Innovations

Formulation and Evaluation of Azadirachta Indica loaded Microsponges

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Abstract

The goal of this project is to establish a method for making microsponges, a herbal composition, as well as methods for assessing its ability.By using the quasi-emulsion solvent diffusion method, herbal extracts, ethyl cellulose used as a polymer, and polyvinyl alcohol as an emulsifier present in the formulation, which is used to prepare microsponge formulations of drugs, were successfully obtained. The developed formulations were taken into consideration for in-depth characterisation. The majority of microsponge preparations are made by putting drugs into them, and the results are repeatable.The FTIR, Differential scanning colorimetry, and Scanning electron microscopy examinations were used to characterise the microsponges, and the total drug content and entrapment efficiency were then determined.The effect of a different drug-polymer ratio on particle size, total drug content, and encapsulation effectiveness was striking. The batch F5 has a 96.23% production yield, a maximum TDC of 97.23%, and an entrapment efficiency of 95.87%.All the results were found to be satisfactory.

Keywords: *Microsponges, Azadirachta indica, Total drug content, Total Drug Content, Entrapment efficiency, FTIR, SEM*

Introduction

Recently, there has been a lot of interest in the development of novel microsponge-based drug delivery systems in an effort to control and modify the release characteristics of the drugs. By adding a medication to a carrier system, one can modify the therapeutic index and timeframe of the drug's activity. The increasing use of ingredients like hydroxy acids and nutritional supplements for topical solutions, which can provide pezrceivable and proven benefits, particularly in elderly or photo-damaged skin, has stoked the public's growing interest in skin care and skin treatment products. Despite the fact that these substances are quite beneficial, irritation is a regular side effect.The symptoms of irritation, which are more prevalent in those with sensitive skin, include burning, stinging, and redness.Once they were aware that this problem existed, the formulators tried employing one of the two approaches to solve it. They reduced the concentration of these components despite sacrificing efficacy in the process. They have also altered the vehicle to make the substance more emollient or skin-friendly. [1,2] This method frequently also lessens the good effects of the final product. The market for innovative pharmaceuticals is expanding, patients are becoming more sensitive to clinical outcomes, and healthcare expenses are on the rise, which is driving the need for alternative drug delivery methods and equipment.Medication delivery systems

that can accurately control release rates or direct release have had a substantial influence on the healthcare sector.Numerous reliable and predictable techniques for administering systemic drugs have been developed for transdermal delivery systems (TDS), which employ the skin as a portal of entry. [3] The efficacy and safety of several drugs that may be administered more efficiently by means of skin contact have improved. TDS, however, cannot be used to transport substances whose final destination is the skin. It has only recently been successful to try controlled release of medications onto the epidermis, with the assurance that the treatment will stay primarily localised and won't enter the systemic circulation in significant amounts.No efficient delivery techniques have been developed for the controlled and directed administration of drugs into the stratum corneum and underlying skin layers, not merely the epidermis.The dermis and epidermis can avoid having too much buildup of chemicals thanks to the microsponge system. Potent drugs may become far less irritable thanks to microsponge technology without losing any of their effectiveness. The empty spheres are then removed by the following washing. These requirements are satisfied by the microsponge delivery technology, which has given rise to a brand-new generation of innovative, highly efficient, and extremely well-tolerated goods. These products frequently take the conventional shapes of creams, gels, or lotions and include a sizable number of chemically active ingredients. [1, 4-5]

Microsponges, a type of polymeric drug delivery system, are constructed from porous microspheres. They are tiny, spongy-like particles with a significant porous surface that are rounded in shape. [6,7] Additionally, they could increase stability, reduce side effects, and favourably change drug release. Microsponge technology is a versatile form of pharmaceutical delivery due to its many advantages.[8] Small, polymer-based microspheres that may suspend or entrap a variety of items are the foundation of Microsponge Systems. After that, these microspheres can be included in a specially created product like a gel, cream, liquid, or powder. The application of microsponge drug delivery devices can efficiently improve the effectiveness of topical active ingredients while improving product stability, safety, and aesthetics. Controlling the rate of delivery of active medications to a particular location in the human body has been one of the biggest challenges facing pharmaceutical scientists. The phrase "transdermal delivery system" (TDS), which employs the skin as the portal of entry, is used to describe a variety of trustworthy and predictable methods for systemic medication administration.[9]Microsponges are compatible with the majority of vehicles and components because of their usual pore size of 0.25 m, where bacteria cannot enter, and are stable throughout a pH range of 1 to 11 and temperature ranges up to 130 °C. They may be affordable and self-sterilizing as well. [10] The efficiency of drugs applied topically is increased by using microsponges methods to solve problems like greasiness and stickiness related to topical formulations. [11,12]A polymeric system comprised of porous microspheres known as the "Microsponge drug delivery system" has gained patent protection. They are made up of a large number of linked voids inside of a noncollapsible, and they have the look of sponges. They are tiny, spherical particles a substantial porous surface that permits the controlled release of active substances [13]The size of the microsponges varies from 5 to 300 micrometres, and a typical 25 micrometre sphere can have up to 250000 pores and an internal pore structure that is 10 feet long, giving them a total pore volume of around 1 millilitre per gramme for significant drug retention. [14] According to the literature review there are many herbal microsponges formulations are already reported but the herbal microsoponges of *A. indica* is not reported till date.

Materials and Methods

Materials

*Azadirachta indica*extract was obtained as a gift sample from AmsarPvt. Ltd., Indore, Madhya Pradesh, India. The reagents including ethyl cellulose were obtained from High Purity Laboratory Chemicals, Mumbai. Polyvinyl alcohol, dichloromethane, methanol, polyvinyl alcohol, propylene glycol, carbopol

were procured from SD Fine-Chem. Limited, Mumbai. All other chemicals were of reagent grades and used as procured.

Methods

Phytochemical Screening

The methanolic extracts of leaves of*Azadirachta indica* were subjected to preliminary phytochemical tests to detect the presence of alkaloids, steroids, saponins, glycosides, anthraquinones, tannins, terpenoids, coumarins, carbohydrates and flavonoids using standard techniques.

Preparation of *Azadirachta indica* Microsponges

The quasi-emulsion solvent diffusion approach was used to create *Azadirachtaindica* (AZI) microsponges. In the internal phase, dichloromethane was mixed with ethyl cellulose (2% w/v). With constant stirring at 600 rpm, the medication (100–500 mg) was progressively added to the EC solution. The aqueous exterior phase containing polyvinyl alcohol (0.5% w/v) was then dropped-by-drop into the internal phase (Table 5.1). The microsponges were created by the system's dichloromethane evaporating after two hours of churning. The microsponges were filtered, dried in a hot air oven at 40 °C until their weight was consistent, and then placed in an airtight container for storage.

Table 1: Composition of different batches of Microsponges formulations

S. No.	Formulation	Azadirachtaindica (mg)	Ethyl cellulose (mg)	Dichloromethane (ml)	Polyvinyl alcohol (g)
1.	**F1**	100	100	5	0.5
2.	**F2**	200	100	5	0.5
3.	**F3**	300	100	5	0.5
4.	**F4**	400	100	5	0.5
5.	**F5**	500	100	5	0.5
6.	**F6**	600	100	5	0.5
7.	**F7**	700	100	5	0.5

Characterization of Microsponges

Particle size analysis

Using a Mastersizer 2000 (Malvern Instruments Ltd.) at 25 °C, the average particle size and polydispersity index (PdI) of all the batches of microsponges were determined.

Total drug content and entrapment efficiency

Total drug content and effectiveness of entrapment with intermittent stirring, the weighed quantities of the drug-loaded microsponges (10 mg) were dissolved in 10 mL of the pH 7.4 methanolic phosphate buffer solution. After the aforementioned sample was properly diluted to 1 mL with methanolic phosphate buffer, the absorbance at 428 nm was measured against a blank using methanolic phosphate buffer solution, where the value of E1% is 0.206. The following equation was used to get the total drug content:

Total drug content = Abs/E1% X dilution factor X 10

The drug entrapment efficiency (%) was calculated as:

%EE = TDC/amount of drug added X 100

where TDC is the total drug content in Microsponges and %EE is the percentage of entrapment efficiency of the Microsponge.

Fourier transform infrared spectroscopy (FTIR)

Using KBr pellets in a Fourier transform infrared spectrophotometer (Shimadzoo), AZI, EC, and AZI microsponges samples were examined using Fourier transform infrared spectroscopy in the wavelength range of 4000 to 400 cm-1.

Differential scanning calorimetry (DSC) analysis

The samples were heated from 30 to 300 °C at a rate of 10 °C per minute for the DSC analysis of CUR and CUR microsponges (Perkin Elmer).

Scanning electron microscopy (SEM)

SEM (Environmental Scanning Electron Microscope, model FEI Quanta 200F with Oxford-EDS system IE 250 Max 80, The Netherlands) was used to evaluate the AZImicrosponges' surface and form after coating. The samples were put on metal grids with double-sided adhesive tape before being seen, and they were then vacuum-coated with gold.

Stability study

According to ICH recommendations, the stability tests on AZI microsponges were performed under accelerated settings. For three months, the microsponge formulations were stored at 40°C± 2 °C and 75 % ±5% RH. Microsponges were examined for their outward appearance, in vitro drug release, and FTIR spectroscopy after three months.

Result and Discussion

Phytochemical test

Phytochemical analysis of *A. indica* aqueous leaf extract indicated the presence of alkaloids, tannins, flavonoids, sterols, terpenoids, and saponins. All of these types of chemicals have been shown to have significant biological activity (Table 1).

Table 2: Preliminary Phytochemical Screening of A. indica

S. No.	Chemical Constituents	Ethanolic	Aqueous	Pet. Ether	Chloroform
1	Alkaloids	+	+	+	+
2	Carbohydrates	+	+	+	+
3	Glycosides	+	+	+	+
4	Steroids	+	+	+	+
5	Flavonoids	+	+	+	+
6	Saponins	+	+	+	+
7	Fixed oils and fats	-	-	-	-
8	Tannins	+	+	+	+
9	Proteins and amino acids	-	-	+	-
10	Terpenoids	-	-	-	-

Abbreviations: (+) is positive; (-) is Negative

Characterization of Microsponges

The creation of AZI microsponges used the quasi-emulsion solvent diffusion process. These microsponges contained carbopol gel and were encased in capsule shells. Different metrics were used to describe the microsponges, and diverse approaches were used to assess the capsules and gel.

Particle Size Analysis

Table 2 displays the manufacturing yield (%) and mean particle size of microsponges. Up to batch F5, which has a drug to polymer ratio of (5:1), it is discovered that increasing the drug to polymer ratio results in an increase in production yield and mean particle size. However, as the drug to polymer ratio is increased to (7:1), a decrease in production yield and entrapment efficiency is observed. This may be caused by a lack of polymer available to entrap the drug, and the rise in mean particle size may be brought on by a higher drug to polymer ratio.

Table 2: Effect of drug to polymer ratio on various parameters

S. No.	Formulation Code	Drug/Polymer ratio	Total drug content (%)	Production yield (%±SD)	Mean particle size (µm)	Entrapment efficiency (%)
1.	F1	1:1	50.23	70.21±0.21	55.23±1.52	53.45
2.	F2	2:1	56.43	75.45±0.43	56.23±1.67	58.12
3.	F3	3:1	63.5	80.20±0.12	57.76±1.32	62.67
4.	F4	4:1	68.20	85.56±0.24	97.12±1.45	86.78
5.	F5	5:1	97.23	96.23±0.21	113.23±1.32	95.87
6.	F6	6:1	74.56	81.34±0.54	105.43±1.78	74.12
7.	F7	7:1	78.20	86.74±0.23	62.24±1.67	76.56

Total drug content (TDC) and entrapment efficiency (EE)

The UV spectrophotometric approach was used to determine TDC and EE in various formulations. The effective molecular association of the drug with the polymers determines the overall drug content and drug entrapment. TDC and EE of the microsponges ranged from 50.23 to 97.23 and 53.45 to 95.87 of various batches, respectively (Table 2). For the formulation F5 with a 5:1 drug to polymer ratio, the values of TDC and EE were determined to be at their highest. As the drug/polymer ratio was raised further, a decline in TDC and EE was seen. The absence of the ideal polymer concentration to coat or entrap the drug molecules may be the likely cause of this drop in TDC and EE.

Fourier transform infrared spectroscopy

The FTIR spectra of AZI, EC, and AZI microsponge are shown in Figure 1. The distinctive transmittance bands for AZI were found in the spectra, as shown in Figure1(a), at 1558.36, 1506.30, 1251.72, and 1157.21cm-1. These wavelengths correspond to the stretching -C=C vibrations of benzene, aromatic -C-O stretching of (-OH and -OMe), and -C-O-C stretching (-OMe). At 3583.49 cm-1, distinctive bands for conjugated ketonic -C=O and phenolic -OH vibrations were also seen. The spectra of EC, shown in Figure1(b), exhibit the characteristic absorption of alcoholic hydroxyl groups at 3448.39cm-1, while the spectra of AZI microsponges, shown in Figure 1(c), exhibit broadening of the band at approximately 3429.20 cm-1, as well as characteristic bands in the range of 1556.45-896.84 cm-1. Comparing the spectra, however, did not reveal any new distinctive peaks in the microsponge, indicating that there were no physical or chemical interactions between the AZI and the carrier polymer.

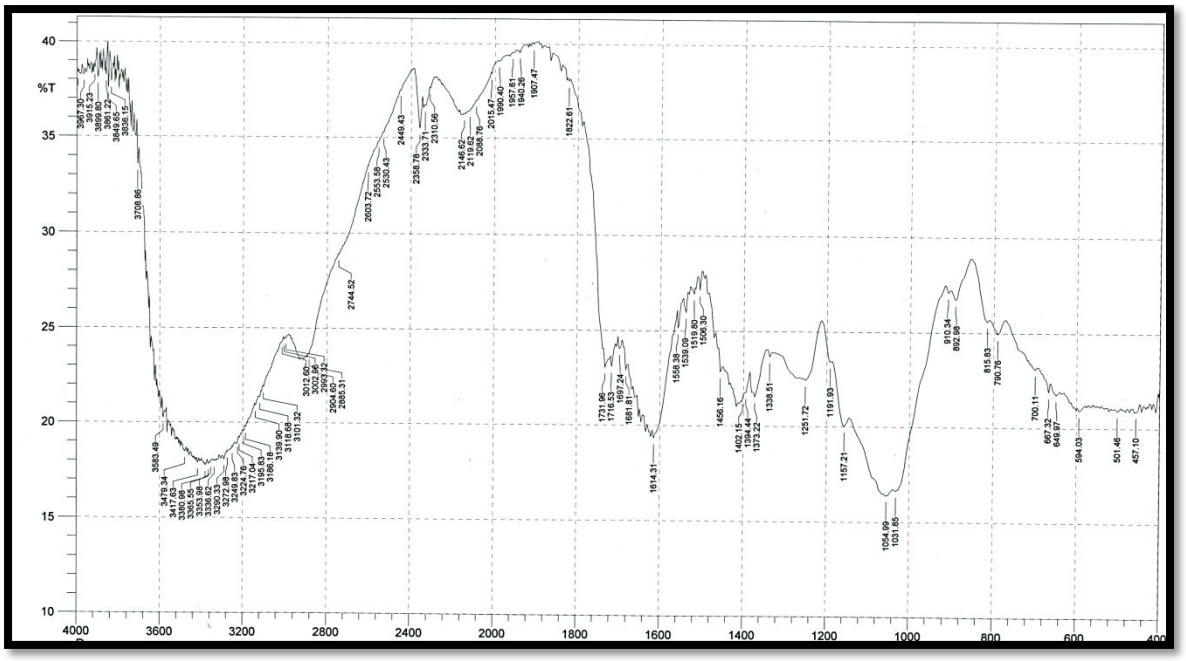

Figure 1(a): FTIR of AZI

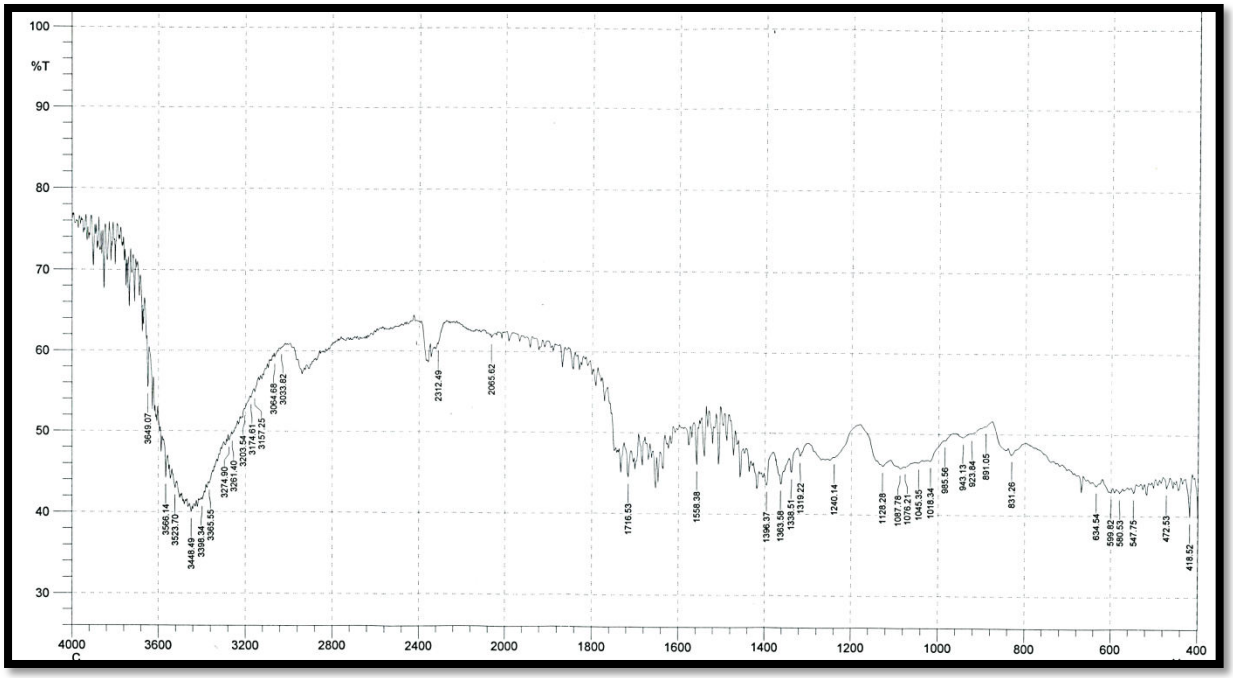

Figure 1 (b): FTIR of Ethyl Cellulose

Figure 1 (c): FTIR of Microsponges

Differential scanning calorimetry

The thermogram of the AZI, EC, and AZI microsponges is shown in Figure 2. Because it is amorphous, ethyl cellulose lacks strong endothermic peaks, but AZI thermogram reveals a sharp exothermic peak at 161.191 °C with a heat of fusion of 296.415 J/g, which corresponds to its melting temperature (Figure 2 (a)). The thermogram of AZI microsponges exhibits aa board exothermic at 162.34°C, and an endothermic at 197.24 °C. Changes have taken place, as evidenced by the shifting of endotherms, the appearance of a new exotherm, and a drop in the heat of fusion (Figure 2 (b)).

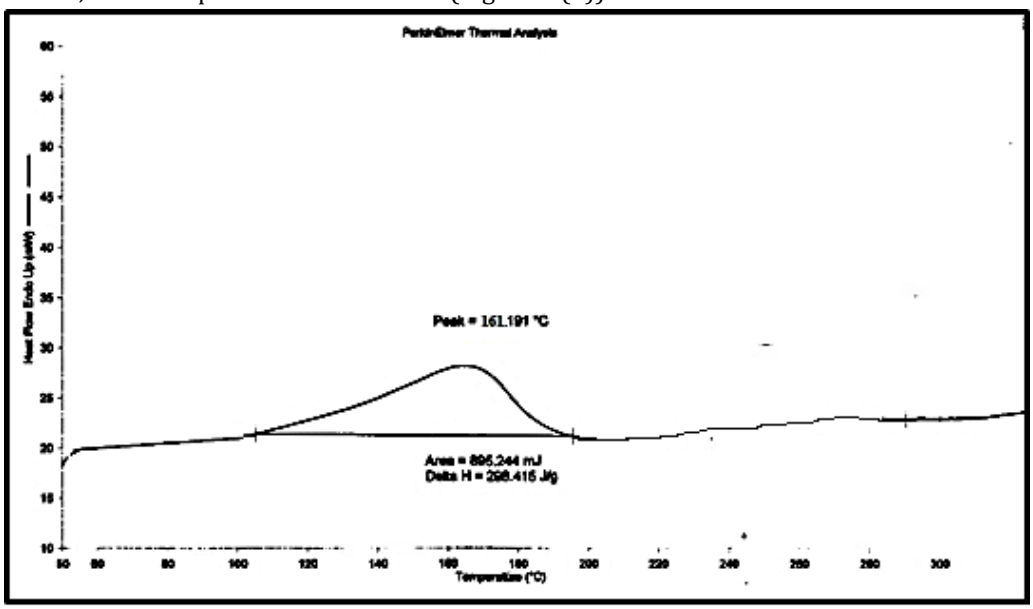

Figure 2 (a): DSC of AZI

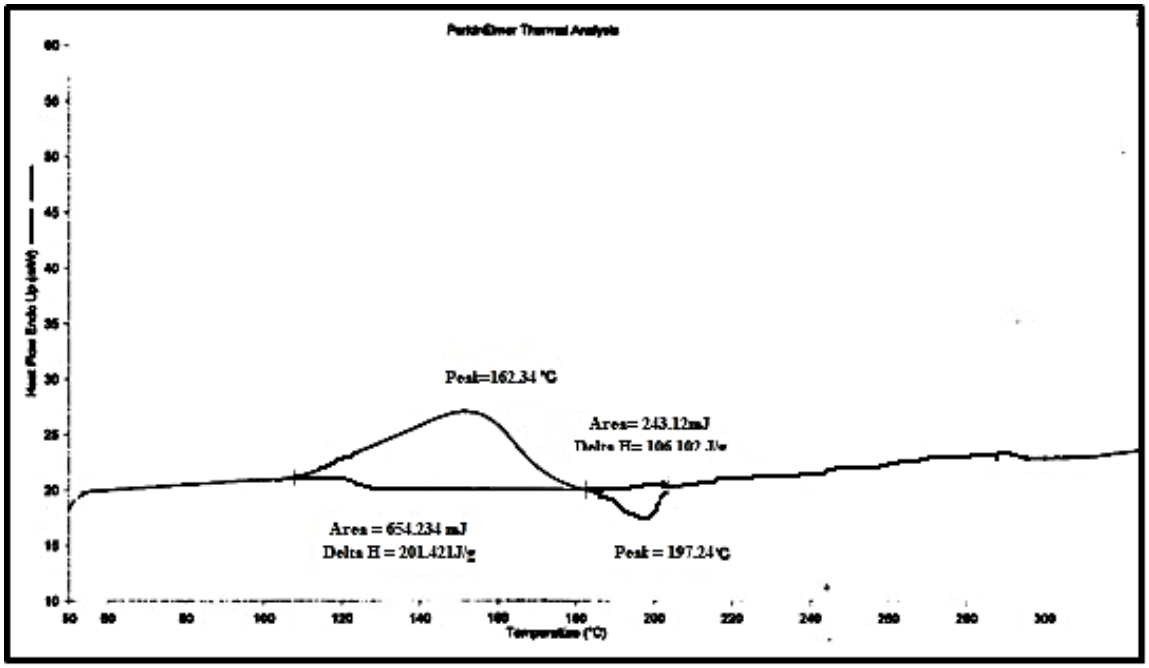

Figure 2 (b): DSC of AZI Microsponges

Scanning Electron Microscopy

The SEM in Figure 3 displays the surface morphology of AZI microsponges. SEM micrographs showed that the majority of the microsponges generated are spherical, and whole AZI crystals are not visible.

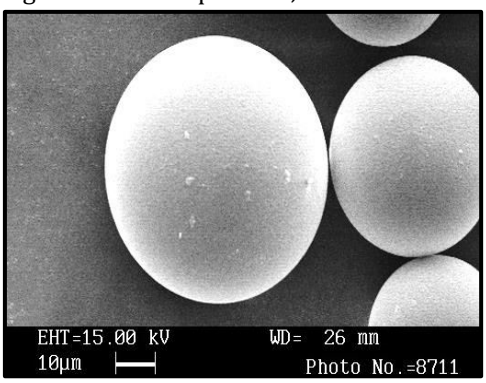

Figure 3. Microsponges of AZI

Stability Studies

The batch F7 was examined for physical appearance, in vitro drug release, and FTIR spectroscopy during a three-month stability investigation under accelerated settings. The formulation was discovered three months later with hardly any visual changes. The in vitro drug release percentage was determined to be 94.56 comparable, and the FTIR spectra showed no indication of instability. All of these factors indicated that the F7 formulation may have a long shelf life.

Conclusion

In order to distribute AZI continuously for a prolonged length of time, lower the frequency of administration, and increase bioavailability, a polymeric microsponge delivery system was developed. Therefore, the quasi-emulsion solvent diffusion approach was used in the current investigation to quickly and easily create AZI microsponges. Studies on the formulation's FTIR, DSC, and SEM properties were conducted. The produced microsponges were then filled with carbopol gel and added to capsule dosage

forms. The effect of a different drug-polymer ratio on particle size, total drug content, and encapsulation effectiveness was striking. The batch F5 has a 96.23% production yield, a maximum TDC of 97.23%, and an entrapment efficiency of 95.87%. Further in future we can use this formulation for the development of different dosage form and the activity of the herbal microsponges.

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Conflict of Interest
Authors have no conflict of interest.

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