Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 30s (2025)
https://www.jneonatalsurg.com

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Formulation and characterization of Methyl Eugenol Nanocapsules

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Cite this paper as: Ms. Pradnya K. Pehere, Dr. Dharmendra Ahuja, (2025) Formulation and characterization of Methyl Eugenol Nanocapsules. *Journal of Neonatal Surgery*, 14 (30s), 792-807.

ABSTRACT

Alzheimer's disease (AD) is a neurodegenerative brain disorder that affects memory, thinking, concentration, and judgment, ultimately hindering a person's ability to perform routine daily activities. It is characterized by progressive memory loss, language deficits, depression, agitation, mood disturbances and psychosis.

On the other hand, it supports the use of herbal medicines due to their nonspecific antioxidant and anti-inflammatory activity and specific cholinesterase inhibitory activity. The popularity of herbal medicines is also increasing due to their perceived effectiveness, safety and affordability. Methyl Eugenol and terpinol are polyphenolic flavonoid that possesses various neuroprotective effects by attenuating the levels of AChE enzyme, accumulated amyloid β, free radicals and neuroinflammation against Alzheimer's disease (AD). Controlled drug release, increased stability of volatile components, active biodegradable packaging and site-specific drug delivery are important areas of this study. Nanocapsules (NCs) are microscopic spherical shells consisting of a core (liquid or solid) in which the drug is enclosed in a cavity by a characteristic polymer membrane. Different methods are used to obtain Nano capsules, but interphase polymerization methods, Nano deposition for a preformed polymer, polymer coating method are mainly preferred. The most important properties in their preparation are particle size and size distribution, which can be evaluated using various techniques such as FTIR, scanning electron microscopy (SEM), DSC, Zeta potential . Nanocapsules with extremely high reproducibility have a wide range of applications in life sciences. They can be used in genetic engineering, cosmetics, cleaning products, wastewater treatment, application of adhesive components, cancer therapy, neuroprotective activity, nano capsule bandages to fight infection, radiotherapy, and as liposomal nano capsules in food science and agriculture. Improved delivery of bioactive molecules through targeted delivery using nano capsules presents many challenges and opportunities for research and future development of new advanced therapies.

Keywords: *Nanocapsules,methyl Eugenol, Neuroprotectants, DSC, FTIR, Release kinetics etc*

1. INTRODUCTION

The chemical composition of the bay laurel leaves has been extensively studied. 1,8-Cineole (30.8%) has been identified to be the main compound followed by-terpinyl acetate (14.9%),-terpineol (8.0%), sabinene (7.9%), terpinen-4-ol (6.0%),-pinene (5.3%), pinene (3.6%), methyleugenol (3.6%), and-terpinene (3.3%) [17]. Nabila et al. [22] reported 1,8-cineole (30.1%),-terpynyl acetate (21.6%) and methyl eugenol (16.9%) as the main compounds in the chemical composition of the essential oil of L. nobilis leaves. It has been demonstrated that-pinene and 1,8-cineole protected U373-MG cells against H2O2-induced oxidative injury by attenuating the loss of cell viability and cell morphology, inhibiting reactive oxygen species (ROS) production and lipid peroxidation, while increasing the endogenous antioxidant status [23]. Also, linalool and 1,8-cineole exhibited anticancer activities in lung cancer A549 cells [24]. Sabinene (8.86%), one of the major compounds from the needles of Abies koreana, contributed to the observed memory-enhancing effect on Sco-induce amnesia in mice.

Eugenol has garnered attention for its potential therapeutic benefits in neurodegenerative conditions, specifically AD [4]. It is believed to work by inhibiting the formation and promoting the disintegration of-amyloid plaques, as well as mitigating the hyper phosphorylation of the tau protein [9]. One limitation of Eugenol is its low solubility and permeability in water,

resulting in low bioavailability and presenting challenges for oral administration [10]. However, by utilizing nanocarrier systems, particularly nanocapsules, it becomes possible to achieve precise drug targeting to specific tissues [11]. This approach enhances drug permeability across physiological barriers, such as the blood–brain barrier, optimizing biodistribution and potentially enhancing the therapeutic efficacy of Eugenol [12–15]. Considering these factors, the objective of this study was to evaluate the effect of Eugenol nanocapsules (NC Curc) on memory loss, neuroinflammation, and oxidative stress reversal in an animal model of AD induced by intracerebroventricular (icv) injection of streptozotocin (2-deoxy-2-(3-(methyl-3-nitrosoureido)-D-glucopyranose) (STZ). Additionally, this study investigated the involvement of acetylcholinesterase (AChE) activity.

Nanotechnology in drug-delivery system provides an opportunity to deliver drugs for a prolonged time with natural affinity [7]. Nanoparticulate systems, dendrimers, micelles, liposomes, and nanoemulsions are some of the nanocarrier systems widely used in drug delivery. These nanocarrier systems help in improving biodistribution of drugs and solubility of hydrophobic compounds, as well as increasing the bioavailability, reducing the number of doses, improving drug targeting, and minimizing toxicity, etc.

Nanocapsules are vesicular systems made up of a polymeric membrane which encapsulating an inner liquid core having size in nanometer. Nanocapsules have varied applications which include medical, nutraceutical, drug delivery, food enhancement, healing materials. Encapsulation ensures protection of drug substances from environment, light, pH and facilitates controlled release and sustained release. Core-shell structure of Lipid Nanocapsule consists of an oil-filled core with a surrounding polymer or lipid. Lipid nanocapsules (LNC) are a new generation of biomimetic nanovectors comprising the oily core of medium chain triglycerides. The core of nanocapsule may be aqueous or composed of lipophilic solvent usually oil and lipids. Since structure of nanocapsule is composed of oily core surrounded by a rigid membrane it is considered as in between polymeric nanocapsule and liposome. Their size can be adjusted below 100nm with narrow distribution. Drug delivery can be targeted to specific cells & location within the body after intravenous & subcutaneous route of injection. An oil surfactant is used in core composition of nanocapsule that is specifically selected to coordinate with the selected drug within a lipidic membrane. The drug must be highly soluble in specific oil and lipid used.

2. MATERIALS AND METHODS

2.1. All research chemicals were purchased from from New Neeta Chemicals, PCMC, Pune. All chemicals used were of analytical grade and used as received.

Preparation of eugenol-loaded nanocapsules

Polymer System:

1% v/v aqueous acetic acid solution was prepared by adding 1 ml acetic acid in 100 ml of water. 300 mg of methyl eugenol and 320 mg of chitosan was added with continuous mixing/ stirring in a water bath at 60°C for 2 h. The pH value of the CAA solution was adjusted to 4.8 using a 2M NaOH solution. 40 mg of Tween 80 was added to the solution. The homogenous emulsion was obtained through magnetic stirring at 60 °C for 1 hr.

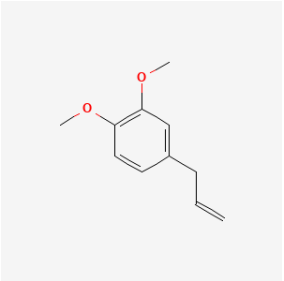
It was stirred under magnetic force for another hour to form a Methyl eugenol and chitosan oil-in-water emulsion at room temperature. Subsequently, 100 mg of Sodium Tripolyphosphate solution (TPP) was included in the emulsion. It underwent 2 hrs. of stirring to crosslink. The sediment was collected via washing at 8000 rpm for 15 min, followed by dispersion in deionized water. The precipitate was collected and vaccum dried and stored in refrigerator at 4C until for testing. The formed nanocapsules was then evaluated for particle size, Zeta potential, PDI, EE etc.

Table1 : Formulation of eugenol-loaded nanocapsules

Sr. no.	Formulation Code	Eugenol: Chitosan: STTP
1	F1	1:2:0.5
2	F2	1:2:1
3	F3	1:1:1.5
4	F4	1:3:0.5
5	F5	1:1:1
6	F6	1:3:1.5
7	F7	1:2:1.5
8	F8	1:3:1

9	F9	1:1:0.5

Characterization

Parameter	Methyl Eugenol
Description	Methyl eugenol is a clear colorless to pale yellow liquid with a spicy earthy odor. Bitter burning taste.
Odor	Mild-spicy, slightly herbal odor
Boiling Point	252°C
Flash Point	98°C
Density	0.9846 g/cm^3
Molecular Weight	178.23 g/mol
Structure	
IUPAC Name	1,2-dimethoxy-4-prop-2-enylbenzene
Molecular Formula	$C_{11}H_{14}O_2$
Solubility	less than 1 mg/mL at 20°C miscible with methanol, ethanol, acetonitrile, Tetrahydrofuran, Chrloroform, Hexane

3. METHODS:

3.1 Particle Size, Particle distribution index and Zeta Potential

Samples were prepared by dispersing in distilled water and detected by a Malvern Zetasizer Nano ZS ZEN3600 analyzer (Malvern Instruments Ltd., Malvern, UK).

Mean particle size, polydispersity index (PDI), and zeta potential were measured (n = 3) after diluting an aliquot of the nanocapsule suspension in ultrapurified water (1:500). All analyses were carried out on the Zetasizer Nanoseries (Malvern Instruments, Malvern, United Kingdom). A one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was performed to verify the statistical difference between the mean values.

3.2 pH Determination

The pH values were obtained by using a digital potentiometer, previously calibrated with pH 4.0 and 7.0 buffer solutions. The pH was measured directly in each colloidal suspension after preparation. The results were expressed as the mean of six different samples.

3.3 DESIGN EXPERT STUDIES – METHYL EUGENOL

Build Information

File Version	13.0.5.0		
Study Type	Response Surface	**Subtype**	Randomized

Design Type	Central Composite	**Runs**	9.00
Design Model	Quadratic	**Blocks**	No Blocks

Factors

Factor	Name	Units	Type	Sub Type	Minimum	Maximum	Coded Low	Coded High	Mean	Std. Dev.
A	Chitosan Conc	mg	Numeric	Continuous	300.00	500.00	-1 ↔ 300.00	+1 ↔ 500.00	400.00	86.60
B	STPP Conc	mg	Numeric	Continuous	50.00	150.00	-1 ↔ 50.00	+1 ↔ 150.00	100.00	43.30

Responses

Response	Name	Units	Observations	Minimum	Maximum	Mean	Std. Dev.	Ratio
R1	Particle Size	nm	9.00	137.2	467.3	291.30	123.40	3.41
R2	Entrapment Efficiency	%	9.00	50.28	64.76	56.90	4.60	1.29

3.4 FTIR

Briker FTIR – Sample mixed with kbr to form a thin film for FTIR analysis. Spectrum recorded from 4000 to 400 $cm^{-1.}$

3.5 UV Spectrophotometer

Standard methyl eugenol was used to make a series of dilution and a calibration curve was constructed. For diluent purpose, a mixture of acetone and water (50:50) was used, as the nanocapsule preparation had a mixture of these solvent system. Lambda max was previously noted at 249 nm. So for current studies, wavelength was taken as 249 nm.

- Preparation of Methyl Eugenol Stock Solution:

1 gm of Methyl Eugenol was accurately weighed in a 100 ml volumetric flask. Final concentration of ME was 10mg/ml (MESS-1). From this solution 1 ml was accurately measured and transferred to 100 ml Volumetric flask and diluent was added. (Final conc. of ME was 100 µg/ml). Label this solution MESS-2.

A calibration curve was constructed with concentrations of 10, 20, 30, 40, 50, and 60 µg/ml methyl eugenol concentration. Refer table below for preparation of Linearity solutions in a 10 ml volumetric flask

MESS-2 in "X" ml	Diluent ml (upto)	Final Conc. µg/ml
1	9	10
2	8	20
3	7	30
4	6	40
5	5	50
6	4	60

Table 2- Preparation of Methyl Eugenol Stock Solution:

3.6 Encapsulation Efficiency %

A UV–Visible Spectrophotometer (Labman 1900 Double beam) was used to measure the concentration of Methyl Eugenol incorporated within chitosan polymer nanocapsule using the technique created by Lalita Keawchaoon. In short, 5 mL of 2M HCl was combined with 200 µL of a sample that had a concentration of 10 mg/mL, and the mixture was then placed in a boiling water bath for 30 minutes. After adding 2 mL of ethanol, the mixture was centrifuged for 5 minutes at room temperature at 7000 rpm. Wavelengths ranging from 250 to 400 nm were used to measure the supernatant's absorbance. The amount was then computed using the standard curve produced from different concentrations of CEO in absolute ethanol at 282 nm, which was the wavelength at which the maximum absorption was observed.

$$EE = \frac{\text{Theoretical drug content} - \text{free drug content}}{\text{theoretical drug content}} X\ 100$$

3.7 SEM – Morphology

The vacuum dried samples (0.5 × 0.5 cm) were fixed on the copper plate then observed after spraying with gold by scanning electron microscopy (S4800, Hitachi, Japan) with an accelerating voltage of 5.00 kV.

3.8 Oil Content

By UV Spectrophotometer and a calibration curve, the % Oil content per 10 mg of the output project was calculated and reported.

3.9 In-Vitro Drug Release.

A dialysis approach was employed to conduct an in vitro release investigation. The dialysis bag was immersed in distilled water to eliminate preservatives and subsequently rinsed with phosphate-buffered saline (PBS) solution. The nanocapsules containing essential oil were redispersed in 3 mL of PBS solution and placed in a dialysis bag, which was surrounded by 50 mL of PBS with 20% ethanol at pH 7.4. A time-dependent release research was conducted over a duration of 0 to 12 hours. All sets were incubated at 37°C with mild agitation. At specific time intervals, 3 mL of the medium was extracted and substituted with new medium, followed by spectrophotometric quantification.

The release was quantified as follows:

Release (%) = [Released oil/Total oil] × 100

4. RESULTS

4.1 pH determination

PH value obtained after preparing the nanocapsules is 5.60

4.2 Methyl Eugenol Assay - Oil Content

Lambda max – 249 nm. Standard methyl eugenol was used to make a series of dilution and a calibration curve was constructed. For diluent purpose, a mixture of acetone and water (50:50) was used, as the nanocapsule preparation had a mixture of these solvent system.

Calibration Curve:

Methyl Eugenol	
Conc. ug/ml	**Absorbance**
10	0.07
20	0.104
30	0.136
40	0.166
50	0.195
60	0.230

Table 3-Methyl eugenol Assay

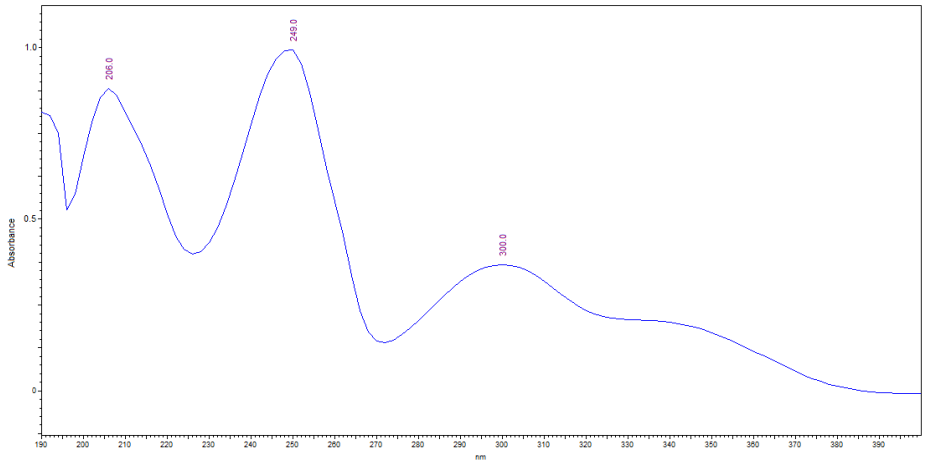


Figure 1: UV Spectrum of Methyl Eugenol

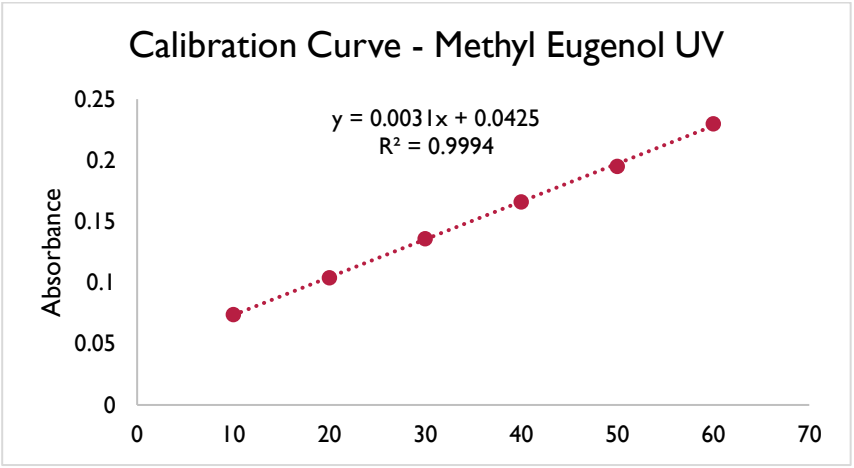


Figure2: Calibration Curve - Methyl Eugenol Uv

Prototype	Absorbance	Methyl Eugenol ug/ml conc.
MENC 1	0.131	28.59
MENC 2	0.142	32.15
MENC 3	0.093	16.32
MENC 4	0.123	26.01
MENC 5	0.189	47.33

Table 4: Absorbance of Methyl Eugenol

4.3 Excipients Compatibility Studies

The individual samples were mixed with diluent (Acetone : Water) 50:50 ratio and the concentration were brough to the range of calibration and analyzed. The content determined are shown in table below:

Prototype	Methyl Eugenol content %
EC1	95.23
EC2	97.65

EC3	96.53
EC4	97.12
EC5	97.02

Table 5: Drug Content

4.4 Design Expert Studies – Methyl Eugenol

Response 1: Particle Size

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	< 0.0001		0.9723	0.9405	
2FI	**0.0393**		**0.9869**	**0.9584**	**Suggested**
Quadratic	0.2342		0.9917	0.9623	
Cubic	0.1156		0.9997	0.9924	**Aliased**

Final Equation in Terms of Coded Factors

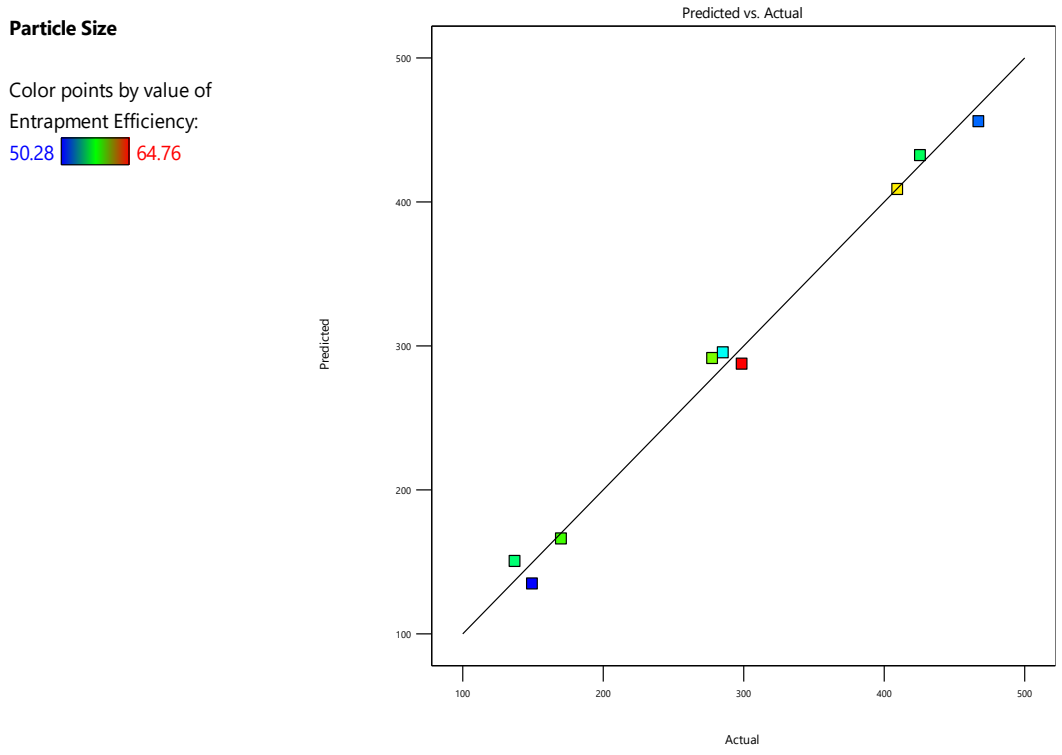
Particle size= 291.30 + 140.95A + 3.95B + 19.57AB

Final Equation in Terms of Actual Factors

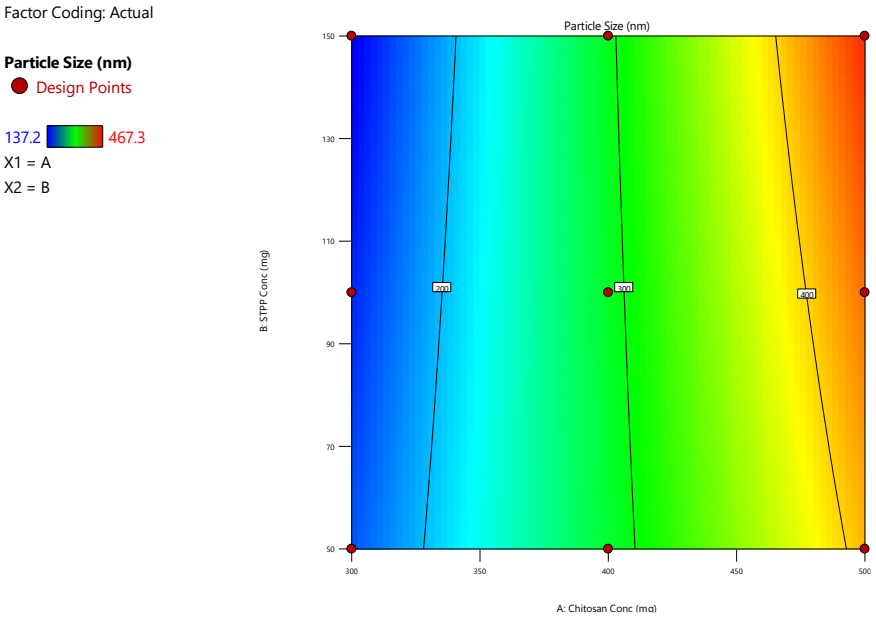
Particle Size=-123.80000+1.01800 Chitosan Conc-1.48700 STPP Conc+0.003915 Chitosan Conc * STPP Conc

The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor. Here, the levels should be specified in the original units for each factor. This equation should not be used to determine the relative impact of each factor because the coefficients are scaled to accommodate the units of each factor and the intercept is not at the center of the design space

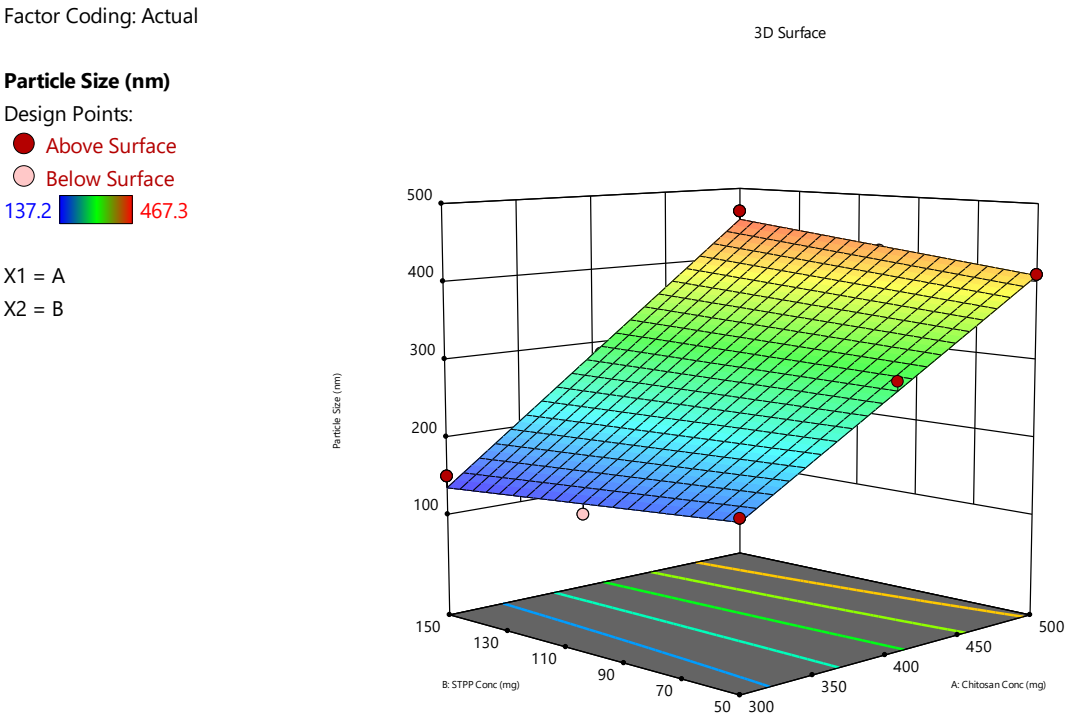
ACTUAL VS PREDICTED PLOT



CONTOUR PLOT



3D SURFACE PLOT



ENTRAPMENT EFFICIENCY

Response 2: Entrapment Efficiency

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	0.0058		0.7607	0.6030	
2FI	0.7803		0.7177	0.2165	

Quadratic	**0.0241**		**0.9607**	**0.8278**	**Suggested**
Cubic	0.3249		0.9876	0.7166	**Aliased**

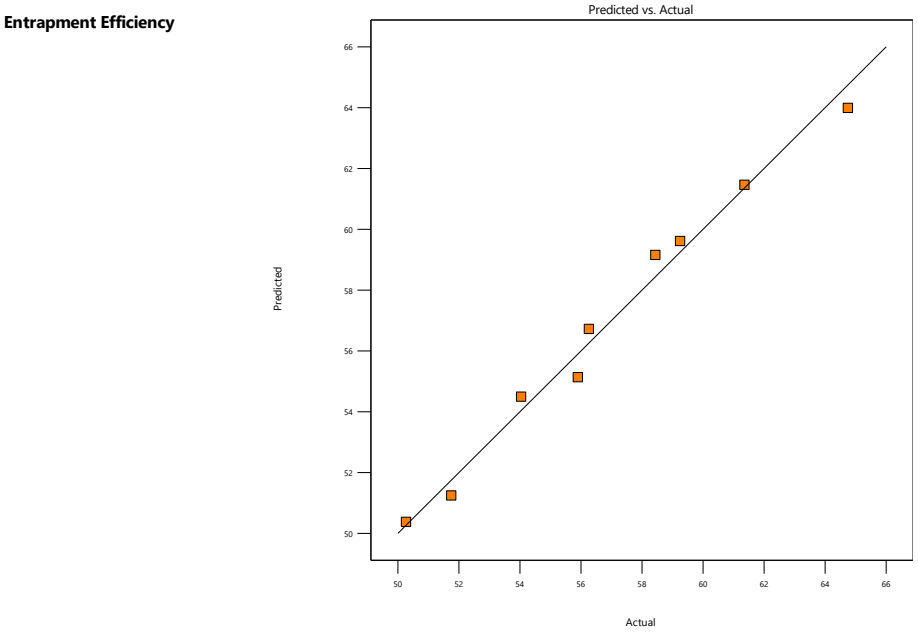
Final Equation in Terms of Coded Factors

Entrapment Efficiency=+59.60+0.7933A-4.75B-0.3600AB-3.68 A^2-0.3683 B^2

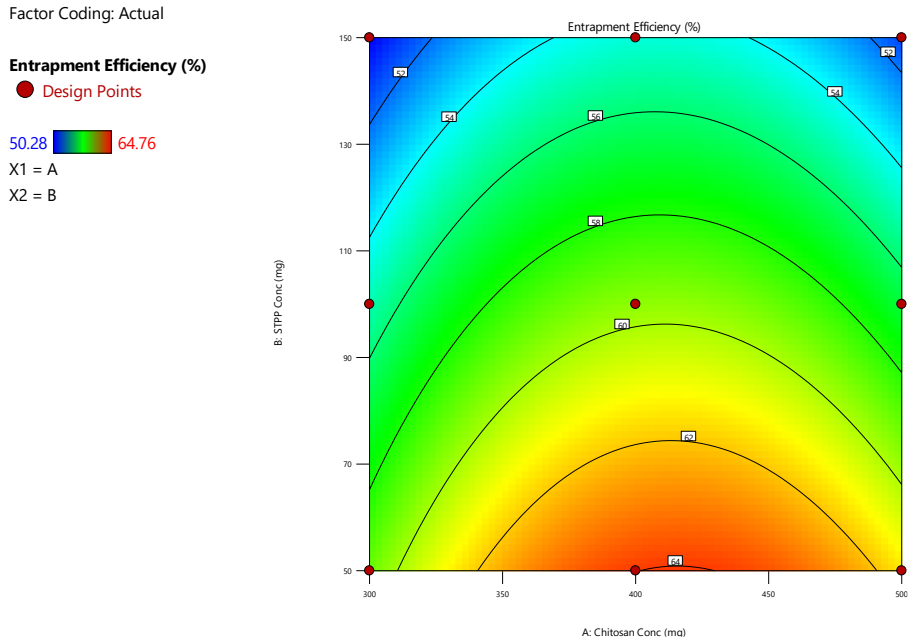
Final Equation in Terms of Actual Factors

Entrapment Efficiency=+2.63889 + 0.309800 Chitosan Conc - 0.036700 STPP Conc - 0.000072 Chitosan Conc * STPP Conc - 0.000368 Chitosan $Conc^2$- 0.000147 STPP $Conc^2$

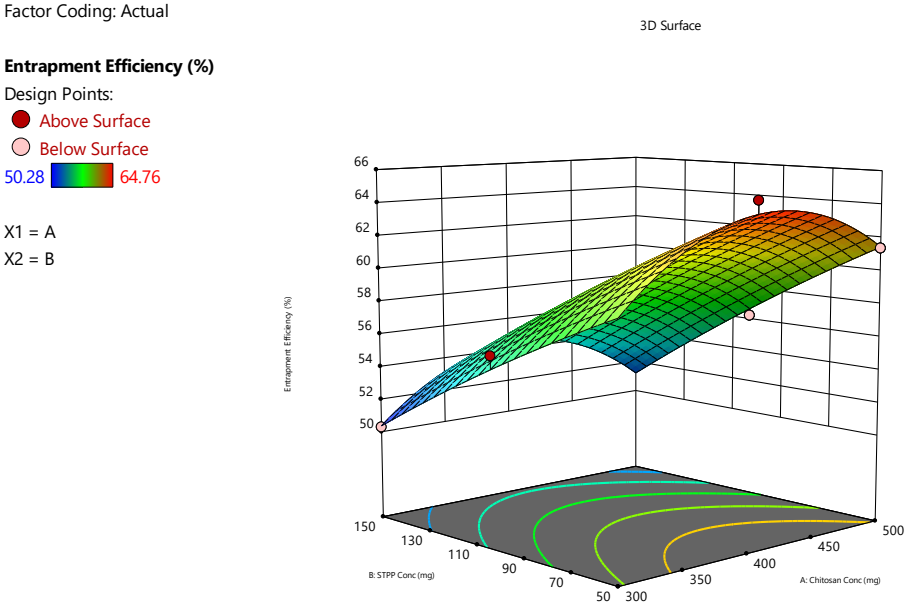
ACTUAL VS PREDICTED GRAPH



CONTOUR PLOT



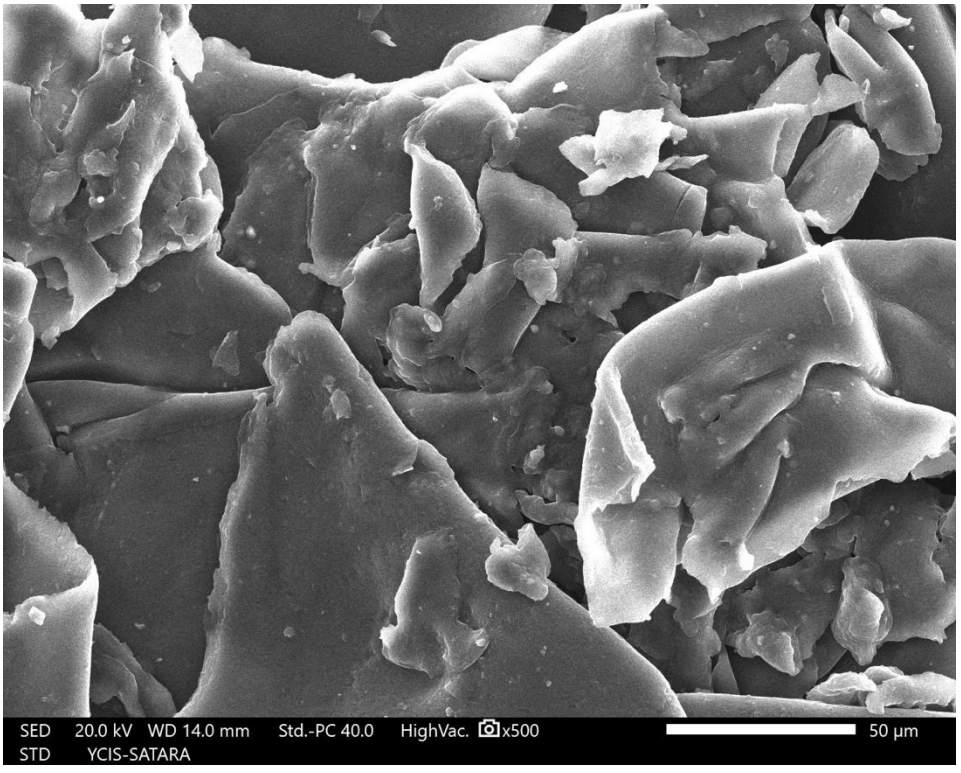
3D SURFACE PLOT



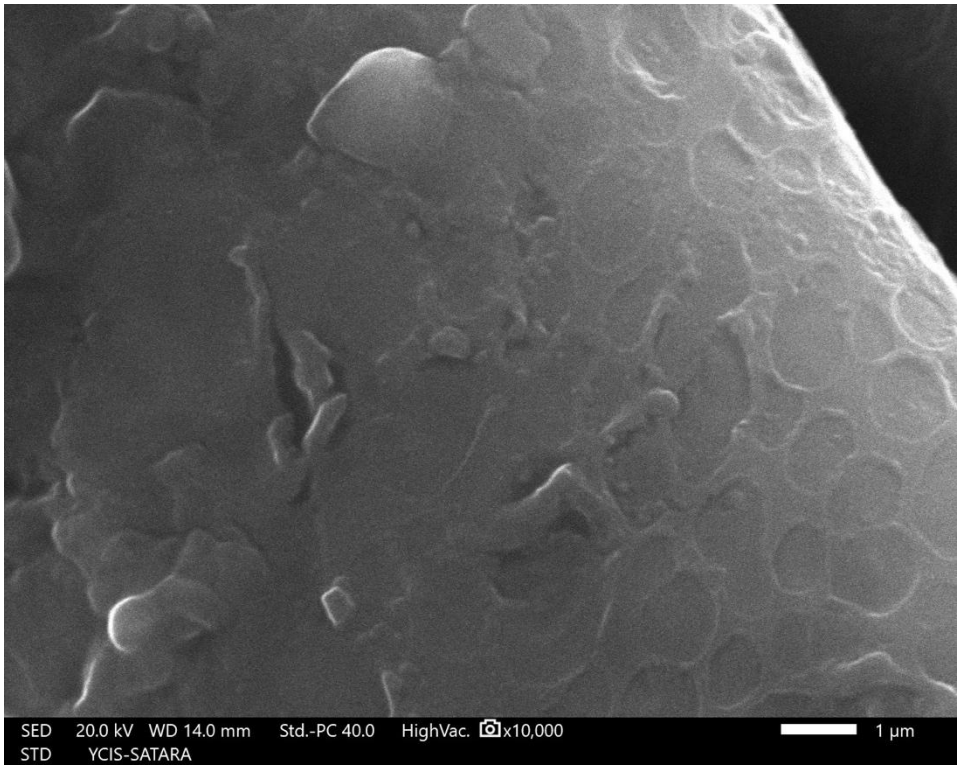
4.5 SEM Imaging

To obtain information about Eugenol oil Nano capsules SEM analysis was performed. This analysis carried out to visualizing size and shape of Nanocapsules. Result shows that shape of NC is spherical and size is below 300 nm.

MENC-5



5 um magnification – MENC-5 – SEM



1 um magnification – MENC-5. -SEM

4.6 FTIR

Below figures shows the FTIR spectra of pure eugenol, pure polymer (chitosan), STTP and Methyl Eugenol nanocapsule formulations. Regarding to eugenol, a stretching bands of OH group at 3,515 cm-1, a C-H signal of CH3 group at 2,968 cm-1, a C=C stretching band at 1,607 cm-1, a CH2 group signal at 1,439 cm-1, and C-O bond signals at 1,269 and 1,032 cm-1 were achieved. For Chitosan C=O stretching vibration band of the carbonyl group at 1,741 cm-1 was assigned. The same signal was found in the FTIR spectra for the nanoformulations. Considering the lactose, stretching vibration bands of OH group at 3,322 cm-1 and C-H group at 2,980; 2,943; and 2,879 cm-1 were observed. No chemical reactions occurred during the nanoencapsulation process**.**

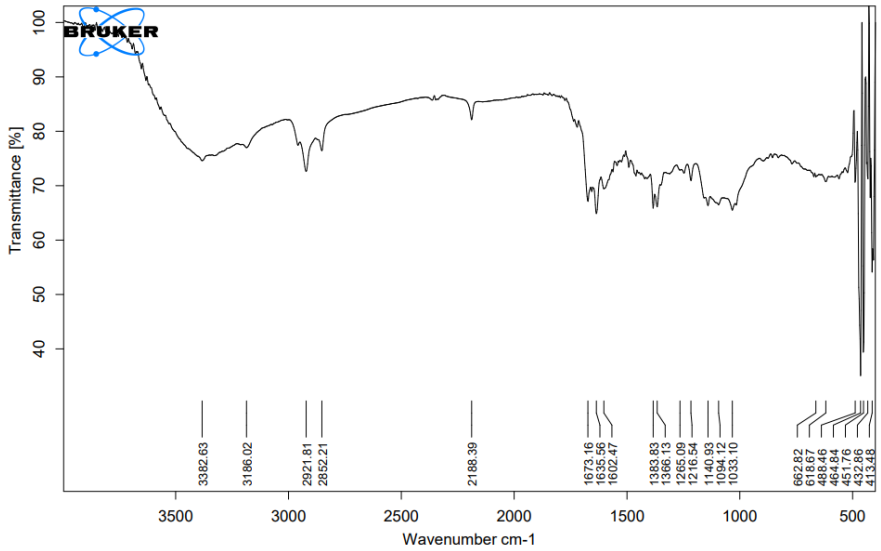


Figure 3 : FTIR of Chitosan

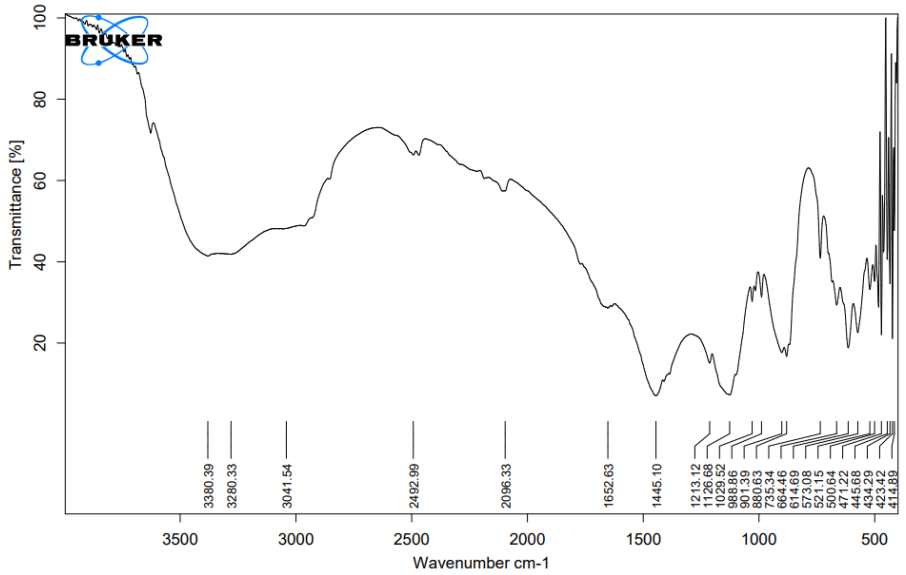


Figure 4: FTIR of STPP

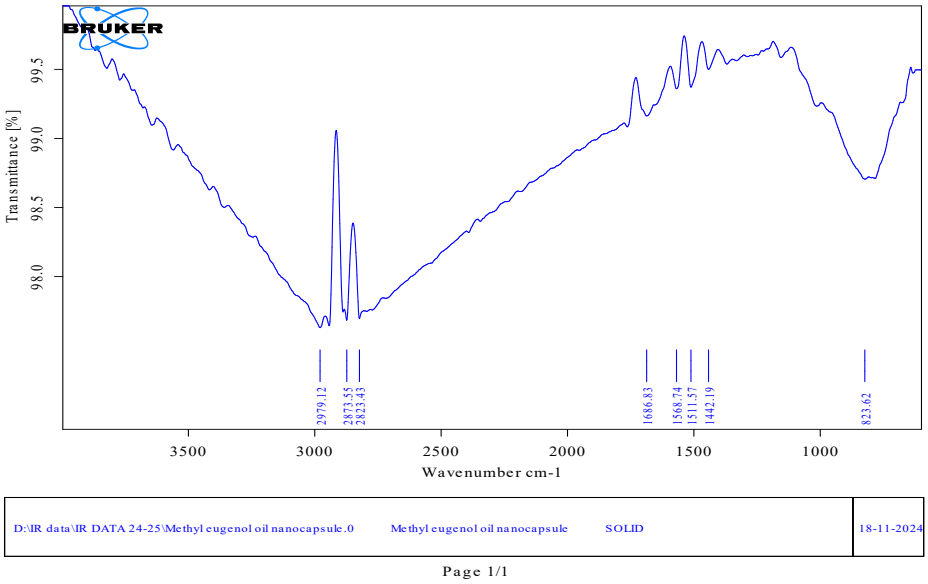


Figure 5: FTIR of Methyl Eugenol Nanocapsule

4.7 Entrapment Efficiency

The EE% was determined using ultraviolet visible spectroscopy. Using a standard graph (y ¼ 0.044x þ 0.030, R2 ¼ 0.991) and (y ¼ 0.0772x þ 2.5194, R2 ¼ 0.9846), the concentrations of the unknown methyl eugenol was determined. Samples were analyzed in triplicates and it was found that 75.91% of the total Methyl eugenol was encapsulated into the nanocapsules. This suggests that our carrier is very well suited for encapsulation of hydrophobic drugs.

Particle size, Zeta Potential, Entrapment Efficiency (%),Drug Content (%)						
Prototype	**Physical Appearance**	**Particle Size (nm)**	**PDI**	**Zeta Potential (mV)**	**Entrapment Efficiency (%)**	**Drug Content (%)**

MENC – 1	off white to slightly Yellow Amorphous powder	298.8 ± 24.8	0.432 ± 0.02	-17.1	64.76 ± 0.82	91.61 ± 2.25
MENC – 2	off white to slightly Yellow Amorphous powder	277.8 ± 22.3	0.243 ± 0.01	-19.3	59.26 ± 0.71	96.82 ± 1.56
MENC – 3	off white to slightly Yellow Amorphous powder	149.6 ± 17.6	0.346 ± 0.02	-25.7	50.28 ± 0.26	97.19 ± 0.92
MENC – 4	off white to slightly Yellow Amorphous powder	409.6 ± 22.6	0.413 ± 0.01	-13.3	61.37 ± 0.61	92.16 ± 3.19
MENC – 5	off white to slightly Yellow Amorphous powder	**136.4 ± 3.2**	**0.25 ± 0.01**	-27.6	**75.91 ± 0.18**	98.73 ± 1.08
MENC – 6	off white to slightly Yellow Amorphous powder	467.3 ± 25.9	0.413 ± 0.01	-8.7	51.76 ± 1.08	90.49 ± 0.81
MENC – 7	off white to slightly Yellow Amorphous powder	285.4 ± 12.9	0.223 ± 0.01	-18.9	54.05 ± 0.56	93.56 ± 1.23
MENC – 8	off white to slightly Yellow Amorphous powder	425.8 ± 13.1	0.313 ± 0.01	-10.7	56.27 ± 0.37	91.11 ± 3.46
MENC – 9	off white to slightly Yellow Amorphous powder	170.2 ± 26.4	0.218 ± 0.01	-21.5	70.45 ± 0.43	95.19 ± 0.52

Table 6 : Characterization of MENC 1-9

Based in the above results, it was incurred that prototype MENC5 had the lowest particle size and was then subjected to SEM studies for morphology understanding.

4.8 In-Vitro Drug Release.

The invitro release data of methyl Eugenol Nanocapsules in phosphate buffer at pH 7.4 are presented in figure 10. The formulation showed biphasic release pattern. The initial burst drug release in 1 hour is 10.49 ± 0.42 %. Afterward it shows a control release pattern from Nanocapsule i.e in 6 hours the release of drug methyl Eugenol Nanocapsules was 93.26± 0.60%.

Release Kinetic Behaviour mathematical model is important tool to understand the drug release kinetics of a dosage form.

The in vitro release data was applied to different kinetic models i.e. zero order, first order, higuchi and korsmeyer peppas to determine the mechanism of release of drug from nanocapsule formulation. Mechanism was interpreted in the form of graphical presentation and evaluated by correlation coefficient (R^2) represented in Table . In the formulation and evaluation of Methyl eugenol loded nanocapsule, the zero order was found to be more suitable than Higuchi model, first-order and Higuchi korsmeyer peppas kinetics models. This indicates that the release of methyl eugenol from the is controlled by diffusion, with the drug molecules moving from the interior of the to the nanocapsule surrounding medium.

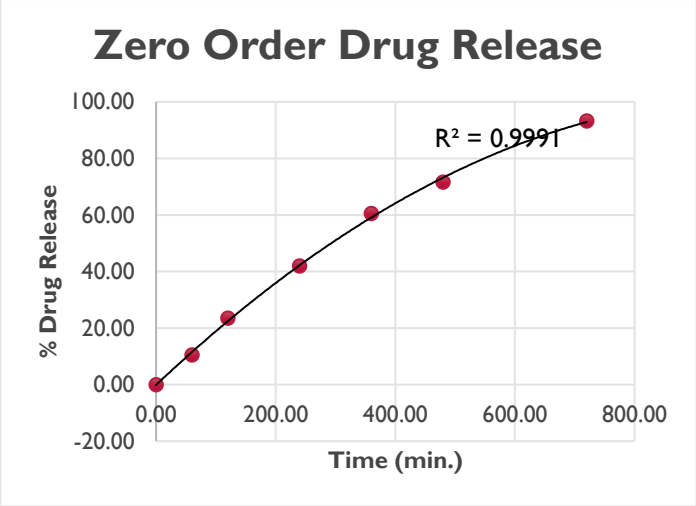


Figure 6: Zero Order Drug Release

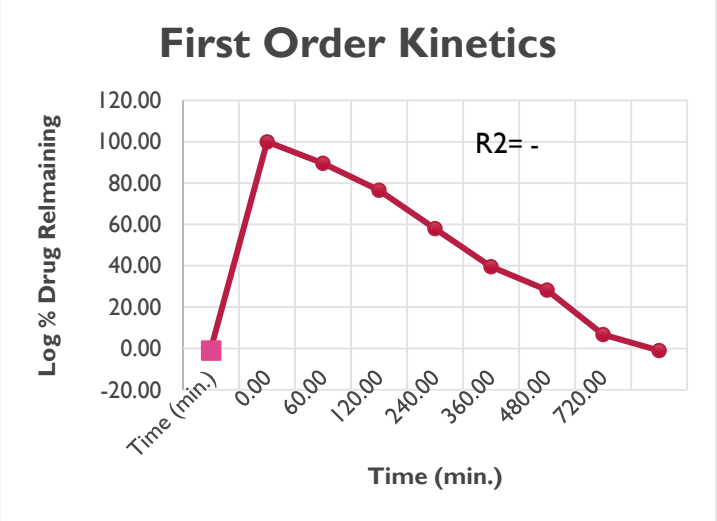


Figure 6: First Order Kinetics

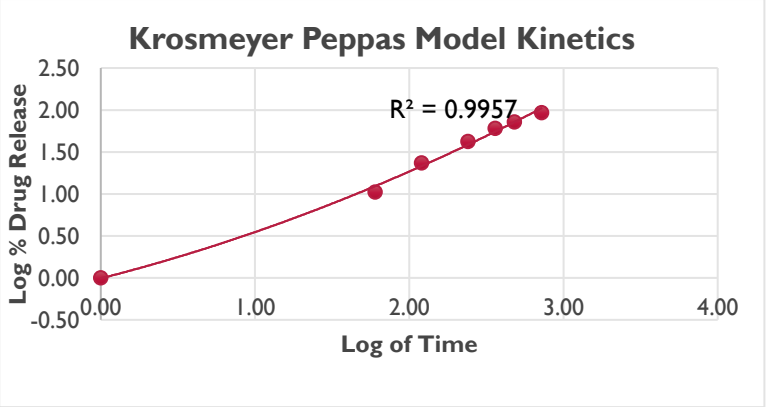


Figure 7: Krosmeyer Peppas Model Kinetics

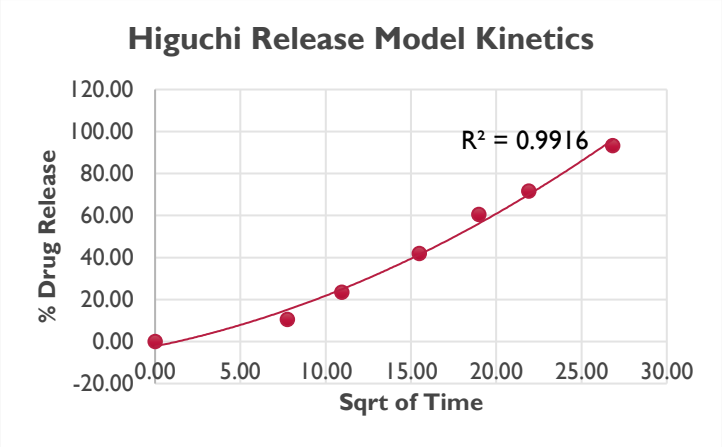


Figure 8: Higuchi Release Model Kinetics

5. CONCLUSION

For enhance the stability and bioavailability of essential oil, methyl eugenol loaded nanocapsules were formulated by method. The prepared nanocapsules were shows an average particle size from 136.4 ± 3.2 to 149.6 ± 17.6. Zeta potential value from -8.7 ± 1.21 to 27.6 ± 1.87. % drug entrapment efficiency was found between 50.28 ± 0.26 to 75.91 ± 0.18. In-vitro drug release of optimized batch i.e. F5 showed biphasic release pattern. In vitro studies confirmed that the methyl eugenol loaded nanocapsules exhibit favourable stability, enhanced bioavailability, and a prolonged release profile, primarily governed by diffusion-controlled mechanisms.

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