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Research article

Development and evaluation of Nanocarrier-based triple antibiotic aerosol spray for targeted treatment of burns and diabetic foot ulcers

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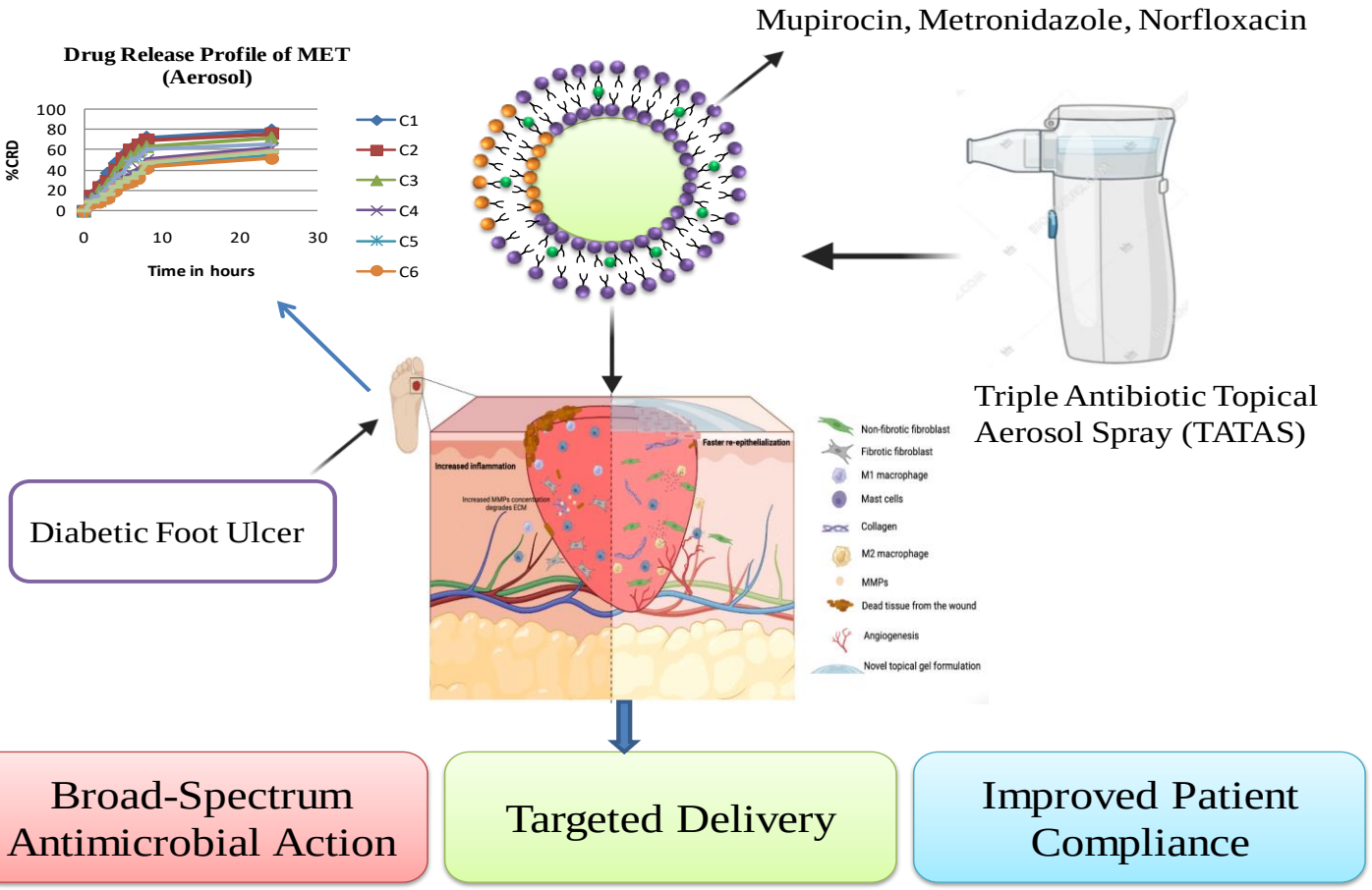
ABSTRACT

This study focuses on developing a nanocarrier-based aerosol formulation for the topical treatment of burns and diabetic foot ulcers (DFUs), which are often associated with severe complications like infections, amputations, and delayed healing.



A Triple Antibiotic Topical Aerosol Spray (TATAS) containing Metronidazole, Mupirocin, and Norfloxacin was formulated to provide broad-spectrum antimicrobial coverage, sustained drug release, and enhanced patient compliance. Eudragit E100 and Isopropyl Myristate (IPM) were selected as the primary polymer and penetration enhancer, respectively, after compatibility and solubility studies. A 3^2 full factorial design was used to optimize the formulation. Among various batches, batch F7 demonstrated cumulative drug release rates of 79.45% (Metronidazole), 80.75% (Mupirocin), and 79.66% (Norfloxacin) over 24 hours. Physicochemical properties of F7, including a pH of 6.32, viscosity of 3.48 cps, and density of 0.798 g/ml, ensured stability and ease of application. The spray exhibited a uniform pattern with a spray angle of 22° and passed all stability tests under ICH guidelines, with no signs of precipitation or crystal growth. These findings highlight the potential of TATAS as an effective and user-friendly treatment for DFUs and burns, offering targeted drug delivery, sustained action, and improved healing outcomes.

Keywords: Nano-carrier, Triple antibiotic, Diabetic foot ulcers (DFUs), Sustained drug release, Topical antimicrobial, Formulation optimization.

INTRODUCTION

Diabetic foot ulcers (DFUs) and burn wounds are major healthcare concerns due to delayed healing, high infection risks, and potential complications like amputations. Conventional topical treatments, such as creams and ointments, often suffer from poor drug penetration, inconsistent bioavailability, and patient discomfort, highlighting the need for advanced drug delivery systems.

Aerosol technology presents a promising alternative for topical drug delivery, ensuring uniform drug distribution, targeted action, and sustained release while minimizing systemic side effects. This study focuses on developing a Triple Antibiotic Topical Aerosol Spray (TATAS) incorporating Metronidazole, Mupirocin, and Norfloxacin to provide broad-spectrum antimicrobial coverage. The formulation employs Eudragit E100 as a pH-responsive polymer and Isopropyl Myristate (IPM) as a penetration enhancer, optimized using a 3^2 full factorial design. Key parameters, including drug release, viscosity, pH, spray angle, and stability, were rigorously evaluated.

By integrating nano-carriers with aerosol delivery, this formulation enhances therapeutic efficacy, reduces dosing frequency, and improves patient compliance, offering a novel strategy for effective wound management [1-2].

MATERIAL AND METHOD

Material

Metronidazole, Mupirocin, and Norfloxacin were obtained from Sigma-Aldrich. Eudragit E100, PVP K30, Carbopol 934, HPMC 100 LV, Ethyl Acetate, and Acetone were procured from HiMedia Laboratories. All chemicals and reagents used in this study were of analytical grade.

Methods

Solubility Study

The solubility of each drug was qualitatively assessed in various solvents. A 50 mg sample of the drug was added to 2 mL of the selected solvent in a capped vial, heated in a water bath at 40°C for 30 minutes, and stirred at 50 rpm at room temperature for 48 hours. The samples were then centrifuged at 5000 rpm for 15 minutes, and the supernatant was analyzed using UV-Visible spectroscopy [3-4].

The solvents tested included ethyl acetate, 0.1 N HCl, 0.1 N NaOH, and water.

UV-Vis Spectroscopy

Standard solutions of Metronidazole, Mupirocin, and Norfloxacin were prepared at a concentration of 10 µg/mL. Stock solutions of the drugs were prepared in their respective solvents and further diluted as needed. Each solution was scanned using a UV-Visible spectrophotometer across the 200–400 nm wavelength range to determine the absorption maxima (λ_{max}). The spectrophotometer was calibrated before analysis to ensure accurate results. The λ_{max} values were recorded and used for drug quantification and compatibility evaluation [5].

Drug Dose Calculation

Metronidazole and Mupirocin are commercially available in 1% w/w topical formulations, while Norfloxacin is primarily marketed as eye drops. For consistency, a 1% w/w drug concentration was selected for all three drugs to prepare a 20 mL aerosol spray batch [6-7].

Method of Preparation of Triple Antibiotic Topical Spray Containing Metronidazole, Mupirocin, and Norfloxacin

A. Selection of solvent and plasticizer

A 50 µg sample of Metronidazole, Mupirocin, and Norfloxacin was dissolved in 2 mL of screened vehicles, heated at 40°C for 30 minutes, and stirred at 50 rpm for 48 hours. The mixtures were then centrifuged at 5000 rpm for 15 minutes, and the supernatants were analyzed via UV-visible spectroscopy to determine drug solubility.

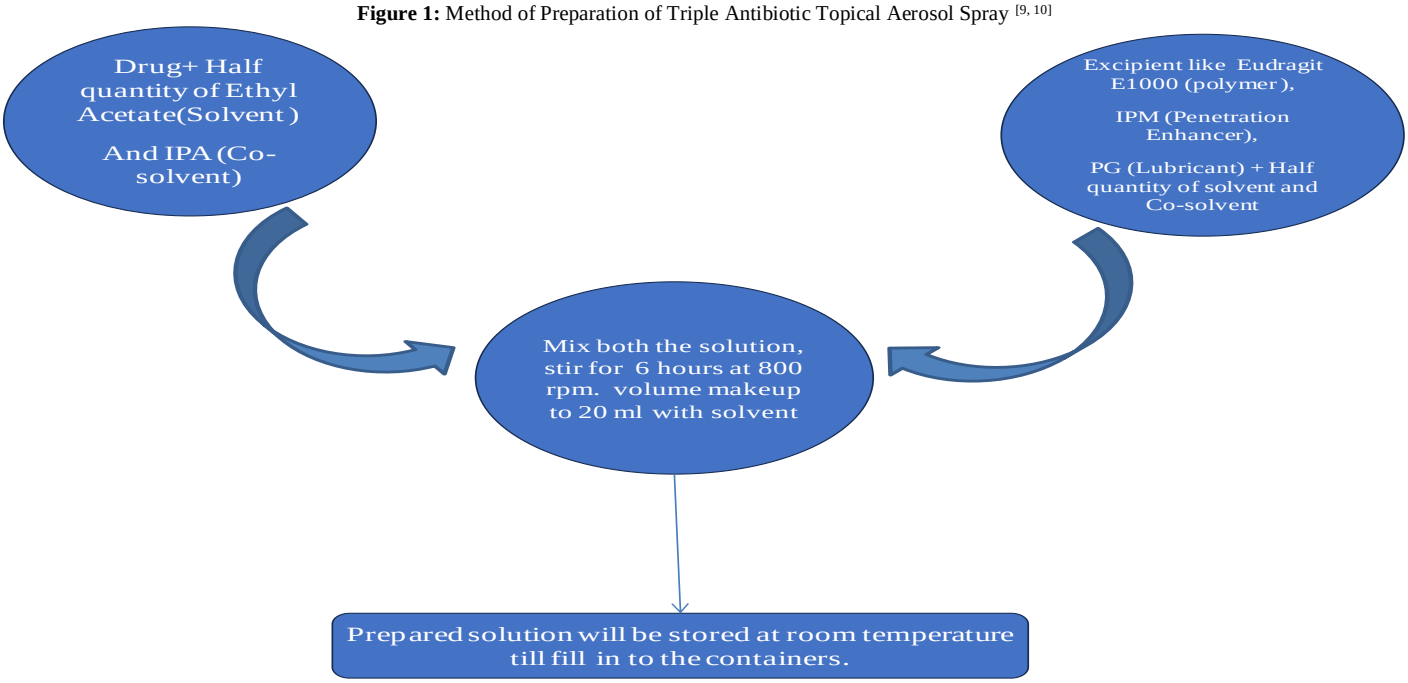
B. Selection of Polymer

A 100 mg polymer sample was dissolved in 2 mL of screened vehicles, heated at 40°C for 30 minutes, and stirred at 50 rpm for 48 hours. After centrifugation at 5000 rpm for 15 minutes, the supernatants were analyzed via UV-visible spectroscopy to assess polymer solubility and drug compatibility.

C. Physical Compatibility of Polymers with Solvents

Polymer-solvent solutions were stored for one week under laboratory conditions and monitored for precipitation or crystal growth to evaluate physical compatibility [8-9].

Figure 1: Method of Preparation of Triple Antibiotic Topical Aerosol Spray [9, 10]



A. Preparation of Preliminary Batches

Preliminary batches were prepared using Eudragit E100, PVP K30, and their 1:1 combination, maintaining constant concentrations of the drug, PG, IPA, and IPM. The compositions are detailed in Table 1, and the formulations were assessed for drug release performance [10].

Based on the results of the preliminary study, Eudragit E100 was selected as the polymer for further formulation development. The selection was made considering its superior diffusion release rate, as evidenced by the comparative analysis [11, 12]. Subsequent optimization focused on refining the formulation variables to enhance drug release and overall performance.

B. Optimization of Batches Based on Preliminary Study

Table 1: Composition of Preliminary Batches

Batch Code	Polymer Used	Polymer Concentration (%)	Each Drug (µg)	IPA (mL)	PG (mL)	IPM (mL)	Ethyl Acetate
C1	Eudragit E100	0.5	200	1	1.5	0.5	16.70
C2		0.75	200	1	1.5	0.5	16.65
C3		1.0	200	1	1.5	0.5	16.60
C4	PVP K30	0.5	200	1	1.5	0.5	16.70
C5		0.75	200	1	1.5	0.5	16.65
C6		1.0	200	1	1.5	0.5	16.60
C7	Eudragit E100 and PVP K30 (1: 1)	0.5	200	1	1.5	0.5	16.70
C8		0.75	200	1	1.5	0.5	16.65
C9		1.0	200	1	1.5	0.5	16.60

C. Concentration of Drugs & Excipients

Table 2: Composition of Drugs and Excipients for Optimization Using 3^2 Factorial Design

Each Drug	Polymer	IPA	IPM	PG	Ethyl Acetate
200 µg	100-200µg	1ml	0.5-1.5 ml	2 ml	Up to 20 ml

Table 3: Variables and levels of 3^2 full factorial designs of Triple Antibiotic Aerosol Spray

Variables and levels of full factorial design 3^2 for Triple Antibiotic Aerosol Spray			
Variables	**Low (1)**	**Medium (0)**	**High (+1)**
Eudragit E100 (mg)	100	150	200
Isopropyl myristate (ml)	0.5	1	1.5

Table 4: Composition of 3^2 full factorial designs of Triple Antibiotic Topical Aerosol Spray

Composition of Triple Antibiotic Topical Aerosol Spray						
Batch Code	**Eudragit E100 (mg)**	**Isopropyl myristate (IPM) (ml)**	**Each Drug (µg)**	**Propylene glycol (ml)**	**Isopropyl Alcohol (IPA) (ml)**	**Ethyl Acetate (ml)**
F1	100	0.5	200	2	1	16.70
F2	150	0.5	200	2	1	16.65
F3	200	0.5	200	2	1	16.60
F4	100	1	200	2	1	16.70
F5	150	1	200	2	1	16.65
F6	200	1	200	2	1	16.60
F7	100	1.5	200	2	1	16.70

F8	150	1.5	200	2	1	16.65
F9	200	1.5	200	2	1	16.60

Evaluation of Topical Spray

A. Evaluation of Physicochemical Characterization of Topical Aerosol

a. Spray pattern

The spray pattern of the optimized aerosol formulation was evaluated by spraying the solution onto a clean glass plate placed at a fixed distance from the nozzle. The spray distribution was visually examined for uniformity and coverage. The procedure was repeated three times for each formulation, and the results were compared to ensure consistency [13, 14].

b. Drug content uniformity

The drug content of the topical spray was determined by withdrawing 1 mL of the solution and diluting it appropriately with a suitable solvent. The solution was analyzed using UV-visible spectroscopy at the respective λ_{max} of the drugs. The study was performed in triplicate, and the mean drug content for each formulation was calculated to ensure uniformity in drug distribution [15, 16].

c. Particle size and zeta potential

The particle size and zeta potential of the aerosol formulation were measured using a dynamic light scattering (DLS) instrument. A sample of the spray solution was diluted with distilled water and analyzed at room temperature. Measurements were performed in triplicate to ensure accuracy and reproducibility. The results were used to assess the stability and uniformity of the formulation [17, 18].

d. Pressure test

The aerosol container pressure was evaluated per USP guidelines at 25°C using a pressure gauge, with readings ranging from 5.0 to 6.0 kg/cm² due to LPG propellant use. The physicochemical properties of formulations (F1–F9) were assessed for topical suitability. The pH ranged from 6.32 to 7.15, ensuring skin compatibility, with F7 having the lowest (6.32 ± 0.05) and F5 the highest (7.15 ± 0.02). Viscosity varied between 3.15 and 4.85 cps, influencing spray consistency, while density ranged from 0.794 to 0.854 g/mL, reflecting formulation differences. These variations aid in selecting an optimal aerosol formulation [19, 20].

e. Flammability and flame extension

The flammability and flame extension tests assessed the safety of aerosol formulations (F1–F9). The flame extension ranged from 55 cm (F3) to 75 cm (F7), with F7 and F4 exhibiting the highest values, classifying them as flammable. Flame flashback values varied between 10 cm and 13 cm, with F2, F3, and F4 showing slightly higher values (12–13 cm). All formulations remained within the acceptable flame extension range (15–75 cm), ensuring compliance with safety standards. These findings emphasize the need for careful formulation optimization to balance flammability risks with product performance [21, 22].

Evaluation of Topical Spray

A. Leak Test

Leak tests were performed to ensure the integrity of the aerosol containers. Two methods were employed as described below [23].

a. Immediate leak test

The aerosol containers were submerged in warm water (approximately 50°C) for 10 seconds immediately after filling. The presence of bubbles in the water indicated leakage in the container [24].

b. Delayed leak test

The leakage test evaluated the sealing efficiency of aerosol containers across all batches (F1–F9). Both immediate and delayed tests showed no leakage, confirming robust sealing and structural integrity. No weight differences were observed after two months of storage, ensuring product stability. These results validate the suitability of the containers for long-term storage and distribution, supporting their reliability for clinical and commercial applications [25, 26, and 27].

c. Spraying angle test

The spraying angle was assessed by releasing the aerosol onto paper from a 1 cm distance. The radius of the resulting spray pattern was measured to calculate the spray angle, determining distribution efficiency. This evaluation provided insights into the formulation's coverage and dispersion performance [28, 29, 30]. The spray angle was calculated from the following equation:

$$\text{Spray angle } (\Theta) = \tan^{-1}(1/R)$$

Where 1 cm is a distance of paper from the nozzle and R is the average radius of the circle.

d. Delivery rate

The delivery rate of the topical spray was evaluated as per USP guidelines. Four aerosol containers were weighed before and after actuating the valve for 5 seconds at 25°C. This process was repeated three times per container, and the average delivery rate was calculated in grams per second [31, 32].

e. Delivery amount

The delivery amount and rate of Triple Antibiotic Topical Spray formulations (F1–F9) were assessed per USP guidelines. The delivery amount ranged from 0.735 g to 0.986 g per 5 seconds, with F7 exhibiting the highest (0.986 g/5 sec) and F3 the lowest (0.735 g/5 sec). The delivery rate varied from 0.147 g/sec to 0.189 g/sec, with F7 showing the highest rate. These variations were influenced by viscosity and density, impacting sprayability. Formulations with lower viscosity and moderate density, such as F7 and F4, achieved optimal delivery performance [33, 34].

f. Minimum fills of topical spray

The minimum fill of the topical spray formulations (F1–F9) was assessed for compliance with quality standards. All formulations exceeded 100% fill, ranging from 100.17% to 100.85%, ensuring regulatory adherence and consumer satisfaction. F7 exhibited the highest fill percentage (100.85 ± 0.89%), while F3 had the lowest (100.17 ± 0.89%). The minimal variation across batches indicates a precise and controlled filling process, ensuring product uniformity and quality [35, 36].

g. Drug content study

The drug content of the topical spray formulations (F1–F9) was evaluated using a validated analytical method. Samples were prepared by diluting the spray solution with a suitable solvent and analyzed via UV-visible spectroscopy or HPLC. The drug content for Metronidazole, Mupirocin, and Norfloxacin ranged from 98.25% to 99.91% of the labeled claim, confirming uniformity and formulation reliability. Triplicate analysis ensured accuracy and precision, demonstrating high consistency across batches.

h. Particle size and size distribution

The particle size and distribution of the topical spray formulations (F1–F9) were analyzed using dynamic light scattering (DLS) to assess uniformity and suitability for topical application. Particle sizes ranged from 67.54 nm (F3) to 110.68 nm (F4), influenced by formulation composition. Smaller sizes, such as F3 (67.54 ± 1.49 nm) and F1 (77.9 ± 1.34 nm), may enhance skin penetration, while larger sizes, like F4 (110.68 ± 1.38 nm) and F8 (110.29 ± 1.64 nm), could impact sprayability. Minimal standard deviations across batches confirmed formulation consistency, highlighting the need for optimization to ensure effective delivery and uniform application.

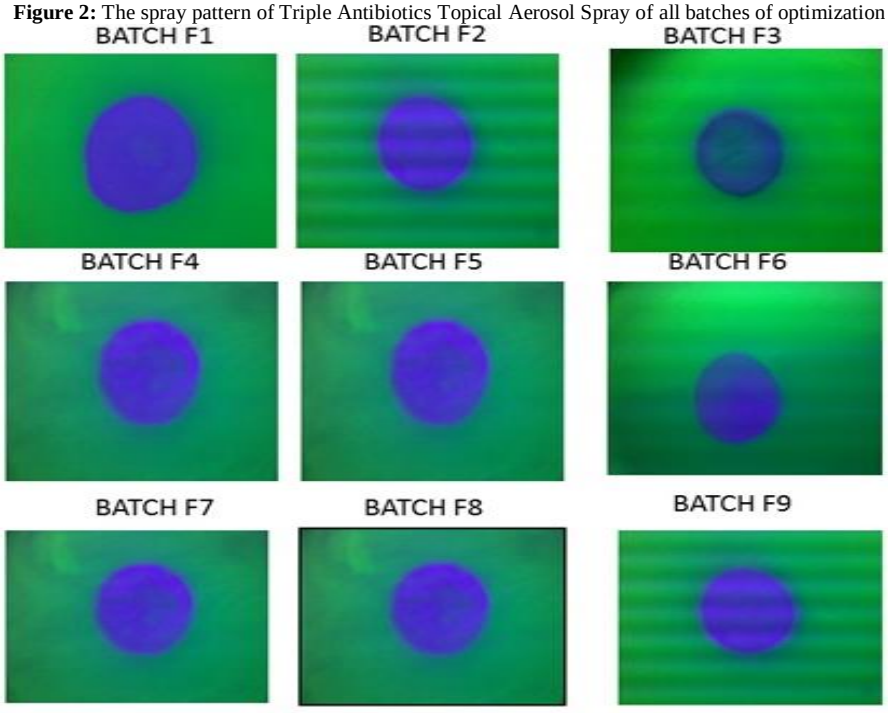
i. *In-Vitro* drug release study

In-vitro drug release studies were performed using a Franz diffusion cell with cellophane membrane and a receptor medium (ethyl acetate: phosphate buffer, pH 6.8, 80:20) at 37°C. A spray solution (10 mg drug) was applied to the donor side, and samples were periodically withdrawn, analyzed via UV spectrophotometry, and replaced to maintain sink conditions. The study ensured consistent and reliable drug release profiling.

j. Stability studies

The optimized topical spray's stability was assessed per ICH guidelines at 40°C ± 2°C and 75% ± 5% RH for 90 days. Periodic evaluations of physical appearance, drug content, and pH confirmed its stability, ensuring long-term efficacy.

Figure 2: The spray pattern of Triple Antibiotics Topical Aerosol Spray of all batches of optimization



RESULTS AND DISCUSSION

Pre Formulation Studies

Physical appearance

The physical appearance and melting points of the selected antibiotics were analyzed to ensure their suitability for the topical spray formulation. Metronidazole appeared as a white to pale yellow crystalline powder with a slight odor, with a melting point of 162 ± 2°C. Mupirocin was observed as a white to off-white crystalline powder, melting at 76 ± 0.5°C. Norfloxacin exhibited a white to pale yellow crystalline appearance, with a melting point of 219 ± 1°C. These findings confirmed the stability and purity of the antibiotics, essential for their effective incorporation into the formulation.

UV spectroscopy of antibiotics

UV spectroscopic analysis of Metronidazole, Mupirocin, and Norfloxacin was performed in different solvent systems to establish calibration curves and assess absorbance behavior. Both ethyl acetate and ethyl acetate: PBS systems demonstrated high

linearity and reliability for quantification, with solvent choice impacting sensitivity. Ethyl acetate enhanced absorptivity, while the PBS mixture improved versatility. Strong correlation coefficients confirmed method robustness, ensuring precise drug quantification for development.

Table 5: Physical appearance and melting point

Antibiotics	Appearance	Melting Point
Metronidazole	White to pale yellow crystalline powder with a slight odor.	162±2 °C
Mupirocin	White to off-white crystalline powder	76±0.5 °C
Norfloxacin	White to pale yellow crystalline powder	219±1 °C

Solubility study

The selection of solvent, plasticizer, and polymer was based on a solubility study. The results are shown in table 6.1 to 6.5.

Table 6.1: Solubility of antibiotics in different solvents (Mean ± SD; n=3)

Drug	Ethyl Acetate	0.1N HCl	0.1N NaOH	Water
Metronidazole	5.96 ± 0.12	0.05 ± 0.01	3.0± 0.08	0.07 ± 0.01
Mupirocin	6.2± 0.15	0.09 ± 0.02	3.8 ± 0.10	0.12 ± 0.02
Norfloxacin	4.5 ± 0.21	0.02 ± 0.01	1.8± 0.15	0.26 ± 0.12

Table 6.2: Selection of different polymers based on solubility study of Metronidazole (Mean ± SD; n=3)

Polymers	Metronidazole + Solvents	Solubility (mg/mL)
Eudragit E100	Ethyl acetate	43.32±0.16
	Acetone	22.23±2.43
	Ethyl acetate: Acetone (2:1)	37.4±2.03
PVP K30	Ethyl acetate	34.5±1.44
	Acetone	38.06±0.45
	Ethyl acetate: Acetone (2:1)	36.45±0.59
Carbopol 934	Ethyl acetate	11.43±1.85
	Acetone	15.66±3.56
	Ethyl acetate: Acetone (2:1)	17.21±2.34
HPMC 100 LV	Ethyl acetate	5.2±2.89
	Acetone	8.6±1.19
	Ethyl acetate: Acetone (2:1)	7.3±2.67

Table 6.3: Selection of different polymers based on solubility study of Mupirocin (Mean ± SD; n=3)

Polymers	Mupirocin + Solvents	Solubility (mg/ml)
Eudragit E100	Ethyl acetate	45.2±0.23
	Acetone	19.23±1.59
	Ethyl acetate: Acetone (2:1)	32.4±2.43
PVP K30	Ethyl acetate	31.5±1.35
	Acetone	34.06±2.15
	Ethyl acetate: Acetone (2:1)	29.45±0.52
Carbopol 934	Ethyl acetate	14.63±0.75
	Acetone	17.62±2.96
	Ethyl acetate: Acetone (2:1)	18.21±1.12
HPMC 100 LV	Ethyl acetate	4.2±1.64
	Acetone	7.6±1.68
	Ethyl acetate: Acetone (2:1)	7.9±2.15

Table 6.4: Selection of different polymers based on the solubility study of Norfloxacin

Polymers	Norfloxacin + Solvents	Solubility (mg/ml
Eudragit E100	Ethyl acetate	46.18±0.19
	Acetone	19.13±1.12
	Ethyl acetate: Acetone (2:1)	38.4±2.32
PVP K30	Ethyl acetate	33.43±1.89
	Acetone	38.73±0.68
	Ethyl acetate: Acetone (2:1)	39.15±0.32
Carbopol 934	Ethyl acetate	13.12±1.85
	Acetone	15.26±4.20
	Ethyl acetate: Acetone (2:1)	16.88±2.44
HPMC 100 LV	Ethyl acetate	8.2±1.92
	Acetone	8.6±1.19
	Ethyl acetate: Acetone (2:1)	7.3±2.71

Table 6.5: Selection of plasticizer based on solubility study (Mean ± SD; n=3)

Drug + Solvent	Propylene Glycol	Glycerin	Castor Oil
Metronidazole + EA	8.6 ± 0.44	2.1 ± 0.21	5.3 ± 0.37
Mupirocin +EA	6.0 ± 0.20	1.8 ± 0.15	4.0 ± 0.25
Norfloxacin +EA	3.0 ± 0.25	0.5 ± 0.10	1.5 ± 0.20

The selection of solvent and plasticizer was based on the solubility of all three antibiotics in solvent and plasticizer. From the screened solvents and plasticizers Ethyl acetate as a solvent, IPA as a co-solvent, and propylene glycol (PG) as a plasticizer were selected due

to the greater solubility of all the three antibiotics in Ethylacetate and PG in the presence of IPA. The selection of polymer was also based on a solubility study. From the study, Eudragit E 100 and PVP K 30 were selected for preliminary optimization batches based on greater solubility and compatibility in Ethyl acetate. A preparation containing Carbopol 934 and HPMC 100LV was rejected due to particle size and crystal growth in Ethyl acetate, acetone, and a mixture of ethyl acetate with acetone.

Formulation Development and Characterization

Aerosol container specification

The aerosol containers (Samples I, II, and III) showed consistent dimensions, with an average height of 97.47 mm and a capacity of 50 ml. Key specifications, including wall thickness (0.992 mm), dip tube (3.016 mm ID, 4.2 mm OD), and valve body opening (0.91 mm), exhibited minimal variation. The uniformity in weight (17.83 g) and dimensions ensures reliable spray performance.

***In- Vitro* drug release data of Preliminary batches C1 to C9 (Metronidazole)**

The *in-vitro* drug release profiles of Metronidazole, Mupirocin, and Norfloxacin were evaluated for preliminary batches (C1 to C9).

Among the formulations, batches containing Eudragit E100 demonstrated a superior release profile, making it the polymer of choice for further optimization. Similar to Metronidazole, the formulations with Eudragit E100 exhibited enhanced drug release, supporting its selection for subsequent studies.

The release behavior of Norfloxacin further reinforced the efficacy of Eudragit E100 in achieving desirable drug release. It was observed that formulations containing PVP K30 polymer produced turbidity (milky appearance), rendering it unsuitable for aerosol formulation and excluding it from further optimization. Based on these findings, subsequent optimization of batches was performed using a 3^2 full factorial design.

Polymer Eudragit E100 was selected from C1, C2, and C3 batches due to a greater release profile. But PVP K30 polymer in aerosol formulation produces turbidity (Milky), therefore not considered for further optimization. Further optimization of batches was done by 3^2 full factorial designs.

Figure 3: *In- Vitro* drug release data of Preliminary batches C1 to C9 (Metronidazole)

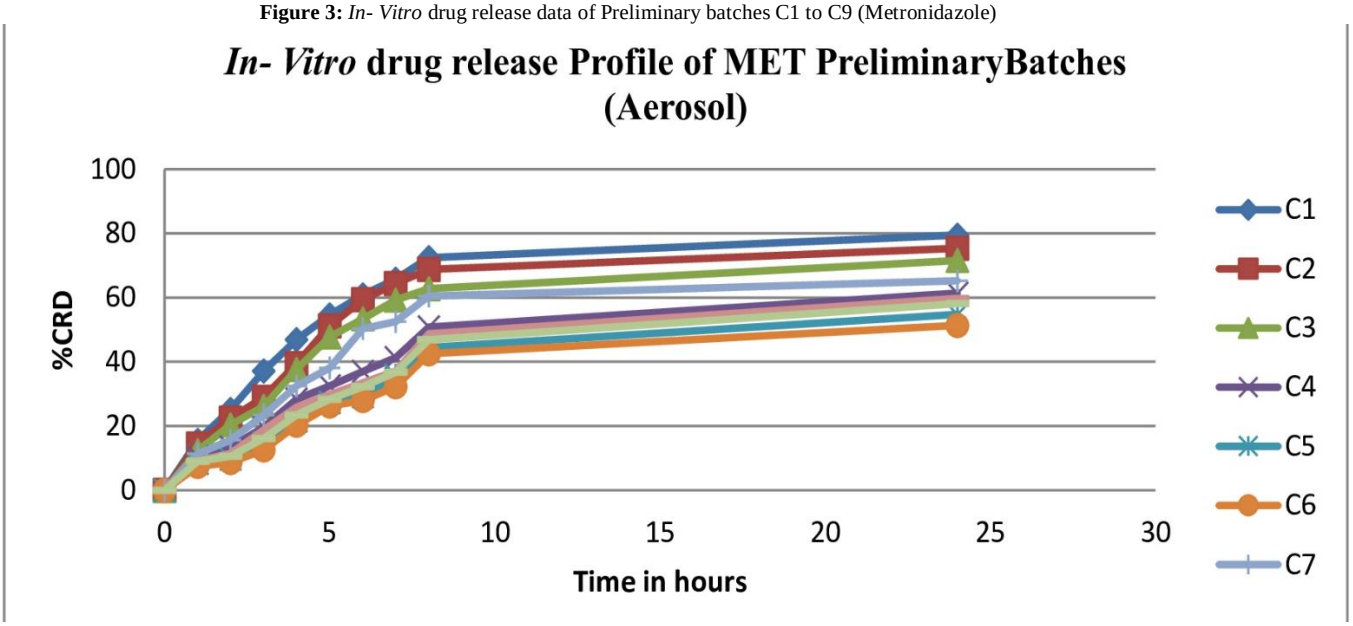


Figure 4: *In- Vitro* drug release data of Preliminary batches of Mupirocin

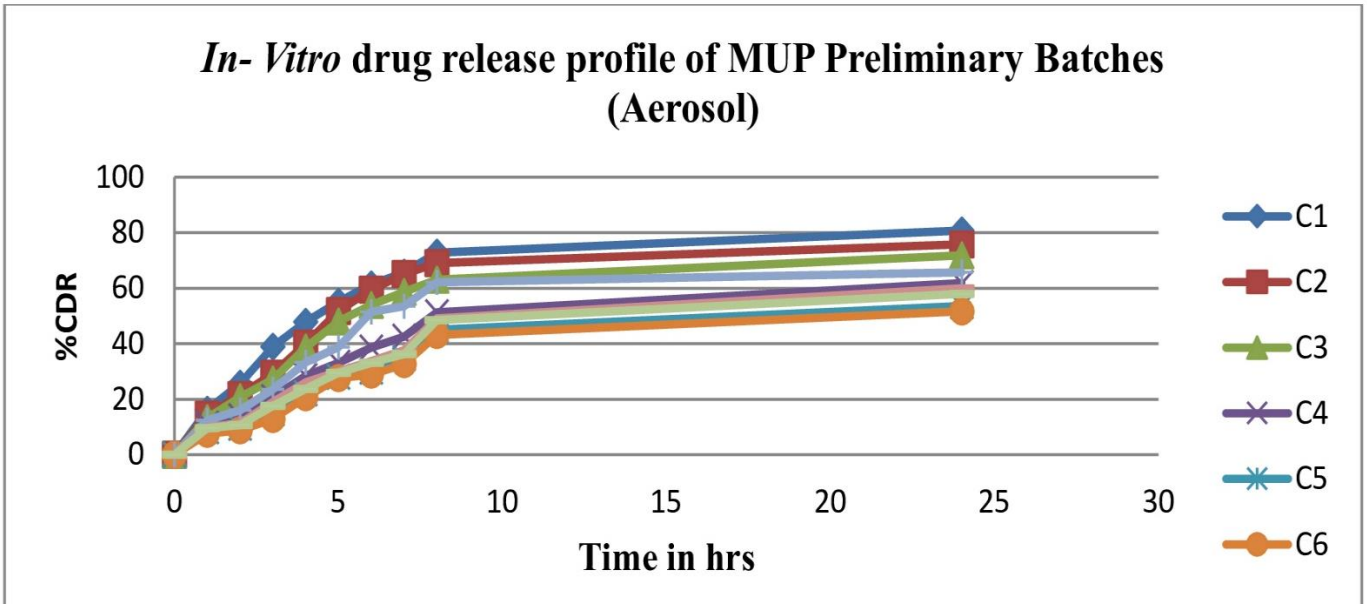
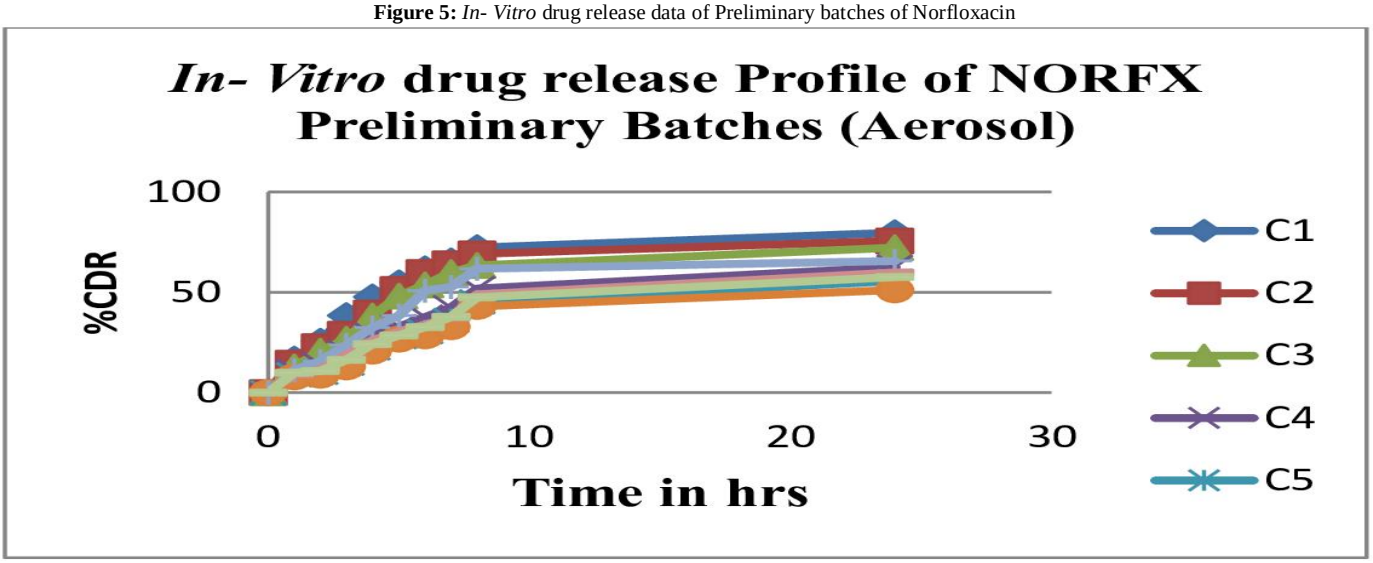


Figure 5: *In- Vitro* drug release data of Preliminary batches of Norfloxacin



Diffusion release Profiles of Optimize batches from F1-F9

Diffusion release of Optimize batches was performed by using the Franz diffusion cell. The results are shown in figures.

Figure 6: Diffusion release profile of optimized batches (F1-F9) (Metronidazole)

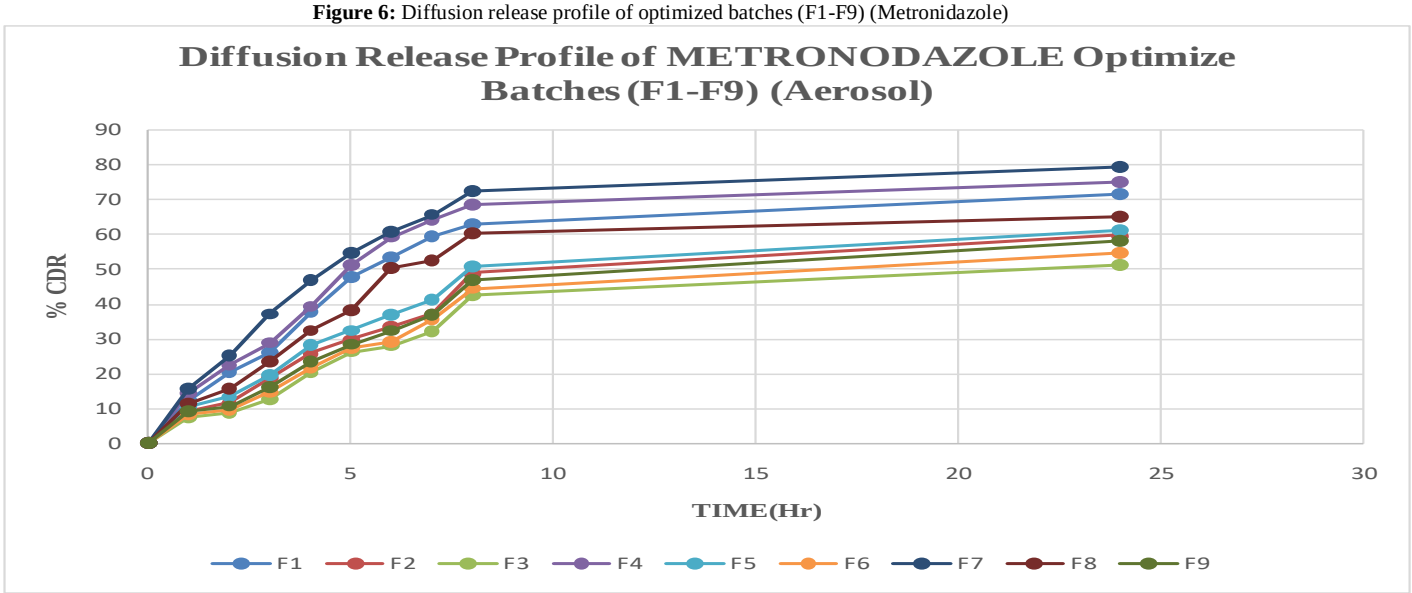


Figure 7: Diffusion release profile of optimized batches (F1-F9) (Mupirocin)

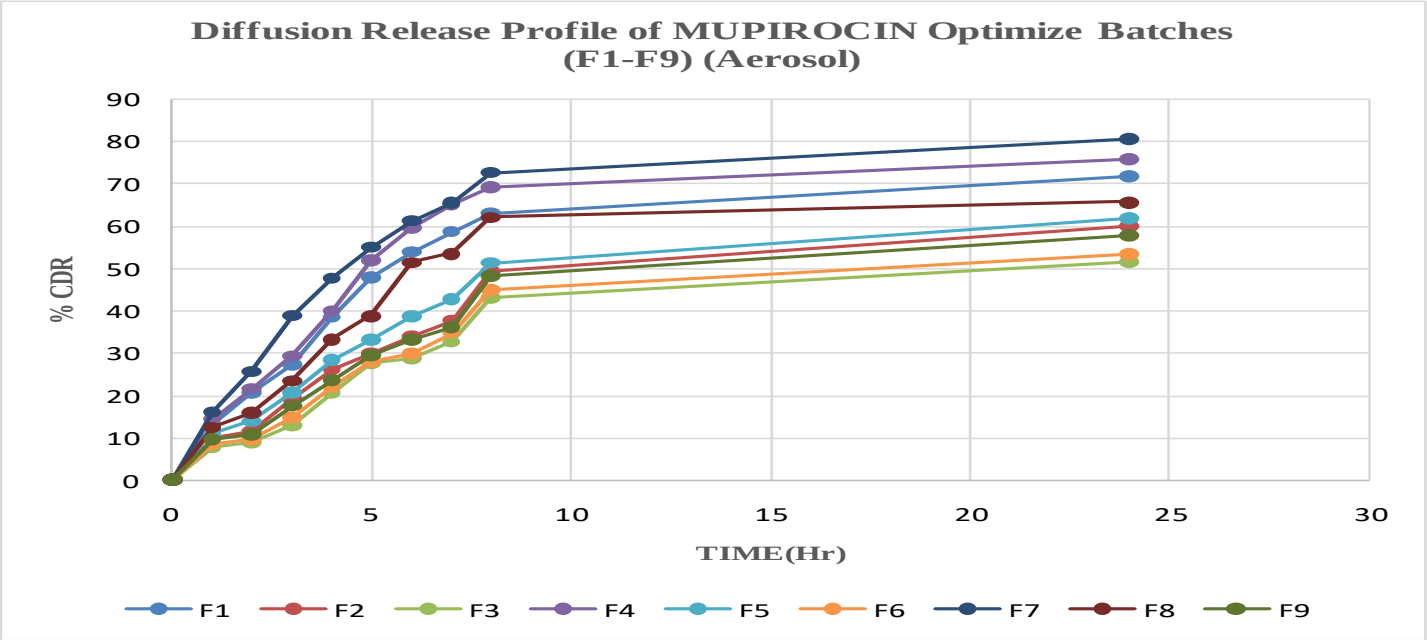
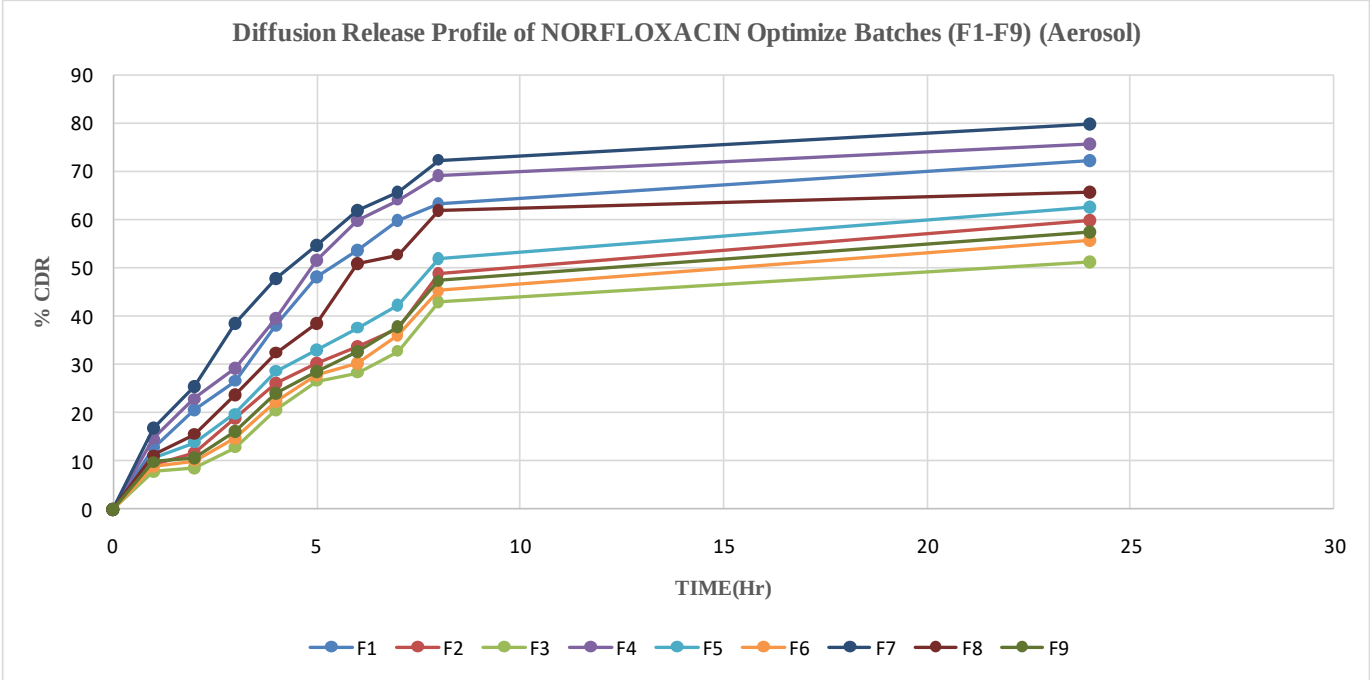


Figure 8: Diffusion release profile of optimized batches (F1-F9) (Norfloxacin)



Formulations F1, F4, F5, **F7** and F8 had greater release profile from F1 to F9 batches. When the concentration of IPM increased and Eudragit E100 decreased then the release of the formulation was also increased. A higher concentration of Eudragit E100 was responsible for less release of the drug from the formulations. From the diffusion release profiles of optimized batches from F1 to F9, F7 showed the highest drug release profile.

Particle Size Analysis

The optimized F7 batch showed a Z-average diameter of 234.7 d.nm with a PDI of 0.254, indicating uniform particle distribution. Zeta potential was -0.705 mV, ensuring formulation stability. The single sharp peak confirmed no aggregation, validating its suitability for topical application.

Figure 9: Particle size of triple antibiotic topical aerosol spray

Results

			Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	234.7	Peak 1:	239.7	96.4	80.70
PdI:	0.254	Peak 2:	4015	3.6	1162
Intercept:	0.693	Peak 3:	0.000	0.0	0.000
Result quality	Good				

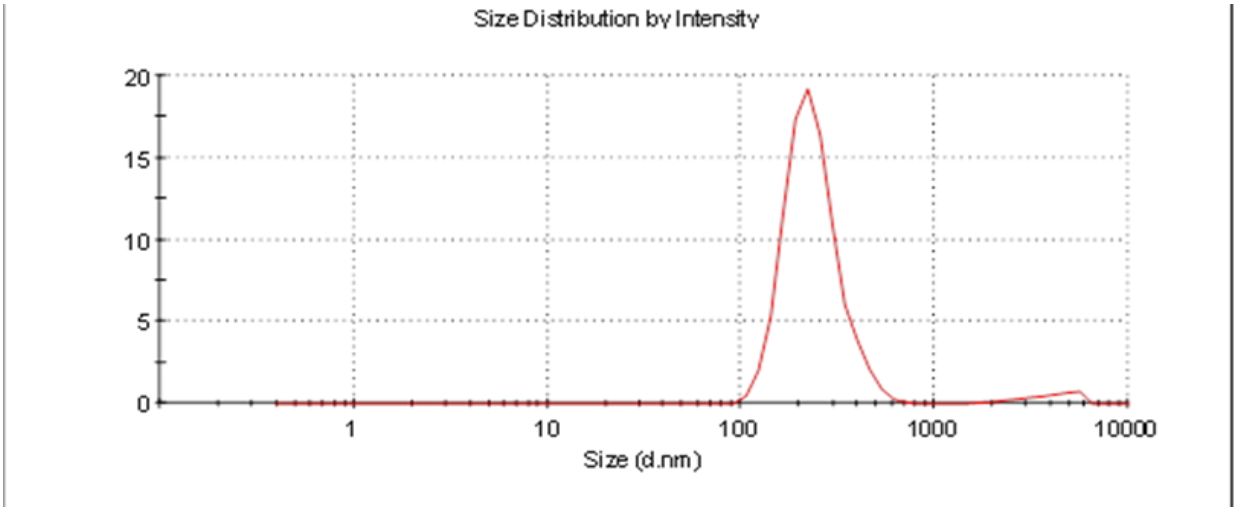
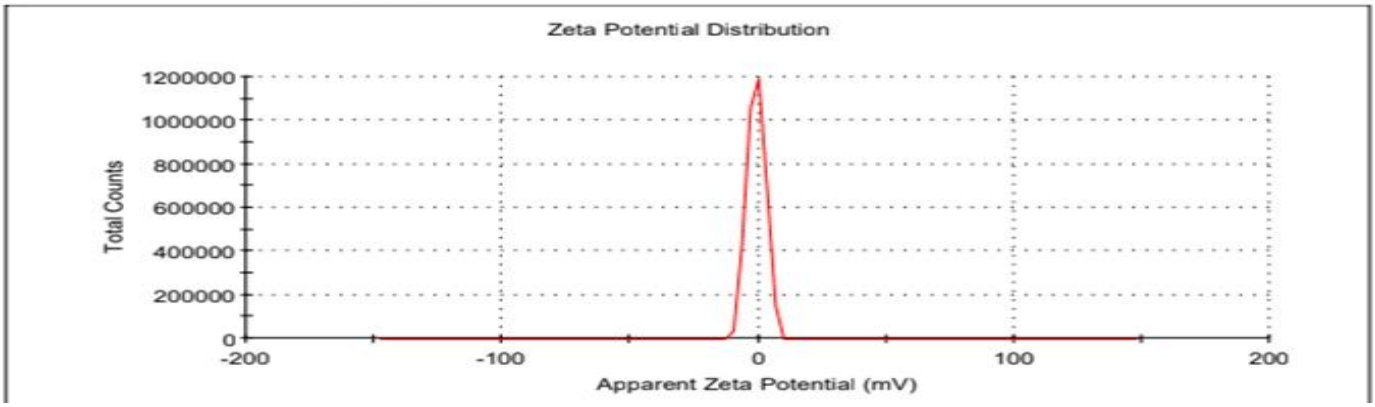


Figure 10: Graph of zeta sizer for particle size and size distribution of Optimized F7 Batch

Results

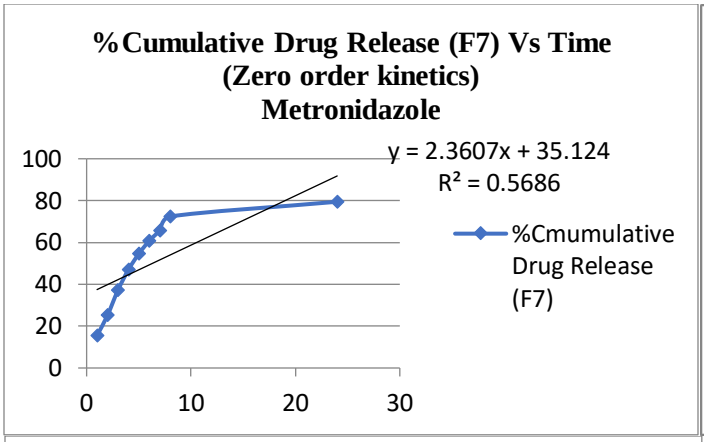
			Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV):	-0.705	Peak 1:	-0.705	100.0	3.53
Zeta Deviation (mV):	3.53	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm):	0.612	Peak 3:	0.00	0.0	0.00
Result quality	Good				

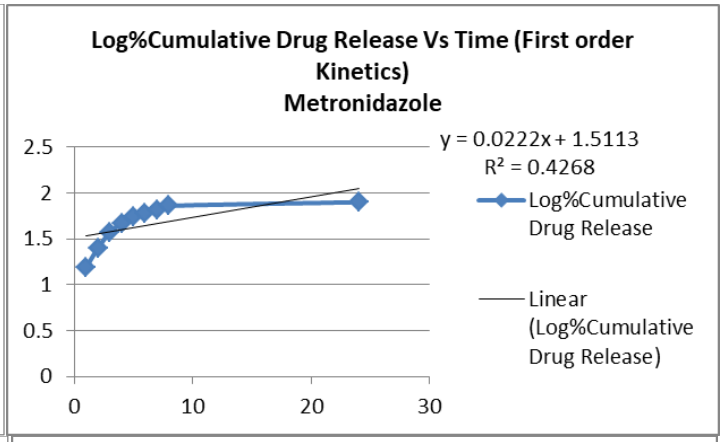


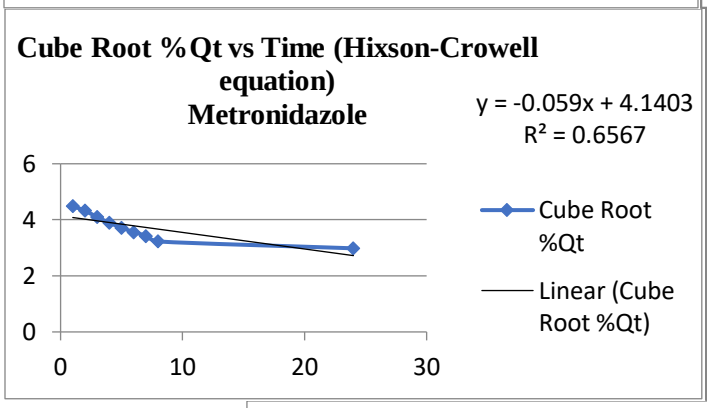
Kinetic studies of the optimized batch F7

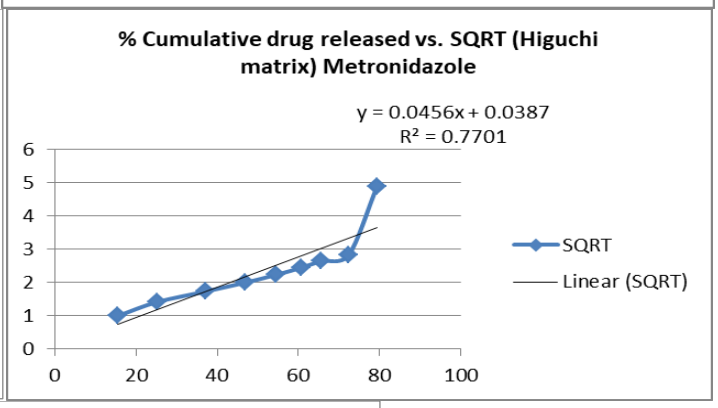
Diffusion data of the optimized batch F7 for all three drugs were applied to various mathematical models and graphs were plotted.

Figure 11: Graphical representation of Diffusion kinetic study of optimized F7 batch (Metronidazole)









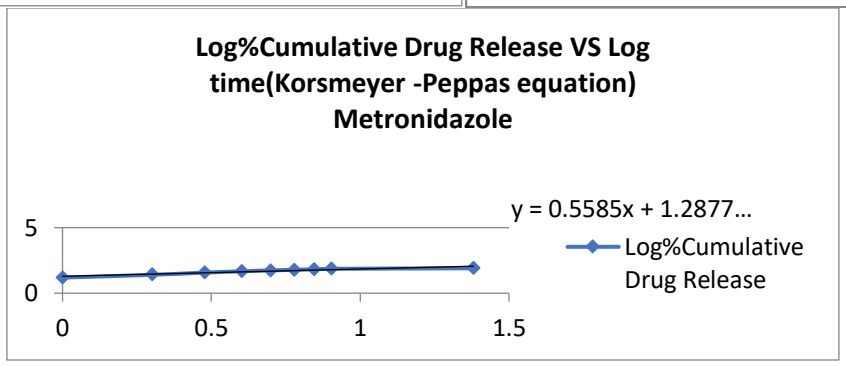
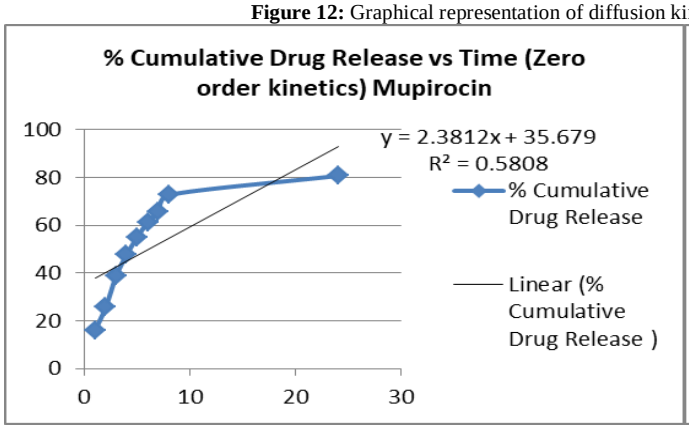
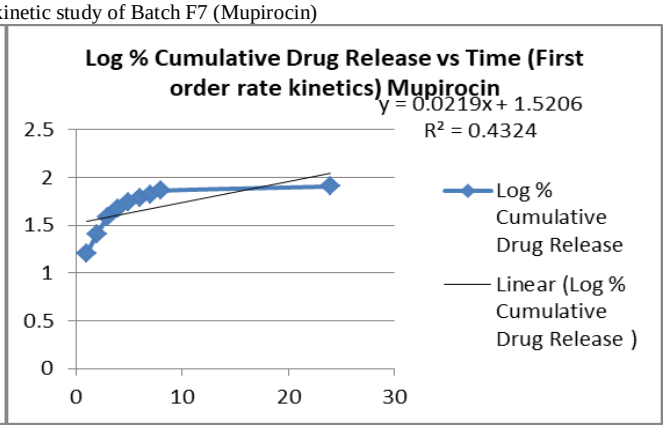
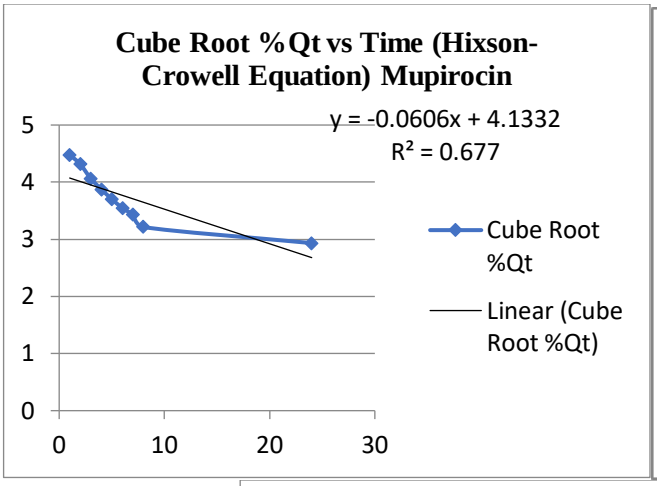
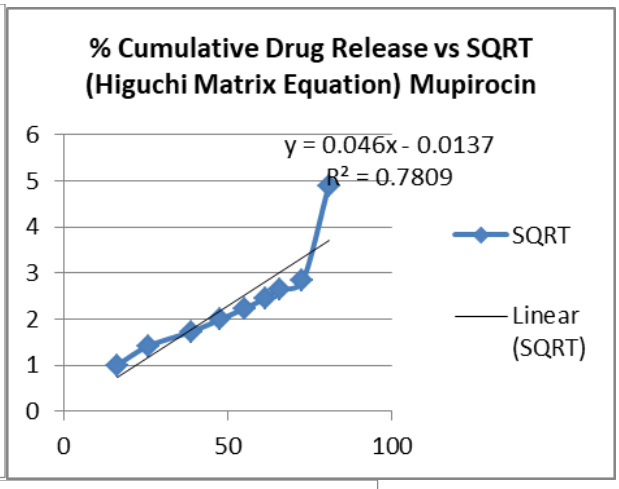


Figure 12: Graphical representation of diffusion kinetic study of Batch F7 (Mupirocin)









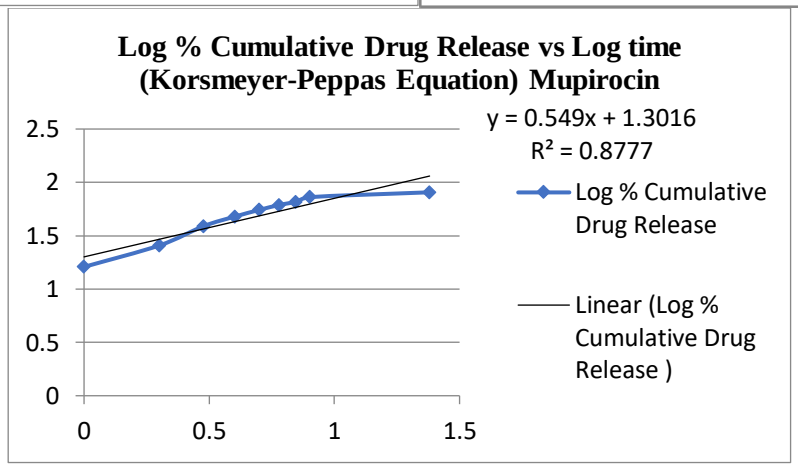
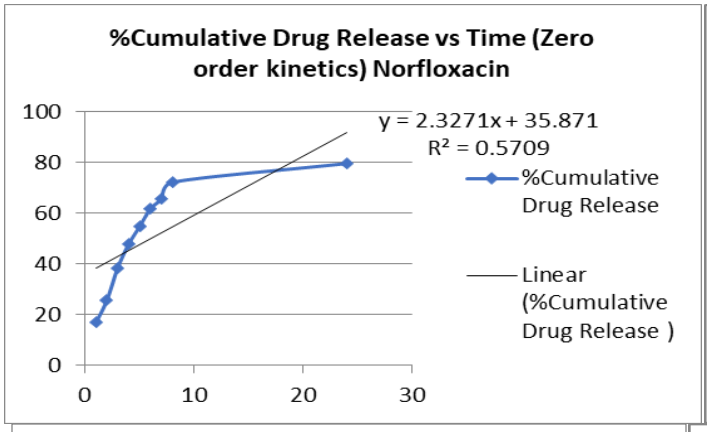
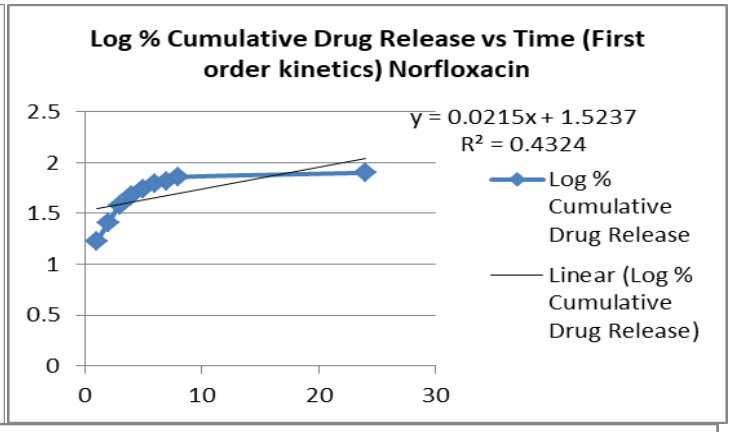


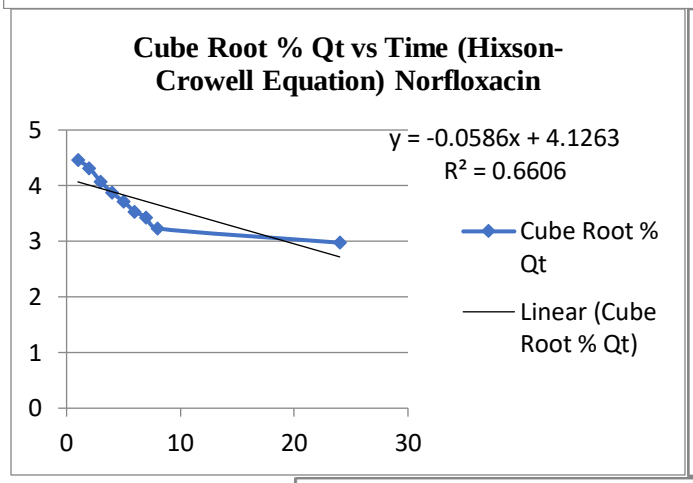
Table 7. Stability study of optimized batch F7

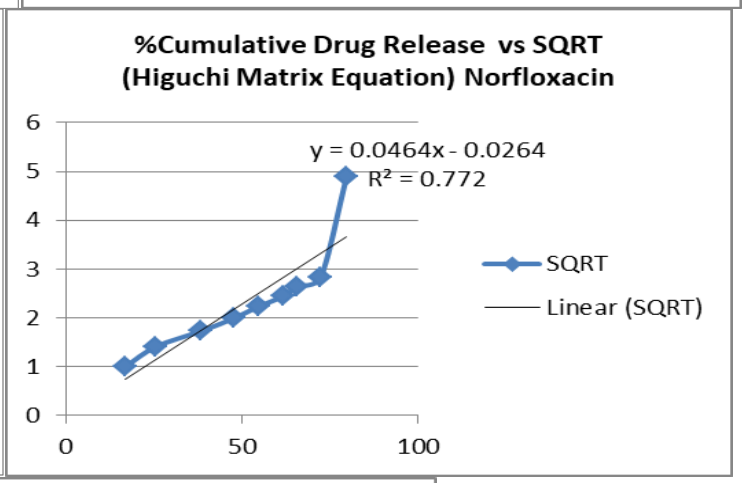
Test	Parameter	Initial	After 3 months
Physico-chemical test	pH	6.32	6.28
	Viscosity	3.48 cps	3.44 cps
	Density	0.798 gm/ml	0.794 gm/ml
	Pressure	5.89 bar	5.87 bar
	Flame Extension	75 cm	72 cm
	Flame Flashback	12 cm	10 cm
Performance test	Spray Angle	22^0	22^0
	Spray Pattern	33.4 mm	32.9 mm
	Delivery Rate	0.145 gm/sec	0.140 gm/sec
	Delivery Amount	0.986gm/5 sec	0.983gm/5 sec
	Drug Content	Metronidazole-99.87% Mupirocin-99.67% Norfloxacin- 99.59%	Metronidazole -99.74% Mupirocin-99.59% Norfloxacin- 99.52%
In-vitro drug diffusion study	%CDR After 24 hrs	Metronidazole-73.55% Mupirocin-74.75% Norfloxacin- 73.66%	Metronidazole-73.29% Mupirocin-74.56% Norfloxacin- 73.18%
Particle size	Particle size zeta potential PDI	234.7 -0.705 0.254	267.5 -0.711 0.276

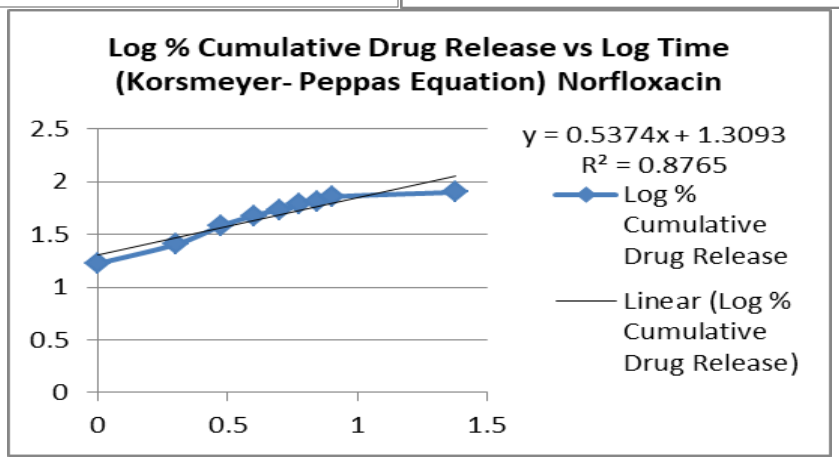
Figure 13. Graphical representation of diffusion kinetic study of Batch F7 (Norfloxacin)











The Korsmeyer-Pappas model best fit the drug release kinetics, with R^2 values of 0.874 (Metronidazole), 0.876 (Norfloxacin), and 0.877 (Mupirocin), suggesting a non-Fickian diffusion mechanism. The Higuchi model also indicated diffusion-controlled release. These findings confirm controlled and sustained drug release for improved therapeutic efficacy.

Stability study

An accelerated stability study of optimized batch F7 was conducted as per the ICH guidelines at a temperature of 40°C±2°C and Relative humidity of 75±5% RH for 3 months [37]. All the test parameters of the physicochemical test, performance test, *in-vitro* diffusion study, particle size, etc. were studied after 3 months**.**

The accelerated stability study of the optimized F7 batch, conducted under ICH conditions (40°C ± 2°C, 75% ± 5% RH) for one month, confirmed the formulation's stability across all evaluation parameters. Physicochemical tests showed minimal changes in pH (6.32 to 6.28), viscosity, and density, pressure, and flame properties, all within acceptable limits. Performance tests indicated consistent spray angle (220°), minor reductions in spray pattern (33.4 mm to 32.9 mm), and slight variations in delivery rate and amount. Drug content remained highly stable, with negligible reductions for Metronidazole (99.87% to 99.74%), Mupirocin (99.67% to 99.59%), and Norfloxacin (99.59% to 99.52%). The in-vitro drug diffusion study showed minimal changes in cumulative drug release over 24 hours, confirming sustained drug availability. Particle size increased slightly (234.7 nm to 267.5 nm), with minor variations in zeta potential (-0.705 to -0.711 mV) and PDI (0.254 to 0.276), maintaining colloidal stability. Overall, the study demonstrated that the Triple Antibiotic

Topical Aerosol (F7) remained stable, with no significant impact on quality, performance, or therapeutic efficacy, validating its robustness for clinical applications.

CONCLUSION

Topical aerosol formulations, packaged under pressure and released as fine liquid droplets through a specialized valve system, offer a novel and efficient approach to delivering antibiotics directly to infected areas. The Triple Antibiotic Topical Aerosol Spray (TATAS) developed in this study addresses key limitations associated with conventional antibiotic dosage forms, including poor skin permeability, systemic side effects, and antibiotic resistance. By forming a thin film over the infection site, the aerosol spray acts as a reservoir, ensuring sustained release and localized drug action.

This formulation has demonstrated promising attributes, including effective drug deposition, enhanced permeability, and reduced systemic absorption, making it an excellent candidate for treating diabetic foot ulcers (DFUs) and burn wounds. Additionally, the aerosol delivery system minimizes pain and irritation during application, significantly improving patient compliance.

The TATAS formulation not only optimizes the therapeutic efficacy of antibiotics but also represents a step forward in addressing the challenges of managing chronic wound infections. It's potential to enhance healing outcomes while reducing the burden of conventional treatments makes it a valuable innovation in the field of topical drug delivery systems. Further clinical studies are warranted to establish its effectiveness in real-world settings and to explore its broader applications in wound care management.

Human and animal rights

No animals or humans were used in this study.

Availability of data and material

The data will be available from the corresponding author, upon request.

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Conflict of interests

The authors declare that they have no conflicts of interest.

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