Section A-Research paper
ISSN2063-5346

DEVELOPMENT AND CHARACTERIZATION OF MEDICATED CHEWING GUM CONTAINING NANOCOMPOSITE LOADED WITH LEAF EXTRACT OF SOLANUM NIGRUM: A STATISTICAL STUDY

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Keywords: Mouth ulcers, Solanum nigrum, aphthous, chewing gum, nanoparticles, FESEM,

ABSTRACT: Mouth ulcer treatment is a challenging task for many civilizations of the world. There are different plants used for the effective treatment of mouth ulcers and aphthous. Drugs for mouth ulcers are obtained from different parts of the plants. Nobility of dosage forms with no side effects is a major development and thus extract of plant was used to prepare such types of dosage form. For the treatment of mouth ulcer and aphthous ulcer, extract of leaves of Solanum nigrum was taken for the study. Polymeric nanoparticles of the leaves extract of Solanum nigrum were prepared using ionic gellation method. Optimization of this formulation was done using 2^2 factoial designs. Characterization of nanoparticles containing Solanum nigrum leaves extract was done using some characterization evaluation parameters like zeta potential, FESEM studies. These prepared nanoparticles were then incorporated into chewing gum taken amount three factors and two levels. Response surface methodology (RSM) and central composite design (CCD) was used for the study. Medicated chewing gum were prepared and evaluated for the low and high levels of the ingredient variables.

INTRODUCTION:

The ulcer is defined as a break in the skin, or on the surface of an organ inside the body, that does not heal naturally. Ulcers are named generally depend on the location. Mouth ulcers or mucosal ulcers are also called as oral ulcers. They occur in the mouth cavity over mucous membrane[1]

Oral Ulcers are characterized by loss of the mucosal layer in the mouth. It generally presents inside the cheek pouch and on the lips. Ulcers may be in the form of lesions, shallow, round or oval and painful. In most of the countries are using medicinal plants as a therapeutic agent for the maintenance of good health since the ancient time[2] .

It has been proved that NPs are more voluntarily absorbed than synthetic drugs[3]. Herbal formulations when inculcated in nanonized composite which penetrate into buccal mucosa rapidly and more efficiently in the systemic circulation.

Herbal drugs and their uses have been since the origin of mankind. Plant leaves of Solanum nigrum belongs to family Solanaceae have shown antiulcer property especially oral or mouth ulcer.

Medicated chewing gum is beneficial than other conventional dosage forms because it offers faster onset of action and an excellent possibility for the delivery of metabolically unstable drugs[4].

Medicated chewing gums if incorporated with mucoadhesive nanoparticulate carriers containing extracts of herbal drugs is a unique milestone in the field of drug therapy and pharmaceutical sciences.

MATERIALS AND METHODOLOGY:

Collection of Crude drugs: Solanam nigram leaves were collected from around 10 km area of my village in Siddharthnagar, U.P. A voucher specimen of the plant has been deposited in the herbarium and remaining leaves (2 kg) were air-dried at room temperature (24 ± 2 °C) with no direct sunlight[5].

Procurements of chemicals used in work: Pure and natural Beeswax was procured from Herian by Amazon India. Polyethylene Glycol (PEG 400) was purchased from BRM chemicals, Delhi. Xylitol, Ethyl alcohol, Peppermint oil, Corn syrup and all other ingredients were of analytical grades. All the chemicals used have no hazardous property reported.

Methodology of the research work:

A. Authentication of drugs: Authentication of Drugs (Solanum nigrum) was done from Botanical Survey of India, Allahabad.

B. Standardization of herbal drug[6]:

Table1. Table showing standardization of leaf extract of Solanum nigrum

S No.	Analysis	Specifications	Results
1.	Appearance	Brown fine powder	Complied
2.	Odor	Characteristics	Complied
3.	Taste	Characteristics	Complied
4.	Assay/ Extract ratio	10:1	Complied
5.	Loss on drying	≤ 5.0 %	3.54 %
6.	Sieve Analysis	Pass 80 mesh	Complied
7.	Bulk density	45-55/100 mL	49.2g/100 mL
8.	Heavy metal	Not more than 20ppm	Complied
9.	Arsenic	Not more than 2ppm	Complied
10.	Total plate count	Not more than 500 cfu/g	Complied
11.	Yeast & mold	Not more than 100 cfu/g	Complied
12.	E. coli	Negative	Complied
13.	Salmonella	Negative	Complied

C. Extraction of herbal drug (Solanam nigrum)[7]: The dried leaves were powdered with electric mixer and powdered leaves were extracted by maceration for 24 hrs at room temperature with 150 mL each of ethanol/water solution in the ratio (10**:**1) (SN1) and water

(SN2), respectively. The extraction procedure was repeated three times, and the solvent extracts were combined and separated from the residue by filtration through Whattmann N.1 filter paper in a Buchner funnel under vacuum. The ethanol was removed under a reduced pressure below 40 ^{0}C by using a rotary evaporator, and the aqueous phase remaining after evaporation of the organic phase was freeze-dried. Extract was dried and collected in tightly closed containers.

Extraction of leaves of the drug e.g. Solanam nigram was done with water and ethyl alcohol in the ratio of (10:1).

D.Formulation of nanoparticles containing leave extract of Solanum nigrum[8]:

Optimization of Formulation variables: Optimization of formulation variables was done using statistical design (Taguchi Method). According to Taguchi Method, L4 orthogonal array was used as formulation design. Two factors with two variables were used in the design L4, 2^3 .This designs consist of up to 2 factors at 2 levels each. There are 4 runs in the design.

Table 2: Table of different formulation variables and levels as L4 design

Run	Columns		
	1	2	3
1	1	1	1
2	1	2	2
3	2	1	2
4	2	2	1

The Orthogonal Array L4 (2^3)

E. Design and Formulation of Nanoparticles Containing Solanum Nigram Extract by Taguchi Method:, Stirrer speed and stirring time were two factors and two levels of these factors were taken for the study.

Factor: 1. Stirrer speed (rpm)

Factor: 2. Stirring time (hr)

Table 3. Formulation of nanoparticles containing Solanum nigrum leaves extract

S.No.	Name of Formulations	SA* (%)	CCl (mM)	SN Extract (%)	S. Speed(rpm)	S. Time(hr.)
1	**F1**	0.03	36	3.5	3000	1
2	**F2**	0.03	36	3.5	3500	1
3	**F3**	0.03	36	3.5	3000	2
4	**F4**	0.03	36	3.5	3500	2

***SA= Sodium alginate, CCl= Calcium chloride, SN= Solanum nigrum, S. speed= Stirring speed, S. time= Stirring time, rpm= round per minute, hr=hours**

F1 formulation was prepared as mentioned in the table 2. Nanoparticles were prepared and evaluated. Particles were examined for particle size analysis and zeta potential by Instrument Malvern mastersizer. Results of particle size analysis and zeta potential are given in the graph 1 and graph 2 respectively. Particle sizes were within the nanoscale range.

F.Characterization of Nanoparticles containing Solanum nigrum leaves extract[9]:

(i) Particle size and Size distribution:

Particle size and size distribution of nanoparticles containing leaves extract of **Solanum nigrum** were done by using Malvern Zetasizer**.** These experiments were done from CIF, LPU, Jalandhar, Punjab and results are shown in Graph 1.

(ii) Zeta potential: Zeta Potential of nanoparticles containing leaves extract of **Solanum nigrum** were done by using Instrument Malvern Zetasizer**.** Experiment was done from CIF, LPU, Jalandhar, Punjab and results are shown in Graph **5**.

(iii) Field Emission Scanning Electron Microscope analysis (FESEM Analysis):

FESEM Analysis of nanoparticles was done using magnification of 25-100000, accelerated voltage 0-1KV to 30KV and secondary electron detectors. FESEM analysis results are shown in figures.

G. Preparations of chewing gum containing nanoparticles of leave extract: Chewing gum containing leaf extract of the plant was prepared.

(i) Formulation of Medicated chewing gum:

Methods of Preparation of Medicated Chewing Gums[10]: Beeswax was melted in a beaker until it becomes liquefied. Pour honey (2%) in the given amount and mixed in liquefied beeswax. PVP and calcium carbonate (0.5 gm) gave a strength and elasticity to liquid mixture. Add Polyethylene glycol 400 (2gm) in the melted mixture and stir thoroughly. Peppermint oil (0.5 ml) was then added and mixed. Powdered sugar and xylitol (4%) were added and mixed for 15 mins.

Drug containing nanoparticles was then added and mixed thoroughly. This semisolid mixture was then poured into pre-lubricated molds of desired sizes and dried in a refrigerator.

Statistical Design: Optimization and analysis of data was done using Design experiment software (8.0.7.1) Stat-Ease Inc., Minneapolis, MN, USA. To obtain the optimum conditions of three independent variables 3-D response surface methodology (RSM) technology with central composite design were used. Total 17 formulations were prepared by using response surface methodology .Coefficient of determination and ANOVA were used to access goodness of fit and regression determination. Amount of quercetin and % friability were taken for two responses e.g. R1 and R2 respectively.

H. Characterization of chewing gum containing nanoparticles of leave extracts:

1. Weight variations: Prepared chewing gums (20) were taken for study. Twenty were weighed on an electronic balance. Average weight of all chewing gums was calculated and then individual weight and standard deviation was calculated. Not more than two individual weights of chewing gums were deviated for more than 5%.

2. Friability: Friability is an ability of a solid compact mass to resist the abrasion and sock during handling and transportation. Test was performed as per IP and chewing gums (10) were randomly selected for study and weighed accurately and placed in a Friabilator (Rocche, USA). Rotation was done at 25 rpm for 100 revolutions. The chewing gums were collected then de-dusted with canvas cloth and reweighed properly. Friability (%) was then calculated using the following equation:

$$\% F = (\text{final weight} - \text{initial weight} / \text{initial weight}) \times 100$$

Where F = friability

The ideal condition is when (%) friability is in between 0.5 to 1.00.

3. In-vitro dissolution studies:

The in-vitro drug release studies were performed for amount of quercetin released as per apparatus described in European Pharmacopoeia. Standard curve of quercetin was taken for calculation. The chamber temperature was maintained at 37±0.5^{o}C. 20 ml of buffer solution (pH 6-8) were filled in a chamber made for chewing having volume of 40 ml and applied a chewing rate of 60 strokes per minute[11]. Samples were collected in an interval of 2 minutes for a period of 30 minutes. 2 ml buffer solution then replaced into dissolution flask. Collected samples then studied with UV spectrophotometer at absorption of 280 nm. Graph of cumulative amount of drug release vrs different formulation has been shown in figure 13.

RESULTS AND DISCUSSION: Results of authentication of Drugs (Solanum nigrum) have shown that the original and authentic drug is taken for this work. All the physico-chemical evaluation data showed standard readings. Physical properties like taste, texture, odor complies with the standard product. Chemical properties showed the positive test for organic acids like Acetic acid, tartaric acid, malic acid and citric acid etc. Leaves extract showed positive test for Polyphenols and flavones. Mucoadhesive nanoparticles were prepared containing plant leaves extract using sodium alginates by ionic gelation method. Characterization of nanoparticles e.g. particle size distribution, zeta potential and FESEM analysis were done. Particle size distribution curve showed maximum particles lie between 200-500 nm size ranges. Zeta Potential value of F2 formulation found out to be maximum value. So it may be more stable nanoparticles than others. FESEM analysis showed that the F2 formulation has more pronounced surface which showed maximum stability and rigidity.

For making chewing gum using best combination of nanocomposite three different formulations variables with three different levels e.g. low, medium and high were taken for statistical analysis using response surface methodology. The obtained results showed a good fit with the regression Equation (1) and the results were adequate with satisfactory R2 values and were statistically acceptable at different p-values. The “fitness” of the models was observed through the lack-of-fit tests ($p > 0.1$), which showed the feasibility of the models to predict the variations accurately[12].

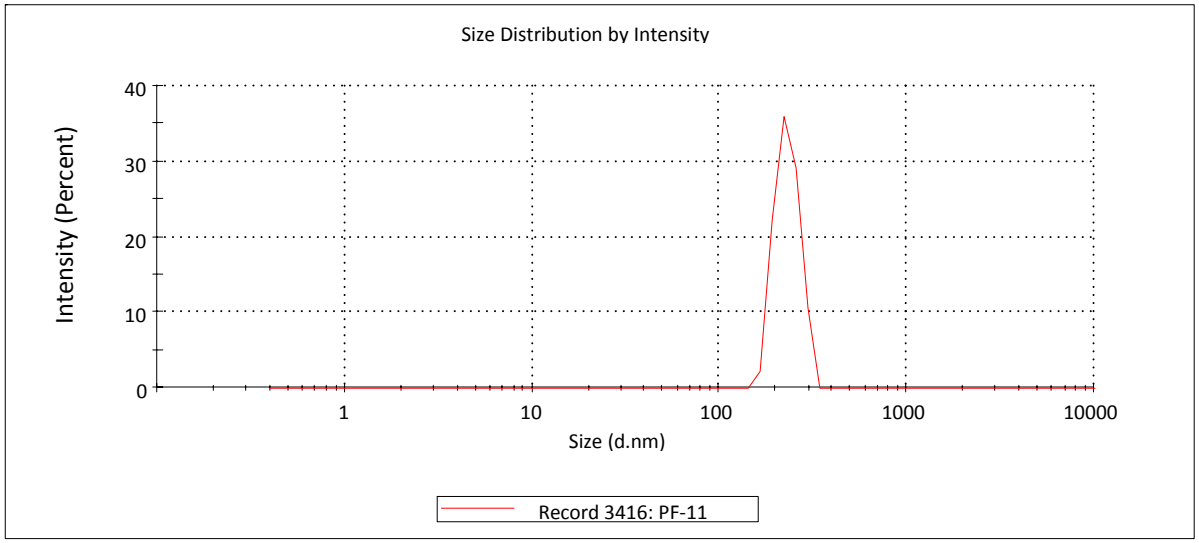


Figure 1: Graph Showing Particle size distribution of F1 nanoparticles

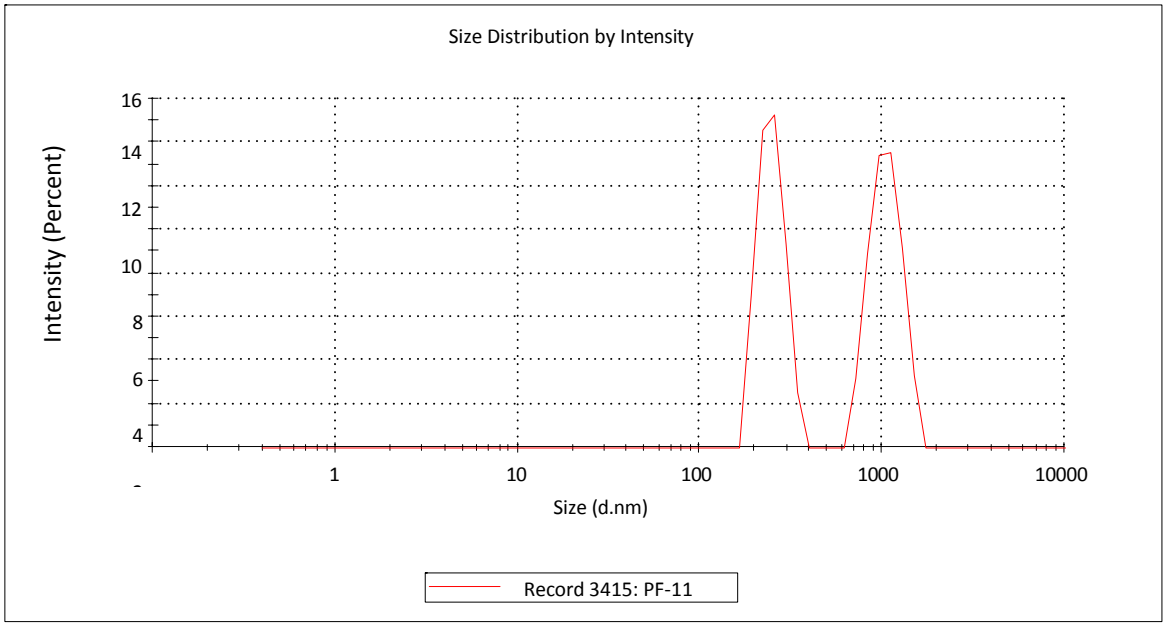


Figure 2: Graph Showing Particle size distribution of F2 nanoparticles

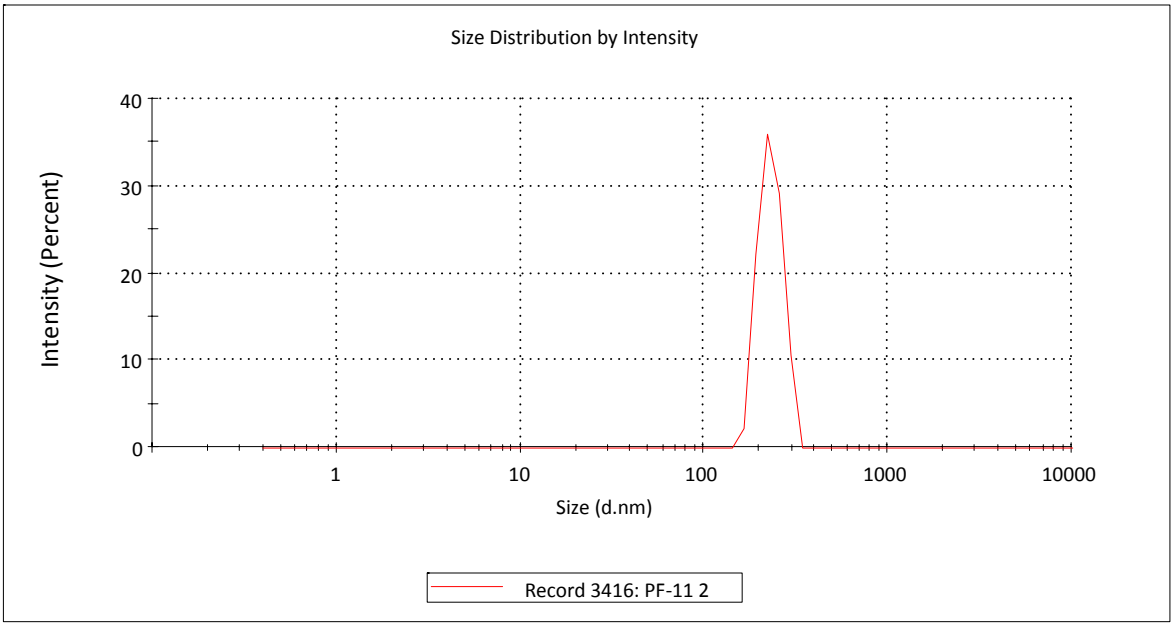


Figure 3: Graph Showing Particle size distribution of F3 nanoparticles

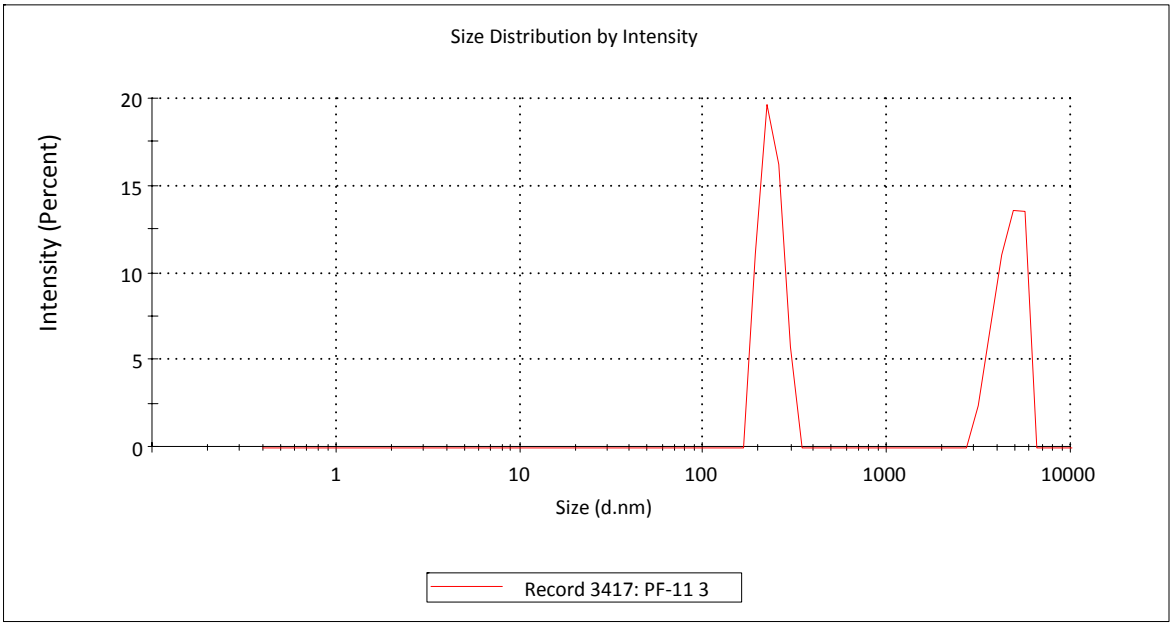


Figure 4: Graph Showing Particle size distribution of F4 nanoparticles

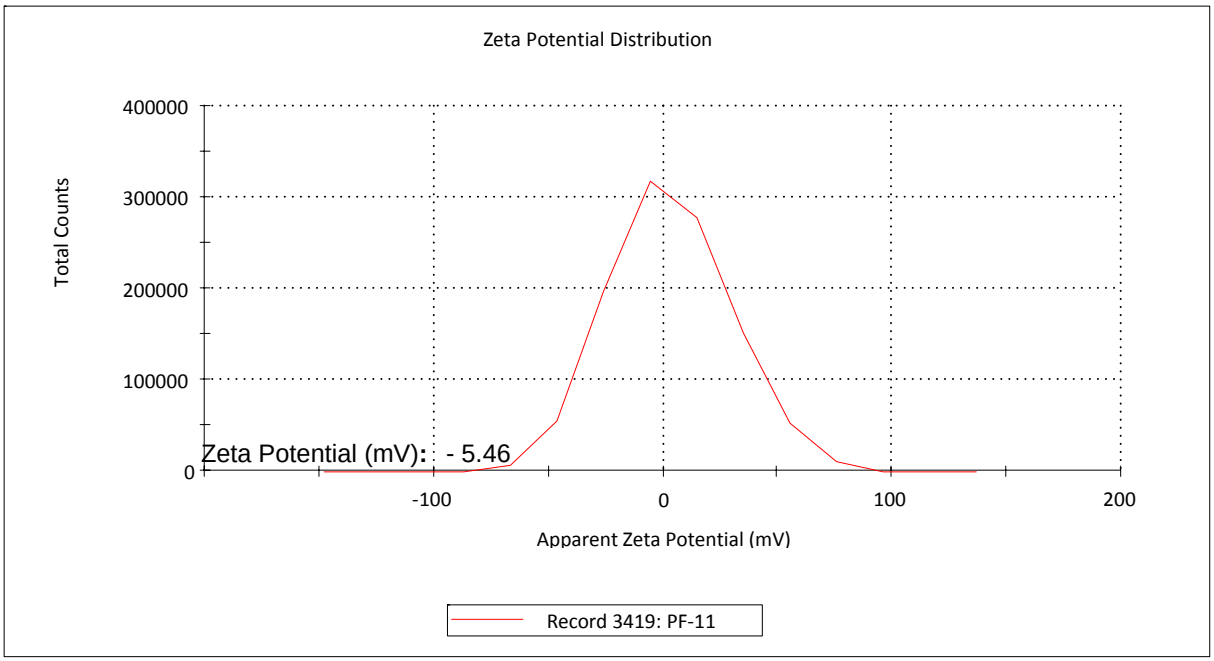


Figure 5: Graph Showing Zeta Potential of F1 nanoparticles

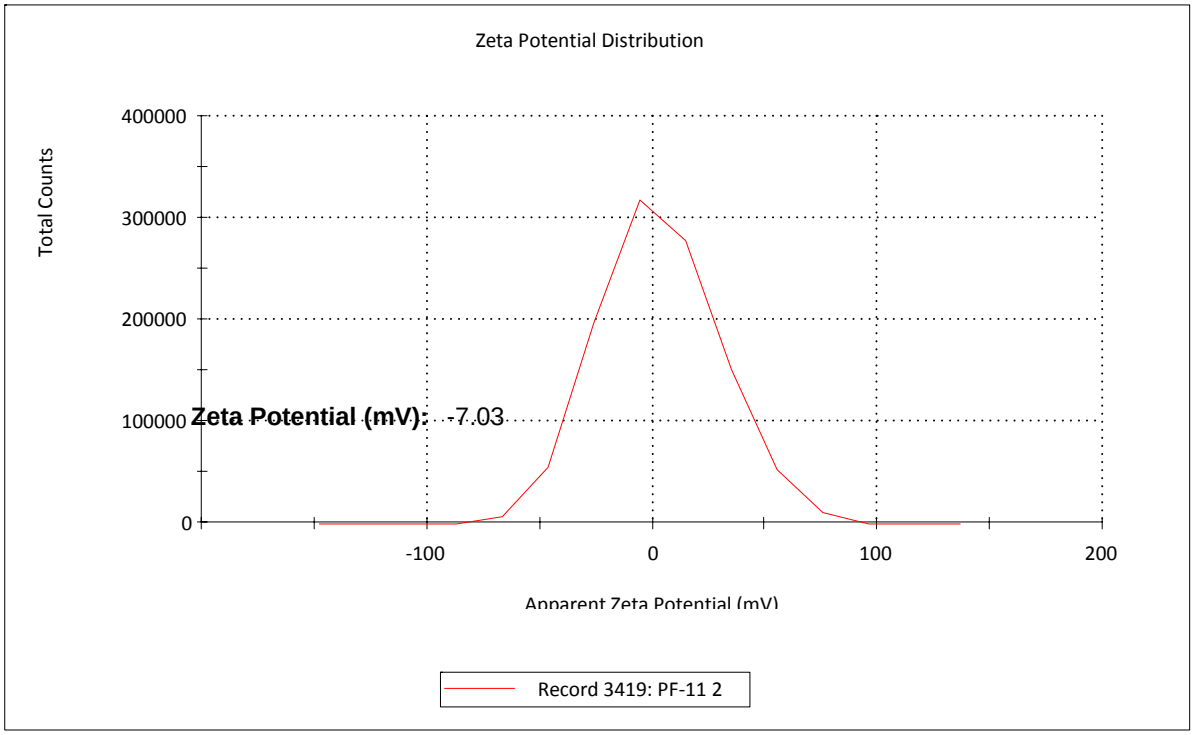


Figure 6: Graph Showing Zeta Potential of F2 nanoparticles

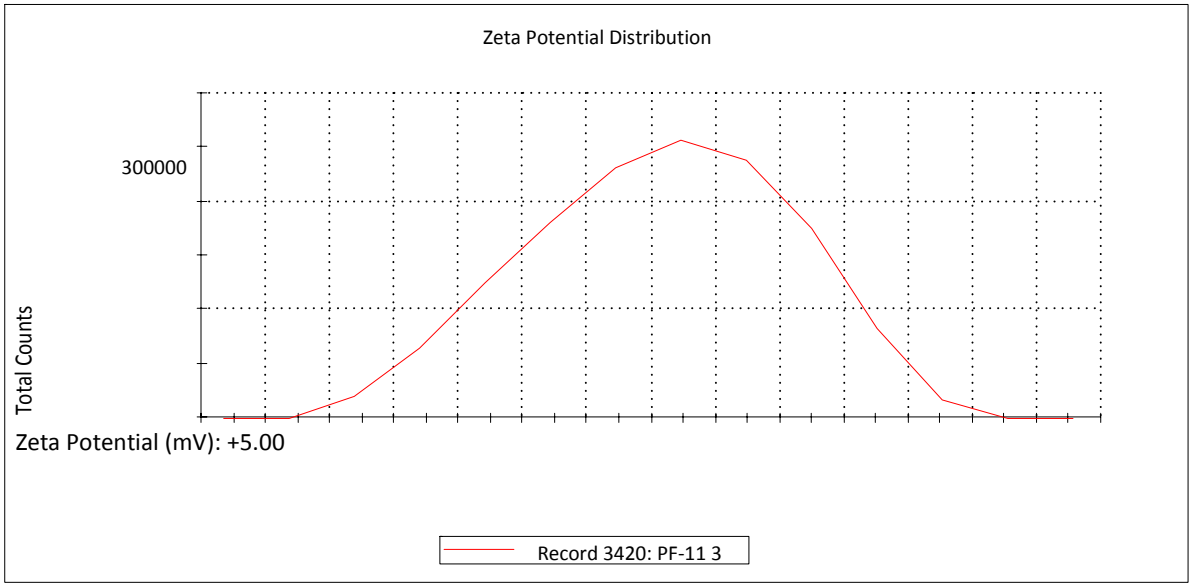
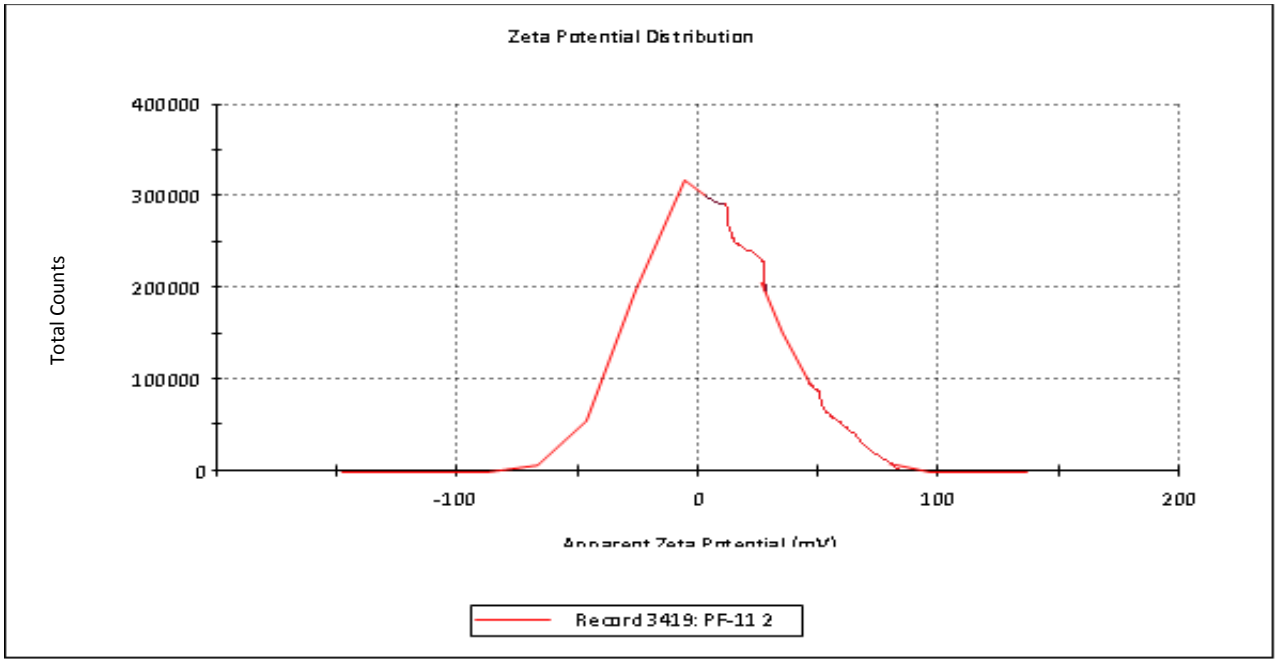


Figure 7: Graph Showing Zeta Potential of F3 nanoparticles



Zeta Potential (mV):-3.05

Figure 8: Graph Showing Zeta Potential of F4 nanoparticles

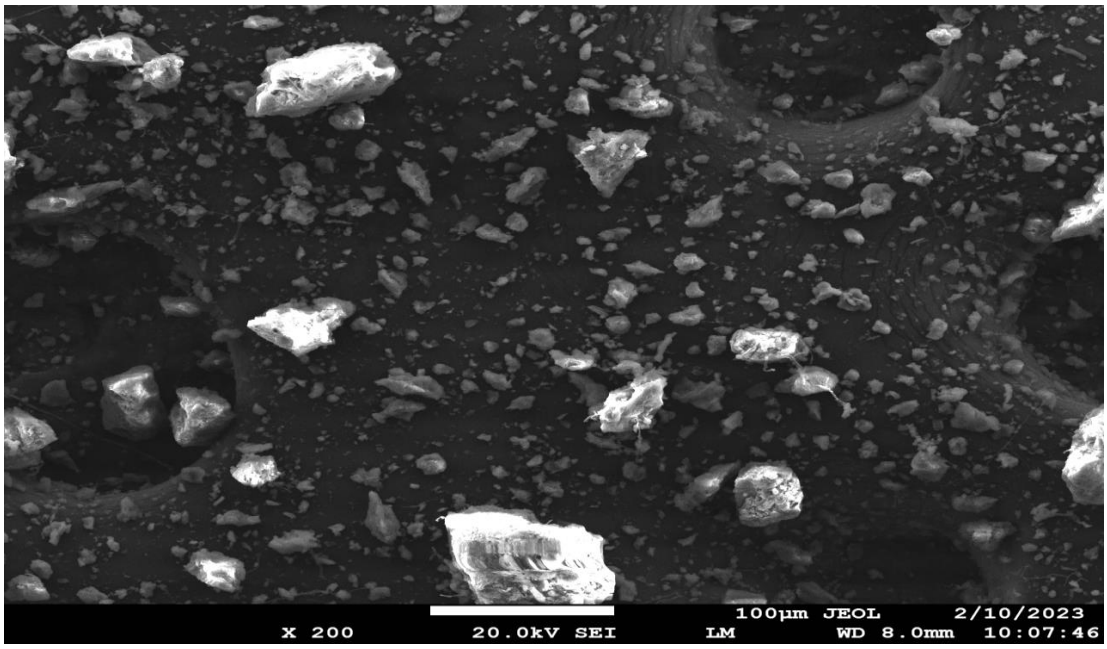


Figure 9: Field Emission Scanning Electron Microscope analysis (FESEM Analysis) of F1 formulation

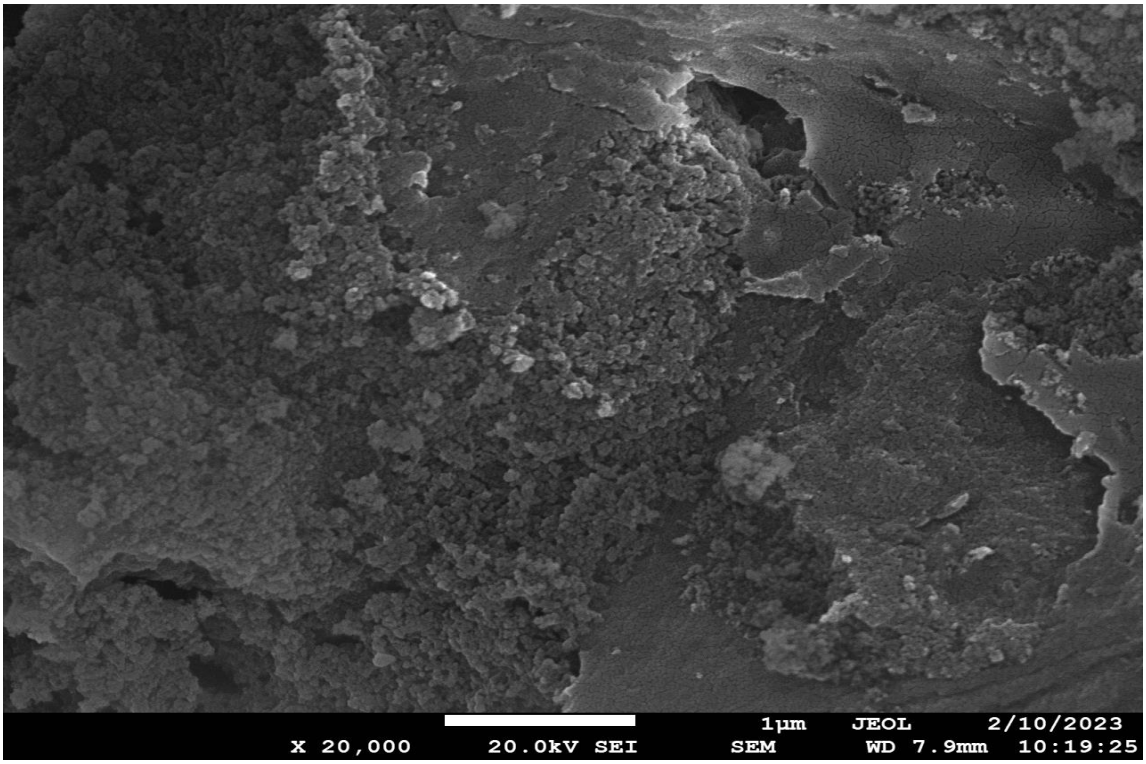


Figure 10: Field Emission Scanning Electron Microscope analysis (FESEM Analysis) of F2 formulation

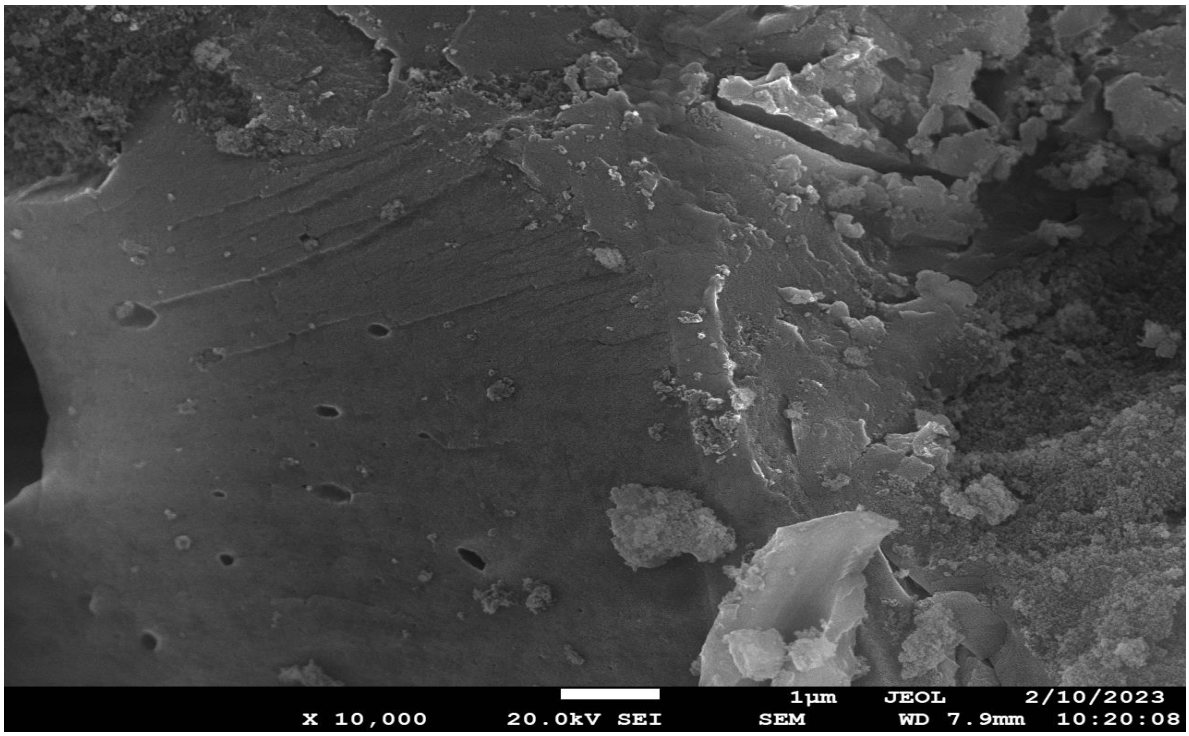


Figure 11: Field Emission Scanning Electron Microscope analysis (FESEM Analysis) of F3 formulation

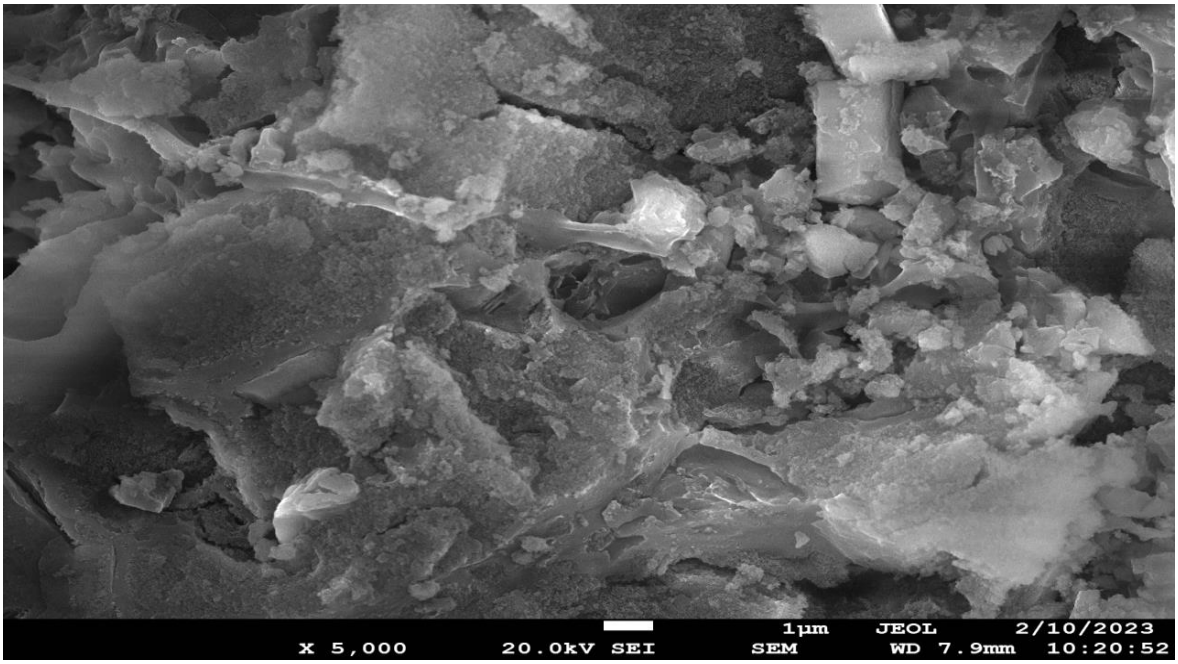


Figure 12: Field Emission Scanning Electron Microscope analysis (FESEM Analysis) of F4 formulation

Table 4: Table showing variables and their run combinations[13]

Run	Factor 1	Factor 2	Factor 3
	A:Amount of Bees wax (gm)	**B:**Amount of Powdered sucrose (gm)	**C:**Amount of PVP (gm)
1.	-0.15	5.89	4.37
2.	1.45	10.39	7.77
3.	3.80	5.89	-1.35
4.	3.80	-1.68	4.37
5.	3.80	5.89	4.37
6.	1.45	10.39	0.97
7.	3.80	5.89	10.09
8.	6.15	1.39	0.97
9.	7.75	5.89	4.37
10.	6.15	10.39	7.77
11.	3.80	5.89	4.37
12.	1.45	1.39	0.97
13.	3.80	5.89	4.37
14.	6.15	1.39	7.77
15.	3.80	13.46	4.37
16.	1.45	1.39	7.77
17.	6.15	10.39	0.97

Table 5: Experimental design of two level independent variables:

S.No.	Group symbol	Factors	Low	Medium	High
1.	**A**	Amount of Bees wax	1.45	3.80	6.15
2.	**B**	Amount of Powdered sucrose	1.39	4.50	10.39
3.	**C**	Amount of PVP	0.97	3.40	7.77

Table 6: Results of 3-D Response Surface Methodology Experimental design (Central composite Design) for three central values

Run	A	B	C	R1	R2
1	-0.15	5.89	4.37	23.37	0.24
2	1.45	10.39	7.77	21.08	0.41
3	3.80	5.89	-1.35	33.26	0.56
4	3.80	-1.68	4.37	42.44	0.32
5	3.80	5.89	4.37	47.88	0.16
6	1.45	10.39	0.97	32.98	1.02
7	3.80	5.89	10.09	37.4	0.08
8	6.15	1.39	0.97	25.23	0.58
9	7.75	5.89	4.37	41.67	0.45
10	6.15	10.39	7.77	41.79	0.78
11	3.80	5.89	4.37	48.07	0.8
12	1.45	1.39	0.97	21.45	0.74
13	3.80	5.89	4.37	46.19	0.18
14	6.15	1.39	7.77	32.89	0.64
15	3.80	13.46	4.37	39.12	0.33
16	1.45	1.39	7.77	28.7	0.46
17	6.15	10.39	0.97	39.04	0.73

Table 7: Results of ANOVA and regression coefficient for amount of Quercetin and % Friability

S.No.	Source	Amount of Quercetin	Friability
1.	γ	+6.91	+0.85
2.	A-Amount of Bees wax	+0.43	+0.057
3.	B-Amount of Powdered sucrose	+0.14	-0.063
4.	C-Amount of PVP	+0.080	-0.075
5.	AB	+0.20	-0.069

6.	AC	+0.17	+0.12
7.	BC	-0.30	-0.056
8.	A^2	-0.54	-0.026
9.	B^2	-0.28	- 0.068
10.	C^2	-0.44	-0.12
11.	R-Squared	0.8569	0.8274
12.	Adj R-Squared	0.6729	0.6056
13.	Pred R-Squared	-0.1161	- 0.3201
14.	Adeq Precision	6.559	6.059
15.	Lack of fit	0.0202	0.0991
16.	Df	9	9
17.	F value	4.66	3.73
18.	Pvalue Prob > F	0.0274	0.0483
19.	Model	Significant	Significant

Where γ = intetcept

Equation:

Sqrt(Amount of Quercetin released) = +6.91 +0.43 * A + 0.14 * B +0.080 * C + 0.20 * A * B + 0.17 * A * C - 0.30 * B * C - 0.54 * A^2 - 0.28 * B^2 - 0.44 * C^2

The Model F-value of 4.66 implies the model is significant. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, A^2, C^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. The "Lack of Fit F-value" of 48.93 implies the Lack of Fit is significant.

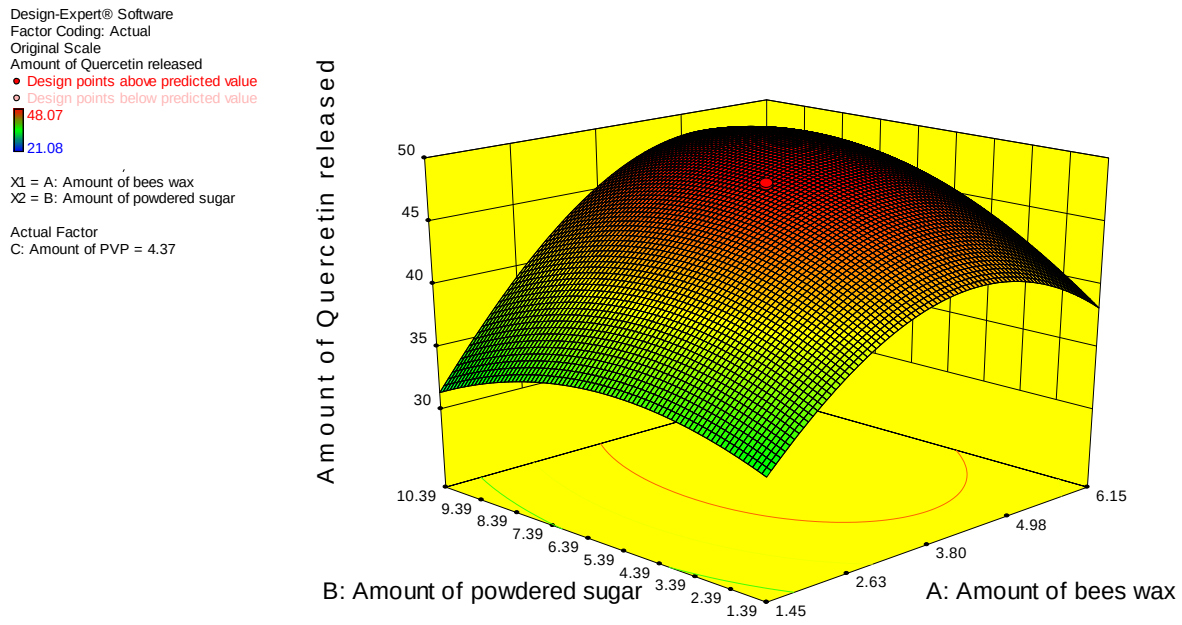


Figure 13: The response surface plot of total amount of Quercetin released

Equation:

Sqrt(% Friability) = + 0.68 + 0.030 * A + 0.022 * B - 0.093 * C

The "Model F-value" of 1.16 implies the model is not significant relative to the noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. The "Lack of Fit F-value" of 0.42 implies the Lack of Fit is not significant relative to the pure error.

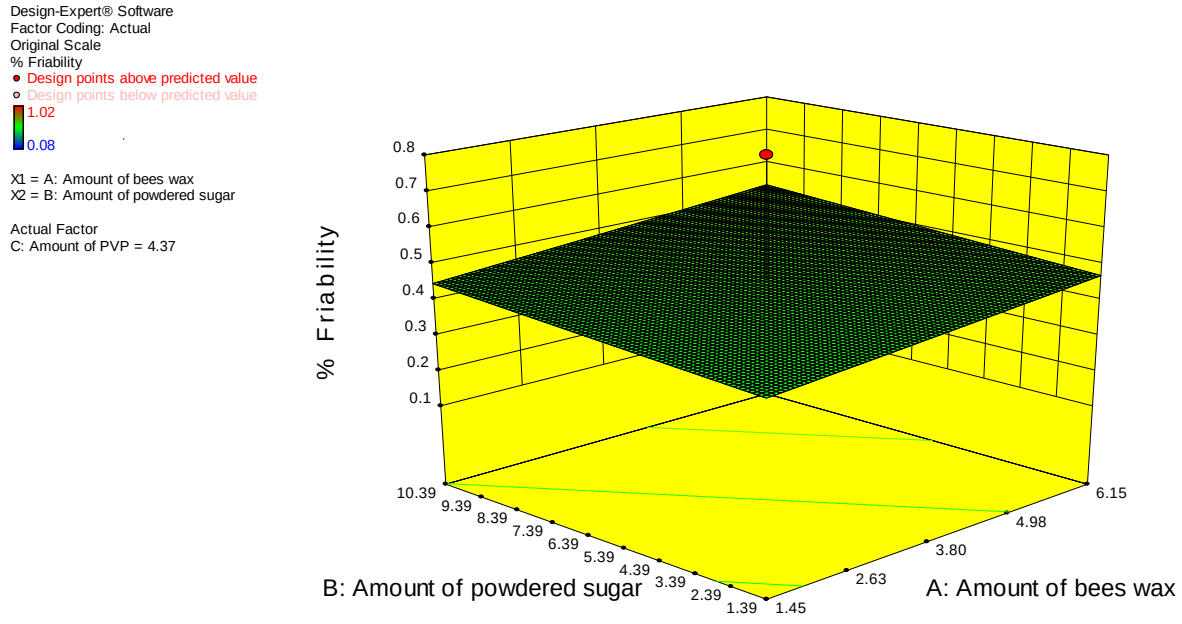


Figure 14: The response surface plot of % Friability found

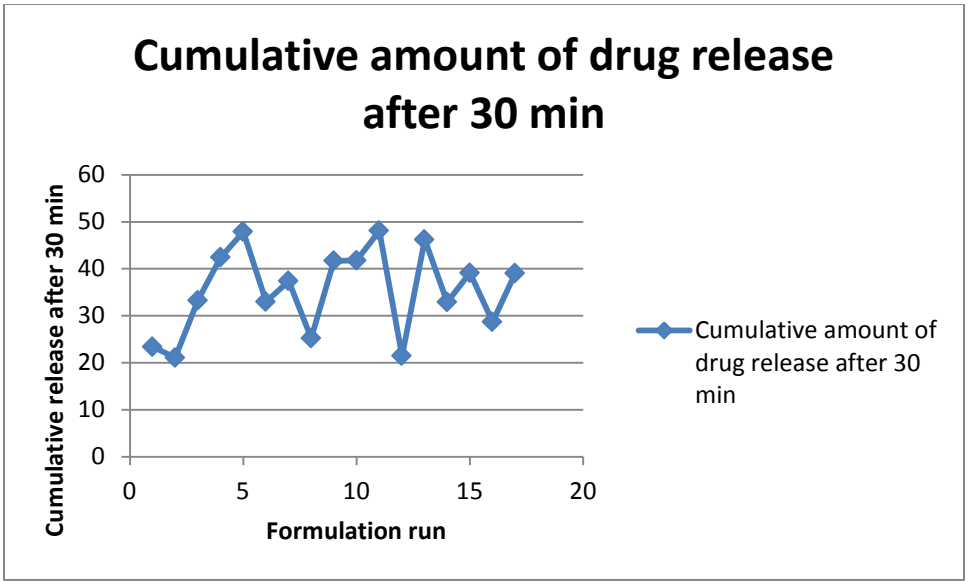


Figure 15: Graph of formulation run and their corresponding cumulative drug release after 30 min

CONCLUSION: This present study was an effort to make a more convenient, efficient, stable, safe, more bioavailable dosage form for mouth ulcers. Dosage forms contain herbal drug for the effective treatment of mouth diseases. Drug extract of plant itself an effective treatment and its nanoparticles form showed better release amount of responses. Unique type of dosage form was developed when these nanoparticles were incorporated in medicated chewing gums. All designed formulations are statistically optimized using different statistical methods e.g. Taguchi method and Response surface methodology. ANOVA and regression analysis studies showed optimum concentrations of variables and their levels. Suitable combination will reflect best responses parameters.

Conflict of interest: There is no funding source for all of the work done and there is no any conflict of interest. This is an original article not any part of the article has been published previously in any journal or magazines.

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