)

Calculation of Entrapment Efficiency

The encapsulation efficiency was evaluated using the centrifugation and ultrafiltration process. Microemulsion formulation was subjected to ultra-centrifugal filter using centrifuge tube (Pall Nanosep® Centrifugal Devices, 0.5mL, cut off 30 kDa). Free boswellic acid was separated with centrifugal force 10,000×g for 10 min(10). The filtrate was collected and adequately diluted with solvent and analyzed. The entrapment efficiency was determined using the following equation, (19)

% Entrapment efficiency (%EE)=Total amount of drug- Free drug /Total amount of drug * 100

Stability Studies of microemulsion

The stability of the microemulsion was evaluated at high temperature conditions, in presence of light, on centrifugation and on application of freeze-thaw cycles. The microemulsion stability at high temperature was evaluated by heating the microemulsion at 80-100 degree Celsius for 4 hours and observing for phase separation. The microemulsion stability was evaluated at 25°C, 30°C and 40 °C. The photostability of microemulsion was evaluated by placing the microemulsion in light and observing for any phase separation or changes in stability. The stability of the microemulsion to centrifugation was evaluated by centrifuging the microemulsion at 3500 rpm for 30 minutes.

The stability of the system to freeze-thaw cycling was evaluated between - 21˚C and +25 ˚C by storing the microemulsion systems at the respective temperatures of not less than 48 hours.

Characterization studies of microemulsion gel

i)pH determination

The pH value of 1 % solution of the prepared microemulsion gel was measured using a digital pH meter.

ii) **Viscosity**

The viscosity of the formulated batches was determined using a Brookfield viscometer with spindle 6 to 10 rpm. The assembly was connected to a thermostatically controlled circulating water bath maintained at 25˚C.The sample was added to a beaker covered with a thermostatic jacket. The spindle was allowed to rotate freely into the microemulsion and the reading was noted The viscosity of formulation was calculated using Brookfield Viscometer Model – R.V.SERIES using formula

Dial Reading X Factor = Viscosity cps

Evaluation of Irritation Potential of Microemulsion and Microemulsion gel

The irritation potential of Boswellia serrata microemulsion and microemulsion gel was evaluated using the HET-CAM test. Incubated eggs after 9th day were used. The samples evaluated were as follows

1. 0.9% w/v sodium chloride in distilled water as negative control
2. N sodium hydroxide in distilled water as positive control
3. Placebo microemulsion formulation
4. Drug loaded microemulsion formulation
5. Placebo gelling system
6. Gel loaded with microemulsion formulation

The egg shell was removed from the incubated eggs at the 9th day and 0.3 ml of the test samples were directly applied to the CAM surface. The effect of the test substance applied on the CAM after 300 seconds was determined. Observations were made for Hemorrhages, vascular lysis and coagulation. The observation for each sample was determined on 3 eggs per sample. (16)

The endpoint observed were for Hemorrhages (Bleeding from the vessel), Vascular lysis (Blood vessel disintegration) and Coagulation (Intra and extravascular protein denaturation). (19)

RESULTS AND DISCUSSION

Standard Plot for Boswellia serrata

A straight line plot was obtained for Boswellia serrata when determined at 249 nm. The standard plot equation obtained was y = 0.0041x + 0.0023 and the regression coefficient was obtained as 0.999. The standard plot was used to evaluate the drug content from the microemulsion gel formulation.

Determination of Boswellic acid content from Boswellia serrata extract

The total boswellic acid content was determined from titre reads obtained for total and mineral acids. The total boswellic acid content was observed to be 73.31 %. The anti-inflammatory activity of Boswellia seratta extract can be contributed to presence of Boswellic acids

Solubility studies of Boswellia serrata extract in oils in combination with Primrose oil and surfactants

Capmul PG with Primrose oil was found to be the most suitable oil system based on the solubility studies of drug in different oil systems in combination with Primrose oil. The selection of optimized oil combination aids to yield a stable microemulsion. Primrose oil being in liquid form was blended with the oil phase of Capmul PG and the solubility of the extract was determined.

Screening of surfactants

Cremaphor EL and Tween 20 was found to be the most suitable surfactant system for Boswellia serrata drug in terms of solubility enhancement.

Plotting of Phase diagrams

The phase diagrams were plotted using Tridraw software.

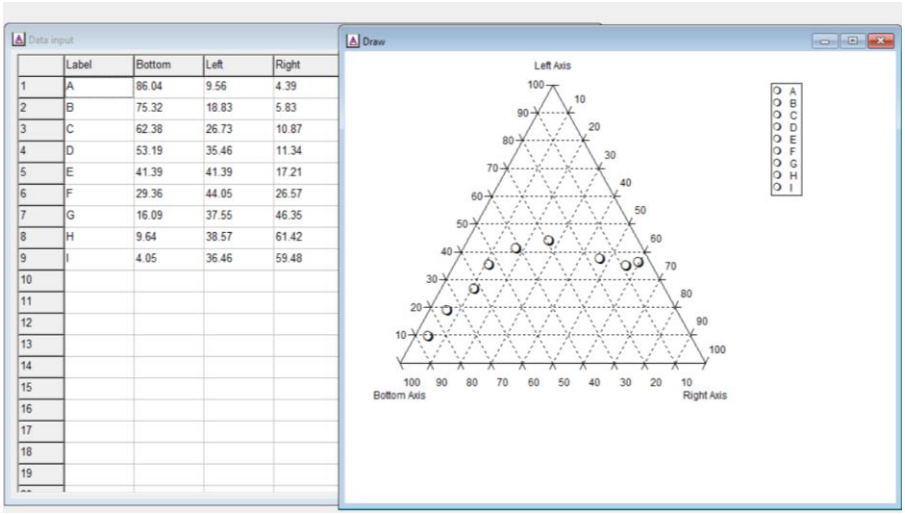

Figure 1: Microemulsion plot

Formulation of Microemulsion containing Boswellia serrata

Microemulsions were prepared by considering the points in microemulsion region from the tridraw microemulsion plot. Microemulsions were formulated considering 3 different quantities of drugs i.e. 50, 100 and 150 mg drug.

Table 1: Formulas for microemulsion formulation containing different amounts of drug

Clear microemulsions were obtained.

Drug (mg)	Primrose oil	Capmul PG	Surfactant Mixture tween 20: KL EL(1:1)	water
50	0.2	0.4	1.85	2.95
100	0.2	0.4	1.85	2.95
150	0.2	0.4	1.85	2.95

Figure 2: Microemulsion containing Boswellia serrata

Formulation of Boswellia loaded microemulsion containing gel

The obtained microemulsion containing Boswellia serrata drug and Primrose oil loaded into a Carbopol 974 gel in a ratio of 1:1 of microemulsion :gel was found to yield a clear gel system.

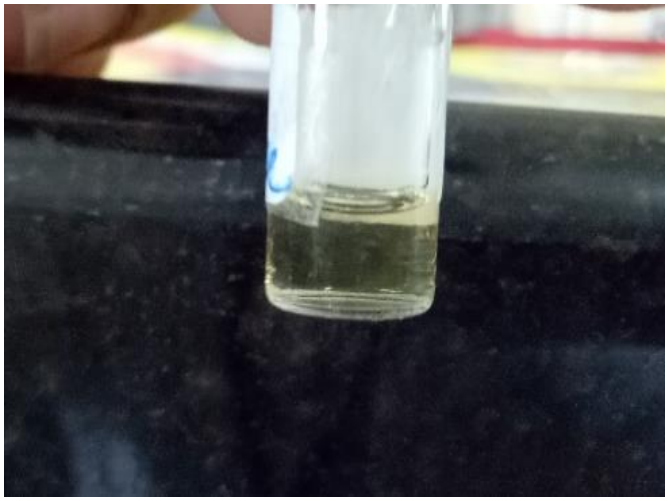

Figure 3: Boswellia serrata microemulsion based gel

Evaluation studies for microemulsion

Globule Size measurement and Polydispersity index

The globule size was found to be 403.1 nm and the polydispersity index was found to be 0.332. The stable nature of microemulsion can be predicted from the globule size and low poly-dispersity index.

Fourier Transform Infrared Spectroscopy

The microemulsion was found to show bands at wavenumbers characteristic of standard drug thus confirming no changes in the drug structure after incorporation in microemulsion.

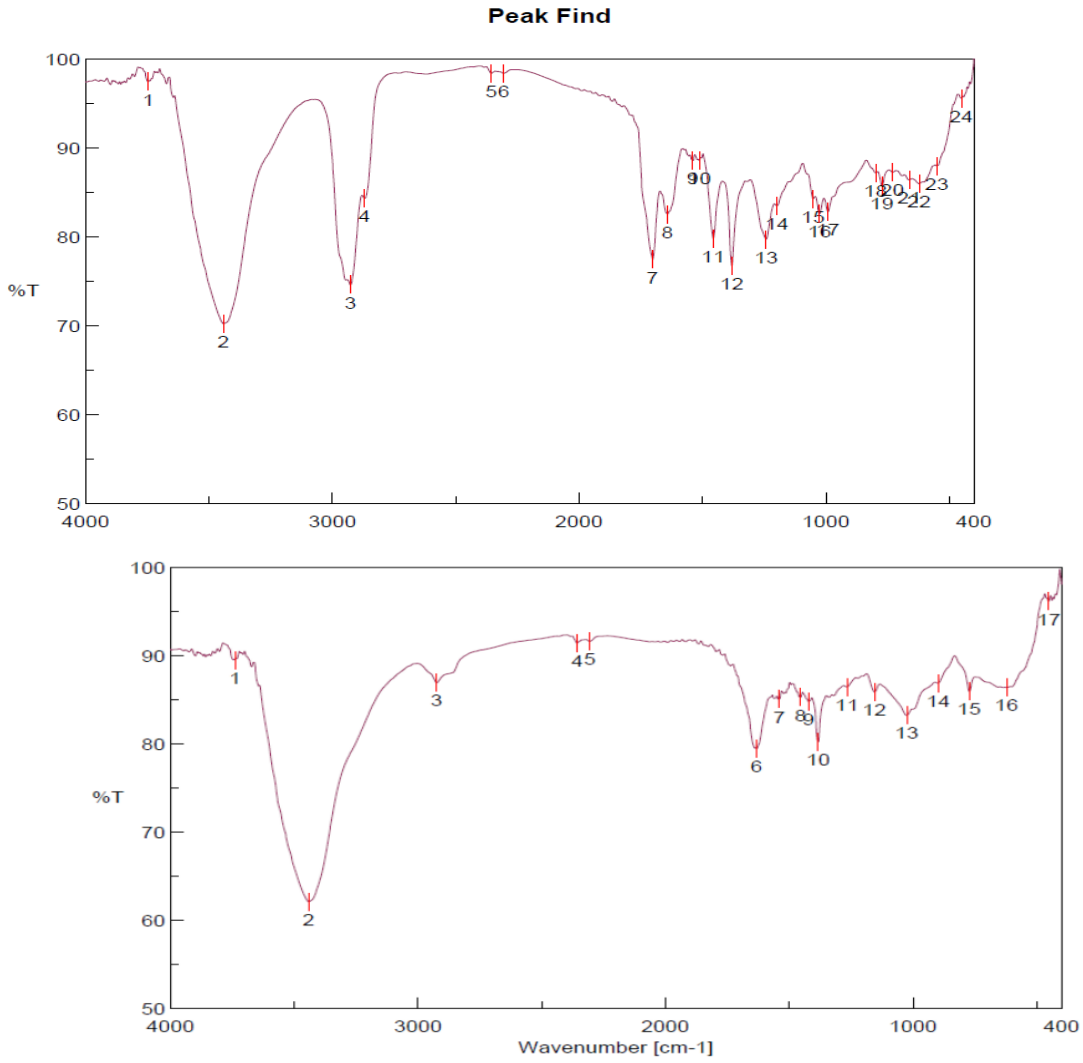

Figure 4: Infrared Spectra for Boswellia serrata drug and microemulsion containing Boswellia serrata drug.

Bands were observed at wavenumbers as observed in Table 2

Table 2: Infrared spectral details for Drug Boswellia serrata(Standard) and Boswellia serrata loaded microemulsion (Reference)

Group	Standard	Reference
0-H stretching	3340	3440.39
C-H Stretching	2926	2926.49
C-H stretch for alkane	2868	2868.59
Alpha beta unsaturated bond	1701	1701.81
C-C multiple bonds for aromatics	1538	1538.92
C-H bending	1455	1455.99

Calculation of entrapment efficiency

The drug entrapment efficiency of the microemulsion system was found to be 91 %.

Stability studies

The microemulsion was found to be stable at all conditions of temperatures at 25°C, 30°C and 40 °C and other conditions like high temperature, light, centrifugation, freeze-thaw cycling and on dilution. (20)

Table 3: Stability study results of microemulsion at 25°C , 30°C and 40 °C

Sr no.	Parameter	Month 1	Month 3	Month 6
1.	Appearance	Clear	Clear	Clear
2.	Globule size	403.1nm	401.11nm	400.93nm

Sr no.	Parameter	Month 1	Month 3	Month 6
1.	Appearance	Clear	Clear	Clear
2.	Globule size	403.1nm	445.22nm	478.21nm

Sr no.	Parameter	Month 1	Month 3	Month 6
1.	Appearance	Clear	Clear	Clear
2.	Globule size	403.1 nm	456.11	500.11

Characterization studies for microemulsion containing gel

pH

The pH of 1% solution of microemulsion gel was found to be 5.15. The desirable pH of topical formulations is required to be 5-6 in order to be non-irritant to skin.

Viscosity

The viscosity of the microemulsion gel was found to be 312.5 cps.

Irritation Studies

Boswellia Serrata drug-loaded microemulsion, microemulsion loaded gel and saline did not show any reaction on CAM and hence the formulations were found to be non-irritant. The positive control was markedly bloody due to hemorrhage and lysis of blood vessels in contact with 0.1N NaOH. HETCAM test demonstrated the non-irritant effect of microemulsion and microemulsion based gel.

Figure 5: i) HETCAM test for Saline ii)HETCAM test for Boswellia serrata containing microemulsion iii)HETCAM test for Boswellia serrata loaded microemulsion based gel

CONCLUSION

A stable microemulsion based gel and microemulsion system were formulated containing Boswellia serrata as the active agent and Primrose oil as the active oil. The microemulsion gel and microemulsion system were found to be non-irritant as confirmed by HETCAM test and were found to show desirable parameters. Microemulsion gel system proved to be an effective drug delivery system for a poorly-soluble drug like Boswellia serrata.

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