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RESEARCH ARTICLE
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DEVELOPMENT AND CHARACTERIZATION OF MICROSPONGE GEL CONTAINING PIPERINE FOR ANTI-BACTERIAL

Mr. Ayush Sidhu[1*], Dr. M.K. Gupta[2], Dr. Anjana Devi[3]

[1*]Research Scholar, Career Point School of Pharmacy, Kota (Rajasthan)
[2]Dean and Principal, Career Point School of Pharmacy, Kota (Rajasthan)
[3]Associate Professor, Department of Pharmacy, Career Point University, Hamirpur,H.P.

***Corresponding Author:** Satbir Singh
*Associate Professor, Pt. LR College of Pharmacy, Faridabad, Email: satbirpharma89@gmail.com, Mobile: 8708977210

Abstract
The polymeric system made up of porous microspheres that can entrap and release Microsponges into the skin over an extended length of time is the highly cross-linked, porous microsphere delivery system used in Microsponges. With less discomfort, increased tolerance, and enhanced thermal, physical, and chemical stability, this delivery technique offers longer release. Emulsion systems or suspension polymerization into a liquid-liquid system are two methods used to create Microsponges. Microsponges are capable of encasing different kinds of drugs and can be used in lotions, creams, powders, and gel formulations. Piperine is found in black pepper, white pepper and long pepper belonging to the family Piperaceae. Piperine represents diverse biological activities, such as anti-inflammatory, anticancer, antiviral, anti-larvicidal, pesticide, anti-Alzheimer's, antidepressant and most importantly piperine is known as the bioavailability enhancer. The current review article aims to explore the Development and Characterization of Microsponge Gel Containing Piperine for Anti-Bacterial effect.

Keywords: Microsponges, Oral administration, Controlled release, Piperine, Antibacterial

INTRODUCTION

Polymeric drug delivery devices called microsponges are made of porous microspheres. Originally, microsponges were created to administer medications topically. Microsponges were large porous beads filled with active substances that typically had a diameter of 10–25 microns. Microsponges are porous microspheres made of polymeric material that are mostly used for extended standard doses. Gels, on the other hand, are semisolid systems made of small or large molecules dispersed in an aqueous liquid medium that are become jelly-like by adding a gelling agent.[1,2] Topical drug administration is a localized drug delivery system anywhere in the body through ophthalmic and skin as topical routes.[3] Skin is the one of the most readily accessible organs on human body for topical administration and is main route of topical drug delivery system.[4] An antibacterial drug represents a chemical substance derived from a biological source or produced by chemical synthesis that is able to destroy or to inhibit the development/growth of bacteria.[5] It is the most important type of antibacterial agent for fighting bacterial infections, and antibiotic medications are widely used in

the treatment and prevention of such infections. They may either kill or inhibit the growth of bacteria.[6]

PIPERINE

Piperine is isolated from the fruit of *Piper nigrum*, the source of plant both black and white pepper. Its melting point 129 °C Piperine is the alkaloid that gives black pepper and long peppers their pungent flavor. Piperine has anti-bacterial property.[7,8] Black pepper (Piper nigrum), long pepper (Piper longum), and other fruits from the Piperaceae family of plants contain the alkaloid piperine, which is one of the most widely used spices. Piperine is responsible for black pepper's unique biting quality. Especially when used to treat chronic illnesses, piperine has a wide spectrum of pharmacological activities and health benefits. Hepatic steatosis therapy and reduced insulin resistance are a couple of these benefits.[9,10]

MATERIALS & METHODS

MATERIALS USED

List of materials & equipments that was used in the formulation are listed in table 1 and 2.

Table 1: MATERIALS USED[11,12]

Sr. No.	Materials
1	Piperine
2	Xanthan Gum
3	Ethyl Cellulose
4	Sodium hydroxide
5	Methanol
6	Iso-propyl alcohol
7	Potassium Dihydrogen orthophosphate
8	Disodium hydrogen orthophosphate
9	HCl
10	n-octanol
11	Chloroform
12	Acetone

EQUIPMENTS USED

Table 2: EQUIPMENTS USED[13,14]

Sr. No.	Name of equipment
1	UV Spectrophotometer
2	Hot Air Oven
3	Cooling Centrifuge
4	Dissolution Apparatus
5	Magnetic Stirrer
6	pH meter
7	Microscope
8	Ultrasonic Bath
9	Water Bath Shaker
10	Mechanical Stirrer
11	Melting Point Apparatus
12	Vortex mixer

Formulation Development

Table 3: Composition of Different Piperine microsponge[15,16]

Formulation Code	Drug (mg)	Ethyl cellulose (mg)	DCM (ml)	PVA (mg)	Water (ml)
F1	200	100	20	0.50%	60
F2	400	100	20	0.50%	60
F3	600	100	20	0.50%	60
F4	800	100	20	0.50%	60
F5	1000	100	20	0.50%	60
F6	1200	100	20	0.50%	60

Composition of Different Piperine Microsponge gels

Table 4: Composition of Different Piperine Microsponge gels[17]

Sr. No.	Composition	Formulation Code		
		F7	F8	F9
1	Piperine microsponge	Equivalent to 10mg of Piperine drug for 10mg of gel		
2	Carbopol 934 (%w/v)	1%	1.50%	2%
3	NaOH	Q.S	Q.S	Q.S

Method of Preparation of Piperine microsponges gels

The improved carbopol 934 was dissolved in 10 ml of distilled water to make a gel formulation, which was left at room temperature for 24 hours. The polymer dispersion was put aside so that the polymer could swell completely. The Microsponges gel was subsequently made by mixing Microsponges with gel dispersion. Drop by drop, sodium hydroxide solution was added to maintain the pH at 7.5. The developed Microsponges gel was evaluated for a variety of aspects.[18-22]

RESULT AND DISCUSSION

PREFORMULATION STUDIES

1. **Organoleptic Properties**

Organoleptic properties of drug are listed in table 5.

Table 5: Organoleptic Properties[23]

Properties	Description
Form	Crystalline Powder
Odour	Odorless
Colour	A white powder

2. Melting point[24]

Table 6: Melting point

Drug	Observed melting point	Reference melting point
Piperine	129.67±0.58°C	128-130 °C

The melting point of Piperine was found to be 129.67± 0.58 °C, the medication sample was devoid of all contaminants.

3. Determination of absorption maxima by UV spectroscopy[25]

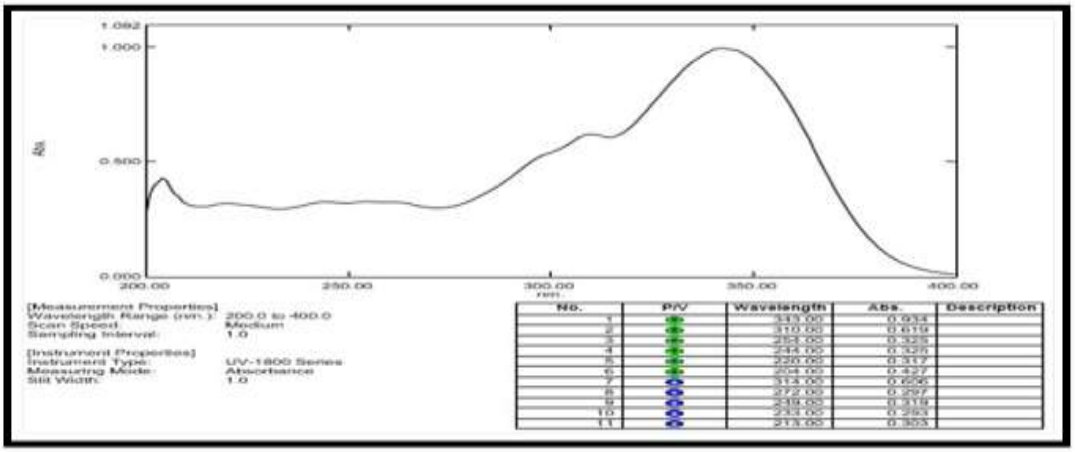

Figure 1: Absorption maxima by UV spectroscopy of Piperine

Table 7: Absorption maxima of Piperine

Name of drug	Absorption maxima (λ_{max})	
	Observed	Reference
Piperine	343 nm	342nm

Piperine was determined to have a maximum wavelength of 343 nm, which is in agreement with reference standards.

Preparation of calibration curve of Piperine in water

A 1000 g piperine standard stock solution was made using water. This solution was diluted to the appropriate concentrations (17µg/ml) with methanol before being analyzed spectrophotometricaly at 343 nm.[26]

Table 8: Calibration curve of Piperine in methanol

Sr.no.	Concentration µg/ml	Absorbance
1	1	0.132±0.002
2	2	0.263±0.001
3	3	0.399±0.001
4	4	0.532±0.001
5	5	0.668±0.002
6	6	0.831±0.002
7	7	0.990±0.005

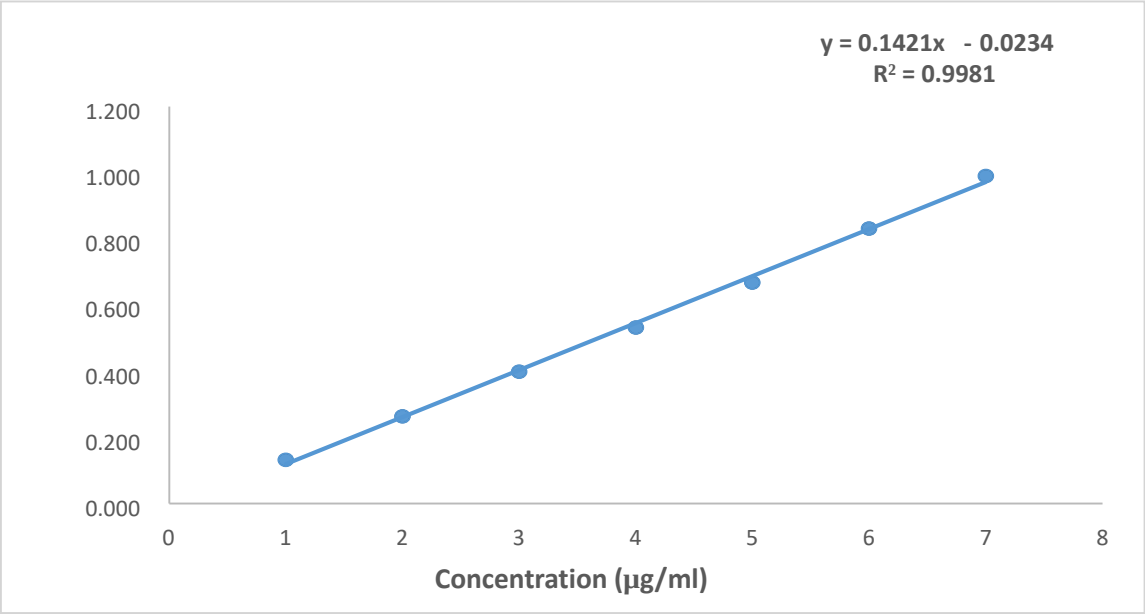

Figure 2: Calibration curve of Piperine in Methanol

Discussion: With piperine concentrations ranging from 1 to 7µg/ml, methanol was used to generate the calibration curve for piperine. At 343 nm, the absorbance was determined. The standard curve for piperine is shown in table 8. The regression equation is given by y= 0.1421x-0.0234 and the R2 value is 0.9981, indicating good linearity.

4. Partition coefficient determination[27]

The shake flask method was used to carry out the investigation on partition coefficient determination.

Table 9: partition coefficient of piperine.

Drug	Solvent system	Log P Values
Piperine	n-octanol:water	2.091± 0.020

Discussion: The partition coefficient of Piperine in n-Octanol: Water; was found to be 2.091±0.020, was consistent with the literature. This exhibits that Piperine has lipophilic properties.

5. Solubility studies[28]

Table 10: solubility profile of Piperine in different solvent

Sr. no.	Solvent	Solubility (mg/ml)	Solubility
1	Ethanol	49.313±0.107	Soluble
2	Methanol	34.886±0.107	Soluble
3	Chloroform	33.713±0.177	Soluble
4	PB pH 7.4	0.041±0.001	Practically insoluble
5	Water	0.033±0.002	Practically insoluble

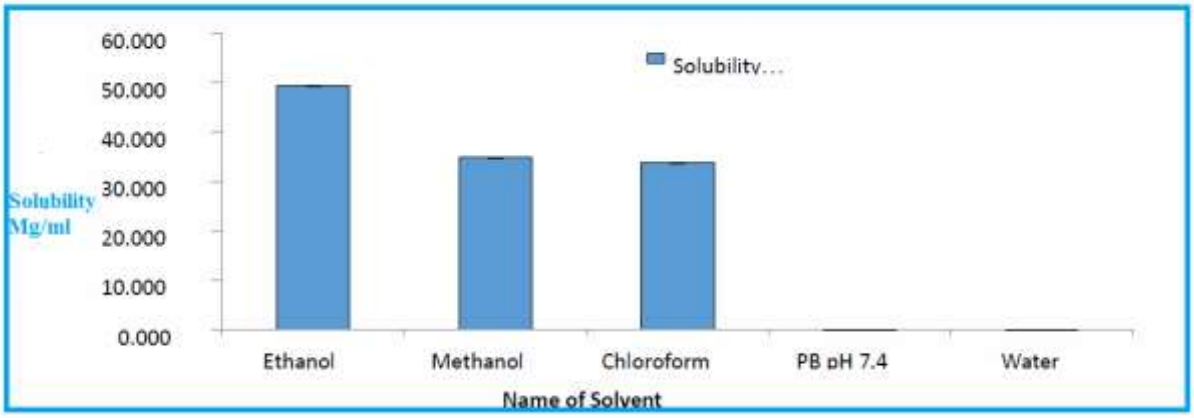

Figure 3: Solubility profile of Piperine in different solvent

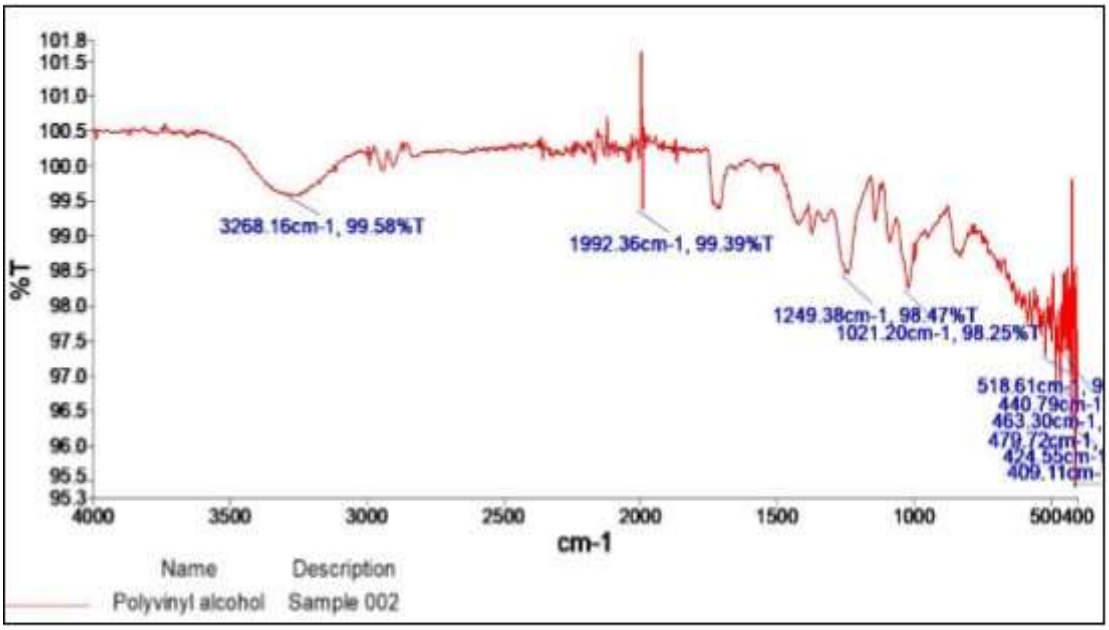

Figure 4: FTIR spectrum of Piperine

6. FTIR Analysis of crude Drug and Excipients[29]

Table 11: Interpretation of FTIR spectrum of Piperine

Reported peak (cm^{-1})	Observed peak (cm^{-1})	Functional group
3000-2800	3009.11	N-H stretching
2840-3000	2940.75	C-H stretching
1900-2000	1992.5	C-H bending (aromatic)
1680	1633.38	C=O stretching
1150-1085	1113.5	C-O stretching

Figure 4 and table 11 both displayed the piperine FTIR spectra. The major N-H stretching, C-H stretching, C-H bending (aromatic), 1633.38 cm-1, C=O stretching, and CO stretching IR absorption peaks of piperine were found. The C-H stretching, C-H bending (aromatic), and 1992.50 cm-1 IR absorption peaks were found to be the next most significant peaks. These big peaks were evidence of the authenticity and purity of the Piperine.

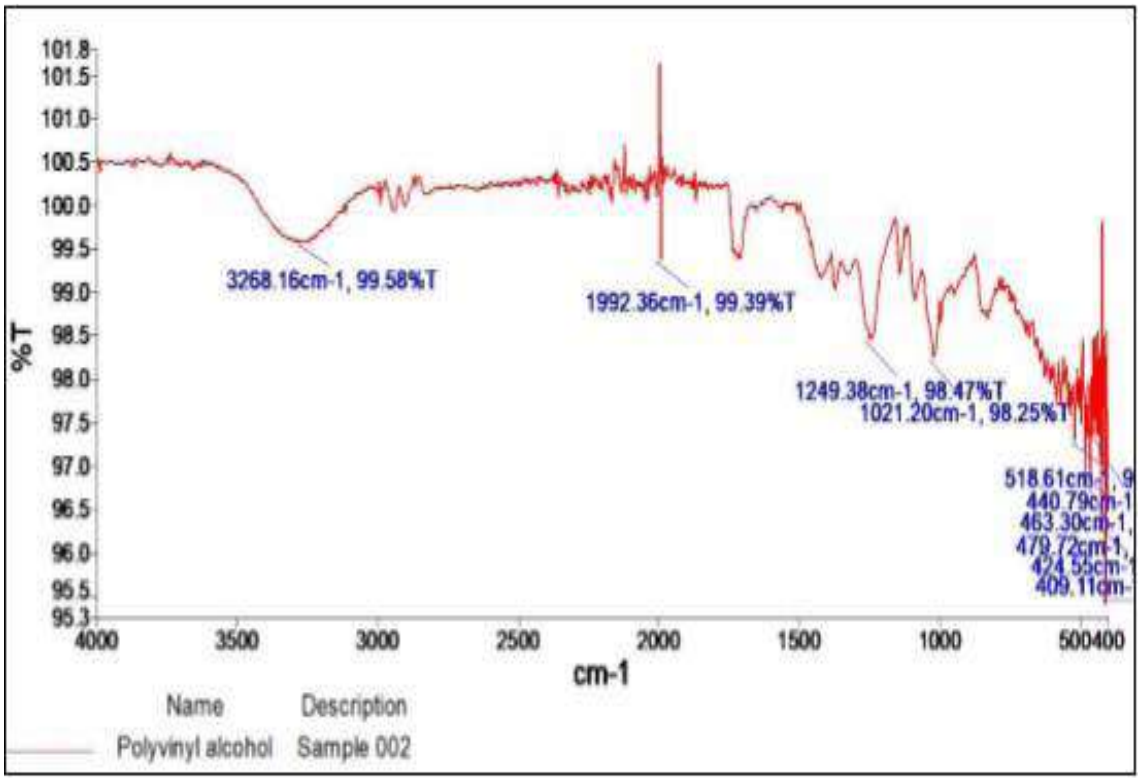

Figure 5: FTIR Spectrum of PVA

Table 12: Interpretation of FTIR spectrum of PVA

Reported peak (cm^{-1})	**Observed peak (cm^{-1})**	**Functional group**
3297.54	3268.16	O-H bending
1076	1059.87	O-H bending
1408	1466.21	C-O symmetric stretching due to Carboxylate anion
1634.75	1602.88	Presence of acetyl group

PVA FTIR spectra are shown in both Figure 5 and Table 12. The major IR absorption peaks of PVA were found to be nearby the peaks mentioned above and were identified as being at 3268.16 cm-1 (O-H bending) , 1602.88 cm-1 (acetyl group presence), 1466.21 cm-1 (C-O symmetric stretching due to carboxylate anion), and 1059.87 cm-1 (O-H bending). These huge peaks were evidence of the PVA's authenticity and purity.

Table 13: Interpretation of FTIR spectrum of Ethyl Cellulose

Reported peak (cm^{-1})	**Observed peak (cm^{-1})**	**Functional group**
3300	3436.9	O-H (phenolic) stretching
1520-1650	1637.62	C-C (aromatic)
1300	1376.83	C-O-C (ether linkage)

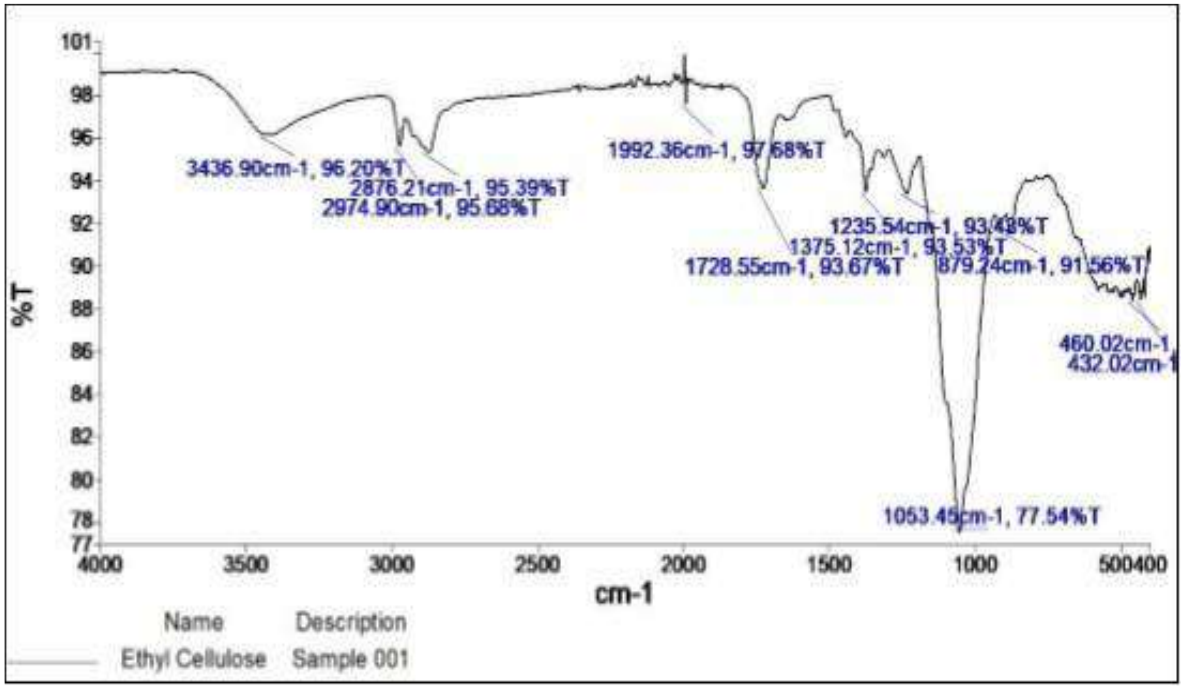

Figure 6: FTIR Spectrum of Ethyl Cellulose

In figure 6 and table 13, the ethyl cellulose FTIR spectra were shown. The principal IR absorption peaks of ethyl cellulose were determined to be 3436.90 cm-1 (O-H (phenolic) stretching), 1637.62 cm-1 (C-C (aromatic)), and 1376.83 cm-1 (C-O-C (ether linkage). These notable peaks demonstrated the authenticity and purity of the ethyl cellulose.

7. Drug excipient compatibility study by FTIR spectroscopy

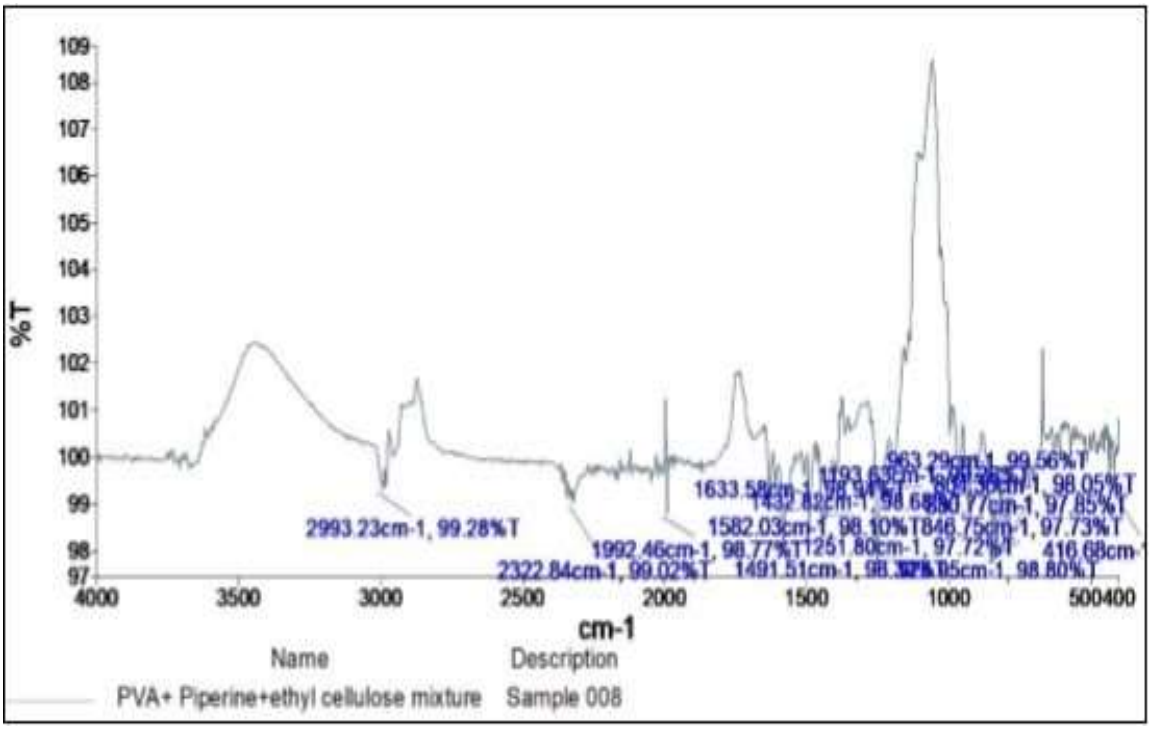

Figure 7: FTIR spectrum of Physical mixture (Piperine, PVA and Ethyl Cellulose)

Table 14: Interpretation of FTIR spectrum of Physical mixture (Piperine, PVA and Ethyl Cellulose)

Reported peak (cm^{-1})	Observed peak (cm^{-1})	Functional group
1240.53	1240.47	C-O Stretching
796.92	769.1	Due to a substituted naphthalene group
1578.31	1578.9	C=C aromatic
1059.87	1071.31	OH bending
1376.83	1398.74	C-O-C (ether linkage)

In order to ascertain the likelihood of an interaction between the drug and the excipients utilized with the analytical method of drug evaluation, the physical mixture was exposed to FTIR analysis (Figure 7 and Table 14). The presence of the excipient peaks and their associated drug peaks in the spectra above was proven by all of the spectrum's peaks**.** There was no interaction in this composition as a result.

8. Evaluation of Piperine Microsponge[27-30]

Microscopic examination and Surface appearance

Table 15: Microscopic examination and Surface appearance

Sr. No.	Formulation code	Visual Appearance
1	F1	Microsponge not formed
2	F2	Microsponge not formed
3	F3	Cluster structure was formed
4	F4	Microsponge are formed
5	F5	Microsponge are formed
6	F6	Irregular shaped microsponge formed

Discussion: According to table no. 15, some formulations had spherical shapes, while others had irregular structures and some demonstrated cluster formation.

Percentage yield

Table 16: Percentage yield

Sr. No.	Formulation Code	Average
1	F1	35.827±0.289
2	F2	40.330±0.193
3	F3	42.733±0.153
4	F4	44.550±0.128
5	F5	53.780±0.121
6	F6	52.827±0.131

Value is expressed as mean ± SD; n = 3

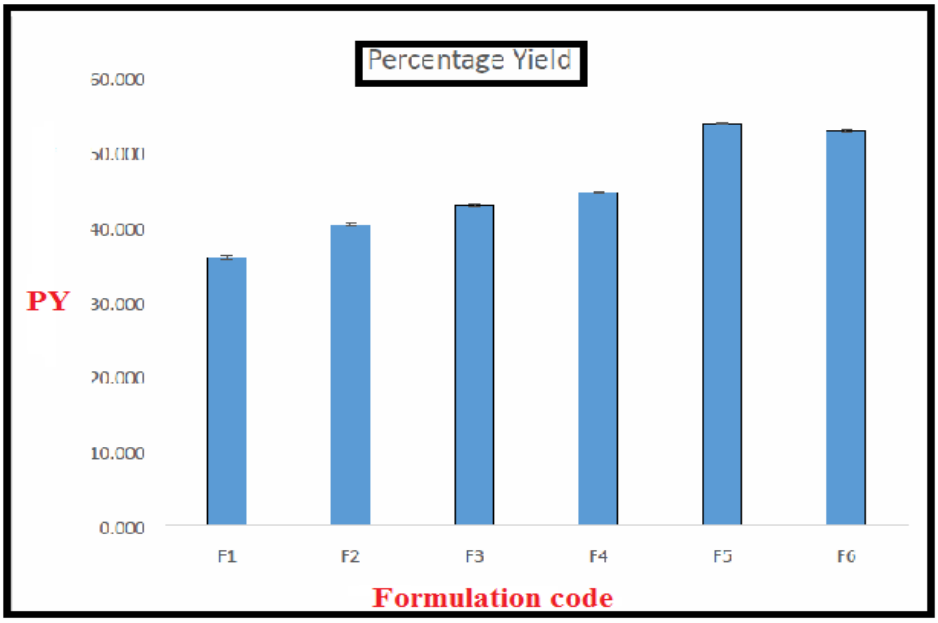

Figure 8: Percentage yield of Piperine microsponges (F1-F6)

Discussion: The percentage yield of every formulation was discovered to range between 35.8270.289 and 53.7800.121.

Percentage Drug Entrapment

Table 17: Drug Entrapment of formulations (F1-F6)

Sr. No.	Formulation Code	% EE
1	F1	63.612±0.107
2	F2	72.503±0.107
3	F3	75.881±0.107
4	F4	88.759±0.146
5	F5	90.823±0.141
6	F6	80.432±0.203

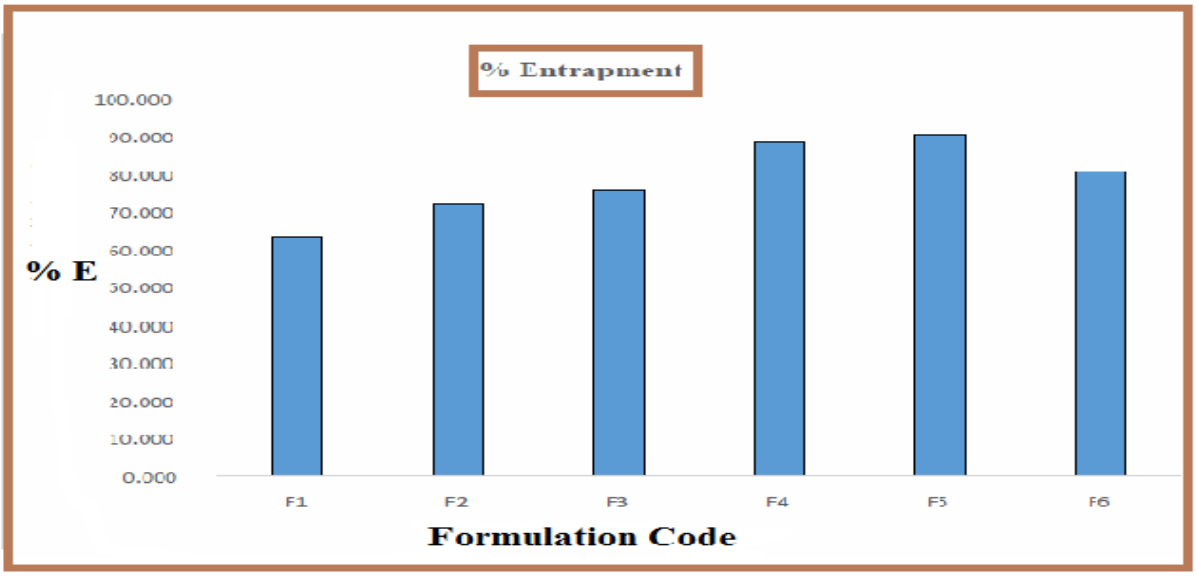

Figure 9: Percentage Drug Entrapment

Discussion: According to the above figure and table, the drug entrapment ranged from 63.612±0.107 to 90.823±0.141%. According to research, formulation F5 has the highest rate of drug entrapment.

Particle size measurement

Table 18: Particle size of formulations

Sr. No.	Formulation Code	Particle Size (µm)
1	F1	44.7±4.509
2	F2	53.3±2.517
3	F3	42.7±1.528
4	F4	36.0±3.000
5	F5	34.3±3.055
6	F6	35.0±2.646

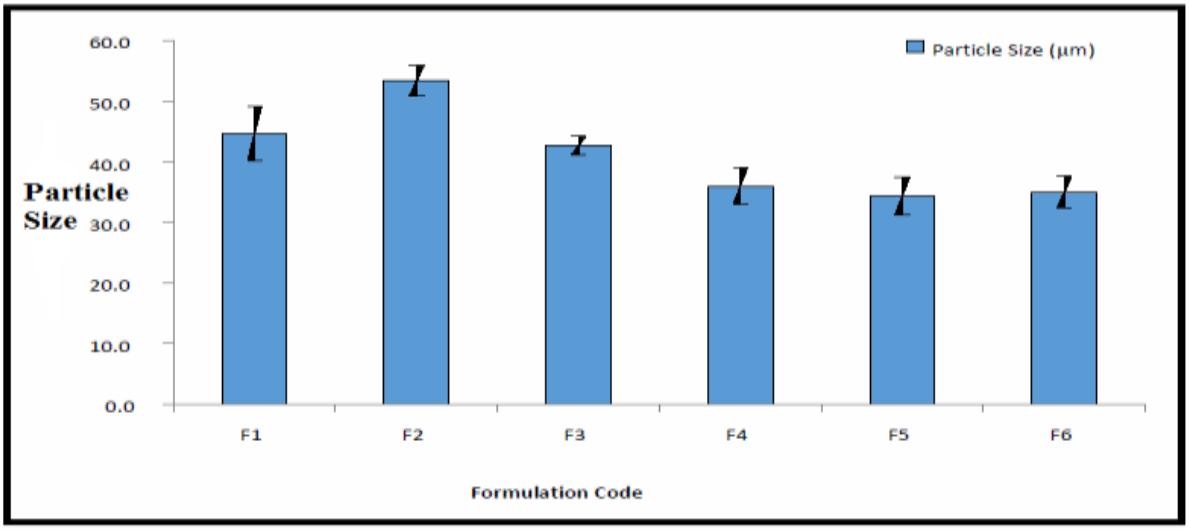

Figure 10: Particle size of formulations (F1-F6) (F1-F6)

Discussion: The Particle Size of all formulations was found to be in the range of 34.3±3.055 to 53.3±2.517 m, according to table 18. These findings clarify why the Microsponges had identical diameters when the polymer concentration was changed.

Scanning electron microscope (SEM) study

The optimised formulation (F5)'s prepared piperine microsponges were discovered to be spherical in shape.

Incorporation of Piperine microsponge into gel for transdermal drug delivery After successfully dispersing the formulation in Carbopol 934 varied concentrations, the gel of the optimized F7 and F8 formulation was created and submitted to characterization.

Evaluation of Piperine Microsponges gel

1. Physical Appearance

Table 19: Visual Appearance of Piperine microsponge gel

Sr. no.	Formulation code	Visual Appearance
1	F7	Milky white gel formed
2	F8	Milky white gel formed
3	F9	Milky white gel formed

The prepared gel was visually inspected for consistency and discovered to have a smooth look. F7 and F8 formulation batches had excellent homogeneity and no lumps whereas, F9 formulation was viscous and having lumps. So, more research was done using F7 and F8 formulation batches.

2. pH determination

Table 20: pH Determination of optimized Formulation

Sr. no.	Formulation code	pH (Mean ± S.D)
1	F7	7.17±0.05
2	F8	7.25±0.05

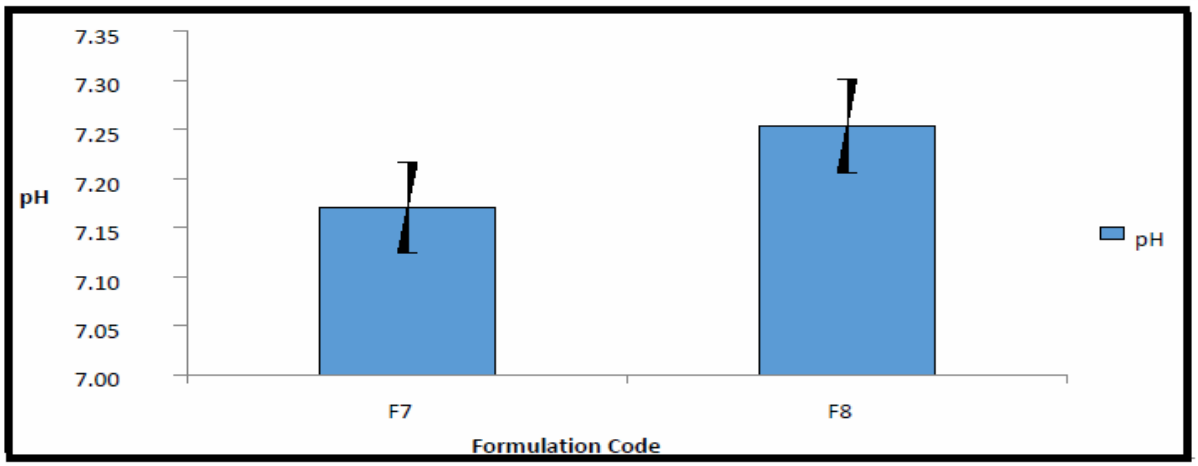

Figure 11: pH of gel

Discussion: According to Table 20 and Figure 11, the pH of formulations F7 and F8 was found to be between 7.17 and 7.2**5.**

3. Rheological studies

Table 21: Viscosity of optimized Formulation

Sr. no.	Formulation code	Viscosity (cps)
1	F7	3778±6.03
2	F8	4129±4.04

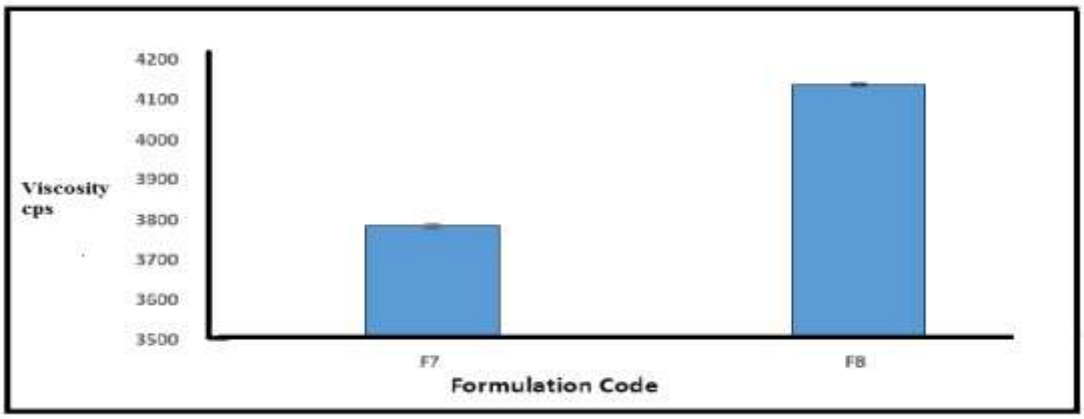

Figure 12: Viscosity Study of formulations (F7-F8)

Discussion: According to Table 21 and Figure 12, the viscosity ranges for formulations F7 and F8 were 3778±6.03 and 4129±4.04, respectively.

4. Spreadability Study

Table 22: Spreadability Study

Sr. no.	Formulation code	Spreadability (g.cm/sec) (mean ± SD)
1	F7	17.589±0.17
2	F8	17.244±0.26

Discussion: The Spreadability of formulations F7 and F8 was in range of 17.589±0.17and 17.244±0.26 (Table 22).

5. Drug content

Table 23: Drug content of formulations (F7-F8)

Sr. No.	Formulation Code	%D.C
1	F7	95.754±1.465
2	F8	87.779±2.471

Discussion: It was discovered that the drug content of gels ranged from 87.779±2.471 to 95.754±1.465%. Formulations F7 and F8's % medication content was deemed to be adequate. As a result, it was determined that the procedure used for gel formulations was appropriate.

6. FTIR Study

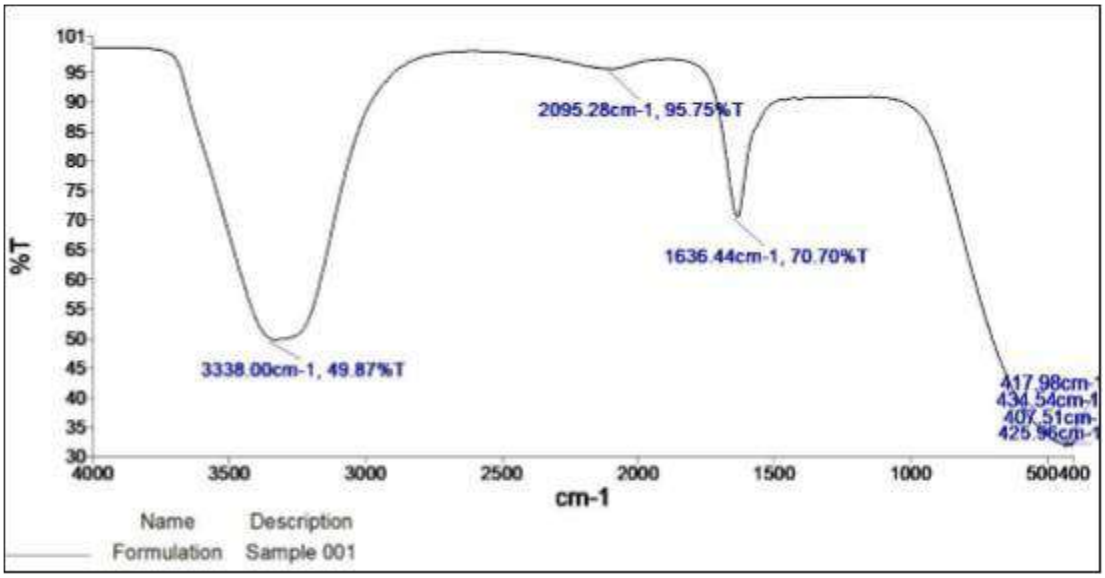

Figure No. 13: FTIR Spectra of Formulation F7

Table 24: Interpretation of FTIR spectrum of Formulation

Reported peak (cm^{-1})	Observed peak (cm^{-1})	Functional group
3338	3340.86	O-H bending
1636.44	1637.62	C-C (aromatic)

Discussion: As shown in Figure 13 of the spectra for the formulation (F7), peaks were discovered at 3340.86 cm-1 (O-H bending) and 1637.62 cm-1 (C-C aromatic), respectively. In the final

formulation F7 FT-IR spectra, some of the piperine peaks were preserved while others experienced minor changes.

7. In vitro drug release studies

Table 25: In vitro drug release of Piperine microsponge gel & control gel formulation

Sr. No.	Time (Hr)	Drug Release of Pure drug (%)	Drug Release of Formulation F7 (%)
1	0	0	0
2	0.25	1.223±0.003	2.663±0.096
3	0.5	3.693±0.003	6.007±0.116
4	1	8.693±0.003	11.302±0.101
5	2	12.583±0.003	17.420±0.076
6	3	16.865±0.004	22.066±0.580
7	4	19.124±0.004	35.493±0.658
8	5	24.764±0.003	42.587±0.791
9	6	29.519±0.004	50.441±0.580
10	8	34.763±0.003	61.968±0.956
11	10	39.367±0.003	72.988±0.956
12	12	41.583±0.003	83.375±1.005

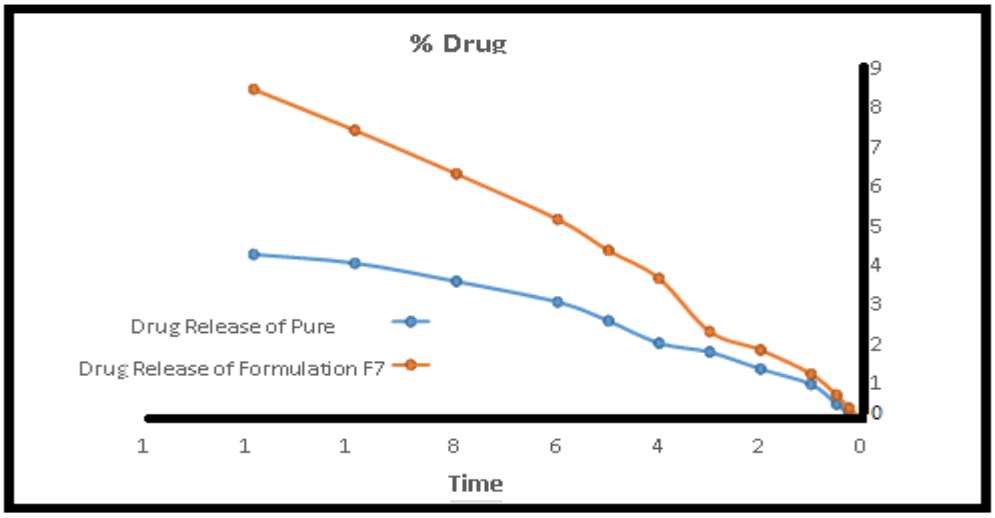

Figure 14: Percentage drug release of Piperine microsponge gel & control gel formulation

Table 25 and Figure 14 provide more information of the in-vitro drug release of the piperine microsponge gel and control gel formulations. The control gel releases 71.4681.222% after 3 hours. The drug release in gel formulationF7, in contrast, showed 83.3751.005% over the course of 12 hours in phosphate buffered saline (pH 7.4). The drug loaded gel formulation F7, which contains 1% Carbopol 934, demonstrated a maximum drug release of up to 83.3751.005% within 12 hours.

Table 26: Drug release kinetic studies

Formulation	Zero order		First order		Higuchi		K. Peppas	
Code	K_0	R^2	K_0	R^2	K_0	R^2	K_0	R^2
F7	7.0823	0.9882	-0.0619	0.9773	25.404	0.957	0.872	0.994

Discussion

It is common practice to compare release characteristics and predict the release mechanism using mathematical models. Within the optimized formulation were plots of the log percent drug release versus time (first order), log percent drug release vs square root of time (Higuchi plot), and log of log% drug release versus log time (Korsmeyer and Peppas Exponential Equation). Every time the graph's R^2 value was calculated, Table 5.22 and Figures 5.20 to 5.23 showed it. The release data was used to find Korsmeyer and Peppas (R2=0.994) after accounting for the determination coefficients. The results showed how to release the drug from the piperine-containing Microsponges gel using a control mechanism.

SUMMARY AND CONCLUSION

After applying linear regression to the piperine absorbance standard curves, the absorbance values were determined. Piperine exhibits good linearity with a correlation coefficient of 0.9981, which is quite close to one. Based on the findings of the preformulation study (FT-IR spectrum, UV spectrum, and melting point), piperine was found to be pure and of good quality, and the estimation technique was found to be dependable and appropriate for formula production. The evaporation process of Quassi - Emulsion Solvent was employed to generate piperine microsponges. To maximize Microsponges, several formulations (F1 through F6) were developed using various polymer dosages. The morphologies of the formulations for Microsponges varied; some had asymmetrical structures, some were spherical, and still others showed signs of aggregation development. All formulations had percentage yields ranging from 35.827±0.289 to 52.827±0.131. A formulation (F7) is considered optimal when it possesses high medication content together with desirable look, features, and size. Using optical microscope measurements of particle size and SEM validation of the enhanced F7 formulation's shape, the majority of the particles were found to be accurately recognized. To create Microspongal gel with Carbopol 934P in three distinct concentrations (1%, 1.5%, and 2%), the ideal formulation (F7) was selected. Upon visual inspection, the consistency of the gels produced seemed to be smooth. Formulations F7 and F8 have good homogeneity and no lumps. It was discovered that the pH range of the optimal gels that were produced was 7.17–7.25. All of the formulations had viscosities between 3778 and 4129 cps. All formulations had spread abilities ranging from 17.589±0.17 to 17.244±0.26g.cm/sec. The drug content in all forms (including the gel formulation) varied between 95.754±1.465 and 87.779±2.471%. Using Franz diffusion cells, drug release from gel formulations was investigated. Formulation (F7) achieved a high of 83.375±1.005% in less than 12 hours, significantly outperforming other gel formulations and the control gel. To determine the exact rate and mechanism of drug release, the in-vitro data were fitted with the zero order, first order, Higuchi, and Korsmeyer-Peppas models. The findings demonstrated that the Korsmeyer Peppas followed the F7 formulation's drug release, which accounts for the sustained method that Piperine microsponge gel uses. It was found that the process of creating the piperine-containing microsponge gel was straightforward and reproducible.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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