ENCAPSULATION OF THYME OIL INTO MICROSPONGES: PREPARATION, CHARACTERIZATION AND *IN VITRO* EVALUATION

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ABSTRACT

Thyme essential oil (TEO) is a prominent essential oil reported to have diverse properties and biological activities including anti-inflammatory. This research aims to encapsulate TEO in prepared microsponges for the purpose of controlled drug delivery and reduce the frequency of drug administration by enhancing its bioavailability. For the preparation of TEO loaded microsponges, the quasi-emulsion diffusion technique was used by employing dichloromethane as solvent, polyvinyl alcohol as a stabilizer and ethyl cellulose as polymer. The formulation variables such as altering the concentration of PVA and EC were used for optimization and characterized by FTIR, SEM and evaluated for particle size, *in vitro* drug release and entrapment efficiency and further subjected for *in vitro* anti-inflammatory activity. results showed that all the dispersions were in the micro-size range with good entrapment efficiency and release profile. The percent inhibition of protein denaturation by TEO loaded microsponges was found within the significant range at various concentrations.

Keywords: Ethyl cellulose, encapsulation, microsponges, quasi-emulsion solvent diffusion, thyme essential oil

INTRODUCTION

Essential oils, commonly called volatile oils, are biodegradable, renewable natural materials that are also non-toxic to human health. *Thymus vulgaris, Mentha piperita, Salvia officinalis, Lavandula angustifolia, Origanum compactum* and *Artemisia absinthium* are some of the plants containing volatile oils broadly known for their fungicidal, antibacterial and anti-inflammatory effects[1]. Thymus, belonging to the Lamiaceae family, is considered as a genus of aromatic, semi-evergreen or evergreen, perennial and herbaceous plants with around 400 species, variations, and sub-varieties. Thyme EO has a wide range of pharmacological effects, including antioxidant, anti-inflammatory, immunomodulating, antibacterial and antifungal. Thyme's infection-fighting and cough-suppressing properties have also been used to treat respiratory problems[2].

In recent years, the increase of innovative microsponge based drug delivery system has received a lot of attention, intending to modify and control drug release behavior. It is possible to change the therapeutic index and duration of activity of medications by inserting them into a carrier system. Microsponge medication delivery is now a simple way to deliver controlled drugs. It is mostly utilized for topical application and enables the longer release of medicinally active components[3].

Microsponges are usually porous tiny microspheres which are 5-300 µm in diameter[4], typically designed to deliver the medicine effectively at a lower dose, thereby improving stability, changing the drug release profile, and reducing side effects[5]. They are strongly cross-linked, non-collapsible, and can entrap, and subsequently release a diversity of active chemicals over time. Due to their spongy texture, they exhibit unique dissolving and compression capabilities. Due to its effectiveness, non-irritating, non-allergic, non-mutagenic and non-toxic characters, they provide better stability with patient compliance. Microsponges are made of a diversity of polymers, including Eudragit, ethylcellulose (EC), methylcellulose, polystyrene and various others. These active microsponges are also available in a range of packaging options and can be utilized in a range of formulations, including creams, lotions, capsules, gels and powders[6].

In the present research work, TEO was successfully incorporated into prepared microsponges using the

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quasi- emulsion solvent diffusion approach. Various batches of microsponges were prepared by varying the concentrations of EC and polyvinyl alcohol (PVA) while keeping the concentration of dichloromethane (DCM) constant and then the formulation was optimized. The objective of the developed polymeric microsponges was to deliver the essential oil in a controlled way for a prolonged period to lessen the frequency of administration and to expand its bioavailability. The effects of EC (polymer) and PVA alcohol (emulsifier) on particle size, entrapment efficiency and cumulative drug release of the prepared microsponges were studied. In order to determine the morphology and shape, field emission scanning electron microscopy (FE-SEM) study was performed. Moreover, FTIR spectroscopy was performed to observe any interaction between the drug and other ingredients. Finally, the optimized TEO-loaded microsponge formulation was evaluated for its *in vitro* anti-inflammatory activity.

MATERIALS AND METHODS

Thyme essential oil was procured from Sigma Aldrich, Saint Louis, USA. Various other chemicals and solvents used such as ethylcellulose, ethanol, dimethyl sulfoxide were obtained from Central Drug House, New Delhi. Dichloromethane, sodium chloride and disodium hydrogen phosphate were purchased from LobaChemie, Mumbai. Polyvinyl alcohol was obtained from Qualigens Pharma, Mumbai. Potassium dihydrogen phosphate and PEG- 400 were obtained from Merck, Mumbai. Several other solvents and reagents used were of analytical grade. Distilled deionized water was used in the entire research work.

Authentication of thyme essential oil

The essential oil was authenticated using gas chromatography-mass spectrometry (GC-MS). The bioactive components found in essential oils can be identified using GC-MS. The essential oil was examined using a Shimadzu QP Plus (Shimadzu Corporation, Japan) system with a thermal desorption system including a split injector. The following circumstances were utilized to test GC-MS: capillary column with fused silica was taken and at a steady flow rate of 1.2 mL min^{-1}, helium gas of about 99% purity was used as the carrier gas. The injector temperature was 260 ºC, while the ion-source temperatures were 230°C and 270 ºC (1:100). The temperature of the oven was set at 50 °C (isothermal for about 2 min), then made to increase at a rate of 3°C min^{-1} to 280 °C, then lowered at 10°C min^{-1} to 260°C, and finally raised to 300°C (for about 10 minutes). For fragments ranging from 40 to 500 Da, the mass spectra were obtained at 70 eV by scanning interval of 0.5 seconds. The active component 's identities were determined by comparing their mass spectra.

Preparation and optimization of TEO loaded microsponges

The preparation of thyme essential oil microsponges was done using a quasi-emulsion solvent diffusion approach[7]. TEO was introduced to an organic internal phase of the polymer (EC) dissolved in the solvent (DCM). Here, DCM functions as a good solvent for dissolving both the polymer and the essential oil. Then, drop by drop addition of internal phase was carried out into an aqueous solution containing PVA, placed in a vessel, and agitated for 3 h at 4000 rpm in an agitator (propeller type). Microsponges were generated after the DCM was removed from the reaction media. The generated microsponges were filtered, rinsed in distilled water and finally dried at normal room temperature. The prepared microsponges were found to be influenced by several parameters. Thus, thyme essential oil-laden microsponges were optimized via altering concentrations of polymer (900 mg, 700 mg) and PVA (1.5%, 1.0%, and0.5%) keeping the DCM (20 mL) steady. Further, particle size, percentage cumulative drug liberation, and entrapment efficiency were calculated to choose the best thyme essential oil-laden microsponges.

Selection of λ_{max} by UV-visible spectroscopy

UV spectrophotometer (Systronic Spectrophotometer UV- 1800, Ahmedabad, India) was used to determine the λ_{max} of the drug. To obtain the maximum wavelength of the thyme essential oil, the prepared stock solution A (0.05 mL of the oil in 500 mL of phosphate buffer, pH 6.4) and B (2mL of ethanol was added into 100mL of distilled water) were scanned within the range of 200 nm-800 nm. Moreover, standard working solution was prepared by pipetting out 1 mL – 5 mL of stock solution A into volumetric flask and then adding stock solution B in each flask to make up the volume up to 10 mL to produce concentrations of 10 µg mL^{-1} – 50 µg mL^{-1} and scanned at above range to obtain the ultraviolet spectrum (Fig. 3a).

Characterization of TEO loaded microsponges

Particle size determination

Optical microscopy was employed for measuring the size of particles of the prepared microsponges. The samples were placed on the mechanical stage after being mounted on certain slides. The mean of more than 100 particles was obtained, and particle size was estimated using the calibrated ocular micrometer. All the measurements were performed in triplicate[8].

Field emission scanning electron microscopy (FE-SEM)

FE-SEM is a vital research method in which the surface of a material is scanned with a focused electron beam to produce a high-resolution and enlarged image. The electrons in the beam interrelate with the sample, creating numerous signals that can be used to acquire information on surface structure and composition. Prepared microsponges were investigated using a scanning electron microscope (Nova Nano SEM 450) at 5.00 kV acceleration for morphology and surface topography. Samples were attached over a metal stud with adhesive tape and coated with gold under vacuum before being detected with an ET detector. The samples were then randomly scanned and photomicrographs taken at different magnifications with FE-SEM.

Zeta potential measurement

The Zeta potential of the TEO-laden microsponges was determined using Malvern Zetasizer(Version. 7.11), in which the particular samples were positioned in clear disposable zeta cells. At 25 °C, the results were recorded. During each experiment, before changing samples, washing of the cells was done using methanol and then rinsed by the sample which is to be measured.

Fourier transform infrared spectroscopy (FTIR)

FTIR spectra of thyme essential oil, blank microsponges as well as drug-laden microsponges along with characteristic peaks were obtained by insertion of the samples on the KBr plate. Within the range of 400cm^{-1} to 4000cm^{-1}, all the samples were scanned and evaluation was done employing FTIR Spectrometer (Perkin Elmer Spectrum software Ver. 10.4.00, U.K.).

Entrapment efficiency (EE)

10 mg of the TEO-laden microsponges was weighed accurately 10 mg and triturated using mortar pestle. The entrapped essential oil was extracted by dispersing the particles in an ethanol solution (10 mL) after trituration. The samples were then sonicated for 10 minutes before being filtered via Whatman filter paper. In a UV-visible spectrophotometer, the absorbance was measured at 272 nm. The following equation was used to calculate entrapment efficiency[9].

$$\%\ \text{Entrapment Efficiency} = \left(\text{CR} \times \frac{\text{VR}}{\text{MMP}} \div \frac{\text{MD} + \text{MP}}{\text{MD}}\right) * 100$$

where CR is the concentration of the drug, VR is the volume, MMP is the microparticles mass, MD is the mass of EO and MP is the mass of the polymer

In vitro drug release

Using USP dissolution test apparatus (USPII), dissolution of different batches of TEO-loaded microsponges was carried out to determine the rate of TEO liberation from microsponges. Briefly, 2 mL of microsponges emulsion (equivalent weight 1.83g), 1.5 mL phosphate buffer solution (pH 6.4) and 0.5 mL ethanol were added in the dialysis membrane-110 (diameter 21.5 mm, flat width 31.13mm, Hi-Media). Paddles were connected with the dialysis sacks and placed into dissolution vessels containing a mixture of phosphate buffer (pH 6.4), and PEG 400 (80:10). Finally, vessels were agitated that contained the release media at a speed of 50 rpm under constant temperature (37 ± 1 °C). As a result of diffusion from the membrane, the drug release in the outer solution was assessed by sampling the solution at determined intervals of time. 3mL of the sample was withdrawn for the analysis at 0, 30, 60, 90, 120 and 150 minutes, and also the same amount of the fresh dissolution medium was replaced. Drug content released from the membrane was measured at 272 nm using UV- visible spectrophotometer[10].

In vitro anti-inflammatory activity

Inhibition of protein denaturation

Anti-inflammatory effect was evaluated using inhibition of protein denaturation assay described by Gambhire et al. with slight modification[11]. The standard drug aspirin was taken and 100 µg mL^{-1} concentrations were prepared. Then the suitable TEO loaded microsponges were taken and various concentrations were prepared such as 100 µg mL^{-1}, 200 µg mL^{-1}, 300 µg mL^{-1}, 400 µg mL^{-1}, and 500 µg mL^{-1}. Freshly prepared phosphate buffer (pH 6.4) was added along with the egg albumin in each of the various prepared concentrations. The prepared mixture was incubated for 15 minutes at 27±1 °C. After that, the reaction mixture was placed in the water bath for enhancing denaturation at 70 °C. Further, absorption was taken at 272 nm using UV- visible spectrophotometer using pure distilled water as the control, and % inhibition was measured using the following formula:

$$\%\ \text{Inhibition} = \left(A_C - \frac{A_S}{A_C}\right) * 100$$

where A_C = Absorbance of control and A_S = Absorbance of the test sample

RESULTS AND DISCUSSION

Authentication of thyme essential oil

Gas chromatography-mass spectrometry (GC-MS)

Authentication of TEO was performed using gas chromatography- mass spectrometry (GC-MS), which is

well recognized technique used for both qualitative and quantitative estimation. In the present study, GCMS was used to discover the volatile components present in the TEO. Many components were found to be present in TEO including delta 3- carene, camphene, 2- beta pinene, beta- myrcene, benzene, D-limonene, gamma- terpinene and phenol (thymol) (Fig. 1). GC-MS can act as a useful tool for analysis of the composition of essential oils and helps in proposing the mechanism of action of volatile oil.

Fabrication and optimization of TEO loaded microsponges

Quasi- emulsion solvent diffusion technique was employed for fabrication of thyme essential oil loaded microsponges. EC was used for the fabrication to provide structural integrity and works as a binder to the microsponges. PVA was used as emulsifier, while the solvent was DCM. Through preliminary trial, various batches were made to determine the concentration of EC and DCM to fabricate non aggregating microsponges. It was found that 20 mL DCM required for the preparation of non-agglomerated microsponges which was maintained throughout the experiment. Variation in the quantity of polymer and emulsifier showed remarkable effects on formulated microsponges. Moreover, the effects of polymer EC and emulsifier. PVA were analyzed on the entrapment efficiency, size of the particles and *in vitro* release of microsponges (Table I).

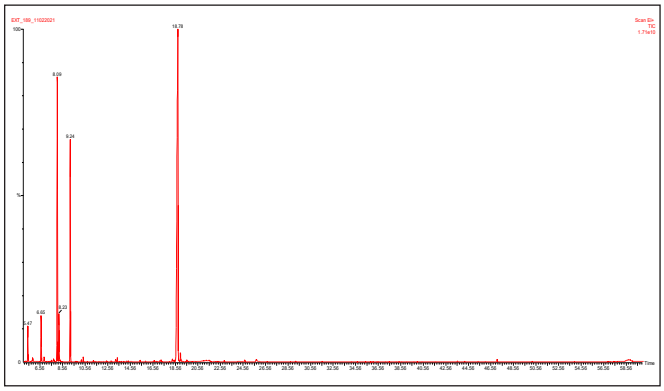

Fig. 1: GC-MS spectra of TEO

The microsponges formulations (F1 to F6) were optimized using design of experiments. The effect of two formulation variables (independent variables) including the amount of EC and PVA was investigated using 2^2 experimental design using Microsoft Excel (version 2007). The dependent variables considered are zeta potential and particle size. The optimization was performed by keeping the stirring speed constant at 4000 rpm. The effect of independent variables on dependent variables was determined using surface response curve. The effects of polymer with their interaction on particle size and zeta potential are shown in Fig. 2.

It has been observed from the surface response plots that formulation F6 shows the lowest particle size as 21.75±1.83 µm and maximum zeta potential value as -11.5±0.42 on decreasing the concentration of EC to 700 mg and PVA to 0.5%. Other formulations showed increased particle size on changing the concentration of EC and PVA.

Selection of λ_{max} by UV-visible spectroscopy

Maximum wavelength was estimated by scanning all the prepared stock solutions within the range of 200-800 nm and the maximum wavelength was found to be at 272 nm. The regression equation as per the calibration curve of the standard TEO was found to be y= 0.042x+0.009. It was found that the solutions show linearity (R^2= 0.999) in absorbance at a concentration of 10-50 µg mL^{-1} and obey Beer- Lambert's Law (Fig. 3b).

Table I: Evaluation of various parameters of TEO loaded microsponges

Batch	Ethyl cellulose (mg)	DCM (mL)	PVA (%)	Particle size (µm)	Entrapment efficiency (%)	Cumulative drug release (CDR%)
F1	900	20	1.5	40.20±2.36	84.07±1.38	63.27±1.06
F2	700	20	1.5	28.32±1.84	67.53±1.16	74.42±1.54
F3	900	20	1.0	32.95±2.51	78.62±1.14	67.81±1.75
F4	700	20	1.0	26.18±3.29	65.61±0.72	77.69±0.83
F5	900	20	0.5	36.47±1.66	69.51±1.32	70.58±1.29
F6	700	20	0.5	21.75±1.83	62.36±0.94	83.63±1.38

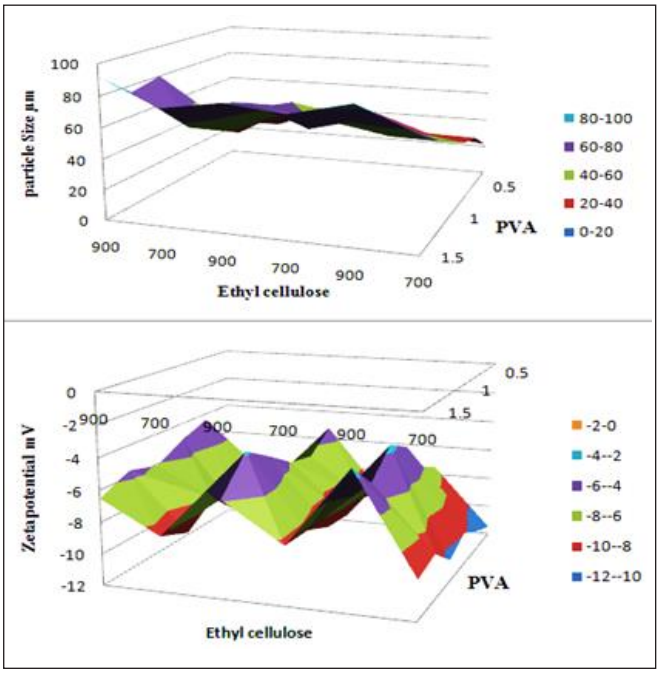

Fig. 2: Optimization of TEO microsponges by varying EC and PVA

PHYSICOCHEMICAL CHARACTERIZATION OF TEO LOADED MICROSPONGES

Particle size determination and FE-SEM analysis

The mean particle size of the prepared microsponges ranged from 21.75±1.83 µm to 40.22±2.36 µm. Visual assessment of all formulations was done using optical microscope, which indicates that the particle size increased with increase in the concentration of polymer. When the quantity of polymer was raised, the size of the particles rose as well. The increase in the size of particles is associated with the creation of a viscous organic phase as a result of a greater polymer content, which results in bigger emulsion droplets. Moreover, by increasing the PVA concentration, the emulsion droplets become more difficult to be divided into smaller droplets, resulting in the production of large-sized microsponges. Hence, it was observed that formulation F6 exhibited minimum particle size i.e., 21.75±1.83 µm because of the presence of the lowest amount of polymer as well as the emulsifier. FE-SEM was employed to examine the texture and surface morphology of the prepared microsponges. It showed that the microsponges were sphere-shaped and porous along with spongy configuration. The captured FE-SEM image of fabricated thyme essential oil loaded microsponges are shown in Fig. 4.

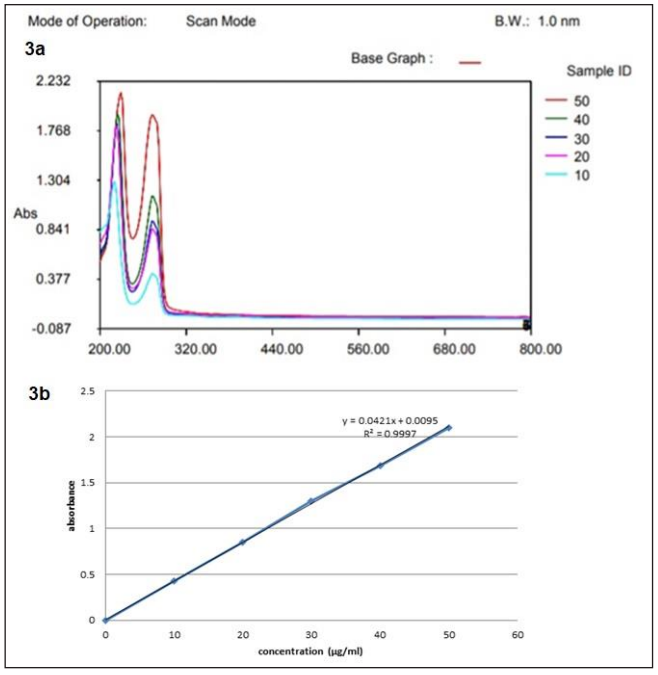

Fig. 3(a-b): Absorption spectra and calibration graph of TEO

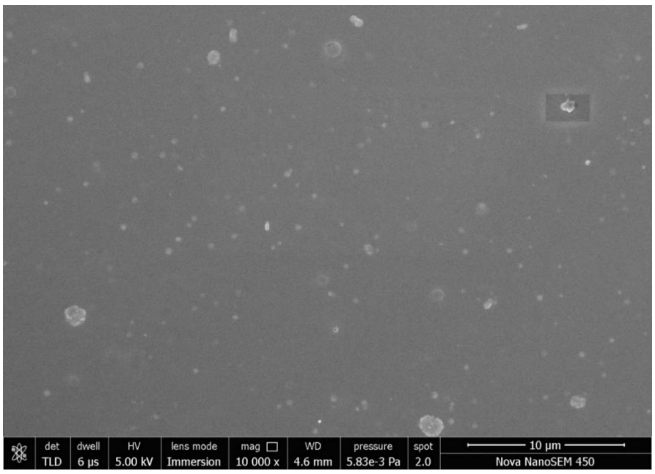

Fig. 4: FE-SEM image of TEO loaded microsponge

Zeta potential measurement

Zeta potential is usually employed for the prediction of particle surface charge and stability. It is the degree of stability of the system due to the charge carried by the microsponges and their tendency to form aggregates. The zeta potential up to +30 or higher is an indication that particles are repellent in nature, repelling each other to stay separate and values of-30 or below indicate not to flocculate and stay dispersed. The zeta potential of prepared TEO-loaded microsponges which was estimated to be -11.5mV indicates that the system is well dispersed and may remain stable for a longer period.

Fourier transform infrared spectroscopy

FTIR spectra of thyme essential oil, blank microsponges as well as drug laden microsponges

along with characteristic peaks were obtained. The FTIR spectrum of thyme essential oil is shown in Fig. 5a. It showed characteristics peak at 3527.98cm^{-1} which is O-H stretch (free OH stretch), 2960.20cm^{-1} and 2872.40cm^{-1} which is C-H stretch, 1619.36cm^{-1} which is C=C stretch (aromatic), 1581.99cm^{-1} and 1514.32cm^{-1} which is aromatic C-C ring stretch, 1454.15cm^{-1} and 1421.74cm^{-1} which is C-H bend (CH_3CH_2), 1288.72cm^{-1} and 1223.50cm^{-1} which is C-O stretch, 1153..45cm^{-1} and 1058.61cm^{-1} which is C-O stretch, 945.32cm^{-1}, 856.64cm^{-1} and 810.19cm^{-1} which is o- substitution, 736.83cm^{-1}, 586.79cm^{-1}, 540.79cm^{-1} and 426.39cm^{-1} which is p- substitution.

The FTIR spectrum of blank microsponges as shown in Fig. 5b, showed characteristics peak at 3338.64cm^{-1} which is O-H stretch (free OH stretch), 2128.26cm^{-1} which is aromatic, 1637.49cm^{-1} which is C=C stretch (aromatic), 1374.66cm^{-1} which is C-H bending, 1070.59 cm^{-1} which is C-O stretch, 599.63cm^{-1} which is p- substitution. The FTIR spectrum of TEO-loaded microsponge as shown in Fig. 5c showed characteristics peak at 3337.60cm^{-1} which is O-H stretch (free OH stretch), 2125.59cm^{-1} which is aromatic, 1637.10cm^{-1} which is C=C stretch (aromatic), 1089.90cm^{-1} which is C-O stretch, 589.14cm^{-1} which is p- substitution. It was found that there were no significant differences among characteristics peak of the drug (thyme essential oil) when compared with the characteristics peak of the drug-loaded formulation. All the peaks were found compatible with other excipients.

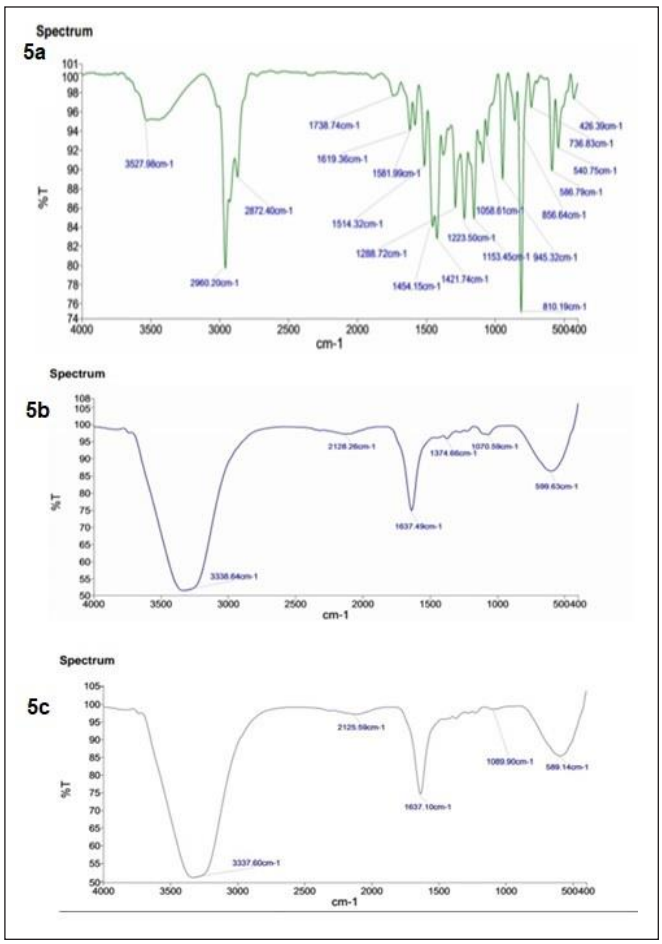

Fig. 5(a-c): FTIR spectra of TEO, blank microsponge and TEO loaded microsponge

Entrapment efficiency

The entrapment efficiency (%EE) of the various prepared batches of TEO laden microsponges ranged from 62.36±0.94% to 84.07±1.38%. Formulation F1 showed maximum entrapment efficiency of 84.07±1.38%, which was then followed by F3 (78.62±1.14%). On increasing the polymer concentration, the availability of polymer for every microsponge to incorporate oil within itself was more. The enhanced amount of polymer increases the intra-molecular forces and also the thickness of the organic phase. Hence, during emulsification, bigger globules are aroused, resulting in larger microsponges and hence can entrap more quantity of EO. With variation in the quantity of polymer, the *in vitro* adsorption also varies and a significant increase was observed because of higher polymer matrix wall thickness.

In vitro drug release profile

In vitro, CDR of fabricated TEO microsponges in phosphate buffer (pH 6.4), and PEG 400 are shown in Table I. The highest drug release (83.63±1.54%) took place from F6, which was then followed by F4 (77.69±0.83%) after the completion of 8 h. As for the given amount of PVA, the microsponges prepared with a minimum amount of EC represented the highest percentage of cumulative drug release. When the amount of EC is increased, the quantity of drug adjacent to the microporous surface of the particle decreases, resulting in an increased amount of drug incorporated in the polymer matrix. As a result of this impact, the drug release rate from the produced microsponges slows down. Further, the rate of drug release slows down with the increase in polymer and also emulsifier. Hence, it was seen that F6 formulation showed maximum cumulative drug release because of the presence of a minimum amount of EC concentration and also of the emulsifier.

In vitro anti-inflammatory activity

The prepared TEO-loaded microsponges were able to inhibit protein denaturation in a concentration-dependent manner. The inhibitory effect of formulation F6 at various concentrations (100 µg mL^{-1} to 500 µg mL^{-1}) on protein denaturation are mentioned in Table II.

Table II: Effect of TEO loaded microsponges on *in vitro* anti-inflammatory activity by inhibition of protein denaturation

Compound	Concentration (µg mL^{-1})	Protein denaturation (% inhibition)
Aspirin (Standard drug)	100	95.25%
Formulation (F6)	100	50.67%
	200	63.09%
	300	69.19%
	400	77.71%
	500	81.73%

On increasing the concentration of the formulation F6, the % inhibition was also increased. The highest percent inhibition was recorded at 500 µg mL^{-1} (i.e.81.73%) whereas standard drug aspirin showed 95.25 % inhibition of protein denaturation at a dose of 100 µg mL^{-1}.TEO is useful in chronic infection as an immunostimulatory agent and thymol and carvacrol are the major anti-oxidants that are present in the oil[12]. The presence of these anti-oxidants might be responsible for the anti-inflammatory effect of essential oil.

CONCLUSION

In the present study, TEO was successfully encapsulated in microsponges using quasi- emulsion solvent diffusion approach. This incorporation of TEO in microsponges resulted in controlled release with good stability and bioavailability including anti-inflammatory effects. TEO was firstly authenticated via GC-MS and then incorporated into EC microsponges. The fabricated microsponges formulations (F1-F6) were optimized and formulation F6 selected on the basis of minimum particle size. Further FTIR, Zeta potential and SEM studies of the fabricated microsponges were performed. SEM study revealed that the microsponges were sphere-shaped having spongy configuration and no interaction was seen in drug and polymer by FTIR and uniform distribution be zeta potential. Highest cumulative drug release make this formulation suitable for preparation of various topical dosage forms. Results of *in vitro* anti-inflammatory activity by inhibition of protein denaturation assay indicate encapsulation process of tested oils in microsponges was successful and can be considered as inexpensive and efficient technique to maintain the anti-inflammatory activity of essential oils. The results obtained from this study suggest that of all formulation techniques, microencapsulation seems to be the best choice for increasing the use of essential oils through offering prolonged release of drug and, hence, would be more useful than conventional formulation therapy in oral as well as in topical drug delivery.

REFERENCES

1. Walentowska J. and Foksowicz-Flaczyk J.: Thyme essential oil for antimicrobial protection of natural textiles, **Int. Biodeterior. Biodegrad.,** 2013, 84, 407-411.
2. Horhat F.G., Gruja A.T., Misca C., Jianu C. and Boruga O.: Thymus vulgaris essential oil: chemical composition and antimicrobial activity, **J. Med. Life,** 2014, 7(3), 56-60.
3. Jyoti J. and Sandeep K.: Innovative and novel strategy: Microsponges for topical drug delivery, **J. Drug Delivery Ther.,** 2018, 8(5), 28-34.
4. Sharma S., Sharma A. and Kaur C.: Microsponges: As a topical drug delivery system, **Int. J. Pharm. Sci. Res.,** 2020, 11(2), 524-534.
5. Singhvi G., Manchanda P., Hans N., Dubey S.K. and Gupta G.: Microsponge: an emerging drug delivery strategy, **Drug Develop. Res.,** 2019, 80(2), 200-208.
6. Bhatia M. and Saini M.: Formulation and evaluation of curcumin microsponges for oral and topical drug delivery, **Prog. Biomater.,** 2018, 7(3), 239-248.
7. Pawar N. and Redhu S.: Development and characterization of microsponge gel for topical delivery of oregano oil, **Int. J. Pharm. Sci. Res.,** 2021, 12(2), 1060-1073.
8. Gulati N., Tomar N. and Nagaich U.: Miconazole Microsponges based topical delivery system for diaper dermatitis, **Ars Pharm.,** 2016, 57(2), 77-87.
9. Arya P. and Pathak K.: Assessing the viability of microsponges as gastro retentive drug delivery system of curcumin: Optimization and Pharmacokinetics, **Int. J. Pharm.** 2014, 460(1-2), 1-14.
10. Wadhwa G., Kumar S., Mittal V. and Rao R.: Encapsulation of Babchi essential oil into microsponges: Physicochemical properties, cytotoxic evaluation and anti- microbial activity, **J. Food Drug Anal.,** 2019, 27(1), 60-70.
11. Gambhire M.,Wankhede S. and Juvekar A.: Anti-inflammatory activity of aqueous extract of Barleria cristata leaves, **J. Young Pharm.,** 2009, 1(3), 220-224.
12. Jadhav N., Patel V., Mungekar S., Bhamare G., Karpe M. and Kadam S. V.: Microsponge delivery system: An updated review, current status and future prospects, **J. Sci. Innov. Res.,** 2013, 2(3), 1097-1110.