Formulation And Evaluation Of Oral Antiulcer Hydrogel Of Berberis Aristata

Saloni V. Mutha[1*], Pallavi M. Chaudhari[2] And Divya J. Karkar[3]

[1]Department of Pharmaceutics, Dr. D. Y. Patil College of Pharmacy, Akurdi, Pune - 411044, Maharashtra, India, Email: salonimutha98@gmail.com, Tel: 9011682670
[2]Department of Pharmaceutics, Dr. D. Y. Patil College of Pharmacy, Akurdi, Pune - 411044, Maharashtra, India, Email: pallavichaudhari@dyppharmaakurdi.ac.in
[3]Department of Pharmaceutics, Dr. D. Y. Patil College of Pharmacy, Akurdi, Pune - 411044, Maharashtra, India, Email: divyakarkar2000@gmail.com

***Corresponding Author:** Saloni V. Mutha
*Department of Pharmaceutics, Dr. D. Y. Patil College of Pharmacy, Akurdi, Pune - 411044, Maharashtra, India, Email: salonimutha98@gmail.com, Tel: 9011682670

Abstract:
Background: Recurrent aphthous ulcer is a disease with unknown etiology that's mostly treated symptomatically and has no definite cure. Ayurveda an ancient medication system that was developed throughout the religious text times, around 5000 years ago. Berberis aristata have been used as medicinal herb due to its antimicrobial, antioxidant, anti-inflammatory, analgesic and healing effects, has been useful in treatment of oral aphthous. **Material and Method:** Therefore, we decided to formulate a Hydrogel with Berberis aristata bark extract to reduce the need for corticosteroid therapy in patients with oral ulcers. Hydrogel was formulated with central composite design using different concentrations of Carbopol 934 and Sodium carboxyl methylcellulose and evaluated for different parameters like pH, viscosity, spredability, drug content, gel strength, percent yield, drug release and antifungal activity. **Result and Conclusion:** The F1 batch was found to be optimized batch as its drug content was 95.58% and drug release was 67.15% and it was stable safe and effective for treatment of mouth ulcers.

Keywords: Ayurveda, Daruhaldi, *Berberis aristata*, Hydrogel.

1. INTRODUCTION:

Topical medicine administration is a localized medicine delivery system anywhere in the body through ophthalmic, rectal, oral, vaginal and skin as topical routes. TDDS gives a more chance of success of medicine delivery over traditional styles like use of injectable and oral solutions. Case adequacy is enhanced bypassing first pass metabolism. Oral health complaint is now a day's major public health problem. [1] An oral ulcer which occurs on the mucous membrane of the oral depression is called aphthous ulcer, or canker sore. Intermittent aphthous stomatitis is a rather common complaint with unknown etiology that's linked by one or further sore ulcers with red spots on the mucous membrane of mouth and is tone- limiting in one or two weeks but can reoccur yearly or a many time. Since this complaint has an unknown cause, its opinion is grounded on clinical signs. Oral aphthous ulcers affect between 5 to 50% of population of any age group. Numerous curatives have been suggested to treat oral ulcers which aim to drop the symptoms of pain and duration of ulcers.[2]

World Health Organization (WHO) has defined herbal drugs are finished, labeled medicinal products that contain active constituents, upstanding or underground corridor of the shops or other factory material or combination. Herbal drugs are the type of drug that uses roots, stems, leaves, flowers, or seeds of the to ameliorate health, help complaint, and treat illness.[3] Herbal drugs are generally used as primary health care due to its artistic adequacy, better adequacy with mortal body and lower adverse effects. Herbal drug with medical parcels has been used for an extended period to help and treat problems associated with oral health. Gels are mainly semi-solid formulations having a liquid phase that has been thickened with some other components. They're applied to the skin or certain mucous membranes for defensive, precautionary or remedial purposes. The Commercially available gels containing synthetic and semi synthetic active agents which have several disadvantages like staining on the teeth, vexation, and burning sensation only because presence of high degree of alcohol content and some organic composites.[4] Hydrogel is defined as a three-dimensional cross-linked polymeric network obtained from synthetic or natural polymers which has the capacity to hold water within its porous structure.[5] This exploration paper deals with use of *Berberis aristata* for the treatment of mouth ulcer in medicinal hydrogel. *Berberis aristata* is commonly known as *Daruharidra, Daru Haldi, Indian barberry, Tree turmeric, Chitra,* belonging to the family Berberidaceae. In ancient times, the plant is used as a tonic, demulcent, diaphoretic, diuretic and to deal with diseases like wound healing, optic problems, dermatological diseases, rheumatism, menorrhagia and jaundice. The main alkaloid component of the plant is Berberine which is present either in plant parts like leaves, roots, rhizomes and stem bark.[6]

2.MATERIALS AND METHOD USED:

2.1Materials:

The stem bark of *Berberis aristata* was purchased and authenticated from Isha agro developers Pvt.Ltd. Carbopol 934, Sodium carboxyl methylcellulose, Propylene glycol, Triethanolamine, Sodium benzoate, Glycerin used in research work were of analytical grade.[7]

2.2Extraction:

The liquid extract of powdered *Berberis aristata* was obtained by decoction. Decoction is method of extraction by boiling plant material [stem, root, bark] to dissolve the chemicals of the material to dissolve the chemicals of the material. In this process powdered *Berberis aristata* was boiled in water for 15 to 30 min. then it was cooled, strained and added enough cold water through the drug to obtain desire volume. Once the decoction was done it's necessary to filter the liquid through the filter paper.[8]

2.3 Phytochemical screening: [9]

The prepared extract was subjected to preliminary phytochemical screening tests to identify the presence of various components, by using different tests and reagent.

Test for Carbohydrate:

Fehling's test: In separate tubes, equal parts of Fehling A and Fehling B were
combined with 2-3 ml of *Berberis aristata* extract and cooked for a short period of time. As the contents got close to boiling, they were stirred together, development of brownish-red precipitates shows presence of carbohydrates.

A. Test for alkaloids:

The extract was dissolved in water, boiled and shaken well then filtered. The filtrate was used to perform following test.
Hager's test: 2-3 ml of *Berberis aristata* extract was mixed with a saturated solution of picric acid and the appearance of a yellow colored precipitate indicated the presence of alkaloids.

B. Test for saponin:

Froth formation test: Two ml of *Berberis aristata* extract were placed in a test tube, shaken, and allowed to stand until foam was created for five minutes (in the presence of saponin). If no foam formed in five minutes, saponin was not present in the extract.

C. Test for Tannin:

Gelatin test: When 1 percent gelatin solution containing 10 percent NaCl added to the *Berberis aristata* extract, tannins caused a buff-colored precipitate to develop.

D. Test for Flavonoids:

Shinoda test: The extract was treated with 10 % NaoH solution, formation of deep yellow color indicates presence of flavonoids in the extract.

E. Test for phenol:

*Ferric trichloride***:** By dissolving the extract in water and adding 8–10 drops of diluted ferric trichloride, a bluish–black shade developed, which showed the presence of phenol.

2.4 Physicochemical analysis of *Berberis aristata*

A. Determination of ash value: Weigh a porcelain dish. Weigh about 1 gm of the powdered drug in porcelain. Place the porcelain on tripod stand. Heat with a burner flame of height 2 cm, heat till vapors almost cease to be evolved, then drop the distance between porcelain and burner flame and heat more strongly until all the carbon burnt off. Cool in desiccator. Weigh the ash. Then calculate the percentage of total ash with regard to standard.

B. Acid insoluble ash value: Wash the ash from the dish used for total ash with 25 mL of dilute hydrochloric acid into a 100 mL beaker. Place beaker on wire gauze over a Bunsen burner and boil for 5 min. Filter ash through an ash less filter paper and collect remnant into the porcelain dish, heat gently until vapors cease to be evolved. Cool in desiccator. Weigh the residue. Then calculate acid-insoluble ash of the crude drug with reference to the standard.

C. Water soluble ash value: The procedure is analogues to acid insoluble ash, only difference is to 25 ml of water rather of dilute hydrochloric acid used.

D. Alcohol soluble extractives**:** 1 gm of herbal extract powder macerated with 100ml alcohol in closed conical flask for 24 hours with frequently shaking. Then it was filtered rapidly and taking precaution while filtering to avoid alcohol evaporation. 25 ml of filtrate is then evaporated in porcelain dish dried at 100 ^{0}C and weighed. The percentage of alcohol soluble extract was calculated with reference to 1 gm herbal extract powder.[10]

2.5 Estimation of *Berberis aristata* extract using UV spectroscopic method:

***A.* Preparation of standard solution:**

An exactly weighed quantity 10mg of *Berberis aristata* was dissolved in methanol and volume was made up to 10ml with methanol in a volumetric flask. Stock solution of *Berberis aristata* was formed by diluting 1 ml of that solution with methanol up to 100ml in volumetric flask to give 10 µg/ml concentration of Berberine.

B. Calibration curve of extract:

Appropriate aliquots of the stock solution were taken and diluted with methanol in separate 10 ml volumetric flasks to prepare standard solutions of Berberine having concentrations ranging from 2 to 20µg/ml of berberine. The absorbance of each standard solution was measured at 348 nm, using methanol as blank. The absorption maxima and Beer's law limit were recorded and data prove the linearity and obey Beer's law limit were noted. The linear correlation between these concentrations (x- axis) and absorbance (y-axis) were graphically presented and slope (b), intercept (a) and correlation coefficient (r^2) were calculated for the linear equation (y=mx+c). [11]

2.6 FORMULATION OF HYDROGEL:

A sufficient amount of Carbopol 934 was soaked in distilled water overnight, with nonstop shifting using a mechanical stirrer. In another beaker dissolve the needed volume of Sodium carboxyl methylcellulose with sodium benzoate and add to above Carbopol result along with propylene glycol and glycerin, also add extract of *Berberis aristata* to blown polymer under constant stirring at 700 RPM.[12] The mixing procedure was kept going on until the clear and homogenous gel phase achieved. The final volume was made up to 50mL. To adjust the pH (6.8- 7) triethanolamine was added dropwise to the resulting solution.

Table 1: Formulation of Hydrogel

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Carbopol 934(g)	1.25	0.5	0.5	1.25	0.18	2.3	2	1.25	2
Sodium Carboxymethylcellulose (g)	2	3	1	3.4	2	2	3	0.5	1
Berberis aristata extract (ml)	1	1	1	1	1	1	1	1	1
Sodium benzoate(gm)	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Propylene glycol (ml)	5	5	5	5	5	5	5	5	5
Glycerine(ml)	q. s	q. s	q. s	q. s	q. s	q. s	q. s	q. s	q. s
Triethanolamine(ml)	q. s	q. s	q. s	q. s	q. s	q. s	q. s	q. s	q. s
Distilled water upto (ml)	50	50	50	50	50	50	50	50	50

2.7 EVALUATION OF HYDROGEL:

1. Visual appearance

The hydrogels were tested for color, clarity, texture, transparency and residence of any gritty particles.[13]

2. pH Measurement

The pH of herbal hydrogel formulations was determined by using digital pH meter. 1 gm of hydrogel was taken and dispersed in 10 ml of distilled water and keep away for 2 h. pH of hydrogel was reported.[14]

3. Homogeneity

All hydrogel preparations were tested for homogeneity by optical inspection after the gels have been set in to the container. They were tested for their presence and appearance of any aggregates.[15]

4. Viscosity test

Viscosities were measured by Brookfield (DV-III) viscometer. Each hydrogel was poured into the container and the spindle no.4 was attached to it because of higher viscosity of formulation. Then the viscosity of all hydrogels was measured in 25°C at 50-250 RPM. Temperature was maintained during viscosity measurement.[16]

5. Spreadability

Spreadability was calculated using the two watch glasses. The 500 mg of the Hydrogel was taken in the watch glass and left steady for some time. The other watch glass was placed on the hydrogel at height of 5cm then measured the spreadability. The spreadability was measured by the force applied and how much gel has spread over the glass. It is measured in g/cm/sec. [17]

6.Gel strength

Gel strength was determined by the time in sec needed by the weight to creep in the gel. A 3.5 gm weight was placed on the surface of 5 gm formulated Hydrogel. Gel strength was determined by reporting time in seconds required by the weight to penetrate 0.5 cm in the gel.[18]

7. Percentage yield

Weighed the empty container in which the Hydrogel was stored then a weighed the container with Hydrogel. To obtain the practical yield subtract the weight of empty container with the container with gel.
Then the percentage yield was calculated by the formula given below:
Percentage yield = (Practical yield/Therotical yield) × 100

8. Drug content

To obtain the drug content the formulation (1g) was dissolved in the 20ml of the phosphate buffer saline for minimum 30 m. The resultant mixture was then filtered through a Whatman filter paper. The filtrate was again diluted with the 10ml of buffer; absorbance of the mixture was taken at 348 nm by the UV-VIS spectrophotometer.[19]

9. In vitro drug release

In vitro drug release studies were carried out with Franz diffusion cell apparatus. The Herbal Hydrogel was taken in the small amount and kept on the membrane which is fixed with receiver. Each of the receivers was filled with the phosphate buffer of pH 6.8 and fixed with the magnetic stirrer to make it warm at the physiological temperature. The drug then comes in the contact of the buffer and getting dissolved in the buffer. The 1 ml of the buffer taken and took the absorbance for the same at 348nm by using UV-VIS spectrophotometer for 6h. Every hour when the drug is withdrawn, same amount of the buffer was replaced by the new one. The in vitro drug release studies done to observe the concentration and drug release was calculated in percentage.[20]

2.8 EXPERIMENTAL RESULTS:

2.8.1 Phytochemical tests of Berberis aristata extract:

Table 2: Preliminary phytochemical screening of extracts of *Berberis aristata*

Sr. No	Phytoconstituents	Test Performed	Inference
1.	Carbohydrate	Fehling's test	+
2.	Alkaloids	Hager's test	+
4.	Saponins	Froth formation test	+
5.	Tannin	Gelatin test	+
6.	Flavonoids	Shinoda test	+
7.	Phenol	Ferric chloride test	+

(+ present, - absent)

Table 3: Physicochemical analysis of *Berberis aristata*

Sr. No	Physico -chemical parameters	Result
1.	Total Ash (%)	46.85± 1.25
2.	Acid-insoluble ash (%)	30.47±1.41
3.	Water-soluble extract (%)	36.87±0.98
4.	Alcohol-soluble extract (%)	5.93±0.36

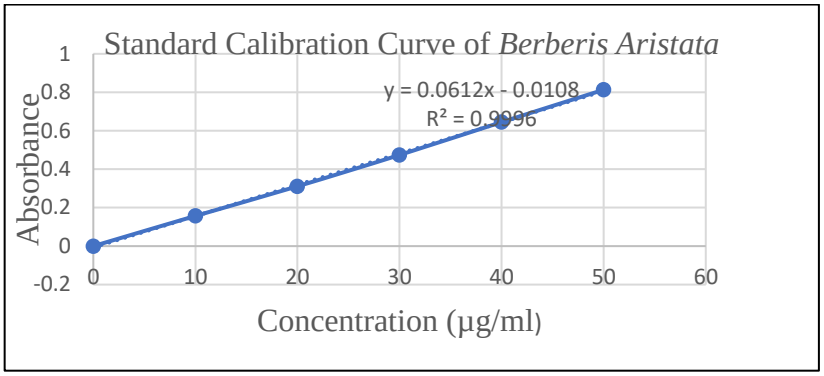
Data represented as± S.D (n=3)

2.8.2 UV analysis of Berberis aristata extract:

Table 4: Absorbance of various concentration of *Berberis aristata*

Sr.no	Concentration (µg/ml)	Absorbance
1.	10	0.157± 0.07
2.	20	0.311±0.13
3.	30	0.474±0.10
4.	40	0.645±0.16
5.	50	0.813±0.11

Data represented± S.D (n=3)



Graph. 1: Calibration curve

Table 5: Summary of Validation Parameter of *Berberis aristata*

Sr.no	Parameters	Result
1	Absorption maxima (ƛmax)	348 nm
2	Standard regression equation	0.0612x-0.0108
3	Intercept	-0.018
4	Slope	0.0612
5	Linearity Range (µg/ml)	2-20

2.9 Appearance of Hydrogel:

Colour, clarity, texture, transparency, appearance and presence of any gritty particles were found to the acceptable limit. All formulation had good consistency and excellent homogeneity, which indicated that good and very stable formulations.

Table 6: Evaluation table

Parameters	F1	F2	F3	F4	F5	F6	F7	F8	F9
Appearance	+++	+++	+++	++	+++	+++	+++	++	+++
pH	6.8±0.05	7±0.1	7.2±0.05	6.9±0.1	7±0.1	6.8±0.15	7.2±0.15	6.9±0.2	7±0.1
Viscosity[cps]	13451±4.5	25789±6.6	48530±9.5	56560±7	20765±6.1	68810±3.6	85084±3	32066±3.05	17779±5.03
Spredability [g/cm/sec]	3±0.5	3.5±0.1	3.593±0.12	3.91±0.22	4.93±0.46	6.5±0.3	5±0.66	3±0.5	5±0.5
Drug content [%]	95.58±0.22	94.21±0.84	92.80±0.34	91.89±0.25	94.70±0.44	95.47±0.43	96.90±0.25	96.45±0.38	95.20±0.40
Gel strength[s]	21±0.76	20±0.50	19±0.76	22±0.50	20±1.25	17±1.0	16±0.5	20±0.53	22±0.68
Yield [%]	94±0.35	92.5±0.25	93±0.36	94±0.42	92±0.50	94±0.55	93.5±0.58	94±0.25	96±0.65
Drug release [%]	67.15±0.25	76±0.50	80±0.37	73±0.56	85±0.30	74±0.40	70±0.50	75±0.47	71±0.79

Data represented, where all test was performed by three times ± S.D (n=3)

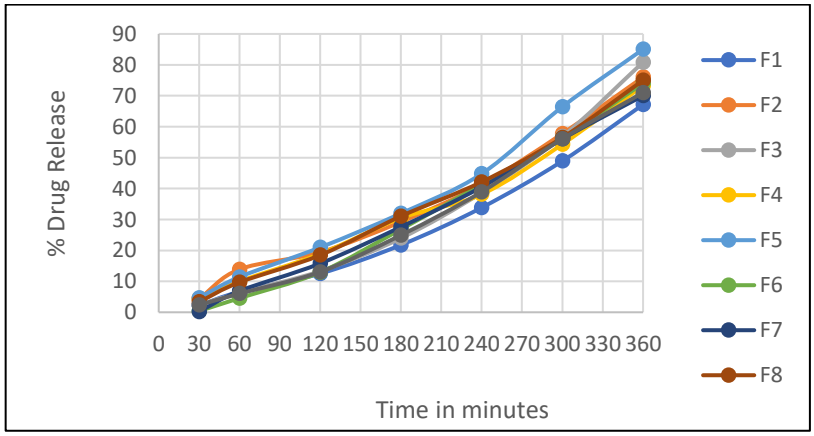


Fig. 1: Drug release profile of different batches

10. Antifungal activity

The antifungal activity of optimized F1 formulation was carried out by Cup-plate method. The antifungal activity was performed by using the microbial strain *Candida albicans*. Nutrient broth was prepared and poured in to sterile petri plates and kept aside for drying and cooling. After that *candida albicans* culture were spread by micron wire loop. By using a sterile cork borer of 6 mm diameter holes was drill in nutrient agar plates. Then place 0.5 g of Hydrogel into these holes. Plates were then nurtured at 27°C for 48 h. Then the zone of inhibition (diameter in mm) was measured. [21]

Table 7: Antifungal activity of F1 batch

Formulation	**Zone of inhibition[mm] *Candida albicans***
Blank	14±1.52
Standard	26± 1
F1 [Optimized batch]	22±1

Data represented± S.D (n=3)

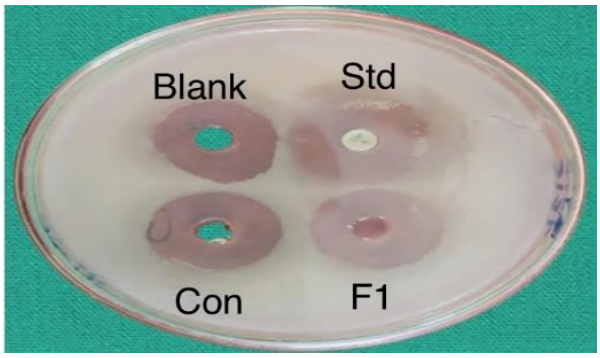


Fig. 2: Antifungal study of Hydrogel

11.Stability study

Stability studies were performed to observe the effect of environmental condition or storage condition on formulation. The optimized F1formulation was kept in accelerated stability at 40°C temperature 75 ± 5% for a period 3 months as per ICH guidelines. [22]

Table 8: **Stability Study at 40°C Temperature 75% ± 5% RH**

Evaluation parameters	**Results after 3 months**
pH	6.8±0.05
Viscosity[cps]	13451± 4.5
Spredability[g/cm/sec]	3±0.72
Gel strength[s]	21±0.72

Data represented± S.D (n=3)

2.10 Finalization of Batch:

Finalization of batch depends on evaluation parameters of all nine (F1-F9) batches like pH, viscosity, spredability, gel strength, drug release. From evaluation parameters it is observed that batch F1 fulfill all evaluation parameters, that batch was selected as optimized batch.

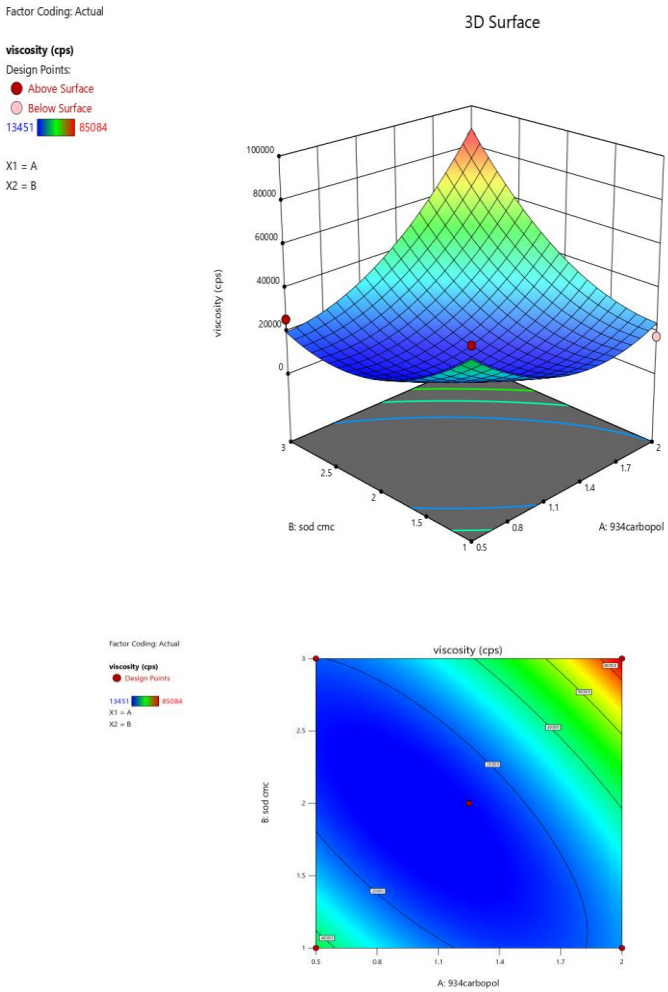
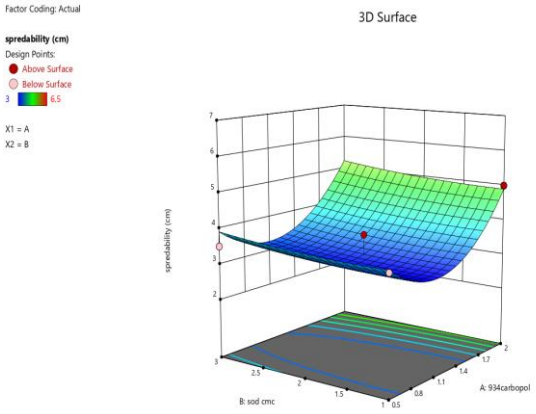


Fig. 2: Response Surface 3-D plots and contour plots showing the effect of concentration of Carbopol 934 and Sodium Carboxymethylcellulose on viscosity



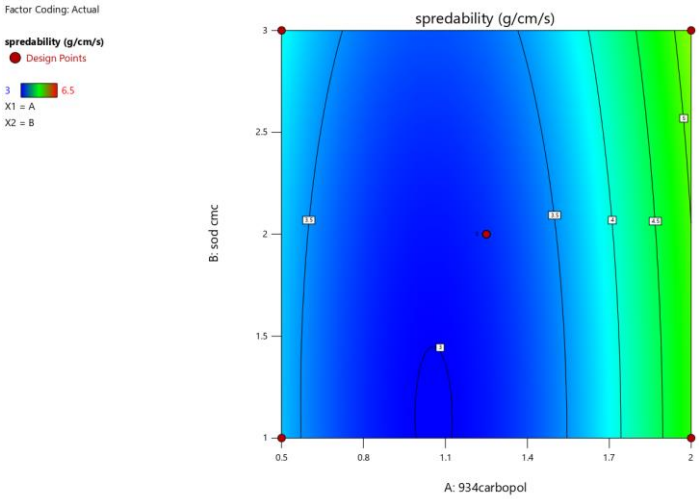


Fig. 3: Response Surface 3-D plots and contour plots showing the effect of concentration of Carbopol 934 *and Sodium Carboxyl methylcellulose on spredability*

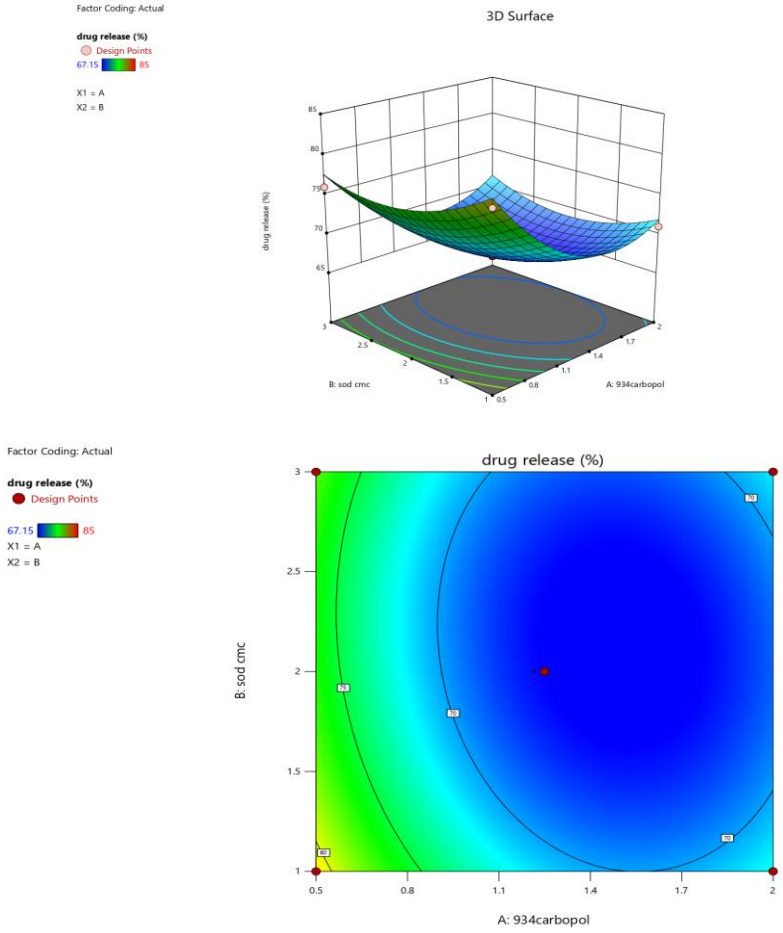


Fig. 4: Response Surface 3-D plots and contour plots showing the effect of concentration of Carbopol 934 and Sodium Carboxyl methylcellulose on percentage drug release

Table 9: Evaluation of Optimized F1 Batch

Parameters	Value
pH	6.8± 0.1
Viscosity[cps]	13451 ± 4.5
Homogeneity	Good
Gel strength[s]	21 ±0.72
Yield [%]	93%±0.80
Spredability[g/cm/sec]	3 ±0.72
Drug content [%]	95.58±0.50
Drug release [%]	67.15± 0.92

Data represented± S.D (n=3)

3. RESULT:

All hydrogel preparations were evaluated for their physical characterization like pH, homogeneity, spreadability, viscosity, drug content, drug release and anti-fungal activity as shown in Table 6. The drug contents of the formulation were in the range of 0.91 - 0.96 % of gel which indicates content uniformity. The pH of all developed formulations was in range of 6.8 -7.2, which fall in normal pH range of the mouth, while viscosity of all gel formulations was in between 13451-85084 cps, along with spreadability values ranges range 3-6.5 (g.cm/s). The F1 formulation had the premier viscosity because of optimum polymer content of Carbopol 934 and Sodium Carboxyl methylcellulose; it is able to remain on mucous surface long enough to release the effect of its active ingredients. Because of uniformity, proper appearance, stability, drug release and acceptable viscosity F1 formulation was selected as the superior formulation for the treatment of oral aphthous ulcers.

4. DISCUSSION:

Aphthous ulcer is one of the most frequent diseases of the oral cavity. Multifactorial elements, including bacterial infection, and genetic-environmental, autoimmune factors, are involved in the development of oral ulcer. The etiology of oral ulcer is unknown in most of the cases, it is generally accepted that it occurs from an imbalance between aggressive factors and the maintenance of mucosal integrity through the endogenous defense mechanism.[23] To regain this balance, different therapeutic agents may be used. *Berberis aristata* is one such plant, used for the treatment of ulcers. Hence, the present research study was oriented to formulate and evaluate Hydrogel from of *Berberis aristata*. Various phytochemical studies were performed to indicate the presence of various phytoconstituents present in stem bark of *Berberis aristata*. By the decoction method the active constituents were extracted from *Berberis aristata*. *Berberis aristata* contains berberine, protoberberine and bis isoquinoline type of alkaloid which possesses activities like anti-pyretic, anti-bacterial, anti-microbial, anti-hepatotoxic, anti-hyperglycemic, anti-cancer, anti-oxidant and anti-lipidemic agent, also its extracts and its formulations are helpful in the treatment of diarrhea, hemorrhoids, HIV-AIDS, pathology, diabetes, eye and ear infections, wound healing, jaundice, skin diseases and protozoal infection.[24]. Hydrogel (also called aquagel) is a lattice of polymer series which are deliquescent, found as a colloidal congeal in which high amount of water is found as the dispersion medium. Hydrogels are highly absorbent (about 99% water) which contain natural or synthetic polymers.[24]Hydrogels possess a degree of flexibility which is similar to natural tissue, due to their significant water content, they are formulated to treat mucosal ulcers. [25] In the present research work different batches of hydrogel from *Berberis aristata* was prepared with varying concentrations of Carbopol 934 and Sodium carboxyl methylcellulose along with other excipients and evaluated for pH, viscosity, spredability, drug content, gel strength, percent yield, drug release and antifungal activity. Hydrogel has the ease of application, good distribution and ability of adhesion and remaining on oral mucosa for a long enough time to release the drug. On the basis of evaluation parameters, F1 batch was selected as optimized batch.[26]

5. CONCLUSION:

As per the study *Berberis aristata* could be an ancient healthful plant employed in Ayurvedic, Chinese and alternative healthcare systems, which possesses anti-bacterial, anti-microbial, anti-hepatotoxic, anti-hyperglycemic, anti-cancer, anti-oxidant and anti-lipidemic activity. The hydrogel of *Berberis aristata* was prepared in different batches using different concentration of Carbopol 934 and Sodium Carboxyl methylcellulose and evaluated for different parameters. On the basis of evaluation parameters, it was found that F1 batch possessed better results with respect to viscosity, spredability and drug release and selected as optimized batch.

6. ACKNOWLEDGEMENT:

None.

7. CONFLICT OF INTEREST:

The author reports no conflicts of interest.

8. REFERENCES:

1. Eswaraiah MC, Kumar TP, "Formulation and evaluation of topical hydrogel containing antifungal drug", Pharm Pharmacol Int J, Vol. 8, no.2, **(2020),** pp 249–254.
2. Bakhle S, Upadhye K, Charde K, "Formulation and evaluation of herbal gel for management of mouth ulcers", Indian J Pharm Pharmacol., Vol .8, no.3, **(2021)** pp.226–230.
3. Chander V, Dobhal R, Uniya DP, "A review on Pharmacological potential of Berberine; an active component of Himalayan Berberis aristata", J. Phytopharm., Vol .6, no.1 **(2017)** pp. 53-58.
4. Singh SK, Dhyani A, Juyal D, "Hydrogel: Preparation, Characterization and Applications", The Pharma Innov J, Vol .6, no.6, **(2017)** pp. 25-32.
5. Bhaskar GR, "A review on hydrogel", World J Pharma and Pharma Res., Vol. 9, no.7**, (2020)**, pp.1288-1298.
6. Upwar NK, Patel R, Waseem N, Mahobia N. Kumar, "Pharmacognostic Evaluation of stem of Berberis aristata," Pharmacognosy J"., Vol. 2, no.17, **(2010)** pp.5-9.

7. Harish N, Prabhu P, charyulu RN, Gulzar MA and Subrahmanyam, "Formulation and Evaluation of in situ Gels Containing Clotrimazole for Oral Candidiasis", Indian. J. Pharm. Sci", Vol.71, no.4, **(2009**), pp. 421-427.
8. Rajasekaran A, Kumar N, "Rasont – A traditional crude drug prepared from Berberis species and its uses", Indian J. tradit. knowl, Vol.8, no.4, **(2009)**, pp.562-563.
9. Shrivastava SK, Khatoon S, Khan MF, "Pharmacognostical and pharmacological aspects of berberis aristata", Nat. Prod. Sci, Vol. 8, no. 4 **(2001**), pp.102-106.
10. Tiwari AK, Ahirwar PK, Chaturvedi A, "Preliminary pharmacognostic and phytochemical analysis of Berberis aristata", J. Chem. Pharm. Res, Vol. 6, no.11 **(2014)** pp.764-770.
11. Joshi H, Kanaki N, 'Quantitative analysis of Berberine in an ayurvedic formulation-Rasayana churna UV Spectrophotometry', J. Pharm. Sci. bio-sci. res, Vol. 3, no.1**(2013)** pp.32-34.
12. Thombre KP, Sharma D, Lanjewa AM, "Formulation and Evaluation Pharmaceutical Aqueous Gel of Powdered Cordia Dichotoma Leaves with Guava Leaves", Am. J. PharmTech res, Vol. 8, no.2, **(2018)** pp.268–277.
13. Raymond C Rowe, Paul J Sheskey, Sian C Owen, Editor Handbook of Pharmaceutical Excipients, 5th ed. Published by the Pharmaceutical Press and the American Pharmacists Association **(2006)**.
14. Akash AK, Dr. Gupta A, "Design and Evaluation of Anti-Microbial Hydrogel of Clitoria Ternatea Leaves", Int. J. Pharm. res appl**,** Vol.7, no.2**, (2022),** pp.2129-2137.
15. Sing R, Bansal S, Mishra MK, "Formulation and evaluation of Herbal Oral Gel Containing Extracts of Powdered Psidium guajava Linn Leaves with Curcuma longa Linn Rhizomes to Treat Mouth Ulcer," Int J. Drug Dev. Res Vol.12, No.2:150, **(2020)**, pp.1-7.
16. Dalavi N, Dange S, Daware S, Bagali R, "Formulation and Evaluation of Antimicrobial Gel from Allium sativum: An Herbal Approach towards Oral Health", Int J Pharm Pharm Res, Vol.25, no.3, (**2022**) pp.340-348.
17. Ramasamy A, Govindarajan A, 2016. "Formulation and evaluation of topical herbal gel for the treatment of arthritis in animal model", Braz. J. Pharma. Sci, Vol .52, no.3**, (2016**) pp.493–507.
18. Sahu R, Jain D, Mehani R, Thawani V, "Novel poly herbal muco-adhesive formulation for treatment of oral aphthous ulcer", Int J. Basic. Clin. Pharmacol, Vol.10, no.8, (**2021)** pp.906-910.
19. Gaire A, Maharajan S, Shrestha, Giri BL, "Formulation & Evaluation of Fluconazole Gel for Topical Drug Delivery System", Am Sci Res J Eng. Tech Sci Vol. 76, No. 1, **(2021),** pp.124-137.
20. Prasad S. Kaur D, "Formulation of Topical Gel from Extract of Berberis aristata DC for Acne" Int J Drug Deliv., Vol. 9, no.2 **(2019**), pp.104–108.
21. Sharmila KJ, Monisha SM, Akila Beevi A, Deebarathi V, "Antibacterial, antioxidant, anticancer effects and GCMS analysis of Berberis aristata", Biomedicine: Vol .40, no.3, **(2020)** pp.286- 293.
22. Singh V, Chaubey N, "Design and Evaluation of Topical Hydrogel Formulation of Aceclofenac for Improved Therapy", J Drug Deli Techno, Vol .9, no.5, **(2019),** pp.118-122.
23. Majumdar S, Battu SK, "Physicochemical Characterization of Berberine Chloride: A Perspective in the Development of a Solution Dosage Form for Oral Delivery", AAPS J, **(2010**), pp.1466–1475.
24. Choudhary S, Kaurav H, Chaudhary MS, "Daruharidra (Berberis aristata): Review based upon its Ayurvedic Properties"; Int. J. Res. appl. sci. biotec, Vol. 8, no.2, **(2021)** pp.98-106.
25. Monica S, Gautami J, "Design and evaluation of topical hydrogel formulation of diclofenac sodium for improved therapy", J Pharm. Sci. Vol. 5, no.5, **(2014)** pp.1973–1980.
26. Mishra P, Banweer J, Tahilani P, Shrivastava PS, "Herbal chewing Gum to Treat Mouth Ulcer using Guava Leaf and Turmeric Rhizomes", Int J Clin Studies Med Case Reports Vol. 21, no.5**, (2022**), pp.1–4.