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Development of erythromycin stearate invasomes for dermal infection

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Abstract
Erythromycin stearate, a macrolide antibiotic, exerts its effect by inhibiting bacterial protein synthesis. Topically Erythromycin stearate is used to treat bacterial skin infections. Erythromycin stearate is a BCS class III Drug (High solubility and low permeability). Hence Invasomes are suitable to increase the penetration of Erythromycin stearate to treat deep skin infections. Invasomes are novel vesicular systems that exhibit improved transdermal penetration compared to conventional liposomes. Invasomes contain phospholipids, ethanol and terpenes. Erythromycin stearate Invasomes are prepared with soy lecithin and various concentrations of terpenes, citral and eugenol. The Fourier-transform infrared spectroscopy study was carried out to determine the compatibility of Erythromycin stearate with the excipients. Zone of inhibition is carried out for Erythromycin stearate against *Staphylococcus aureus*. The Erythromycin stearate Invasomes are prepared by mechanical dispersion method. Formulated Invasomes are evaluated for particle size, zeta potential, entrapment efficiency, drug content, *In vitro* drug release and anti-microbial activity.

Keywords: Invasomes, Fourier-transform infrared spectroscopy, *Staphylococcus aureus*, anti-microbial activity

Introduction
The transdermal route is essential for localized or systemic effects. The stratum corneum, or top layer of skin, acts as a vital barrier to prevent skin penetration for many medications. Many approaches have been developed to overcome this challenge, such as the use of methods like iontophoresis, electroporation, and ultrasound that change the continuity of the stratum corneum (SC) and the use of vehicles and nanocarriers to improve drug penetration. Many kinds of nanocarriers have recently been created to improve the cutaneous and transdermal distribution of drugs. Because the vesicular system's physiochemical properties, such as their deformability, size, and charge can be adjusted by changing the lipid content and production methods, they appear to be suitable carriers [1]. Topical drug delivery systems enable targeted medication delivery through ophthalmic, vaginal, cutaneous, and rectal channels to any part of the body.

Topical formulations include a broad range of formulations designed for application to both healthy and damaged skin, either cosmetically or dermatologically. The physiochemical nature of these formulations varies from solid to semisolid to liquid. Substances used in drug formulations are rarely given on their own; instead, they are combined with one or more non-medicated agents to fulfill a variety of specific and specialized pharmaceutical purposes. If the drug substance is in solution, has a favorable lipid/water partition coefficient, and an electrolyte, its absorption via the skin is improved. Pharmaceutical preparations applied topically are primarily designed to have a localized effect; as such, they are designed to offer extended local contact with a low degree of systemic drug absorption. Antiseptics, antifungal agents, skin emollients, anti- inflammatory, analgesics, and protectants are among the medications that are applied topically to have a localized effect. Since ancient times, localized skin diseases and pain relief have been treated with topical medication delivery devices. While the drug primarily functions at related regions, topical applications of the drug may have the advantage of delivering the drug directly to the site of action and allowing it to stay there for a longer amount of time.

The drug's mean residence time and contact time are extended by the topical delivery method, on the other hand [2].

Macrolide antibiotics are having a macrocyclic lactone ring with attached sugars. Erythromycin was obtained from *Streptomyces erythreus*. Roxithromycin, Clarithromycin, and azithromycin are semi- synthetic macrolides. Erythromycin has proven to be effective, in combating strains such as *Streptococcus pyogenes, Streptococcus pneumoniae, Neisseria gonorrhea, Clostridium perfringens, Corynebacterium diphtheriae, Listeria, Mycoplasma, Legionella, Chlamydia trechomatis* and *Bordetella pertussis.* However, it does not show efficacy against *Bacteroides fragilis.* Additionally, it is worth noting that erythromycin exhibits solubility in water and requires refrigeration, for stability. Mechanism of action for erythromycin, when erythromycin concentrations are high, it becomes bactericidal (For specific bacteria exclusively) and loses its bacteriostatic properties. When bound to the bacterial 50s ribosomal subunit, erythromycin and other macrolides prevent the production of new proteins. An alkaline pH increases their activity. The organism in question and how quickly it multiplies determines the bactericidal activity. The reason for the great susceptibility of sensitive gram- positive bacteria to erythromycin is that they store the antibiotic intracellularly via active transport. Due to the drug's preference for its non-ionized (Penetrable) form at higher pH levels, activity is multiplied several times in alkaline situations.

The antibiotic erythromycin inhibits the synthesis of proteins in bacteria by combining with subunits of 50s ribosomes and interfering with their translocation. This means that when a peptide bond forms between a newly attached amino acid and the nacent peptide chain at the acceptor (A) site, the elongated peptide is translocated back to the peptidyl (P) site, freeing up the A site for the next aminoacyl tRNA attachment. Erythromycin prevents this process preventing the ribosome from moving along the mRNA to reveal the next codon. Inadvertently, peptide chains may end prematurely: bigger protein synthesis is particularly inhibited.

Invasomes were initially used in 2002 by a research team under the direction of Professor Alfred Fahr [3]. Invasomes are liposomal vesicles that contain terpenes or terpene combinations and small amounts of ethanol. They perform better skin penetration as possible carriers. The skin penetration rate of invasomes is higher than that of ethosomes and liposomes. Among the many advantages that invasomes provide are increased patient comforts, compliance as well as increased therapeutic efficacy [4, 5]. A synergistic effect between terpenes and ethanol on the percutaneous absorption has been significantly observed. To create a topical formulation with improved Erythromycin skin delivery and provide action against dermal infection [6]. Ethanol is a good penetration enhancer while terpenes have also shown potential to increase the penetration of many drugs by disrupting the tight lipid packing of the stratum corneum [7].

Penetration mechanism of Invasomes: As penetration enhancers that increase the permeability of the invasomes, terpenes and ethanol in the invasomes induce the vesicles to become deformable and rupture the stratum corneum bilayer skeleton. According to Dragicevic - Curic *et al*., terpenes, phospholipid segments, and single phospholipid molecules are released from the vesicle during invasome penetration. These components facilitate penetration and cause the stratum corneum to become more fluid. Penetrating through the stratum corneum intact are smaller invasome vesicles that do not break down [8].

Effect of Composition on the Physicochemical Characteristics of Invasomes

Effect of Ethanol

Adding ethanol to lipid nanovesicles is a good way to make the domains structure, a drop in the lipids transition temperature, and as a result, fluidization and disruption of the densely packed Stratum corneum lipids [9]. Stratum corneum lipids can become fluidized and disturbed by ethanol-based nanocarriers [10]. Because of the lipid acyl chains' freedom to rotate, the presence of ethanol increases the intercellular lipid matrix's flexibility. So, ethanol makes the lipids in the vesicle structure more fluid, giving it a softer, less rigid shape than regular liposomes [11]. Apart from its increased capacity for penetration, ethanol also produces a net negative surface charge and inhibits vesicle aggregation as a result of electrostatic repulsion, which makes invasomes more stable when stored [12, 13].

Effect of Terpenes

Effect of Terpene on Penetration

Terpenes improve drug penetration by rupturing the tight bilayers and lipid packing in the Stratum corneum [14]. Furthermore, Stratum corneum lipids are extracted by breaking hydrogen bonds [15], increasing lipid fluidity to improve the partition into the Stratum corneum [16] and increasing diffusion via the intercellular lipid's terpenes enhances drug permeability through several pathways [17].

Effect of terpene on the size of the invasomes

Particle size analysis revealed a direct relationship between the size of the invasomes and the number of terpenes; as terpene levels rise, so does the size of the invasomes. Skin's lipid bilayer more fluid. Ethanol's interaction with the lipid elements in the polar group area of the Stratum corneum causes changes in the keratinized or lipophilic [18].

Materials and Methods

These are the materials used in these formulations (Drug: Mupirocin), (Source: Hema pharmaceutical PVT LTD), (Excipients: Soy lecithin), (Source: Kanon laboratories), (Terpene: Citral, Eugenol), (Source: Sri Mahalakshmi Scientific Company).

Methodology

Drug and terpene or mixtures of terpenes are dissolved in ethanolic phospholipid solution. The mixture was vortexed for 5 min and then sonicated for 5 min to obtain a clear solution. Phosphate buffer saline (PBS) (pH: 7.4) was added to the solution by a syringe under constant vortexing. The vortexing was continued for an additional 5 min to obtain final invasomal preparation.

Table 1: Composition of Erythromycin stearate invasomes

Ingredients	F_1	F_2	F_3	F_4	F_5	F_6
Erythromycin stearate	100 mg	100 mg	100 mg	100 mg	100 mg	100 mg
Soy lecithin	0.1 g	0.1 g	0.1 g	0.1 g	0.1 g	0.1 g
Citral	0.5%	1%	2%	-	-	-
Eugenol	-	-	-	0.5%	1%	2%
Ethanol	3.3 ml	3.3 ml	3.3 ml	3.3 ml	3.3 ml	3.3 ml
Phosphate buffer	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml

Evaluation of Invasomes

Drug entrapment

5ml of the sample was centrifuged at 5000rpm at 40 ˚C for 60 minutes. The supernatant liquid was separated without disturbing the sediment layer using a micropipette. The sample withdrawn was diluted using phosphate pH 7.4 and analyzed by UV Spectrophotometer at 285nm.

Drug entrapment = (Total drug content - Drug content in supernatant liquid) X 100 / Total drug

Drug Content

1 ml of Invasome preparation was taken in a 100ml volumetric flask. 2ml of methanol was added and volume was made up with phosphate buffer pH 7.4. Samples were filtered through Whatman filter pape r number 40 and diluted with phosphate buffer pH 7.4. Drug content was determined spectrophotometric ally at 285 nm.

In vitro Drug release

In vitro drug release can be determined using Franz's diffusion cell with receiver cell volume and effective permeation area of 10 ml and 0.196 cm^2 respectively. The donor cell containing the invasomal formulation was placed over the receptor cell in which phosphate buffer saline (pH 7.4) was filled. The experiment was conducted for 24 h at a temperature of 37±1 ℃ with constant magnetic stirring at 600 rpm. The samples were withdrawn at 1, 2, 3, 6, 24 & 30 hours and replaced. The same volume of buffer was replaced to maintain the sink condition. The sample was estimated using a UV-visible spectrophotometer at 285nm.

Antibacterial activity

The antibacterial activity of prepared invasomes was determined by measuring the zone of inhibition against Staphylococcus aureus. The microorganism was inoculated on a compatible sterilized nutrient agar medium. The test composites were transferred on square plastic grids or disks on the solidified agar medium and incubated for 24h at 37 °C. After the incubation period, the zone of inhibition was observed in four directions for each tested sample.

Zeta Potential &Vesicle Size: The Zeta potential & vesicle size of the prepared invasomes was analyzed using the Horiba Nanoparticle analyzer SZ-100 [19].

Results and Discussion

Determination of solubility

Soluble in water at 2mg/ml and ethanol at 50 mg/ml.

Determination of melting point

The melting point of Erythromycin stearate was determined by the capillary tube method, which was found to be 135 °C. This value matched with the literature reference standard drug value of Erythromycin stearate.

Table 2: Determination of melting point

Compound name	Melting point	
	Observed	Reported
Erythromycin stearate	135 °C	132 °C - 137 °C

Standard Calibration curve for Erythromycin stearate

Erythromycin stearate was dissolved in ethanol and suitable dilutions were made to prepare 2, 4, 6, 8 and 10 µg/ml. The absorbance of each solution was measured at 285nm against ethanol as a blank. A graph of the concentration of the drug versus absorbance was plotted. The absorbance obtained was found to obey Beer's Lambert's law with a regression coefficient R^2 value of 0.998 in ethanol.

Table 3: Calibration graph curve of Erythromycin stearate

S. No.	Concentration (µg/ml)	Absorbance at 285 nm
1.	0	0
2.	0.2	0.009
3.	0.4	0.017
4.	0.6	0.025
5.	0.8	0.032
6.	1.0	0.041

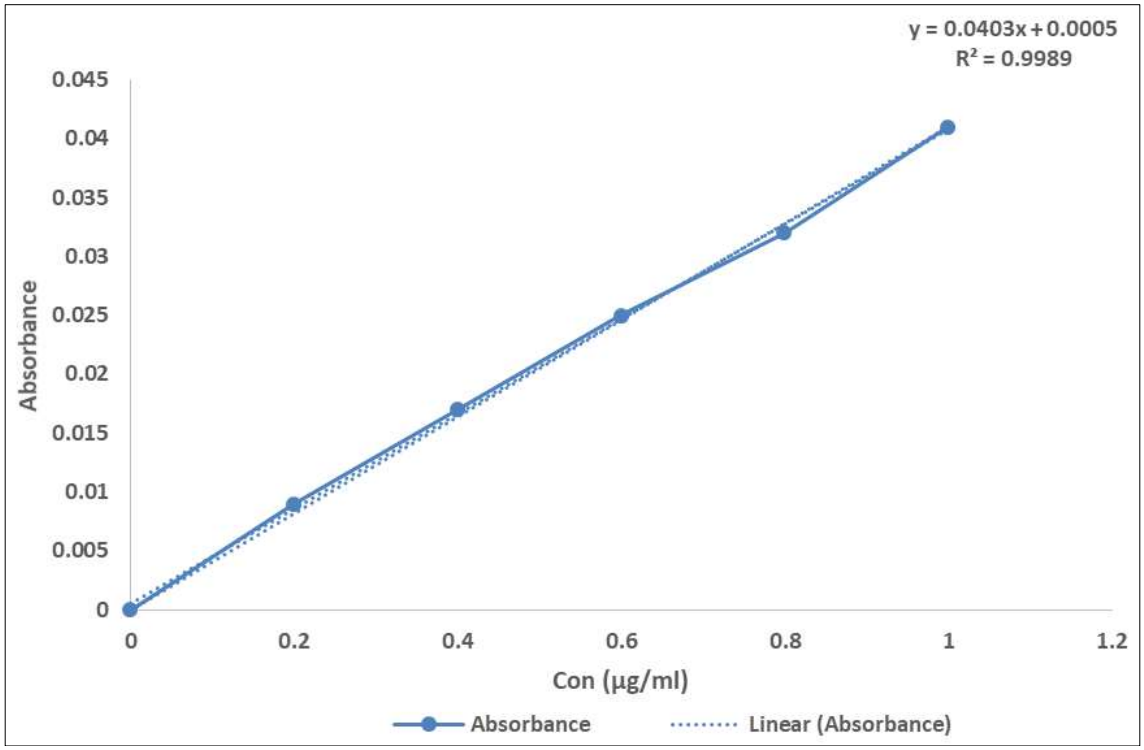

Fig 1: Calibration graph curve of erythromycin stearate

Compatibility studies by FTIR spectroscopy

The IR spectra of the drug and its other excipients were shown. The FT-IR study showed no major change in the position of the peak obtained in the drug and the mixture of the drug with excipients, thus indicating no interaction between the drug and excipients

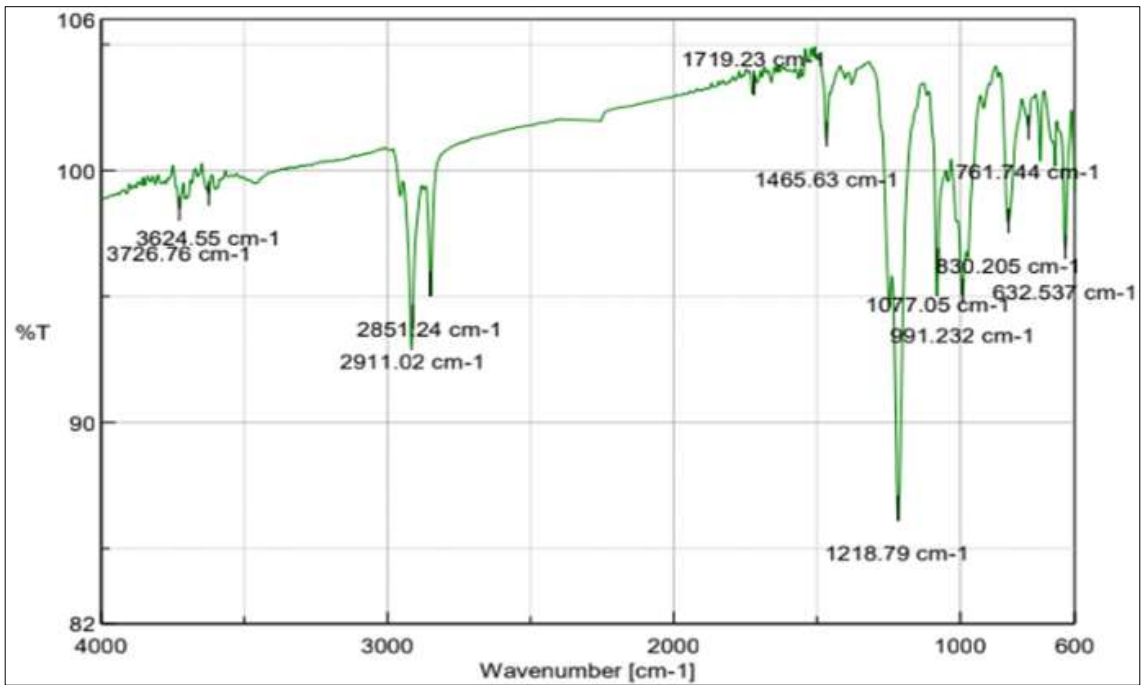

Fig 2: FTIR spectra of erythromycin stearate

Table 4: Interpretation FTIR spectra erythromycin stearate

S. No.	Peak Obtained (cm^{-1})	Range (cm^{-1})	Characteristic group
1.	830	790 - 830	C-H Bending
2.	1719	1705 - 1725	C=H Stretching
3.	2851	2850 - 2950	CH and CH_2Stretching
4.	3624	3500 - 3700	O-H Stretching

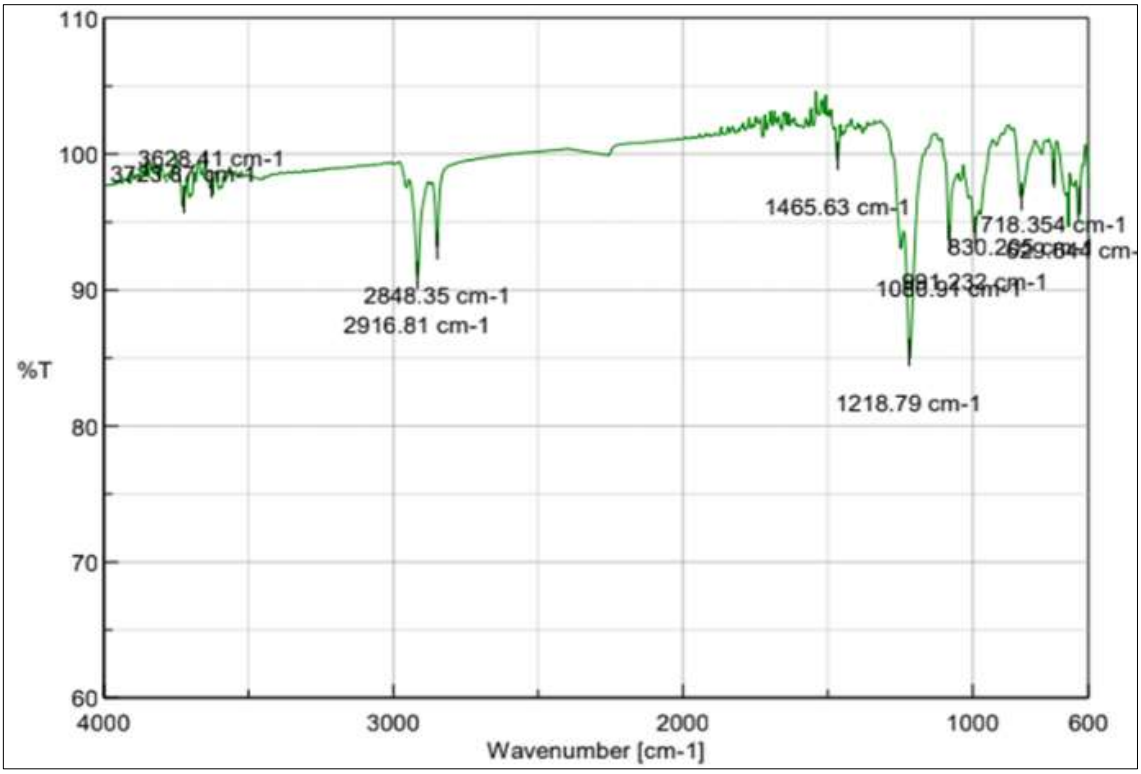

Fig 3: FTIR spectra of the physical mixture of erythromycin stearate and soy lecithin

Table 5: Interpretation of FTIR spectra of erythromycin stearate and soy lecithin

S. No	Peak Obtained	Range	Characteristic group
1	718cm	600 - 800	C=C Bending
2	1080cm	1000 - 1200	C-N Stretching
3	1218cm	1200 - 1300	C-O Stretching

Antibacterial activity

The antibacterial activity of Erythromycin stearate invasome was carried out by the Disc diffusion method against *Staphylococcus aureus*. Nutrient agar plates were prepared and solidified. After solidification, the bacterial culture (*Staphylococcus aureus)* was spread on the plate using a sterile cotton swab. Then sterile disc was soaked in the Erythromycin stearate invasome solution and placed on the medium using sterile forceps. After incubation, the clear zone formation indicates antibacterial activity.

Fig 4: Erythromycin stearate shows antibacterial activity against gram-positive bacteria (F_1 to F_3)

Fig 5: Erythromycin stearate shows antibacterial activity against gram-positive bacteria (F_4 to F_6)

The above-mentioned formulations (F_1, F_2, F_3, F_4, F_5, F_6) show antibacterial activity against *Staphylococcus aureus* by the Disc diffusion method.

Characterization Erythromycin stearate

Particle size: The particle size of all the formulations was analyzed by using the Horiba Nanoparticle analyzer. The formulations were placed in the sample holder and particle size was measured. The results are given in the table.

Table 6: Particle size of invasome formulations

S. No.	Formulation	Particle size
1.	F_1	150 nm
2.	F_2	154.3 nm
3.	F_3	1186.0 nm
4.	F_4	175.8 nm
5.	F_5	322 nm
6.	F_6	836 nm

The mean particle size of the formulation F_1 to F_6 is found to be in the size range of 150 nm - 1186 nm.
The polydispersibility index of the formulation F_1 to F_6 is found to be in the range of 0.333 - 0.736 that concluded the homogenous preparation of vesicles. A lower polydispersibility index indicates a more homogenous sample with particles that are more uniform in size.

Zeta potential: The zeta potential of the invasome was measured using a Horiba Nanoparticle analyzer SZ -100. The zeta analysis, software produces a frequency spectrum from which the electrophoretic mobility hence the zeta potential, is calculated. Zeta potential of the formulations was found to be -10.5mV, -1.0mV, -21.0mV, -4.9mV, -21.9mV, -1.3mV for the formulation F_1 to F 6 respectively. The negative potential of the vesicles can enhance the penetration rate of the drug as well as the stability of the formulation.

Table 7: Zeta potential of invasome formulations

S. No.	Formulation	Zeta potential
1.	F_1	-10.5 mV
2.	F_2	-1.0 mV
3.	F_3	-21.0 mV
4.	F_4	-4.9 mV
5.	F_5	-21.9 mV
6.	F_6	-11.3 mV

Drug entrapment

Table 8: Entrapment efficiency of prepared invasomes

Formulation code	Entrapment efficiency (%)
F_1	73
F_2	79
F_3	82
F_4	67
F_5	69
F_6	74

The entrapment efficiency percentage of all the formulations is calculated from the absorbance obtained from the supernatant after centrifugation. All the formulations showed a percentage entrapment in the range of (67 - 82%).
It was observed that the F_3 Formulation showed the maximum entrapment when compared to other formulations.

Drug content

The % drug content of Invasome preparation ranges from (98.41 -99.36%). F_3 formulation showed the maximum drug content of Erythromycin stearate at 99.36%.

Table 9: Percentage Drug Content

Formulation code	Drug content (%)
F_1	99.24
F_2	98.58
F_3	99.36
F_4	98.41
F_5	98.79
F_6	99.15

In vitro drug release

Table 10: *In vitro* drug release study of Erythromycin stearate invasome (F_1 to F_6)

Time	F_1	F_2	F_3	F_4	F_5	F_6
0	0	0	0	0	0	0
0.5hr	605	276	377	251	655	503
1hr	706	326	453	326	782	630
2hr	933	529	807	433	984	807
3hr	1531	857	1009	883	1692	1338
6hr	2198	1034	1540	1009	2223	2198
24hr	6421	3715	5005	3412	6573	5384
30hr	8292	5586	6168	5460	8444	7989

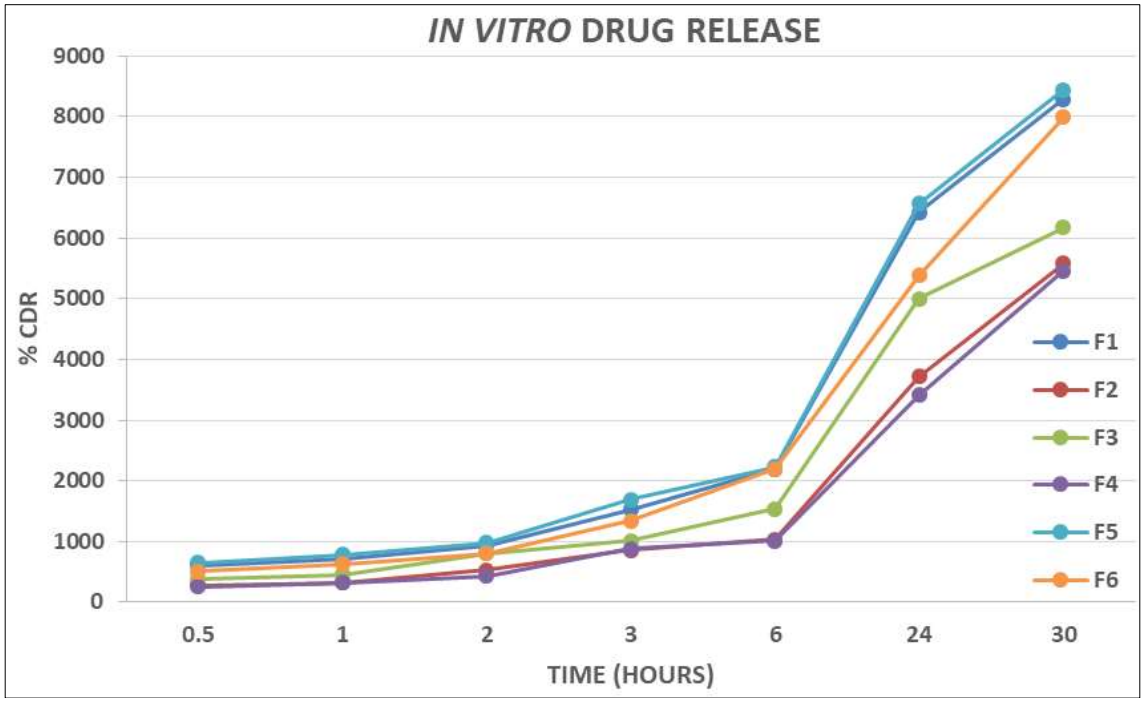

Fig 6: *In vitro* drug release study of Erythromycin stearate invasome (F_1 to F_6)

In vitro drug release of Erythromycin Stearate invasomes was found to be 85.23%, 79.47%, 77.49%, 72.86%, 70.89%, 80.54% for the formulation F_1 to F_6 respectively at the end of 30 hrs.

Conclusion

In this study, Erythromycin stearate invasome was prepared by mechanical dispersion method using Soy lecithin, Citral and Eugenol. The mean particle size of the formulation of invasomes containing citral was found to be 150, 154.3 and 1186 nm for F_1 (0.5% citral), F_2 (1% citral) & F_3 (2% citral) respectively. The mean particle size of the formulation of invasomes containing eugenol was found to be 175.8, 322, and 836 nm for F_4 (0.5% eugenol), F_5 (1% eugenol) & F_6 (2% eugenol) respectively. It indicates that an increase in the terpene concentration reduced the particle size of the invasomes. The polydispersibility index of the formulation F_1 to F_6 is found to be in the range of 0.333 - 0.736. It concluded the homogenous preparation of vesicles. A lower polydispersibility index indicates a more homogenous sample with particles that are more uniform in size. Zeta potential of the formulations was found to be in the range of -1.0mV to -21.9mV. The negative potential of the vesicles can enhance the penetration rate of the drug as well as the stability of the formulation. The percentage of drug entrapment efficiency was between 67% to 82%. The formulation F 3 possess maximum entrapment efficiency (82%). The drug content was found to be between 98.41% to 99.36%. The *In vitro* drug release of the Erythromycin stearate invasome was found to be between 70.39% to 85.23% at the end of 30 hrs. The anti-microbial study for the formulated invasomes was carried out with Staphylococcus aureus. The formulated invasome of erythromycin stearate possess an anti-bacterial activity with a zone of inhibition of 16, 20, 19, 16, 15, 17 mm for F_1 to F_6 respectively. From the above results, the formulation F 3 can be optimized for further studies. Finally, according to the results of the study, we can conclude that Erythromycin stearate invasome enhances the penetration of the drug.

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