RESEARCH PAPER

Formulation and Evaluation of Mucoadhesive Film-Forming Spray of Metronidazole for Localised Treatment of Periodontitis

Ms. Khushi Patel[1*], Ms. Pallavi Gholap[2], Mr. Sahil Hire[3], Ms. Dipsha Patra[4], Mr. Dhiraj Pagare[5], Ms. Gautami Tak[6] and Ms. Angeleena Lamb[7]

Dr. D. Y. Patil College of Pharmacy, Akurdi, Pune-411044
**Corresponding Author: Ms. Khushi Patel*
khushipatel0310@gmail.com

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ABSTRACT

Background: Periodontitis is a chronic inflammatory disease with a global prevalence of over one billion individuals, and the hallmark of the disease is progressive destruction of the tooth-supporting periodontium. Systemic metronidazole (MTZ) treatment produces subtherapeutic levels in the periodontal pocket and may result in gastrointestinal side effects and the development of antimicrobial resistance. Mucoadhesive film-forming delivery through a spray provides a non-invasive, patient-friendly delivery option.

Aim: To formulate and characterize a mucoadhesive film-forming spray (FFS) of metronidazole (0.25% w/v) based on polyvinyl alcohol (PVA) and carboxymethyl cellulose (CMC) as film-forming/mucoadhesive polymers, along with xanthan gum (XG) and propylene glycol, PEG 400 as the plasticizers in a hydroalcoholic system as vehicle.

Methods: A series of in total 13 formulations (B1–B13) were obtained by adjusting the polymer type and concentration, according to the established procedure. The preparations were tested for pH, viscosity, spray pattern, film-forming, uniformity of dosage, drying time, mucoadhesive strength and in vitro-release of drug (using semi-permeable membrane in phosphate buffer pH 6.8) through Franz diffusion cell. Quantification was performed by UV spectrophotometry at 277 nm.

Results: The pH of all the batches was within the physiological range of saliva (6.12–6.30). Viscosity (12 rpm) was between 24.6 and 82.5 cP, and consistent elliptical spray patterns were formed by B1–B5 and B7–B13. The batch B 12 (PVA 0.3%, CMC 0.3%, XG 0.1%, PEG 400 20%) showed the best results: drying time 28 ± 2s; mucoadhesive strength 0.42 ± 0.02 N/cm²; uniformity of dose 0.249 ± 0.003 mg/spray and biphasic drug release of 24.1% at 1 h and 57.3% at 4 h, more chronologically consistent with Higuchi diffusion kinetics.

Conclusion: The optimized mucoadhesive film-forming spray (Batch B12) can be considered as an efficient and patient-compliant actuation device for delivery of localized metronidazole for treatment of periodontitis, which quickly forms film in situ with prolonged antimicrobial activity and supports non-invasive adjunctive local drug delivery to periodontitis sites.

Keywords: *Periodontitis; Metronidazole; Mucoadhesive film-forming spray; Polyvinyl alcohol; Carboxymethyl cellulose; Xanthan gum; Local drug delivery; In vitro drug release.*
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1. INTRODUCTION

1.1 Periodontitis: Epidemiology and Clinical Significance

Periodontitis, which is a polymicrobial-induced chronic inflammatory disease triggered by a dysbiotic subgingival microbial biofilm that interacts with a preactivated host immune response and results in the loss of the teeth's supporting tissues, including the periodontal ligament, cementum and alveolar bone [1,2]. Its clinical features are bleeding on probing, deepening pockets (>4 mm), clinical attachment loss (CAL), bone loss observed radiographically, and potentially tooth mobility or loss. In addition to the mouth, strong bidirectional relationships have been demonstrated between periodontitis and systemic disease such as diabetes mellitus, atherosclerotic cardiovascular disease, adverse pregnancy outcomes, and rheumatoid arthritis [1,3].

Worldwide, severe periodontitis is the sixth most common condition, with a global prevalence of around 1 billion. The increase in the global prevalence rate by an estimated

**Author for Correspondence: khushipatel0310@gmail.com*

91.3% to 12,498 per 100,000 population occurred mainly due to population growth and ageing [4]. Most of the disease burden is found in regions with lower sociodemographic indices. Known risk factors include inadequate oral hygiene, smoking, poorly controlled diabetes mellitus, IL-1 gene cluster polymorphisms, obesity and psychological stress [4,5].

1.2 Components of the Periodontium

The periodontium contains the following four specialized tissues:

- **Gingiva:** Soft tissue surrounding the teeth, organized as the free (marginal) gingiva, the attached gingiva, and the interdental papillae; the depth of the sulcus is about 1 to 3 mm in health.
- **Periodontal Ligament (PDL)**: Fibrous Connective Tissue: The PDL is a highly specialized dense connective tissue (0.15–0.38 mm thick) that connects the cementum covering the root of the tooth to the alveolar bone proper via Sharpey's fibers; it acts as a viscoelastic shock absorber, a sensory organ, and a source of progenitor cells.
- **Cementum:** Avascular, acellular and mineralized tissue covering the root surface which can be either acellular (cervical) or cellular (apical).
- **Alveolar Bone:** Special bone consisting of bundle and trabecular bone, well-known as a highly turnover tissue modulated by mechanical and inflammatory signals.

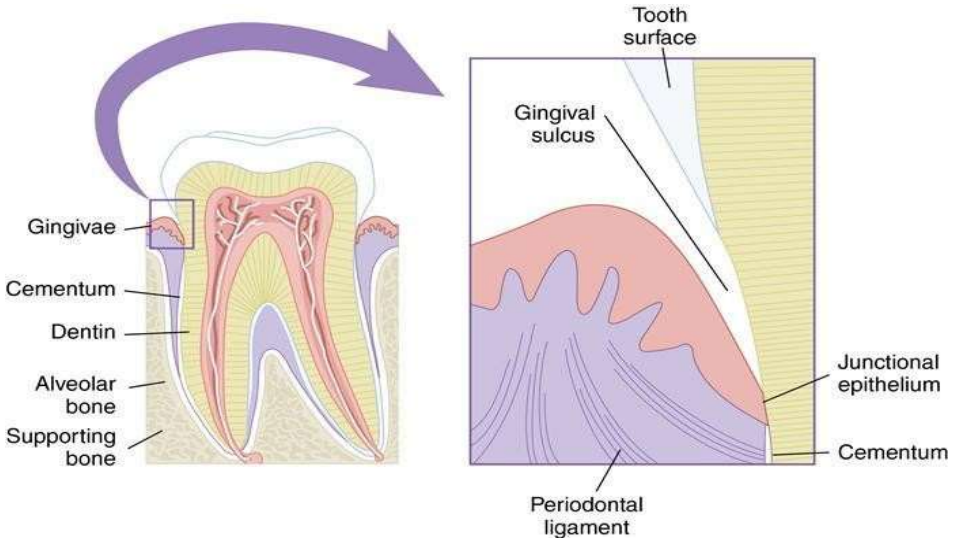

Figure 1: Illustrates the healthy periodontium, depicting the spatial relationships among the gingiva, periodontal ligament, cementum, and alveolar bone.

Table 1: Components of the Periodontium and Their Functions

Component	Structure	Function
Gingiva	Soft tissues around teeth	Protective barrier and seal
Periodontal Ligament	Fibrous connective tissue	Shock absorption and attachment
Cementum	Mineralized root covering	Anchors PDL fibers
Alveolar Bone	Jawbone socket	Support and stabilizes teeth

1.3 Periodontitis Pathogenesis

Periodontitis is a polymicrobial dysbiotic disease. Major periodontopathogens (P. gingivalis, T. forsythia, T. denticola, F. nucleatum) secrete lipopolysaccharides, gingipains, and volatile sulfur compounds that escape host defenses and stimulate hyperinflammation [6, 2]. Production of pro-inflammatory mediators, such as IL-1β, TNF-α, PGE_2, MMP-8/9, because of activation of pattern recognition receptors (TLR2/4) expressed on epithelial and immune cells. The linked RANKL/OPG dysregulation-dependent osteoclast activation - which leads to the destruction of alveolar bone and is irreversible - is driven by this process [1,2].

1.4 Classification: 2017 World Workshop Staging

The 2017 World Workshop classification categorizes periodontitis by severity and complexity [7]:

Table 2: Clinical Features of Periodontitis Stages

Stage	Pocket Depth	Bone Loss	Clinical Signs
Stage I (Initial)	1-3 mm	Minimal (<15% root length)	Mild inflammation
Stage II (Moderate)	4-5 mm	Moderate (15-33%)	Bleeding gums
Stage III (Severe)	>6 mm	Severe (>33%)	Tooth mobility
Stage IV (Advanced)	>7 mm	Extensive	Tooth loss, bite collapse

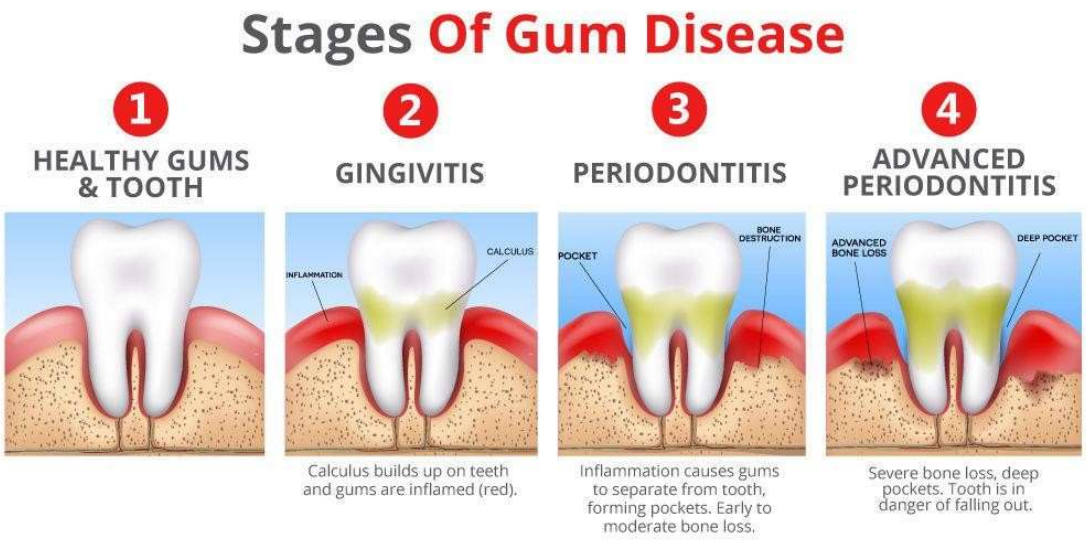

Figure 2: This illustration represents the stages of gum disease development, starting with healthy gums, then gingivitis, moderate periodontitis, and the advanced disease with deep pockets and severe bone loss.

1.5 Limitations of Traditional Therapy

Scaling and root planing (SRP) is the basic, most effective, and generally sufficient step in the therapy of periodontal diseases; however, in deeper pockets (>5 mm), in furcation areas, and in dentinal tubules, residual pathobionts remain [8,9,10]. Systemic antibiotics, although previously used as adjuncts, do not reach sufficient concentrations for therapeutic effect in periodontal pockets, have associated risks of gastrointestinal toxicity, and promote antimicrobial resistance [8]. These constraints also highlight the clinical relevance of developing site-specific controlled-release local delivery vehicles.

1.6 Metronidazole in Periodontal Therapy

Metronidazole (MTZ) is a 5-nitroimidazole antimicrobial that possesses selective bactericidal activity against obligating anaerobes by intracellular reductive activation (by ferredoxin/ flavodoxin), generating cytotoxic free radicals that cause DNA strand breakage [11,12]. Its MIC for the main periodontopathogens is also clinically favorable: P. gingivalis 2—8 µg/mL; F. nucleatum 4—16 µg/mL. Oral administration (500 mg TID) is associated with metallic dysgeusia, gastrointestinal disturbance, disulfiram-like reactions, and increasing microbial resistance [12], despite efficient systemic availability (~80%). Localized delivery approaches address these limitations by producing pocket concentrations 100–1000-fold higher than the MIC with minimal systemic exposure [12,19,23].

1.7 Film-Forming Sprays for Local Drug Delivery Systems

The film-forming sprays are a novel platform, based on which a hydroalcoholic polymer solution aerosol is sprayed on the delivery site where the solvent rapidly evaporates forming a thin flexible mucoadhesive film. This in-situ generated film prolongs residence time (24–48 h), provides zero-order or biphasic release of drug and adapts to the irregular anatomical shape of the periodontal pocket without the need of insertion or removal, which are added values when compared to pre-formed films, chips, or gel syringes [2,13]. This study is designed on a rational basis to take advantage of this platform for targeting the delivery of MTZ.

2. MATERIALS AND METHODS

2.1 Materials

Table 3: List of Materials and Their Roles

Sr. No.	Ingredient	Grade/Source	Role
1.	Metronidazole (MTZ)	API	Antibacterial drug
2.	Propylene Glycol (PG)	PharmGrade	Plasticizers, permeation enhancer, co-solvent
3.	Polyvinyl Alcohol (PVA)	PharmGrade	Film-forming polymer, viscosity enhancer
4.	Carboxymethyl Cellulose (CMC)	PharmGrade	Mucoadhesive polymer, thickening agent
5.	Xanthan Gum (XG)	PharmGrade	Mucoadhesive polymer, thickening agent
6.	Polyethylene Glycol 400 (PEG 400)	PharmGrade	Plasticiser, film flexibility enhancer
7.	Ethanol	AR Grade	Volatile solvent, drug solubiliser

8.	Distilled Water	Purified	Aqueous Vehicle
9.	Peppermint Oil	PharmGrade	Flavouring, mild antimicrobial
10.	Mentha Oil	PharmGrade	Flavouring, breath freshener
11.	Sodium Benzoate	PharmGrade	Antimicrobial preservative

2.2 Drug Profile: Metronidazole

Table 4: Physicochemical Profile of Metronidazole

Parameter	Description
IUPAC Name	2-(2-methyl-5-nitro-1H-imidazole-1-yl)ethan-1-ol
Molecular Formula	$C_6H_9N_3O_3$
Molecular Weight	171.15 g/mol
Melting Point	158-160°C
Appearance	White to pale yellow crystalline powder
Solubility in Water	~10 g/L at 25°C
Solubility in Ethanol	4-7
Mechanism of Action	Reductive activation → cytotoxic free radical formation →DNA strand breakage
Drug Class	Nitroimidazole antibacterial and antiprotozoal

2.3 Pre-Formulation Studies

2.3.1 Organoleptic Characterization

The visual and sensory evaluation of the color, smell, taste, and texture of MTZ was carried out under white light.

2.3.2 Determination of melting point

Rates using the capillary method. The observations were compared with the USP/BP specification (158–161°C) and thus determined the purity and no polymorphism.

2.3.3 Solubility Testing

The solubility of MTZ in distilled water and 40% v/v ethanol and the most optimized hydroalcoholic vehicle was assessed at 37° using the test tube method.

2.3. 4 UV-Visible (λmax Evaluation)

A methanolic standard solution of MTZ (10 µg/mL) was scanned in the range 200–400 nm with a UV-visible spectrophotometer (UV-1800, Shimadzu, Japan). The wavelength of maximum absorbance (λmax) was identified and was used as the analytical wavelength for all the subsequent estimations.

2.3.5 Construction of the calibration curve

The MTZ standard solutions in phosphate buffer pH 6.8 with concentrations 0, 1, 2, 3, 4 and 5 µg/mL were prepared. The absorbance was taken at 277 nm and the calibration curve was prepared by using absorbance against concentration. The linearity was evaluated by the determination coefficient (R^2).

Table 5: Calibration Data of Metronidazole at 277 nm

Concentration (µg/mL)	Absorbance (277 nm)
0	0.000
1	0.082
2	0.162
3	0.245
4	0.322
5	0.401

Linear regression: Absorbance = 0.0801 x C + 0.0002; R^2 = 0.9998

2.4 Formulation of Mucoadhesive Film-Forming Spray

2.4.1 Composition of Formulation Batches

Table 6: Composition of Formulation Batches

Batch	MTZ (% w/v)	PG (% v/v)	PVA w/v	CMC w/v	XG (% w/v)	PEG 400 (% v/v)	Ethanol (v/v)	Distilled water q.s. (mL) to
B1	0.25	5	0.2	-	-	10	40	100
B2	0.25	5	0.3	-	-	10	40	100
B3	0.25	5	-	0.2	-	10	40	100
B4	0.25	5	-	0.3	-	10	40	100
B5	0.25	5	-	-	0.2	10	40	100
B6	0.25	5	-	-	0.3	10	40	100

B7	0.25	5	0.2	0.2	-	10	40	100
B8	0.25	5	0.2	0.3	-	10	40	100
B9	0.25	5	0.3	0.2	-	10	40	100
B10	0.25	5	0.3	0.3	-	10	40	100
B11	0.25	5	0.3	0.3	0.1	10	40	100
B12	0.25	5	0.3	0.3	0.1	20	40	100
B13	0.25	5	0.3	0.3	0.1	30	40	100

2.4.2 Method of Preparation Procedure

The film-forming spray was manufactured according to a validated 10-step process in accordance with ICH Q8(R2) guidelines:

1. **Xanthan gum dispersion:** XG was gradually added into distilled water and stirred at 80^◦C for 10 min on a magnetic stirrer, followed by stirring at 25 ± 2^◦C for 2 h to get a viscous lump-free solution [14].
2. **Dissolution of PVA:** PVA was slowly added to distilled water and stirred at 25 ± 2°C for 45 min; mild heating was applied when necessary to a temperature of 60–70°C, let to cool to room temperature [14].
3. **Solubilization of CMC:** Dissolve sodiumCMC in distilled water with constant moderate stirring at 25 ± 2°C for 30-60 min to prepare a clear fy ~~ous solution [14].
4. **Preparation of the polymer blend:** The solutions of the individual polymers were mixed in the specified ratios of the formulation and stirred at moderate speed for 15–30 min [14].
5. **Plasticizer addition:** To the blend propylene glycol (5% v/v) was added and stirred for 10–15 min and then PEG 400 was added and stirred for additional 10–15 min [14].
6. **Preparation of drug solution:** MTZ (0.25% w/v) was weighed accurately and dissolved in ethanol under stirring till clarity [14].
7. **Drug loading:** MTZ solution was emulsified with the polymeric blend by adding drop by drop under moderate/heavy turbulence stirring for a time of 10-15 min to facilitate homogeneous distribution [14].
8. **Volume correction:** The volume was made up to 100 mL with distilled water (qs) and stirred for 15-20 min to dissolve completely to yield a clear/slightly opalescent solution [14].
9. **Degassing:** The solution rested for 30–60 min at 25 ± 2°C without being disturbed to allow the entrapped air to be released [14].
10. **Storage:** The prepared formulation was kept in clean, airtight, amber colored bottles at 25 ± 2°C in darkness [14].

2.5 Evaluation Parameters

2.5.1 Appearance

The initial samples and the stored samples were visually inspected under ordinary light to determine its color, clarity, uniformity, any phase separation, and precipitation.

2.5.2 Determination of pH

pH was determined by a calibrated digital pH meter (Systronics, India) at 25°C after standard buffer calibration. Permissible range: 5.5–7.0 (the physiological pH of saliva) [15,16].

2.5.3 Viscosity

At 25°C, three different rotational speeds (12, 30, and 60 RPM) and suitable spindle were spun on a Brookfield viscometer (LV-DV-II+Pro). The decreasing_ viscosity with increasing_ RPM was used_ to evaluate the shear-thinning property [15,17].

2.5. 4 Spray Pattern Analysis

The formulations were sprayed from a distance of 10 cm on grade-1 Whatman filter paper. After photographing, the resultant spray pattern was analyzed for uniformity of distribution, shape (elliptical/round), size and satellite droplets, if any [14].

2.5.5 Film Formation and Integrity

Each formula volume 0.1 mL was spread on a clean glass plate and dried under environmental conditions. The film was evaluated for uniformity, clarity, pliability, absence of cracks and surface bonding [18,19].

2.5.6 Dose Uniformity

Dose uniformity was evaluated by sampling ten consecutive actuations of the spray, dissolving in a predetermined amount of pH 6.8 phosphate buffer, and determining the MTZ content spectrophotometrically at 277 nm. Mean dose and %RSD were calculated [14,15].

2.5.7 Drying Time

The preparation was sprayed on a vertically positioned glass slide. At 5-s intervals, there was slight fingertip pressure until the transfer of materials to the finger ceased. The time between application and conversion into non-tacky state was noted (n = 3) [14,20].

2.5.8 Mucoadhesive Strength

The mucoadhesive strength was determined by a modified physical balance method. Freshly excised porcine buccal mucosa (rested in phosphate buffer pH 6.8) was mounted on a glass slide. The formulation was applied and after 5 min of film formation, the detachment force (N/cm²) was measured [21,15].

2.5.9 in Vitro Drug Release (Franz Diffusion Cell)

In vitro drug release was carried out in a Franz diffusion cell (diffusion area: 3.14 cm²) across a dialysis membrane (MWCO 12,000 Da) which was pre-equilibrated with

phosphate buffer pH 6.8. The receptor compartment was loaded with 15 mL of pH 6.8 phosphate buffer at 37 ± 0.5°C with stirring (magnetic) at 100 RPM. ALIQOUTS (of 1 mL) were collected at 0.25, 0.5, 1.0, 2.0, and 4.0 h and replenished with the fresh buffer. The content of MTZ was estimated by UV spectrophotometry at 277 nm. The cumulative % drug release (Mean ± SD, n = 3) is computed [13,15,21].

3. RESULTS

3.1 Pre-Formulation Studies

3.1.1 Organoleptic Characteristics

Table 7: Organoleptic Characteristics of Metronidazole

Characteristic	Observation	USP/BP Compliance
Appearance	White to slightly yellow crystalline powder	Compliant
Odour	Odorless	Compliant
Taste	Slightly bitter	Compliant
Color	White or pale yellow	Compliant
Texture	Crystalline, slightly gritty powder	Compliant

3.1.2 Melting Point

MTZ was found to melt at 158–160°C (sharp melting, without decomposition), which was within the range specified by USP/BP 158–161°C, indicating that the drug was of high purity with no polymorphic transformation.

3.1.3 Solubility Profile

Table 8: Solubility Profile of Metronidazole at 37°C

Solvent System	Solubility	Significance
Distilled Water	9-10 mg/mL	Slightly soluble; base vehicle
40% Ethanol (v/v)	25-35 mg/mL	Enhanced solubility; rapid evaporation for film formation
Optimized Hydroalcoholic Vehicle	50-70 mg/mL	Excellent solubility; no crystallization; ideal spray ability

3.1.4 UV Spectral Study and Calibration Plot

MTZ had a pronounced peak at λmax = 277 nm in ethanol and phosphate buffer pH 6.8 according to the literature [19,23]. The calibration curve was highly linear in the range 0–5 µg/mL (R2 = 0.9998), indicating that it obeyed the Beer–Lambert's law. The regression equation was: Absorbance = 0.0801× C + 0.0002.

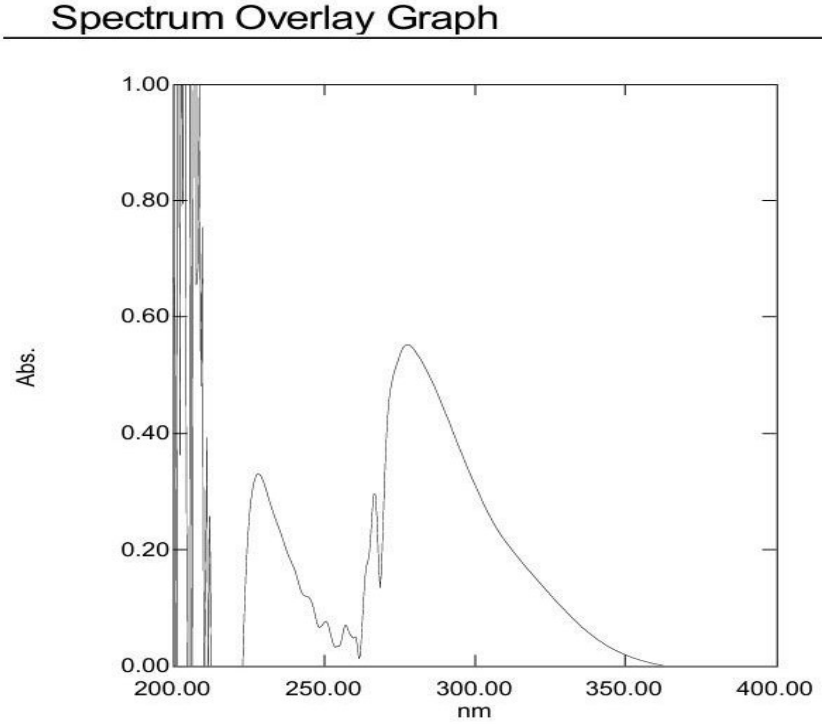

Figure 3: UV Spectrum of metronidazole

3.2 Evaluation of Formulation Batches

3.2.1 Appearance:

Table 9: Visual Appearance of Optimized Formulation (B12)

Characteristic	Observation
Clarity	Clear, no phase separation
Color	Colorless
Odour	Characteristic peppermint/mentha
Nature	Homogeneous, uniform

All B1–B13 batches were clear, homogeneous, colorless solutions without visible particles or precipitation, which means physicochemical compatibility was maintained among all excipients.

3.2.2 Determination of pH

Table 10: pH Values of All Formulation Batches

Batch	pH
B1	6.30
B2	6.28
B3	6.18
B4	6.22
B5	6.20
B6	6.12
B7	6.27
B8	6.24
B9	6.26
B10	6.23
B11	6.21
B12	6.25
B13	6.24

All formulation batches showed pH in the range 6.12–6.30 and it is compatible to physiological pH of saliva 5.5–7.0 for the mucosa and stable enough for drug [15,16].

3.2.3 Viscosity

Table 11: Viscosity of All Formulation Batches at 25°C (cP)

Batch	12 RPM	30 RPM	60 RPM	Behavior
B1	24.6	20.8	18.2	Newtonian-like
B2	33.4	28.7	25.3	Slightly shear-thinning
B3	46.8	40.5	35.6	Shear-thinning
B4	37.2	32.1	28.7	Shear-thinning
B5	58.9	50.1	45.2	Shear-thinning
B6	82.5	72.4	65.3	Strongly shear-thinning
B7	50.1	43.6	38.5	Shear-thinning
B8	41.3	36.3	32.5	Shear-thinning
B9	55.8	48.7	42.6	Shear-thinning
B10	48.5	41.8	36.7	Shear-thinning
B11	63.7	55.4	48.5	Shear-thinning
B12	**59.5**	**51.2**	**45.5**	**Optimal shear-thinning**
B13	53.1	46.2	40.7	Shear-thinning

All the formulations showed pseudoplastic (shear-thinning) behaviour, which is the preferred behaviour for an oral spray system (high viscosity at rest condition retains film and low viscosity at shearing condition enhances atomization and spray delivery) [17,22].

3.2.4 Spray Pattern

Batches B1–B5 and B7–B13 generated consistent and elliptical spray (diameter: 4.5–5.0 cm) from 10 cm with uniform droplet distribution without satellite formation. Batch B6 showed a mildly irregular pattern due to its high viscosity (82.5 cP at 12 RPM), which did not allow optimal atomization.

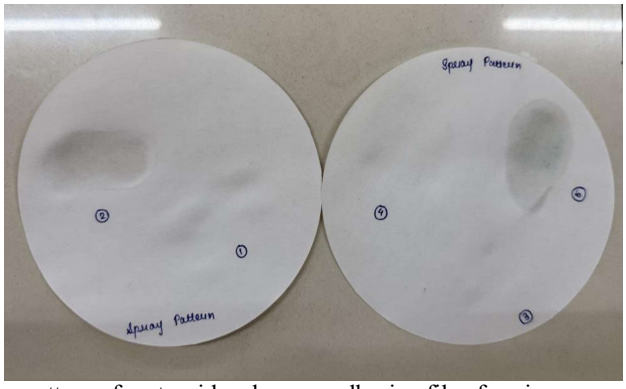

Figure 4: Spray pattern of metronidazole mucoadhesive film-forming spray on filter paper.

3.2.5 Dose Uniformity

All the batches (B1–B13) had consistent delivery of 0.249 ± 0.003 mg MTZ per actuation (%RSD, 2%), which indicates a reliable pump performance and that the drug solution is evenly dispersed throughout the spray. It meets the requirement of USP for a nasal/oral spray concerning dose uniformity ≤25% RSD [2,15].

3.2.6 Drying Time

Batch B12 showed a drying time of 28 ± 2s, which was the ideal drying time. Increased drying times were observed for higher XG contents due to its greater hydrophilicity, and single-polymer batches (B1–B2) formed films in shorter times (18–22 s) but with poor flexibility and adhesion.

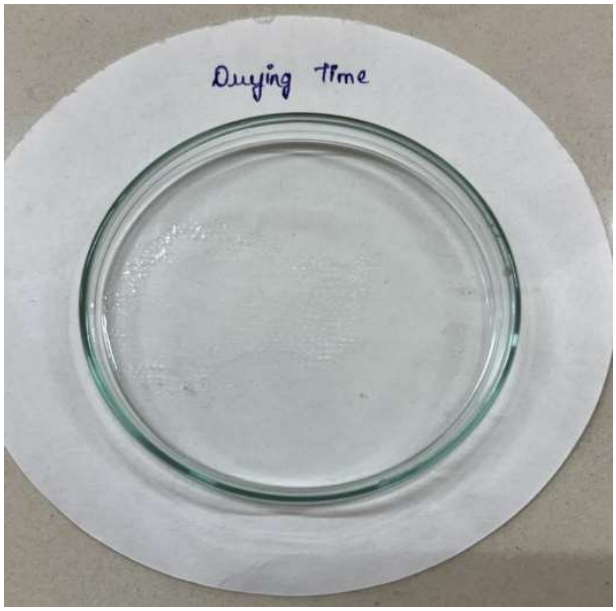

Figure 5: Drying time determination of mucoadhesive film-forming spray on glass surface.

3.2.7 Mucoadhesive Strength

Table 12: Mucoadhesive Strength of All Formulation Batches (N/cm^2, Mean ± SD, n=3)

Batch	Mucoadhesive Strength (N/cm^2)
B1	0.18 ± 0.02
B2	0.21 ± 0.02
B3	0.25 ± 0.03
B4	0.28 ± 0.03
B5	0.32 ± 0.04
B6	0.35 ± 0.03
B7	0.30 ± 0.02
B8	0.33 ± 0.03
B9	0.34 ± 0.03

B10	0.37 ± 0.04
B11	0.40 ± 0.03
B12	**0.42 ± 0.02**
B13	0.41 ± 0.03

Mucoadhesive strength was increased steadily from single-polymer batches (B1: 0.18 N/cm 2) to ternary polymer blends (B12: 0.42 N/cm 2) due to synergistic hydrogen bonding and interpenetration of the PVA, CMC and XG chains with the mucin glycoproteins. The mucoadhesive strength of batch B12 was maximal at 0.42 ± 0.02 N/cm².

3.2.8 In Vitro Drug Release

Table 13: Cumulative % Drug Release at 1 h, 2 h and 4 h (Mean ± SD, n=3)

Batch	1 h (%)	2 h (%)	4 h (%)
B1	32.3 ± 1.8	48.6 ± 2.1	68.2 ± 2.3
B2	30.7 ± 1.6	46.5 ± 2.0	65.7 ± 2.4
B3	28.5 ± 1.7	44.2 ± 1.9	63.2 ± 2.2
B4	26.9 ± 1.5	42.1 ± 1.8	59.4 ± 2.0
B5	24.3 ± 1.4	39.8 ± 1.7	56.9 ± 1.9
B6	23.7 ± 1.3	37.5 ± 1.6	55.3 ± 1.9
B7	28. 6 ± 1.5	45.3 ± 1.9	62.5 ± 2.5
B8	27.2 ± 1.5	43.0 ± 1.8	61.2 ± 2.1
B9	27.6 ± 1.4	44.1 ± 1.9	62.4 ± 2.0
B10	26.4 ± 1.4	41.7 ± 1.7	60.1 ± 2.1
B11	25.1 ± 1.3	39.6 ± 1.6	58.4 ± 1.9
B12	**24.1 ± 1.2**	**38.5 ± 1.5**	**57.3 ± 1.8**
B13	27.8 ± 1.5	43.2 ± 1.8	60.7 ± 1.8

Table 14: Detailed In-Vitro Drug Release Profile – Optimized Batch B12 (Mean ± SD, n=3)

Time (h)	Cumulative % Drug Released
0.25	11.8 ± 0.8
0.50	18.2 ± 1.0
1.0	24.1 ± 1.2
2.0	38.5 ± 1.5
4.0	57.3 ± 1.8

Batch B12 exhibited a typical biphasic pattern of drug release: an initial burst of ~24% within 1h (providing immediate concentrations therapeutically above the MTZ MIC of 2–16 µg/mL against the major periodontopathogens), followed by extended release of 57.3% at 4 h, showing consistency with Higuchi diffusion kinetics from the matrix system [13,23].

4. DISCUSSION

4.1 Effects of polymer type and concentration.

Selection of polymers and concentration used was based on previous work, and in this study, incorporating different types of polymers in various concentrations resulted in batches B1 to B13. The 13 batches, each with a different concentration of PVA, CMC, and XG, was a key in providing a holistic perspective on the effect the formulation components had on performance. In terms of mucoadhesion and the slow delivery of the drug, the single-polymer systems (B1–B6) were the worst. Ternary mixtures (B7–B10) showed intermediate results, whereas the tertiary system comprising three polymers (B11–B13) was able to obtain the best mucoadhesion and controlled release by complementary mechanisms: PVA is an elastic film-forming agent by –OH hydrogen bonding with mucin [15,20]; CMC shows ionic mucoadhesion by –COONa interaction with mucosal glycoproteins [13,22]; and XG through its pseudoplastic rheology (shear-thinning) promotes atomization during actuation and its helix–coil transition in aqueous media enables entanganglement with mucin chains [17,22].

4.2 Role of Plasticizers

Propylene glycol (5% v/v) had a twofold role: as a plasticizer of the polymer matrix (Tg reduction ~20°C) and as a penetration enhancer of the buccal/periodontal mucosa [16]. Due to the added pliability and flexibility imparted to the film by PEG 400, it prevented the brittle cracking of the in-situ formed film. Increasing the concentration of PEG 400 from 10 to 20 to 30% for the three formulations (B11, B12, B13) resulted in a visible viscosity decrease (63. 7 → 59.5 → 53.1 cP at 12 RPM), enhancing atomization with no impact on the integrity of film. Batch B12 (20% PEG 400) was chosen as most

suitable efficiency of drug release, 风flexibility of film and viscosity of B12 (20% PEG 400) was considered to be the best among the formulations tested. [16,18].

4.3 pH and Compatibility of the Mucosa

The pH values of all the batches (6.12–6.30) were in the range of physiological pH of saliva which is important to (a) avoid mucosal irritation which may affect patient compliance; (b) maintain MTZ stability (stable pH 4-7); (c) realize that polymers maintain efficiency for the hydrogen bonding [15,16].

4.4 Drug Release Kinetics and Clinical Relevance

The biphasic release profile of Batch B12—an initial burst release and a sustained release—is beneficial in clinical application. The burst delivery rapidly achieves concentrations of MTZ above the MIC (2–16 µg/mL) for P. gingivalis, T. denticola and F. nucleatum within minutes, sustained delivery for 4h maintains these concentrations at inhibitory levels along the length of treatment [12,13,23]. The Higuchi model fit (√t-dependent release from a matrix) indicates that drug diffusion within the swollen polymer matrix is the rate-controlling step.

4.5 Comparison with literature

In contrast to chitosan/PCL films described by El-Kamel et al. [21], the present spray system offers non-contact application and no need for removal. Sustained buccal release of drug for 12 h was reported by Perioli et al. [15] using compressed tablets; here, a similar sustained release profile is achieved in a system which can be applied more easily to the non-uniform shape of pockets. Singh et al. [12] reported encapsulation of MTZ drug in PLGA nanoparticles along with suitable periodontal results; nevertheless, the spray delivery of this study enhances clinical practicability by removed manual insertion.

4.6 Excipient Rationale Summary

Table 15: Compatibility and Rationale Summary

Component	Concentration in B12	Functional Justification
Metronidazole	0.25% w/v	Bactericidal against anaerobes; optimal local dose
PVA	0.3% w/v	Flexible film former; strong mucoadhesion via-OH bonds
CMC	0.3% w/v	Iconic mucoadhesion; viscosity enhancement
Xanthan Gum	0.1% w/v	Shear-thinning rheology; matrix sustainment
PEG 400	20% v/v	Plasticization; improved atomization
Propylene Glycol	5% v/v	Plasticizer; permeation enhancer
Ethanol	40% v/v	Drug solubilization; rapid film formation via evaporation
Peppermint + Mentha Oil	Trace	Taste masking; mild antimicrobial; TRPM8-medicated sensation
Sodium Benzoate	0.1% w/v	Antimicrobial preservation; pH 5-7 compatible

5. CONCLUSION

A mucoadhesive film-forming spray of metronidazole (0.25% w/v) was successfully formulated and characterized across thirteen systematically designed batches. The optimized formulation, **Batch B12** (PVA 0.3%, XG 0.1%, PEG 400 20%, PG 5%, ethanol 40% v/v), demonstrated:

- pH 6.25 (physiologically compatible with oral mucosa)
- Pseudoplastic viscosity of 59.5 cP (12 RPM), enabling efficient atomization
- Uniform elliptical spray pattern (4.8 cm diameter)
- Rapid in-situ film formation within **28 ± 2 seconds**
- Consistent dose delivery of 0.249 ± 0.003 mg MTZ/actuation (%RSD <2%)
- Biphasic drug release: 24.1% at 1 h and 57.3% at 4 h, following Higuchi kinetics

This non-invasive, patient-friendly platform addresses the critical limitations of systemic MTZ therapy and existing invasive local delivery devices by providing high localized antimicrobial concentrations, minimizing systemic exposure, and enabling compliance self-administration. The formulation holds significant promise as an adjunctive therapy in non-surgical management of Stage I-III periodontitis.

Future investigations should include accelerated stability (ICH Q1A), ex vivo permeation and mucoadhesive on human gingival tissue, antimicrobial efficacy testing against P. gingivalis and F. nucleatum, and randomized controlled clinical trials to validate therapeutic outcomes in human subjects.

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REFERENCE

1. Hajishengallis G, Chavakis T, Lambris JD. Current understanding of periodontal disease pathogenesis and targets for host-modulation therapy. Periodontol 2000. 2020;84(1):14-34.
2. Hajishengallis G. Immunomicrobial pathogenesis of periodontitis: keystones, pathobionts, and host response. Trends Immunol. 2014;35(1):3-11.

3. Viglianisi G, Santonocito S, Lupi SM, Amato M, Spagnuolo G, Pesce P, et al. Impact of local drug delivery and natural agents as new target strategies against periodontitis: new challenges for personalized therapeutic approach. Ther Adv Chronic Dis. 2023;14:20406223231191043.
4. Sanz M, Herrera D, Kebschull M, Chapple I, Jepsen S, Beglundh T, et al. Treatment of stage I–III periodontitis — The EFP S3 level clinical practice guideline. J Clin Periodontol. 2020;47(Suppl 22):4-60.
5. Kassebaum NJ, Bernabé E, Dahiya M, Bhandari B, Murray CJL, Marcenes W. Global burden of severe periodontitis in 1990–2010: a systematic review and metaregression. J Dent Res. 2014;93(11):1045-1053.
6. Lamont RJ, Koo H, Hajishengallis G. The oral microbiota: dynamic communities and host interactions. Nat Rev Microbiol. 2018;16:745-759.
7. Tonetti MS, Greenwell H, Kornman KS. Staging and grading of periodontitis: framework and proposal of a new classification and case definition. J Periodontol. 2018;89 (Suppl 1):S159-S172.
8. Schwach-Abdellaoui K, Vivien-Castioni N, Gurny R. Local delivery of antimicrobial agents for the treatment of periodontal diseases. Eur J Pharm Biopharm. 2000;50(1):83-99.
9. Vyas SP, Sihorkar V, Mishra V. Controlled and targeted drug delivery strategies towards intraperiodontal pocket diseases. J Clin Pharm Ther. 2000;25(1):21-42.
10. Budală DG, Luchian I, Tatarciuc M, Butnaru O, Armencia AO, Virvescu DI, et al. Are local drug delivery systems a challenge in clinical periodontology? J Clin Med. 2023;12(12):4137.
11. Kapoor G, Saigal S, Elongavan A. Action and resistance mechanisms of antibiotics: a guide for clinicians. J Anaesthesiol Clin Pharmacol. 2017;33(3):300-305.
12. Khan G, Yadav SK, Patel RR, Nath G, Bansal M, Mishra B. Development and evaluation of biodegradable chitosan films of metronidazole and levofloxacin for the management of periodontitis. AAPS PharmSciTech. 2016;17(6):1312-1325.
13. Labib GS, Aldawsari HM, Badr-Eldin SM. Metronidazole and pentoxifylline films for the local treatment of chronic periodontal pockets: preparation, in vitro evaluation and clinical assessment. Drug Des Devel Ther. 2014;8:1189-1198.
14. Ilaf J. Atoosh, Mowafaq M., Ghareeb. Optimizing Mucoadhesive Film-Forming Spray for Efficient Oral Delivery of Fluconazole in Candidiasis Treatment. DOI 10.7759/cureus.70359.
15. Perioli L, Ambrogi V, Rubini D, Giovagnoli S, Ricci M, Blasi P, et al. Novel mucoadhesive buccal formulation containing metronidazole for the treatment of periodontal disease. J Control Release. 2004;95(3):521-533.
16. Rowe RC, Sheskey PJ, Quinn ME, editors. Handbook of pharmaceutical excipients. 6th ed. London: Pharmaceutical Press; 2009.
17. Petri DFS. Xanthan gum: a versatile biopolymer for biomedical and technological applications. J Appl Polym Sci. 2015;132(23).
18. Laffleur F, Keckeis V. Advances in drug delivery systems: work in progress still needed? Int J Pharm X. 2020;2:100050.
19. Patel VF, Liu F, Brown MB. Advances in oral transmucosal drug delivery. J Control Release. 2011;153(2):106-116.
20. Cilurzo F, Cupone IE, Minghetti P, Selmin F, Montanari L. Fast dissolving films made of maltodextrins. Eur J Pharm Biopharm. 2008;70(3):895-900.
21. El-Kamel AH, Ashri LY, Alsarra IA. Micromatricial metronidazole benzoate film as a local mucoadhesive delivery system for treatment of periodontal diseases. AAPS PharmSciTech. 2007;8(3):E75.
22. Baranov N, Popa M, Atanase LI, Ichim DL. Polysaccharide-based drug delivery systems for the treatment of periodontitis. Molecules. 2021;26(9):2735.
23. Kilicarslan M, Koerber M, Bodmeier R. In situ forming implants for the delivery of metronidazole to periodontal pockets: formulation and drug release studies. Drug Dev Ind Pharm. 2014;40(5):619-624.
24. Amato M, Santonocito S, Polizzi A, Tartaglia GM, Ronsivalle V, Viglianisi G, et al. Local delivery and controlled release drug systems: a new approach for the clinical treatment of periodontitis therapy. Pharmaceutics. 2023;15(4):1312.
25. Rençber S, Karavana SY, Şenyiğit ZA, Eraç B, Limoncu MH, Baloğlu E. Mucoadhesive in situ gel formulation for vaginal delivery of clotrimazole: formulation, preparation, and in vitro/in vivo evaluation. Pharm Dev Technol. 2017;22(4):551-561.