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# Development and Evaluation of Cellulose Derivative and Pectin Based Swellable pH Responsive Hydrogel Network for Controlled Delivery of Cytarabine

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**Abstract:** In the present study, pH-sensitive, biodegradable, and biocompatible Na-CMC/pectin poly(methacrylic acid) hydrogels were synthesized using an aqueous free radical polymerization technique and encapsulated by cytarabine (anti-cancer drug). The aim of the project was to sustain the plasma profile of cytarabine through oral administration. Sodium carboxymethyl cellulose (Na-CMC) and pectin were cross-linked chemically with methacrylic acid (MAA) as a monomer, using methylene bisacrylamide (MBA) as cross-linker and ammonium per sulfate (APS) as an initiator. Prepared hydrogel formulations were characterized for their texture, morphology, cytarabine loading efficiency, compositional and structural properties, thermal nature, stability, swelling response, drug release profile (pH 1.2 and pH 7.4), and in-vivo pharmacokinetic evaluation. Cytarabine-loaded hydrogels were also evaluated for their safety profile by carrying out toxicity studies in rabbits. Results demonstrated efficient encapsulation of cytarabine into the prepared network with loading ranging from 48.5–82.3%. The highest swelling ratio of 39.38 and maximum drug release of 83.29–85.27% were observed at pH 7.4, highlighting the pH responsiveness of the grafted system. Furthermore, cytarabine maximum release was noticed over 24 h, ensuring a sustained release response for all formulations. Histopathological studies and hemolytic profiles confirmed that the prepared hydrogel system was safe, biocompatible, and non-irritant, showing no symptoms of any toxicities and degeneration in organs. Moreover, pharmacokinetic estimation of the cytarabine-loaded hydrogel showed a remarkable increase in the plasma half-life from 4.44 h to 9.24 h and AUC from 22.06 µg/mL.h to 56.94 µg/mL.h. This study revealed that the prepared hydrogel carrier system has excellent abilities in delivering the therapeutic moieties in a controlled manner.

**Keywords:** drug delivery; ocular; cytarabine; anticancer; free radical polymerization; hydrogel; health care; drug discovery



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## 1. Introduction

Recently, numerous smart drug delivery systems have been introduced to treat cancer using biocompatible polymers, showing sensitivity towards multiple physiological factors

including pH and temperature. At different pH, pH-sensitive hydrogels show different responses in terms of swelling, shrinking, bending, and degradation. Particularly for controlled drug release, different polymer-based drug delivery systems have been formulated such as polymeric micelles, polymersomes, nanospheres, hydrogels, liposomes, dendrimers, and polymeric films. Their fundamental strategy is to preferentially kill cancerous parts without damaging the normal healthy tissues and the host immune system [1]. The hydrogels are versatile, and smart drug delivery systems can be utilized in numerous fields like formulation of controlled drug delivery devices, removal of metal ions and dyes, pH sensors, biosensors, tissue engineering, preparation of wound dressings, contact lenses, and spinal cord reformation by injectable hydrogel, e.g., supercapacitor hydrogels [2].

Cytarabine is an anticancer, low molecular weight hydrophilic drug with a short half-life of 1–3 h [3]. Oral administration of cytarabine is challenging due to its unstable nature and low bioavailability of less than 20% (BCS class III); thus, it is ineffective by this route and is therefore administered as IV infusion [4]. The usual dose of cytarabine is 3 g/m<sup>2</sup> administered every 3 h to treat leukemia [5]. It is metabolized rapidly in the presence of enzymes to an inactive moiety, i.e., 1- $\beta$ , D-arabino-furanosyl uracil, and is eliminated through urine as uracil arabinoside [6].

Sodium carboxymethyl cellulose (Na-CMC) is a hydrophilic ether derivative of cellulose. It is a polysaccharide with remarkable biodegradability and biocompatibility. It has diverse applications in topical and oral pharmaceutical formulations. It offers considerable advantages to be used in hydrogel preparations because it is a polyelectrolyte and is said to be a “smart” cellulose derivative because of its responsiveness towards pH and ionic variations. Additionally, it contains so many reactive hydroxyl groups (–OH) in its polymeric chain that allow its use preferentially in hydrogel formulations. Furthermore, the occurrence of carboxylate groups in the macromolecular chain makes hydrogel preparation feasible via multivalent ionic cross-linking. Overall, Na-CMC is widely used in various fields like cosmetics, food, medicine, and agriculture as gelling agents, thickening agents, stabilizers, and suspending agents [7,8].

Pectin is a natural biodegradable polymer with a number of ideal characteristics, making it a suitable polymer to be used in hydrogel preparations. It is a hydrophilic anionic polysaccharide that is mainly collected through the plant cell walls and has multiple applications including formulation of targeted delivery devices [9]. It is comprised of D-galacturonic acid units joined through  $\alpha$ -(1–4) glycosidic linkage and  $\alpha$ -D-galactopyranosyluronic acid units with variable numbers of methyl esterified carboxyl groups. Its structure contains neutral sugars and galacturonan units and has rhamnose in small amounts in its backbone. A few other neutral sugars like arabinose, xylose, and galactose also exist in the side chains [10,11]. It presents dual responsiveness in that it responds to pH alterations as well as microbial enzymatic degradation, making it advantageous to be used in designing smart hydrogels [12].

In the current project, the stable hydrogels were developed using a better technique and a novel combination of biodegradable, biocompatible polysaccharide polymers, pectin from natural origin, and Na-CMC derived synthetically. The study aimed to assess pH-specific oral controlled delivery of cytarabine through developed hydrogels focusing on improving the half-life and bioavailability of cytarabine.

## 2. Results and Discussion

All hydrogel formulations (NP-1–NP-12) were developed through a free radical polymerization technique using specified proportions of Na-CMC, pectin, methacrylic acid, and MBA, as presented in Table 1. The detailed method of the Na-CMC/pectin-g-poly (methacrylic acid) network synthesis has been described in Section 4.2.

**Table 1.** Composition of Na-CMC/pectin-g-poly (methacrylic acid) hydrogels (NP-1 to NP-12).

Formulations	Na-CMC (g/100 g)	Pectin (g/100 g)	Methylene Bisacrylamide (g/100 g)	Methacrylic Acid (g/100 g)	Ammonium Persulphate (g/100 g)
NP-1	0.5	0.25	0.15	10	0.15
NP-2	1.0	0.25	0.15	10	0.15
NP-3	1.5	0.25	0.15	10	0.15
NP-4	0.5	0.55	0.15	10	0.15
NP-5	0.5	0.75	0.15	10	0.15
NP-6	0.5	1.0	0.15	10	0.15
NP-7	0.5	0.25	0.15	15	0.15
NP-8	0.5	0.25	0.15	20	0.15
NP-9	0.5	0.25	0.15	25	0.15
NP-10	0.5	0.25	0.17	10	0.15
NP-11	0.5	0.25	0.19	10	0.15
NP-12	0.5	0.25	0.21	10	0.15

### 2.1. Physical Evaluation of Na-CMC/Pectin-g-Poly (Methacrylic Acid) Hydrogels

Formulations of the Na-CMC/pectin-g-poly (methacrylic acid) network were effectively fabricated using varying amounts of components. It was noted that raising the polymers ratios (formulations NP1–NP6) produced a glossy texture, darker color, reduced roughness, and adhesiveness of hydrogel formulations. Similarly, an increased monomer concentration (NP7–NP9) developed transparent hydrogels with non-adhesive nature and reduced abrasion. Whereas preparations having increased amount of cross-linker (formulation NP10–NP12) were brittle and transparent in color (Figure 1). All prepared formulations maintained their integrity while drying at different temperatures, thus confirming their stability.

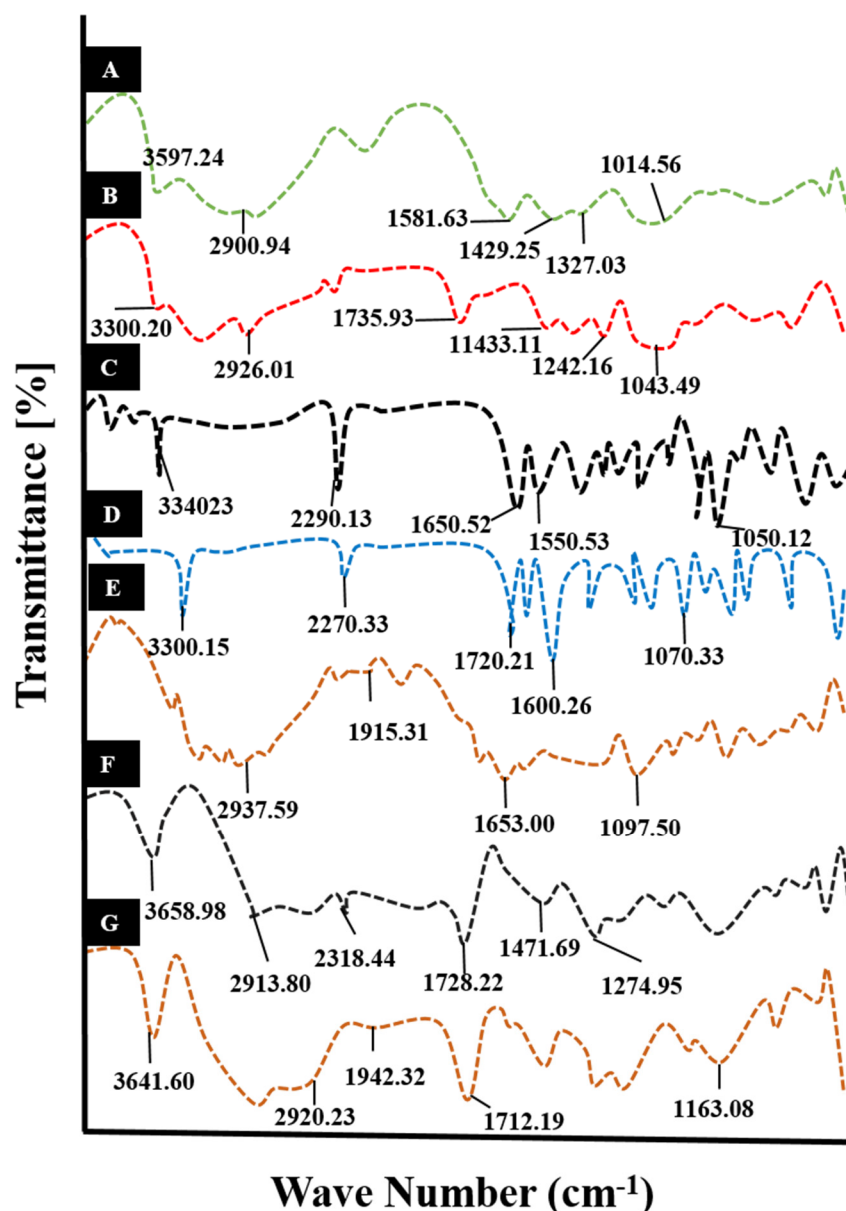
**Figure 1.** Developed Na-CMC-g-poly (MAA) hydrogel discs.

### 2.2. FTIR Studies

FTIR spectra of pure Na-CMC, pectin, cytarabine, methacrylic acid, MBA, physical mixtures, and prepared Na-CMC/pectin-g-poly (methacrylic acid) hydrogels were recorded, as displayed in Figure 2, to verify cross-linking between polymers (Pectin, Na-CMC) and monomer.

The FTIR spectrum of Na-CMC showed numerous peaks at  $3597.24\text{ cm}^{-1}$  and  $2900.94\text{ cm}^{-1}$  due to  $-\text{OH}$  stretching vibrations of the polymer. The peaks at  $1581.63\text{ cm}^{-1}$ ,  $1429.25\text{ cm}^{-1}$ , and  $1327.03\text{ cm}^{-1}$  were attributed to the stretching vibrations of  $-\text{COO}$ ,  $-\text{CH}_2$  scissoring, and  $-\text{OH}$  bending vibrations of the polymer, respectively. Sugar ring vibrations of Na-CMC were observed in the range of  $1000\text{ cm}^{-1}$  and  $1250\text{ cm}^{-1}$ . An absorption band appeared at  $1014.56\text{ cm}^{-1}$  due to C-O stretching vibrations, as shown in Figure 2A.

The spectrum of pure pectin revealed a prominent peak at  $3300.20\text{ cm}^{-1}$  due to  $-\text{OH}$  stretching, as shown in Figure 2B. Other peaks appeared at  $2926.01\text{ cm}^{-1}$ ,  $1735.93\text{ cm}^{-1}$ ,  $1242.16\text{ cm}^{-1}$  to  $1433.11\text{ cm}^{-1}$ , and  $1043.49\text{ cm}^{-1}$ , which were associated with C-H stretching vibrations, C=O group,  $-\text{CH}_2$  stretching vibrations, and C-O stretching, respectively.



**Figure 2.** FTIR spectra of (A) Na-CMC, (B) pectin, (C) cytarabine, (D) MBA, (E) physical mixture, (F) unloaded hydrogel, and (G) cytarabine-loaded hydrogel.

In case of cytarabine, prominent peaks were displayed at  $3340.23\text{ cm}^{-1}$  ( $-\text{NH}$  stretching),  $2290.13\text{ cm}^{-1}$  ( $-\text{CH}$  stretching movements),  $1650.52\text{ cm}^{-1}$  ( $\text{C}=\text{O}$  stretching),  $1550.53\text{ cm}^{-1}$  ( $-\text{CN}$  bending), and  $1050.12\text{ cm}^{-1}$  ( $-\text{CO}$  stretching) on the IR spectrum, as shown in Figure 2C.

The FTIR spectrum of methylene bisacrylamide demonstrated various peaks at different wave numbers, i.e.,  $3300.15\text{ cm}^{-1}$ ,  $3000\text{ cm}^{-1}$ ,  $2900\text{ cm}^{-1}$ ,  $2270.33\text{ cm}^{-1}$ ,  $1720.21\text{ cm}^{-1}$ ,  $1600.26\text{ cm}^{-1}$ , and  $1070.33\text{ cm}^{-1}$ , that corresponded to the vibrations of  $-\text{NH}$  stretching,  $-\text{CH}_2$  asymmetric motions,  $-\text{CH}_2$  symmetric motions,  $-\text{CO}$  stretching,  $-\text{CH}$  stretching,  $\text{C}=\text{O}$  vibrations due to acrylamide group, and  $-\text{CO}$  stretching vibrations, respectively, as shown in Figure 2D.

No significant variation was seen on the physical mixture spectrum, as displayed in Figure 2E. The IR spectrum of the fabricated Na-CMC/pectin grafted poly (methacrylic acid) hydrogel system, as shown in Figure 2F, displayed different configurations from their individual constituents; characteristic peaks that appeared at  $3658.96\text{ cm}^{-1}$  and  $2931.80\text{ cm}^{-1}$  were due to stretching vibrations of the  $-\text{OH}$  and  $\text{C}-\text{H}$  groups of the polymers.

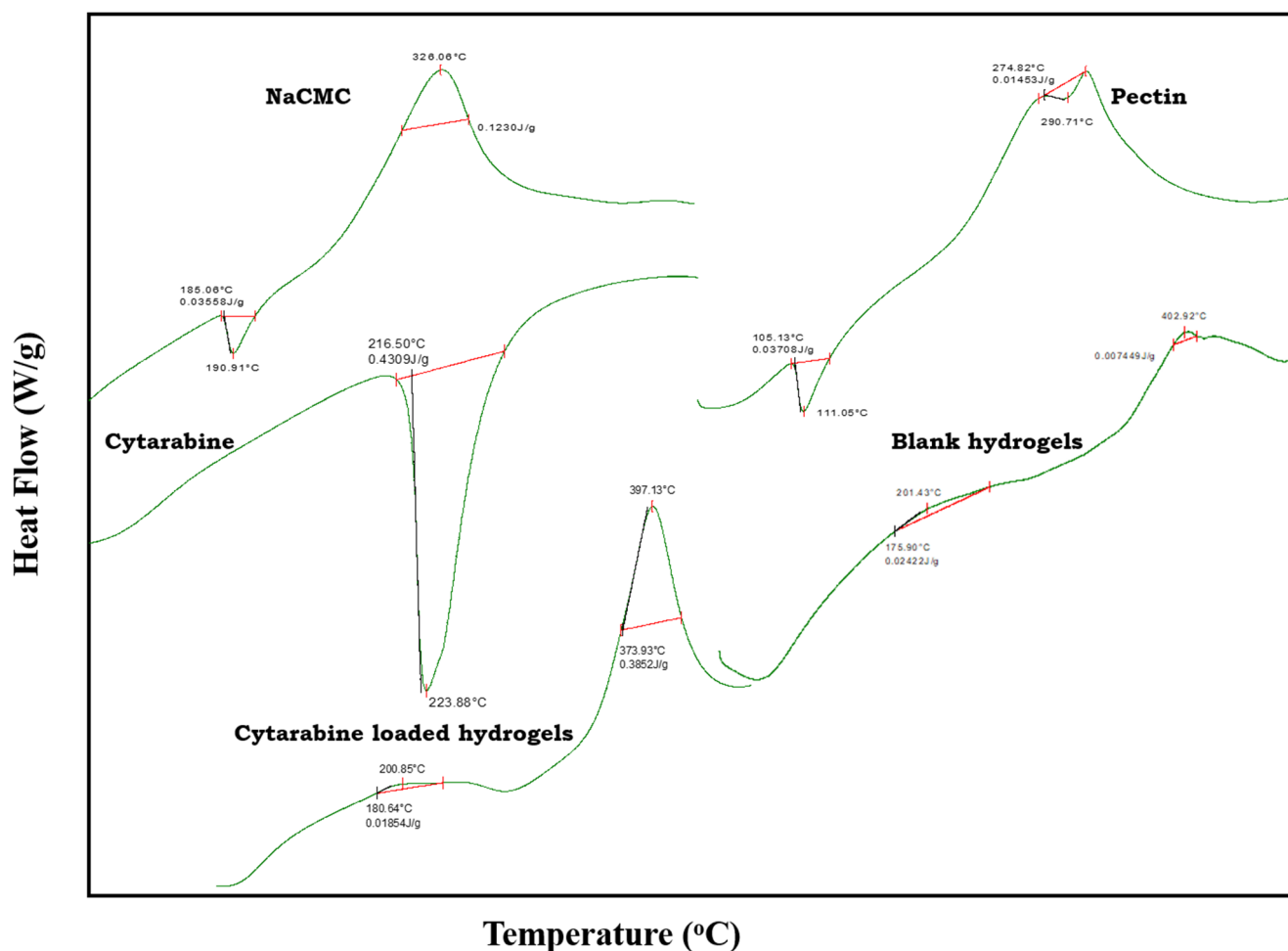
Similarly, absorption bands appeared in the range of  $2300\text{ cm}^{-1}$ – $2700\text{ cm}^{-1}$ , which were representing the  $-\text{CH}$  stretching vibrations. An absorption band at  $1728.22\text{ cm}^{-1}$  was attributed to the  $-\text{COOH}$  group ( $\text{C}=\text{O}$  stretching movements) of Na-CMC and pectin. While peaks found at  $1471.69\text{ cm}^{-1}$  and  $1274.95\text{ cm}^{-1}$  corresponded to the  $-\text{COO}$  and  $-\text{CO}$  groups symmetrical stretching vibrations, revealing the efficient crosslinking of methacrylic acid within polymeric chains.

Cytarabine peaks at  $1050.12\text{ cm}^{-1}$ ,  $1650.52\text{ cm}^{-1}$ , and  $3340.23\text{ cm}^{-1}$  due to the  $\text{C}-\text{O}$  group,  $\text{C}=\text{O}$  stretching vibrations, and  $\text{N}-\text{H}$  stretching vibrations were shifted towards  $1163.08\text{ cm}^{-1}$ ,  $1712.79\text{ cm}^{-1}$ , and  $3641.60\text{ cm}^{-1}$  on the IR spectrum of cytarabine-loaded hydrogels, confirming the complex formation, as shown in Figure 2G.

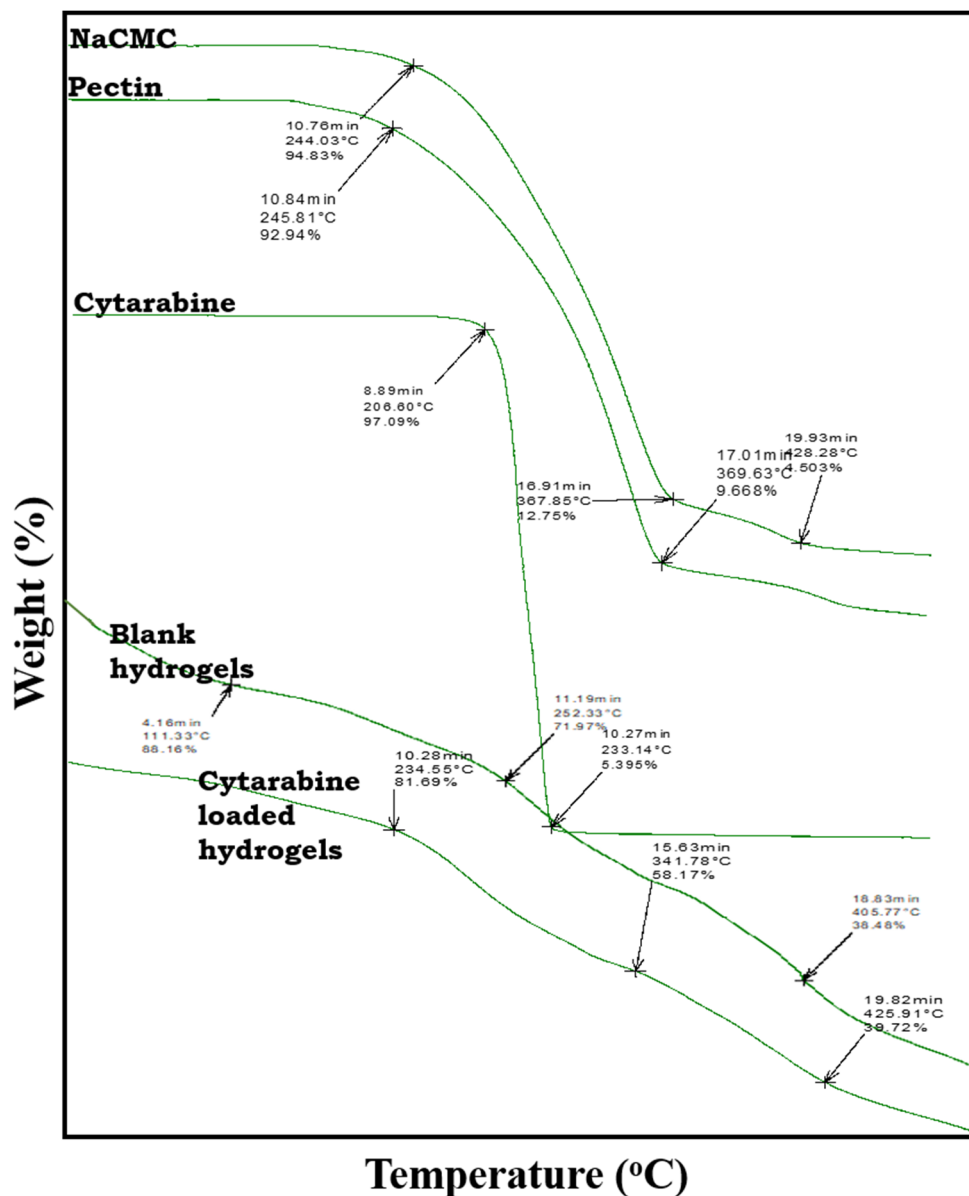
FTIR findings were in line with the observations of Dharmalingam and co-workers (2019). They verified the fabrication of a cross-linked structure either by merging or shifting of functional unit bands participating in polymerization [13].

### 2.3. Thermal Analysis

Thermal studies (DSC and TGA) were performed to monitor the effects of changing temperature on the stability of the developed network. For this purpose, pure cytarabine, polymers (Na-CMC and pectin), and drug-loaded network were processed through the analysis and results are demonstrated in Figure 3 (DSC) and Figure 4 (TGA).



**Figure 3.** DSC thermograms of Na-CMC, pectin, cytarabine, blank hydrogel, and cytarabine-loaded hydrogel.



**Figure 4.** TGA thermograms of Na-CMC, pectin, cytarabine, blank hydrogel, and cytarabine-loaded hydrogel.

2.3.1. DSC Studies

In differential scanning calorimetry, the thermal analytical procedure is used to evaluate influence of temperature and heat flow associated with shifts of functional groups in prepared hydrogels with time and temperature. Absorption or emission of heat results in a change of heat flow and the variation of heat is scanned as a peak. The area under peak represents the heat of enthalpy and the direction of the peak demonstrates the thermal behaviour of the component [14].

In the DSC thermogram of Na-CMC, an endothermic peak appearing at 190.91 °C can be associated to complete moisture loss of Na-CMC, whereas an exothermic peak at 326.06 °C showed complete combustion of the polymer.

The DSC thermogram of pectin showed endothermic peaks at 111.05 °C and 290.71 °C. These endothermic peaks represented loss of moisture and complete combustion of pure pectin, respectively. For cytarabine, a peak at 216.50 °C appeared due to solvent evaporation and an endothermic peak at 223.88 °C was due to the melting point of cytarabine.

The DSC thermogram of the blank developed hydrogel network presented a slight exothermic deflection at 201.43 °C that might be due to any moisture retained within the network, and a broad exothermic event was noted at 402.92 °C related to the combustion of the developed network.

In the thermogram of the cytarabine encapsulated network, a new exothermic peak appeared at 397.13 °C, hence confirming stability of cytarabine in the fabricated polymeric hydrogel system. Results are agreed to the work of Deepa and co-researchers (2018) who observed removal of a clear cytarabine peak and the presence of a new absorption peak in the 250–260 °C range, showing the amorphous distribution of cytarabine in a chitosan-based hydrogel network [15].

### 2.3.2. TGA Studies

Thermogravimetric analysis, as shown in Figure 4, displayed weight loss of polymers, cytarabine, and the prepared hydrogel with increasing temperature.

The thermogram of Na-CMC showed 6.17% and 96.5% weight loss at 244.03 °C and 428.28 °C, respectively, due to moisture loss and polymer decomposition with continuous increase in temperature. The thermogram of pectin showed 8.06% weight loss due to moisture loss at 245.81 °C, and 91.34% weight loss was observed at 369.63 °C because of polymeric fragmentation. Whereas in the TGA spectrum of cytarabine, 2.91% weight loss was noticed at 206.60 °C due to moisture removal and weight loss of 94.60% was measured at 233.14 °C because of polymer chain fragmentation.

As observed in thermogram of cytarabine-loaded hydrogel, the drug retained was 81.69%, 58.17%, and 39.72% at 234.55 °C, 341.78 °C, and 425.91 °C, respectively, thus confirming greater stability of the cytarabine-loaded fabricated hydrogel system as compared to its individual components.

Meanwhile, the TGA thermogram of the blank hydrogel network presented an initial mass loss of 11.84% at 111.33 °C, and a second degradation event occurred at 252.33 °C with a mass loss of 28.03%. At a temperature above 400 °C, more than 35% of the mass of the developed network was in intact form, thus confirming the stability of the network at elevated temperatures.

Our conclusions are supported by the results of Wang et al., (2010), where displacement of peaks to a higher temperature (234.33–425.91 °C) confirmed the new covalent bonds formed in the grafted co-polymeric system with improved thermal stability [16].

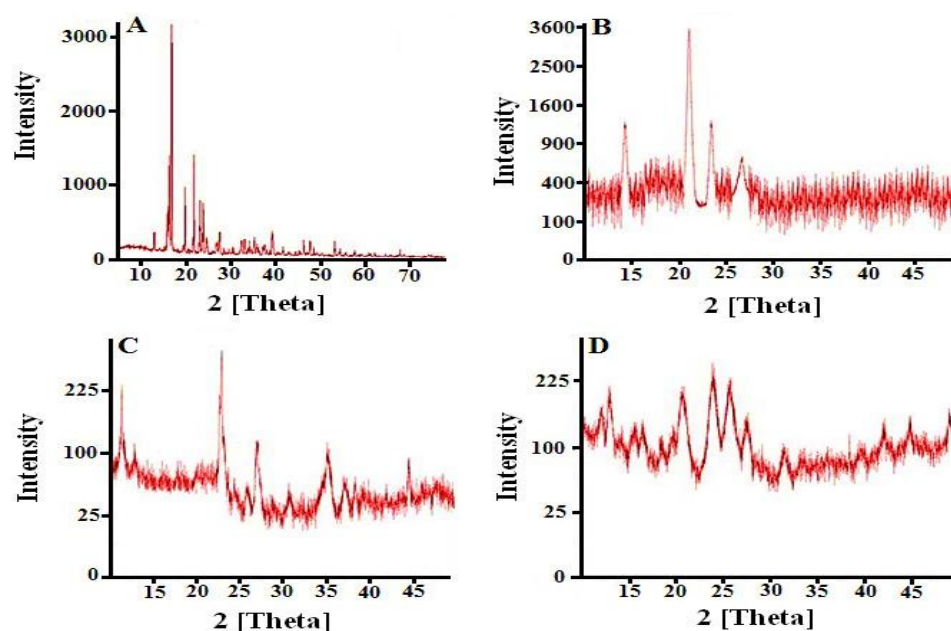
### 2.4. XRD Studies

XRD diffractogram of pure cytarabine showed intense and sharp peaks at  $2\theta = 16.5^\circ$ ,  $19.5^\circ$ ,  $22.4^\circ$ , and  $24.3^\circ$ , confirming the crystal habit of the pure drug (Figure 5A). Diffractogram of Na-CMC showed four conspicuous peaks at  $2\theta = 14.5^\circ$ ,  $21.2^\circ$ ,  $23.5^\circ$ , and  $26.5^\circ$ , showing the semi-crystalline behavior of Na-CMC (Figure 5B).

X-ray diffractogram of pectin also showed semi-crystalline nature displaying high and low-intensity peaks at  $2\theta = 12.2^\circ$ ,  $23.1^\circ$ ,  $27.5^\circ$ ,  $36.2^\circ$ , and  $45.1^\circ$  (Figure 5C).

XRD diffractogram of the cytarabine-loaded Na-CMC/pectin copolymerized network showed low-intensity peaks at  $2\theta = 13.2^\circ$ ,  $21.4^\circ$ ,  $24.5^\circ$ ,  $26.1^\circ$ , and  $28.2^\circ$ , proving amorphous behavior of the developed hydrogel network (Figure 5D).

These results showing transformation of peaks are similar to the observations of Eleamen et al., (2017) who synthesized an aminothiophene derivative complex with 2-hydroxypropyl- $\beta$ -cyclodextrin and reported the transformation of crystalline behavior of drug into an amorphous one during XRD studies [17].



**Figure 5.** XRD diffractograms of (A) cytarabine, (B) Na-CMC, (C) pectin, and (D) prepared hydrogel.

### 2.5. Elemental Analysis (EDX)

Elemental analysis of the fabricated hydrogel system was performed using the Energy Dispersive X-ray technique. Specific weights of nitrogen (N), oxygen (O), and carbon (C) noticed in cytarabine powder were 11.9%, 27.93%, and 60.18%, respectively. In blank polymeric hydrogel, the observed weights for carbon and oxygen were 39.08% and 60.32%, respectively. In the cytarabine-loaded hydrogel preparation, 63.06%, 10.31%, and 26.63% was seen for the weight of carbon, nitrogen, and oxygen, respectively.

Nitrogen, being an essential component of cytarabine, did not show a peak in the case of the unloaded network but emerged clearly in the spectrum of the cytarabine-loaded hydrogel. The presence of the nitrogen peak in the grafted spectrum evidenced the efficient encapsulation of cytarabine in the prepared Na-CMC/pectin-g-poly (methacrylic acid) hydrogel (Figure 6). Our results correspond to observations by Moghaddam et al., (2019); they noticed a new peak in the EDX spectrogram after drug loading in a developed hydrogel system during the preparation of a pectin-based interpenetrating system for delivering tetracycline [18].

### 2.6. Scanning Electron Microscopy

Na-CMC/pectin co-polymeric synthesized hydrogel was tested for surface morphology using scanning electron microscopy (SEM). The external surface was smooth and shiny, having numerous pores, as shown in Figure 7. These pores offer a pathway for water uptake when the hydrogel discs come in contact with water and other fluids, leading to the swollen system. The observed structure was compact, which confirmed compatibility among reactants. Thus, a stable hydrogel network was achieved.

### 2.7. Sol–Gel Fraction

The effect of ingredients on gel percentage of the network was investigated. It was noticed generally that gel fraction was pronounced with the rise of ingredients; however, their intensity was variable with respect to the type of ingredients.



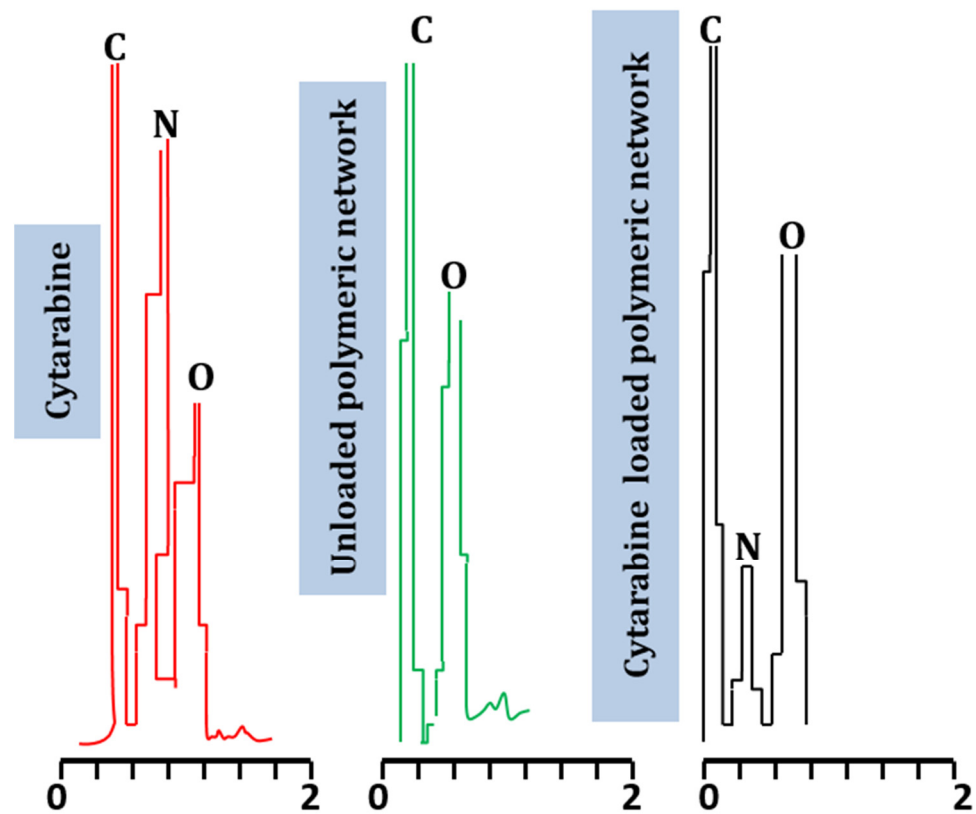


Figure 6. EDX spectra of cytarabine, unloaded network, and cytarabine-loaded network: nitrogen (N), oxygen (O), and carbon (C).

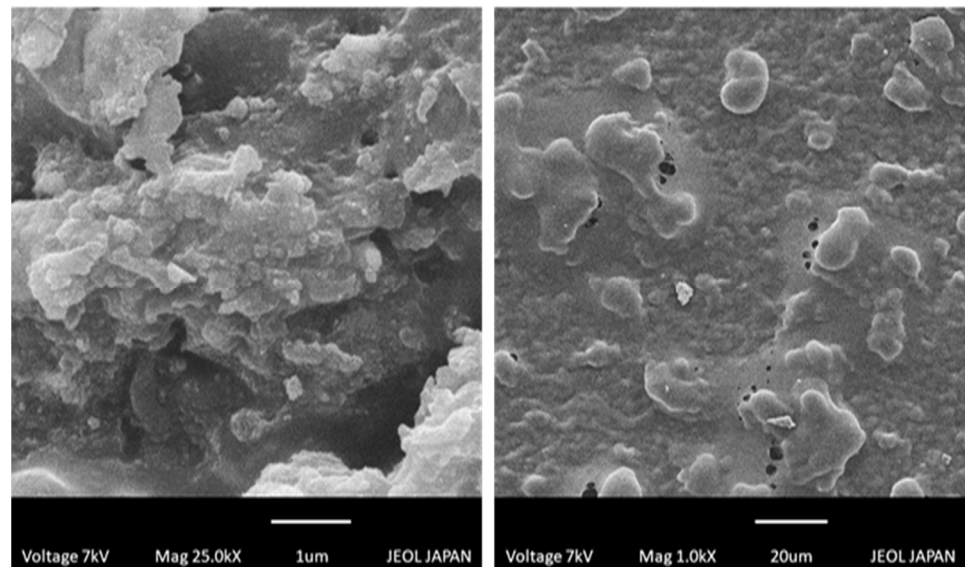
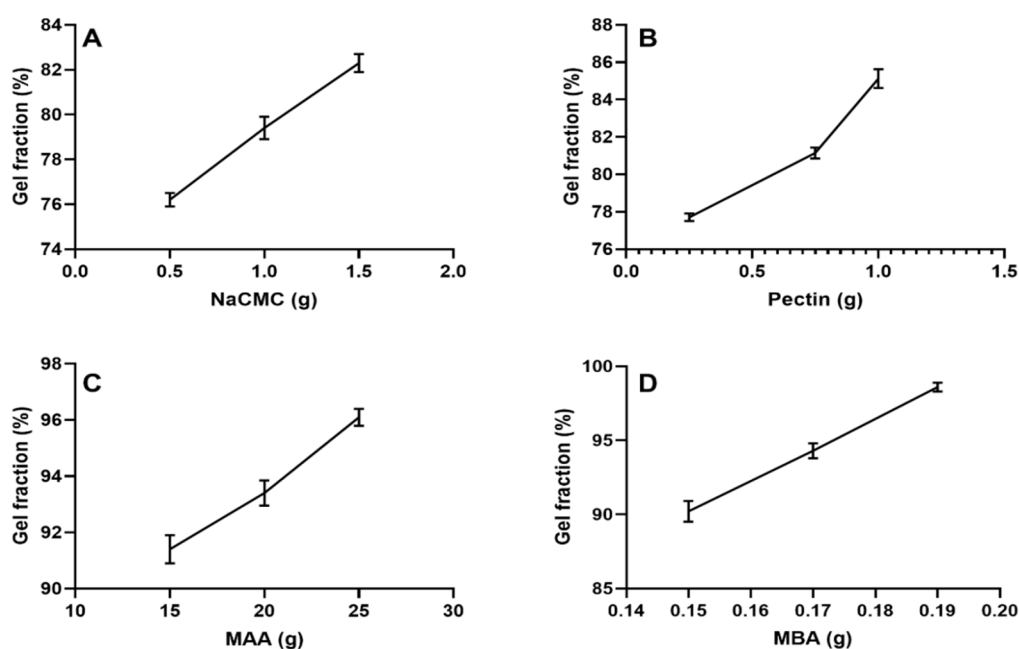


Figure 7. SEM photomicrographs of Na-CMC/pectin-co-poly (methacrylic acid) hydrogel.

Formulations containing increasing contents of Na-CMC (NP-1 to NP-3) showed gel fraction results of 76.2 to 82.3% (Figure 8A). This increased gel fraction could be justified as the availability of more carboxylic acid (-COOH) groups from Na-CMC. These groups get involved in cross-linking with neighboring moieties when ionized and hence promote the gel fraction of the network. Khan and Anwar (2021), developed gelatin and CMC-based polymeric networks for the targeted delivery of 5-fluorouracil. In gel fraction determinations, they have revealed similar gel fraction responses with respect to this polymer [19].



**Figure 8.** Effect of (A) Na-CMC, (B) pectin, (C) methacrylic acid (MAA), and (D) methylene bisacrylamide (MBA) on gel fraction.

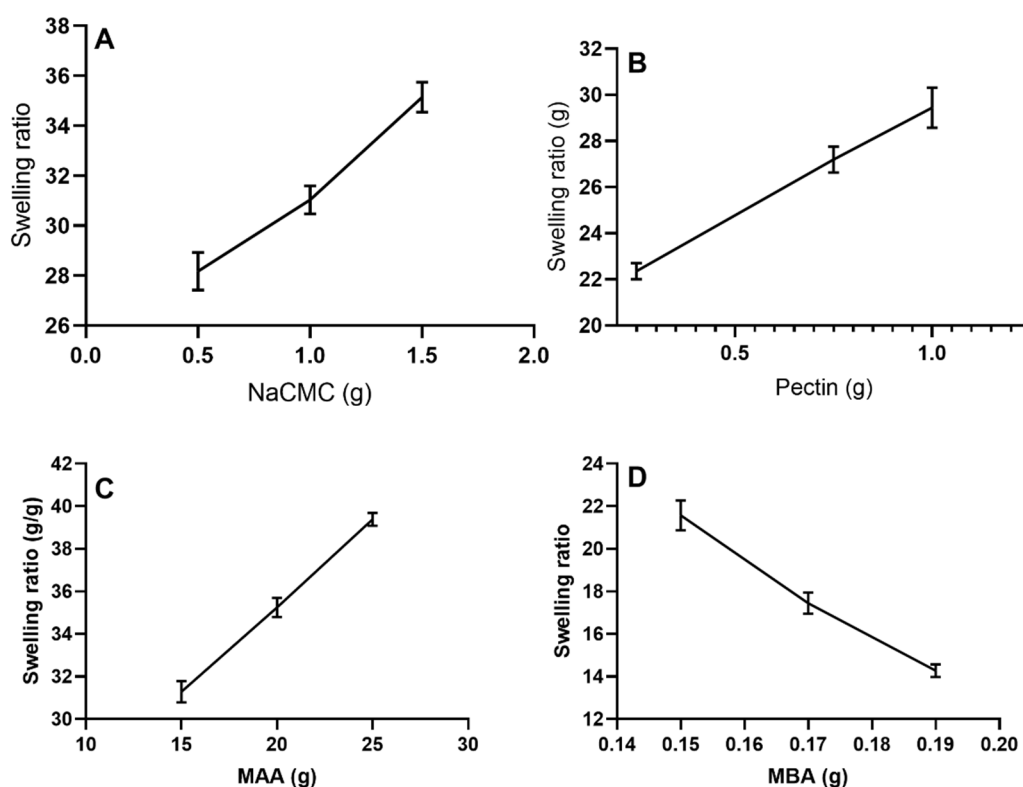
Formulations containing variable contents of pectin also exhibited a rise in gel fraction results, i.e., 77.7% to 85.12% (NP-4 to NP-6) (Figure 8B). The promoted gel percentage may be the result of the active participation of the polymer resulting in higher cross-linking density. Minhas et al. (2020) have developed pectin-based hydrogels. In their results of gel fractions, a direct relation was obtained with the pectin contents, as noticed in our findings [20]. Likewise, formulations (NP-7 to NP-9) with increasing contents of methacrylic acid revealed a promoted gelling, i.e., 91.40% to 96.10% (Figure 8C), which is attributed to the presence of carboxylate groups within methacrylic acid. As the number of these groups increases, more and more carboxylate ions participate in the polymerization reaction and hence a higher gel content is obtained. Similar conclusions are found in a work of Shabir et al. (2017) [21]. Similarly, in formulations (NP-10 to NP-12) containing increasing contents of MBA, the gel fraction was also promoted, i.e., 90.20% to 98.60% (Figure 8D). MBA promotes higher cross-linking density, and hence more gel fraction was noted. Similar findings are also noted in the research of Suhail et al. (2021) [22].

### 2.8. Swelling Studies

The swelling properties of the fabricated Na-CMC/pectin-co-polymeric device were evaluated at pH 7.4, using phosphate buffer at 37 °C.

The swelling response was calculated by varying the proportions of compositional contents such as pectin, Na-CMC, MAA, and methylene bisacrylamide, while changing a single component amount at one time; the other constituent's weight was kept unchanged, as shown in Figure 9.

By increasing concentrations of polymers, as presented in Figure 9A,B, a pronounced swelling degree of the grafted network occurred, i.e., 28.17–35.15 for NP-1–NP-3 with increased concentration of Na-CMC and 22.36–29.45 for NP-4–NP-6 with changing concentrations of pectin, respectively, as shown in the composition Table 1. Our findings correspond to observations of Guner et al. (2018), that swelling was increased on increasing the pectin content, until constant weight was achieved [23]. Tulain et al. (2018) also confirmed that by using carboxymethyl cellulose in increasing ratios, there was promoted swelling behavior of the grafted hydrogel system [14].



**Figure 9.** Effect of different hydrogel contents on the swelling behavior of hydrogels at pH 7.4. Effect of (A) Na-CMC, (B) pectin, (C) methacrylic acid (MAA), and (D) methylene bisacrylamide (MBA).

Formulations (NP-6–NP-9) with increasing methacrylic acid content, as shown in Figure 9C, showed an increased swelling ratio from 31.29 to 39.38. Enhanced swelling might be associated with the availability of excessive  $-\text{COOH}$  groups in methacrylic acid.

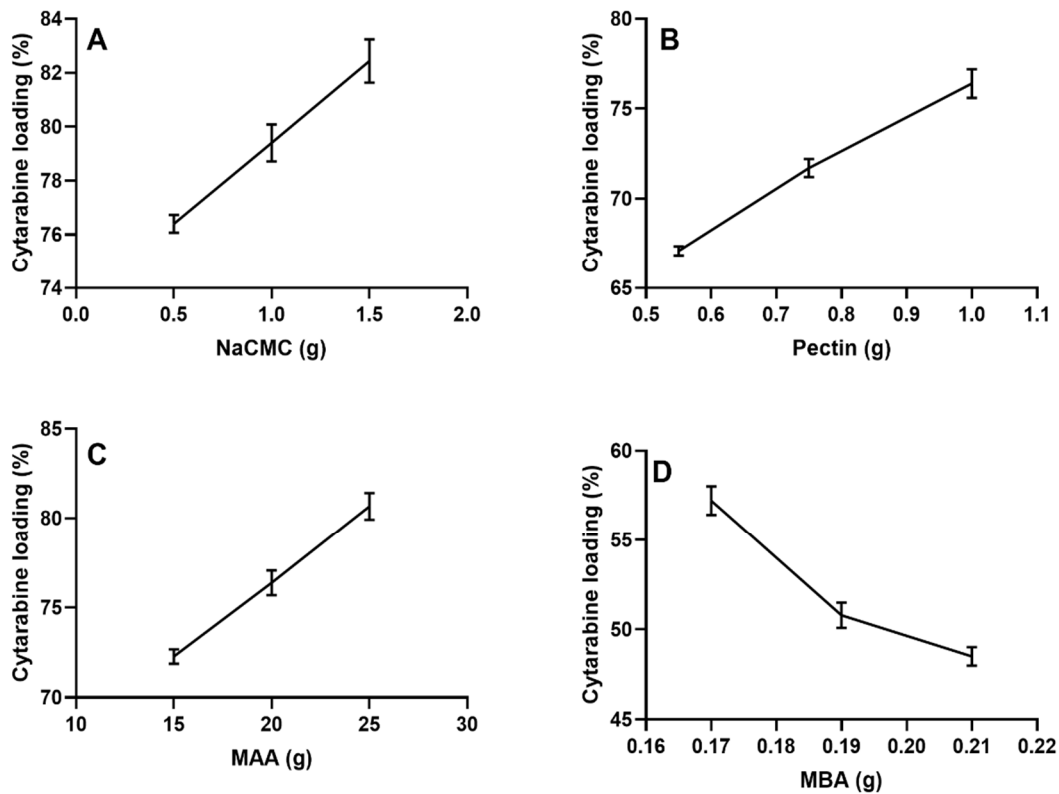
Similarly, an effect with varying concentrations of cross-linker (MBA) on the swelling ratio of the network system was also observed. A decrease in the swelling ratio was seen with the increased amount of methylene bisacrylamide, i.e., 21.57–14.28 (NP-10–NP-12), respectively, as shown in Figure 9D. This effect of MBA in formulations can be compared to the findings of Akalin et al., (2018), who noticed that the reduction in swelling was because polymer chains rendered immobile at an increased cross-linker density; thus, fewer molecules of water penetrated the hydrogel structure [24].

### 2.9. Cytarabine Loading (%)

Cytarabine was loaded in the Na-CMC/pectin grafted polymeric hydrogel by a swelling-assisted diffusion process. Maximum drug loading of 82.3% and 76.4% were observed for formulations NP-3 (Na-CMC) and NP-6 (pectin), respectively, with increased concentrations of polymers. Increased drug loading was observed with increased concentration of MAA, i.e., 80.67% in formulation NP-9.

The lowest drug loading (48.5%) was seen in formulation NP-12, owing to less fluid penetration due to the dense network by the increased quantity of methylene bisacrylamide.

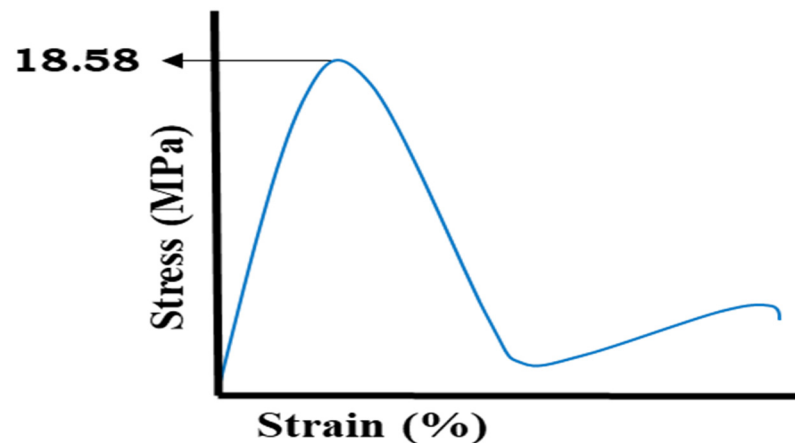
Our results resemble a study conducted by Khanum et al. (2018); they demonstrated that more drug was encapsulated in the grafted polymeric hydrogel with increased concentration of polymers and monomer, as shown in Figure 10A–C, but cytarabine incorporation was increased inversely with raising the cross-linker concentration, as shown in Figure 10D [25].



**Figure 10.** Effect of ingredients (A) Na-CMC, (B) pectin, (C) MAA, and (D) MBA on cytarabine loading (%).

### 2.10. Mechanical Properties

Tensile tests were conducted to find out the mechanical strength of the developed Na-CMC/pectin-based hydrogel system, as shown in Figure 11. The fabricated co-polymeric network exhibited mechanical characteristics with a tensile strength of 18.14 N/mm<sup>2</sup>. The newly prepared hydrogel system demonstrated unusual rigidity, with 141.34 N/mm<sup>2</sup> Young's modulus and 18.586% of final elongation found. The maximum force of 2.10 N was noticed at the breaking point of hydrogel network. The surface of the developed hydrogel remained uniform during the process. Mishra et al. (2008) showed good tensile properties of fabricated hydrogel membranes of pectin, similar to the current developed hydrogel formulation [26].



**Figure 11.** Tensile stress-strain curve.

### 2.11. Cytarabine Release Profile and Kinetic Modeling

Cytarabine release studies were carried out at pH 1.2 and 7.4 to explore the pH dependent nature of the developed network. At acidic pH, negligible release of cytarabine was noticed because carboxylic groups remain in the unionized state at this pH and polymeric chains do not repel each other, thus inhibiting the penetration of gastric fluid. Maximum and prolonged release was observed at basic pH as the carboxylic groups get ionized to carboxylate ions, repel each other, create voids, and promote uptake of the intestinal media for dissolution and release of the incorporated moiety. Cytarabine release was also affected by varying the amounts of each component in the developed hydrogel system, as demonstrated in Figure 12.

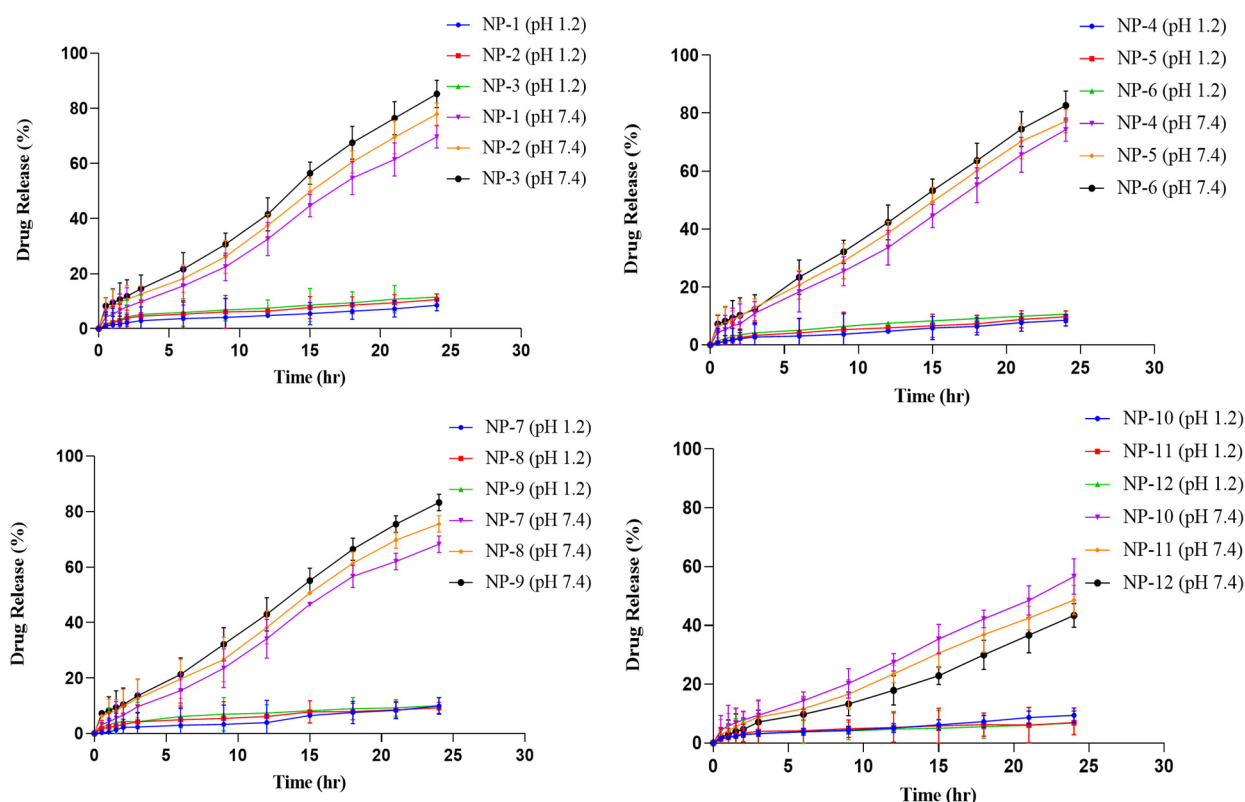


Figure 12. Cytarabine release studies in acidic (pH 1.2) and basic medium (pH 7.4).

Cytarabine release, i.e., 69.67–85.27%, was observed by using an increased concentration of Na-CMC in a co-polymeric grafted hydrogel network (NP-1–NP-3) with unaltered concentrations of other constituents. Dhanaraju et al., (2009), reported improved drug release by having Na-CMC in increasing proportions during release studies of Na-CMC polymeric beads containing Diclofenac sodium [27].

When the concentration of pectin was increased, cytarabine release was also promoted to 74.27–82.67% (NP-4–NP-6), while other constituents remained unchanged. Similarly, with an increased concentration of methacrylic acid in NP-7–NP-9, cytarabine release was also increased, i.e., 68.24–83.29%, while keeping other constituents constant.

A rapid decrease in the drug release, i.e., 56.61–43.38%, was noticed when cross-linker (MBA) content was increased in formulations NP-10–NP-12. Gupta et al. (2007) reported a similar effect of methacrylic acid and methylene bisacrylamide concentrations on drug release as those found in our observations [28].

Maximum cytarabine release, i.e., 85.27%, was noted for the formulation NP-3 containing an increased concentration (1.5 g) of Na-CMC.

It was seen that increasing the concentration of ingredients resulted in an increase in drug release initially, but after a certain time period, constant drug release at pH 7.4 was

observed. This might be due to higher cross-linking within the formulations with increased concentration, which decrease capillary action of the polymeric network and thus reduce fluid uptake from surroundings.

Kinetic modeling revealed zero order release kinetic patterns for all formulations based on  $R^2$  values, i.e., 0.9759–0.9991 (Table 2). Cytarabine release mode from the grafted system was assessed from the exponent “n” value of the Korsmeyer–Peppas model that was more than 0.89, confirming super Case II transport, i.e., diffusion and erosion taking place simultaneously.

**Table 2.** Cytarabine release kinetics.

Formulations	Zero Order	1st Order	Higuchi	Korsmeyer–Peppas	
	$R^2$	$R^2$	$R^2$	$R^2$	n
NP-1	0.9963	0.9831	0.8536	0.9965	1.013
NP-2	0.9958	0.9767	0.87	0.9943	0.945
NP-3	0.9968	0.976	0.8833	0.9945	0.9
NP-4	0.9977	0.9619	0.857	0.9979	1.014
NP-5	0.9987	0.9678	0.8867	0.997	0.904
NP-6	0.9993	0.9671	0.8916	0.9977	0.89
NP-7	0.9982	0.9782	0.8854	0.996	0.905
NP-8	0.9973	0.9812	0.8764	0.996	0.936
NP-9	0.9923	0.9677	0.8353	0.9948	1.089
NP-10	0.9966	0.9708	0.8553	0.9966	0.998
NP-11	0.997	0.9653	0.879	0.9956	0.92
NP-12	0.9986	0.9642	0.8881	0.9971	0.898

### 2.12. Acute Oral Toxicity

During toxicity studies, all test animals (rabbits) were observed carefully regarding their dietary habits, body weight, and any sign of common disease, for example, diarrhea, itching, fever, and other toxicities. The body weight of all experimental animals was checked carefully before and after administering the developed polymeric network and weight change was insignificant.

No symptom was found after consuming a definite dose of the fabricated network. No hypersensitivity, ocular, or dermal signs of toxicity were observed. Fluid intake was also normal. All biochemical and hematological values were within range, as shown in Tables 3–5.

**Table 3.** Medical interpretation of acute oral toxicity.

Interpretations	Group A (Control)	Group B (Administered with Hydrogel (2 g/kg))
Signs of illness	Nil	Nil
<b>Weight (kg)</b>		
Pretreatment	2.11 ± 2.1	2.12 ± 1.7
At day 1	2.13 ± 1.9	2.14 ± 1.4
At day 7	2.14 ± 2.2	2.16 ± 2.0
At day 14	2.16 ± 2.0	2.18 ± 2.3
<b>Fluid intake (mL)</b>		
Pretreatment	176.51 ± 1.19	189.20 ± 0.04
At day 1	190.61 ± 1.8	188.31 ± 1.48
At day 7	212.21 ± 2.17	192.19 ± 1.41
At day 14	216.49 ± 2.19	207.41 ± 2.09

**Table 3.** *Cont.*

Interpretations	Group A (Control)	Group B (Administered with Hydrogel (2 g/kg))
<b>Diet intake (g)</b>		
Pretreatment	73.21 ± 1.31	74.31 ± 1.13
At day 1	74.35 ± 1.07	75.18 ± 1.17
At day 7	71.51 ± 1.25	73.51 ± 1.31
At day 14	73.10 ± 1.16	75.53 ± 1.13
<b>Others</b>		
Ocular toxicity	Nil	Nil
Skin rashes	Nil	Nil
Death	Nil	Nil

Note: All values are presented as mean ± SD (n = 3).

**Table 4.** Biochemical analysis of rabbits' blood.

Parameter	Group A (Control)	Group B (Treated with Hydrogel 2 g/kg)
Hemoglobin (g/dL)	12.28 ± 0.33	12.34 ± 0.32
pH	7.49 ± 0.15	7.66 ± 0.13
White blood cells ( $\times 10^3$ uL <sup>-1</sup> )	12.19 ± 0.29	12.43 ± 0.57
Red blood cells ( $\times 10^6$ uL <sup>-1</sup> )	6.15 ± 0.142	6.22 ± 0.29
Platelets ( $\times 10^3$ uL <sup>-1</sup> )	252 ± 2.01	261 ± 0.23
Monocytes (%)	4.42 ± 0.05	4.51 ± 0.06
Neutrophils (%)	27.29 ± 2.48	27.56 ± 2.21
Lymphocytes (%)	58.13 ± 1.02	58.2 ± 0.51
Mean corpuscular volume (fl)	73.59 ± 2.14	75.52 ± 0.18
Mean corpuscular hemoglobin (pg/cell)	25.14 ± 2.27	25.23 ± 0.07
Mean corpuscular hemoglobin concentration (g/dL)	32.57 ± 1.34	33.2 ± 0.19

Note: All values are given as mean ± SD (n = 3).

**Table 5.** Biochemical findings of the Na-CMC/pectin-co-polymeric (methacrylic acid) hydrogel after oral administration to rabbits.

Biochemical Analysis	Group A (Control)	Group B (Treated with Hydrogel (2 g/kg))
Creatinine (mg/dL)	1.5 ± 0.26	1.6 ± 0.3
Urea (mmol/L)	62.28 ± 1.9	63.46 ± 2.02
Bilirubin mg/dL	0.92 ± 0.28	0.94 ± 0.26
ALT (IU/L)	66.56 ± 4.06	67.51 ± 2.31
AST (IU/L)	71.51 ± 1.54	72.57 ± 1.61
ALK Phos (IU/L)	22.47 ± 2.53	23.48 ± 2.56

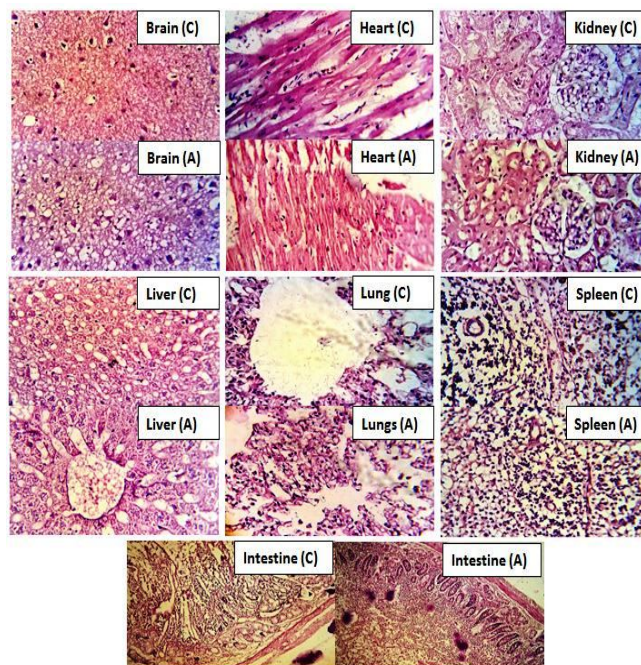
Note: All values are mentioned as mean ± SD (n = 3).

### Histopathological Observations

After 2 weeks, all experimental animals of both groups were weighed carefully and slaughtered to isolate key organs to analyze any symptoms of toxicity of the administered polymeric network (Na-CMC/pectin), as shown in Figure 13.

All isolated organs were weighed, and no difference was noticed in the weight of control and treated group rabbits. A definite amount of blood was withdrawn using a 22-gauge needle and was subjected to centrifugation for 15 min at 5000 rpm to isolate plasma. No sign of disease, redness, or any ailment was noticed during the histopathological observation of separated organs under a light microscope. Organs of animals were not displaying any signs of tissue destruction. The mortality rate was zero during the whole study. Cardiomyocytes of heart tissues were properly organized, maintaining their

shape, and showing no damage or ballooning. Microscopic evaluation of liver tissues of both groups displayed no degeneration. Moreover, hepatic cord and lobules were properly arranged. Monocytes, macrophages, neutrophils, and lymphocytes were also arranged precisely without invasion.



**Figure 13.** Histopathological images of key organs of rabbits following Na-CMC/pectin-g-(methacrylic acid) hydrogel network administration (NP-1–NP-12) in control (C) and treated (A) groups.

Lungs were normal without having any sign of destruction in their bronchioles. Similarly, rabbit kidney tissue showed some renal cells containing nuclei without any symptom of cell damage or hemorrhage, as observed microscopically. Spleen cells were observed to be arranged symmetrically, maintaining their shape in both the control and tested group. Brain and intestine cells were also normal, showing no sign of destruction or abnormality.

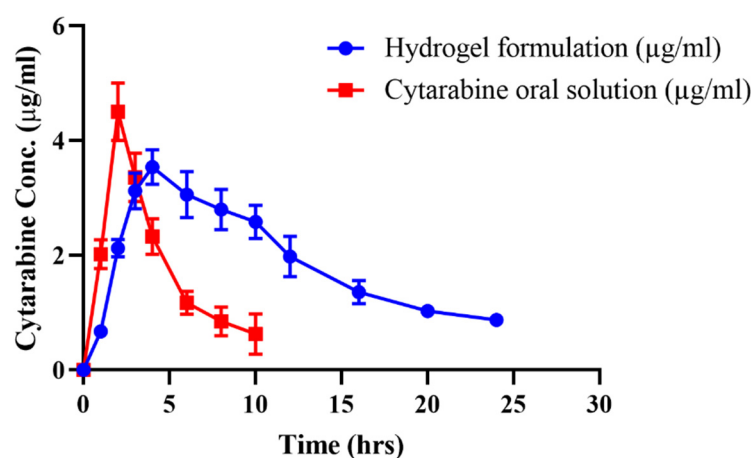
Therefore, the Na-CMC/pectin-g-poly (methacrylic acid) network was approved as a non-toxic and biocompatible platform for the oral administration of therapeutic agents. Rehman et al. (2022) performed toxicity studies of pH responsive hydrogels in healthy rabbits and concluded the safety and biocompatibility of grafted system similar to our observations [29].

### 2.13. In-Vivo Studies

In-vivo performance of cytarabine from hydrogel was investigated by performing pharmacokinetic studies in rabbits. Cytarabine oral solution and the optimized drug loaded formulation (NP-3) in equal doses were administered to both groups (A and B) of animals. At specified time intervals, cytarabine concentrations were determined in plasma using the validated HPLC method. The plasma profile of cytarabine after giving oral solution and hydrogels is presented in Figure 14, and pharmacokinetic parameters are computed in Table 6. The maximum plasma concentration ( $C_{max}$ ) of cytarabine was found to be  $4.50 \mu\text{g/mL}$  attained after 2 h ( $t_{max}$ ) of administration of oral solution. For the cytarabine-loaded Na-CMC/pectin-g-poly (methacrylic acid) network, the  $C_{max}$  was observed to be  $3.53 \mu\text{g/mL}$ , which was achieved after 4 h ( $t_{max}$ ) of administration and sustained for 24 h. Abrupt release of the drug from the hydrogels was noticed within the initial half hour due to the initial rapid release from the surface, which was then sustained for up to 24 h. The



half-life ( $t_{1/2}$ ) and mean residence time (MRT) of cytarabine were 4.44 h and 6.12 h, which were extended after taking drug in the form of hydrogels to 9.24 h and 15.54 h, respectively. Moreover, the AUC was also prolonged from 22.06  $\mu\text{g}/\text{mL}\cdot\text{h}$  to 56.94  $\mu\text{g}/\text{mL}\cdot\text{h}$ . Better results in terms of the half-life, MRT, and AUC were found owing to the steady release of cytarabine from the network, evidencing its controlled release response in plasma. Hence, the Na-CMC/pectin-g-poly (methacrylic acid) network was confirmed to be a good and efficient carrier for a sustained effect of cytarabine and other anticancer moieties. Zhang et al. (2016) conducted in-vivo studies in rats to find out the pharmacokinetic parameters of cytarabine and its conjugates with colic acid. They concluded that giving drug in the form of conjugates resulted in an increase in its  $t_{\text{max}}$  (2.5–3.6 h), AUC (16603.7–34857.0 ng h/mL), and  $t_{1/2}$  (from 4–15 h) in plasma [30]. Our results also reported an increase in half-life, AUC, and a decrease in the  $C_{\text{max}}$  of the drug from prepared formulations, as presented in Table 6.



**Figure 14.** Combined plasma concentration graph of pure cytarabine powder and cytarabine-loaded Na-CMC/pectin-g-poly (methacrylic acid) hydrogel, after oral administration.

**Table 6.** Pharmacokinetic parameters of cytarabine solution and cytarabine encapsulated Na-CMC/pectin-g-poly (methacrylic acid) hydrogel after oral administration.

Parameters	Cytarabine Oral Solution	Hydrogel Formulation
$C_{\text{max}}$ ( $\mu\text{g}/\text{mL}$ )	4.50	3.53
$C_{\text{last}}$ ( $\mu\text{g}/\text{mL}$ )	0.139	0.246
$t_{\text{max}}$ (h)	2	4
$t_{\text{last}}$ (h)	24	24
$\text{AUC}_{0-24}$ ( $\mu\text{g}/\text{mL}\cdot\text{h}$ )	18.03	45.30
$\text{AUC}_{0-\text{inf}}$ ( $\mu\text{g}/\text{mL}\cdot\text{h}$ )	22.06	56.94
$\text{AUMC}_{0-\text{inf}}$ ( $\mu\text{g}/\text{mL}\cdot\text{h}^2$ )	135.17	885.196
MRT (h)	6.12	15.54
$t_{1/2}$ (h)	4.44	9.24
$V_z$ ( $\text{mg}/(\mu\text{g}/\text{mL})$ )	2.90	2.34
$\text{Cl}$ ( $\text{mg}/(\mu\text{g}/\text{mL})/\text{h}$ )	0.453	0.175

### 3. Conclusions

Na-CMC/pectin-g-poly (methacrylic acid) hydrogel was synthesized successfully as a pH responsive carrier and cytarabine was successfully loaded into this network. Newly formed hydrogels were assessed for different parameters by performing cytarabine loading efficiency, swelling studies, structural and compositional tests, DSC and TGA analysis, and drug release experiments. All formulations exhibited a pH sensitive response by showing maximum swelling and cytarabine release at alkaline pH (7.4). Formulation of NP-3 having the maximum ratio of Na-CMC demonstrated optimized results showing highest swelling, loading, and cytarabine release at pH 7.4. Thus, the optimum formulation

was selected for carrying out oral toxicity studies in healthy rabbits to verify the safety of the network system. Animals showed no pathological change and ailment in their vital organs and blood. Furthermore, pharmacokinetic experiments were also performed in rabbits; the results revealed an extended half-life, MRT, and AUC of cytarabine when administered in the form of Na-CMC/pectin-g-poly (methacrylic acid) hydrogel. Thus, the newly developed carrier was proven to be safe, and has potential for oral controlled delivery of cytarabine with higher bioavailability.

## 4. Materials and Methods

### 4.1. Materials

Cytarabine (99.8%) was purchased from Beijing Mesochem Technology Co., Ltd., China. Pectin (99.97%), Na-CMC (99.96%), methacrylic acid (99.78%), ammonium persulfate (APS) (99.69%), N, N-methylene bisacrylamide (MBA) (99.6%), n-hexane (99.71%), and ethanol (99.74%) were purchased from Sigma-Aldrich Co., St Louis, MO, USA. Sodium hydroxide and monobasic potassium phosphate were purchased from Dae-Jung, Korea. Distilled water was prepared in the Pharmaceuticals lab, Faculty of Pharmacy, The University of Lahore. All chemicals utilized in this project were of analytical-grade.

### 4.2. Synthesis of Na-CMC/Pectin-g-Poly (Methacrylic Acid) Hydrogel

Various formulations of hydrogels were synthesized by adopting the aqueous free-radical polymerization technique. Specified quantities of polymers (as mentioned in composition Table 1) were dissolved separately in 10–20 mL of distilled water on a hot plate magnetic stirrer to obtain a clear solution. APS solution prepared in 5–10 mL of distilled water was poured gradually with continuous stirring to the polymer solutions, followed by the addition of a measured amount of monomer (methacrylic acid) in small portions while mixing. Afterwards, the cross-linker (MBA) solution prepared in warm distilled water (45 °C) was mixed in the above combination. The whole mixture was subjected to sonication for 5 min to remove any air bubbles and was shifted to washed and dried test tubes. These tubes were capped with aluminum foil and placed in a digital water bath initially at 50 °C for 2 h, which was raised to 60 °C sequentially for up to 16 h. After this, tubes containing hydrogel formulations were taken out from the instrument and kept at room temperature. Hydrogel-formed test tubes were broken with a test tube holder under tap water. Cylinder-shaped hydrogels were obtained that were converted into discs having 9mm length by a sharp-edged blade. Obtained discs were immersed in a distilled water and ethanol (50:50) mixture for 30 min to remove untreated components. Washed discs were kept at 40 °C in a dry heat oven until complete drying and were preserved in air-tight containers for further use [31]. The proposed scheme for possible polymerization among reactants is given in the following Figure 15.

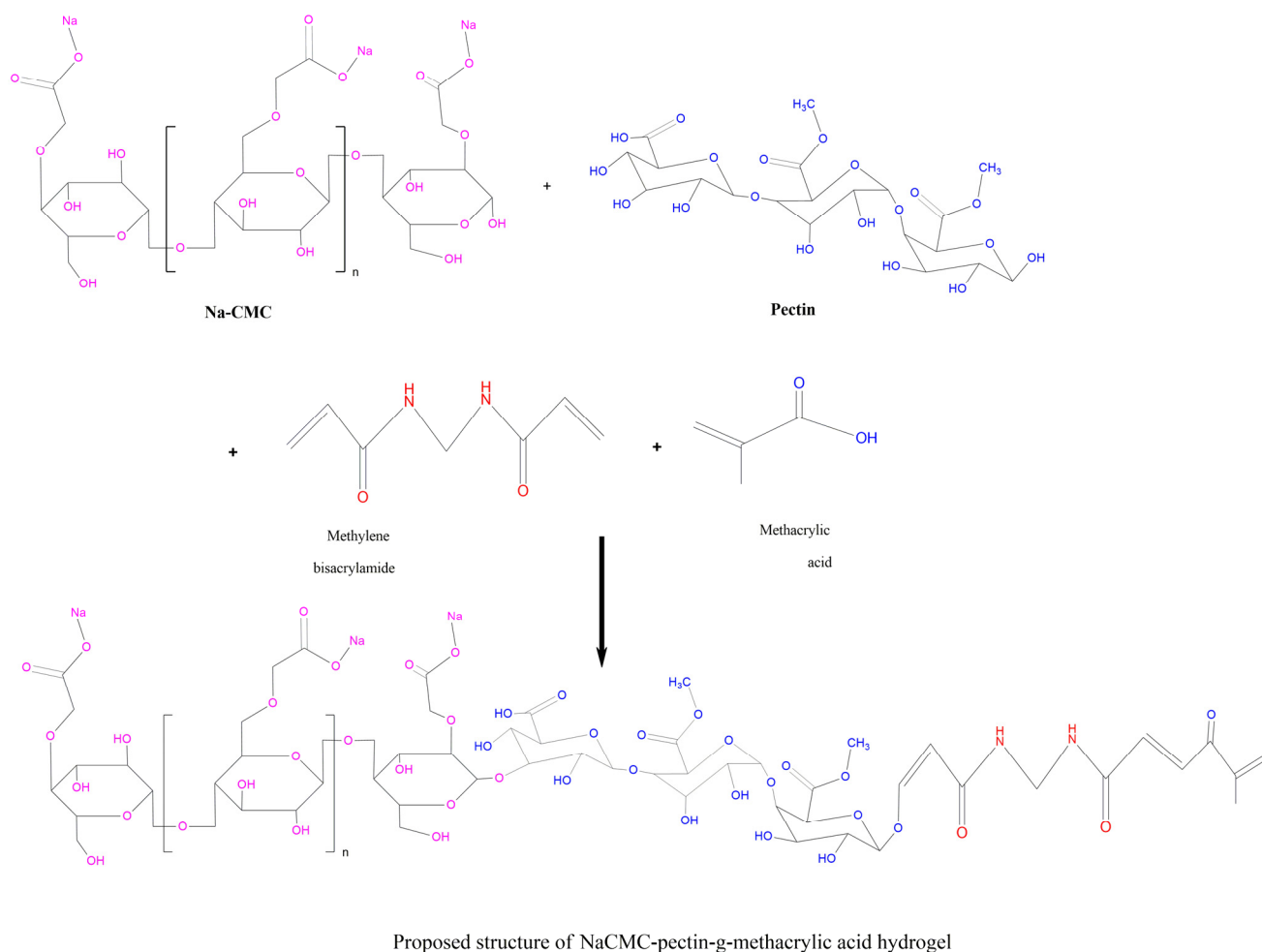
### 4.3. Characterization

#### 4.3.1. Fourier Transform Infrared Spectroscopy (FTIR)

To analyze incompatibility among components of network, the FTIR spectra of the pure drug, polymers, and unloaded- and cytarabine-loaded hydrogels were obtained. Samples were pulverized with a motor and pestle, and analysis was continued in the 700–4000  $\text{cm}^{-1}$  range by Attenuated Total Reflectance FTIR (Bruker Tensor 27, Germany) [32].

#### 4.3.2. Differential Scanning Calorimetry (DSC)

The DSC study was conducted by using a thermo-gravimetric analyzer (West Sussex, UK of Q5000 series). Samples of drug, polymers, and the cytarabine-loaded polymeric system were weighed up to 0.5–3 g into a pan of platinum linked to microbalance in order to collect DSC thermograms. By passing a nitrogen gas stream from 0–600 °C at a heating rate of 20 °C/min, samples were scanned and thermograms were collected [33].



**Figure 15.** The proposed scheme for possible polymerization among reactants.

#### 4.3.3. Thermogravimetric Analysis (TGA)

TGA was carried out to confirm the thermal stability of Na-CMC/pectin-g-poly (methacrylic acid) hydrogel and its comparison with individual components of the network. The same instrument was used for analysis as in DSC, employing similar processing parameters [34].

#### 4.3.4. XRD Studies

XRD studies were carried out to explore the crystallinity or amorphous nature of the compound. Samples of pure cytarabine, pectin, Na-CMC, and blank and cytarabine-loaded fabricated hydrogel network were subjected to XRD to analyze their structural properties. X-ray analytical Xpert powder diffractometer was used to collect diffractograms. XRD designs were kept in the  $2\theta$  scope, in the scanning range of  $10\text{--}100^\circ$ , and  $3^\circ/\text{min}$  check speed using Copper  $k\alpha$  as a radiation source having wavelength of  $1.542 \text{ \AA}$  [35].

#### 4.3.5. EDX (Energy Dispersive X-ray Study)

Oxford instrument UK was used for conducting EDX to analyze the percentages of different elements in the individual components, prepared polymeric cross-linked networks, and to confirm the drug encapsulation within the network. X-ray radiations emitted by the samples were used and assaulted with an electronic beam to collect spectrograms verifying elements on qualitative and quantitative basis [36].

#### 4.3.6. Scanning Electron Microscopy (SEM)

Surface morphology of hydrogels was analyzed by A Quanta 400 SEM by FEI Company, Cambridge, UK. Samples were shaped to fine discs of appropriate dimension and placed on an aluminum stump by pasting with double adhesive tape. Upon the aluminum stump, a thin layer of gold up to ~3000 Å was coated using a vacuum evaporator with the help of gold sputter. Samples were scanned, followed by photomicrographs. These were then studied to explore the morphological characteristics of the prepared hydrogel [37].

#### 4.3.7. Sol-Gel Fraction

Gel fraction (%) was calculated for determining the extent of reactants utilization during the synthesis of cross-linked system. For this, fabricated dried hydrogels were sized into pieces by motor and pestle. The extraction procedure was continued by Soxhlet apparatus carrying boiled water ( $95 \pm 5$  °C) for 4 h. After the specified time, the crushed hydrogel was removed from flask, kept at room temperature, followed by drying in the oven at 40 °C [38].

Gel fraction (%) was determined as follows:

$$\text{Gel fraction (\%)} = \frac{W_o - W_i}{W_o} \times 100$$

Here,  $W_o$  = Initial weight before extraction and  $W_i$  = final weight after extraction and drying

#### 4.3.8. Swelling Ratio

Swelling experiments were carried out at a pH value of 7.4. Discs of dried hydrogels were weighed (1 g) and dipped in 100 mL of phosphate buffer solution (pH 7.4) at 37 °C. These discs were taken out at predetermined time intervals and reweighed after swabbing with tissue paper.

The equilibrium swelling ratio was calculated as:

$$\text{Equilibrium swelling ratio} = W_f / W_i$$

Here,  $W_f$  = Weight of swollen discs at specified time while,  $W_i$  = Initial weight of dried discs [39].

#### 4.3.9. Cytarabine Loading (%)

All prepared hydrogels were loaded with cytarabine by the diffusion-aided swelling method. Pre-weighed dried discs were taken and saturated individually in 1% solution of drug till they achieved maximum swelling. Inflated hydrogel discs were then rinsed with distilled water to clean the surface from excessive adhered drug. These were subjected to drying by placing in a dry heat oven (Mettler, Japan) at 35 °C until attainment of constant weight [40].

#### 4.3.10. Mechanical Strength

Based on the American Society of Testing Materials guidelines, a tensile strength apparatus equipped with TIRA software and a 10 kN load was used to measure the tensile strength. For this, a cross-linked hydrogel developed in a rod-shaped form in glass tube was used having dimensions of 40 mm length and 4 mm diameter. A sample from the fine surface was cut and fixed within the two jaws of the tester. The apparatus was operated by maintaining 50 mm/min speed in a controlled environment. The slope of tensile stress-strain curve was used to find out the Young's modulus. Likewise, stress ( $\sigma$ ) and strain ( $\epsilon$ ) were determined as follows:

$$\sigma = \frac{F}{\pi r^2}$$

where,  $F$  = Original stress applied on hydrogel and  $r$  = radius of sample

$$\epsilon = (lf - li) / li$$

Here,  $lf$  = Final length and  $li$  = initial length [41].

#### 4.3.11. Cytarabine Release Studies

The release profile of cytarabine from the Na-CMC/pectin-based network was assessed by conducting dissolution studies using the United States Pharmacopoeia (USP) apparatus II. Nine-hundred-milliliters of acidic and basic media (0.1 N HCl (pH 1.2) and phosphate buffer (pH 7.4)) were used in each basket for dissolution study. Temperature and paddle speed of the instrument were kept at  $37 \pm 0.5$  °C and 50 rpm, respectively. Sampling was done at specified time intervals and cytarabine content was analyzed at wavelength 281 nm by a UV spectrophotometer (UV-1600, Shimadzu, Germany). Kinetic analysis was done by processing the data through different models, i.e., zero order, first-order, Korsmeyer–Peppas and Higuchi [42,43].

#### 4.3.12. Acute Oral Toxicity Studies

To confirm the safety and biocompatibility of the formed network, toxicity studies were carried out following OECD rules. Study design and protocols were checked and approved by the Institutional Research Ethics Committee of the Faculty of Pharmacy, The University of Lahore vide notification no. IREC-2020-29. Physical factors of animals were noticed during the studies for estimation of hydrogel effects on animals.

Eighteen albino rabbits were placed in bird cages for 7 days in a dark/light cycle, i.e., 12 h for accommodation to pet environment with an adequate availability of a proper diet. Six rabbits were considered as the control group (A) and six as the treated group (B). Rabbits in treated group were fasted overnight and administered hydrogel in powder form, dosed at 2 g/kg. Animals were kept under close monitoring for 2 weeks regarding any physical changes, body weight, food water intake, and any ailment. After 1 week, hematology, renal profile, AST, and ALT levels were measured by drawing out a 3–4 mL blood samples from ear's marginal vein of the animal using a 3cc syringe and placing it into EDTA tubes. Analysis was made after centrifugation (5000 rpm) of these blood samples.

After 2 weeks, all rabbits were reweighed and anesthetized by giving 1 mL/kg dose of xylazine and ketamine (30:70). All vital organs of animals were removed after slaughtering them, i.e., heart, brain, kidney, liver, stomach, spleen, lungs, and intestine. These were preserved in a 10% formalin solution after washing under tap water, stored in individual labeled plastic jars, and their histopathology were performed [44].

#### 4.3.13. In-Vivo Studies

Eighteen healthy albino male rabbits (2–2.5 kg) were chosen according to standard protocol by Institutional Research Ethics Committee, Faculty of Pharmacy, The University of Lahore, Punjab, Pakistan vide notification no. IREC-2020-29. Three groups of animals were made ( $n = 6$ ; A, B and C) to perform in-vivo studies following a cross-over design. Groups A and B were fasted for approximately 12 h prior to experimentation but had access to water. The dose was calculated according to FDA guidelines (2005), at a human intermediate dose of cytarabine 5 mg/kg body weight [45]. Rabbits of group A were given solution of pure cytarabine powder (10 mg/kg dose) with water. Cytarabine-loaded hydrogel at an equivalent dose was converted to small particles using a pestle and mortar and filled in hard gelatin capsules to administer to the animals of group B. Group C animals were kept as controls. Cytarabine concentration in plasma was quantified by drawing out a 3-mL blood sample from rabbit's jugular vein with a 3-mL syringe (Injekt®) at the defined time periods (0, 0.5, 1, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 9.0, 12.0, 15.0, 18.0, 21.0, and 24.0 h) and transferred in tubes containing anticoagulant immediately. These blood samples were subjected to centrifugation (5000 rpm) for 10 min to isolate the plasma. Plasma was carefully separated and frozen at  $-20$ °C in an ultra-low freezer (Sanyo-Japan,  $-80$  °C). A frozen sample was thawed at room temperature before use [46].

#### 4.3.14. HPLC Estimation of Cytarabine Concentration in Plasma

Solvent extraction technique was used for deproteinization in plasma. For this, 1 mL of isolated plasma was mixed with an equivalent volume acetonitrile in a glass centrifuge tube for plasma protein precipitation. To ensure uniform agitation, tubes were subjected to vortex mixing for 2–4 min and centrifugation was carried out (5000 rpm) for 10 min. The upper layer was collected, filtered, and diluted with mobile phase and 20  $\mu$ L volume was used for quantitative analysis. A developed HPLC method was employed for cytarabine analysis using a UV visible spectrophotometer linking with a Pentium IV computer. Column (Hypersil Octadecylsilane C<sub>18</sub>) dimensions were 250  $\times$  4.6 mm (Thermo Electron cooperation, Torrance, CA, USA), with a 5  $\mu$ m particle size. The mobile phase used was a mixture of acetonitrile and water (50:50) flowing at 1.2 mL/min rate. A run time of 15 min was selected and cytarabine was detected at  $\lambda_{\max}$  272 nm [47,48].

**Author Contributions:** Conceptualization, methodology N.B., R.M.S., and A.M.; software, investigation S.A.; writing—original draft preparation, supervision U.R. and M.Z.; writing—review and editing, H.A.G.; project administration, D.M.A. All authors have read and agreed to the published version of the manuscript.

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**Institutional Review Board Statement:** The study was conducted in accordance with the Declaration of Helsinki, and was approved by the Institutional Research Ethics Committee of Faculty of Pharmacy, The University of Lahore, Pakistan videnotification no. IREC-2019-137A.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** All data are reported in the manuscript.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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