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# A novel pH-responsive hydrogel system based on *Prunus armeniaca* gum and acrylic acid: Preparation and evaluation as a potential candidate for controlled drug delivery

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ABSTRACT

pH-responsive hydrogels have become effective and attractive materials for the controlled release of drugs at predetermined destinations. In the present study, a novel hydrogel system based on *Prunus armeniaca* gum (PAG) and acrylic acid (AA) was prepared by a free radical mechanism using N, N-methylene bisacrylamide (MBA) as cross-linker and potassium persulfate (KPS) as initiator. A series of hydrogels varying PAG, AA, and MBA concentration was developed to determine the impact of these components. Formulated hydrogels were characterized for pH-responsive swelling, drug release, gel content, and porosity. Structural analysis was performed by FTIR, XRD, and SEM analysis. TGA study was applied to assess thermal stability. Oral acute toxicity and in vivo drug release were performed in rabbits. Hydrogels exhibited pH-dependent swelling and drug release. Swelling, drug loading and release, and porosity increased by increasing PAG and AA concentration while decreased by increasing MBA. The gel content of formulations was increased by increasing all three components. FTIR studies confirmed the development of copolymeric networks and the loading of drug. XRD studies revealed that hydrogels were amorphous, and the crystalline drug was changed into an amorphous form during loading. TGA results indicated that hydrogels were stable up to 600 °C. Acute oral toxicity results confirm that hydrogels were nontoxic up to a dose of 2 g/kg body weight in rabbits. The pharmacokinetic evaluation revealed that hydrogels prolonged the availability of the drug and the peak plasma concentration of the drug was obtained in 6 h as compared to the oral solution of the drug. Tramadol hydrochloride (THC) was used as a model drug. Hence, pH-responsive swelling and release, nontoxic nature and improved pharmacokinetics support that PAG-based hydrogels may be considered as potential controlled-release polymeric carriers.

## 1. Introduction

Hydrogels have emerged as extremely diverse and effective materials for a wide variety of applications. Their unique architectural features impart high hydrophilicity, biocompatibility, and soft physical texture analogous to living tissues (Koetting et al., 2015). The stimuli-responsive hydrogels have become the subject of keen interest due to their controlled release ability in response to external environmental cues. Equipped with an array of triggering mechanisms these "smart" hydrogels can show a precise and unprecedented level of control over the fundamental properties of the material under consideration (Koetting et al., 2015). They exhibit remarkably different swelling behavior towards various stimuli (pH, temperature, solvent) and serve as controlled release carriers for the delivery of a variety of molecules (Mohd Amin et al., 2014). Amongst these stimuli-responsive systems, pH-sensitive hydrogels are the extensively studied systems

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and are used for targeted delivery to improve the efficacy ratio and bioavailability of the existing drugs (Kumar et al., 2010; Quintanilla de Stéfano et al., 2020). Polymer-based hydrogels are considered extremely beneficial for oral controlled delivery because of less dosage frequency and minimal side effects associated with certain drugs (Mohd Amin et al., 2014).

The development of modified hydrogels by the amalgamation of natural and synthetic materials has made possible groundbreaking advances in polymer science and healthcare treatments. Grafting specific monomers to the polymer's backbone is an effective and attractive method to impart certain fascinating properties to the parent structure without interfering with their inherent biodegradable properties (Pourjavadi et al., 2008). Numerous natural polymers have been investigated in combination with ionic monomers to engineer pH-responsive hydrogels (Mohd Amin et al., 2014). Several pH-responsive drug delivery systems have been developed by copolymerizing acrylic acid (AA) with natural polymers (Kumar et al., 2010). AA is a hydrophilic monomer extensively employed for the preparation of polymeric hydrogels due to its remarkable water-absorbing ability (Elliott et al., 2004). Copolymeric networks containing AA due to their polyanionic nature swell appreciably at the physiological pH (7.4) due to deprotonation of carboxylic acid groups causing pH-responsiveness. Their actual pH-controlled release abilities can be modified by changing the polymeric composition (Bukhari et al., 2015).

The suitability of these hydrogels for biomedical applications depends on certain properties which in turn are controlled by their cross-linked structure. These properties can be modified by the variation of the polymer, monomers, and cross-linking agent used for preparation (Singh and Sharma 2014). A lot of nature-based polymers have been employed to fabricate pH-responsive hydrogels due to the availability of ample ionic moieties in their skeleton. These ionic groups facilitate the direct modification of the chemical properties of polymers by introducing new functional groups such as carboxylate, amide, phosphate, and sulfate (Camponeschi et al., 2015).

Natural gums and their modified forms have become the materials of prime choice for the development of pharmaceutical dosage forms (Bhardwaj et al., 2000). Gum-based stimuli-responsive hydrogels have been proven potential candidates for targeted drug delivery. The drawback of the rapid release of drugs from swollen gums can be overcome by cross-linking with other suitable polymers. A three-dimensional polymeric network created by intermolecular interactions between two types of polymeric chains can contain and transport drugs without any damage to the predetermined site (Singh et al., 2020).

*Prunus armeniaca* gum (PAG) is a polysaccharide composed of rhamnose, mannose, xylose, D-galactose, and L-arabinose, (Fathi et al., 2016). PAG has been prescribed by many traditional healers for the treatment of a variety of diseases including lung infections, anemia, fever, and antidot (Bouaziz et al., 2016). It has emulsifying, stabilizing, binding, and antioxidant potential (D. Salarbashi et al., 2021). Previously, it has been used to develop many therapeutic formulations. PAG alone and in combination with other gums have been used as sustained-release matrix tablets for some therapeutic agents (Azam Khan et al., 2012; Saha et al., 2013). PAG-based nanoparticles exhibited sustained release of curcumin for up to 6 h (D. Salarbashi et al., 2021). Microspheres prepared by cross-linking PAG and alginate in calcium chloride solution by ionotropic gelation have shown a sustained release of Tramadol hydrochloride (THC) for up to 12 h (Noureen et al., 2022).

To the best of our knowledge, this is the first report on the synthesis of a pH-responsive copolymer system based on PAG. Previously gums obtained from Prunus dulcis and *Prunus cerasifera* have been employed to design grafted copolymer systems (Hussain and Jaisankar 2017; Shi et al., 2019; Singh et al., 2022). The present study was undertaken to prepare pH-responsive hydrogels from PAG and AA combination by using the free radical polymerization method. A series of hydrogel formulations was prepared by varying the PAG, AA, and cross-linker N,

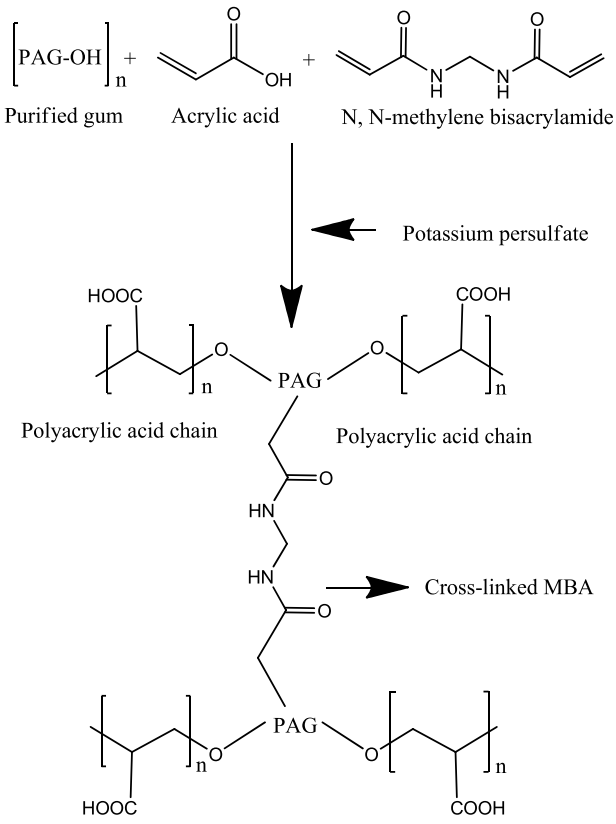


**Fig. 1.** Schematic diagram for preparation of PAG-co-AA copolymeric hydrogels.

N-methylene bisacrylamide (MBA) concentration. The impact of content variation was evaluated by investigating the swelling behavior, porosity, gel content, and release profile using THC as a model drug. Formulation with the best swelling and release was structurally characterized by FTIR, XRD, SEM, and thermal stability. In vivo, studies were conducted to determine the oral acute toxicity and pharmacokinetic behavior of the formulated hydrogels.

## 2. Materials and methods

### 2.1. Materials

The gum was collected from the trunk of the *Prunus armeniaca* tree in July 2021. Tramadol Hydrochloride was received as a gift from The Searle Company Pvt, Ltd. Pakistan. Ethanol, Acrylic acid, N, N-methylene bisacrylamide, Potassium persulfate, Acetonitrile, triethylamine, and Potassium dihydrogen phosphate were purchased from Sigma-Aldrich. All other chemicals and reagents were of analytical grade and used without further purification.

### 2.2. Extraction of pag

For extraction raw gum was collected and cleaned manually to remove impurities and dried to constant weight. Pulverized gum was soaked in distilled water and stirred for 2 h at 160 rpm. The suspended material was kept overnight at 4 °C to hydrate completely. The insoluble part was decanted through a muslin cloth. The soluble fraction was centrifuged for 20 min at 3000 × g and the supernatant was collected. The soluble fraction of PAG was extracted with ethanol by precipitation. Precipitated gum was dried, powdered, and passed 80 mesh sieve (Fathi et al., 2016).

**Table 1**
Composition of components in PAG-co-AA hydrogels.

| Formulations code | Polymer (g/100 mL) PAG | Monomer (g/100 mL) AA (g) | Cross-linker (g/100 mL) MBA (g) | Initiator (g/100 mL) KPS (g) |
|---|---|---|---|---|
| PAF1 | 0.5 | 15 | 0.25 | 0.2 |
| PAF2 | 0.5 | 17.5 | 0.25 | 0.2 |
| PAF3 | 0.5 | 20 | 0.25 | 0.2 |
| PAF4 | 0.5 | 20 | 0.35 | 0.2 |
| PAF5 | 0.5 | 20 | 0.45 | 0.2 |
| PAF6 | 0.5 | 20 | 0.50 | 0.2 |
| PAF7 | 0.3 | 20 | 0.25 | 0.2 |
| PAF8 | 0.75 | 20 | 0.25 | 0.2 |
| PAF9 | 1.5 | 20 | 0.25 | 0.2 |

### 2.3. Preparation of hydrogels by free radical polymerization

A series of hydrogels formulations was prepared using different compositions of polymer PAG, cross-linker N, N-methylene bisacrylamide (MBA), and monomer acrylic acid (AA) via free radical mechanism choosing potassium persulfate (KPS) as initiator. A schematic diagram for the preparation of PAG-co-AA copolymeric hydrogels is given in Fig. 1. PAG powder was suspended to dissolve in distilled water at 70 °C on a magnetic stirrer. KPS was separately dissolved in a minimum quantity of distilled water and poured into the PAG solution. The mixture was stirred for ten minutes to generate free radicals and then cooled down to room temperature. A solution of cross-linker MBA in AA was prepared and added to the above combination while stirring. The required volume was adjusted by adding distilled water. The whole mixture was allowed to homogenize thoroughly by stirring for further five minutes. Final solutions were transferred in test tubes and placed in a preheated water bath at 50 °C. The temperature gradually increased from 50 to 70 °C by 10 °C per hour and was fixed at 70 °C for the next eight hours. The test tubes were removed after the prescribed time and were cooled to room temperature. Cylindrical shape hydrogels formed were cut into 5.00 mm discs and washed to get rid of non-reacting components. Discs were washed constantly until the pH of the washing medium and distilled water became equal. Discs were initially dried at room temperature and then in an oven at 45 °C to get constant weight (Ijaz and Tulain 2019). The composition of formulations is listed in Table 1.

### 2.4. Swelling studies

The swelling behavior of PAG-based hydrogels in two different media (phosphate buffer pH 7.4, acidic buffer pH 1.2) was evaluated by determining dynamic and equilibrium swelling (Singh et al., 2007; Nasir et al., 2019). Pre-weighted discs of each formulation were soaked in media (100 mL) maintained at 37±0.5 °C. Dynamic swelling was determined by weighing swollen discs after pre-decided time intervals for up to 72 h. Each time discs were removed, blotted off carefully to remove surface water, and weighed. The dynamic swelling ratio was calculated by the formula:

$$Dynamic\ swelling\ \text{ratio} = \frac{W_1}{W^*} \quad (1)$$

where $W_1$ is the weight of the swollen disk and $W_0$ is the weight of the dry disk.

The equilibrium swelling ratio of hydrogels was determined by retaining the soaked discs at the same conditions of temperature and media pH till constant weight.

$$Equilibrium\ swelling\ ratio = \frac{W_1}{W^*} \quad (2)$$

where $W_2$ is the weight of the fully swollen disk at equilibrium swelling and $W_0$ is the weight of the dry disk.

### 2.5. Pulsatile behavior

The reversible swelling of a hydrogel controls the release of the drug from formulated matrix. Changes in the pH of the external environment can reversibly initiate or stop drug release from a pH-responsive hydrogel. Selected formulation from PAG-co-AA hydrogels was tested for pH-sensitive reversible swelling in pH 7.4 and 1.2 medium in a cyclic manner at 37±0.5 °C. The swelling medium of pre-weighted discs was replaced successively after every forty-five minutes in four cycles. The weight of the discs was determined to calculate the swelling ratio (Sadeghi 2011).

### 2.6. Sol-gel analysis

Freshly prepared hydrogel discs were dried in an oven at 50 °C to get constant weight before washing. After weighing, a non-reacted fraction of components was extracted with distilled water for 72 h. Thoroughly washed discs were dried in an oven at 50 °C till constant weight (Ranjha et al., 2014). Sol-gel part was calculated by the formulae:

$$Sol\,fraction\ (\%) = \frac{W_1 - W_2}{W_1} \times 100 \quad (3)$$

$$Gel fraction\ (\%) = 100 - sol\ fraction \quad (4)$$

where $W_1$ is the initial weight of dried disk before washing and $W_2$ is the weight of the extracted disk after drying.

### 2.7. Porosity measurement

The space-to-overall volume ratio is illustrated as porosity. The solvent displacement method was applied to assess the porosity (Shabir et al., 2017). Pre-weighed dried discs were socked in 50 mL of absolute ethanol and kept at 37±0.5 °C for 48 h to attain equilibrium. After the prescribed time discs were taken out, superficially dried to remove excess ethanol, and reweighted. Porosity was determined by the following formula:

$$Porosity\ (\%) = \frac{M_2 - M_1}{\rho V} \times 100 \quad (5)$$

where $M_2$ is the final mass of disk, $M_1$ is the initial mass of disk, V is disk volume, and $\rho$ is density of absolute ethanol.

### 2.8. Drug loading

The drug loading was carried out by simple diffusion. Pre-weight dried discs were soaked in a 1.00% (w/v) solution of THC in ethanol-water (50% v/v) at 37 ± 0.5 °C till equilibrium swelling. Swollen discs were removed, rinsed with distilled water to eliminate the surface-entrapped drug, and dried in an oven at 45 °C to obtain constant weight (Hussain et al., 2011).

Two methods (weight difference and extraction) were used to evaluate the drug-loading capacity of hydrogels. In the weight method difference in weight of loaded and unloaded dry discs was determined by using the formula:

$$Loaded\ drug = W_2 - W_2 \quad (6)$$

while $W_1$ is the weight of the unloaded disk and $W_2$ is the weight of the loaded disk.

In the extraction method, loaded dry discs were ground in a clean dried pestle and mortar, and 100 mg of each formulation was extracted in 500 mL of pH 7.4 buffer. The extraction was repeated with a fresh medium until the complete extraction. Drug loading was measured by measuring absorbance using a UV–VIS spectrophotometer at 271 nm. The drug in all fractions was added to obtain the total loaded drug (Ijaz and Tulain 2019).

### 2.9. In vitro drug release

In vitro release pattern of THC from hydrogel formulations was studied using a USP dissolution test apparatus II at 37±0.5 °C and 50 rpm. The release was investigated in different external media resembling GIT conditions. Simulated gastric fluid (acidic buffer pH 1.2) and simulated intestinal fluid (phosphate buffer pH 7.4) were used. Each container was filled with 900 mL of medium and pre-weighted THC-loaded hydrogels discs were put in. After pre-decided time intervals, 5.00 mL of fluid was withdrawn and reinstated with an equal volume of fresh medium to retain a similar environment. Withdrawn aliquots were subjected to UV–VIS spectrophotometric analysis at 271 nm. In vitro experiment was executed thrice for both media and final results were computed as the mean of three values (Hussain et al., 2011).

### 2.10. Kinetic modeling

Results obtained during in vitro release studies of hydrogel formulation were evaluated by fitting to various mathematical models to forecast the order and release mechanism. The best-fitted mathematical model was confirmed by comparing the values of the squared correlation coefficient ($R^{2)}$. Zero order, First order, Higuchi, Hixson Crowell, and KorsmeyerPeppas models were applied for kinetic evaluation (Ijaz and Tulain 2019).

### 2.11. Structural characterization

Physical interactions and chemical modifications alter the overall structural features of the parent polymer. So, structural characterization is needed to trace the desired modifications, drug-polymer compatibility, and behavior of the formulations (Pal et al., 2009; Guilherme et al., 2015).

#### 2.11.1. Fourier transform infrared spectroscopy

Copolymerization and drug loading was confirmed by FTIR data of polymer, AA, THC, unloaded and THC-loaded hydrogel formulations on an Agilent Cary 630 FTIR spectrophotometer between the spectral ranges of 4000–650 $cm^{-1}$. Spectra were recorded as an average value of 20 scans at a resolution of 4 $cm^{-1}$ as transmittance (Bolade et al., 2018).

#### 2.11.2. X-ray diffraction

The crystalline or amorphous nature of PAG, pure drug, unloaded and drug-loaded hydrogels was analyzed by X-ray diffractometer (Bruker, AXS/8, Berlin, Germany). Powder samples were sealed in the sample holder and scanning was run between 5° to 80° at a step of 0.05° (Rezaei et al., 2016).

#### 2.11.3. Thermal analysis

The thermal analysis of PAG, AA, and hydrogel formulation was investigated by a thermal analyzer SDT Q 600. The measured quantity of sample powder was sealed in an airtight aluminum pan with a gradual temperature rise of 10 °C/minute from 25 °C to 600 °C. An nert environment was obtained by passing nitrogen gas at a flow rate of 10.00 mL/minute (Fadavi et al., 2014).

#### 2.11.4. Scanning electron microscopy

Scanning electron microscopy was employed to observe the morphological features of hydrogels. To examine a finer microstructure, fully swollen discs in pH 7.4 buffer were freeze-dried in a freeze-dryer (Christ-Alpha 1–4 LD plus, Germany). Microstructure was observed after sputter-coating gold and photographed using SEM (JEOL. JSM-5910, Tokyo, Japan) at various magnifications (Lim et al., 2005).

### 2.12. Toxicological analysis

Oral acute toxicity of the hydrogel formulation was tested using rabbits. Animals chosen for toxicity study were handled according to the recommendations of "Guide for the Care and Use of Laboratory Animals". The entire experimental protocol was further approved by the Biosafety and Ethical Review Committee of University of the Sargodha via reference no (Ref: SU/ORIC/394/22/09/2022).

#### 2.12.1. Animal selection

Healthy albino rabbits weighing 1.5–2 kg were selected and housed in the animal house of the University of Sargodha for one week in a well-ventilated and hygienic place under a 12-hour light and dark cycle for acclimatization. All the animals were given free access to water and a standard diet. Before the dose administration, rabbits were randomly divided into two groups of control and treated ($n = 3$) and labeled for individual identification during the study.

#### 2.12.2. Dose administration

PAG-based hydrogel formulation showing maximum release was evaluated for safety profile. The control group was given water alone. Each rabbit in the treated group was orally given a dose of 2 g/kg body weight powder formulation as aqueous suspension via a 5.00 mL finely cut syringe at the needle end. After dose administration 10.00 mL water was provided to each animal using an oral tube containing a syringe.

#### 2.12.3. General observation

After dose administration rabbits were housed again for two weeks with free access to water and standard food. Rabbits were observed daily for mortality, and signs of illness. Food and water intake, and body weight were noted on day one, seven and fourteen.

#### 2.12.4. Hematological, biochemical, and histopathological analysis

At the end of the study, after two weeks blood samples were withdrawn for biochemical and hematological analysis. Finally, rabbits were sacrificed, and vital body organs (heart, liver, spleen, kidney, and stomach) were obtained, weighed, and preserved in formalin solution (10% v/v). Organs were cut to prepare slides for histopathological analysis (Erum et al., 2015).

### 2.13. In vivo drug release

Formulated drug carrier systems are often tested for their in vivo drug release ability to evaluate the correlation with in vitro release profile (Sundararaj et al., 2016).

#### 2.13.1. Experimental design

Healthy albino rabbits (2 kg-2.5 kg) of either sex were housed in a hygienic and airy place at the animal house of the University of Sargodha, Pakistan with free food and water supply, under controlled temperature and humidity and 12:12 h light and dark cycle. All the rabbits were deprived of food and fasted for 12 h before the study and were given free water access. Before dose administration rabbits were randomly divided into two groups ($n = 10$), $A$=control and $B$=hydogel treated. On study day each rabbit from group A was given orally an aqueous solution of THC (4 mg/kg), while the treated group was given a calculated dose of THC-loaded PAG hydrogels (PAF9) having an equal dose of drug using a silicone rubber gastric intubation tube with gavage. About 3.00 mL of blood samples were collected from the jugular vein at 0, 1, 2, 3, 4, 6, 8, 10, and 12 h after dozing and saved in heparinized centrifuge tubes. Withdrawn blood was centrifuged at 5000 rpm for 10 min to separate plasma and stored at −20 °C till further analysis.

#### 2.13.2. High-performance liquid chromatographic analysis

High-performance liquid chromatography (HPLC) was employed to measure the THC content in rabbit plasma by adopting a previously described method. The quantification was carried out using HPLC, Shimadzu, Japan, at wavelength 218 nm. The column selected was of silica C18 (4.6mm×250 mm) with a mobile phase 0.01 M phosphate

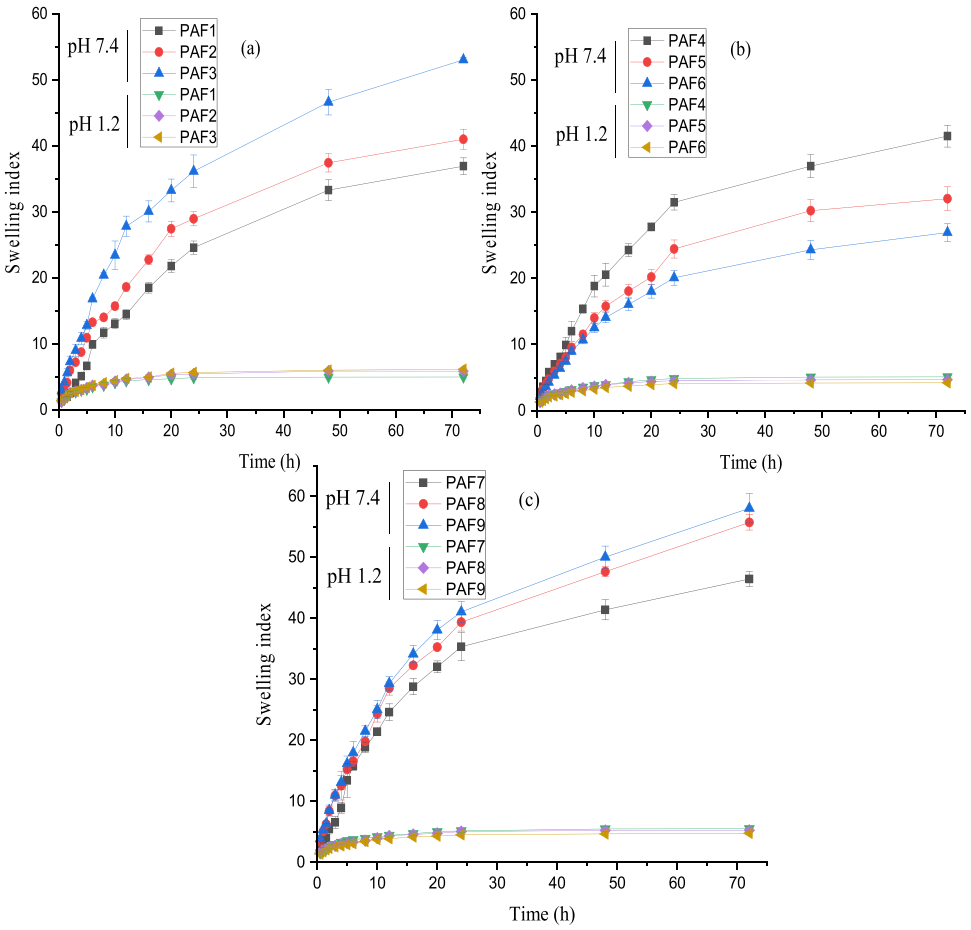


**Fig. 2.** Effect of components on swelling index of PAG-co-AA hydrogels (a) AA, (b) MBA, (c) PAG.

buffer (70%): Acetonitrile (30%) whose pH was adjusted at 3 by adding 0.5 mL of triethylamine (0.05%). The mobile phase was filtered using a 0.45 μm 47 mm membrane filter, degassed by sonication before running, and eluted at the flow rate of 1.00 mL/min. A calibration curve for THC was developed between the ranges of 50–1000 ng/mL. The adopted HPLC method was fully validated by the guidelines of the FDA/ICH. Accuracy and precision were calculated using three different concentrations in triplicate to record the responses for the intra-day and inter-day variations and represented in terms of the percent coefficient of variation (% CV). The injection volume for samples was 100 μL. Before injection, a mixture of 1.00 mL THC-containing plasma and 700 μL of acetonitrile were agitated on a vortex for 1. 00 min and then centrifuged at 5000 rpm. The clear supernatant separated was filtered through 0.22 μm 13 mm nylon syringe filters. The area under peak was observed for all concentrations (Danuta et al., 2014).

#### 2.13.3. Pharmacokinetic and statistical analysis

THC concentration in rabbit plasma was quantified by using Microsoft Office Excel 2019 program. Pharmacokinetic parameters for THC: $T_{max}$ (Time to reach peak plasma concentration), $C_{max}$ (peak plasma concentration), $T_{1/2}$ (Half-life), MRT (mean residence time), $AUC_{0\text{-}t}$ (area under the curve from zero to t time), and $C_{last}$ (last observed conc.) were calculated by using a non-compartmental model in PKSolver software package.

### 2.14. Stability studies

Stability studies were performed for optimized formulation according to the ICH guidelines. PAG-co-AA (F9) discs were saved in a humidity chamber for three months at 40 ± 2 °C, 75 ± 5% RH. After the decision period in vitro drug release capability was determined to appraise the stability of the hydrogel formulation (Ijaz et al., 2018).

### 2.15. Mucoadhesion studies

The mucoadhesive ability of the PAG-co-AA hydrogels was estimated by the rheometer (MCR 302, Anton Paar). Mucin (the main protein component of the mucus layer) at a concentration of 10% w/v was thoroughly dissolved in Dulbecco's phosphate-buffered saline (DPBS) at 37±0.5 ℃. Mucin was applied on the lower plate of the rheometer in the form of an even layer. The prehydrated hydrogel disk was fixed at the upper plate of the rheometer. The upper plate was lowered slowly to touch the mucin layer with the contact force of 5 N and held for 2 min. The maximum force of detachment to separate the disk and mucin layer was recorded by lifting the upper plate at a rate of 10 μm/s (Oh and Kim 2020).

## 3. Results and discussions

### 3.1. Copolymerized hydrogels

The structural modifications via grafting on the polymer chains may lead to an updated version which enables them to earn a new domain of application than their parent components (Nayak et al., 2018). Hydrogels of PAG with AA (PAG-co-AA) were obtained by free radical polymerization using MBA as cross-linker and KPS as initiator. The stable, opaque hydrogels formulations prepared were analyzed by further studies.

**Table 2**
pH-dependent equilibrium swelling of hydrogels of PAG.

| PAG-co-AA | Equilibrium swelling | |
|---|---|---|
| Formulation code | pH 7.4 | pH 1.2 |
| 9PAF1 | 41.62±1.77 | 4.98±0.18 |
| PAF2 | 46.07±1.70 | 5.86±0.12 |
| PAF3 | 57.28±1.34 | 6.14±0.17 |
| PAF4 | 46.72±2.77 | 5.10±0.17 |
| PAF5 | 34.76±1.95 | 4.67±0.25 |
| PAF6 | 28.09±1.62 | 4.20±0.11 |
| PAF7 | 50.87±2.35 | 5.50±0.42 |
| PAF8 | 59.60±1.41 | 5.24±0.19 |
| PAF9 | 62.03±2.53 | 4.77±0.28 |

### 3.2. Swelling studies

The pH of the external environment is the key stimulus to control the swelling ability of a hydrogel (Ijaz et al., 2022). All the PAG-based hydrogel formulations exhibited a pH-dependent swelling behavior. A dramatic increase in swelling ratio was observed by increasing the pH from 1.2 to 7.4. At lower pH, less swelling ratio is attributed to the protonation of ionized -$COO^-$and hydrogen bond formation in -OH, -COOH, and -NHCO groups of polymer chains. Contrarily, at 7.4 pH ionization of -COOH groups and hydrogen bond breaking leads to electrostatic repulsion in polymer chains resulting in higher water absorption and increased swelling (Wang et al., 2010). The swelling ratio quickly increased up to 72 h then slowly attained constant weight within 7–10 days.

Swelling ratio was also influenced by feed composition as reported by many researchers (Hosseinzadeh 2010; U.R. Tulain et al., 2018; Suhail et al., 2022). Formulated hydrogels were grouped into three sets to appraise the effect of monomer, cross-linker, and polymer concentration on the swelling behavior. Both the dynamic and equilibrium swelling ratio of hydrogels was affected by changing the components.

The dynamic swelling ratio of PAG-co-AA formulations having different concentrations of AA was evaluated at 1.2 and 7.4 pH. At 7.4 pH, the swelling ability was increased by increasing the concentration of AA from 15 to 20% (w/v). It can be explained based on increased water intake due to the addition of more AA population and ionization of groups causing chain repulsion. An increase in the dynamic swelling ratio in F1-F3 from 36.93 to 53.04 (Fig. 2a) confirmed that water absorption increased by increasing AA. These results are in accordance with previously reported results. More AA enriches the parent polymer chains with ionizable -COOH groups and enhances the pH sensitivity and hydrophilicity of newly formed hydrogels. Ionized groups create electrostatic repulsion between chains causing more penetration of water molecules into the porous network (Hosseinzadeh 2010). Dynamic swelling was also increased in pH 1.2 medium from 4.98 to 6.14 as reported earlier (U.R. Tulain et al., 2018).

The welling ratio decreased by adding more cross-linker (F4-F6) as shown in Fig. 2(b). This decrease is associated with the creation of a compact, less porous structure due to higher cross-linking leading to lower water absorption (Çaykara and Turan 2006).

An increase in PAG concentration (0.5 to 1.5%) enhanced the swelling ability of hydrogels from 46.44 to 58.03 Fig. (2). At higher concentrations, more polymer chains provide more vacant sites to attach monomer molecules and reduce the possibility of the formation of homo polymers from leftover AA molecules (Finkenstadt and Willett 2005). Additionally, more polymer chains have more ionizable carboxylate and amide groups which cause electrostatic repulsion and thus more water absorption (Murthy et al., 2006).

The equilibrium swelling ratio of PAG-based hydrogels was notably higher in pH 7.4 medium than in 1.2. Like dynamic swelling equilibrium swelling was also increased by increasing AA and PAG and decreased by increasing MBA (U.R. Tulain et al., 2018). Equilibrium swelling ratios of all PAG-co-AA formulations are given in Table 2.

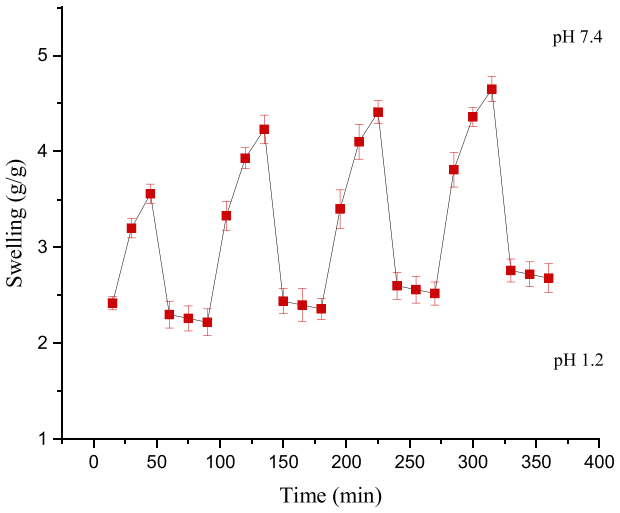


**Fig. 3.** Pulsatile behavior of PAG-co-AA hydrogels.

### 3.3. Pulsatile behavior

The on-off swelling of the formulated hydrogels by varying the pH of the external environment was studied at 7.4 and 1.2 pH. Fig. 3 points toward a rapid change in the water absorption ability of hydrogel by changing the pH from 7.4 to 1.2. There was an appreciably high swelling ability at pH 7.4 which quickly dropped down by lowering the pH to 1.2. This swelling-deswelling is attributed to ionization (pH 7.4) and protonation (pH 1.2) of hydrophilic groups of polymer chains and attached AA. This on-off swelling behavior verifies the pH-responsive reversible swelling nature of PAG-based hydrogels (An et al., 2010).

### 3.4. Sol-gel analysis

Sole-gel analysis helps to determine the consumption of components and unreacted fraction after hydrogel formation. It can be inferred from Fig. 4 that the gel content of PAG-co-AA hydrogels increased by increasing the components monomer, polymer, and cross-linker. An increase in component molecules increases the grafting hence gel fraction in a hydrogel forming a stable network by more cross-linking (Mohd Amin et al., 2014). More polymers provide more free radicals creating more opportunities for PAG and AA reactions. Similarly, monomer AA also increases grafting to form compact structure (Samanta and Ray 2014). A high concentration of cross-linker led to a denser network as a result of chain elongation between PAG and AA units (Hussain et al., 2011).

### 3.5. Porosity measurement

The porosity study of a hydrogel is important as the porous structure of a cross-linked hydrogel is responsible for the diffusion of water molecules that sequentially controls the absorption and release of entrapped molecules (Akhlaq et al., 2020). The results depicted in Fig. 5 indicate that an increase in PAG and AA concentration created more porous hydrogels. On the other hand, more MBA concentration decreased the porosity by increasing the cross-linking density resulting in reduced pore size (Babaei et al., 2019; Chen et al., 2019).

### 3.6. Drug loading and in vitro release

The drug loading ability of different PAG-co-AA hydrogels described in Table 3 reveals that more swell-able hydrogels loaded more quantity of drug in their polymer network. These outcomes are in line with the previously reported studies (Hussain et al., 2011).

The drug release ability of PAG-co-AA hydrogels was evaluated by

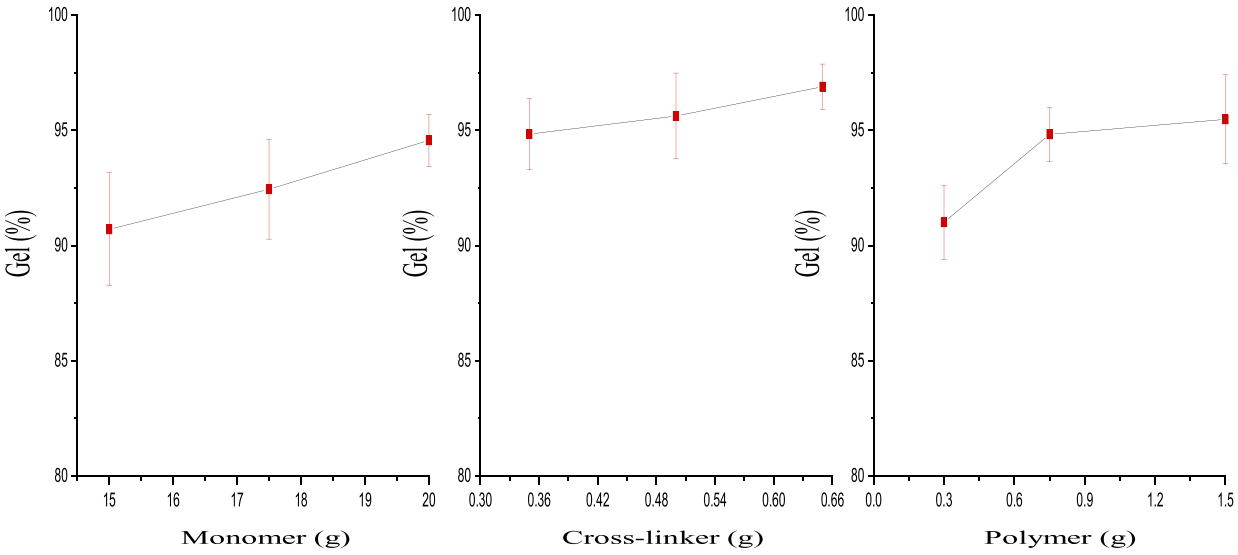


**Fig. 4.** Effect of components concentration on gel% of PAG-co-AA hydrogels.

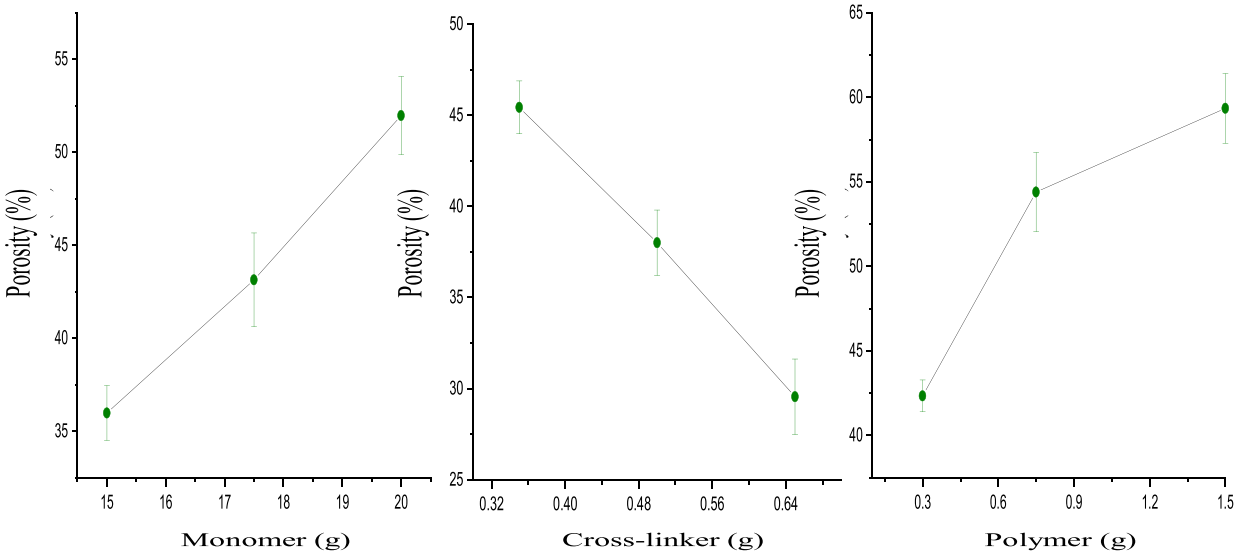


**Fig. 5.** Effect of components concentration on porosity of PAG-co-AA hydrogels.

**Table 3**
Quantity of THC loaded.

| Formulation code | Loaded drug | |
|---|---|---|
| | Weight method | Extraction |
| PAF1 | 162.61±9.71 | 158.62±7.74 |
| PAF2 | 196.74±5.81 | 192.26±3.87 |
| PAF3 | 210.02±2.13 | 205.32±4.61 |
| PAF4 | 197.68±9.87 | 193.41±11.43 |
| PAF5 | 158.59±7.85 | 154.11±6.93 |
| PAF6 | 130.10±7.63 | 124.16±7.61 |
| PAF7 | 193.67±9.76 | 189.97±9.40 |
| PAF8 | 209.22±8.49 | 204.47±10.43 |
| PAF9 | 211.17±10.85 | 205.11±11.86 |

determining the cumulative percent release from the developed polymeric system. Initially, the release was carried out at 1.2 pH for two hours then the medium was replaced, and the study was continued at 7.4 pH for the next 22 h maintaining other conditions. Figs. 6 (a-c) are evidence that the release percent of PAG-co-AA formulations was affected by both pH and composition (monomer, cross-linker, and polymer). All formulations released a very small quantity of the drug at 1.2 pH in the first two hours which can be attributed to lower swelling ability in an acidic medium (Shabir et al., 2017).

Fig. 6(a) demonstrates that by increasing the AA concentration from 15 to 20% increased the cumulative release of THC from 89.21 to 95.26% up to 24 h. Similarly, polymer concentration positively affects the release percent of the loaded drug (86.33–96.49%) from fabricated hydrogels of PAG as shown in Fig. 6(b). These release results are consistent with swelling studies as the swelling index was also increased by increasing AA and PAG concentration. Hydrogel formulations having more AA and polymer concentrations are enriched with more ionizable groups causing more swelling as well as drug release. The relaxation of polymer network during swelling cause diffusion of drug (Ijaz et al., 2022).

The addition of more cross-linker (0.35–0.65%) decreased the cumulative release percent from 92.30 to 86.65% as shown in Fig. 6(c). It is because increased cross-linking agent increased the physical entanglement among polymer chains resulting a compact less porous structure

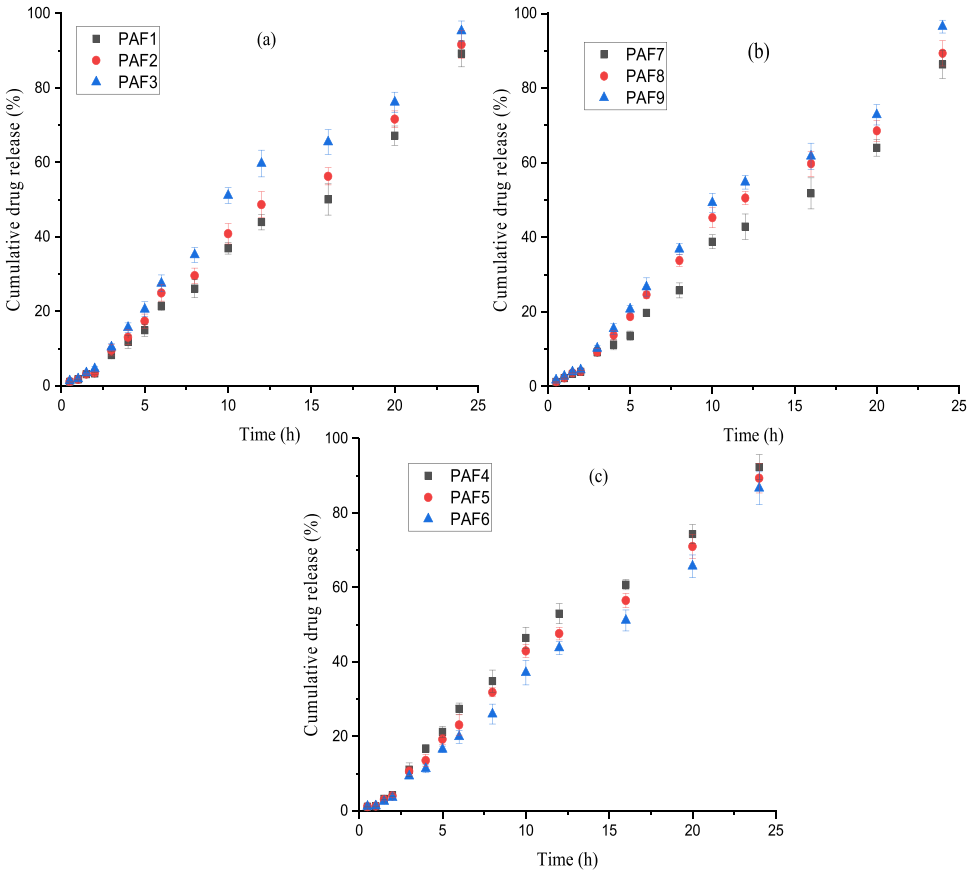


**Fig. 6.** Effect of cross-linker concentration on drug release percent from PAG-co-AA hydrogels.

**Table 4**
Results of kinetic modeling of in vitro release profile of THC from PAG-co-AA hydrogels.

| F.C | Zero-order | | First-order | | Higuchi | | Hixson-Crowell | | Korsmeyer-Peppas | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | $R^2$ | $k_0$ | $R^2$ | $k_1$ | $R^2$ | $k_H$ | $R^2$ | $k_{HC}$ | $R^2$ | $k_{kp}$ | n |
| PAF1 | 0.9876 | 3.477 | 0.9294 | 0.049 | 0.7770 | 12.523 | 0.9526 | 0.015 | 0.9898 | 2.729 | 1.086 |
| PAF2 | 0.9912 | 3.729 | 0.9415 | 0.055 | 0.8014 | 13.535 | 0.9643 | 0.016 | 0.9906 | 3.562 | 1.016 |
| PAF3 | 0.9760 | 4.128 | 0.9485 | 0.066 | 0.8260 | 15.158 | 0.9700 | 0.019 | 0.9777 | 5.134 | 0.922 |
| PAF4 | 0.9848 | 3.941 | 0.9579 | 0.061 | 0.8332 | 14.472 | 0.9772 | 0.018 | 0.9868 | 4.853 | 0.926 |
| PAF5 | 0.9908 | 3.709 | 0.9525 | 0.055 | 0.8155 | 13.525 | 0.9727 | 0.016 | 0.9903 | 3.942 | 0.978 |
| PAF6 | 0.9904 | 3.431 | 0.9395 | 0.048 | 0.7866 | 12.390 | 0.9610 | 0.015 | 0.9913 | 2.895 | 1.060 |
| PAF7 | 0.9883 | 3.406 | 0.9381 | 0.048 | 0.7847 | 12.302 | 0.9594 | 0.014 | 0.9890 | 2.874 | 1.060 |
| PAF8 | 0.9831 | 3.759 | 0.9564 | 0.057 | 0.8231 | 13.767 | 0.9745 | 0.017 | 0.9835 | 4.406 | 0.943 |
| PAF9 | 0.9769 | 3.954 | 0.9593 | 0.062 | 0.8361 | 14.564 | 0.9770 | 0.018 | 0.9806 | 5.164 | 0.905 |

F.C= Formulation code; $R^2$= Correlation coefficient; n= Diffusion exponent.

leading to less swelling, less loading and release of drug (Sohail et al., 2014).

### 3.7. Kinetic modeling

The drug release kinetics was assessed by fitting the release data in various kinetic models like zero order, first order, Higuchi, Hixson Crowell, and Korsmeyers Peppas in DDSolver software. The results obtained from the curve fitting of the release profile of PAG-co-AA hydrogels as shown in Table 4 indicated that maximum formulations followed the Korsmeyer Peppas model as $R^2$ values for the Korsmeyer Peppas model were higher than the rest of the applied models (0.9777–0.9913). This model explains the possible relationship of the polymer relaxation in a solvent followed by the diffusion of the drug through the polymer matrix (Trongchuen et al., 2018). $R^2$ values for the Higuchi model were greater than 0.5 which shows that the release of the drug was diffusion based. The value of diffusion exponent 'n' explains the type of diffusion (Suhail et al., 2022). For the PAG-co-AA hydrogels (F1-F9), the diffusion exponent n value was observed between 0.905 −1.086, indicating that the THC release from hydrogels followed the super case II transport mechanism controlled through swelling and relaxation of the polymeric network. Results of the swelling study suggested that hydrogel formulations extensively absorb water during the dynamic swelling period of 72 h due to quick polymeric network relaxation followed by water absorption. It means the chain relaxation rate of polymeric structure controls the drug release from these hydrogel formulations (Das and Subuddhi 2013). PAG-based hydrogels showed similar release kinetics as developed and studied linseed polysaccharides-based hydrogels (Shabir et al., 2017).

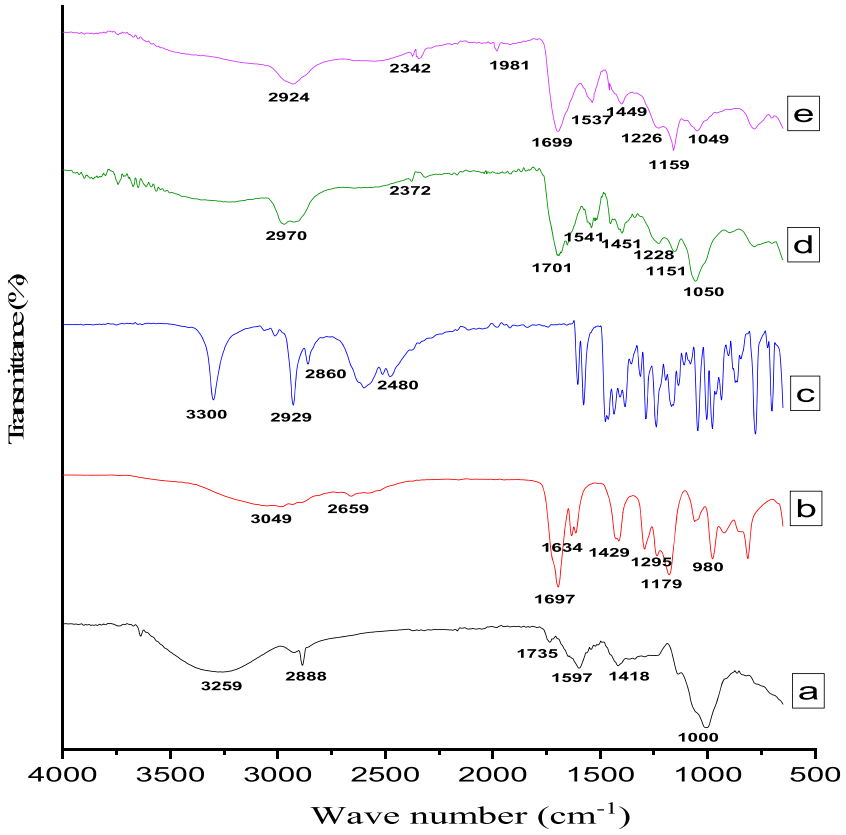


**Fig. 7.** FTIR spectra of (a) PAG, (b) AA, (c) THC, d) unloaded PAG-co-AA, (e) loaded PAG-co-AA hydrogels.

### 3.8. Structural characterization

#### 3.8.1. Fourier transforms infrared spectroscopy

Copolymerization of polymer chains and AA can be confirmed by FTIR examinations of formulated hydrogels and components used for preparation. FTIR is a reliable technique to find out any change in bond positions, which proves the interaction (Sohail et al., 2014). The FTIR spectral data of PAG, AA, THC, unloaded, and THC-loaded PAG-co-AA were recorded. Pure PAG (Fig. 7) showed a band at 3259 $cm^{-1}$confirming the O—H bond movements. A peak at 2888 $cm^{-1}$ specified the C—H vibration. A small absorption peak at 1735 $cm^{-1}$ corresponded to COOH presence. Two peaks at 1457 $cm^{-1}$ and 1418 $cm^{-1}$ are referred to as the vibration of the $CH_3$ and $CH_2$ bond. Peaks between 1200 and 800 $cm^{-1}$ are particularly related to characteristic carbohydrate structure (Fathi et al., 2016). The FTIR spectra of AA exhibited a peak at 3049 $cm^{-1}$ for O—H vibration, while absorption at 1697 $cm^{-1}$ and 1634 $cm^{-1}$is corresponded to C = O vibrations of COOH. Similarly, the stretching movements of C—C and C—O were demonstrated by the band at 1295 $cm^{-1}$ and 1179 $cm^{-1}$ (Haider et al., 2017). FTIR spectra of PAG-co-AA exhibited various modifications in parent spectra of PAG and AA. Some peaks totally disappeared such as 3259 $cm^{-1}$ of PAG backbone and 2659 $cm^{-1}$ of AA, while some were modified such as 2888 $cm^{-1}$ of PAG was changed to 2970 $cm^{-1}$ and 1697 $cm^{-1}$ of AA was adjusted to 1701 $cm^{-1}$ in the hydrogel. A new peak appeared at 2372 $cm^{-1}$. The formation of new peaks, the disappearance of earlier ones and the alteration in some peaks authenticated the grafting of AA on the chains of PAG (Suhail et al., 2022). The pure drug exhibited distinguishing peaks at 3300 $cm^{-1}$ for O—H while at 2929 $cm^{-1}$, and 2860 $cm^{-1}$ for C—H movements. Characteristic fingerprint spectra were shown within the range of 1600–650 $cm^{-1}$ (Kamel and Abbas 2013). FTIR results of THC-loaded formulation showed that drug peaks overlapped with a minor band shift confirming the loading to the hydrogel network (Kausar et al., 2021). These findings confirm that formulated hydrogels are compatible with model drug THC (Cirri et al., 2012)

#### 3.8.2. X-ray diffraction

Copolymerization and changes in the crystalline nature of hydrogel formulations were investigated by XRD analysis. Fig. 8(a) describes comparative PXRD results of pure PAG and PAG-co-AA hydrogel. Both pure PAG and PAG-hydrogel had an analogous structure without any sharp peaks indicating the retention of the structural integrity of the PAG backbone during copolymerization (Sharma et al., 2014). The absence of sharp peaks points towards the amorphous structure of polymer and formulated hydrogel. A less intense PXRD pattern of hydrogels specifies an enhanced amorphous nature of formulation as compared to the parent polymer (Kaity et al., 2013). A comparative view and overlay of THC, unloaded, and loaded PAG-co-AA is described in Figs. 8(b) and (c). A distinctive pattern of sharp peaks in XRD is used to identify the crystalline drugs [Khan et al., 2013]. PXRD of THC exhibited a significant number of well-defined characteristic peaks at 2θ=10.35°, 13.1°, 16.7°, 18.55°, 20.55°, 24.45°, 30.85 °confirming its crystalline structure. All these peaks disappeared in THC-loaded hydrogels confirming the dispersion of the drug in the formulation network in its amorphous form (Sohail et al., 2014).

#### 3.8.3. Thermal analysis

The TGA curve of AA showed a sharp weight loss and almost degraded up to 110 °C (Rashid et al., 2020). TGA of polymer and PAG-co-AA hydrogel, both exhibited three successive weight losses showing three-step degradation from 25 °C to 600 °C. In the PAG curve, an initial weight loss was observed between 30 and 243 °C showing moisture loss and some decomposition of the polymer network. A second weight loss of up to 307 °C may be due to the thermal degradation of the polymer. A third weight loss can be associated with the conversion of polymeric mass to burned residue (Sharma et al., 2020). In the case of PAG-co-AA hydrogel similar weight losses were observed between 30

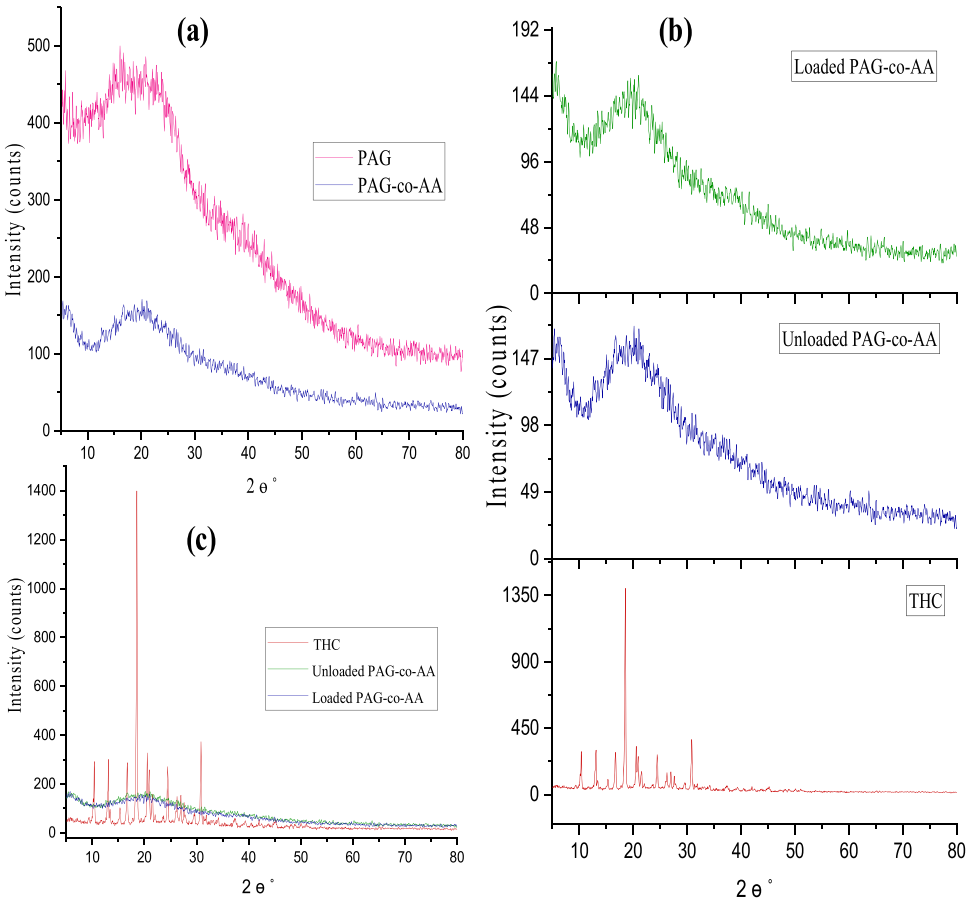


**Fig. 8.** XRD of (a) PAG and PAG-co-AA (b-c) overlays of XRD of THC, unloaded and loaded PAG-co-AA hydrogels.

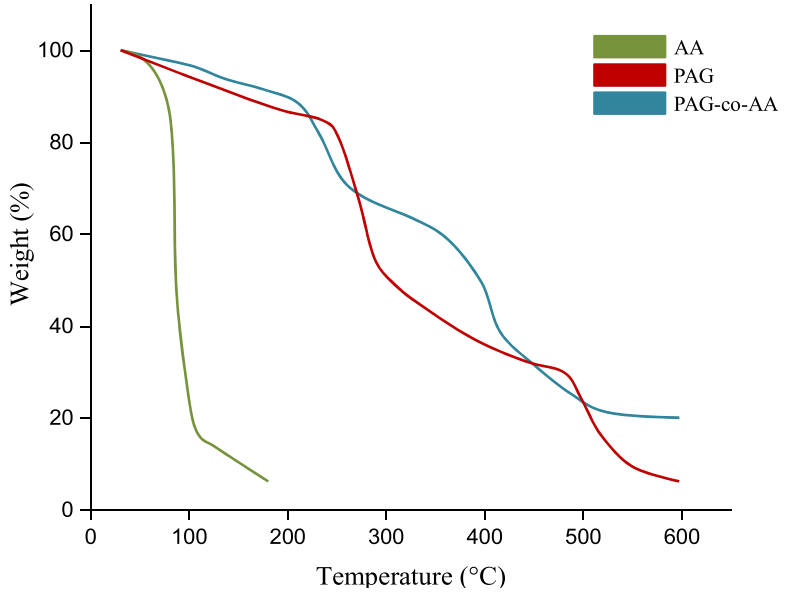


**Fig. 9.** TGA of PAG, AA, and PAG-co-AA hydrogel.

and 258 °C for the first weight loss, up to 386 °C for the second weight loss and a third weight loss to form residual mass. At the end of the study up to 600 °C residual mass for PAG was 10.17% and for PAG-co-AA hydrogel was 19.16% as depicted in Fig. 9. As hydrogel formation was more stable than the parent polymer so we can infer that grafting of AA on PAG has increased the thermal stability of the polymer (Ijaz and Tulain 2019).

### 3.8.4. *Scanning electron microscopy*

Freeze-dried PAG-co-AA hydrogels showed a porous, irregular surface under a scanning electron microscope as shown in Fig. 10. It had a heterogeneous, folded structure with characteristic cracks and wrinkles.

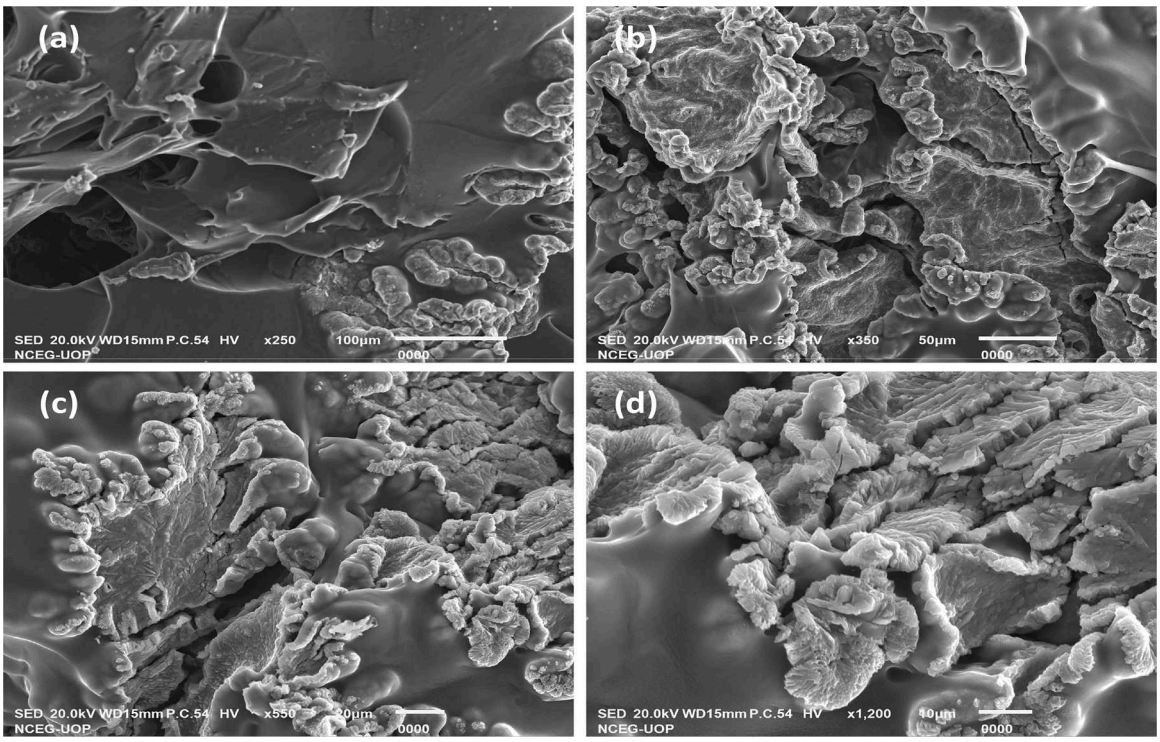


**Fig. 10.** SEM micrograph of PAG-co-AA at 100, 50, 20, and 10 µm.

**Table 5**
General observation for acute toxicity in control and PAG-co-AA hydrogel treated groups of rabbits.

| General observations | Control group | Treated group |
|---|---|---|
| Mortality | Nil | Nil |
| Sign of illness | Nil | Nil |
| Body weight (g) | | |
| Pretreatment | 1293±49 | 1342±67 |
| Day 1 | 1291±48 | 1336±66 |
| Day 7 | 1302±50 | 1349±73 |
| Day 14 | 1321±53 | 1359±74 |
| water intake (mL) | | |
| Pretreatment | 133±7.09 | 137±12.50 |
| Day 1 | 135±7.02 | 134±11.01 |
| Day 7 | 141±7.57 | 139±3.60 |
| Day 14 | 153±5.50 | 145±7.09 |
| Food intake (g) | | |
| Pretreatment | 143±8.50 | 161±16.82 |
| Day 1 | 142±7.93 | 153±18.92 |
| Day 7 | 152±8.73 | 162±17.05 |
| Day 14 | 161±9.07 | 169±14.97 |

Wrinkled surfaces with cracks may result from the partial collapsing of the hydrogel network during the drying process (Malik et al., 2021). The structure had many interconnected channels having a mixture of rough and smooth inner walls (Kausar et al., 2021). These structural features confirm a compatible cross-linked network. High porosity offers more water permeation leading to more swelling, and drug loading/releasing capacity (Khanum et al., 2018).

### 3.9. Toxicological analysis

#### 3.9.1. General observations

During the two weeks trial period, all the rabbits were carefully inspected daily for illness or any sign of abnormality due to the toxic effect of the formulation dose. Body weight, food and water intake were measured on days one, seven and fourteen after treatment. All the rabbits were active and healthy with no considerable difference in food and water intake and body weight between the control and treated groups as mentioned in Table 5. There were no signs of illness or any abnormality during the study period. These observations prove that there were no adverse effects on the physical activities, general behavior, and growth of rabbits treated with PAG-based hydrogels up to 2 g/kg body weight (Pour et al., 2011).

**Table 6**
Hematological and biochemical analysis of control and polymers treated rabbits.

| Parameters | Control group | Treated group |
|---|---|---|
| Hematology | | |
| Hb (g/dl) | 13.34±0.71 | 13.86±0.66 |
| Total WBCs (103/µl) | 11.03±0.21 | 10.10±0.72 |
| RBC (106/µl) | 5.86±0.21 | 5.30±0.36 |
| Platelets (103/µl) | 151±5.13 | 145±4.16 |
| Liver profile | | |
| AST (U/L) | 24.67±6.65 | 24.67±4.93 |
| ALT (U/L) | 85±5.29 | 60.35±5.03 |
| ALP (U/L) | 43±7.21 | 37±4.58 |
| Renal profile | | |
| Urea (mg/dl) | 24.33±4.04 | 19±1 |
| Creatinine (mg/dl) | 0.67±0.06 | 0.7±0.26 |
| Uric acid (mg/dl) | 3.7±0.5 | 3.80±0.50 |
| Lipid profile | | |
| Cholesterol (mg/dl) | 105±5.56 | 94±7 |
| Triglyceride (mg/dl) | 119±6.11 | 115±7.93 |

#### 3.9.2. Hematological and biochemical analysis

The hematological and biochemical assessment of withdrawn blood from both groups was carried out to find any difference in the blood chemistry. Hematology, biochemistry, liver, renal, and lipid profiles of both groups have been listed in Table 6. A comparative review of all results indicates that all the parameters tested for both the control and treated group were very close to each other and within the standard reference range proving that PAG-co-AA hydrogels are nontoxic in nature (Nasir et al., 2019). Administration of these hydrogels did not change any hematological and biochemical parameters out of standard

**Table 7**
Vital organs weight of control and polymers treated groups.

| Group | Liver | Stomach | Heart | Kidney | Spleen |
|---|---|---|---|---|---|
| Control group | 35.16 ±2.84 | 17.76 ±0.95 | 3.4±0.2 | 4.16 ±0.50 | 0.45 ±0.05 |
| Treated group | 33.84 ±1.13 | 18.03 ±1.17 | 3.56 ±0.41 | 4.33 ±0.25 | 0.42 ±0.05 |

reference ranges proving their nontoxic nature.

### 3.9.3. Histological analysis

At the end of the study, rabbits were sacrificed to collect vital organs and the weight of organs of both groups was noted. Results (Table 7) show that there was no significant difference in the weight of the liver, stomach, heart, kidney, and spleen of the two groups proving the nontoxic effect of hydrogel formulation. Microscopic examination of these vital organs indicated a consistency in control and treated group animals. There were no pathological lesions seen on vital organs as evident in Fig. 11. This can be attributed to the normal functioning of vital organs in both groups (Malik et al., 2020).

### 3.10. In vivo release analysis

In vivo drug release was studied by administering an equal amount of THC loaded in PAG-co-AA and its aqueous solution (4 mg/kg body weight) orally in rabbits. Optimized formulation with the best results was selected. The control group received an aqueous oral solution of THC and the treated groups received a hydrogel formulation of THC-loaded PAG hydrogels. The mean plasma concentration of THC after oral administration of its solution and THC-loaded hydrogels is depicted in Fig. 12. Quick absorption of oral suspension THC was observed in the control group, giving a peak concentration of 84.04±4.26 ng/mL at 2 h. However, in the hydrogel-treated group, THC absorption was extended and a peak concentration of 98.34±4.53 ng/mL was attained at 6 h. Hence Tmax of THC-loaded PAG-co-AA hydrogel is significantly higher (6 h) than the oral solution of THC (2 h). These results suggest that PAG-based hydrogels have prolonged the THC delivery in selected animal model than its oral suspension indicating their potential as controlled release polymer system (Aamir et al., 2011). Additionally, higher plasma concentration was observed in the case of PAG-hydrogels as compared to oral solution.

The pharmacokinetics parameters of the oral solution of THC and THC released from PAG-co-AA formulations in rabbit plasma were analyzed employing the PKSolver software package and are written in Table 8. The peak plasma concentration of THC was higher for hydrogel formulation with an extended period. The elimination half-life ($t_{1/2}$) was

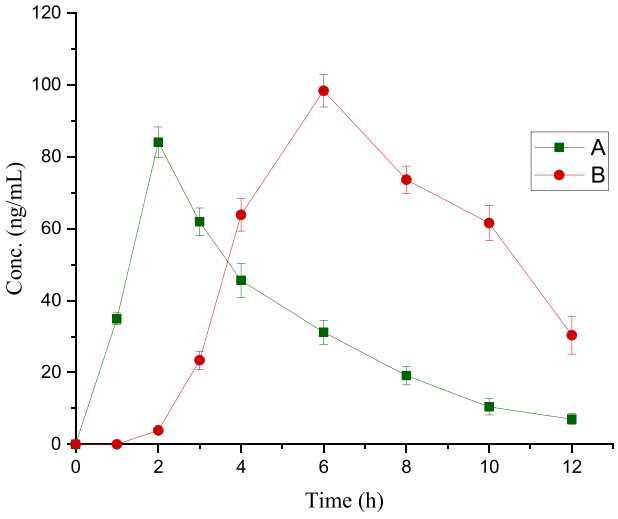


**Fig. 12.** Mean plasma concentration of (A) oral solution of THC (B) PAG-co-AA hydrogels formulation.

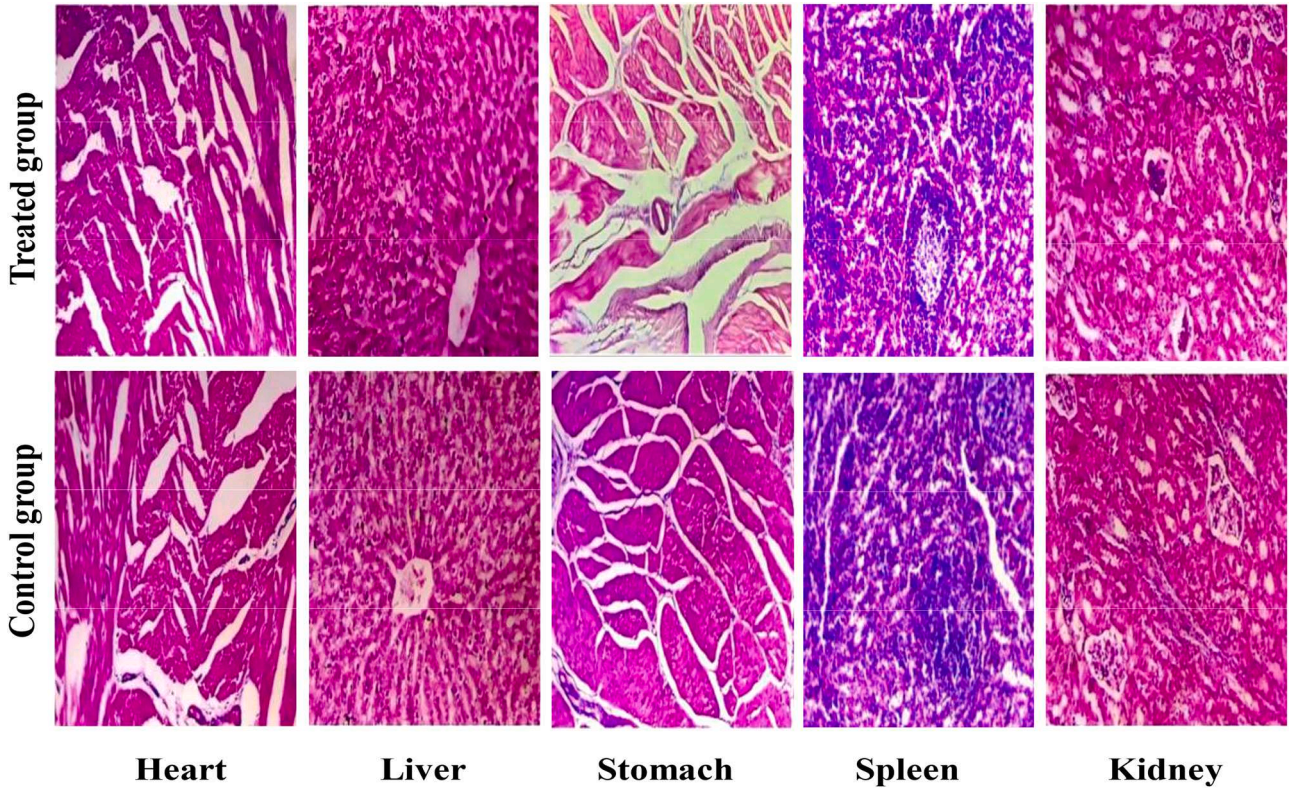


**Fig. 11.** Histological evaluation of control and hydrogels treated groups.

**Table 8**
A comparative descriptions of pharmacokinetic parameters of oral solution of THC and PAG-co-AA hydrogels.

| Parameter | Group-A Control | Group-B Hydrogel treated |
|---|---|---|
| $C_{max}$(ng/mL) | 84.04 | 98.34 |
| $T_{max}$ (h) | 2 | 6 |
| $AUC_{0-t}$ (ng/mL*h) | 377.645 | 622.305 |
| MRT(h) | 5.145 | 9.248 |
| $C_{last}$ (ng/mL) | 6.99 | 30.37 |
| $T_{1/2}$ (h) | 2.81 | 3.74 |

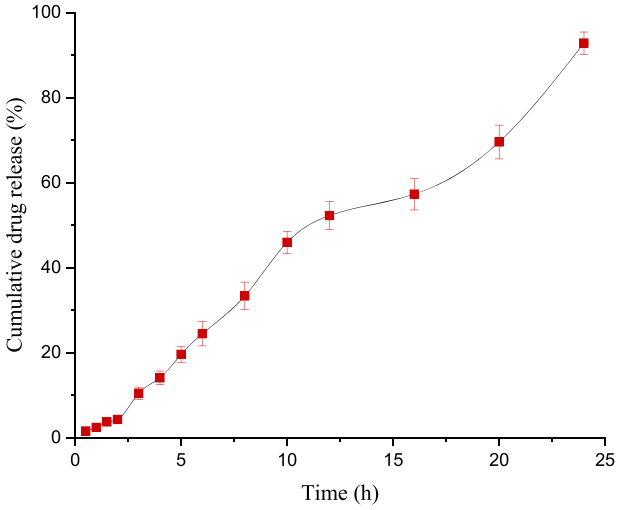


**Fig. 13.** Stability studies of optimized formulation of PAG-co-AA for three month.

higher for hydrogel formulation than for oral solution. The area under the curve (AUC) and mean residence time (MRT) were also significantly higher for formulation.

### 3.11. Stability studies

Stability study was conducted for optimized PAG-co-AA formulation by keeping the hydrogel for three months at 40 ± 2 °C, 75 ± 5% RH. After 3 months, in vitro drug release study was performed in a dissolution apparatus. Results shown in Fig. 13 showed no significant change in percent release of THC from the saved formulation network. it means the formulation retained its stability for three months under prescribed conditions as reported earlier for hydrogels (Ijaz et al., 2018; Ijaz et al., 2019)

### 3.12. Mucoadhesion studies

The mucoadhesive ability of orally administered drug carriers can enhance the bioavailability of drugs by prolonging the contact of membranes and drug carriers which improve therapeutic efficacy. Anionic hydrophilic polymers, like AA and its analogues, have been reported to boost the mucoadhesion of polymeric pharmaceutical formulations due to hydrogen bond forming groups (Alsarra et al., 2009; Ahmad et al., 2014). The mucoadhesive ability of the polymeric drug carriers is controlled by the nature and quantity of mucoadhesive polymers (Ramadan et al., 2018). The mucoadhesion test was applied to evaluate the effect of PAG concentration on the mucoadhesiveness of PAG-co-AA hydrogels. The maximum force required for detachment was determined using the rheometer. Results shown in Fig. 14 demonstrate that PAG-co-AA hydrogels are mucoadhesive in nature and mucoadhesiveness increased by increasing the PAG concentration. The

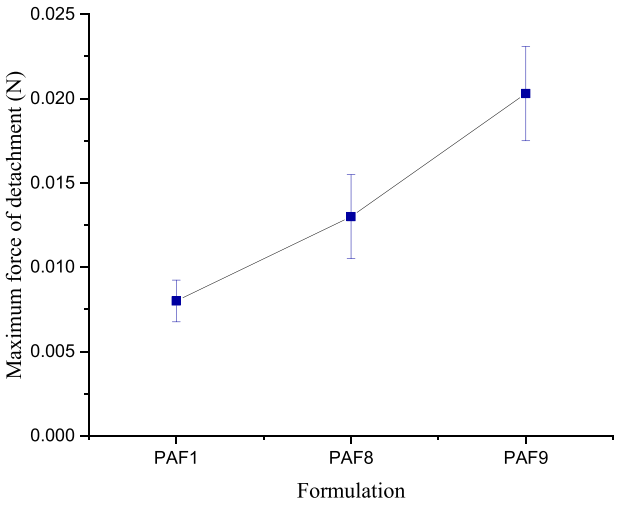


**Fig. 14.** Mucoadhesiveness of PAG-co-AA hydrogels having various concentrations of PAG.

enhancement of mucoadhesiveness by increasing PAG concentration can be attributed to the availability of more hydrophilic groups like amine and carboxylate and adequate polymer chain flexibility which are key requirements for mucoadhesiveness (Oh and Kim 2020).

## 4. Conclusion

PAG and AA based novel pH-responsive hydrogels were synthesized for controlled release of THC. The free radical polymerization method was applied using MBA as a cross-linker and KPS as an initiator. Fabricated hydrogels showed pH-dependent swelling and drug release behavior which increased by increasing PAG and AA but decreased by increasing MBA. THC release profile results were found to be the best fit in the Korsmeyers Peppas model. The release mechanism was super case II transport controlled through swelling and relaxation of the polymeric network. Thermally stable hydrogels showed a highly porous structure under SEM. Acute oral toxicity studies performed in rabbits exhibited no noticeable change in general behavior, food and water intake, hematological, and biochemical profile of the control group and hydrogels treated group. The pharmacokinetic evaluation confirmed that developed formulations enhanced the bioavailability of the selected drug for a prolonged period as compared to the oral solution of the drug. pH-responsive swelling and drug release, nontoxic nature, and ability to release drug for longer time support that hydrogels prepared by PAG and AA could be used as controlled-release drug delivery systems.

## CRediT authorship contribution statement

**Shazia Noureen:** Conceptualization, Supervision, Writing – review & editing. **Sobia Noreen:** Methodology, Writing – original draft, Investigation, Visualization. **Shazia Akram Ghumman:** Methodology, Investigation, Writing – review & editing. **Ehab A. Abdelrahman:** Methodology, Investigation, Writing – review & editing. **Fozia Batool:** Supervision, Investigation, Writing – review & editing. **Afeefa Aslam:** Methodology, Investigation, Writing – review & editing. **Muhammad Mehdi:** Methodology, Investigation. **Bahareh Shirinfar:** Methodology, Investigation, Writing – review & editing. **Nisar Ahmed:** Supervision, Writing – review & editing, Funding acquisition.

## Data availability

No data was used for the research described in the article.

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