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FORMULATION AND EVALUATION OF ALLIUM SATIVUM OIL LOADED NANOSPONGES FOR THE TREATMENT OF RHEUMATOID ARTHRITIS

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Abstract

Allium Sativum loaded nanosponges were prepared successfully that belongs to class II of Biopharmaceutical Classification System, that shows that it has low permeability and high solubility. Nanosponges shows sustained release and controlled release property in formulation so as to enhance the effect of allium sativum for the management of rheumatoid arthritis for longer duration. The formulation of allium sativum loaded nanosponges were done by emulsion solvent evaporation method, in which two phases were prepared i.e., the organic phase and the aqueous phase that is further mixed at 1000 rpm for production of nanosponges. The formulated nanosponges were evaluated with particle size, drug entrapment, percentage drug release and percentage yield, by which optimized formulation was obtained using optimization technique Box-Behnken Design. The optimized formulation was subjected to SEM analysis and FT-IR study. The particle size of the optimized formulation was obtained as 290.5 nm, with 80.1% drug release, 86% drug entrapment, and 82.58% yield.

Keywords: Nanotechnology, Nanosponges, Garlic oil, Allium Sativum.

Introduction

Nanotechnology has great role in pharmaceutical drug delivery system in improvement of newer formulations for diagnosis, prevention, and treatment of various kind of disease(1). Nanotechnology helps to improve wide range of formulations for drug delivery system, drug targeting, to improve technique drug delivery techniques, enhance the efficacy of drug as well as to reduce to toxicity and amount of drug required to produce effect(2). Nanosponges are tiny nanoparticles with porous structure. The size of nanosponges is below 1µm. These tiny sponges of nano size consist of drug incorporated to a specific kind of dosage form(3). The drug releases from nanosponges in a very controlled manner in order to enhance the duration of action of that particular drug and also helps in improving the systemic level of the drug(4). The inflammatory synovial membrane that characterizes rheumatoid arthritis (RA), a chronic systemic autoimmune inflammatory disease, causes progressive articular cartilage degradation, bone erosion, and associated abnormalities. Due to synovial hyperplasia and pannus development, it presents as heated, swelling, and tender joints that are also accompanied by a loss of mobility and grip strength. WHO studies indicate that the majority of RA diagnosis occur between the ages of 20 and 40, which negatively impacts quality of life. (5).

Although there have been improvements in pharmaceutical treatments, controlling RA still poses difficulties, typically accompanied by negative consequences linked to prolonged use of medication. Therefore, there is an urgent need for novel therapeutic strategies that provide enhanced effectiveness and fewer adverse effects. Nanotechnology has become a potential method for delivering drugs in recent years, providing improved bioavailability, precise targeting, and less overall toxicity. Nanosponges have gained significant attention due to their adaptability, stability, and capacity to encapsulate various therapeutic substances, making them stand out among the many types of nanocarriers. In addition, incorporating natural chemicals like garlic oil, which is well-known for its strong anti-inflammatory and antioxidant effects, into nanosponge compositions offers a promising approach for treating RA. This study aimed to explore the formulation and evaluation of garlic oil-loaded nanosponges as a novel therapeutic approach for RA.

Materials And Method

Garlic Oil was purchased from PARAS ENGINEERS MARK OF QUALITY. Ethyl Cellulose, Dichloromethane, Polyvinyl alcohol was obtained from CDH private limited. The Garlic oil solubility studied in alcohol such as methanol and ethanol. Garlic oil is soluble in some other organic solvent like Acetone, chloroform, ether, dichloromethane and hexane(6)**.**Weighed out 0.01 ml of oil in a volumetric flask, added 9 ml of ethanol and 1 ml of PBS (pH of 7.4), and then mix it well. After adding 2 ml of ethanol and 100 ml of volumetric flask, top up the volume with distilled water. Pipetted out. Stock solution in millilitres: 0.05 ml, 0.1ml, 0.015 ml, 0.02 ml,0.02.5 into each volumetric flask with a 10 ml capacity, add stock sol. B until the volume reaches 10 ml. This yields a concentration of 1000 µg/ml, 1500 µg/ml, 2000 µg/ml, 2500 µg/ml, and 3000 µg/ml(7).Formulation of Allium sativum oil loaded nanosponges was prepared by emulsion solvent diffusion method. In this method, two phases were prepared i.e., the organic phase, and the aqueous phase. Initially Allium sativum oil and ethyl cellulose was mixed and dissolved in 20 ml of dichloromethane to produce organic phase. Into another beaker, 100ml of distilled water was taken, to which, desired quantity of polyvinyl alcohol was added to produce aqueous phase. The aqueous phase was kept as magnetic stirrer at 1000 rpm for 2hours with continuous mixing of organic phase for the formulation of nanosponges(8).

Optimization Of Garlic Oil Loaded Nanosponges by Design Expert (Version 12)

The design of Garlic Oil loaded nanosponges was implemented using Design-Expert® software (trial version 12, Stat-Ease), and a total of 15 runs were done. For the final optimization of Garlic Oil loaded nanosponges, a surface response approach, Box-Behnken design with three level, three factor, was implemented. The drug and polymer ratio, were taken as independent factors(9,10). Whereas, % entrapment efficiency, % release and % Yield and Particle Size were considered as dependent responses(9,10),

Table 1: Factors in Box Behnken Design with their used levels

Factor	**Name**	**Lower level (-1)**	**Upper level (+1)**
A	Garlic Oil	0.2	0.5
B	Ethyl Cellulose	20	250
C	Polyvinyl alcohol	10	300

A – Garlic Oil, B – Ethyl Cellulose,C- Polyvinyl alcohol

Characterization of Nanosponges

In each formulation the DLS method i.e., dynamic light scattering was used to determine the particle size in the formulation(11).In this method, nanosponges required quantity was taken, equal to drug quantity. The nanosponges were dissolved in 0.1N HCl and centrifuged for two hours. After completion, of centrifugation, 0.1ml of clear liquid was taken into volumetric flask of 10 ml, volume was made up with 0.1N HCl. By UV spectroscopy, absorbance was calculated at 240nm(12).Percentage drug release of all the formulations was calculated by determining the absorbance of sample(13)**.**The percentage yield was measured by determining raw material initial weight and Nanosponges final weight.

% yield= (Nanosponges weighed practically / Theoretical mass) X 100

Results and Discussion

Solubility Study:

Table 3: Solubility of Garlic Oil

SOLUBILITY	**OBSERVATION**	**REFERENCE**
Methanol	Slightly soluble	Slightly soluble
Ethanol	Soluble	Soluble
Acetone	Soluble	Soluble
Chloroform	Soluble	Soluble

Hexane	Soluble	Soluble
Water	Insoluble	Insoluble

Physical Properties of Garlic Oil

Table 4: Physical Properties of Garlic Oil

PROPERTY	OBSERVATION	REFERENCE
Colour	Pale yellow	Yellow
Odour	Pungent	Pungent
Taste	Pungent	Pungent
pH	6.4	6.3-6.7
Specific Gravity	0.920gm/cm^3	0.885 – 0.940 gm/cm^3
Refractive index	1.560	1.560
Saponification value	189 mg KOH/gm	186-195 mg KOH/gm
Acid value	2.5	2.4-2.6

FTIR of Garlic Oil

The FTIR studies revealed characteristic peak values from3500-1000 cm^{-1} for various functional compounds like hydroxyl, carbonyl, carboxylic, organic sulphur and aromatic compounds in allium sativum oil (Figure.1).

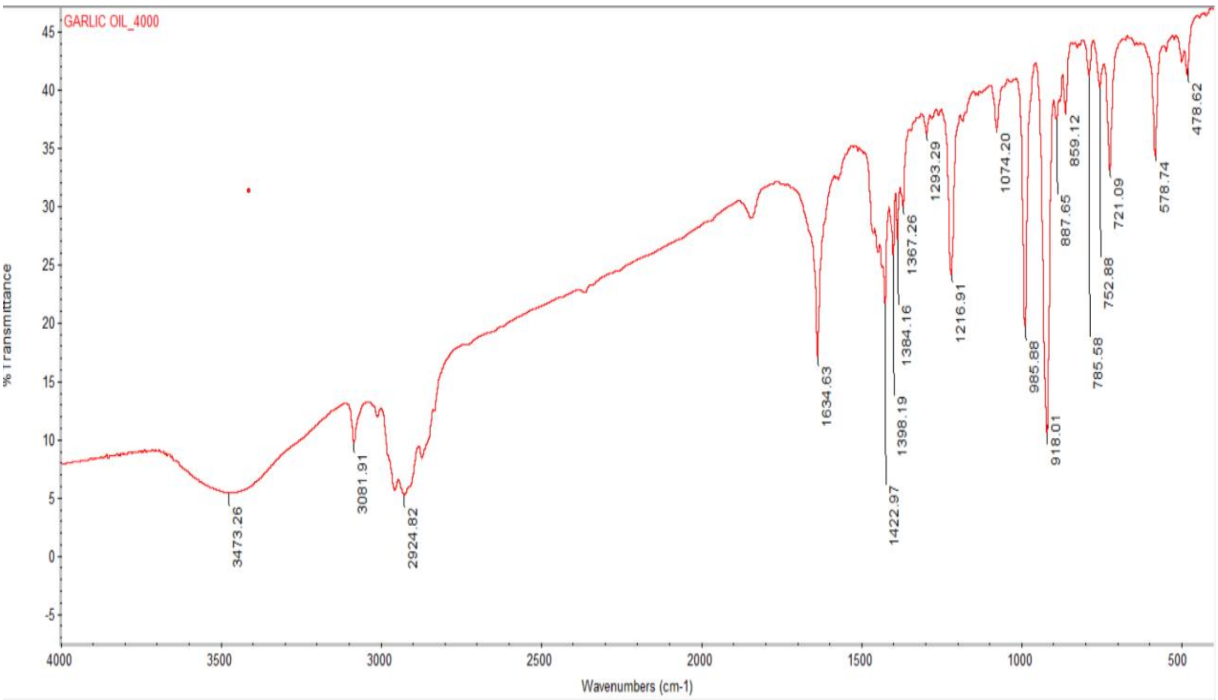

Figure 1: FTIR OF GARLIC OIL

The particle size, % Drug Entrapment, % Drug Release, and % Yield of each formulation was determined and the observed date for analysis in given in the table below:

Table 5: Formulation Response

Std	RUN	Factor-1 A: Garlic Oil (in ml)	Factor-2 b: Ethyl Cellulose (mg)	Factor-3 C: Polyvinyl alcohol (mg)	Response 1 Particle size (nm)	Response2 % Drug Entrapment	Response 3 % Drug Release	Response 4 % Yield
7	4	0.2 ml	250 mg	300 mg	210.5	65.2	71.2	69
1	6	0.2 ml	100 mg	200 mg	212.5	65.3	72	69.5
3	8	0.2 ml	20 mg	20 mg	220.2	66.8	73.2	70.2
5	10	0.2 ml	20 mg	40 mg	228.2	70.6	76.3	72.1
10	1	0.2 ml	20 mg	60 mg	236.7	71.3	77.2	74.8
12	3	0.2 ml	20 mg	80 mg	240.1	72.1	79.5	74.99
11	5	0.5 ml	40 mg	20 mg	252.6	73.6	79.9	76.1
9	7	0.5 ml	60 mg	20 mg	271.8	76.5	82.1	80.2
15	9	0.5 ml	80 mg	20 mg	280.3	78.2	84.2	80.9
14	12	0.5 ml	100 mg	20 mg	290.5	80.1	86	82.58
13	13	0.5 ml	15 mg	10 mg	302.5	83.6	86.3	83.2
2	2	0.5 ml	25 mg	10 mg	311.3	85.2	87.2	85.3
8	11	0.5 ml	30 mg	10 mg	323.56	87.1	88.9	86.8
6	14	0.5 ml	30 mg	10 mg	340.8	89.2	93.5	89.8

4	15	0.5 ml	35 mg	10 mg	352.6	91.5	95.6	93.5

2.1.MODEL ANALYSIS

Table 6: Fit Statistics

Std. Dev.	10.50	**R^2**	0.9326
Mean	361.33	**Predicted R^2**	0.3500
% C.V.	2.91	**Adjusted R^2**	0.8084
		Adeq-Precision	8.8059

ANOVA-Quadratic model

Table 7: Response-1: Particle Size

Source	Squares Sum	Df	Mean Square	F-value	p-value	
Model	7491.44	9	832.38	7.55	0.0192	significant
A-GARLIC OIL	6716.40	1	6716.40	60.94	0.0006	
B-ETHYL CELLULOSE	308.76	1	308.76	2.80	0.1550	
C-PVA	122.46	1	122.46	1.11	0.3401	
AB	23.57	1	23.57	0.2134	0.6635	
AC	10.56	1	10.56	0.0958	0.7694	
BC	38.44	1	38.44	0.3488	0.5805	
A^2	52.19	1	52.19	0.4732	0.5221	
B^2	0.3703	1	0.3703	0.0034	0.9560	
C^2	229.47	1	229.47	2.08	0.2086	
Residual	551.05	5	110.21			
Lack of Fit	290.00	3	96.67	0.7406	0.6182	not significant
Pure Error	261.05	2	130.52			
Cor Total	8042.58	14				

Coded: Factor coding.
Type III – Partial: Sum of squares

The **model F-value** of 7.55 indicates that particular model is important. Only 1.92% chance that due to noise, the large F-value can be appear.

Equation 367.367 + 28.975 * A + 6.2125 * B + 3.9125 * C + -2.425 * AB + -1.625 * AC + 3.1 * BC + -3.75833 * A^2 + 0.316667 * B^2 + -7.88333 * C^2

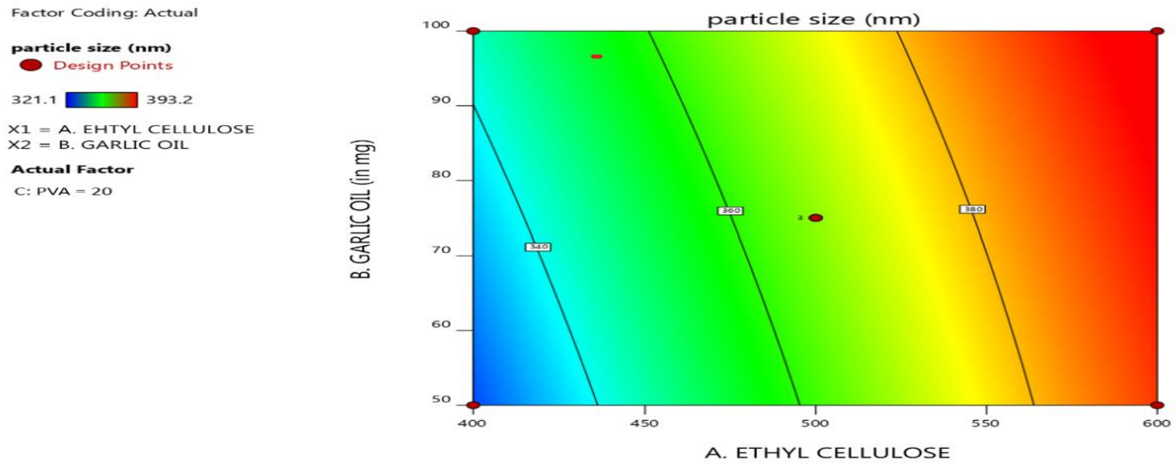

Figure 2: Contour plot for effect of particle size by ratio of A and B fluctuations

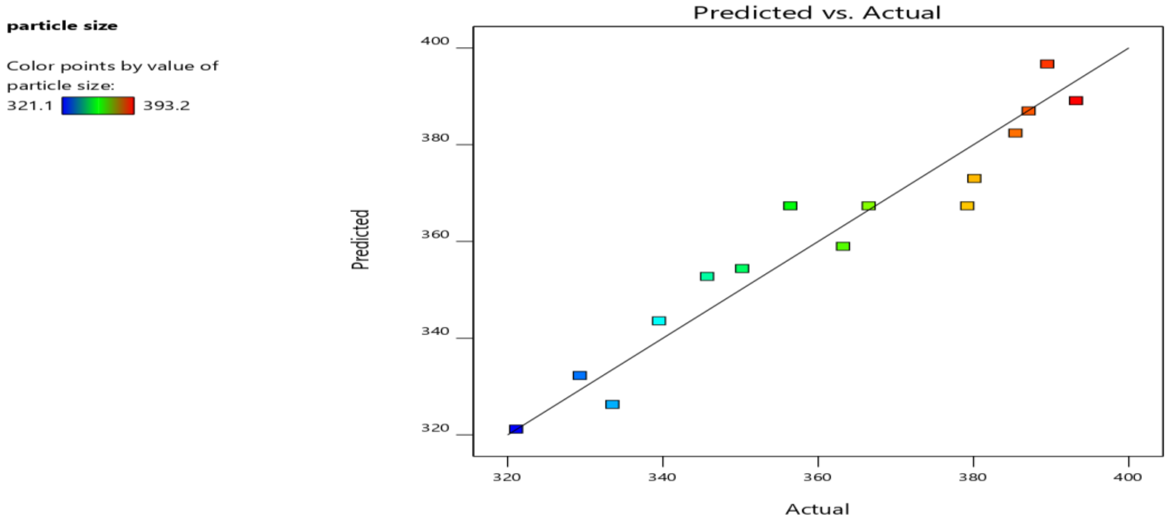

Figure 3: The report of predicted v/s actual value designed using software

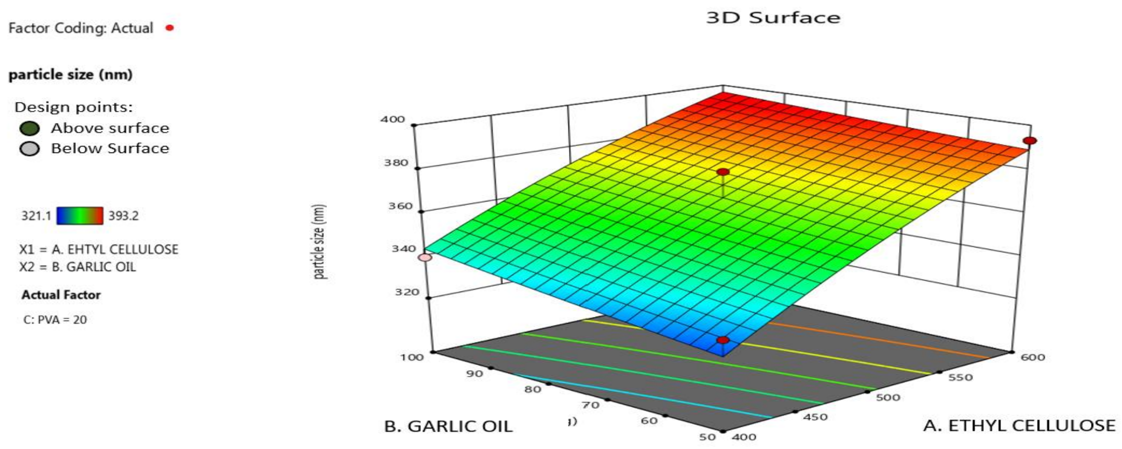

Figure 4: 3D surface diagram of particle size effect on ratio of variable A and B

ANOVA-Quadratic model

Table 8: Response-2: Drug Entrapment

Source	Squares Sum	df	Mean-Square	F-value	p-value	
Model	1082.28	9	120.25	13.18	0.0055	significant
A-GARLIC OIL	972.40	1	972.40	106.61	0.0001	
B-ETHYL CELLULOSE	39.61	1	39.61	4.34	0.0916	
C-PVA	23.81	1	23.81	2.61	0.1671	
AB	6.55	1	6.50	0.7129	0.4370	
AC	0.3028	1	0.3025	0.0332	0.8626	
BC	2.40	1	2.40	0.2634	0.6297	
A^2	0.1078	1	0.1078	0.0118	0.9177	
B^2	2.49	1	2.49	0.2727	0.6238	
C^2	32.77	1	32.77	3.59	0.1165	
Residual	45.63	5	9.12			
Lack of Fit	37.60	3	12.53	3.13	0.2514	not significant
Pure Error	8.01	2	4.00			
Cor Total	1127.97	14				

Coded: Factor coding.
Type III – Partial: Sum of squares

The **model F-value** of 13.18 indicates that particular model is important. Only 0.55% chance that due to noise, the large F-value can be appear.

Table 9: Fit Statistics

Std. Dev.	3.02	**R^2**	0.9598
Mean	77.07	**Predicted R^2**	0.4509
% C.V.	3.92	**Adjusted R^2**	0.8867
		Adeq-Precision	11.6789

Equation 78.1333 + 11.025 * A + 2.225 * B + 1.725 * C + -1.275 * AB + -0.275 * AC + 0.775 * BC + 0.170833 * A^2 + 0.820833 * B^2 + -2.97917 * C^2

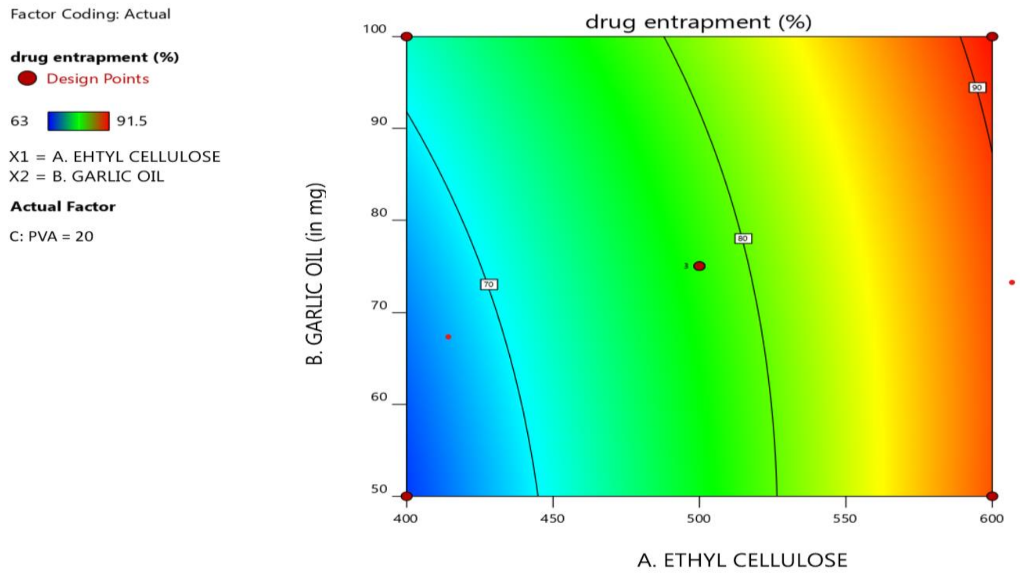

Figure 5: Contour plot for effect of % drug entrapment by ratio of A and B fluctuations.

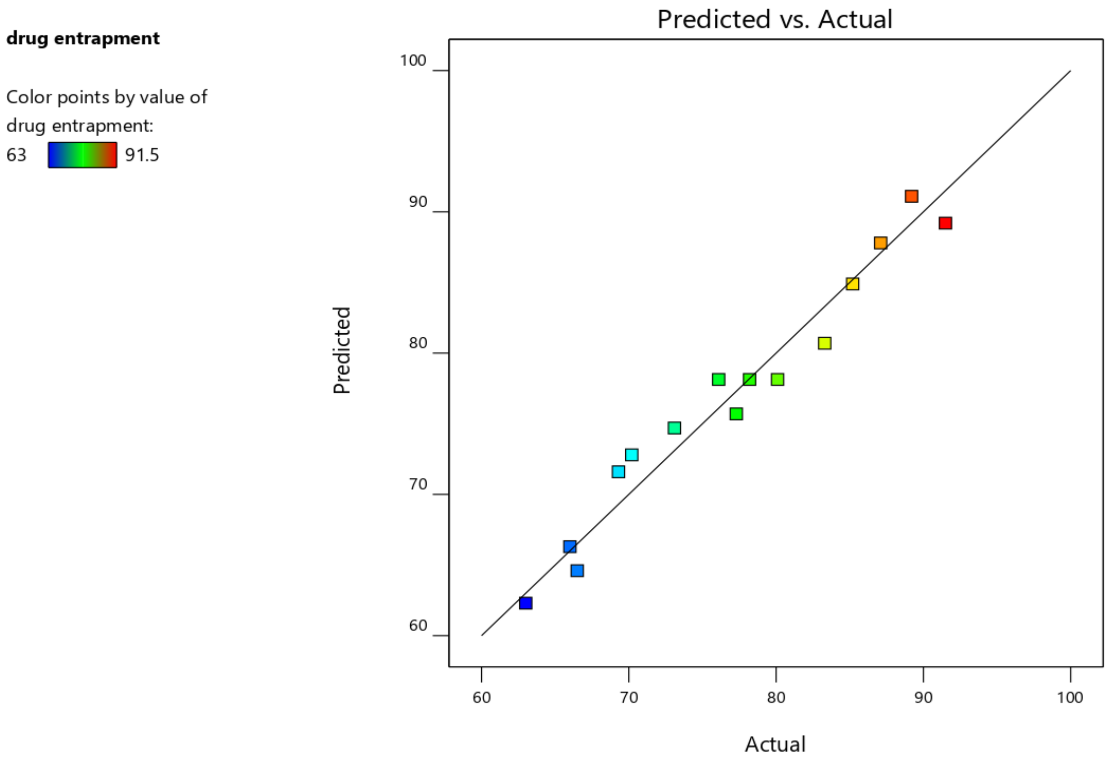

Figure.6: The report of predicted v/s actual value designed using software

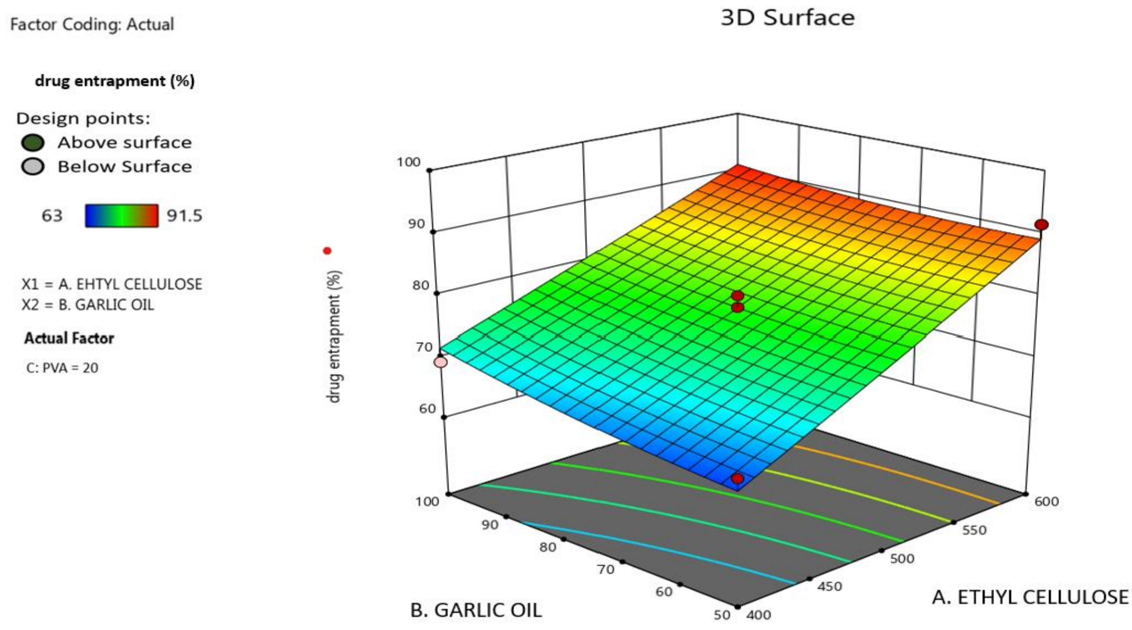

Figure 7: 3D surface diagram of drug entrapment effect on ratio of variable A and B

ANOVA-Quadratic model

Table 10: Response-3: Drug Release

Source	Squares Sum	df	Mean-Square	F-value	p-value	
Model	891.74	9	99.08	12.20	0.0066	significant
A-GARLIC OIL	780.12	1	780.12	96.09	0.0002	
B-ETHYL CELLULOSE	16.82	1	16.82	2.07	0.2096	
C-PVA	15.68	1	15.68	1.93	0.2233	
AB	10.24	1	10.24	1.26	0.3124	
AC	2.29	1	2.25	0.2772	0.6211	
BC	0.8100	1	0.8100	0.0998	0.7649	
A^2	0.3510	1	0.3510	0.0432	0.8435	
B^2	10.84	1	10.78	1.33	0.3014	
C^2	50.32	1	50.32	6.20	0.0552	
Residual	40.59	5	8.12			
Lack of Fit	18.07	3	6.02	0.5346	0.7031	not significant
Pure Error	22.53	2	11.26			
Cor Total	932.44	14				

Coded: Factor coding.
Type III – Partial: Sum of squares

The **model F-value** of 12.20 indicates that particular model is important. Only 0.66% chance that due to noise, the large F-value can be appear.

Table 11: Fit Statistics

Std. Dev.	2.85	**R^2**	0.9567
Mean	82.34	**Adjusted R^2**	0.8778
% C.V.	3.46	**Predicted R^2**	0.6351
		Adeq Precision	11.1553

Equation 83.2333 + 9.875 * A + 1.45 * B + 1.4 * C + -1.6 * AB + 0.75 * AC + 0.45 * BC + 0.308333 * A^2 + 1.70833 * B^2 + -3.69167 * C^2

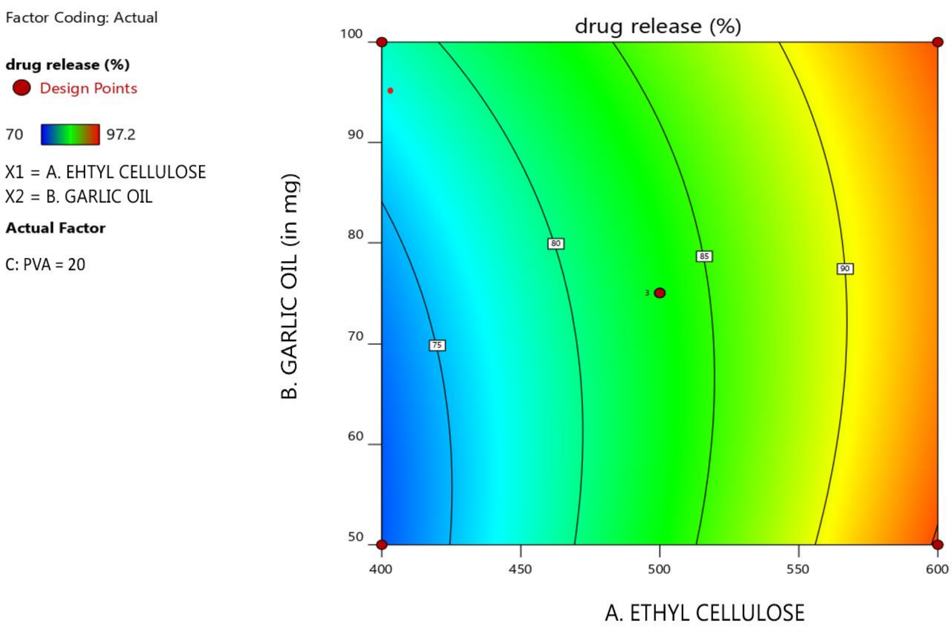

Figure 8: Contour plot for effect of % drug release by ratio of A and B fluctuations.

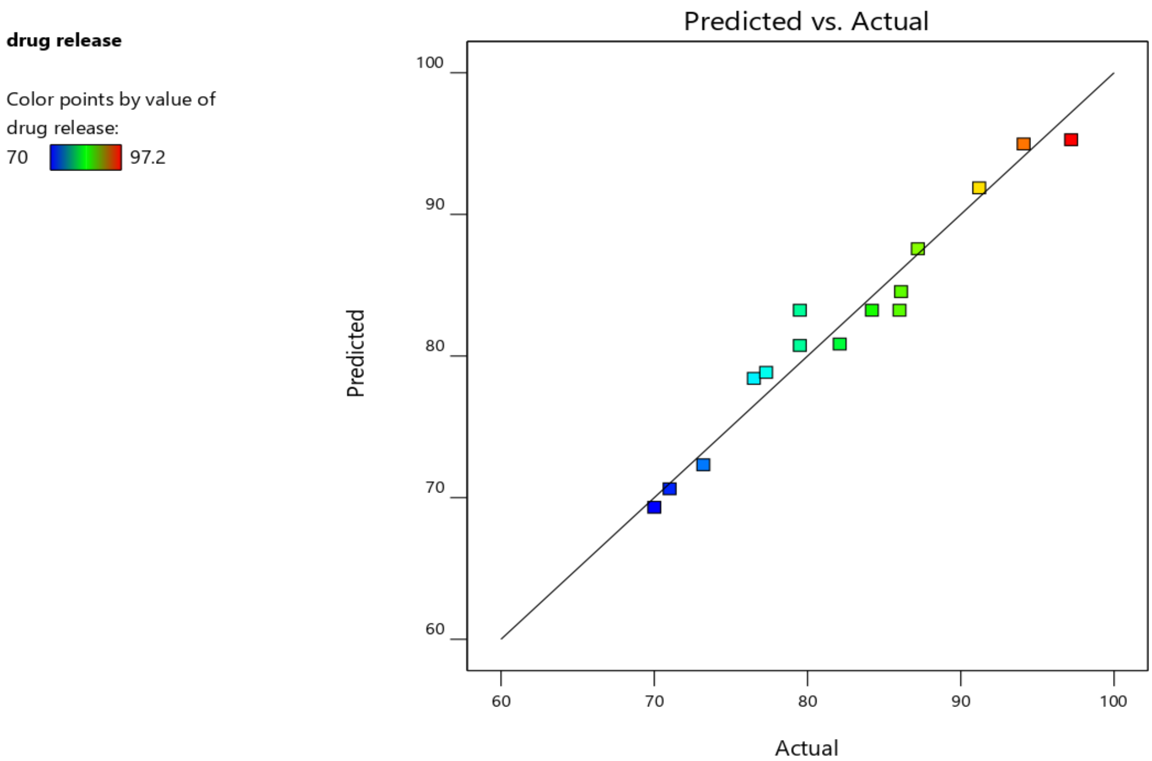

Figure 9: The report of predicted v/s actual value designed using software

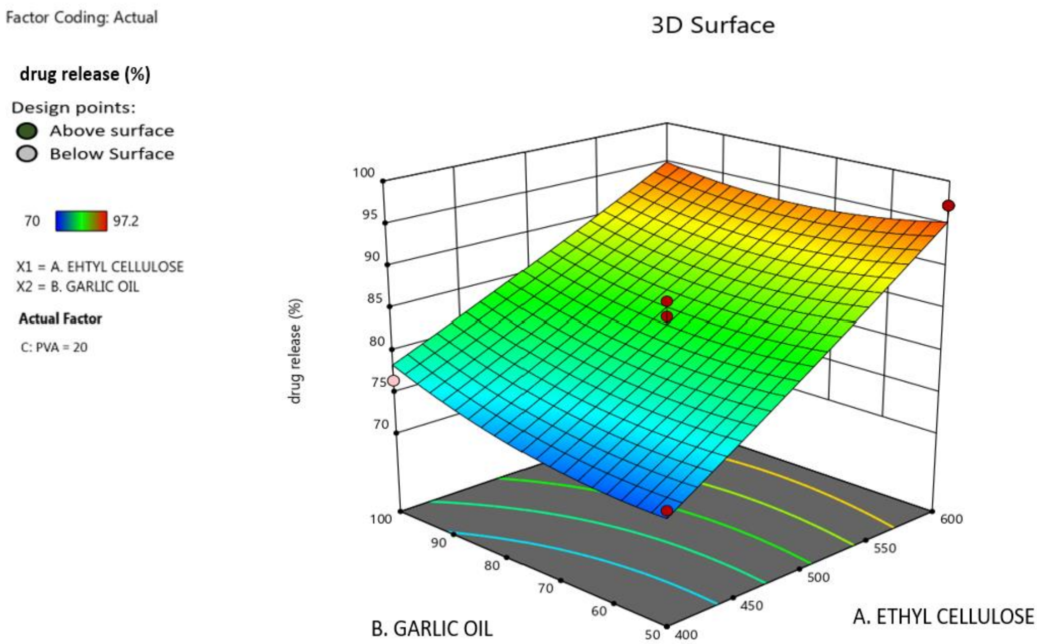

Figure 10: 3D surface diagram of % drug release effect on ratio of variable A and B

ANOVA-Quadratic model

Table 12: Response-4: % Yield

Source	Squares Sum	df	Mean-Square	F-value	p-value	
Model	808.87	9	89.87	10.30	0.0097	significant
A-GARLIC OIL	710.64	1	710.64	81.41	0.0003	
B-ETHYL CELLULOSE	26.17	1	26.17	3.00	0.1439	
C-PVA	6.00	1	6.00	0.6877	0.4447	
AB	3.42	1	3.42	0.3921	0.5587	
AC	0.2025	1	0.2025	0.0232	0.8849	
BC	3.122	1	3.19	0.3650	0.5721	
A^2	2.58	1	2.59	0.2970	0.6092	
B^2	18.23	1	18.20	2.09	0.2083	
C^2	33.89	1	33.89	3.88	0.1059	
Residual	43.65	5	8.73			
Lack of Fit	34.34	3	11.45	2.46	0.3021	not significant
Pure Error	9.31	2	4.65			
Cor Total	852.57	14				

Coded: Factor coding.
Type III – Partial: Sum of squares

The **model F-value** of 10.30 indicates that particular model is important. Only 0.97% chance that due to noise, the large F-value can be appear.

Table 13: Fit Statistics

Std. Dev.	2.95	**R²**	0.9489
Mean	78.58	**Predicted R² ²**	0.8568
% C.V.	3.76	**Adjusted R**	0.3313
		Adeq-Precision	10.8089

Equation 78.5667 + 9.425 * A + 1.80875 * B + 0.86625 * C + -0.925 * AB + -0.225 * AC + 0.8925 * BC + 0.837917 * A^2 + 2.22042 * B^2 + -3.02958 * C^2

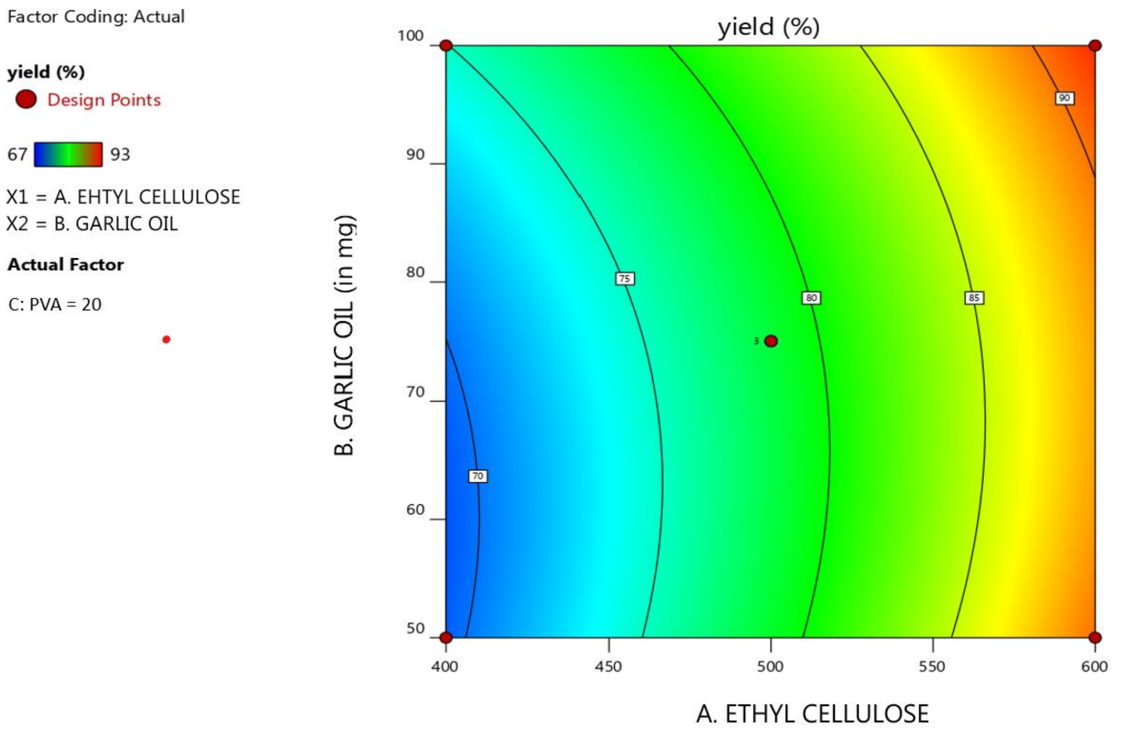

Figure 11: Contour plot for effect of % yield by ratio of A and B fluctuations

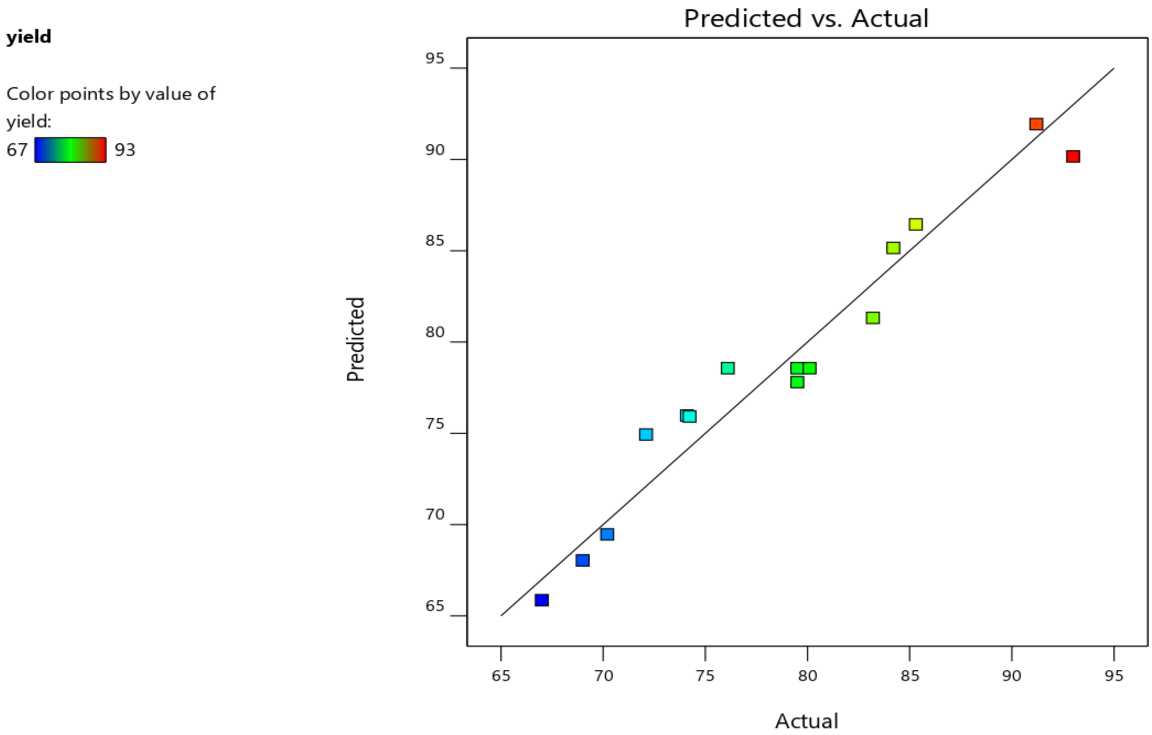

Figure 12: The report of predicted v/s actual value designed using software

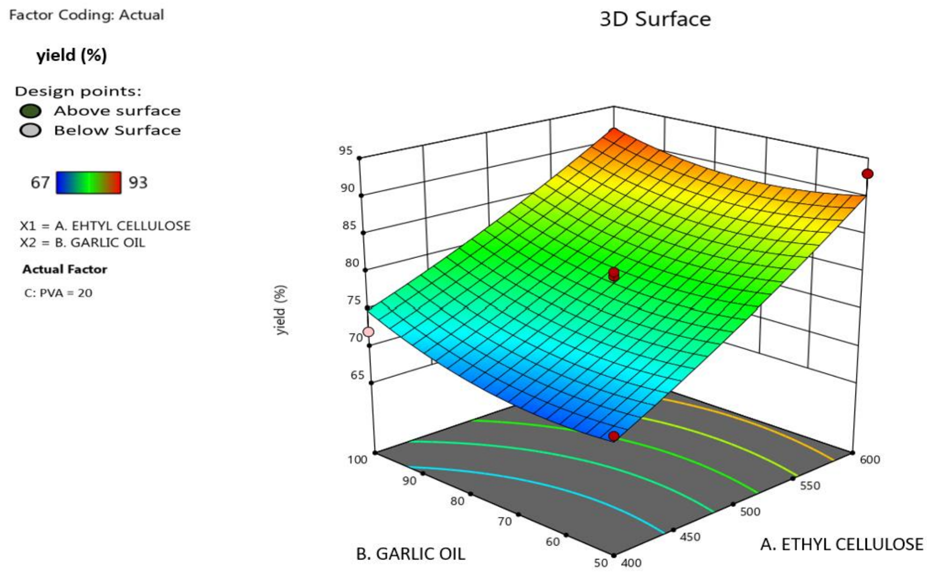

Figure 13: 3D surface diagram of % yield effect on ratio of variable A and B

FT-IR of Optimized formulation

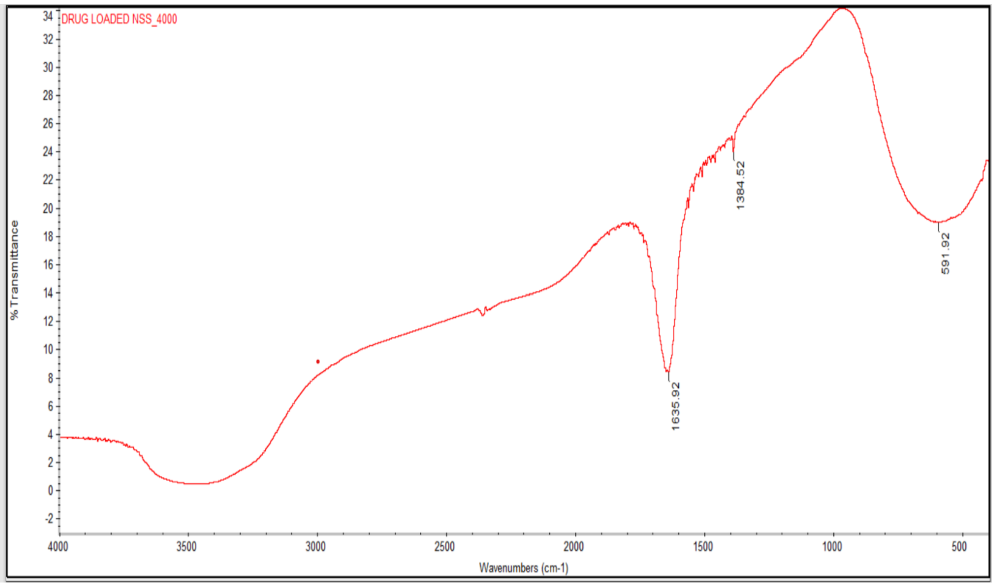

Figure 14: FT-IR of garlic oil loaded optimized formulation

2.1.MORPHOLOGY AND SURFACE TOPOGRAPHY:

The morphological properties of Garlic Oil loaded nanosponges were studied by SEM analysis and evaluated. The shape of nanosponges were examined as spherical shaped. The droplet size observed as 1 nm and 11 nm (w) and distributed evenly in nanometre range. The observed image was shown in the figure-

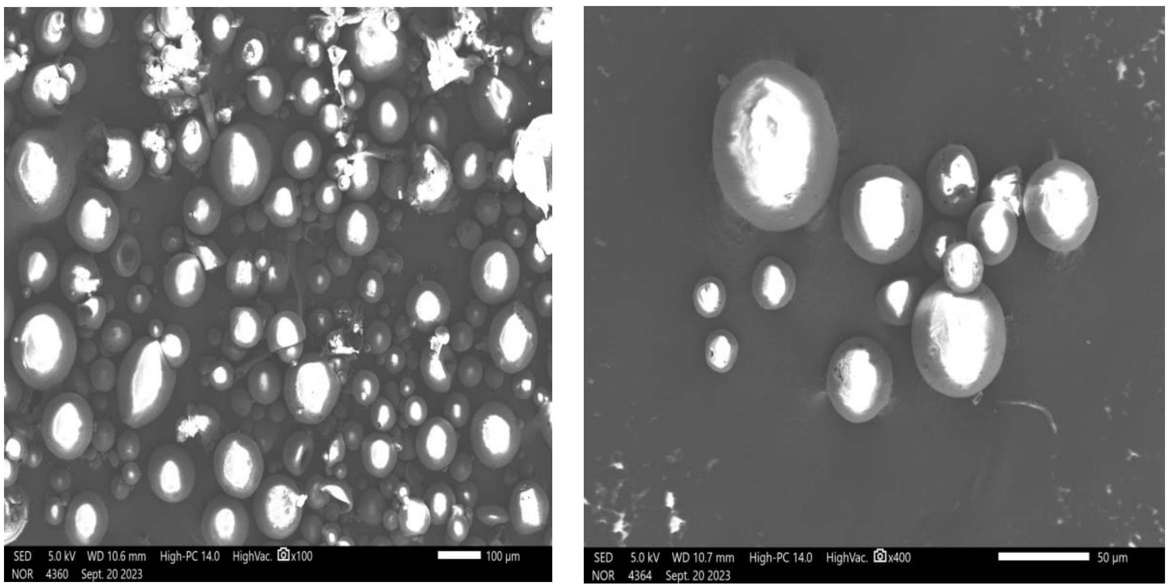

Figure 15: Figure showing SEM analysis of nanosponges of optimized formulation at 3000X and 3000X resolution.

CONCLUSION:

Successfully formulated garlic oil loaded nanosponges, to enhance the controlled and sustained release property of the formulation. Use of experimental design i.e. (Box-Behnken Design) in formulating garlic oil loaded nanosponges has a significant role. The formulation of garlic oil loaded nanosponges was done by the method of emulsion solvent diffusion method. In this method, Ethyl Cellulose was dissolved in organic solvent and polyvinyl alcohol was dissolved in aqueous solvent. Using different compositions, varieties of formulations were prepared which were subjected to study parameters such as determination of particle size, % drug release, % drug entrapment, and % yield. Box- Behnken design was implemented to get optimized formulation. The SEM analysis was done to study the morphological properties of the optimized formulation. The particle size of the optimized formulation was obtained as 290.5 nm, with 80.1% drug release, 86% drug entrapment, and 82.58% yield.

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