doi.org/10.36721/PJPS.2026.39.8.230.1

Formulation and *in-vitro* characterization of okra mucilage-based controlled release hydrogels

Mriba Khaliq[1], Muhammad Irfan Siddique[2], Samreen Soomro[2], Mamoona Tariq[3], Rabia Khokhar[1], Rabia Suleman[1], Anam Ijlal[1,4], Arslan Javed[5], Hafiz Awais Nawaz[1] and Muhammad Usman[1]*

[1]Institute of Pharmaceutical Sciences, University of Veterinary and Animal Sciences, Lahore, Pakistan
[2]Department of Pharmaceutics, Faculty of Pharmacy, Northern Border University, Rafha, Saudi Arabia
[3]School of Pharmacy, Shanghai Jiao Tong University, China
[4]Department of Pharmaceutics, Green International University, Lahore, Pakistan
[5]Punjab University College of Pharmacy, University of the Punjab, Lahore, Pakistan

Abstract: **Background**: Certain drugs at therapeutic doses can cause gastrointestinal toxicity, which controlled-release formulations help to address. Natural polymers are widely used in drug delivery due to their non-toxic, biodegradable, and biocompatible properties. Okra mucilage powder (OMP) is especially promising because of its strong gelling ability and pH-sensitive behavior. **Objectives**: This study aimed to develop OMP-based hydrogels for the controlled release of methotrexate (MTX) over extended periods via free-radical polymerization. **Methods**: Acrylic acid (AA) was used as the monomer with OMP and gelatin as polymers, N, N-methylene bis-acrylamide (MBA) as the crosslinker, and ammonium persulfate (APS) as the initiator. SEM analysis revealed a porous, rough structure that facilitates MTX loading. Thermal stability and chemical compatibility were confirmed by TGA and FTIR analyses. **Results**: The hydrogels exhibited pH-dependent swelling, with the highest at pH 7.4 and the lowest at pH 1.2. Formulation OG3 with increased gelatin (1.6%) and MBA (0.6%) showed the best stability and controlled release (r^2=0.996). **Conclusion**: Drug release followed the Korsmeyer-Peppas model ($r^2 \geq 0.996$), demonstrating successful development of stable, pH-responsive hydrogels for MTX delivery.

Keywords: Hydrogel; *In-vitro* drug release; Okra mucilage; pH-responsive

Submitted on 26-07-2024 – Revised on 17-02-2025 – Accepted on 20-09-2025

INTRODUCTION

Despite the rapid advancements in drug development, cancer remains the second leading cause of global deaths and the need for the development of plant-based medicinal products remains. To this end, a vast number of experiments and techniques have been carried out for its treatment and the improvement of human health (Dehshahri *et al.,* 2020). Methotrexate (MTX) is a folate antagonist and has proven to be one of the best choices for the treatment of many different types of cancers (Tekko *et al.,* 2020). It is an analog of folic acid with a molecular weight of 454.5 g/mol. MTX dissolves in distilled water at 20 °C with a pH-dependent solubility of 0.01 mg/mL. For its maximum stability, the ideal pH range is 6.6 to 8.2 (Abolmaali *et al.,* 2013). The stability of such drugs can be improved by an appropriate pH-responsive carrier system (Dehshahri *et al.,* 2020).

MTX is known to induce gastrointestinal toxicity, particularly intestinal mucositis, which is a significant dose-limiting side effect. This toxicity arises because MTX targets rapidly dividing cells, including those in the gut, leading to symptoms such as diarrhea and abdominal pain that can severely affect a patient's quality of life and treatment adherence (Hamed *et al.,* 2022). The rapid clearance of MTX necessitates frequent high dosing to maintain therapeutic levels, which in turn increases the risk of adverse effects, particularly gastrointestinal complications, which are one of the primary reasons for treatment interruption in patients receiving MTX (Zhou *et al.,* 2018). The polymeric hydrogels can provide a promising strategy for improving MTX delivery by enabling sustained drug release, protecting MTX from degradation in the stomach and potentially allowing for targeted release in the intestinal environment. This approach aims to reduce systemic exposure and enhance therapeutic outcomes while mitigating adverse effects associated with conventional dosing regimens (Di Francesco *et al.,* 2021).

Polymeric hydrogels represent one such type of controlled-release preparation. They are considered a safe drug delivery strategy for oral administration due to their mucoadhesive properties, which prolong drug release and absorption (Ghasemiyeh and Mohammadi-Samani 2019). Hydrogels offer effective delivery systems that mitigate the limitations of conventional drug delivery methods, thereby maximizing the therapeutic benefits of the drug (Tabassum *et al.,* 2022). The near-constant release rates of hydrogels make them ideal for controlled drug delivery. A hydrogel is composed of hydrophilic polymers that form a three-dimensional (3-D) structure. The cross-links formed by polymeric network chains enable them to retain and absorb

**Corresponding author:* e-mail: usman.ips@uvas.edu.pk

large volumes of biological fluids or water without dissolving (Bashir *et al.,* 2020). Hydrogels serve a key role in biomedical applications due to their unique characteristics, which include hydrophilicity, biocompatibility, controlled drug release, biodegradability and smart drug delivery. (Bashir *et al.,* 2020). Traditional drug delivery systems require multiple administrations or greater doses for poorly soluble and low-permeable drugs to attain a therapeutic effect, which results in decreased patient compliance and efficiency as well as substantial adverse effects and toxicity. For this purpose, various types of controlled-release dosage forms have been devised, prepared and tested in the past few years (Tabassum *et al.,* 2022). This study explores the use of Okra mucilage (OM) as a novel, biocompatible and cost-effective natural polymer for the controlled release of MTX. Compared to traditional MTX tablets, the OM-co-poly acrylic acid (AA) hydrogel offers the potential for sustained drug release, reduced gastrointestinal irritation and improved patient adherence due to less frequent dosing. The sustained release achieved with the OM-co-poly (AA) hydrogel could potentially improve MTX bioavailability by maintaining therapeutic drug levels over a longer period and reducing the peak plasma concentration, which is often associated with adverse effects (Kurra *et al.,* 2022).

Okra is one of the most important vegetables with mucilaginous properties. It belongs to the Malvaceae family, commonly identified as lady finger and biologically as Abelmoschus esculentus (Elkhalifa *et al.,* 2021). Extraction of Okra holds numerous bioactive benefits like antitumor, antioxidant, antimicrobial, antidiabetic, immunomodulator and anti-ulcer as well as removes toxins from the liver (Nampuak and Tongkhao 2020). This study focuses on OM-co-poly(AA) hydrogels designed to provide controlled release of MTX, aiming to address issues of low solubility and limited permeability. Different hydrogels with different concentrations of polymer and crosslinker were formulated. Their characteristics, such as sol-gel fraction, swell ability studies and in-vitro release kinetics were analyzed to verify their drug release properties.

MATERIALS AND METHODS

Materials

Okra fruits were obtained from the local market of Lahore. Acrylic acid was procured from Samchun Chemicals (Seoul, Korea). N, N-methylene bis-acrylamide and ammonium persulfate (APS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Gelatin was obtained from Glentham Life Sciences (Corshan, UK). Demineralized water was used in the preparation of buffers and the preparation of hydrogels. All of the above-mentioned chemicals were of analytical grade and used as received, without further purification. Hydrogel synthesis was carried out in a temperature-controlled water bath (Grant Instruments, Cambridge, UK). The pH of buffer solutions was measured using a calibrated digital pH meter (Mettler Toledo, Columbus, OH, USA). The morphology of the prepared hydrogels was examined by scanning electron microscopy (SEM; JEOL JSM-6490, Tokyo, Japan). Fourier-transform infrared (FTIR) spectroscopy (Shimadzu IRTracer-100, Kyoto, Japan) was used to characterize the hydrogels. Thermal analysis was performed using thermogravimetric analysis (TGA; TA Instruments Q500, New Castle, DE, USA) and differential scanning calorimetry (DSC; TA Instruments Q20, New Castle, DE, USA). Drug content and release studies were conducted using a UV-Vis spectrophotometer (Shimadzu UV-1800, Kyoto, Japan). Dissolution testing was performed using a USP dissolution apparatus (Electrolab TDT-08L, Mumbai, India).

Extraction of Okra mucilage powder

Okra fruits were collected, washed carefully, dried in the shade for 24 hours, then dried again in a hot-air oven at 40-45°C until a constant weight was achieved. A grinder was used to reduce the particle size to achieve Okra powder. Powdered fruit was then placed in an airtight container after passing through sieve #22 (stainless steel standard testing sieve) and further processing was performed by mucilage extraction and mucilage isolation (Farooq *et al.,* 2013). For extraction, the granulated powder was suspended in 500 mL of distilled water and stirred continuously at 60 °C for 4 hours. Muslin cloth was used to filter the concentrated solution and then it was cooled at 4°C-6°C in the refrigerator. Mucilage was isolated by precipitation in acetone. Precipitated mucilage was filtered through the muslin cloth and washed with acetone. It was then again filtered through muslin fabric. Pressed mucilage was then dried in a hot-air oven at 35-45°C until a constant weight was achieved or until completely dried. The obtained hardcake of mucilage was ground, passed through a #100 sieve, and placed in an airtight container for further use (Kale 2020).

Development of OM-co-poly (AA) hydrogels

A total of 6 types of hydrogels were prepared with 1.6% gelatin and without gelatin using a cross-linking agent MBA with different proportions, that is 0.2%, 0.4% and 0.6%, while the concentration of OM (3.2%), acrylic acid 24% and APS (0.32%) were kept the same in all formulations. The OM and gelatin were weighed (2:1 wt%) with a precision of 0.01 g using an electronic balance. Both polymers were dispersed in 5 mL of demineralized water under constant stirring (200 RPM) and heated to 50°C until dissolved and mixed to form a single polymer solution. The monomer (AA), 20% by weight, was carefully added (dropwise) to the polymer mixture already formed. The prepared APS solution was added to the polymer dispersion to initiate polymerization. Finally, the prepared solution of MBA was added dropwise into it and the resulting mixture of the polymeric blend was transferred into a clean and labeled test tube. Heating was applied in a hot air oven at 55°C and 45°C for 2 hours and 24 hours,

respectively, and allowed to cool at room temperature and then washed with demineralized water to remove any unreactive ingredients. The cylindrical hydrogels were cut into 8 mm discs using sharp blades and dried in the oven for 48 hours at 40°C (Maryam *et al.,* 2022). The composition of formulated hydrogels is described in Table 1.

Characterization determination

Drug loading

MTX loading was done by the diffusion-driven swelling method, in which the preparation of 1% weight-by-volume drug solution was prepared in a phosphate buffer solution of pH 7.4 (Bernardes *et al.,* 2021). Hydrogel discs were weighed and placed into the MTX solution for 48 hours. The hydrogel discs were then removed, blotted on filter paper and kept in the oven at 40°C until the discs were completely dried (Hussain *et al.,* 2022). Drug loading was found by the dry weight method, in which the dried hydrogel discs were weighed again to determine the quantity of loaded drug in the hydrogel. The loaded amount was determined by comparing the weights of hydrogel discs before and after immersion in the drug solution. Hydrogels soaked in PBS solution at pH 7.4 have been used as control and weight changes in control groups have been optimized in experimental groups (not mentioned in the results). Drug loading is estimated using the given equation 1.

$$\text{Drug loading (\%)} = \frac{\text{final dry weight-initial dry weight}}{\text{final dry weight}} \times 100 \text{.....Eq.1}$$

Sol-gel analysis

In this characterization, the hydrogel discs were precisely weighed and placed in distilled water for 72 hours at room temperature. The individual discs were taken out after 72 hours and dried completely. After reweighing the discs, the percentages of the sol and gel fractions were calculated using equations 2 and 3 (Akhlaq *et al.,* 2021).

$$\text{Sol fraction (\%)} = \frac{W_1 - W_2}{W_1} \times 100 \text{..... Eq. 2}$$

$$\text{Gel fraction (\%)} = 100 - \text{Sol fraction} \text{.....Eq. 3}$$

where W_1 and W_2 are the weights of the disc before and after immersing in water, respectively.

Thermal stability

A thermogravimetric analysis (TGA) was carried out for OM, MTX-unloaded hydrogels and MTX-loaded hydrogels using a simultaneous thermal analyzer (SDT-Q600). The thermal stability of all the samples was investigated using TGA. The samples were examined while being continuously flooded with nitrogen. All samples were subjected to constant heat between 0°C and 500°C. (Bashir *et al.,* 2018).

Scanning electron microscopy (SEM) analysis

The SEM analysis was performed to evaluate the surface morphology and porosity of the gel texture. The sample was dried and gold-coated before analysis using a scanning electron microscope (Zeiss EVO LS 10). This was done at an accelerated electron-beam voltage of 20 kV to determine the surface morphology of OM-co-poly (AA). (Masood 2022).

FTIR spectroscopic analysis

Any potential chemical interactions between the drug, MTX and the polymeric components of hydrogels were examined through an FTIR spectrophotometer, over the wave number range of 400-4000 cm^{-1}. Infra-red (IR) spectra of MTX, empty hydrogel and MTX-loaded hydrogels were recorded following the method previously reported in the study. A change in the material composition is indicated by modifications to the distinctive absorption band pattern. (Pal *et al.,* 2019).

Swelling behavior

The dried hydrogel formulations (OG1-OG6) were weighed and immersed in the buffer solutions of pH 1.2 & pH 7.4 separately for 24 h at room temperature to quantify the pH-dependent swelling characteristics of hydrogels. The hydrogels were taken out at certain time intervals from the buffer solution and the surface of the hydrogel was wiped off. The hydrogels' weight was calculated, and they were returned to the same medium. This procedure was repeated until a constant weight was achieved. The pH-dependent equilibrium swelling (ES) was examined by employing equations 4 and 5 (Bashir *et al.,* 2018).

$$\text{Swelling ratio (Sr)} = \frac{W - Wo}{Wo} \text{.....Eq. 4}$$

$$\text{\% Swelling} = Sr \times 100 \text{.....Eq. 5}$$

Where "W" is the weight of swollen hydrogel and Wo is the Weight of dried hydrogel.

Drug release kinetics studies

Dissolution studies were done using USP Apparatus-II with 900 mL of dissolution media at pH 7.4 (intestinal) added to each basket of the dissolution apparatus. The hydrogel discs were immersed in the medium at 100 RPM and 37°C. At specified intervals, 5 mL of sample was taken from each basket, and the basket was replaced with an equal volume of fresh medium. Samples were detected using a UV-visible spectrophotometer (UV-1602, BMS) at 292 nm, and release kinetics were analyzed using different models, including Zero order, First order, Higuchi model, and Korsmeyer-Peppas model, to determine the drug release mechanism. (Hussain *et al.,* 2022).

Statistical analysis

Drug swelling behavior of hydrogel groups (OG1 to OG6) has been compared statistically by unpaired t-test within each group at both pH values and using one-way ANOVA with post hoc Tukey's t-test across the groups at pH 7.4.

RESULTS

Isolation of OM from Okra plant

The OM was successfully extracted from the Okra fruits, which showed a fibrous nature changed into an amorphous light brown colored powder after drying and passing through sieve number 80 (Fig. 1). The percentage yield of

OM was 14.2%, which is close to the previously reported yield (Senthil *et al.* 2010).

Synthesis of OM-gel-AA hydrogel and drug loading

Hydrogels (OG1-OG6) were formulated by altering MBA concentration with or without the presence of gelatin as described in Table 1. Formulations (OG1-OG3) were more elastic due to the presence of gelatin. The compact structure of the hydrogel and the elasticity loss were noticed as the concentration of the cross-linker was changed, especially in formulations (OG4-OG6) and also due to the absence of gelatin (Maryam *et al.,* 2022).

After drying, the hydrogels were added to a 1% MTX solution and left to swell for 48 hours for drug loading. MTX loading (%) in the formulated hydrogels is described in table 2. The loading percentage was higher in formulations with low concentrations of crosslinking agents. As the concentration of MBA increases, the loading percentage decreases, as in OG1-OG3 and OG4-OG6. Percentage loading in formulations without gelatin (OG4-OG6) was relatively higher than in those with gelatin (OG1-OG3), probably due to the less dense structure of hydrogels.

Sol-gel analysis

A sol-gel approach was used to identify the non-cross-linked polymer in the formulation during polymerization. The sol-gel proportion is directly proportional to the amount of the cross-linker and the concentration of the gelatin. It was noted that the sol-gel percentage for OG1 to OG6 was 91.12 ± 0.13%, 92.66 ± 0.74%, 94.47 ± 0.40%, 90.92 ±0.40%, 91.99 ±0.32% and 93.28 ± 0.56% respectively as shown in Table 3. It was observed that increasing the concentration of the crosslinking agent increases the gel fraction, and vice versa. Also, the presence of gelatin polymer contributes to a higher gel fraction than in hydrogels without gelatin. For each of the six formulations, sample OG3 had the highest level of sol-gel fraction (94.47%) due to the high concentration of MBA and the presence of gelatin, as shown in Fig. 1. Sample OG1 had a lower level, as it contains a low concentration of MBA and sample OG4 showed the lowest level of sol-gel fraction due to the absence of gelatin and low concentration of the crosslinker (Akhlaq *et al.,* 2021).

FTIR spectroscopic analysis

FTIR analysis (400-4000 cm^{-1}) was performed to check for chemical interactions between MTX) and the hydrogel polymer blend. The MTX spectrum showed characteristic peaks at 3343 cm^{-1} (O-H stretching), 3124 cm^{-1} (N-H stretching) and 1651 cm^{-1} (C=O stretching) (results not shown). The polymer blend displayed C-H stretching at 2922 cm^{-1} (AA), methylene stretching at 2852 cm^{-1} (gelatin), C=O stretching at 1712 cm^{-1} (AA) and polysaccharide bands in the 1016-1553 cm^{-1} range (Okra gum). Slight variations in component peaks confirmed the formation of a cross-linked polymeric blend. The presence of MTX peaks at 3130 cm^{-1} in the drug-loaded hydrogel confirmed successful drug incorporation and its stability within the hydrogel matrix, with no indication of chemical interaction (Fig. S1).

Thermal stability

Thermal analysis of OM, MTX and OM-co-poly (AA) formulation was examined, and a correlation between weight changes and temperature changes was found by thermogravimetric analysis (TGA). Water evaporation caused MTX weight loss at 70°C, followed by a phase transition and functional group degradation, resulting in a major weight loss at 320°C. However, MTX was stable between 350 and 490°C. TGA of OM results indicate that a small mass loss occurred at 80°C. The second weight loss was observed above 250°C. Mass loss occurs in three stages at high temperatures. The first weight loss was observed below 180 °C. The second loss of mass occurs within the 270-330 °C temperature range. At 375 °C, there is a significant loss that shows the formulation's complete decomposition, as shown in Fig. 2. Results show that the blended OM-co-poly (AA) hydrogel has a high degree of heat stability (Dharmalingam and Anandalakshmi 2019).

Scanning electron microscopy (SEM)

To evaluate the surface morphology of the OM-co-poly (AA) hydrogel, SEM was performed at various magnifications (Fig. S2). The hydrogel presented various flat sheets of differing sizes and shapes, having a textured surface and uneven spaces (Shahbazizadeh *et al.,* 2022). The presence of interconnected pores within the hydrogel was due to the water evaporation by the heated medium during the polymerization reaction. This could facilitate the flow of water molecules when the material is exposed to water, potentially resulting in expansion or swelling. Diffusion of water molecules into and out of the hydrogel may also contribute to the loading of drug solutes. It also encourages and contributes to the progressive drug release from the hydrogel.

Swelling studies

Swelling profiles of OM-co-poly (AA) hydrogels formulated by changing the concentrations of gelatin and cross-linker were examined at pH 1.2 and pH 7.4 at 37°C. Since the swelling percentage of OM-Gel-poly (AA) hydrogels was much higher at pH 7.4 than at pH 1.2, swelling experiments showed that simulated intestinal fluid (SIF) is a viable choice for these pH-sensitive drug delivery formulations. The percentage swelling of hydrogels, along with the standard deviation, is given in Table 4. The greater swelling of OM-co-poly (AA) hydrogels in the basic environment is due to the electrostatic repulsion between negatively charged hydrophilic groups (-COOH) in the polysaccharide chains. This repulsion forces the polymer chains to spread apart, allowing more water to enter the hydrogel (Fig. 3) elling of OM-co-poly (AA) hydrogels in the basic environment is due to the electrostatic repulsion between negatively charged hydrophilic groups (-COOH)

in the polysaccharide chains. This repulsion forces the polymer chains to spread apart, allowing more water to enter the (Bashir *et al.,* 2018). In an acidic environment, the hydrophilic groups of polymeric networks were unable to become ionized. This decreased the electrostatic repulsion between the groups, reducing hydrogel swelling. The presence of gelatin makes the polymeric structure more compact, which accounts for the less water uptake than the hydrogels without gelatin (OG1-OG3). Also, with the increasing MBA concentration in OG2, OG3, OG5, and OG6, hydrogel swelling was decreased owing to increased cross-linking density (Fig. 3).

In-vitro drug release and kinetic modeling

The dissolution investigations were carried out using the USP Apparatus II and 900 mL of dissolving medium. The selected medium had a pH of 7.4, similar to the intestinal environment. The samples were taken out for examination at predetermined intervals and release of MTX through OM-co-poly (AA) polymeric blends was determined by applying different models upon dissolution data, which includes Zero order, First order, Higuchi and Korsmeyer-Peppas kinetic models (Hussain *et al.,* 2022). These models are powerful tools to predict concentration independent (zero order), concentration dependent (first order), diffusion controlled (Higuchi) or to identify the mechanism when multiple processes govern drug release (Korsmeyer-Peppas). R2 (regression coefficient) values, along with standard deviations (SD), were examined, and it was discovered that all six formulations (Okra Gel 1 to 6) showed ranges between 0.960 and 0.996. These results suggested that the release behavior of these formulations, as shown in Table 5, was best described by the Korsmeyer-Peppas release model with the highest r^2 value and the lowest standard deviation (SD) values, which indicates good experimental design, low random error and shows that experimental measurements are highly reproducible. These results imply that all formulations adhered to the Korsmeyer-Peppas release model and exhibited both diffusion ($n < 0.45$) and dissolution ($n > 0.45$) mechanisms. The model has been well documented as an explanation of drug kinetics in pH-driven hydrogels. Formulation 3 was selected for its highest regression value (0.996) and the best AIC and MSC values, among other criteria. The value of "n" showed the diffusion mechanism, where the values between 0.49 and 0.89 represent the non-Fickian release mechanism. As shown in Fig. 4, this kinetic model efficiently demonstrates how the active drug is released through hydrogels (George *et al.,* 2020).

DISCUSSION

Hydrogels have attracted a lot of interest because of their distinct characteristics and diverse range of applications (Bashir *et al.,* 2020). They are 3-D network structures that crosslink to form a polymeric network chain. Hydrogels are incapable of dissolving but can greatly expand when exposed to water (Varghese *et al.,* 2020) and are being used due to having biocompatibility and biodegradability in physiological environments. Due to having structural similarity to macromolecular components of the human body, hydrogels are considered more biocompatible (Lee and Mooney 2001). A lot of studies have been published in the past years on the synthesis of natural hydrogels (Burdick and Prestwich 2011) as well as on synthetic hydrogels (Moghadam and Pioletti 2016) that have wide applications in the biomedical field. However, a well-established strategy is to synthesize hydrogels by using both natural and synthetic components. This technique yields hydrogels with improved stiffness and degradation resistance. Hydrogels show unique characteristics, including hydrophilicity, controlled drug release and smart drug delivery. The combination of natural and synthetic polymers makes hydrogels more responsive to surface molecules in body tissue for improving biological functions (Geckil *et al.,* 2010).

OM, a natural polysaccharide found in Okra pods, is being investigated for its potential applications in the pharmaceutical industry. Okra has been assessed for various applications, spanning both the food and non-food industries. Okra polysaccharides show great promise due to their significant water-retention capacity and functional properties, making them suitable for drug delivery applications (Deka *et al.,* 2023). By keeping in view the above-mentioned advantages of biopolymers and OM, in current research, a hydrogel is prepared using both natural and synthetic polymers, OM powder and acrylic acid, respectively, for the controlled release of the model drug at the intestinal pH.

The percentage yield of OMP was calculated to be 14.2%. This mucilage was fabricated into a hydrogel with gelatin and acrylic acid (AA) as a copolymer, and methylene bis-acrylamide (MBA) asa a crosslinking agent. Three formulations were synthesized using this combination (OG1-OG3) by varying the MBA concentration. In OG1, 0.2% MBA concentration was used; in OG2, 0.4%; and in OG3, 0.6%. The concentrations of OM, gelatin, and AA were 3.2%, 1.6%, and 24%, respectively. Ammonium persulfate is used in a concentration of 0.32% as the initiator. The other three formulations (OG4-OG6) were synthesized in the same manner as OG1-OG3, but without gelatin. MTX was used as a model drug and loaded in the hydrogel using the diffusion-driven swelling method (Hussain *et al.,* 2022). The drug loading percentage was higher in formulations with lower concentrations of the crosslinking agent. As the concentration of the crosslinking agent increased, the loading percentage decreased. This trend was observed in both formulations with gelatin (OG1-OG3) and without gelatin (OG4-OG6). The higher loading percentage in formulations without gelatin is probably due to the less dense structure of the hydrogel.

Table 1: Composition of OM-co-poly (AA) hydrogels

Formulations	OM (wt%)	Gelatin (wt%)	AA (wt%)	MBA (wt%)	APS (wt%)
OG 1	3.2	1.6	24	0.2	0.32
OG 2	3.2	1.6	24	0.4	0.32
OG 3	3.2	1.6	24	0.6	0.32
OG 4	3.2	-	24	0.2	0.32
OG 5	3.2	-	24	0.4	0.32
OG 6	3.2	-	24	0.6	0.32

Table 2: MTX loading in OM-co-poly(AA) hydrogels (mean ± S.D)

Formulations	Weight before loading (g)	Weight after loading (g)	Loading (%)
OG 1	0.572 ± 0.007	0.770 ± 0.008	25.671 ± 1.742
OG 2	0.565 ± 0.006	0.747 ± 0.008	24.353 ± 0.185
OG 3	0.565 ± 0.007	0.740 ± 0.013	23.707 ± 0.590
OG 4	0.564 ± 0.006	0.762 ± 0.010	26.017 ± 0.710
OG 5	0.563 ± 0.006	0.754 ± 0.011	25.287 ± 0.340
OG 6	0.582 ± 0.009	0.731 ± 0.003	24.021 ± 0.673

Table 3: Percentage sol-gel fraction of formulation OG1-OG6 (mean ± S.D)

Formulations	Sol fraction (%)	Gel fraction (%)
OG1	8.87	91.12 ± 0.13
OG2	7.33	92.66 ± 0.74
OG3	5.52	94.47 ± 0.40
OG4	9.07	90.92 ± 0.40
OG5	8.00	91.99 ± 0.32
OG6	6.71	93.28 ± 0.56

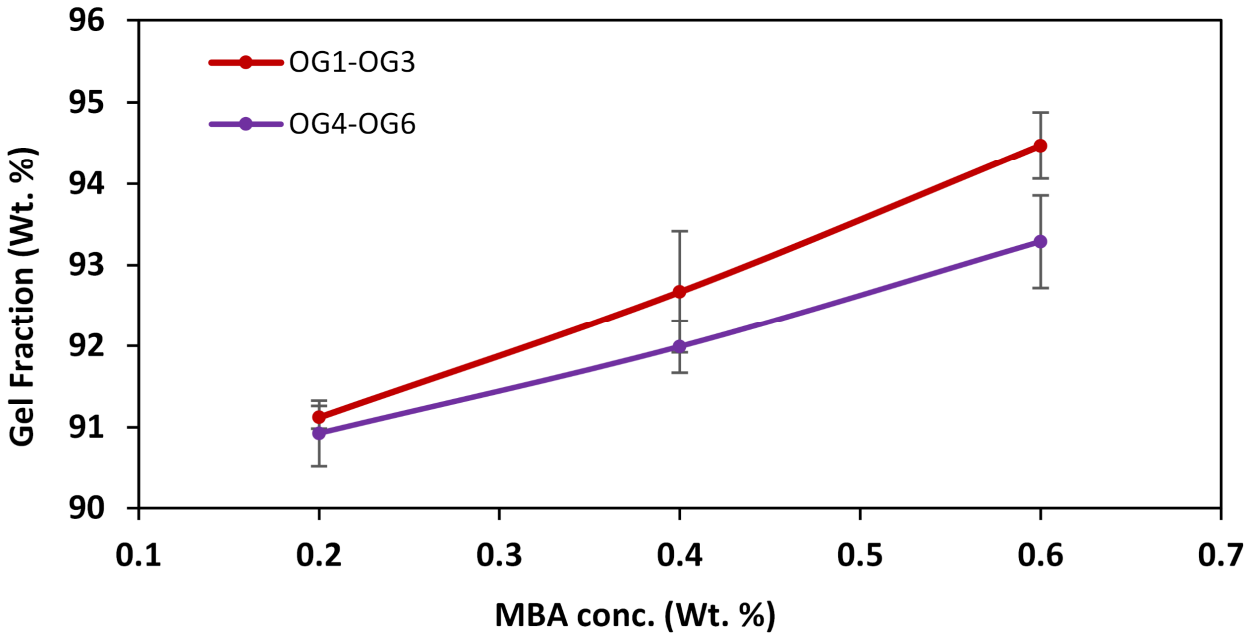

Fig. 1: Percentage gel fraction of OM-co-poly (AA) with gelatin (OG1-OG3) and without gelatin (OG4-OG6) with increasing concentration of MBA. Asterisk indicates statistically significant results (α=0.05).

Table 4: Swelling percentage of OM-Gel-poly (AA) hydrogels at pH 1.2 & pH 7.4 (mean ± S.D)

Formulations	%age swelling of hydrogels		
	pH 1.2	pH 7.4	*p-values*
OG 1	83.12 ± 2.84	1383.12 ± 79.25	5.43 e-12
OG 2	100.03 ± 11.87	999.92 ± 37.37	1.91 e-13
OG 3	74.36 ± 7.40	790.30 ± 24.71	2.87 e-14
OG 4	95.67 ± 7.47	1395.93 ± 22.17	2.80 e-17
OG 5	83.95 ± 8.40	1167.47 ± 53.75	7.84 e-13
OG 6	82.46 ± 11.07	815.51 ± 34.65	6.99 e-13

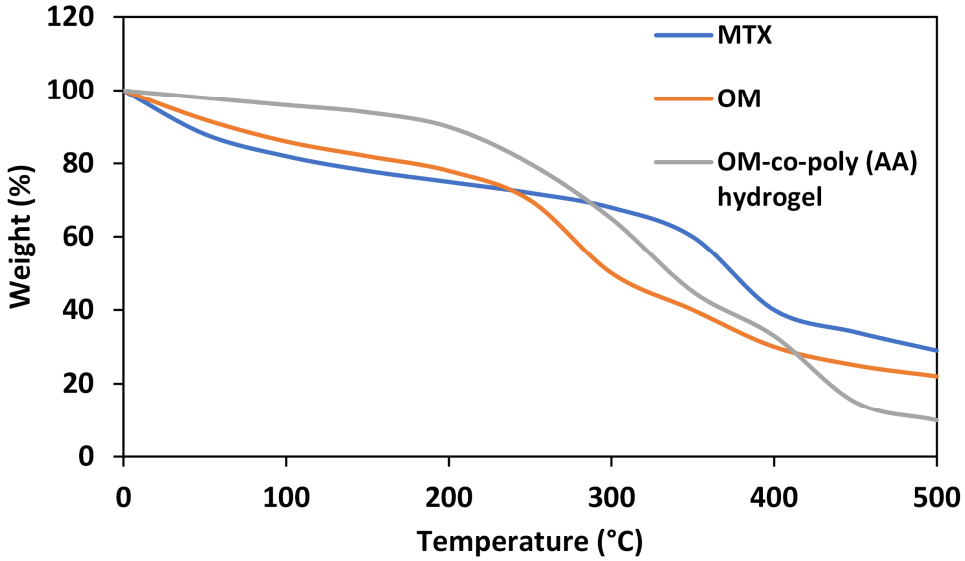

Fig. 2: Thermogravimetric analysis of MTX, OM and OM-co-poly (AA) hydrogel

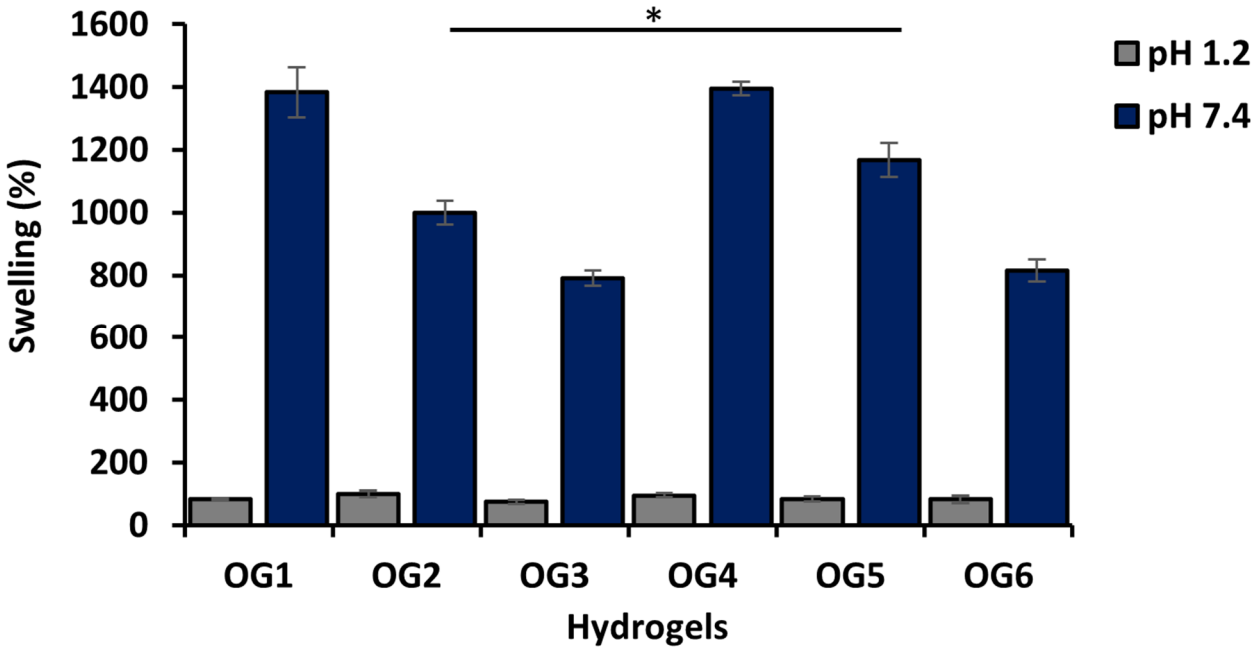

Fig. 3: Percentage swelling of OM-Gel-poly (AA) hydrogels at pH 1.2 and pH 7.4. Asterisk indicates statistically significant results (α=0.05)

Table 5: Drug release kinetics of formulations OG 1 - OG 6)

Formulations	Zero order $r^2 \pm SD$	First order $r^2 \pm SD$	Higuchi $r^2 \pm SD$	Korsmeyer-peppas $r^2 \pm SD$	n
OG 1	0.784 ± 0.0905	0.973 ± 0.319	0.967 ± 0.351	0.991 ± 0.179	0.613
OG 2	0.859 ± 0.0676	0.971 ± 0.304	0.946 ± 0.419	0.991 ± 0.171	0.666
OG 3	0.722 ± 0.0913	0.936 ± 0.436	0.983 ± 0.222	0.996 ± 0.108	0.577
OG 4	0.906 ± 0.2056	0.893 ± 0.116	0.804 ± 0.659	0.988 ± 0.159	0.329
OG 5	0.903 ± 0.1664	0.755 ± 0.840	0.957 ± 0.352	0.984 ± 0.209	0.415
OG 6	0.813 ± 0.1510	0.763 ± 0.814	0.951 ± 0.370	0.960 ± 0.333	0.437

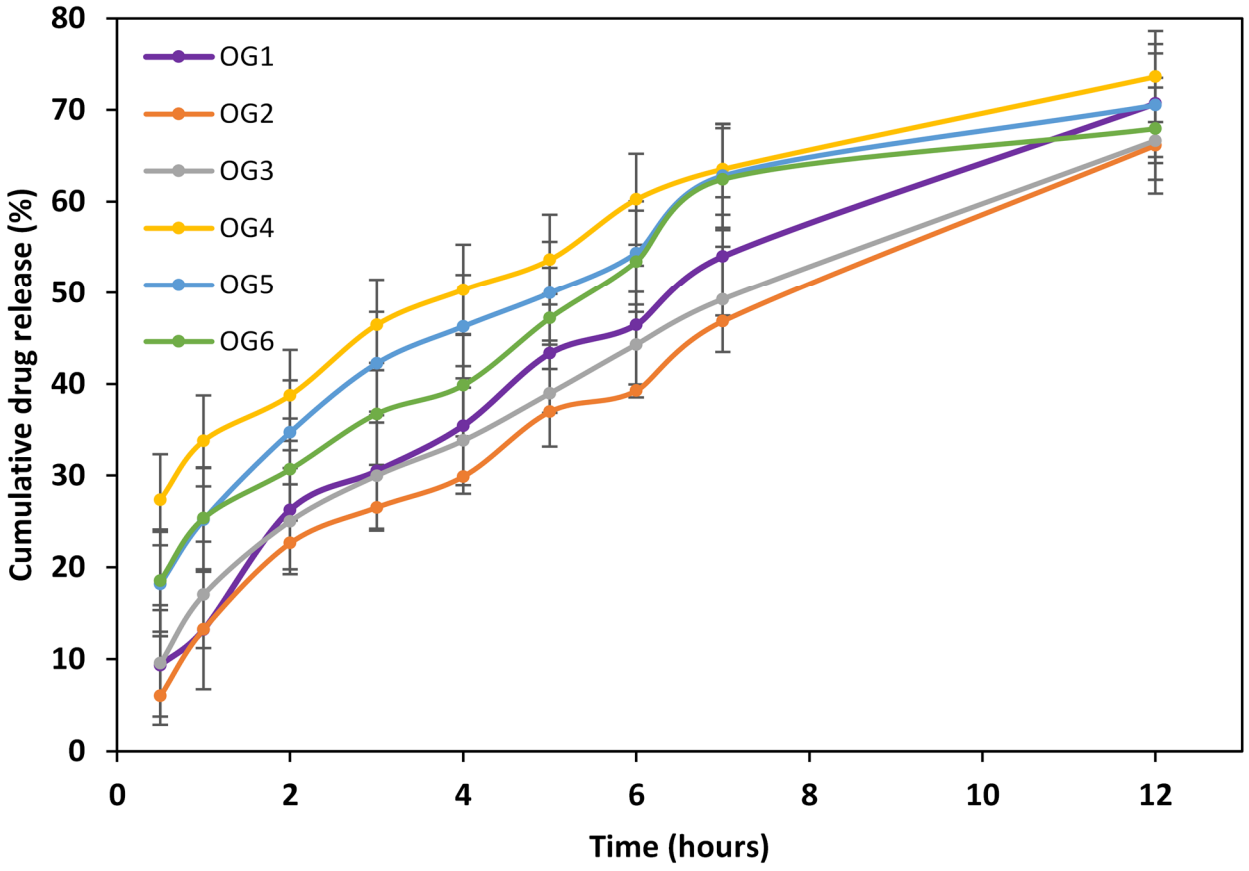

Fig. 4: Cumulative drug release (%) of different formulations of OM-co-poly (AA)-MTX

The swelling percentage of OM-co-poly (AA) hydrogels was notably higher at pH 7.4 than at pH 1.2, indicating that SIF is a suitable choice for these pH-sensitive drug delivery formulations. Swelling was much higher (very low *p-values*, Table 4) in the basic environment (pH=7.4) in comparison to the acidic environment (pH=1.2). Also, swelling was significantly different across the groups in the basic environment ($p<0.001$, Fig. 3). In a basic environment, the increase in swelling can be attributed to the electrostatic repulsion between the negatively charged -COOH group within the polysaccharide chains. This repulsion causes the polymer chain to separate, allowing more water to penetrate (Bashir *et al.,* 2018). Whereas, in an acidic environment, the hydrophilic groups do not ionize effectively, resulting in reduced electrostatic repulsion between the groups and consequently, reduced hydrogel swelling.

The SEM analysis revealed that the hydrogel had a textured surface with uneven spaces of different shapes and sizes (Shahbazizadeh *et al.,* 2022). The interconnected pores in hydrogel can allow water molecules to flow through, causing the hydrogel to expand or swell. The diffusion of water molecules into and out of the hydrogel helps to release the drug over time. MTX-loaded hydrogels showed fewer pores than unloaded hydrogels. This is because the MTX was successfully loaded into the pores of the hydrogel. However, the rough surface of the loaded hydrogel would allow solvent ingress and promote MTX release.

The FTIR of the unloaded hydrogel disc revealed distinct peaks, indicating that a successful polymeric structure was formed during polymerization. Notably, a small AA peak was observed at 2922 cm^{-1}; this peak was likely due to C-H stretching. Significant peaks were visible at 2852 cm^{-1} and 1712 cm^{-1} in the FTIR spectrum, showing the methylene stretching of gelatin and the presence of the carboxylic group (COOH) from the AA group (Hussain *et al.,* 2022). The unloaded hydrogel disc also exhibited alkenyl stretching (C=C) at approximately 1650 cm^{-1}. The presence of Okra gum polysaccharides is responsible for the distinct bands observed in the wavenumber range of 1016-1553 cm^{-1}. The loaded OM-co-poly (AA) hydrogel disc exhibits an MTX peak at 3130 cm^{-1}, indicating that the drug is stable in the hydrogel mixture.

During the identification of non-crosslinked polymers in the formulations by sol-gel analysis, the sample OG3 exhibited the highest sol-gel fraction, reaching 94.47%. This can be attributed to the elevated MBA concentration and the presence of gelatin. On the other hand, sample OG4 demonstrated the lowest sol-gel fraction, primarily due to the absence of gelatin and the low concentration of the crosslinker (Akhlaq *et al.,* 2021). It was observed that increasing the concentration of the crosslinking agent increases the gel fraction, and vice versa. Also, the presence of gelatin polymer contributes to a higher gel fraction than in hydrogels without gelatin.

During thermal analysis, the MTX thermogram exhibited three distinct degradation patterns as the temperature increased. At first, MTX at 70°C lost weight primarily due to water evaporation. At 320°C, a phase change and functional group degradation were observed, resulting in a considerable weight loss. However, it was shown that between 350°C and 490°C, MTX remained steady. The TGA findings of OM revealed a small mass loss at 80°C, likely due to water evaporation. However, above 250°C, a more considerable weight loss occurred, demonstrating the significant durability of Okra samples against high temperatures. When exposed to high temperatures, the TGA of the OM-co-poly(AA) hydrogel mixture showed an initial mass loss below 180°C, with a second mass loss occurring between 270°C and 330°C. Significant loss was seen at 375°C, which shows that the formulation completely decomposed (Dharmalingam and Anandalakshmi 2019). It was observed that OM-co-poly (AA) showed weight loss at relatively higher temperatures than the individual components. These findings demonstrate the remarkable thermal stability of the blended OM-co-poly(AA) hydrogel.

The release behavior of these formulations, when evaluated by zero order, first order, Higuchi and Korsmeyer-Peppas model, was best described by the Korsmeyer-Peppas model. These results indicate that all formulations followed the Korsmeyer-Peppas release model and exhibited both diffusion and dissolution mechanisms. Formulation 3 was selected for its highest regression coefficient (0.996) among the formulations. The value of "n" showed the release mechanism, where the values between 0.49 and 0.89 represent the non-Fickian release mechanism, which efficiently demonstrates how the active drug is released through gels (George *et al.,* 2020).

Based on the *in-vitro* release profiles, it is hypothesized that the OM-co-poly (AA) hydrogel formulation would likely decrease the peak plasma concentration (Cmax) of MTX compared with immediate-release formulations. The sustained release would also be expected to increase the time to reach peak concentration (Tmax), resulting in a more prolonged therapeutic effect. The Korsmeyer-Peppas release kinetics suggest a diffusion-controlled mechanism, which would lead to slower, more sustained MTX release, thereby affecting Cmax and Tmax.

CONCLUSION

The OM-co-poly (AA) hydrogels developed in this study demonstrate pH-dependent swelling and controlled release of MTX *in-vitro*. The swelling index increased with higher MBA concentrations and the presence of gelatin. The drug release kinetics suggested controlled release of the drug from the polymeric system, following a non-Fickian diffusion mechanism. TGA results showed a high degree of heat stability of the developed hydrogel. In conclusion, the OM-co-poly (AA) hydrogels show potential for the controlled release of MTX and other drugs. While these results are promising, further studies are needed to evaluate the *in-vivo* performance and toxicity of these hydrogels before they can be considered for clinical applications.

Acknowledgments
The authors extend their appreciation to the Deanship of Scientific Research at Northern Border University, Arar, KSA, for funding this research work.

Authors' contributions
MK: Performed data curation, investigation, resources, and writing – original draft; MIS: Performed extraction, reviewed and edited the manuscript and managed resources; SS: Performed FTIR analysis and interpreted the results; MT: Contributed to the development of hydrogels and manuscript revision; RK: Developed hydrogels and performed characterization; RS: Contributed in hydrogels development and characterization and also contributed in writing initial draft; AI: Facilitated in sol-gel, thermal stability and SEM analysis. AJ: Performed drug release kinetics; HAN: Supervised study, managed resources and provided all technical guidance; MU: Supervised study and finalized the manuscript.

Funding
Funding was provided by the Higher Education Commission (HEC) of Pakistan via grant No. 20-14989.

Data availability statement
The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Ethical approval
This study did not involve human participants, animals, or any *in-vivo* experiments; therefore, ethical approval was not required.

Conflicts of interest
There was no conflict of interest in performing this study.

Supplementary data
https://www.pjps.pk/uploads/2026/06/SUP1780405943.pdf

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