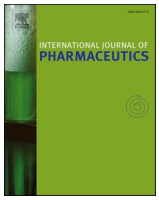
International Journal of Pharmaceutics 661 (2024) 124461

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm



Association of mucoadhesive polymeric matrices and liposomes for local delivery of miconazole: A new approach for the treatment of oral candidiasis

Angela Abruzzo [a,1], Elisa Corazza [a,1], Barbara Giordani [a], Fiore Pasquale Nicoletta [b], Beatrice Vitali [a], Teresa Cerchiara [a], Barbara Luppi [a], Federica Bigucci [a,*]

[a] Department of Pharmacy and Biotechnology, University of Bologna, Via San Donato 19/2, 40127 Bologna, Italy
[b] Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, Edificio Polifunzionale, 87036 Arcavacata di Rende, Cosenza, Italy

ARTICLE INFO

Keywords:
Miconazole nitrate
Liposomes
Freeze-dried matrices
Mucoadhesion
Buccal administration
Candida albicans

ABSTRACT

Since the local treatment of oral candidiasis usually requires long-term administration of the antifungal drug, an ideal dosage form should be able to maintain the drug release over an extended period, assuring an adequate concentration at the infection site. In this context, we have considered the possibility of a buccal delivery of miconazole nitrate (MN) by mucoadhesive polymeric matrices. The loading of the antifungal drug in a hydrophilic matrix was made possible by taking advantage of the amphiphilic nature of liposomes (LP).

The MN-loaded LP were prepared by a thin film evaporation method followed by extrusion, while solid matrices were obtained by freeze-drying a suspension of the LP in a polymeric solution based on chitosan (CH), sodium hyaluronate (HYA), or hydroxypropyl methylcellulose (HPMC).

MN-loaded LP measured 284.7 ± 20.1 nm with homogeneous size distribution, adequate drug encapsulation efficiency (86.0 ± 3.3 %) and positive zeta potential (+47.4 ± 3.3). CH and HYA-based formulations almost completely inhibited *C. albicans* growth after 24 h, even if the HYA-based one released a higher amount of the drug. The CH-based matrix also provided the best mucoadhesive capacity and therefore represents the most promising candidate for the local treatment of oral candidiasis.

1. Introduction

Fungal infections are common diseases that affect the oral mucosa. In fact, some fungal organisms such as *Candida albicans* populate the healthy oral cavity in a large part of the human population. Changes in the oral and/or systemic environment can cause an overgrowth of these microorganisms, leading to oral and pharyngeal clinical infections. Predisposing factors include local or systemic immunosuppression in HIV or after chemotherapy, modifications in the oral microflora caused by antibiotic therapy, and hyposalivation/xerostomia due to disease, medication, or radiation therapy (Lalla and Bensadoun, 2011).

Although systemic treatment has provided new clinical options, topical therapy is still the main recommended treatment for oral candidiasis due to its high efficacy, low cost, and fewer side effects (Ré et al., 2021). In fact, localized therapy improves the drug safety profile by limiting systemic exposure, which is responsible for adverse systemic reactions and resistance development. Similar to antibiotics for bacterial infections, the emergence of antifungal resistance among *Candida* species is a serious threat to public health worldwide (Bhattacharya et al., 2020).

Since the treatment of oral candidiasis usually requires long-term administration of the antifungal drug, an ideal dosage form should be able to maintain the drug release over an extended period, assuring an adequate concentration at the infection site and the reduction of the dosage frequency. One of the most exploited strategies to achieve this goal involves the use of materials with adhesive properties, such as mucoadhesive polymers (Laffleur, 2014). Indeed, their ability to create strong adhesive contacts with the mucosa increases the drug residence

* Corresponding author at: Department of Pharmacy and Biotechnology, University of Bologna, Via San Donato 19/2, 40127 Bologna, Italy.
E-mail addresses: angela.abruzzo2@unibo.it (A. Abruzzo), elisa.corazza7@unibo.it (E. Corazza), barbara.giordani4@unibo.it (B. Giordani), fiore.nicoletta@unical.it (F.P. Nicoletta), b.vitali@unibo.it (B. Vitali), teresa.cerchiara2@unibo.it (T. Cerchiara), barbara.luppi@unibo.it (B. Luppi), federica.bigucci@unibo.it (F. Bigucci).
[1] These authors contributed equally to the work.

https://doi.org/10.1016/j.ijpharm.2024.124461
Received 16 April 2024; Received in revised form 19 June 2024; Accepted 9 July 2024
Available online 10 July 2024

time at the target site (Fonseca-Santos and Chorilli, 2018). In this context, freeze-dried matrices for buccal administration based on hydrophilic polymers such as pectin, cellulose derivative, sodium hyaluronate, gelatin, chitosan, sodium alginate, gums, or a mixture of these polymers were developed by our research group (Abruzzo et al., 2021a; Giordani et al., 2019). These matrices exhibit mucoadhesive properties and the freeze-drying process ensures high porosity and specific surface area and, consequently, rapid hydration and development of a viscous network capable of prolonging the drug release. Moreover, freeze-dried matrices could meet the current demand for child-friendly medicines by exploiting advantages of solid dosage forms, such as dose accuracy and high physicochemical stability, together with the possibility of ensuring easy administration and subsequent removal if the matrix is not completely biodegradable. Finally, the fast hydration after contact saliva could positively influence dosage form perception and improve formulation comfort and paediatric patient acceptability (Krampe et al., 2026; Abruzzo et al., 2021a).

Miconazole nitrate (MN) is among the most widely used topical antifungals in adults (Salazar et al., 2020). It is an imidazole derivative that has a broad spectrum of activities against dermatophytes and yeasts. Despite being a nitrate salt, it is slightly soluble in water and has an octanol–water partition coefficient of about 6.25 (Bhalekar et al., 2009; Shahid et al., 2022). This poor solubility in water limits its therapeutic outcome and represents a challenge in the formulation process, especially in the case of mucoadhesive preparations based on hydrophilic polysaccharide polymers. Currently, MN is available on the market in various topical forms, including oral gels and buccal tablets (Xiao et al., 2022). In addition, new delivery systems for buccal administration have been studied to overcome some limitations of traditional topical therapies like poor patient compliance for treatments that require administrations several times a day. Studies for buccal administration of MN include, among others, microemulsions (Talianu et al.,), nanostructured lipid carriers (Hosny et al., 2022), polymeric sponges (Ossama et al., 2021), solid lipid microparticles (De Caro et al., 2021), polymeric films (Mady et al., 2018; Tejada et al., 2018, 2017a, 2017b), polymeric microparticles (Cartagena et al., 2017), and cysteamine conjugated β-cyclodextrin (Ijaz et al., 2015).

The aim of this work is the formulation of innovative dosage forms capable of remaining for a prolonged period on the oral mucosa and guaranteeing the local action of MN inside the mouth without having to resort to a high frequency of administration. In this context, we have considered the possibility of delivering MN to the buccal mucosa by loading it into liposomes embedded into mucoadhesive polymeric matrices. This strategy could be an interesting option to load a lipophilic drug in a hydrophilic matrix. In fact, liposomes are consolidated and attractive biomimetic nanocarriers widely used in drug delivery (Bozzuto and Molinari, 2015) and their structural versatility could also be exploited for loading MN. To the best of our knowledge, up to now, very few studies have been published on the association of mucoadhesive polymeric systems and lipophilic carriers for the buccal administration of MN. Murgia reported bioadhesive matrix tablets loaded with lipophilic nanoparticles (Murgia et al., 2019) and Hosny studied hydrogel loaded with drug self-nanoemulsion (Hosny et al., 2019).

The first part of this work describes the preparation of MN-loaded liposomes with a thin film evaporation method followed by extrusion, and their characterization in terms of size, polydispersity index (PDI), ζ-potential, encapsulation efficiency, and *in vitro* drug release. Subsequently, the study focused on the preparation of polymeric matrices by freeze-drying a polymeric solution based on chitosan (CH), sodium hyaluronate (HYA), or hydroxypropyl methylcellulose (HPMC) containing a liposome suspension. These polymers have been chosen for well-known characteristics such as biocompatibility, biodegradability, mucoadhesive properties, and gelling capacity in an aqueous environment. Then the properties of the final formulations, including water-uptake ability, mucoadhesive capacity, and *in vitro* drug release, were evaluated. Finally, the ability of the formulations to exert anti-*Candida* activity was tested.

2. Materials and methods

2.1. Materials

L-α-phosphatidylcholine from egg yolk (purity ~ 60 %), miconazole nitrate (MN), chitosan from shrimp shells (CH; MW 150 kDa, degree of deacetylation 96–98 %), and mucin type II from porcine stomach were purchased from Sigma-Aldrich (Milan, Italy). Sodium hyaluronate (HYA; MW 800–1500 kDa) as well as lactic acid 80 % were provided from Farmalabor (Canosa di Puglia, Italy), whereas glacial acetic acid and hydroxypropylmethylcellulose Benecel™ K100M (HPMC; MW 1000 kDa) were supplied by VWR International (Milan, Italy) and Ashland Industries Europe GmbH (Schaffhausen, Switzerland), respectively. Sabouraud dextrose (SD) broth was from Difco (Detroit, MI, USA). All other chemicals and solvents were of analytical grade and purchased by Sigma-Aldrich (Milan, Italy). Buffer solutions were prepared as follows: 8.38 mM $Na_2HPO_4{\cdot}12H_2O$, 7.35 mM KH_2PO_4, 94.11 mM NaCl for phosphate buffer at pH 6.8 (PBS) which simulates human saliva pH (Marques et al., 2011); lactic acid 1.6 % (v/v) in ultra-filtered water (18.2 MΩcm, MilliQ apparatus by Millipore, Milford, MA, USA) and adjusted to pH 5.6 with NaOH 5 % (w/v) for lactate buffer; sodium acetate 0.1 M adjusted to pH 3.0 with glacial acetic acid for acetate buffer used during drug quantification. *Candida albicans* DSM70014 and *Candida albicans* DSM1386 were purchased from the German Collection of Microorganisms and Cell Cultures GmbH (DSMZ, Braunschweig, Germany).

2.2. Liposome preparation

Liposomes were prepared according to the thin film hydration method described in the literature (Jøraholmen et al., 2020), but with slight modifications. L-α-phosphatidylcholine (200 mg) was dissolved in chloroform (2.5 mL) and, once a clear solution was obtained, an equal volume of methanol was added. Removal of the organic solvent was achieved through rotary evaporation (Rotavapor Heidolph Laborota-4000, Heidolph instruments, Scwabach, Germany) under vacuum (−0.9 bar) at 60 °C for 90 min. Subsequently, lactate buffer (10 mL) was added to the dry lipid film and stirred at 210 rpm for 1 h to produce vesicles. The liposome suspension was then extruded 15 times through a polycarbonate membrane with a pore size of 200 nm (manual syringe extruder Avestin LF-1, Avestin Europe GmbH, Ottawa, Canada), and stored at + 4–8 °C prior to further use. Drug-loaded liposomes (LP-MN) were developed following the procedure described for plain vesicles (LP), but with an additional step: MN was solubilized in methanol (16 mg/mL) and added to the solution of phosphatidylcholine in chloroform.

2.3. Liposome characterization

LP and LP-MN underwent a physical characterization step in terms of size, polydispersity index (PDI), and ζ-potential. Moreover, LP-MN were further examined to evaluate their encapsulation efficiency (EE) and drug release.

2.3.1. Size, PDI and ζ-potential

Liposome size and PDI were determined by PCS (photon-correlation spectroscopy) using a Brookhaven 90-PLUS (Brookhaven Instruments Corporation, New York, USA) with a He-Ne laser beam at a wavelength of 532 nm (scattering angle of 90°). LP and LP-MN suspensions were diluted 1:2000 v/v in ultra-filtered water (pH 6.0) and, for each sample, the average diameter (nm) and the PDI were measured. ζ-potential was evaluated after the sample dilution in ultra-filtered water (1:2000 v/v), through a potential analyzer (NanoBrook Omni, Brookhaven Instrument Corporation, New York, USA) setting the electrical field level at 10 V/

cm. For both analyses, the temperature of the cuvettes was set at 25 °C.

2.3.2. Encapsulation efficiency

The percentage of MN entrapped into liposomes (EE %) was investigated by exploiting the dialysis method (Abruzzo et al., 2020; Frank et al., 2012). Briefly, an aliquot of 0.5 mL of LP-MN was poured into a Visking Tubo Dialysis membrane (Medicell International Ltd., London, UK) with a cut-off size of 12,000–14,000 Dalton, and the suspension was dialyzed against 50 mL of lactate buffer pH 5.6 (external phase), which was maintained under stirring (100 rpm) at room temperature for 24 h. Afterwards, the MN retained in the vesicles was quantified. Precisely, the whole content of the dialysis bag was collected, diluted 1:100 v/v with methanol, and kept under stirring for another 24 h to break the vesicles. The free drug was detected through HPLC assay and the EE % was calculated as follows:

$$EE\% = (\text{Amount of MN in the dialysis tube}) \times 100/(\text{Total amount of MN})$$

2.3.3. Chromatographic conditions for HPLC assay

HPLC analytical assay was performed using a Shimadzu (Milan, Italy) LC-10ATVP chromatographic pump and a Shimadzu SPD-10AVP UV–vis detector set at 273 nm. Separation was obtained on a Phenomenex (Torrance, CA, USA) SinergyTM 4 μm Hydro-RP 80 Å LC column (150 × 4.60 mm) coupled to a Phenomenex Security Guard C18 guard cartridge (4 × 3.0 mm i.d., 5 μm). The mobile phase consisted of a mixture of methanol/acetonitrile/sodium acetate pH 3.0 (60:30:10, v/v/v) and it was flushed at a rate of 0.5 mL/min (Heneeda et al., 2012). Manual injections were made using a Rheodyne 7125 injector with a 20 μL sample loop and data analysis was carried out through the CromatoPlus software (Shimadzu Italia, Milan, Italy). Since drug quantification was performed for different purposes, more than one calibration curve was developed. The calibration curve of MN in methanol, obtained with drug concentrations ranging from 0.32 μg/mL to 394 μg/mL, showed good linearity ($R^2 = 1$) and it was used to evaluate the EE %. The calibration curve of MN in PBS pH 6.8/ethanol 60:40 (v/v) mixture was set up in the 0.31–386 μg/mL range, characterized by a linearity coefficient (R^2) of almost 1, and employed during the release studies.

2.3.4. In vitro release study from liposomes

The release profile of MN from liposomes was investigated as reported by a previously published method (Abruzzo et al., 2018), with some adjustments. An aliquot of 0.35 mL of LP-MN suspension was transferred in a beaker containing 5 mL of PBS pH 6.8/ethanol 60:40 (v/v) mixture and the system was kept under stirring (100 rpm) for up to 6 h at room temperature. At predetermined time points (30, 60, 120, 180, 240, 300, and 360 min) aliquots of the sample were taken from the medium, filtered with syringe filters cut-off 0.22 μm (cellulose acetate syringe filter, Sanford, USA), and diluted 1:10 v/v with methanol prior to quantification through HPLC analytical assay. A control consisting of the pure drug (1.4 mg) placed in the same medium was also tested.

Due to the complexity of the formulation in terms of composition and structure, we believed to preliminarily analyze its functional properties (water-uptake ability, mucoadhesion, drug release) in a simple medium such as phosphate buffer at pH 6.8, which simulates human saliva pH (Marques et al., 2011). In subsequent studies, we will consider using simulated salivary fluid which, as reported by several authors (Farias and Boateng, 2018; Farias and Boateng, 2020), more faithfully reproduces physiological conditions and is more predictive of the *in vivo* behaviour of the matrices.

2.4. Development of freeze-dried polymeric matrices containing liposomes

Freeze-dried polymeric matrices containing LP or LP-MN were obtained using three different polymers, selected *in virtue* of their peculiar gelling and mucoadhesive properties, and because of their diverse charge: CH (positively charged), HYA (negatively charged), and HPMC (electrically neutral). Polymeric solutions were prepared by dissolving the polymer at 1.5 % (w/v) in 1.6 % (v/v) of lactic acid aqueous solution for CH, or directly in lactate buffer for HYA and HPMC. The complete solubilization was achieved after 24 h under stirring at room temperature and, in the case of the CH solution, it was necessary to adjust the pH to 5.6 through the addition of NaOH 5 % (w/v). The pH value of 5.60 was found suitable because it satisfied two requirements: the need to have a final pH closer to that of the oral cavity (pH 6.8), and the presence of the proper conditions for the ionization and solubilization of chitosan chains (pKa = 6.50) (Wang et al., 2006). LP and LP-MN suspensions were then added dropwise (1 drop/sec) to the different polymer solutions (volume ratio 1:1) under stirring (400 rpm) and the resulting mixture was left in these conditions for 2 h. Subsequently, 0.7 g of the liposome suspensions incorporated into the different polymeric solutions, as well as the solutions containing only the polymers, were placed into each cavity (diameter 15 mm) of a blister pack (Farmalabor, Canosa di Puglia, Italy), frozen overnight at −20 °C, freeze-dried at 0.01 atm and −45 °C (Freeze Dryer ALPHA 1–2, Christ, Milan, Italy) and stored in a desiccator until use.

2.5. Polymeric matrix characterization

Polymeric matrices made of the sole polymer, named CH, HYA, and HPMC, as well as the same matrices containing LP or LP-MN, indicated as CH/HYA/HPMC-LP and CH/HYA/HPMC-LP-MN respectively, were subjected to Differential Scanning Calorimetry (DSC) analysis and characterized in terms of morphology, diameter, thickness, weight, hydration ability and mucoadhesive properties. Lastly, the drug release process from the freeze-dried matrices was investigated similarly to the liposome dispersion.

2.5.1. Dimension and weight

The polymeric matrices produced by freeze-drying were characterized in terms of thickness and diameter through an electronic digital caliber (art. 1367 E 2900; Shanghai ShangErBo Import & Export Company, Shanghai, China) and weight by using an electronic balance (Sartorius ED221S, Muggiò, Milano, Italia).

2.5.2. Differential scanning Calorimetry analysis (DSC)

DSC experiments were performed on freeze-dried matrices to evaluate the drug's solid state. Measurements were carried out using a DSC200 PC differential scanning calorimeter (Netzsch, Selb, Germany), placing the samples in aluminium pans that were sealed with aluminium lids. Thermal analyses were performed from 25 °C to 350 °C under a dry nitrogen atmosphere and a heating rate of 10 °C/min.

2.5.3. Morphology

The inner morphology of polymeric matrices made of the sole polymer as well as the same matrices containing LP and LP-MN were assessed by means of scanning electron microscopy (SEM). Samples were fixed on the sample holder with double-sided adhesive tape and examined using a Nova NanoSEM 450 (FEI Company, Hillsboro, Oregon) in a low-vacuum configuration and operating at 8.00 kV accelerating voltage.

2.5.4. Water-uptake study

The *in vitro* water-uptake study was performed to investigate the hydration ability of the polymeric matrices. Moreover, to examine the eventual influence of the liposomes and the drug on the hydration process, measurements were carried out on both the matrices containing LP and LP-MN. Specifically, a sponge (6.2 cm x 9.7 cm x 2.2 cm) was immersed in 500 mL of PBS at pH 6.8 within a glass Petri dish (diameter 22 cm, height 7 cm) for 30 min. Then a cellulose nitrate filter (Sartorius Stedim Biotech GmbH, Goettingen, Germany; diameter = 4.7 cm, pore size = 0.45 mm) was placed on the top of the hydrated sponge for other

30 min to get soaked (Giordani et al., 2019). Lastly, one freeze-dried matrix was positioned on the hydrated filter and the weight of the matrix-filter system was measured at different time points up to 360 min. The matrices' water uptake ability over time was determined by using the following equation:

$$Wateruptake\% = (W_A - W_B) \times 100/W_B$$

where W_A is the weight (g) of the hydrated matrix at a certain time point (subtracted by the weight of the hydrated filter) and W_B is the weight of the dry matrix.

After 360 min of hydration with PBS at pH 6.8, the pH value of the matrix surface was measured with an indicator strip pH 6 – 7.7 (Macherey-Nagel, Düren, Germany) to evaluate its compatibility with the buccal mucosa after application.

2.5.5. Mucoadhesion test

The mucoadhesive potential of the samples was assessed by measuring the displacement of the matrices on an agar-mucin inclined plane. The method was adapted from Bertram and Bodmeier (2006). A graduated scale (the markings are every 0.1 cm) was drawn on the vertical diameter of a Petri dish to measure the displacement of the sample. The matrix was positioned at the top of the scale where an area with a diameter of approximately 10 mm was highlighted. A hot solution of agar (1 % w/v) and mucin (0.5 % w/v) in PBS at pH 6.8 was cast on Petri dishes (diameter 8.8 cm) and left at room temperature for 30 min to cool down. Then, the Petri dishes were wrapped with parafilm to prevent water evaporation from the medium and left at 4 °C overnight. Prior to the analysis, the plates were stored for 1 h at room temperature to equilibrate to the test conditions. Just before placing the matrix on the Petri dish, the highlighted area was hydrated with 25 μL of PBS at pH 6.8. After an adhesion time of 3 min, the Petri dishes were placed in an upright position with an angle of 30° and, using a peristaltic pump (Minipuls 3, Gilson, Middleton, USA), PBS at pH 6.8 was dripped (flow rate of 0.5 ml/min) at a distance of 1.5 cm from the upper portion of the matrix. The displacement of formulations over different time intervals was recorded. The maximal measurable displacement on the Petri dishes was 4.5 cm.

2.5.6. In vitro release study from polymeric matrices

The drug release process from polymeric matrices was evaluated following the procedure described for the *in vitro* release study from liposomes (see Section 2.3.4) with a modification. The different polymeric matrices containing loaded liposomes were attached on a glass slide using cyanoacrylate adhesive and the slide was immersed in 40 mL of PBS pH 6.8/ethanol mixture 60:40 (v/v). The attachment on the glass slide is necessary to avoid the matrix floating during the test (Abruzzo et al., 2021a). At predetermined time points (30, 60, 120, 180, 240, 300, and 360 min) aliquots of the sample were taken from the medium and replaced with fresh medium. The samples were filtered with syringe filters cut-off 0.22 μm (cellulose acetate syringe filter, Sanford, USA) and the drug was quantified through HPLC analytical assay.

2.6. Antimicrobial activity

The antifungal activity towards planktonic cultures of two *Candida albicans* strains (DSM70014 and DSM1386) was assessed adapting the method reported in Giordani et al. (2019). *C. albicans* was grown aerobically in SD medium at 30° C for 24 h. A fungal suspension, prepared from a broth culture in log phase of both candida strains, was used to inoculate Erlenmeyer flasks containing 40 mL of SD medium (T0 inoculum: about 5 log CFU/mL). Viability of *C. albicans* alone (control) was compared with viability of the same strain cultured in the presence of MN (1.4 mg) in different forms: (i) 350 μL of free MN (stock solution of 4 mg/mL in ethanol); (ii) 350 μL of LP-MN; (iii) one of each polymeric matrices containing LP-MN (CH/HYA/HPMC-LP-MN). The viability of *C. albicans* strains inoculated with not-loaded carriers (LP, CH/HYA/HPMC-LP and CH/HYA/HPMC) was also evaluated, as well as the effects of ethanol and lactate buffer. Counts of viable *C. albicans* cells were carried out on SD agar plates at the inoculum time (T0) and after 6 h (T6) and 24 h (T24) of incubation at 37 °C (human body temperature). Plates were incubated aerobically at 30 °C for 48 h.

2.7. Statistical analysis

All experiments were performed in triplicate; results are expressed as mean ± SD. The *t*-test or ONE-way ANOVA followed by Tukey correction for multiple comparisons were used to determine the statistical significance of the studies. The criterion for statistical significance was $p < 0.05$.

3. Results and discussion

As described in the introduction section, MN is a hydrophobic imidazole antifungal agent characterized by a very slight solubility in water and high lipophilicity (Shahid et al., 2022). Its low solubility can determine a poor availability of the drug at the infection site and scarce therapeutic effectiveness. It follows the need for multiple administrations and decreased patient compliance (Kenechukwu et al., 2018). Moreover, another important aspect linked to low drug solubility is the limited number of technological approaches to deliver it to the site of infection. Among all the formulation strategies, the inclusion of hydrophobic drugs inside liposomes represents an interesting approach to enhance drug solubility (Soliman, 2017). Furthermore, it has been reported that liposomes, besides their ability to encapsulate both hydrophilic and hydrophobic molecules, can favour the interaction of the delivered drug with pathogens, like *C. albicans*, as well as the penetration into the extracellular matrix of the preformed biofilm (Abruzzo et al., 2021b; Gbian and Omri, 2021), resulting in the optimisation of the antimicrobial effect. Taking these observations into account, in this study, we first prepared a liposomal formulation capable of encapsulating MN and subsequently, we evaluated its inclusion in different polymeric matrices able to adhere to the buccal mucosa and control the drug release.

3.1. Liposome characterization

3.1.1. Size, PDI, and ζ-potential

LP and LP-MN were characterized for their size, PDI, and ζ-potential. The results are summarized in Table 1. The obtained sizes were in agreement with the method used for liposome preparation, which consisted of the thin film hydration followed by the extrusion employing polycarbonate filters of 200 nm. The higher size of vesicles with respect to the filter pore dimension could be attributed to a reversible elastic deformation of the vesicles (Hinna et al., 2016). A little increase in size was observed for loaded liposomes (284.7 ± 20.1 nm) with respect to the unloaded ones (239.0 ± 11.7 nm; $p < 0.05$), as a consequence of the presence of MN inside the vesicle structure. Moreover, the developed formulations presented a low PDI value (less than 0.3), as a result of the good sample homogeneity (Chen et al., 2011). Finally, ζ-potential values of liposomes were measured since it is recognized that ζ-potential values higher and lower than + 30 and −30 mV, respectively, could limit liposome aggregation thanks to the repulsive forces between the charged vesicles (Müller et al., 2001). Our results demonstrated that

Table 1
Size, PDI, and ζ-potential of unloaded (LP) and loaded liposomes (LP-MN).

	LP	LP-MN
Size (nm)	239.0 ± 11.7	284.7 ± 20.1
PDI	0.289 ± 0.002	0.282 ± 0.021
ζ-potential (mV)	− 50.6 ± 1.1	+ 47.4 ± 3.3

unloaded liposomes were characterized by a negative ζ-potential (−50.6 ± 1.1 mV), which can be correlated to the negative charges of phosphatidylcholine phosphate groups (Ascenso et al., 2013). On the other hand, loaded liposomes showed a positive ζ-potential value (+47.4 ± 3.3 mV), probably due to the presence of positive charges of MN (weak base with pKa = 6.7; Abdel-Rashid et al., 2022) on their surface. Similar results were obtained in other previous studies (Qushawy et al., 2018; Shah et al., 2016). Qushawy and colleagues developed MN-loaded transferosomes and attributed the positive values of vesicles' ζ-potential to both the presence of cationic nitrogen atoms in the structure of MN and the neutral charge of the non-ionic surfactants used for the transferosome preparation. The positive ζ-potential of vesicles containing MN was also reported by Shah and co-workers, who prepared MN-loaded solid lipid nanoparticles and found that the unloaded particles were negative, while the inclusion of MN lent a highly positive ζ-potential.

3.1.2. Encapsulation efficiency

The possibility to encapsulate hydrophobic drugs inside vesicle suspensions, such as liposomes, and their consequent solubility improvement plays a crucial role in the development of formulations able to guarantee the drug therapeutic dose of the drug at the target site (Bhalani et al., 2022). Liposomes showed an EE % equal to 86.0 ± 3.3 %, thus demonstrating the excellent ability of vesicles to incorporate the hydrophobic drug inside their structure.

3.1.3. In vitro release study from liposomes

For MN release studies, it was chosen as a release medium a mixture of PBS at pH 6.8 and ethanol at a volume ratio equal to 60:40 able to guarantee a detectable amount of drug in solution and maintain the sink condition. Liposome's ability to release MN over time was tested and compared with a control consisting of the pure drug in the same medium. As shown in Fig. 1, the control provided the immediate release of the drug in the medium, reaching the maximum cumulative amount (%) after 1 h. Conversely, LP-MN provided the release of a lower amount of drug over time with respect to the control, probably due to the presence of the bilayers through which the drug must diffuse before reaching the release medium. Particularly, in the case of the LP-MN release profile, a biphasic behaviour was observed: the first step was characterized by a fast drug release occurring within 1 h, which was probably due to both the rapid diffusion in the release medium of the free drug in the liposome suspension and the drug on the vesicle surface; the second step was featured by a more gradual drug release, likely as a result of the partitioning process of the encapsulated drug through the different layers of the liposomes.

3.2. Development of freeze-dried polymeric matrices containing liposomes

Although the inclusion of MN inside the liposomal structure improves drug solubility, their application in the buccal cavity suffers from some disadvantages including the short residence time. In this study, freeze-dried matrices were prepared employing mucoadhesive polymers such as CH, HYA and HPMC, to increase the residence time of LP-MN on the buccal mucosa. The employment of polymeric matrices as the final dosage form for buccal delivery shows additional advantages, like the easy manageability and the ability to rapidly gel upon contact with the saliva, thus guaranteeing a high patient comfort level and consequently increasing compliance.

3.3. Polymeric matrix characterization

3.3.1. Dimension and weight

All the selected polymers allowed the production of matrices without LP, matrices with unloaded LP, and matrices with LP-MN. The matrices were easily handled and removable from the blister cavity. They showed a cylindrical shape with a smooth surface. Table 2 reports the diameter, thickness and weight of the formulations. The diameter, in accordance with the dimensions of the blister cavity in which the matrices were prepared, was approximately 13 mm and the thickness did not vary when comparing formulations made up of the same polymer. Finally, the weight of the matrices of CH, HA or HPMC alone, matrices with LP, and

Table 2
Diameter, thickness and weight of the developed matrices.

	Diameter (mm)	Thickness (mm)	Weight (mg)
CH	13.8 ± 0.4	4.5 ± 0.8	14.8 ± 0.8
CH-LP	13.0 ± 0.6	4.2 ± 0.1	21.5 ± 0.7
CH-LP-MN	13.5 ± 0.5	3.9 ± 0.3	23.2 ± 0.5
HYA	12.8 ± 0.2	2.7 ± 0.7	15.2 ± 0.1
HYA-LP	11.9 ± 0.8	2.7 ± 0.5	20.8 ± 1.8
HYA-LP-MN	12.4 ± 0.7	2.9 ± 0.7	22.8 ± 0.6
HPMC	14.3 ± 0.4	4.7 ± 0.5	16.0 ± 0.5
HPMC-LP	14.3 ± 0.2	4.6 ± 0.4	22.3 ± 0.4
HPMC-LP-MN	14.1 ± 0.1	4.8 ± 0.1	23.3 ± 0.6

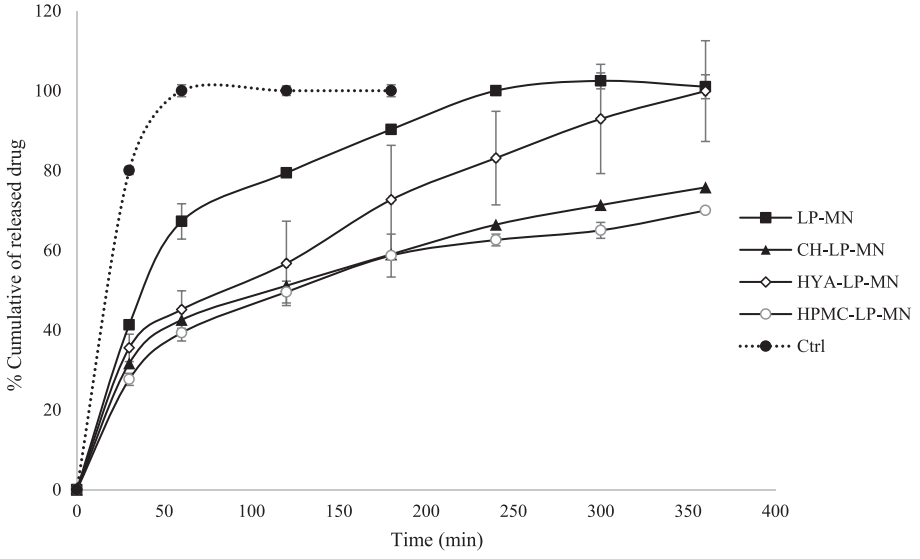


Fig. 1. Cumulative drug amount (%) released from the different freeze-dried matrices, LP-MN and control (Ctrl) plotted as a function of time. Data are expressed as means ± SD, n = 3.

matrices with LP-MN was different for the same polymer in accordance with the preparation method.

3.3.2. Differential scanning Calorimetry analysis (DSC)

DSC analysis provided insights into the solid state of the drug inside the matrices. Fig. 2 shows the DSC profiles of MN, matrices without LP, matrices with LP, and matrices with LP-MN. The pure drug showed an endothermic peak of around 184 °C due to its melting point in agreement with previous findings (Umeyor et al., 2021). Differently, the thermograms of freeze-dried matrices generally showed one endothermic peak at a temperature immediately upper than 100 °C, corresponding to the water loss, and an exothermic peak beyond 200 °C, related to the polymer decomposition. The endothermic peak of the drug disappeared when was incorporated into matrices containing LP-MN, indicating that the drug was in the amorphous state in the formulations. The drug amorphization can represent an advantage in terms of an increase in drug solubility (Rumondor et al., 2016).

3.3.3. Morphology

Fig. 3 shows the SEM images of polymeric matrices made of HPMC, CH and HYA and of HPMC, HPMC-LP and HPMC-LP-MN. Fig. 3A, 3B and 3C highlight that matrices based on sole polymer showed a porous structure. Fig. 3D, 3E and 3F show that the inclusion of LP and LP-MN determined a different morphology which appeared more compact and homogeneous. This observation was in agreement with a previous work (Nour et al., 2023) in which the presence of liposomes inside polymeric films determined a different organisation between the polymeric chains, thus creating more compact and tighter structures with respect to films without liposomes (Nour et al., 2023).

3.3.4. Water uptake study

The hydration ability was measured in reason of its impact on both the matrix's adhesiveness to the buccal mucosa and the release of the drug. In fact, it has been reported that matrix hydration causes disentanglement and relaxation of the polymer chains and promotes penetration of the mucus chains during adhesion. Moreover, the presence of the polymer in the gelled form can facilitate drug diffusion and release. All these aspects can finally determine the increase of drug availability at the infection site and consequently improve the therapeutic outcome. Fig. 4A and 4B show the *in vitro* water uptake profiles of freeze-dried matrices composed of the sole polymer and matrices with LP and LP-MN, respectively. The porous structure allowed a quick hydration in contact with the aqueous medium. Moreover, the water uptake ability of matrices was influenced by the polymeric composition. All three polymers have groups capable of establishing hydrogen bonds with water and CH and HYA, depending on the environmental pH, can present different degrees of ionization. Particularly, the CH matrix showed the lowest hydration ability among all the formulations ($p < 0.05$), reaching a water-uptake % equal to 646 ± 22 % after 360 min. This result can be probably due to the presence of a low number of free charges on CH chains (pKa = 6.5) at pH 6.8, which consequently determined the entry of a limited amount of water. On the other side, the HYA-based matrix showed the highest hydration ability and after 360 min a value of 1987 ± 124 % was measured, as a consequence of its high hydrophilicity and the presence of negatively charged carboxylic groups of the polymer (pKa = 2.9) under the tested conditions (Bigucci et al., 2015). Moreover, HPMC which is a neutral polymer has a hydration capacity comparable to that of CH (after 360 min water-uptake % equal to 735 ± 96 %; no significant difference with respect to CH matrix, $p > 0.05$).

Finally, as can be seen in Fig. 4B, the presence of phosphatidylcholine inside the matrices generally determined a significant reduction in the water uptake with respect to matrices made of the sole polymer ($p < 0.05$), probably because its lipidic nature influenced in a negative manner the hydrophilicity of system. For the same reason, the inclusion of the lipophilic drug MN determined a further reduction in the hydration ability with respect to the matrices prepared with unloaded LP ($p < 0.05$). These results were in agreement with previous findings which reported a reduced water-uptake when liposomes were loaded inside polymeric films with respect to the formulations without liposomes, as a consequence of the reduced hydrophilicity of the final dosage form (Nour et al., 2023). Moreover, the formation of a compact structure in the presence of liposomes, as observed in morphological studies (see Section 3.3.3), could further limit the entry of water.

Another important property that was observed during this study regarded the aspect of the matrices obtained during the hydration period. Specifically, matrices based on CH gelled maintaining their cylindrical shape even after 360 min, while matrices composed of HYA and HPMC rapidly gelled losing their initial structure. The inclusion of LP or LP-MN inside the matrices did not influence this behaviour in the case of CH, while in the case of HYA and HPMC matrices, it slowed down the gelling process.

The surface pH of all matrices after hydration with PBS pH 6.8 was 7.0 ± 0.3. In the literature (Patel et al., 2006), a pH within the limit of 7.0 ± 1.5 is considered suitable for buccal formulations because it avoids the appearance of irritation.

3.3.5. Mucoadhesion test

The mucoadhesion ability of a solid dosage form is a crucial property to ensure prolonged contact with the buccal mucosa and consequently to provide high drug concentration at the administration site. Several

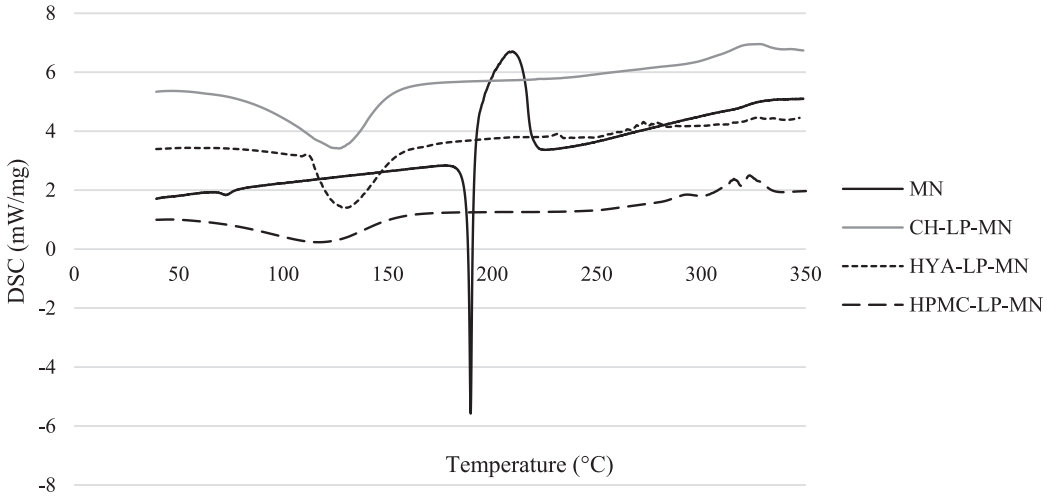


Fig. 2. DSC thermograms of MN and the different matrices containing LP-MN.

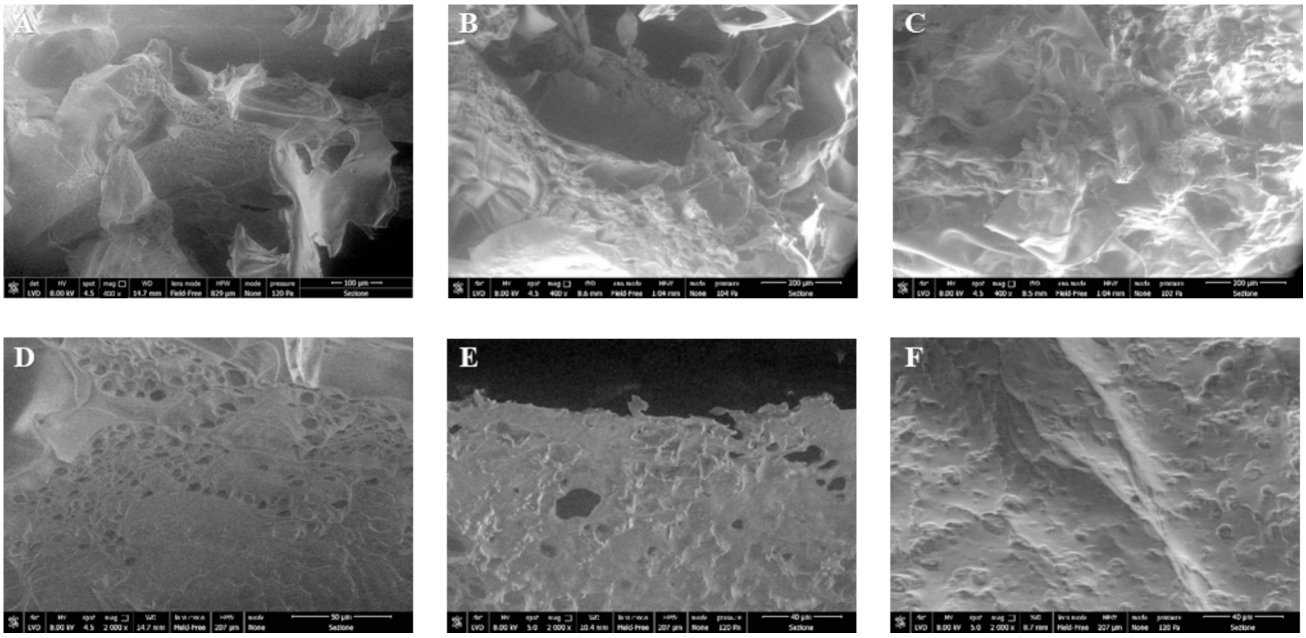


Fig. 3. SEM images of polymeric matrices made of HPMC (A), CH (B) and HYA (C) (400 x) and of HPMC (D), HPMC-LP (E) and HPMC-LP-MN (F) (2000 x).

mechanisms contribute to this adhesion, including wettability, physical entanglement of the polymeric chains, electrostatic interaction, and multiple low-affinity bonds, such as hydrogen bonds and Van der Waal's forces as well as the hydrophobic interactions between the hydrophobic segments of the molecules (Andrews et al., 2009). The mucoadhesive ability of the polymeric matrices was studied by measuring their movement over time on an agar-mucin inclined plane which is reached by a continuous flow buffer pH 6.8. The mucoadhesion ability was inversely related to the displacement of the matrix.

The results showed different behaviours for the three types of polymers (Fig. 5). The HYA and HPMC matrices hydrated, gelled, and then solubilized. The former gelled rapidly and travelled 1.2 cm in 30 min before dissolving, while for the latter the gelation process was slower and travelled 2 cm in 180 min before dissolving. HYA-based formulations, even if they hydrate more than HPMC ones, adhered less because the presence of negatively charged carboxyl groups (pKa = 2.9) induces repulsive electrostatic interactions with sialic acid residues (pKa = 2.6) and mucin sulfate. On the contrary, the HPMC chains after hydration were capable of interpenetrating the mucin chains and establishing numerous hydrogen bonds. The CH formulation, despite the poor hydration, maintained its initial shape and remained in the application area for the entire duration of the experiment (no displacement was observed), showing the best mucoadhesive capacity (data not shown in Fig. 5). This behaviour is attributable to the electrostatic interactions between its protonated amino groups (pKa = 6.5) and the sialic acid and sulphonic acid of glycoproteins (Bravo-Osuna et al., 2007, Sogias et al., 2008).

Finally, matrices based on polymer alone (HYA and HPMC) are those that travel the shortest distances at the same time, while matrices loaded with LP and LP-MN cover larger distances, exhibiting a lower mucoadhesive capacity. The results highlight that a lower hydration capacity of the matrices in the presence of both liposomes and the drug (see Section 3.3.4) produced lower mucoadhesion. The results also proved that, despite the greater lipophilicity and the lower capacity to hydrate, the CH-based matrices containing LP and LP-MN maintained the same ability to adhere to the mucin-agar plate of the matrices made up of polymer alone (no displacement was observed). These formulations therefore represent the best candidates to guarantee a prolonged residence time on the buccal mucosa after application.

3.3.6. *In vitro release study from polymeric matrices*

As previously described, the drug release process can be influenced by different factors, such as the solid state of the drug and its solubility in the medium, the drug diffusion from the lipidic phase and the gelled matrices.

Fig. 1 shows the *in vitro* release profiles obtained with the different freeze-dried matrices containing LP-MN. As can be seen, the freeze-dried matrices provided the release of a lower amount of drug with respect to LP-MN ($p < 0.05$). This result can be attributed to the different vehicles through which the drug must diffuse prior to reaching the release medium. Particularly, besides the diffusion of the drug through the lipidic phase, in the case of freeze-dried matrices, the drug must also diffuse through the gelled matrix. Additionally, a different performance was observed as a consequence of the polymeric composition. In fact, the CH-based matrix determined the release of a lower amount of MN over time with respect to HYA ($p < 0.05$). This result can be correlated to the different water-uptake behaviour of the formulations. In fact, as previously described, CH-based matrices slowly hydrated and maintained their initial structure; both these factors limited the drug release. As observed for water-uptake results, also in the case of drug release profiles, no significant difference was observed between CH and HPMC matrices ($p > 0.05$).

In all cases, a biphasic release profile was observed in which a first fast-release step and subsequent gradual drug release were evident. The first step, which occurred within 30 min, can be attributed to the release of the free drug or the portion of the drug on the vesicle surface (as for LP-MN release), the second one was probably linked to the diffusion of the drug firstly through the lipidic phase and then through the gelled polymers. The prolonged drug release can bring advantages in the treatment, like the reduction of the frequency of administration, which consequently can increase patient compliance and minimize the drugs' side effects.

3.4. *Antimicrobial activity*

Among *Candida* species, *C. albicans* is the major causative agent of oral candidiasis. MN has been used widely in treating this disease *in virtue* of its high efficacy and few reports of resistance (Zhang et al., 2016).

Here, in order to assure that the antifungal activity of MN was

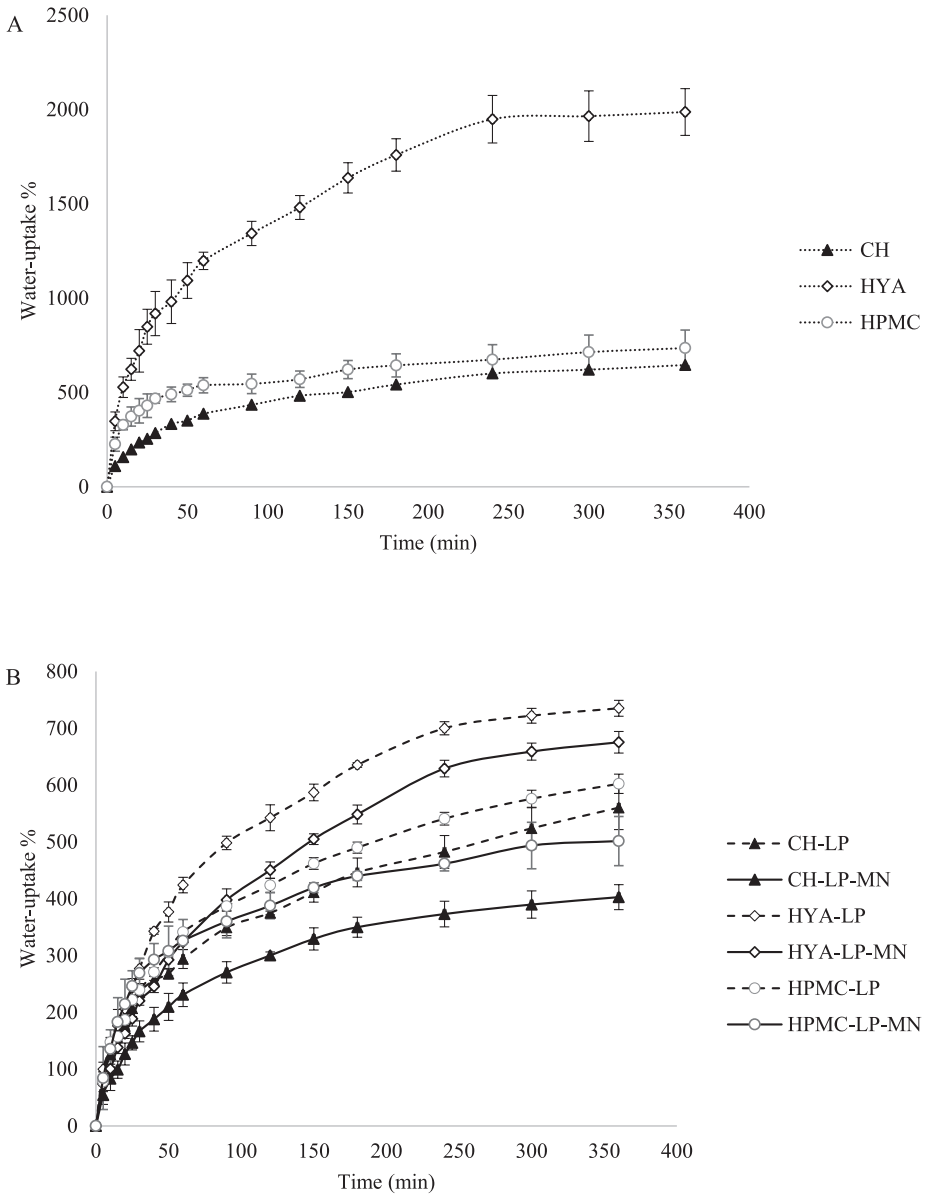


Fig. 4. In vitro hydration profiles for matrices composed of the sole polymer (A) and with LP or LP-MN (B). Data are expressed as means ± SD, n = 3.

maintained after the loading in liposomes and their further incorporation inside freeze-dried polymeric matrices, the proposed formulations were tested towards two *C. albincans* strains isolated from human sputum, as depicted in Fig. 6. In the control cultures (sole medium or medium added with lactate buffer and ethanol) yeast concentration similarly increased over the time ($p > 0.05$) (T0: 4.99–5.05 log CFU/mL; T6: 5.94–6.15 logCFU/mL; T24: 6.66–7.14 logCFU/mL). On the contrary, the viability of *C. albicans* DSM70014 incubated with free MN was abolished (<1 log CFU/mL) after 6 h of incubation (Fig. 6A). MN in liposomes retained a strong fungicidal activity, being able to reduce the growth of *C. albicans* DSM70014 by 2.2 log CFU/mL after 6 h of incubation and to completely inhibit yeast proliferation in 24 h. However, the antifungal effect was slightly delayed compared to free MN ($p < 0.05$) and it was compatible with the sustained release of the drug. CH-LP-MN and HYA-LP-MN showed activity against *C. albicans* DSM70014 similar to that of LP-MN ($p > 0.05$). Indeed, after 24 h no viable cells were detected while the survival was reduced by 1.9 logCFU/mL and 2.2 logCFU/mL after 6 h of incubation with CH-LP-MN and HYA-LP-MN, respectively. Instead, the incorporation of LP-MN in HPMC matrices significantly limited the antifungal activity of LP-MN both at 6 h and 24 h, likely due to a slower diffusion of MN through the HPMC polymeric matrix and, consequently, a lower release of the drug in the medium, as observed in release studies (Fig. 1). Consistently with the data from release studies, after 6 h no significant differences were observed between CH-LP-MN and HPMC-LP-MN, while HYA-LP-MN resulted more effective than HPMC-LP-MN ($p < 0.05$).

A complete killing of *C. albicans* DSM1386 was achieved only after 24 h of incubation with free drug or LP-MN (Fig. 6B), suggesting that this strain was less susceptible to the action of MN than *C. albicans* DSM70014. Consistently with this observation, polymeric matrices resulted less effective than LP-MN in counteracting *C. albicans* DSM1386 proliferation ($p < 0.05$), as they allowed for the release of a lower amount of drug over the time. Nevertheless, fungal growth was strongly impaired after 24 h of culture in the presence of CH-LP-MN, HYA-LP-MN and HPMC-LP-MN, as demonstrated by the low cell concentrations found (1.77 logCFU/mL, 2.40 CFU/mL and 2.98 CFU/mL, respectively).

In general, no relevant differences ($p > 0.05$) in activity towards *C. albicans* DSM70014 and *C. albicans* DSM1386 were observed between CH-LP-MN and HYA-LP-MN even if HYA-LP-MN provided the release of a higher amount of drug (see Section 3.3.6). This can be due to the mild

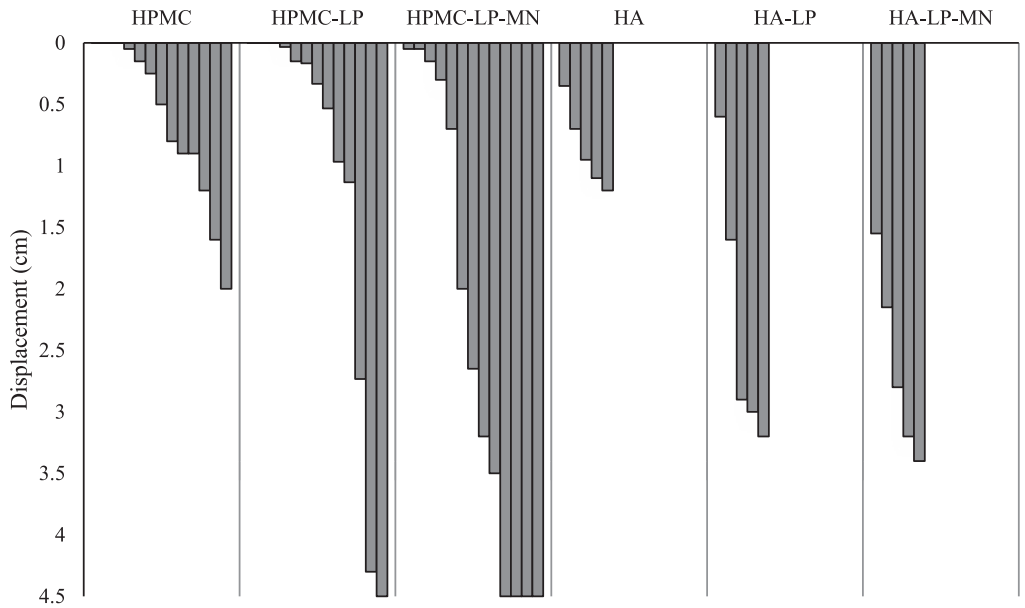


Fig. 5. Displacement of matrices on agar-mucin gels after 5, 10, 15, 20, 30, 40, 50, 60, 75, 90, 120, 150, and 180 min (from left to right). Data are expressed as means ± SD, n = 3.

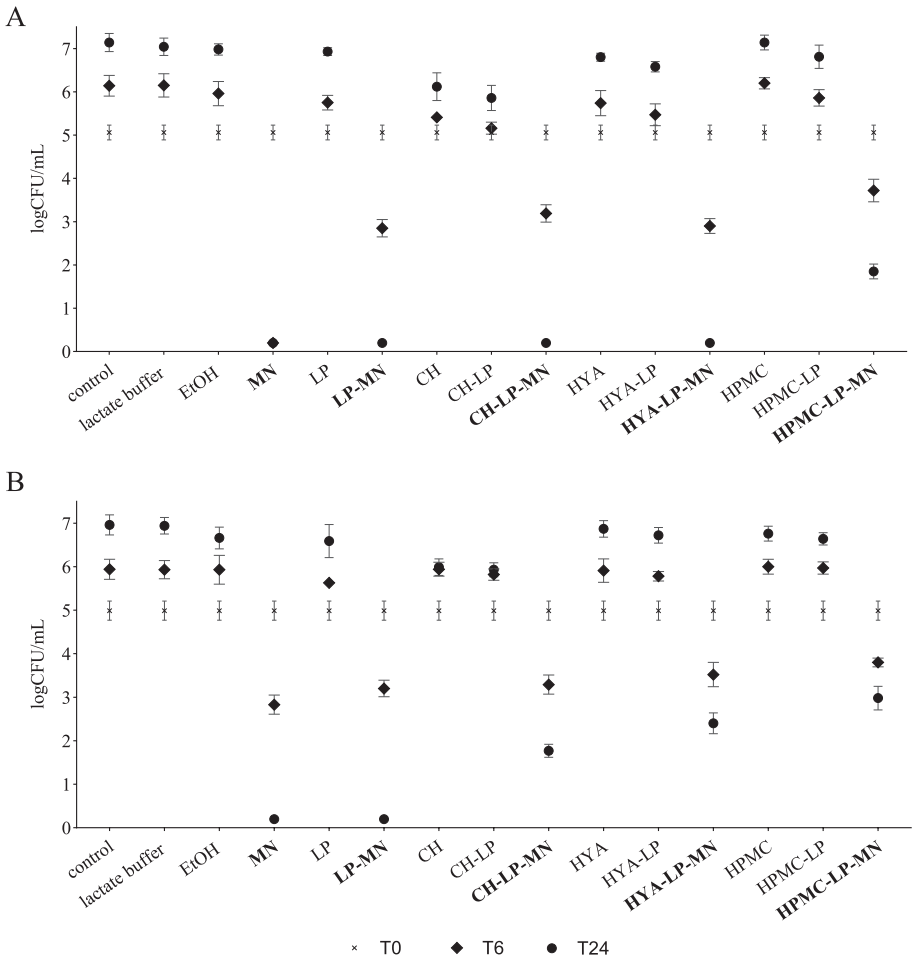


Fig. 6. Viability (log CFU/mL) of C. albicans DSM70014 (A) and C. albicans DSM1386 (B) assessed at the inoculum time (T0) and after 6 h (T6) and 24 h (T24) of incubation in sole SD medium (control) or in the presence of lactate buffer, ethanol (EtOH), not-formulated MN (MN), liposomes (LP/LP-MN) or polymeric matrices (CH/HYA/HPMC, CH/HYA/HPMC-LP, CH/HYA/HPMC-LP-MN). Data are expressed as mean ± SD, n = 3.

anti-*Candida* activity exerted by chitosan empty matrices (CH/CH-LP) themselves, which caused a significant reduction of fungal growth of around 1 logCFU/mL after 24 h of incubation. The antifungal activity of chitosan is mainly ascribed to the presence of cationic amino groups able to interact with anionic components of cell walls, as previously reported by other authors (Anderson et al., 2017). Even though in other studies also the hyaluronic acid was reported to be active towards *Candida* spp. (Giordani et al., 2019), in the present experimental conditions HYA/HYA-LP, as well HPMC/HPMC-LP and LP, did not exert any inhibitory activity against the fungal strains tested.

4. Conclusions

The treatment of oral candidiasis requires a local administration of the antifungal drug for an extended period of time. An ideal dosage form should therefore be able to prolong drug release, assuring an adequate concentration at the infection site and the reduction of the dosage frequency. To evaluate possible new dosage forms with these characteristics, we have developed formulations that exploit the combination of a mucoadhesive hydrophilic matrix with liposomes loaded with the lipophilic drug MN. The matrices were able to rapidly hydrate and develop a viscous network capable of prolonging the drug release. Moreover, the selection of the more suitable polymer for matrix preparation allowed for modulation of the functional properties. Specifically, CH and HYA-based formulations showed the best activity towards *C. albicans* without relevant differences, even if the HYA-based one released a higher amount of the drug. Furthermore, CH-LP-MN provided the best mucoadhesive capacity, making the formulation a promising candidate for prolonged antifungal activity in the treatment of oral candidiasis.

CRediT authorship contribution statement

Angela Abruzzo: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Elisa Corazza:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Barbara Giordani:** Writing – original draft, Validation, Methodology, Investigation, Formal analysis. **Fiore Pasquale Nicoletta:** Writing – original draft, Validation, Methodology, Investigation, Formal analysis. **Beatrice Vitali:** Writing – review & editing, Methodology. **Teresa Cerchiara:** Writing – review & editing. **Barbara Luppi:** Writing – original draft, Validation, Methodology, Investigation, Formal analysis. **Federica Bigucci:** Writing – review & editing, Writing – original draft, Supervision, Resources, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

The authors would like to thank Elisa Battistini and Carlotta Dell'Omo for their valuable contributions.

References

Abdel-Rashid, R.S., Helal, D.A., Alaa-Eldin, A.A., Abdel-Monem, R., 2022. Polymeric versus lipid nanocapsules for miconazole nitrate enhanced topical delivery: *in vitro* and *ex vivo* evaluation. Drug Deliv. 29, 294–304. https://doi.org/10.1080/10717544.2022.2026535.

Abruzzo, A., Giordani, B., Parolin, C., Vitali, B., Protti, M., Mercolini, L., Cappelletti, M., Fedi, S., Bigucci, F., Cerchiara, T., Luppi, B., 2018. Novel mixed vesicles containing lactobacilli biosurfactant for vaginal delivery of an anti- Candida agent. Eur. J. Pharm. Sci. 112, 95–101. https://doi.org/10.1016/j.ejps.2017.11.012.

Abruzzo, A., Cappadone, C., Farruggia, G., Luppi, B., Bigucci, F., Cerchiara, T., 2020. Glycyrrhetinic acid liposomes and hyalurosomes on Spanish Broom, flax, and hemp dressings to heal skin wounds. Molecules 25, 2558. https://doi.org/10.3390/molecules25112558.

Abruzzo, A., Crispini, A., Prata, C., Adduci, R., Nicoletta, F.P., Dalena, F., Cerchiara, T., Luppi, B., Bigucci, F., 2021a. Freeze-dried matrices for buccal administration of propranolol in children: Physico-chemical and functional characterization. J. Pharm. Sci. 110, 1676–1686. https://doi.org/10.1016/j.xphs.2020.10.033.

Abruzzo, A., Giordani, B., Parolin, C., De Gregorio, P.R., Foschi, C., Cerchiara, T., Bigucci, F., Vitali, B., Luppi, B., 2021b. Lactobacillus crispatus BC1 biosurfactant delivered by hyalurosomes: An advanced strategy to counteract candida biofilm. Antibiotics 10, 33. https://doi.org/10.3390/antibiotics10010033.

Andersen, T., Mishchenko, E., Flaten, G.E., Sollid, J.U., Mattsson, S., Tho, I., Škalko-Basnet, N., 2017. Chitosan-based nanomedicine to fight genital Candida infections: Chitosomes. Mar. Drugs 15, 64. https://doi.org/10.3390/md15030064.

Andrews, G.P., Laverty, T.P., Jones, D.S., 2009. Mucoadhesive polymeric platforms for controlled drug delivery. Eur. J. Pharm. Biopharm. 71, 505–518. https://doi.org/10.1016/j.ejpb.2008.09.028.

Ascenso, A., Cruz, M., Euletério, C., Carvalho, F.A., Santos, N.C., Marques, H.C., Simões, S., 2013. Novel tretinoin formulations: a drug-in-cyclodextrin-in-liposome approach. J. Liposome Res. 23, 211–219. https://doi.org/10.3109/08982104.2013.788026.

Bertram, U., Bodmeier, R., 2006. In situ gelling, bioadhesive nasal inserts for extended drug delivery: In vitro characterization of a new nasal dosage form. Eur. J. Pharm. Sci. 27, 62–71. https://doi.org/10.1016/j.ejps.2005.08.005.

Bhalani, D.V., Nutan, B., Kumar, A., Singh Chandel, A.K., 2022. Bioavailability enhancement techniques for poorly aqueous soluble drugs and therapeutics. Biomedicines 10, 2055. https://doi.org/10.3390/biomedicines10092055.

Bhalekar, M.R., Pokharkar, V., Madgulkar, A., Patil, N., Patil, N., 2009. Preparation and evaluation of miconazole nitrate-loaded solid lipid nanoparticles for topical delivery. AAPS PharmSciTech 10, 289–296. https://doi.org/10.1208/s12249-009-9199-0.

Bhattacharya, S., Sae-Tia, S., Fries, B.C., 2020. Candidiasis and mechanisms of antifungal resistance. Antibiotics 9, 312. https://doi.org/10.3390/antibiotics9060312.

Bigucci, F., Abruzzo, A., Saladini, B., Gallucci, M.C., Cerchiara, T., Luppi, B., 2015. Development and characterization of chitosan/hyaluronan film for transdermal delivery of thiocolchicoside. Carbohydr. Polym. 130, 32–40. https://doi.org/10.1016/j.carbpol.2015.04.067.

Bozzuto, G., Molinari, A., 2015. Liposomes as nanomedical devices. Int. J. Nanomedicine 10, 975–999. https://doi.org/10.2147/IJN.S68861.

Bravo-Osuna, I., Vauthier, C., Farabollini, A., Palmieri, G.F., Ponchel, G., 2007. Mucoadhesion mechanism of chitosan and thiolated chitosan-poly(isobutyl cyanoacrylate) core-shell nanoparticles. Biomaterials 28, 2233–2243. https://doi.org/10.1016/j.biomaterials.2007.01.005.

Cartagena, A.F., Lyra, A.M., Kapuchczinski, A.C., Urban, A.M., Esmerino, L.A., Klein, T., Nadal, J.M., Farago, P.V., Campanha, N.H., 2017. Miconazole nitrate-loaded microparticles for buccal use: Immediate drug release and antifungal effect. Curr. Drug Deliv. 14, 1144–1153. https://doi.org/10.2174/1567201813666161006115041.

Chen, M., Liu, X., Fahr, A., 2011. Skin penetration and deposition of carboxyfluorescein and temoporfin from different lipid vesicular systems: In vitro study with finite and infinite dosage application. Int. J. Pharm. 408, 223–234. https://doi.org/10.1016/j.ijpharm.2011.02.006.

De Caro, V., Giannola, L.I., Di Prima, G., 2021. Solid and semisolid innovative formulations containing miconazole-loaded solid lipid microparticles to promote drug entrapment into the buccal mucosa. Pharmaceutics 13, 1361. https://doi.org/10.3390/pharmaceutics13091361.

Farias, S., Boateng, J.S., 2018. Development and functional characterization of composite freeze dried wafers for potential delivery of low dose aspirin for elderly people with dysphagia. Int. J. Pharm. 553, 65–83. https://doi.org/10.1016/j.ijpharm.2020.119807.

Farias, S., Boateng, J.S., 2020. *In vitro*, *ex vivo* and *in vivo* evaluation of taste masked low dose acetylsalicylic acid loaded composite wafers as platforms for buccal administration in geriatric patients with dysphagia. Int. J. Pharm. 589, 119807 https://doi.org/10.1016/j.ijpharm.2020.119807.

Fonseca-Santos, B., Chorilli, M., 2018. An overview of polymeric dosage forms in buccal drug delivery: State of art, design of formulations and their in vivo performance evaluation. Mater. Sci. Eng. C 86, 129–143. https://doi.org/10.1016/j.msec.2017.12.022.

Frank, K.J., Westedt, U., Rosenblatt, K.M., Hölig, P., Rosenberg, J., Mägerlein, M., Brandl, M., Fricker, G., 2012. Impact of FaSSIF on the solubility and dissolution-/permeation rate of a poorly water-soluble compound. Eur. J. Pharm. Sci. 47, 16–20. https://doi.org/10.1016/j.ejps.2012.04.015.

Gbian, D.L., Omri, A., 2021. The impact of an efflux pump inhibitor on the activity of free and liposomal antibiotics against Pseudomonas aeruginosa. Pharmaceutics 13, 577. https://doi.org/10.3390/pharmaceutics13040577.

Giordani, B., Abruzzo, A., Musazzi, U.M., Cilurzo, F., Nicoletta, F.P., Dalena, F., Parolin, C., Vitali, B., Cerchiara, T., Luppi, B., Bigucci, F., 2019. Freeze-dried matrices based on polyanion polymers for chlorhexidine local release in the buccal and vaginal cavities. J. Pharm. Sci. 108, 2447–2457. https://doi.org/10.1016/j.xphs.2019.02.026.

Heneedak, H.M., Salama, I., Mostafa, S., El-Sadek, M., 2012. HPLC and chemometric methods for the simultaneous determination of miconazole nitrate and nystatin. J. Chromatogr. Sci. 50, 855–861. https://doi.org/10.1093/chromsci/bms127.

Hinna, A., Steiniger, F., Hupfeld, S., Stein, P., Kuntsche, J., Brandl, M., 2016. Filter-extruded liposomes revisited: a study into size distributions and morphologies in relation to lipid-composition and process parameters. J. Liposome Res. 26, 11–20. https://doi.org/10.3109/08982104.2015.1022556.

Hosny, K.M., Aldawsari, H.M., Bahmdan, R.H., Sindi, A.M., Kurakula, M., Alrobaian, M. M., Aldryhim, A.Y., Alkhalidi, H.M., Bahmdan, H.H., Khallaf, R.A., El Sisi, A.M., 2019. Preparation, optimization, and evaluation of hyaluronic acid-based hydrogel loaded with miconazole self-nanoemulsion for the treatment of oral thrush. AAPS PharmSciTech 20, 297. https://doi.org/10.1208/s12249-019-1496-7.

Hosny, K.M., Sindi, A.M., Ali, S., Alharbi, W.S., Hajjaj, M.S., Bukhary, H.A., Badr, M.Y., Mushtaq, R.Y., Murshid, S.S.A., Almehmady, A.M., Bakhaidar, R.B., Alfayez, E., Kurakula, M., 2022. Development, optimization, and evaluation of a nanostructured lipid carrier of sesame oil loaded with miconazole for the treatment of oral candidiasis. Drug Deliv. 29, 254–262. https://doi.org/10.1080/10717544.2021.2023703.

Ijaz, M., Matuszczak, B., Rahmat, D., Mahmood, A., Bonengel, S., Hussain, S., Huck, C. W., Bernkop-Schnürch, A., 2015. Synthesis and characterization of thiolated β-cyclodextrin as a novel mucoadhesive excipient for intra-oral drug delivery. Carbohydr. Polym. 132, 187–195. https://doi.org/10.1016/j.carbpol.2015.06.073.

Jøraholmen, M.W., Johannessen, M., Gravningen, K., Puolakkainen, M., Acharya, G., Basnet, P., Škalko-Basnet, N., 2020. Liposomes-in-hydrogel delivery system enhances the potential of resveratrol in combating vaginal Chlamydia infection. Pharmaceutics 12, 1203. https://doi.org/10.3390/pharmaceutics12121203.

Kenechukwu, F.C., Attama, A.A., Ibezim, E.C., Nnamani, P.O., Umeyor, C.E., Uronnachi, E.M., Gugu, T.H., Momoh, M.A., Ofokansi, K.C., Akpa, P.A., 2018. Surface-modified mucoadhesive microgels as a controlled release system for miconazole nitrate to improve localized treatment of vulvovaginal candidiasis. Eur. J. Pharm. Sci. 111, 358–375. https://doi.org/10.1016/j.ejps.2017.10.002.

Krampe, R., Visser, J.C., Frijlink, H.W., Breitkreutz, J., Woerdenbag, H.J., Preis, M., 2016. Oromucosal film preparations: points to consider for patient-centricity and manufacturing processes. Expert Opin. Drug Deliv. 13, 493–506. https://doi.org/10.1517/17425247.2016.1118048.

Laffleur, F., 2014. Mucoadhesive polymers for buccal drug delivery. Drug Dev. Ind. Pharm. 40, 591–598. https://doi.org/10.3109/03639045.2014.892959.

Lalla, R.V., Bensadoun, R.-J., 2011. Miconazole mucoadhesive tablet for oropharyngeal candidiasis. Expert Rev. Anti-Infec. Ther. 9, 13–17. https://doi.org/10.1586/eri.10.152.

Mady, O.Y., Donia, A.M., Al-Madboly, L.A., 2018. Miconazole-urea in a buccal film as a new trend for treatment of resistant mouth fungal white patches. Front. Microbiol. 9, 837. https://doi.org/10.3389/fmicb.2018.00837.

Marques, M.R.C., Loebenberg, R., Almukainzi, M., 2011. Simulated biological fluids with possible application in dissolution testing. Dissolution Technol. 18, 15–28. https://doi.org/10.14227/DT180311P15.

Müller, R.H., Jacobs, C., Kayser, O., 2001. Nanosuspensions as particulate drug formulations in therapy. Adv Drug Deliv. Rev. 47, 3–19. https://doi.org/10.1016/S0169-409X(00)00118-6.

Murgia, D., Angellotti, G., D'Agostino, F., De Caro, V., 2019. Bioadhesive matrix tablets loaded with lipophilic nanoparticles as vehicles for drugs for periodontitis Treatment: Development and Characterization. Polymers 11, 1801. https://doi.org/10.3390/polym11111801.

Nour, E.M., El-Habashy, S.E., Shehat, M.G., Essawy, M.M., El-Moslemany, R.M., Khalafallah, N.M., 2023. Atorvastatin liposomes in a 3D-printed polymer film: a repurposing approach for local treatment of oral candidiasis. Drug Deliv. and Transl. Res. 13, 2847–2868. https://doi.org/10.1007/s13346-023-01353-4.

Ossama, M., Lamie, C., Tarek, M., Wagdy, H.A., Attia, D.A., Elmazar, M.M., 2021. Management of recurrent aphthous ulcers exploiting polymer-based muco-adhesive sponges *: in-vitro* and *in-vivo* evaluation. Drug Deliv. 28, 87–99. https://doi.org/10.1080/10717544.2020.1858999.

Patel, V.M., Prajapati, B.G., Patel, J.K., Patel, M.M., 2006. Physicochemical characterization and evaluation of buccal adhesive patches containing propranolol hydrochloride. Curr. Drug Deliv. 3, 325–331. https://doi.org/10.2174/156720106777731082.

Qushawy, M., Nasr, A., Abd-Alhaseeb, M., Swidan, S., 2018. Design, optimization and characterization of a transfersomal gel using miconazole nitrate for the treatment of Candida skin infections. Pharmaceutics 10, 26. https://doi.org/10.3390/pharmaceutics10010026.

Ré, A.C.S., Martins, J.F., Cunha-Filho, M., Gelfuso, G.M., Aires, C.P., Gratieri, T., 2021. New perspectives on the topical management of recurrent candidiasis. Drug Deliv. and Transl. Res. 11, 1568–1585. https://doi.org/10.1007/s13346-021-00901-0.

Rumondor, A.C.F., Dhareshwar, S.S., Kesisoglou, F., 2016. Amorphous solid dispersions or prodrugs: Complementary strategies to increase drug absorption. J. Pharm. Sci. 105, 2498–2508. https://doi.org/10.1016/j.xphs.2015.11.004.

Salazar, S.B., Simões, R.S., Pedro, N.A., Pinheiro, M.J., Carvalho, M.F.N.N., Mira, N.P., 2020. An overview on conventional and non-conventional therapeutic approaches for the treatment of candidiasis and underlying resistance mechanisms in clinical strains. J. Fungi 6, 23. https://doi.org/10.3390/jof6010023.

Shah, R.M., Bryant, G., Taylor, M., Eldridge, D.S., Palombo, E.A., Harding, I.H., 2016. Structure of solid lipid nanoparticles produced by a microwave-assisted microemulsion technique. RSC Adv. 6, 36803–36810. https://doi.org/10.1039/C6RA02020H.

Shahid, M., Hussain, A., Khan, A.A., Alanazi, A.M., Alaofi, A.L., Alam, M., Ramzan, M., 2022. Antifungal Cationic nanoemulsion ferrying miconazole nitrate with synergism to control fungal infections: In vitro, ex vivo, and in vivo evaluations. ACS Omega 7, 13343–13353. https://doi.org/10.1021/acsomega.2c01075.

Sogias, I.A., Williams, A.C., Khutoryanskiy, V.V., 2008. Why is chitosan mucoadhesive? Biomacromolecules 9, 1837–1842. https://doi.org/10.1021/bm800276d.

Soliman, G.M., 2017. Nanoparticles as safe and effective delivery systems of antifungal agents: Achievements and challenges. Int. J. Pharm. 523, 15–32. https://doi.org/10.1016/j.ijpharm.2017.03.019.

Talianu, M.T., Dinu-Pîrvu, C.E., Ghica, M.V., Anuta, V., Prisada, R.M., Popa, L., 2024. Development and characterization of new miconazole-based microemulsions for buccal delivery by implementing a full factorial design modelling. Pharmaceutics 16, 271. https://doi.org/10.3390/pharmaceutics16020271.

Tejada, G., Barrera, M.G., Piccirilli, G.N., Sortino, M., Frattini, A., Salomón, C.J., Lamas, M.C., Leonardi, D., 2017a. Development and evaluation of buccal films based on chitosan for the potential treatment of oral candidiasis. AAPS PharmSciTech 18, 936–946. https://doi.org/10.1208/s12249-017-0720-6.

Tejada, G., Piccirilli, G.N., Sortino, M., Salomón, C.J., Lamas, M.C., Leonardi, D., 2017b. Formulation and in-vitro efficacy of antifungal mucoadhesive polymeric matrices for the delivery of miconazole nitrate. Mater. Sci. Eng. C Mater. Biol. Appl. 79, 140–150. https://doi.org/10.1016/j.msec.2017.05.034.

Tejada, G., Lamas, M.C., Svetaz, L., Salomón, C.J., Alvarez, V.A., Leonardi, D., 2018. Effect of drug incorporation technique and polymer combination on the performance of biopolymeric antifungal buccal films. Int. J. Pharm. 548, 431–442. https://doi.org/10.1016/j.ijpharm.2018.07.023.

Umeyor, C.E., Okoye, I., Uronnachi, E., Okeke, T., Kenechukwu, F., Attama, A., 2021. Repositioning miconazole nitrate for malaria: Formulation of sustained release nanostructured lipid carriers, structure characterization and in vivo antimalarial evaluation. J. Drug Deliv. Sci. Technol. 61, 102125 https://doi.org/10.1016/j.jddst.2020.102125.

Wang, Q.Z., Chen, X.G., Liu, N., Wang, S.X., Liu, C.S., Meng, X.H., Liu, C.G., 2006. Protonation constants of chitosan with different molecular weight and degree of deacetylation. Carbohydr. Polym. 65, 194–201. https://doi.org/10.1016/j.carbpol.2006.01.001.

Xiao, Y., Yuan, P., Sun, Y., Xu, Y., Deng, X., Wang, X., Liu, R., Chen, Q., Jiang, L., 2022. Comparison of topical antifungal agents for oral candidiasis treatment: a systematic review and meta-analysis. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. 133, 282–291. https://doi.org/10.1016/j.oooo.2021.10.023.

Zhang, L.W., Fu, J.Y., Hua, H., Yan, Z.M., 2016. Efficacy and safety of miconazole for oral candidiasis: a systematic review and meta-analysis. Oral Dis. 22, 185–195. https://doi.org/10.1111/odi.12380.