Periodate-Modified Gum Arabic Cross-linked PVA Hydrogels: A Promising Approach toward Photoprotection and Sustained Delivery of Folic Acid

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ABSTRACT: The chemically oxidized gum arabic was prepared and used as a naturally derived nontoxic and pH-responsive cross-linker to develop smart polyvinyl alcohol (PVA)-based hydrogels for the first time. The formulated hydrogels exhibited high mechanical properties, good porosity, and pH sensitivity, which facilitated their application as promising biomaterials for sustained delivery of folic acid. Further, the synthesized cross-linked PVA hydrogels displayed no cytotoxicity toward the human embryonic kidney cell line and exhibited higher blood compatibility. The hydrolytic degradation study confirmed their biodegradable nature. While the sustained delivery along with photoprotective properties of these hydrogels confirmed their multifunctional characteristics, these results suggest that these hydrogels may act as an efficient photoprotective material and find their application in the field of drug delivery.

1. INTRODUCTION

In recent years, periodate modification of biopolymers like hyaluronic acid,1 cellulose,2 gum arabic (GA),3 alginates4 and so forth have received an enormous interest in synthesizing the most active, green, nontoxic, and biocompatible cross-linking agent, which have been used in the formation of hydrogels. These periodate-modified polysaccharides having certain advantages over conventional aldehyde-based cross-linkers as these are higher molecular weight macromolecules having larger size which prevents them from evaporation, thereby reducing environmental toxicity.5 Among these, GA being highly water soluble, readily available, sustainable, and easily processable is found to be the suitable material for the formulation of hydrogel.6,7 The periodate oxidation of GA proceeds with the C2–C3 bond cleavage of its glucose residues resulting in the formation of di aldehyde groups per monomeric unit. These aldehyde groups of oxidized GA (OGA) can cross-link with NH2/OH groups of other polymers resulting in the formation of hydrogels via Schiff base/acetal linkages.8 For instance, Nishi and Jayakrishnan9 used OGA as cross-linking agent in the synthesis of injectable polymer conjugate-based hydrogel used for drug delivery. The fabricated hydrogel exhibited sustained release behavior. Recently, Sarika et al.10 have used OGA as the main precursor as well as an internal cross-linker in the preparation of hydrogel scaffolds. The developed hydrogel showed no cytotoxicity toward the cells and act as a suitable material for drug screening and cancer therapy. However, literature reveals that the cross-linking efficiency of OGA with OH group containing polymers has not been explored till date, which needs to be further investigated.

The hydrogels based on polyvinyl alcohol (PVA) have been extensively used in various biotechnological applications like drug delivery, tissue engineering, articular cartilage, and wound dressings,10,11 because of their nontoxic, recyclable, water-soluble, eco-friendly, and biodegradable nature. In addition, PVA possesses excellent film-forming ability, favorable thermal property, and flexibility.12 However, poor stiffness and hydrophilicity limit its applications. These drawbacks can be overcome through the combination of PVA with other biopolymers using various methods like cross-linking and blending.11,13 The physical cross-linking methods like thawing, freezing, and high-temperature treatment have been used, which facilitates the formation of weak interactions within the hydrogel and also their cross-linking mechanism is not clearly understood.14,15 The chemical cross-linking approach is found to be the most promising one because of the involvement of chemical interactions between the PVA and the cross-linker resulting in the formation of more stable hydrogels. These interactions can significantly enhance the absorption and mechanical properties of PVA in aqueous solutions and physiological fluids thus, enhance their biological activities. The various conventional chemical cross-linking agents like anhydrides, epichlorohydrin, and aldehydes (formaldehyde, glutaraldehyde, and benzalde-
hyde), used for the cross-linking of PVA has a synthetic origin which is associated with high level of toxicity causing harmful effects on environment and living cells. Besides, these low-molecular weight molecules have small size, which may penetrate easily through many portals into living systems. Therefore, for environmental safety and biomedical application, there is higher requirement for biocompatible green and nontoxic cross-linkers derived from natural polymers which have the tendency to function as a substitute to these lower molecular weight and toxic cross-linking agents.

In this regard, present study highlights the utilization of a naturally derived cross-linker, that is OGA, for the design and formulation of cross-linked PVA-based hydrogels using folic acid (FA) as sample drug and investigate its photoprotection ability and sustained delivery. Structural, mechanical, and physico-chemical properties of PVA cross-linked hydrogels such as chemical structure, morphological observations, swelling behavior, and tensile properties were characterized. The hemolytic assay and in vitro biocompatibility of these cross-linked hydrogels were also investigated to confirm their nontoxic nature.

2. RESULTS AND DISCUSSION

The GA was successfully oxidized to form a promising cross-linking agent (OGA). The OGA was used to synthesize highly cross-linked PVA-based hydrogels.

2.1. Preparation and Structural Analysis of OGA.

Oxidation of GA was carried out using 4.67 mmol concentrations of NaIO₄ to form OGA (Figure 1a) with 46.16% degree of oxidation and 4.37 mmol/g aldehyde content. The formation
of OGA was successfully confirmed from Fourier transform infrared (FTIR) and NMR analysis (Figure 1b,d). FTIR spectra of GA showed a stretching peak OH group at 3255 cm$^{-1}$, asymmetric CO stretching frequency at 1600 cm$^{-1}$ and $\text{CH}_2$ stretching band at 2927 cm$^{-1}$. The occurrence of the peak at 1417 cm$^{-1}$ can be ascribed to $\text{CH}$ and $\text{CH}_2$ wagging vibrations arising because of skeletal motions of the carbon rings.29 While as the OGA showed new peaks at 1729 and 876 cm$^{-1}$ which correspond to the CO stretching of aldehyde and formation of hemiacetal bands, respectively.30

The GA protons showed almost similar chemical shifts in the $^1$H NMR spectrum as that reported by Ali et al. and on comparison an acceptable matching of signal was achieved17 (Figure 1c). The $^1$H NMR spectrum of OGA (Figure 1d) also confirms its successful formation. The presence of a small peak at 9.48 ppm was detected, which can be to aldehydic protons of OGA.30

The solid $^{13}$C NMR spectra of GA comprise the peaks at 13.7 ppm (C-6, Rhap), 60.1 ppm (C-2, C-5, Galp-Rhaf), 100–120 ppm (C-1), and 165.1 ppm (C-6, Glap A); these peaks are in well agreement with that reported in the literature.31 A clear and pure $^{13}$C NMR spectrum of OGA in the solution form was difficult to be obtained because of its poor D$_2$O solubility, which therefore showed the poorly resolved signal of carbonyl in the range of 175–180 ppm (not given here). From solid-state $^{13}$C shifts of OGA, a peak at 174 ppm was observed (Figure 2) which may be ascribed to the carbonyl carbon of the aldehyde group.

2.2. Formulation and Structural Analysis of Cross-linked PVA Hydrogels. The cross-linked PVA hydrogels were formulated via acetal linkage between the OH groups of PVA and aldehyde of OGA (Figure 3a). The FTIR spectra of the pure PVA film (F0) consist of a peak at 3390 cm$^{-1}$, which is due in stretching vibration of OH groups of PVA, the bands between 2910 and 2921 cm$^{-1}$ can be due to asymmetric stretching vibration of the C−H bond (Figure 3b). While the peak at 1630 and 1087 cm$^{-1}$ corresponds to C=O and C−O stretching of acetyl groups, respectively.32 The FT-IR spectra of F0 and cross-linked hydrogels (F1, F2, and F3) showed differences in characteristic peaks with increase in concentration of OGA (Figure 3b). The large broad band was observed in the frequency range of 3250–3390 cm$^{-1}$ because of intermolecular and intramolecular stretching vibrations of OH groups. It has been observed that the intensity of OH groups decreased with the increased cross-linking density indicating more involvement of −OH groups in acetal bond formation. The peaks observed at 2840–2859 and 1705–1724 cm$^{-1}$ can be attributed to O−C−H and C═O stretching of unreacted aldehyde and their intensities relatively decrease with the increased concentration of OGA. The band is observed at 1005–1040 cm$^{-1}$ with gradual increase in the peak intensity upon increasing the concentration of OGA. This can be ascribed to the presence of O−C═O acetal group vibrations.33

2.3. X-ray Analysis. The X-ray diffraction (XRD) spectra of F0 and cross-linked hydrogels F1, F2, and F3 hydrogels are shown in (Figure 4). It is observed that the F0 film showed two distinct diffraction peaks at 19.6° and 40.3° which are due to (101) and (111) planes of the monoclinic unit cell, respectively.34 The semicrystalline nature of F0 is because of the intermolecular H-bonding. However, upon cross-linking the intensity of the peak decreases at 19.6°, while the peak at 40.3° disappeared. This can be attributed to the acetal bond formation which substitutes the hydrogen bonding. Therefore, increase in cross-linker density increases amorphousness, thus, F3 hydrogel exhibited more amorphous nature than other hydrogels.

2.4. Morphology of PVA Cross-linked Hydrogels. Scanning electron microscopy (SEM) was used to visualize the surface morphologies of cross-linked hydrogels. The hydrogel (F1) exhibits a highly porous surface morphology as shown in Figure 5 (F1 and F1′ at two different resolutions).
However, when the OGA content increases, the porosity and the pore size decrease, and the average pore size of F1 hydrogel was in the range of 181.6 μm and that of F2 and F3 was 94.92 and 91.99 μm, respectively. Thus, F3 hydrogel which has higher OGA concentration resulted in the formation of highly compact gel with low porosity. Therefore, the increased cross-linking lowers the available free volume in the hydrogels that may reduce the swelling properties of the resultant hydrogel.

2.5. Mechanical Strength. The mechanical strength (elongation break and tensile strength) of F0 and cross-linked hydrogels (F1, F2, and F3) were investigated at 25 °C. From the strain−stress curve (Figure 6), it can be concluded that F0 displays a tensile strength of 21.03 MPa with percent elongation of 314%. However, when the concentration of OGA was increased, the % elongation of the hydrogels decreased while the value of tensile strength increased, and the maximum value was 43.99 MPa in case of the F3 hydrogel which has higher OGA concentration. Cross-linking helps in the formation of inter- and intra-molecular linkages and thus helps in lengthening of PVA molecules. However, the interaction among the molecules could be enhanced, which results in enhancement in tensile strength and decline in % elongation in case of F1 with low cross-linking higher sliding of PVA molecules may take place. The continuous increase in the cross-linking concentration avoids the sliding of PVA molecules because of higher inter- and intra-molecular interactions, which cause decrease of % elongation. Thus, F1 hydrogel which has lower concentration of OGA (30 mg/mL) shows higher values of tensile strain and lower value of tensile stress than other cross-linked hydrogels.

2.6. Differential Scanning Calorimetry of Cross-linked Hydrogels. The $T_g$ and $T_m$ of F0 and F1, F2, and F3 cross-linked hydrogels were analyzed with the help of the second heating thermograms (Figure 7). The F0 showed $T_g$ at around 67 °C and $T_m$ at 215 °C which is in well agreement with that reported in the literature. It has been observed from the differential scanning calorimetry (DSC) graph that by increasing OGA concentration, $T_g$ of hydrogels increases from 72.5 °C for F1 and 78.37 and 86.13 °C for F2 and F3, respectively. The increase in the values of $T_g$ of these cross-linked PVA hydrogels are very comparable to that already reported in literature. The increasing of $T_g$ with increasing cross-linking concentration can be ascribed to the chemical interactions between OH groups of PVA and aldehyde groups of OGA which limits polymer chain mobility. However, it was also observed that by increasing OGA concentration, the melting endotherm peaks shifted slightly to lower temperature. The decrease in $T_m$ may be related to the decline of crystallization because of cross-linking reaction between the PVA and OGA which suppresses the hydrogen bonding interaction in the PVA polymer chains and leads to the amorphous nature in the hydrogels. These results are in accordance with that obtained from XRD analysis which shows amorphous nature of highly cross-linked hydrogel.

2.7. Swelling Studies. The swelling behavior of cross-linked hydrogels (F1, F2, and F3) was studied at pH 7.4 and 2.1. It has been observed from the swelling plots (Figure 8) that the equilibrium degree of swelling for all cross-linked hydrogels films was achieved in 9 h. The hydrogels with lower OGA cross-linked showed a higher degree of swelling than the hydrogel with higher cross-linker concentrations. The higher concentration of OGA favors the formation of highly cross-linked networks in the hydrogel films, which restrict the mobility of the polymeric chains and thus limits their exposure to the water molecules. Besides, the OH groups of PVA are hydrophilic and can easily undergo hydration with water. As the cross-linking density increases, more hydroxyl groups are participated in acetal bond formation; hence the capacity of OH groups to form hydrogen bonds with water decreases, which led to the reduction in the swelling capacity of hydrogels. Further, the higher percentage of swelling occurred at pH 7.4 than at pH 2.1 for all hydrogel because at higher pH, more OH⁻ ions are generated that

Figure 5. SEM micrographs of PVA cross-linked hydrogels F1, F2, and F3.

Figure 6. Stress−strain graph of cross-linked hydrogels.

Figure 7. DSC thermogram of cross-linked PVA hydrogels.
hydrolyze the acetate groups of PVA. Therefore, the chains become more ionic possessing similar charges which create repulsive forces that repel the chains, thereby increasing the space for incoming solution and improves the swelling ratio.37 The date of swelling plots (Figure 8) has been used for the evaluation of the cross-linked network of these hydrogels. Various parameters like MC, ξ, and ρ are used for characterizing the porous structure of hydrogels which are necessary for the transport of various drugs. These parameters can easily be determined from swelling equilibrium theory given by Wright and Peppas.38 On the application of this theory, it has been observed that the MC and ξ values are found to be higher in cross-linked PVA hydrogels with low cross-linking concentration as shown in Table 1.

Table 1. Molecular Weight between Cross-links, Mesh Size, and the Cross-Linking Density of Cross-linked PVA Hydrogels

| Hydrogel | MC (g/mL) | ξ (Å) | ρ (mol/cm³ × 10¹⁷) |
|----------|-----------|-------|-------------------|
| F1       | 1766 ± 1122 | 102 ± 25 | 3.7               |
| F2       | 1368 ± 8932 | 76 ± 31  | 11.23             |
| F3       | 833 ± 102   | 55 ± 15  | 19.34             |

2.8. Biocompatibility Studies. The biocompatibility studies carried out using MTT assay on HEK-293, revealed that cross-linked hydrogel film and FA-loaded hydrogel (F1) do not show any significant decline in the cell viability and did not induce any cytotoxicity in the dose range of 8–64 μg/mL up to 48 h (Figure 9d). However, these hydrogels showed a marginal cytotoxicity (less than 20%) at a higher dosage (128 μg/mL), which is not considered as higher toxicity. Further, the results revealed that the original cell morphology was not affected by the treatment with FA loaded and unloaded hydrogel (Figure 9a–c) even at the maximum concentration (i.e. 128 μg/mL).

2.9. Hemolytic Assay. The application of hydrogels in various biomedical fields like drug delivery involves the usage of living beings, thus it is important to determine their blood compatibility. The blood compatibility in vitro conditions of FA-loaded hydrogel (F1) was carried out with three different concentrations (50, 150, and 250 mg/mL) via hemolysis tests. It was observed that the hemolysis percentage of 1.64% was found at 250 mg/mL concentration followed by 1.57 and 1.11% at 150 and 50 mg/mL concentrations, respectively (Figure 9e). Therefore, with the increase in concentration, the hemolysis percentage increased and was found to be lower than 2%, confirming the nontoxic nature of hydrogels.38

2.10. Hydrolytic Degradation. The hydrolytic degradation of cross-linked hydrogels (F1, F2, and F3) was investigated in the buffer solution (pH 7.4), which causes the hydrolysis of acetal linkages and enhances the biodegradability. The degradability investigations revealed that initially an increase in the weight of hydrogels was observed through swelling. After achieving equilibrium swelling, successive weight loss was detected. The F1 hydrogel showed more weight loss as compared to F2 and F3 because of the presence of lower cross-linker concentration, which causes improvement in the water uptake ability, thus favors its hydrolytic degradation. All the hydrogels showed higher hydrolytic stability and start to degrade after 2 days. The enhancement in the rate of degradation of F3 hydrogel can be attributed to the formation highly stable acetal linkage between the OGA and OH groups of PVA. The F1 hydrogel shows about 98% weight loss after 18 days which is 40% greater than F3 (Figure 10).

2.11. Loading and in Vitro Release of FA. The loading of FA in cross-linked hydrogel showed a higher % of loading in case of F1 (29.11 and 21.03%) compared to F2 (24.11 and 17.27%) and F3 (17.21 and 13.42%) after 72 h at both pH 7.4 and 2.1. The loading of FA was observed to be relatively lower in F2 and F3 at both pH, which can be due to formation of highly cross-linked networks in these hydrogels, thereby lowering the available space in the hydrogel matrix. The loaded FA hydrogel films (~50 mg) were kept in 20 mL of solution (pH 7.4 and 2.1) at room temperature. Samples were withdrawn periodically, replaced with fresh medium to keep the constant volume of the dissolution medium same. The amount of FA released from the hydrogel samples were measured using the spectrophotometer. The release behavior of FA was investigated at acidic and basic medium, which exists in stomach and intestines, respectively. From the release behavior, it was found that higher percentage of release occurred at pH 7.4 than at pH 2.1 Assadpour et al.39 and Madziva et al.40 have observed similar release profiles for FA at different pH. The higher release of FA at higher pH could be ascribed to pH-dependent behavior of FA-loaded hydrogels. More over FA shows more solubility with increasing pH.41 Thus, it can be suggested that loaded FA may exhibit a scarce release in the acidic environment, that is, stomach, but shows a higher release rate in the alkaline pH (pH 7.4) which prevails in the small intestine, where its absorption takes place. From cumulative release studies (Figure 11) it was observed that nearly about 78% of FA was released in case of F1 at pH 7.4, followed by 68 and 28% in F2 and F3 hydrogels, respectively. However, the % release of FA was low in hydrogels at pH 2.1 which may be possibly due to lower swelling ability of hydrogels.
at lower pH as confirmed from their swelling behavior studies and lesser solubility of FA at low pH. Further, it was also observed that higher release of FA occurs in the first 5 h at both pHs and then showed gradual increase up to 7 h. The highest release of 21 and 78.87% of FA was observed in case of F1 hydrogel after 1 and 5 h, respectively, at pH 7.4. This presents extended release times as compared to that of commercially available FA tablets, which at same time and pH showed a release rate of 60 and 90%. Other systems used for FA delivery presented a range of release rate and time, depending upon the materials used (Table 2).

### Table 2. Percentage of FA Released in Different Delivery Systems

| system                              | release % | time (h) | pH of solution | refs |
|-------------------------------------|-----------|----------|----------------|------|
| microcapsules based on ethyl cellulose | 32 and 70 | 1 and 6  | 7.4            | 42   |
| commercial FA tablet                | 60 and 90 | 1 and 5  | 7.4            | 43   |
| electro spun fibres of sodium alginate pectin poly(ethylene oxide) | 90–100    | 2        | 7.4            | 44   |
| crosslinked PVA hydrogels           | 21–78     | 1 and 5  | 7.4            | this work |

Figure 9. Inverted phase contrast microscopic image of HEK-293 cells (a) control (b) F1 and (c) FA-loaded F1 hydrogel after 48 h of incubation of 128 µg/mL highest concentration at 40X. Cellular viability of F1- and FA-loaded F1 hydrogel films using MTT assay for 48 h (d). Percentage hemolysis of FA-loaded F1 hydrogel and photographs of precipitated RBC’s treated with three different concentrations of F1 (e).

Figure 10. Hydrolytic degradation of F1, F2, and F3 hydrogels.

Figure 11. Cumulative release study graph of FA-loaded cross-linked PVA hydrogel films at pH 2.1 and 7.4.
The release mechanism of FA from cross-linked hydrogels can be predicted to take place in three steps (Figure 12). In the first step, the FA-loaded hydrogel has less volume of water as a result of which the hydrogel shows lower flexibility, small pore size, and limited FA mobility. In the second step, diffusion of water takes place which causes relaxation in the hydrogel, induces flexibility, and increases hydrogel pore size, leading to the enhancement in the FA mobility. In the third step, these hydrogels became fully hydrated and relaxed, increases the rate of diffusion because of larger pore size.

The drug release from the matrix of hydrogel generally occurs via either non-Fickian, Fickian, or case II diffusion which relies upon mechanical strength of hydrogels, their chemical properties, and external stimulus. In order to study the FA transport mechanism through cross-linked PVA hydrogels, different diffusion models were applied for fitting the experimental date and among these models, Korsmeyer-Peppas gives an idea of the FA release mechanism through cross-linked PVA hydrogels, while as the Higuchi model explains the release mechanism completely. For all cross-linked hydrogels (F1, F2, and F3), the n values (Table 3) were found in between 0.51–0.78, signifying different model fitted values of in vitro FA release at pH 2.1

| crosslinked hydrogel | zero-order model R² | first-order model R² | Higuchi model R² | Korsmeyer–Peppas model R² | n |
|----------------------|---------------------|----------------------|-----------------|--------------------------|---|
| F1                   | 0.8822              | 0.9413               | 0.9816          | 0.9613                   | 0.51 |
| F2                   | 0.8793              | 0.9492               | 0.9887          | 0.9628                   | 0.55 |
| F3                   | 0.8917              | 0.9411               | 0.9819          | 0.9672                   | 0.58 |

that the release of FA from hydrogel takes place via non-Fickian diffusion. It was also observed from R² values (Table 3) that FA release from cross-linked hydrogel follows the Higuchi model which indicates that diffusion is responsible for release of FA from these hydrogels. It can be clearly observed that at higher pH (7.4), the n values more closely approach to 0.89 at which the FA release is governed by the relaxation of hydrogels. The n values for cross-linked hydrogels are found to be smaller at lower pH (Table 4) than at higher pH. This can be due to the collapsed form of these hydrogels in acidic pH as a result of which the FA is unable to disseminate fast from the hydrogel. Thus, it can be concluded that at lower pH FA release depends less on hydrogel relaxation.

2.12. Photostability of FA-Loaded Hydrogel. FA (Vit-B9) is vital ingredient required for all living organisms. Human body is unable to synthesis this vitamin; thus, we depend entirely on our foods for its supply. FA deficiency may lead to various types of diseases such as neural tube defects, Alzheimer’s disease, complications related to pregnancy and cancers. It is sensitive to UV radiations and acidic conditions, which cleave the C₉=N₁₀ bonds to form biologically inactive (p-amino benzoyl)glutamic acid and pteridine. This may result in the lower bioavailability of FA in food materials and thus large concentrations are required to ensure good health of humans. Therefore, one of the key purposes of this work was to investigate the photoprotection property of FA-loaded hydrogels. The pure FA solution and the FA-loaded hydrogel (F1) was subjected to UV radiation and their absorption spectra was compared with the nonirradiated samples. The absorption spectra of FA displayed two main peaks at 280 and 350 nm, which can be because of the occurrence of pteridine and p-aminobenzoic glutamate moieties. However, after 2 h of irradiation the FA spectrum changed, thus signifying degradation of FA as shown in Figure 13. The absorption spectra of UV-irradiated free FA shows peaks at 355, 310, and 272 nm which can be attributed due to the formation of 6-formylpterin and pterine-6-carboxylic acid which are biologically inactive photoproducts of irradiated FA. While as the UV–visible spectra of FA-loaded hydrogel did not show any notable changes after 2 h of UV exposure.

Figure 12. Schematic representation of the process of FA release from cross-linked hydrogel.

Figure 13. UV–visible spectra of pure FA and loaded FA before and after UV irradiation.
3. CONCLUSIONS

The OGA was successfully synthesized through periodate oxidation and used as an effective cross-linker for the formulation of cross-linked PVA hydrogels. The swelling and release study of the said hydrogels was performed. It was observed that the PVA-based hydrogels exhibited higher swelling ratio at higher pH. While the release studies revealed that the release rate of FA was found to be higher in F1 (78 and 32%) as compared to F2 (69 and 22%) and F3 (30 and 18%) at pH 7.4 and 2.1, respectively. The increase in the swelling ratio is due to the decrease in cross-linking density of these hydrogels. The in vitro haemolytic and cytotoxicity studies of hydrogels and FA-loaded hydrogels showed biocompatible and nontoxic nature. Further, the hydrogels displayed UV-photoprotection property and prevented the loaded FA from UV degradation. These results suggest that these hydrogels may act as a promising photoprotective material and are suitable candidates for sustained delivery of FA.

4. MATERIALS AND TECHNIQUES

4.1. Materials. PVA (molecular weight = 85 000–124 000, degree of hydrolysis = 86–89%) was procured from S.D. Fine Chemicals, Mumbai, India, GA (molecular weight = 953 900 g/mol), hydroxy amine hydrochloride (NH₂OH·HCl) ethylene glycol, sodium periodate (NaIO₄), ethanol, sodium thiosulphate, hydrochloric acid (HCl), and sodium hydroxide (NaOH) were purchased from Merck. FA was obtained from CDH Chemicals. These chemicals were used as such without further purification.

4.2. Synthesis of OGA. Oxidation of GA was carried out to synthesize the cross-linker aldehyde GA (OGA) using NaIO₄ as oxidizing agent following reported in the literature.¹⁷ GA (1.0 g) was added in 20 mL of deionized water. NaIO₄ (0.5 g) was dissolved in 10 mL of deionized water and was added dropwise to the GA solution, then the reaction mixture was allowed to stir in dark for 24 h at 20°C. After, ethylene glycol (5 mL) was added to the solution to neutralize the unreacted periodate, finally, the reaction mixture was precipitated out by the addition of excess ethanol (150 mL) which was filtered, washed several times with ethanol water mixture, and then subjected to freeze-drying to obtain OGA powder. The oxidation degree was calculated with the help of periodate that remain unreacted in the reaction mixture using iodometric titration.¹⁷ Sodium bicarbonate solution (10 mL) was used to neutralize the 5 mL aliquot from the reaction mixture. The liberation of iodine occurs by adding 2 mL of potassium iodide solution and 1 M sulphuric acid (5 mL) to the reaction mixture. The solution was kept away from light in dark conditions for 30 min and the periodate present in the reaction mixture was estimated by titration of the liberated iodine with 0.1 N sodium thiosulphate in the presence of starch indicator.

4.3. Aldehyde Content Determination. The aldehyde content of OGA was evaluated by the conversion of aldehyde groups into oxime via Schiff base reaction with the help of hydroxylamine hydrochloride.¹⁷ OGA (0.3 g) was dissolved in 20 mL of deionized water (pH 5.0) then, 20 mL (0.72 mol/L) of NH₂OH·HCl was mixed to this solution under stirring for 4 h at 40°C. The amount of HCl released was estimated by titrating the solution with standard solution of NaOH (1.0 M) in the presence of phenolphthalein as the indicator. The aldehyde content was calculated using following equation

\[
\text{Aldehyde content (%) = } \left( \frac{V_o - V_b}{V_o} \right) \times 8 \times m/M \times 100
\]

where \(V_o\) and \(V_b\) are the volume of NaOH consumed in the presence of OGA and blank, respectively. \(C\) is concentration of NaOH, \(m\) is weight of OGA, and \(M\) is the approximate molecular weight of the monomeric OGA (162).¹⁷

4.4. Preparation of PVA Cross-linked Hydrogels. Hydrogels were formed by the chemical cross-linking involving the aldehyde functionality of OGA and OH groups of PVA. Various concentrations (30, 50, and 70 mg/mL) of OGA were dissolved in 10 mL of deionized water and poured dropwise into 10% aqueous solution of PVA in the presence of a catalyst (2 mL 1 M HCl). The mixtures were kept under the slow and controlled stirring for 3 h at 60 °C and transferred into the casting material. The casted hydrogel films were air-dried at room temperature and washed thoroughly to remove the unreacted material. The hydrogels prepared with 30, 50, and 70 mg/mL concentration of OGA were named as F1, F2, and F3, respectively, while the pure PVA film was formulated by dissolving PVA (1 g) in 10 mL of deionized water on stirring for 3 h at 60°C and then transferred to the casting material for film formation. The pure PVA hydrogel film was named as F0.

4.5. Characterizations. FTIR spectroscopy (PerkinElmer Cetus Instruments, Norwalk) was used to identify the aldehydic groups of OGA and acetal bond formation of cross-linked PVA hydrogels. Proton NMR spectra (Bruker 300 MHz spectrometer, Billerica, USA) of GA and OGA in D₂O were obtained to investigate the presence of aldehydic protons on OGA. The presence of aldehydic carbon on OGA was confirmed using the solid-state ¹³C NMR technique (JEOL, 400 MHz FT-NMR). XRD patterns of cross-linked hydrogels were recorded with the help of Rigaku Ultima (IV) XRD (40 kV, Tokyo, Japan) having a scanning speed of 8°/min with 2θ ranging from 10° to 70°. To observe the morphology of hydrogels in swelling, the samples were kept in double distilled water for 24 h and then dried in vacuum after that these were analyzed with Zeiss, EVO 18 SEM instrument. The mechanical tests of the cross-linked PVA hydrogel films in the swelling state were conducted using the universal testing machine (Instron 8871) with the 10 N load cell. The hydrogel films with thickness and width of 30–35 μm and 10 mm, respectively, were used for mechanical studies. The tests were performed in 25 °C and under the prestrain of 0.05–0.1 N. The samples were conditioned in the measuring environment one day before their testing. UV spectrophotometer (Lambda, 650) was used for the detection of FA release behavior and to study the photoprotection property of hydrogel samples. The glass transition temperature (T_g) and melting temperature (T_m) of F0 and cross-linked F1, F2, and F3 hydrogels were investigated with the help of DSC (DSC-60 Plus, Shimadzu). These hydrogel samples were analyzed between 0 and 250 °C temperature range under nitrogen atmosphere with 10 °C/min of scanning rate.

4.6. Swelling Studies. The swelling behavior of cross-linked hydrogel was determined gravimetrically in water by evaluating their water uptake. Initially, these dried hydrogels were weighed and immersed in 30 mL of deionized water for different periods at 25 °C. The hydrogels were taken out and blotted using tissue paper to wipe off the surface water. The amount of water absorbed by the hydrogels was determined with the help of following equation

\[
\text{Swelling percentage } = \left( \frac{W_i - W_d}{W_d} \right) \times 100
\]

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where \( W_s \) and \( W_d \) and are weight of hydrogels in swollen and dry states, respectively.

### 4.7. Determination of Molecular Weight among Cross-links (MC), Cross-linking Density (\( \rho \)), and Mesh Size (\( \xi \)).

The determination of MC, \( \rho \), and \( \xi \), these hydrogel samples were cut instantly after cross-linking and weighed in heptane and air. Afterward, these hydrogels were submerged in double distilled water at room temperature till the equilibrium swelling was achieved, then again weighed in heptane and air. Finally, these hydrogels were dried in oven at 25 °C for 4 days and then again weighed in heptane and air. From these weights, the hydrogel volume fraction can be calculated.\(^{18}\)

From swelling data, the MC can be calculated using following equation.\(^{20}\)

\[
\frac{1}{MC} = \frac{2}{M_n \nu_2 r((v_2, s/v_2, r)^{1/3} - 1/2(v_2, s/v_2, r))}
\]

(3)

where \( M_n \) represents PVA number average molecular weight of before cross-linking (85 000), \( \nu \) is the specific volume of the polymer (0.788 cm\(^3\)/g), \( v_1 \) signifies the molar volume of water (18.1 cm\(^3\)/mol), \( v_2, r \) denotes volume fraction of the cross-linked PVA hydrogel in the relaxed state, \( v_{2,s} \) is designated as the volume fraction of the PVA hydrogel in the swollen form. \( \chi \) denotes the Flory PVA hydrogel—solvent interaction parameter which is about 0.494 for PVA/water.

The \( \xi \) of hydrogel is the linear distance among successive cross-links. It represents the space that is available for the diffusion of solute or drug molecules and can be calculated with the help of following equation.\(^{20}\)

\[
\xi = v_{2s}^{-1/3} C_i (2MC/Mr)^{1/2}
\]

(4)

where \( C_i \) is Flory ratio (8.3), \( l \) is the bond angle between C–C bond (1.54 Å), \( Mr \) is the molecular weight of repeating units of PVA, and MC represents molecular weight of cross-links.

The \( \rho \) of these hydrogels were determined with the help of following equation.\(^{18}\)

\[
\rho \chi = \frac{1}{vMC}
\]

(5)

### 4.8. Maintenance of Cell Line.

The in vitro biocompatibility of FA-loaded cross-linked PVA hydrogel having higher loading percentage of FA (F1) was determined with the help of human embryonic kidney cell line (HEK-2930). The epithelial morphological cell line is a most extensively used cell line for the determination of biocompatibility or cytotoxicity. The culturing of cells was carried out using the T-25 culture flask in Dulbecco’s modified Eagle’s medium (HiMedia) at 37 °C, which comprises penicillin/streptomycin and 10% fetal bovine serum (HiMedia) and CO\(_2\) (5%) in a humidified chamber (Nuaire, incubator, USA). Biocompatibilities of F1 and F1-loaded FA were confirmed using MTT assay, which is considered as the most active assay for conduction of biocompatibility under in vitro conditions for any synthetic or biological materials.

### 4.9. MTT Assay of Hydrogels.

In the evaluation of biocompatibility through MTT assay, the FA-loaded hydrogels were subjected to freeze-drying and then crushed using mortar and pestle. The working solution of 1 mg/mL concentration was prepared by dispersing the crushed powder of hydrogel films in distilled water. The prepared samples were subjected to ultrasonication at high speed for about 1 h before their treatment with cells. The T-25 flask of HEK 293 cells was trypsinized with the help of trypsin (0.25%) and the cells were calculated with the help of the Neubauer chamber. Total of 1 × 10\(^4\) cells were seeded in a 96-well plate in a flat bottom. The treatment was given after 24 h to these cells with the dose range of 8–128 \( \mu \)g/mL for 48 h. After 48 h, the medium was removed and cells were subjected to incubation in the presence of MTT solution (5 mg/mL) at 37 °C for 4 h. Formazan crystals formed through the reduction reaction of succinate dehydrogenase enzyme were dissolved in dimethyl sulfoxide solution (150 \( \mu \)L). Furthermore, the absorbance at 570 nm was recorded after incubation for 15 min using iMark microplate (Bio-Rad, USA). Percentage of cell viability was evaluated as the fraction of control.

### 4.10. Blood Compatibility by Haemolytic Activity.

The hemolytic assay for FA-loaded F1 hydrogel was performed by following the reported protocol with slight modifications.\(^{21}\) Fresh human blood from a healthy donor was collected in a centrifuge tube containing anticoagulant (ethylenediaminetetra-acetic acid) and allowed for centrifugation at 2000 rpm for 15 min. The erythrocytes were collected and then washed with phosphate-buffered saline (PBS) (pH 7.4) three times. A 10% erythrocytes/PBS suspension was prepared and 0.95 mL erythrocyte solution was taken in a 1.5 mL centrifuge tube with 0.05 mL of the sample. Total hemolysis was achieved with the help of 1% Triton X-100 as the positive control. These tubes were kept for incubation for 1 h at 37 °C and then centrifuged at 2000 rpm for 10 min at 20 °C. The supernatant (150 \( \mu \)L) was shifted to a flat-bottomed Bio-Rad microplate and the absorbance at 570 nm was measured with the help of a UV–visible spectrophotometer. These experiments were carried out in triplicates and their mean values were considered.

### 4.11. Biodegradability Studies.

Hydrolytic degradation of hydrogels was conducted at pHs 7.4 at 25 °C. The amount of weight loss of these cross-linked hydrogels was investigated regularly at a definite interval of time (1, 2, 3, 4, 5, 6, 7, ... 20 days) with the help of the gravimetric technique. The degradability tests were conducted in triplicate and average values were considered. The ratio of degradability was determined by calculating the differences of mass loss of these hydrogels by using the following equation.\(^{22}\)

\[
\% \text{ mass loss} = \frac{W_0 - W_t}{W_0} \times 100
\]

(6)

where \( W_0 \) mass loss of hydrogel and \( W_t \) is the equilibrium swollen states at time \( t \).

### 4.12. FA Loading.

Loading of FA in the cross-linked PVA hydrogels was studied by immersing the preweighed hydrogels in 30 mL of buffer solution (pH 7.4 and 2.1) containing FA (5 mg/mL). After attaining the equilibrium degree of swelling, FA-loaded hydrogel films were carefully removed and washed with PBS followed by heating at 25 °C in a vacuum oven till the constant weight was attained. The maximum FA loading was determined after 24 h, on attaining the yellow colored transparent hydrogels. The % FA was calculated with the help of following equation.\(^{23}\)

\[
\% \text{ loading of FA} = \left( \frac{\text{weight of FA in hydrogel}}{\text{weight of hydrogel}} \right) \times 100
\]

(7)

### 4.13. In Vitro FA Release Study.

The in vitro release studies of FA were conducted by immersing the loaded hydrogel (F1) in 10 mL solutions of pH 2.1 and 7.4 in a beaker for 24 h at 25 °C. At different intervals of time (1, 2, 4, 6, 9, 12 and 24 h), 5 mL solution was withdrawn followed by the addition of the same
The quantity of FA released from cross-linked PVA hydrogels during different intervals of time was fitted using different kinetic models such as zero order, first order, Korsmeyer–Peppas, and Higuchi’s models to characterize release mechanism FA from cross-linked hydrogels.

I. The zero-order kinetic model: it represents the system in which the release rate does not depend on the drug concentration.

\[ Q_t = Q_0 + K \cdot t \]  
(9)

where \( Q_t \) represents the quantity of drug dissolved in time \( t \), \( Q_0 \) is the initial amount of drug, and \( K \) is the release rate constant.

II. First order kinetics: it depicts the system in which the release rate of drug depends on concentration of drug.

\[ \log Q_t = \log Q_0 + K \cdot t/2.303 \]  
(10)

where \( Q_t \) represents the dissolved amount of drug in time \( t \), \( Q_0 \) is the initial concentration of drug, and \( K \) is the rate constant for release.

III. Higuchi model: it explains the fraction of drug released from hydrogel directly related to the square root of time.

\[ M_t / M_{\infty} = K \cdot t^{1/2} \]  
(11)

where, \( M_t \) and \( M_{\infty} \) is the cumulative fraction of drug released during time \( t \) and \( K_{\text{Higuchi}} \) is Higuchi constant.

IV. Korsmeyer–Peppas model: this explains the fractional amount drug release exponentially equal to drug release time.

\[ M_t / M_{\infty} = K \cdot t^{n} \]  
(12)

where, \( M_t / M_{\infty} \) represents fraction release of drug during time \( t \) and \( K \) is the constant and \( n \) is the diffusional exponent. The value of \( n \) is used for characterizing various release processes. If \( n \leq 0.45 \), then Fickian diffusion predominates while the value of \( n \) in between \( n \leq 0.45 \)–\( 0.89 \) designates non-Fickian or anomalous diffusion and when \( N > 0.89 \), then case II diffusion applies.

4.14. FA Release Kinetics and Various Mechanism Models. The quantity of FA released from cross-linked PVA hydrogels during different intervals of time were fitted using different kinetic models such as zero order, first order, Korsmeyer–Peppas, and Higuchi’s models to characterize release mechanism FA from cross-linked hydrogels.

The FA release studies were carried out in triplicates and their average values were considered using following equation.

\[ \% \text{ release of FA} = \left( \frac{\text{concentration} \times \text{dilution factor}}{1000} \right) \]

where, \( \% \text{ release of FA} \) is the percentage of FA released, \( \text{concentration} \) is the concentration of FA, and \( \text{dilution factor} / 1000 \) is the dilution factor.

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