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Original Article

Modified curdlan-based hydrogels containing ornidazole for vaginal delivery

Emine Saldamlı[1,2], Melike Sessevmez[2], Tilbe Çevikelli[3], Erdal Cevher[2], Genada Sinani[4]

[1]İstanbul University, Institute of Graduate Studies in Health Sciences, İstanbul, Türkiye
[2]İstanbul University, Faculty of Pharmacy, Department of Pharmaceutical Technology, İstanbul, Türkiye
[3]Bahçeşehir University, Faculty of Pharmacy, Department of Pharmaceutical Technology, İstanbul, Türkiye
[4]Altınbaş University, Faculty of Pharmacy, Department of Pharmaceutical Technology, İstanbul, Türkiye

ABSTRACT

Background and Aims: This study aimed to prepare and characterize, as well as compare the potential of biopolymer-based hydrogels for topical administration of ornidazole, a commonly used drug against vaginal infections. Hydrogels were successfully prepared using curdlan (Crd), carboxymethyl curdlan (CMCrd), hydroxypropyl methyl cellulose (HPMC), and xanthan gum (XG) as biopolymers, which were used alone or blended. In addition, carboxymethylation of Crd, a natural polysaccharide polymer that is attractive in the pharmaceutical field, was carried out in-house.

Methods: The structure of the synthesised CMCrd was analysed by Fourier-transform infrared spectroscopy(FT-IR). The physicochemical, mechanical, and mucoadhesive properties of hydrogels were evaluated, then the drug release patterns from the hydrogels were examined in a simulated vaginal environment.

Results: The hydrogels exhibited a uniform appearance and were pH-compatible with the vaginal environment. The viscosity, spreadability, and drug release characteristics were dependent on the polymer type and total amount of polymer present in the hydrogels. The texture profile analysis results indicated that all formulations exhibited appropriate mechanical characteristics (hardness, compressibility, cohesiveness, and elasticity) for vaginal administration, while also demonstrating mucoadhesive properties and good stability. Carboxymethylation improved mucoadhesion of Crd.

Conclusion: The results obtained indicate that the hydrogels developed in this study can be considered promising candidates for the local treatment of vaginal infections.

Keywords: Hydrogel, Polymer, Curdlan, Ornidazole, Vaginal infection, Texture analysis

INTRODUCTION

Vaginal infections, including those caused by yeast, bacteria, and parasites, are highly prevalent among women of reproductive age worldwide. These infections can lead to vaginitis, and delayed treatment may result in serious clinical consequences, including pelvic inflammatory disease and infertility (Palmeira-de-Oliveira R, Palmeira-de-Oliveira A & Martinez-de-Oliveira, 2015; Ravel, Moreno, & Simón, 2021). Several products formulated as solutions, semisolids, foams, and vaginal tablets are used to treat vaginal infections. However, the successful vaginal delivery of drugs either for systemic or local effects faces challenges due to several factors, including the pH of the vagina, physiological changes in the vaginal epithelium, and the presence of a mucus barrier, which can lead to poor absorption. Furthermore, the natural self-cleaning of the vagina, which acts as a defence mechanism against external pathogens, limits the contact time of locally delivered drugs at the target site. Consequently, local drug administration frequently results in a reduction in the efficacy of the treatment, a decrease in patient compliance, and the necessity for repeated administration (Swingle, Riccardi, Peranteau, & Mitchell, 2023). The design and development of patient-friendly formulations with mucoadhesive properties can overcome the problems related to the delivery of therapeutic agents *via* the vaginal route, thereby increasing treatment efficacy.

Hydrogels are widely preferred for vaginal application in the treatment or prevention of infections due to their ease of application, good spreading ability over a large surface area, and low production costs. Moreover, polymeric hydrogels exhibit mucoadhesive properties, thus allowing a higher drug retention time in vaginal tissue and possibility for controlled drug release

Corresponding Author: Genada Sinani **E-mail:** genada.sinani@altinbas.edu.tr
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(Gosecka & Gosecki, 2021). To date, a variety of natural, semi-synthetic, and synthetic biopolymers, including hydroxypropyl methylcellulose and chitosan, have been used to fabricate hydrogels containing antimicrobial drugs for vaginal administration. Biopolymers exhibit biocompatible, biodegradable, and non-toxic properties, rendering them suitable for use in drug delivery, as evidenced by numerous studies (Ahuja, Singh & Kumar,, 2013; Al-barudi, Sinani & Ulker, 2024; Osmałek et al., 2021).

In this study, polymeric hydrogels were developed for the vaginal delivery of ornidazole, an antibiotic currently used in clinical practice for the treatment of infections caused by both anaerobic bacteria and protozoa (Vaghani, Patel & Satish, 2012). Curdlan (Crd), carboxymethyl curdlan (CMCrd), hydroxypropyl methylcellulose (HPMC), and xanthan gum (XG) were used as biopolymers in the preparation of hydrogels. HPMC is a hydrophilic polymer derived from cellulose and has a wide range of applications in drug delivery, including its use in formulations to control release of drugs over extended periods of time. It is extensively studied to form hydrogel-based systems due to its gelling properties in aqueous environments. Moreover, HPMC exhibits good adhesiveness to mucosal surfaces (Caramella, Rossi, Ferrari, Bonferoni & Sandri, 2015). On the other hand, (XG) is a naturally occurring, non-toxic anionic biopolymer. This heteropolysaccharide exhibits viscous properties even at very low concentrations. Furthermore, XG exhibits high stability in acidic and alkaline environments, rendering it safe for use in pharmaceutical formulations for a variety of applications, including the formulation of vaginal hydrogels (Jadav, Pooja, Adams, & Kulhari, 2023). Crd is a biopolymer with a well-known linear (1→3)-β-glucan structure derived from *Agrobacterium* species, which exhibits unique rheological and thermal gelling properties. Several studies have demonstrated the suitability of this biopolymer for use in the food, cosmetic, and pharmaceutical industries. Furthermore, the bioactive properties of Crd, including antimicrobial and anti-inflammatory effects, have been identified (Lin et al., 2021). Nevertheless, its poor solubility in water represents a significant limitation for its utilisation, particularly in the context of drug delivery applications. Consequently, modification approaches have been employed to enhance its water solubility. The introduction of hydrophilic carboxymethyl groups into the Crd structure *via* carboxymethylation results in the formation of a Crd derivative (*i.e.* CMCrd) with good aqueous solubility. Moreover, carboxymethylation can enhance the physicochemical and bioactive properties of native Crd, making it a potentially advantageous polymer for drug delivery applications (Chen & Wang, 2020).

Hydrogels containing ornidazole were prepared using the aforementioned biopolymers in various amounts, either alone or blended. CMCrd was synthesised in-house and subjected to characterisation. The physiochemical, mechanical, and mucoadhesive properties of the hydrogels were evaluated. In addition, the drug release patterns and kinetics from hydrogels were determined. The potential of using the hydrogels of ornidazole as alternative dosage forms was discussed. To the best of our knowledge, this is the first study to evaluate the textural and mucoadhesive properties of Crd-based hydrogels in simulated vaginal fluids and, to explore their potential use in the local treatment of infections.

MATERIAL AND METHODS

Materials

Ornidazole was kindly gifted from Deva İlaç, Türkiye and HPMC (Benecel™ K4M) was gifted from Ashland, Türkiye. Crd was obtained from Kirin Company, Japan. CMCrd was synthesised in-house. XG was purchased from Jungbunzlauer, Austria. Methylparaben and propylparaben were from Doğa İlaç, Türkiye. Porcine mucin type II was obtained from Sigma-Aldrich, Germany. All other chemicals were of analytical grade.

Quantification of ornidazole

The analysis of ornidazole was carried out using high-performance liquid chromatography (HPLC), as previously described in the literature, with some modifications (Baloğlu et al., 2006). The drug quantification was performed using a C18 column with a particle size of 5 µm (VP-ODS C18, 250 mm x 4.6 mm). A mixture of phosphate buffer (pH 4.5):acetonitrile:methanol (55:15:30, v/v/v) was used as the mobile phase. The analysis was conducted at a flow rate of 1.0 mL min^{-1}, wavelength of 318 nm, oven temperature of 40°C, and injection volume of 20 µL. The correlation coefficient (r^2) was found to be 0.999 (n=3). None of the other ingredients in the formulation interfered with the ornidazole peak.

Synthesis and characterisation of carboxymethyl curdlan

The CMCrd polymer was synthesised in-house as previously described (Sessevmez, Sinani, Okyar, Alpar & Cevher, 2023). Briefly, Crd in 2-propanol was stirred at room temperature for 30 min. Sodium hydroxide solution (30% w/v) was added, stirred for a further 90 min, and sodium chloroacetate was added. After stirring at 55°C for 5 h, the product was obtained by filtration. Subsequently, the product was subjected to a series of washings with methanol:acetic acid (7:3, v/v), methanol:water (4:1, v/v), and methanol and acetone. Thereafter, the final product was dissolved in water and subjected to dialysis (12-14 kDa, Visking®, Serva, Germany) against purified water at 4°C for 4 days. CMCrd was freeze-dried and stored in a desiccator at room temperature until further use. The successful synthesis of CMCrd was confirmed by FT-IR (Spectrum 100 FT-IR, Perkin Elmer), and the spectra were recorded over a scan range of 400-4000 cm^{-1} at room temperature.

Preparation of ornidazole-containing hydrogels

Hydrogels were prepared using varying amounts of polymers, either alone or in combination, as detailed in Table 1. Firstly, CMCrd, XG, or HPMC and 150 mg ornidazole were dispersed in 10 mL of purified water. Subsequently, 20% (w/w) propylene glycol and 10% (w/w) glycerine were added under constant stirring. Finally, methylparaben (0.03% w/w) and propylparaben (0.01% w/w) were added to all the formulations. As gelling of Crd requires heating above 55°C, Crd-containing hydrogels were prepared by the same method via heat treatment (Jin, Zhang, Yin & Nishinari, 2006).

Physicochemical characterisation of hydrogels

Visual inspection

The physical appearance of each hydrogel formulation was visually observed at room temperature against a dark background, and homogeneity, transparency, clarity, and colour were assessed.

Viscosity and pH

The viscosity of the formulations was evaluated using a viscometer (Brookfield DV-II+ Pro Viscometer, USA) as specified in the manufacturer's instruction manual (Brookfield, 2015). The pH values of the hydrogels were determined using a calibrated digital pH meter (InfoLab pH 720, Germany).

Spreadability

To evaluate the spreadability of the hydrogels, 0.5 g of each formulation was placed in a pre-marked 1 cm diameter circle on a glass plate. A second glass plate was placed on top of the first, and a 500 g weight was applied for 5 min. At the end of the period, the increase in diameter resulting from the spread of the gels on the plate was determined (Bachhav & Patravale, 2009).

Content uniformity

To quantify the ornidazole content in the formulations, the hydrogels were first extracted with ethanol and then centrifuged at 4000 rpm for 20 min. The supernatant was filtered through a 0.45 µm membrane filter, and the ornidazole content of the sample was quantified by HPLC using a previously described method.

Texture profile analysis

Texture profile analysis (TPA) was performed to investigate the mechanical properties of hydrogels using Texture Analyser (Stable Micro Systems, UK) equipped with a 5-kg weighted load cell (Cevher, Taha, Orlu & Araman, 2008). Hydrogel samples from each formulation (25 g) were collected, and air bubbles were removed using an ultrasonic water bath. A cylindrical analytical probe with a diameter of 10 mm was used to compress each sample twice at a speed of 2 mm s^{-1} and at a specified depth of 15 mm, with a delay period of 15 s allowed between the two compressions. Each sample was analysed in triplicate at 37±0.5 °C. Hardness (the maximum peak force during the first compression cycle, F), compressibility (the work required to deform the gel in the first pass of the probe and calculated the value as Area 1:3), adhesiveness (the work required to overcome the attractive forces generated between the sample and the surface of the probe and calculated the value as Area 3:4), cohesiveness (the ratio of the area under the force–time curve obtained on the second compression cycle to that of the first compression cycle after a defined recovery time and calculated the value as Area 4:6/Area 1:3), and elasticity (the rate at which a deformed sample returns to its original, undeformed shape upon removal of stress and calculated the value as the ratio of the distance between anchors 4:5 divided by the distance between anchors 1:2) were determined using the force-time graphs. A typical force-time graph with the annotated properties of two-cycle texture profile analysis (TPA) is shown in Figure 1.

Mucoadhesion studies

The mucoadhesion of hydrogels was tested using TA-XT*Plus* texture analyser (Stable Micro Systems, UK) as described earlier (Cevher et al., 2008). A filter membrane disc wetted with 10 µL of 8 % (w/v %) mucin dispersion in pH 5.5 phosphate buffer saline (PBS) was attached to the probe and used as an *in vitro* simulated mucosal membrane (de Araújo et al., 2019; Şenyiğit et al., 2014). The probe was lowered onto the surface of each sample (25 mg) at a constant speed of 0.1 mm s^{-1} and contact force of 0.1 N for 120 s. The probe was then moved vertically upward at a constant speed of 0.1 mm s^{-1}. All measurements were performed at 37 ± 0.5 °C. The mucoadhesion work was determined using the equation given below. Each experiment was performed in triplicate. A typical force-time graph with the annotated properties of the mucoadhesion test is shown in Figure 2.

$$Work\ of\ mucoadhesion\left(\frac{mJ}{cm^2}\right) = \frac{AUC}{\pi r^2} \qquad (1)$$

AUC: Area under the curve between anchors 1:2

πr^2: the artificial mucosal surface in contact with hydrogel

Fourier transform infrared (FT-IR) spectroscopy

The FT-IR spectra of pure ornidazole alone and hydrogel samples containing the drug were recorded by scanning at a wavelength range of 400-4000 cm^{-1} at room temperature using a Spectrum 100 FT-IR spectrophotometer (Perkin Elmer).

Table 1. Polymeric composition of hydrogels

Formulation (w/w%)	Crd	CMCrd	HPMC	XG
Crd-1	4.25	-	-	-
Crd-2	4.5	-	-	-
Crd-H1	3.25	-	1	-
Crd-H2	3.5	-	1	-
CMCrd-H1	-	1	1	-
CMCrd-H2	-	1.5	1	-
H1	-	-	2	-
H2	-	-	3	-
H3	-	-	4	-
XG1	-	-		3.5
XG2	-	-		4
XG3	-	-		5

Note: Each hydrogel formulation contains 150 mg ornidazole, propylene glycol (20% w/w), glycerine (10% w/w), methylparaben (0.03% w/w), and propylparaben (0.01% w/w).

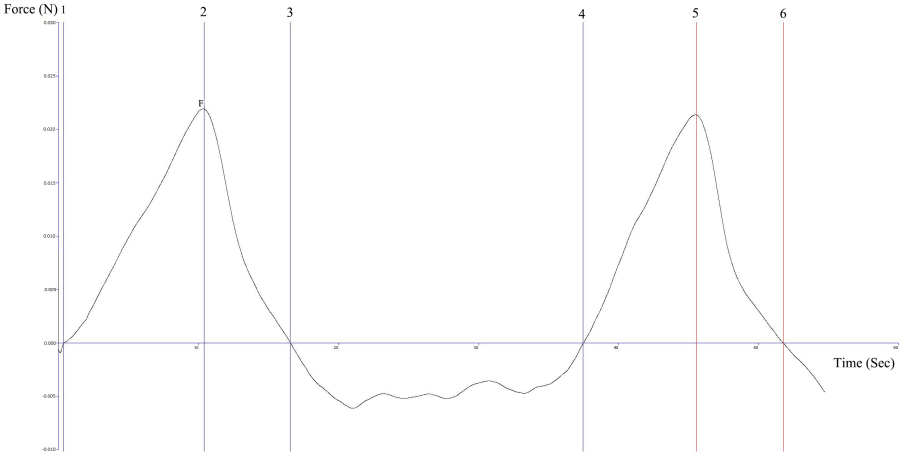

Figure 1. Typical force-time graph with annotated properties for two-cycle texture profile analysis (TPA)

Microstructure analysis

Samples of ornidazole-containing hydrogels were lyophilised using a freeze dryer (Virtis (SP Scientific, USA)), and the surfaces and cross sections of the lyophilised hydrogels and ornidazole were examined using a scanning electron microscope (Phenom ProX, Phenom-World B.V.) at 10 kV and 70 Pa.

In vitro drug release and kinetics

The drug release from hydrogels was determined by dialysis-based testing with some modifications (Enggi et al., 2021). Each formulation (1 g) was placed in dialysis tubes and subjected to drug release studies in 100 mL of pH 4.5 lactic acid buffer at 37°C to simulate vaginal fluid (Singh et al., 2017). At predetermined time intervals, 2 mL of sample was taken and replaced with an equal volume of fresh medium. The withdrawn samples were analysed by HPLC, as previously described. Studies were conducted in triplicate for each formulation.

To investigate drug release kinetics, data obtained from drug release studies were fitted to zero-order, first-order, Higuchi, and Korsmeyer-Peppas models (Sethi et al., 2020). The kinetic model was selected based on the largest r^2 value obtained in each linear regression analysis.

Short-term stability studies of hydrogels

Hydrogels were subjected to a short-term stability study at 25 ± 2°C and 65 ± 5 % relative humidity according to the

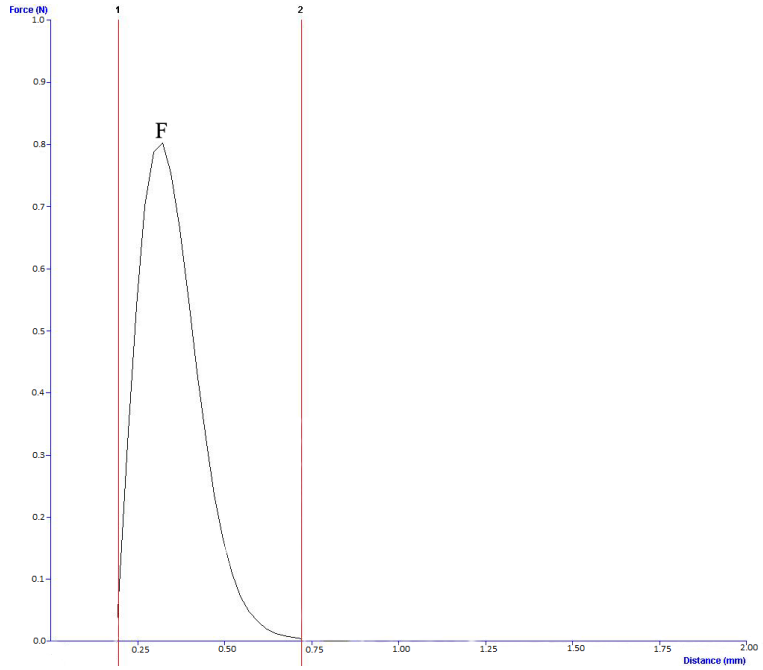

Figure 2. Typical force-time graph with the annotated properties of mucoadhesion test

storage conditions stated in the International Conference on Harmonisation (ICH) guideline (ICH Q1A (R2), 2003) for 1 month. At the end of the study, the physical appearance and drug content of the hydrogels were examined.

RESULTS AND DISCUSSION

Structural characterisation of carboxymethyl curdlan

The structure of CMCrd synthesised in-house was confirmed by FT-IR analysis (Figure 3). The spectra are dominated by a broad band of approx. 3323 cm^{-1} assigned to the stretching vibration modes of the OH groups. This band tended to shift to a higher wavenumber upon chemical modification. The peak at 1627 cm^{-1} observed in the spectrum of Crd is attributed to the presence of water in the structure of Crd (Jin et al., 2006). On the CMCrd spectrum, a new peak at 1583 cm^{-1} resulting from the stretching vibration of the carboxylate $RCOO^-$ group was observed. In particular, the absorption band at 1404 cm^{-1}, corresponding to the symmetric vibration of $RCOO^-$, became more intense after the addition of the carboxymethyl group, indicating that CMCrd was successfully synthesised. The FT-IR spectra are similar to previous reports (Jin et al., 2006; Rafigh et al., 2016). In our previous study, the carboxymethylation of Crd synthesised by the same method was confirmed by ^{13}C NMR in our previous study (Sessevmez et al., 2023).

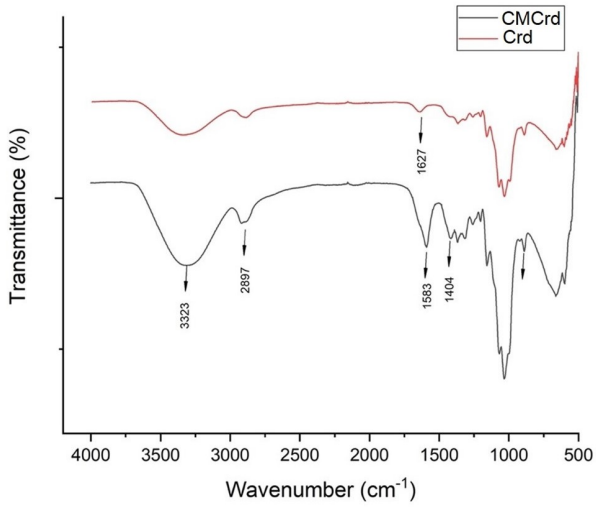

Figure 3. FT-IR spectrum of CMCrd and Crd

Preparation and physicochemical characterisation of hydrogels

Different classes of excipients, including gelling agents, humectants, and preservatives, are used in the composition of hydrogel formulations. Among them, several polymers can have function both as gelling and mucoadhesive agents. They also can prolong the retention time of hydrogels at mucosal sites (Caramella et al., 2015; Cook & Brown, 2018). In this study, hydrogels containing ornidazole as the active ingredient, propylene glycol

and glycerine as humectants, and methylparaben and propylparaben as preservatives were successfully prepared using different biopolymers from natural sources. Hydrogels comprising CMCrd, XG, and HPMC were spontaneously formed in an aqueous solution at defined concentrations given above. The carboxymethylation of Crd resulted in a notable improvement in its water solubility, enabling the successful preparation of hydrogels in purified water at room temperature. In contrast, the preparation of Crd hydrogels involved heat treatment to achieve gelation. It is acknowledged in the literature that the introduction of carboxymethyl groups into the structure of various polysaccharides can alter their physicochemical properties and consequently enhance their water solubility (Jin et al., 2006). In our previous study, we synthesised a carboxymethyl derivative of pullulan, a natural non-derivatised biodegradable polysaccharide, with the objective of enhancing its solubility in water at room temperature. It was utilised in the formulation of hybrid nanoparticles with chitosan derivatives as vaccine carriers (Sessevmez et al., 2023). Also, the carboxymethylation of the cationic polysaccharide chitosan resulted in an improvement of the polymer's water solubility and enhancement of its bioactivity. Consequently, carboxymethyl chitosan has emerged as a versatile polymer for the delivery of antimicrobial drugs (Zhang et al., 2023) and gene therapy (Sinani, Durgun, Cevher & Özsoy, 2023). In another study, carboxymethyl laminarin demonstrated enhanced *in vitro* bioactivity compared with its unmodified form, sulphated or aminated derivatives (Malyarenko, Usoltseva, Rasin & Ermakova, 2023). Thus, that the utilisation of CMCrd could be more advantageous than that of its native polymer for several drug delivery applications.

The physicochemical parameters of the prepared hydrogels, including their physical appearance, pH, viscosity, spreadability, and drug content, are presented in Table 2. The hydrogels were observed to be colourless and free of air bubbles. The XG formulations exhibited an opaque appearance, in contrast to the transparent nature of the other hydrogels. The high drug content in each formulation indicated that ornidazole was homogeneously dispersed within the hydrogels. Healthy vaginal pH is moderately acidic, with a typical range between 3.8 and 4.5, and infection can cause an imbalance in vaginal pH by increasing the pH levels (Machado, Palmeira-de-Oliveira A, Martinez-de-Oliveira & Palmeira-de-Oliveira R, 2017). Ideally, the pH of the formulation should be maintained at a level that ensures the stability of the drug and should be as close as possible to the pH of the application area to avoid irritation. The pH values of all hydrogels ranged from 4.0 to 5.5, which is consistent with the pH values of other semi-solid commercial vaginal products already in clinical use (Machado et al., 2017). While the pH range of hydrogels demonstrates satisfactory pH compatibility with vaginal environments, ornidazole remains stable in acidic environments (pH < 6) (Pyka-Pająk, 2023).

Viscosity is another factor that can influence the clinical performance of hydrogels due to its effect on drug release as well as applicability of hydrogels and contact time with the vaginal epithelium. Although a low viscosity improves spreadability, a reduction in residence time at the application site which can decrease treatment outcomes can be observed. On the other hand, vaginal hydrogels should maintain their viscosity when subjected to increased shear rates during application or when diluted with vaginal fluids (Machado et al., 2017). Thus, the optimal viscosity of vaginal hydrogels is difficult to define. The hydrogels prepared in this study exhibited a viscosity range of 6200–350000 cP. As expected, the hydrogel viscosity was influenced by the type and total amount of polymers in the formulation and increased in a concentration-dependent manner, in agreement with previous studies on many polymers (Cook & Brown, 2018; Mikušová, Ferková, Žigrayová, Krchňák & Mikuš, 2022). The blending of Crd and HPMC in hydrogels prepared with a total amount of solid polymers of 4.25-4.5% resulted in an increase in hydrogel viscosity compared to hydrogels prepared with Crd alone at the same solid polymer content. Nevertheless, composite CMCrd-HPMC hydrogels demonstrated higher viscosity than hydrogels containing Crd and HPMC alone at all the concentrations studied.

Spreadability is another parameter that indicates the ability of hydrogels to spread over a surface upon application and demonstrates how easily the topical formulation can be spread to administer a standard dose. As shown in Table 2, the spreadability values varied between 4.5 and 6.5 cm. However, the results revealed that the spreading ability and viscosity of hydrogels are correlated. Increasing the polymer concentration led to a decrease in spreadability, as expressed by the smaller diameter of the spreading circle, which can be further explained by the increased hydrogel viscosity, i.e., increased viscosity leads to a lower spreading ability. Similar results have been reported in other studies (Arpa et al., 2020; Cook & Brown, 2018).

Texture characterisation

Texture profile analysis enables evaluation of the texture properties of semi-solid products and prediction of their behaviour upon application. It is essential that vaginal gel formulations exhibit the requisite hardness, compressibility, adhesiveness, cohesiveness, and elasticity properties to ensure that they provide maximum benefits to the patient. The mechanical properties of the hydrogels were evaluated using texture profile analysis and were calculated from the resultant force-time curve (Table 3) (Cevher et al., 2008).

Hardness is measured as the maximum peak of the first compression cycle and is expressed as the maximum compressive force. The hardness of hydrogels must be evaluated to assess the necessary force required for the deformation of hydrogels. Low hardness values are preferred for ease of application to the desired site (Cevher et al., 2008). The hardness values of

Table 2. Physical observation, pH, viscosity, spreadability, and drug content of hydrogels

Formulation	Physical evaluation		pH (±SD)	Viscosity (cP) (±SD)	Spreadability (cm±SD)	Drug content (%±SD)
	Appearance	Clarity				
Crd-1	Transparent/Clear		4.00±0.08	8500±170	4.7±0.1	98.5±1.4
Crd-2	Transparent/Clear		4.13±0.05	12000±235	4.2±0.3	98.3±1.6
Crd-H1	Transparent/Clear		4.12±0.02	35270±423	3.7±0.2	98.8±2.9
Crd-H2	Transparent/Clear		4.18±0.03	39140±282	4.0±0.3	99.0±1.3
CMCrd-H1	Transparent/Clear		5.33±0.05	64803±362	5.2±0.9	98.8±1.9
CMCrd-H2	Transparent/Clear		5.50±0.08	66406±292	4.7±0.3	100.0±0.1
H1	Transparent/Clear		4.14±0.00	6200±38	5.0±0.5	99.2±1.8
H2	Transparent/Clear		4.13±0.00	14000±120	4.4±0.3	99.8±3.6
H3	Transparent/Clear		4.11±0.00	51650±145	3.9±0.1	99.7±0.7
XG1	Opaque/Clear		4.57±0.06	9133±75	4.9±0.7	100.8±1.6
XG2	Opaque/Clear		4.69±0.18	190000±365	4.8±0.5	98.7±1.6
XG3	Opaque/Clear		4.77±0.07	350000±315	4.1±0.3	99.3±0.2

Data presented as average ± SD (n = 3).

Table 3. Mechanical properties of ornidazole-containing hydrogels (n=3)

Formulation	Hardness (N ± SD)	Compressibility (mJ ± SD)	Adhesiveness (mJ ± SD)	Cohesiveness (± SD)	Elasticity (± SD)
Crd-1	0.024±0.002	0.154±0.136	0.035±0.013	0.677±0.007	1.041±0.092
Crd-2	0.043±0.021	0.065±0.004	0.056±0.022	0.708±0.027	1.032±0.088
Crd-H1	0.025±0.003	0.059±0.001	0.008±0.007	0.762±0.057	1.057±0.007
Crd-H2	0.038±0.022	0.059±0.002	0.011±0.004	0.880±0.029	0.998±0.000
CMCrd-H1	0.030±0.000	0.069±0.001	0.011±0.002	0.848±0.045	1.019±0.027
CMCrd-H2	0.030±0.000	0.069±0.001	0.017±0.007	0.859±0.033	1.039±0.038
H1	0.024±0.003	0.070±0.002	0.023±0.004	0.952±0.044	0.966±0.006
H2	0.024±0.000	0.058±0.003	0.029±0.010	1.019±0.075	1.000±0.000
H3	0.050±0.035	0.061±0.000	0.070±0.004	1.025±0.036	1.030±0.021
XG1	0.067±0.005	0.138±0.006	0.062±0.013	0.768±0.013	0.981±0.010
XG2	0.078±0.012	0.151±0.004	0.069±0.006	0.780±0.029	0.968±0.027
XG3	0.091±0.015	0.163±0.008	0.127±0.012	0.780±0.004	0.947±0.035

the prepared hydrogels exhibited a range from 0.024 to 0.091 N, contingent on the polymer type and its concentration. The H2 formulation exhibited the lowest hardness value, while the XG3 formulation had the highest hardness. The XG-based hydrogels also resulted in higher hardness values than Crd-based hydrogels. It was observed that for all hydrogels, an increase in the polymer concentration was accompanied by an increase in hardness, as expected.

A similar trend was observed when the compressibility of hydrogels was investigated. All hydrogels exhibited low compressibility values in the range of 0.058–0.163 mJ (mJoule, N.mm), and the highest values were measured for the XG hydrogels, ranging from 0.138 to 0.163 mJ. As compressibility indicates the necessary work for deformation of hydrogels and influences the removal of the formulation from the container and spreading at the application side, low compressibility values are favourable (Arpa et al., 2020). Overall, the low compress-

ibility values of all hydrogels indicate that they are convenient for vaginal application.

Furthermore, a product intended for mucosal application should possess good adhesive properties, as this can enhance the localisation of the drug at the application site, thereby improving clinical efficacy (Caramella et al., 2015). In texture profile analysis, adhesiveness is commonly expressed as the work required to overcome attraction forces between the sample and probe surface (Cevher et al., 2008). In all formulations, adhesiveness increased with increasing polymer content, with values ranging from 0.008 to 0.127 mJ. The composite CMCrd-HPMC hydrogels exhibited lower adhesiveness compared to Crd or HPMC hydrogels alone. This may be attributed to the lower total amount of polymers present in the formulation: 2% for CMCrd-H1 and 2.5% for CMCrd-H2, in contrast to 4.25% for Crd1, 4.5% for Crd2, 3% for H2, and 4% for H3, all expressed as weight-to-weight percentages. In accordance with previous investigations on the adhesiveness of different polysaccharide-based hydrogels, it can be concluded that a direct correlation exists between the total polymer quantity and the adhesiveness of the hydrogels (Mikušová et al., 2022).

While adhesion describes the ability of a material to bind to a surface, cohesion indicates the strength with which the hydrogel coheres under external shear. The degree of cohesion in a hydrogel depends on the strength of the intermolecular attractive forces within the hydrogel network. The lowest values for cohesiveness were observed in Crd-based hydrogels. HPMC hydrogels exhibited higher cohesion, which was found to depend slightly on the polymer concentration in a linear fashion. Similarly, previous studies have indicated that hydrogel cohesiveness tends to increase at high HPMC concentrations (Karavana, Güneri & Ertan, 2009). The blending of Crd with HPMC resulted in enhanced cohesiveness compared to hydrogels containing Crd alone. Additionally, the HPMC hydrogels demonstrated superior cohesiveness compared to Crd or XG hydrogels. The cohesiveness of Crd-HPMC and CMCrd-HPMC blend hydrogels was found to be comparable.

In addition to the aforementioned characteristics, an ideal vaginal hydrogel should exhibit appropriate elasticity to respond to physiological and mechanical effects upon application. Elasticity refers to the rate at which a deformed sample returns to its former state after the applied force is removed (Rençber et al., 2017). The hydrogel elasticity ranged from 0.947 to 1.057. All elasticity values were found to be comparable among the formulations, with no significant differences observed in the elastic properties of the hydrogels due to varying polymer content. Comparable elasticity values were observed for clotrimazole-containing hydrogels intended for vaginal administration (Rençber et al., 2017).

It is noteworthy that the mechanical characteristics of hydrogels may be influenced by the properties of the polymer, including its molecular weight, structure, and concentration. The results of texture profile analysis conducted in this study for the developed hydrogels indicate that none of the formulations exhibited excessive hardness or compressibility that would hinder their application. On a more comparative basis with widely used HPMC and XG hydrogels, the mechanical properties of the novel Crd and CMCrd hydrogels support their use as vaginal products.

Mucoadhesion studies

Mucoadhesion studies are widely conducted to investigate the ability of hydrogels to adhere to mucosal surfaces. Hydrogels with mucoadhesive properties are advantageous for vaginal drug delivery. They can enhance therapeutic outcomes by increasing the residence time of the drug in the vaginal mucosa and by reducing hydrogel leakage. Thus, they allow sufficient drug release from the formulation at the vaginal mucosa, thereby enhancing the local action of the drug. The mucoadhesiveness of hydrogels can be affected by several factors related to the polymer characteristics, including the type, structure, molecular weight, and concentration of the polymer within the hydrogel (Palmeira-de-Oliveira et al., 2015). Furthermore, it is essential that the same mucoadhesion testing method and experimental conditions are employed to perform a comparative evaluation of the results (Bayer, 2022). The mucoadhesion data of the hydrogels prepared in this study were obtained using Texture Analyser and is presented in Figure 4. As anticipated, alterations in the polymer content of hydrogels resulted in changes in mucoadhesion.

HPMC is commonly used in vaginal hydrogels because of its mucoadhesive properties (Cook & Brown, 2018). The mucoadhesion of the hydrogels increased with increasing concentration of HPMC. However, the incorporation of HPMC with Crd did not enhance the mucoadhesive properties of the Crd hydrogels. The work of mucoadhesion was found to be lower for Crd-HPMC blend hydrogels than for Crd hydrogels alone. Furthermore, the mucoadhesive properties of Crd hydrogels were found to be superior to those of HPMC hydrogels. Crd polymer exhibits better mucoadhesive properties in a simulated vaginal environment than HPMC. To the best of our knowledge, no previous reports have described the mucoadhesion properties of Crd for vaginal application. In previous studies, Crd derivatives were combined with chitosan to form nanocarriers for the nasal administration of antigens. One of these studies (Zhang et al., 2018) reported that curdlan sulfate–O–linked quaternised chitosan nanoparticles, prepared via polyelectrolyte complexation, probably had mucoadhesive capacity and could be used as a mucoadhesive agent. Also, the results from our earlier studies indicated that chitosan-CMCrd composite nanoparticles exhibit optimal properties for nasal immunisation of protein antigens, suggesting that these nanocarriers have mucoadhesive properties (Sessevmez et al., 2023). A comparison of the mucoadhesion between the CMCrd-HPMC and Crd-HPMC

blend hydrogels revealed almost a 2-fold increase in the work of mucoadhesion for the former, indicating a positive impact of carboxymethylation on the mucoadhesive properties of the hydrogels. In line with these results, it was also reported that the use of carboxymethylated guar gum enhanced the mucoadhesive properties of native guar gum (Giri & Singh, 2020), whereas in another study, carboxymethylation increased the mucoadhesive strength of gellan gum (Ahuja et al., 2013).

The work of mucoadhesion was found to be inversely proportional to the polymer concentration in XG hydrogels. A similar trend was previously observed by our group. It was observed that mucoadhesion decreased with increasing XG content in the formulation (Cevher et al., 2014). A similar fashion was observed by other researchers who evaluated vaginal gel for treating mixed vaginal infections. They showed that a reduction in the XG content of vaginal gels, either alone or in combination with other polymers (*e.g.* HPMC or sodium alginate), enhanced bioadhesive strength (Ahmad, Alam, Khan, Khar & Ali, 2008). The inherit mucoadhesive properties of XG have been already acknowledged in the literature (Jadav et al., 2023). In this study, the XG hydrogels demonstrated superior mucoadhesiveness to HPMC hydrogels. It is noteworthy that the mucoadhesion results were comparable to those obtained for the Crd-based hydrogels.

The results obtained from the mucoadhesion tests in this study provide a comparative evaluation of the mucoadhesive properties of Crd-based hydrogel formulations and hydrogels prepared with widely used polymers, namely HPMC and XG. Furthermore, these findings contribute to a deeper understanding of the mucoadhesive characteristics of Crd-based pharmaceutical formulations, particularly those intended for vaginal administration.

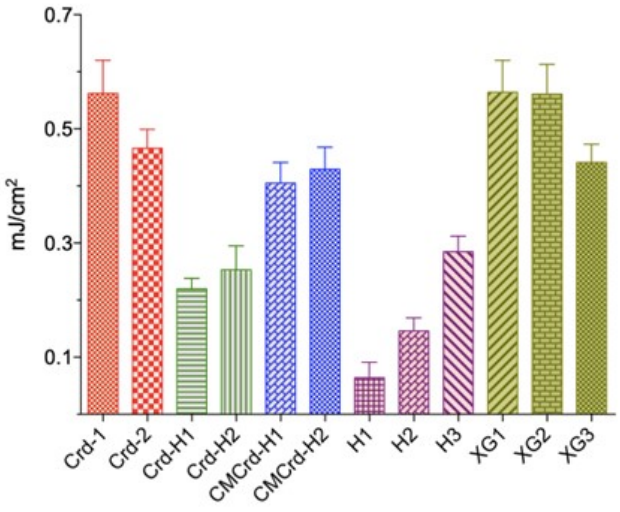

Figure 4. Work of mucoadhesion of ornidazole-containing hydrogels (n=3)

Fourier transform infrared (FT-IR) spectroscopy analysis of ornidazole-containing hydrogels

The FT-IR studies were conducted to assess potential interactions between the drug and other components in the hydrogels. As shown in Figure 5, the FT-IR spectrum of ornidazole reveals bands due to O–H stretch at 3109 cm^{-1}, C–H stretch at 3086 cm-1, asymmetric NO2 stretch at 1539 cm^{-1}, symmetrical NO2 stretch at 1363 cm^{-1} and 1273 cm^{-1}, C–H-dependent stretching at 1190 cm^{-1} to and stretching at 831 cm^{-1} is attributed to C–N and NO2. All of these peaks were identified in the FT-IR spectrum of ornidazole-containing hydrogels, confirming the presence of the drug in the formulation without any significant interaction with other components (Vaghani et al., 2012).

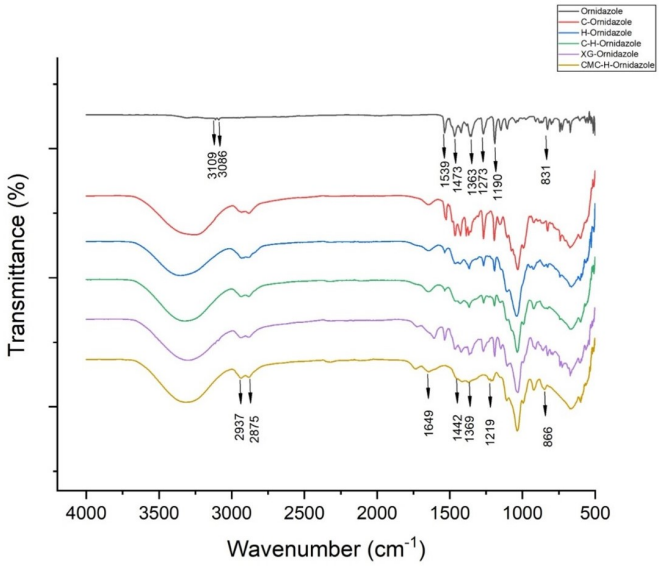

Figure 5. FT-IR spectra of ornidazole-containing hydrogels

Microstructure analysis

SEM is a widely used technique to observe surface morphology and characterise the hydrogel structure at the micrometer scale (Antonietti, Caruso, Göltner & Weissenberger, 1999). SEM images of ornidazole and hydrogels are presented in Figure 6.The surfaces of all hydrogels appeared heterogeneous. HPMC hydrogels exhibited a random circular or ellipsoidal pore shape, whereas Crd-HPMC hydrogels displayed smaller and denser pores. Zhang et al. (Zhang, Nishinari, Williams, Foster & Norton, 2002) observed that gels containing HPMC and Crd exhibited greater porosity and smaller pore sizes than gels containing only HPMC. Furthermore, increasing Crd amount in the formulations resulted in an increase in porosity and a reduction in pore size in the hydrogels. Blending Crd with HPMC may increase the viscosity and gel strength, which could lead to the formation of more compact structures. In contrast, the Crd hydrogel exhibited a dense, heterogeneous, and slightly rough

surface. The observed compact network structure can be attributed to hydrogen bonds (Tao et al., 2021). It is pertinent to highlight that the XG hydrogels exhibit greater porosity than the other hydrogels. The SEM images revealed distinct differences in surface morphology between the hydrogels and ornidazole.

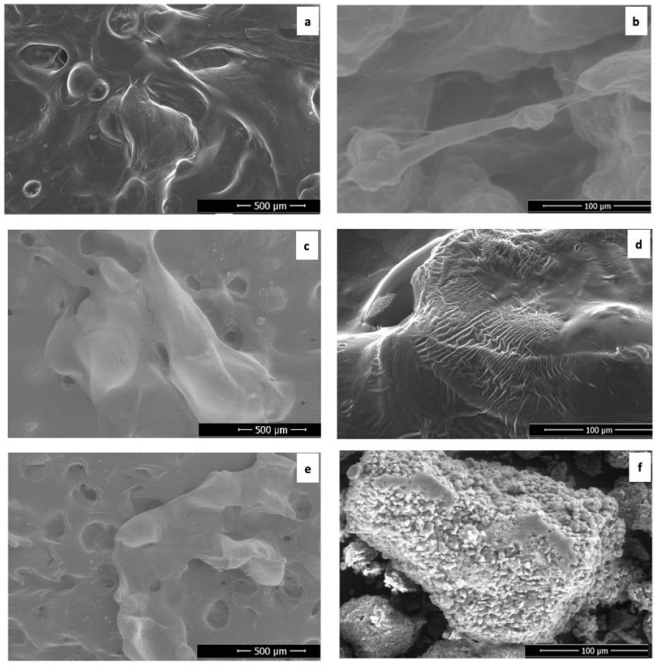

Figure 6. SEM images of ornidazole-containing hydrogels (a) HPMC, (b) Crd, (c) Crd-HPMC, (d) CMCrd-HPMC, (e) XG, and (f) pure ornidazole

In vitro drug release and release kinetics

A drug release profile characterised by controlled release can provide a favourable approach to improve the success of therapy with antimicrobial drugs (Osmałek et al., 2021). Overall, the drug-release pattern of hydrogels depends on the type and amount of polymer present in the hydrogel (Figure 7). For all hydrogels, fast drug release was observed during the first hour. Crd-based hydrogels released most of the ornidazole content within 2 h. The blending of HPMC and Crd (C-H1 and C-H2) resulted in a slight decrease in drug release, indicating that it is possible to alter drug release by modifying the hydrogel composition. In a previous study, it was reported that the release of tetracycline hydrochloride from Crd-phosphorylated Crd hydrogels crosslinked with 1,4-butanediol diglycidyl ether was also influenced by the composition of the hydrogels, and the equilibrium of drug release was achieved after 3.5 h in PBS pH 6.8 (Suflet, Popescu, Prisacaru & Pelin, 2021). CMCrd-based hydrogels exhibited faster drug release due to the increased solubility of CMCrd in the aqueous environment, which results in greater swelling and consequently loosening of the polymer network, allowing faster drug release. Among the various hydrogels studied, the XG- and HPMC-based hydrogels exhibited a more sustained drug release profile, with approximately 75-85% of ornidazole content released within 6 h.

The drug release data of ornidazole-containing hydrogels were fitted using various release kinetic models, including zero-order, first-order, Higuchi and Korsmeyer-Peppas models (Table 4). Application of the Korsmeyer-Peppas kinetic model also enables identification of the release mechanism by calculating the n exponent (Baloğlu et al., 2006; Sethi et al., 2020). Kinetic modelling of drug release from hydrogels showed that the drug release data best fit to the first-order kinetic model. The first-order kinetics describes drug release from the system as proportional to the amount of drug remaining in the system over time. Thus, according to this model, drug release decreases with decreasing concentration gradient (Barradas, Senna, Cardoso, de Holanda e Silva & Elias Mansur, 2018). A recent paper reviewing the modelling of drug release from hydrogel-based systems outlined that first-order equations are widely employed to describe drug release from hydrogels (Caccavo, 2019). Hydrogels composed of natural polymers have been reported to show drug release according to first-order model (Khan & Ranjha, 2014; Khanum, Ullah, Murtaza & Khan, 2018).

Short-term stability study

Stability studies were performed to evaluate the ability of hydrogels to remain stable at 25±2 °C and 65±5% relative humidity conditions, as specified in the relevant ICH guidelines (ICH Q1A (R2), 2003). At the end of the study, the physical appearance and percentage of drug content of the hydrogel formulations were evaluated. As illustrated in Figure 8, no alterations were observed in the percentage drug content of hydrogels at the end of the stability study. Furthermore, the physical appearance of the hydrogels after one month was comparable to that observed at the beginning of the study. It can be concluded that the polymeric hydrogels developed in this study exhibit good stability when stored at room temperature.

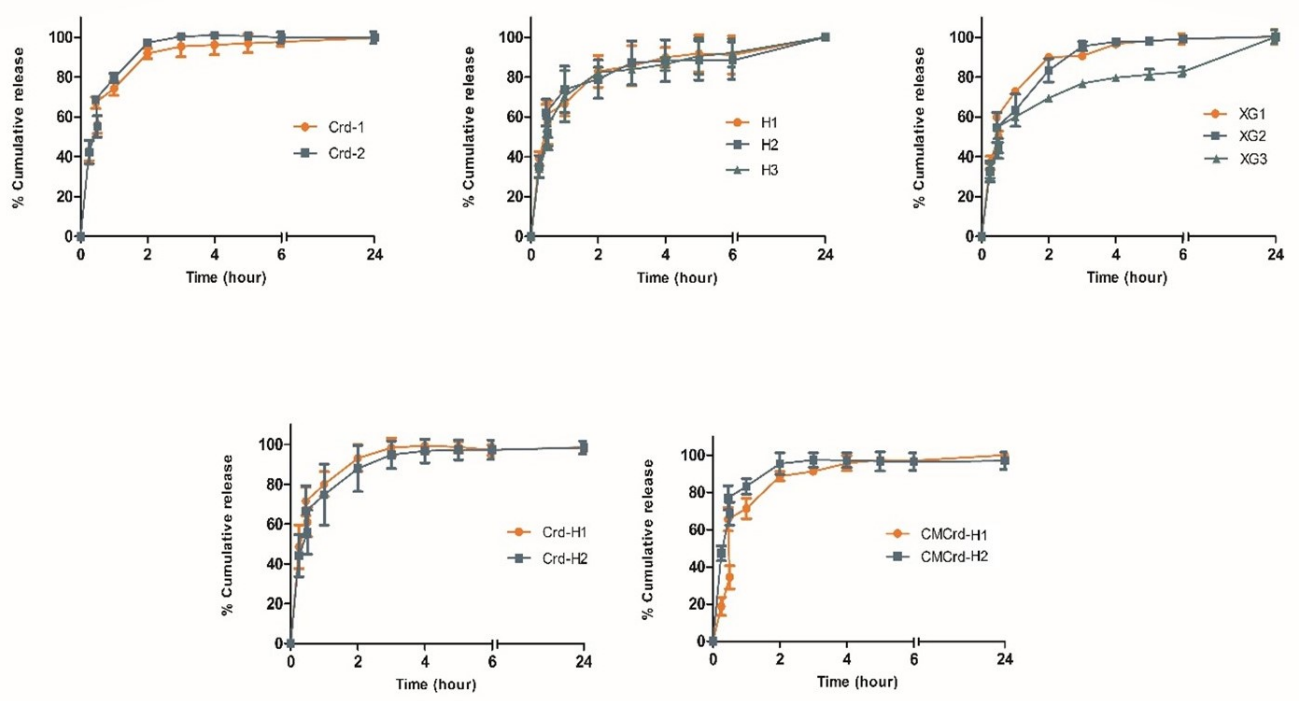

Figure 7. Drug release profile of ornidazole from hydrogels (mean ± SD, n = 3)

Table 4. Kinetic models of ornidazole release from hydrogels

Formulation	Zero order	First order	Higuchi	Hixson–Crowell	Korsmeyer-Peppas	
	r^2	r^2	r^2	r^2	r^2	n
Crd-1	0.824	0.970	0.897	0.937	0.723	0.508
Crd-2	0.806	0.979	0.884	0.981	0.672	0.536
Crd-H1	0.784	0.992	0.878	0.958	0.715	0.439
Crd-H2	0.739	0.924	0.858	0.875	0.705	0.462
CMCrd-H1	0.806	0.918	0.898	0.885	0.798	0.356
CMCrd-H2	0.811	0.964	0.900	0.940	0.799	0.710
H1	0.746	0.928	0.866	0.902	0.839	0.422
H2	0.702	0.945	0.827	0.906	0.789	0.696
H3	0.746	0.918	0.862	0.866	0.796	0.556
XG1	0.754	0.984	0.870	0.936	0.761	0.569
XG2	0.797	0.978	0.904	0.933	0.818	0.457
XG3	0.783	0.910	0.885	0.878	0.773	0.407

r^2: correlation coefficient, n: diffusional exponent

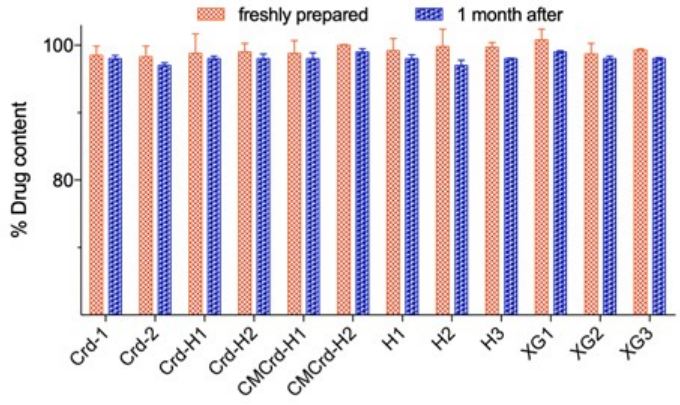

Figure 8. Drug content in hydrogels initially and after one month

CONCLUSION

In this study, hydrogels of ornidazole were prepared using Crd, CMCrd, and their combinations with HPMC, as well as XG alone. The physicochemical characterisation studies, mechanical and mucoadhesive properties and drug release characteristics were determined. The preparation process was straightforward, and the results demonstrated that all hydrogels exhibited favourable properties (*i.e.* physicochemical and textural properties) for use as vaginal formulations for the treatment of infections. In particular, the mucoadhesive properties of the Crd-hydrogels and CMCrd-HPMC hydrogels were comparable to those of the XG hydrogels and superior to those of the HPMC hydrogels in simulated vaginal environment. Consequently, these vaginal hydrogels can be considered promising mucoadhesive systems capable of prolonging the retention time of drug at the application site. They can be alternative formulations for the local treatment of vaginal infections. It is important to mention that the use of vaginal delivery systems that can provide therapeutically relevant levels of antimicrobial drugs in the vaginal tissue can overcome issues related to the use of high-dose drugs required for oral therapy. Moreover, reduced administration frequency and increased patient compliance could be potential positive outcomes. Nevertheless, further studies are required to evaluate the biological activity and safety of these biopolymer-based hydrogels.

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ORCID IDs of the authors

Emine Saldamlı 0000-0003-0405-5271
Melike Sessevmez 0000-0002-6233-9574
Tilbe Çevikelli 0000-0002-0881-0644
Erdal Cevher 0000-0002-0486-2252
Genada Sinani 0000-0003-0372-631X

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