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FORMULATION AND EVALUATION OF HERBAL NANOGEL USING *SESBANIA GRANDIFLORA* LEAVES EXTRACT

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ABSTRACT

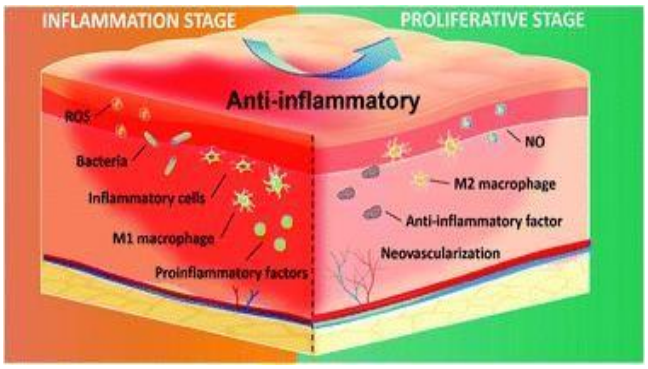
Interest in usage of herbal remedies have significantly increased in recent years, even in areas where access to modern treatment is available. Phytochemicals and herbal remedies have gained popularity recently a lot of attention because medicinal plants are the primary source of bioactive molecules utilized in both conventional and medicine of the modern age. The present research has been undertaken with the aim to formulate and evaluate the herbal Nanogel containing *Sesbania grandiflora* leaves extract. Firstly, the preparation of Nanoparticle was carried out by using chitosan polymer. Nanogel formulation was designed by using ethyl acetate extract in varied concentrations and was evaluated using physiological measurements. The maceration procedure was used to prepare the ethanolic extracts. In next step involved stirring continuously as you thoroughly combined each ingredient with the Carbopol 940 Nanogel was prepared. The Nanogel was prepared by using Carbopol 940, Sodium CMC, Sesbania grandiflora extract, Chitosan Nanoparticle, Glycerin, Methyl paraben, Propyl paraben and required amount of distilled water. Then skin pH (6.8-7) was maintained by drop wise addition of tri-ethanolamine. The physiochemical parameters of formulations (pH, viscosity, spreadability etc.) were determined. The results showed that formulation F3 was one of the best formulations among the all-other formulations.

KEYWORDS: Herbal Nanogel, *Sesbania grandiflora*, Anti-inflammatory activity.

INTRODUCTION

The term nanogel refers to highly crosslinked nano-sized hydrogels occurring in the form of either polymers or monomers. The average size of the nanogel matrix spans from 20 to 200nm. Some salient characteristics of these nanocarriers include pronounced thermodynamic stability, high solubility degree, relatively low viscosity, and maintenance of the structure under sterilization process. Anti-inflammatory or antiphlogistic is the property of a substance or treatment that reduces inflammation or swelling. Anti-inflammatory drugs, also called anti-inflammatories, make up about half of analgesics. These drugs remedy pain by reducing inflammation as opposed to opioids, which affect the central nervous system to block pain signaling to the brain.

Sesbania grandiflora is a small, loosely branching tree that grows up to 8- 15 m tall and 25-30 cm in diameter; stems tomentose, unarmed; roots normally heavily nodulated with large nodules; the tree can develop floating roots. Leaves alternate and compound; pinnate, 15-30 cm long with 12-20 pairs of oblong, rounded leaflets, 3-4 cm long and about 1 cm wide; leaves borne only on terminal ends of branches; leaves turn bright yellow before shedding.



MATERIALS AND METHODS PREPARATION OF PLANT POWDER

The plant was dried under shade and then powdered coarsely with a mechanical grinder. The powder was passed through sieve no. 40 and stored in an air-tight container for further use.

PREPARATION OF EXTRACTS

Extract was prepared by continuous hot extraction using Soxhlet apparatus with solvent ethanol. Each extraction was continued for 6-8 hrs. About 50 gm of accurately weighed homogenized powder was placed in thimble and solvent was poured on it. The extract was

settled at bottom in the flask. The filtrate was transferred to a tarred petri-dish and evaporated to dryness on a hot plate. The residue was dried till its weight become constant, cooled and weighed immediately.

PREPARATION OF NANOPARTICLES

Plant extract and chitosan were prepared by separately dissolving in chloroform at room temperature using a magnetic stirrer. After that, 8g of plant extract and chitosan was combined at respective amounts. Sodium dodecyl sulphate was then added into solution. The mixture was agitated with a magnetic stirring until the complete evaporation of organic solvent was accomplished. After that, the protective excipients glucose (50 - 400 mg) and lactose (100 - 300 mg), as aqueous solutions, were added to the nanoparticle dispersions. The nanoparticle dispersions were frozen at -32°c for a minimum of 12h and freeze-dried at -55°c and 0.5 kpa for 24 hrs.

PREPARATION OF NANOGEL

The nanogel is prepared from modified emulsion solvent diffusion method. It is having 4 steps. The first step accurately weighed quantity of extract is dissolved in ethanol and propylene glycol with stirring (organic phase). The second step aqueous phase is prepared by using Carbopol -940 dissolved in water with continuous stirring and heat for a 20min in a magnetic stirring. And the drug phase (extract) is sonicated under Ultrasonic Bath Sonicator for 10min. The drug phase (extract) is added drop by drop into aqueous phase during high-speed homogenization for 30 min at 6000rpm to from emulsion. The emulsion is converted into nanodroplet by homogenizer results in o/w emulsion formed. Step 4 in this step o/w emulsion is homogenized for 1 hour at 8000rpm and triethanolamine is added with continues.

Tab no. 1: Formulation of herbal Nanogel (F1).

S.NO	Ingredients	Formulation
1	Extract	2gm
2	Carbopol 940	0.5gm
3	Ethanol	10ml
4	Propylene glycol	4ml
5	Triethanolamine	4ml
6	Water	Q.S.

Tab no. 2: Formulation of herbal Nanogel (F2).

S.No	Ingredients	Formulation
1	Extract	2gm
2	Carbopol 940	1gm
3	Ethanol	10ml
4	Propylene glycol	4ml
5	Triethanolamine	4ml
6	Water	Q.S.

Tab no: 3 Formulation of herbal Nanogel (F3).

S.No	Ingredients	Formulation
1.	Extract	2gm
2.	Carbopol 940	1.5gm
3.	Ethanol	10ml
4.	Propylene Glycol	4ml
5.	Triethanolamine	4ml
6.	Water	Q.S.

SUMMARY AND DISCUSSION

A) Appearance

The Prepared Nanogel were Inspected Visually for Clarity, Colour and the Presence of any Particles.

Tab No. 4: Organoleptic Properties.

Physical Appearance	F1	F2	F3
Colour	Greenish	Greenish	Greenish
Texture	Smooth	Smooth	Smooth
Homogeneity	Homogenous	Homogenous	Homogenous

B) pH

The pH values of the formulated Nanogel (F1,F2, and F3) was found to be between 6 to 7 which is in the skin pH range. The pH range is considerable to avoid the skin irritation after application to the skin.

Tab no. 5: pH.

Formulation	pH
F1	6.37
F2	6.79
F3	7

C. Spreadability

The Spreadability diameter for different formulation F1, F2, and F3show good Spreadability. ie, Nanogel is easily spreadable. The Spreadability plays an important role in patient compliance and ensures uniform application of nanogel to the skin surface.

Tab No. 6: Spreadability Data.

Formulation	Spreadability
F1	16.93
F2	17.25
F3	20.22

D. Extrudability

The extrudability of formulated nanogel (F1, F2 and F3) for extrudability was based upon the quantity in percentage of gel and gel extruded from lacquered Aluminum collapsible tube on application of weight in grams required to extrude at least 0.5 cm ribbon of gel in 10 seconds.

Tab No. 7: Extrudability Data.

Formulation	Extrudability
F1	Good
F2	Good
F3	Excellent

E. Viscosity

All the fomulation of nanogel were subjected to brookfield viscometer used to measure the viscosity (in cps) by dropping a cone attached to a holding rod from distance of 10cm in such a way that, it should fall on center of the glass cup filled with nanogel.

Tab No. 8: Viscosity Data.

Formulation	Viscosity
F1	0.3821
F2	0.3864
F3	0.3896

F. In Vitro Studies

By using Tranz diffusion cell apparatus, drug release of Nanogel formulation was observed. The collected aliquots were scanned using UV-visible spectrophotometer, and % cumulative drug release was calculated. % cumulative drug diffusion was shown.

Standard curve of Plant extract

The standard calibration curve of plant extracts by plotting absorbance vs. Concentration at 273 nm.

Tab No. 9: Plant Extract Calibration Curve at 273nm.

S.No	Concentration (µg/ml)	Absorbance
1.	Blank	0.000
2.	0.2	0.082
3.	0.4	0.163
4.	0.6	0.242
5.	0.8	0.314
6.	1.0	0.383

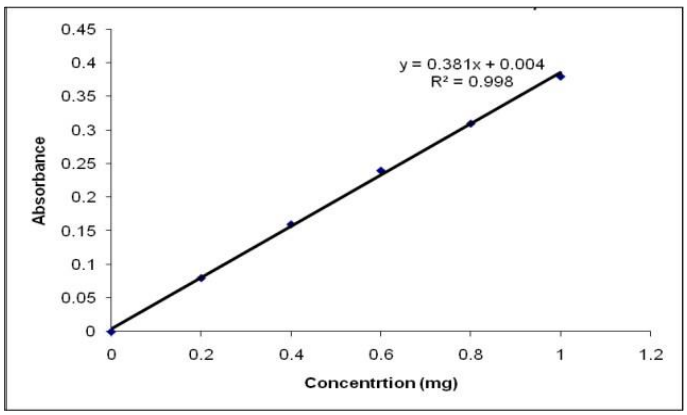


Fig. no. 1: Plant extract calibration graph.

Tab. No. 10: Drug Release of Formulation at 273nm.

Time	F1	F2	F3
0	0	0	0
0.5	11.2	11.02	9.91
1	18.24	19.04	13.4
2	28.97	27.32	21.6
3	34.06	36.01	28.9
4	43.25	44.8	31.6
6	54.06	55.2	37.2
8	76.98	77.6	39.9
10	87.32	88.1	40.19
12	92.03	99.9	40.5

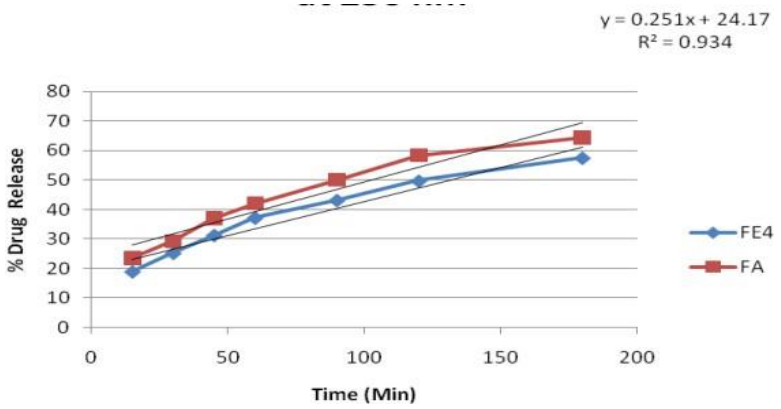


Fig no. 2: Graph for drug release(F1,F2 and F3).

G. FT-IR Studies

From the FTIR spectra of leaves extract and physical mixture of the extract, polymer and other ingredients, it was observed that the peaks of major functional groups of *sesbania grandiflora* leaves extract were also observed in spectrum of physical mixture.

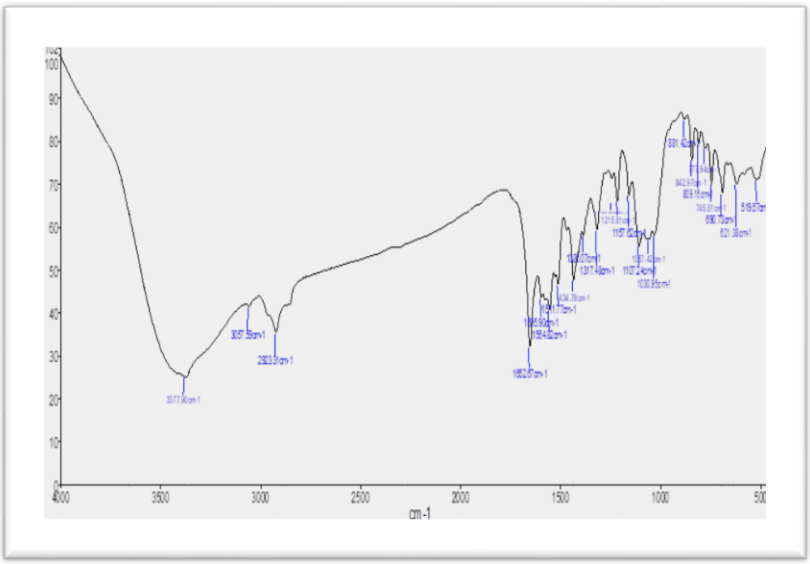
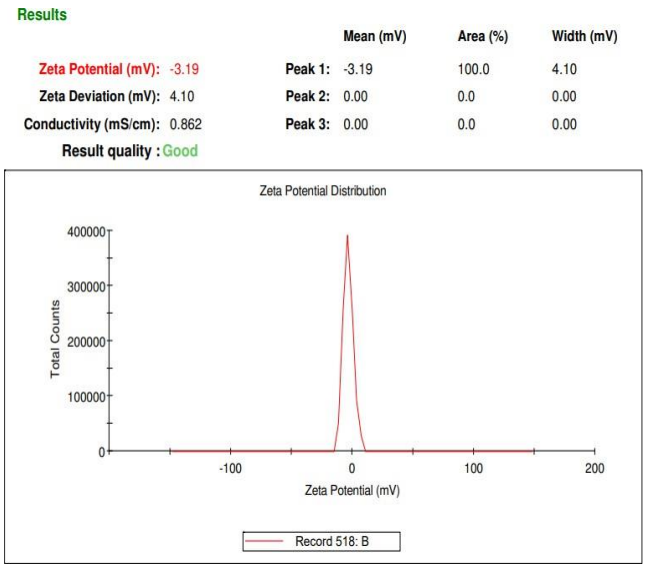


Fig no. 3: FTIR.

H. Zeta Potential

Zeta potential measurement has shown that dispersions with a charge close to zero – whether exhibiting a positive or negative charge – tend to yield shorter shelf-lives, with an inclination towards coagulation or flocculation of emulsions. Conversely, emulsions with a surface activity greater than ~ +/- 30mv are inclined towards improved system stability and low aggregation. The zeta potential of formulated nanogel was discussed below:

Zeta potential: 4.10 (mv) Conductivity: 0.862(ms/cm)



I. Determination of Absorption maximum (λ-max)

The UV-visible spectroscopy is used to characterize the excitation spectra of the samples, which is useful to prove the presence of nanoparticles. The UV-visible spectra gives an absorption band at 273nm.UV-visible spectroscopy is very useful to identify the formation of nanogel in reaction mixture.

Tab no: 13 UV studies.

Concentration	Absorbance
0	0
50	0.182
100	0.276
150	0.511
200	0.681
250	0.88

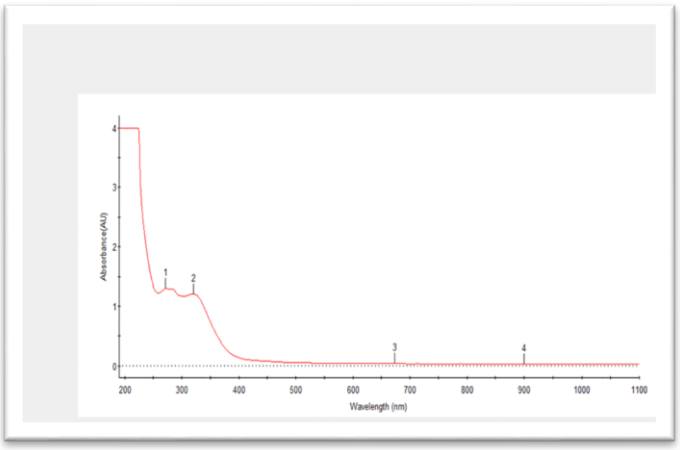


Fig. no: 4 Determination of λ-max.

J. Scanning Electron Microscope

Using scanning electron microscopy, the surface morphology of the GTE equipped chitosan nanoparticle was investigated. The research gave a better understanding of the morphological features of the nanoparticle. At higher cross-linking time, the SEM image of dried chitosan nanoparticles, little forwarded but small spherical nanoparticles were obtained in formulation F3.

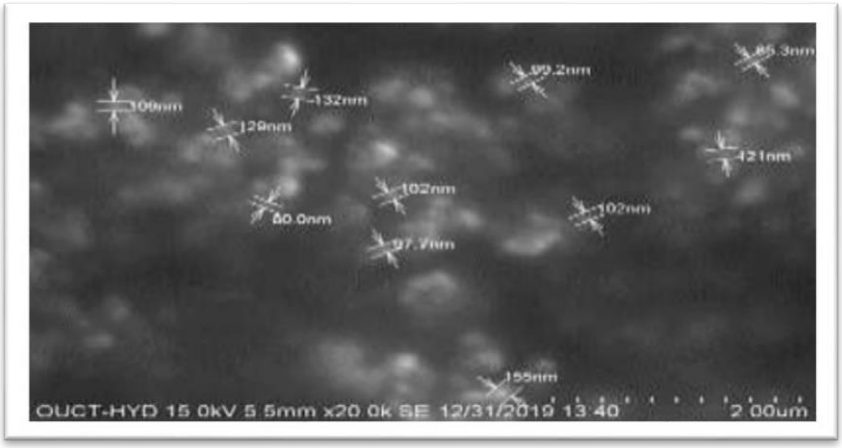


Fig no: 5 SEM image.

K. Invitro anti-inflammatory activity

Proteinase inhibitory activity

The maximum inhibition was observed from ethanolic extract (97 %). The standard aspirin (80 %) drug showed the maximum proteinase inhibitory action.

Tab no. 14: Invitro anti-inflammatory activity of sesbania grandiflora by proteinase denaturation.

S.NO	Concentration (µg/ml)	Inhibition
1	50	72%±0.052
2	100	97%±0.090
3	200	78%±0.056
4	300	86%±0.55
5	500	87%±0.056
6	Standard drug (Aspirin 250µg/ml)	80%±0.050

L. Stability Studies

The optimized formulation is subjected to stability study for the period of 45 days. The sample is stored in described condition as per ICH guidelines. After the stability study sample is subjected to evaluation test and compared to test values just before to that of stability study.

Tab no. 15: Stability testing at 25°C ± 2°C/60% ± 5% RH (3rd months) of Nanogel of *Sesbania grandiflora* leaves extract.

Formulation	Color	Appearance	Spreadability (g.cm/sec)	pH
F1	Greenish	Homogenous	16.93	6.37
F2	Greenish	Homogenous	17.25	6.79
F3	Greenish	Homogenous	20.22	7

Tab no. 16: Stability testing at 30°C ± 2° C/65% ± 5% RH (3rd months) of Nanogel of *Sesbania grandiflora* leaves extract.

Formulation	Color	Appearance	Spreadability (g.cm/sec)	pH
F1	Greenish	Homogenous	15.32	6.86
F2	Greenish	Homogenous	18.34	7
F3	Greenish	Homogenous	21.65	7

Tab no. 17: Stability testing at 40°C ± 2°C/75% ± 5% RH (3rd months) of Nanogel of *Sesbania grandiflora* leaves extract.

Formulation	Color	Appearance	Spreadability (g.cm/sec)	pH
F1	Greenish	Homogenous	15.36	6.91
F2	Greenish	Homogenous	17.96	7
F3	Greenish	Homogenous	21.63	7

CONCLUSION

The selection of Nano drug delivery system was carried out because of having large number of advantages over the drug delivery system. Formulation batches were subjected to number of evaluation test, Organoleptic properties, pH, Homogeneity, Spreadability, Viscosity, Extrudabaility, FTIR studies, SEM, Zeta potential, Invitro drug release test, stability studies and Invitro anti-inflammatory activity of formulation batches were carried out by considering all of the evaluating parameters. The formulation batch which followed best results for majority of evaluation test, is considered to be optimized formulation. Hence F3 was one of the best formulations among the all-other formulations. Enhanced solubility; Improved drug loading and Bioavailability; slow release of drug. There are many types of natural penetration enhancer. Nanogels, being a flexible and versatile drug carrier, have numerous applications in the pharmaceutical domain.

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