

FORMULATION & EVALUATION OF ANTI-ACNE NANOEMULSION GEL FROM BROMELAIN ENZYME (PINEAPPLE EXTRACT) & AEGLE MARMELOS (BAEL LEAVES) WITH ESSENTIAL OIL

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ABSTRACT

The present research emphasises on developing and evaluation of an anti-acne nanoemulsion gel made from Aegle marmelos (Bael leaves) extract and Bromelain enzyme (derived from Ananas comosus fruit extract) combined with eucalyptus essential oil. Its objective was to formulate a stable, natural, effective nanoemulsion gel to treat acne vulgaris. Tween 80 was used as the surfactant and eucalyptus oil as the oil phase to create the nanoemulsion. The physicochemical characteristics, particle size, zeta potential, viscosity, pH, drug content, spreadability, stability, and antibacterial activity of the optimised nanoemulsion were assessed after it was added to a carbopol-based gel. The formulation demonstrated nanoscale droplet size (120 nm), good stability, skin-compatible pH, and strong antibacterial activity against *Propionibacterium acnes* and *Staphylococcus aureus*, according to the results. Based on the study's conclusion, the nano-emulsion gel represents an appropriate herbal alternative for managing acne.

KEYWORDS: Bromelain, *Aegle marmelos*, Eucalyptus oil, Nano-emulsion, Anti-acne gel, Herbal formulation, Triethanolamine (TEA), *Propionibacterium acnes* and *Staphylococcus aureus*.

➤ INTRODUCTION

Acne- One of the most common dermatological conditions is acne vulgaris. A substantial part of the worldwide population has been impacted, including over 85% of teenagers. It can show up as a range of skin lesions, such as inflammatory lesions like papules, pustules, nodules, and scars, as well as non-inflammatory comedones like blackheads and whiteheads. Acne can psychologically lead to despair and a decrease in self-confidence. Seborrhea, follicular hyperkeratinization, microbial overgrowth (particularly that of *Cutibacterium acnes*, and *Staphylococcus epidermidis*), and inflammatory responses constitute some of the variables that contribute to the pathophysiology of acne.

An excess of oil in the sebaceous glands is the cause of acne, a skin ailment. Acne vulgaris, occasionally referred to as "Common Acne," is the most prevalent kind of acne. In relation to septicity, the skin becomes red. Hair follicles are obstructed by oil glands and dead skin cells. Oil accumulates underneath the clogged pores. Then, bacteria can proliferate rapidly. An infection causes the skin to swell and get red, making it obvious. The face, chest, back, and upper arm are the body areas where acne most frequently appears. The bacteria that change from bacteria to comedons produce the inflammatory factors. Acne often appears throughout puberty, when a person is transitioning from children to puberty.

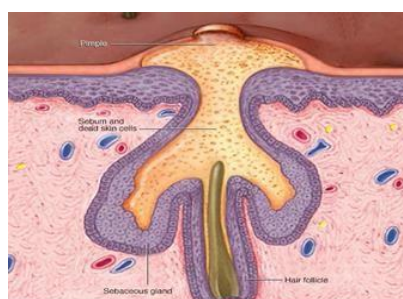


Fig. 2: Acne Causing to Skin.

Types of pimples

- **Whiteheads:** These are kinds of minor pimples that lie under the skin's surface.
- **Blackheads:** The dirt inhibits them from growing. It appears black and develops on the skin's surface. Blackheads are black in color, not because of the dirt. The protein known as keratin is often oxidized by air.
- **Papules:** These are noticeable, tiny, delicate pink pimples that appear on the skin's surface.

- **Pustules** are skin lesions that appear on the skin's surface. They are red at their bottom and consist of pus on top.
- **Nodules** are large, painful, solid pimples that are easily visible on the skin's surface but are located deep within the skin.
- **Cysts** are very painful and visible on the skin.



Fig. 2: Type of Pimples.

Nano-Emulsion Gel- The topical drug delivery system (TDDS) that has gained popularity recently is nanoemulgel, which features two release control systems: hydrogel and nanoemulsion. Nanoemulsions are **nano-sized droplets (10-100 nm)** that penetrate swiftly and deeply to release active chemicals. The addition of a nanoemulsion system to a gel matrix that improves skin penetration is called nanoemulgel.

Additionally, compared to creams and ointments, they are less sticky and much more permeable, which promotes patient compliance. Pharmaceutical formulations known as nanoemulgel (NEG) are becoming more and more well-liked due to their dual functionality as a gel and a nanoemulsion. These products are well-known for their user-friendliness, spreadability, controlled release, and ability to moisturize dry skin. It has been demonstrated that natural essential oils increase topical formulations' cutaneous permeability, promoting both the safety and effectiveness of medications.

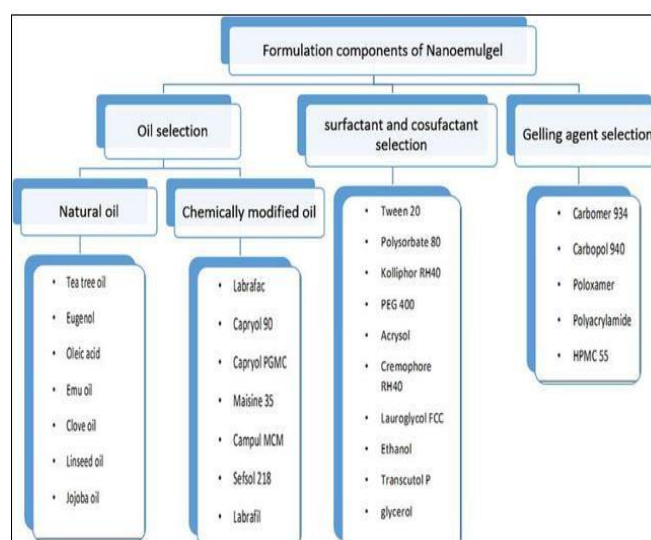


Fig. 3: Potent formulation component of nanoemulgel.

➤ Drug & Excipient Profile with Source

1. Bael leaves (*Aegle Marmelos*)

Synonyms. Bengal Quince and Indian Bael.

Biological Source: The plant known as *Aegle Marmelos* produces the unripe or partially ripe fruits and leaves that make up bael.

Family Rutaceae.

Uses: Due to its strong antibacterial, anti-oxidant, anti-inflammatory, antifungal, and anti-acne properties, it is used to treat a variety of skin conditions.



Fig. 4: Bael Leaf.

2. Pineapple (*Ananas comosus*)- The fruit and stem extracts are known as fruit and stem ‘bromelain’ that are rich sources of cysteine proteases bearing the same name bromelain

Synonyms. *Ananas ovatus*, *Ananas ananas (L.) Bromelia ananas L.*

Biological Source: Brazil and Paraguay; naturalized in parts of Asia, Africa, Australia, Mexico, Central America, the West Indies, northern South America, and various islands in the Pacific.

Family- Bromeliaceae

Uses: Anti-inflammatory agent in soft tissue injury, rheumatoid arthritis, hematomas and dissolving necrotic tissues.



Fig. 5: Ethanolic Bromelain Powder.

Table 1: Drug & Excipient Profile.

S.No.	Drug & Excipient	Source	Properties
1.	Bromelain	Extract from Pineapple	Anti Acne Agent
2.	Bael leave extract	Bael leaves	Anti-oxidant Agent & Anti-inflammatory agent
3.	Eucalyptus oil	BRM chemical	Anti-Scaring Agent, moisturizer
4.	Carbopol 940	Nutrose life-science	Gelling agent
5.	Methylparaben	Nutrose life-science	Preservative
6.	Propylparaben	Nutrose life-science	Preservative
7.	Triethanolamine	BRM chemical	Neutralizer
8.	Propylene glycol	BRM chemical	Co- Surfactant
9.	Tween 80	BRM chemical	Surfactant
10.	Distilled water	-----	Vehicle

➤ MATERIALS AND METHODS

Materials- Pineapple, Bael leaf, Eucalyptus oil, Methylparaben, Propylparaben, TEA, Propylene glycol, Distilled water, Magnetic Stirrer, Homogeniser, pH meter, Sonicator.

Methodology

1. Extraction drug
2. Preformulation study of drug
 - a. Yield Percent
 - b. Identification
 - c. Phytochemical analysis
3. Formulation of nano-emulgel.
4. Evaluation

1. Extraction of Drug

- i. **Pineapple Extract-** Using tap water to thoroughly wash the pineapple. Cut the chosen pieces into small pieces after separating the peel, core.

In a mixer, combine the pineapple fragments with a similar volume of cold distilled water or phosphate buffer. To homogenize the mixture, blend for around three minutes.

To prevent the enzyme from denaturing, a refrigerator blender is the best option. Simplicia was extracted with two litres of 96% ethanol for 120 hours. To get rid of big solid particles, strain the combined mixture using a fine screen or coarse cheesecloth. Fill centrifuge tubes with the strained liquid (the crude extract). To separate insoluble materials, centrifuge at a high speed (10,000 rpm) for 10 to 20 minutes at a low temperature. Using a pipette, carefully collect the supernatant, or top liquid layer. The product was filtered, and the ethanol was extracted using a water bath and a rotary evaporator for three days.

- ii. **Bael Leaf Extract-** After collection, the fresh bael leaves were cleaned under running water and allowed for drying in indirect sunshine. then ground into fine powders and passed through a 60 # sieve. To create an extract, desired amounts of herbal medication were weighed, macerated, soaked in 100 ml of 95% ethanol, and shaken on a platform shaker adjusted to 150 rpm at 250° C. For full extraction, the soaking method was repeated three days. After three days, the material was removed using a straightforward filtration technique, and the filtrates were gathered in a vessel. The extract was filtered twice using a funnel and basic filter paper. Filtrates were left in an evaporating pan to evaporate at an electronic water bath was utilised for evaporation. Until the desired extract concentration was reached, filtrates are allowed to evaporate in an evaporating pan set at 600 C.

2. Pre-formulation- Organoleptic Properties, Solubility profile.

a). Yield Percentage

$$\text{Yield Percentage} = \frac{\text{Weight of Extract}}{\text{Initial weight of Sample}} \times 100$$

- b). **Infrared spectroscopy of Bromelain.** The potassium bromide dispersion technique, which involves adding dry components and potassium bromide in a sample cell and using an FTIR Spectrophotometer to record the infrared spectrum, was used to record the FTIR absorption spectra of bromelain.

c). Phytochemical analysis- To determine the secondary metabolites in various plant materials, phytochemical screening was performed. Tannins, alkaloids, flavonoids, triterpenoids, sterols, glycosides, coumarins, and saponins were the secondary metabolites examined using previously defined procedures.

- i. Test for alkaloids-** An equal volume of the ethanolic extract of the sample was mixed with two millilitres of dilute hydrochloric acid (HCl). Wagner's reagent was carefully added after the solutions had been mixed thoroughly. Alkaloids were found to be present when the solution took on a reddish-brown colour.
- ii. Test for flavonoids-** The researchers used an alkaline reagent test to identify flavonoids. To do this, a few drops of NaOH was added to the test solution. When a few drops of diluted acid were added, the bright yellow colour that had developed to indicate the presence of flavonoids is disappeared.
- iii. Test for saponin-** 50 mg of extract is diluted with distilled water to create a 20 mL solution. After that, the mixture was agitated for 15 minutes in a graduated cylinder. after this process, 2 cm layer of foam appears, confirming the presence of saponins.
- iv. Test for tannins-** The ferric chloride test was used to identify the presence of tannins. In particular, they stirred 2 ml of a 1% HCl solution with 1g of the extract. The presence of tannins was shown by the resulting formation of a red precipitate.
- v. Protein (Biuret test)-** A few drops of Biuret reagent should be poured to an amount of the extract. The key component of bromelain, protein, is confirmed by a blue-purple colour that indicates the presence of peptide chains.

3. Formulation of Nano-Emulsion gel

Table 2: Formulation Table.

S.No.	Material	F-1 (gm)	F-2 (gm)	F-3 (gm)	F-4 (gm)
1.	Bromelain	2	2	2	2
2.	Beal Leaf Extract	1	1	1	1
3.	Eucalyptus oil	1	1	1	1
4.	Tween 80	4.5	5.5	6.5	7.5
5.	Carbopol 940	1.5	1.4	1.6	1.9
6.	Methylparaben	0.05	0.05	0.05	0.05
7.	Propylparaben	0.05	0.05	0.05	0.05
8.	Triethanolamine	0.05	0.06	0.04	0.05
9.	Propylene glycol	3.7	3.7	3.7	3.7
10.	Distilled water	36.15	35.24	34.06	32.75
	Total	50	50	50	50

3.1. Preparation of Nano-emulsion

- Tween 80 and Propylene glycol were combined with eucalyptus oil in a 2:1 ratio.
- Bromelain and Bael leaf extract were diluted in distilled water to prepare the aqueous phase.
- **Nano-emulsion Formation:** Under constant stirring (1000 rpm), the oil phase was gradually added to the aqueous phase. An ultra-sonicator was used to sonicate the resultant emulsion for 15 minutes in order to produce nanosized droplets.

3.2. Preparation of Nano-emulsion Gel

- **Gel Base Formation:** Triethanolamine (TEA) was used for neutralising the 1% carbopol 940 that was dissolved in water and allowed to swell.
- **Nano-emulsion Incorporation:** For the formation of a uniform nano-emulsion gel, the prepared nano-emulsion was stirred gently into the gel base.



Fig. 6 Final Product.

4. Evaluation of Nano- Emulsion Gel

- 4.1. Organoleptic test-** Organoleptic tests were performed on nanoemulgel, and color, smell, and shape changes were visually observed.
- 4.2. Homogeneity Test-** A certain amount of samples were applied to a glass object in order to execute the homogeneity test on nano-emulgel. There must be no visible coarse particles and the sample must exhibit a uniform arrangement.
- 4.3. Viscosity measurement-** The aim of viscosity measurement is to ascertain the viscosity of preparations and nano-emulgel. The brooksfeld viscometer is used to measure viscosity. The preparation was put in a 50 ml beaker and the proper spindle was used to measure the viscosity.
- 4.4. pH Measurement:-** The pH of nano-emulgel was obtained using a pH meter, which was first calibrated using a buffer solution with a pH of 7.01 and 4.01 until the instrument showed the pH value.

4.5. Physical stability study of nano-emulgel- The objective of a cycling test is to determine stability. The cycling test can be accelerated by maintaining the preparation at 24 hours at $4\pm 20^\circ\text{C}$, followed by a 24-hour shift to $40\pm 20^\circ\text{C}$. There is just one cycle of this treatment. Phase separation was noted after the procedure was performed four times. Physical conditions prior to and following the experiment were compared.

4.6. Spreadability Test:- A weight is applied, a predetermined amount of sample is placed between two parallel plates, and the spread that results is measured or the top plate is pulled a predetermined distance beneath the weight.

- A flat surface, such as a plate or a glass slide, is covered with a particular mass of the sample.
- The sample can be placed between two plates by placing a second plate on top of it.
- For a certain period of time, a weight is applied to the top plate in order to eradicate air bubbles and create a uniform film.

$$Si = \frac{m \times l}{t}$$

Where,

- **S** = Spreadability (e.g., $\text{g}\cdot\text{cm}/\text{s}$, $\text{g}\cdot\text{cm}^{-1}\cdot\text{sec}^{-1}$, or $\text{g}\cdot\text{cm}^2/\text{s}$)
- **M** = Weight tied to the upper glass slide (grams)
- **L** = Length of the glass slides (cm)
- **T** = Time taken to separate the slides (seconds)

4.7. Antibacterial activity- Nanoemulgel formulations were tested for antibacterial activity. This test was carried out using the agar diffusion method (Kirby-Bauer). After adding 0.1 mL of the inoculum to a sterile petri dish, 15 mL of nutrient agar (NA) media was added at the temperature between 45 and 50°C and homogenised until solid. A paper disc with the test solution drip-coated on it was placed inside each petri plate. A calliper was used to measure the diameter of the clear zone, which was expressed in millimetres, following a 24-hour incubation period at 36 – 37°C . Triple testing is done.

➤ RESULT AND DISCUSSION

1. Result of evaluation parameter of preformulation study of drug

- Organoleptic Properties
- Yield Percentage
- Infrared spectroscopy of Bromelain

d. Phytochemical analysis

2. Result of evaluation parameter of evaluation parameter of nanoemulgel

- a. Organoleptic Properties.
- b. Homogeneity Test.
- c. Viscosity
- d. Ph test
- e. Physical Stability
- f. Spreadability
- g. Anti-Bacterial Activity

1. Result of evaluation parameter of preformulation study of drug

a. Organoleptic Properties

- Colour of ethanolic extract of bromelain: Pale yellow
- Odour of ethanolic extract of bromelain: Characteristic odor
- pH of ethanolic extract of bromelain: 5.5 to 7.5
- Solubility ethanolic extract of bromelain: The solubility test we observed the following solubility result as table no.3

Table 3: Solubility of Ethanolic Bromelain.

Solvent	Solubility
Water	Readily Soluble
Ethanol	Insoluble
Methanol	Insoluble
Chloroform	Partially Soluble

b. Yield Percentage- We determined the yield value of ethanolic extract of pineapple is –

$$\text{Yield Percentage} = \frac{\text{Weight of Extract}}{\text{Initial weight of Sample}} \times 100$$

$$\text{Yield Percentage} = \frac{90.682 \text{ gm}}{200 \text{ gm}} \times 100$$

Yield%= 45.341%

c. Infrared spectroscopy of Bromelain- Bromelain's FTIR spectrum typically shows distinctive absorption bands associated with its protein and amino acid composition.

The IR bands at **1425–1256 cm⁻¹** are assigned to the distinctive **C–N** stretch vibration frequencies of monoalkyl guanidinium. **C=O** groups are seen in the band at **1660–1630 cm⁻¹** (amides at **~1629 cm⁻¹**). **O–H** and **N–H** stretching are visible in the band at **3700–3000**

cm⁻¹ this verifies the presence of hydroxyl and amine/amide groups in the main protein chain and side chains of amino acids. **N-H bending** and **C-N stretching** bands at **1546–1535 cm⁻¹** (Amide II)

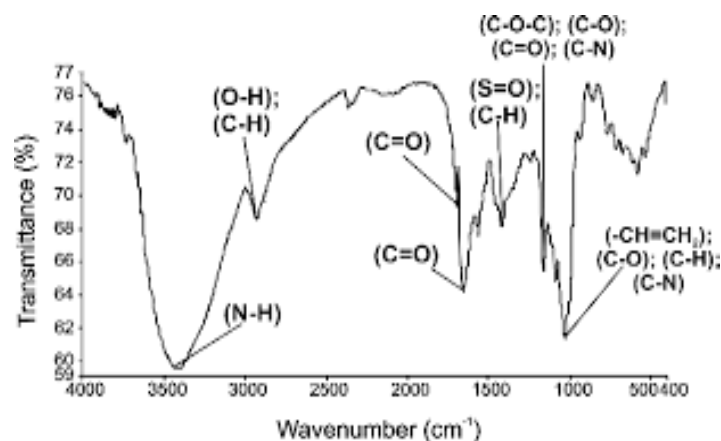


Fig. 6: FTIR spectrum.

d. Phytochemical Analysis

Table 4: Phytochemical Analysis Result.

Phytochemical	Test Method	Observation (Positive Result)
Proteins	Biuret Test	+
Tannin	Ferric Chloride Test	+
Alkaloids	Wagner's Test	+
Flavonoids	Sodium Hydroxide test	+
Saponins	Foam test	+

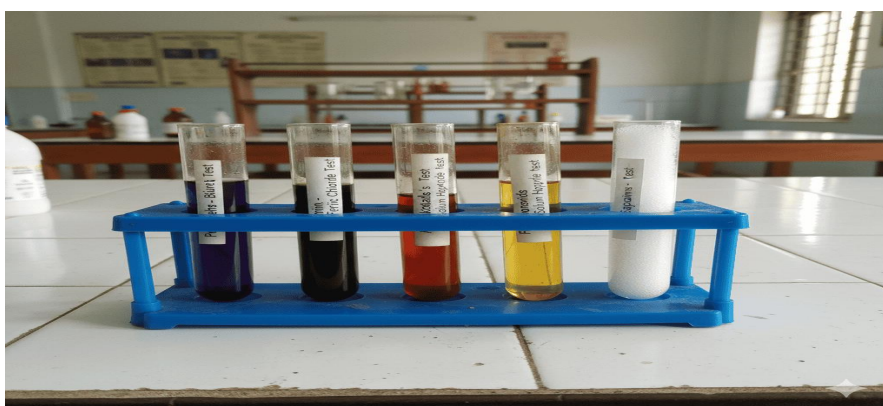


Fig. 7: Phytochemical Analysis Result.

2. Result of evaluation parameter of evaluation parameter of nano-emulgel

a. Organoleptic Properties

Visual organoleptic evaluations were conducted on all batches, assessing color, odor, and appearance.

Table 5: Oragnoleptic Test.

Formulation	Color	Appearance	Odor
F-1	Light Green	Transparent	Characteristics
F-2	Light Green	Transparent	Characteristics
F-3	Light Green	Transparent	Characteristics
F-4	Light Green	Transparent	Characteristics

b. Homogeneity Test- We found that every formulation had a smooth texture in the homogeneity test.

Table 6: Viscosity Test.

Formula	Viscosity in cps
F-1	3238
F-2	3318
F-3	3569
F-4	3116

c. Ph test - The purpose of the pH test is to determine the formulation's stability and safety in preventing skin irritation. Table No. 7 contains the results of the pH measurements.

Table 7: pH Test.

Formula	Ph
F-1	5.75
F-2	5.69
F-3	5.76
F-4	5.50

**Fig. 8: pH Test.**

d. Physical Stability –Physical observations of nanoemulgel preparations have revealed no phase separation, colour change, or odour change. following a 24-hour shift to $40 \pm 20^\circ\text{C}$ after completing the cycling stability test (24 hours at $4 \pm 20^\circ\text{C}$). The nanoemulgel and preparations were shown to be stable in storage. We have tested the optimised F-2's

physical stability. following a 24-hour shift to $40 \pm 20^\circ\text{C}$ after completing the cycling stability test (24 hours at $4 \pm 20^\circ\text{C}$).

Table 8: Physical Stability Test.

Parameter of F-2	Before Storage	After performing Cycling Test
Colour	Light Green	Light Green
Odour	Characteristics Odour	Characteristics Odour
Appereance	Transparent	Transparent
Spreadability	28.17/9.27gm.cm/min	28.17/9.27gm.cm/min
Viscosity	3318	3325

- e. **Globule Size-** After the preparation was finished, an E-SEM test was used to measure the nanoemulgel particles. The outcomes of the measurements of globule size. It is an important parameter because of their larger surface area and better penetration capabilities, which offset destabilisation effects, smaller globule sizes typically result in faster absorption rates and improved stability. So F-2 is more convenient formulation to other one.

Table 9: Globule Size.

Formulation No.	Particle Size Range
F-1	75.00 nm
F-2	69.52 nm
F-3	74.30 nm
F-4	80.28 nm

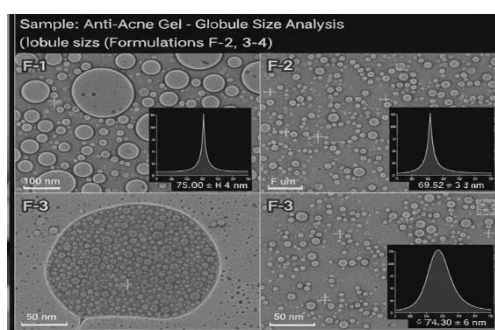


Fig. 9: E-SEM Globules Size Result.

- f. **Spreadability-** The rate and length of shear produced during smearing, as well as the target location's temperature, are crucial factors to take into account when evaluating a formulation's spread ability because a nanoemulsion gel with high spreadability spreads readily and evenly across the skin, increasing the rate of drug absorption at the site of action and reducing systemic adverse effects.

Table 10: Spreadability Test.

Formulation No.	spreadability Value
F-1	20.13/4.06gm.cm/min
F-2	29.82/7.08gm.cm/min
F-3	25.42/8.02gm.cm/min
F-4	26.17/9.27gm.cm/min

g. Anti-Bacterial Activity**Fig.10: Propionibacterium acnes.****Table No. 11: Antibacterial activity.**

Formulation No.	Inhibition Zone Diameter (mm)
	<i>Propionibacterium acnes</i>
F-2	15.58

➤ CONCLUSION

The study effectively created and assessed an anti-acne nanoemulsion gel that contained eucalyptus essential oil, *Aegle marmelos* extract, and the enzyme bromelain. All of the formulations exhibit the same organoleptic qualities, but the F-2 formulation performs better in every parameter mentioned in the above table, including Viscosity Ph test, Physical Stability, Spreadability, and Anti-Bacterial Activity.

➤ Future Scope

Further studies including in-vivo testing, clinical trials, and long-term stability assessments can be conducted to establish its commercial feasibility and patient compliance.

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