

Chitosan-celluloses wafers containing emulsified curcumin: a nano-in-matrix approach for buccal delivery

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ABSTRACT

Oral surgical and periodontal procedures generate localized tissue damage that favors microbial colonization and delayed healing. Local mucoadhesive drug delivery systems capable of prolonged release may overcome the limitations of systemic therapies. Curcumin (CUR) exhibits antimicrobial and anti-inflammatory activities but is hindered by poor aqueous solubility and physiological instability. This study aimed to develop and characterize polymeric buccal wafers containing a CUR-loaded nanoemulsion as a nano-in-matrix system for sustained local delivery and antimicrobial protection in the oral cavity. Chitosan (CH)-based hydrogels, alone or combined with cellulose derivatives (HPMC or HPC), were loaded with a CUR-nanoemulsion and lyophilized to obtain porous wafers. Nanoemulsification improved CUR aqueous dispersibility and modulated hydrogel rheology, shifting the viscoelastic profile toward a more structured behavior. Among the formulations, CH:HPMC_{low} wafers had optimal performance, combining mucoadhesive strength ($F_{\max}$ 0.46 N), adequate puncture resistance, and sustained CUR release for up to 48 h. X-ray diffraction indicated reduced CUR crystallinity within the matrix, while FTIR confirmed physical entrapment without chemical interactions. CUR-loaded gels and wafers inhibited the growth of *P. gingivalis* and *S. aureus* (MIC of 6.25 and 100 $\mu\text{g/mL}$, respectively). Wafer extracts showed cytocompatibility with TR146 cells in all tested concentrations (up to 40 $\mu\text{g/mL}$). Despite increased water uptake, the wafers remained intact after 4 months of refrigerated storage, retaining 98.31% of CUR content. These findings support the potential of the developed wafers for further pre-clinical studies as buccal delivery systems for post-surgical oral care and prevention of microbial proliferation.

1. Introduction

Oral surgical procedures disrupt the integrity of the oral mucosa and create transient portals for microorganisms (Alghamdi et al., 2024; Kumbhar et al., 2025). In this context, pathogens may penetrate underlying tissues and delay healing leading to persistent infection. In addition, polymicrobial interactions play a critical role in amplifying inflammatory responses and tissue damage following mucosal injury (Rajasekaran et al., 2024). Periodontopathogens such as *Porphyromonas gingivalis* (Yoshimasu et al., 2018; Paul et al., 2021), as well as opportunistic bacteria such as *S. aureus* (Teow et al., 2016), have been

associated with enhanced virulence, immune evasion, and persistence in compromised oral tissues.

Conventional management of oral infections relies on use of mechanical debridement, systemic antibiotics, corticosteroids, analgesics, and topical antiseptics such as chlorhexidine (Alghamdi et al., 2024; Eltay and Van Dyke, 2023; Ahmadi et al., 2021; Alves et al., 2021). Non-invasive local dosage of these actives in the form of gels, mouthwashes, pastes, and sprays, are widely used; however, their clinical performance is often limited by rapid salivary washout, mechanical stress, and insufficient residence time at the application site (Andrews et al., 2024; Boateng et al., 2012). Although innovative commercial products such as

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dental hemostatic dressings (e.g., Hemospon®, Maquira, collagen sponge) and mucoadhesive patches (e.g., Ora-Aid®, DZA Health, cellulose-based film) improve local protection, they primarily act as physical barriers and lack pharmacological activity against infection or inflammation, highlighting a gap in these therapies.

Among the experimental substances for treatment, the polyphenolic from the turmeric *Curcuma longa* has emerged as a promising multifunctional bioactive for oral applications due to its antimicrobial, anti-inflammatory, antioxidant, and wound-healing properties (Tang et al., 2021; Amalraj et al., 2016; Muglikar et al., 2013). Clinical studies using curcumin (CUR) in formulations have reported reduction in plaque accumulation and gingival inflammation comparable to chlorhexidine, supporting CUR's potential as an adjunct in oral care (Kandwal et al., 2015; Mali et al., 2012; Al-Maweri et al., 2022). In addition, CUR enhances tissue repair with local application increasing collagen organization and reepithelialization at injury sites, supporting its biocompatibility and wound-healing potential (Sha and Garib, 2019).

Despite this growing body of clinical evidence, most CUR-based oral formulations reported to date are designed primarily for short-term management of pain or inflammation and are administered as liquid or semi-solid systems (Al-Maweri et al., 2022; Maulina et al., 2018; Pandya et al., 2023; Anil et al., 2019). Nevertheless, CUR clinical translation is hindered by poor aqueous solubility, chemical instability at physiological pH, and low bioavailability in the dynamic oral environment (Cui et al., 2021; Mai et al., 2020; Kotha and Curcumin, 2019).

Nanoemulsion-based delivery systems have been explored to overcome these limitations, improving CUR solubility, stability, and mucosal permeation (Ye et al., 2024; Tavares et al., 2023). Incorporating CUR-loaded nanoemulsions into solid mucoadhesive platforms represents a rational strategy to enhance local retention with protection of the active compound (Feitosa et al., 2019). Using wafers as a solid matrix provides greater mechanical robustness, higher porosity and prolonged mucosal retention compared to oral films (Costa et al., 2019; Liew et al., 2016). Also, wafers are formed by lyophilization, with matrix freezing and drying at lower than ambient temperatures. These features inhibit matrix separation (compared to heat drying) and are an advantage to maintain CUR stability. The addition of an occlusive ethylcellulose backlayer to wafers reduces salivary washout, drug leakage and possible enzymatic degradation (Boateng et al., 2012; Costa et al., 2019).

Regarding wafer composition, a variety of polymers were used in experimental literature. Chitosan (CH) is the most frequent natural polymer used, offering mucoadhesion, antimicrobial activity, and potential hemostatic benefits (de Sousa et al., 2020; Bertolo et al., 2020; Nasaj et al., 2024; Korelc et al., 2023; Martins et al., 2023). Among the semisynthetic excipients, hydroxypropyl methylcellulose (HPMC) and hydroxypropyl cellulose (HPC) contribute to mechanical strength, hydration control, and release modulation (da Silva et al., 2023; Laffleur and Egeling, 2020; Vlad et al., 2025).

Although CUR-loaded lyophilized wafers have been previously investigated, these systems were primarily developed for topical skin applications and wound healing rather than oral delivery (Adel et al., 2021). The few buccal platforms reported to date have focused mainly on physicochemical characterization and drug release, without assessing activity against clinically relevant oral pathogens (Hazzah et al., 2015). Furthermore, formulation strategies with greater translational potential, including polymer blending to tailor wafer performance and the incorporation of a backing layer for unidirectional delivery and prolonged protection against salivary washout, remain unexplored.

Therefore, this study aimed to develop and characterize a buccal nano-in-matrix wafer composed of CH and cellulose derivatives containing a CUR-loaded nanoemulsion and an occlusive backing layer. The proposed system was designed to provide sustained local delivery, extended residence time, antimicrobial activity and biocompatibility, supporting its potential application in post-surgical oral care and prevention of microbial colonization of adjacent tissues.

2. Material and methods

2.1. Material

Chemical components: Curcumin C3 Complex® (kindly donated by Sabinsa Brasil; contains minimum 95% curcuminoids, of which curcumin was 70% – 80% w/w; demethoxycurcumin 15–25% w/w; and bisdemethoxycurcumin 2.5–6.5% w/w); low chitosan molecular weight (CH) (Sigma-Aldrich, ref 448869, 50–190 kDa, degree of deacetylation 75–85%); Super Refined™ Polysorbate 80 (Croda Brasil, Tween-80 HP-LQ-(MH), ref SD43361); Propylene Glycol Monocaprylate NF (Gattefosse, Capryol® 90, ref 3254); diethylene glycol monoethyl ether (Gattefosse, Transcutol® HP, ref 3265); hydroxypropyl methylcellulose (HPMC) (Ashland; Benecel™ K200M 1200 kDa, ref 895603; and Benecel™ E4M 400 kDa, ref 821895); hydroxypropyl cellulose (HPC) (Ashland, Klucel™ HXF Pharm 1150 kDa, ref 414380); Super Refined™ Castor Oil (Croda Brasil, Castor Oil-LQ-(MH); and ethylcellulose (Kerry Group, ref 20421672). For antimicrobial assays: Schaedler broth (ref M292), agar powder (Bacteriological Grade, ref GRM026), Tryptone Soy Agar (TSA, ref M290), and Mueller Hinton broth (MHB, ref M391), all from HiMedia (PA, USA). Defibrinated sheep blood from Anilab Brazil (batch 42/25). All other materials (glacial acetic acid, ethanol, methanol, DMSO, glycerol) were of reagent grade.

2.2. Nanoemulsion preparation

Similarly to Shakeel et al., 2013 (Shakeel et al., 2013), 20% w/w polysorbate-80 was solubilized in 50% w/w deionized water for 45 min/600 rpm magnetic stirring/room temperature (RT). In a separate beaker, 20% w/w diethylene glycol monoethyl ether was mixed with 10% w/w propylene glycol monocaprylate (600 rpm/30 min/RT). CUR (12.5 mg/g of emulsion) was solubilized in the oil phase for the loaded formulations. Nanoemulsion was then formed by slowly dropping the aqueous phase into the oil phase with a Pasteur Pipette, followed by the same stirring conditions for 60 min.

2.3. Gel preparation

For CH gels, the polymer was dissolved in acetic acid 0.8% v/v (350 rpm/16 h/RT). For HPMC and HPC gels, polymers were solubilized in deionized water under manual stirring (spatula), and left to rest 16 h/RT to ensure a clear, bubble-free gel. The following day, glycerol 2% w/w was added to each gel. The mixture of CH and the cellulose polymers were prepared as 1:1 mixture by manual stirring, in order to minimize air entrapment and ensure formulation homogeneity.

2.4. Freeze-drying

Wafers were prepared by sequential incorporation of the nanoemulsion (with or without CUR) into the cellulose-based gel, followed by addition of the CH gel under gentle stirring. Because prolonged storage of the CUR-loaded nanoemulsion led to curcuminoids precipitation after approximately 72 h, the preparation protocol was standardized to ensure that the freshly prepared emulsion was incorporated into the polymeric gel within a few hours prior to freeze-drying. This approach ensured homogeneous API distribution in the gel matrix and prevented phase instability during processing.

The final formulation (2.8 g, 1.4 mg of CUR per unit) was poured on top of a thin back layer (509 µL; 3% w/v of ethylcellulose and 1.5% v/v castor oil in ethanol, pre-dried in oven at 40°C/1h on a silicone ice mold). A 60-h lyophilization period was then undertaken (Lyostar 3, SPScientific, USA). The steps included: freezing –30°C/14 h, primary drying –30°C/16 h, secondary drying 0°C/22 h, and finally, 10°C/8h, with 100 mTorr vacuum for drying steps.

2.5. Particle size of CUR emulsion

Nanoparticle Tracking Analysis, NTA (Nanosight NS300, Malvern Instruments Ltd, UK) was performed at 25°C to determine the size, polydispersity, and concentration of nanoparticles in suspension. Analyses included 60 s-captures of five different populations of CUR emulsion and CUR release from CH:HPMC_{low} optimized wafer, with samples diluted in ultrapure water until around 30–100 particles per frame (dilution factor: 10.000 and 200, respectively), in duplicate. The nanoparticle size distribution data (polydispersity) were obtained by calculating the Span index, according to the Equation (1), where D10 means that 10% of the sample is smaller than this particle size, D50 refers to 50% and D90 to 90%.

$$\text{Span index} = (D90 - D10)/D50 \quad (1)$$

Span index values below 0.5 were indicative of homogeneous (monodisperse) distribution, and values greater than 1.0 were considered polydisperse.

2.6. Surface charge and pH of nano-in-matrix systems

Zeta potential was measured on a Zetasizer Nano ZS equipment (Malvern Instruments, Malvern, UK) using the technique of electrophoretic light scattering. The samples (polymers in association with nanoemulsion without CUR) were measured in suspension immediately after preparation and diluted 1:100 in 10 mM NaCl solution. The pH was measured in triplicate using a digital pH meter by reading diluted samples (1 g of gel to 9 g of ultrapure water).

2.7. Rheology of nano-in-matrix systems

Dynamic rheology experiments were performed using a plate-plate geometry (P35 Ti) on a Haake MARS 60 rheometer at 25°C, with Peltier-based temperature control. Samples were equilibrated for at least 5 min before conducting experiments. The oscillatory experiments were performed within the linear viscoelastic range as determined by strain sweep measurements starting from 10^{-2} to 10^2 Pa. Thus, the frequency sweep proceeded with constant shear stress equal to 1 Pa from 10^{-2} to 10^2 Hz. For flow curves (apparent viscosity as function of the shear rate) the samples were pre-sheared for 60s at 10^{-3} s^{-1} to attenuate the inertial effects at low shear rates and, then, step-sheared from 10^{-3} to 1000 s^{-1} with 30 steps distributed in logarithmic scale. The storage modulus (G') and loss modulus (G'') were determined as a function of temperature using a temperature sweep from 25 to 80°C at a fixed frequency of 1 Hz and a constant stress of 1 Pa.

2.8. Freeze-drying microscopy of nano-in-matrix systems

Collapse temperature (CT) of the samples was determined using a microscope (Olympus BX51, Japan) coupled to a lyophilization module (Lyostat 2, model FDCS 196, Linkam Instruments, Surrey, UK), equipped with a liquid nitrogen freezing system, temperature controller and Linksys32 software. The equipment was calibrated with aqueous NaCl solution. Samples were cooled at a controlled rate of 10°C/min to −60°C, followed by heating to 5°C (10°C/min, 0.100 Torr). The CT was defined as the temperature at which the frozen structure collapsed during lyophilization.

2.9. Wafer 1H time-domain NMR (TD-NMR)

To analyse the rehydration profile of wafers, samples (0.05 g of wafer per analysis) were cut and inserted into glass tubes of 10 mm diameter, with 1 mL of artificial saliva (prepared as described in topic 2.13. *Wafer in vitro disintegration and CUR release profile*). The experiments were performed on a Bruker Minispecmq 20 NMR analyzer (Bruker BioSpin,

Rheinstetten, Germany) with a temperature probe at 37°C. Carr-Purcell Meiboom-Grill (CPMG) Sequence Pulse was used by measuring the T2 relaxation time, in which the 90° and 180° proton pulses were 8.89 μs and 17.78 μs, respectively, and an echo time of 0.3 ms. Measurements were taken every 10 min for 3 h total. Data were acquired from 30,000 echoes with 16 scan repetitions and recycle delay of 15 s. The relaxation decays were evaluated by distributed exponential fit analysis using Peakfit software (version 4.0, Systat Software, Chicago, USA) by the Inverse Laplace Transform algorithm.

2.10. Wafer puncture strength

The puncture strength (Ps) of wafers was evaluated on a texture analyzer (TA-XT Plus – Stable Micro Systems) with a spherical-ended puncture probe (5 mm, Film Support Rig – HDP/FSR), similarly to Meneguim et al, 2017 (Meneguim et al., 2017). Briefly, the samples (n = 3) were supported on a metallic plate with a circular hole (diameter = 10 mm), while the probe was moved down at a constant velocity (0.1 mm/s), with a trigger force of 0.005 kg. The Ps was calculated accordingly Equation (2):

$$\text{Puncture strength (Ps)} = F/A \quad (2)$$

where F is the maximum force required to fracture the film (burst strength, in Newtons) and A (mm²) is the sectional area of the film ($A = 2rh$, in which r is the hole radius and h is the film thickness).

2.11. Wafer ex vivo mucoadhesiveness

In accordance with the original authors' protocol Pestana et al., 2024 (Pestana et al., 2024), swine jaws were commercially obtained from a local slaughterhouse (Angelelli Ltda., Piracicaba – SP, Brazil, certified by the Secretariat of Agriculture and Supply of the State of São Paulo – SIF 2259), immediately after slaughter. The buccal mucosa of the animals was separated from the adjacent tissue with the aid of a scalpel and washed with standard PBS (8 g/L sodium chloride NaCl, 0.2 g/L potassium chloride (KCl), 0.24 g/L potassium monobasic phosphate KH₂HPO₄, 1.44 g/L disodium phosphate Na₂HPO₄, with pH adjusted to 7.4).

After being immersed in distilled water at 60°C/2 min, the epithelium was carefully separated from the underlying connective tissue (lamina propria) with a Molt detacher. The intact epithelia were kept in artificial saliva at 37°C until required. The artificial saliva solution for this test was prepared with 8 g/L NaCl, 0.19 g/L KH₂HPO₄, 2.38 g/L Na₂HPO₄, with pH adjusted to 6.8.

The mucoadhesiveness of the wafers were measured using a TA-XT Plus texture analyzer (Stable Micro Systems) with a A/MUC mucoadhesion rig in tension mode. Briefly, the freeze-dried formulations (n = 5) were attached to the probe with a double sided adhesive tape and lowered (1 mm/s) until full contact (0.5 N/600 s). Mucoadhesiveness was expressed as the force required to separate the sample from the biological substrate.

2.12. CUR quantification

A CUR calibration curve was generated by UV spectrometry. A stock solution (CUR 400 μg/mL in ethanol 70% v/v) was diluted to 2–20 μg/mL in ethanol 50% v/v in a volumetric flask. Samples (100 μL) were then transferred to a microplate and the absorbance UV-Vis was read with BioTek's Epoch™ Microplate Spectrophotometer (425 nm). The calibration curve was evaluated for linearity, selectivity, limit of detection and limit of quantification, in accordance with ANVISA's RDC N° 166/2017 (Online Resource, Fig. S1, Table S1, and Fig. S2).

2.13. Wafer *in vitro* disintegration and CUR release profile

The disintegration and CUR release profile from the wafers were performed in an orbital shaker (60 rpm/ 37°C, Benchmark Incu-Shaker mini), similarly to previous works (de Sousa et al., 2020; Barati et al., 2018). Briefly, in a 50 mL beaker, 5 mL of artificial saliva was poured on top of the samples ($n = 3$), with the backlayer facing upwards. Aliquots of 500 μ L were collected in fixed time intervals (1 h, 2 h, 4 h, 8 h, 24 h, 32 h and 48 h), and replaced by the same volume of fresh artificial saliva. These aliquots were diluted in ethanol and centrifuged at 5000 rpm / 5 min / RT. Finally, 100 μ L of the supernatant absorbance was read in the UV-Vis BioTek's Epoch™ Microplate Spectrophotometer. The absorbance intensity values were converted into concentration (μ g/mL) using the analytical calibration curve. The cumulative amount of CUR released from the wafers was then calculated using the Equation 3:

$$\text{CUR release (\%)} = \left[\frac{\text{amount of released CUR (\mu g)}}{\text{total amount of CUR within the wafer (\mu g)}} \right] \times 100 \quad (3)$$

To prepare the artificial saliva for this assay, 0.2% w/v of methylparaben was first solubilized in ultrapure water at 70°C / 15 min. After this time, the solution was brought to RT, only then the salts (1.594 g/L NaCl, 0.636 g/L KH₂PO₄, 0.202 g/L KCl, and 0.198 g/L urea) and polysorbate-80 1% v/v were added. The pH was adjusted to 6.8 (Sarkar et al., 2019; Bilhan et al., 2007).

Additionally, to compare the release profiles of CH against CH:cellulose derivative wafers, the similarity factor (f_2) was calculated using the Equation 4:

$$f_2 = 50 * \log_{10} \left\{ 100 * \left[1 + \frac{1}{n} * \sum_{t=1}^n (R_t - T_t)^2 \right]^{-0.5} \right\} \quad (4)$$

Where n is the number of time points and R_t and T_t are the mean percentages of the released drug from the (CH) and (CH:cellulose) wafers, respectively, at the t time point. The similarity factor reaches values from 0 to 100, in which $f_2 > 50$ suggests similarity of the dissolution profiles according to the EMA and FDA guidelines (Muselík et al., 2021).

2.14. Wafer average weight, thickness, and residual moisture

The wafers were weighted on an analytical balance (Shimadzu ATY224, Kyoto, Japan) immediately after completion of the freeze-drying process. The height and width of the wafers were measured using a caliper ($n = 3$). Residual moisture (Rm, loss on drying, Pharmacopeia USP (General Chapters, 2025) was assessed by recording the mass of wafers periodically, while they were kept in an oven drying at 40°C / 48 h. The Equation (5) used to calculate the water content loss:

$$Rm (\%) = \left[\frac{(W_i - W_f)}{W_i} \right] \times 100 \quad (5)$$

where W_i is the initial weight and W_f is the final weight after 48 h oven-drying. Values were reported as mean $\pm$ SD.

For the final formulation, residual moisture was also determined by thermogravimetric analysis (TGA-50 M, Shimadzu, Kyoto, Japan). Samples were weighed into alumina pans using a microanalytical balance (MX5, Mettler Toledo, Schwerzenbach, Switzerland) and heated from 25°C to 150°C at 10°C/min under a nitrogen flow of 50 mL/min.

2.15. Wafer morphological analysis

A scanning electron microscope (SEM; Quanta FEI Thermo®, Oregon, USA) was used to capture the morphological characteristics of the systems with and without CUR. Initially, the freeze-dried material was deposited over a double-sided tape and coated with colloidal gold under argon atmosphere. Then images of both sides (upper and backlayer-side) were taken at 100x magnification. ImageJ software was used to count

the amount of pores on wafers, as well as its total area (%), according to the following: image 8-bit > threshold > fill holes & erode (binary) > set measurement > analyse particles.

2.16. Wafer crystallinity

The crystallinity patterns of CUR powder and CUR-wafer were analyzed by X-ray diffractometer (Philips Xpert MPD, PW1811/16) at RT with a step size of 0.05°/2 θ , a scan speed of 1.05 s/step over a 2 θ range of 5–50°, and CuK α radiation (40 mV / 40 mA). The data obtained was plotted using Origin software (OriginLab Corporation, Northampton, MA, USA).

2.17. Wafer Fourier Transform Infrared Spectroscopy (FTIR)

CUR, CH:HPMC_{low}, CH:HPMC_{low} + blank emulsion (BL, without CUR), CH:HPMC_{low} + CUR wafers were analyzed using Fourier Transform Infrared Spectroscopy (FTIR) in an ATR mode (Jasco 6100, Japan) at a wavelength of 550–4000 cm⁻¹ with a reading of 100 scans. Since it was an ATR scan, the laser passed through the wafer, part without a connected backlayer. The test was performed to verify if there is an interaction between curcumin and the wafer.

2.18. Antibacterial activity of formulations

Determination of the minimum inhibitory concentration (MIC) was made by the broth microdilution method in 96-well plates as described by the Clinical and Laboratory Standards Institute (CLSI, M11A7 and M07A9) (Clinical and Laboratory Standards Institute, Ninth Edition; Clinical and Laboratory Standards Institute, Seventh Edition). Treatments were prepared prior to the addition of the bacterial suspension. Control wells included: 1) medium only (broth, sterility control), 2) medium with bacteria, 3) medium with bacteria and DMSO 2% v/v, and 4) medium with bacteria and amoxicillin (positive inhibition control, from 20 μ g/mL to 0.03 μ g/mL).

Porphyromonas gingivalis ATCC 33277 and *Porphyromonas gingivalis* W83 were anaerobically cultured using the Whitley A20 Workstation (80% N₂, 10% CO₂, 10% H₂, Don Whitley Scientific) at 37°C up to 5 days, on supplemented Schaedler agar (Schaedler Broth, Agar powder 1.5% w/v, and 5% v/v defibrinated sheep blood). Resulting dark brown colonies grown on the agar plates were investigated by Gram staining. *Porphyromonas gingivalis* colonies were transferred from the agar to Schaedler broth and incubated anaerobically for 24 h at 37°C. The optical density of the culture was adjusted to OD₆₆₀ = 0.5, followed by a 10-fold dilution for the inoculum.

For *S. aureus* ATCC 29213 (Methicillin Susceptible, MSSA) and *S. aureus* 43,300 (Methicillin Resistant, MRSA), the species were cultured aerobically on Tryptone Soy Agar for 24 h/37°C. The colonies were then transferred to Mueller Hinton Broth, the OD₆₆₀ was adjusted to 0.1 right away, followed by a 100-fold to generate the inoculum.

A 100- μ L volume of the bacterial suspension was added to 96-well plates containing 100 μ L of each previously prepared treatment. After a 48-h incubation for *P. gingivalis* and 20-h incubation for *S. aureus*, OD readings were taken at 660 nm, and 30 μ L of 0.01% w/v resazurin aqueous solution was added for 20 min. Bacterial viability was then assessed visually and by measuring absorbance at 570 nm.

In addition to the MIC assay performed in 96-well plates, a macrodilution adaptation (based on CLSI M07-A8) was conducted in 24-well plates to evaluate the antibacterial activity of blank (BL, without CUR) and CUR-loaded wafers. Wafers were cut into circular sections of 1.0, 0.8, 0.6, and 0.4 cm diameter. Each section was individually weighed, and the corresponding CUR content was estimated by proportionality from the experimentally determined CUR loading of the whole wafer, assuming homogeneous drug distribution throughout the matrix. Bacterial inocula (1 mL) were placed on top of the wafer sections and immersed in culture medium (1 mL), resulting in a final assay volume of

2 mL. Incubation conditions, controls, and result interpretation followed the same procedures described previously. The equivalent CUR concentration ($\mu\text{g/mL}$) was calculated based on the theoretical CUR content of each wafer section and the final assay volume (2 mL). All MIC assays were performed using duplicate wells for each tested condition, and the experiments were independently repeated on two different days using separate microplates.

2.19. Preparation of wafer extracts for the cytocompatibility assays

Blank and CUR-loaded wafers were individually weighed, finely cut, and immersed in 5 mL of FluoroBrite™ medium (Thermo Fisher Scientific, ref. A1896701) supplemented with 2% (v/v) fetal bovine serum (FBS; Cultilab Brazil, ref. 63) and 1% (v/v) penicillin–streptomycin (Sigma-Aldrich, ref. P4333). The samples were maintained at 200 rpm/4h/37°C and later centrifuged at 10000 rpm/10 min/20°C. The supernatants were collected and saved for the following tests.

The CUR concentration in the extracts was determined by UV–Vis spectrophotometry at 425 nm using the calibration curve previously established for CUR content determination. Briefly, 100 μL of extract was mixed with 300 μL of 3% (v/v) acetic acid and vortexed for 30 s. Subsequently, 125 μL of this solution was diluted with 500 μL of methanol and vortexed for an additional 30 s. An aliquot of 100 μL was transferred to a microplate, and absorbance was measured at 425 nm. The CUR concentration in each extract was calculated considering the corresponding dilution factors.

2.20. TR146 cell culture and cytocompatibility of wafer extracts

Cells from passages 26–29 were cultured in high-glucose Dulbecco's Modified Eagle Medium (DMEM high glucose, Sigma-Aldrich, ref. D6429) supplemented with 10% (v/v) FBS and 1% (v/v) penicillin–streptomycin, at 37°C in a humidified atmosphere containing 5% CO_2 . Cells were subcultured upon reaching approximately 80% confluence using trypsin–EDTA (Cultilab Brazil, ref. 1057).

The cytotoxicity of the CUR-loaded wafers was evaluated through MTT and Neutral Red assays. Serial dilutions of the wafer extracts were done in complete DMEM supplemented with 5% (v/v) FBS and 1% (v/v) penicillin–streptomycin to obtain final CUR concentrations ranging from 40 to 0.6 $\mu\text{g/mL}$. Free CUR dissolved in DMSO was diluted in complete DMEM to the same concentrations, resulting in a final DMSO concentration of 0.5% (v/v). Complete mediums without and with 0.5% (v/v) DMSO were used as negative and vehicle controls, respectively.

TR146 cells were seeded in 96-well plates at a density of 2.3×10^4 cells/well and incubated for 24 h to allow cell attachment. The culture medium was then replaced with the respective treatments, and cells were incubated for more 24 h. Between the treatment and subsequent assay steps, wells were gently washed 2–3 times with pre-warmed PBS (pH 7.4). For each plate, nine wells were allocated to the untreated cell control (complete DMEM, $n = 9$), three wells to the vehicle control (0.5% DMSO, $n = 3$), and two wells to each of the eight tested concentrations ($n = 2$). Three independent experiments were performed on three different days, using different passages and independently prepared samples.

For the MTT assay, cells were incubated with 100 μL of DMEM containing 10 μL of MTT solution (5 mg/mL; Thiazolyl Blue Tetrazolium Bromide, Sigma-Aldrich, ref. M5655) for 3 h at 37°C. The resulting formazan crystals were dissolved in 100 μL of DMSO, and absorbance was measured at 570 nm. For the NR assay, cells were incubated with 200 μL of Neutral Red solution (50 $\mu\text{g/mL}$; Sigma-Aldrich, USA, ref. N7005) for 3 h at 37°C. After removal of the staining solution and washing with pre-warmed PBS, the incorporated dye was extracted with 150 μL of a destaining solution consisting of 50% ethanol, 49% deionized water, and 1% glacial acetic acid (v/v). Absorbance was measured at 540 nm. Cell viability was expressed as a percentage relative to the untreated cell control according to the following equation:

$$\text{Cell viability (\%)} = \left(\frac{\text{OD of treated cells}}{\text{average OD of untreated cells}} \right) \times 100 \quad (6)$$

2.21. Short term stability of wafers

After production, the wafers were vacuum sealed in plastic bags and stored under refrigeration (4°C), protected from light. Residual moisture (Rm, %, loss on drying) and CUR recovery were evaluated at 2 and 4 months, in triplicate. Wafers were finely cut into small pieces, transferred to a 25 mL volumetric flask with 3% v/v acetic acid and magnetically stirred 600 rpm/90 min/RT, hydrating and breaking the polymer network. Subsequently, 4 mL of methanol was added to 1 mL of the suspension, followed by an additional 15 min stirring to promote CUR extraction. After centrifugation (5000 rpm/10 min/20°C), 100 μL aliquots were transferred to a 96-well plate, and absorbance was measured at 425 nm using a UV/Vis spectrophotometer. Remaining CUR content (%) was calculated as the ratio between the CUR content at time t and the CUR content at $t(0)$, multiplied by 100.

2.22. Statistical analysis

Samples were evaluated as mean $\pm$ standard deviation. The statistical significance in the differences between samples was determined using the One-way Analysis of Variance (ANOVA), with the Tukey's Honest Significant Difference (HSD) post-hoc test in Excel. The differences were considered significant at $p\text{-value} < 0.05$. Plots were made using Excel, Origin, and GraphPad Prism softwares.

3. Results and discussion

3.1. Development of matrices for buccal wafers

The selection of the polymeric matrix was guided by the need to combine mucoadhesion, mechanical integrity, and compatibility with lyophilization, while avoiding materials prone to rapid enzymatic degradation, microbial fermentation, or structural collapse upon drying. Natural fibers and food-grade polysaccharides were initially screened but failed to form cohesive hydrogels or mechanically stable wafers (data not shown). In contrast, CH combined with cellulose derivatives provided reproducible gel formation, adequate flexibility, and structural robustness after freeze-drying, supporting their selection for further optimization and incorporation of the CUR-loaded nanoemulsion. To support the selection of the polymeric composition, an initial screening was performed using Active Pharmaceutical Ingredient-free wafers (blank, BL), focusing on polymer type and concentration and their impact on post-lyophilization morphology and structural integrity (Table 1).

Similarly to a previous report, wafers prepared with 1% w/w CH showed fragile structures, whereas formulations containing 2% polymer exhibited improved handling properties (Adel et al., 2021). Cellulose blends containing CH exhibited superior structural integrity and prolonged disintegration time, compared to single-cellulose matrices. Formulations combining CH with low-molecular-weight HPMC or HPC were then prioritized due to their better homogeneity. High-molecular-weight HPMC was excluded due to viscosity-related limitations and handling (persistent trapped bubbles even at low shear mixing). Therefore, formulations 2 (CH), 10 (CH:HPMC_{low}), and 11 (CH:HPC) were selected for further studies. The subsequent formulation screening was based on the results of rheological, mechanical, mucoadhesive, and drug-release characterization, allowing formulations with less favorable performance to be excluded at each stage. The rationale for the final selection of CH:HPMC_{low} over CH and CH:HPC is discussed in the corresponding characterization sections.

Table 1

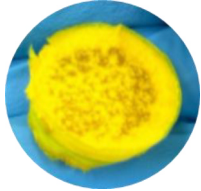
Screening of polymer type and concentration for the development of buccal wafer matrixes.

Wafer number	Polymer composition*	Concentration of polymer (% w/w)	Visual morphology evaluation after freeze-drying	Disintegration time
1	CH	1	fragile, thin, homogeneous	> 48 h
2	CH	2	intact, demoldable, thick, homogeneous	
3	CH	3	intact, demoldable, thick, homogeneous	
4	HPMC _{high}	1	fragile, thin, shrunk, homogeneous	> 6h
5	HPMC _{high}	2	demoldable, thin, shrunk, homogeneous	
6	HPMC _{high}	3	highly shrunk, homogeneous	
7	HPMC _{low}	2	intact, thin, demoldable, homogeneous	< 6 h
8	HPC	2	thin, demoldable, homogeneous	> 6 h
9	CH:HPMC _{high} (1:1)	2	intact, demoldable, thick, heterogeneous	> 48 h
10	CH:HPMC _{low} (1:1)	2	intact, demoldable, thick, homogeneous	> 48 h
11	CH:HPC (1:1)	2	intact, demoldable, thick, homogeneous	> 48 h

*CH = chitosan, HPMC = hydroxypropyl methylcellulose (low and high molecular weight), HPC = hydroxypropyl cellulose.

Table 2

Emulsion screening for CUR incorporation on CH:HPMC_{low} wafers.

Emulsion number	CUR in emulsion (mg/g)	CUR solubility (first hours)	CUR solubility (after 3 days)*	Emulsion in gel (% w/w)	Visual aspect of wafers after freeze-drying (1.4 mg CUR/wafer)*
1	10.0	+	Little precipitation	5	detached backlayer, heterogeneous, partially hollow
					
2	12.5	+	Average precipitation	4	adhered backlayer, homogeneous intact, heterogeneous
					
3	14.29	+	High precipitation	3.5	adhered backlayer, intact, homogeneous
					

*Uncropped images are presented in Online Resource (Fig. S3 and S4).

3.2. Development of formulations with CUR

Given the poor aqueous solubility of CUR, its direct incorporation into the hydrogel matrix would be unfavorable for homogeneous distribution and sustained release. Therefore, a lipid nanoemulsion was selected as a solubilization and delivery strategy prior to wafer incorporation.

The final CUR loading had formulation constraints, so the highest concentration that could be incorporated into the nanoemulsion without visible drug precipitation was 1.4 mg CUR per wafer. Based on the available literature, a minimum loading concentration of 700 µg would provide sufficient CUR content for antibacterial activity against a broad range of bacterial strains (Kumbar et al., 2025; Le et al., 2023; Gunes et al., 2016). Since we expected sustained release and consequent drug distribution over time, we maintained the maximum physical load as the target dose for further evaluations.

Formulation screening (Table 2) demonstrated that higher emulsion contents resulted in oily, mechanically fragile wafers, while excessive CUR loading promoted precipitation during short-term storage. An emulsion concentration of 12.5 mg CUR/g, corresponding to 4% w/w of emulsion in the hydrogel, provided the optimal balance between CUR solubilization, physical stability, and wafer integrity, yielding homogeneous matrices with adequate adhesion to the backlayer (emulsion number 2, Table 2).

3.3. Particle size of optimized CUR emulsion

Nanoparticle permeation across the buccal mucosa can occur via two main routes: paracellular, i.e., through the intercellular space, and transcellular, involving transport directly across epithelial cells (Pestana et al., 2024). Since the paracellular route is strongly size restricted by tight junctions, particles below 200 nm are generally considered favorable for mucus diffusion and epithelial interaction (Madhav et al.,

2009). For example, mucoadhesive zein/ β -cyclodextrin nanoparticles with an average size of 140 nm demonstrated stability and strong potential for buccal delivery of CUR (Esposito et al., 2020). In addition to size, transcellular transport is influenced by surface properties and formulation excipients, including surfactants, lipids, and mucoadhesive polymer (Zheng et al., 2024).

In this study, the nanoparticle tracking analysis (NTA, Online Resource, Fig. S5) confirmed the nanometric size distribution of the CUR-loaded emulsion. The formulation exhibited a mean $\pm$ standard error particle size of 123.7 ± 2.2 nm, with D10, D50, and D90 values of 82.7 ± 0.9 nm, 110.1 ± 2.4 nm, and 185.1 ± 5.6 nm, respectively, indicating a relatively broad but predominantly sub-200 nm population.

The modal particle size was 94.2 ± 2.3 nm, while the total particle concentration reached 1.28×10^{13} particles/mL. The span value of 0.93 indicates a relatively homogeneous nanosized population within the range considered suitable for mucosal delivery.

3.4. Rheology of gels with and without emulsion

Shear flow and oscillatory rheology were employed to characterize the viscoelastic behavior of the hydrogels and to evaluate the impact of cellulose derivatives and CUR nanoemulsion on network organization and flow properties (Budai et al., 2023; Dabbaghi et al., 2021). Amplitude sweep experiments at 1 Hz (Fig. 1, A row) were conducted to define

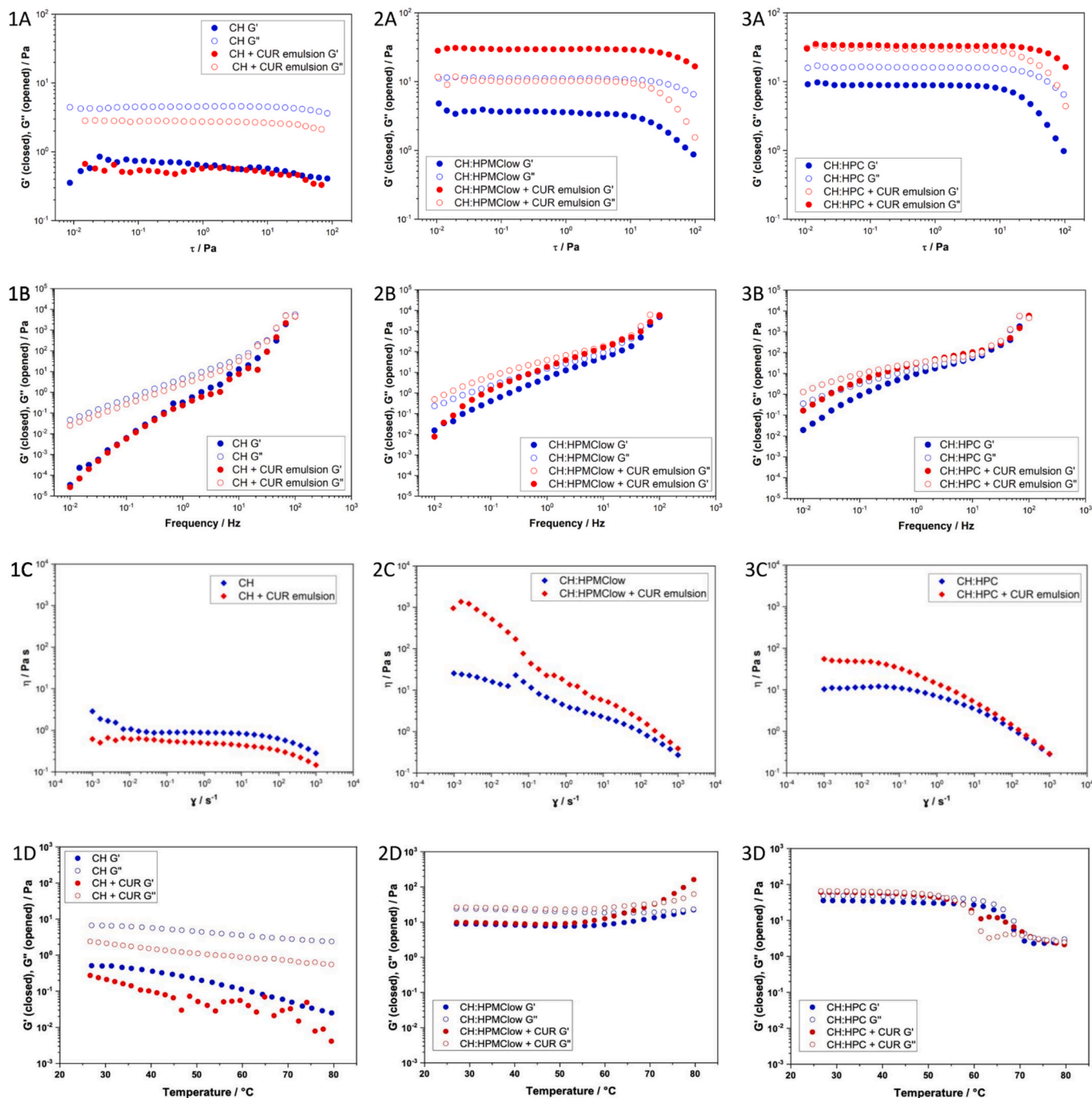


Fig. 1. Rheological profiles of (1) CH, (2) CH:HPMC_{low}, and (3) CH:HPC gels under (A) amplitude sweep, (B) frequency sweep, (C) viscosity tests, and (D) temperature oscillation tests. Formulations without (blue) and with (red) CUR-loaded nanoemulsion.

the linear viscoelastic region (LVR) and the mechanical stability of the gels. CH-based formulations exhibited predominantly viscous behavior ($G'' > G'$), confirming a weakly structured network. The incorporation of the CUR-loaded nanoemulsion had a limited effect on CH alone, indicating minimal reinforcement of its polymeric matrix.

In contrast, both cellulose-containing formulations showed a clear shift toward a more elastic response upon CUR emulsion incorporation. For CH:HPMC_{low}, G' exceeded G'' already within the plateau region, indicating selective strengthening of the elastic network prior to structural breakdown. For CH:HPC, both G' and G'' increased and approached convergence, suggesting enhanced mechanical strength accompanied by increased viscous dissipation. All formulations displayed a well-defined plateau followed by a sharp decrease in moduli at stresses around 20 Pa, corresponding to the yield stress. Notably, the presence of the nanoemulsion increased the maximum stress tolerated before yielding (20–30 Pa), indicating improved resistance to deformation and structural collapse.

Oscillatory frequency sweep analysis (Fig. 1, B row) further elucidated the time-dependent viscoelastic behavior of the gels. All formulations exhibited weak-gel characteristics, with G'' remaining higher than G' throughout the evaluated frequency range, indicating dominance of viscous behavior governed by loosely entangled polymer chains and reversible physical interactions (Sipos et al., 2025). Both moduli (G' and G'') increased with frequency, consistent with weak polysaccharide-based hydrogels reported in the literature (Andrews et al., 2024).

The addition of the CUR-loaded nanoemulsion shifted the crossover point ($G' = G''$) toward lower frequencies, reflecting a modest increase in elastic contribution. Among the systems, CH alone exhibited a larger separation between G' and G'' at low frequencies, whereas cellulose-containing formulations showed closer moduli, indicating partial reinforcement of the polymer network. Although CH:HPC presented an earlier crossover frequency compared to CH:HPMC_{low}, this behavior does not necessarily reflect a more structured gel. Instead, it likely results from weaker intermolecular interactions, leading to an earlier transition from viscous- to solid-like response under oscillatory deformation.

Steady shear viscosity measurements (Fig. 1, C row) revealed that incorporation of cellulose derivatives into the CH matrix markedly increased gel viscosity, an effect further amplified by the addition of the CUR nanoemulsion. CH:HPMC_{low} displayed higher viscosity than CH:HPC, consistent with stronger hydration and chain entanglement associated with HPMC_{low}. All formulations exhibited shear-thinning (pseudoplastic) behavior, characterized by decreasing viscosity with increasing shear rate, a desirable property for processing and application and in agreement with previous reports on CH-based hydrogels (Bertolo et al., 2020).

Temperature-dependent oscillatory measurements (25–80°C, Fig. 1, D row) were conducted to probe the thermal stability and temperature-induced transitions of the polymeric networks. All formulations exhibited a viscous-dominated response ($G'' > G'$), consistent with weak hydrogel behaviour. This profile indicates that the networks are primarily governed by reversible physical interactions rather than permanent crosslinks.

For CH alone, G' progressively decreased with increasing temperature, reflecting thermal thinning of the polymer network. This behaviour is typical of polysaccharide solutions, in which increased thermal motion disrupts hydrogen bonding and chain entanglements, weakening transient intermolecular associations (dos Santos Carvalho et al., 2022; do Amaral Sobral et al., 2022).

The incorporation of cellulose derivatives introduced composition-dependent thermal transitions. In CH:HPC formulations, a pronounced decrease in both viscoelastic moduli was observed around $\sim 60^\circ\text{C}$,

indicating loss of structural integrity. This transition is attributed to the lower critical solution temperature (LCST) behaviour of HPC, where reduced polymer–water affinity at elevated temperatures promotes dehydration of hydroxypropyl groups and favours polymer–polymer interactions. As a consequence, phase separation into HPC-rich and water-rich domains occurs, leading to a drop in viscoelastic moduli and network cohesion (Guido and Grizzuti, 1995). Notably, the presence of the CUR nanoemulsion shifted this transition to slightly lower temperatures, suggesting that the dispersed lipid phase interferes with polymer hydration and accelerates thermal destabilisation.

CH:HPMC_{low} formulations exhibited a distinct response. Rather than a decrease in moduli, an increase in G' was observed near 70°C , consistent with thermogelation behaviour. At elevated temperatures, enhanced hydrophobic interactions among methoxy groups promote chain aggregation and network reinforcement, yielding a more solid-like response (Almeida et al., 2014; Perez-Robles et al., 2022). Although both HPMC_{low} and HPC display reduced aqueous solubility with increasing temperature, their rheological outcomes diverge markedly: HPC undergoes phase separation, whereas HPMC_{low} retains water within a cohesive polymer network, resulting in increased elasticity. Again, incorporation of the CUR nanoemulsion shifted the onset of this transition to lower temperatures, likely due to disruption of water–polymer interactions and surfactant-mediated modulation of chain mobility.

From a formulation perspective, these temperature-induced transitions are relevant for processing robustness, as they may influence steps such as mixing, degassing, freeze-drying behaviour, and stability under temperature fluctuations during storage and application.

3.5. Collapse temperature of pre-lyophilized formulations

The collapse temperature (T_c) of the nano-in-matrix system was determined as -23.1°C , while the onset of freezing occurred at -24.4°C . These values were fully compatible with the freeze-drying protocol adopted for wafer preparation, in which the freezing and primary drying steps were conducted at -30°C . Operating below the experimentally determined T_c ensured structural stability of the frozen matrix, preventing pore collapse, surface shrinkage, and heterogeneity during sublimation (Abdelwahed et al., 2006; Tang and Pikal, 2004). Consequently, the selected lyophilization conditions were suitable to preserve the porous architecture of the wafers and to promote efficient water removal, resulting in homogeneous and mechanically consistent dried systems.

3.6. pH and zeta potential of pre-lyophilized formulations

The pH and surface charge of the emulsion-loaded hydrogels were evaluated before freeze-drying. All formulations exhibited slightly acidic pH values, ranging from 4.62 to 4.91, which favor protonation of CH amino groups and contribute to the formation of a positively charged polymeric network. This measurement was performed in a 1:10 dilution due to viscosity, which may have overestimated the pH value in approximately a 0.5 unit of pH, since this property consistently rises by about 0.5 units per ten-fold dilution of weak acids in water.

The positively charged CH was confirmed by the zeta potential measurements, which remained positive for all samples. The incorporation of cellulose derivatives significantly reduced the zeta potential of the systems, from $+14.03 \pm 0.76$ mV for CH alone to $+7.23 \pm 1.51$ mV and $+6.00 \pm 1.68$ mV for CH:HPMC_{low} and CH:HPC, respectively ($p = 7.78 \times 10^{-4}$). Noteworthy, the zeta potential was measured in a 1:100 diluted sample, therefore does not reflect the zeta potential of the concentrated solution, but it is adequate for comparison among diluted samples. It has been reported that cross-linking of CH with other

polymers decreases bioadhesion due to the reduction in positive charges (İkinci et al., 2002), highlighting the importance of electrostatic interactions between protonated amine groups of CH and negatively charged sialic acid residues in mucin (de Sousa et al., 2020; Kulkarni et al., 2025). In this present study, zeta potential analysis confirmed that mixing CH with cellulose derivatives (1:1 ratio) reduced its surface charge by approximately 50%.

3.7. Impact of adding emulsion on wafers puncture strength

Puncture strength and elongation are two complementary parameters used to describe the mechanical performance of polymeric wafers. Puncture strength reflects the maximum force required to fracture the matrix, serving as an indicator of structural resistance and rigidity, while elongation represents the extent to which the material can deform before failure, reflecting its flexibility and extensibility (Meneguín et al., 2017).

In gels, these properties are often correlated with rheological parameters such as G' , G'' , and yield stress, where higher elastic contributions (G') usually predict stronger and less deformable structures. However, after freeze-drying, the mechanical behavior of the resulting wafers is not dictated solely by the viscoelasticity of the precursor gel but also by the drying-induced architecture (e.g. pore size, interconnectivity, and density). Consequently, systems like pure chitosan, classified rheologically as weak gels ($G'' > G'$), can still yield robust and mucoadhesive wafers with high puncture strength once converted into solid matrices, as shown in Fig. 2.

All formulations exhibited puncture strength values up to 17 N/mm² and elongation at break between 50 and 72%, which fall within the

range reported for similar buccal systems. For instance, Meneguín et al (2017) (Meneguín et al., 2017) reported values of 8–24 MPa for nanocellulose-reinforced films, although with markedly lower elongation (<10%). The higher extensibility observed in the CH-only wafers likely reflects its plastic-like behavior, since protonated amino groups in acidic conditions favor strong intermolecular hydrogen bonding and the formation of a homogeneous three-dimensional network (Korelc et al., 2023; Kanimozhi et al., 2016; Nie et al., 2016). In contrast, mixing with cellulose derivatives introduces bulky substituents that can interfere with this network, restricting chain mobility during stress, especially observed for the CH:HPMC_{low} formulation compared with CH alone.

Nonetheless, formulations exhibited a good balance between mechanical resistance and flexibility, which is crucial for buccal applications. The wafers must withstand handling during placement while remaining soft and conformable to irregular mucosal surfaces. In this context, the observed tensile properties are adequate for ensuring ease of application, patient comfort, and stable adhesion under dynamic intraoral conditions (da Silva et al., 2023; Fernandes et al., 2018). In the present study, texture analysis confirmed the reinforcing effect of the nanoemulsion, with all formulations showing increased puncture strength and reductions in elongation. This indicates that the emulsified systems became more resistant to fracture without compromising deformability.

3.8. Wafers CUR release profile

To ensure reliable quantification of CUR, analytical validation was performed prior to release studies, including calibration, selectivity and recovery assays (Online Resource, Fig. 1, Fig. 2, and Table S1). The

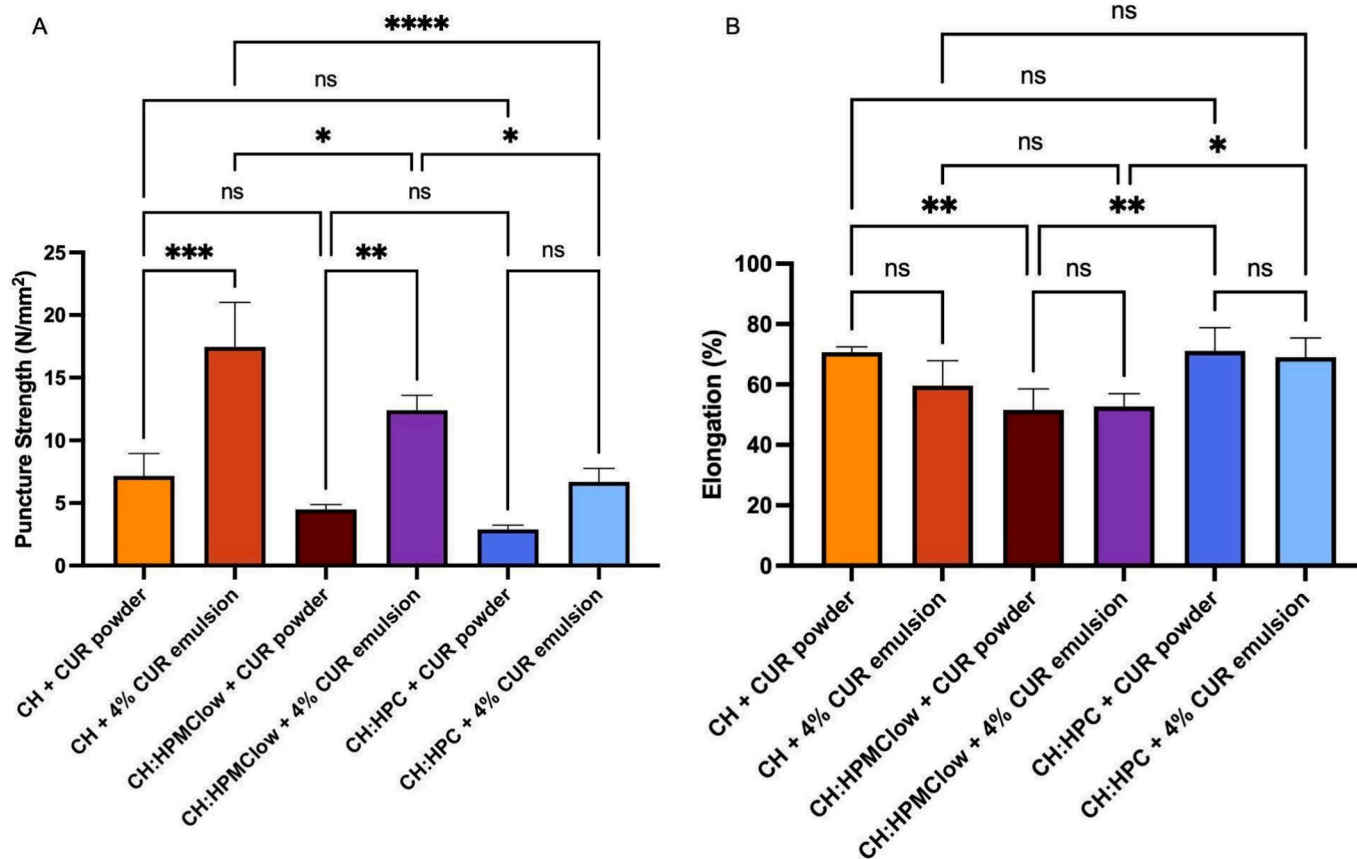


Fig. 2. Puncture strength (A) and elongation (B) of the wafers: comparison of CH, CH:HPMC_{low} and CH:HPC formulations containing CUR powder versus CUR emulsion, in triplicate (n = 3). One-way ANOVA with Tukey's post hoc test was used for statistical analysis. For (A), from left to right, bottom to up: ****P = 0.0001; **P = 0.0010, ns, not significant = 0.3604; ns = 0.0608; ns = 0.8041; *P = 0.0239; *P = 0.0110; ns = 0.1035; ****P < 0.0001. For (B): ns = 0.1546; ns > 0.9999; ns = 0.5724; **P = 0.0082; **P = 0.0071; ns = 0.2716; *P = 0.0227; ns = 0.9997; ns = 0.9948.

method showed linearity in the 2–20 $\mu\text{g/mL}$ range, and no interference from the polymeric matrices was detected at 425 nm.

Release experiments were conducted in artificial saliva to mimic the buccal environment, while carefully accounting for the intrinsic solubility limitations of CUR. The 48-h release period was selected as an experimental timeframe to characterize the extent of CUR release from the wafers and to assess their physical behavior under prolonged hydration. Preliminary tests showed that dispersion of the CUR-loaded nanoemulsion in the original saliva formulation resulted in visible precipitation, reflecting the insufficient solubilizing capacity of the medium. The addition of 1% (v/v) polissorbate 80 effectively prevented emulsion destabilization and CUR precipitation, whereas crystalline CUR still precipitated under the same conditions, confirming the critical role of the lipid nanoemulsion in enhancing the apparent solubility of the bioactive compound.

Under these conditions, CUR release from the wafers increased progressively over time, reaching a plateau after approximately 32 h (Fig. 3), which was characteristic of release under sub-sink conditions. The dissolution medium exhibited a solubilization capacity close to the administered CUR dose, below the conventional sink criterion of 3–10x (Hate et al., 2025). Increasing the surfactant concentration to enforce sink conditions was deliberately avoided, as higher polissorbate-80 contents would substantially alter the surface tension and composition of artificial saliva, thereby compromising the physiological relevance of the model.

Distinct release profiles were observed among the CH, CH:HPMC_{low}, and CH:HPC formulations. The CH-based wafer released the lowest CUR amounts, while incorporation of cellulose derivatives facilitated this release. The higher release observed for CH:HPC can be attributed to its faster hydration and reduced matrix cohesion, which facilitate earlier structural relaxation and CUR diffusion. In contrast, CH:HPMC_{low} exhibited a more restrained release profile, consistent with a more cohesive polymeric network. In a statistical comparison, the f_2 similarity factor was 34.01 for CH versus CH:HPMC_{low}, and 22.38 for CH versus CH:HPC, further confirming the dissimilarity of the release profiles according to the conventional criterion (similar only if $f_2 > 50$). Noteworthy, although we calculated similarity, it has limited interpretation due to the number of replicates per time point (Muselík et al., 2021). Altogether, these results indicate that incorporation of the cellulose derivatives modified not only the extent of CUR release but also its temporal release pattern.

Importantly, the *in vitro* timeframe should not be interpreted as a 48-h residence time in the oral cavity. *In vivo* adhesion would likely be shorter (one day) and in contact with the wound, which presents a different medium for drug release than saliva. Therefore, the 48-h profile primarily demonstrates the capacity of the matrix to sustain CUR

availability under prolonged hydration and the differences between formulations, rather than a preview of biological performance. Further clinical studies would be needed to determine the residence time of the dosage form and CUR pharmacokinetics. Although the release method was discriminative between formulations, the subsink condition was not adequate for release kinetic modelling, and the reason why this property was not evaluated here.

Importantly, despite the slightly acidic pH of the precursor hydrogels, the wafers did not significantly alter the pH of artificial saliva after 48 h ($\Delta\text{pH} < 0.04$), indicating compatibility with the buffered oral environment. Also, the NTA measurements of aliquots collected after 24 and 48 h release (Online Resource, Table S2) demonstrated that the nanoemulsion preserved its nanosized population after release from the optimized CH:HPMC_{low} wafer matrix. Mean particle sizes remained within 124–139 nm, with D50 values close to 100–115 nm throughout the assay. Although span values increased after release, indicating broader particle size distributions, no marked shift toward micron-sized populations was observed under these conditions. These findings suggest that the nano-in-matrix system was capable of sustaining the release of nanostructured CUR over time.

3.9. Wafers *ex vivo* mucoadhesion assay

In the present study, both CH and CH:HPMC_{low} wafers showed mucoadhesiveness, with maximum peak forces of 0.52 ± 0.13 N and 0.46 ± 0.09 N, respectively (Online Resource, Fig. S6). In contrast, the CH:HPC wafer showed a significantly lower force (0.15 ± 0.01 N) and was therefore classified as non-mucoadhesive, similarly to Imosec®, a commercial lactose-based tablet used as a non-mucoadhesive reference ($F_{\text{max}} \approx 0.2$ N) (Pestana et al., 2024). This classification was further supported by the need to mechanically reinforce adhesion to the probe prior to testing.

HPMC_{low} and HPC are non-ionic cellulose derivatives, meaning they do not exhibit dissociable functional groups across the physiological pH range (Laffleur and Egeling, 2020; Vlad et al., 2025). As such, their interactions with mucosal surfaces and other polymers occur primarily through hydrogen bonding, van der Waals forces, and physical entanglement (interpenetration of the polymer chains into the mucin oligosaccharide side chains), rather than electrostatic attraction (Pestana et al., 2024; Serra et al., 2009). Despite this chemical neutrality, the cellulose derivatives tested demonstrated distinct mucoadhesive behaviors.

The superior performance can be attributed to HPMC_{low}'s functional group composition, which includes both hydroxyl and methoxyl moieties. These groups enhance water uptake and enable the formation of hydrated gel networks, allowing close contact and interpenetration with the mucosal surface, thus favoring adhesion (Serra et al., 2009). Conversely, HPC contains bulkier and more hydrophobic hydroxypropyl substituents, which limit hydration and impair the formation of cohesive hydrated networks, resulting in poor mucoadhesive performance. Notably, the higher molecular weight of HPC did not translate into increased adhesion, reinforcing that molecular weight alone is not a reliable predictor of mucoadhesion. The relatively high variability observed in the measurements is likely associated with intrinsic heterogeneity of the *ex vivo* porcine mucosa, including differences in tissue thickness and animal origin.

Since the physicochemical characterization tests were being conducted simultaneously with the optimization of the formulation, before observing the loss of stability of the 14.29 mg CUR/g emulsion (3.5% w/w) after 3 days, the mucoadhesion assay had been already carried out in quintuplicate. As mentioned, the concentration was then kept at 4% w/w for future experiments. As the texturometer results have no significant difference between the most relevant formulations CH and CH:HPMC_{low} (Online Resource, Fig. S6), increasing the percentage of emulsion would not significantly affect mucoadhesion in *ex vivo* porcine tissue.

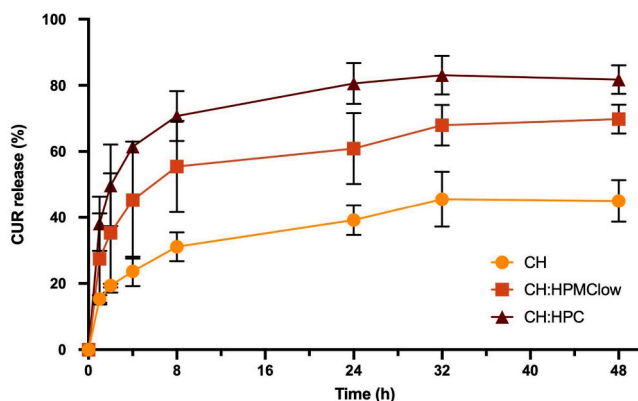


Fig. 3. CUR release profile of CH, CH:HPMC_{low} and CH:HPC wafers, up to 48 h in artificial saliva at 37°C/60 rpm, pH of 6.8 (n = 3, mean value $\pm$ standard error).

3.10. Final wafer physicochemical analysis

Following the initial formulation screening and the subsequent exclusion of less suitable systems (CH:HPMC_{high} and CH:HPC) based on their performance, the morphological and physical characteristics of the final selected formulations were further evaluated. This analysis focused exclusively on CH and CH:HPMC_{low} wafers (Fig. 4), which were produced under standardized conditions using silicone molds and controlled mass per well. Uncropped images are presented in Online Resource, Fig. S7.

Overall, the mean weight of 2 x 2 cm² square wafers was 159.47 ± 2.10 mg and 158.37 ± 4.69 mg for the CH and CH:HPMC_{low} formulations, respectively. Inter-batch CUR content uniformity was assessed using two to three wafers from each of three independent batches, relative to the theoretical CUR content of 1.4 mg per wafer. The mean CUR content was 100.30 ± 6.14%, 103.61 ± 4.23%, and 100.61 ± 1.54% of the theoretical value for batches 1, 2, and 3, respectively. All batch content values were within the 90–110% range commonly used as an acceptance range for drug content consistency, indicating low inter-batch variability and good reproducibility of CUR incorporation into the optimized wafers.

Both formulations exhibited residual moisture levels considered acceptable for freeze-dried products (up to 5%), consistent with efficient water removal and suggesting suitable physical stability for subsequent handling and storage (General Chapters, 2025). Table S3 from Online Resource summarizes the mass, thickness, and residual moisture content of these formulations, providing quantitative support for their morphological uniformity.

From TGA analysis, a mass loss (%) versus temperature curve was plotted (Online Resource, Fig. S8). Up to 150°C, a reduction of nearly 15% of mass loss was observed, most likely corresponding to water. For comparison, the residual moisture content was calculated considering the sample mass at 100°C (water boiling point). The resulting value was 6.63%, which is consistent with that obtained by oven-drying loss.

The optimized formulations were also assessed for morphology using Scanning Electron Microscopy (SEM). At 100x magnification (Fig. 5), the CH wafers revealed more defined pores than the CH:HPMC_{low}, which had fewer pores in quantity and size (surface area %, Online Resource, Table S4), as well as a brittle backlayer.

These findings can be interpreted in light of the rheological properties of the precursor gels. The addition of HPMC_{low} significantly increased the viscosity of the system, which likely limited ice crystal growth during freezing. Since ice crystals act as porogens in the lyophilization process, reduced crystal size leads to a denser and less porous final matrix. Moreover, the bulky methoxyl and hydroxypropyl

substituents of HPMC may interfere with CH chain packing, further restricting the formation of large, open pores.

The formulation selection followed a stepwise screening process in which formulations were progressively excluded according to their processability and performance across rheological, mechanical, mucoadhesive, and drug-release assessments. High-molecular-weight HPMC was initially excluded because its excessive viscosity hindered hydrogel handling and resulted in persistent trapped bubbles even under low-shear mixing. Among the remaining CH, CH:HPC, and CH:HPMC_{low} systems, the latter provided the most favorable overall balance of wafer properties, particularly mechanical performance and mucoadhesion, while also enabling higher CUR release compared with CH alone. Accordingly, CH:HPMC_{low} was selected as the optimized formulation for subsequent characterization.

3.11. Crystallinity and molecular interactions of CH:HPMC_{low} wafers

XRD analysis was performed to investigate the solid-state organization of CUR within the CH:HPMC_{low} wafers. Commercial CUR powder showed a crystalline structure, evidenced by intense and well-defined diffraction peaks at 8.946°, 12.241°, 14.572°, 17.359°, 18.215°, 24.624° and 25.619° (Fig. 6A), in agreement with literature reports (Tolun et al., 2025). In contrast, these peaks were markedly attenuated and broadened in the CUR-loaded wafers, indicating a substantial reduction in crystallinity. This behavior suggests that CUR is predominantly present in an amorphous or molecularly dispersed state within the polymeric matrix, likely due to nanoconfinement and restricted molecular mobility imposed by the CH:HPMC_{low} network.

FTIR spectroscopy (Fig. 6B) was used to further assess the molecular organization of the CH:HPMC_{low} wafers, and to investigate possible interactions between CUR, the lipid nanoemulsion and the polymeric matrix. The characteristic band of chitosan (black) was detected at 854 cm⁻¹, indicating bending at C1-H, while for HPMC (blue), it was found at 926 cm⁻¹ for a vibration of –C-O-C-. Some bands were found to overlap between the two polymers (gray), notably at 3317 cm⁻¹, 2926–2867 cm⁻¹, 1038 cm⁻¹, referring to O–H, C–H stretching and alkyl-substituted cyclic ring containing ester linkages (HPMC), and N–H Stretching, H–C–H (CH₂) Bending, C₃-O Stretching (chitosan) (dos Santos Carvalho et al., 2022).

Shortly, the overlapping bands confirm CH:HPMC_{low} molecular interpenetration and network homogeneity, while the absence of new peaks or relevant shifts indicates physical incorporation of the emulsion and CUR without detectable chemical modification by FTIR.



Fig. 4. Wafers seen from the top, bottom (backlayer) and side, containing: (A) CH and (B) CH:HPMC_{low}, both with 4% w/w CUR-emulsion (1.45 mg CUR/wafer).

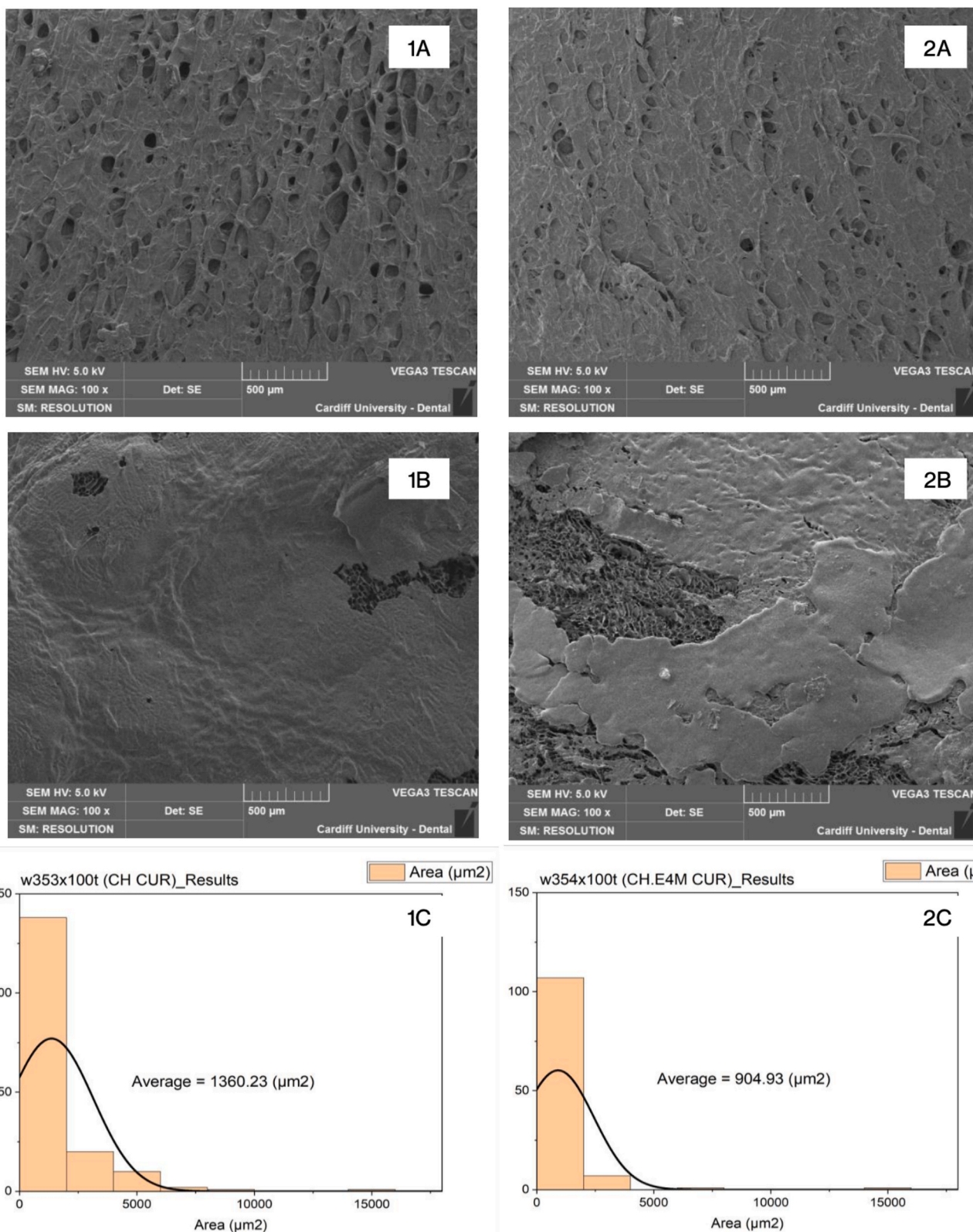


Fig. 5. Comparison between the (1) CH and (2) CH:HPMC_{low} wafers, both containing 4% w/w CUR emulsion. Images taken from the top (A) and bottom surface with backlayer (B) at 100x magnification. The histograms (C) show the pore count in terms of area (µm²), including the average area.

3.12. Water mobility and rehydration dynamics of CH:HPMC_{low} wafers

To investigate rehydration behaviour of the wafers, time-domain NMR was used to monitor water mobility within the polymeric matrix under biorelevant conditions. Both CH:HPMC_{low} wafers, without and

with nanoemulsion, showed a rapid water uptake profile (Fig. 7), similarly to findings of Hazzah et al, 2015 (Hazzah et al., 2015).

Notably, the wafer without nanoemulsion exhibited an almost instantaneous increase in the intensity of the relaxation peak at approximately 300 ms, indicating faster initial hydration. In contrast,

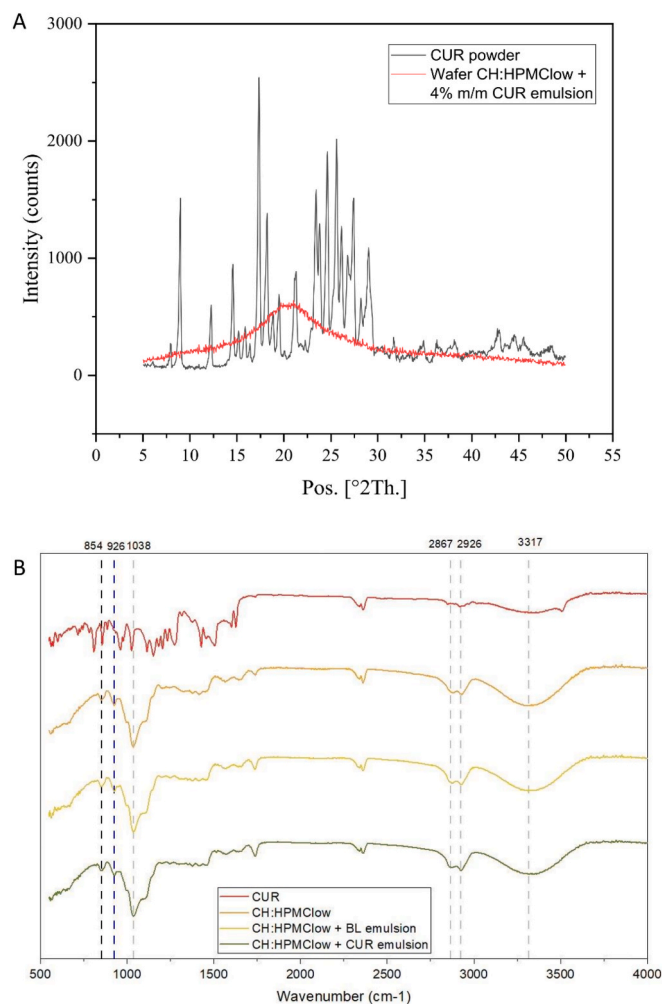


Fig. 6. (A) The XRD diffractograms of the pure CUR powder show characteristic peaks, indicative of its crystalline nature. The CH:HPMC_{low} wafer containing CUR emulsion, on the other hand, showed an amorphous pattern, with low intensity. (B) FTIR spectra of CUR powder, and wafers made of: CH:HPMC_{low}, CH:HPMC_{low} + blank emulsion (BL), CH:HPMC_{low} + CUR emulsion.

the nanoemulsion-loaded wafer showed a more gradual increase in this signal, suggesting a delayed water penetration into the matrix. This was evidenced by a shift of the rightmost relaxation peak (free/bulk water) towards a reduced area under the curve, while the intermediate T_2 peak (associated with water confined in smaller pores) progressively increased over time. Such redistribution indicates that absorbed water quickly penetrates into the polymer network, filling mesopores and micropores rather than remaining as free water. Despite the differences in hydration kinetics, both formulations exhibited a similar overall rehydration pattern, indicating that the nanoemulsion modulates the rate, but not the mechanism, of water uptake.

3.13. Antibacterial activity

The MIC values obtained for the test bacteria are presented in Table 3.

For Gram-negative *P. gingivalis* ATCC 33277 and *P. gingivalis* W83, free CUR exhibited potent antibacterial activity (MIC = 6.25 $\mu\text{g/mL}$), establishing the baseline efficacy of the bioactive compound. The blank nanoemulsion showed inhibitory activity (equivalent to 12.5 $\mu\text{g/mL}$ of CUR), likely due to surfactant-mediated membrane disruption. Incorporation of CUR into the nanoemulsion maintained this activity (MIC = 6.25 $\mu\text{g/mL}$), indicating that the nanocarrier preserved its antimicrobial properties.

The CH:HPMC_{low} gel alone inhibited bacterial growth at higher equivalent concentrations (25 $\mu\text{g/mL}$), which can be attributed to the intrinsic antimicrobial activity of chitosan. As a cationic polysaccharide, CH interacts electrostatically with negatively charged bacterial membranes, disrupting membrane integrity and increasing permeability (Nasaj et al., 2024; İkinici et al., 2002). When combined with the blank nanoemulsion, the MIC decreased to 12.5 $\mu\text{g/mL}$, suggesting an additive effect between the polymeric matrix and surfactant system. Further incorporation of CUR into the gel-nanoemulsion system reduced the MIC to 6.25 $\mu\text{g/mL}$. When formulated as wafers, CH:HPMC_{low} systems maintained inhibitory activity against *P. gingivalis*, although at higher equivalent CUR concentrations (17.06 $\mu\text{g/mL}$). Blank wafers required substantially higher doses, confirming that CUR contributes to antimicrobial efficacy.

For *S. aureus* strains (ATCC 29213 and ATCC 43300), free CUR and CUR-loaded nanoemulsions exhibited MICs of 100 $\mu\text{g/mL}$, similarly to Adel et al, 2021 (Adel et al., 2021). Although the exact mechanism remains not fully elucidated, CUR has been associated with disruption of bacterial membrane integrity, ultimately leading to cell death (Tyagi

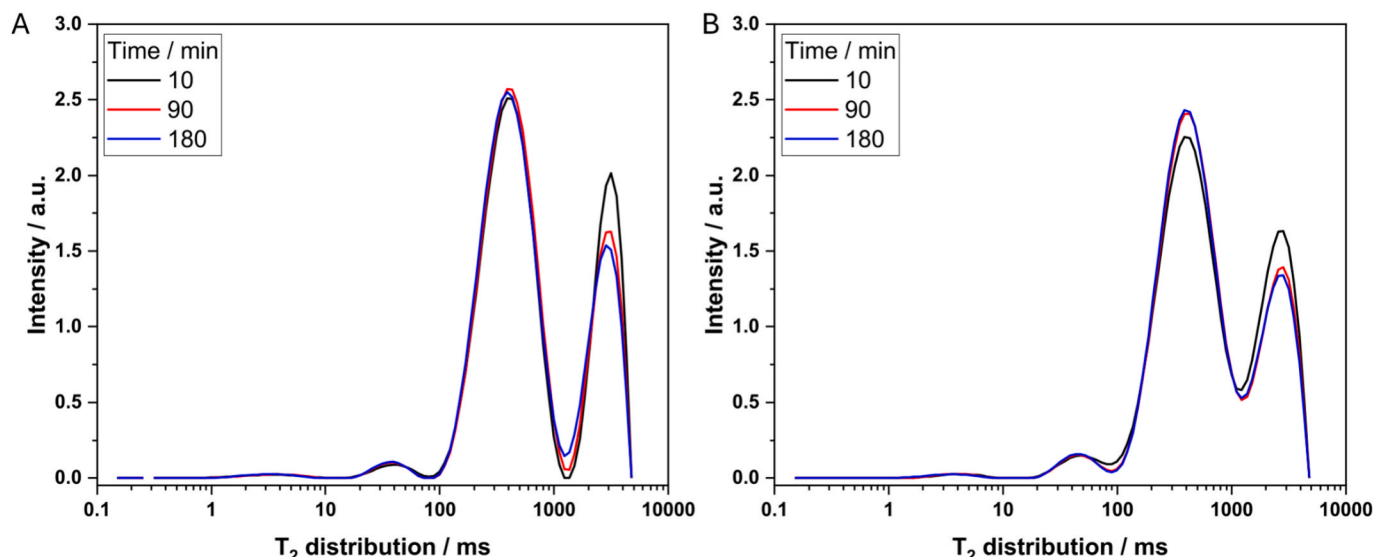


Fig. 7. T_2 distribution of CH:HPMC_{low} wafers (A) without and (B) with CUR emulsion, after the addition of artificial saliva.

Table 3

Minimum Inhibitory Concentration (MIC) values of free CUR, CUR-loaded emulsion, and CUR-loaded wafer formulations, and their respective blanks, against oral bacteria.

Condition*	MIC (µg CUR/mL**)			
	<i>P. gingivalis</i> W83	<i>P. gingivalis</i> ATCC 33277	<i>S. aureus</i> ATCC 29213	<i>S. aureus</i> ATCC 43300
CUR in DMSO 2%	6.25	6.25	100	100
BL emulsion	dilution equivalent to 12.50 µg/mL of CUR	dilution equivalent to 12.50 µg/mL of CUR	dilution equivalent to 100 µg/mL of CUR	dilution equivalent to 200 µg/mL of CUR
CUR emulsion	6.25	6.25	100	100
CH:HPMC _{low} gel	0.18% w/w polymer	0.18% w/w polymer	0.36% w/w polymer	0.36% w/w polymer
CH:HPMC _{low} gel + BL emulsion	(equivalent to 25.00 µg/mL of CUR) dilution equivalent to 12.50 µg/mL of CUR	(equivalent to 25.00 µg/mL of CUR) dilution equivalent to 12.50 µg/mL of CUR	(equivalent to 50.00 µg/mL of CUR) dilution equivalent to 12.50 µg/mL of CUR	(equivalent to 50.00 µg/mL of CUR) dilution equivalent to 25.00 µg/mL of CUR
CH:HPMC _{low} gel + CUR emulsion	6.25	12.50	< 6.25	< 6.25
Amoxicillin (control)	< 0.03	< 0.03	1.25	10
CH:HPMC _{low} wafer + BL emulsion (24 well plate)	0.8 cm diameter	0.8 cm diameter	1.0 cm diameter	1.0 cm diameter
CH:HPMC _{low} wafer + CUR emulsion (24 well plate)	(equivalent to 78.79 µg/mL of CUR) 17.06	(equivalent to 78.79 µg/mL of CUR) 17.06	(equivalent to 124.55 µg/mL of CUR) 124.55	(equivalent to 124.55 µg/mL of CUR) 78.79
	(0.4 cm diameter)	(0.4 cm diameter)	(1.0 cm diameter)	(0.8 cm diameter)

*CUR = curcumin, BL = blank (without CUR), CH: chitosan, HPMC_{low} = hydroxypropyl methylcellulose. The vehicle control containing 2% v/v DMSO showed no antimicrobial effect, and sterility controls remained negative throughout the experiments. **CUR concentrations for wafers were calculated from the mass of each section and the experimentally determined CUR content of the whole wafer, normalized to the final assay volume (2 mL).

et al., 2015). The CH:HPMC_{low} gel alone showed moderate activity (50 µg/mL equivalent), while incorporation of the blank nanoemulsion reduced MIC values (12.5–25 µg/mL), again suggesting a contribution from surfactant-mediated effects. Notably, the combination of CH:HPMC_{low} gel with CUR-loaded nanoemulsion markedly enhanced activity, with MIC values below 6.25 µg/mL, suggesting a combined effect between CH-mediated membrane perturbation and CUR action. CUR-loaded wafers also inhibited *S. aureus* growth, albeit at higher equivalent CUR concentrations (78.8–124.6 µg/mL).

In brief, the MIC assays were discriminatory, demonstrating that antimicrobial performance depends on both bacterial strain and formulation architecture. While gels provided lower MIC values due to immediate availability of actives, wafers retained measurable antibacterial activity under diffusion-limited conditions, supporting their potential for localized and sustained oral delivery.

3.14. Cytocompatibility

The intended use of the formulation on buccal wounds led us to test its cytocompatibility in TR146 cells. This human buccal squamous carcinoma cell line is used as an *in vitro* model for oral drug delivery and buccal mucosal studies (Lin et al., 2020; Sander et al., 2013; Nielsen and Rassing, 2002; Jacobsen et al., 1996).

Wafer extracts were prepared to avoid physical interference of the intact solid formulation with cell monolayers, allowing the evaluation of the biological effects of the released components. The quantification of the recovered CUR by UV–vis spectroscopy indicated that approximately 50% of the drug content (140 µg/mL) was released during the 4 h-extraction period. This saturated solution was then diluted in complete DMEM to 80 µg/mL CUR, followed by serial dilution. Although representing a limitation in evaluating the effects of higher concentrations of the wafer extract, this approach ensured that the treatment solutions applied to the cells consisted predominantly of complete DMEM supplemented with 5% FBS and 1% penicillin–streptomycin (v/v). The MTT and Neutral Red assays, shown in Fig. 8, strengthened the interpretation on cell viability by assessing different cellular functions – mitochondrial metabolic activity and lysosomal integrity, respectively.

The untreated and vehicle controls showed high cell viability, with no significant difference between them, confirming that 0.5% DMSO did not affect TR146 viability under the experimental conditions. In contrast, free CUR reduced cell viability at 20 and 40 µg/mL, whereas the CUR wafer extracts maintained cell viability above the predefined cytotoxicity threshold of 70% (ISO 10993-5) across the evaluated concentration range. This difference suggests that incorporation of CUR into the wafer matrix attenuates the cytotoxic effect observed for the free compound. Those values are particularly relevant because antibacterial activity against *P. gingivalis* was observed at a lower concentration (6.25 µg/mL) while cell viability remained preserved in TR146 cells, indicating a concentration range in which antimicrobial activity and epithelial viability were simultaneously achieved.

These findings are consistent with previous studies evaluating the cytocompatibility of polymeric buccal delivery systems. A recent study of CUR-loaded buccal films evaluated cytotoxicity after 24 h exposure and reported cell viability above 70% for both blank and CUR-loaded films in L929 mouse fibroblast cells (Ünükür Sevim et al., 2026). In another study using TR146 cells, chitosan buccal films also showed acceptable cell viability following 2 and 4 h of exposure (Rayya et al., 2026). Although the shorter exposure period in that study limits direct comparison with the present 24 h treatment, the absence of a relevant cytotoxic effect after the longer exposure period used here further supports the tolerability of the developed wafer extracts.

From a translational perspective, the combination of antimicrobial activity and cellular cytocompatibility is particularly relevant for a locally applied formulation. Prolonged local release may help maintain drug availability and could be advantageous for preventing microbial persistence and recolonization. Advanced delivery platforms have similarly been proposed as enabling strategies for localized biofilm control by combining controlled release and enhanced local exposure (Costea et al., 2026; Grari et al., 2025). Further studies using established biofilms and dynamic oral models would be required to determine whether the observed release profile translates into prolonged antibiofilm activity under physiologically relevant conditions.

3.15. Short term stability of wafers

Short-term stability studies demonstrated that CUR could be reliably quantified in CH:HPMC_{low} wafers over the evaluated period, despite non-ideal storage conditions. Visually, the wafers remained intact, demoldable, homogeneous, and showed no detectable color changes after 2 and 4 months of storage, indicating preserved macroscopic integrity.

Efficient CUR recovery was achieved using magnetic stirring extraction in an acetic acid/methanol mixture, a strategy widely reported to enhance mass transfer and disrupt weak intermolecular interactions with poorly water-soluble compounds such as CUR (Wichitnithad et al., 2009). CUR content remained essentially unchanged during storage (Online Resource, Table S5), corresponding to 100.32% and 98.31% of the initial value after 2 and 4 months, respectively. The quantity observed after 2 months falls within the expected

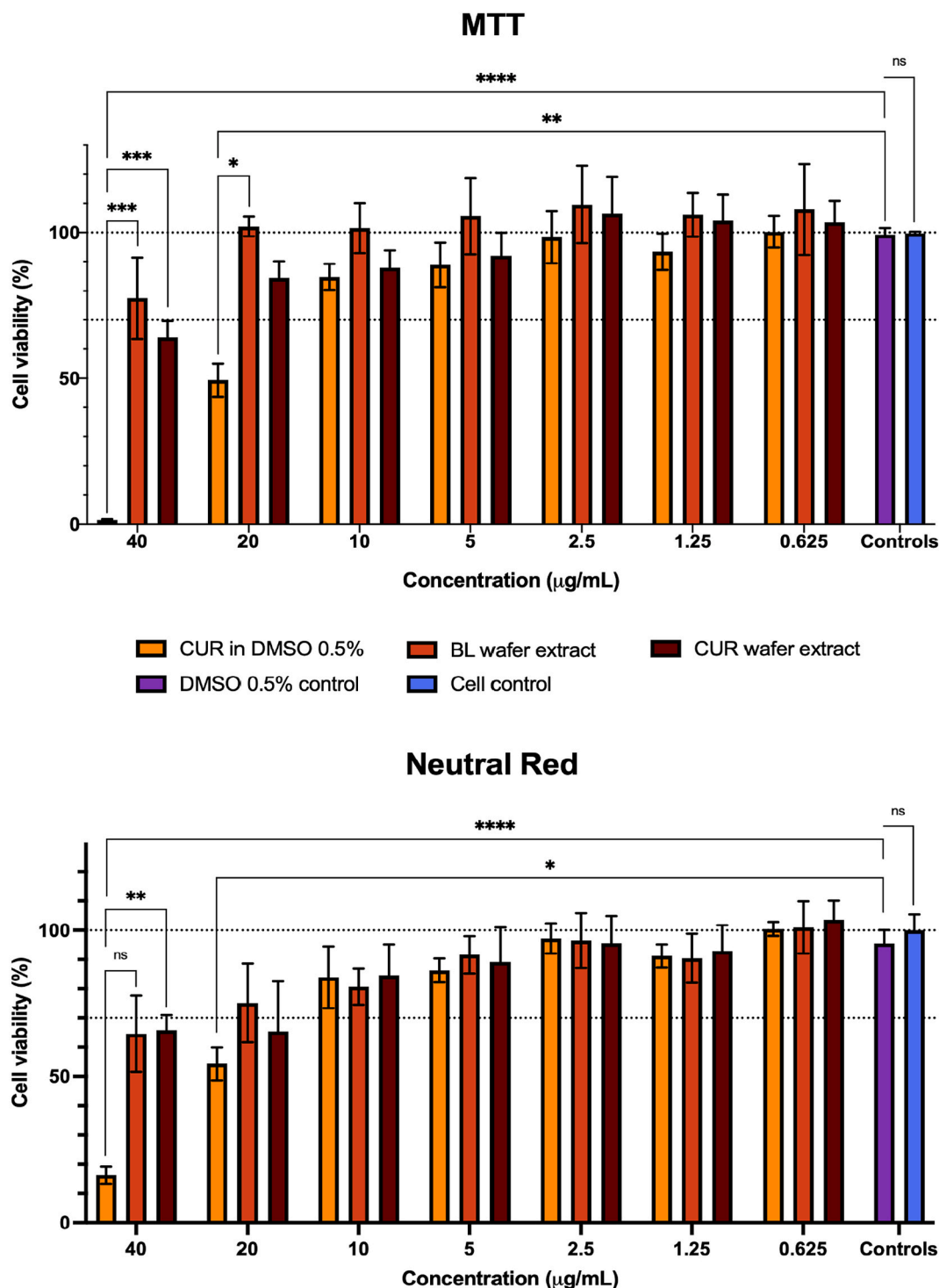


Fig. 8. Cell viability of the TR146 cell line determined by MTT and Neutral Red (NR) assays. Data are expressed as mean $\pm$ standard error ($n = 6$). One-way ANOVA with Tukey's post hoc test was used for statistical analysis to compare samples against their respective controls and between treatment groups at matched concentrations (free CUR versus extracts). For MTT assay (left to right, bottom to up), *** $P = 0.0002$; *** $P = 0.0002$; * $P = 0.0405$; ** $P = 0.0055$; **** $P < 0.0001$; ns, not significant > 0.9999 . For Neutral Red assay: ns = 0.1645; ** $P = 0.0094$; * $P = 0.0254$; **** $P < 0.0001$; ns > 0.9999 . Untreated cell controls (complete DMEM) and vehicle controls (0.5% DMSO) showed viabilities of $99.75 \pm 0.51\%$ and $99.20 \pm 2.36\%$ for the MTT assay; and $100.00 \pm 5.51\%$ and $95.56 \pm 4.50\%$ for the NR assay, respectively. None of the extracts presented a significant difference in comparison to the untreated control, as well as when comparing BL versus CUR extracts at the same concentration.

experimental variability associated with the manufacturing and analytical procedures and does not indicate an actual increase in CUR content.

Residual moisture increased to $7.72 \pm 0.47\%$ after 2 months and $8.06 \pm 0.37\%$ after 4 months, exceeding the commonly accepted

threshold for freeze-dried systems (around 5%). This increase is attributed to the highly porous structure of the wafers, which readily absorbs environmental moisture, as previously evidenced by the rapid rehydration behavior observed in TD-NMR analyses. Nevertheless, no visual deterioration or significant loss of CUR content was observed.

Considering the hygroscopic nature of the formulation, future studies should investigate moisture-protective packaging, such as aluminum blisters, together with long-term and accelerated stability studies under ICH-recommended conditions, including room temperature, to establish product shelf life and support routine distribution. Taken together, the CH:HPMC_{low} wafer demonstrated satisfactory physicochemical stability over four months under refrigeration, supporting its potential as a robust buccal nano-in-matrix delivery platform.

4. Conclusion

Lyophilized buccal wafers based on chitosan and cellulose derivatives were successfully developed as a nano-in-matrix platform for local CUR delivery. The incorporation of a CUR-loaded nanoemulsion improved the aqueous dispersibility of CUR without compromising matrix integrity, while the combination of CH with HPMC_{low} provided a balanced profile of mucoadhesion, mechanical strength, and sustained active ingredient release under biorelevant sub-sink conditions, while preserving antimicrobial activity against key oral pathogens. Importantly, the developed wafers showed antimicrobial activity against oral bacteria and opportunistic pathogens, whereas their extracts revealed cytocompatibility in TR146 cells at all tested concentrations. Despite increased water uptake, the optimized wafers remained physically intact during 4 months of storage, retaining 98.31% of their initial CUR content. In light of these findings, the optimized wafer emerges as a promising non-antibiotic strategy for further studies aiming localized management of oral infections, particularly post-surgery recovery, where mucoadhesive patches with sustained drug exposure can accelerate healing and inhibit local infection spread.

CRediT authorship contribution statement

Elina Sawa Akioka Ishikawa: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Maria Teresa Iorio de Moraes:** Writing – review & editing, Investigation. **Victória Soares Soeiro:** Writing – review & editing, Investigation, Formal analysis. **Guilherme Henrique Vedovello Silva:** Writing – review & editing, Investigation, Formal analysis. **Edvaldo Sabadini:** Writing – review & editing. **Michelle Franz-Montan:** Writing – review & editing, Methodology. **David Wynne Williams:** Writing – review & editing, Resources, Methodology. **Karina Cogo-Müller:** Writing – review & editing, Resources, Methodology. **Laura de Oliveira-Nascimento:** Writing – review & editing, Visualization, Supervision, Resources, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Maria Teresa Iorio de Moraes reports financial support was provided by National Council for Scientific and Technological Development. Victoria Soares Soeiro reports financial support was provided by State of Sao Paulo Research Foundation. Elina Sawa Akioka Ishikawa reports financial support was provided by Coordination for the improvement of Higher Education Personnel. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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UNICAMP). Curcumin C3 Complex® was kindly donated by Sabinsa Brasil; contains minimum 95% curcuminoids.

Consent for publication

All authors agreed with the content of this manuscript and approved the submitted version.

Ethics approval

This study was exempt from approval by the Research Ethics Committee (CEP/CONEP system) of the Universidade Estadual de Campinas (UNICAMP), as stated in the Official Letter CEP No. 099/2024. The exemption was granted because the experimental work was limited to *in vitro* and *ex vivo* assays using commercially available porcine buccal tissue, as well as commercial microbial strains, with no involvement of human participants, human biological samples, or live animals. Clinical trial number: not applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijpharm.2026.127396>.

Data availability

Data will be made available on request.

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