Development of mucoadhesive vaginal films for metronidazole delivery Using methacryloylated, crotonoylated, and itaconoylated gelatin blends with poly(vinyl alcohol)

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RESEARCH ARTICLE

Development of Mucoadhesive Vaginal Films for Metronidazole Delivery Using Methacryloylated, Crotonoylated, and Itaconoylated Gelatin Blends with Poly(vinyl alcohol)

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Abstract

Purpose This work reports the development and characterisation of polymeric films composed of gelatin or its chemically modified derivatives (crotonoylated, itaconoylated, and methacryloylated gelatins) blended with polyvinyl alcohol (PVA). Metronidazole served as an antimicrobial drug in these formulations.

Methods The films were produced by casting aqueous solutions of polymers, followed by solvent evaporation. Their structure and physicochemical characteristics were studied using Fourier transform infrared spectroscopy, scanning electron microscopy, and mechanical testing. The thickness of the films, their folding endurance, the surface pH, and transparency were also evaluated. The mucoadhesive performance of the films was evaluated through an *ex vivo* detachment technique involving freshly excised sheep vaginal tissues. *In vitro* cumulative drug release studies were conducted using Franz diffusion cells.

Results The results demonstrate that incorporating unsaturated functional groups into gelatin improves its mucoadhesive properties compared to native gelatin. The drug release experiments conducted *in vitro* showed that the cumulative release from pure gelatin/PVA films was found to be 49 ± 2%, whereas modified gelatins/PVA (70:30) films released ~ 64–71%.

Conclusion These findings suggest that modified gelatins could serve as effective excipients in designing mucoadhesive formulations for vaginal administration, with potential applications extending to other transmucosal drug delivery systems.

Keywords crotonoylated gelatin · itaconoylated gelatin · methacryloylated gelatin · mucoadhesion · vaginal films

Introduction

Water-soluble polymers, both of charged and neutral nature, have attracted significant interest in drug delivery applications [1]. Natural polymers, known for their biodegradability, biocompatibility, and non-toxicity, have gained particular interest as materials for mucoadhesive applications. They are produced through environmentally friendly and cost-effective processes and are easily amenable to chemical modification. Mucoadhesive materials have shown great potential in enhancing drug bioavailability and retention at mucosal surfaces, making them valuable in drug delivery systems [2]. However, natural polymers also face some limitations, such as their potential biological contamination (e.g. microbial contamination or organic materials), batch-to-batch variability, and challenges associated with isolation and purification processes [3, 4]. Alginates, chitosan, and guar gum are examples of natural polymers with good mucoadhesive properties [5].

Gelatin, a water-soluble polyampholyte biopolymer produced from animal skin and bones [6, 7], is distinguished by its biocompatibility, biodegradability, non-immunogenicity and non-toxicity. These attributes make it a compelling candidate for tissue engineering, wound healing, and developing adhesive biomaterials [8–11]. Moreover, gelatin has been classified as a GRAS (Generally recognised as safe) biopolymer by the United States

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Food and Drug Administration (FDA) [12]. However, gelatin exhibits weak mucoadhesive properties [13–15]. Consequently, enhancing gelatin's bio/mucoadhesive properties through chemical modifications is becoming an area of significant interest. These modifications could provide stronger adhesion to mucosal surfaces by forming covalent linkages with cysteine-containing subdomains of mucin, potentially leading to prolonged contact with mucosal membranes with enhanced drug absorption and reduced dosing intervals [16, 17].

Polymeric films have widely been studied in drug delivery applications owing to their potential to offer controlled release and effective adhesion to mucosal tissues [18]. Particularly, gelatin has been extensively studied as a component of film formulations both individually and in combination with other natural polymers [19]. However, gelatin-based films are usually brittle, have relatively weak mechanical properties, and are sensitive to temperature and relative humidity [20]. Mixing gelatin with synthetic polymers is considered as a potential solution to enhance the mechanical properties of these materials [21].

Polyvinyl alcohol (PVA) is a synthetic water-soluble polymer known for its excellent mechanical strength [20], good hydrophilicity, thermal stability, and biocompatibility. Notably, PVA is among the few synthetic polymers that fully degrade under both aerobic and anaerobic environments [22] and it offers a cost advantage over other synthetic polymers [23]. These characteristics make PVA an excellent material in biomedical applications [24]. However, as a non-ionic water-soluble polymer, PVA exhibits weak mucoadhesive properties [25].

Blending gelatin with PVA has the potential to improve the mechanical characteristics of the resulting films [26–29]. However, despite improvement in the physicochemical characteristics of gelatin-PVA blends, these films are still expected to demonstrate limited mucoadhesive properties similar to their weakly mucoadhesive parent materials.

Previously, Bianco-Peled and colleagues [30–32] reported the use of acryloylated polymers as a novel group of pharmaceutical materials that exhibit superior mucoadhesive properties compared to their underivatised counterparts. Later we also demonstrated similar improvements in the mucoadhesive performance of various polymers (such as chitosan, gellan gum, polyoxazolines, etc.) by functionalising them with methacryloyl groups [16, 33, 34]. In our latest study, we further showed that the chemical modification of gelatin with crotonic, itaconic and methacrylic anhydrides can substantially improve the mucoadhesive properties of this biopolymer [35]. This enhancement results from the ability of unsaturated functional groups to form covalent linkages with thiols found on mucosal surfaces through Michael-type addition reactions driven by thiol-ene click mechanisms that occur under physiological conditions.

In this study, polymeric films were prepared by blending either gelatin or its chemically modified derivatives (Gel-CA, Gel-IA, and Gel-MA) with PVA in varying ratios through casting aqueous polymer solutions. The physicochemical properties of these blends were studied using Fourier transform infrared (FTIR) spectroscopy, mechanical testing, and scanning electron microscopy (SEM). These films were employed in the formulation of metronidazole for vaginal drug administration. The cumulative drug release from the polymeric films was examined *in vitro*. The mucoadhesive properties of these films were evaluated using a tensile (detachment) test involving *ex vivo* sheep vaginal tissues. Films were chosen over other solid dosage forms, such as tablets, due to their flexibility, thin structure, better comfort, faster rehydration, improved adhesion to the mucosa and drug release at the side of application, which enhance the therapeutic efficacy of vaginal route of drug administration.

Materials and Methods

Materials

Type A gelatin sourced from porcine skin (gel strength ~175 g Bloom), polyvinyl alcohol (PVA, Mw 31,000–50,000, 98–99% hydrolysed), and metronidazole were purchased from Sigma-Aldrich (Gillingham, UK). Phosphate-buffered saline (PBS) tablets were purchased from Fisher Scientific (Loughborough, UK). All other chemicals were of analytical grade and were used without further modification.

Methods

Chemical Modification of Gelatin

Crotonoylated (Gel-CA), itaconoylated (Gel-IA), and methacryloylated (Gel-MA) gelatins were synthesised following the protocol described in our previous report [35]. Briefly, 0.5 g of gelatin was dissolved in 100 mL of PBS (pH 7.40) at 50 °C until formation of a clear solution. Then, specific quantities of crotonic (0.2 mL; 1.350 mmol), itaconic (0.2 mL; 2.248 mmol), or methacrylic (0.2 mL; 1.343 mmol) anhydrides were added. Unsaturated anhydrides were added at their highest molar excess relative to the available free amino groups (0.434 mmol) per gram of gelatin, as specified in the reaction protocol. The reaction mixture was maintained at 50 °C under constant stirring for 12 h, with the pH adjusting to 8.50 using 4 M NaOH solution. The reaction was quenched by adding 50 mL of PBS. The resulting products were subsequently purified using dialysis against deionised water (5 L with 8 water changes) for 48 h in the dark. Then, the products were lyophilised and stored in a

freezer (Figure S1A in Supporting Information displays the reaction scheme).

The modification of gelatin was confirmed using ^{1}H NMR spectroscopy, Briefly, 20 mg/mL of gelatin and its chemically modified derivatives were dissolved in warm D_2O. ^{1}H NMR spectra of samples were recorded using a 500 MHz Bruker Avance III NMR spectrometer (Coventry, UK) at 37 °C with 128 scans per spectrum (see Figure S1B in Supporting Information). The quantification of the degree of functionalisation (DoF) of gelatin derivatives was done similarly as described in our previous study [35]. The percentage of incorporated unsaturated functional groups was calculated by comparing the integrated intensities of the double bond peaks to those of the aromatic residues present in gelatin side chains. The data on calculated DoF are presented in Table S1 in Supporting Information.

Preparation of Films

Solutions of gelatin and its derivatives (Gel-MA_6, Gel-CA_6, Gel-IA_{10}) with 5.0% (w/v) were prepared by dispersing the polymers in deionised water while stirred at 50 °C until fully dissolved. Separately, a 1.0% (w/v) PVA solution was prepared in deionised water, maintaining the temperature at 80–85 °C and stirring continuously until complete dissolution. 100 mg/mL metronidazole (MTZ) solution was prepared by dissolving the drug powder in deionised water.

Polymeric films without MTZ were prepared by mixing the polymer solutions in the following volume ratios: each gelatin/PVA, Gel-CA_6/PVA, Gel-IA_{10}/PVA, and Gel-MA_6/PVA at 90:10, 70:30, and 50:50% v/v. Additionally, control films were cast from individual polymer solutions (% v/v): gelatin (100), Gel-CA_6 (100), Gel-IA_{10} (100), Gel-MA_6 (100), and PVA (100). The polymer mixtures (20 mL) were then poured into Ø 40 mm Petri dishes and left to air-dry until a constant weight was achieved.

The films composed of blends of either gelatin or its derivatives with PVA and containing MTZ were prepared using the solvent evaporation method as described above. Briefly, a 1.0 mL solution of MTZ (100 mg/mL) was added to the polymer blend and stirred for 2 h to ensure uniform drug distribution. The resulting mixture (20 mL) was poured into Ø 40 mm Petri dishes and dried in air to a constant weight. All films with MTZ were prepared at 70:30 (% v/v) volume ratio.

Fourier Transform Infrared (FTIR) Spectroscopy

A Nicolet iS10 spectrometer (Thermo Scientific, UK) equipped with an iTX ATR accessory and a diamond crystal was used to record FTIR spectra. The spectra were recorded by averaging 16 scans between 4000 and 500 cm^{-1} range with a resolution of 4 cm^{-1} in the transmittance mode.

Transparency

An Analytik Jena Specord® 210 Plus UV/Vis spectrophotometer (Jena, Germany) was used to assess the transparency of the polymeric films by determining the light transmittance for each 1 × 4 cm strip of the film sample at λ 400 nm, with air serving as the reference.

Surface pH Measurements

Each film with 10 mm diameter discs were placed in plastic Petri dishes (Ø 40 mm). The films were then allowed to swell in contact with 1.5 mL of deionised water at room temperature for 30 min. The surface pH was determined using a LE438 electrode connected to a FiveEasy F20 Mettler-Toledo pH meter (Switzerland), which was positioned on the surface of the swollen films. Each sample was measured in triplicate, and the mean values with standard deviations were calculated.

Scanning Electron Microscope (SEM)

SEM imaging used a ZEISS Crossbeam 540 scanning electron microscope (Carl Zeiss Micrography GmbH, Jena, Germany) with an acceleration voltage set to 5 kV. The fracture surfaces (cross-sections) of polymeric films were photographed after freezing the materials in liquid nitrogen and sputter-coating with a gold layer.

Mechanical Testing

Mechanical testing of films was conducted to measure puncture strength (PS) and elongation at break (EB) using a TA.XT Plus Texture Analyser (Stable Micro Systems Ltd, UK) operating in compression mode. Film samples (30 × 30 mm) were used for the tests. A digital calliper was used to evaluate the thickness of the films [36], with six measurements taken at different locations on each sample to calculate the mean values. The thickness of the films was about 0.51 ± 0.04 mm. Each film sample was secured with screws between two plates with a cylindrical hole of Ø 10 mm (sample holder area: $Ar_s = 78.54 mm^2$), and compressed with a 5.0 mm diameter spherical stainless steel ball probe (P/5S) moving at a rate of 1.0 mm/sec. The probe moved steadily until the rupture occurred. Testing was conducted with the following settings: a test speed of 1.0 mm/sec; target mode set to distance, trigger type set to auto, and 0.049 N trigger force. The puncture strength values were calculated using the following equation:

$$PS = \frac{F}{Ar_s} \quad (1)$$

where F represents the maximum applied force recorded during strain.

Elongation at break (EB) is defined as the ratio of the film's extension at rupture to its initial length, expressed in percentage [37]:

$$EB = \left(\frac{\sqrt{a'^2 + b^2} + r}{a} - 1 \right) \times 100\% \quad (2)$$

where a' is the original length of the film sample unaffected by the probe; b is the vertical displacement (depth of penetration) caused by the probe; r is the radius of the probe; a is the radius of the film within the sample holder opening.

Each experiment was carried out in triplicate, and the mean values ± standard deviations were calculated and evaluated statistically.

In Vitro Study of Drug Release

The release kinetics of metronidazole (MTZ) from films formulated with gelatin and its derivatives, as well as their blends with PVA, were studied using a Franz diffusion cell (FDC) maintained in a water bath at 37 °C [38]. The experiment was conducted in a phosphate-buffered saline (PBS, pH 6.50). A cellulose membrane (12–14 kDa) was placed between the donor and acceptor compartments of the FDC. The acceptor chamber of the FDC contained 32 mL of buffer solution and stirred at 100 rpm. Polymeric film samples were placed in the donor compartment of the diffusion cell on a dialysis membrane. At predetermined time intervals over a 10-h period, 1.0 mL aliquots were aspirated from the acceptor compartment. Following each withdrawal, 1.0 mL of fresh PBS was replaced to keep a constant volume. PBS solution was kept at 37 °C throughout drug release studies in a water bath.

The drug released at each time interval was evaluated spectrophotometrically with a UV/Vis spectrophotometer (Analytic Jena Specord® 200 Plus, Jena, Germany) at λ = 320 nm. The percentage of MTZ released was evaluated with a standard calibration curve (Figure S2 in Supporting Information).

Ex Vivo Mucoadhesion Studies

Adhesion of the polymeric films to the mucosal surface was assessed using a detachment (tensile) method with a TA.XT Plus Texture Analyser (Stable Micro Systems Ltd, UK) with a cylindrical aluminium probe P/25 (25 mm in diameter) [25, 39]. Freshly excised sheep vaginal tissues were acquired from Altyn-Orda Abattoirs (Almaty, Kazakhstan) following animal slaughter and used within 24 h. The vaginal tissue sample, with the mucosal side facing downward, was attached to a cylindrical probe using double-sided adhesive tape. The probe was then mounted onto the mobile arm of the texture analyser. Another vaginal tissue sample, with the mucosal side facing upward, was firmly secured to the mucoadhesion rig. Before commencing each test, the mucosal surfaces were hydrated with vaginal fluid simulant (VFS, pH 4.0). Spherically shaped (Ø 4 cm) polymeric film samples were quickly immersed in VFS solution before being placed onto the surface of the vaginal tissue, which was affixed on the mucoadhesion rig. The testing parameters were set as follows: a testing speed of 0.5 mm/sec and a contact time of 30 sec. The recipe to prepare VFS (pH 4.0) is described in Table S2 in the Supporting Information [40]. VFS solution was kept at 37 °C throughout mucoadhesion studies using a water bath. Data obtained from these experiments were then used to evaluate the mucoadhesive performance of the polymeric films, specifically the force of mucoadhesion (F_M; the maximum force required to achieve detachment) and the total work of adhesion (W_{ADH}; the area under the force/distance curve) values. All experiments were carried out at 37 °C with 100% relative humidity. Each measurement was performed 3 times, and the results were expressed as mean ± standard deviations.

Statistical Analysis

All experiments were conducted at least 3 times, and the results are shown as mean ± standard deviation. Statistical analysis was done using GraphPad Prism software (version 8.0; San Diego, USA). Data were evaluated through a two-tailed Student's *t*-test and one-way analysis of variance (ANOVA), followed by Bonferroni *post hoc* testing, where the level of $p \leq 0.05$ was set as a criterion of statistical significance.

Results and Discussion

Preparation and Characterisation of Films

Polymeric films based on gelatin and its derivatives (Gel-MA, Gel-CA, Gel-IA), as well as their blends with PVA in various ratios, were prepared using the casting method from aqueous polymer solutions. The resulting polymeric films based on gelatin and Gel-MA_6, Gel-CA_6, Gel-IA_{10} blended with PVA demonstrate good homogeneity with no microcracks or tears, as well as adequate elasticity and satisfactory flexural strength. The flexibility of polymeric materials is crucial for films intended for intravaginal administration, as they must maintain integrity without breaking. An established approach for evaluating the flexibility of films involves measuring their folding endurance, which refers to the maximum number of consecutive folds a sample can withstand at the same location without experiencing fracture. According to Tighsazzadeh *et al.* [36, 41], films demonstrating folding endurance values exceeding 300 are classified as

having good flexibility. All our polymeric films withstood more than 300 bends while remaining intact. All films had thicknesses ~ 0.51 ± 0.04 mm. The measurements of the surface pH of the hydrated/swollen films revealed that these materials exhibit a weakly acidic to near-neutral pH. Specifically, drug-free films showed a surface pH ~ 6.20–6.52, while drug-loaded films exhibited slightly lower values (pH ~ 6.05–6.20). The data on surface pH measurement of each polymeric film are presented in Table S3 in Supporting Information. The films are likely to be pH-compatible for vaginal administration. They should not significantly alter the vaginal pH, particularly in cases where the pH is already elevated due to pathological conditions (bacterial vaginosis or trichomoniasis). However, in a healthy vaginal environment (pH is typically between 3.80–4.50 and maintained by lactic acid from resident microbiota), while a slight transient increase in pH may occur immediately post-administration, the natural regulatory mechanisms of the vaginal microbiota are expected to restore the pH to its physiological range without exacerbating the condition.

Gelatin, as a biopolymer composed of various amino acids, has a great potential to form miscible blends with polymers bearing carboxylic, phenol, or alcohol hydroxyl groups [6, 7]. Gelatin has numerous carbonyl groups, known for their proton-accepting properties, forming intermolecular hydrogen bonding with proton-donating hydroxyl groups of PVA. To establish the possibility of hydrogen bonding in blend films of gelatin/PVA and gelatin derivatives/PVA, all samples were characterised using FTIR spectroscopy (Fig. 1).

All samples exhibited similar absorption bands in the range of 3000–3600 cm^{-1}, attributed to the stretching vibrations of the –OH groups. The broad peak observed around 3290 cm^{-1} in all spectra is related to the stretching vibrations of –OH and N–H groups [42]. Additionally, the peaks at 2932 cm^{-1} and 2942 cm^{-1} were identified in all analysed films, corresponding to –CH and $–CH_2$ groups stretching vibrations.

The FTIR spectrum of pure gelatin is characterised by three distinctive absorption bands: amide I, amide II, and amide III [43, 44]. The amide I band appeared at 1628 cm^{-1} is associated with C = O stretching vibrations; the amide II band peak observed at 1543 cm^{-1} is assigned to N–H bending plus C–H stretching vibrations; and the amide III band peak at 1235 cm^{-1} corresponds to the in-plane C–N stretching coupled to N–H bending vibrations, including the vibrations of CH_2 groups from glycine [45], an amino acid abundantly present in gelatin, are in good agreement with the previous studies [19, 46, 47].

In the FTIR spectra of gelatin/PVA, Gel-CA_6/PVA, Gel-IA_{10}/PVA, and Gel-MA_6/PVA films, shifts in the absorption bands corresponding to amide I, amide II, and amide III were observed: amide I at 1631 and 1647 cm^{-1}, amide II at 1546 cm^{-1}, and amide III at 1236 and 1245 cm^{-1}. These shifts indicate the formation of hydrogen bonds and intermolecular interactions between gelatin and its derivatives with PVA, suggesting good compatibility between the polymers. Peaks at 1713 and 1372 cm^{-1} are characteristic of PVA. The peak at 1713 cm^{-1} is due to the stretching C = O and C–O from acetate group remaining in PVA [48]. Absorption bands near 1416 and 1239 cm^{-1} are due to O–H deformation and C–O–C stretching vibrations, respectively [49, 50]. The spectra of polymer blends exhibit all the characteristic absorption bands of their components, with the intensity and shape of these bands dependent on the polymer ratios within the mixture.

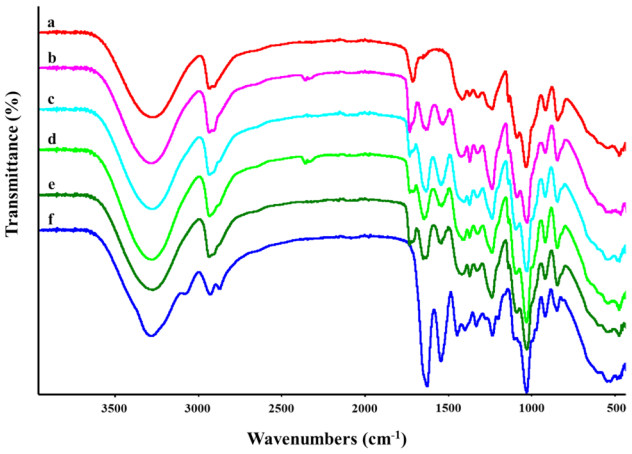

Fig. 1 FTIR spectra of polymer blend films (% v/v): pure PVA 100 (**a**), Gel-CA_6/PVA 70:30 (**b**), Gel-IA_{10}/PVA 70:30 (**c**), Gel-MA_6/PVA 70:30 (**d**), gelatin/PVA 70:30 (**e**), and native gelatin 100 (**f**). Gel-CA – crotonoylated gelatin; Gel-IA – itaconoylated gelatin; Gel-MA – methacryloylated gelatin

All the films without drug were homogeneous, transparent and smooth exhibiting complete miscibility of native gelatin and its modified derivatives with PVA. However, the films containing MTZ were slightly opaque.

The evaluation of film transparency was conducted by measuring light transmittance at 400 nm using a spectrophotometric technique. All drug-free films showed light transmission values greater than 85%, confirming their good transparency. On the other hand, films containing MTZ demonstrated much lower transparency, with transmission values between ~ 39% and ~ 47% at 400 nm. Inadequate transparency of films could be a serious drawback in some applications, particularly in ophthalmic drug delivery, where reduced optical clarity could impair vision. Unlike other routes of administration, vaginal delivery does not require dosage forms to exhibit a particular level of transparency. The observed reduction in transparency in drug-containing films indicates that MTZ content exceeds its solubility threshold within these polymers in the solid state, leading

to partial crystallisation. Table I presents the data on the transparency of modified or unmodified gelatin/PVA blend films with varying ratios.

The morphological characterisation of the specimens was conducted with scanning electron microscopy (SEM). The SEM microphotographs are depicted in Fig. 2. Additional SEM images of the surface of gelatin and its modified derivative films blended with PVA are illustrated in Figure S3 in Supporting Information. The cross-sectional morphological analysis demonstrated a structurally uniform composition, without observable phase separation or distinct boundary formation. These results demonstrate a high level of compatibility between gelatin and its derivatives with PVA in the solid state at various component ratios.

Generally, films based on pure PVA exhibit great puncture resistance and tensile strength due to their good film-forming ability [51, 52]. In this work, films formulated with individual polymers, i.e. gelatin and its chemically modified derivatives exhibited comparatively lower strength values, characterised by pronounced brittleness and reduced elasticity. The mechanical characteristics of the polymeric films were assessed using a TA.XT Plus Texture Analyser at room temperature (Fig. 3). The analysis of the mechanical properties of films based on gelatin and its derivatives blended with PVA, revealed that increasing the PVA content in the film samples results in a gradual improvement in both puncture strength and flexibility (elongation), specifically, due to increased intermacromolecular interaction within the polymer matrices. This effect is particularly evident in the enhanced elongation at break observed in PVA-containing films, compared to films without PVA. Thus, the inclusion of PVA into the polymeric films contributes to their enhanced elasticity by acting as a plasticiser.

Drug Release from Polymeric Films

In order to assess the *in vitro* cumulative release of metronidazole (MTZ) from polymeric films comprising gelatin or its modified derivatives blended with PVA, experiments were conducted using the Franz Diffusion Cell in phosphate-buffered saline (PBS, pH 6.50) at 37 °C. MTZ is an antiprotozoal and antimicrobial agent belonging to the nitroimidazole class, commonly employed in medical practice to treat various infections caused by microorganisms such as *Trichomonas vaginalis, Entamoeba histolytica*, and *Giardia lamblia*, as well as anaerobic bacterial infections [53]. In gynaecology, it is frequently used to address conditions such as vaginal trichomoniasis, bacterial vaginosis, and other infections associated with anaerobic bacteria [54].

The physiological pH of the vaginal environment in healthy women typically varies from 3.80 to 4.50 [55]. This mildly acidic condition is regulated by lactic acid secreted by the resident microbiota. However, deviations from this pH range can occur under pathological conditions such as bacterial vaginosis, trichomoniasis, or infections by group B streptococcus and other pathogenic organisms, leading to an elevated pH [56]. To simulate a pathological vaginal environment, the release of MTZ from gelatin-based films, including derivatives and their blends with PVA was assessed at pH 6.50. Figure 4 depicts the cumulative release profile of MTZ from different polymeric films.

Table I Transparency Values of Polymeric Films with Varying Ratios of Gelatin/PVA, Gel-CA_6/PVA, Gel-IA_{10}/PVA, and Gel-MA_6/PVA

Composition (% v/v)	Transparency			
	MTZ free films			
	Gelatin/PVA	Gel-CA_6/PVA	Gel-IA_{10}/PVA	Gel-MA_6/PVA
100/0	86.6	85.2	86.1	85.7
90/10	86.9	86.1	86.8	86.4
70/30	87.9	87.2	87.5	87.2
50/50	88.1	87.8	88.2	87.3
0/100	88.9	88.1	88.6	88.2
	MTZ-loaded films			
	Gelatin/PVA	Gel-CA_6/PVA	Gel-IA_{10}/PVA	Gel-MA_6/PVA
100/0	39.1	40.3	38.5	39.7
90/10	40.3	40.8	39.4	40.3
70/30	41.4	42.2	41.3	41.5
50/50	46.3	42.8	40.9	42.8
0/100	46.9	43.4	42.7	43.2

The polymeric films cast from individual polymers at 100% v/v, including pristine gelatin, Gel-CA_6, Gel-IA_{10}, and Gel-MA_6, exhibited a slight yellowish colour, whereas the PVA film remained colourless.

Gel-CA, Gel-IA, and Gel-MA are crotonoylated, itaconoylated, and methacryloylated gelatins, respectively; PVA, polyvinyl alcohol; MTZ, metronidazole

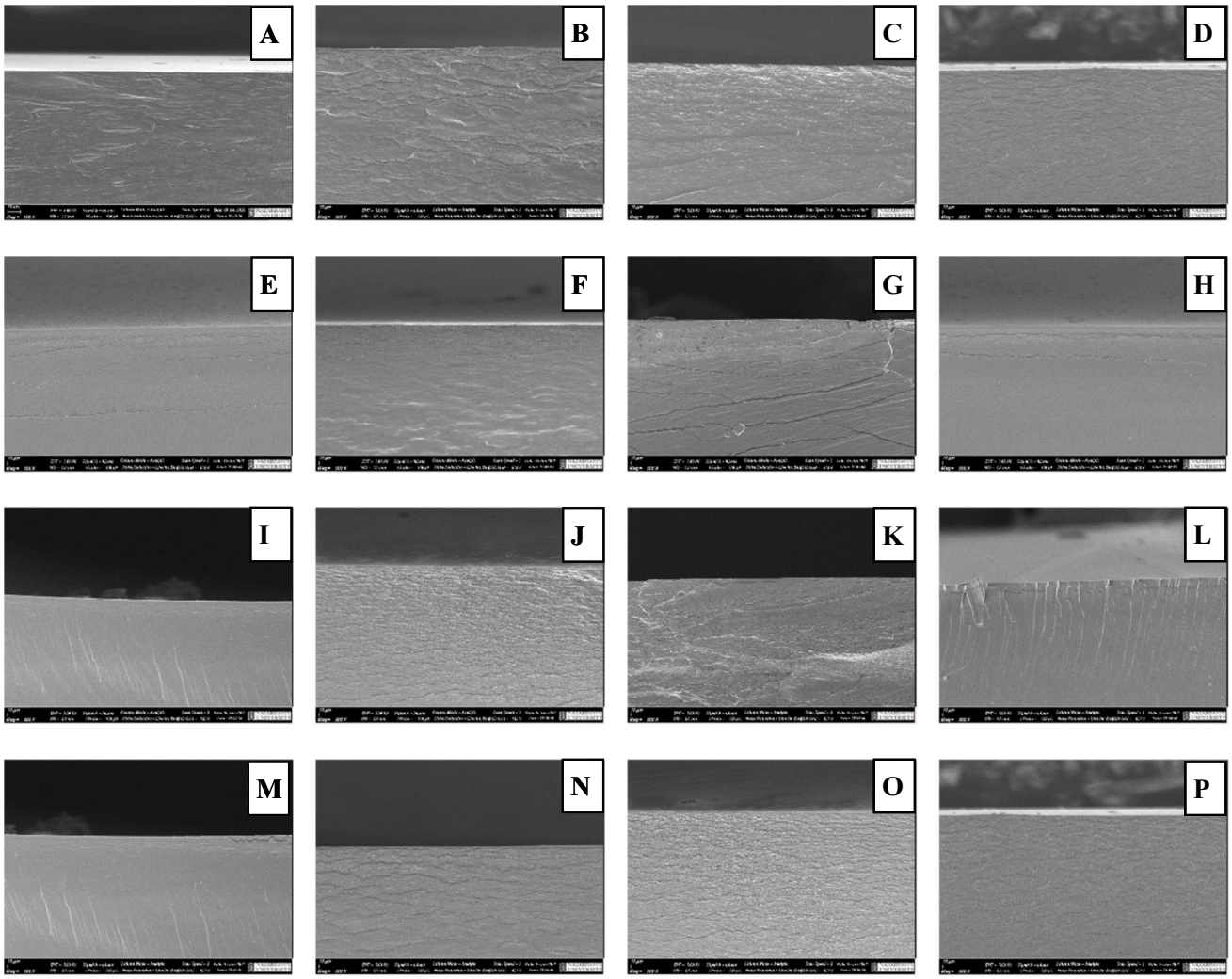

Fig. 2 SEM images of cross-sections of pristine gelatin and its methacryloylated (Gel-MA), itaconoylated (Gel-IA), and crotonoylated (Gel-CA) derivatives and their blends with PVA (% v/v). Gelatin 100 (**A**); gelatin/PVA 90:10 (**B**), 70:30 (**C**), 50:50 (**D**). Gel-MA_6 100 (**E**); Gel-MA_6/PVA 90:10 (**F**), 70:30 (**G**), 50:50 (**H**). Gel-IA_{10} 100 (**I**); Gel-IA_{10}/PVA 90:10 (**J**), 70:30 (**K**), 50:50 (**L**). Gel-CA_6 100 (**M**); Gel-CA_6/PVA 90:10 (**N**), 70:30 (**O**), 50:50 (**P**). Scale bars are 10 µm

Polymeric films composed of gelatin, Gel-MA_6, Gel-IA_{10}, and Gel-CA_6 exhibit significant hydrophilicity, resulting in the rapid release of MTZ from the polymer matrix under physiological conditions (37 °C). This property is evidenced by their high release rates, exceeding the total release of 70% in 10 h. In contrast, the films prepared by blending native gelatin, Gel-MA_6, Gel-IA_{10}, and Gel-CA_6 with PVA exhibited a slower release of MTZ, with less than 50% of the drug being released. This reduced release rate is likely attributed to slower swelling and diminished dissolution rates of the polymer matrix. This approach facilitates a more prolonged drug release, which is crucial for controlled drug delivery applications.

For all film samples, the drug release rate reaches equilibrium after 2 h. Films based on pure gelatin and its derivatives, as well as pure PVA-based films, exhibit a burst drug release within the first 2 h, achieving > 80–90% saturation within 8 h. In contrast, films comprising a mixture of PVA with gelatin and its derivatives demonstrate a more prolonged drug release profile, reaching > 70% saturation within 10 h. This extended release enhances the effectiveness of the drug and maintains therapeutically relevant concentrations within the vaginal cavity for a longer period after intravaginal administration. The slower release of MTZ from films containing a mixture of gelatin and its derivatives blended with PVA could potentially improve drug retention within the vaginal cavity.

Mucoadhesion to Ex Vivo Sheep Vaginal Mucosa

The mucoadhesive performance of polymeric films were assessed using a detachment (tensile) technique. Figure 5

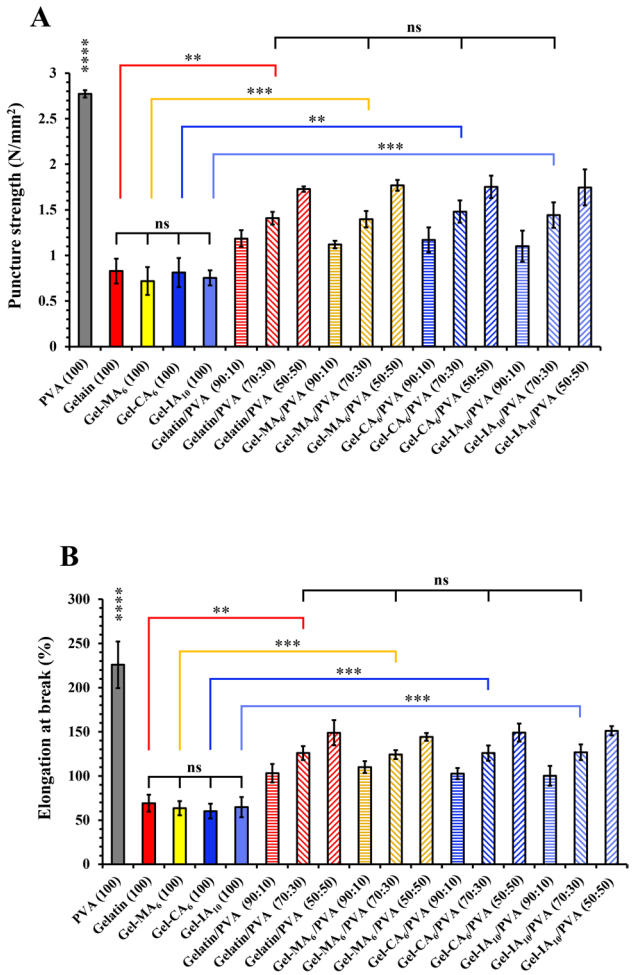

Fig. 3 Mechanical analysis of films based on gelatin and its modified derivatives blended with polyvinyl alcohol (PVA): puncture strength (**A**) and elongation at break (**B**). Gel-CA – crotonoylated gelatin; Gel-IA – itaconoylated gelatin; Gel-MA – methacryloylated gelatin. Data are presented as the mean ± standard deviation values (n = 3). Statistically significant differences are given as: * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0,001$; **** = $p < 0.0001$; ns represents no significance

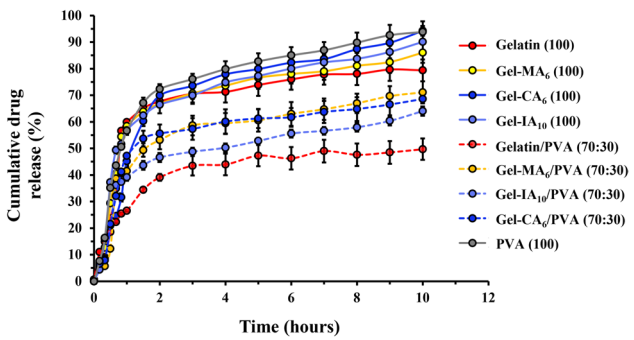

Fig. 4 *In vitro* cumulative release profiles of metronidazole from the films based on gelatin and its derivatives blended with PVA at 70:30% v/v ratio. Gel-CA – crotonoylated gelatin; Gel-IA – itaconoylated gelatin; Gel-MA – methacryloylated gelatin; PVA – polyvinyl alcohol

presents the detachment profiles of films from sheep vaginal mucosa. These measurements provided two mucoadhesive parameters, such as the force of mucoadhesion (F_M) and the total work of adhesion (W_{ADH}).

Gelatin exhibits relatively weak mucoadhesive properties compared to its derivatives ($p < 0.001$) as displayed in Fig. 5. The incorporation of unsaturated functional groups, such as crotonoyl, itaconyloyl, and methacryloyl into the gelatin structure significantly enhances the mucoadhesive properties of films based on these modified gelatins. Notably, no statistically significant differences were observed among the films composed of gelatin modified with crotonoyl (Gel-CA_6), itaconyloyl (Gel-IA_{10}), and methacryloyl (Gel-MA_6) groups, as they displayed very similar values of F_M and W_{ADH}. The enhanced adhesion to sheep vaginal mucosa observed in gelatin derivatives is due to Michael-type addition reaction of unsaturated groups in modified gelatins with the thiol groups present in mucin, resulting in the formation of stable covalent bonds. Additionally, the protonated primary amino groups in gelatin can engage in electrostatic interactions with mucins due to mild acidic pH of vaginal fluid simulant (pH 4.0) used to moisturise the mucosal surfaces.

The incorporation of polyvinyl alcohol (PVA) into gelatin-based films significantly reduces their mucoadhesive properties compared to films made from pristine gelatin and its derivatives. This reduction in adhesion force correlates with a decrease in the overall work of adhesion, highlighting the impact of PVA on the mucoadhesive effectiveness of the films. PVA as a non-ionic water-soluble polymer was expected to reduce mucoadhesive properties of these films. This study demonstrates that the derivatisation of gelatin with unsaturated functional groups substantially enhances its mucoadhesive properties. This advancement is crucial for developing more effective drug delivery systems with mucoadhesive properties, which hold significant potential for improving therapeutic interventions in gynaecology.

Conclusions

Polymeric films composed of gelatin and its derivatives (Gel-MA_6, Gel-IA_{10}, and Gel-CA_6) with PVA were produced as flexible and transparent films. Structural characterisation using FTIR spectroscopy and SEM demonstrated that the films exhibited a uniform structure without phase separation, indicating effective compatibility of the components. Analysis of mechanical properties revealed that increasing the PVA content in the films enhances both tensile strength and puncture resistance. Metronidazole-loaded films exhibited ability to swell in aqueous solutions, facilitating rapid drug release at 37 °C. However, the incorporation of PVA, served as a plasticiser, resulted in slower drug release rates, which is advantageous for prolonged drug delivery. *Ex vivo*

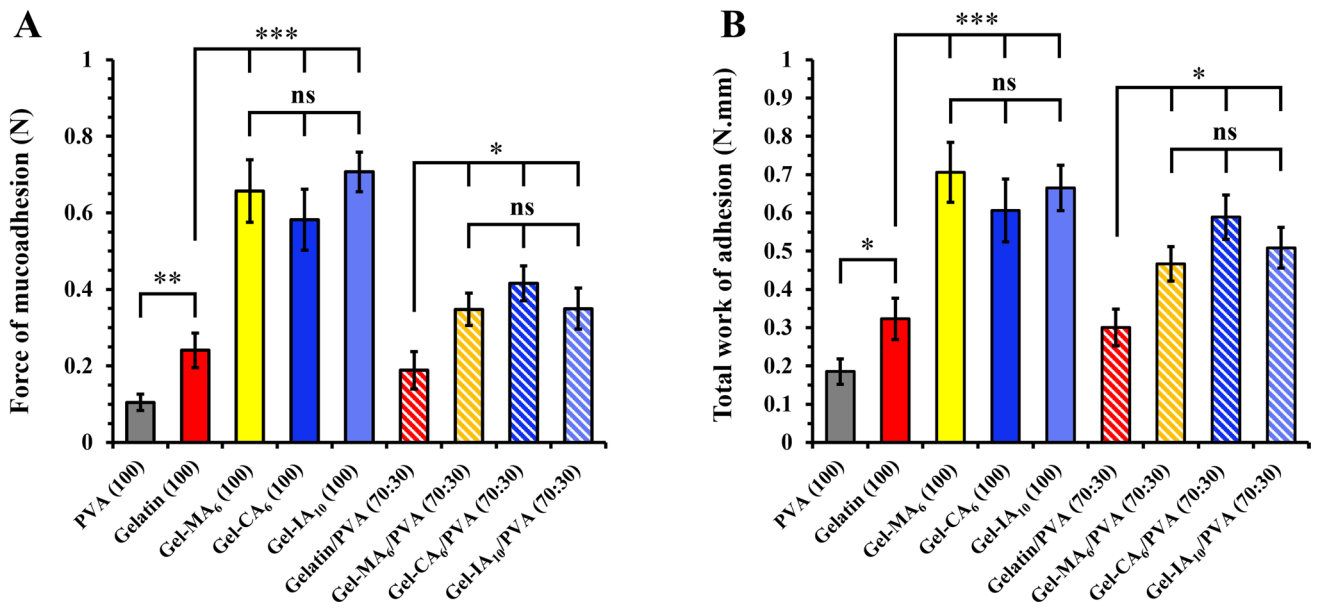

Fig. 5 Force of mucoadhesion (**A**) and the total work of adhesion (**B**) of polymeric films based on gelatin and its chemically modified derivatives, and their blends with polyvinyl alcohol (PVA) at 70:30% v/v ratio. Gel-CA – crotonoylated gelatin; Gel-IA – itaconoylated gelatin; Gel-MA – methacryloylated gelatin. Data are expressed as the mean ± standard deviation values (n = 3). Statistically significant differences are given as: * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; ns denotes no significance

mucoadhesion studies demonstrated that films made from native gelatin had reduced mucoadhesive strength. In contrast, films containing modified gelatins with unsaturated functional groups demonstrated substantially improved mucoadhesive properties. Nonetheless, the presence of PVA in the films resulted in reduced mucoadhesion, attributed to weak adhesion characteristics of PVA.

Gelatin derivatives functionalised with unsaturated groups represent promising new excipients with superior mucoadhesive properties, potentially useful for developing mucoadhesive dosage forms intended for vaginal administration. Furthermore, Gel-CA_6, Gel-IA_{10}, and Gel-MA_6 could also find applications in other fields of transmucosal drug delivery, including the formulation of gels, microspheres, or nanoparticles, etc.

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Data Availability Data is available in Supporting information.

Declarations

AI technology was used to refine language.

Conflict of Interest The authors declare no conflicts of interest in relation to the work presented in this paper.

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