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Porous Polymeric Carrier System for Modified Drug Release of Boswellic Acid

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Abstract

Microsponges being the polymeric drug delivery systems consist of porous microspheres that can entrap a wide range of active ingredients. Boswellic acid is a pentacyclic triterpenoid having anti-inflammatory, anti-hypertensive, anti-cancer activities.

The aim of the study was to develop, characterize and formulate *microsponge* delivery for topical application. The *Microsponges* were prepared by quasi emulsion solvent diffusion method using QbD approach. Drug excipient compatibility study was carried out by FT-IR, DSC and XRD. The prepared *Microsponges* were further evaluated for its physicochemical properties by scanning electron microscopy (SEM), photomicroscopy, transmission electron microscopy (TEM), particle size, zeta potential, and porosity analysis. The optimized *Microsponges* were incorporated into gel base formulation and evaluated for *in-vitro* drug release, dissolution studies and *ex-vivo* permeability studies. Further, *microsponge* formulations, subjected for animal studies for skin irritation test and clinical efficacy.

The present study confirmed the formation of *Microsponges* of Boswellic acid. It also proves the sustained release of drug through *microsponge* formation.

Keywords: • Boswellic acid • Microsponge • Gel formulation • Diffusion

Abbreviations: BAF: Boswellic Acid Fraction • FTIR: Fourier Transform Infrared • DSC: Differential Scanning Calorimetry • XRPD: X-ray Powder Diffraction • SEM: Scanning electron microscopy • TEM: Transmission electron microscopy • PSA: Particle size analysis, QbD: Quality by design • DCM: Dichloromethane

Introduction

Boswellic acids are pentacyclic triterpenoids are the major constituents of the gum resin derived from the plant Boswellia Serrata belonging to the family Burseraceae [1]. It consist of various Boswellic acids namely β-Boswellic acid, 11-keto-β-boswellic acid (KBA) and other corresponding acetates like acetyl β-Boswellic acids (ABA), acetyl-11-keto-β-boswellic acid (AKBA), acetyl-α-boswellic acid [2].

Boswellia has traditionally been used for a number of topical applications, including treatment of bacterial and fungal infections, boils, acne wound healing, scars, and varicose veins [3]. It has a lot of medicinal value like anti-inflammatory [4], diuretics [5], anti-cancer, peptic ulcer diseases, analgesic and sexually transmitted diseases (STDs) [6].

Boswellic acid readily penetrates through the hydrophobic stratum corneum while the lower epidermal and dermal layers are hydrophilic in nature, which limits its solubility and transfer of the drug from stratum corneum [7]. Both KBA and AKBA are highly lipophilic drug, they have relatively poor absorption through GIT but high retention. The elimination half-life of 11-keto-β-boswellic acid (KBA) is approximately 6 h. This implies that Boswellic acids should be taken every 6 h postoperative to achieve maximum plasma levels. BAs should be taken along with fatty meal as it significantly increases their plasma concentration [8].

To achieve targeted and sustained release of drugs, microparticles and nanoparticles being increasingly investigated. *Microsponges* are porous, polymeric and tiny, sponge-like spherical particles. It contains a number of interconnecting voids within a non-collapsible structure that imparts a large porous surface [9].

Thus, the aim of the present investigation was to design ethyl cellulose *Microsponges* as a novel carrier for the controlled topical delivery of Boswellic acid fraction (BAF). This novel carrier is expected to prevent the excessive accumulation of the drug in the skin, improve its efficacy and decrease the frequency of application and the systemic absorption. The present work included the preparation, optimization and evaluation of BAF *Microsponges*. A QbD factorial design assisted in the statistical optimization. The optimized *Microsponges* were incorporated into a gel and evaluated for their performance. Studies revealed that no study carried out to formulate sustained release medication containing Boswellic acid.

Materials and Methods

Materials

Boswellic acid fraction (BAF) was received as a gift sample from Central India Pharmaceuticals Ltd. Nagpur, Maharashtra, India. All other chemical solvents were of laboratory grade and were used as procured.

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Methods

The organic internal phase containing BAF and ethyl cellulose in dichloromethane was gradually added into external phase, which contained PVA as emulsifying agent. The mixture was stirred at 500 rpm for a specific period of time at room temperature to remove dichloromethane from the reaction flask. The formed *Microsponges* were filtered, washed with distilled water, and dried at room temperature. *Microsponges* were weighed, and production yield was determined [10].

Drug content analysis

Microsponge powder equivalent to 10 mg of drug was weighed and dissolved in 50 ml of ethanol under sonication for about 10 mins at 30°C. It was analysed spectrophotometrically, after further dilution in ethanol to obtain 20 μg/ml concentration solutions. The drug content and entrapment efficiency were calculated by using following equations: [11].

Drug content (%) = $M_{act}/M_{ms} \times 10$

Entrapment efficiency (%) = $M_{act}/M_{the} \times 100$

Where,

M_{act} is the actual amount of drug in the weighed quantity of *Microsponges*

M_{ms} is the weighed quantity of *microsponge* powder

M_{the} is the theoretical amount of drug in the *Microsponges*

Optimization of formulation parameters and process variables

Preliminary trials were undertaken to establish the effect of drug/ethyl cellulose ratios on the physical characteristics of *Microsponges*. To optimize dependent variables such as drug: polymer ratio, emulsifier concentration and stirring time, a series of formulations were prepared by factorial design study using Design Expert 11 version software using stirring rate as independent variable [12,13].

Characterization techniques

Formed *Microsponges* were viewed at a magnification of 40X using photomicroscopy (Model: eclipse E200, Make: Nikon). Surface topography was studied by using a scanning electron microscope (SEM), (Model: EVO special edition, make: Zeiss).

For transmission electron microscopy (TEM) a small amount of BAF *microsponge* was negatively stained with 2% uranic acid and placed on copper grid (Make: Jeol, Model: JEM 2100, SAIF, Cochin University). Spectra were determined to understand the drug-excipient compatibility, by Fourier transform-IR (FT-IR).

The sample crystallinity was determined using X-ray diffraction using voltage 40 kV; current 20 mA; scanning speed 1/min. The results were recorded over a range of 5–60° (2 θ) using the Cu-Anode X-ray tube and scintillation detector (Model: Rigaku, Ultima IV, National Institute of Oceanography, Donapaula). Particle size studies were carried out using photon correlation spectroscopy with dynamic light scattering using Zetasizer 2000 (Model: Nano series, S90 Zetasizer Malvern, Sinhgad Institute of Pharmacy, Pune). Electrophoretic mobility of *Microsponges* were determined by zeta potential. The porous properties were determined by Mercury Intrusion Porosimeter (Model: Nova Station A, Quantochrome, Shivagi University) by using adsoption-desorption isotherms.

Dissolution studies

In the *in-vitro* dissolution study, accurately weighed samples equivalent to 50 mg of drug was added to the surface of the stirred dissolution medium (900 mL phosphate buffer, pH 6.8) at the beginning of the study in a USP type II dissolution apparatus.

The dissolution was carried out at 100 rpm at 37°C. Samples were withdrawn at regular time intervals. The filtered samples were analysed by UV spectroscopy at 203nm for BAF respectively [14,15].

Preparation of BAF microsponge loaded gel

Carbopol gel based was prepared by dispersing Carbopol 934 in a mixture of water and soaked overnight.

The dispersed mixture was neutralized to a pH 7.0 with triethanolamine to form a gel base. 50 mg of drug equivalent BAF *microsponge* was taken and uniformly dispersed in the gel base to obtain BAF *microsponge* loaded gel [16] (Table 1).

Table 1. Batches of gel formulation *In-vitro* release studies of gel.

Ingredients	B1	B2	B3	B4
Carbopol 934	1.0%	1.5%	2.0%	2.5%
Drug Concentration	1%	1%	1%	1%
Propylene Glycol	2.5%	2.5%	2.5%	2.5%
Triethanolamine	q.s	q.s	q.s	q.s
Methyl Paraben	0.1%	0.1%	0.1%	0.1%
Propyl Paraben	0.01%	0.01%	0.01%	0.01%
Distilled Water	q.s	q.s	q.s	q.s

The *in-vitro* release study was carried out using Franz-diffusion cells with a receptor compartment volume of 50 mL and an effective diffusion area of 3.14 cm^2. Dialysis membrane was soaked overnight in phosphate buffer (pH: 7.4). A predetermined amount of BAF *Microsponges* gel was placed on the donor side. The receptor medium was continuously stirred at 150 rpm to ensure homogeneity and maintained at 37 ± 0.5°C. At predetermined time interval 5 mL of release medium was withdrawn for analysis and was compensated by equal volume of fresh buffer. The drug release data were analysed to determine the release kinetics (zero-order and first-order) as well

as diffusion controlled mechanism (Higuchi model, Peppas, Hixson-Crowell and Korsmeyer-Peppas) using linear regression analysis.

Primary skin irritation studies

Primary skin irritation studies of the optimized formulations were performed using Wistar Albino rats in accordance with the guidelines of OECD 404. Group I served as the control (gel without drug) and group II received gel respectively. The scores were recorded after 1, 3, 5 and 7 days for reactions such as erythema and edema [17].

Ex-vivo permeation study

Wistar albino rat was anesthetized using excessive dose of thiopental through i.v route and sacrificed by cervical dislocation to procure the dorsal side of rat skin. The excised skin was washed and mounted on Franz diffusion cell with stratum corneum facing the donor compartment and the dermis side facing the receptor compartment. A weighed quantity of formulation gel was placed on to the skin in the donor compartment and was immersed slightly in 50 mL of receptor medium. The cell content was stirred on magnetic stirrer at a temperature 37 ± 0.5ºC. An aliquot of 5 mL was withdrawn at specific time intervals up to 8 h, and was estimated spectrophotometrically at 203 nm for BAF. After each withdrawal, the diffusion medium was replaced with an equal volume of fresh diffusion medium [18].

Results and Discussion

Formulation and optimisation of *Microsponges* is shown in Table 2 below.

Table 2. Formulation batches of BAF.

Formulation Code	Ratio	Solvent	Pva	Time	% Yield	Drug Content	Entrapment Efficiency
F1	01:01	2	30	60	76.45	25.78	51.56
F2	07:01	2	30	60	71.8	80.5	91.875
F3	01:01	8	30	60	78.3	39.05	78.125
F4	07:01	8	30	60	70.2	54	61.5
F5	01:01	2	50	60	61.55	41.56	83.125
F6	07:01	2	50	60	68.85	45	51.5
F7	01:01	8	50	60	92.8	32.5	65
F8	07:01	8	50	60	92.1	75.65	86.25
F9	01:01	2	30	120	62.8	42.65	85.3
F10	07:01	2	30	120	79.1	37.55	42.5
F11	01:01	8	30	120	90.15	37.5	75
F12	07:01	8	30	120	76.6	78.947	90
F13	01:01	2	50	120	84.9	38.437	76.5
F14	07:01	2	50	120	66.54	49.616	56.56
F15	01:01	8	50	120	87.1	38.281	76.56
F16	07:01	8	50	120	96.1	46.05	52.5
F17	04:01	5	40	90	79.25	60.385	80.31
F18	04:01	5	40	90	77.25	54.981	73.125
F19	04:01	5	40	90	84.5	53.846	70

The effects of various independent variables i.e. drug: polymer ratio, solvent volume, surfactant concentration and stirring time on the dependent variables were illustrated in Figures 1 and 2. In Figure 1A the Pareto charts explains about the variables being significant only when it crosses the t-value limit. In this case only the solvent volume had a significant impact on the % yield whereas other variables could be changed in order to maintain the hierarchy. The Figure 1B explains the 3-D effect and the interrelation between the drugs: polymer ratio, solvent volume and % yield. Figure 2A explains that the dependent variables had a positive effect on the drug content. Figure 2B represents that the drug: polymer ratio and the drug content go hand in hand. The contour plots explained the correlation of the axis at the center of the surface. Thus it can be seen that the experimental values were in very close agreement with the predicted values, indicating the success of the design for the evaluation and optimization of the formulations.

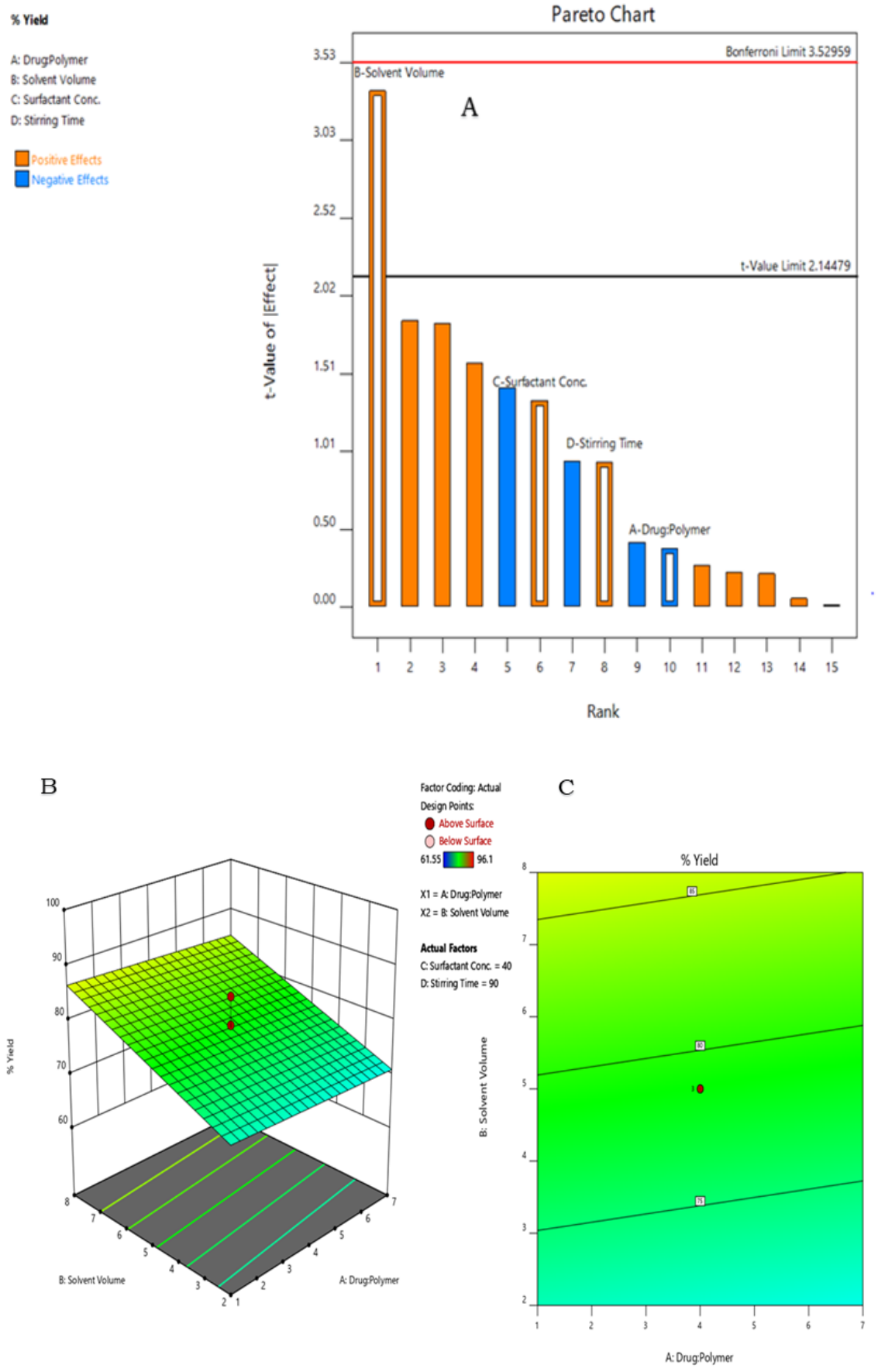

Figure 1. (A) Pareto chart (B) 3-D and (C) contour plot for the % yield for BAF.

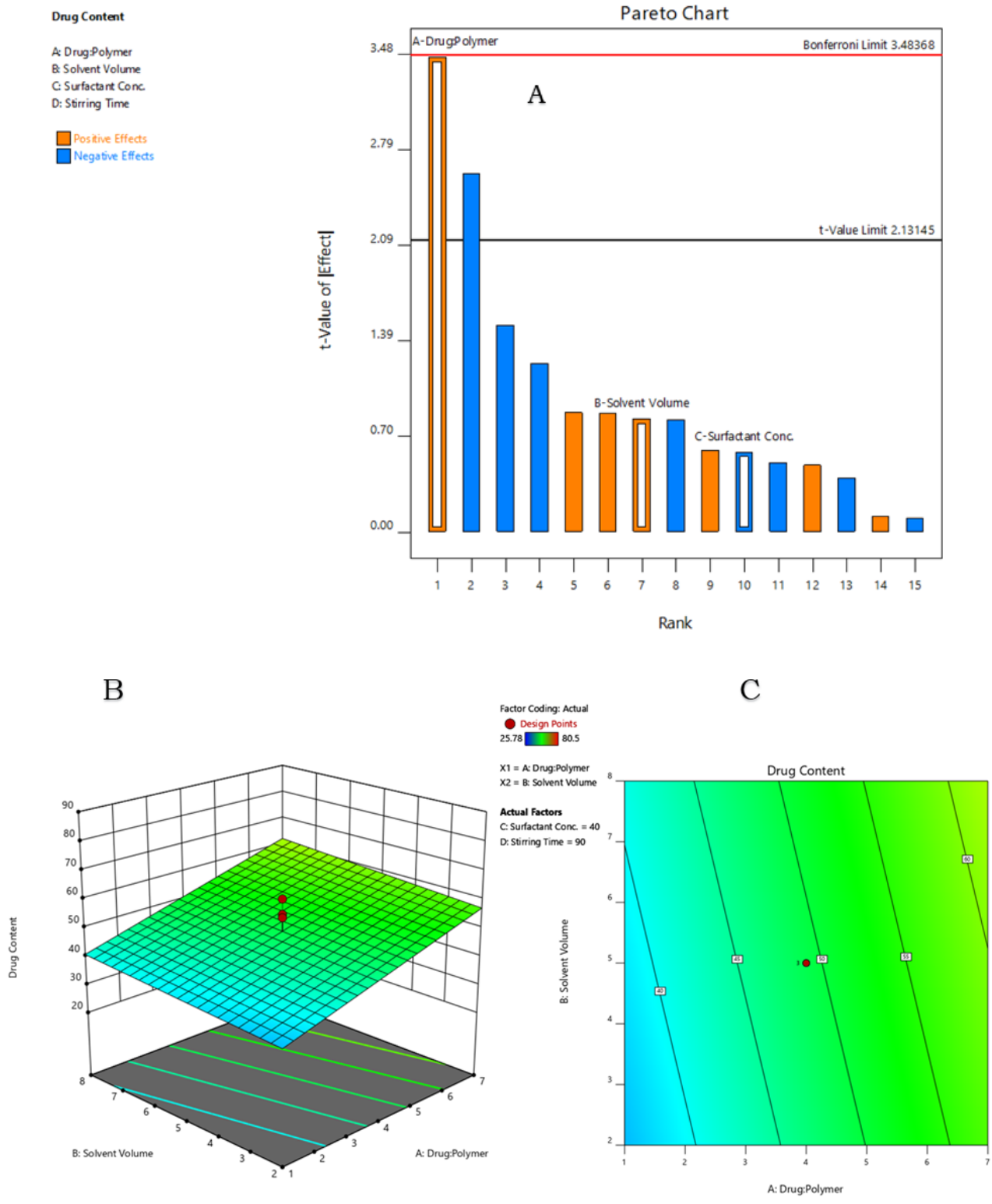

Figure 2. (A) Pareto chart (B) 3-D and (C) contour plot for the drug content for BAF

Characterization of Microsponges

Analysis of IR results (Figures 3-6) suggested that there was chemical compatibility between drug and excipients during *microsponge* preparation. It also confirmed the stability of the drug with successful entrapment in the *microsponge* formulation. Spectroscopic study discovered neither appearance of any new peak nor disappearance of existing peaks [19].

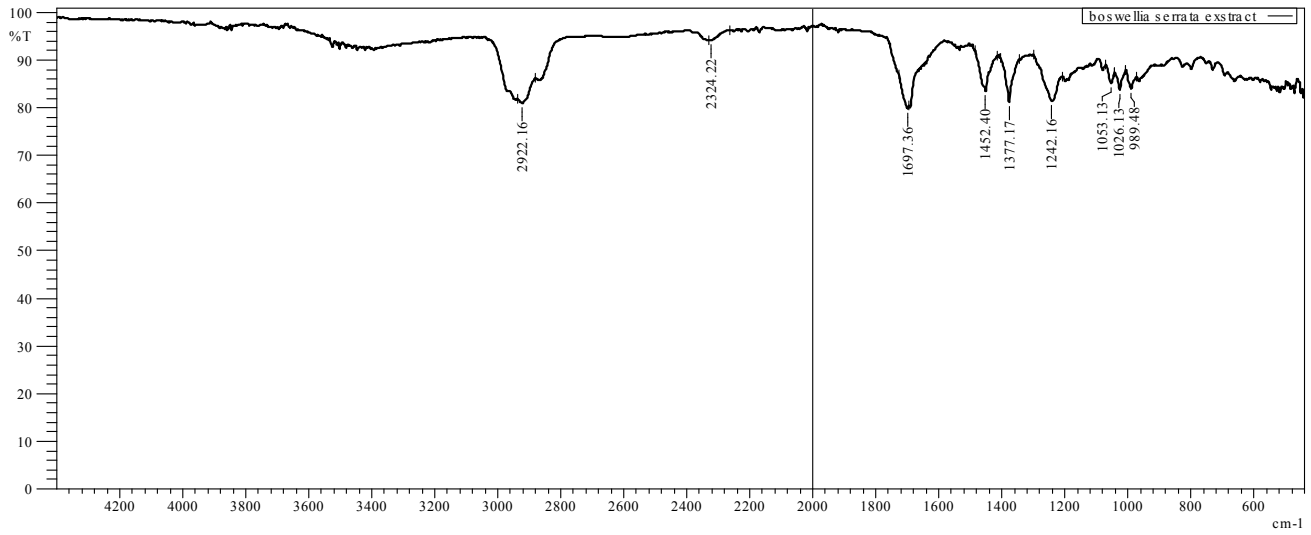

Figure 3. IR spectra of BAF.

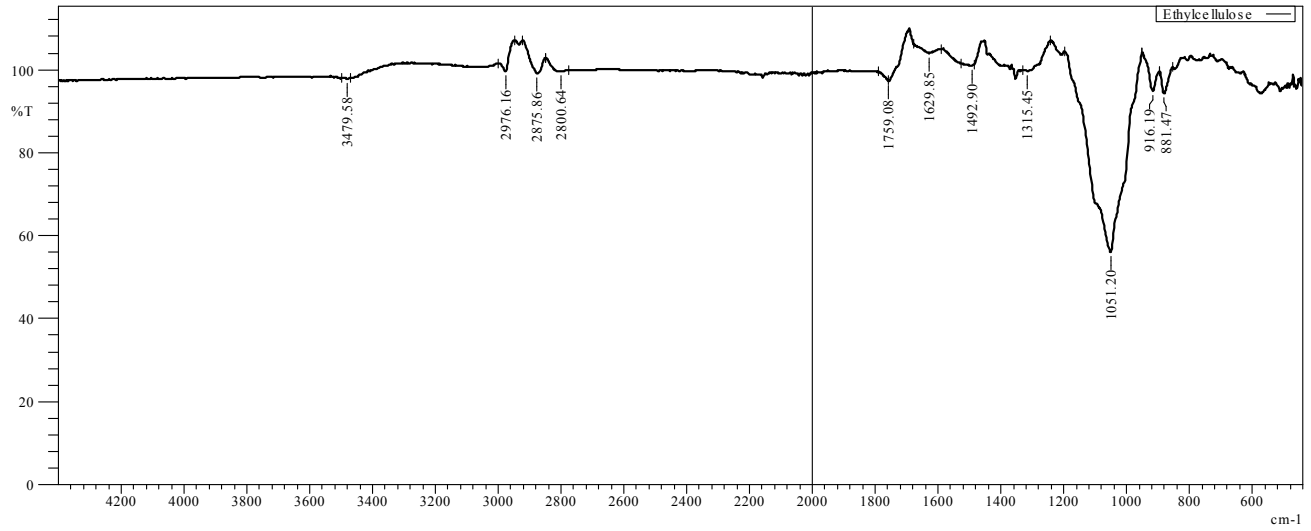

Figure 4. IR spectra of Ethyl cellulose.

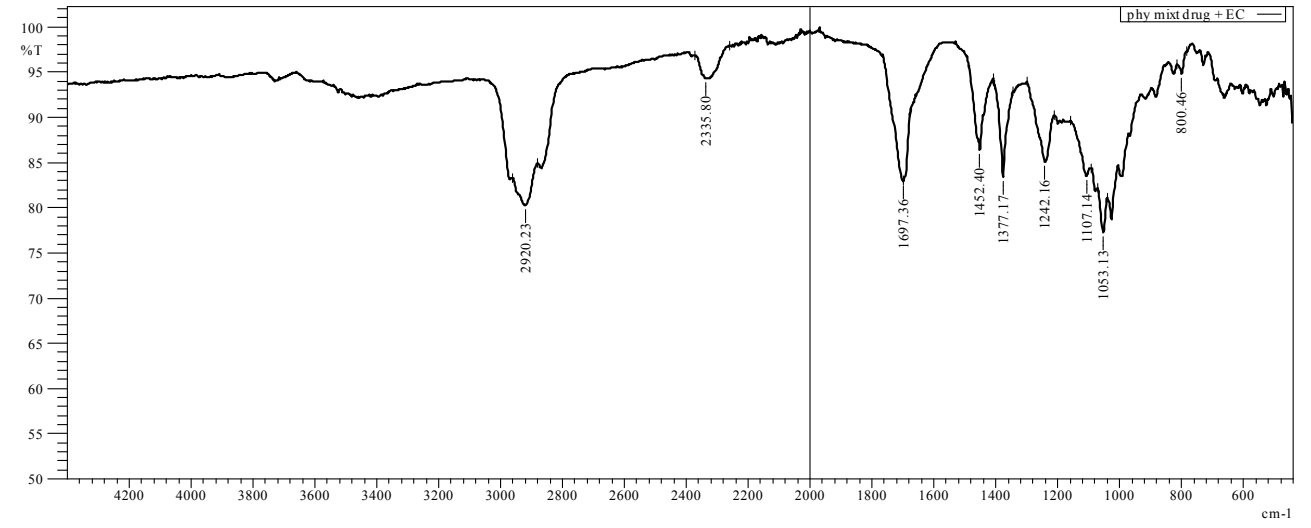

Figure 5. IR spectra of BAF and ethyl cellulose.

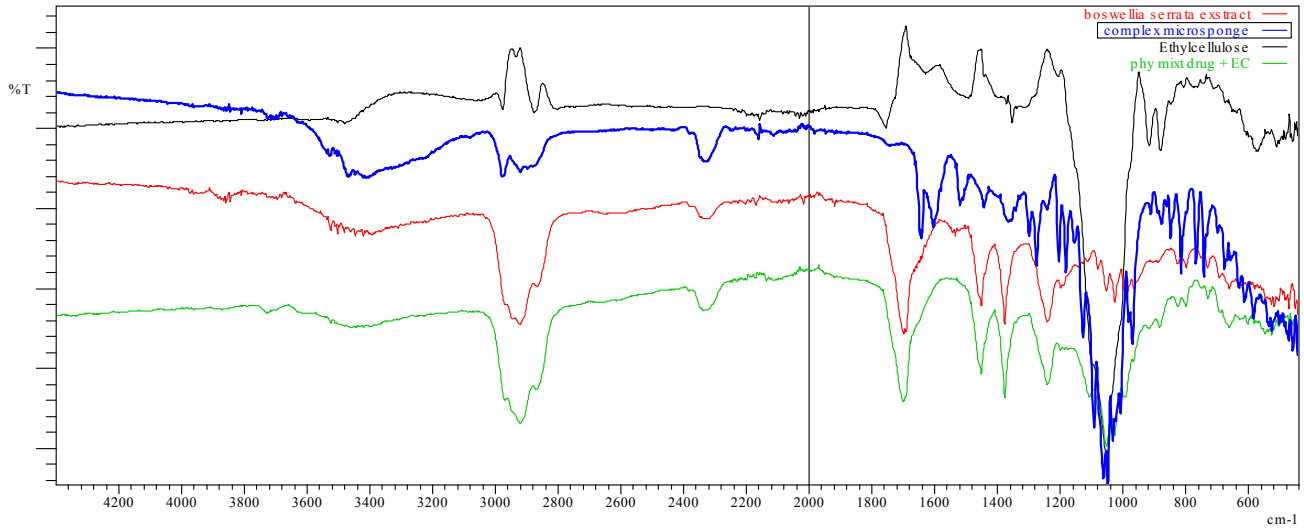

Figure 6. Comparison of IR spectra of BAF.

DSC studies were carried out to confirm the compatibility of BAF *microsponge.* The thermograms, showed a sharp endothermic peak at 91.05°C (Figure 7) corresponding to the melting point of BAF drug in the crystalline form.

The DSC curve of physical mixture of BAF drug and polymers (Figure 8) exhibited the characteristic peak of the drug [20].

The result for *Microsponges* for BAF (Figure 9) is indicative of the compatibility between drug and the polymers and thus provides the suitability of preparation process [21].

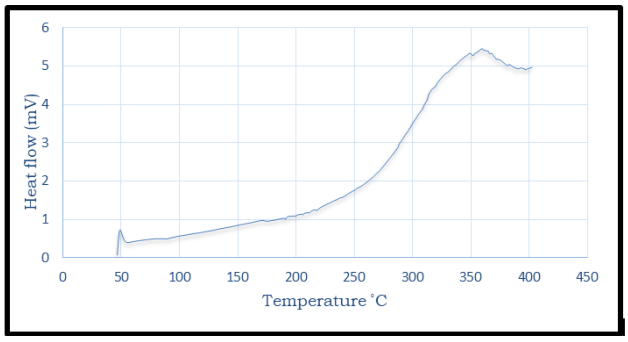

Figure 7. Thermogram of BAF.

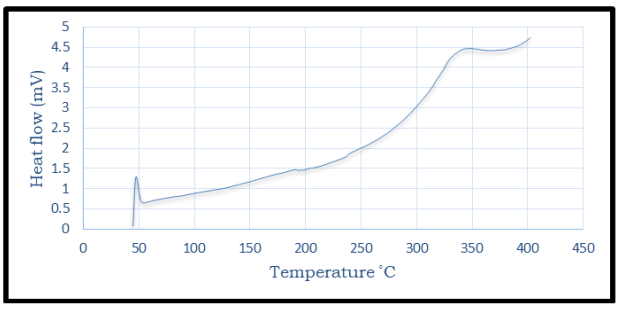

Figure 8. Thermogram of BAF and ethyl cellulose.

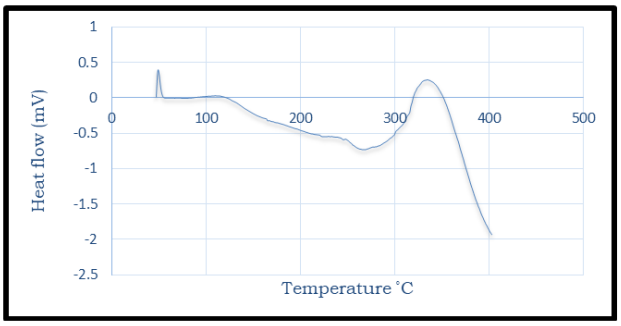

Figure 9. Thermogram of BAF Microsponge.

In the XRD the optimized *Microsponges* retained the peaks corresponding to the crystalline structure of BAF.

Therefore, it was possible to confirm that the crystalline structure of the BAF was maintained, indicating that neither the excipients nor the process affected the crystallinity of the drugs (Figures 10-12).

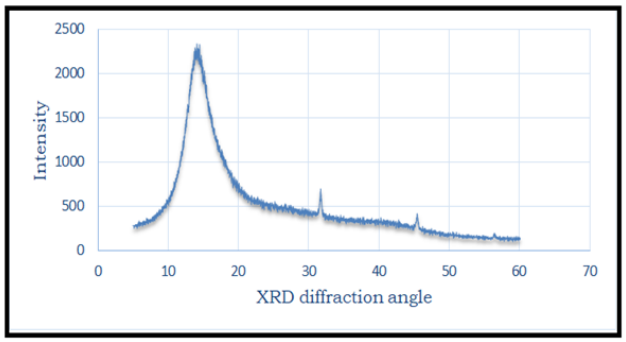

Figure 10. XRD study of BAF.

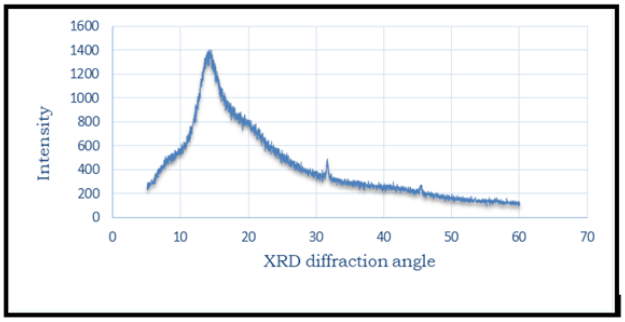

Figure 11. XRD study of Physical mixture of BAF and ethyl cellulose.

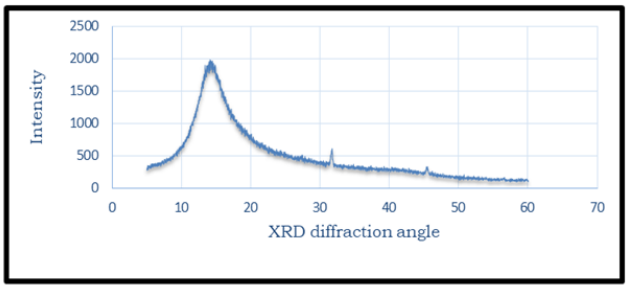

Figure 12. BAF Microsponge.

The microscopic view showed the presence of spherical structures of the *Microsponges.*

The Figure 13 represented the *Microsponges* formed with the drug and the polymer, which resulted in the intercalation.

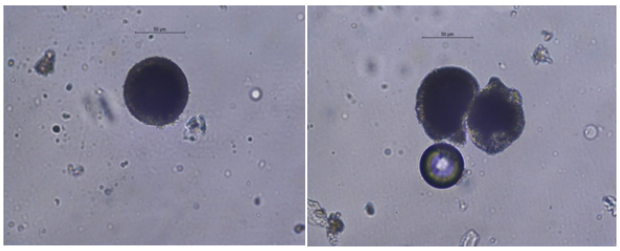

Figure 13. Microscopic images of BAF.

SEM of drug loaded *Microsponges* as shown in Figure 14 revealed that the *Microsponges* were uniform, predominantly spherical in shape, and highly porous in nature.

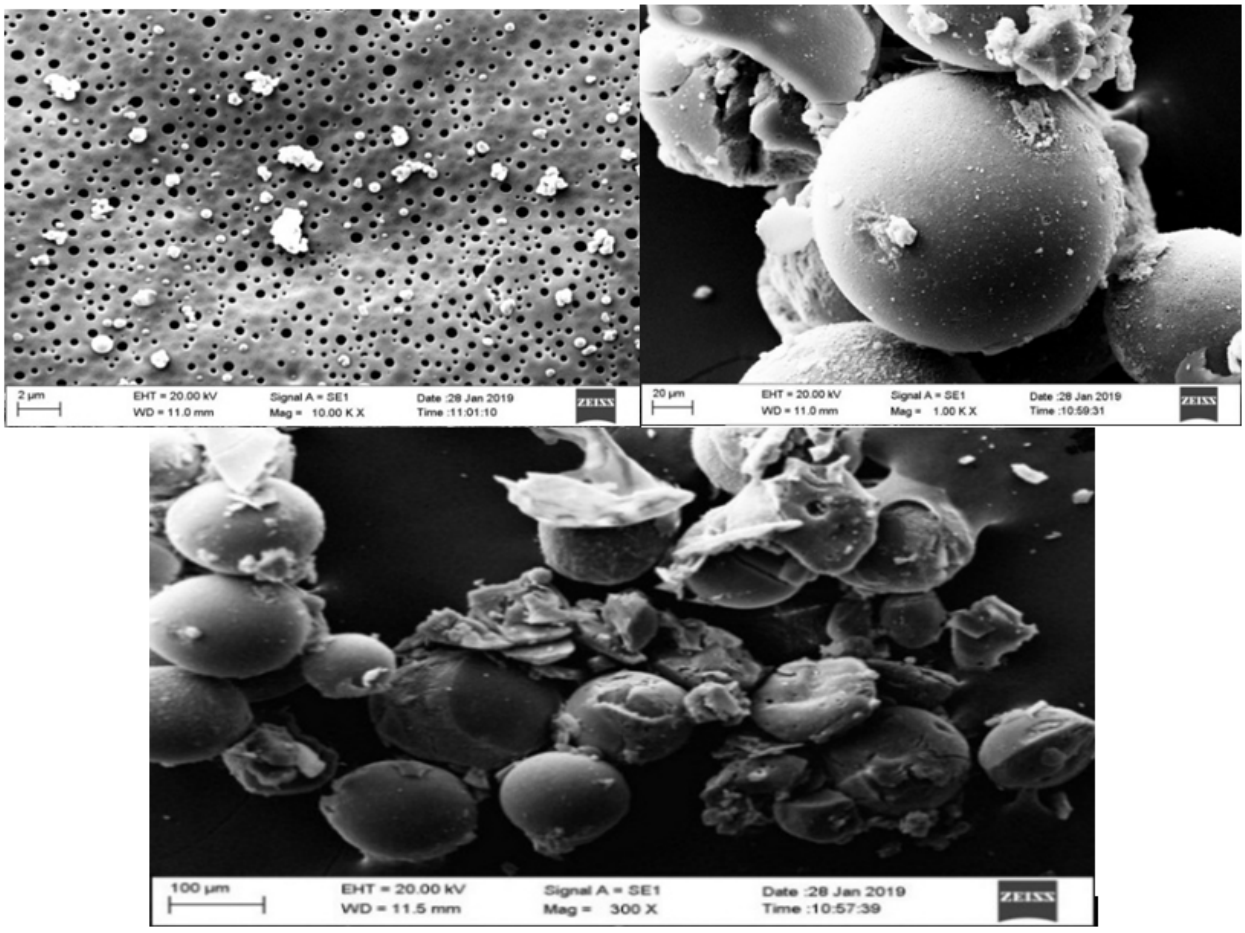

Figure 14: SEM images of BAF drug.

TEM images of BAF complex indicated that based on the TEM results, the *Microsponges* had the most spherical shape and thus exhibited the internal structure of the prepared *Microsponges* (Figure 15).

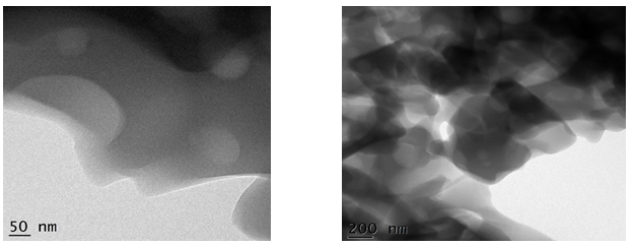

Figure 15. TEM images of BAF microsponge.

Dissolution studies

Figure 16 depicted the dissolution in the phosphate buffer (pH 6.8) at the end of 8 hr. The dissolution studies data was subjected to various release models namely (Figures 17-21), zero order, first order, Higuchi, Peppas, Hixson-Crowell and Korsmeyer-Peppas, and best fit model was decided by highest r2 value. The dissolution drug release showed highest regression value for the zero order model (0.9815 for BAF) and was found to be best fit for the formulation.

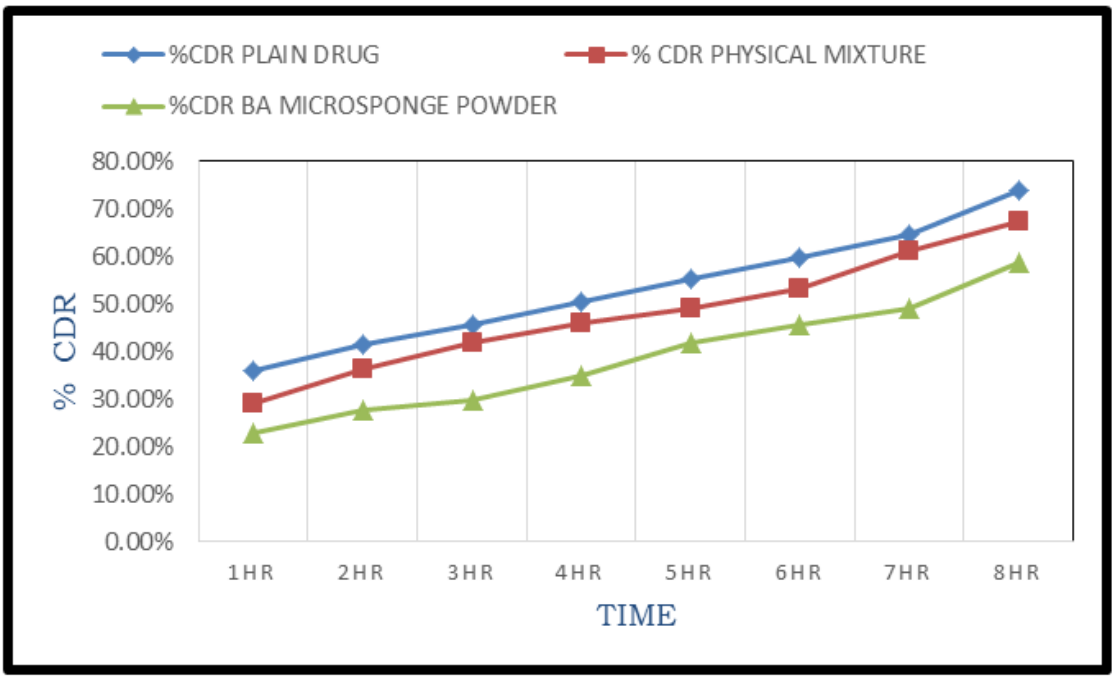

Figure 16. Dissolution study of BAF microsponge.

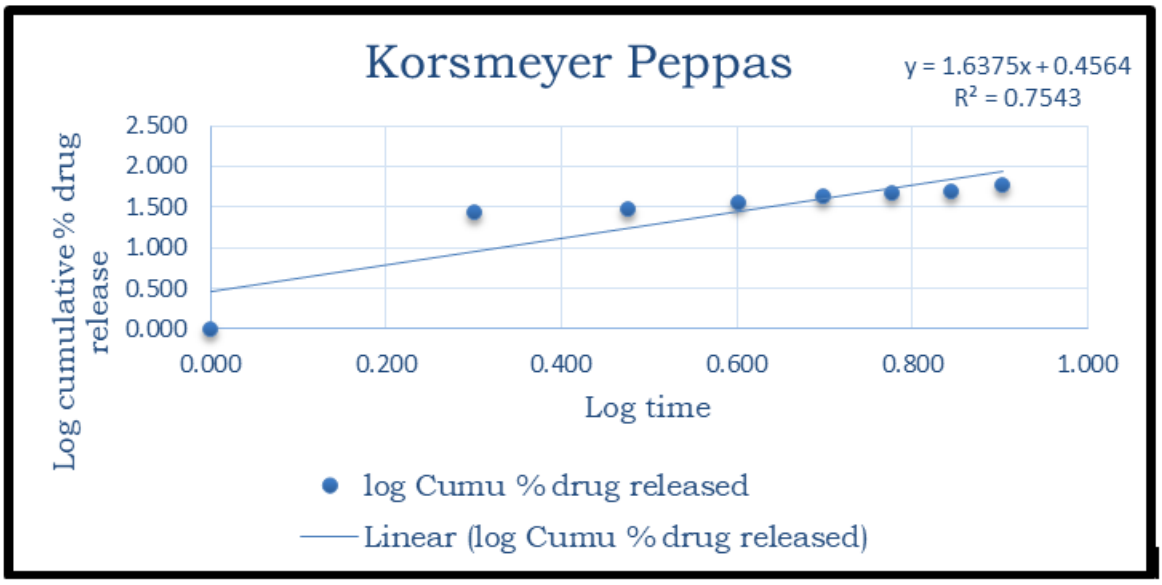

Figure 17. BAF microsponge release model.

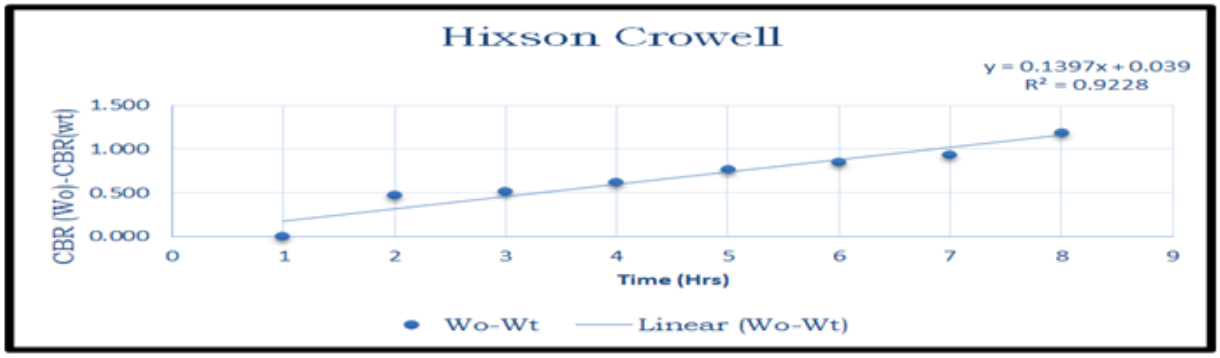

Figure 18. BAF microsponge release model.

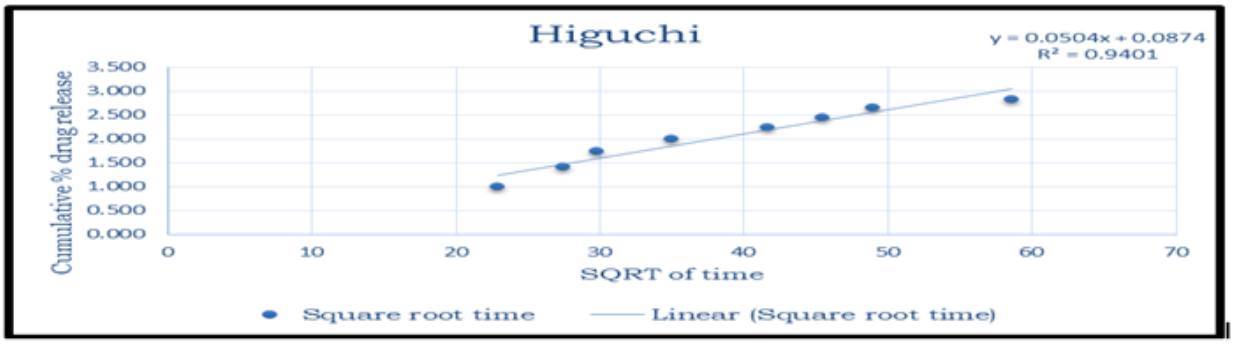

Figure 19. BAF microsponge release model.

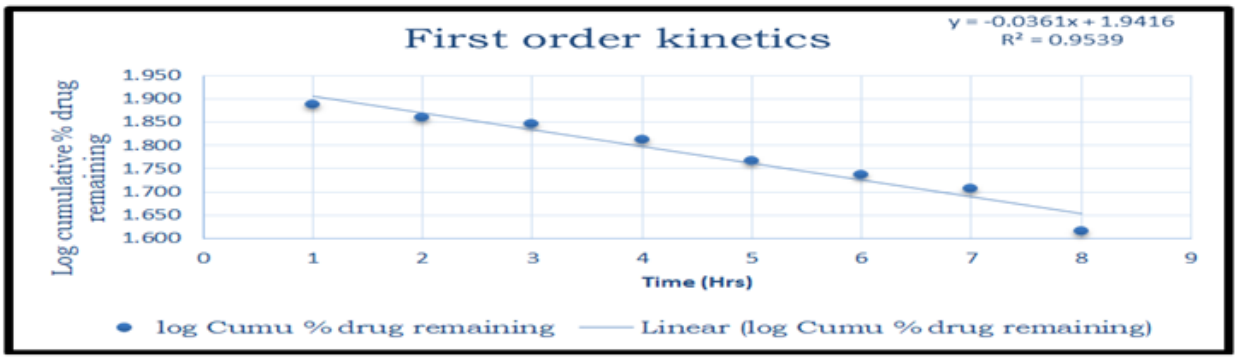

Figure 20. BAF microsponge release model.

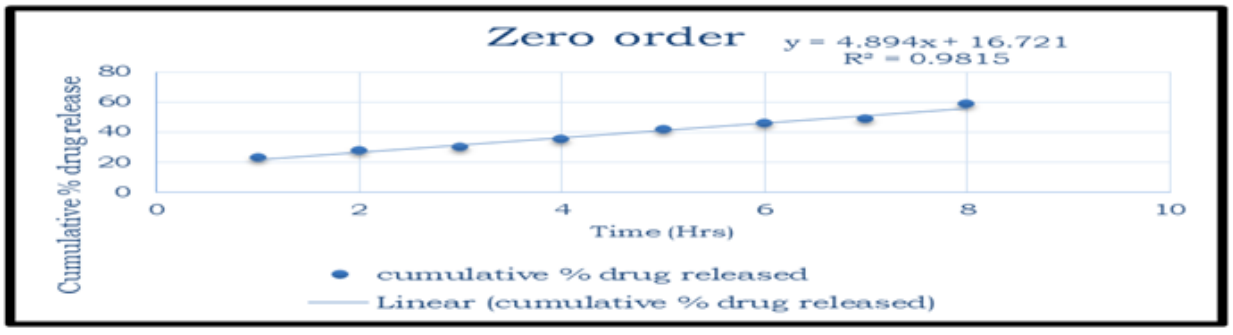

Figure 21. BAF microsponge release model.

In-vitro release study of BAF gel

The influence of composition and vehicle on release profile of different formulations was investigated using a dialysis membrane. *In-vitro* release profiles of BAF *microsponge* from different formulations shown in Figure 22, indicated that the highest drug release i.e. 37.90% was found for the formulation B3, while the lowest 30.34% for B2. Thus indicating the sustained release action of the drug.

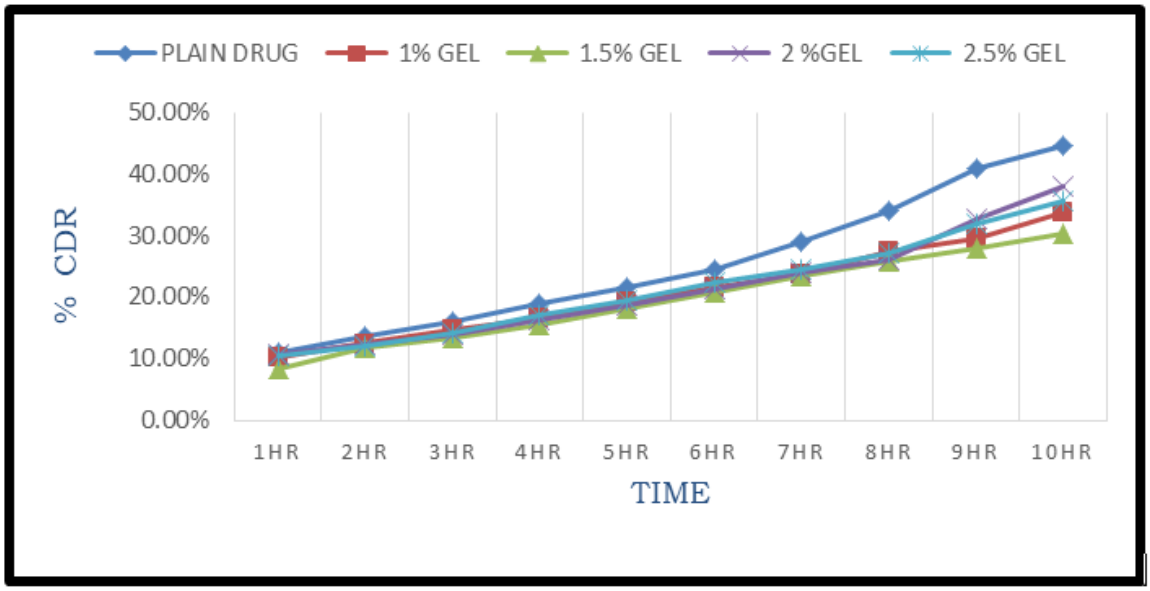

Figure 22. *In-vitro* drug release study of BAF gel.

Primary skin irritation study

The developed formulation of *microsponge* gels and the plain drug of BAF showed no erythema or edema on the intact and abraded rat skin when they were monitored for seven days. Thus the prepared formulations were found to be non-irritant to the rat skin [22].

Ex-vivo permeation study

Amount of BAF *microsponge* deposited in excised rat skin from different formulations at different time intervals has been shown in Figure 23. Hence optimized *microsponge* gel showed lower release rate than control gel. This indicates that *Microsponges* improved the drug residence in skin.

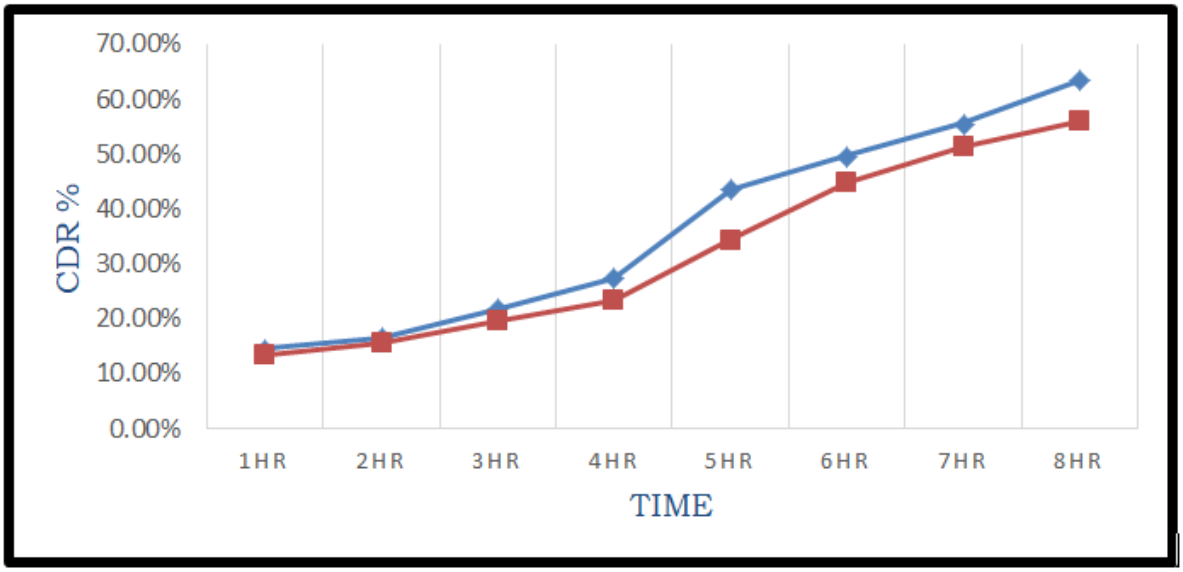

Figure 23. *Ex-vivo* permeability of BAF gel

Conclusion

The present study reported development of BAF loaded *Microsponges* using ethyl cellulose by quasi-emulsion solvent diffusion method. The aim behind developing a polymeric *microsponge* delivery system was to deliver BAF in a sustained manner for an extended period of time, to reduce frequency of administration and to improve its bioavailability. Therefore, in present study, sustained release formulation of BAF was prepared by incorporating it in polymeric *Microsponges.* Prepared *Microsponges* were then incorporated into a gel dosage form. The quasi-emulsion solvent diffusion method implemented was found to be simple, reproducible and rapid. Formed *Microsponges* were spherical in shape, had high porosity and good flow. Varied drug-polymer ratio reflected remarkable effect on particle size, drug content and encapsulation efficiency and stirring time. The dissolution studies showed the highest regression value for zero order kinetics. The formulation B_2 was selected as optimized formulation as it showed lowest % cumulative drug release. Skin irritation test was carried out on rats and the formulation showed no adverse reaction such as redness, erythema and edema. *Ex-vivo* permeation study for BAF was carried out. The maximum amount of gel that permeated during 8 hrs of the study in case of plain gel was 63.32% CDR whereas the BAF optimized gel was 55.94 % CDR.

Thus, BAF *Microsponges* prepared in this study was found to be promising new delivery system offering prolonged release of BAF and hence would be more useful than conventional formulation therapy.

Acknowledgement

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