DEVELOPMENT OF MICROMATRICIAL BIODEGRADABLE MOXIFLOXACIN PARTICLES FORMING IN-SITUGEL AS A POTENTIAL TREATMENT FOR PERIODONTITIS

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Abstract

Periodontal disease is a prevalent oral health issue caused by gram-negative bacterial infections in the periodontal pocket. This condition leads to inflammation of subgingival plaque and the deterioration of alveolar bone, teeth, dental cementum, and periodontal ligaments. It affects around 80% of adults in the United States and over 50% of the population in India which are affected by this chronic inflammatory condition, highlighting the seriousness of periodontitis. Current treatments aim to reduce bacterial infection and promote healing of the damage caused by inflammation. This study investigates a localized drug delivery system utilizing Moxifloxacin, a fourth-generation fluoroquinolone antibiotic, specifically targeting periodontal infections. The approach centers on designing and evaluating Eudragit-coated pectin microspheres to achieve controlled drug release and maintain effective concentrations within the periodontal pocket. These microspheres were formulated through the emulsion dehydration method, with pectin as the primary polymer and Eudragit RS100 as the coating material. Formulation B2 was characterized for particle size, morphology through scanning electron microscopy (SEM), swelling index (3.32%), drug entrapment efficiency (80.25%), and drug release rate (90.32%) in a pH 6.8 phosphate buffer. Results indicate that Eudragit-coated Moxifloxacin pectin microspheres are promising for periodontal therapy, effectively maintaining localized drug concentrations over an extended period, and enhancing patient compliance while minimizing adverse effects.

Keywords: Periodontitis, Moxifloxacin, In-situ Gel.

1. Introduction

Periodontitis

Early-stage gingivitis is characterized by swollen gums, bleeding, and bad breath. In its more severe form, it leads to gum inflammation and degeneration of the alveolar bone and dental cementum, a condition known as periodontitis. This disease involves progressive bone loss around the teeth, resulting in looseness and eventual loss of teeth, along with the formation of periodontal pockets, which typically range from 3 to 5 mm in depth. There are two types of periodontal pockets: suprabonyand infrabony. Periodontitis often develops from pre-existing gingivitis and is caused by microorganisms that adhere to the surfaces of teeth. Key periodontal pathogens, including *Porphyromonasgingivalis*, *Prevotella intermedia*, and *Actinobacillus actinomycetemcomitans*, contribute to tissue destruction.

Periodontal disease is one of the most common chronic diseases worldwide, with approximately 80% of American adults experiencing some form of it and about 50% of the Indian population affected. The prevalence of periodontal disease with pocket formation increases with age, with rates of about 1% at age 10, 10% at age 20, 20% at age 35, and 40% at age 50. Treatment for periodontitis primarily aims to reduce the overall bacterial load. Conventional approaches include both surgical and non-surgical methods, such as mechanical scaling and root planing, often combined with antimicrobial agents. These strategies focus on eliminating bacterial flora while ensuring prolonged drug-microbial contact.

The incidence and severity of gingival recession tend to rise with age. The pathogenesis of gingival recession is complex and can result from various factors, including inflammatory destruction, improper tooth brushing techniques, incorrect occlusal relationships, inadequate flossing, plaque buildup, and traumatic injuries. Chronic and aggressive forms of periodontitis are multifactorial diseases primarily driven by dental plaque microorganisms, with significant contributions from other local and systemic factors.

Fig.1. Diagram illustrating a healthy versus an infected tooth.

Traditional drug formulations like gels and emulsions frequently face challenges with short retention times in the mouth, influenced by factors such as saliva production, swallowing, food consumption, and the abrasive actions of soft tissues. Consequently, products that do not adhere well to oral surfaces provide limited effectiveness shortly after application. Bioadhesive systems applied to the oral mucosa could potentially increase drug retention and enhance treatment outcomes.

The usual approach to treating periodontitis includes mechanical methods, such as supragingival and subgingival scaling and root planing, often accompanied by antibiotic therapy.administered systemically or locally. The periodontal pocket serves as a natural reservoir, making it an ideal site for drug delivery systems. Intra-pocket delivery systems are particularly advantageous due to their potential for reducing side effects, improving treatment effectiveness, and enhancing patient compliance. Local drug delivery allows for higher concentrations of antimicrobial agents at the affected sites, offering significant benefits over systemic administration. Such devices generally consist of a drug reservoir and a controlling element that regulates the rate of drug release, ensuring sustained concentrations of active ingredients at the treatment site despite clearance from crevicular fluid.

Various agents, including tetracycline, chlorhexidine, and metronidazole, have been formulated into local delivery devices. These devices can maintain high local drug concentrations over extended periods, with several types, such as fibers, films, dental implants, and gels, currently in use. Dental implants can be easily placed in the periodontal pocket, delivering therapeutic concentrations of Moxifloxacin for prolonged periods at lower doses. It is essential for the drug to remain on the mucosal surface to facilitate controlled release at the local site. The administered microspheres convert to a gel-like structure in the periodontal pocket, thereby increasing the residence time of the formulation at the site of action.

Materials and Methods:

Materials: Moxifloxacin was kindly provided by Laborate Pharmaceuticals Ltd, based in Paonta Sahib, H.P., India. The polymers, pectin and Eudragit, were obtained from SD Fine-Chem Limited in New Delhi. All other chemicals used were of analytical grade and sourced from commercial suppliers.

Preparation of Moxifloxacin Microspheres:

Emulsion-Dehydration Method: The drug and polymer were dissolved in distilled water and stirred continuously for 10 hours. This drug-polymer solution was then dispersed in light liquid paraffin containing Span 80, with continuous stirring at 1000 rpm to form a water/oil emulsion. The mixture was rapidly cooled to 15 ºC, after which acetone was added to dehydrate the pectin droplets. Stirring continued at 1000 rpm and 25 ºC for 30 minutes to ensure complete solvent evaporation. The microspheres were then washed with acetone and stored in a desiccator.

Coating of Pectin Microspheres Loaded with Moxifloxacin: The Moxifloxacin-loaded pectin microspheres were encapsulated using an emulsion-evaporation method. A 100 mg sample of pectin microspheres was dispersed in 20 ml of a coating solution, which was prepared by dissolving different ratios of Eudragit in an ethanol-acetone mixture (2:1). This organic phase was then introduced into 12.5 ml of light liquid paraffin containing 1% wt/vol Span 80. Emulsification was carried out for 2 hours at 1000 rpm using a mechanical stirrer. The resulting Eudragit-coated microspheres were collected, washed with n-hexane, and dried.

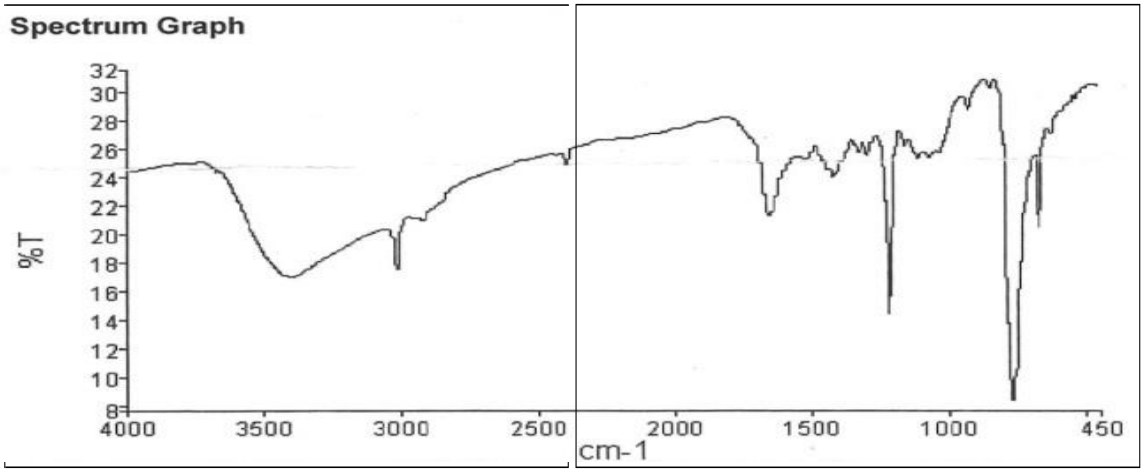


FIG.2: SpectralGraph of FTIR of Moxifloxacin

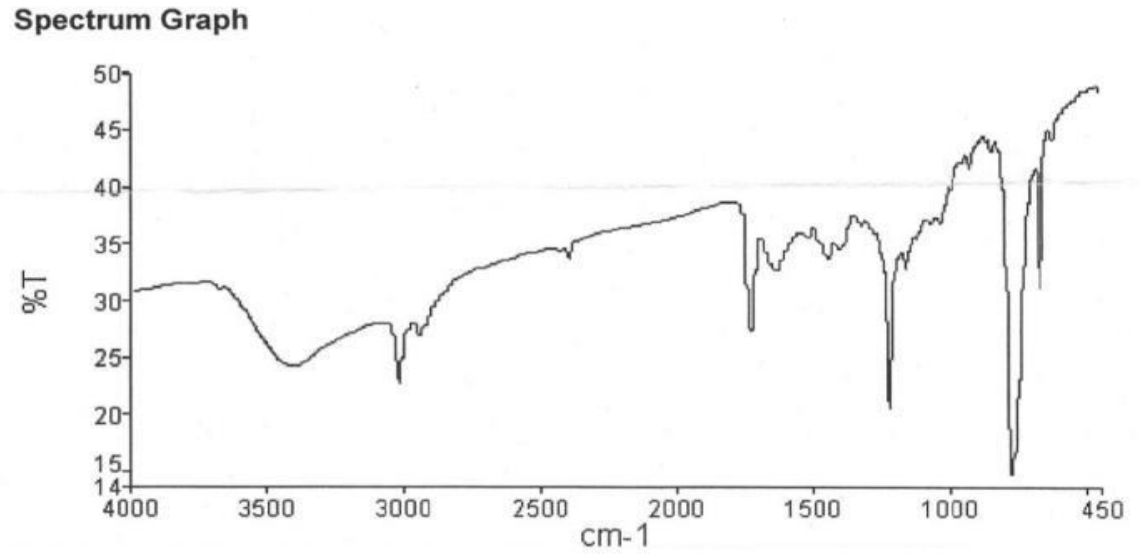


Fig. 3: IR spectra of drug, pectin, and eudragit

RESULTSANDDISCUSSION:

Drug-Excipient Interaction Study: The drug and polymer were assessed for their interaction using Fourier Transform Infrared Spectroscopy (FTIR). The FTIR spectral lines of the pure drug is presented in Fig. 2, while the spectral lines of the mixture of the drug with the polymers, shown in Fig. 3, revealed no substantial shifts at the position of the distinctive peak. This finding indicates compatibility between the polymer and the drug.

Table 1: Physicochemical Evaluation Results of the Microspheres

S. no.	Batch	Code of Formulation	Particle Size (given in µm)	Swelling Index (given in %)	Percentage yield (given in %)	Drug content (given in %)	Drug Entrapment Efficiency(given in %)	Cumulative % Drug Release
1	A-1	F-1	310.22±0.23	2.52±0.06	54.54±0.12	11.36±0.99	70.49±0.32	88.94±0.23
2		F-2	363.88±0.14	2.39±0.12	54.16±0.04	10.41±1.08	71.57±0.37	87.51±0.57
3		F-3	377.43±0.01	1.26±0.02	54.81±0.02	10.47±1.09	73.52±0.33	83.96±0.91
4	A-2	F-4	308.08±1.1	2.96±0.01	56.54±0.13	10.86±0.47	72.74±0.24	79.88±0.45
5		F-5	433.26±0.02	3.29±0.01	55.75±0.06	10.00±1.00	75.45±0.45	81.25±0.27
6		F-6	334.35±0.29	3.32±0.01	55.55±0.96	8.92±0.61	80.25±0.55	90.84±1.42
7	A-3	F-7	355.63±0.10	1.93±0.03	53.75±0.03	10.43±1.55	72.48±0.31	87.23±1.15
8		F-8	343.56±0.45	1.90±0.02	57.27±0.02	9.61±1.11	77.51±0.05	81.12±0.66
9		F-9	321.09±0.57	2.39±0.01	55.70±0.52	8.62±1.13	79.64±0.49	74.69±0.83

Morphology(Shape and Surface): The shape and surface characteristics of the microspheres were analyzed using by scanning electron microscopy (SEM). To prepare the samples, the microspheres were carefully placed on double-sided adhesive tape attached to an aluminum stub, which was then coated.

Swelling Index: The minimum swelling index of the microspheres was recorded at 1.26% in formulation F3 of batch B1, while the maximum swelling index was observed at 3.32% in formulation F6 of batch B2 when tested in 6.8 phosphate buffer. Since pectin is soluble in water, a higher concentration of pectin leads to an increased swelling index. Detailed results of the swelling index are presented in Table 1.

Percentage Yield: The high concentration of pectin and Eudragit polymers in formulation F8 resulted in a maximum percentage yield of 57.27%, while formulation F7 of batch B3 had a minimum yield of 53.27%. Detailed results of the percentage yield are provided in Table 1.

Drug Content: In formulation F6, the concentration of Eudragit was high, resulting in a minimum drug content of 8.92% for batch B2. The samples were coated with gold using a sputter coater under high vacuum and high voltage. Scanning electron microscopy (SEM) images of the uncoated

microspheres are shown in Fig. 4, the coated microspheres in Fig. 5, and the images after drug release microspheres in Fig.6

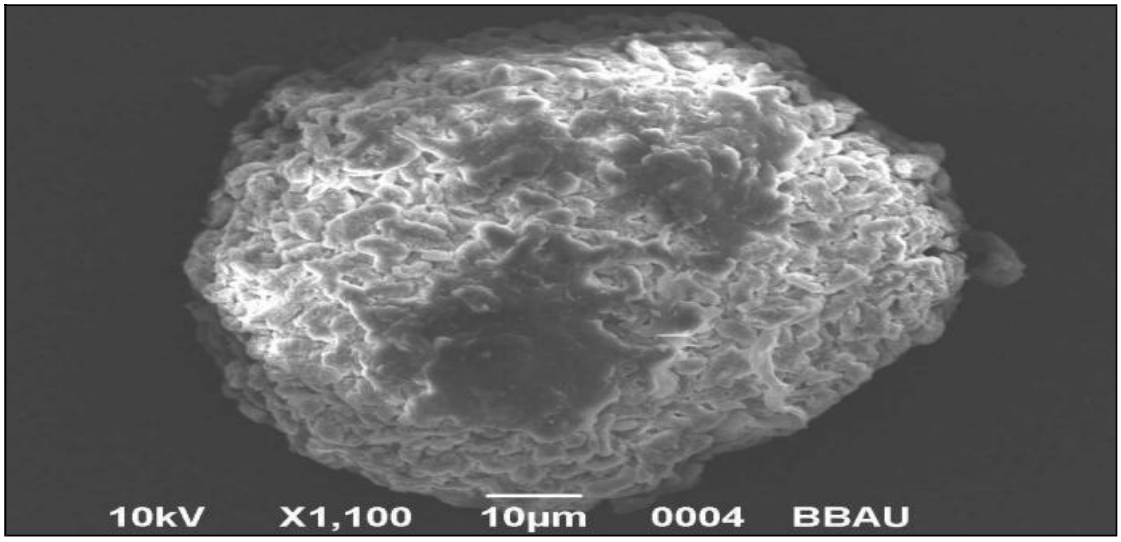
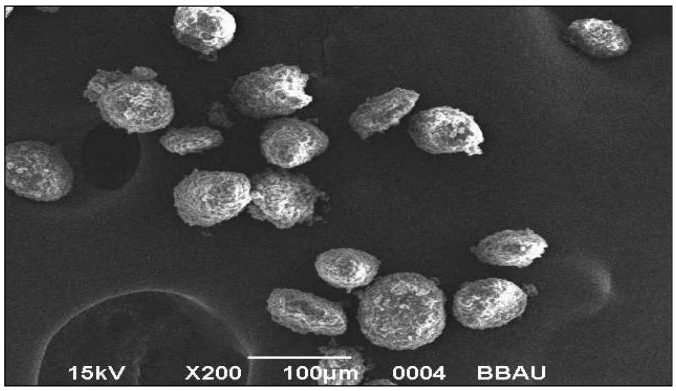


FIG.4: SEMIMAGEUNCOATEDMICROSPHERES

Concentration of eudragit was less in F1 formulation, and drug content was found to be a maximum of 11.36% in batch B1. The detail result of drug content is given in Table 1.



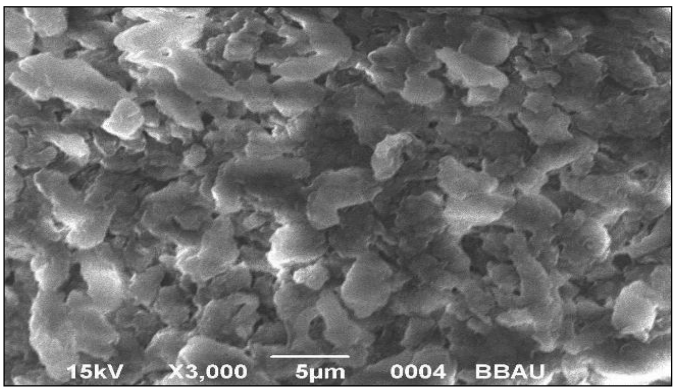


FIG. 5:SEM IMAGE OF COATED MICROSPHERES **FIG.6:SEMIMAGE Before drug release After Drug Release**

Efficiency of Drug Entrapment: The drug entrapment efficiency was measured using a UV spectrophotometer, revealing a maximum value of 80.25% in formulation F6 of batch B2, attributed to the high concentration of pectin. Conversely, a lower concentration of pectin in formulation F1 of batch B1 resulted in a minimum entrapment efficiency of 70.49%. Detailed drug entrapment efficiency results are presented in Table 1.

In-vitro Drug Release Study of Microspheres: The maximum cumulative drug release from the microspheres was observed to be 90.84% in formulation F6 of batch B2 after 8 hours, attributed to the low concentration of Eudragit. Conversely, the minimum cumulative drug release was 74.69% in formulation F9 of batch B3 after 8 hours, due to a higher concentration of Eudragit. The drug release study was conducted in phosphate buffer at pH 6.8, and a comparison of drug release for formulations F4, F5, and F6 is shown in Fig. 7. Detailed percentages of cumulative drug release for different batches are provided in Table1.

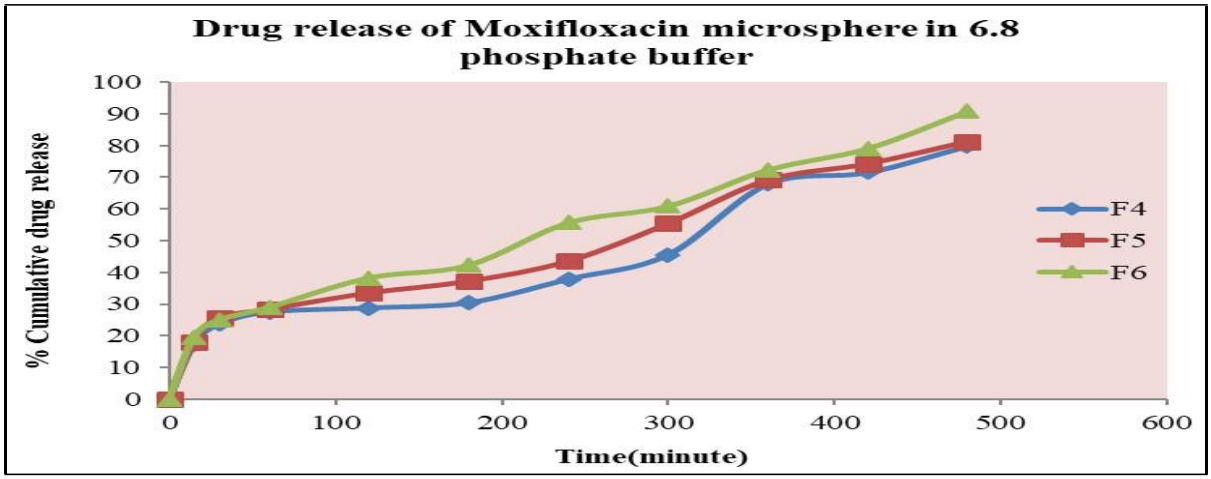
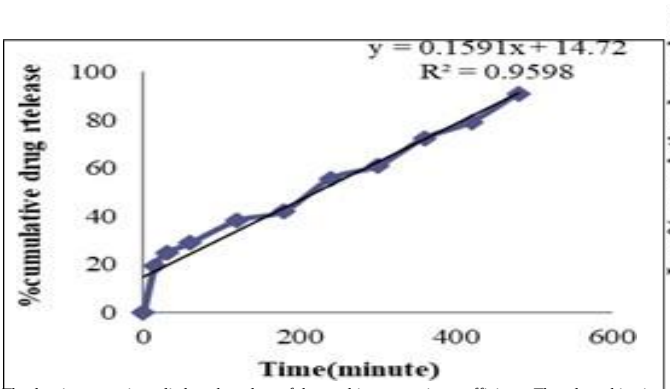
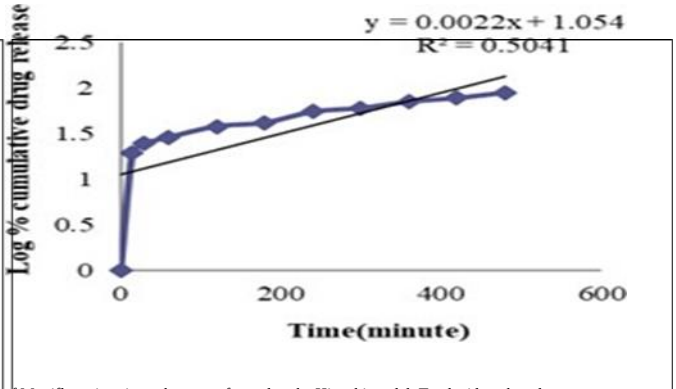


Fig. 7: In-vitro Release Study Graph of Optimized Formulations (f4, f5 & f6)

Mechanism of Release: The in-vitro release data were analyzed using various equations and kinetic models to describe the release kinetics of Moxifloxacin from the microspheres. The kinetic models employed included the zero-order equation shown in Fig. 8, the first-order equation in Fig. 9, the Higuchi model in Fig. 10, and the Korsmeyer-Peppas model in Fig. 11.





The data interpretation relied on the values of the resulting regression coefficients. The release kinetics of Moxifloxacin microspheres conformed to the Higuchi model. To elucidate the release mechanism of Moxifloxacin from the microspheres, the drug release data were analyzed using the Higuchi model, which yielded the highest regression coefficient value ($R^2 = 0.9714$), suggesting a non-Fickian diffusion mechanism for drug release. The regression coefficient values for all models are presented in Table 2.

Table 2: In-vitro Release kinetics Standardsfor code offormulation (f6)

Batch	ZeroOrderRelease Kinetics		FirstOrderRelease Kinetics		HiguchiEquation		Korsmeyer's PeppasEquation	
F-6	K	R^2	K	R^2	K	R^2	N	R^2
	0.1591	0.9598	0.0022	0.5041	3.7586	0.9714	0.6454	0.9319

Table 3: Antimicrobial effectiveness of moxifloxacin microspheres against S. Aureus.

Time(inmin)	Zoneofinhibition(cm)(*S.aureus*)
0	0
60	1.0
120	1.2
180	1.4
240	1.4
300	1.8
1440	2.0

The antibacterial effectiveness of Moxifloxacin-loaded Eudragit-coated microspheres was evaluated by determining the minimum inhibitory concentration (MIC) against the pathogenic strain Staphylococcus aureus (S. aureus). Agar plates were prepared and inoculated with S. aureus. After 48 hours of incubation at 37 °C, samples with various dilutions were transferred to freshly inoculated agar plates and incubated for an additional 48 hours. The zones of inhibition were observed at different time intervals on the agar plates, with an inhibition zone measuring 1 cm recorded. The antimicrobial results are presented in Table 3 below.

Stability Studies: Stability studies of formulated microspheres was carried out by storing the formulation F6 at 4 °C ± 1 °C in refrigerator and at 40 °C ± 2 °C, RH 75% ± 5 in humidity control oven and room temperature and humidity (25 °C ± 2 °C / RH 60% ± 5%) for a period of 2 months (according to ICH guidelines) refer the values of drug content in Table 4, 5 and 6.

Table4:Physicochemical evaluation of formulation F6 during stability studies conducted at 4°C ± 1°C.

S.no.	Standards	Day 0	Day 15	Day 30	Day 45	Day 60
1	Physical Standards	+	+	+	+	+
2	Active Ingredient Concentration	10.86±0.47	10.86±0.47	10.82±0.43	10.82±0.43	10.76± 0.38

Table5:Physicochemical evaluation of formulation F6 during stability studies conducted at 25°C ± 2°C with 60 ± 5% relative humidity.

S.no.	Standards	Day 0	Day 15	Day 30	Day 45	Day 60
1	Physical Standards	+	+	+	+	+
2	Active Ingredient Concentration	10.86±0.47	10.86±0.47	10.79± 0.39	10.79±0.39	10.75±0.32

Table6:Physicochemical evaluation of formulation F6 during stability studies conducted at 40°C ± 2°C and 75% relative humidity ± 5%.

S.no.	Standards	Day 0	Day 15	Day 30	Day 45	Day 60
1	Physical Standards	+	+	+	+	+
2	Active Ingredient Concentration	10.86±0.47	10.81±0.47	10.79±0.41	10.75± 0.36	10.75±0.42

CONCLUSION: The limitations of systemic antibiotic therapy have sparked interest in developing localized drug delivery systems that can deliver effective antibiotic concentrations directly to the periodontal site with fewer side effects. Moxifloxacin microspheres, prepared using the emulsion-dehydration technique, have proven to be a valuable method for creating microspheres. By ensuring the drug remains at the site of absorption, this approach can maintain drug concentration and therapeutic efficacy, in contrast to systemic antibiotic administration. The results suggest that Eudragit-coated pectin microspheres of Moxifloxacin could serve as a promising drug delivery system for targeting the periodontal pocket.

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CONFLICT OF INTEREST: Nil

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