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Development and evaluation of *in situ* thermoresponsive nasal gel system for *Nardostachys jatamansi*

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ABSTRACT

Nardostachys jatamansi is conventionally used indigenous plant as decoction for sedative effect. The purpose of present studies was to formulate and evaluate thermoresponsive nasal gel of *N. Jatamansi* extract. Cold method was used for formulation of gel containing pluronic PF 127 with dried ethanolic *N. Jatamansi* extract, PEG 400, PEG 4000 as gelling point modifiers and methyl paraben as preservatives. Most potent ethanolic extract used in the formulation was screened by assessing righting reflex using rat as animal model. ³ Factorial design was applied for factors of amount of Pluronic F 127 and PEG4000 and were evaluated for studying their effect on gelation temperature and p^H . Optimized batches showed gelation point at $34^{\circ}C$ and $37^{\circ}C$. p^H between 4.1-5.3, Spreadability between 0.35-0.8cm, Mucoadhesive strength was 1524.44 and 1720.44 dyne/cm² across freshly excised sheep nasal mucosa. Rheological studies indicated viscous and Newtonian behavior signifies spreadability and increased residence time. The IR spectroscopic studies indicate possibility of jatamansone in the extract and formulation. HPTLC studies revealed the presence of jatamansone similar component. *N. Jatamansi* was successfully formulated into stable *in situ* thermoresponsive nasal gel system revealed by stability studies. It may enhance patient compliance by increasing bioavailability and reducing side effects.

Keywords: *Nardostachys jatamansi*, Pluronic F 127, nasal thermoresponsive gel, Rheology.

INTRODUCTION

Roots of *Jatamansi* morphologically are similar to the matted hair of the Himalayan ascetics, hence named *Jatamansi*. Its flesh resembles dreadlock. *Jatamansi* possess wider pharmacological actions principally on nervous system with Hysteria, nervousness, epilepsy, Parkinson's and insomnia like indications. It had been extensively used to clear pitta, calm vata and reduce kapha. Because of its benefiting majja dhatu-agni it improves intellect and induces mental clarity. Also it has penetrating quality which is useful in treating headaches and muzzy head syndrome. *Jatamansi* is also indicated in heart for Palpitations, angina, essential hypertension, in GIT problems, Gynecological conditions like dysmenorrhoea, lung problems and it has special use for promoting hair growth, preventing hair loss and graying hair. In the present study we focused on CNS effects of *N. Jatamansi*. [1]

The human nose has the potential to be an alternative route for the systemic delivery of a wide range of therapeutic agents. The richness of vasculature in nasal mucosa coupled with its high drug permeation makes the nasal route attractive for many drugs, including proteins and peptide to achieve faster and higher level of drug absorption.[2]

Also the olfactory region of the nose provides a potential for a drug to be available to the central nervous system.[3,5] However the liquid nasal dosage form has limitation of draining out, although gels are available, they have problem of patient compliance because of its semi solid nature and difficulty in application. It can be overcome by preparing a formulation that is liquid at lower temperature and forms a gel at body temperature. [4]

Gels increases the contact time of drug at the administered site, hence increasing the absorption of drug, due to its mucoadhesive property through the polymeric network. However, it shows less patient compliance owing to its semi solid nature and difficulty in application. Our objective was to formulate a thermoresponsive nasal gel system containing N. Jatamansi root extract which will overcome these limitations and will have fewer side effects.

Thermo reversible gels can be formulated using environmentally responsive polymer poloxamers. Lutrol F grades are block copolymers referred to as poloxamers, consisting of Polyoxyethylene (POE) and polyoxypropylene (POP) units. Higher molecular weight poloxamer has the ability to form thermoreversible gels. In particular poloxamer 407 (Lutrol F 127) has been used in number of applications including nasal drug delivery, where the increase in viscosity at body temperature, increases the residence time of the drug in the nose. [6] Hence, a formulation that would increase residence time in the nasal cavity and at the same time increase absorption of the drug would be highly beneficial in all respects.

MATERIALS AND METHODS

Materials: Pluronic F 127(BASF Mumbai) was provided as gift sample, PEG400 (Molichem Ltd), PEG 4000 (ACME Chemicals Mumbai), methyl paraben (ACME chemicals, Mumbai), Ethanol (ChangshuYangshuan Chemicals, China) of high purity were procured.

Animals: Adult male Swiss albino rats (Wt. 200–250 g) were obtained from the animal house of the Department of Pharmacology, Bharati Vidyapeeth College of pharmacy, Shivaji University, Kolhapur, MS, India. Activity was approved by Institutional Animal Ethics Committee (Approval No. BVCPK/CPCSEA/IAEC/02/10).

Collection, Identification and authentication:

The rhizomes of *Nardostachys Jatamansi* were collected from authorized ayurvedic store from Kolhapur, (MS). Identification and authentication was done by Dr. M. Y. Bachulkar, Department of Botany, B. Y. College of Arts, Science and Commerce, Pethwadgaon (MS).

Extraction:

The air dried rhizomes (1kg) were coarsely powdered and extracted with distilled water, ethanol and ethanol: water mixture (70:30) for 48 hours by using Soxhlet apparatus. [7,8] The collected extract was filtered; the filtrate was concentrated in rotary vacuum evaporator.

Screening of extracts for sedative activity by loss and regaining of righting reflex:

Sedative property of *Jatamansi* extract in rats by loss and regaining of righting reflex was assessed using 'restrainer' as described by Ponomarev and Crabbe (2002). Activity was approved by Institutional Animal Ethics Committee (Approval No. BVCPK/CPCSEA/IAEC/02/10). Animals were divided in 4 groups of 6 rats each, Group 1 serves as control received normal saline and Group 2, 3, 4 received alcoholic, aqueous, and hydro alcoholic extract of *Jatamansi* (i.v.) respectively. The extracts were dissolved in ethanol (5%), and PEG 400 (5%), completely which was further dissolved in sterile normal saline water and volume was made up with the same. Dose was adjusted 6mg/0.5ml as a single dose calculated in accordance with the Guidance for Industry, Center for Drug Evaluation and Research (CDER), July 2005 Pharmacology and Toxicology. These solutions were administered intravenously in tail vein. The restrainer was then gently rotated on its axis through 90° every 3 s to assess for the righting reflex response. Initially for first few such tests the mouse immediately righted itself. The rat was considered to have lost its righting reflex, if it was no longer able to right itself within 5 s from its supine position and regained righting reflex if they could either right themselves from a supine position within 5 s period (cut off period) or could not be placed on their backs after eight successive 90° turns of the restrainer. [9]

Preparation of plain pluronic F127 gel and its effect on gelation point:

Solutions of pluronic F-127 was prepared according to the cold method described by Schmolka et al. Required quantity of polymer was weighed and added to deionised water at about 4°C and stirred. It was then refrigerated overnight to ensure complete dissolution of the polymer in water resulting in clear transparent solution at 4°C.¹⁰ Five

different concentrations viz. 15, 16, 17, 18, 19 and 20% were prepared; gelation temperature of each prepared gel was determined by modified Millar et al technique.

Preparation of gels:

Aqueous gels containing 16.5%, 17.5% and 18.5% of Pluronic PF 127 were prepared by using cold method described by Schmolka et al. The dried ethanolic extract of *Nardostachys Jatamansi* (screened as most active after assessment of righting reflex) (1.3gm) was dissolved in ethanol (0.25 ml) and PEG400 (0.30 ml), this was then added to the solution of polymer followed by addition of PEG 4000 (dissolved in deionised water before addition) and methyl paraben (0.001%).

3² Factorial design:

A 3² randomized full factorial design was used in this study. Experimental trials were performed at all 9 possible combinations. The amount of PluronicF127 (X₁) and amount of PEG4000 (X₂) were selected as independent variables. A statistical model incorporating interactive and polynomial term was used to evaluate the responses.

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1X_1 + b_{22}X_2X_2 \quad (\text{Eq.1})$$

Where Y is dependent variable, b₀ is the arithmetic mean response of the 9 runs, and b_i (b₁, b₂, b₁₂, b₁₁ and b₂₂) is the estimated coefficient for the factor X_i.

Evaluation of gel:

Gelation and gel melting: A modified Millar et al technique using 2ml of gel in test tubes sealed with Aluminum foil immersed in water bath at 4⁰ C was used. Water bath temperature was increased by 1⁰ C and left to equilibrate. Gelation occurred when the meniscus would no longer move upon tilting to 90⁰ C. Gel melting temperature was recorded when gel starts flowing. [10]

p^H of the gel: The pH of each batch was measured using digital pH meter.

Spreadability: A mucoadhesive formulation that is having high Spreadability and high surface tension will adhere strongly to the mucus membrane. The spreadability in terms of flow ability of various mucoadhesive thermoreversible gels was determined. For assessing spreadability a rectangular, glass slide (10x4cm) was used. [11] The sheep nasal mucosa from serosal side was tied on the surface of slide with thread. The slide was kept in a hot air oven and temperature was maintained at 37⁰ C. One drop of formulation was placed on mucosa at an angle of 120⁰ C and the distance traveled by drop before it gets converted into gel was determined. Measurements were repeated three times for each of the gel preparation.

Bioadhesion strength: The Bioadhesive force of was determined by using measuring device in Figure 1. In brief, a section of nasal mucosa was cut from the sheep's nasal cavity and instantly secured with mucosal side out onto each glass vial (C) using rubber band. The vials with the nasal mucosa were stored at 37°C for 5 min. Next, one vial with a section of mucosa (E) was connected to the balance (A) and the other vial were placed on a height adjustable pan (F). Formulation (D) was added onto the nasal mucosa on the other vial, one by one. Then, the height of vial was adjusted so that the gel could be placed between the mucosal tissues of both vials. The weight (B) was kept raised until two vials were attached. Bioadhesive force, the detachment stress, was determined from the minimum weight that detached two vials. The nasal mucosa was changed for each measurement. [12]

Rheological studies: Rheological measurements of thermoresponsive nasal gel system for *N. Jatamanasi* were performed using a controlled stress rheometer (Cone and plate). Fresh sample was used for every test all measurements were carried out at 37°C. [13]

1. Viscometry: The samples were exposed to increasing stress (0.1-100 Pa) and relation between shear stress and shear rate was studied.

2. Oscillation stress sweep: Oscillation stress sweep was performed to determine constant Linear Viscoelastic Region (LVR) in stress range of 0.1 to 1000 Pa and constant frequency of 1Hz. The G' values were plotted in

logarithmic scale. This test allows the determination of the Linear Viscoelastic Regime (LVR) of the sample, and therefore the consequent choice of the stress value to use in the other oscillation tests.

3. Oscillation frequency sweep: The samples were exposed to increasing frequency (0.1- 10 Hz) at a constant stress in LVR. The frequency range and the $G'G''$ values were plotted in logarithmic scale.

4. Creep-recovery: The test was carried out at a constant stress in LVR, which was maintained constant for 100 s. It was then instantly removed and the recovery was followed for 200 s. The Creep compliance J_C (defined as the ratio between the measured strain and the applied stress) is monitored against time.

FT-IR studies

FTIR studies were done to assess whether any possible interaction among drug, polymer and extract and this was done by FTIR spectrophotometer (Jasco-4100). Infrared spectrums of pure drug, physical mixture of ingredients of the formulation, and optimized batch were recorded.

Stability study:

The stability study was performed as per ICH guidelines. The formulated gel were stored at temperature and humidity condition of $4^{\circ}\text{C} \pm 2^{\circ}\text{C} / 60\% \pm 5\% \text{RH}$ for a period of three months and studied for color, appearance, pH

Thin Layer Chromatography of *N. jatamansi* extract:

TLC of *N. jatamansi* oil was performed using silica gel for Thin Layer Chromatography. The solvent used was methanol. Mobile phase used was Acetone and petroleum ether with varying proportions.

High Performance Thin Layer Chromatography:

A Camag HPTLC system equipped with an automatic TLC sampler ATS 4, TLC scanner 3 and integrated software Win-CATS version 1.2.3 was used for the analysis. HPTLC was performed on a pre-coated silica gel HPTLC 60F254 (10 · 10 cm) plate of 0.20 mm layer thickness. The optimized batch of the gel, oil and dried ethanolic extract were extracted in methanol and 10 μl of supernatant were applied to the plate as 6 mm wide bands on the HPTLC plate. Chromatographic development was carried out using Acetone: Petroleum ether (3:1) as mobile phase. Scanning was carried out at 340 nm.

RESULTS AND DISCUSSION

The ethanolic extract obtained after extraction was more potent which was assessed by righting reflex in rats.

Righting reflex:

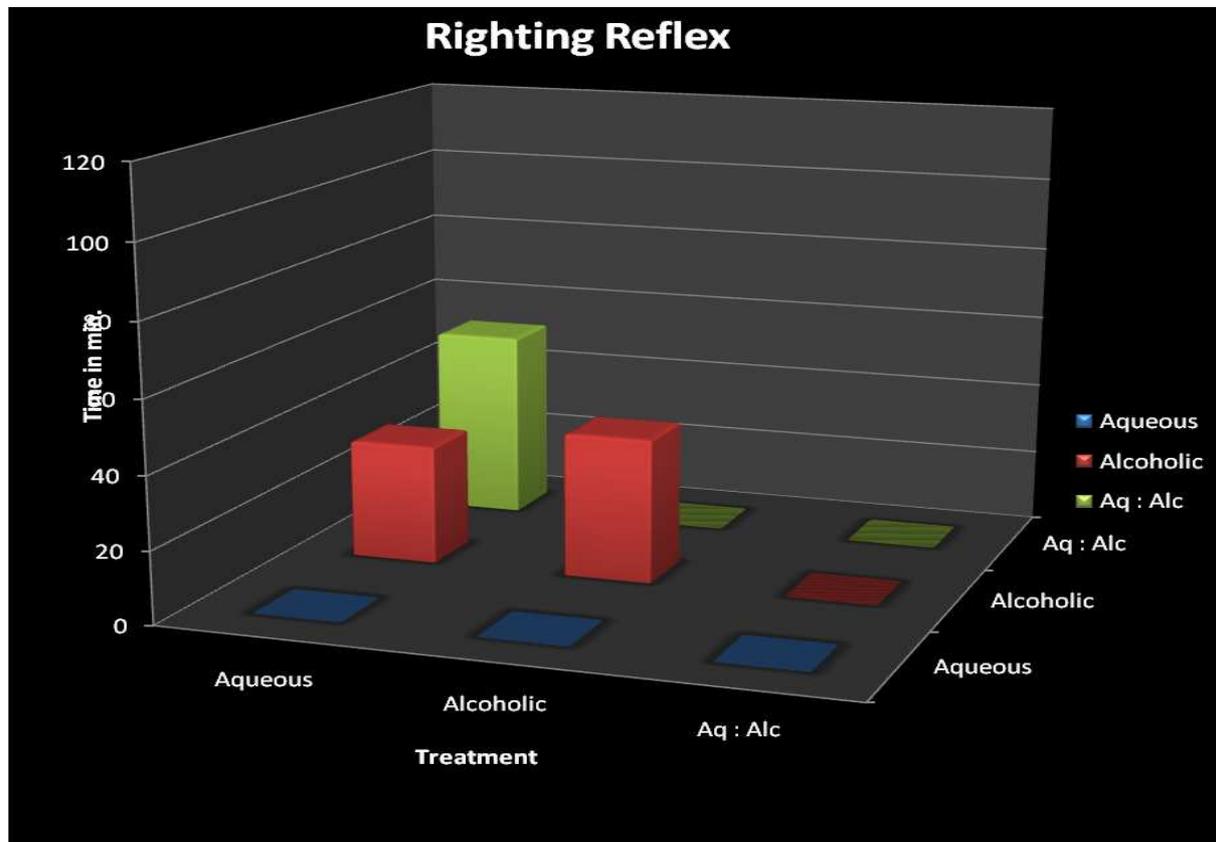
The assessment of extracts by righting reflex on rats was done; observations are depicted in the Table 1.

Table no.1- Assessment of sedative activity of *Nardostachys jatamansi* by righting reflex in rat (RR: righting reflex, TP: Tail pinch, PW: Pedal withdrawal reflex, NM: Not measurable.)

Experimental Group	Treatment	Time to loss reflex (min)			Time to regaining reflex (min)		
		RR	TP	PW	PW	TP	RR
1	Aqueous	NM	NM	NM	NM	NM	NM
2	Alcoholic	33min	40min	NM	NM	NM	NM
3	Aq : Alc	52min	NM	NM	NM	NM	NM

Results show the inhibition of mobility measured in terms of tail pinch in rat was more with the alcoholic extract i.e. after 40 min as compared to aqueous and aqueous: alcoholic extract. Also righting reflex was observed in alcoholic and aq: alc extract, but it was earlier in alcoholic extract. Righting reflex was not observed in aqueous extract (Fig. 1).

Fig.1- Response surface plot showing screening of extracts by Righting reflex on rats



Therefore most active ethanolic extract was further used for the formulation of gels.

Characterization of extract using Infrared Spectroscopy:

Infrared absorption spectrum of dried ethanolic extract, physical mixture and optimized batch (A5) was taken. The spectrum shows all prominent peaks of Jatamansone. IR spectrum indicated that characteristics peaks belonging to major functional groups described in Table no.2. In FTIR study of extract and developed batch shows all prominent peaks of Jatamansone.

Table 2- IR interpretation

For Extract		
Sr. No.	Peaks (cm ⁻¹)	Groups
1	2875.34(2872-2962)	-CH ₃
2	1731.44 (1670 – 1820)	Cyclic six membered C=O
For Physical Mixture		
1	2877.27 (2926– 2853)	C-H2
2	1698.98, 1731.7 (1670 – 1820)	Cyclic six membered C=O
3	2905 (2872-2962)	-CH3
For optimized formulation		
1	2881.13 (2926-2853)	CH2
2	1692.23, 1753.9 (1670 – 1820)	Cyclic six membered C=O
3	2960.2935 (2872-2962)	-CH3

Hence it can be concluded that there were no any significant changes and behavior in the physical mixture of extract and polymer i.e. pluronic F127, PEG400, PEG4000 and the presence of jatamansone in extract as well as in formulation.

Gelation temperature and gel melting:

The minimum concentration of PF-127 that formed gel below 38°C was 17% w/w indicated by preliminary studies. The gelation temperature of different concentration of pluronic F-127 is shown in Table 3.

Table no.3 - Effect of concentration of pluronic F127 on gelation temperature

Conc. Of Pluronic (%w/w)	Gelation point
15%	No gelling up to 100°C
16%	
17%	38°C ± 0.2
18%	32°C±0.1
19%	29.5°C±0.3
20%	28°C±0.2

It was observed that as the concentration of pluronic F-127 increased, the gelation temperature decreased as shown in the table 3. As the concentration of pluronic F127 increases, there is micelle formation, followed by micellar aggregation. The gel phase can only occur when the concentration is above the micellar concentration. When the material is in cold water, hydrogen bonding between POP chains and water keeps the hydrophobic portions of the pluronic apart. Increase in temperature leads to disruption of the hydrogen bonds and gel is formed due to hydrophobic interactions. Therefore, the gelling properties of the pluronic are dependent on percentage of hydrophobic portion. As the concentration of pluronic F127 increases, the hydrophobic portion also increases resulting in formation of gel at lower temperature. [12]

The different formulations were prepared to get the gelling temperature between the range of 30-37°C since if the gelation temperature is below 30°C, might be lead to formation of gel at room temperature also can cause problems regarding patient compliance and difficulties in administration and if its more than 37°C, it can easily drain out of the nasal cavity as the temperature of nasal cavity is 34°C.

Formulations were prepared by dissolving the most potent ethanolic extract in ethanol, as it is soluble in ethanol thus incorporation of drug is achieved. However it may dehydrate the micellar gel system and ultimately lowering of gelation temperature. Also the amount of ethanol used was least possible as it leads to nasal irritation in higher concentration. So the solubility of extract was enhanced by addition of PEG 400. Gel-sol transition temperature is modified by using water soluble polymer PEG. It may interfere with process of micellar association of the polymer PF127. As PEG comprises same hydrophilic polyoxyethylene moiety, it is likely to be enhancing dehydration and results into entanglement within micelles. This could be the reason of increase in gelation temperature. This completely dissolved extract was added to the aqueous solution of pluronic F-127 of 17% concentration as at this concentration plain gel shows gelation at 38°C, followed by addition of methyl paraben. This batch showed gelation temperature at 16 °C.

So attempts were made to increase the gelation point of the formulation by adding varying amounts of inert polymer PEG 4000. For 2% of PEG 4000 concentration gelation point was 22°C, for 3% gelation point was 29°C and for 4%, it was above 50°C.

Because of the sensitivity of gelation point for PEG4000 the concentrations between 3% and 4% of PEG4000 were tried also amount of ethanol was further reduced as it lowers the gelation point. Also 3² factorial designs were applied to the batches by considering concentrations of Pluronic F 127 and PEG4000 as dependent variables and gelation point as independent variable. Ultimately the batch A5 and A6 showed gelation point at 34°C and 37°C respectively.

Table no.4- Formulation batches. (PF-127: Pluronic F127)

Batches	Concentration of PF-127 (X ₁)	Concentration of PEG 4000 (X ₂)	Gelation point °C	Gel melting °C	p ^H
A1	16.5	2.25	16°C	65°C	4.9
A2	16.5	4.25	18°C	58°C	5.1
A3	16.5	6.25	42°C	62°C	4.3
A4	17.5	2.25	14°C	Solid at 95°C	5.3
A5	17.5	4.25	34°C	80°C	4.4
A6	17.5	6.25	37°C	76°C	5.2
A7	18.5	2.25	14°C	Solid at 95°C	4.7
A8	18.5	4.25	18°C	Solid at 95°C	4.6
A9	18.5	6.25	15°C	Solid at 95°C	5.1

The data clearly indicate that the gelation point strongly dependent on the selected independent variables. The fitted equation relating the response gelation point to the transformed factors is shown in equation 1 and 2.

$$T_1 = 29.70 - 3.975X_1 + 8.333X_2 - 5.15X_1X_2 - 6.975X_1X_1 - 2.05X_2X_2 \quad \text{Eq.1}$$

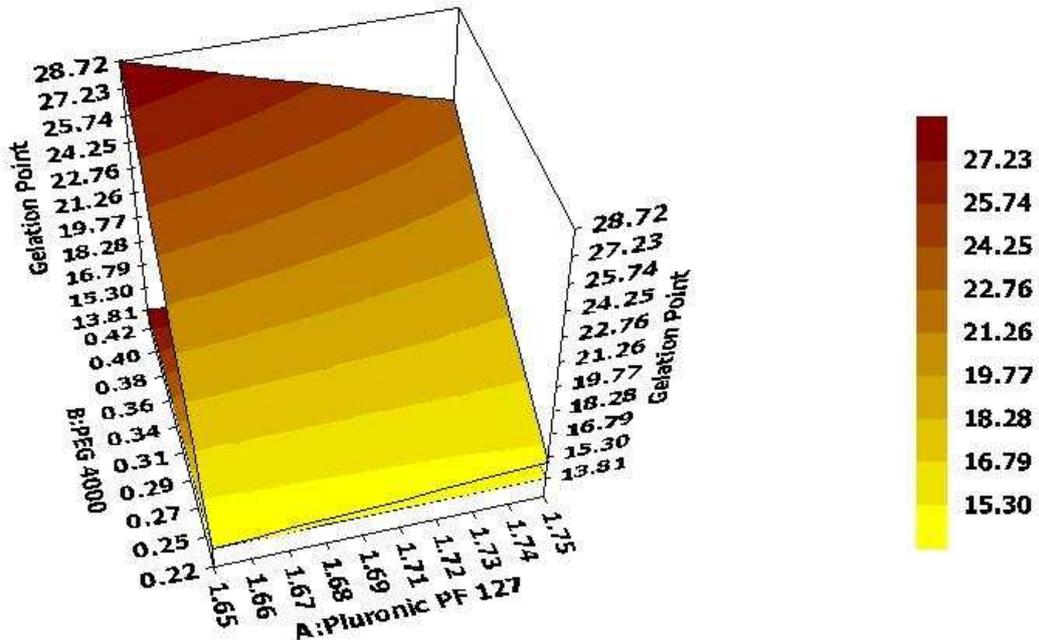
$$T_1 = 29.70 - 3.975X_1 + 8.333X_2 \quad \text{Eq. 2}$$

(R² = 0.7953)

Surface plot of Pluronic F 127 and PEG4000 against gelation point.

Fig 2 shows the surface plot of the amount of pluronic F 127 (X₁) and amount of PEG4000 (X₂) versus gelation point respectively. The plot was drawn using DOE software, Reliasoft Corporation, India.

Fig.2 -Surface plot of Pluronic F 127 and PEG4000 against gelation point



The data demonstrate that both X₁ and X₂ affect the gelation point. It may also be concluded that the low level of X₁ (amount of pluronic F 127) and the higher level of X₂ (amount of PEG4000) favors the occurrence of gelation point at the physiological temperature of nasal cavity. It can be concluded that the gelation time pattern may be changed by appropriate selection of the X₁ and X₂ levels.

An increase in the concentration of pluronic F 127 (X₁) and amount of PEG4000 (X₂), decrease and increase gelation point of the thermoresponsive nasal gel system of the *Nardostachys jatamansi*. All batches of gels showed various gelation points but the formulation A5 showed the gelation points at the physiological temperature of nasal cavity and batch A6 showed gelation point close to the physiological temperature of nasal cavity. (Table no 4)

p^H of the gel:

Though normal physiological p^H of human nose is in range of 4.5-6.5, nasal mucosa can tolerate p^H between 3-10. All the formulations showed p^H between 4.1- 5.3. (Table No. 4)

Table 5 : p^H of all batches

Sr. No.	Batches	p ^H
1	A1	4.9
2	A2	5.1
3	A3	4.3
4	A4	5.3
5	A5	4.4
6	A6	5.2
7	A7	4.7
8	A8	4.6
9	A9	5.1

Spreadability:

Spreadability considered as the ability of the formulation to flow and in terms of flow ability it was determined by placing a drop of formulation on sheep nasal mucosa at angle of 120° and the distance travelled by gel was determined. Spreadability may be related to the viscosity of nasal gels, Assessment of spreadability is done in terms of distance travelled by gels. It reveals that more the viscosity more will be the residence time and lesser the distance travelled by gel on the surface of nasal mucosa kept at 37°C. Distance travelled by formulated batches of gel determined was 0.35 to 0.8 cm. The optimized batches have viscous nature confirmed by oscillation stress sweep study in rheological investigation explained later. Sufficient spreadability of the batches may be because of its viscous nature and thereby may have enhanced residence time.

Bioadhesion strength:

Nasal membrane is rich in glycoproteins, having ability to interact with various materials. The –OH and etheral oxygen of the pluronic polymer structure is capable of extensive hydrogen bonding with nasal glycoproteins. [10] Results reveal that variable PEG 4000 is having effect on mucoadhesive strength. It shows that as level of PEG4000 increases, mucoadhesive strength also increases. Bioadhesion increased with polymer concentration due to extensive bonding with glycoproteins..The Bioadhesion strength of optimized batches A5 and A6 was observed as 1524.44 and 1720.44 dyne/cm² respectively, which indicates the prepared formulations possess sufficient Bioadhesion property and may have desired residence in nasal cavity.

$$\text{Detachment stress (dyne/cm}^2\text{)} = \frac{m \times g}{A} \tag{Eq. 3}$$

Where, m =Weight required for detachment of two vials in grams, g = Acceleration due to gravity [980cm/s²], A = Area of tissue exposed.

Rheological studies:

Rheological studies of therrmosensitive gel system were performed to investigate viscoelastic properties of the gel formed at 37°C.

Viscometry: The shear rate and shear stress profiles of the optimized batch A5 are shown in Fig. 3 respectively. Direct relationship between shear rate and shear stress was observed which signifies Newtonian relationship in both gel systems.

Fig.3 -Rheogram presenting relationship between shear stress and shear rate (Viscometry) (Batch A5)

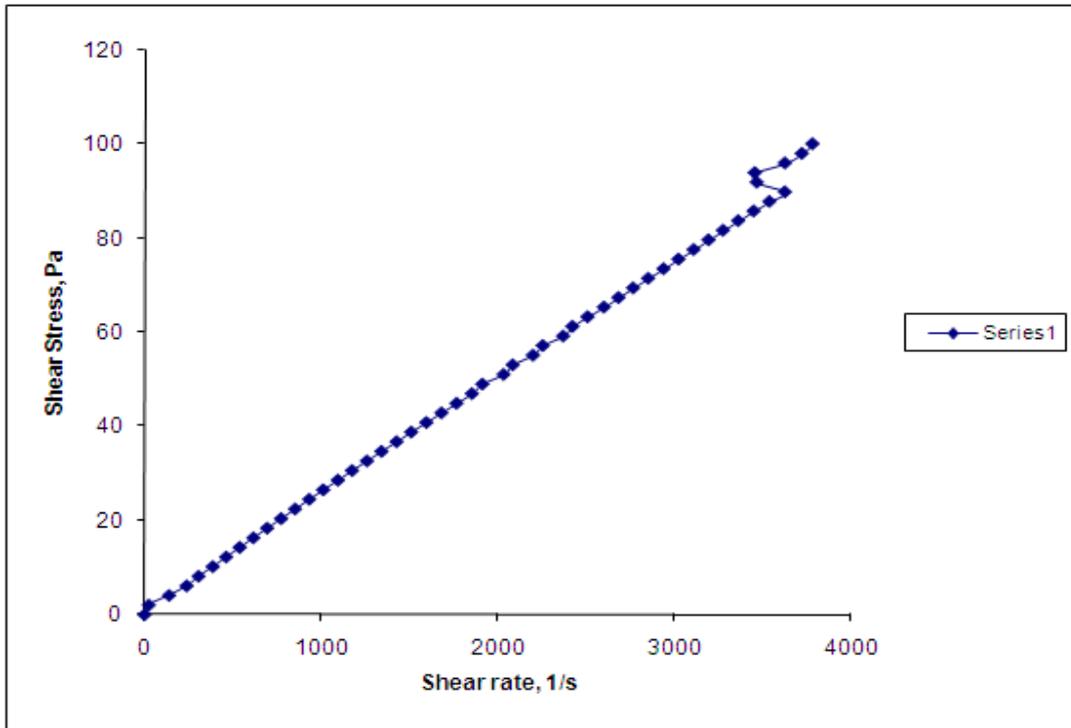
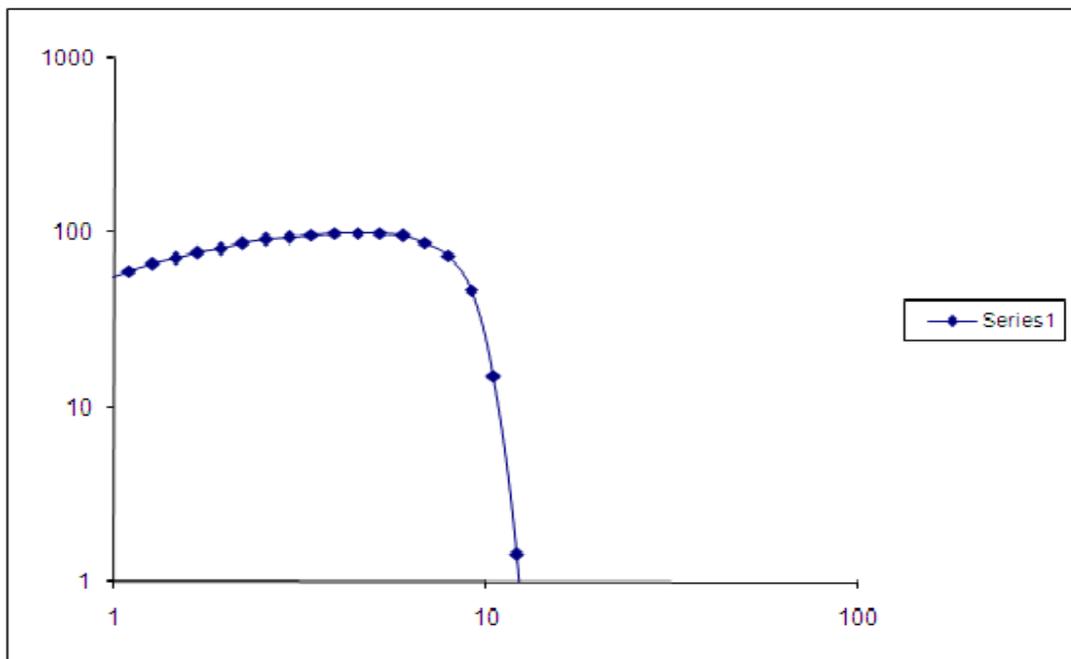


Fig.4- Stress against G' (Oscillation Stress Sweep) (Batch A5)

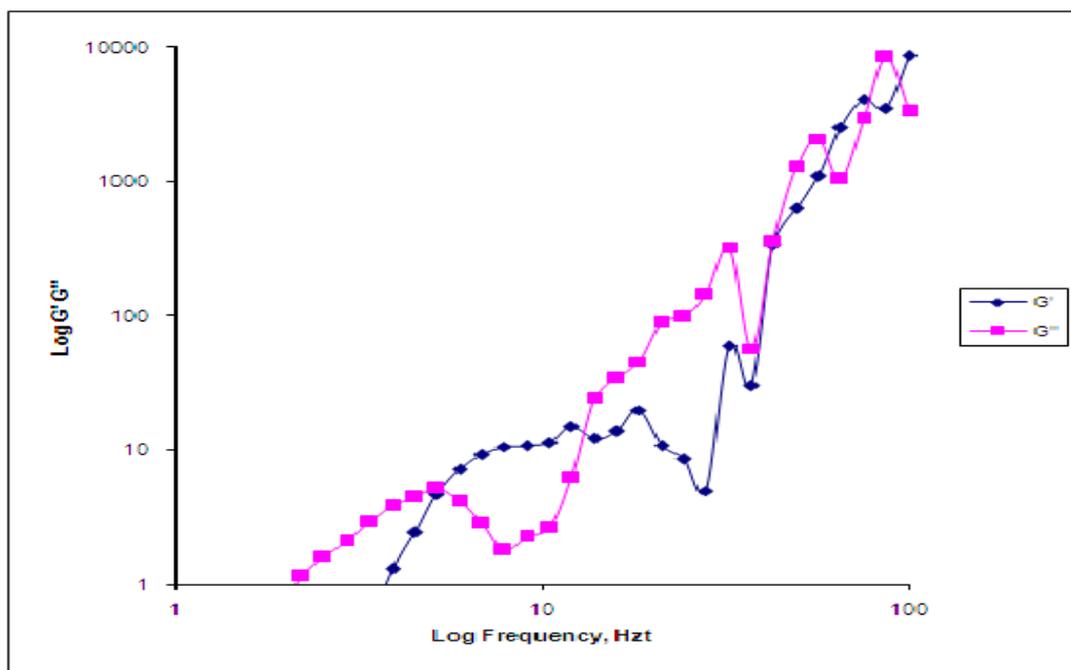


Oscillation stress sweep: The samples were exposed to increasing stress at a constant frequency; at 20°C, 1Hz frequency and different ranges of stresses (0.1 to 100Pa). Fig. 4 shows the changes in G' over applied range of stress. Well interpreted LVR region was observed.

Oscillation frequency Sweep: The samples were exposed to a stepwise of increasing frequency at a constant stress, 0.1–100 Hz frequency range, in the field of linear viscoelasticity. If G'' is higher than G' then sample is viscous and if G' dominates G'' then sample is elastic.

Fig.5 (batch A5) clearly indicates G'' greater than G' at lower frequency, as frequency increased G'' show little decrease and finally G'' dominates G' , indicating viscous nature of system.

Fig.5 - Frequency sweep for G' and G'' (Oscillation frequency Sweep) (Batch A5)

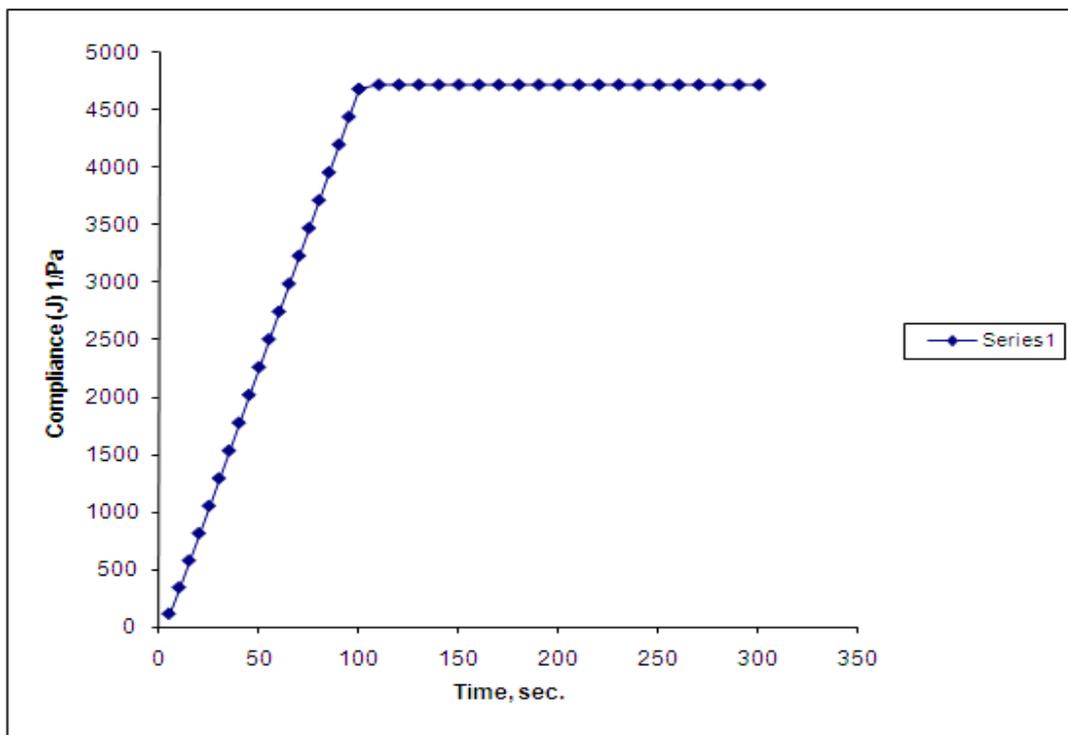


Creep Recovery: Ability of system to regain its original structure after removal of stress is studied. Initially stress of 3.4 Pa for 100 seconds was applied and then removed it completely by giving negligible stress of 0.000001 Pa for 200 seconds. The creep compliance, J (defined as ratio between measured strain and applied stress) was recorded against time. Initially under stress the batch lost their structure up to 100 seconds and was not able to regain their original structure as the graph shows linear region parallel to X-axis. So it can be interpreted from the Fig. 6 of creep recovery that sample have viscous properties and absence of elastic recovery. Thus the absence of recovery component in creep recovery justified thermal gelation ability of both samples as a recovering system will interfere with the structure of the pluronic gel formed at 37°C by virtue of its structure retaining ability.

As previously described, the rheological characterization pointed out an increasing sample viscosity at increasing temperature and on gelation properties of pluronic F 127 and *N. Jatamansi* extract in PEG 400, PEG 4000 and double distilled water. Storage modulus (G'), loss modulus (G'') was monitored. The elastic or storage modulus represents the elastic storage of energy and this is a measure of how well structured a material is. The loss or viscous modulus represents the viscous energy dissipation and it will be large when the sample is predominantly viscous.

The creep-recovery test performed on the gel samples confirms the feature of a viscous system of this sample characterized by absence of elastic component of a system, J (compliance) which is the total recovered elasticity of a system and data obtained from viscometry test performed on the gel samples shows the linearity between the shear rate and shear stress which confirms the Newtonian behavior.

Fig.6- Compliance (J) against Logarithmic Time (Creep Recovery) (Batch A5)



Stability studies:

Stability studies under accelerated condition showed satisfactory results. It can be concluded that gel containing N. jatamansi extract showed good consistency, homogeneity and stability and has wider prospect for nasal preparations (Table 5).

Table no 6- Stability studies

Sr. no.	Batches	Months	Appearance	Color	pH
1	A4	0	Homogeneous	Brownish	4.4
		1	Homogeneous	Brownish	4.4
		2	Homogeneous	Brownish	4.5
		3	Homogeneous	Brownish	4.6

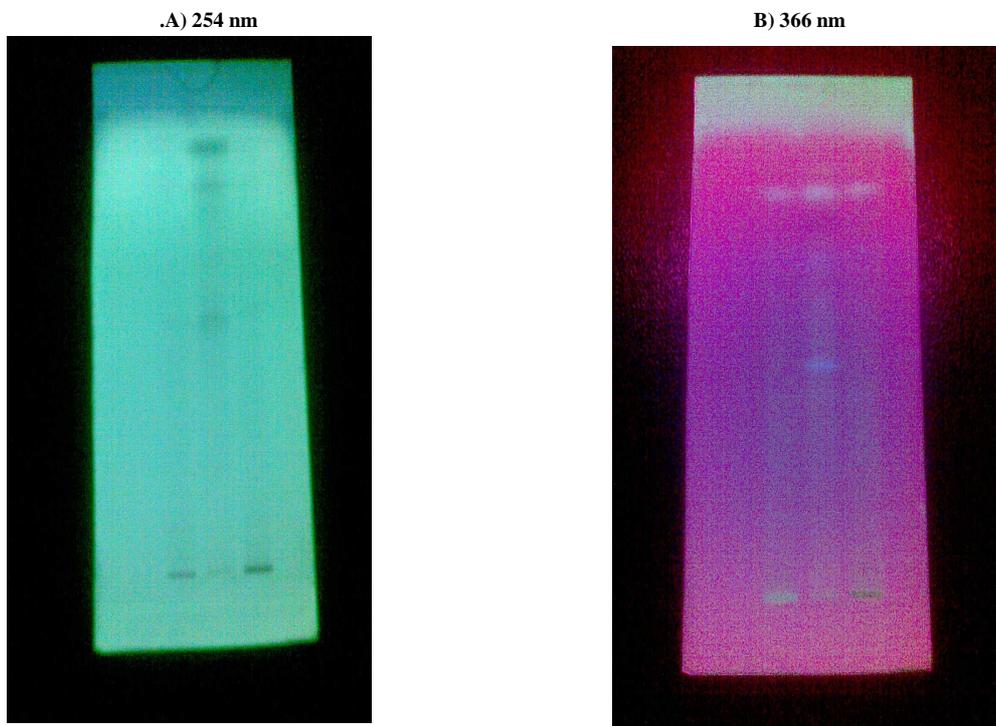
Thin Layer Chromatography:

The observation from the TLC data shows that the proportion of mobile phase as 3:1, Acetone: Petroleum ether shows good separation of the components with R_f values 0.29, 0.62, 0.69 and 0.82.

High Performance Thin Layer Chromatography

Jatamansone being highly soluble in methanol, methanol was selected for preparing its solution. The resolved HPTLC profile of Optimized gel, oil, dried ethanolic extract has been presented in Fig.8.

Fig 7- HPTLC profile of extracted samples of Track 1-Gel extract, Track 2-Oil extract, Track 3-Dried ethanolic extract of *N. jatamansi* developed in solvent system (Acetone: Petroleum ether 3:1 v/v).A) 254 nm B) 366 nm.



The spots with R_f value 0.45 or 0.79 may belong to jatamansone. The spot with R_f value of 0.45 was clearly seen in the HPTLC profile of all the three tracks. It indicates that there may be presence of jatamansone in the ethanolic extract, oil and ultimately in the developed batch of thermoresponsive gel.

CONCLUSION

In situ thermoresponsive nasal gel containing *Nardostachys jatamansi* root extract was successfully prepared by cold method showing the gelation point at the physiological temperature of nasal cavity. Animal studies showed the ethanolic extract was most active among the aqueous and aqueous- alcoholic extract. Rheological studies indicate the viscous property of the developed batch which increases the residence time of the formulation in nasal cavity.

HPTLC profile indicated there may be presence of jatamansone in the formulation but the quantitation was not done due to unavailability of marker. Formulation of jatamansone in nasal gel is needed to reduce the dose and it will help in quantitation. Further EEG studies in humans are needed to carry out to assess sleep depth and duration of the prepared thermoresponsive gel.

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