Innovative Formulation and Development of Nanogels with Clove Oil and Tannic Acid for Oral Antimicrobial Delivery

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ABSTRACT:

The present study aims to formulate and evaluate a nanogel system incorporating Clove oil and Tannic acid, two bioactive compounds known for their potent antimicrobial, anti-inflammatory, and antioxidant properties. The nanogel was prepared by incorporation of Clove oil and Tannic acid into a suitable polymer matrix, i.e. chitosan to enhance their stability, bioavailability, and controlled release. The formulation was characterized in terms of its physicochemical properties, including particle size, pH, viscosity, spreadability, and in vitro release study. This study highlights the potential of Clove oil and Tannic acid-based nanogels for pharmaceutical and cosmetic applications, offering a novel approach for the delivery of natural bioactive agents.

Keywords: Clove oil, Tannic acid, Poloxamer, Chitosan, Nanogel.

INTRODUCTION:

The growing demand for natural, safe, and effective therapeutic agents has led to significant interest in plant-based bioactive compounds for use in pharmaceuticals, cosmetics, and healthcare products. Clove oil, derived from the dried flower buds of the clove tree, and tannic acid, a polyphenolic compound found in various plants, are two such bioactive substances known for their wide range of biological activities. Clove oil is renowned for its antimicrobial, anti-inflammatory, analgesic, and antioxidant properties, making it a valuable ingredient in various therapeutic and cosmetic formulations. Similarly, tannic acid, with its potent antioxidant and antimicrobial properties, has been widely studied for its potential in wound healing, skin care, and as an anti-inflammatory agent. [1-2]

Despite their promising biological effects, the poor solubility, stability, and bioavailability of Clove oil and tannic acid limit their widespread use in topical formulations. To address these challenges, nanotechnology has emerged as a powerful tool for enhancing the delivery and efficacy of bioactive compounds. Nanogels, which are nanoscale hydrogel particles, are particularly attractive for the delivery of such active ingredients. Due to their high surface area, ability to encapsulate both hydrophilic and hydrophobic compounds, and controlled release capabilities, nanogels provide an ideal system for the delivery of bioactive agents like Clove oil and tannic acid.

The formulation of nanogels containing Clove oil and tannic acid could significantly enhance the therapeutic effects of these natural compounds by improving their stability, solubility, and controlled release at the site of action. Furthermore, nanogels can offer improved skin penetration, sustained activity, and reduced irritation, making them an excellent candidate for topical applications. The objective of this study is to develop and evaluate a nanogel system incorporating Clove oil and tannic acid, assessing its physicochemical properties, stability, as well as its potential for therapeutic use in periodontal disease. This work aims to contribute to the development of novel, effective, and safe delivery systems for natural bioactive compounds.

MATERIALS AND METHOD:

Tannic acid and Clove oil was procured from JK chemical, Vapi and Shiva exports, UP respectively. Chitosan and Poloxamer were procured from Himedia and Sigma Aldrich pvt ltd respectively. All the chemicals used in the study were of analytical grade.

Chitosan is dissolved in 10 mL of 0.5% (v/v) acetic acid solution. This acidic medium helps dissolve the chitosan, and constant stirring ensures it dissolves uniformly. A separate solution containing tripolyphosphate (TPP), tannic acid (0.1%), and clove oil (0.75%) is prepared in

10 mL of deionized water. To this solution, add chitosan solution drop wise under constant magnetic stirring for 2 hours. During stirring, the chitosan nanoparticles form due to ionic interactions between chitosan and TPP. The solution becomes turbid, indicating nanoparticle formation. After the formation of nanoparticle, poloxamer is added to the solution that helps to convert the solution of nanoparticle into nanogel. The system is stirred for about 30 minutes to ensure the uniform distribution of poloxamer in the solution. [3]

Experimental design by Design Expert software Version 11

1**. Objective:**

- To assess the influence of three independent variables on two dependent variables in the preparation of a nanogel.

2. Independent Variables (Factors):

- Polymer amount (X1): The amount of polymer (chitosan) used.

- Stirring speed (X2): The speed at which the solution is stirred.

- Sonication time (X3): The duration of sonication applied to the solution.

3. Dependent Variables (Responses):

- Particle size (Y1): The size of the nanoparticles formed in the nanogel.

- Entrapment efficiency (Y2): The amount of material entrapped in nanogel.

4. Design Approach:

- A 3^2-level Central Composite Design (CCD) was chosen for experimentation. This design allows for the study of multiple variables at various levels.

5. Experimental Setup:

- The nanogel samples were prepared according to the experimental design matrix, which was generated using Design-Expert® Software Version 11.

6. Purpose of CCD:

- To systematically analyze how the independent variables interact and affect the response variables, helping to identify optimal conditions for the nanogel formulation.Values of independent variables are in table 1.

Evaluation of nanogel:

- **Particle Size**

The particle size distribution and morphology are the primary determinants of NPcharacterization. Particle size was determined at 25℃ by the photon correlation spectroscopy technique. This analysis measures the particle size of particles suspended inliquids in the range of 0.6 nm to 10µm with sample suspension concentrations from 0.00001to 40%. All the data presented are produced under identical production conditions.

- **Drug content**

The nanoparticles were analyzed for drug content by transferring 1 mL of formulation in 100 mL volumetric flask. In this volumetric flask, 50 mL of phosphate buffer with pH 6.8 was added, followed by continuous shaking until the gel was totally dispersed to give a clear solution. Final volume was adjusted to 100 ml with the help of phosphate buffer saline pH 6.8 and filtered the solution. Drug concentration in filtrated solution was determined

spectrophotometrically at 272nm and 282nm for tannic acid and clove oil (eugenol) respectively using UV-Visiblespectrophotometer.

- **Viscosity**

  - **Prepare the Nanogel Sample**: Ensure the nanogel is homogeneously mixed.
  - **Set up the Brookfield Viscometer**: Attach the appropriate spindle (usually a low viscosity spindle) and set the speed.
  - **Measure the Viscosity**: Immerse the spindle into the nanogel sample and allow the viscometer to run at a constant speed.
  - **Record the Reading**: Note the viscosity value (in cP) displayed on the viscometer. [4]

- **Measurement of pH**

  - **Prepare the Nanogel Sample**: Ensure the nanogel is well-mixed and homogenous.
  - **Calibrate the pH Meter**: Calibrate the digital pH meter with standard buffer solutions (usually pH 4, 7, and 10).
  - **Measure pH**: Immerse the pH electrode into the nanogel sample and allow the reading to stabilize.
  - **Record the pH**: Note the pH value displayed on the pH meter.[5]

- **Spreadability**

  - **Prepare the Nanogel Sample**: Place a small amount of the nanogel onto a glass plate or substrate.
  - The spreadability of the nanogel was evaluated by placing 0.5 g of the gel on a 2 cm diameter circle marked on a glass plate.
  - A second glass plate was then positioned on top, and a 500 g weight was placed on the upper plate for 5 minutes.

- Following this, the diameter of the spread gel was measured.[6]

➢ ***In-vitro*** **drug release study**

The drug release of the formulation was estimated using Franz Diffusion Cell. The diffusion cell consists of receptor and donor compartments and a dialysis membrane was placed between receptor and donor compartments. 1 gram of gel was applied uniformly on the dialysis membrane which is previously soaked in a phosphate buffer saline pH 6.8and the membrane was then fixed to one end of the tube. The entire assembly was positioned so that the lower end of the tube containing the gel touched the surface of the PBS 6.8pH. The setup was placed on the magnetic stirrer and the temperature was maintained at 37°±1°C.6,9 Samples were withdrawn at different time intervals and then analyzed at 272nm and 282nm for tannic acid and clove oil (eugenol) respectivelyusing UV-Visible Spectrophotometer. [7]

➢ **Stability studies**

Drug decomposition or degradation occurs during stability, because of chemical alteration of the active ingredients or due to product instability, lowering the concentration of the drug in the dosage form. The stability of pharmaceutical preparation should be evaluated by accelerated stability studies. Stability studies of periodontal nanogel were carried out to determine the effect of contents on the stability of the drug. The accelerated stability studies were carried out according to ICH guidelines by storing the samples at 40 + 2°C and 75 + 5% RH for 6 month using stability chamber (Remi, India). The optimized formulation was evaluated for clarity, pH measurement, drug content, spreadability, and drug release.

RESULTS AND DISCUSSION

The evaluation of tannic acid and clove oil nanogel formulations revealed notable variations in entrapment efficiency and particle size depending on the formulation parameters. Particle

size ranged from 421 nm to 604 nm, with larger particles typically forming under higher stirring speeds and polymer amounts. Entrapment efficiency ranged from 46.35% to 60.05%, with the highest values observed in formulations with moderate polymer amounts (75 mg), stirring speeds of 1250 rpm, and sonication times of 7.5 to 12 minutes (Runs 2, 3, 8, 9, and 18). Higher polymer amounts (100 mg) generally resulted in lower entrapment efficiencies, particularly at longer sonication times, while lower polymer amounts (50 mg) produced acceptable efficiencies with slightly smaller particle sizes. These results suggest that optimal entrapment efficiency and particle size are achieved when the polymer amount is balanced with stirring speed and sonication time.

The viscosity, pH, and spreadability of the tannic acid and clove oil nanogel formulations showed slight variations across different runs. Viscosity values ranged from 3100 to 3900 cP, with the highest values observed in formulations with higher polymer amounts (e.g., Run 13, 117 mg). The pH of the formulations remained relatively consistent, ranging from 6.35 to 6.64, indicating good stability within the desired pH range for skin application. Spreadability values were also similar, with most formulations showing a spreadability range of 5.4 to 6.2, which suggests that the formulations have good spreadability characteristics suitable for topical use.

Formulations with moderate stirring speeds (1250 rpm) and sonication times (7.5–12 min) tended to show a balanced combination of viscosity, pH, and spreadability, making them optimal for further development. However, formulations with lower polymer amounts (50 mg) tended to have slightly better spreadability but lower viscosity. Overall, these properties suggest that the formulations are well-suited for topical application, with optimal conditions requiring a balance of polymer concentration and processing parameters.

The in-vitro drug release results for tannic acid and clove oil from the nanogel formulations show varying release profiles based on the formulation parameters. For both tannic acid and clove oil, the highest release percentages were observed in formulations with moderate polymer amounts (75 mg), stirring speeds (1250 rpm), and sonication times (7.5–12 min), with a maximum release of approximately 84.45% for tannic acid and 80.33% for clove oil (e.g., Runs 2, 3, 8, 9, and 18). These formulations exhibit a favorable release rate, suggesting optimal encapsulation and the controlled release of both compounds.

In contrast, formulations with lower polymer amounts (e.g., 50 mg) or shorter sonication times (e.g., Run 10) resulted in comparatively lower release rates. For instance, Run 5, with 100 mg polymer and a longer sonication time, showed a significant reduction in the release of both tannic acid (61.56%) and clove oil (57.44%). This might be due to stronger encapsulation that inhibits the drug release. Additionally, the lowest releases were observed for the formulation with the highest polymer concentration (Run 13, 117 mg), with a release of 70.35% for tannic acid and 66.23% for clove oil.

The overall trend suggests that intermediate polymer concentrations, moderate stirring speeds, and optimal sonication times are key factors for maximizing the release of both tannic acid and clove oil. These findings indicate that the formulation's release behavior can be finely tuned by adjusting these processing parameters to achieve the desired drug release rate.

Table 1: Independent variables with their effect on dependent variable along with results of evaluation of Viscosity, pH, spreadability, and *invitro* drug release study on nanogel

Run	Factor:1	Factor:2	Factor:3	Response:1	Response:2	Viscosity	pH	Spreadability	In-vitro drug release	
	A: Polymer Amount (mg)	B: Stirring Speed (rpm)	C: Sonication Time (min)	Entrapment efficiency (%)	Particle Size (nm)				Tannic acid	Clove oil
1	75	1670	7.5	54.2	504	3500	6.35	5.8±0.05	74.35	70.46
2	**75**	**1250**	**7.5**	**60.05**	**604**	**3450**	**6.45**	**5.9±0.80**	**84.45**	**80.33**
3	**75**	**1250**	**7.5**	**60.05**	**604**	**3450**	**6.45**	**5.9±0.80**	**84.45**	**80.33**
4	75	1250	3	56.25	486	3700	6.5	5.6±1.10	72.85	68.73
5	100	1000	10	46.35	425	3700	6.5	5.6±1.10	61.56	57.44
6	100	1500	10	53.55	485	3750	6.52	5.5±1.15	72.25	68.13
7	50	1500	10	54.75	490	3300	6.37	5.9±0.95	75.55	71.43
8	**75**	**1250**	**7.5**	**60.05**	**604**	**3450**	**6.45**	**5.9±0.80**	**84.45**	**80.33**
9	**75**	**1250**	**7.5**	**60.05**	**604**	**3450**	**6.45**	**5.9±0.80**	**84.45**	**80.33**
10	50	1000	5	51.95	470	3250	6.35	6.0±0.80	70.45	66.33
11	100	1000	5	54.05	490	3600	6.45	5.6±0.80	74.76	70.64
12	75	1250	12	53.55	488	3500	6.48	6.0±0.95	73.88	69.76
13	117	1250	7.5	51.6	475	3900	6.52	5.4±0.80	70.35	66.23
14	33	1250	7.5	50.9	468	3100	6.35	6.2±1.15	69.25	65.13
15	50	1000	10	47.3	425	3275	6.4	6.0±0.95	62.22	58.1
16	100	1500	5	46.85	421	3650	6.48	5.6±0.95	67.11	62.99
17	50	1500	5	58.3	515	3275	6.64	6.0±0.95	77.45	73.33
18	**75**	**1250**	**7.5**	**60.05**	**604**	**3450**	**6.45**	**5.9±0.80**	**84.45**	**80.33**
19	**75**	**1250**	**7.5**	**60.05**	**604**	**3450**	**6.45**	**5.9±0.80**	**84.45**	**80.33**
20	75	830	7.5	49.2	465	3515	6.48	5.9±0.95	67.22	63.1

CONCLUSION

Nanogels were formulated using a Central Composite Design (CCD) approach with the Design Expert software, resulting in 20 different formulations, including 6 center points. Polymer concentration, stirring speed, and sonication time were chosen as the independent variables, while entrapment efficiency and particle size were selected as dependent variables. The formulations corresponding to runs 2, 3, 8, 9, 18, and 19, which consisted of 75 mg polymer concentration, a stirring speed of 1250 rpm, and a sonication time of 7.5 minutes, exhibited an optimal entrapment efficiency of 60% and particle size of approximately 604 nm. These formulations also demonstrated favorable rheological properties and excellent in-vitro drug release profiles, with 84% release of tannic acid and 80% release of clove oil. These results suggest that this specific formulation combination offers balanced performance in terms of encapsulation efficiency, particle size, and drug release characteristics.

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