"FORMULATION AND EVALUATION OF EMULGEL INCORPORATED WITH BRYONIA LACINIOSA SEED EXTRACT FOR ITS ANTI-INFLAMMATORY PROPERTIES"

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ABSTRACT

The plant scientifically known as *Bryonia laciniosa* is belonging to the Cucurbitaceae family. *Bryonia laciniosa* was extracted using methanolic solvent. Phytochemical physio-chemical evaluation of the extract was done. FT-IR studies were carried out to check the compatibility. The dosage form known as Emulgel is created when gel and emulsion are mixed. Using the most recent NDDS method, Emulgel has twofold control release property. Herbal emulgel is developed using Carbopol 940 and span20, according to professional design, in different concentrations of extract medicines. The emulgel was design via the W/O approach. The emulgel was successfully prepared by combining emulsion with the gel base in 1:1 ratio using homogenizer. The DOE method was used preparation of 9 batches of herbal emulgel. The F4 shows good results of consistency, homogeneity and washability with semi solid appearance and non-greasy in nature. It appearance was light green in colour, spreadability 4.8 cm, Viscosity was 4320cPs, PH 6.29. By performing in-vitro anti-inflammatory studies the IC50 of standard drug & Plant extract was found to be 68.55% & 94.35% respectively. The %cumulative drug release by performing Drug permeation through eggshell membrane was observed to be 73.30% after 300 minutes.

Keywords: anti-inflammatory activity, *Bryonia Laciniosa* leaf

Introduction

A wound refers to any physical injury produced by an event that causes the skin to crack or break. Inflammation is a response of the body to injury or infection, the cardinal features of which are redness, swelling, and pain. On the other hand, chronic inflammation also contributes to different health conditions, such as autoimmune diseases and tissue degradation. Controlling inflammation early is important for avoiding lasting health problems.[1]

Herbal medicine is the use of plants for medicinal purposes and to promote general health and well-being. Herbal medicine makes great use of the whole plant, in contrast to synthetic drugs, so healing is more holistic. The plant name Bryonia laciniosa. The plant name Bryonia laciniosa, common around the forest is called Shivlingi is a member of the Cucurbitaceae family of medicinal plants. Because of its many pharmacological qualities, such as anti-inflammatory, analgesic, and antimicrobial effects, it has long been utilized in Ayurveda. The plant's medicinal effects are aided by the presence of bioactive substances like bryonin.[2]

One such topical medication delivery system that combines the advantages of gel and emulsion is called Emulgel, which offers a dual release control mechanism. Emulgel's primary goal is to deliver hydrophobic medications through the skin so they can take advantage of gels' special qualities. Emulgel made from bryonia laciniosa uses the plant's inherent anti-inflammatory qualities to reduce swelling and discomfort locally while minimizing systemic side effects. This formulation is especially helpful because it improves stability, increases drug penetration, and extends contact time, making it especially helpful in the treatment of inflammatory diseases.

Cotton dressings, gauze, and lint are used in traditional wound care. Ancient societies, like the Egyptians, made extensive use of natural remedies to heal wounds. Infected wounds are frequently treated with antimicrobials, such as preparations based on iodine and substances that release silver. By attacking bacteria on several levels, these substances reduce bacterial resistance. In conclusion, while both conventional therapies and herbal remedies have their uses, the development of drug delivery systems, like emulgels, holds great promise for improving treatment efficacy, particularly when it comes to managing inflammation.[3]

Material & Methodology

Extraction process

Collection and Authentication:

Bryoniosa laciniosa fruits of plant collected area of Ajra, Dist- Kolhapur. The plant identification and authenticated by Mr.S.S. Patil Sir. Identification and authantication of completed in shivraj College of Arts, Commerce and D. S. Kadam Science College, Gadhinglaj.

Extraction Procedure: [4]

Bryoina laciniosa seed were separated and drying of seeds was done using shade drying. Dried seeds were crushed in an electrical grinder and then powdered. To fill the thimble, 250gm of precisely weighted dried plant material were used. 500 millilitres of methanol were used as the solvent in a round-bottom flask that was attached to a Soxhlet extractor and condenser. Crushed plant material is placed inside the thimble of the Soxhlet extractor. The sidearm had glass wool trailing along it. The heating mantle is used to heat the solvent, which begins to evaporate as it passes through the apparatus. The solvent pours back into the flask to restart the cycle when it reaches the siphon level. The procedure went through 8-10 cycles and ran for a total 15 hours. 8 gm of extracted plant material was left in the round bottom flask after solvent evaporated.
% Yield was calculated by using formula-
%Yield=W1/W2 ×100 Where, WI= Wt. of dry extract, W2=Wt. of dry plant Extract was collected by this method 16.5gm. By using this formula % yield of extracts was found 33%

Phytochemical Screening:

The phytochemical screening for Alkaloids, Glycosides, Flavonoids, Saponins, tannins and phenolic compunds were performed as per procedure.

General Description

The *bryonia laciniosa* seed extract was observed the physical test, colour, and odour.

Compatibility study:

Fourier Transform Infrared (FTIR) spectroscopy investigation was used to check the compatibility of the *bryonia laciniosa* seed extract and excipients before they were selected for the emulgel formulation.

Fourier Transform Infrared Spectroscopy (FTIR):[6]

Fourier transform infrared spectrometer (Shimadzu corporation analytical instrument division, Japan) was used to examine the compatibility of the *bryonia laciniosa* seed extract and polymer. FTIR is useful determining drug purity. The FTIR peaks display corresponds the chemical composition. *bryonia laciniosa* leaf extract and carbopol 934 and physical mixture of extract and Carbopol 934 is FTIR spectrum was captured using the FTIR. The FTIR spectrum was recorded between wave numbers 4000 and 1000cm-1

Optimization Batch:

Experimental Design:

Nine Emulgel formulations were prepared according to a 32 factorial design employing the qualitative 2 factors and 3 levels shown in Table. Generation and evaluation of the experimental design was carried out using Design Expert software Expert® DX 13.0 (StatEase Inc., MN). Two independent variables were evaluated: Amount of Carbapol 934 (X), Span 20 (X2). Viscosity (R1) and Spreadability (%) (R2) were selected as the dependent variables.

3 ^{2} full factorial design: factors, factor levels and responses for Emulgel formulations.

Table No.01: 3 ^{2} full factorial design

Independent Variables						
Factors	**Coded Values**			**Actual Values (%w/w)**		
	Low (-1)	**Medium (0)**	**High (+1)**	**Low (-1)**	**Medium (0)**	**High (+1)**
Carbopol 934 (X)	-1	0	+1	1.5	1	0.5
Span 20 (X2)	-1	0	+1	0.3	0.2	0.1
Responses (Dependent variable)						
R1 = Spreadiability						
R2 = Viscosity						

Formulation table of Emulgel

Composition of 2 [3] factorial design batches of Emulgel.

Table No.02: Formulation table of Herbal Emulgel

Sr. No.	Ingredients (%w/w)	F1	F2	F3	F4	F5	F6	F7	F8	F9
1	*Bryonia laciniosa l. Extract* (mg)	40	40	40	40	40	40	40	40	40
2	Carbapol 940 (gm)	1.5	1	1	0.5	1	0.5	1.5	0.5	1.5
3	Span20 (ml)	0.65	1	0.65	0.3	0.3	0.65	0.3	1	1
4	Methyl paraben (gm)	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
5	Propyl paraben (gm)	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
6	triethanolamine (ml)	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
7	Liquid paraffin (ml)	3	3	3	3	3	3	3	3	3
8	Tween 80 (ml)	1	1	1	1	1	1	1	1	1
9	Propylene glycol (ml)	3	3	3	3	3	3	3	3	3
10	Distilled water (ml)	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s

Formulation of herbal Emulgel: [8]

Formulation of Gel:

Gelling agents were soaked in water for 24 hours. Triethnolamine was added to the soaked gel and pH was adjusted.

Formulation of Emulsion

Dissolving span 20 add light liquid paraffin while the aqueous phase was prepared by dissolving tween 20 in purified water. Methyl parabens were dissolved in propylene glycol whereas *bryonia laciniosa* seed extract was dissolved in purified water, and both solutions were mixed with the aqueous phase. The water phase was added to the oil phase with continuous stirring.

Formulation of emulgel

Emulsion was mixed with the gel vigorously to obtain the emulgel.

Evaluation of herbal Emulgel formulation-

Physical evaluation-[9]

Emulgel was observed: Colour, Odour and Texture

Solubility-[10,11]

The solubility of *bryonia laciniosa* emulgel in using solvent determined by using shake flask method. The obtained result was noted.

pH determination-[12]

1gm of gel was dissolved in 100 ml of distilled water and it was placed for 2 hr and then dip the glass electrode into an emulgel. The measurement of pH of each formulation was done in triplicate and average values were calculated.

Spreadability-[13]

One gram of each sample was added to a pre-marked 1 cm diameter in order to test the emulgel's Spredability. On a glass block, draw a circle. A second glass block that had been previously weighed was placed on top, applying a weight of about 100 g for five minutes. The distribution of the emulgel was determined by measuring the ensuing rise in diameter. Spredability was calculated by using formula:

Spredability = Wt. tied to upper slide × Length of glass slide / Time

Where, M= weight tied to upper slide (10g), L = length of glass slide (6.8cm), T = Time taken to separate the slides

Viscosity-[15]

Initially viscosities of freshly prepared nine formulations were determined using brook field viscometer with spindle no 04. The spindle was lowered perpendicular into the center of emulgel formulation placed in a beaker taking care that spindle did not touch the bottom of the beaker and rotated at the speed of 2.5 rpm for 5 min. The viscosity reading was noted.

Skin permeation test[16]

Take a fresh egg and break it slowly from the upper side and remove all the content inside it wash the egg with distilled water. Dip this egg into conc. HCL which degrades the outer shell and inner eggshell membrane is obtained Wash this membrane and allow it to dry at room temperature. Add the formulation to the donar compartment (cylinder) and mount it with egg membrane. Fill the receiver compartment with the suitable buffer solution (pH 6.4). Place the donar compartment above the receiver compartment such that the egg membrane dips in the buffer solution. Place this apparatus on a magnetic stirrer and stirr it continuously. Maintain the temperature constant at 37 ± 0.5°C. Withdraw 5ml sample from the receiver compartment and add 5ml fresh buffer solution in the compartment again at a intervel at each 60 minutes. Carry this process for 180 minutes and then analyse the sample via UV Spectrophotometry.

Result and Discussion-

Table No. 03: Percentage yield of *Senna auriculata*

Plant	% Yield
leaf extract of *bryonia laciniosa*	33% W/W

Organoleptic characters:

Table No.04: Organoleptic characters

Sr. No.	Physical properties and tests	Methods	Description of *bryonia laciniosa* seed extract
1.	Physical state	Visual observation	Solid (powder)
2.	Color	Visual observation	Dark green

3.	Odour	By smelling	Bitter

Table No.05 Table: Solubility

Sr. No.	Solvents	Solubility of *bryonia laciniosa* leaf extract
1.	**Water**	soluble
2.	**Methanol**	soluble
3.	**Acetone**	Slightly soluble
4.	**Ethanol**	Slightly soluble
5.	**Chloroform**	Slightly soluble

Phytochemical screening

Table No.06: Phytochemical screening

Sr. No.	Constituents	Test	Observation	Inference of *bryonia laciniosa* leaf extract
1.	**Alkaloids**	Hagers test	Yellow colour	Alkaloids present
		Wagners test	Brown or raddish brown colour	Alkaloids present
		Fehling test	Red preceipitate	Carbohydrates present
2.	**Glycosides**	Lugols test	Pink colour	Glycosides present
3.	**Flavonoids**	Lead acetate test	Flocculant white precipitate	Flavonoids present
4.	**Saponins**	Foam test	1 cm of foam formation	Saponins present
5.	**Tannins**	Braemers test	Dark blue colour	Tannins present
6.	**Phenolic compounds**	Ferric chloride test	Violet colour	Phenolic compounds present

FTIR (Fourier Transform Infrared Spectroscopy):

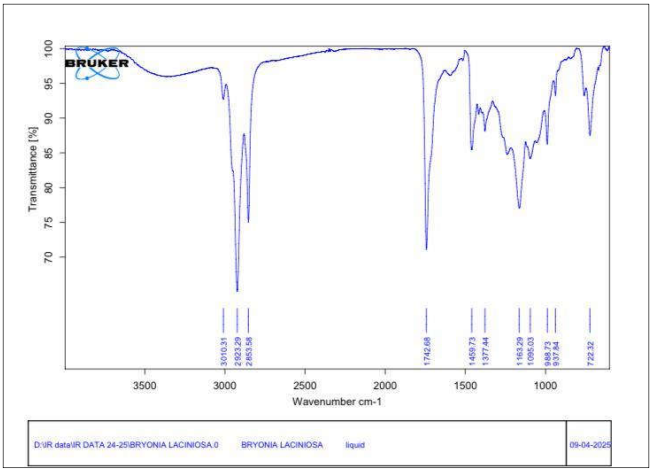


Fig No.01 FTIR of *bryonia laciniosa* seed extract

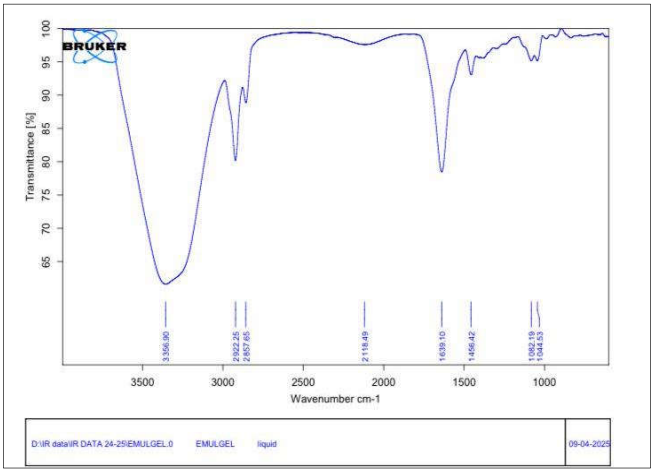


Fig No.02 FTIR of *physical mixture*

Batch Emulgel formulation:

Fig No.05 Batch Emulgel formulation

Evaluation tests of Emulgel:

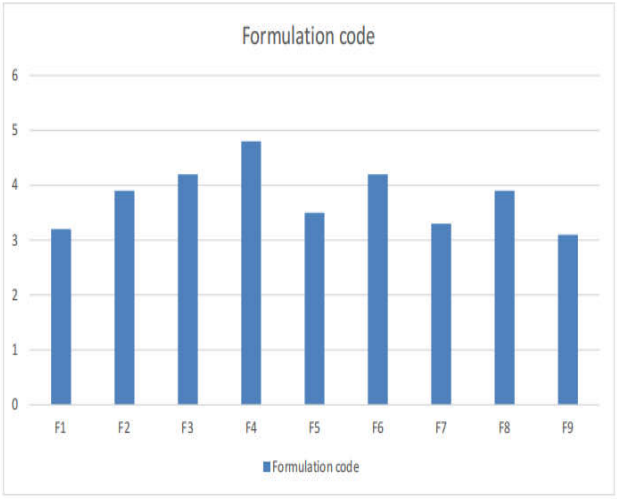
Table No.08 Evaluation tests of Emulgel

Formulation	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
Colour	Greenish	Greenish	Greenish	Greenish	Greenish	Greenish	Greenish	Greenish	Greenish
Odour	Bitter	Bitter	Bitter	Bitter	Bitter	Bitter	Bitter	Bitter	Bitter
Texture	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth

Evaluation of Emulgel formulation:

Table No. 09 Evaluation of Emulgel formulation

Batc h	pH	Spreadability (gm.cm/sec)	Viscosity (cps)
F1	6.66±0.11	3.2	14360
F2	6.49	3.9	13240
F3	6.06	4.2	9400
F4	5.87	4.8	4320
F5	6.23	3.5	15760
F6	5.73	4.2	2720
F7	6.36	3.3	9760
F8	7.0	3.9	3120
F9	5.45	3.1	24240



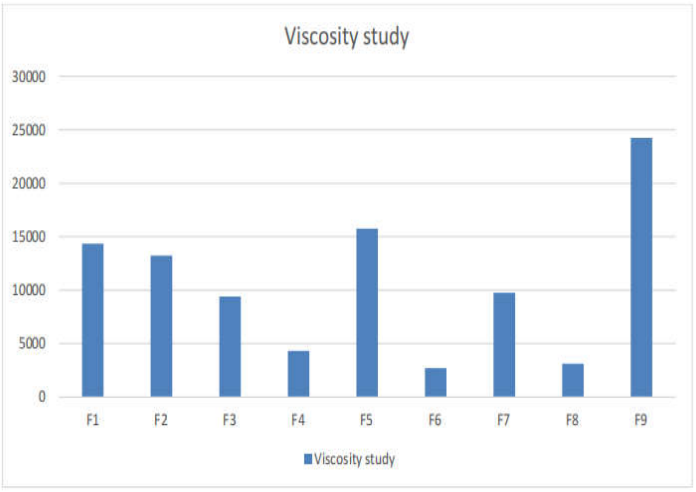


Fig No.06 Spreadability graph **Fig No.07 Viscosity graph**

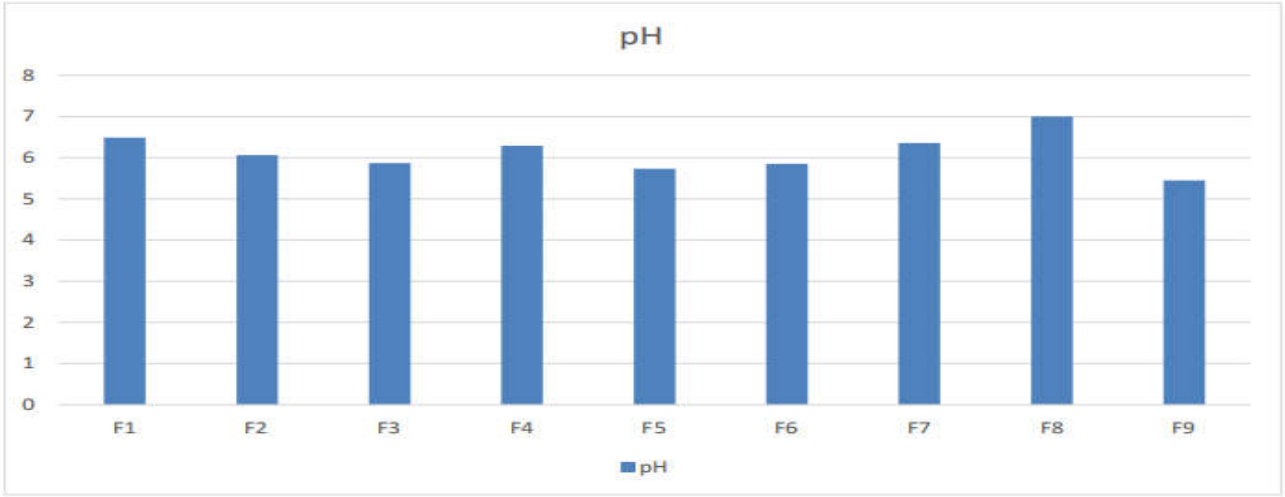


Fig No.08 pH graph

Table No. 10 Optimization of Formulation:

Std	Run	Factor 1	Factor 2	Response 1	Response 2
		A: Carbopol	B: Span 20	Spreadability	Viscosity
		% w/w	% w/w	cm	cP
6	1	1.5	0.65	3.2	14360
8	2	1	1	3.9	13240
9	3	1	0.65	4.2	9400
1	4	0.5	0.3	4.8	4320
7	5	1	0.3	3.5	15760
5	6	0.5	0.65	4.2	2720
2	7	1.5	0.3	3.3	9760
3	8	0.5	1	3.9	3120
4	9	1.5	1	3.1	24240

Statistical Analysis

Statistical Analysis of the central composite design batches was performed by multiple regression analysis using Design of Experiment (version 10) software, results of ANOVA shown below table 11 **ANOVA for Quadratic model for Spreadability (R1):**

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1.90	2	0.9483	9.00	0.0156	significant
A-Carbopol	1.81	1	1.81	17.22	0.0060	
B-Span 20	0.0817	1	0.0817	0.7750	0.4125	
Residual	0.6322	6	0.1054			
Cor Total	2.53	8				

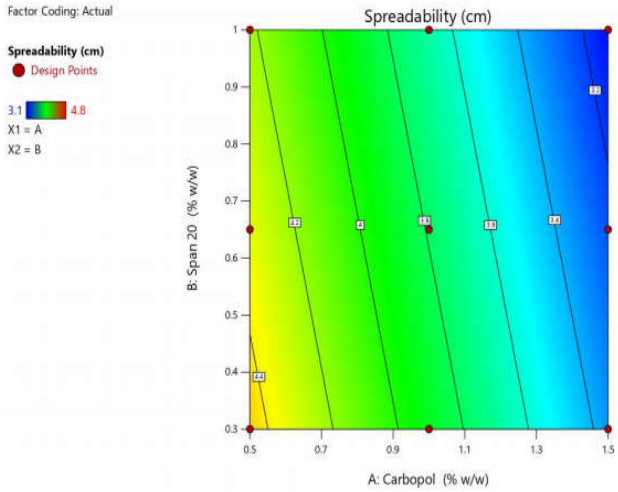
The **Model F-value** of 9.00 implies the model is significant. There is only a 1.56% chance that an F-value this large could occur due to noise. **P-values** less than 0.0500 indicate model terms are significant. In this case A is a significant model term. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

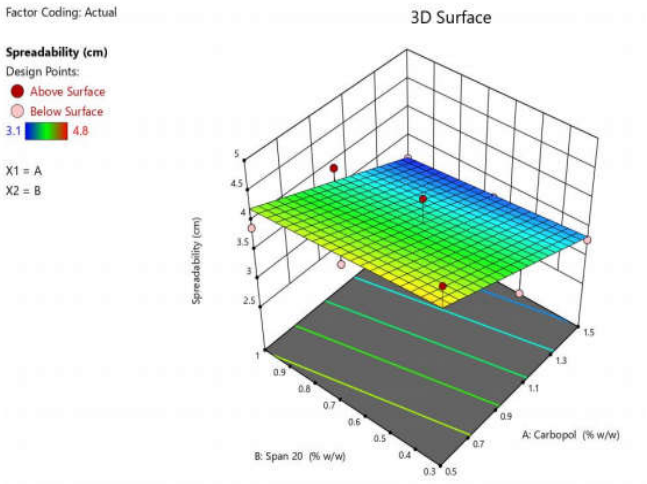
Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	**0.0156**		**0.6667**	**0.4460**	**Suggested**
2FI	0.3230		0.6775	0.2971	
Quadratic	0.8441		0.5199	-0.9187	
Cubic	0.5681		0.5351	-9.5899	**Aliased**

The **Predicted R^2** of 0.4460 is not as close to the **Adjusted R^2** of 0.6667 as one might normally expect; i.e. the difference is more than 0.2. This may indicate a large block effect or a possible problem with your model and/or data. Things to consider are model reduction, response transformation, outliers, etc. All empirical models should be tested by doing confirmation runs.

Final Equation in Terms of Coded Factors:

Spreadability	=
+3.79	
-0.5500	A
-0.1167	B





ANOVA for Quadratic model for Viscosity (R2):

Table No. 12 ANOVA for the quadratic model for Viscosity (R2)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	3.240E+08	3	1.080E+08	7.80	0.0247	significant
A-Carbopol	2.432E+08	1	2.432E+08	17.58	0.0086	
B-Span 20	1.930E+07	1	1.930E+07	1.39	0.2908	
AB	6.147E+07	1	6.147E+07	4.44	0.0889	
Residual	6.919E+07	5	1.384E+07			
Cor Total	3.932E+08	8				

The **Model F-value** of 7.80 implies the model is significant. There is only a 2.47% chance that an F-value this large could occur due to noise. **P-values** less than 0.0500 indicate model terms are significant. In this case A is a significant model term. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

Table No. 13 Fit statistics of Viscosity (R2)

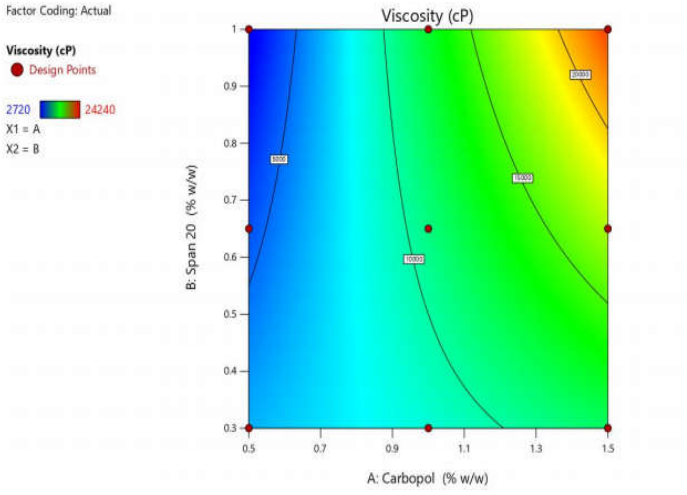
Std. Dev.	3719.84	**R²**	0.8240
Mean	10768.89	**Adjusted R²**	0.7184
C.V. %	34.54	**Predicted R²**	0.4356
		Adeq Precision	8.2961

The **Predicted R²** of 0.4356 is not as close to the **Adjusted R²** of 0.7184 as one might normally expect; i.e. the difference is more than 0.2. This may indicate a large block effect or a possible problem with your model and/or

data. Things to consider are model reduction, response transformation, outliers, etc. All empirical models should be tested by doing confirmation runs.

Final Equation in Terms of Coded Factors:

Viscosity	=
+10768.89	
+6366.67	A
+1793.33	B
+3920.00	AB



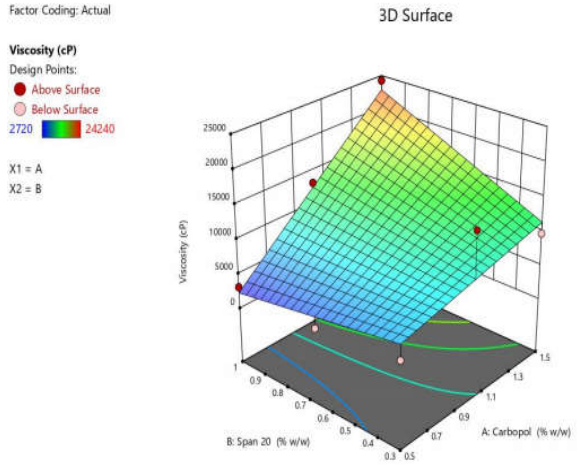


Fig No.14 Contour plot for Viscosity (R2) Fig No.15 3D surface plate plot for Viscosity (R2 Predicted Vs. Actual for Viscosity (R2) Perturbation for Viscosity (R2)

Table No. 18 Stability study:

Test	**1st week**	**2nd week**	**3rd week**
Physical evaluation	Good	Good	Good
pH	6.23±0.02	6.03±0.02	5.94±0.02
Spreadability	10.3±0.15	9.33±0.5	9.22±0.11

Summary and Conclusion:

The *Bryonia Laciniosa* leaves were collected and authenticated. The drug was shade dried and grinded. The extraction was done by using methanolic extract that was subjected to Soxhlet extraction. Concentrated the solvent. The extract obtained was used to study the phytochemical and physiochemical parameters. Phytochemical tests were performed to determine the presence of alkaloids, flavonoids, glycosids, tannins, saponins, phenolic compounds. The *bryonia laciniosa* showed presence of alkaloids,flavonoids, glycosids, tannins, saponins, phenolic compounds. FTIR studies of extract, gelling agent and mixture of extract and gelling agent was taken. A mixture, known as an emulsion, was created. The emulsion was then transformed into a gel called emulgel, using a gelling agent called Carbopol- 934. The concentration of the gelling agent was determined using a factorial design approach. A total of 9 different formulations of the Emulgel were developed. F1-F9 formulations were

subsequently assessed for various characteristics, including color, pH level, greasiness, spreadability, viscosity, and appearance. The prepared batches exhibited a greenish colour. All the batches had a non-greasy nature. The washability of the formulations was deemed good. The viscosity of the formulations ranged from 2720 to 24240 cP, and the pH of the formulations ranged from 5.45 to 7.0 cp. The spreadability of the formulations fell within the range of 3.1 to 4.8 cm. The result of optimized batch was found to be pH 6.29, viscosity 4320, cp Spreadiability 4.8 in cm. In-vitro anti-inflammatory activity of formulation was accessed. protein, denaturation method was used to access anti-inflammatory activity.

AUTHORS CONTRIBUTIONS: All authors have contributed equally.

CONFLICTS OF INTERESTS: All authors have declared no conflict of interest.

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