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Original article

Synthesis and characterization of Guar gum based biopolymeric hydrogels as carrier materials for controlled delivery of methotrexate to treat colon cancer

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ABSTRACT

Guar Gum has been evaluated for its importance in food and pharmaceutical industry. A blended biopolymeric hydrogel was prepared by solution casting technique using guar gum (GG), chitosan (CS), polyvinyl alcohol (PVA), chemically crosslinked with tetra orthosilicate (TEOS) and impregnated with methotrexate (MTX) to assess its drug carrying capacity against colon cancer (HCT-116). The surface morphology, chemical bonding, hydrophilicity and water absorbing capacity were analyzed by atomic force microscopy (AFM), Fourier transform infrared spectroscopy (FTIR), contact angle measurements and swelling properties in variable conditions. Furthermore, degradation, drug release kinetics, hemocompatibility, and cytotoxicity of MTX-loaded hydrogel was tested. The release of MTX from GG/CS/PVA biopolymeric blend occurred in sustained manner. Results displayed that in 7 h 25 min duration 96% of the drug was released in phosphate buffer saline (PBS) at pH 7.4. These blends were non-hemolytic, and antiproliferative against HCT-116. Furthermore, the MTT assay has revealed that MTX-loaded hydrogel had prominently decreased the cell viability (with IC50 11.7 μg/ml) as compared to free MTX (with IC50 21.57 μg/ml). Hence, these results suggest that guar gum based hydrogels are potential biomaterials for colon cancer treatment.

1. Introduction

Natural occurring polymers are abundant, biocompatible and biodegradable with modifiable properties. For many years drug delivery system based on polymer is becoming a popular tool for controlled and targeted drug delivery. Hydrogels, rods, wafers, drug-eluting films, and nanoparticles are examples of polymer delivery vehicles. It ensures drug bioavailability to the target site, improved drug solubility, and fewer systemic adverse effects (Liow et al., 2016). Polymeric hydrogels have gained attention in the past few years as ideal drug carriers and tools for tissue engineering, regenerative medicines, and biomedical applications (Dou et al., 2014; Drury and Mooney, 2003; Jerome and Croisier, 2013; Toh and Loh, 2015). These hydrogels swell and gain size without dissolving in water (Kopeček and Yang, 2007). Hydrogel mechanical strength can occasionally be impaired despite their high absorption capacity; however, this can be fixed by the use of appropriate crosslinkers. Crosslinkers enhance porosity, mechanical properties, 3-D structure, an affinity for an aqueous environment, and drug carrying ability Sugar monomers are linked together to form large molecules in natural gums. Which are easily accessible, abundant, non-toxic, and cheap (Rana et al., 2011). Most of the active compounds in medicines have been derived from the natural products. 80% of pharmacological compounds were obtained from a natural compound (Selamoglu, 2018). Guar gum (GG) is a non-ionic high molecular weight branched polymer with a ratio of 2:1 (linear β(1–4) mannose and α(1–6) galactose) obtained from Cyamopsis tetragonoloba. GG forms a viscous solution at low temperatures, capable of swelling, and stable between pH 5–7 (Iqbal et al., 2020). It is a unique thickener and stabilizer

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because of its ability to make hydrogen bonds with water and makes aqueous solution that is highly viscous. Due to these characteristics, it has numerous uses in the food, pharmaceutical, textile, oil, paint, paper, explosive, and cosmetic sectors. Its low price is another factor contributing to its industry-wide popularity (Mudgil et al., 2014). It is rich in several essential minerals and phytochemicals, including saponin and flavonoids (Hassan et al., 2013). Among the numerous and varied phenolic compounds originating from the higher plants, flavonoids are the largest and most diversified category. These are strong bioactive chemicals which have antioxidant (Selamoglu, 2017a) and anticarcinogenic properties inhibiting the onset growth and cancer progression by regulating cellular proliferation, apoptosis and metastasis (Selamoglu, 2017b).

Chitosan (CS) is a non-toxic, biocompatible, and biodegradable compound composed of N-acetyl-D glucosamine and D-glucosamine subunits. It possesses high water and fat binding capacity, as well as antibacterial and antifungal properties (Kweon et al., 2003). CS is stable between pH 0.6 to 7.4 (Fan et al., 2009). Therefore, it can be used to make blends with synthetic polymers PVA as an example (Bahrami et al., 2003). Poly Vinyl Alcohol (PVA) is a water soluble synthetic polymer comprising of film forming ability and excellent tensile strength. Due to its non-toxicity and high biodegradability PVA has been extensively used in pharmaceutical and biomedical applications. It has been proven to be effective in site-specific controlled drug delivery system (Jaganathan et al., 2019) which dissolves more readily in hot water than in cold water. To overcome this hindrance a crosslinker can be added. For this purpose tetraethoxysilane (TEOS) is an ideal crosslinker of polymers. Compared to previously used cross-linkers such as glutaraldehyde, epichlorohydrin, borate, and tripolyphosphate, it is new and safe. The polymers employed as biomaterials are frequently cross-linked with TEOS (Khan et al., 2021).

The combination of CS and PVA possesses good mechanical strength and shows non-toxic, biocompatible, and biodegradable behaviour (Wei et al., 2009). These properties can be enhanced by the addition of guar gum. Since it can establish hydrogen bonds with water molecules, it is widely used as thickener stabilizer. In addition it helps in the treatment of various health related problems such as bowel irregularities, diabetes, heart disease and cancer (Mudgil et al., 2014).

In the current study, GG, CS, and PVA were used to make biopolymeric Methotrexate (MTX) loaded hydrogels with three different doses of tetraethoxysilane (50 µl, 100 µl, and 150 µl) as a cross-linker. MTX is a potent chemotherapeutic drug that is presently in use to treat colon cancer, head and neck cancer, lung cancer, breast cancer, and choriocarcinoma (Bleich et al., 1975). In addition, MTX is a strong anti-inflammatory drug that can be used to treat inflammation in future studies.

We sought to develop a combination of natural and synthetic polymers (GG/CS/PVA) while keeping in mind the benefits of on-site drug delivery and the drawbacks of chemotherapeutic treatment. A good biodegradable and biocompatible, pH-sensitive blended hydrogel using a solution casting method for sustained drug release which can support onsite delivery of methotrexate (MTX) against colon cancer (HCT-116). An excellent biodegradable and biocompatible, pH-sensitive blended hydrogel has prepared by using solution casting technique for prolonged drug release that can allow onsite administration of methotrexate (MTX) against colon cancer (HCT-116).

2. Material and methods

2.1. Materials

GG, CS, PVA, and TEOS (Merck Germany). Ethanol, acetic acid, PBS, Sodium chloride (NaCl), Hydrochloric acid (HCl), Sodium hydroxide (NaOH), Triton x-100, Dimethyl sulfoxide (DMSO), (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) (MTT) (Sigma Aldrich). Fresh RBCs from the donor, RBC solution, Desiccator, and Distilled water (DW) were taken from the research lab at Government College University Faisalabad (GCUF), and Methotrexate was purchased from Mehboob Medicals Allied Hospital Faisalabad.

2.2. Methods

2.2.1. Synthesis of hydrogel

2.2.1.1. Preparation of GG/PVA/CS hydrogel. GG, CS, and PVA based hydrogels were prepared by previously reported solution casting method (Khan et al., 2021). The concentration of the crosslinker (TEOS) was varied while PVA was kept the same. In brief, 1 g polymeric gel was cast in which CS (0.2 g, 0.4 g, and 0.6 g) was dissolved in 30 ml distilled water, acetic acid (2%), and stirred for 1 h at 300–350 rpm at 50℃ to get a clear solution. A consistent amount of PVA (0.2g) was added to 10 ml of deionized water and heated at 80℃. GG (0.2g, 0.4g, and 0.6g) was dissolved in 20 ml deionized water and stirred for 2 h at 50℃ to obtain GG solution and then added into CS solution. To get a homogenized mixture blended solution was stirred at 55℃ for 1 h. Different concentrations of TEOS (50 µl, 100 µl, and 150 µl) were mixed with ethanol (5 ml) and added into the blend of GG/CS/PVA drop-wise and stirred for 3 h at 55℃.

2.2.2. Fabrication and formulation of hydrogel with crosslinker

Different concentrations of Guar Gum and Chitosan were mixed to make a blend with a consistent amount of PVA named F0, F1, F2, and F3 (Table 1). Various amounts of TEOS were added to the blend and formulations were named F1a, F1b, and F1c.

After the addition of the cross-linker, the blended mixture was stirred for 3 h at 50℃ and then hydrogel was poured into the petri dish and stored at room temperature. To obtain dry films, hydrogels were kept in a vacuum oven at 55℃ for 4 h. Dried films were kept in plastic bags for future analysis.

2.2.3. Characterization

2.2.3.1. Swelling studies.

2.2.3.1.1. Swelling analysis. Swelling analysis was performed in PBS because it mimics the natural environment, Distilled Water (DW) to show the hydrophilic properties, acid and basic media, and at different temperatures to verify the behavior of hydrogels in a cancer environment. The procedure of swellings is as follows:

2.2.3.1.2. Swelling in PBS. The sample was weighed after being cut into 1 cm pieces of dried hydrogels. Following this, the pieces were maintained in Petri dishes with PBS solution for 10 min until equilibrium was attained (Khan et al., 2021). PBS was removed and the swelled hydrogel was dried and weight was noted after 10 min regular intervals. The experiment was carried out three times for precise results. The equation below was used to determine swelling %.

$$Swelling\% = \frac{Ws - WD}{WD} X100 \quad (2)$$

Where W_S and W_D represents the swollen and dried weights of the prepared hydrogels respectively.

2.2.3.1.3. Swelling at different temperature and pH. Small pieces of dried hydrogels were taken and dipped in solution with varying pH (4, and 10) and temperatures (37°, and 43°). Readings of the swollen hydrogels were taken after each 10 min and the fluid was replaced each time. The experiment was carried out three times for a precise outcome. The swelling % was calculated by using the formula (1).

Table 1
Formulations used for the preparation of hydrogels.

Formulations	GG	CS	PVA	Cross linker (TEOS)
F0	0.1 g	0.7 g	0.2 g	0
F1	0.2 g	0.6 g	0.2 g	0
F2	0.4 g	0.4 g	0.2 g	0
F3	0.6 g	0.2 g	0.2 g	0
F1a	0.2 g	0.6 g	0.2 g	50 µl
F1b	0.2 g	0.6 g	0.2 g	100 µl
F1c	0.2 g	0.6 g	0.2 g	150 µl

2.2.4. FTIR spectrum

FTIR spectra were taken using a Shimadzu IR Prestige-21 in transmission mode with a 4000–600 cm^{-1} wavenumber range to reveal the molecular structure information of the hydrogel. A total of 100 scans were performed for each spectrum at a resolution of 4 cm^{-1} with air being the background.

2.2.5. Atomic force microscopy (AFM)

AFM of the prepared hydrogels was performed using Nano-Solver, NT-MDT, Russia, to examine the surface roughness. For this, the dried hydrogel was placed in the sample holder, and the silicon nitride tip was used to scan the desired area of the sample in ambient conditions. The selected area of hydrogel 5 µm × 5 µm was analyzed using Nova-Px software.

2.2.6. Contact angle measurement

For wetting analysis, static contact angle measurements were taken using (SEO-Phoenix-MT(A), Korea) in ambient conditions. The three different test surfaces were used to obtain the contact angle results. Measurements were taken automatically within a few seconds after placing the droplet. The average contact angle was calculated from the left and right contact angles.

2.2.7. Drug loading into GG/CS/PVA hydrogel

Synthesized hydrogel GG/CS/PVA was loaded with Methotrexate (MTX). The solution of MTX was prepared in distilled water with a concentration of 1 mg/ml. MTX was added dropwise and stirred the mixture for 1 h at 55℃. TEOS was added dropwise into the polymeric mixture and stirred at 55℃ for 3 h. Drug-loaded hydrogel was then poured in to the Petri dish and allowed it to dry at 55℃ in an oven to obtain dry film. To prevent contamination, the drug loaded hydrogel was stored in plastic bag for further drug release analysis.

2.2.8. Drug release analysis

At 37 °C, the dried (drug loaded) hydrogel was dipped in a 100 ml PBS solution. 5 ml solution was replaced every 10 min to maintain the volume. Sample collection was continued for 360 min. The absorbance was calculated by using UV–visible spectroscopy at a wavelength of 321 nm (wavelength of MTX).

2.2.9. Drug release kinetics

Drug release kinetics was used to evaluate the drug release in PBS pH 7.4 at 37 °C. The mathematical models listed below were used to assess release kinetics.

$$Zero-order: Mt = Mo + Kot \quad (2)$$

$$Firstorder: logCo - kt/2.303 \quad (3)$$

$$Korsmeyer-Peppas: model: lnMt/Mo = nlnt + lnK \quad (4)$$

$$Higuchimodel: ft = Q = KH \times t\frac{1}{2} \quad (5)$$

2.2.10. Hemolysis

Blood from a healthy donor was centrifuged at 3500 rpm for 5 min to separate the red blood cells (RBCs), which were then twice washed in PBS. For 5% (v/v) suspension, blood cells were suspended in PBS. The hydrogel was added to this (100 µl) suspension of RBCs. PBS was used as negative and Triton-X was used as the positive control. The suspensions were incubated for 1 h at 37C and then centrifuged again at 3500 rpm for 5 min (Hoque et al., 2017). The supernatant was transferred into the cuvettes and OD was recorded at 540 nm. % of hemolysis was calculated as follows:

$$\%\text{hemolysis} = \frac{A - Ao}{Atotal - Ao} X100 \quad (6)$$

Where A = Absorbance of the hydrogel, A_O = absorbance of negative control, A_{total} = absorbance of positive control.

2.2.11. Cell culture

The cells were grown in DMEM along with 10% FBS and 20ul/ml penicillin–streptomycin. The cultures were incubated at 37 °C in a humidified atmosphere of 5 % CO_2. Before testing, cells were kept under logarithmic growth conditions and allowed to reach 70 percent confluence (Rasul et al., 2012).

2.2.11.1. Cell viability assay. The anti-proliferation activity of various cell lines in the presence of MTX as an anti-cancer medication was tested using the micro tetrazolium (MTT) assay. The MTT test is a colorimetric technique that uses live cells to convert tetrazolium salt to formazan. The cells (Hct-116) were collected and counted using a hemocytometer in a cell culture flask. In each well of a 96-well plate, 1x104 cells were planted, and 100 l medium was added. The 96-well plate was incubated at 95 percent humidity, 5% CO2, and 37Celsius until 70–80% confluency was achieved. A 1X PBS solution was used to wash every well of the 96-well plate. Cells were treated with a variety of conditions DMSO as a control, F1b hydrogel loaded with MTX by serial dilution. The cells were cultured for 24 h after being exposed to various environments. In each well of the 96-well plate, 100 l solubilization buffer was added while formazan crystals developed during incubation. To accomplish full solubilization, the culture plate was gently swirled. The absorbance at 570 nm wavelength was measured using an ELISA plate reader (Rasul et al., 2012).

2.2.12. Statistical analysis

The results were statistically analysed using ANOVA. The data was presented in triplicates (n = 3 and p-value < 0.05).

3. Results

3.1. Swelling analysis

Swelling studies have been performed to determine the swelling behavior of synthesized hydrogels. Different swelling patterns of prepared hydrogels with various concentrations of GG have been shown in (Figs. 1a–1d). Swelling tests were carried out in different media (PBS and distilled water), at different temperatures (37 °C and 43 °C), and at different pH values i.e. 4 and 10 respectively. Among these hydrogels (F0, F1, F2, and F3) the best results were shown by F1 which was further named (F1a, F1b, and F3c) based on the addition of various concentrations of crosslinker TEOS (50 µl, 100 µl, and 150 µl). A gradual increase in swelling can be seen by F1. A maximum swelling peak of 2232% was obtained in PBS afterward swelling rate was decreased. In distilled water (DW) an abnormal pattern was noticed by F2 and F3 whereas no prominent swellings were shown by F0 (Fig. 1a). F1 showed consistent swelling behavior in all media and at 37 °C and 43 °C (Fig. 1b).

At pH4 and 10, F1 showed a consistent swelling rate (Fig. 1c). This result explains that hydrogel was pH-sensitive in an acidic medium.

Based on excellent swelling results F1 was used for further analysis by adding various amounts of TEOS and named F1a, F2b, and F3c (Fig. 1d). The development of bonds between the chains of a hydrogel network was caused by an increase in –OH groups as a result of cross-linking agents, which might result in the maximum swelling when the amount of cross-linking agents was increased (100 µl). But more increase in the amount of crosslinker (150 µl) resulted in a decreased swelling. The swelling study was carried out three times, and the outcomes were consistent.

3.2. FT-IR analysis

A variety of functional groups can be found using FT-IR, which is also sensitive to changes in molecule structure. It offered details based on the formulation's chemical make-up and physical state, such as the key modifications brought about by polymerization or cross-linking.

FT-IR spectra of prepared hydrogels F1 a, b and c are shown in (Fig. 2). Broadband observed between 3212 and 3258 cm^{-1} is attributed to –OH stretching, while characteristic peaks of –CH, and C-O-C correspond to 2920 and 1246 cm^{-1}. The two characteristic peaks at 1400 and 1500 cm^{-1} show –NH stretching. The presence of siloxane linkage at 1065 cm^{-1} confirms the formation of intermolecular hydrogen bonding between chitosan, PVA, and Guar gum along TEOS.

3.3. Atomic force microscopy

AFM technique is used to investigate the surface roughness of hydrogel F1b. The average surface roughness and root mean square roughness was calculated to be 5.474 nm and 7.397 nm, respectively (Fig. 3). The low surface roughness resulted from the appropriate quantity of cross-linker TEOS and was further lowered with the addition of guar gum.

3.4. Wetting analysis

Wetting analysis is required to determine the hydrophobicity or hydrophilicity of the hydrogel, which is an important parameter in drug delivery and proliferation. The contact angles measured for hydrogels F0, F1, F2, F3 are 107°, 104° and 106° (Fig. 4). The hydrogels F1a, F1b and F1c contains different amount of TEOS (cross-linker). It is observed that adding TEOS sufficiently increased the hydrophilicity of the gels as the contact angle reduced greatly as compared to F1, F2 and F3 hydrogels. The water contact angle measured for hydrogels F1a, F1b and F1c are 71.28°, 76.58° and 95.77°, respectively. The reason is the addition of TEOS cross-linker that encourages more compact cross-linking (Thakur et al., 2018) and hence resulting in lower water contact angle and increased hydrophilicity.

3.5. Hemolysis

Hemolytic analysis was performed to check the compatibility of hydrogel with mammalian cells. The hemolysis % in relation to the very toxic surfactant triton-X was measured after hydrogel was exposed to human erythrocytes for hemocompatibility analysis as described before (Hoque et al., 2016). Remarkably, hydrogel F1b showed negligible hemolysis (hemolytic index = <5%) (Fig. 5). This showed that hydrogel is hemocompatible against human red blood cells. For better intuition, treated erythrocytes were also observed under the microscope. Treated cells had the typical round morphology of healthy blood cells whereas the positive control (TX-100 and Distilled water) showed zero survival, no cells were observed under the microscope.

3.6. In vitro drug release analysis

The release of the drug was analyzed in PBS at 7.4 pH (Fig. 6) shows the graph between time and % cumulative release. Almost 50% drug release by F1b was observed in the first 5 h and a sustained drug release of 96% in 7.25 h. This result makes F1b hydrogel a promising applicant for slow-controlled drug carrier. A UV–visible spectrometer was used for measuring the absorbance. The calibration curve defines the cumulative drug release.

3.7. Drug release kinetics

To comprehend drug release behaviour, mathematical models (Equations (2) – (5)) were used to evaluate the drug release kinetics. The results are shown in Fig. 7. The polymeric structure of controlled and sustained drug release is supported by hydrogel. Drug release kinetics are affected by degradation and swelling, and various fitting models (such as zero and first order, Higuchi, and Peppas) were used to study curve fitting. The MTX-loaded hydrogel

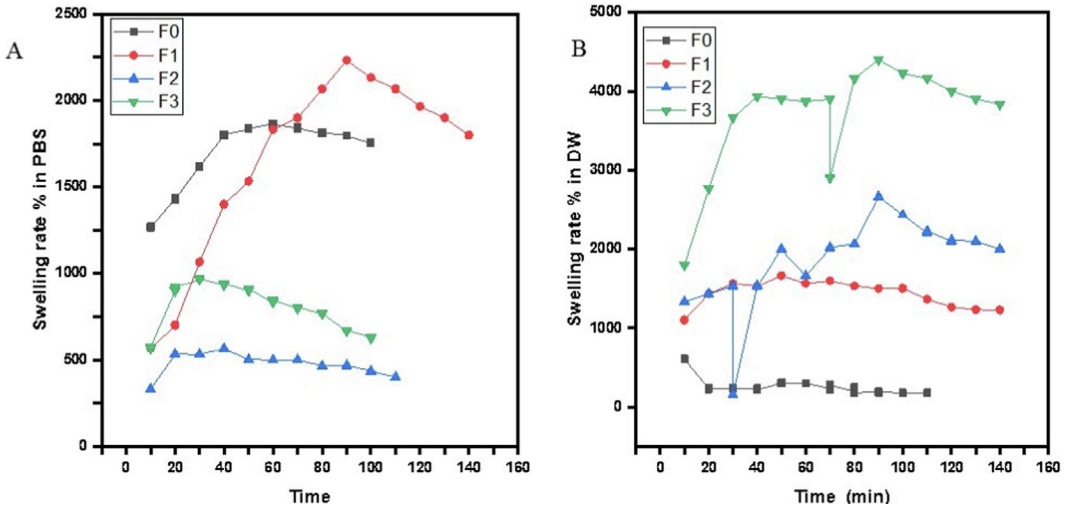

Fig. 1a. Swelling rate % (A) in PBS and (B) in Distilled Water.

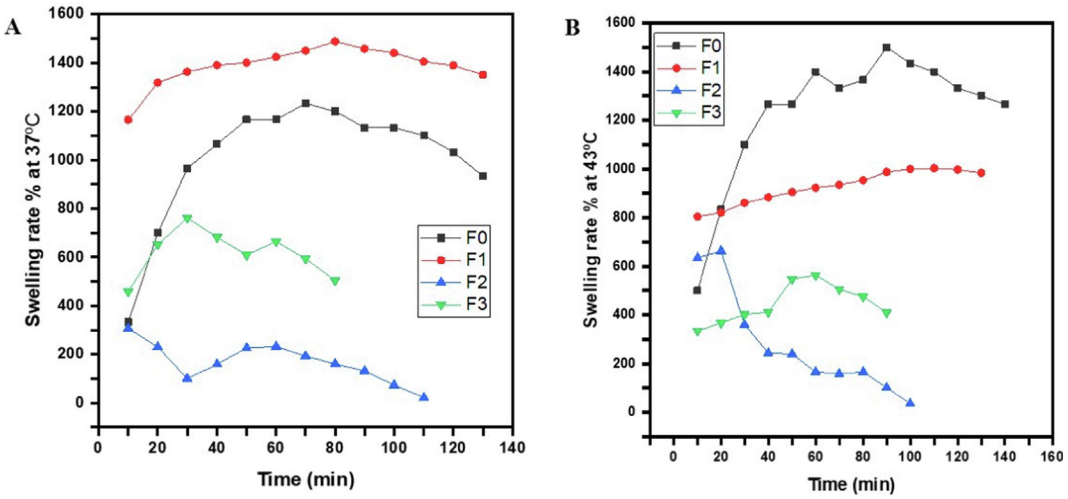

Fig. 1b. Swelling rate % (A) at 37 °C, (B) at 43 °C.

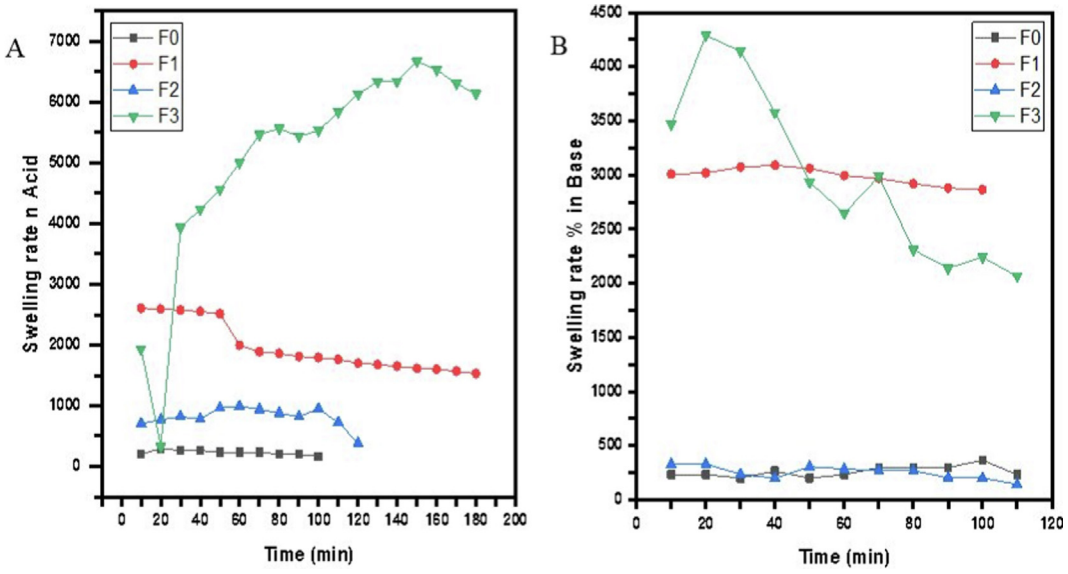

Fig. 1c. Swelling rate % at different pH (A) pH4 (B) pH10.

had various kinetic release behaviours, in Table 2 regression coefficient (R^2 = 0.9835) is presented with other data.

3.8. Cell viability assay

MTT assay was performed to evaluate the antiproliferative effects of MTX-loaded hydrogels. HCT-116 cells were cultured in a medium that contains different concentrations of free MTX, F1b, and F1b + MTX to see if F1b has improved therapeutic effectiveness. Our results (Fig. 8) exhibited (>50% cell viability) measured for F1b at the concentration of 225 µg/ml. free MTX has an IC50 of 21.5 µg/ml. A large amount of MTX is used to get reach its target due to its passive movement through the plasma membrane (De Graaf et al., 1996; Morsy et al., 2022). Whereas F1b + MTX has shown the synergistic effect of 50% growth inhibition (with IC50 11.7 µg/ml) at concentration of 2.34 µg/200 ml. Hence it can be concluded by our results that F1b hydrogel enhances the drug efficacy in meager amounts.

4. Discussions

In the current study, we have synthesized and characterized Guar Gum based biopolymeric hydrogel as a vehicle for controlled drug delivery of Methotrexate to treat colon cancer. Guar legumes are enriched with flavonoids and other numerous polyphenolic compounds which are having strong antioxidant properties potentially important in various diseases such as chronic.

fatigue syndrome, myocardial infarction, Parkinson's and Alzheimer's disease (Hassan et al., 2013; Selamoglu, 2018).Presence of multiple hydrophilic groups such as –OH, -SO3H, –NH2, –COOH, etc. as well as osmotic pressure and capillary action, enable hydrogels to absorb significant amount of water (Ullah et al., 2015).

We characterized the prepared hydrogels via swellings at different conditions, FT-IR, AFM, wetting analysis. Drug release kinetics, hemocompatibility, and cytotoxicity of MTX-loaded hydrogel against HCT-116 was analyzed.

Hydrogel expands and begins to absorb water when its polymeric network is introduced to a solvent. External stimuli (such

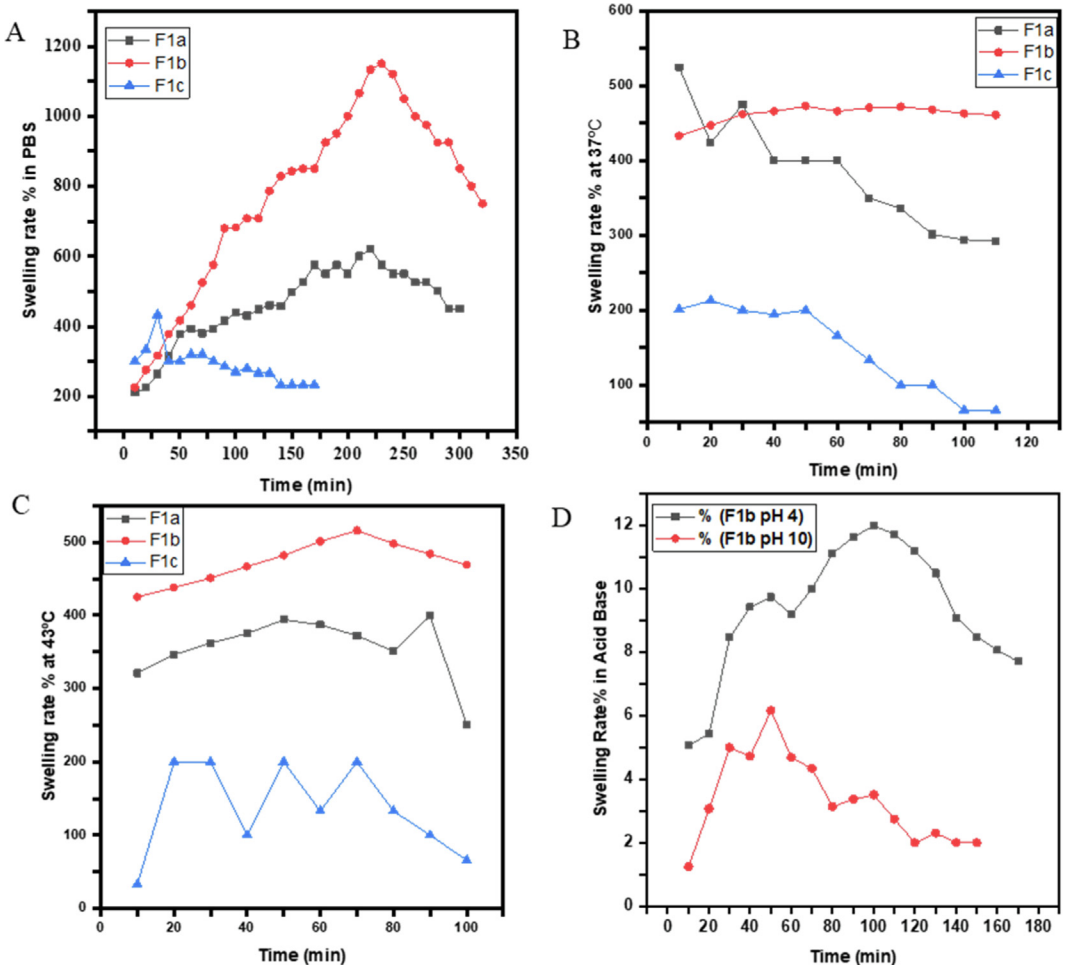

Fig. 1d. **(A)** Swelling rate % of F1a, F1b, and F1c (A) in PBS with different concentrations (50 µl, 100 µl, and 150 µl) of TEOS, (B and C) Swelling rate % at different temperatures (37 °C and 43 °C), (D) Swelling rate % of F1b at different pH.

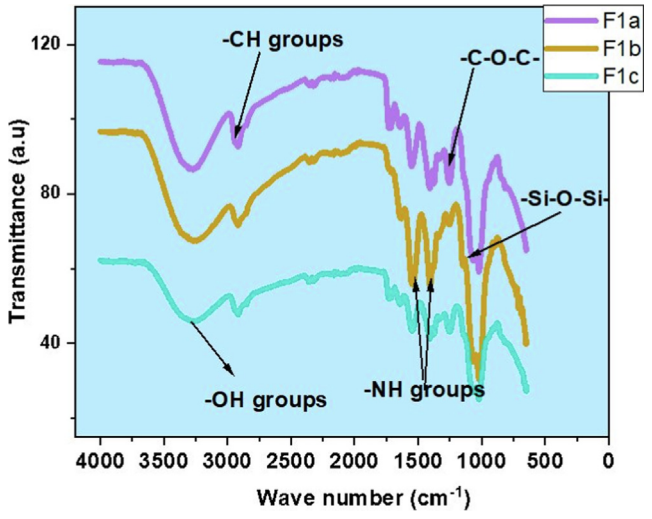

Fig. 2. FTIR spectra of F1a, F1b and F1c hydrogels.

as temperature, pH and ionic strength) have an impact on how hydrogel swelling behaves. Since hydrogels have a large surface area, they respond quickly to swelling capacity and enable greater fluid interactions with the environment. Among the prepared hydrogels (F0, F1, F2 and F3), F1 has been shown the fascinating swelling results. Iqbal et al., reported a hydrogel blend made of low concentration guar gum with a prominent porous structure which is promising for future biopolymeric hydrogels (Iqbal et al., 2020). Goyal et al., prepared different formulations of GG and CS. In this study he preferred GG coated compositions as they have comparable viscosity, fluidity, and moisture-keeping properties (Goyal et al., 2015). It is concluded that GG is an excellent candidate for keeping moisture content.

After the addition of different concentrations (50 µl, 100 µl, and 150 µl) of TEOS in F1, hydrogels were named (F1a, F1b and F1c). F1b has shown the consistent swelling behavior in PBS and Distilled water, at different temperatures (at 37 °C and 43 °C), and at different pH (4 and 10). The gaps between the hydrogel network reduce by increasing the amount of crosslinker. Maximum quantity (0.02%) of TEOS resulted in the reduced swelling. Because water molecules couldn't pass through the hydrogel's polymeric structure, the swelling was reduced (Khan et al., 2021). This tendency was in line with the typical pattern of decreased swelling with rising TEOS concentration. Generally, it can be assumed that the degree of crosslinking affects the hydrogel's capacity to swell (Zhou and Wu, 2011).

A gradual increase in swelling pattern has been observed at pH 4 which confirms the pH sensitivity of the hydrogel in an acidic medium. The pH-dependent release behaviour of the drug entrapped in hydrogel can be linked to increased swelling rate in acidic environments is volume-dependent (Khan et al., 2021). In pH-dependent hydrogel, the cationic group of Chitosan is responsi-

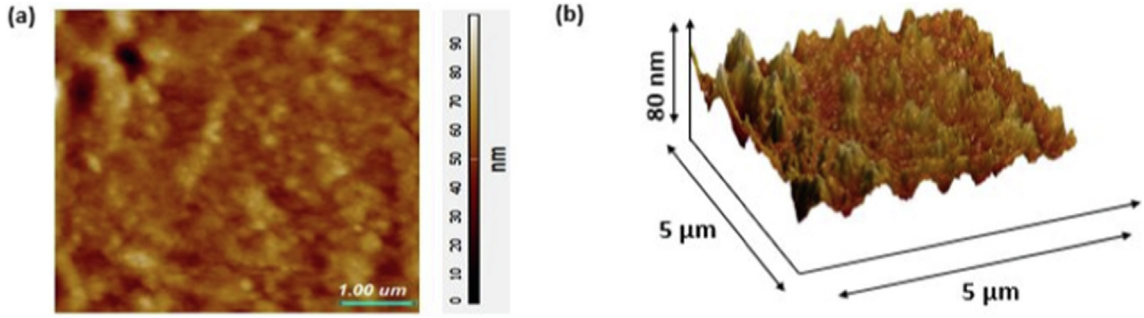

Fig. 3. AFM images of the prepared hydrogel F1b.

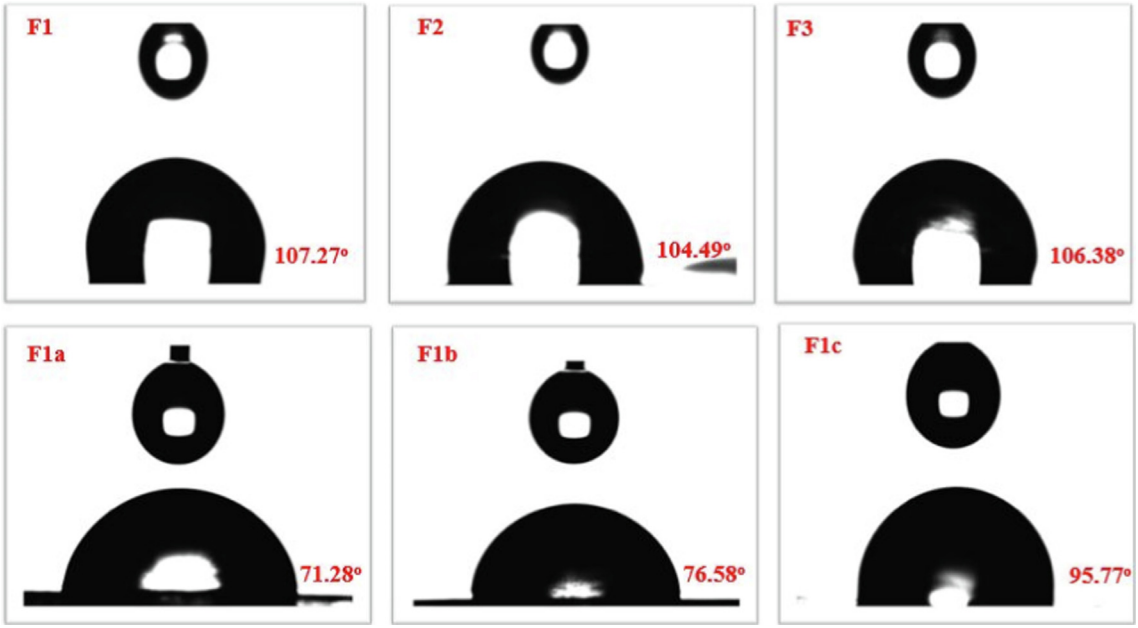

Fig. 4. Wetting analysis of the prepared hydrogels.Fig. 5**:** Hemolysis analysis of F1b hydrogel. (A) Graphical representation, n = 3, results are significant with $p < 0.05$. (B, C) Representative pictures of hemolysis. (i) + ve control (ii) Hydrogel F1b, (iii) –ve control.

ble for swelling. At acidic pH, the swelling was increased because of the positive charge of the polymeric network due to the protonation of NH3+ (Rizwan et al., 2017). A significant increase in the swelling behavior of hydrogel can be linked to pH-dependent properties. An acidic environment enhances the swelling ratio and the release behavior of entrapped drugs is improved (Khan et al., 2021).

Our FT-IR results show the presence of GG, CS, PVA and TEOS. The –OH/–NH2 molecular hydrogen bonds in Chitosan and Guar Gum respectively are visible in the broadband, 3000–3500 cm^{-1}. (Mansur et al., 2009; Maqsood et al., 2020). Successful crosslinking of TEOS with CS, GG and PVA had been confirmed when the presence of Si-O-Si was indicated by the absorbance peak at 1065 cm^{-1}.

When it comes to surface roughness, AFM images of the prepared hydrogels displayed an amazing behaviour. 5.474 nm and 7.397 nm, respectively, were computed as the average surface roughness and root mean square roughness. Guar gum has hydroxyl functional groups that provided a compact structure and hence a smooth surface area, resulting in decreased surface roughness (Thombare et al., 2016).

Wetting is a crucial aspect of drug administration since it reveals whether a substance is hydrophilic or hydrophobic. To examine the effectiveness of the hydrogel's surface water adsorption, the wetting behaviour toward water was assessed. Usually, the hydrogel with a water contact angle of<90° is considered hydrophilic. As the contact angle increases above 90°, the hydrogels start to become hydrophobic and above 150° they are considered superhydrophobic (Law, 2014). The contact angles measured for hydrogels F1, F2, F3 are 107°, 104° and 106° No doubt, guar gum has hydroxyl groups that encourage strong hydrogen bonding (Thakur et al., 2018). But we still need cross-linker to increase the hydrophilicity of the hydrogels. Prepared hydrogels F1a, F1b, and F1c have water contact angles of 71.28°, 76.58°, and 95.77°, respectively. In addition to increasing the close packing of the structure, the increased cross-linking also produces a surface that is more compact and smooth (Kankala et al., 2018). As a result, the hydrophilicity behaviour changes from hydrophilic to hydrophobic as cross-linking intensity rises. Hydrogels that are closely packed limit water absorption.

In order to assess the hydrogels' hemocompatibility, human red blood cells (hRBC) were placed on the hydrogel surfaces and the amount of hemolysis caused by the very toxic surfactant triton-X was measured. The treated cells had round morphology indicating that they were healthy blood cells. However, no cells were seen under a microscope in the wells treated with TX-100, which did not exhibit any RBC survival. The facts stated above further demonstrated the non-cytotoxic character of the adhesive surfaces appropriate for biomedical applications (Hoque et al., 2017).

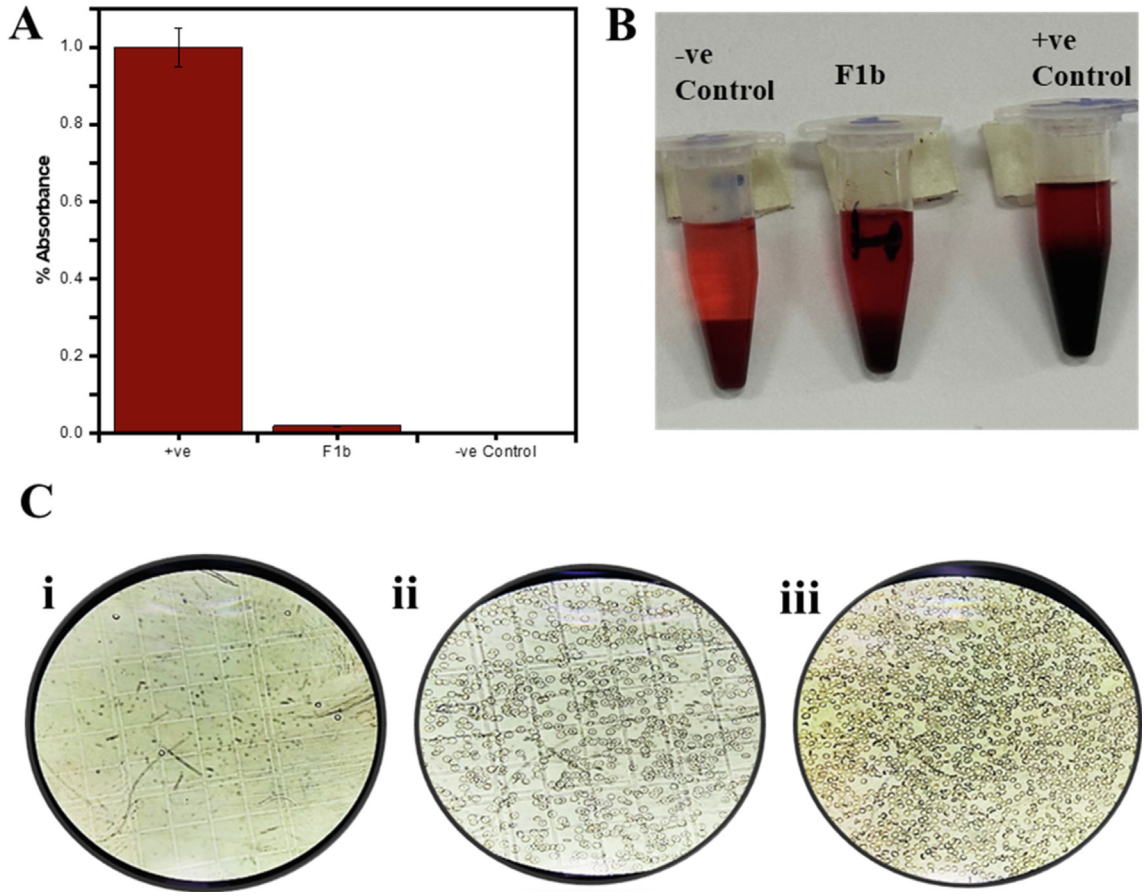

Fig. 5. Hemolysis analysis of F1b hydrogel. (A) Graphical representation, n = 3, results are significant with $p < 0.05$. (B, C) Representative pictures of hemolysis. (i) + ve control (ii) Hydrogel F1b, (iii) –ve control.

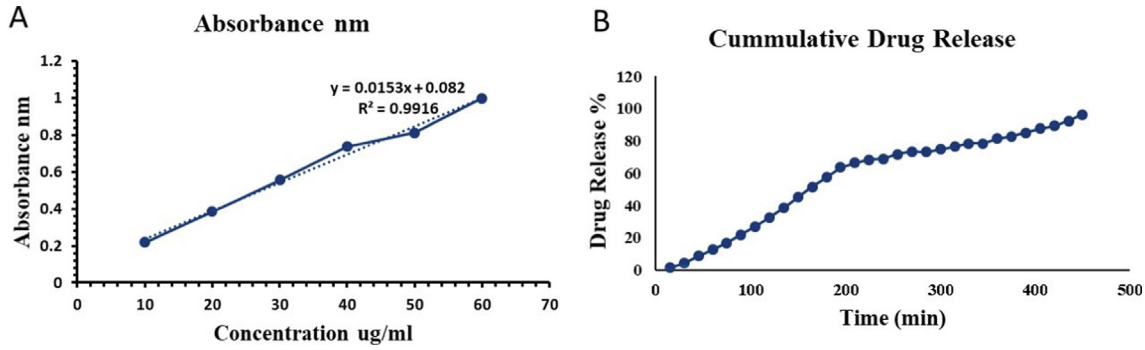

Fig. 6. (A) Absorbance (nm) of MTX. **(B)**The calibration of MTX.

Our findings are in agreement with earlier research that have shown similar results that Diffusion is always responsible for the drug release mechanism, depending on the porosity of the hydrogel, drug molecules release from the polymeric network (Khan et al., 2021). The Higuchi model predicted on the following hypotheses: (i) compared to the drug solubility, the initial matrix drug concentration is much higher. (ii) Since drug dispersion only occurs in one dimension, there must be minimal edge impact; (iii) dissolution and matrix swelling are negligible; (iv) continuous drug diffusivity and optimum sink conditions are always met in the release environment (Raucci et al., 2014). Due to the hydrogel's improved physicochemical behaviour, the sustained drug release of MTX in F1b makes it appropriate for controlled drug delivery applications. The release of the drug (MTX) was analyzed in PBS at 7.4 pH. The results have shown the release of 96% drug (MTX) 435 min (7 h 25 min) time interval. Shahid et al., reported GG as a potential candidate in controlled drug release (Shahid et al., 2013). The hydrogel loaded with MTX follows the Peppas model and is referred to as the "Power-law" since it has the highest regression coefficient (R^2 = 0.9835), and confirms that the drug is released from the polymeric system (Zhu et al., 2010).

Injectable thermosensitive hydrogels exhibit a transition in sol–gel phase in response to temperature which makes them an attractive drug delivery system. Such hydrogels have recently gained a significance in cancer therapy by offering high local drug concentration, low toxicities, prolonged release, and negligible invasive-

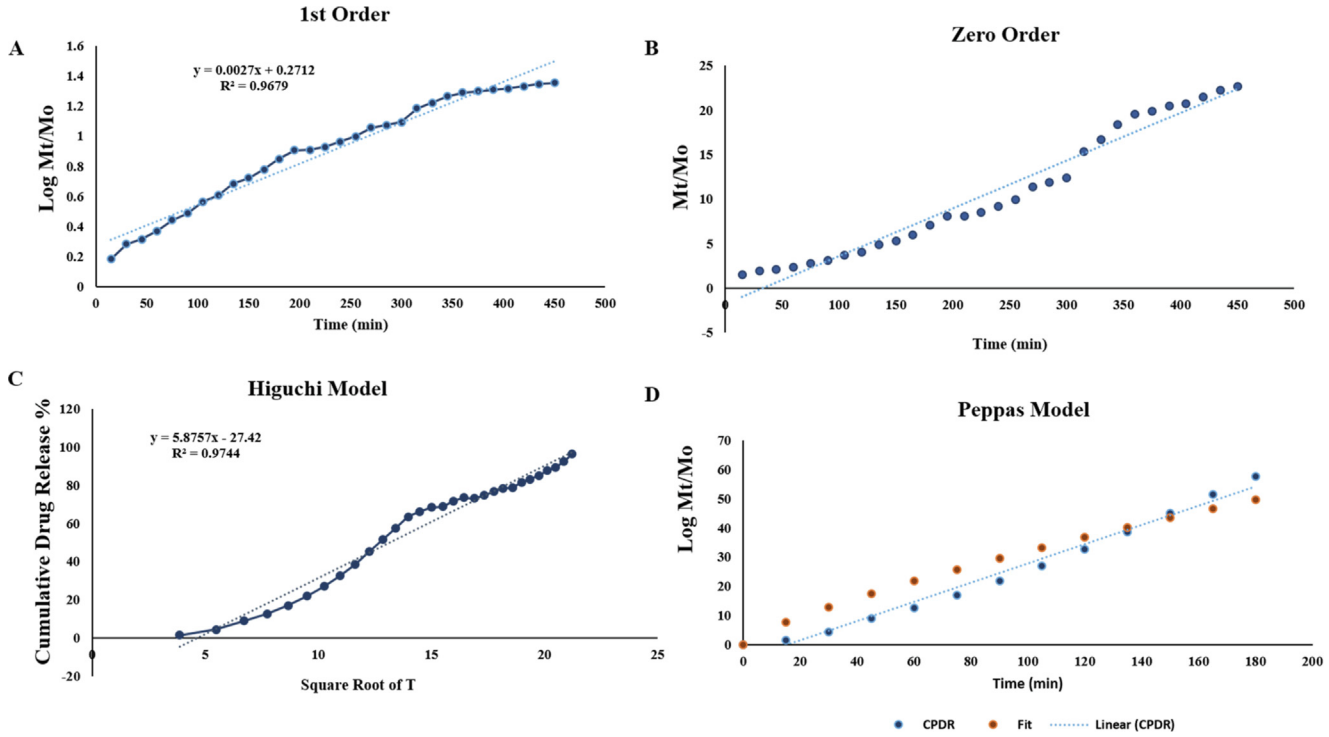

Fig. 7. Drug release kinetics of prepared hydrogel with different mathematical models (**A)** Zero-order, (**B)** First-order, (**C)** Higuchi, and (**D)** Korsmeyer-Peppas model to analyze drug release behavior.

Table 2
Drug release kinetics by different models.

Models	Intercept	Slope	R^2
Zero order	1.7978	0.0538	0.9655
First order	0.2712	0.0027	0.9679
Korsmeyer-Peppas model	5.0432	0.3285	0.9835
Higuchi model	27.42	5.8757	0.9744

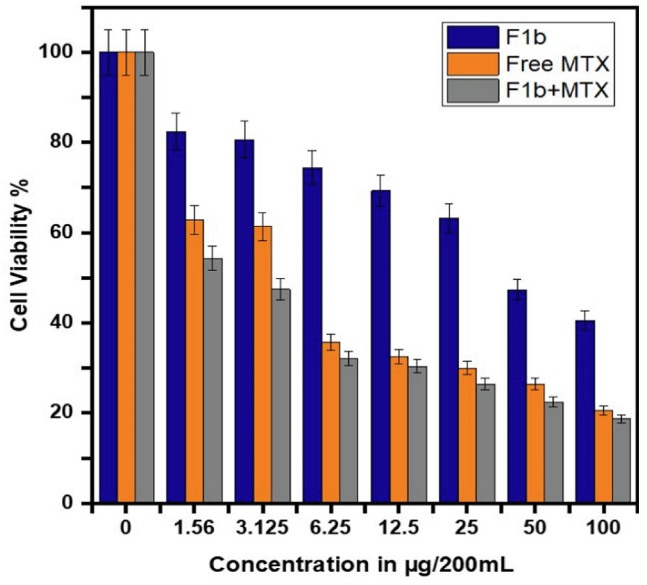

Fig. 8. Cytotoxic potential of MTX alone (Free MTX), F1b, and MTX loaded F1b (F1b + MTX) against HCT-116 cell line at different concentrations after 48 h of treatment. Cell viability decreased gradually, and a sudden decrease of viable cells was observed after IC50 of MTX loaded F1b hydrogel and MTX alone. MTX loaded F1b hydrogel (F1b + MTX) has shown the synergistic/ combinatorial effect which has enhanced its antiproliferative activity against HCT-116. The collected data were calculated in triplicate (n = 3 and p-value < 0.05).

ness (Xiao et al., 2021). F1b + MTX was found to have a considerably higher cytotoxicity than F1b and MTX alone. The proposed reason of this effect is the Flavonoids present in one of the polymers Guar Gum used in our hydrogel, already known for their antioxidant and anticancer potential (Selamoglu, 2017a, 2017b). The MTX loaded F1b hydrogel together resulted in increasing cytotoxic effects of 50% growth inhibition (with IC50 11.7 µg/ml) at concentration of 2.34 µg/200 ml against colon cancer cell line (HCT-116) (Morsy et al., 2022).

5. Conclusion

In this study, we prepared GG/CS/PVA hydrogel crosslinked by TEOS and loaded with Methotrexate. Characterization of hydrogel with different ratios of polymers (GG, CS, and PVA) with various amounts of crosslinker showed the effect on pore size, swelling patterns, and water holding capacity. FTIR and AFM provide proof of preparation and show the morphological behavior of the hydrogel. All the prepared hydrogels showed different swelling patterns in PBS, distilled water, at different pH, and different temperatures. Among these hydrogels overall persistent results were shown by F1. It exhibited maximum swelling capacity (2232%) after 1 h and 30 min in PBS. Sustained swelling was shown in acidic and basic media and at different temperatures (37 °C and 43 °C). The characteristics of polymers are improved by crosslinkers. Without crosslinker hydrogels disintegrated after 120 min. The addition of TEOS overcame this drawback. It improved the tensile strength, solution absorbing capacity, and also swelling time of the hydrogels. The amount of TEOS has been found to correlate with the increase of water contact angle while having the opposite effect on degradation and water retention. Poor moisture loss was seen by F1 which makes it a potential candidate for holding more water and sustained drug delivery. Based on swelling results, hydrogel F1b was further used for various analysis including the hemolytic

activity, drug release, and cell viability test. F1b has shown negligible hemolysis (<5%) which represents its biocompatible nature.

In the present study F1b hydrogel was used to load the chemotherapeutic drug Methotrexate (MTX) against colon cancer cell line (HCT-116). It is proved by results that F1b hydrogel is a potential candidate for consistent drug release about 96% MTX release was observed in 7 h 25 min in PBS media. MTX loaded hydrogel further increased the anticancer activity than the MTX alone. MTT assay revealed the (IC50 11.73 μg/ml) of drug-loaded GG/CS/PVA.

Thus, after analyzing all the above data, it is concluded that thermo-sensitive and pH-dependent GG/CS/PVA hydrogel with sustained drug release property is a promising vehicle for future biomedical applications.

Declaration of Competing Interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2023.103731.

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