THE FORMULATION AND EVALUATION OF SALICYLIC ACID-LOADED POLYMERIC NANOPARTICLES USING EUDRAGIT RS-100 FOR ANTIBACTERIAL ACTIVITY

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ABSTRACT

The present study investigates the formulation and evaluation of salicylic acid-loaded polymeric nanoparticles using Eudragit RS-100, prepared via the solvent evaporation method. This approach involved emulsification, solvent removal, and lyophilization, resulting in nanoparticles designed for enhanced antimicrobial and anti-biofilm performance. Comprehensive physicochemical characterization was performed, including particle size analysis, zeta potential measurement, entrapment efficiency, and morphological assessment via scanning electron microscopy (SEM). The nanoparticles exhibited sizes ranging from 97.23 to 342.22 nm, with F4 formulation demonstrating the smallest average particle size. Zeta potential values (−0.1 to +5.3 mV) indicated colloidal stability, while entrapment efficiency ranged from 68.16% to 92.33%, with F4 again showing the highest encapsulation. Antibacterial activity was assessed against *Bacillus subtilis* (Gram-positive) and *Pseudomonas aeruginosa* (Gram-negative) using the agar well diffusion assay. The nanoparticles displayed notable zones of inhibition, with F4 formulation achieving the highest antibacterial efficacy. Anti-biofilm activity was further evaluated using the test tube assay and quantified via crystal violet staining and spectrophotometry. Results demonstrated substantial biofilm inhibition at higher concentrations (125 µg/mL), highlighting the nanoparticles' ability to impede bacterial adhesion and biomass formation. These findings underscore the potential of Eudragit RS-100-based polymeric nanoparticles as a viable platform for controlled drug delivery and effective antimicrobial interventions, particularly in targeting resistant bacterial strains and biofilm-associated infections.

KEYWORDS: Formulation, Salicylic Acid, Nanoparticles, Eudragit RS-100, Polymeric nanoparticles

1. INTRODUCTION

Nanoparticles, typically ranging in size from 10 to 1000 nanometers, have emerged as promising carriers in drug delivery systems due to their ability to dissolve, entrap, encapsulate, or bind active pharmaceutical ingredients. The method of preparation determines the structural form of the nanoparticles, such as nanospheres or nanocapsules. Nanospheres are characterized by uniform dispersion of the drug throughout the polymer matrix, whereas nanocapsules encapsulate the drug within a distinct polymeric shell. Among various types of nanoparticles, biodegradable polymeric nanoparticles have garnered significant attention, especially when coated with hydrophilic polymers like poly(ethylene glycol) (PEG), for their capabilities in prolonging systemic circulation, enabling targeted delivery, and supporting gene therapy applications.

A major focus in nanoparticle-mediated drug delivery lies in the precise control of particle size, surface properties, and release kinetics to enhance therapeutic efficacy. Liposomes have been widely studied for drug delivery due to their biocompatibility and ability to reduce drug toxicity; however, they often face challenges such as low encapsulation efficiency and instability. In contrast, polymeric nanoparticles offer advantages including controlled drug release, improved stability, and the ability to deliver a wide range of therapeutic agents, including proteins and peptides.

Among the polymeric materials used for nanoparticle formulation, Eudragit RS-100—a biocompatible and water-insoluble polymer—has shown potential for sustained drug release. The solvent evaporation method is commonly employed for formulating Eudragit-based nanoparticles, involving emulsification, solvent removal, and lyophilization to yield stable nanosystems suitable for pharmaceutical applications **(Vila et al., 2002; Mu, et al., 2003).**

In the present study, polymeric nanoparticles were prepared using Eudragit RS-100 via the solvent evaporation method. Their antibacterial potential was evaluated against Bacillus subtilis (Gram-positive) and Pseudomonas aeruginosa (Gram-negative) using the well diffusion assay on Nutrient Agar Media (NAM). Additionally, the anti-biofilm efficacy of the nanoparticle formulations was assessed through a test tube-based biofilm inhibition assay, employing crystal violet staining and spectrophotometric analysis to quantify bacterial adhesion and biofilm formation **(Lsanger, 2000; Lee et al., 2005).**

2. MATERIALS AND METHODS

2.1 Formulation of nanoparticle

The solvent evaporation method was used for the preparation of Eudragit RS-100 nanoparticles. Firstly, the emulsification of the polymeric solution was done in an aqueous solution containing a surfactant. Then, the evaporation of the polymeric solution was done by precipitation of the polymer. In the solution, of methanol drug was dissolved. With constant stirring using a magnetic stirrer, the organic solution was added to an aqueous phase containing polyvinyl alcohol (PVA). The emulsion was sonicated using a sonicator for the nano size of the emulsion. The organic solvent was then evaporated using constant stirring on a magnetic stirrer for about 4-5 hrs. After centrifugation (30 min, 10000 rpm), the nanoparticles were collected. The prepared emulsion was then kept for lyophilization for 48 hrs. **(Saharan et al., 2019).**

Table 1 : Ingredients used in nanoparticle formulation

Ingredients	Formulation 1	Formulation 2	Formulation 3	Formulation 4	Formulation 5
Salicylic acid (mg)	100	100	100	100	100
Eudragit RS 100 (mg)	50	75	100	125	150
Polyvinyl alcohol (%)	0.1	0.2	0.3	0.4	0.45
Sonication Time (Min.)	15	15	15	15	15
Methanol (ml) (Solvent)	10	10	10	10	10
Water	10	10	10	10	10

2.2 Evaluation parameter of Nanoparticle formulation

2.2.1. Particle size

The particle size is one of the most important parameters for the characterization of nanoparticles. The size of the nanoparticle was measured using a Malvern Zeta sizer (Malvern Instruments). The dispersions were diluted with Millipore filtered water to an appropriate scattering intensity at 25°C, and the sample was placed in a disposable sizing cuvette **(Sharma et al., 2011; Balla et al., 2020)**

2.2.2. Zeta potential

The zeta potential was measured for the determination of the movement velocity of the particles in an electric field and the particle charge. In the present work, the nanoparticle was diluted 10 times with distilled water and analyzed by Zeta sizer Malvern instruments. All samples were sonicated for 5-15 minutes before zeta potential measurements **(Kumar et al., 2011; Penjuri et al., 2016).**

2.2.3. Entrapment efficiency

%Entrapment efficiency was determined by indirect estimation. Drug -loaded nanoparticles were centrifuged at 15,000 rpm for 30 min using REMI Ultra Centrifuge. The non-entrapped drug (free drug) was determined in the supernatant solution using UV spectrophotometer. The peak area was determined and amount of free drug is determined by extrapolating the calibration curve. And drug

entrapment calculated by using below equation **(Balla et al., 2020).**

Entrapment efficiency % = Total drug conc. - Supernatant drug conc. / total drug conc.*100

2.2.4. Scanning Electron Microscopic (SEM)

The electron beam from a scanning electron microscope was used to attain the morphological features of the optimized drug-loaded alginate beads were coated with a thin layer (2–20 nm) of metal (s) such as gold, palladium, or platinum using a sputter coater under vacuum. The pre-treated specimen was then bombarded with an electron beam and the interaction resulted in the formation of secondary electrons called Auger electrons. From this interaction between the electron beam and the specimen's atoms, only the electrons scattered at 90° were selected and further processed based on Rutherford and Kramer's Law for acquiring the images of surface topography.

2.3 Antibacterial Activity of Polymeric Nanoparticles

2.3.1. Antibacterial Activity by Well Diffusion Assay

2.3.1.1. Preparation of Nutrient Media

28 g of nutrient agar media was dissolved in 1 liter of distilled water. pH of media was checked before sterilization. Media was sterilized in autoclave at 121^{o}C at 15 lbs pressure for 15 minutes. After sterilization, media was allowed to be cool but not solidify. Nutrient media was poured into plates and placed in the laminar air flow until the agar was get solidified.

2.3.1.2. Well Diffusion Assay

Bacterial cultures were spread on Nutrient Agar Media (NAM). A 1 mL volume of *Betula utilis* methanolic and ethyl acetate extracts was taken directly from the stock. A standard solution of salicylic acid was prepared by dissolving 10 mg in 10 mL of distilled water (1 mg/mL). Inocula of *Bacillus subtilis* (MTCC 736, Gram-positive) and *Pseudomonas aeruginosa* (MTCC 8076, Gram-negative) were prepared by suspending 1 g of each in 10 mL of nutrient broth and standardizing to 10^{8} CFU/mL **(Mohammadi-Sichani *et al.*, 2012)**. These cultures were incubated in a shaker. From each standardized broth culture, 50 µL of inoculum was transferred onto sterile solidified agar plates and spread uniformly using a sterile spreader. Four wells of 6 mm diameter were created in each plate using a sterile cork borer. Wells were filled with different concentrations (F1–F5) of the test samples, and one well on a separate plate was filled with 100 µL of the standard drug. Plates were left at room temperature for 30 minutes to allow diffusion, then incubated at 37°C for 18–24 hours. After incubation, zones of inhibition (ZOI) were observed around the wells. The diameters of the clear inhibition zones were measured in millimeters, including the diameter of the wells, using a ruler placed behind the inverted Petri dish against a black, non-reflective background **(Manandhar *et al.*, 2019).**

2.4 Anti-biofilm activity by the Test tube method

2.4.1 Effect of F4 solution on the bacterial biofilm formation

Biofilm formation was assessed in sterile test tubes, where five appropriate concentrations (125,62.5,31.25,15.62, and 7.81 µg/ml) of polymeric nanoparticles (1mg/ml) were prepared from a serial two-fold dilution method in NAM broth, and five tubes were inoculated with 1 ml of the 0.5 McFarland turbidity standard (bacterial culture) and incubated for 4-5 hours to allow cell attachment, and then add 1ml of each concentration was added to each tube. All the tubes were further incubated for 24 hours at 37°C, the eighth tube contained bacteria and NAM broth only (negative control).

2.4.2 Ability of adherence bacteria

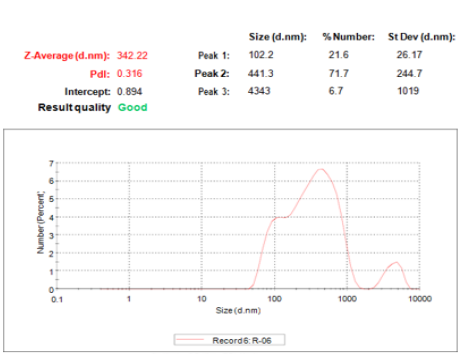
The adhered cell biomass was determined using 1% crystal violet staining. At first, test tube was emptied and washed three times with sterile Phosphate Buffered Saline (PBS). The tubes were air-dried and then oven-dried at 60 ℃ for 45 min. then the tubes were stained with1ml of 1% crystal violet and incubated at normal temperature for 15 min after which the tubes were washed 5 times with sterile distilled water to remove unabsorbed stain after that 5ml of ethanol was added to each tube and the absorbance was determined against blank at 540nm using a spectrophotometer **(Al-Gbouri et al., 2018).** The percent of biofilm inhibition was calculated by the following formula:

$$\textbf{Biofilm Inhibition (\%)} = \textbf{ODc -ODt} \times \textbf{100} \div \textbf{ODc}$$

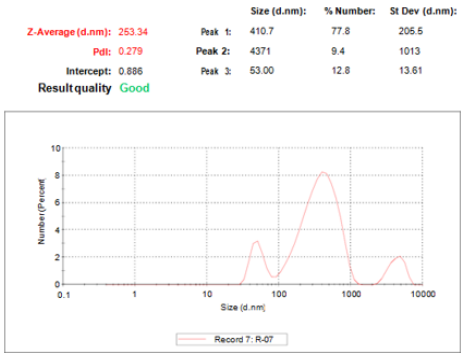
3. RESEARCH AND DISCUSSION

3.1 Evaluation parameter of drug loaded Nanoparticle

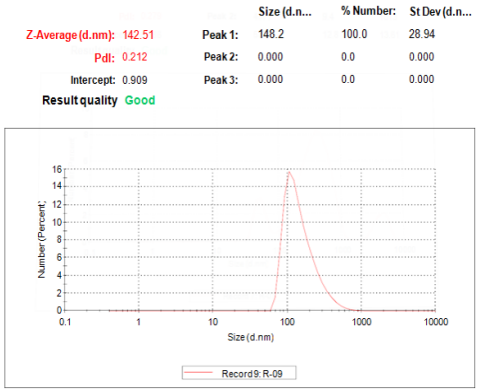
3.1.1.Particle size determination



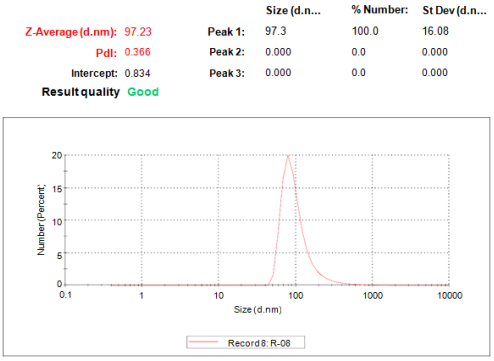
Graph 1 : Particle Size (F1)



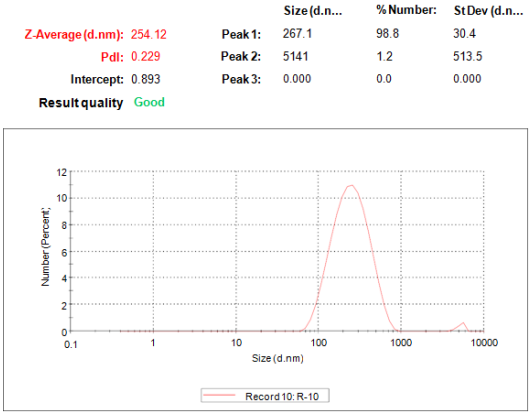
Graph 2: Particle Size (F2)



Graph 3 : Particle Size (F3)



Graph 4 : Particle Size (F4)



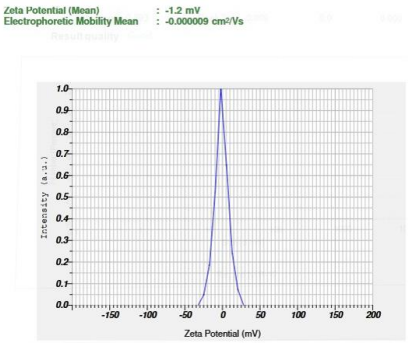
Graph 5: Particle Size (F5)

Table 1 : Result of Particle size of all formulations

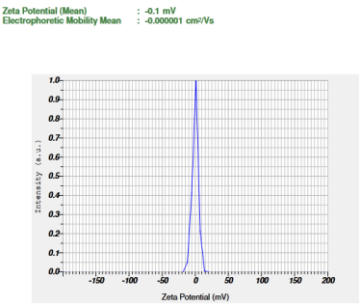
S. No	Formulations	Particle size (nm)	PDI Value
1.	F1	342.22 nm	0.316
2.	F2	253.34 nm	0.279
3.	F3	142.51 nm	0.212
4.	**F4**	**97.23 nm**	**0.366**
5.	F5	254.12 nm	0.229

The particle size is one of the most important parameters for the characterization of nanoparticles. The average particle sizes of the prepared Salicylic acid-loaded nanoparticle formulation were measured using Malvern Zeta Sizer. Particle size analysis showed that the average particle size of the nanoparticle was found to be range of 97.23 to 342.22 nm. These particle size values indicate that the formulated nanoparticle is within the range of nanoparticle and F4 is the lowest particle size of all formulations.

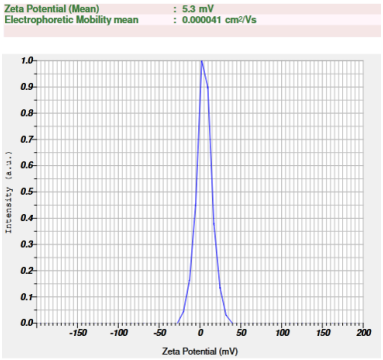
3.1.2. Zeta potential



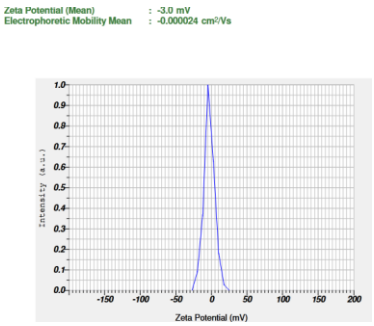
Graph 6: Zeta potential (F1)



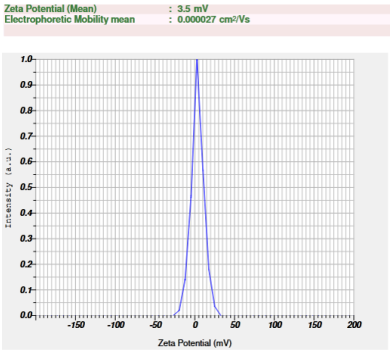
Graph 7 :Zeta Potential (F2)



Graph 8: Zeta Potential (F3)



Graph 9: Zeta Potential (F4)



Graph 10: Zeta Potential (F5)

Table 2: Result of Zeta potential of all formulation

S. No	Formulation	Zeta potential
1	Nanoparticle (F1)	-1.2 mV
2	Nanoparticle (F2)	-0.1 mV
3	Nanoparticle (F3)	5.3 mV

4	Nanoparticle (F4)	-3.0 mV
5	Nanoparticle (F5)	3.5 mV

Zeta potential analysis is carried out to find the surface charge of the particles. The magnitude of zeta potential is predictive of the colloidal stability. Zeta potential was found to be all formulation range -0.1 to 5.3 mV with peak area of 100% intensity. These values indicate that the all-formulated Nanoparticle is stable. Results show in above table 15.

3.1.3 Entrapment efficacy

Table 3: Entrapment efficacy

S. No.	Formulations	Entrapment efficacy (%)
1.	Nanoparticle (F1)	68.16
2.	Nanoparticle (F2)	79.63
3.	Nanoparticle (F3)	83.92
4.	**Nanoparticle (F4)**	**92.33**
5.	Nanoparticle (F5)	90.52

This might be due to the fact that the variation in entrapment efficiency was due to the changes in the polymer and solvents concentration. The prepared Salicylic acid loaded Nanoparticle formulation possesses high drug entrapment efficiency (92.33) of F4 formulation due to change in polymer concentration and all formulation EE % found to be in the range of **68.16 to 92.33 %.**

3.1.4 SEM analysis

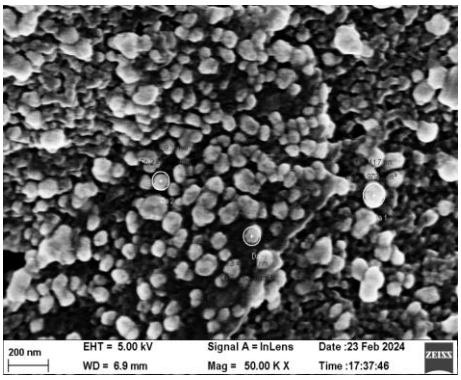


Figure 1: SEM analysis of Nanoparticles

SEM analysis was performed to determine their microscopic characters (shape & morphology) of prepared nanoparticle. Nanoparticle were prepared and dried well to remove the moisture content and images were taken using scanning electron microscopy. Scanning electron micrograph of the prepared nanoparticles at 50.00 kx magnification showed that the nanoparticle was porous with a smooth surface morphology and spherical shape. The nature of nano sponges was clearly observed in the SEM images.

3.2 Result of antibacterial activity of Polymeric Nanoparticles

3.2.1. Antibacterial activity of Polymeric Nanoparticles against *Bacillus subtilis*

Table 4: Antibacterial activity of Polymeric Nanoparticles against *Bacillus subtilis*

Name	Zone Of Inhibition plate 1 (mm)	Zone Of Inhibition plate 2 (mm)	Zone Of Inhibition plate 3 (mm)	Mean ± SD (mm)
F1	26mm	20mm	16mm	20.66 ± 4.10
F2	25mm	23mm	15mm	21± 4.32
F3	24mm	25mm	20mm	23± 2.16
F4	26mm	27mm	23mm	25.33± 1.69
F5	25mm	23mm	18mm	22± 2.94

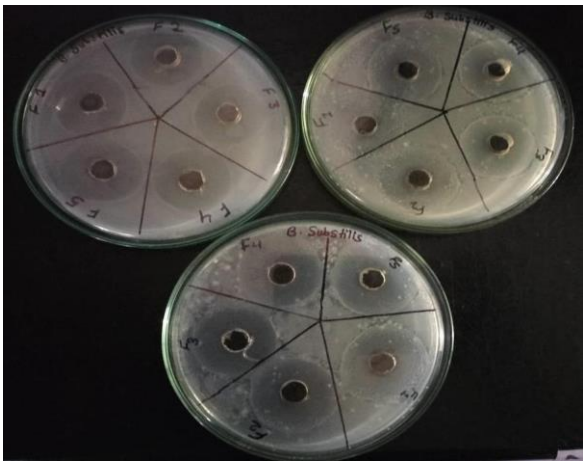


Figure 2: Antibacterial activity of Polymeric Nanoparticles against *Bacillus subtilis*

3.2.2 Antibacterial Activity of Polymeric Nanoparticles against *Pseudomonas aeruginosa*

Table 5: Antibacterial activity of Polymeric Nanoparticles against *Pseudomonas aeruginosa*

Name	Zone Of Inhibition plate 1 (mm)	Zone Of Inhibition plate 2 (mm)	Zone Of Inhibition plate 3 (mm)	Mean ± SD (mm)
F1	15mm	13mm	13mm	13.66± 0.94
F2	12mm	11mm	11mm	11.33± 0.47
F3	13mm	14mm	10mm	12.33± 1.69
F4	10mm	15mm	14mm	13± 2.16
F5	14mm	16mm	13mm	14.33± 1.24

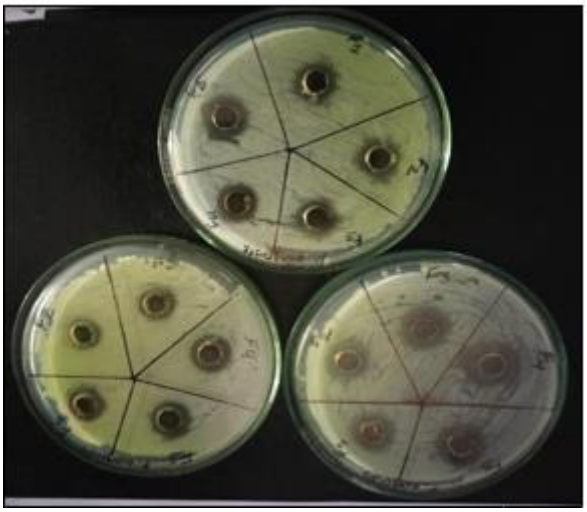
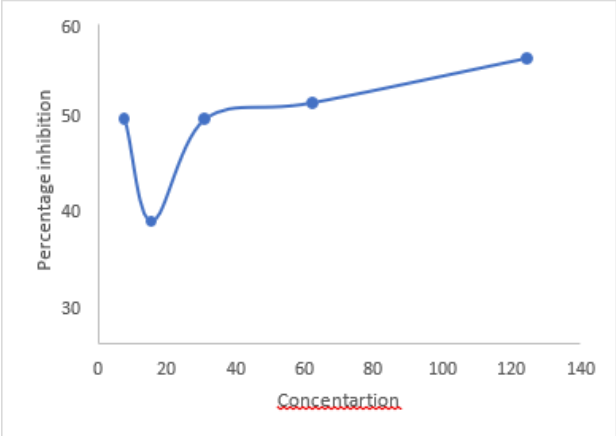


Figure 3 : Antibacterial activity of Polymeric Nanoparticles against Pseudomonas aeruginosa

Nanoparticles, due to their small size, offer a larger contact surface with bacterial cells, increasing their penetration and bactericidal effect. Polymeric nano-formulations have been used to increase efficacy and reduce adverse reactions by altering the physicochemical properties of antimicrobials. Bacteria have diverse defense systems against toxic xenobiotics, and some enzymes can modify or destroy active compounds. Polymeric nanoparticles exhibit good antimicrobial activity, with salicylic acid showing a 23mm zone of inhibition against Bacillus subtilis and 14mm zone against Pseudomonas aeruginosa..

3.3 Result of Anti biofilm activity by Test tube method

3.3.1. Anti biofilm activity of Polymeric Nanoparticles against *Bacillus subtilis*



Graph 11: Graph represents anti biofilm activity of *Bacillus subtilis*

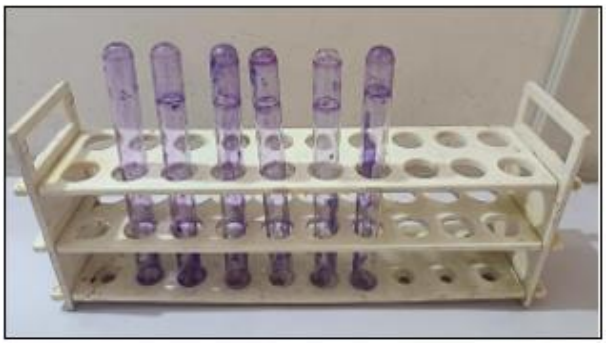
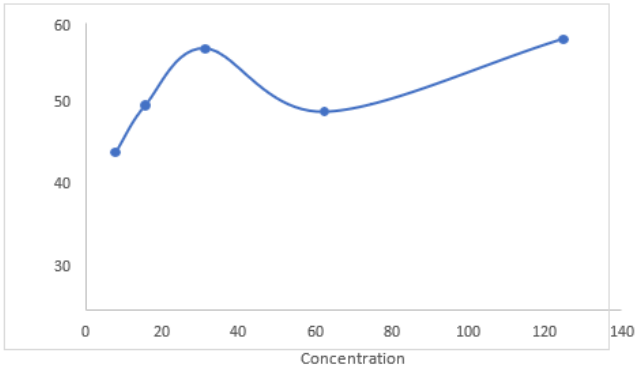


Figure 4: Anti biofilm activity of Polymeric Nanoparticles against *Bacillus subtilis*

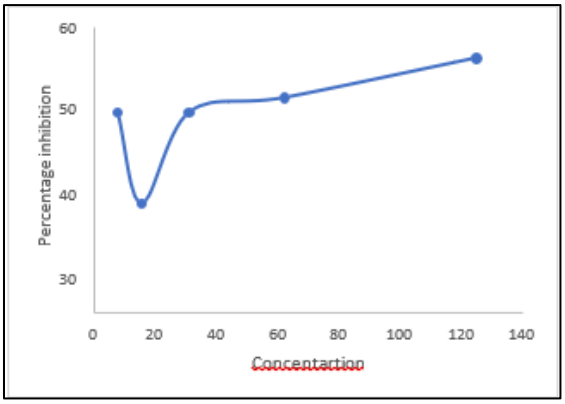
3.3.2. Anti-biofilm activity of Polymeric Nanoparticles against *Pseudomonas aeruginosa*



Graph 12: Graph represents anti biofilm activity of *Pseudomonas aeruginosa*

Figure 5: Anti-biofilm activity of Polymeric Nanoparticles against *Pseudomonas aeruginosa*

The anti-biofilm activity of the nanoparticle was assessed using the test tube method. The maximum percentage inhibition of bacterial growth at 125µg\ml highlights the potential effectiveness of a compound or formulation against two significant pathogens. exhibited a ***Bacillus subtilis*** and ***Pseudomonas aeruginosa*** remarkable inhibition rate 53.47% and 56.57% and showed a zone of inhibition.



Graph 13: Graph represents anti biofilm activity of *Bacillus subtilis*

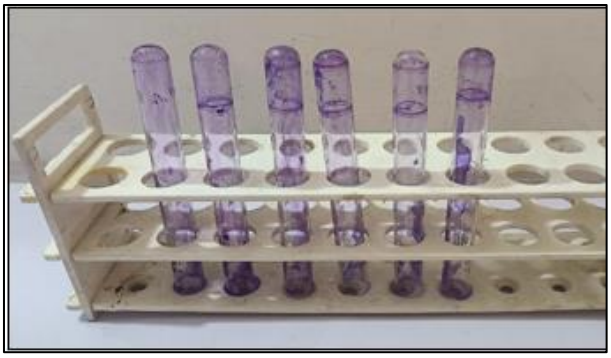
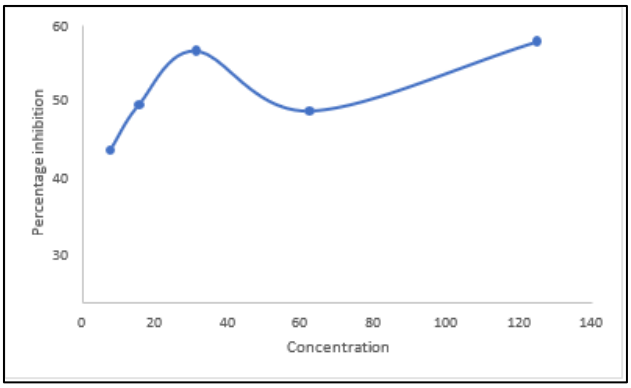
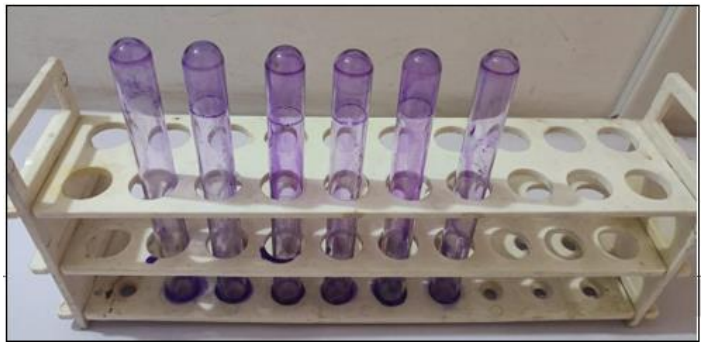


Figure 6: Anti biofilm activity of Polymeric Nanoparticles against *Bacillus subtilis*

3.3.3. Anti-biofilm activity of Polymeric Nanoparticles against *Pseudomonas aeruginosa*



Graph 13: Graph represents anti biofilm activity of *Pseudomonas aeruginosa*



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4. CONCLUSION

The study demonstrates the potential of Eudragit RS-100 polymeric nanoparticles as effective drug delivery systems, particularly for antibacterial and anti-biofilm applications. The solvent evaporation method proved efficient in formulating stable nanoparticles with controlled release properties. The nanoparticles exhibited significant antibacterial activity against both Gram-positive (Bacillus subtilis) and Gram-negative (Pseudomonas aeruginosa) bacteria, as evidenced by the well diffusion assay. Furthermore, the test tube-based biofilm inhibition assay highlighted their efficacy in preventing bacterial adhesion and biofilm formation, underscoring their promise in combating bacterial infections. The advantages of polymeric nanoparticles, including controlled drug release and enhanced stability, make them a superior choice compared to liposomes, which often suffer from low encapsulation efficiency and instability. The use of biodegradable and biocompatible polymers like Eudragit RS-100, along with hydrophilic coatings such as PEG, enhances their systemic circulation and targeting capabilities, making them ideal for a range of therapeutic applications, including gene therapy.

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