Formulation & Evaluation of Ethambutol Nanoparticle

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Abstract: Objective: Tuberculosis (TB) is a bacterial infection caused by Mycobacterium tuberculosis, which usually affects the lungs. Tuberculosis is the deadliest infectious disease in the world.

The main objective of this study is to develop a drug delivery system that uses polymer nanoparticles to maintain the release of ethambutol (ETB) while reducing the dosing frequency.

Methods: A preformulation study of ETB drugs was conducted based on physical characteristics. Determination and estimation of melting point by UV spectrophotometry. ETB loaded nanoparticles were prepared by double emulsion (W/O/W) diffusion technique provided by particle size, dispersion index, zeta potential, drug polymer compatibility study, in vitro release and drug release kinetics.

Keywords: Mycobacterium tuberculosis, Ethambutol (ETB), Polymeric nanoparticle.

INTRODUCTION

Nanoparticles are revolutionizing tuberculosis (TB) treatment through targeted drug delivery. Research shows that periodic treatment with first and second line drugs available in synthetic or natural polymer carriers[1]. Can effectively prevent tuberculosis. This method enhances drug delivery. Reduce side effects and improve treatment results Especially against drug-resistant dormant bacteria, polymer nanoparticles, liposomes, dendrimers, etc., nanocarriers release drugs slowly[2]. This increases the organelle's plasma concentration. This innovative approach allows flexible delivery of chemotherapy, reduced doses, and fewer side effects. The main drugs available include isoniazid, rifampicin, ethambutol, pyrazinamide, streptomycin, moxifloxacin, and linezolid, aimed at combating drug-resistant and dormant bacteria. Nanoparticles are therefore likely to improve the results of tuberculosis therapy[3-5.]

Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-1000 nm. Nanotechnology has transformed the treatment of tuberculosis and increased efficiency in reduced side effects[23]. Health outcomes have been improved through streamlined disease management. Thus, nanotechnology opened the new window of innovation for the treatment of tuberculosis: this is better patient outcomes and enhanced management of the disease[24]. Nanotechnology helps in the treatment process of tuberculosis through specially designed nanoparticles, stability, higher pharmacokinetics, and versatility of application. The administration routes can now be flexible, including oral administration or inhalation, thus providing higher absorption of the drug, and less frequency of treatment is required. This newly evolved treatment method eventually maximizes patient compliance, thereby obtaining better health outcomes and effective treatment of TB[25,26].

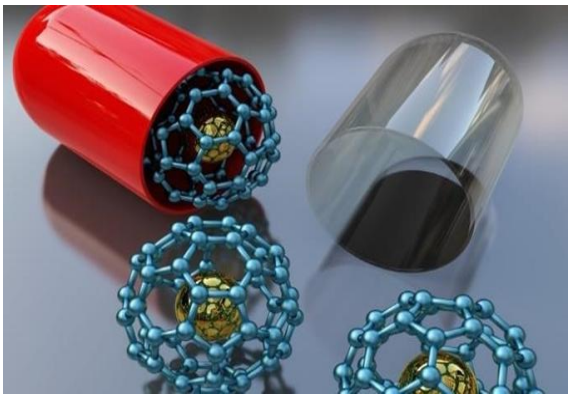


Fig: Nanoparticals

Tuberculosis is highly infectious and chronic as a result of Mycobacterium tuberculosis and Mycobacterium Bovis. It is the leading killer of the world's infectious diseases other than HIV/AIDS in terms of mortality rates[6]. This disease spreads within the air, primarily affecting parts with high oxygen concentrations, such as the lungs. In addition, it can affect other parts of the body. This bacterium continues to be a major global health threat, exposing significant health risks around the globe[7].

Multi-drug treatment helps maintain a balance between bactericidal and sterilizing effects to ensure minimal side effects and drug resistance. A widely accepted regimen includes two essential drugs coupled

with two supplementary drugs that have activity against different stages of bacterial growth[13]. Treatment objectives include eradicating viable bacteria and inactivating non-viable bacteria, preventing relapse, and shortening the treatment period. One has to start with the appropriate drugs, since this is one of the critical requirements of TB, carefully selecting medications and their management so that only drugs that have powerful bactericidal and sterilizing effects are selected and that minimize side effects combined with concomitant medications. All these ensure comprehensive treatment against TB, the fight against it, and restoration of proper health to the patients[14].

Ethambutol is a type of medication that is very effective in the fight against a certain bacteria called mycobacterium[8]. It is now commonly used to treat tuberculosis as a drug of first choice. Researchers have discovered that it is an inhibitor of mycobacterial arabinosyl transferases that mediate the polymerization reaction of arabinoglycan, an essential component of the cell wall of mycobacteria. It works very well in vitro and in vivo against Mycobacterium tuberculosis[9,10].

Need of nanoparticles:

- Improved efficiency: Improve the efficiency and effectiveness of products such as drug delivery or energy storage.
- Targeted Solution: Small size allows targeted delivery in medical applications. And reduce side effects.
- Environmental protection: Helps reduce pollution and improve processes. Makes cleaning the environment more efficient.
- Material Strength: Nanoparticles can increase the mechanical properties of materials. As a result, the product is stronger and lighter.
- Cost effectiveness: Can reduce the amount of materials required for certain applications. This may help reduce costs.
- Innovation: Nanotechnology has led to the development of new technologies and applications. That has never happened before.

Ideal properties of nanoparticles:

Nanoparticles should be biodegradable, stable, non-immunogenic, non-thrombogenic, non-toxic, easy to fabricate, cost-effective, and able to release their payloads only at the target site [11].

In this research, we tried to develop a novel nanoscopic drug delivery system called polymeric nanoparticles with ETB to see how well it could fight tuberculosis in lab tests.

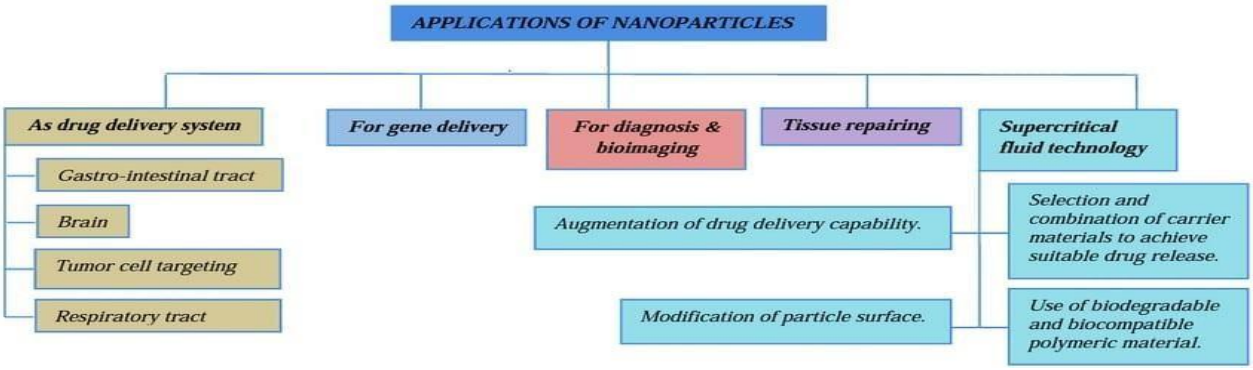
Advantages of nanoparticles:

- Increased surface area: A high surface area to volume ratio increases reactivity and efficiency in chemical processes.
- Improved Properties: Nanoparticles can exhibit unique optical, electrical, and mechanical properties not found in bulk materials.
- Targeted Delivery: In the medical field Nanoparticles can be designed for targeted drug delivery. Improve treatment efficiency and reduce side effects.
- Improved Catalysts: They can act as effective catalysts by speeding up chemical reactions in industrial processes.
- Environmental applications: Nanoparticles can be used for environmental remediation, such as removing pollutants from water or air.
- Lightweight material: Can make the material lightweight. This is useful in the aerospace and automotive industries. And strengthen the material.
- Biomedical imaging: Nanoparticles could improve imaging techniques. Provides better resolution and contrast in diagnostic procedures.

Disadvantage of nanoparticles:

- Toxicity: Adversely affects human health and the environment.
- Regulatory Challenges: Difficulty in establishing safety standards and regulations.
- Production Issues: Challenges in achieving consistency and scalability in production.
- Biological Interactions: Unintended interactions with biological systems can pose health risks.
- Cost: High manufacturing and testing costs may limit widespread use.
- Stability: Some nanoparticles can degrade or change their properties over time. Which affects efficiency

Application of nanoparticles:



Polymeric Nanoparticle:

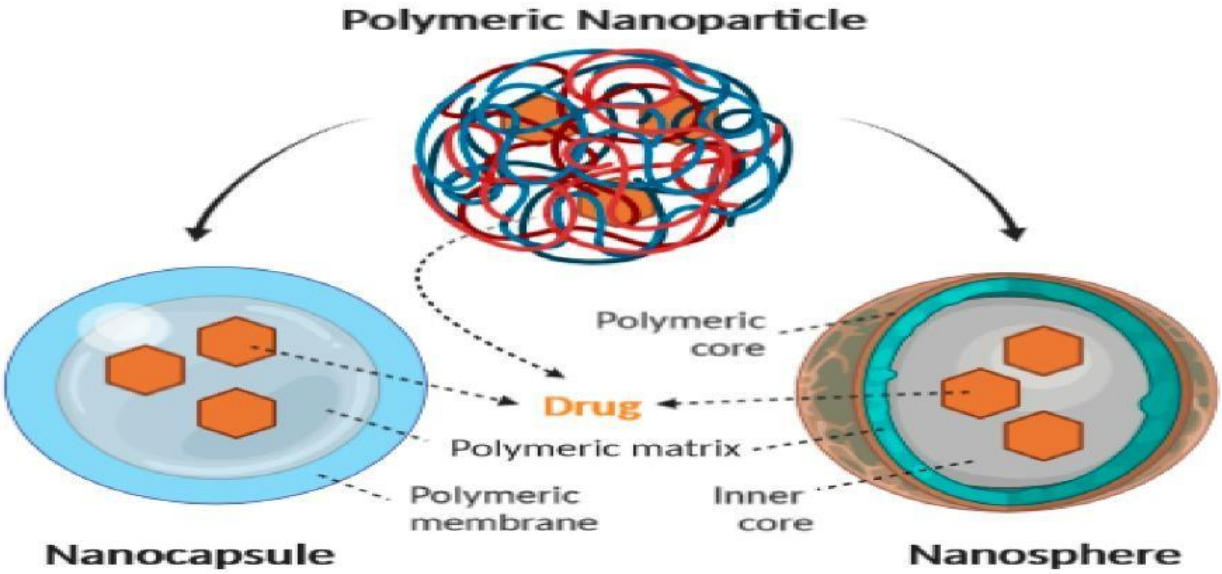


Fig: polymeric Nanoparticle

PNs are drug carrier systems that have a diameter of less than 1 µm. The term nanoparticles include Nano-spheres (NSs) and Nano-capsules (NCs), which differ according to composition and structural organization[15].

Polymeric nanoparticles have performed well because they are compatible with the body. Research has discovered that they are the best options for delivering medication. Polymeric nanoparticles are made stronger by using different types of polymers. They noted the presence of these substances. Different types of polymers can help in treating tuberculosis by using polymeric nanoparticles, through varies site of administration [16-18].

Preparation of ETB loaded polymeric nanoparticles:

The ETB loaded nanoparticles were prepared using a double-emulsion (W/O/W) diffusion technique.

Materials:

Sr. No.	Material
1.	Ethambutol
2.	Quillaja saponin
3.	Oil (soybean or peanut oil)
4.	pH adjustment agents (e.g. HCl, NaOH)
5.	Ethanol
6.	Water

Procedure:

1. Primary emulsification process: First mix ethambutol in ethanol (1-5% concentration). Then, combine it with Quillaja saponin solution (1-5% concentration). Create an emulsion by using sonication or mixing at high speed.

2. Second round of emulsification: Mix the main emulsion with soybean oil in a step called adding the primary emulsion to an oil phase.

Use sonication or high-speed mixing to emulsify the ingredients. To make the secondary mixture, gently pour water while stirring.

3. Diffusion: Let it sit for 30 minutes to an hour to allow diffusion.
4. Adjusting pH: - Use HCl or NaOH to adjust the pH to a range of 6.5-7.5.
5. Mixing: - Keep mixing for half an hour to help create nanoparticles.
6. Centrifugation: - To separate the mixture, use a centrifuge at 15,000 rpm for 30 minutes.
7. Cleaning: - Clean the nanoparticles by rinsing them with water or ethanol.
8. Freeze- Drying: - Freeze the nanoparticles and dry them to get a powder.

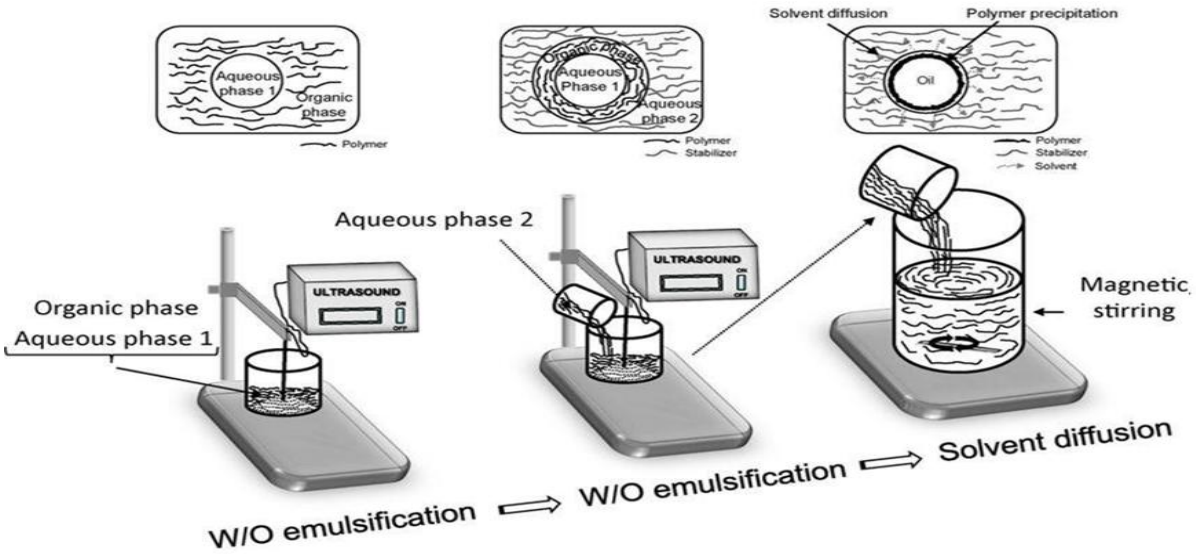


Fig: Set up used for preparation of nanoparticles by the double emulsion diffusion method.

Preformulation study: -

1) physical characteristics of drug:
   - Solubility: Suitable solvents for preparation
   - Stability: Temperature, pH sensitivity of light
   - Particle size and distribution: Optimizing for effective drug delivery
   - Crystallinity: Ensuring maximum drug release and bioavailability
   - Hygroscopicity: Storage and handling requirements
   - Compatibility: Interactions with excipients and other nanoparticle components
   - Toxicity: Safe use in a biological system.

2) Drug Polymer Compatibility Study:

Compatibility studies of drugs and excipients. It is an important process in developing a constant solid volume. application form Incompatibility of drugs and excipients can change. Stability and bioavailability of drugs This affects the safety and efficacy of the drug. To check for interactions between drugs and excipients, Fourier transform infrared (FTIR) spectroscopy and X-ray diffraction (XRD) analysis was performed for drugs, polymers, Physical Mixing and Formulation.

3) Melting point determination:

The capillary melting point apparatus detects the melting point of a drug by heating a small sample in a sealed capillary tube and noting when the first solid melts and the melting ends, hence providing good information on the physical properties of the drug[19].

4) Ultraviolet Spectrophotometric Studies:

Max absorption wavelength (λmax) of Ethambutol in various solvents such as phosphate buffer, hydrochloric acid buffer, and distilled water was determined through a UV spectrophotometric study.

Standard solutions of ETB at concentrations 1000, 100, and 10 µg/mL were scanned in the range of 200-400 nm using a UV-VIS spectrophotometer with guidelines of Indian Pharmacopoeia 2010 followed for all. This study will determine λmax for analyzing ETB in various pharmaceutical formulations, which is crucial to understand the chemical structure and the properties of the compound as well as quantitative analysis of drug[20].

5) XRD Analysis:

X-ray diffraction image of the physical mixture ETB, Eudragit RS-100. & formulation using an X-ray diffractometer (Rigaku MiniFlex II, Tokyo, Japan) to check the physical condition of the ETB and Interaction with other ingredients in the formula Source Copper X-rays Kα (γ=1.5405 °A) are monochromatic. Operating at 30 kV and 15 mA, the sample was scanned in 2 Theta range: 10°80° [21].

6) FT-IR spectroscopy

FT-IR spectrum of the physical ingredients of ETB, Eudragit RS-100 and the drug
& polymer (1:1) are observe the mixing formula. KBr was measured using a FTIR spectrophotometer (FTIR-4100, Jasco, Tokyo, Japan). Process: The samples were lightly crushed with anhydrous KBr and it is compressed into tablets. The scanning range is 400 and 4000 cm-1 respectively[21].

Evaluation of ETB Loaded Polymeric Nanoparticles:

1) Particle size:

Particle size and distribution are two of the most important nanoparticle parameters, which impact stability, dosage, and drug release. Valuable management is essential for optimal nanoparticle-based drug delivery. Several methods determine particle size and distribution:

a. Photon Correlation Spectroscopy: Quantitative measurement.
b. Electron Microscopy (EM): Measures individual particles. It requires gold coating 30-50 nm thickness for non-conductive materials.
    - Scanning Electron Microscopy: Fast and efficient.
    - Transmission Electron Microscopy: Allows the differentiation of nanocapsules, nanoparticles, and emulsion droplets.
    - Freeze Fracture Technique: Internal morphological structure observable.
c. Atomic Force Microscopy (AFM): Describes nanospheres; possible imaging with water, which provides insights into the nanoparticle behavior under biologic conditions.

2) Surface Area:

An instrument called a sorptometer is used to determine the specific surface area of freeze-dried nanoparticles. The specific surface area can be calculated using the following equation. $A = 6/pd$
where, A = specific surface area of nanoparticles p = density of medium d = diameter of nanoparticle
However, sometimes surfactant residues attached to nanoparticles cause deviations from measured and calculated values.

3) Surface Charge:

The zeta potential is a measure of the electrical properties of nanoparticles, and surface charge is one way of expressing these measurements. Zeta potential is usually dependent upon composition and diffusion medium and it is measured using a zetameter. Good values must be above ±30 mV, which prevents agglomeration and therefore maintains suspension stability. Such a measurement not only specifies surface charge but also determines the electrically charged state of the drug, such as whether it resides in the core or is surface-adsorbed within the nanoparticle. The stability and lack of aggregation of the nanoparticle toward effective drug delivery were ensured by the zeta potential of above ±30 mV.

4) Density:

The basic idea of density distribution in a nanoparticle matrix can be derived from structural defects. This can be viewed with SEM or TEM and freeze fracture techniques. The density of nanoparticles can be determined using a gas pycnometer. (using air or helium)

5) Molecular Weight:

The molecular weight of nanoparticle polymers can be determined by gel permeability chromatography (GPC) using a refractive index detector.

6) Recovery of Nanoparticle:

Nanoparticle yield is given in terms of % and is calculated by the following equation,

Nanoparticle yield=

(Conc. of drug in the nanoparticles / Conc. of recovered nanoparticles) □100

7) Drug Incorporation Efficacy (DIE):

DIC of nanoparticle =

Quality of drug entrapped in the nanoparticles / Total amount of drug used

Drug loading into nanoparticles can be done in two ways.

(a) Drug incorporation during nanoparticle production.
(b) Incubation of the fabricated nanoparticles with concentrated drug solution.

8) In vitro release:

In vitro drug release from nanoparticles can be calculated by:

There are Two Methods:

1. Diffusion Cells (Propagation Cells): Two-chamber device, with a hydrophilic membrane which separates the two chambers.

Donor Chamber: suspension of the nanoparticles.

Acceptor Chamber: phosphate buffer.

2. Ultrafiltration: Ultra filtration cell filled with buffer. Suspension of nanoparticles added, Buffer fractions filtered periodically on membrane. Free drug analysed in order to quantify its rate of release

9) Drug release:

One important reason for pursuing nanotechnology is drug delivery. Therefore, it is important to understand the types and scope in which drug molecules are released in order to obtain such information Most release procedures require separating the drug and dispensary. It is the simplest method for separating soluble substances from insoluble drugs in traditional solid forms. For example, A filtering step is sufficient to create this separation.

CONCLUSION

Nanoparticulate drug delivery systems utilizing Quillaja saponin have transformed the pharmaceutical landscape by overcoming the limitations of conventional drugs, including high toxicity, non-specific delivery, poor solubility, and short circulating half-lives. Quillaja saponin-based nanoparticles enhance pharmacological and therapeutic properties, enabling prolonged, targeted, and controlled drug delivery. Various novel methods, such as supercritical fluid technology, emulsification solvent evaporation, solvent diffusion, and coacervation, have been successfully employed for nanoparticle development. With vast applications in prolonged drug delivery, targeted drug delivery, gene delivery, oral delivery, and drug delivery to the brain, Quillaja saponin-based nanoparticulate systems hold immense promise for improving treatment outcomes. As nanotechnology advances, the future of drug delivery research is bright, with Quillaja saponin-based nanocarriers poised to revolutionize healthcare.

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