Research Article

Development Of Throat Nano-Spray Formulation For The Treatment Of Streptococcus Pyogenes Tonsillitis

Robin Singh[1], Sanjar Alam[1*], Swamita Arora[1], Yatendra Kumar[1], Nazli Shahin[2], Mohammad Rashid[1]

[1]R.V. Northland Institute, G.B. Nagar, Dadri, Greater Noida. U.P. 203207
[2]Pharmacy Consultant, Dubai
*Correspondence Author
Prof. (Dr) Sanjar Alam
Principal, R. V. Northland Institute
sanjaralam10@gmail.com

ABSTRACT

Background:
Tonsillitis is an inflammatory condition of the tonsillar tissues, commonly caused by Streptococcus pyogenes, leading to pain and difficulty in eating. Conventional treatments such as gargles and ointments often suffer from poor patient compliance due to inconvenience. In contrast, nano oral sprays offer a more convenient and patient-friendly alternative by enhancing drug delivery and retention on the oral mucosa.
Materials & Methods:
Allicin, an active constituent of Allium sativum (garlic), was selected due to its potent antimicrobial properties. It has a molecular weight of 162.28 g/mol and limited water solubility (0.8%). A nanoemulsion-based oral spray was developed using the water titration method and pseudo-ternary phase diagrams. Oleic acid was selected as the oil phase due to its high solubility (200 mg/ml). Various surfactant ratios (1:1, 2:1, 3:1) were optimized. Mucoadhesive potential was evaluated using Gum Ghatti (GG) at different concentrations.
Results:
The optimized nanoemulsion showed a pH of 6.95 ± 0.58, viscosity of ~71 mPas, and refractive index of 1.463 ± 0.35. The particle size was 227.5 nm with a PDI of 0.270, indicating good uniformity. The spray exhibited a discharge rate of 60 ± 1.5 µL per actuation. GG (5% and 10% w/v) demonstrated superior mucoadhesion compared to CMC-Na. Drug release followed the Higuchi model. The nano spray showed a significantly higher antibacterial activity (zone of inhibition: 25.0 ± 1.00 mm) compared to marketed formulations. Stability studies confirmed good physical and thermodynamic stability, with a calculated shelf life of approximately 13.99 months.
Conclusion:
The developed allicin nano oral spray demonstrated enhanced antibacterial efficacy, improved bioavailability, and strong mucoadhesive properties. It provides prolonged residence time, better patient compliance, and reduced side effects, making it a promising alternative for effective tonsillitis management.

Keywords: Tonsillitis, Allicin, Nano spray, Mucoadhesion, S. pyogenes, Nano-formulation.

1. INTRODUCTION

Acute tonsillitis is a prevalent, curable, and potentially severe long-term disease that affects nearly every child in their lifetime. It is primarily managed by pediatricians and primary care doctors and is most prevalent in young children.[1] Bacterial pathogens are the most prevalent etiologic factors, with Streptococcus pyogenes being the most significant. Other bacterial and viral agents can cause acute tonsillar inflammation, but the streptococcus bacterium (S. pyogenes) can also cause this form of tonsillitis.[2]
Recurrent tonsillitis, also known as a throat infection, is more common in young children and can be treated with antibiotics.[3] Surgical removal of the tonsils may be recommended in cases where symptoms are more frequent. The reason why certain children are more susceptible to recurrent tonsillitis is not well understood. To understand their condition, Dr. Shane Crotty's team studied tonsil tissue samples from 86 children with sleep apnea and 66 children with recurrent tonsillitis.[4]

T-follicular helper cells (TFH) in children with recurrent tonsillitis have higher concentrations and fewer B cells, affecting defense-enhancing antibodies against microbes and viruses.[5]

Allicin is a compound found in garlic that helps ease irritation and block loose radicals, which can damage cells and tissues.[6] It is a key ingredient in garlic and is produced by the enzyme alliinase when the clove is minced or crushed. Allicin bioavailability for enteric tablets ranges from 36 to 104% but decreases when consumed with a high-protein meal. Non-enteric tablets significantly increase Allicin bioavailability (80-111%), while garlic powder pills increase it by 26-109%.[7] Allicin has a half-life of 2.5 days at room temperature and has limited solubility in water (0.8%).[8] However, with a bioavailability of around 40%, it is orally active and easily absorbed after oral administration.

Allicin is an effective and well-tolerated drug for treating tonsillitis, available under various brand names like Alli-Max, Garlique, AlliUltra, and Diaba-life. Common adverse effects include stomach upset, bleeding risk, dizziness, bad breath, heartburn, gas, and diarrhea. Its healing efficacy may be reduced when combined with Benzthiazide, Bepridil, Betamethasone, Aripiprazole, Acebutolol, and Acetazolamide. Its effectiveness may be increased or decreased when used in combination with Acetazolamide.[9] Allicin is an anti-microbial, anti-inflammatory, and anti-cancer medication that can be taken in two or three doses of 500 mg + 400 mg each. It is safe for most individuals to use for a short period when administered topically or orally. However, drug interactions can reduce its pharmacological effect, increase the risk of hypoglycemia when combine with Benzthiazide, and increase the risk of hyperglycemia when combined with Betamethasone.

Nanoemulsions are emulsions with droplets ranging from 20 to 200 nanometers, formed when two immiscible liquids are combined.[6][10] These droplets offer several advantages over conventional emulsions, such as enhanced stability, improved optical clarity, and increased bioavailability. They also have higher kinetic stability, making them less likely to separate or coalesce over time.[11] Nanoemulsions are used in various sectors, including medicine, cosmetics, food, and agriculture. In the pharmaceutical industry, Nanoemulsions enable improved absorption and targeted drug delivery, while in skincare, they increase active ingredient distribution and sensory qualities.[12]

In the food sector, Nanoemulsions can encapsulate flavors, enhance bioactive ingredient solubility, and extend product shelf life. In agriculture, Nanoemulsions are used for pesticide delivery and controlled nutrient release. High-energy processes like ultrasonication, high-pressure homogenization, and micro fluidization are often used to create Nanoemulsions.[13] Research and development in Nanoemulsions are ongoing, with new applications and technologies emerging. The average Nanoemulsion droplet diameter is 500 nm, and due to their small droplets, they have a clear or blurrier appearance compared to coarse emulsions.

Nanoemulsions disrupt conventional destabilization processes, resulting in long-lasting physical stability. Kinetic instability caused by gravity or viscosity is compensated by Brownian motion.[14] Nanoemulsions are used to solubilize drugs, shield them from the environment, target specific organs, and circumvent the reticuloendothelial system. Hydrophobic medications are solubilized using Nanoemulsions orally to increase absorption and systemic bioavailability. Nanoemulsions bypass metabolism, increasing bioavailability and reducing hepatic transformation.[15]

They effectively check medicines for metallic or harsh aftertaste, reducing nausea and noncompliance. Surfactant usage is lower for Nanoemulsions compared to traditional colloidal dispersions.[7] A spray formulation is a product or medication delivered through a spray mechanism, typically containing a liquid or solution within a pressurized container or spray bottle. When pressed, the liquid is expelled as a fine mist or spray.[16] These formulations are used for various purposes, including medical treatments, personal care products, and household cleaners. The formulation of a spray product can vary depending on its intended use and desired properties.[17]

Mucoadhesive Drug Delivery System concentrates medication to specific body areas using polymer adhesion capabilities, resulting in longer usage and long-lasting benefits.[18] This biological adhesion occurs when materials are held together using interface forces, including biological ones. Mucoadhesion also occurs between artificial fabrics and organic compounds, involving polymer attachment to mucin layers in mucosal tissue.[19]

The most ideal transportation method for any medicine is an oral route. The following categories of delivery by the oral cavity membranes are possible:

- Sub-lingual delivery: It is the systemically delivery of drugs through the oral mucosal membranes.[20]
- Buccal delivery: The delivery of medication through the buccal mucosa.[21]
- Oral transport: The oral cavity is where the medication is transported.[22]

The buccal mucosa is a crucial channel for managing medication distribution within the mouth mucosal canal. Its wide clear muscle area and immobile mucosa make it ideal for systemic transmucosal pharmaceutical delivery. Sublingual mucosa is more porous, but continuous delivery is preferred for buccal mucosa. A sophisticated mucous adhesive polymeric medication administration system can be created to target specific areas of the gastrointestinal tract, promoting greater absorption and higher drug concentrations.[23] The design of confined targeted delivery depends on factors like pH level, length, area, and enzyme activity.[24]

2. MATERIAL AND METHODS

2.1. Drug & Excipients:

The selection of a drug for oral-throat system formulation involves several parameters, including lipophilicity, molecular weight, potency, non-irritation, low melting point, irreversible binding to stratum corneum tissues, pH, treatment duration, and first-pass metabolism. Allicin was chosen for a 24-hour oral throat Nanoemulsion-based system due to its low molecular weight, melting point, no throat irritation history, stability in neutral and alkaline mediums, and extensive first-pass metabolism. This low water solubility necessitates nano-emulsions to improve solubility and oral mucosal penetrability.

The material used in this study includes tween 80/20, allicin, carbopol, ethanol, octanol, disodium hydrogen phosphate, potassium dihydrogen phosphate, distilled water, water HPLC grade, Gum Ghatti, and PEG 200. All these materials are provided by R.V. Northland Institute Store.

2.2. Preformulation studies:

2.2.1. Identification & marking of drug:

The drug was first screened according to its physicochemical characteristics. The taste, color, and odour were recorded and examined in light of the data that was already available. The use of UV spectrophotometry was carried out as Using the UV-1700 Corporation equipment from Kyoto, Japan, of Brand Shimadzu, the pharmaceutical solution, prepared in a buffer of 0.01% w/v, was exposed to UV scanning within the 200–400 nm range, and the results were properly reported. A mixture of KBr and ALLICIN was pressed in a specified ratio to make pellets for the Fourier transform infrared (FTIR) examination. The spectrum results were recorded.

2.2.2. Solubility studies

The solubility of Allicin was determined in water and phosphate buffer (pH 7.2), since this buffer was used as release media in permeation studies. Three flasks, each containing water and phosphate buffer (pH 7.2), were taken, each carrying an excessive quantity of the medication. Samples incubated at 30°C for 48 hours, centrifuged at 10,000 rpm for 10 min (Jiang et al.) The aliquots of supernatant were filtered through a 0.1µm membrane filter. After appropriate dilutions with Ethanol, solubility of Allicin was determined using UV at 240 nm.

2.2.3. Determination of partition coefficient

The partition coefficient was calculated by dissolving a specific quantity of Allicin in a 5 mL separating funnel of Octanol and Water (1:1) combination. The system was agitated for 30 minutes to achieve equilibrium, and then it was left unattended overnight, separated, and centrifuged for 15 minutes, Allicin content was evaluated by a UV-Visible spectrophotometer, evaluating absorbance at 240 nm. The partition coefficient is frequently measured using the shaking flask method and computed using the following formula:

$P (o/w) = C_1 (oil)/ C_2 (water)$

Where, C_1 (oil) = Conc. of solute in organic phase.

C_2 (water) = Conc. of solute in aqueous phase.

P (o/w) = Partition coefficient, Log P = log (o/w)

2.2.4. Analytical Methodology

HPLC analysis of Allicin

a. **Equipment-** Shimadzu HPLC with an UV detector
b. **Column-** Phenomenex C_{18} reverse – phase column (particle size 5 micrometer, 25cm * 4.6mm)

c. **Mobile phase-** Acetonitrile: Water: Ethanol (50: 41: 9)
d. **Flow rate-** 1 ml/min.
e. **Detector-** 240nm, UV detector
f. **Syringe-** 20 micro liter
g. **Run time-** 10 minutes
h. **Retention time-** 3.717 ± 0.1 min.

The quantitative analysis of Allicin was performed using High Performance Liquid Chromatography (HPLC). The chromatographic system consisted of a Shimadzu HPLC system equipped with a UV-Visible detector.

Chromatographic separation was achieved on a Phenomenex C18 reverse-phase column (250 mm × 4.6 mm, 5 µm particle size). The mobile phase comprised of Acetonitrile: Water: Ethanol in the ratio of 50:41:9 v/v/v. The mobile phase was filtered through a 0.45 µm membrane filter and degassed by sonication prior to use.

The flow rate was maintained at 1.0 mL/min, and the injection volume was 20 µL. The detection wavelength was set at 240 nm, which corresponds to the maximum absorbance (λmax) of Allicin. The total run time for each analysis was 10 minutes.

Under these conditions, Allicin exhibited a retention time of 3.717 ± 0.1 minutes, allowing for clear resolution and quantification. Standard and sample solutions were prepared in the mobile phase, filtered using 0.45 µm syringe filters, and analyzed in triplicate to ensure repeatability.

This method provided a reliable and reproducible means for the identification and quantification of Allicin in formulation matrices or biological samples.

2.2.5. DSC (Differential Scanning Colorimetry) of Allicin

To look for potential physical changes in allicin during spray congealing, a DSC analysis was done. To detect possible changes throughout the process, the extract was combined with a melted carrier to create a 5% weight/weight extract combination. The DSC curve of allicin exhibited several endothermal processes about 50 °C and 80-120 °C. The presence of volatile chemicals might explain the large peak at 50 °C. Endothermic peaks at 80 and 120 °C signify the extract's components melting in order.

2.3. Method of Preparation of Nanospray formulation:

2.3.1. Development of optimised formulation:

Excipients Screening The solubility of poorly soluble pharmaceuticals is essential for the evaluation of components in oils, surfactants, and cosurfactants. Evaluation of oil for Nanoemulsion. The solubility of allicin in several oils, including oleic acid, olive oil, isopropyl myristate, castor oil, jojoba oil, babachi oil, labrafac oil, and soybean oil, was evaluated using cremophore EL. Two milliliters of oil were poured in vials, with an excess of medication added. Vials were sealed, agitated, then centrifuged at 10,000 rpm for a duration of 10 minutes. (Azeem et al., 2009) Following suitable dilution with ethanol, the resultant solution was isolated, filtered, and solubility was evaluated.

Drug solubility, phase behaviour, and Nanoemulsion area were used to determine which formulations from the Nanoemulsion area should be introduced into the oil phase. These formulations were selected based on the drug's effect on phase behaviour and the use of minimal amounts of co- and surfactants.

Maximum area is seen in the pseudo ternary phase diagrams. A variety of Nanoemulsion with diverse formulae were chosen in the ratios of 1:1, 2:1, and 3:1. Phase diagrams' range of Nanoemulsion occurrence was studied, and oil compositions with less surfactant were chosen. A translucent liquid was created by taking the oil phase, adding surfactant, and then adding water. The sealed Nanoemulsion were kept at room temperature for storage.

Pseudo ternary Phase Diagram Development

Initial phase involved oleic acid as an oil phase, Tween 80 as a surfactant, and carbitol as a cosurfactant. Distilled water was used as an aqueous phase. Pseudoternary phase diagrams were created using the water titration methodology. Five different weight ratios (1:0, 1:1, 1:2, 1:3, and 1:4) were used to combine the surfactant and cosurfactant. 14 different ratios of oil and Smix (1:9, 1:8, 1:7, 1:6, 1:5, 6:4, 1:3, 3:7, 1:2, 4:6, 5:5, 6:4, 8:2, and 9:1) were created. Slow titration with aqueous phase was carried out for each weight ratio. Visual observations were made for each aqueous phase addition to the oil. The physical condition was depicted on a fictitious three-component phase diagram. Simple observations and a pseudo-ternary phase diagram were tabulated. Distinct figures were made to plot Nanoemulsion points for formulation development.

2.3.2. Testing of placebo Nanoemulsion for thermodynamic stability

Investigations into the thermodynamic stability of placebo Nanoemulsion were done in order to identify stable ones and weed out unstable or metastable ones. (43)

Freeze thaw cycle

Placebo nanoemulsions was stored in a deep freezer (-20^{O} C) for 24 hours. The nanoemulsions were removed after 24 hours and stored at room temperature. Within 2-3 minutes, the thermodynamically stable nanoemulsions were able to regain their original form. Similar cycles were repeated several times.

Fig. 1: Freeze thaw cycle application over formulations

Centrifugation studies

Placebo Nanoemulsion were centrifuged for 10 minutes at 10,000 rpm after undergoing a freeze-thaw cycle. There was no phase separation or turbidity in the stable formulations.

2.3.3. Selection of Allicin drug concentration for Nanoemulsion formulation

We conducted few preliminary trails by selecting concentration range from 2 mg to 20mg. It was found that the permeability of Allicin at 10 mg was similar to that at 20 mg, below which the permeability started getting decreasing. Therefore, 10 mg was selected as the Allicin concentration to be loaded in the Nanoemulsion formulation.

2.3.4. Drug loaded formulation of Nanoemulsions

To create medication-loaded nanoemulsions, 10 mg of Allicin was dissolved in the oil phase. The required amount of surfactant was added, and then water was added drop by drop until a clear and transparent liquid was created, much like with placebo nanoemulsion formulations. The nanoemulsions were stored at room temperature and hermetically sealed.

2.3.5. Drug-loaded Nanoemulsions undergo thermo-dynamic stability testing.

To ensure the drug-loaded Nanoemulsions remain stable after loading, they were submitted to freeze-thaw cycles and centrifugation tests. Over time, physical stability was regularly assessed. At room temperature, several features such as phase separation, turbidity were noticed.

2.4. Methods of Characterization of Nanospray formulation:

2.4.1. Determination of Particle size

Droplet size distribution of the nanospray was determined by photon correlation spectroscopy, which analyzes the fluctuations in light scattering due to Brownian motion of the particles, using a Zetasizer 1000 HS (Malvern Instruments, UK). Light scattering was monitored at 25°C at a 90° angle.

2.4.2. Determination of Surface morphology by Transmission electron microscopy

Morphology and structure of the nanospray were studied using transmission electron microscopy (TEM; Morgagni 268D SEI, USA) operating at 200 KV and capable of a 0.18 nm point to point resolution. Combination of bright field imaging at increasing magnification and of diffraction modes was used to reveal the form and size of nanospray

droplets. In order to perform the TEM observations, a drop of the nanospray was directly deposited on the holey film grid and observed after drying.[30]

2.4.3. Zeta potential

Malvern Zetasizer device is used to measure it. Zeta potential is measured by diluting nano emulsion and extrapolating its value from the electrophoretic mobility of oil droplets. It is thought that a zeta potential of 30 mV is adequate to guarantee the physical stability of a nano emulsion.

2.4.4. Refractive Index –

Refractive index of the nano emulsion can be determined by Abbes type refractometer at 25±0.5° by placing a drop of nano emulsion on slide and comparing it with refractive index of water (1.333).

2.4.5. Viscosity Determination-

Measurement of viscosity by Brookfield DV III ultra V6.o RV co-nano emulsion and plate rheometer (Brookfield Engineering Laboratories, Inc., Middleboro, MA) is used to measure it at a spindle temperature of 25±0.5^{0}c. Rheocalc V2.6 was the programme utilised for the computation**.**

Capillary viscometers determine viscosity by measuring the flow rate of a fluid via a small-diameter capillary while being affected by gravity or an applied pressure to determine the viscosity. The time it takes for the fluid to flow through the capillary is monitored, and viscosity is estimated using the capillary size and fluid characteristics. Viscometers that measure viscosity by watching the drop of a sphere through a fluid are known as falling ball viscometers. The ball is launched from a certain height, and its final velocity is measured. The viscosity of a fluid is defined by its qualities, the size of the ball, and the time required for the ball to descend a specific distance.

2.4.6. pH –

A digital pH meter (Elico) was used to test the formulation's apparent pH in triplicate at 25±10c.

Dilution test by diluting a nano emulsion with oil or water, this kind may be demonstrated.

2.4.7. Weight Checking

It is accomplished by periodically adding to the filling line tared empty containers, which after being filled are accurately weighted and no or little deviation in weight was examined on each simultaneous filling.

2.4.8. Discharge Rate

Discharge rate of oral spray was found to be 60± 3.5 microliters per one push.

2.5. Ex-vitro oral mucosa permeation study

Ex vivo oral mucosa permeation studies were performed on a modified Keshary Chien-diffusion cell with an effective diffusion area of 3.0 cm^2 and 20 mL of receiver chamber capacity, using goat mucosa. The full thickness of goat mucosal membrane was excised from the mucosal region. The subcutaneous tissue was removed surgically and the dermis side was wiped with isopropyl alcohol to remove adhering fat. The cleaned mucosal membrane washed with distilled water and stored at 21ºC until further use.[21]

2.6. In vitro kinetics analysis of Nanoemulsion.

The drug release study utilized the dialysis membrane diffusion technique, a USP apparatus, and a release medium. The drug-loaded formulation was placed inside a pre-soaked membrane, immersed in phosphate buffer saline (PBS) at 37°C with continuous stirring. Samples were analyzed spectrophotometrically or via HPLC to determine drug concentration. The cumulative percentage of drug release was calculated and plotted against time. The release data were fitted into various kinetic models to evaluate the release mechanism and rate constants. The study aimed to determine the release mechanism and rate constants. The in vitro drug release data were fitted into various kinetic models to determine the release mechanism and order of the reaction, including zero-order, first-order, Higuchi, and Korsmeyer–Peppas models.

2.7. Determination of adhesive strength using different adhesives.

A 45mm-diameter by 25mm-deep container was filled with a 30 millilitre sample of the solution. An oral mucosa-like surface was created using a 20mm Teflon plunger. The sample solution and plunger surface made contact after the plunger was lowered to a depth of 1mm and raised at a rate of 1mm/s. When the plunger disengaged from the surface of the sample solution, the adhesive force was gauged. In-vitro release kinetic study.

2.8. Determination of Antimicrobial activity

Disc diffusion technique was selected for antimicrobial study. These tests were carried out using cultures of S. Pyogenes in Sabouraud-dextrose agar media. An amount of 15 ml of the media with 24-hour subculture of S.

Pyogenes was distributed in each petridish (10-cm diameters) and allowed to solidify. On solidification, microbial suspension was spread with the help of sterilized cotton swab on the surface of the media. The filter paper disc was prepared from Whatman's filter paper, which then impregnated with drugs having allicin in Nanospray. Zone of inhibition diameter (mm) were then recorded for the test and control (28˚C and 18–24 hours). [31] The study was carried out in an aseptic area. The experiment was performed in sterilized condition.

2.9. Stability Study:

To evaluate the stability of the Nanoemulsion spray, samples were stored at different conditions—refrigerator (4°C), room temperature (25°C), and elevated temperature (40°C ± 2°C)—for up to 3 months. At regular intervals (0, 15, 30, 60, and 90 days), physical parameters such as appearance, phase separation, globule size, PDI, pH, and viscosity were observed. Centrifugation and freeze-thaw cycles were also performed to assess thermodynamic stability. No significant change in these parameters indicated good physical and formulation stability. For shelf-life prediction, the degradation data (e.g., droplet size or drug content) was analyzed using Arrhenius kinetics, and the shelf life was extrapolated based on the time required for 10% degradation (t90). A stable formulation showing minimal change under accelerated conditions was considered to have a shelf life of at least 2 years under recommended storage conditions.

3. RESULT AND DISCUSSION

The drug (API) was characterized based on a monograph. The allicin is usually in the garlic bud. Allicin is a chemical constituent of garlic (Allium Sativum) with various biological properties.

Molecular Weight: 162.28 g/mol

Molecular Formula: $C_6H_{10}OS_2$

Color: Pale yellow liquid or a colorless

Melting Point: 24-27 °C (75-81 °F)

Physical appearance: Powder

Oduor: Strong pungent garlic-like odor.[25]

3.1. Pre-formulation studies

3.1.1. Solubility studies

Table 3.1: **Solubility Profile of Drug & Polymer**

S. No	Sample compound	Freely Soluble	Slightly Soluble	Very Slightly Soluble	Insoluble
1.	Allicin	Methanol, Ethanol	Ether, Acetone	Water	----------
2.	Carbopol 934	Water	----------	----------	
3.	Gum Ghatti	Water	Methanol	Alcohol	Ethyl alcohol

Table 3.2: Allicin's solubility in various oils, surfactants, and co-surfactants

S.NO.	Solvents	24 hrs	48hrs
1	Water	Soluble	
2	0.1N HCl	Soluble	
3	7.2 pH Phosphate Buffer	Soluble	
4	Span 20	Insoluble	Soluble
5	Tween 20	Insoluble	Soluble
7	Oleic acid	Soluble	
8	Olive oil	Insoluble	Soluble

9	Glycerol	Insoluble	Insoluble
10	Cween 80	Insoluble	Soluble
11	Ethanol	Soluble	

The results of the aforesaid investigation showed that Allicin medication was essentially insoluble in the release study medium, 7.2 pH phosphate buffer. As a result, the addition of a cosolvent was made necessary. After evaluating the oils for allicin solubility, it was discovered that oleic acid has the highest solubility. As a result, oleic acid was selected as the oil phase. Another advantage of combining oleic acid with Cween 80 is that it has been recognized as a potent booster for buccal mucosa distribution.

Table 3.3: Allicin's solubility in various oils mg/ml.

S.NO	Oils	Solubility (mg/ml)
1	Oleic acid	200
2	Isopropyl myristate (IPM)	3
3	Olive oil	4
4	Triacetin	0.4
5	Castor oil	0.01
6	Labrafac	0.8
7	Soyabean oil	0.5
8	Coconut Oil	0.04
9	Sefsol	0.4

3.1.1.1. Partition coefficient

Table 3.4: Allicin's partition coefficient in octanol/buffer.

Total amount of drug (µg)	Amount in organic phase(µg)	Amount in aqueous phase(µg)	Partition Coefficient
25000	24992.65	3.1205	(logP=1.545)

3.1.2. UV Spectroscopy Standard Calibration Curves

The maximum was measured at 240 nm. The standard calibration curve for Allicin was developed using the regression coefficient values of phosphate buffer (R^2 = 0.994), HCl (R^2 = 0.996), and water (R^2 = 0.9972). Within the concentration range of 3-6 g/ml of allicin, the relationship between drug concentration and absorbance is linear, and the curve obeys Beer-Lambert's law. This calibration curve was used to calculate entrapment efficiency and in vitro drug release as shown in Figure 2 & 3.

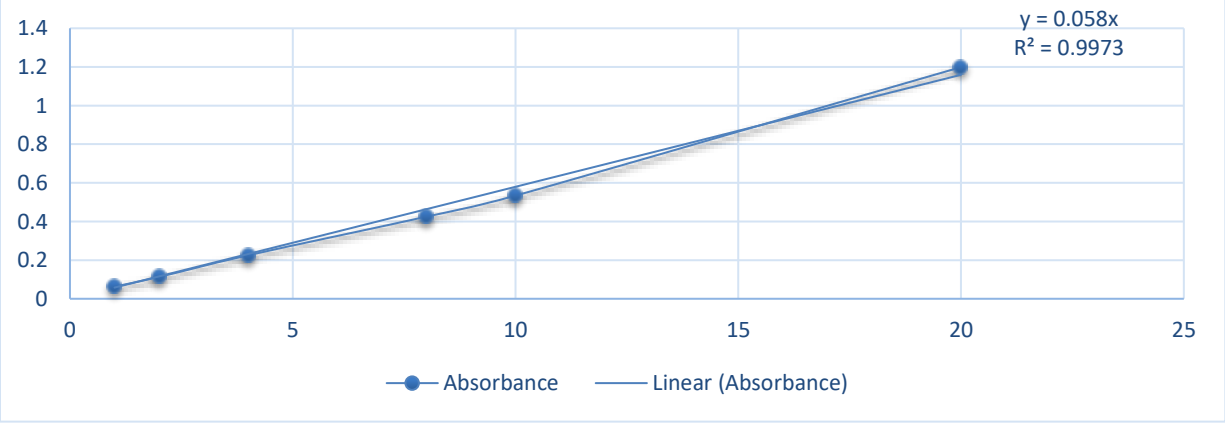


Fig. 2: Calibration plot of allicin in 7.2 pH phosphate buffer (UV)

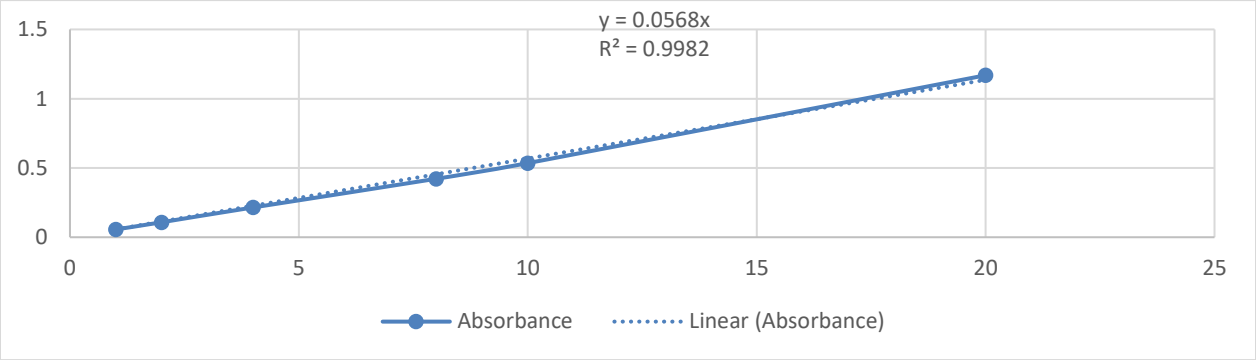
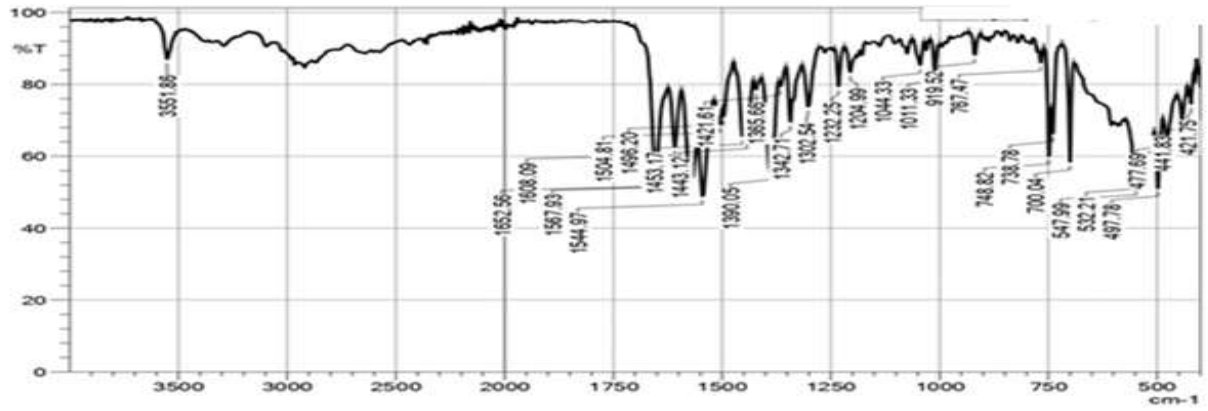


Fig. 3: Calibration plot of allicin in 0.1N HCl (UV)

3.1.3. Fourier Transform Infrared Spectroscopy (FTIR)

The assay results were found satisfactory with the percentage recovery ranging from 99.93 % to 99.99 % (mean 99.96) indicating that the drug was undecomposed and stable in the formulation, as shown in figure 4.



	Item	Value
2	Sample name	
3	Sample ID	
4	Option	
5	Intensity Mode	%Transmittance
6	Apodization	Happ-Genzel
9	No. of Scans	25
10	Resolution	4 cm-1

	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Comment
1	421.75	74.59	4.82	424.62	416.91	187.917	19.111	
2	441.83	70.24	8.23	453.31	430.35	578.188	83.970	
3	477.69	65.19	7.22	486.30	459.05	619.761	95.037	
4	497.78	51.17	17.40	506.38	486.30	758.443	132.463	
5	532.21	53.34	2.77	535.07	506.38	1184.476	63.648	
6	547.99	51.46	4.15	569.50	543.68	1026.229	29.293	
7	700.04	58.47	29.28	718.69	691.44	512.501	204.937	
8	738.78	66.38	10.22	743.08	718.69	416.662	-12.002	
9	748.82	60.06	16.98	760.29	743.08	411.063	98.469	
10	767.47	86.05	3.61	771.77	760.29	134.208	16.874	
11	1011.33	88.21	6.36	933.87	905.18	217.857	61.872	
12	1011.33	83.40	8.69	1021.38	999.86	237.565	66.498	
13	1044.33	85.37	6.88	1062.98	1035.72	295.320	68.208	
14	1204.99	83.42	6.15	1217.90	1187.78	389.163	70.502	
15	1232.25	79.48	10.70	1240.86	1217.90	313.681	89.845	
16	1302.54	73.81	13.33	1315.45	1278.15	666.723	215.110	
17	1342.71	69.55	9.66	1352.75	1336.97	376.102	67.716	
18	1365.66	79.44	2.55	1369.96	1352.75	314.435	21.708	
19	1390.05	55.71	25.80	1408.70	1369.96	1213.513	496.687	
20	1421.61	78.54	2.93	1427.34	1408.70	365.714	22.957	
21	1443.12	64.37	7.21	1448.86	1427.34	614.341	68.824	
22	1453.17	65.58	6.49	1464.64	1448.86	435.259	47.269	
23	1496.20	71.04	3.73	1499.07	1473.25	526.839	6.375	
24	1504.81	68.86	5.25	1517.72	1499.07	525.955	52.054	
25	1544.97	48.88	19.64	1557.88	1517.72	1510.808	323.858	
26	1567.93	53.89	14.89	1593.75	1557.88	1329.350	315.379	
27	1608.09	62.64	16.15	1623.87	1593.75	852.668	214.342	
28	1652.56	53.87	27.61	1699.90	1635.35	1331.914	436.761	
29	3551.86	86.99	8.25	3606.37	3517.43	645.510	262.741	

Fig. 4: FTIR spectra of Allicin

3.1.4. HPLC (High Performance Liquid Chromatography)

Based on the solubility profile of the drug, co-solvent in the release medium along with phosphate buffer (pH 7.2). The drug exhibited λ_{max} in the 240 nm range in the release medium. The intrinsic stability of drug in release medium showed that drug was stable in all the solutions as no shift in λ_{max} or significant change in absorbance was observed. Therefore, the drug samples were stored at room temperature. Table 3.5. HPLC method was used for analyzing the samples of in vitro studies and for assay of drug as shown in figure 5.

Table 3.5: Area- concentration values.

Concentration	Area
1	5408299
2	10571320
4	27742676
8	48613388
10	59486856
20	112404364

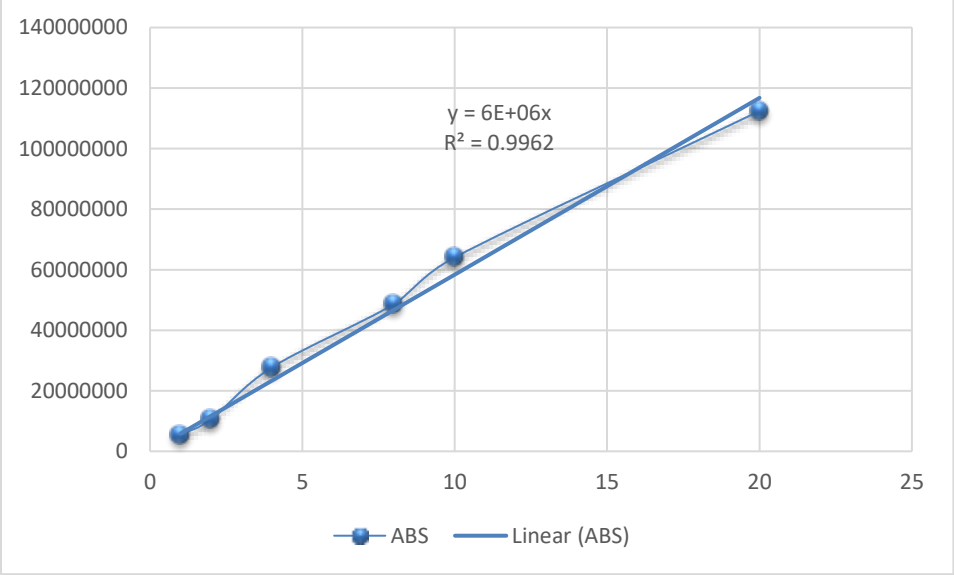


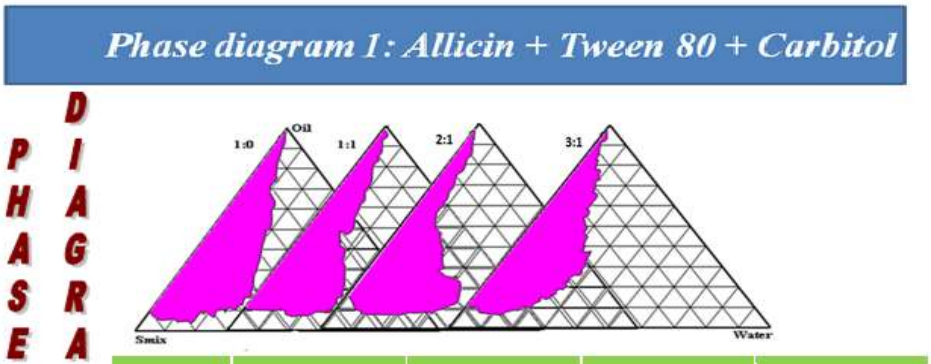
Fig. 5: HPLC Calibration curve of Allicin

3.2. Preparation of spray Nanoemulsion

3.2.1. Formulation & selection of placebo Nanoemulsion

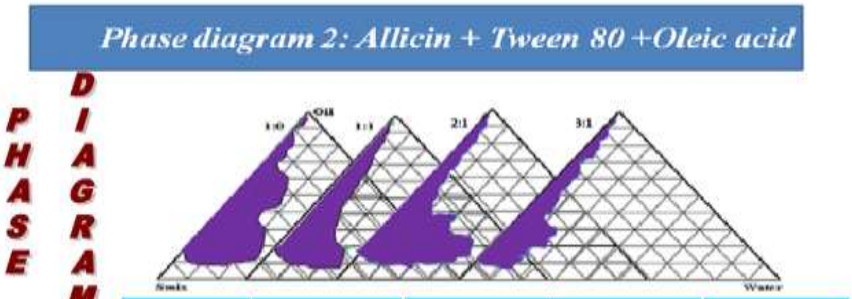
The maximum area is seen in the pseudo-ternary phase diagrams. A variety of nano-emulsions with diverse formulae were chosen in the ratios of 1:1, 2:1, and 3:1. Phase diagrams' range of nanoemulsion occurrence was studied, and oil compositions with less surfactant were chosen. A translucent liquid was created by taking the oil phase, adding

surfactant, and then adding water. The sealed nano-emulsions were kept at room temperature for storage. These formulations were selected based on the drug's effect on phase behaviour and the use of minimal amounts of surfactant and co-surfactants as shown in Figure 6 & 7.



Ratios	1:0	1:1	2:1	3:1
Area (Sq. cm)	29.46+0.72	32.82+2.17	37.25+2.19	32.96+1.71

Fig. 6: Phase diagram of Allicin + Tween 80 + Carbitol



Ratios	1:0	1:1	2:1	3:1
Area(Sq. cm)	31.55±1.41	30.34±0.82	33.05±1.02	21.34±1.47

Fig. 7: Phase diagram of Allicin + Tween 80 + Oleic acid

3.2.2. Drug loaded formulation of Nanoemulsions

To create medication-loaded Nanoemulsions, 10 mg of Allicin was dissolved in the oil phase. The required amount of surfactant was added, and then water was added drop by drop until a clear and transparent liquid was created, much like with placebo Nanoemulsion formulations table 3.6,3 .7, 3.8. The Nanoemulsions were stored at room temperature and hermetically sealed.

Table 3.6: Nanoemulsions with 1:1 S/CoS ratio composition.

Nanoemulsion	Oleic acid (% v/v)	Tween 80+ Carbitol	Distilled water (%v/v)	Allicin (mg) 1%w/v

		(% v/v)		
NE1	2	18	80	10
NE2	4	36	60	10
NE3	6	42	52	10
NE4	8	48	44	10
NE5	10	50	40	10
NE6	15	60	25	10

Table 3.7: Nano-emulsions with 2:1 S/CoS ratio composition.

Nanoemulsion	Oleic acid (%v/v)	Tween 80+ carbitol (%v/v)	Distilled water (%v/v)	Allicin (mg) 1%w/v
NE1	2	18	80	10
NE2	4	36	60	10
NE3	6	42	52	10
NE4	8	48	44	10
NE5	10	50	40	10
NE6	15	60	25	10

Table 3.8: Nanoemulsions with 3:1 S/CoS ratio composition.

Nanoemulsion	Oleic acid (%v/v)	Tween 80 (%v/v)	Distilled water (%v/v)	Allicin (mg) 1%(w/v)
NE1	2	18	80	10
NE2	4	36	60	10
NE3	6	42	52	10
NE4	8	48	44	10
NE5	10	50	40	10
NE6	15	60	25	10

3.3. CHARACTERIZATION OF OPTIMIZED NANOEMULSION

3.3.1. pH: pH was found to be 6.95±0.58 by using a pH meter.

3.3.2. Viscosity:

The viscosity of the resulting Nanoemulsion is measured by a concentric cylinder Brookfield viscometer having a viscosity of 82 centipoises. Viscosity of the developed spray was about 70.9 ± 3.58 mPas at 25°C.

3.3.3. Refractive Index

The refractive index was found to be 1.463 ± 0.35 by using Abbe's refractometer at 25ºC.

3.3.4. Surface Morphology

Transmission electron microscopic (TEM) analysis was used to establish the morphology of the dispersed phase. On a copper grid with a mesh size of 300, a diluted Nanoemulsion was applied, dried for one minute, and then inverted

for ten seconds. PTA was applied, and any leftover PTA was filtered out using paper. The grid was then examined between 60 and 80 KV at a magnification of 1550x. The TEM images are shown in Figure 8.

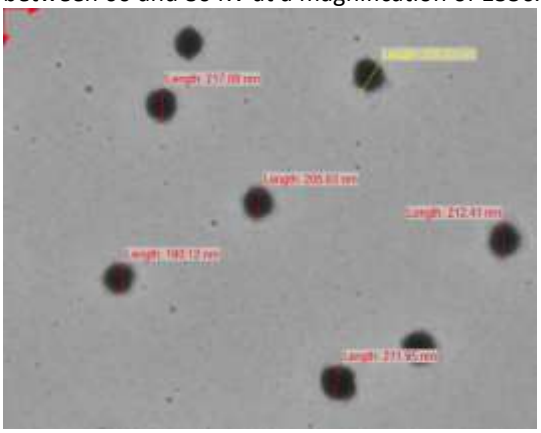


Fig. 8: TEM images of optimized Nanospray

Droplet Size:

Fig 9: Particle size analysis of optimized Nanospray

Dynamic light scattering (DLS) analysis showed that the Nanoemulsion spray had a Z-average particle size of 227.5 nm with a polydispersity index (PDI) of 0.270**,** indicating a moderately uniform droplet distribution. The major intensity peak was observed at **192.5 nm**, contributing to **100% intensity**, suggesting monodispersity. The result quality was marked as 'Good'**,** and the intercept value was 0.903, supporting reliable measurement. The size distribution graph further confirmed a narrow and sharp peak, consistent with stable and well-dispersed nanoemulsion droplets, shown in Figure 9.

3.4. Characterization of the developed oral nano spray

3.4.1. Viscosity measurement

Viscosity of the developed spray was about 71.1± 2.3 mPas at 25°C.

3.4.2. Weight Checking

It is accomplished by periodically adding to the filling line tared empty containers, which after being filled are accurately weighted and no or little deviation in weight was examined on each simultaneous filling shown in table 3.9.

Table 3.9: Weight variation after spray

S.No.	Container weight of 5 ml	Before spray	After spray
1	22.731	5000µl	49940µl
2	22.731	5000µl	49939µl
3	22.731	5000µl	49941µl

3.4.3. Leak Testing

A mean of checking the crimping of the valve was closely done to prevent defective containers due to leakage. None of the container showed any leakage when they undergo dye leakage test as shown in figure in 10 & 11.

Fig. 10: Sample immersed in dye

Fig. 11: Clear solution free from leakage

3.4.4. Density

A pycnometer is a specialized glass container used to measure the density of liquids or fine powders. The empty pycnometer is weighed, then filled with the substance whose density is to be determined. After weighing the filled pycnometer, the density of a material is calculated by dividing the difference in mass by the pycnometer's volume. For drug density, a 50-cm^3 pycnometer was used. The following equation (R^2 = 0.994) provided the best match for the collected density data: r = 1.1109 0.0010 T, where r represents density (g/cm^3) and T represents temperature (C).

3.4.5. Discharge Rate

Discharge rate of oral spray was found to be 60 ± 1.5 microliters per one push.

3.4.6. Determination of the sample solution's adhesive strength using different adhesives

The ability of GG solution to adhere to oral mucosa was evaluated using a creep meter. A 1% w/v CMC-Na solution was used as the control solution. In comparison to CMC-Na solution, the adhesive force of 5 and 10% w/v GG solutions was higher. However, the adhesive force remained constant between 5 and 10% w/v GG. As a result,

Spray in this investigation was based on a addition of 5% GG solution which shows better mucoadhesive strength as shown in figure 12.

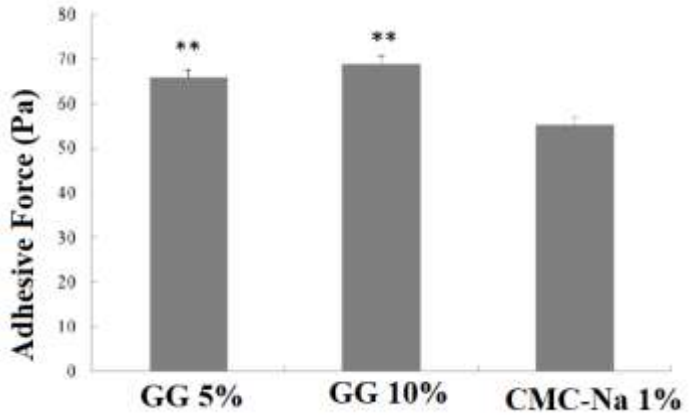


Fig 12: Adhesive strength of Gum Ghatti

3.4.7. In Vitro Release Kinetics Study

Kinetic analysis of the in vitro release profile of the optimized allicin nanospray was done to ascertain release order shown in Tables 3.10 & 3.11.

Table 3.10: Kinetics analysis of allicin-loaded Nanospray

Time (min)	Square root of time	Log time	Percentage of drug release	Fraction drug release	Log percentage of drug release	Percentage of drug remaining	Log percentage of drug remaining
0	0	0	0	0	0	100	2
10	3.162278	1	5.4	0.054	0.732394	94.6	1.975891
20	4.472136	1.30103	16.2	0.162	1.209515	83.8	1.923244
30	5.477226	1.477121	26.8	0.268	1.428135	73.2	1.864511
60	7.745967	1.778151	35.1	0.351	1.545307	64.9	1.812245
120	10.95445	2.079181	47.4	0.474	1.675778	52.6	1.720986
240	15.49193	2.380211	66.2	0.662	1.820858	33.8	1.528917
360	18.97367	2.556303	78.3	0.783	1.893762	21.7	1.33646
480	21.9089	2.681241	83.1	0.831	1.919601	16.9	1.227887
720	26.83282	2.857332	**89.1**	0.891	1.949878	10.9	1.037426

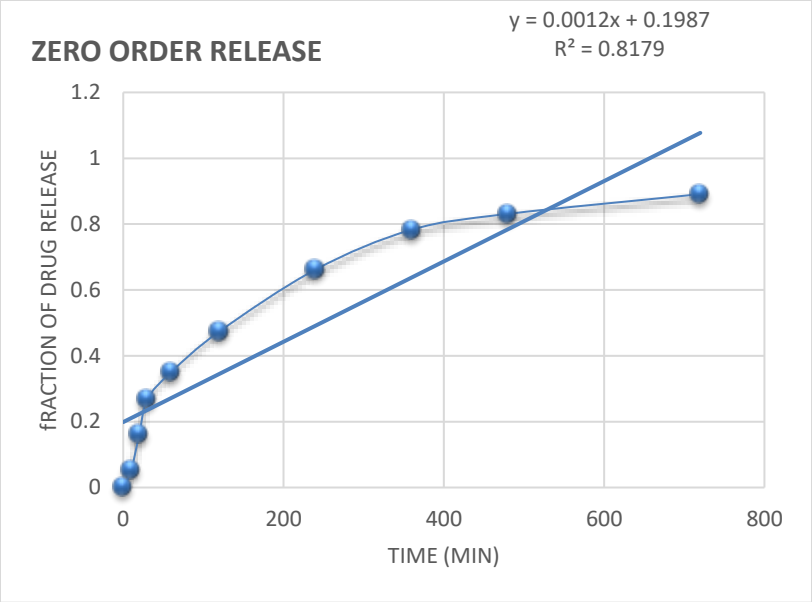


Fig. 13: The kinetics of zero order release of an optimised formulation

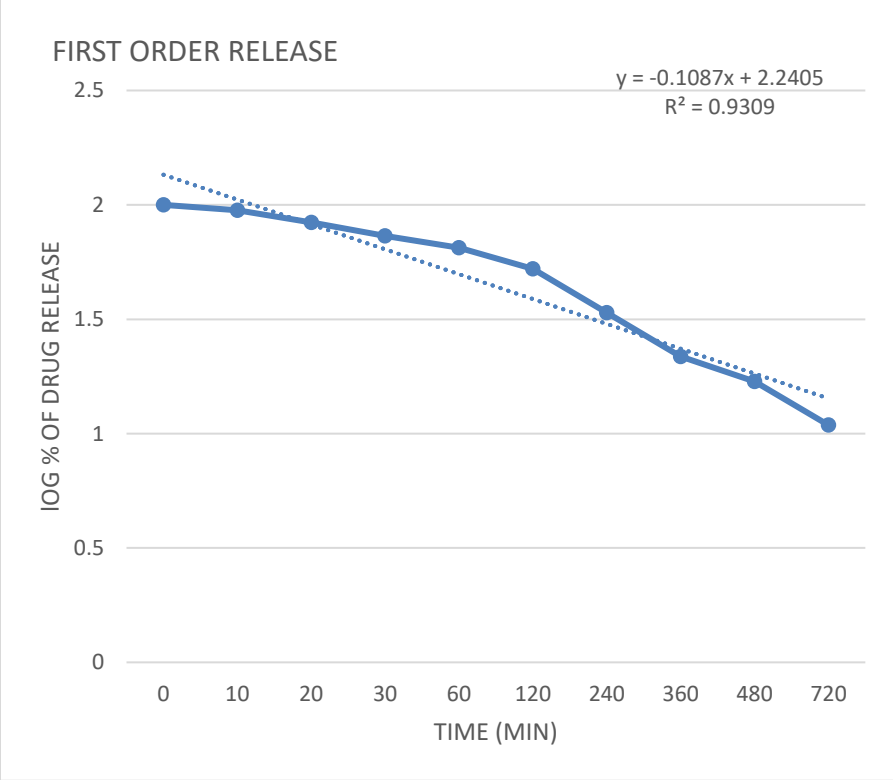


Fig. 14: The kinetics of first order release of an optimised formulation

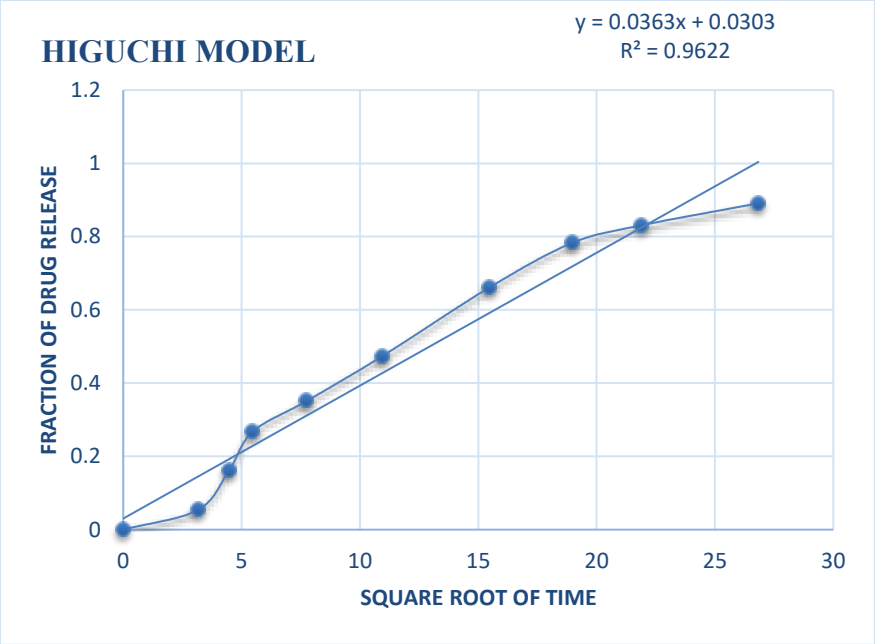


Fig. 15: Higuchi's plot of optimized formulation

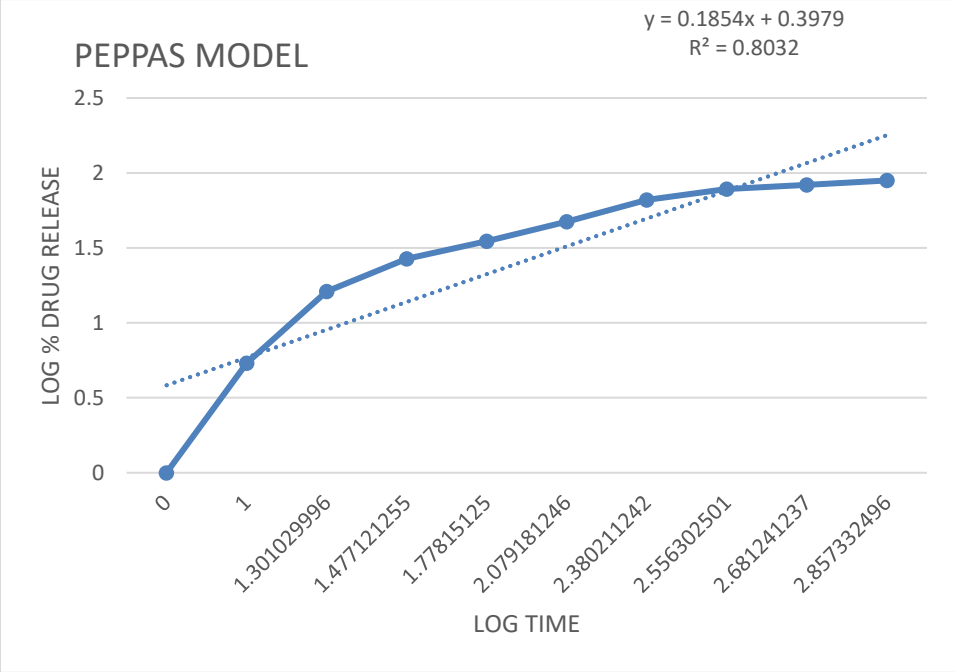


Fig. 16: Korsemeyer peppas plot of optimized formulation

Table 3.11: Model fitting of the formulation's medication release profile

Release Model	R^2 (Goodness of Fit)	Slope	Rate Constant (k)	Interpretation
Zero-order	0.8179	0.0012	0.0012	Poor fit; slow constant release

First-order	0.9309	-0.1087	0.2501	Good fit; concentration-dependent release
Higuchi model	0.9622	0.0363	0.0363	Best fit; diffusion-controlled release
Korsmeyer–Peppas model	0.8032	0.1854 (n)	1.5325	Moderate fit; slope n=0.1854 n = 0.1854 indicates Fickian diffusion if thin film

Interpretation of Release Models (In Vitro)

The drug release kinetics were evaluated using various mathematical models. The Higuchi model exhibited the best linearity with an R^2 value of 0.9622, indicating that the drug release followed a diffusion-controlled mechanism. The first-order model also showed a good fit (R^2 = 0.9309), suggesting a concentration-dependent release pattern. The Korsmeyer–Peppas model yielded a release exponent n=0.1854n = 0.1854n=0.1854, which is characteristic of Fickian diffusion, though the fit (R^2 = 0.8032) was comparatively poor, shown in Figure 13 -16.

3.5. ANTIMICROBIAL DATA

Table 3.12: 1% Allicin has antimycotic action in vitro when combined with Staphylococcus and the disc diffusion method. As a test organism, pyogene

S.NO.	COMPOUNDS	Zone of inhibition (mm) for Staphylococcus pyogene					
		1%			2%		
		I	II	III	I	II	III
1	Nano oral spray	25	24	26	24	26	25
2	Marketed Formulation	17	18	17	19	17	18
3	Control	6	7	7	8	7	7

Fig. 17: Graphical representation of Zone of inhibition (mm) for Staphylococcus pyogene by Nano oral spray (1% & 2%)

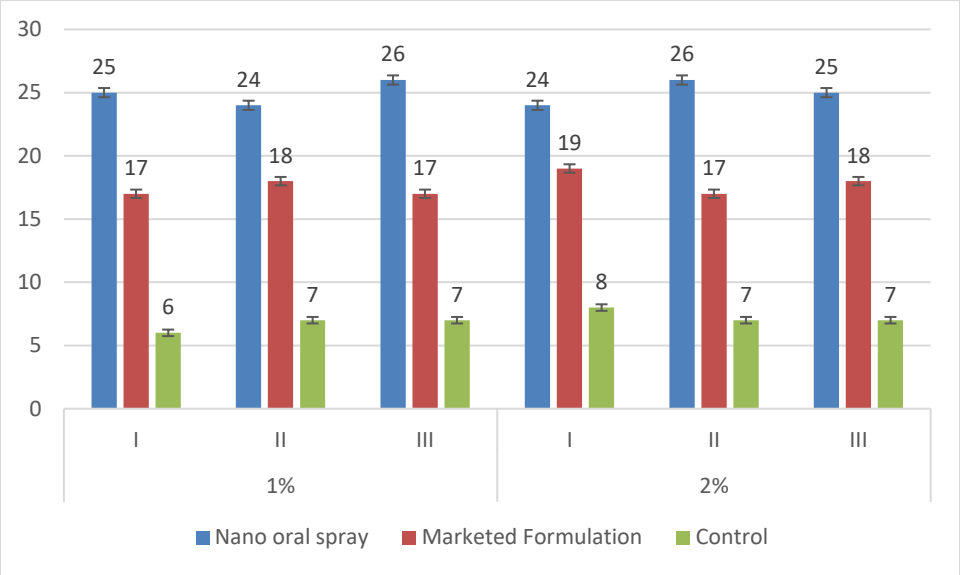


Fig. 18: Comparison graph for antimicrobial activity of Nanospray with marketed preparation and control formulation.

The antimicrobial activity of the Nano Oral Spray formulation was assessed against Staphylococcus pyogenes using the agar well diffusion method. At both 1% and 2% concentrations, the Nano Oral Spray exhibited a significantly larger zone of inhibition (25.0 ± 1.00 mm) compared to the marketed formulation (17.33 ± 0.58 mm at 1% and 18.0 ± 1.00 mm at 2%). The control showed minimal inhibition shown in Figure 17. These results suggest that the nano-formulation offers superior antibacterial activity, likely due to enhanced bioavailability and surface interaction of the nano-sized drug particles.

From the mentioned zone of inhibition values test with the two controls, observe the Nanoemulsion oral spray had the highest zone of inhibition both at 100ppm and 200ppm shown in Figure 18. It may be therefore concluded that incorporation of the drug in Nanoemulsion oral spray exhibit better antimycotic activity against Staphylococcus pyogenes in comparison with marketed formulation and control. The inhibition zone of Nanospray was observed to be 25 and 27.66 mm respectively for 100 & 200 ppm concentrations as in Table 3.12.

3.6. Stability Study & Shelf-Life Prediction:

The Nanoemulsion spray was stored at various temperatures for three months and evaluated at regular intervals for appearance, phase separation, globule size, PDI, pH, and viscosity. No significant changes indicated good physical stability. Thermodynamic stability was also confirmed using centrifugation and freeze-thaw cycles, shown in Figure 19 & Table 3.13.

Table 3.13: Stability Study & Shelf Life of the final formulation:

Time (Days)	Temp.(^{0}c) ±0.5	Drug content (mg)	Drug degraded	% Drug remaining	Log drug remaining (Log C)	k1 (rate constant)
0	30	100	0	1	2	0.000460600
30	30	99.4	0.6	99.4	1.99738638	
60	30	98.1	1.9	98.1	1.99166901	

90	30	96.7	3.3	96.7	1.98542647	
Time (Days)	**Temp.(^{0}c) ±0.5**	**Drug content (mg)**	**Drug degraded**	**% Drug remaining**	**Log drug remaining (Log C)**	**k2 (rate constant)**
0	40	100	0	1	2	0.0006909000
30	40	98.2	1.8	98.2	1.99211149	
60	40	96.6	3.4	96.6	1.98497713	
90	40	94.3	5.7	94.3	1.97451169	
Time (Days)	**Temp.(^{0}c) ±0.5**	**Drug content (mg)**	**Drug degraded**	**% Drug remaining**	**Log drug remaining (LogC)**	**k3 (rate constant)**
0	50	100	0	1	2	0.0011515000
30	50	97.1	2.9	0.971	1.98721923	
60	50	93.2	6.8	0.932	1.96941591	
90	50	90.3	9.7	0.903	1.95568775	
Time (Days)	**Temp.(^{0}c) ±0.5**	**Drug content (mg)**	**Drug degraded**	**% Drug remaining**	**Log drug remaining (LogC)**	**k4 (rate constant)**
0	60	100	0	1	2	0.0018424000
30	60	95.2	4.8	0.952	1.97863695	
60	60	90.2	9.8	0.902	1.95520654	
90	60	85.2	14.8	0.852	1.93043959	
Time (Days)	**Temp.(^{0}c) ±0.5**	**Drug content (mg)**	**Drug degraded**	**% Drug remaining**	**Log drug remaining (LogC)**	**k4 (rate constant)**
0	70	100	0	1	2	0.0025333000
30	70	92.1	1.56	0.921	1.96425963	
60	70	85.1	2.6	0.851	1.92992956	
90	70	79.1	4.56	0.791	1.89817648	

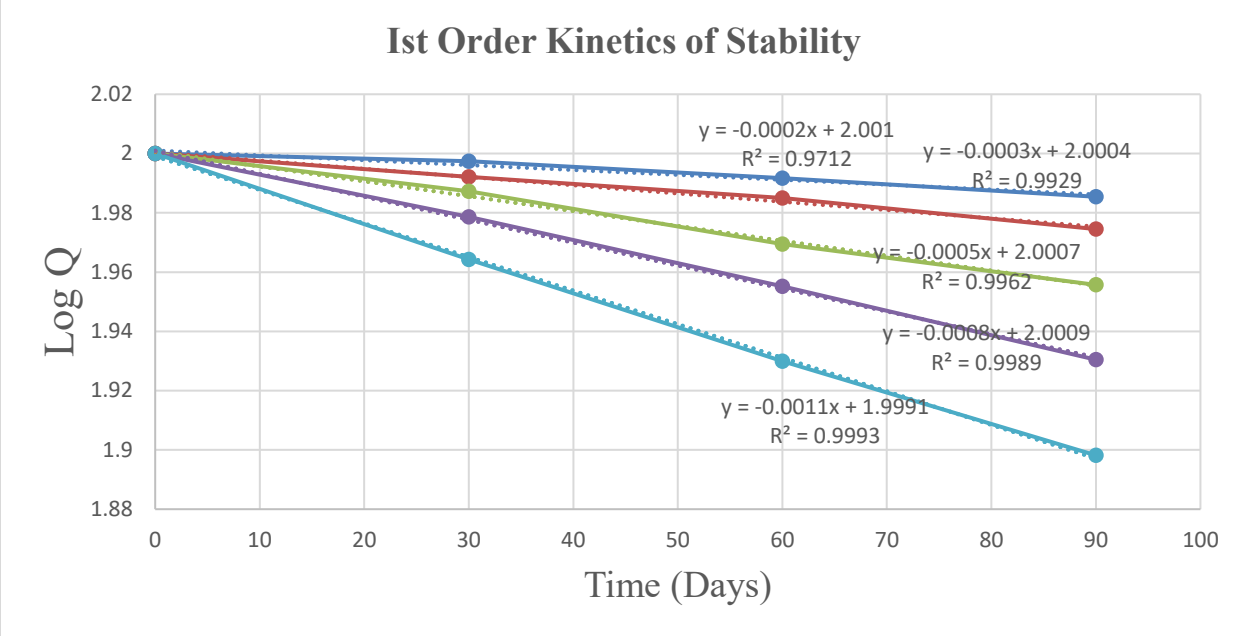


Fig. 19: Plot for Kinetics of degradation of drug via Nanospray

Table 3.14: Temperature vs log k values of stability study

Temp ©	TEMP(K)	1/T	k (rate constant)	Log k
30	303.15	0.0032987	0.000460600	-3.33668
40	313.15	0.00319336	0.0006909000	-3.16058
50	323.15	0.00309454	0.0011515000	-2.93874
60	333.15	0.00300165	0.0018424000	-2.73462
70	343.15	0.00291418	0.0025333000	-2.59631
25	**298.15**	**0.00335402**	**0.000349501**	**-3.45655**

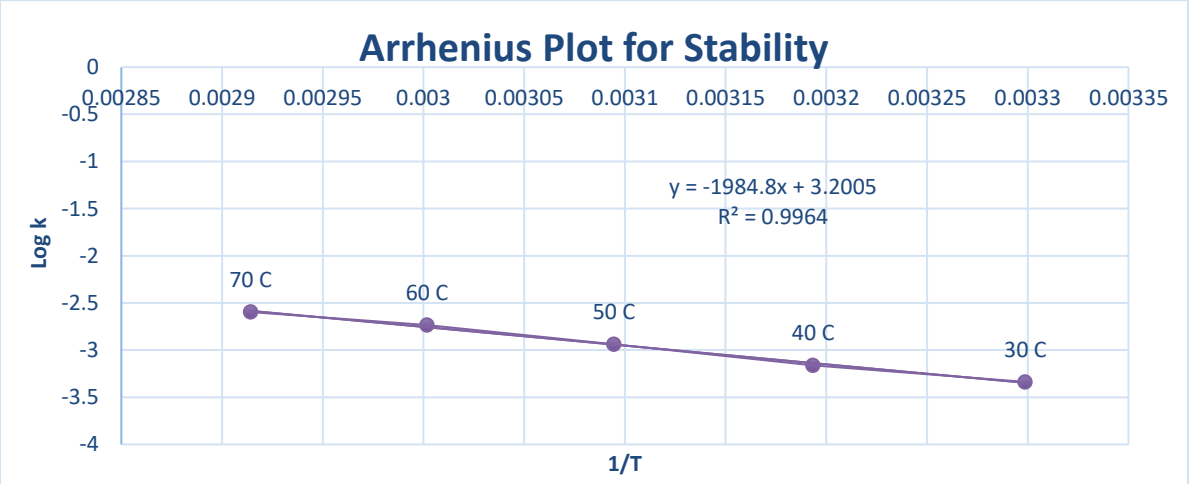


Fig. 20: Arrhenius Plot for Stability

$t_{0.9}$= 0.1488/k25 = 425.75 days = 13.997 months = 1.1664 years.

Using Arrhenius kinetics, the shelf life (t0.9) was calculated to be approximately 425.75 days, which equals 13.99 months or about 1.17 years. This suggests the formulation remains stable for over a year under recommended storage conditions shown in Figure 20 and Table 3.14.

CONCLUSION

The size of developed optimized Nanospray was found to be 227 nm and has shown remarkable result via delivering through oral mucosa. Encapsulation into Nanoemulsion has increase the residence time leading to improved therapeutic efficacy better dispersibility and good storage stability with increased bioavailability and lower irritability and lower side effects. Therapeutic dose and dosing frequency of developed oral nano spray was decreased as compared to the available conventional preparation. The optimized oral spray shows inhibition zone of 25 and 27.66 mm respectively for 100 & 200 ppm concentrations and provide sustained action in the treatment of tonsillitis by inhibiting the growth S. pyogenes. Therefore, the developed oral nano spray has proved to be a better formulation in the treatment of tonsillitis.

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Authors Contribution: Robin Singh: Conceptualization, methodology, software, data curation, visualization, investigation, writing — original draft preparation. Dr. Sanjar Alam: Conceptualization, methodology, visualization, investigation, reviewing, supervision.Dr. Swamita Arora: Conceptualization, methodology, software, data curation, visualization, investigation, writing — original draft preparation. Dr. Md. Rashid: Data curation, visualization, writing — reviewing, editing. All authors read and approved the final version of the manuscript.

CONFLICTS OF INTEREST

There is no any conflict of interest among the authors.

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