ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Development and Characterization of Microsponge of Amphotercin B for Topical Drug Delivery.

SG Killedar[1], DA Bhagwat[1], Avinash Choudhari[2], JK Saboji[3], Pankaj C Chougule[4], and Sunil T Galatage[4]*.

[1]BVCP College of Pharmacy Kolhapur
[2]Dayanand Institute of Pharmacy Latur
[3]K.L.E.Society's College of Pharmacy Akol road, Nipani- 591237, Karnataka India
[4]SantGajananMaharaj College of Pharmacy Mahagoan

ABSTRACT

The aim of present research work to formulate microsponge of Amphotericin B to control the delivery rate of Amphotericin B to a predetermined site in human body with with control and maximum therapeutic drug delivery. Controlled release of drugs onto the epidermis with assurance that the drug remains primarily localized and does not enter the systemic circulation is the biggest challenge. To overcome this we Developed microsponges of amphotericin-B evaluated for compatibility studies, morphology and surface topography, Particle size, Percentage yield, Loading efficiency, Drug release, Diffusion study and antimicrobial activity. The prepared microsponge of amphotericin-B was found to be satisfactory. Formulation F2 of Amphotericin B wasbest in the class of Eudragit RS- 100 as a retarding polymer. The developed microsponges improve therapeutic potential of antifungal drug amphotericin-B in the treatment of Candida albicans and Aspargillus fungates and it is proved by antifungal activity.

Keywords: Microsponge, Amphotersin B, Anti-fungal, Quasi-emulsion diffusion method.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Topical drug delivery system can be defined as the drug application containing formulation to the skin to directly treat cutaneous disorders or the cutaneous manifestation of a general disease [1, 2].

Microsponge is polymeric delivery system consisting of porous microspheres that are mostly used for extended topical administration of a variety of active ingredients such as emollients, sunscreens, anti-infective, antifungal, and inflammatory agents. Microsponge offers many advantages such as delivering the active ingredients at minimum does, enhanced stability, reduced side effects, and the ability to modify drug release profiles [3].

Drug loading in microsponges can take place in two ways, single step process or by two-step process titled as liquid-liquid suspension polymerization and quasi emulsion solvent diffusion techniques. The microsponges were prepared by quasi emulsion solvent diffusion techniques using different polymer concentrations [4].

Antifungal Drugs [5]:

Mycology infections also have a part to play in allergic and inflammatory diseases.There are some treatment options are available for fungal infections. The majority of infections appear to be in low income, middle income countries and countries with low health system.

Amphotericin B: It is derived from Streptomyces nodosus in 1956. It is an amphoteric polyene macrolide antibiotic insoluble in water. It has antifungal activity, which includes Candida albicans, Histoplasmacapsulatum, Cryptococcus neoformans, Coccidioidesimmitis, Blastomycosisdermatitidis, Sporothrixschenkii and many strains of Aspergillosis. It is also active on Leishmania.

MATERIALS AND METHOD

Materials:

Amphotericin B obtained as gift sample from Lifecare Innovations Pvt. Ltd. Vadodara, Eudragit RS100 was obtained from Evonik industries, Mumbai, Polyvinyl alcohol from Loba Chemicals. Pvt.Ltd, Mumbai.

Method of Preparation of microsponge [6, 7]

Preparation of Blank Microsponge:

Microsponges were prepared by quasi-emulsion solvent diffusion method. The internal phase consists of Eudragit RS-100 (200 mg) and triethyl citrate (1% v/v) dissolved in 5 ml of dichloromethane. Triethyl citrate was then added to enhance the plasticity of the polymer. The mixture was then poured into 0.5% w/v aqueous solution of polyvinyl alcohol (PVA) which served as the external phase. The mixture was stirred at speed 500 rpm. After 8 hr. of stirring, microsponges were formed due to the removal of dichlormethane from the system by evaporation. The microsponges were washed with water, filtered and dried at 40^0C for 12 hr. in hot air oven.

Method of preparation of microsponges:

The required amount of Drug and Polymer were weighed accurately and dissolved in 20 ml of DCM: Methanol (1:1), this constitutes the internal phase. The surfactant PVA was weighed accurately and dissolved in distilled water at 60^0C, which is the external phase. The external phase was allowed to cool to attain room temperature. The internal phase was taken in a burette and added drop wise to the external phase. During addition the emulsion was stirred using a Remi mixer at 800 rpm. Mixing was continued for about 2 hrs. to achieve complete diffusion of the external phase. The microsponges formed were filtered and dried in hot air oven at 40^0C for period of 12 hrs.

Preformulation studies:

Melting point determination [8]:

Melting point of drug sample was determined by digital melting point apparatus and temperature range at which the drug melts is noted.

Drug-Excipients Compatibility study:

FTIR Spectroscopy [9, 10]:

Potassium bromide was mixed with drug & polymer and the spectra were taken using FTIR spectroscopy.

X-Ray Diffraction [11]:

For characterization of crystalline state, the X-ray diffraction (XRD) patterns for drug alone and in combination with Eudragit RS-100 and Ethyl cellulose were determined using X-ray diffractometer with a copper target, at a voltage of 40 KV and current of 20MA. The rate of the scanning was 0.30°C /min.

Differential Scanning Calorimetry (Thermal analysis) [12]:

DSC has been one of the most widely used calorimetric techniques employed to characterize the solubility and physical state of drug in the complex. The samples (5 mg) were thematically sealed in flat bottomed aluminum pans and heated over a temperature range of 100-300° C at a rate of 10° k/min using alumina as a reference standard.

Formulation of Microsponge gel [13, 14]:

Accurately weighed amount of carbopol 940 was taken and dissolved in water using propeller. In another beaker, microsponges containing Amphotericin B (free or entrapped, equivalent to) drug dissolved in ethanol and addition of PEG 400. Neutralized the carbopol solution by slowly adding triethanolamine solution with constant stirring until the gel is formed. The pH of the final gel formed was determined.

EVALUATION OF MICROSPONGE:

Compatibility studies [15]:

Compatibility of drug with reaction adjuncts can be studied by Fourier Transform Infra-red spectroscopy (FT-IR). For DSC approximately 5 mg samples was accurately weighted into aluminium pans and sealed and it was run at a heating rate of 15^0C/min over a temperature range 25-430^0C in atmosphere of nitrogen.

Morphology and surface topography of microsponges [16]:

For morphology and surface topography, the prepared microsponges was coated with gold-palladium under an argon atmosphere at room temperature and then the surface morphology of the microsponges was studied by scanning electron microscopy (SEM).

Particle size determination [17]:

Particle sizes of the prepared Microsponge formulations were determined by optical microscopy. The diameters of 50 micro particles were measured randomly by optical microscope.

Percentage production yield [18]:

The percentage production yield was calculated from the weight of Microsponge recovered from each batch in relation to the sum of the initial weight of starting materials.

$$\% \text{ yield} = \frac{\text{Practical Mass (Microsponge)}}{\text{Theoretical Mass (Polymer + Drug)}} \times 100$$

Determination of drug loading Efficiency [19]:

Drug loaded Microsponge (100 mg) were digested with 10 ml of acetonitrile at room temperature for 12 h. After filtration and suitable dilution, Sumatriptan Succinate present in the solution was determined at 229 nm using a UV visible spectrophotometer.

Drug Release profile [19]:

The study was performed for 6 hrs, and cumulative drug release was calculated at different time intervals. To compute the mechanism of drug release the experimental data was fitted in to four different kinetic models namely, Zero order kinetics, First order kinetics, Higuchies classical diffusion model, and Peppas model.

Diffusion study [20]:

The in vitro release of microsponge formulations were studied using cellophane membrane using modified apparatus. The dissolution medium used was neutralizing phosphate buffer, freshly prepared (pH 5.4). One gram of formulation (equivalent to 1000 mg of Amphotericin B) was accurately placed into this assembly. The dissolution medium was stirred at 100 rpm speed using Teflon coated magnetic bead. The aliquots were suitably diluted with the receptor medium and analyzed by UV-Visible spectrophotometer at 415 nm using neutralizing phosphate buffer as blank.

Antimicrobial Activity [21]:

Antimicrobial activity of optimum formulation F2 was carried out on AspargillusFungates and Candida albicans specie using disc diffusion method. In this method different concentration of formulation F2 was prepared and checked the activity against fungus species.

RESULT AND DISCUSSION

Preformulation Studies:

Melting point of Amphotericin B

Melting point of Amphotericin B in literature is 170ºC, after estimation it was found to be 168-171ºC which indicates drug sample is pure.

FTIR Spectra of Amphotericin B, ethyl cellulose and Eudragit RS-100

The FTIR spectrum of pure Amphotericin B, ethyl cellulose and Eudragit RS-100 shows characteristic absorption peaks as given in Figure No.01, 02 and 03.

FTIR Spectra of Amphotericin B and Ethyl Cellulose complex

The FTIR spectrum of Amphotericin B and ethyl cellulose shows characteristic absorption peaks seemed to be retained at almost the same wave number with the same intensity in the spectra of pure Amphotericin B, Which signify the absence of any potential physical or chemical interaction between the drug and polymer. Hence, the polymer was found to be compatible with the drug.

ISSN: 0975-8585

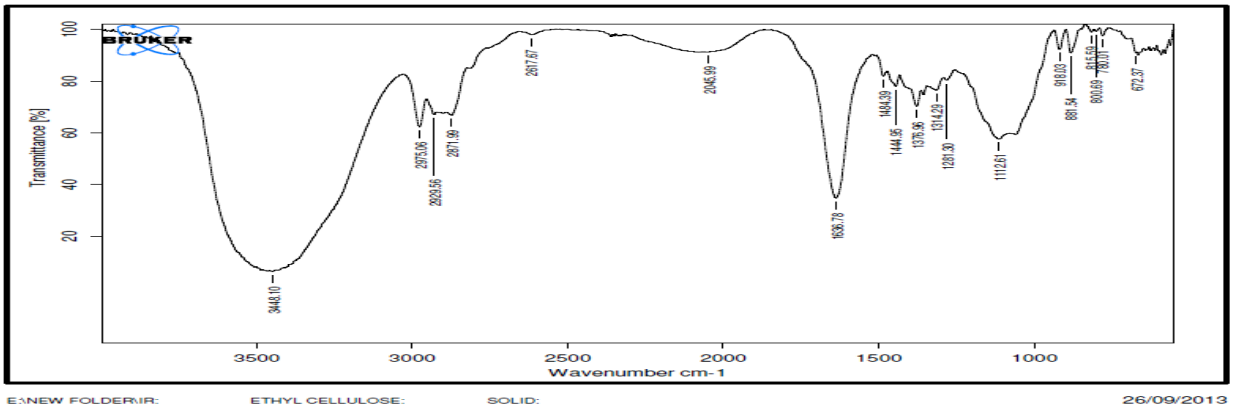

Fig 1: FTIR Spectra of Amphotericin B

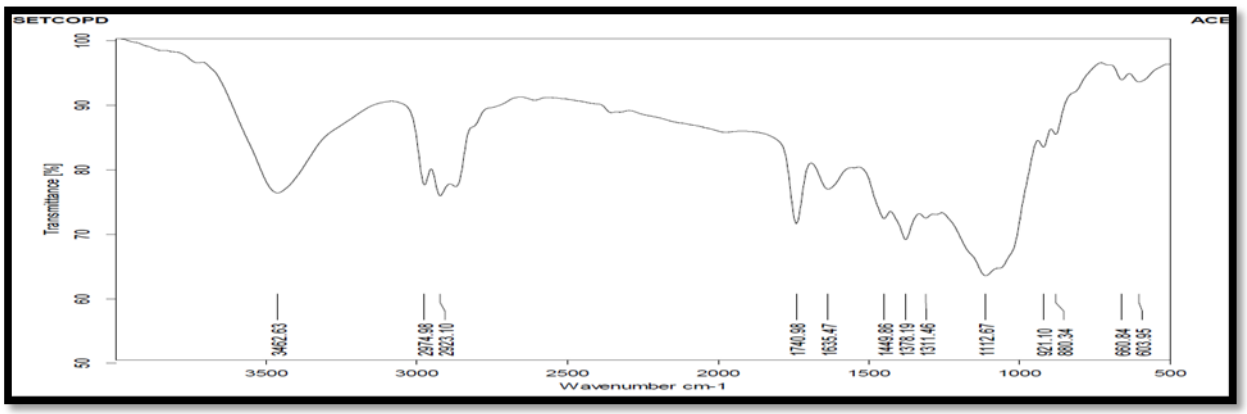

Fig 2: FTIR Spectra of Ethyl Cellulose

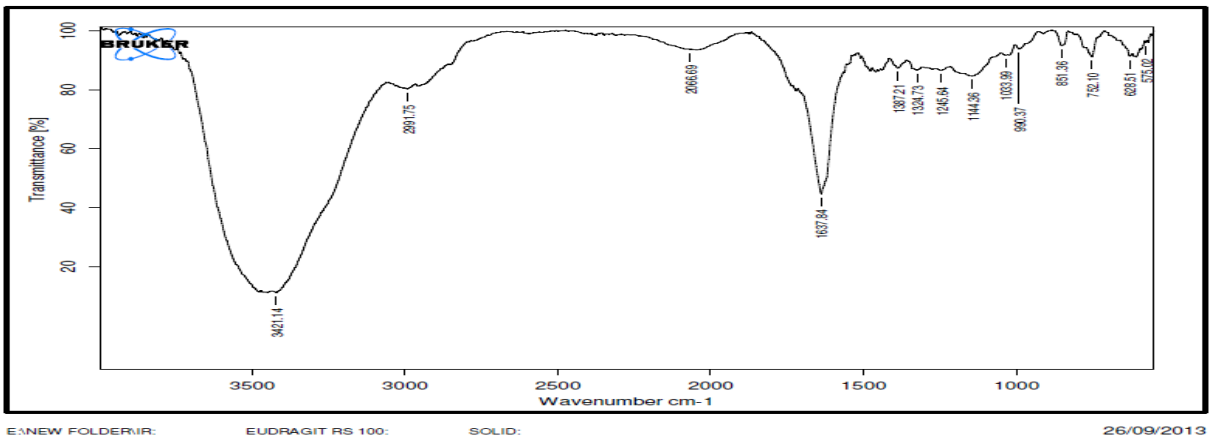

Fig 3: FTIR Spectra of Eudragit RS-100

ISSN: 0975-8585

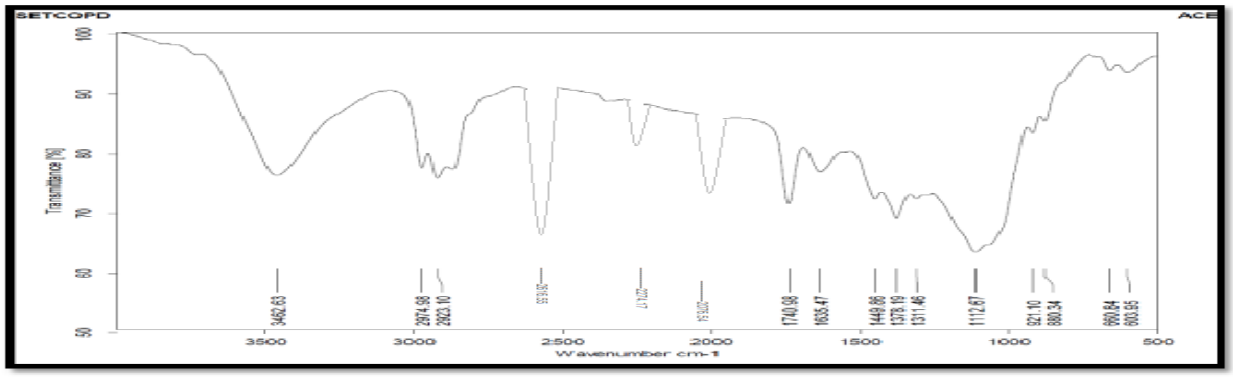

Fig 4: FTIR Spectra of Amphotericin B and Ethyl Cellulose

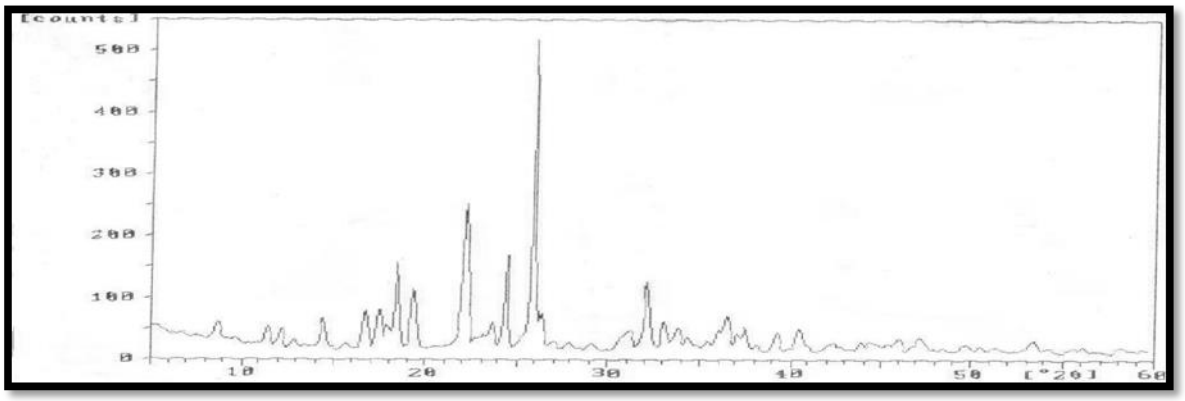

Fig 5: X-Ray Diffraction of Amphotericin B

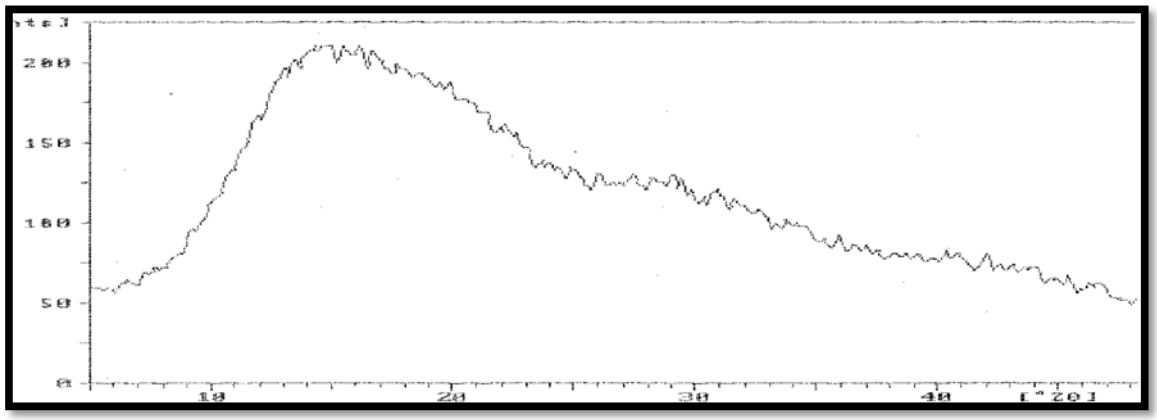

Fig 6: X-Ray Diffraction of Eudragit RS-100

X-Ray Diffractometry of Amphoteresin B and Eudragit RS 100:

X- Ray diffraction study for pure Amphotericin B powder, pure Eudragit RS 100 polymer shown in following figure no.05 & 06.

DSC Studies of Amphotericin B, Eudragit RS- 100 and Ethyl Cellulose:

The DSC study of pure Amphotericin B, Eudragit RS-100 and ethyl cellulose shows characteristic curves as given in Figure No.07 08 and 09 respectively. The melting temperature for Amphotericin B, Eudragit RS 100 and Ethyl cellulose were 170˚C, 165˚C and 175˚C respectively.

ISSN: 0975-8585

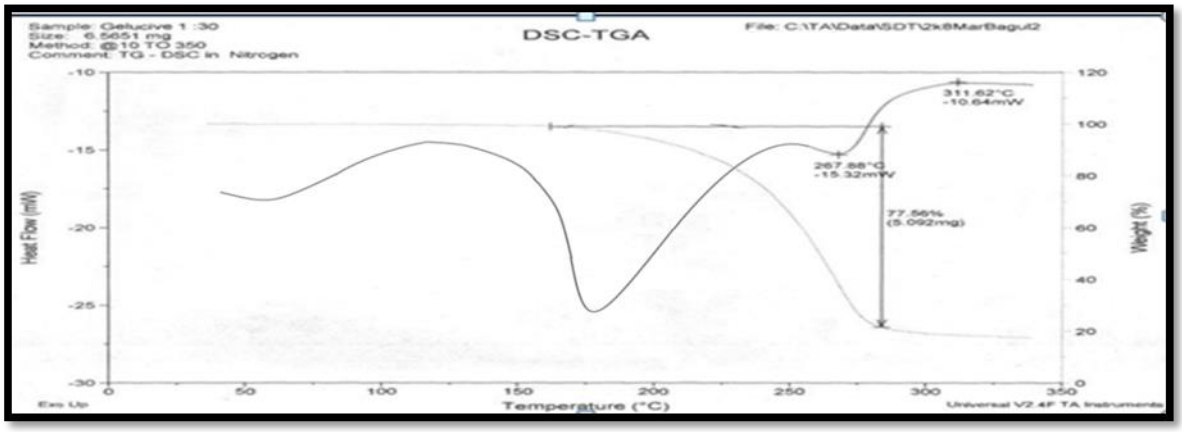

Fig 7: DSC Studies of Amphotericin B

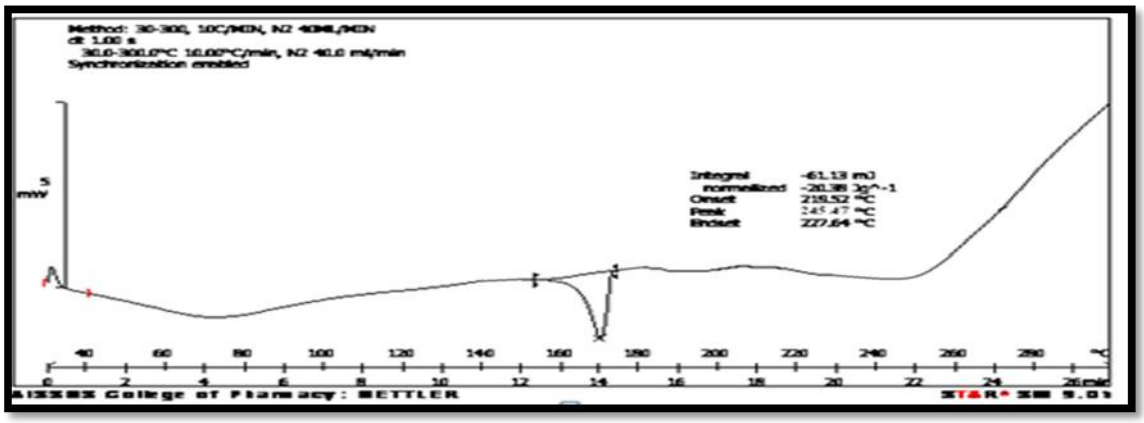

Fig 8: DSC Studies of Eudragit RS- 100

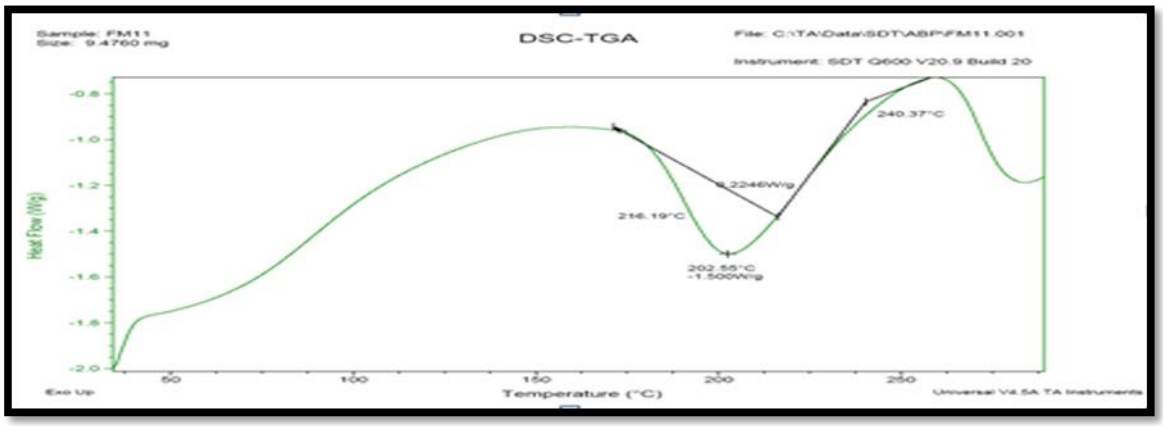

Fig 9: DSC Studies of Ethyl Cellulose

ISSN: 0975-8585

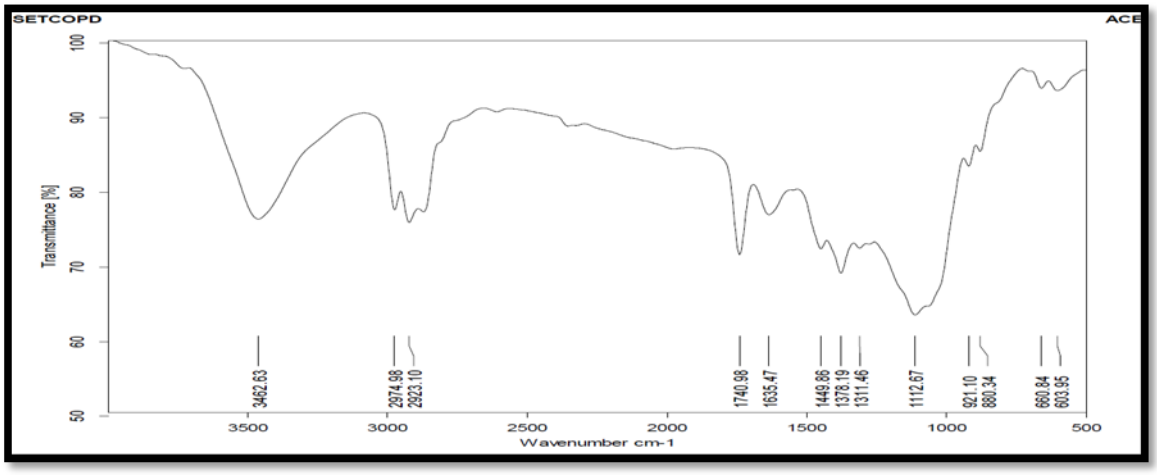

Fig 10: IR Spectra of Amphotericin B Formulation F2

Evaluation of prepared Microsponges

Compatibilities Studies:

FTIR Spectra of Amphotericin B Formulation

The IR spectrum obtained from Amphotericin B formulation F2 shows characteristic peaks for functional group as that of FTIR spectrum of pure Amphotericin B. It Concluded that the excipients shows compatibility with Amphotericin B in the formulation. The FTIR spectra of Amphotericin B Formulation F2 were shown in fig. No 10.

X-Ray Diffraction of Formulation

The internal structures of pure Amphotericin B are shown in Figure No 05 and X-ray diffraction pattern of the selected formulationF2 is shown in Figure no.11. The data indicated that the initial crystalline behavior of Amphotericin B has been altered marginally in the formulation. This may be attributed to the effect of amorphous characteristics of polymer and may be due to less availability of drug for diffraction pattern in the formulation.

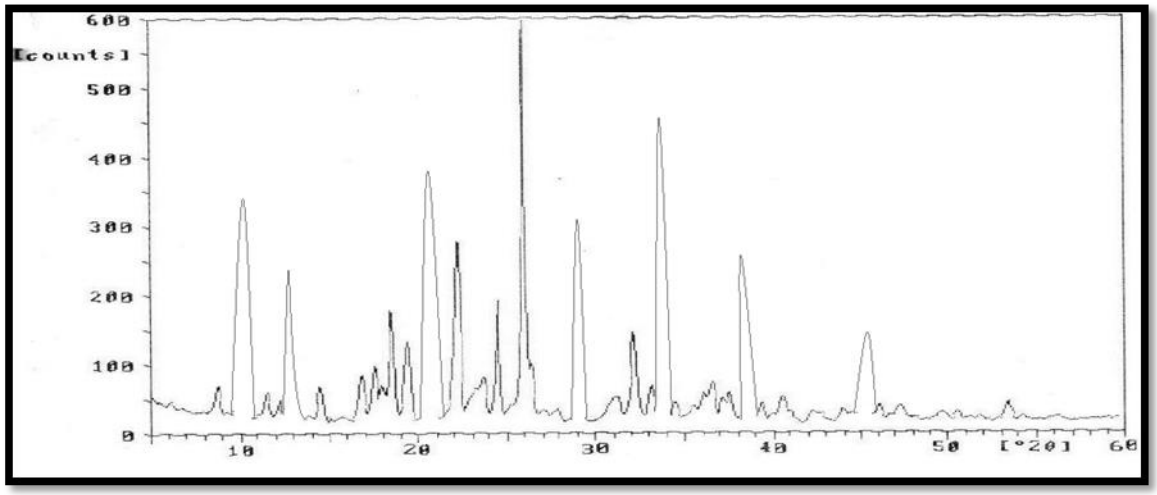

Fig 11: XRD Studies of Amphotericin B Formulation F2

ISSN: 0975-8585

DSC Studies of Formulation

The results of Differential Scanning Calorimetric analysis showed that the melting temperature for Amphotericin B, Eudragit RS 100 and Ethyl cellulose were 170˚C, 165˚C and 175˚C. The integrity of drug was unaffected when developed in to Microsponge, this is confirmed by DSC of formulationF2 where the composite melting peaks of Amphotericin B, Eudragit RS 100 and ethyl cellulose were found to be at 171˚C, 168˚C and 178.53˚C indicating compatibility between drug polymer and processing conditions.

Scanning Electron Microscopy of microsponge formulation

Microsponge of formulation F2 was spherical and their surface was smooth and devoid of cracks giving them good appearance. The SEM data obtained on the drug-loaded microsponge are shown in Figure no.13.

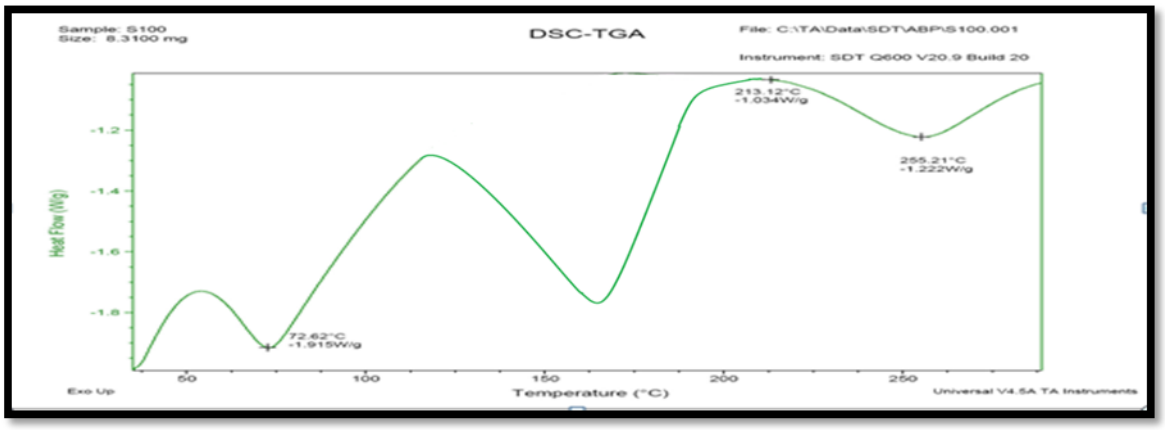

Fig 12: DSC study of Amphotericin B Formulation F2

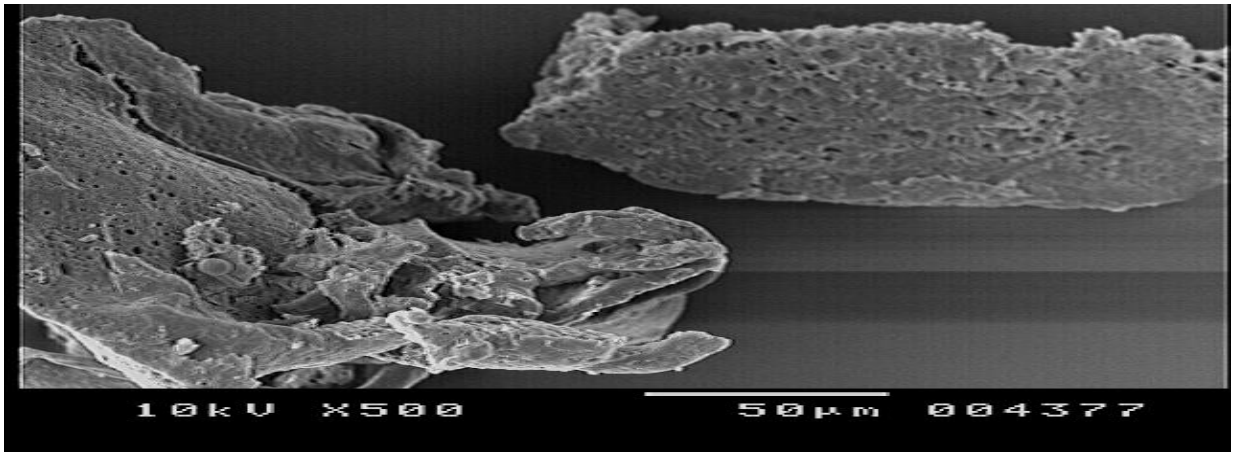

Fig 13: SEM of formulation F2

Particle size of Microsponge:

Particle sizes of all formulations were found to be decreased with increase in drug: polymer ratio that is lowest particle size found at highest drug polymer ratio. The lowest particle size found for F2 formulation 41.28µm. The results were shown in table no. 02.

ISSN: 0975-8585

Table 1: Formulation Table of Microsponge of Amphotericin B

Formulation	PVA	Eudragit RS-100	Ethyl cellulose	Amphotericin B	Dichloromethane	Water	Methanol
F1	0.75%	1g	_	200mg	10ml	90ml	10ml
F2	0.75%	3g		200mg	10ml	90ml	10ml
F3	0.75%	2g		200mg	10ml	90ml	10ml
F4	0.75%	_	1g	200mg	10ml	90ml	10ml
F5	0.75%	_	2g	200mg	10ml	90ml	10ml
F6	0.75%		3g	200mg	10ml	90ml	10ml

Percentage Yield:

Percentage yield calculated for all microsponges, ranged from 84.18-89.34%, it is indicated that increasing the drug: polymer ratio increased the percentage yield. The percentage yield shown in Table no.02.

Table 2: Evaluation of microsponge for particle size, percentage yield & drug loading efficiency

Formulations	Particle Size (μm)	Percentage yield	Drug loading efficiency (%)
F1	71.19±1.5	86.18±2.8	77.07±3.5
F2	41.28±2.9	89.34±2.1	94.11±3.6
F3	62.26±2.8	88.16±1.4	89.31±1.0
F4	61.11±2.4	84.18±2.1	79.71±2.8
F5	53.93±3.1	86.58±1.5	83.07±2.9
F6	69.66±1.9	88.09±2.8	86.19±3.5

*Each value represents mean ±SD of three observations

Drug loading efficiency:

From the results it was found that as drug: polymer ratio increases the drug loading efficacy also increases. The loading efficacy of all 6 formulations were found in the range of 77.07-94.11 as shown in table no.02

Drug Release profile:

It was observed that the drug release from the formulations slightly increases as the particle size of the formulation does decrease. The exact release mechanism was justified based on statistical calculations. The results of statistical analysis showed that the release mechanism is zero order kinetic, in which the rate drug release is independent of concentration of drug. % Cumulative drug release of microsponge gel is shown in table no.03 and Fig No.14.

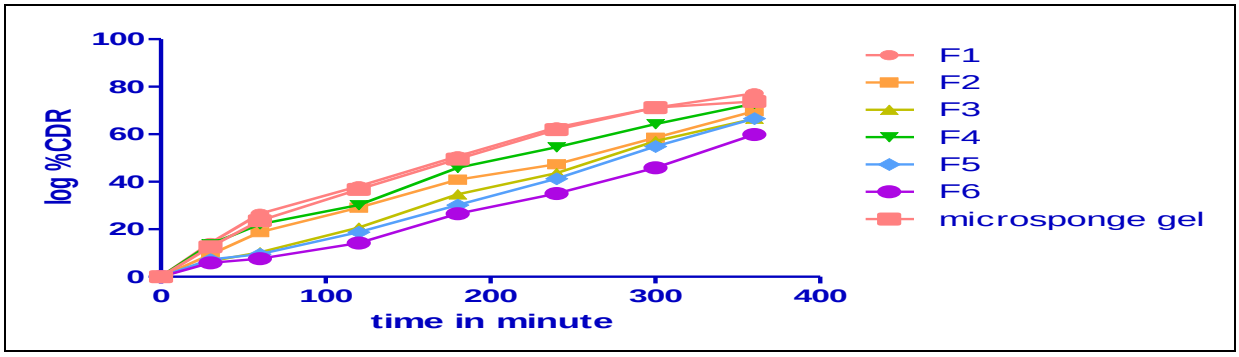

Fig 14: Log % CDR vs Time

ISSN: 0975-8585

Table 3: % CDR of formulations

Formulations	% CDR for 30 min	60min	120 min	180 min	240 min	300 min	360 min.
F1	9.35±0.5	18.81±0.9	29.11±1.4	50.68±1.4	47.35±2	58.5±1.6	66.51±2.2
F2	14.24±0.5	26.56±0.8	38.14±1.2	64.09±2.1	62.9±1.2	71.22±1.7	77.24±2.5
F3	6.52±0.2	10.3±0.6	20.72±0.8	34.74±1.5	43.61±1.2	57.16±2.4	69.7±1.1
F4	13.71±0.5	22.1±0.9	30.18±0.9	45.9±1.6	54.51±1.2	64.28±1.8	72.88±2.3
F5	7.33±0.4	9.56±0.9	18.81±1.6	30.18±1.5	41.33±1.7	54.9±2.5	66.62±2.1
F6	5.84±0.9	7.6±1.4	14.24±1.2	26.56±1.6	35.06±1.8	45.9±2.1	59.91±2.4
Microsponge Gel	12.64±0. 8	23.64±0.7	36.76±1.6	49.51±1.4	61.94±2.6	71.2±2.2	73.77±2.5

*Each value represents mean ±SD of three observations

Diffusion study of microsponge gel:

The exact release mechanism was justified based on statistical calculations. The results of statistical analysis showed that the release mechanism is zero order kinetic, in which the rate drug release is independent of concentration of drug.

Antifungal activity:

In-Vitro antifungal activity of Amphotericine B microsponge optimized batch F1 carried out

By disc diffusion method. Fungus species used for biological evaluation were Candida albicans and Aspargillusfungates. Antifungal activity of Ampotersinewas shown in figNo.15 and 16 and table no.04.

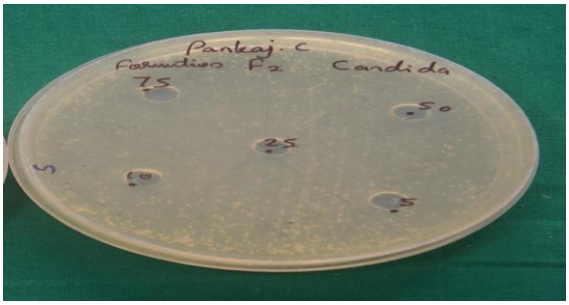

Fig 15: Antifungal activity of Amphoterecin B on Candida albicans

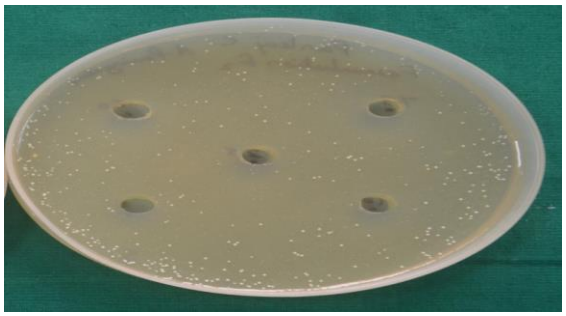

Fig 16: Antifungal activity of Amphoterecine B on Aspargillus fumigates

Table 4: Antifungal activity of formulation F2

Sr. No	Sample	75 µl/ml	50 µl /ml	25 µl /ml	10 µl/ml	05 µl/ml
01	Candida albicans	18mm	15mm	13mm	R	R
02	A.Fumigatus	18	15	13	10	R

Note: R- Resistant

CONCLUSION

Topical polymeric microsponge formulation of Amphotericin B using two types of polymers Eudragit RS-100 and Ethyl cellulose was formulated. From the study the following conclusions can be drawn.

- By considering the solubility of the drug and polymer and the rate of diffusion of the solvent used, the internal phase suitable for the preparation of microsponges was found to be Dichloromethane and Methanol in the ratio 1:1 and the external phase was found to be water.
- The ratio of drug: polymer required to produce microsponges with good encapsulation efficiency was found to be 1:3 ratio below this ratio microsponges formed had low capacity encapsulation of the drug, hence it was concluded that 1:3 ratios of drug: polymer to produce good microsponges.
- The ratio of drug: polymer to produce microsponges with good percentage yield was found to be F2 formulations because of varying ratios of polymers as compare to other formulations.
- The particle size range increases as increase in amount of polymer in the formulation.
- SEM analysis of microsponge revealed that all the formulations were smooth and spherical with ideal surface morphology.
- Finally we may conclude that formulation F2 of Amphotericin B were best formulations in the class of Eudragit RS- 100 as a retarding polymer.

ACKNOWLEDGEMENT

The authors are also thankful to Principal SGMCP Mahagaon, Department of Pharmaceutics SGMCP Mahagaon ,Trustees of SGMCP Mahagaon and Principal KLE college of pharmacy nippani for providing required guidance and support.

Conflict of Interest: Myself Mr. SUNIL T.GALATAGE, as the author of the manuscript and don't have a direct financial relation with the commercial identities mentioned in your paper that mingt lead to a conflict of interest for any of the authors.

REFERENCES

[1] K. Krantikumar. "Formulation and evaluation of diacerein cream." AJPCR, 2011; 4(2).
[2] D. Meenakshi." Emulgel: A novel approach to topical delivery." IJPBS, Jan; 4(1): 847-856.
[3] Viral Shaha, Hitesh Jain." Microsponge drug delivery: a review." IJRPS, 2010; 1(2): 212-218.
[4] HibahAldawsari, Shalmaa M." Microsponge as promising vehicle for drug delivery and targeting: preparation, characterization and applications." AJPP, 2013; 7(17): 873-881.
[5] Head, M. G. et al. "Systematic analysis of funding awarded for mycology research to institutions in the UK", 1997−2010. BMJ Open 4, e004129.
[6] Saboji J.,Manvi F. "Formulation and evaluation of ketoconazole microsponge gel by quasi emulsion solvent diffusion." JCTR,2011; 11(1): 2691-2696.
[7] Chandramouli Y., Shaikh F. "Preparation and evaluation of microsponge loaded controlled release topical gel of Acyclovir sodium.",IJB,2012; 3(2): 96-102.
[8] H H Willard; L LMerrit (Jr.); J A Dean. Instrumental Methods of Analysis, 7th Edition, CBS Publishers and Distributors, New Delhi, (1986); 287-320.
[9] SkoogDA, HollerFJ, NiemanDA. Principle of Instrumental Analysis, 6 Edition Reprint, Thomson Brooks/Colepublication, (2004); 300-351.

[10] International conference on harmonization October: Text on Validation of Analytical Procedures Q 29(A) (1994).
[11] Raju R., Kumar S. "Standadization of process parameters involved erythromycin microsponges by quasi emulsion solvent diffusion method.
[12] Patel E.,Oswal R. "Nanosponge and microsponges: A novel drug delivery system." IJRPC, 2012; 2(2): 2231-2781.
[13] Saboji J.,Manvi F. "Formulation and evaluation of ketoconazole microsponge gel by quasi emulsion solvent diffusion." JCTR, 2011; 11(1): 2691-2696.
[14] Chandramouli Y.,Shaikh F. "Preparation and evaluation of microsponge loaded controlled release topical gel of Acyclovir sodium." IJB, 2012; 3(2): 96-102.
[15] Pattnaik S., Swain K. "Effect of casting solvent on crystallinity of ondoestron in transdermal films" IJP, 2001; 106-110.
[16] E F Fiese; T A Hagen. The Theory and Practice of Industrial Pharmacy, (L Lachman; H. A. Liebermann; J LKanig. Eds), 3rdEdition, Varghese Publishing House, New Delhi, (1987);180
[17] S. Srinivas, Y. Anand Kumar, A. Hemanth, M. Anitha; Preparation and evaluation Of Niosomes containing Aceclofenac, Digest Journal of Nanomaterials and Biostructures,(2010);5(1):249-254.
[18] Fabrication of Novel Types of Colloidosome Microcapsules for Drug Delivery Applications Materials Research Society Symposium Proceedings. Volume 845, 2005. NanoscaleMaterials Science in Biology and Medicine, Held in Boston, MA on 28 November-2 December 2004.
[19] ChawdaHimmat Singh, Jain C P, BairwaNarendra Kumar; Formulation Characerization, Stability and invitro evaluation of NimusulideNiosomes, pharmacophore an international research journal,(2011)2(3):168-185.
[20] Abrar B.,Aniss. S. "Formulation and in vitro evaluation of NSAID gel. IJCPR, 2012; 4(3): 56-58.
[21] Clinical Microbiology procedures handbook. HeneryD.Hesenberg.Volume 1. American society of microbiology/ Washington D.C, 1992.