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Original Research Article

Development of a hydrogel containing metronidazole-loaded Eudragit RS 100 nanoparticles for buccal drug delivery

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Abstract

Purpose: *To formulate Eudragit RS 100-based nanoparticles containing metronidazole (MTZ NPs) and incorporate them into hydrogels using different gelling agents for controlled release in the buccal mucosa.*
Method: *MTZ NPs were fabricated using the nanoprecipitation method. MTZ NPs (1 % w/w) were introduced into hydrogels using different gelling excipients. Physicochemical properties of MTZ NPs, mucoadhesive strength and spreadability of the hydrogels, and ex vivo permeation of MTZ were evaluated.*
Results: *MTZ NPs were spherical in shape and had a mean particle size of 201.9 ± 5.6 nm, polydispersity index (PDI) of 0.092 ± 0.014, and encapsulation efficiency of 46.28 ± 1.18 %. Hydroxyethyl cellulose (HEC) hydrogel containing MTZ NPs showed homogenous and smooth appearance, mucoadhesive strength of 2789.7 ± 13.4 dyne/cm^2, and spreadability of 5.32 ± 0.77 cm. The cumulative amount of drug permeated from MTZ hydrogel and MTZ-containing NP hydrogel through porcine buccal mucosa was 1104.33 ± 28.97 and 397.62 ± 16.13 µg/cm^2, respectively, after 7 h ($p < 0.05$).*
Conclusion: *HEC hydrogel containing MTZ NPs demonstrates high mucoadhesive strength, good spreadability, and sustained ex vivo permeation of MTZ. Thus, HEC hydrogel is a promising pharmaceutical formulation for controlled drug release in the buccal mucosa.*

Keywords: *Metronidazole, Nanoparticle, Eudragit RS 100, Hydrogel, Buccal delivery*

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INTRODUCTION

Periodontitis, a chronic inflammation of the gum and periodontium, affects the supporting structure of the teeth and may lead to tooth loss. In early stages, gingivitis (mild form of periodontal disease) occurs. If not promptly treated, gingivitis progresses to periodontitis. The disease is often initiated by opportunistic infections and harmful bacteria in the plaque, and tartar accumulation is the main cause of periodontitis. Various strategies, including mechanical, pharmacological, and surgical

methods (in severe conditions), have been applied to treat the disease [1].

Systemic administration of antibiotics has some limitations such as erratic antibiotic concentration in target tissues, rapid decrease in plasma drug concentration below the therapeutic index, or antibiotic resistance. Alternatively, topical routes of administration might be used to avoid such problems and are recommended for treatment of periodontitis. Particularly, gel is a pharmaceutical form that delivers high concentrations of antibiotics to the periodontal pocket and ensures a long time of contact between the bactericidal drug and treatment site. Furthermore, these gel-based products have better patient acceptability owing to their special properties, such as convenient removal, non-greasy texture, and wide spreadability [1]. Thus, hydrogels are promising pharmaceutical forms for buccal drug delivery. Topical application of bioadhesive gels in the oral cavity provides prolonged retention time, proper drug permeation, and high efficacy and patient compliance [2].

Metronidazole (MTZ), an antibiotic of the nitroimidazole class, has good efficacy against anaerobic bacteria, which are the main contributors to periodontal disease. Reduction of the nitro group of MTZ to a nitrosohydroxyl amino group leads to the disruption of the structure of microbial DNA, which eventually inhibits the synthesis of bacterial proteins [3]. Although MTZ is effective in the treatment of periodontitis, its rapid release from classical gels is an obstacle in buccal delivery. Nanotechnology has been used to counteract this disadvantage by prolonging the duration of action of MTZ and enhancing its bioavailability. Eudragit RS 100 (EUD) is insoluble at physiological pH, and EUD-based nanoparticles (NPs) are promising candidates for ensuring controlled drug release at the site of action [4].

Therefore, this study was aimed at preparing a sustained-release dosage form for the buccal delivery of MTZ from EUD-based NPs embedded in hydrogels.

EXPERIMENTAL

Materials

MTZ was purchased from Hubei Hongyuan Pharmaceutical Technology Co. Ltd. (China). EUD was purchased from Enovik (Germany). Sodium carboxymethyl cellulose (NaCMC) was obtained from Daicel Corporation (Osaka, Japan). Carbopol® 934 (CBP) was purchased from Lubrizol Corporation (Ohio, USA) and hydroxypropyl methyl cellulose K15M (HPMC) from Colorcon (PA, USA). Hydroxyethyl cellulose (HEC) was procured from Ashland Inc. (DE, USA). Tween 80 was purchased from Xilong Scientific Co. Ltd., (Guangdong, China). All other chemicals were of high-performance liquid chromatography (HPLC) or analytical grade.

Preparation of MTZ-containing NPs

MTZ-containing NPs (MTZ NPs) were fabricated using the nanoprecipitation approach [5]. MTZ and EUD were dissolved in acetone. This phase was subsequently added to the aqueous phase containing Tween 80 at a flow rate of 2 mL/min, while simultaneously employing magnetic stirring at 1000 rpm (IKA, Germany) for 30 min. Acetone was removed using a vacuum evaporator (Buchi, Switzerland) at 50 °C for 1 h. The resultant NPs were washed thrice with distilled water using a centrifugal filter (Millipore, USA) with molecular weight cut-off of 10 kDa at 3000 × *g* for a duration of 30 min.

MTZ suspension was lyophilized using a freeze-dryer (Coolsafe 55 – 4 PRO, Scanvac, Denmark). The dispersion underwent a preliminary freezing process at a temperature of -70 °C for a period of 12 h. Subsequently, it was subjected to lyophilization at -50 ± 2 °C for 24 h with a vacuum pressure of 0.120 ± 0.020 mbar.

Preparation of hydrogel formulations containing MTZ NPs

MTZ NPs (MTZ, 1% w/w) were introduced into hydrogels using different gel-forming excipients (CBP, NaCMC, HPMC, HEC), glycerin (5%, w/w) as a moisturizer, and nipagin (0.18%, w/w) and nipasol (0.02%, w/w) as preservatives. Triethylamine was used to adjust the pH between 6 and 7 (Table 1). The hydrogels were left undisturbed overnight and subsequently stored at 4 °C for further investigations [6].

Characterization of MTZ-containing NPS

Particle size and polydispersity index (PDI)

The particle size and PDI of NPs were determined using dynamic light scattering, employing the Zetasizer Nano 90 instrument manufactured by Malvern Instruments (UK). Triplicate measurements were performed with each sample [6].

Table 1: Formulations of hydrogels containing MTZ NPs

MTZ (%, w/w)	CBP (%, w/w)	TEA (%, w/w)	NaCMC (%, w/w)	HEC (%, w/w)	HPMC (%, w/w)
1.0	0.2	q.s	0	0	0
1.0	0.3	q.s	0	0	0
1.0	0.5	q.s	0	0	0
1.0	0	0	2.0	0	0
1.0	0	0	3.0	0	0
1.0	0	0	4.0	0	0
1.0	0	0	0	2.0	0
1.0	0	0	0	3.0	0
1.0	0	0	0	0	1.0
1.0	0	0	0	0	3.0
1.0	0	0	0	0	4.0

Morphological characterization

Hitachi S-4800 instrument (Tokyo, Japan) was utilized for scanning electron microscopy (SEM) analysis to examine the morphology of NPs. To prepare the samples for imaging, they were deposited onto aluminum foil and allowed to air-dry at a controlled temperature of 25 ± 2 °C. Before conducting SEM analysis, a 3 nm layer of platinum was applied to the surface of the NPs [6].

Fourier transform infrared spectroscopy (FT-IR)

Samples (MTZ, EUD, Physical mixture (PM), and MTZ NPs) were compressed into KBr tablets and analyzed using a Shimadzu Prestige-21 FTIR spectrometer (Shimadzu Prestige-21, Japan) over a range of 4000–500 cm^{-1} [6].

X-ray powder diffraction (XRPD) analysis

Spectra of samples, including MTZ, EUD, PM, and MTZ NPs, were captured using a diffractometer (D8 ADVANCE, Bruker Corporation, MA, USA). The instrument parameters were set as follows: Cu Kα radiation (λ = 1.5406); 2θ, 10°–60°; step size, 0.03°; total measurement time, 1497 s; current, 40 mA; voltage, 40 kV; measuring temperature, 25 ± 2 °C [6].

Encapsulation efficiency

MTZ (2 ml) nanosuspension was introduced into a centrifugal filter (10 kDa, Millipore, USA) and subjected to centrifugation at 3000 × *g* for 30 min. The amount of free MTZ (Wf) was determined using HPLC, and the encapsulation efficiency (EE) of the drug was calculated using Equation 1 [6].

$$EE\ (\%) = \{(Wi–Wf)/Wi\} \times 100 \quad \ldots\ldots\ldots\ldots (1)$$

where Wi is the weight (in mg) of the initial drug.

Metronidazole content

HPLC analysis was employed using a Shimadzu Prominence system (Kyoto, Japan) to analyze the MTZ content. The analysis utilized an InertSustain C18 column measuring 15 cm × 4.6 cm, with a particle size of 5 µm (GL Sciences Inc., Japan). A mixture of methanol and distilled water in a ratio of 20:80 (v/v) was used as the mobile phase. A UV detector set at a wavelength of 320 nm was used for detection. The flow rate was set at 1.0 mL/min, and a 30 µL injection volume was employed for each analysis [7].

Characterization of MTZ-NP hydrogels

Visual appearance and pH

Fabricated gel formulations containing MTZ-loaded NPs were visually examined for their appearance, presence of particulate matter, and uniformity. Each hydrogel formulation (1 g) was dispersed in 10 mL of distilled water, and the pH of the resulting dispersion was measured using a pH meter (pH Sension PH3 HACH, Spain) *[6].*

Mucoadhesive strength

Mucoadhesive strength was determined using the physical balance method of Bansal *et al* [8] with minor modifications. The apparatus is a two-arm balance (Figure 1), with one arm containing two glass vials and the other a container. Porcine buccal tissues were obtained from a local abattoir (Thua Thien Hue, Vietnam). Fresh tissue was attached to both glass vials. The hydrogel formulation (0.1 g) was laid over the tissue as a thin layer on a lower vial. The upper vial was then attached on the lower vial with an applied weight of 50 g for 5 min over the upper vial. Water was dropped from a burette into the container of the remaining arm of balance at a dropping rate of 10 mL/min until the upper and

lower vials were detached. Its weight was recorded for determining the mucoadhesive strength (dyne/cm^2).

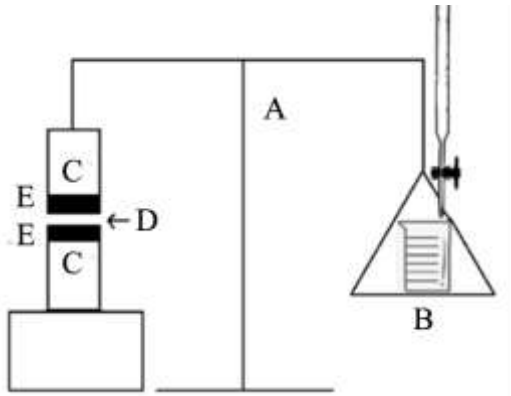

Figure 1: Illustration of the device for measuring mucoadhesive strength of hydrogels (A: balance; B: water container; C: glass vial; D: hydrogels; E: porcine buccal tissue)

Spreadability test

The spreadability of the prepared hydrogels was measured by adding the hydrogel (0.5 g) on one glass plate in a pre-marked circle (1 cm in diameter). A second glass plate was placed upon the hydrogel mass. A 100 g load was put on the upper glass plate for 5 min and the spreadability was calculated by measuring the increase in diameter of the hydrogels [9].

Ex vivo *permeation studies*

The permeation studies were performed using a diffusion system (Hanson Research, CA, USA) comprising a working diffusion area of 1.767 cm^2 and a receptor chamber that had a release volume of 7.0 mL. Within 3 hours of sacrificing the pig, porcine buccal mucosa was collected using surgical scissors. After immersing in the release medium (phosphate-buffered saline, pH 6.8) for 1 h, the MTZ-containing NP hydrogel (0.3 g) was applied to the surface of the tissue. As a control, an MTZ hydrogel (1%, w/w) was used. The release medium was kept at a temperature of 37 °C and constantly agitated at 400 rpm using a magnetic shaker. Two milliliter of release medium was withdrawn at each defined time point. Fresh medium was immediately added to maintain the initial volume. The quantification of MTZ content was performed through HPLC analysis [6].

The structural analysis of the porcine oral mucosa was performed after hematoxylin and eosin staining using standard tissue processing protocols. The stained sections were examined using a Leica light microscope (Model DM750, Germany) [6].

Statistical analysis

The results are reported as mean ± standard deviation based on triplicate experiments. Statistical comparisons between groups were performed using Student's *t*-test or ANOVA at a confidence level of 95%.

RESULTS

Characteristics of MTZ-containing NPs

Particle size and morphology

After evaluating the physicochemical properties of MTZ-containing NPs prepared with different formulation factors, the most favorable formulation was selected for further development (*data not shown*). This formulation consisted of 5 mg/mL polymer, 1% (w/v) Tween 80, a polymer to drug ratio of 9:1 (w/w), and an oil phase to aqueous phase ratio of 1:1 (v/v). MTZ NPs were spherical and polydispersed, with a particle size of 201.9 ± 5.6 nm (Figure 2 A), PDI of 0.092 ± 0.014, and EE of 46.28 ± 1.18%. SEM images of the MTZ NPs are shown in Figure 2 B.

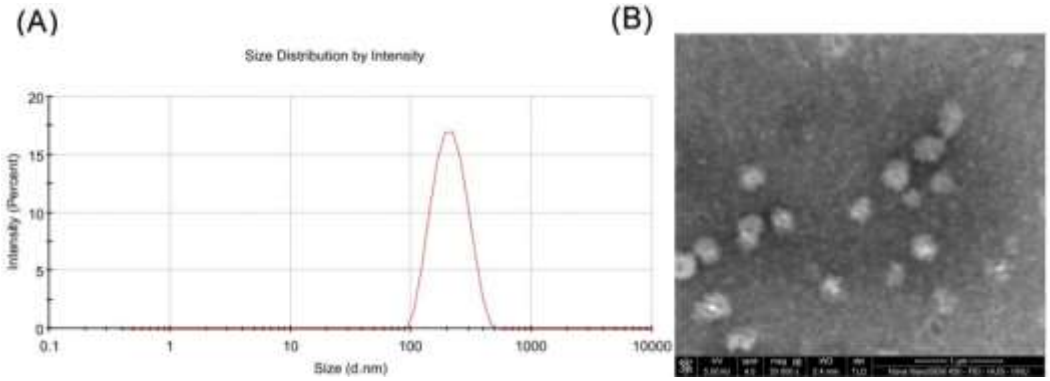

Figure 2: (A) DLS analysis, and (B) Morphology of MTZ NPs by SEM (Scale bar = 1 µm)

FT-IR spectra

The characteristic peaks of MTZ, EUD, PM, and NPs were identified using FTIR spectroscopy. The characteristic peaks of MTZ were assigned to OH stretching vibration at 3221 cm^{-1}, C=CH stretching at 3098 cm^{-1}, NO stretching at 1535 cm^{-1}, -CN at 1186 cm^{-1}, and C-OH stretching at 1074 cm^{-1} (Figure 3). EUD showed characteristic peaks at 1730.15 cm^{-1} related to carbonyl ester group and at 2993.52 cm^{-1} corresponding to C-H stretching. These peaks were present in the spectra of MTZ NPs and PM. The FTIR results indicate no chemical interaction between MTZ and other excipients [3].

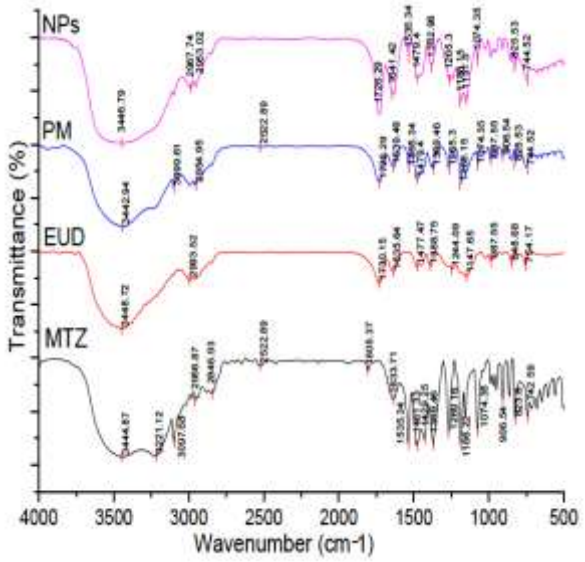

Figure 3: FT-IR spectra of MTZ, Eudragit RS 100 (EUD), the physical mixture (PM), and the nanoformulations (NPs)

X-ray powder diffraction

The XRPD patterns of MTZ, EUD, NPs, and PM are shown in Figure 4. The crystallinity of MTZ was observed through numerous sharp peaks at 2θ angles of 12.2°, 13.8°, 24.7°, 27.8°, and 29.3° [10]. The XRPD profile of EUD exhibited no sharp peaks owing to its amorphous state [11]. In the XRPD pattern of NPs, sharp peaks corresponding to MTZ either disappeared or their intensities were noticeably reduced. Results show that in NPs, MTZ mostly changes into an amorphous state.

MTZ-containing NP hydrogel

Gel formulations were prepared to overcome the obstacles encountered with EUD-based NPs, including their low viscosity and mucoadhesive properties. The hydrogel texture gradually thickened when the concentration of gelling agent was increased. Owing to their homogenous and smooth appearance, hydrogel formulations using CBP at a concentration of 0.3% (w/w), NaCMC at a concentration of 3% (w/w), and HEC at a concentration of 3% (w/w) were used in further investigations.

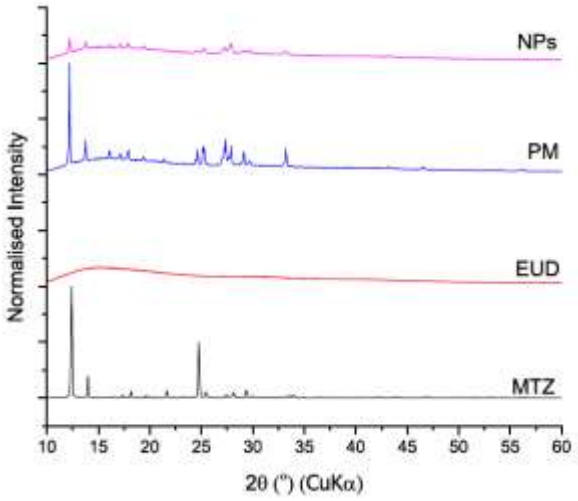

Figure 4: XRPD patterns of MTZ, Eudragit RS 100 (EUD), physical mixture (PM), and nanoformulations (NPs)

Mucoadhesive strength

The mucoadhesive strength of hydrogel formulations containing MTZ NPs were 2196.3 ± 44.8, 2543.3 ± 49.4, and 2789.7 ± 13.4 dyne/cm^2 for 0.3% CBP, 3% NaCMC, and 3% HEC, respectively. The 3% (w/w) HEC-based formulation had the highest detachment stress from all three formulations ($p < 0.05$). Thus, the 3% (w/w) HEC-based formulation was chosen to prepare the MTZ-containing NP hydrogel.

Spreadability

Spreadability refers to the spread of the hydrogel onto the application area and is a critical parameter for high therapeutic efficacy and patient compliance. The HEC-based formulation had good spreadability, with a diameter of 5.32 ± 0.77 cm. In addition, HEC-based formulation had a pH of 6.34 ± 0.35 and drug content of 0.988 ± 0.012 % (w/w).

Ex vivo permeation

The *ex vivo* skin permeation profiles were assessed for the developed hydrogel formulations. Their drug permeation data across porcine buccal mucosa at pH 6.8 (in phosphate buffer) are illustrated in Figure 5. For enhancing drug permeation in the buccal cavity and achieving pharmacological efficacy, the

residence time is an essential parameter that needs to be controlled. The smooth layer and no physiological changes in the porcine buccal mucosa were observed, which could ensure the performance of the study (Figure 5 A). As shown in Figure 5 B, the MTZ-containing NP hydrogel exhibited lower permeability through the porcine buccal mucosa than the MTZ hydrogel. The permeability profile of the MTZ-containing NP hydrogel demonstrated a prolonged-release pattern, indicating a sustained and controlled drug permeation over time. After a 7-hour period, the cumulative amount of permeated drug from MTZ hydrogel and MTZ-containing NP hydrogel was determined to be 1104.33 ± 28.97 and 397.62 ± 16.13 µg/cm^2, respectively ($p < 0.05$).

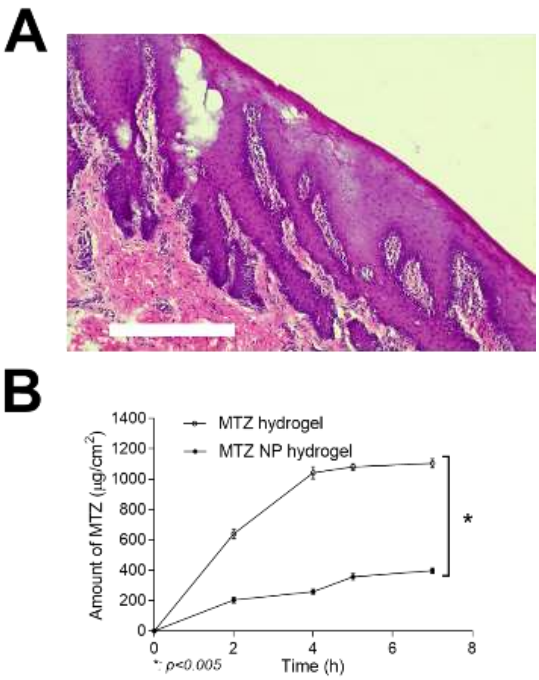

Figure 5: (A) Hematoxylin and eosin staining image of porcine buccal epithelium before permeation study (Scale bar = 500 µm); (B) Amount of MTZ permeated from MTZ hydrogel and MTZ NP hydrogel at pH 6.8 (in phosphate buffer, n = 3)

DISCUSSION

EUD is a pH-independent, biocompatible, and insoluble copolymer that is widely used to control drug release in various drug delivery systems [4]. Various formulations of EUD-based NPs were developed via nanoprecipitation by altering different variables. The best formulation of NPs was further introduced into hydrogels to overcome the challenges of drug delivery to the oral cavity, such as the movement of saliva fluid and surrounding tissues [2].

Bioadhesive hydrogels are often fabricated for oral drug administration. Bioadhesive products could prolong the residence time of drugs at the oral site. A variety of hydrogel-based bioadhesive formulations for modified drug release have been summarized in literature [12]. In this study, the influence of gelling agents (CBP, NaCMC, HEC, and HPMC) in a buccal drug delivery system with MTZ-containing EUD-based NPs was investigated.

The hydrogel prepared at a CBP concentration of 0.3 % (w/w) exhibited a uniform and smooth texture. Similar results were obtained by Singh *et al* [13] who prepared hydrogels containing acetazolamide-poly (lactic-co-glycolic) acid NPs using CBP concentrations ranging from 0.1 to 0.5 %. Increasing the concentration of NaCMC from 2 to 4 % (w/w) led to a gradual thickening of hydrogels. The hydrogel formed using 3 % (w/w) NaCMC had a smooth and homogeneous texture. Mohammed *et al* [14] also used this NaCMC concentration for developing hydrogels containing diclofenac. A smooth, thick, and homogeneous hydrogel was formed using 3 % (w/w) HEC. Bansal *et al* [15] reported similar results at HEC concentrations of 2 – 3 %. Thus, for further investigations, hydrogels containing 0.3 % (w/w) CBP, 3 % HEC, and 3 % (w/w) NaCMC was prepared.

Among the three formulations, the 3 % HEC-based formulation had the highest adhesive strength. HEC has a strong adhesive capacity owing to its good swelling in water and good adhesion to the mucous layer. This result is supported by the findings of Andrews *et al* [16], who showed that HEC has higher adhesion than NaCMC. In this study, the HEC-based formulation also exhibited good spreadability.

Porcine buccal mucosa is considered suitable for performing *ex vivo* permeability studies owing to its similar structure and composition to those of human mucosa [17]. The integrity of porcine buccal mucosa was checked and there were no physiological changes before using it in the permeation study [6].

When compared with hydrogel containing free MTZ, the hydrogel containing MTZ-loaded EUD-based NPs showed a longer release through porcine buccal mucosa ($p < 0.05$ at 7 h), indicating a prolonged release profile. The existence of positively charged ammonium groups in EUD can enhance mucoadhesive properties of the hydrogel by ionic interaction with mucin layer having negative charge within the mucosa. Thus, it can ensure a close contact between the hydrogels and the buccal mucosa and can extend the duration of contact [4]. The sustained release from hydrogel containing MTZ-

loaded EUD-based NPs has potential for local administration, avoiding the systemic absorption of drug.

CONCLUSION

MTZ NPs have been successfully fabricated using nanoprecipitation method with an EUD carrier. HEC-based hydrogel containing MTZ-loaded EUD-based NPs exhibit high mucoadhesive strength, good spreadability, and extended *ex vivo* drug permeability. Thus, the developed HEC-based hydrogel containing MTZ-loaded EUD-based NPs is a promising pharmaceutical formulation for local drug administration to the buccal mucosa.

DECLARATIONS

Acknowledgements

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Hoang Nhan Ho performed conceptualization, methodology, formal analysis, investigation, writing – original draft, resources, and supervision, while Van Anh Tuan Nguyen, Nguyen Anh Thu Ho, and Hoang Hao Le participated in the Methodology, formal analysis, and investigations.

REFERENCES

1. *Wroblewska M, Szymanska E, Szekalska M, Winnicka K. Different types of gel carriers as metronidazole delivery systems to the oral mucosa. Polymers (Basel) 2020; 12(3).*
2. *Salamatmiller N, Chittchang M, Johnston T. The use of mucoadhesive polymers in buccal drug delivery. Adv Drug Deliv Rev 2005; 57(11): 1666-1691.*
3. *Zupančič Š, Potrč T, Baumgartner S, Kocbek P, Kristl J. Formulation and evaluation of chitosan/polyethylene oxide nanofibers loaded with metronidazole for local infections. Eur J Pharm Sci 2016; 95: 152-160.*
4. *Di Prima G, Conigliaro A, De Caro V. Mucoadhesive polymeric films to enhance barbaloin penetration into buccal mucosa: A novel approach to chemoprevention. AAPS PharmSciTech 2019; 20(1): 18.*
5. *Aisha AFA, Abdulmajid AMS, Ismail Z, Alrokayan SA, Abu-Salah KM. Development of polymeric nanoparticles of Garcinia mangostana xanthones in Eudragit RL100/RS100 for anti-colon cancer drug delivery. J Nanomater 2015; 2015: 1-12.*
6. *Ho HN, Le HH, Le TG, Duong THA, Ngo VQT, Dang CT, Nguyen VM, Tran TH, Nguyen CN. Formulation and characterization of hydroxyethyl cellulose-based gel containing metronidazole-loaded solid lipid nanoparticles for buccal mucosal drug delivery. Int J Biol Macromol 2022; 194: 1010-1018.*
7. *United States Pharmacopeial Convention. Metronidazole. The United States Pharmacopoeia. 41 ed. MD, USA: United Book Press, Inc.; 2018.*
8. *Bansal M, Mittal N, Yadav SK, Khan G, Gupta P, Mishra B, Nath G. Periodontal thermoresponsive, mucoadhesive dual antimicrobial loaded in-situ gel for the treatment of periodontal disease: preparation, in-vitro characterization and antimicrobial study. J Oral Biol Craniofac Res 2018; 8(2): 126-133.*
9. *Labib GS, Farid RM. Osteogenic effect of locally applied Pentoxyfilline gel: in vitro and in vivo evaluations. Drug Deliv 2014; 22(8): 1094-1102.*
10. *Budai-Szűcs M, Léber A, Cui L, Józó M, Vályi P, Burián K, Kirschweng B, Csányi E, Pukánszky B. Electrospun PLA fibers containing metronidazole for periodontal disease. Drug Des Devel Ther 2020; 14: 233-242.*

11. Kuzminska M, Pereira BC, Habashy R, Peak M, Isreb M, Gough TD, Isreb A, Alhnan MA. Solvent-free temperature-facilitated direct extrusion 3D printing for pharmaceuticals. Int J Pharm 2021; 598: 120305.
12. Aggarwal G, Nagpal M. Pharmaceutical polymer gels in drug delivery. In: Thakur VK, Thakur MK, Voicu SI, editors. Polymer gels. Gels horizons: From science to smart materials: Springer, Singapore; 2018. p. 249-284.
13. Singh J, Chhabra G, Pathak K. Development of acetazolamide-loaded, pH-triggered polymeric nanoparticulate in situ gel for sustained ocular delivery: in vitro, ex vivo evaluation and pharmacodynamic study. Drug Dev Ind Pharm 2013; 40(9): 1223-1232.
14. Mohammed FA. Topical permeation characteristics of diclofenac sodium from nacmc gels in comparison with conventional gel formulations. Drug Dev Ind Pharm 2001; 27(10): 1083-1097.
15. Bansal K, Rawat MK, Jain A, Rajput A, Chaturvedi TP, Singh S. Development of satranidazole mucoadhesive gel for the treatment of periodontitis. AAPS PharmSciTech 2009; 10(3).
16. Andrews GP, Gorman SP, Jones DS. Rheological characterisation of primary and binary interactive bioadhesive gels composed of cellulose derivatives designed as ophthalmic viscosurgical devices. Biomaterials 2005; 26(5): 571-580.
17. Pinto S, Pintado ME, Sarmento B. In vivo, ex vivo and in vitro assessment of buccal permeation of drugs from delivery systems. Expert Opin Drug Deliv 2019; 17(1): 33-48.