

## Research Article

# Formulation, In Vitro Evaluation, and Toxicity Studies of *A. vulgaris*-co-AAm Carrier for Vildagliptin

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This study investigated the use of *Artemisia vulgaris* L. seed mucilage as a new excipient for sustained delivery of Vildagliptin. Copolymeric carrier of *A. vulgaris* seed mucilage-co-AAm was devised by using acrylamide (AAm) as a monomer, methylene-bis-acrylamide (MBA) as a crosslinker, and potassium persulfate (KPS) as an initiator through free radical polymerization. Different formulations of *A. vulgaris*-co-AAm were devised by varying contents of polymer, monomer, crosslinking agent, initiator, and reaction temperature. Copolymeric structures were characterized through XRD analysis, Fourier transform infrared (FTIR) spectroscopy, TGA and DSC analysis, and scanning electron microscopy. Porosity, gel fraction, and Vildagliptin loading capacity of copolymers were also established. Swelling and in vitro drug release studies were conducted. XRD evaluation showed the alteration of the crystalline structure of Vildagliptin into an amorphous form. FTIR analysis confirmed the successful grafting of AAm to *A. vulgaris* seed mucilage backbone. Porosity was increased with increasing polymer concentration and reaction temperature while it was decreased with an increasing amount of AAm, MBA, and KPS. Gel content was decreased with increasing polymer concentration and reaction temperature while it was increased with an increasing amount of AAm, MBA, and KPS. Acute oral toxicity of copolymeric network was done in animal models to evaluate the safety. Copolymers showed the same swelling behavior at all pH 1.2, 4.5, 6.8, and 7.4. Vildagliptin release from copolymer showed a cumulative trend by increasing polymer content and reaction temperature, while a declining trend was observed with increasing contents of monomer, crosslinking agent, and initiator. Sustained release of Vildagliptin was observed from copolymers and release followed the Korsmeyer-Peppas model. From the acute oral toxicity studies, it is evident that newly synthesized copolymeric carriers are potentially safe for eyes, skin, and vital organs.

## 1. Introduction

Naturapolyceutics is based on interdisciplinary approaches that combine natural polymer and pharmaceuticals for advancement in drug delivery design [1]. Since the primal epoch, plants frisked a vibrant character in human daily life from purposeful food to medication [2]. Plant-derivative excipients are constant applicants, which display a spirited part in pharmaceutical product development. Furthermore, the marvelous direction of formulation scientists towards

the development of plant instigated excipient offerings a new edge to ascertain, extract, and refine such compounds. Plant polysaccharides, such as gums and mucilages, are commonly used in pharmaceutical, biomedical, and cosmetic industries. Moreover, seed coats of several plants extrude mucilage on potential connection with water [3]. This mucilage comprises complex polysaccharides, which are plentiful with a high degree of biocompatibility, biodegradability, and ability to imitate the natural extracellular matrix (ECM) microenvironment. Due to their diverse nature, these

TABLE 1: Composition of formulations.

| Formulation code | Polymer (g/100 g) | Monomer (g/100 g) | Initiator (g/100 g) | Crosslinker (g/100 g) | Temperature      |
|------------------|-------------------|-------------------|---------------------|-----------------------|------------------|
| P1               | 1                 | 15                | 0.4                 | 0.4                   | 70°C             |
| P2               | 1.5               | 15                | 0.4                 | 0.4                   | 70°C             |
| P3               | 2.0               | 15                | 0.4                 | 0.4                   | 70°C             |
| M1               | 2.0               | 10                | 0.4                 | 0.4                   | 70°C             |
| M2               | 2.0               | 20                | 0.4                 | 0.4                   | 70°C             |
| M3               | 2.0               | 25                | 0.4                 | 0.4                   | 70°C             |
| M4               | 2.0               | 30                | 0.4                 | 0.4                   | 70°C             |
| C1               | 2.0               | 15                | 0.4                 | 0.5                   | 70°C             |
| C2               | 2.0               | 15                | 0.4                 | 0.75                  | 70°C             |
| C3               | 2.0               | 15                | 0.4                 | 1.0                   | 70°C             |
| I1               | 2.0               | 15                | 0.5                 | 0.4                   | 70°C             |
| I2               | 2.0               | 15                | 0.6                 | 0.4                   | 70°C             |
| I3               | 2.0               | 15                | 0.7                 | 0.4                   | 70°C             |
| T1               | 2.0               | 15                | 0.4                 | 0.4                   | 70°C             |
| R1               | 2.0               | 15                | 0.4                 | 0.4                   | Room temperature |

functional materials are in great demand to ripen sustained/controlled/targeted formulations in design and drug delivery [4].

To overcome the drawbacks of the conventional drug delivery system in recent times, the current research area is to develop modified dosage systems to give a more stable and economical dosage form. Among different drug delivery systems, hydrogels are under consideration, which not only decreases the demerits of the conventional dosage form but also provides a stable, more convenient, and biocompatible drug delivery system [5, 6]. Hydrogels have gain attention from the past years due to their extensive applications in medical, biological, and pharmaceutical disciplines [7, 8].

Hydrogels are the copolymeric networks that can swell and keep water within its polymeric network without dissolving in water [7]. Graft copolymers represent surplus benefits, especially stimuli-responsive polymers, such as higher acid-base and thermal resistance and lower crystallinity of natural polymers. Graft copolymers are prepared by first creating free radicals on the biopolymer backbone and then permitting these radicals to function as macroinitiators [9]. As compared to conventional drug delivery systems, hydrogels have prolonged and sustained action. They are biocompatible, biodegradable and provide site-specific drug delivery. This results in improved patient compliance due to reduced frequency of dosing and side effects [8, 10].

*A. vulgaris* L., commonly known as mugwort, belongs to family Compositae and is native to Europe, Asia, and northern Africa. *Artemisia* species are used throughout the world for their different kinds of medicinal properties, e.g., anti-inflammatory, antimicrobial, antioxidant, and antimalarial. *Artemisia vulgaris* seed mucilage, which shows amazing swelling in water, shows stimuli-responsiveness in different physiological conditions, solvents, and electrolytes [11].

Acrylamide is one of the favourite choices due to its excellent compatibility, ease of preparation, noncarcinogenicity, low cost, biocompatibility, and biodegradability [12].

The integral parts of copolymeric carrier synthesis are polymer, monomer, crosslinking agent, and initiator. The swelling behavior of hydrogels depends upon the nature of the polymer, monomer, initiator, and crosslinking agent as well as their concentrations and reaction temperature. The applications of hydrogels mainly depend upon the swelling behavior of the polymeric network of the hydrogel. These formulation parameters are used to alter the swelling behavior of hydrogels to modify the drug release. Extensive studies have been reported to modify swelling properties of cross-linked hydrogels by varying the formulation parameters such as the concentration of polymer, monomer, crosslinking agent, initiator, and various reaction temperatures [13–18].

Vildagliptin is an effective, selective, and orally dynamic dipeptidyl peptidase-4 (DPP-4) inhibitor, which inhibits inactivation of incretion hormones by inhibiting DPP-4 [19]. Its biological half-life is 1 to 3 hours as a result; it entails recurrent management to retain optimum plasma drug level. For the mentioned purpose, a sustained release drug delivery system improves patient compliance by dropping frequency of dosing. Thus, there is a strong clinical prerequisite and market prospective for a dosage form that will provide Vildagliptin in a sustained fashion to a patient demanding therapy. So it could be a potential candidate for safe and effective sustained drug delivery from an ideal dosage form for the treatment of type II diabetes [20].

The present work was undertaken to prepare hydrogels from *Artemisia vulgaris* mucilage and acrylamide blend by the application of free radical polymerization. The purpose of the current study was to evaluate mucilage obtained from *Artemisia vulgaris* as a new excipient of natural origin for producing smart drug delivery systems such as graft copolymer.

## 2. Materials and Methods

**2.1. Materials.** Seeds of *Artemisia vulgaris* were procured from Seed Needs, LLC. Acrylamide, potassium persulfate,

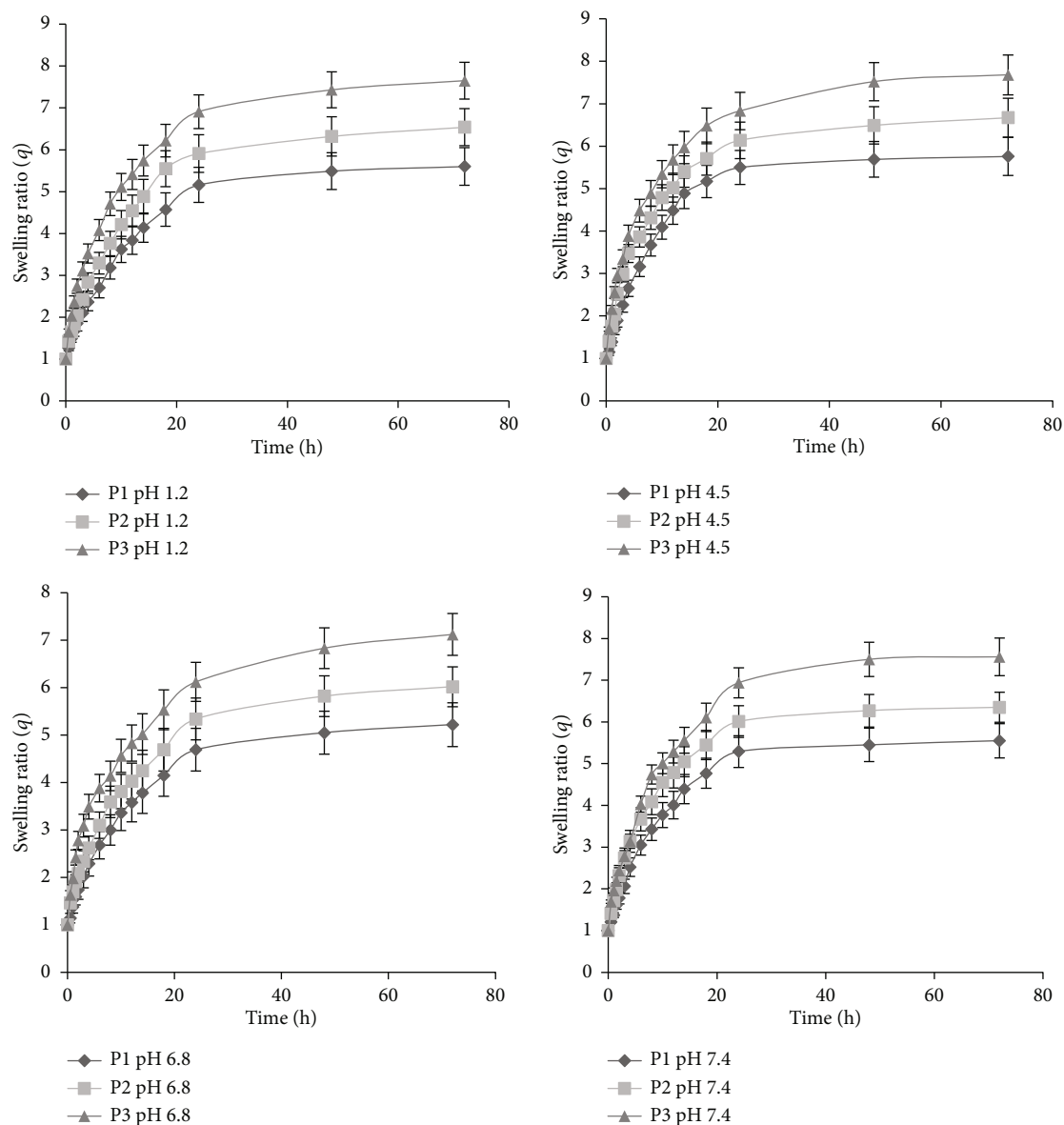


FIGURE 1: Comparative swelling ratios of *Artemisia vulgaris*-AAm copolymers with varying *seed* mucilage concentrations at pH 1.2, 4.5, 6.8, and 7.4.

sodium hydroxide, and potassium dihydrogen phosphate were purchased from Sigma-Aldrich, Germany. Methylene-bis-acrylamide was purchased from Fluka, Switzerland. Hydrochloric acid and absolute ethanol were purchased from Riedel-de Haen, Germany. The drug Vildagliptin (99.5% purity) was obtained from M/S Fuxin Long Rui Pharmaceutical Co. Ltd. Distilled water was obtained from the distillation unit of the University of Sargodha. All other chemicals used in this study were of analytical grade.

**2.2. Extraction of *Artemisia vulgaris* Seed Mucilage.** The *Artemisia vulgaris* mucilage was obtained by a hot water extraction method. Extraneous materials were removed by picking and sieving of seeds (200 g). Uncontaminated seeds were soaked in distilled water (1:9 ratio) at room temperature for 48 hours. Swollen seeds of *Artemisia vulgaris* were

passed by 40 mesh sieve later heated at 80°C for 30 minutes. The thick exudate was separated by nylon mesh. Defatted mucilage was obtained by n-Hexane ( $\geq 98.0\%$  purity, Sigma-Aldrich, Germany) treatment; the resultant was later washed thoroughly with distilled water (repeated thrice) to collect pure mucilage. Dried *Artemisia vulgaris* mucilage was triturated to obtain even powder of extract and stored in vacuum desiccators [21].

**2.3. Determination of Purity of *Artemisia vulgaris* Seed Mucilage.** Aqueous extract was prepared by dissolving *Artemisia vulgaris* Seed Mucilage powder in distilled water. Molisch's reagent and sulphuric acid were used to identify the presence of carbohydrates. Molisch's reagent was added in the aqueous extract of mucilage; then sulphuric acid was added (Malviya et al. [22]). Amino acid presence in extracted

powder was checked by dissolving aqueous extract with Ninhydrin reagent (Farooq et al. [23]).

**2.4. Synthesis of *Artemisia vulgaris*-AAM Copolymers.** AV seed mucilage was suspended in distilled water with constant stirring at 70°C. KPS was added to it to generate free radicals. A solution of AAm (monomer) and MBA was prepared and transferred to the reaction mixture. The reaction mixture was placed in a water bath for polymerization with continuous rise in temperature from 45 to 70°C by 10°C/h. The temperature was kept at 70°C to complete the reaction. Copolymers attained were cut into discs of 0.5 cm. Ethanol and water (30:70) were used to remove an unreacted monomer followed by drying in an oven at 50°C. These discs were kept in a desiccator till further use. A similar method was followed to prepare copolymers at room temperature as a reaction temperature [24].

The composition of all formulations with varying contents is summarized in Table 1.

**2.5. Swelling Studies.** The swelling ratio (dynamic swelling) of copolymers was performed up to 72 h, and equilibrium swelling of copolymers was continued for 2-3 weeks until a constant weight was achieved in 100 mL buffer solutions of different pH, respectively [25, 26]. Dynamic and equilibrium swelling was calculated from the following formulas:

$$q = \frac{W_s}{W_d}, \quad (1)$$

$$\%ES = \frac{W_s - W_d}{W_s} \times 100,$$

where  $q$  is the swelling ratio, %ES is the % equilibrium swelling,  $W_s$  is the swollen disc weight at time  $t$ , and  $W_d$  is the weight of dried disc [24, 27].

**2.6. Gel Fraction.** Nonwashed dried copolymer discs were soaked in water for 48 h to remove the insoluble part of copolymer at room temperature followed by drying in an oven at 50°C until constant weight was achieved. Gel fraction was calculated by

$$\text{Gel fraction(\%)} = \frac{W_1}{W_0} \times 100, \quad (2)$$

where  $W_1$  is the initial weight of dried disc of copolymer and  $W_0$  is the weight of the dried insoluble part of copolymer after extraction with water [24, 26].

**2.7. Porosity Measurement.** Dried copolymer discs were soaked in absolute ethanol overnight. Excess ethanol on the disc was removed by using a filter paper. Then, the weight of dried disc was taken. The following formula was used to calculate the porosity:

$$\text{Porosity} = \frac{(M_2 - M_1)}{\rho V} \times 100, \quad (3)$$

where  $M_1$  is the weight of dried disc before soaking and  $M_2$  is

TABLE 2: Percent equilibrium swelling (%ES).

| Formulations code | % equilibrium swelling (%ES) |        |        |        |
|-------------------|------------------------------|--------|--------|--------|
|                   | 1.2 pH                       | 4.5 pH | 6.8 pH | 7.4 pH |
| P1                | 69.15                        | 68.44  | 67.98  | 69.19  |
| P2                | 75.02                        | 74.74  | 73.93  | 74.72  |
| P3                | 82.22                        | 82.57  | 82.85  | 81.88  |
| M1                | 84.66                        | 84.38  | 84.89  | 84.12  |
| M2                | 77.69                        | 77.42  | 77.81  | 77.58  |
| M3                | 72.07                        | 72.53  | 72.65  | 72.38  |
| M4                | 68.26                        | 68.34  | 68.32  | 68.34  |
| C1                | 77.88                        | 77.45  | 77.62  | 77.57  |
| C2                | 72.12                        | 72.44  | 72.21  | 72.38  |
| C3                | 65.36                        | 65.24  | 65.16  | 65.53  |
| I1                | 79.64                        | 79.52  | 79.48  | 79.73  |
| I2                | 75.28                        | 75.49  | 75.73  | 75.64  |
| I3                | 70.12                        | 70.20  | 70.36  | 70.54  |
| T1                | 82.22                        | 82.57  | 82.85  | 81.88  |
| R1                | 53.04                        | 53.32  | 53.19  | 53.43  |

the weight of disc after soaking in ethanol,  $V$  is the volume of the disc, and  $\rho$  is the density of absolute ethanol [28].

**2.8. Fourier Transform Infrared (FTIR) Spectroscopy.** FTIR spectroscopic analysis of *A. vulgaris* seed mucilage, acrylamide, Vildagliptin (VLG), loaded, and unloaded copolymer was carried out by using a FTIR spectrometer (IR prestige-21 Shimadzu) between the ranges of 4000-400  $\text{cm}^{-1}$  [28].

**2.9. Thermogravimetric Analysis.** Thermogravimetric analysis (TGA) and differential scanning calorimetric (DSC) analysis were done for the evaluation of thermal changes in pure drug, loaded, and unloaded copolymeric networks at a temperature range of ambient to -600°C under nitrogen atmosphere [29, 30].

**2.10. Determination of Surface Morphology by Scanning Electron Microscopy (SEM).** Surface morphology of the copolymeric network was examined through a scanning electron microscope (FEI Quanta 250). Copolymer discs were allowed to swell in distilled water at room temperature to constant weight and then freeze-dried. Samples were placed on carbon stub, and after fixing on carbon stub, discs were examined under an electron microscope [31].

**2.11. X-Ray Diffraction Analysis (XRD).** XRD of Vildagliptin, loaded, and unloaded copolymers was performed through an X-ray diffractometer (Panalytical, Germany). The XRD was performed by exposing samples to Cu-K $\alpha$  radiation at the angle between 0 and 50°, and the scan rate was set at 1° per minute at 2 $\theta$  [29].

**2.12. Loading of Drug.** The copolymer discs were soaked in a 1% drug solution and allowed to swell for 24 hours at room temperature. After that, the discs were taken out and washed with distilled water. Finally, the drug-loaded discs were

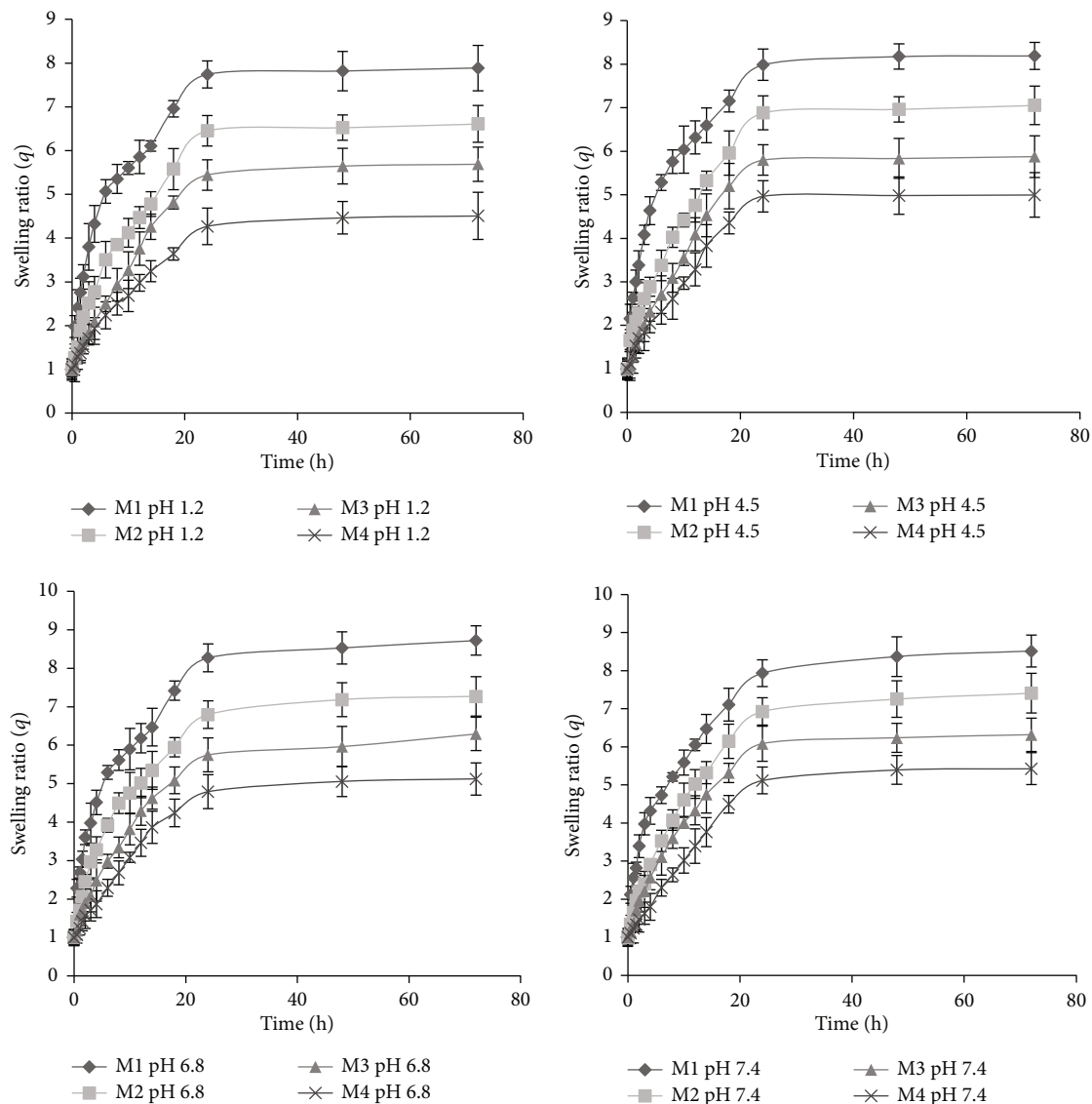


FIGURE 2: Comparative swelling ratios of *Artemisia vulgaris*-AAm copolymers with varying acrylamide concentrations at pH 1.2, 4.5, 6.8, and 7.4.

placed in an oven to dry at 50°C until the uniform weight was achieved [32, 33].

2.13. *Weight Method.* The % age drug loading (DL%) was calculated by the weight method after 24 hours by using the following formula:

$$DL\% = \frac{W_{dg}}{W_g} \times 100, \tag{4}$$

where  $W_{dg}$  is the quantity of drug entrapped in the copolymer and  $W_g$  is the weight of the dried copolymer discs [34, 35].

2.14. *In Vitro Drug Release Measurement.* USP-dissolution apparatus II was used to conduct dissolution studies at  $37 \pm 0.5^\circ\text{C}$  in buffer solution (500 mL) of pH 7.4 by keeping 50 rpm as a stirring rate. Aliquots of 5 mL were pulled out

at 0.5, 1, 2, 4, 6, 8, 10, 14, 18, 22, and 24 hours and replenished with fresh media. The samples were filtered, suitably diluted, and analyzed using a UV spectrophotometer at  $\lambda_{max} = 207$  nm [33, 35].

2.15. *Drug Release Percentage.* The drug release percentage from copolymers was measured by using the following formula:

$$\% \text{drug release} = \frac{F_t}{F_{load}} \times 100, \tag{5}$$

where  $F_t$  shows the amount of Vildagliptin released at any time  $t$  and  $F_{load}$  shows the quantity of a drug that was loaded in copolymer discs [26].

2.16. *Assessment of Kinetics of Drug Release.* The model-dependent approach was used to analyze the release kinetics

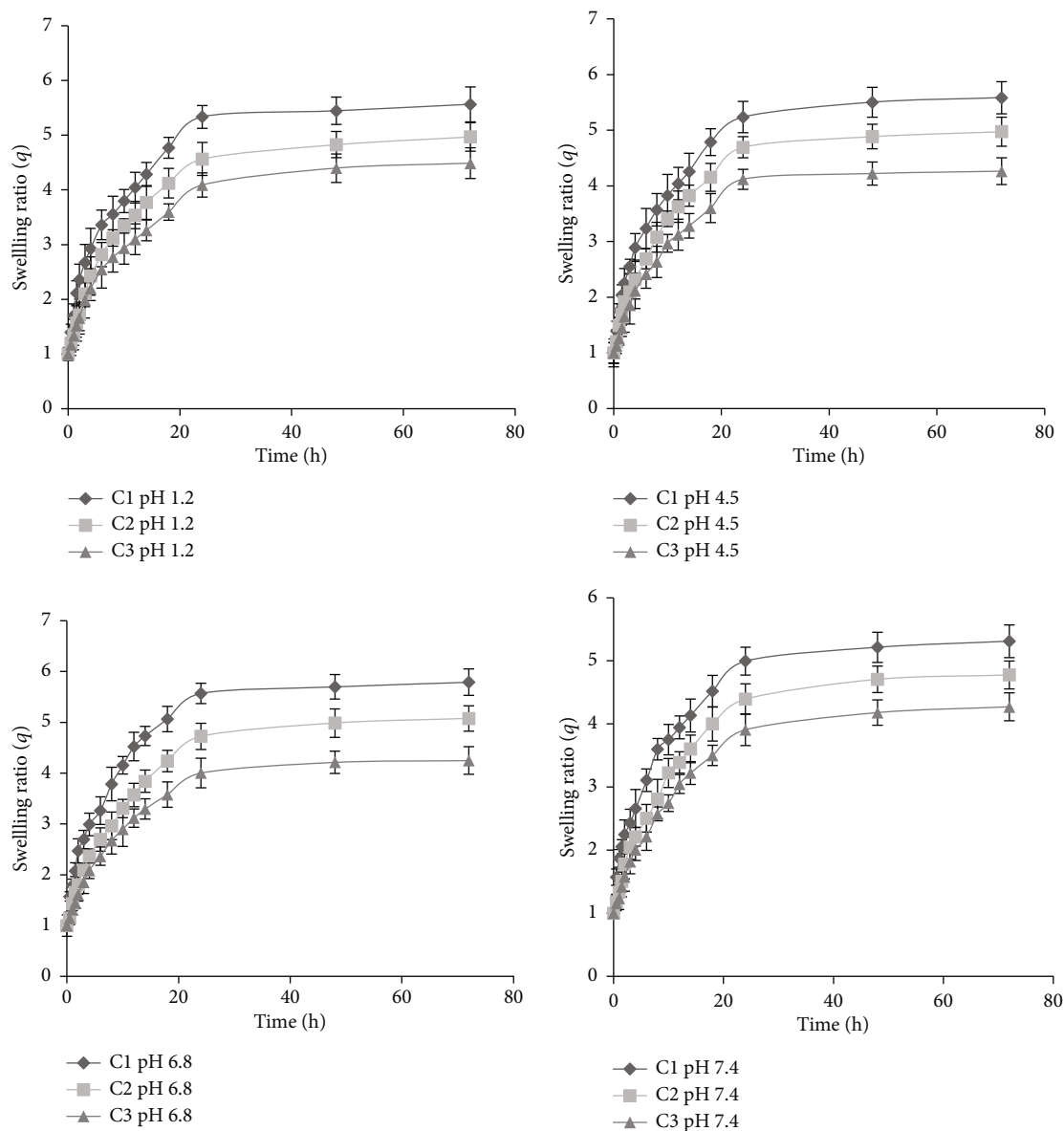


FIGURE 3: Comparative swelling ratios of Artemisia vulgaris-AAm copolymers with varying MBA concentrations at pH 1.2, 4.5, 6.8, and 7.4.

of Vildagliptin by using a DD solver. The following equations are used for Zero order, First order, Higuchi, Korsmeyer-Peppas, and Hixon-Crowell models [33, 36, 37].

**2.17. Acute Oral Toxicity.** Swiss female albino mice with  $32 \pm 5$  g weight were allocated into four groups ( $n=5$ ) and were kept in a controlled environment having free access to water and food. A copolymeric network was given a dose of 1 mg/kg, 3 mg/kg, and 5 mg/kg to group 2, group 3, and group 4, respectively, while N/S was taken by group 1 which acted as a control group. The mice of all groups were observed for 48 h for any change in weight and visual sign of mortality, behavioral pattern alteration (skin and fur, the color of urine, fecal consistency, respiration, eyes, salivation, sleep pattern, mucosal membrane variation, convulsion, and finally come), changes in the physical appearance, and further signs of illness on each day while the study was

conducted [38]. Fourteen days later, the serum biochemistry analysis was performed, and for tissues (heart, liver, kidney, gastric, small intestine, and colon) of histology studies, mice were sacrificed [39, 40].

### 3. Results and Discussion

**3.1. Synthesis of Artemisia vulgaris-AAm Copolymers.** After many trials, it was determined that the optimum concentration of Artemisia seed mucilage for Artemisia vulgaris-AAm copolymers was 2%.

#### 3.2. Swelling Studies

**3.2.1. Effect of Polymer Concentration.** It was found that copolymers with varying contents of Artemisia vulgaris seed mucilage showed an increase in dynamic and equilibrium



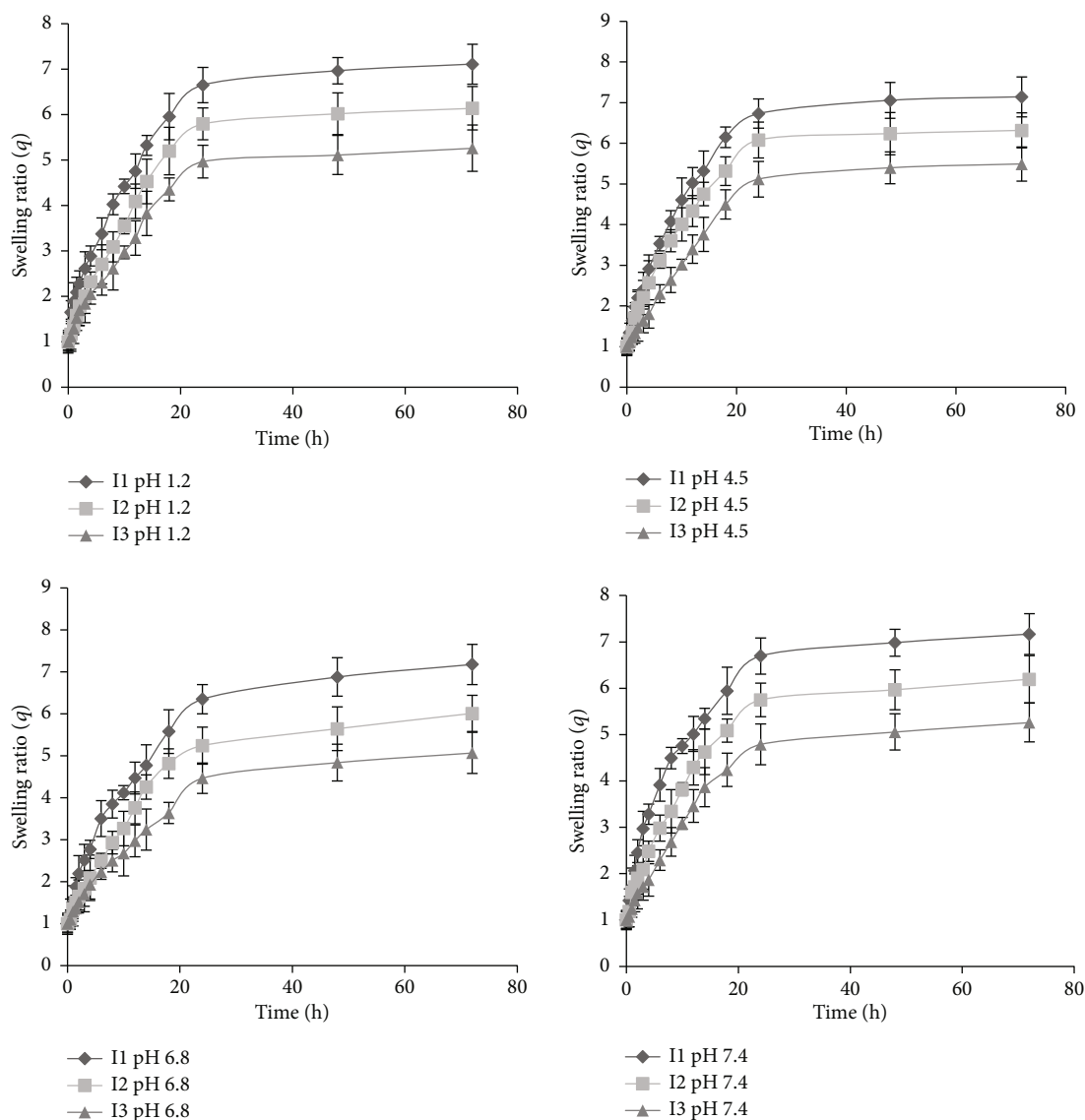


FIGURE 4: Comparative swelling ratios of *Artemisia vulgaris*-AAm copolymers with varying KPS concentrations at pH 1.2, 4.5, 6.8, and 7.4.

swelling with an increase in polymer content (Figure 1, Table 2). This can be explained by the fact that an increase in polymer content results in enhanced pore volume which ultimately causes the penetration of water in the polymer network, thus resulting in enhanced swelling ability as well as an increase in polymer concentration lead to increase in viscosity of solution that results in a reduction in movement of free radicals which ultimately lead to impaired polymerization associated with low crosslinking density. Thus, the formation of interconnected channels results in the high porosity of *Artemisia vulgaris*-AAm copolymer [41, 42].

**3.2.2. Effect of Monomer Concentration.** M1, M2, M3, and M4 copolymers are prepared with varying contents of acrylamide, and it was observed that the increase in monomer concentration results in heat-induced auto-crosslinking and branching reaction that causes a reduction in swelling and also inter- and intramolecular self-cross linkage by secondary binding

forces is increased due to increase in monomer concentration which is associated with a diminished porous gel structure which prevents the accessibility of more solvent into the polymeric matrix which can be the reason of reduction in the swelling capacity of a copolymer (Figure 2, Table 2) [43–45].

**3.2.3. Effect of Crosslinker Concentration.** The effect of varying contents of MBA on dynamic swelling was studied, as the crosslinking agent concentration is directly related to the crosslinking density, which markedly affects the dynamic swelling behavior of hydrogels [46]. A clear picture can be seen in Figure 3 (dynamic swelling) and Table 2 (equilibrium swelling) that as the concentration of MBA was increased in feed mixture, the swelling ratio was decreased. This was since there was an increase in the stability of the network and a decrease in network mesh size as the crosslinking agent content was increased in the feed mixture. The eventuating highly cross-linked rigid structure could not be expanded, so this behavior

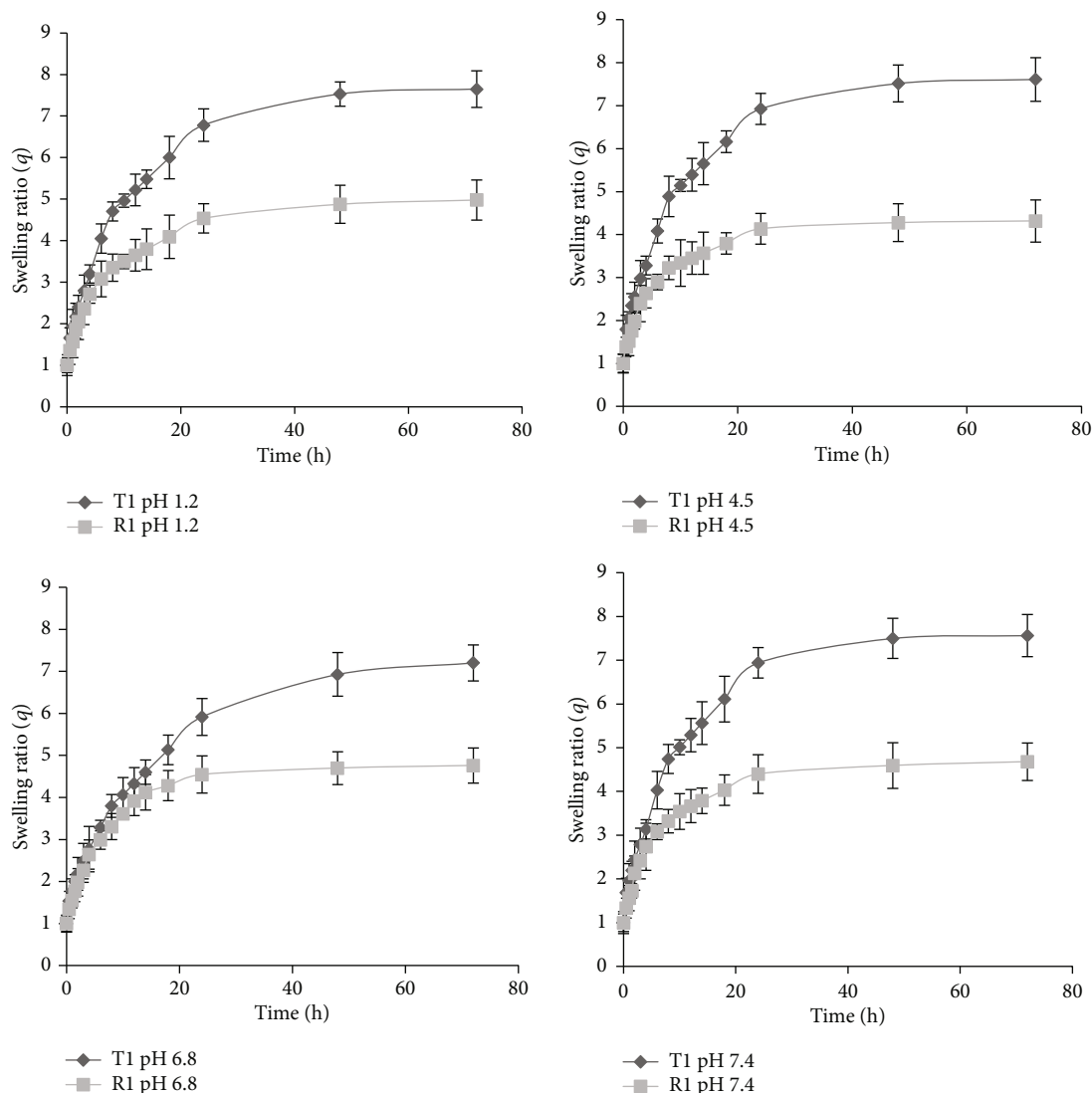


FIGURE 5: Comparative swelling ratios of *Artemisia vulgaris*-AAM copolymers with varying reaction temperatures at pH 1.2, 4.5, 6.8, and 7.4.

caused a low uptake of fluid by the copolymer and ultimately leads to a lower swelling ratio as well as equilibrium swelling ([47] #84). Findings of other similar studies reported by pioneer researchers revealed that a less porous network structure was produced by a high degree of crosslinking of copolymers which has a low swelling ratio [24, 33, 41].

**3.2.4. Effect of Initiator Concentration.** The effect of varying initiator concentrations at all pH is presented in Figure 4 and Table 2. The obtained results indicate that by increasing initiator content in feed mixture, swelling ratio, as well as equilibrium swelling, was decreased. This can be attributed to the reason that by increasing initiator contents, the molecular weight of resulting crosslinked copolymers was decreased, which leads to a shortening of macromolecular chains due to the formation of too many radicals and reducing the available free space within the copolymeric network. This causes a decrease in the swelling ratio clearly [48, 49].

**3.2.5. Effect of Reaction Temperature.** The impact of varying reaction temperatures on swelling ratio (Figure 5) and equilibrium swelling (Table 2) was studied. It was noticed that by increasing reaction temperature, the swelling ratio was increased. The reason for this action is a decomposition of peroxides as a result of irradiation of the base polymer in the air leading to an initial increase in grafting, making the requisite radicals available for grafting. The further decrease in grafting is due to the increased molecular motion with an increase in temperature that causes the increase in radical decay [50]. Besides the optimum increase in temperature, poor selectivity, various hydrogen abstraction, and chain transfer reactions can be the leading cause of graft copolymerization that results in a decrease of percent grafting [51].

**3.2.6. Effect of pH on Swelling.** There are comparative swelling ratios of different formulations of *Artemisia vulgaris*-AAM copolymer with varying component concentrations at



pH 1.2, 4.5, 6.8, and 7.4. When the pH medium is varied from acidic to basic, swelling behavior did not depict major differences which can be observed in Figures 1–4. This manner is fascinating for the applications of the copolymer. This suggests a high specific surface area of acrylamide, which ensures better solvent interactions and proper swelling. There also was no significant difference in swelling of these hydrogels on varying pH. This could be due to the nonionogenic character of polyacrylamide that leads to pH-independent swelling [52, 53].

**3.3. Percent Gel Content.** The percent gel content of all *Artemisia vulgaris*-AAM copolymers is given in Table 3. In all formulations, gel content was decreased with increasing polymer concentration and reaction temperature due to less crosslinked networks while it was increased with increasing contents of AAM, MBA, and KPS. The reason for increased gel content with these respective parameters is that there will be more crosslinking which will ultimately increase the gel strength and hence gel fraction [42, 51].

**3.4. Determination of Porosity.** Porosity measurement of all *Artemisia vulgaris*-AAM copolymer formulations is given in Table 3. According to results, the porosity of copolymers was increased with increasing polymer content and decreased with increasing content of acrylamide, MBA, and KPS. This can be explained as the increasing amount of polymer leads to increased viscosity of feed mixture which hindered the free radical movement and caused impair polymerization. So the resulting copolymer network has interconnected channels with low crosslinking density and high porosity [41]. By increasing the amount of AAM, MBA, and KPS, porosity was decreased. As molecular entanglements are increased between a polymer and monomer by increasing crosslinking density which results in a decrease in mesh size of the copolymer and ultimately the porosity ([41, 54]), the porosity of copolymer was increased by increasing reaction temperature from room temperature to 70°C. Grafting is reduced by an increase in temperature, and a copolymer with reduced crosslinked density is developed [51].

**3.5. Fourier Transform Infrared (FTIR) Analysis.** FTIR spectra of pure *Artemisia vulgaris* mucilage, acrylamide, Vildagliptin, unloaded, and loaded *Artemisia vulgaris*-AAM copolymer were recorded. *Artemisia vulgaris* seed mucilage (Figure 6(a)) showed peaks at 3795.91 corresponding to OH stretching vibration. NH stretching vibration was observed at 3196.05 and 3263.56/cm ([55]), whereas Vildagliptin spectrum (Figure 6(b)) showed characteristic peaks at 3286.70/cm (OH and NH stretching), 2245.14/cm (CN nitrile stretching), 1660.71/cm (C=O tertiary amide), 1232.51/cm (CN stretching), 1145.72/cm (C-O(H) stretching), and 1037.70/cm (C-O(H) cycloalkane 3-hydroxyadamantane stretching) [56, 57]. FTIR spectroscopic analysis of loaded *Artemisia vulgaris*-AAM copolymer (Figure 6(c)) showed Vildagliptin peaks were overlapped with a slight band shift which indicated the absence of any interaction between drug and

TABLE 3: Porosity and gel fraction of all *Artemisia vulgaris*-AAM copolymer.

| Formulation code | Porosity measurement $\pm$ SEM | %gel content $\pm$ SEM |
|------------------|--------------------------------|------------------------|
| P1               | 35.38 $\pm$ 0.28               | 81.86 $\pm$ 0.65       |
| P2               | 41.24 $\pm$ 0.26               | 77.38 $\pm$ 0.54       |
| P3               | 48.06 $\pm$ 0.31               | 65.58 $\pm$ 0.48       |
| M1               | 49.12 $\pm$ 0.34               | 63.58 $\pm$ 0.56       |
| M2               | 40.23 $\pm$ 0.22               | 73.77 $\pm$ 0.62       |
| M3               | 36.09 $\pm$ 0.33               | 80.45 $\pm$ 0.51       |
| M4               | 31.98 $\pm$ 0.24               | 86.25 $\pm$ 0.45       |
| C1               | 42.14 $\pm$ 0.34               | 68.98 $\pm$ 0.55       |
| C2               | 39.86 $\pm$ 0.23               | 73.02 $\pm$ 0.63       |
| C3               | 31.47 $\pm$ 0.28               | 82.53 $\pm$ 0.57       |
| I1               | 43.16 $\pm$ 0.34               | 69.58 $\pm$ 0.45       |
| I2               | 38.34 $\pm$ 0.26               | 77.38 $\pm$ 0.52       |
| I3               | 32.88 $\pm$ 0.28               | 82.82 $\pm$ 0.61       |
| T1               | 48.06 $\pm$ 0.31               | 65.58 $\pm$ 0.48       |
| R1               | 23.80 $\pm$ 0.27               | 83.27 $\pm$ 0.62       |

polymer [57, 58]. FTIR spectroscopic analysis also showed the grafting of AAM on to the polymer (Figure 6(d)) due to the presence of a characteristic peak at 1637.56/cm was due to the C=O stretching of amide [59, 60]. Other peaks observed were 3169.04 and 1319.31/cm corresponding to NH stretching and CN stretching [61, 62].

**3.6. TGA and DSC.** It is evident from the TGA curve (Figure 7) that VLG showed 43.75% weight loss at 315.59°C. At the same temperature, drug-loaded *Artemisia vulgaris*-AAM copolymer showed a 21.75% weight loss. At 544°C, the drug showed 76.77% weight loss while at this stage drug-loaded *Artemisia vulgaris*-AAM copolymer showed 71.53% weight loss. Higher remaining mass indicated that the thermal stability of the drug was increased upon loading to copolymer [63]. The results of the DSC analysis are presented in (Figure 8). It can be evident from the results that VLG showed initially flat profile but, when it entered its melting range, a sharp endothermic peak was observed at 164. However, in VLG-loaded *Artemisia vulgaris*-AAM copolymer, this peak was not evident clearly due to a rigid copolymer network; these observations indicate that VLG was molecularly dispersed in copolymer formulation on loading. These observations indicated that drug and polymer were compatible with each other [56, 64].

**3.7. Scanning Electron Microscopy.** For determining morphological characteristics of a prepared copolymer, scanning electron microscopy was performed for formulations of *Artemisia vulgaris*-AAM copolymer with the best swelling results. SEM images (Figure 9) showed dense and compact structure with porous interconnected channels [44, 45, 65].

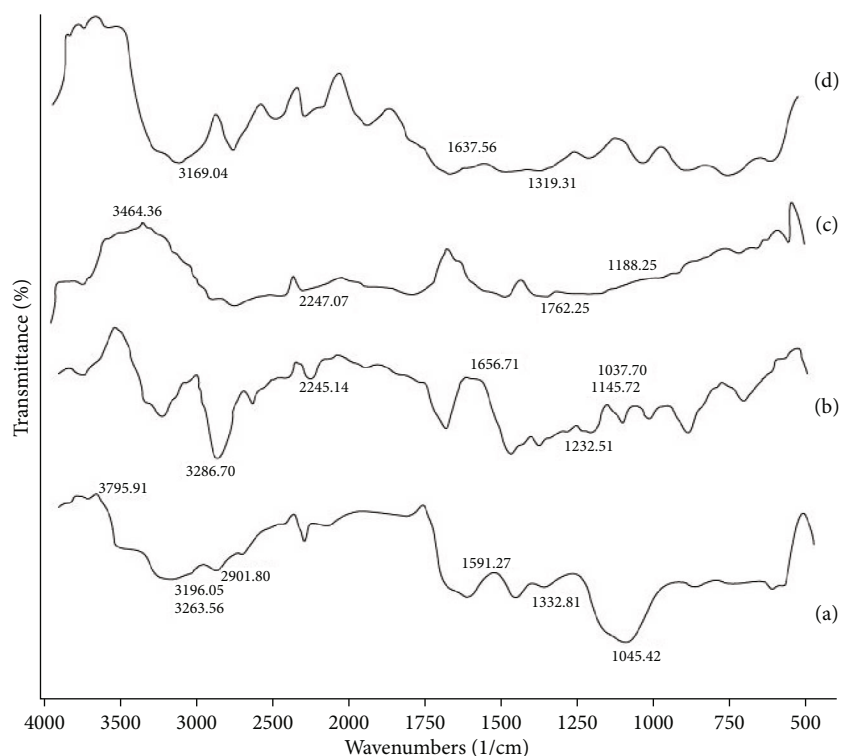


FIGURE 6: FTIR overlays of (a) *Artemisia vulgaris* mucilage, (b) Vildagliptine, (c) loaded *Artemisia vulgaris*-co-AAm, and (d) unloaded and loaded *Artemisia vulgaris*-co-AAm.

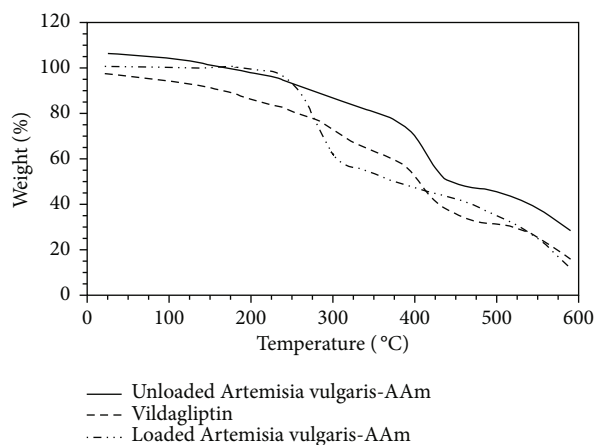


FIGURE 7: TGA for unloaded *Artemisia vulgaris*-AAm copolymer (S1), Vildagliptin (S4), and loaded *Artemisia vulgaris*-AAm copolymer (S3).

**3.8. X-Ray Diffraction Analysis.** It was determined that the crystalline nature of the drug was altered to an amorphous state after its entrapment in the copolymer. An overlay of XRD (Figure 10) of *Artemisia vulgaris*-AAm copolymer revealed that characteristic peaks of Vildagliptin showed a clear decrease in its intensity. Therefore, results have indicated that Vildagliptin underwent amorphous dispersion [57, 58]. For more characterization and applications of *A. vulgaris* mucilage, readers are referred to following works of peers [11, 66].

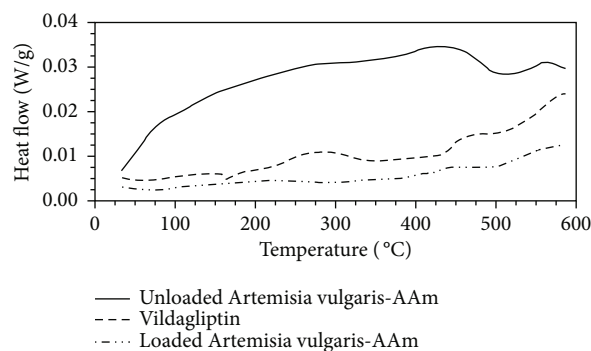


FIGURE 8: DSC for unloaded *Artemisia vulgaris*-AAm copolymer (S1), Vildagliptin (S4), and loaded *Artemisia vulgaris*-AAm copolymer (S3).

**3.9. Loading of Drug.** The drug loading capacity of different formulations of *Artemisia vulgaris*-AAm copolymer is given in Table 4.

**3.10. In Vitro Drug Release Measurement.** *In vitro* release profiles of *Artemisia vulgaris*-AAm copolymers with varying polymer, monomer, crosslinker, initiator, and reaction temperatures at pH 7.4 are presented in Figure 11. Formulations with varying polymer contents showed an increase in drug release with increasing contents of the polymer. As for P1, P2, and P3, Vildagliptin release after 24 hours was 76.84%, 81.81%, and 86.58%, respectively. This significant drug release was observed due to increased porosity of the network

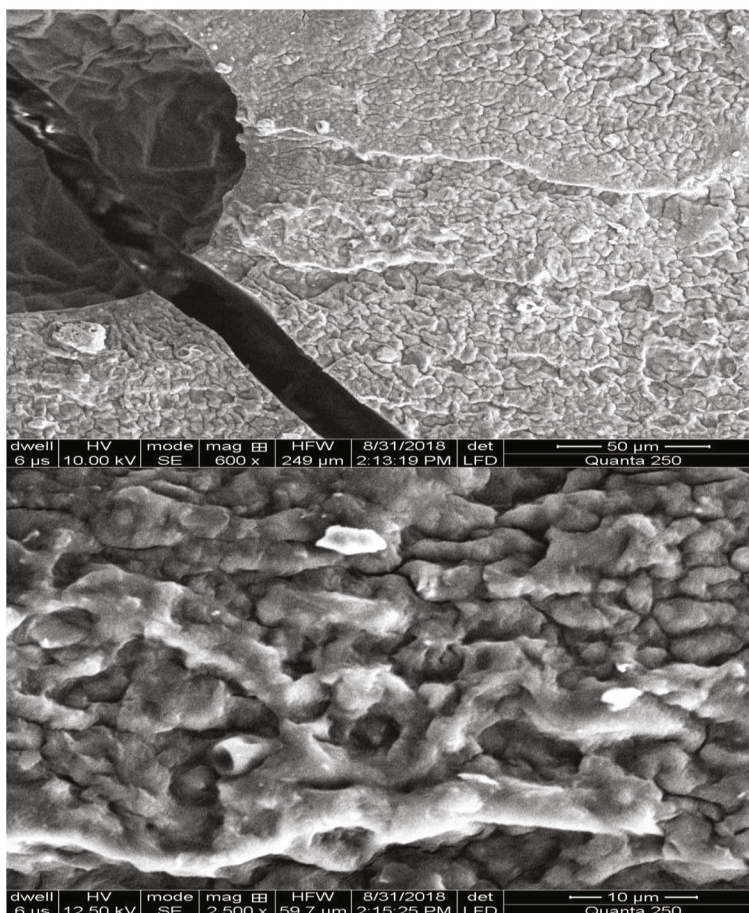


FIGURE 9: SEM image of *Artemisia vulgaris*-AAm copolymer (a) with 50  $\mu\text{m}$  scale and (b) with 10  $\mu\text{m}$  scale.

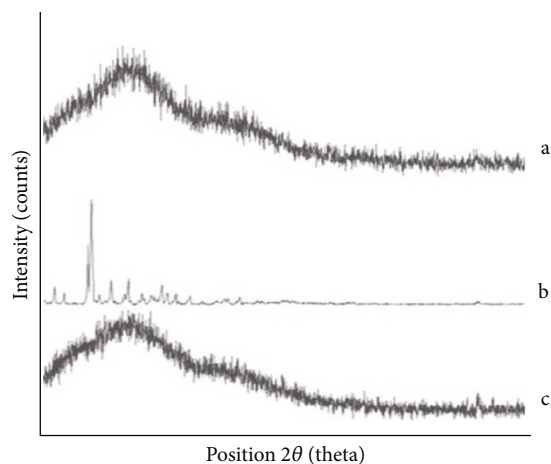


FIGURE 10: Overlaid XRD spectra of (a) unloaded *Artemisia vulgaris*-AAm copolymer, (b) Vildagliptin, and (c) Vildagliptin-loaded *Artemisia vulgaris*-AAm copolymer.

with increasing content of polymer [67]. Formulation with varying contents of monomer showed a decrease in drug release with increasing contents of acrylamide. As for M1, M2, M3, and M4, the percentage cumulative drug release observed after 24 h was 89.58%, 79.36%, 75.58%, and 69.41%, respectively. A similar behavior was observed with

TABLE 4: Drug loading capacity in all formulations of *Artemisia vulgaris*-AAm copolymer.

| Formulation code | Vildagliptin loaded (mg/g) disk |
|------------------|---------------------------------|
| P1               | 68 $\pm$ 0.9                    |
| P2               | 75 $\pm$ 1.3                    |
| P3               | 102 $\pm$ 0.8                   |
| M1               | 110 $\pm$ 0.7                   |
| M2               | 84 $\pm$ 1.4                    |
| M3               | 70 $\pm$ 1.2                    |
| M4               | 58 $\pm$ 0.9                    |
| C1               | 86 $\pm$ 0.8                    |
| C2               | 75 $\pm$ 1.2                    |
| C3               | 52 $\pm$ 1.4                    |
| I1               | 95 $\pm$ 0.9                    |
| I2               | 77 $\pm$ 1.3                    |
| I3               | 68 $\pm$ 1.2                    |
| T1               | 102 $\pm$ 0.9                   |
| R1               | 55 $\pm$ 1.5                    |

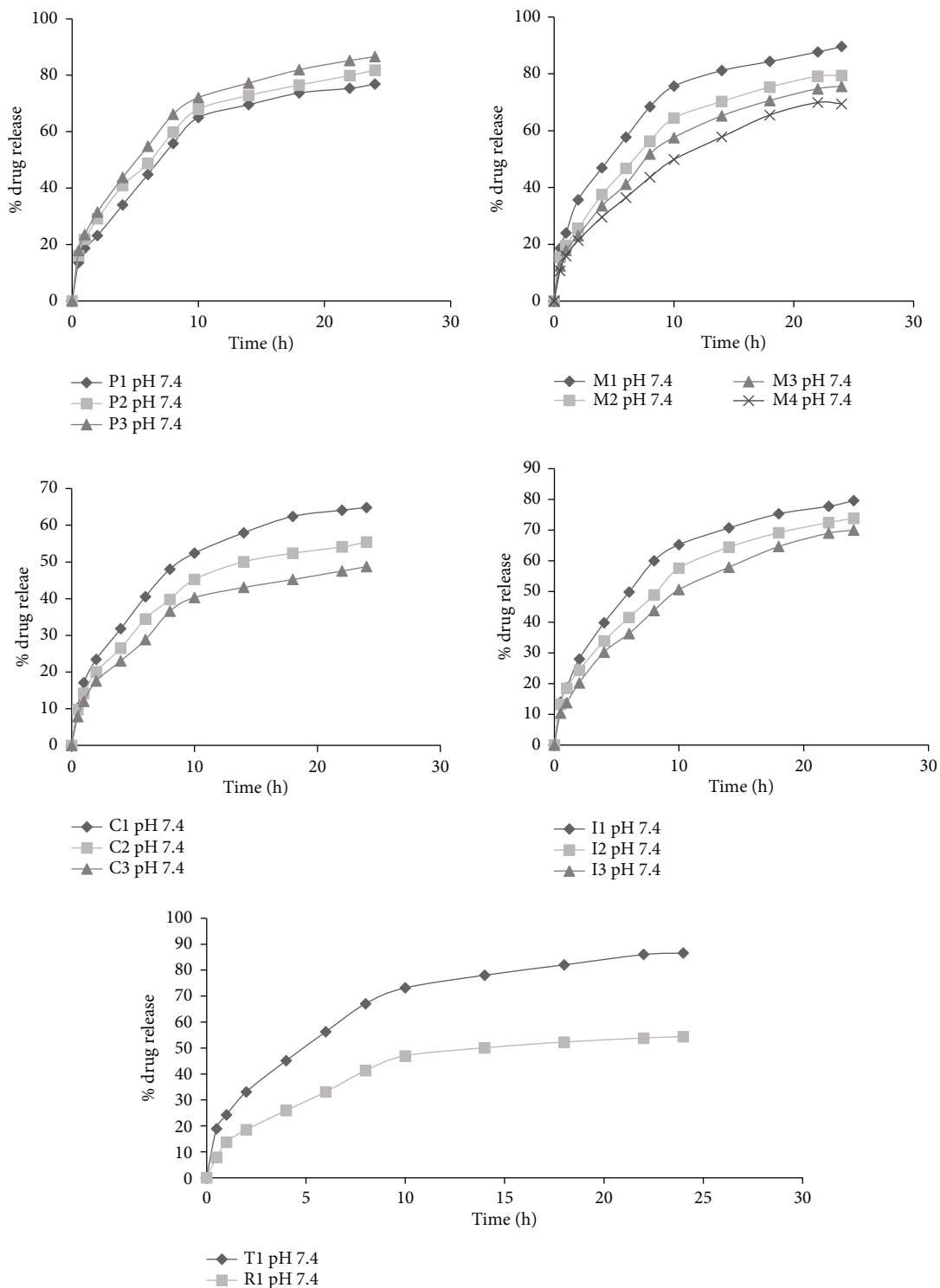


FIGURE 11: Percent drug release: (a) varying polymer contents, (b) varying acrylamide, (c) MBA, (d) varying KPS, and (e) varying reaction temperature.

MBA and KPS. By increasing the contents of the above-mentioned parameters, a more crosslinked network is formed, this behavior of network causes a decrease in chain relaxation and drug release [59, 68, 69]. Also, the impact of varying reaction temperatures was determined. It can be seen

in Figure 11 that in the formulation prepared by varying the reaction temperature (T1 and R1), the highest percent of Vildagliptin release was shown by T1 formulation. The drug release pattern was strongly linked with the swelling pattern of copolymers [36].

TABLE 5: Hematology and blood chemistry parameters of mice.

| Blood parameter            | Normal ranges                  | Group 1 (control) | Group 2       | Group 3       | Group 4       |
|----------------------------|--------------------------------|-------------------|---------------|---------------|---------------|
| CBC (complete blood count) |                                |                   |               |               |               |
| TLC                        | 8.1-21.5 × 10 <sup>3</sup> /μL | 13.51 ± 0.03      | 11.05 ± 0.11  | 15.23 ± 0.17  | 9.55 ± 0.21   |
| RBC                        | 3.8-7.9 × 10 <sup>6</sup> /μL  | 5.17 ± 0.03       | 4.63 ± 0.07   | 6.29 ± 0.15   | 6.61 ± 0.13   |
| Hb (hemoglobin)            | 9.4-17.4 g/dL                  | 11.81 ± 0.23      | 10.43 ± 0.21  | 14.53 ± 0.19  | 11.67 ± 0.12  |
| HCT (PCV)                  | 35-40%                         | 37.31 ± 0.21      | 36.25 ± 0.15  | 35.63 ± 0.23  | 38.81 ± 0.25  |
| MCV                        | 50-75 fL                       | 62.05 ± 0.11      | 53.33 ± 0.16  | 56.29 ± 0.13  | 54.45 ± 0.11  |
| MCH                        | 18-24 pg                       | 22.87 ± 0.17      | 19.43 ± 0.16  | 22.51 ± 0.04  | 20.56 ± 0.07  |
| MCHC                       | 27-34 g/dL                     | 35.29 ± 0.18      | 32.53 ± 0.15  | 30.65 ± 0.21  | 28.13 ± 0.23  |
| Platelet count             | 250-650 × 10 <sup>3</sup> /μL  | 371.67 ± 1.77     | 341.03 ± 1.13 | 531.33 ± 2.35 | 277.05 ± 2.33 |
| Neutrophils                | 34-70%                         | 44.71 ± 0.24      | 52.63 ± 0.25  | 38.17 ± 0.12  | 41.87 ± 0.07  |
| Lymphocytes                | 30-70%                         | 39.13 ± 0.21      | 41.21 ± 0.23  | 45.25 ± 0.18  | 48.29 ± 0.27  |
| Monocytes                  | 0-3%                           | 1.35 ± 0.03       | 1.83 ± 0.02   | 2.33 ± 0.02   | 2.61 ± 0.03   |
| Eosinophils                | 0-1%                           | 0.55 ± 0.01       | 0.35 ± 0.01   | 0.73 ± 0.01   | 0.81 ± 0.01   |

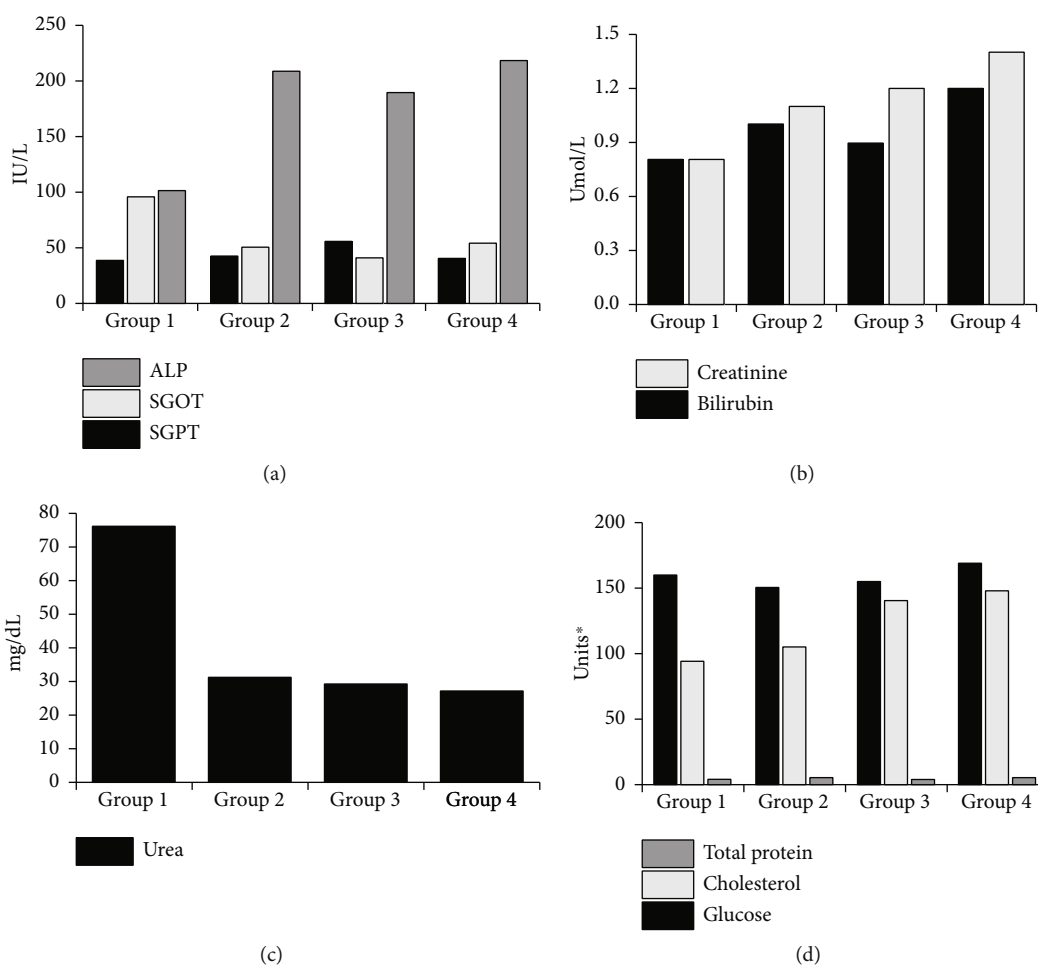


FIGURE 12: Serum biochemistry of Swiss albino mouse blood evaluating acute oral toxicity; (a) liver function tests, (b) bilirubin and creatinine, (c) urea, and (d) serum biochemistry analysis were performed in accordance with OECD 425 guidelines for acute oral toxicity (\*units for glucose (mg/dL), cholesterol (mg/dL), and total protein (g/dL)).



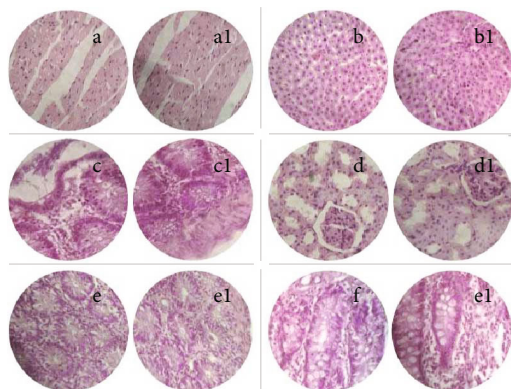


FIGURE 13: Microscopic assessment of tissue histology; (a) control heart and (a1) treated sample of the heart, (b) control liver and (b1) treated sample of the liver, (c) control gastric tissue and (c1) treated sample of the gastric tissue, (d) control kidney and (d1) treated sample of the kidney, (e) control small intestine and (e1) treated sample of the small intestine, and (f) control colon and (f1) treated sample of the colon attained from Swiss albino mice after being euthanized.

**3.11. Assessment of Drug Release Kinetics.** The correlation coefficient and release rates of all formulations were determined. Based on the highest regression coefficient value ( $R^2$ ), the best fit model found was Korsmeyer-Peppas. This model is applied to determine the release mechanism of drugs, i.e., Fickian diffusion and non-Fickian diffusion [70, 71]. According to the value of the release exponent, i.e., less than 0.5, the drug release mechanism from copolymer was Fickian [72]. Changez et al. prepared poly(acrylic acid)-gelatin hydrogel and reported that gentamicin follows Fickian diffusion from hydrogel [73].

**3.12. Acute Oral Toxicity Evaluation.** The acute oral toxicity was carried out on Swiss albino mice for the copolymeric network [38]. After 48 h of observation following administration, no change was visible on the skin, fur, and behavioral pattern in all observed groups. Later on, the same parameters were subsequently observed for 14 days to ascertain toxic events macroscopically. There was no significant change in body weight, and no mortality was observed for 14 days. Furthermore, on completion of 14 days, the blood sample was withdrawn from each mouse to conduct CBC and serum biochemistry analysis [74].

**3.13. Serum Biochemistry Analysis.** Serum biochemistry of Swiss albino mouse blood evaluating acute oral toxicity was determined. Hematology and blood chemistry parameters of mice are shown in Table 5; all the values hemoglobin, liver profile, renal profile, and serum biochemistry shown graphically in Figure 12 are comparable to control and within normal range thus verifying the safety of the copolymeric network [75, 76].

**3.14. Histopathological Evaluation.** The macroscopic examination of the heart, gastric tissue, kidney, liver, small intestine, and colon did not display any visible changes or lesion in the tissues of these organs. For further investigation, slides

were prepared for each organ (Figure 13) mentioned above for histological exploration. The microscopic examination proved that slides of the heart, gastric tissue, kidney, small intestine, and colon did not show any lesions; the liver was normal and did not present any fatty change or necrosis when compared to control. Hence, the copolymeric network did not turn out to be toxic for these organs and back up the provisions of RFTs and LFTs, thus indicating the safety of the copolymeric network. Conclusively, no gross difference in histopathological observation was found between the control and treatment groups similar to hematological and biochemical biomarkers, credited to the normal functioning of vital organs. The outcomes of acute oral toxicity assessment presented that there was no toxic reaction or histopathological vicissitude instigated after the maximum dose level of *A. vulgaris* seed mucilage-co-AAm copolymer. Thus, this drug delivery system might be a safe nominee for use in biomedical field, particularly in an oral drug delivery system [77, 78].

## 4. Conclusions

*Artemisia vulgaris* mucilage-based copolymers were successfully fabricated by a free radical polymerization method. Among various graft copolymer formulations prepared with varying contents of the polymer, monomer, crosslinker, initiator, and reaction temperature, M1 presents superior properties regarding swelling and sustained drug release. *Artemisia vulgaris*-based copolymers were proved to be a suitable carrier for sustained drug delivery of Vildagliptin. The results of acute oral toxicity evaluation showed that there was no toxic response or histopathological changes caused by *A. vulgaris* seed mucilage-co-AAm. Thus, the copolymer prepared might be a safe candidate for application in the biomedical field, especially in the oral drug delivery system. The mucilage obtained from *Artemisia vulgaris* can be used as a new excipient of natural origin for producing smart drug delivery systems such as graft copolymer.

## Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

## Conflicts of Interest

The authors declare no conflict of interest.

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and UOS were utilized for animal acute oral toxicity evaluation.

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