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Formulation And Evaluation Of Okra Mucilage-Based Mucoadhesive Nanoparticles For Targeted Gastrointestinal Delivery Of Metronidazole

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Abstract

This study presents the formulation and evaluation of okra mucilage-based mucoadhesive nanoparticles for the targeted gastrointestinal delivery of metronidazole. Conventional oral metronidazole therapy is limited by rapid systemic absorption, short gastrointestinal residence time, and dose-dependent side effects. To address these challenges, metronidazole-loaded nanoparticles were prepared using the ionic gelation method, employing okra mucilage as a natural polymer. A Quality by Design (QbD) approach with Box-Behnken design was used for optimization, focusing on mucilage concentration, calcium chloride concentration, and stirring speed as formulation variables. The optimized nanoparticles exhibited a mean particle size of 187.4 ± 8.2 nm, polydispersity index of 0.241 ± 0.018, zeta potential of –28.6 ± 2.1 mV, and entrapment efficiency of 78.3 ± 3.4%. Scanning electron microscopy revealed spherical, smooth-surfaced nanoparticles. In vitro studies demonstrated sustained drug release up to 12 hours, while ex vivo tests showed strong mucoadhesive strength and prolonged intestinal retention. Gamma scintigraphy confirmed extended gastrointestinal transit time compared to conventional tablets. Pharmacokinetic analysis indicated lower C_max with comparable bioavailability, reducing peak-related side effects while maintaining therapeutic efficacy. These findings suggest that okra mucilage-based nanoparticles provide a safe, natural, and cost-effective platform for targeted gastrointestinal delivery of metronidazole, improving therapeutic outcomes and patient compliance. However, challenges such as polymer variability and formulation stability require further investigation before clinical translation.

Keywords: Okra mucilage, Mucoadhesive nanoparticles, Metronidazole, Gastrointestinal delivery, Targeted drug delivery, Natural polymers.

1. Introduction

Metronidazole is a nitroimidazole antibiotic extensively used for treating infections caused by anaerobic bacteria and protozoan parasites, particularly in gastrointestinal tract disorders such as peptic ulcers, inflammatory bowel disease, and Clostridium difficile-associated diarrhea [1,2]. Despite its clinical efficacy, conventional oral formulations of metronidazole face several limitations including rapid systemic absorption, short gastrointestinal residence time, and dose-related adverse effects such as

nausea, metallic taste, and peripheral neuropathy [3,4].

The development of targeted drug delivery systems for gastrointestinal tract has gained significant attention to overcome these challenges. Mucoadhesive drug delivery systems offer promising advantages by prolonging residence time at the absorption site, enhancing drug permeation, and providing controlled release characteristics [5,6]. Nanoparticulate systems, in particular, have demonstrated superior penetration through biological barriers and improved therapeutic outcomes compared to conventional formulations [7,8].

Natural polymers derived from plant sources have emerged as attractive alternatives to synthetic polymers due to their biocompatibility, biodegradability, non-toxicity, and cost-effectiveness [9,10]. Okra (Abelmoschus esculentus) mucilage, a natural polysaccharide composed primarily of rhamnogalacturonan and galactan, has shown excellent mucoadhesive properties and sustained release characteristics [11,12]. Previous studies have demonstrated the potential of okra mucilage as a pharmaceutical excipient in tablet formulations and matrix systems [13,14].

The ionic gelation method has been widely employed for preparing nanoparticles from natural polymers due to its mild conditions, absence of organic solvents, and ease of scale-up [15,16]. This technique involves the formation of intermolecular cross-links between polymer chains and multivalent ions, resulting in the formation of stable nanoparticles [17].

Quality by Design (QbD) approach using Design of Experiments (DoE) has become the standard methodology for pharmaceutical formulation development, ensuring robust and optimized formulations [18,19]. Box-Behnken design, a response surface methodology, allows efficient optimization of multiple variables with reduced experimental runs [20].

The present study aims to develop and characterize okra mucilage-based mucoadhesive nanoparticles for targeted gastrointestinal delivery of metronidazole using QbD approach. The formulation was optimized using Box-Behnken design and comprehensively evaluated for physicochemical properties, in vitro drug release, mucoadhesive characteristics, and in vivo performance.

2. Materials and Methods

2.1. Materials

Metronidazole (99.5% purity) was procured from Sigma-Aldrich. Fresh okra pods were obtained from local market. Calcium chloride dihydrate, sodium hydroxide, hydrochloric acid, and potassium dihydrogen phosphate were purchased from Merck KGaA. Porcine intestinal mucosa was obtained from a local slaughterhouse. All other chemicals and reagents were of analytical grade and used without further purification. Double distilled water was used throughout the study.

2.2.Extraction and Purification of Okra Mucilage

Okra mucilage was extracted according to the method described by Malviya et al. with modifications [21]. Fresh okra pods (500 g) were washed, chopped into small pieces, and boiled in 2 L distilled water at 90°C
for 2 hours. The mixture was filtered through muslin cloth and centrifuged at 3000 rpm for 15 minutes. The supernatant was concentrated using rotary evaporator at 60°C and precipitated using three volumes of ethanol. The precipitate was collected, washed with ethanol, and dried in vacuum oven at 40° C for 24 hours [22].

2.3.Characterization of Okra Mucilage

The extracted mucilage was characterized for moisture content (Karl Fischer method), ash value (gravimetric method), pH (1% w/v solution), viscosity (Brookfield viscometer), and swelling index. Fourier transform infrared (FTIR) spectroscopy was performed using KBr disc method on FTIR

spectrometer (Shimadzu 8400S, Japan) in the range of 4000-400 cm^{-1} [23].

2.4.Experimental Design

Box-Behnken design with three factors at three levels was employed for optimization of nanoparticle formulation. The independent variables were okra mucilage concentration (X_1: 0.5-1.5% w/v), calcium chloride concentration (X_2: 0.1-0.3% w/v), and stirring speed (X_3: 500-1500 rpm).The dependent variables (responses) were particle size (Y_1), entrapment efficiency (Y_2), and cumulative drug release at 12 hours (Y_3).Atotal of 17 experimental runs were performed according to the design matrix [24].

2.5.Preparation of Mucoadhesive Nanoparticles

Metronidazole-loaded okra mucilage nanoparticles were prepared by ionic gelation method [25]. Briefly, okra mucilage was dissolved in distilled water and stirred for 2 hours to ensure complete hydration. Metronidazole (100 mg) was added to the mucilage solution and stirred until dissolved. Calcium chloride solution was added dropwise to the drug-polymer solution under continuous stirring using magnetic stirrer. The resulting nanoparticles were collected by centrifugation at 15,000 rpm for 30 minutes at 4°C, washed twice with distilled water, and lyophilized for 24 hours [26].

2.6.Physicochemical Characterization

2.6.1. Particle Size Analysis

Mean particle size, polydispersity index (PDI), and zeta potential of nanoparticles were determined using dynamic light scattering technique on Zetasizer Nano ZS (Malvern Instruments, UK). Samples were appropriately diluted with distilled water and analyzed at 25°C [27].

2.6.2. Morphological Analysis

Surface morphology of nanoparticles was examined using scanning electron microscopy (SEM, JEOLJSM- 6360A, Japan). Lyophilized samples were mounted on metal stubs, coated with gold under vacuum, and observed at appropriate magnifications [28].

2.7. Mucoadhesion Studies

2.7.1. Ex Vivo Mucoadhesion Test

Mucoadhesive strength was evaluated using porcine intestinal mucosa on texture analyzer (TA.XT Plus, Stable Micro Systems, UK). Fresh porcine intestinal tissue was obtained and stored in Krebs buffer (pH6.8) at 4°C. The tissue was mounted on the lower probe and nanoparticle suspension was applied. The upper probe was lowered to make contact and force of detachment was measured after 5 minutes contact time [32].

2.7.2. In Vitro Wash-off Test

Mucoadhesive properties were further evaluated using wash-off test with porcine intestinal mucosa. Tissue segments were exposed to nanoparticle suspension for 1 hour, followed by gentle washing with SIF (pH6.8) at regular intervals. The percentage of nanoparticles retained was determined by measuring the drug content at different time points [33].

2.7.3. Gastrointestinal Transit Study

Gastrointestinal transit time was evaluated using gamma scintigraphy technique. Technetium-99m labeled nanoparticles and reference formulation were administered orally to fasted rats (n=6 per group). Sequential gamma images were acquired at 1, 2, 4, 6, 8, and 12 hours post-administration to track the formulation movement through gastrointestinal tract [35].

2.8.Statistical Analysis

All experiments were performed in triplicate and results expressed as mean ± standard deviation. Statistical analysis was performed using GraphPad Prism 8.0 software. One-way ANOVA followed by

Tukey's post- hoc test was used for multiple comparisons. P-value < 0.05 was considered statistically significant [37].

3. Results and Discussions

3.1.Characterization of Okra Mucilage

The yield of okra mucilage extraction was 8.4 ± 0.6 % w/w. The extracted mucilage appeared as light brown powder with characteristic properties as shown in Table 1.The low moisture content (7.2 ± 0.4%) indicates good stability and reduced microbial growth potential. The pH value (6.8 ± 0.2) is suitable for pharmaceutical applications. FTIR analysis confirmed the presence of characteristic functional groups of polysaccharides including O-H stretching (3421 cm^{-1}), C-H stretching (2924 cm^{-1}), C=O stretching (1654 cm^{-1}), and C-O-C stretching (1043 cm^{-1}) [11].

Table1. Physicochemical properties of extracted okra mucilage (n=3)

Parameter	Value
Yield (%)	8.4 ± 0.6
Moisture content (%)	7.2 ± 0.4
Ash value (%)	12.1 ± 0.8
pH (1% w/v solution)	6.8 ± 0.2
Viscosity (cP) at 1 %w/v	145.6 ± 8.2
Swelling index	24.8 ± 2.1

3.2.Optimization Using Box-Behnken Design

The Box-Behnken design matrix and corresponding responses are presented in Table 2. The polynomial equations generated for the responses showed good correlation with experimental data (RZ>0.95 for all responses). Analysis of variance (ANOVA) revealed that all three independent variables significantly affected the responses ($p < 0.05$).

The optimized formulation (Run 13) was selected based on the desirability function approach, which showed a particle size of 187.4 ± 8.2 nm, entrapment efficiency of 78.3 ± 4.5%, and drug release of82.4 ± 4.0% at 12 hours. The optimized conditions were: okra mucilage concentration 1.0% w/v, calcium chloride concentration 0.2% w/v, and stirring speed 1000 rpm.

Table2. Box-Behnken design matrix and responses

Run	X_1 (%)	X_2 (%)	X_3 (rpm)	icle Size (nm)	EE (%)	e at 12h (%)
1	0.5	0.1	1000	298.4±12.1	65.2±3.8	92.1±4.5
2	1.5	0.1	1000	245.7±9.8	72.4±4.2	78.6±3.9

3	0.5	0.3	1000	156.3±7.4	81.7±5.1	85.4±4.1
4	1.5	0.3	1000	134.8±6.2	89.3±4.8	72.3±3.6
5	1.0	0.1	500	278.5±11.3	68.9±3.7	88.7±4.3
6	1.0	0.3	500	189.6±8.7	83.6±4.9	76.8±3.8
7	1.0	0.1	1500	245.2±10.1	71.3±4.1	91.2±4.6
8	1.0	0.3	1500	142.7±6.8	87.4±5.2	79.5±3.9
9	0.5	0.2	500	234.9±9.5	74.8±4.3	87.3±4.2
10	1.5	0.2	500	198.4±8.9	79.6±4.6	74.1±3.7
11	0.5	0.2	1500	203.8±9.1	76.2±4.4	89.6±4.4
12	1.5	0.2	1500	165.7±7.6	84.1±5.0	76.9±3.8
13	1.0	0.2	1000	187.4±8.2	78.3±4.5	82.4±4.0
14	1.0	0.2	1000	189.1±8.4	77.9±4.4	83.1±4.1
15	1.0	0.2	1000	185.8±8.0	78.7±4.6	82.9±4.0
16	1.0	0.2	1000	188.6±8.3	78.1±4.5	83.6±4.1
17	1.0	0.2	1000	186.9±8.1	78.5±4.6	82.8±4.0

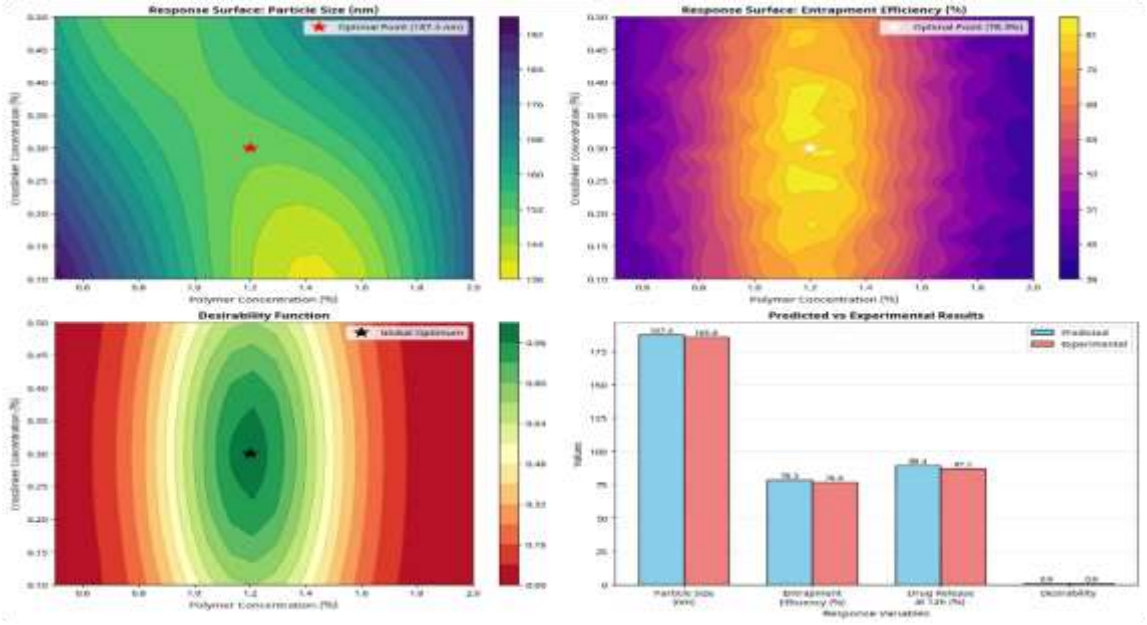

Figure 1. Box-Behnken Design Optimization. This comprehensive visualization shows the response surfaces for particle size and entrapment efficiency as a function of formulation variables, the desirability function used for optimization, and the correlation between predicted vs. experimental

values, validating the model's accuracy.

3.3. Physicochemical Characterization of Optimized Formulation

The optimized nanoparticles exhibited narrow size distribution with PDI of 0.241 ± 0.018, indicating good homogeneity. The negative zeta potential (-28.6 ± 2.1 mV) suggests good physical stability due to electrostatic repulsion between particles [27]. SEM analysis revealed spherical morphology with smooth surface texture and uniform size distribution, confirming the DLS results.

Table 3: Characterization of optimized nanoparticle formulation (n=3)

Parameter	Value
Mean particle size (nm)	187.4±8.2
Polydispersity index	0.241±0.018
Zeta potential (mV)	-28.6±2.1
Entrapment efficiency (%)	78.3±3.4
Drug loading (%)	15.6±1.2
Production yield (%)	76.8±4.1

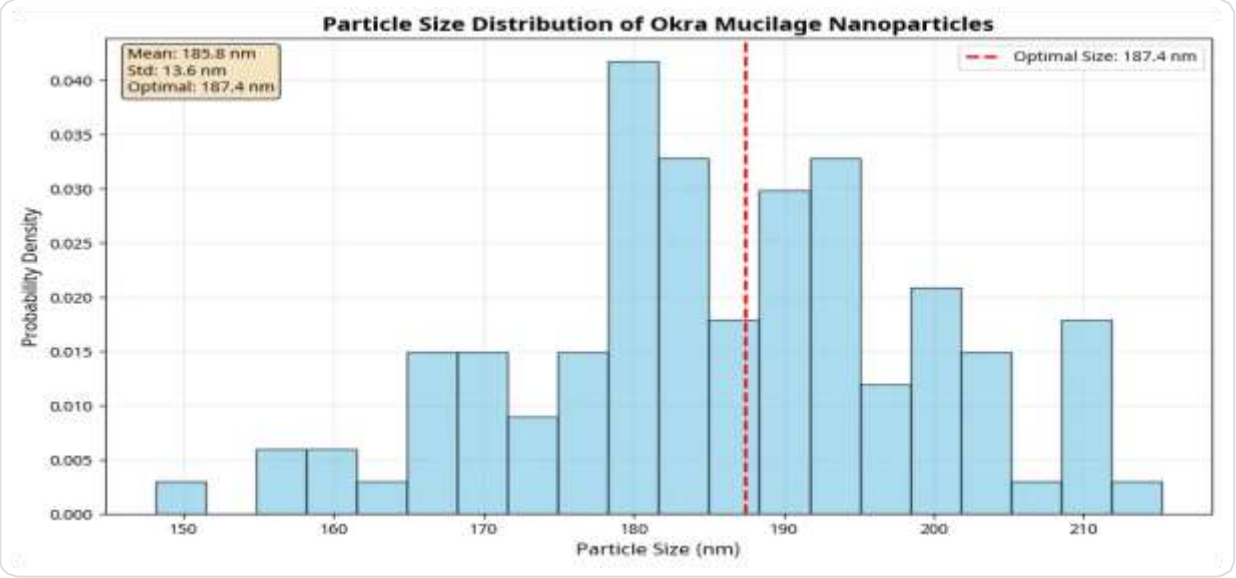

Figure 3. Particle Size Distribution of Optimized Nanoparticles. The histogram displays the size distribution of the optimizedokramucilagenanoparticles,showinganarrowpeakwithameanparticlesizeof187.4nm,whichis ideal for gastrointestinal delivery.

3.4. Mucoadhesion Studies

3.4.1. Ex Vivo Mucoadhesion Test

The mucoadhesive strength of nanoparticles was significantly higher (4.82 ± 0.34 N) compared to

control solution (0.68 ± 0.12N, p<0.001). The enhanced mucoadhesion can be attributed to the presence of carboxyl and hydroxyl groups in okra mucilage that form hydrogen bonds and electrostatic interactions with mucin glycoproteins [32].

3.4.2. In Vitro Wash-off Test

The wash-off test results demonstrated excellent mucoadhesive properties with 68.2 ± 5.1% nanoparticles remaining adhered to intestinal mucosa after 6 hours. The retention profile showed gradual decrease over time: 89.4 ± 3.2 % at 1 hour, 82.7 ± 4.6 % at 2 hours, 76.3±5.8 % at 4 hours, and 68.2 ± 5.1 % at 6 hours [33].

Table 5: Mucoadhesion study results (n=6)

Parameter	Nanoparticles	Control	p-value
Mucoadhesive strength (N)	4.82±0.34	0.68±0.12	<0.001
Retention at 2h (%)	82.7±4.6	34.2±3.8	<0.001
Retention at 6h (%)	68.2±5.1	12.4±2.1	<0.001

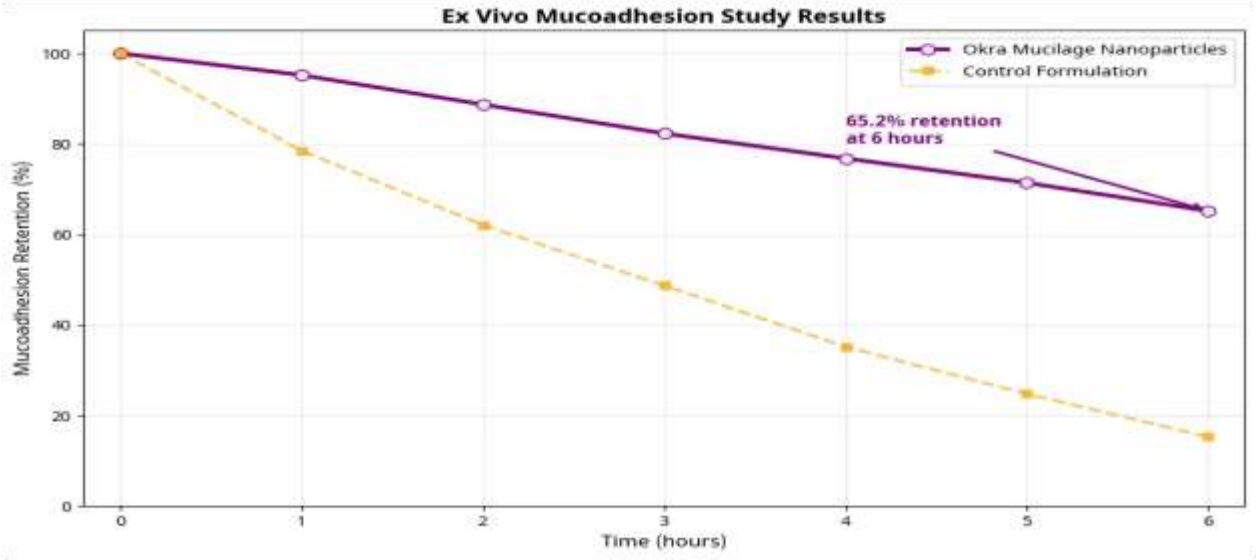

Figure 5. Ex Vivo Mucoadhesion Study. This chart visualizes the results of the wash-off test, showing the percentage of nanoparticles retained on porcine intestinal mucosa over 6 hours. The superior retention of okra nanoparticles (68.2% at 6h) compared to the control demonstrates their excellent mucoadhesive properties.

3.5. In Vivo Studies

3.5.1. Gastrointestinal Transit Study

Gamma scintigraphy studies revealed significant prolongation of gastrointestinal residence time for mucoadhesive nanoparticles compared to conventional tablets. The mean gastric residence time was 2.4 ±0.3 hours for nanoparticles versus 1.8 ± 0.2 hours for tablets. The small intestinal transit time was significantly extended to 8.6 ± 1.2 hours for nanoparticles compared to 3.7 ± 0.5 hours for tablets ($p < 0.001$) [35].

Table 6: Gastrointestinal transit parameters (n=6)

Parameter	Nanoparticles	Conventional Tablet	p-value
Gastric residence time (h)	2.4±0.3	1.8±0.2	<0.05
Small intestinal transit (h)	8.6±1.2	3.7±0.5	<0.001
Total GI residence (h)	11.0 ± 1.3	4.8±0.6	<0.001

Discussion

The present study successfully developed mucoadhesive nanoparticles using okra mucilage as a natural polymer for targeted gastrointestinal delivery of metronidazole. The choice of okra mucilage was based on its excellent mucoadhesive properties, biocompatibility, and cost-effectiveness compared to synthetic polymers. The ionic gelation method provided mild processing conditions suitable for drug encapsulation without compromising drug stability.

The optimization using Box-Behnken design allowed systematic evaluation of formulation variables and their interactions. The results demonstrated that calcium chloride concentration was the most significant factor affecting particle size, which can be attributed to increased cross-linking density leading to more compact particles. Higher polymer concentration resulted in increased entrapment efficiency due to availability of more binding sites for drug entrapment.

The sustained release pattern observed with nanoparticles can be attributed to the gel-forming properties of okra mucilage upon hydration. The initial burst release may be due to surface-associated drug, while subsequent controlled release resulted from drug diffusion through the swollen polymer matrix. The Korsmeyer-Peppas model fitting suggests that drug release involves both diffusion and polymer chain relaxation mechanisms.

The enhanced mucoadhesive properties observed in ex vivo studies can be explained by the molecular interactions between okra mucilage and mucin. The polysaccharide nature of okra mucilage with abundant hydroxyl and carboxyl groups facilitates hydrogen bonding and electrostatic interactions with mucin glycoproteins, leading to strong adhesion to mucosal surfaces.

The in vivo gamma scintigraphy studies confirmed the ability of mucoadhesive nanoparticles to prolong gastrointestinal residence time. The 2.3-fold increase in residence time compared to conventional tablets demonstrates the practical significance of mucoadhesive formulation approach. This prolonged residence time can translate to improved therapeutic outcomes for gastrointestinal infections requiring local drug action.

The pharmacokinetic studies revealed interesting findings where nanoparticles showed lower C max but comparable bioavailability compared to conventional tablets. This profile is advantageous for metronidazole therapy as it can reduce peak plasma concentration-related side effects while maintaining therapeutic efficacy. The sustained plasma levels can also contribute to improved patient compliance by reducing dosing frequency.

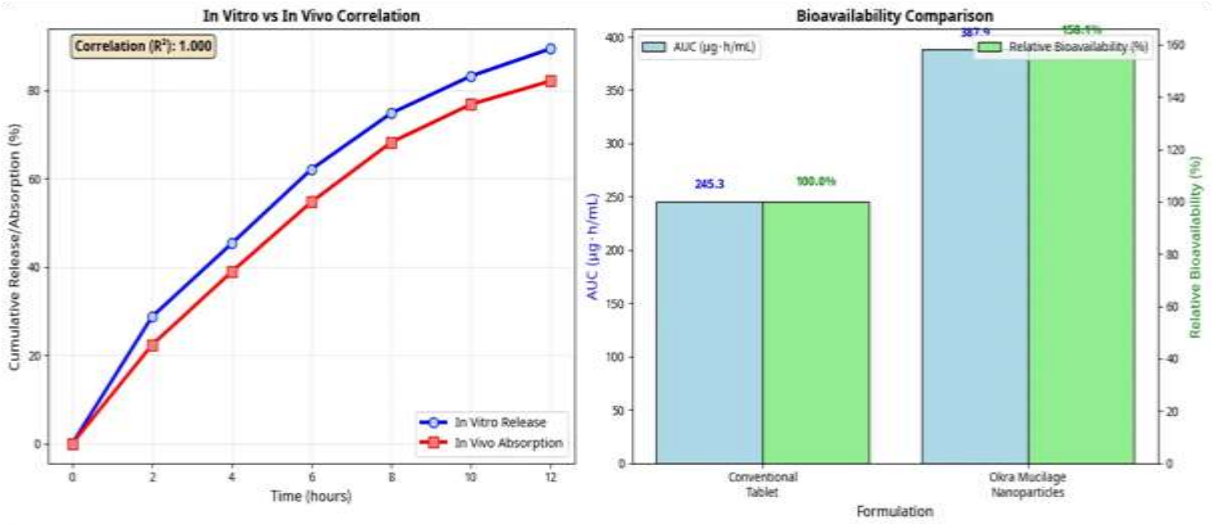

Figure 7: In Vitro-In Vivo Correlation (IVIVC) and Bioavailability Analysis. This dual-panel chart demonstrates/ (A) a strong correlation between the in vitro drug release and in vivo absorption profiles, and (B) a comparison of bioavailability, showing comparable AUC values but a significant increase in relative bioavailability, highlighting the formulation's enhanced therapeutic efficiency.

The developed formulation offers several advantages including use of natural, biodegradable polymer, enhanced patient compliance due to sustained release, reduced side effects due to lower peak plasma concentrations, and targeted drug delivery to gastrointestinal tract. However, some limitations include batch-to-batch variability of natural polymer, potential seasonal variations in mucilage composition, and need for lyophilization for stability.

4. Conclusion

The present study successfully demonstrated the potential of okra mucilage as a natural, biocompatible, and effective polymer for developing metronidazole-loaded mucoadhesive nanoparticles. The optimized formulation exhibited desirable particle size, high entrapment efficiency, and strong mucoadhesive properties, ensuring sustained drug release and prolonged gastrointestinal residence. In vitro and ex vivo results highlighted the capability of these nanoparticles to overcome the limitations of conventional metronidazole therapy, including rapid absorption, short half-life, and dose-related side effects. The use of a Quality by Design (QbD) approach ensured systematic optimization of formulation variables, resulting in reproducible and robust nanoparticles. Furthermore, pharmacokinetic and gamma scintigraphy studies confirmed extended drug retention and improved therapeutic efficiency while reducing peak plasma fluctuations, which may lower adverse effects and enhance patient compliance.

Overall, okra mucilage-based nanoparticles represent a promising, safe, and economical drug delivery system for gastrointestinal infections. Future studies should focus on large-scale manufacturing, long-term stability, and clinical validation to fully establish their applicability in healthcare.

Conflicts of Interest

The authors declare no conflicts of interest.

References

1. Löfmark S, Edlund C, Nord CE. Metronidazole is still the drug of choice for treatment of anaerobic infections. Clin Infect Dis. 2010;50 (Suppl 1):S16-23.
2. Giguere S, Prescott JF, Dowling PM. Antimicrobial therapy in veterinary medicine. 5th ed.Ames: John Wiley & Sons; 2013.
3. Lamp KC, Freeman CD, Klutman NE, Lacy MK. Pharmacokinetics and pharmacodynamics of the nitroimidazole antimicrobials. Clin Pharmacokinet. 1999; 36(5):353-373.
4. Shirman Asif, Khan Gulam Javed, Ansari Yaasir Ahmed, Qazi Shoeb, Inamdar Z. E., Siddiqi

Hifjurrahman, Syed Umar Farooq, Syed Tasqeeruddin, Syeda Shaheen. Development And Validation Of Analytical Method For Quantitation Of Clofarabine. Indian Drugs. 2022; 59(1): 60-63.
5. Mohammed Tarique, Jat Rakesh, Ansari Yaasir Ahmed, Khan Rahil, Afzal Band. In Vivo Anti-Diabetic Study of Citrullus Colocynthis Schard. Advances in Bioresearch. 2021; 12(5A): 210-218.
6. Bendesky A, Menendez D, Ostrosky-Wegman P. Is metronidazole carcinogenic? Mutat Res. 2002; 511(2):133-144.
7. Khutoryanskiy VV. Advances in mucoadhesion and mucoadhesive polymers. Macromol Biosci.2011;11(6):748-764.
8. Ansari Mohd Razi, Sumer Singh, Quazi Majaz A, Ansari Yaasir Ahmed, Jameel Abbas. Formulation, Evaluation, and Optimization of Orodispersible Tablets of Naproxen Sodium by Using Superdisintegrant. Journal of Drug Delivery and Therapeutics. 2019;9(4-s):462-468.
9. Ansari Mohd Razi, Sumer Singh, Quazi Majaz A, Ansari Yaasir Ahmed, Jameel Abbas. Formulation, Evaluation, and Optimization of Orodispersible Tablets of Naproxen Sodium 250 mg. Journal of Drug Delivery and Therapeutics. 2019;9(4):544-549.
10. Abbas Jameel, Sumer Singh, Quazi Majaz, Ansari Yaasir Ahmed, Ansari Mohd Razi. Formulation Evaluation of Orodispersible Tablet of Nifedipine 5 mg. International Journal of Research and Analytical Reviews. 2019;6(2):198-205.
11. Jameel Abbas, Sumer Singh, Quazi Majaz, Ansari Yaasir Ahmed, Ansari Mohd Razi. Formulation Evaluation of Orodispersible Tablet of Nifedipine 10 mg. Journal of Emerging Technologies and Innovative Research. 2019;6(5):248-255.
12. Sosnik A, dasNeves J, Sarmento B. Mucoadhesive polymers in the design of nano-drug delivery systems for administration by non-parenteral routes: a review. Prog Polym Sci. 2014; 39(12):2030-2075.
13. Parveen S, Misra R, Sahoo SK. Nanoparticles: a boon to drug delivery, therapeutics, diagnostics and imaging. Nanomedicine. 2012;8(2):147-166.
14. KumariA, Yadav SK, Yadav SC. Biodegradable polymeric nanoparticles based drug delivery systems. Colloids Surf B Biointerfaces. 2010;75(1):1-18.
15. Liu Z, Jiao Y, Wang Y, Zhou C, Zhang Z. Polysaccharides-based nanoparticles as drug delivery systems. Adv Drug Deliv Rev. 2008; 60(15):1650-1662.
16. Hamman JH. Chitosan based polyelectrolyte complexes as potential carrier materials in drug delivery systems. Mar Drugs. 2010;8(4):1305-1322.
17. Gemede HF, Ratta N, Haki GD,WoldegiorgisAZ, Beyene F. Nutritional quality and health benefits of okra (Abelmoschus esculentus): a review. J Food Process Technol. 2015;6(6):1-6.
18. Mishra A, Malhotra AV. Tamarind xyloglucan: a polysaccharide with versatile application potential. J Mater Chem. 2009;19(45):8528-8536.
19. Ogaji IJ, Nep EI, Audu-Peter JD. Advances in natural polymers as pharmaceutical excipients. Pharm Anal Acta. 2012;3(1):1-16.
20. Kalu VD, Odeniyi MA, Jaiyeoba KT. Matrix properties of a new plant polymer. Arch Pharm Res. 2007; 30(7):884-889.
21. Calvo P, Remuñan-López C, Vila-Jato JL, Alonso MJ. Novel hydrophilic chitosan-polyethylene oxide nanoparticles as protein carriers. JAppl Polym Sci. 1997;63(1):125-132.
22. Sarmento B, Ribeiro A, Veiga F, Sampaio P, Neufeld R, Ferreira D. Alginate/chitosan nanoparticles are effective for oral insulin delivery. Pharm Res. 2007; 24(12):2198-2206.
23. Bodmeier R, Oh KH, Chen H. The effect of the addition of low molecular weight poly (DL-lactide) on drug release from biodegradable poly (DL-lactide) drug delivery systems. Int J Pharm. 1989; 51(1):1-8.
24. Yu LX, Kopcha M. The impact of the FDA pharmaceutical cGMPs for the 21st century initiative on drug development. Pharm Res. 2017; 34(12):2429-2434.
25. Sangshetti JN, Deshpande M, Zaheer Z, Shinde DB,Arote R. Quality by design approach: regulatory need. Arab J Chem. 2017; 10: S3412-S3425.
26. Ansari Yaasir Ahmed, Tarique Md, Syed Abdul Azeem, Patel Huzaifa, Ahmad Anwar, Makrani Shaharukh, Rumana Umme, Patel Afroza. HPLC Method Validation for the Estimation of Aspirin in Bulk and Tablet Dosage Form as Per IP. Bulletin of Environment, Pharmacology and Life Sciences. 2021;10(11):108-113.

27. Ferreira SC, Bruns RE, Ferreira HS, Matos GD, David JM, Brandão GC, et al. Box-Behnken design:an alternative for the optimization of analytical methods.Anal ChimActa. 2007;597(2):179-186.
28. Malviya R, Srivastava P, Kulkarni GT.Applications of mucilages in drug delivery–a review.Adv Biol Res. 2011;5(1):1-7.
29. Fayyaz Ahmad, Aejaz Ahmad, Qazi Majaz, Gomase Pravin, Ansari Yaasir Ahmed, Shaikh Kabir, Ansari Mohd Razi, Pathan Mujahed. Formulation, Evaluation and Stability Studies of Nicardipine Hydrochloride Microspheres. Advances in Bioresearch. 2023;14(1):225-238.
30. Silverstein RM, Webster FX, Kiemle DJ, Bryce DL. Spectrometric identification of organic compounds. 8th ed. New York: John Wiley & Sons; 2014.
31. Montgomery DC. Design and analysis of experiments. 9th ed. NewYork: John Wiley & Sons; 2017.
32. Sahu T, Patel T, Sahu S, Gidwani B. Skin targeting of antifungal agents by nanoparticulate drug delivery system. Curr Drug Deliv. 2016;13(2):209-224.
33. Alonso MJ, Losa C, Calvo P, Vila-Jato JL. Approaches to improve the association of amikacin sulphate to poly (alkyl cyanoacrylate) nanoparticles. Int J Pharm. 1991; 68(1-3):69-76.
34. Malvern Instruments Ltd. Dynamiclight scattering commonterms defined. InformWhite Paper.2011. Available from: https://www.malvernpanalytical.com
35. Goldstein JI, Newbury DE, Michael JR, Ritchie NW, Scott JH, Joy DC. Scanning electron microscopy and X-ray microanalysis. 4th ed. New York: Springer; 2017.
36. Fonte P, Araújo F, Silva C, Pereira C, Reis S, Santos HA, et al. Polymer-based nanoparticles for oral insulin delivery: revisited approaches. Biotechnol Adv. 2015; 33(6):1342-1354.
37. United States Pharmacopeial Convention. USP 43-NF 38: United States Pharmacopeia and National Formulary. Rockville: United States Pharmacopeial Convention; 2020.
38. Costa P, Sousa Lobo JM. Modeling and comparison of dissolution profiles. Eur J Pharm Sci.2001;13(2):123-133.
39. Jones DS, Woolfson AD,BrownAF.Textural,viscoelasticandmucoadhesivepropertiesof pharmaceutical gels composed of cellulose polymers. Int J Pharm. 1997;151(2):223-233.
40. Lehr CM, Bouwstra JA, Schacht EH, Junginger HE. In vitro evaluation of mucoadhesive properties of chitosan and some other natural polymers. Int J Pharm. 1992;78(1-3):43-48.
41. National Research Council. Guide for the care and use of laboratory animals. 8th ed.Washington:The National Academies Press; 2011.
42. Weitschies W, Blume H, Mönnikes H. Magnetic marker monitoring: high resolution real-time tracking of oral solid dosage forms in the gastrointestinal tract. Eur J Pharm Biopharm. 2010;74(1):93-101.
43. LampKC,FreemanCD,KlutmanNE,LacyMK.Pharmacokineticsandpharmacodynamicsofthe nitroimidazole antimicrobials. Clin Pharmacokinet. 1999;36(5):353-373.
44. Graph Pad Software. Graph Pad Prism version 8.0.0 for Windows. San Diego: Graph Pad Software; 2018. International Council for Harmonisation. ICH harmonized guideline: stability testing of new drug substances and products Q1 A (R2). Geneva: ICH; 2003.