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Research Article

RADIATION PREPARATION OF SMART HYDROGEL HAS ANTIMICROBIAL PROPERTIES FOR CONTROLLED RELEASE OF CIPROFLOXACIN IN DRUG DELIVERY SYSTEMS

NABILA AHMED MAZIAD[1], SABRNAL EL-HAMOULY[2], EBRHEM ZIED[3], TAMER A EL KELANI[4], NADA RABIE NASEF[2]

[1]Department of Polymer Chemistry, National Centre for Radiation Research and Technology, Atomic Energy Authority, Egypt. [2]Department of Chemistry, Faculty of Science, Manoufia University, Egypt. [3]Department of Organic Chemistry, Faculty of Science, Manoufia University, Egypt. [4]Department of Microbiological, National Centre for Radiation Research and Technology, Atomic Energy Authority, Egypt.
Email: Nabila_Maziad@yahoo.com

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ABSTRACT

The objective of the present work was to synthesize copolymer hydrogel composed of poly acrylic acid (PAAc) and PAAc/pectin, which are very sensitive to environmental stimulus, this feature is important for their application in biomedical applications, due to its unique properties, which can resemble human living organs, wound dressing, drug delivery systems. Gamma radiation induces synthesis and modification of monomer to polymer hydrogel was studied. The effect of different parameter onto preparation of smart hydrogel such as monomer concentration, radiation dose on to swelling percent of the prepared copolymer hydrogel have been studied, gel fraction have been studied as a function of swelling ratio. Structure characterization of the prepared copolymer hydrogel have been investigated using Fourier transform infrared spectroscopy, The morphological structure using X-ray diffraction analysis and scanning electron microscopy have been studied. The swelling properties of the prepared copolymers have been studied at different time and pH. It was found that the swelling percent increases as the time increase and increases as pH increase and the maximum swelling occurs at pH 6 with the value of 19,000% for PAAc hydrogel and 10,000% for PAAc/pectin hydrogels after 24 hrs. Drug loading measurements using ciprofloxacin (CPFX) drug at pH 7 for PAAc hydrogel after 24 hrs and at pH 11 for PAAc/pectin hydrogels. Studies of drug-releasing of CPFX as drug model have been investigated, at different time and pH and it was found that the drug release incases as pH increase and the maximum release occurs at pH 4 for PAAc and pH (3,8) for PAAc/pectin hydrogels, the antimicrobial activity of the synthesized copolymeric hydrogel under study was evaluated based on the diameters of clear zone surrounding the polymeric substance (disk diffusion test) this proved that polymeric hydrogel can be used as antibacterial agent.

Keywords: Radiation, Copolymerization, Pectin, Acrylic monomers, Drug release.

INTRODUCTION

Hydrogels are three-dimensional (3D) cross-linked polymeric networks capable of absorbing and retaining large amounts of water and physiological fluids while remaining insoluble in aqueous solutions. In general, these hydrogels at equilibrium comprise 60-90% fluid and only 10-30% polymer. Due to characteristic properties such as swell ability in water, high water content and elastic nature similar to natural tissue, biocompatibility and lack of toxicity, hydrogels have been utilized in a wide range of biological, medical, pharmaceutical and environmental applications [1-3]. Hydrogels are polymers characterized by hydrophilicity and insolubility in water. Hydrogel in water, they swell to an equilibrium volume but preserve their shape. The hydrophelicity is due to the presence of water solubilizing groups, such as –OH, –COOH and –CONH. The insolubility and stability of the shape are due to the presence of 3D network structures in the hydrogel [4-6]. Hydrogels are usually made of hydrophilic polymer molecules, which are cross-linked either by chemical bonds or other cohesion forces such as ionic interaction, hydrogen bonding or hydrophobic interaction [6]. Hydrogels are used extensively in medicine and pharmacy as drug delivery systems, and one of the most important applications of hydrogels is in controlled release systems and for targeting drug to specific areas of the body [7]. When contact is established to the target site, the rate and duration of drug release depends on the swelling behavior of the hydrogels [4]. Drug delivery systems have been the subject of great interest because they can effectively deliver a drug to the target site, maximize the efficiency of drug, minimize the side-effects, and reduce dosing frequency by prolonging the release time [8,9]. Thus far numerous technologies and materials have been developed to maximize various benefits of drug delivery formulations [10,11]. Among them, the "intelligent" or "smart" hydrogels have received considerable attention in drug delivery systems because they can regulate drug release through the volume phase change of gel induced by the environmental stimuli, such as pH [9,12,13]. pH-sensitive hydrogel can rapidly response to the external pH stimuli, and was developed as the most effective carrier of gastrointestinal drugs [2]. Pectin, a plant polysaccharide play important role in gastroenterological medicine, for use in drug carriers for oral drug delivery [14,15].

Hence, the pH-sensitive hydrogels were produced from various sources as promising, intelligent drug delivery systems [16,18], and the polysaccharide based systems show a unique prospect by virtue of their advantages such as non-toxicity, inexpensive, biodegradability, and biocompatibility [19,20]. Pectin is a naturally occurring biopolymer that is finding increasing applications in the pharmaceutical. Also have several unique properties that have enabled it to be used as a matrix for the entrapment and delivery of a variety of drug. Furthermore, cross-linked polymers from pectin can form hydrogels that are able to absorb and retain hundreds of times their weight of water and are known as superabsorbent [21,22].

Pectin is known as a miracle polymer of natural origin because of its excellent biodegradable and biocompatible nature. Pectin has been widely investigated for targeted drug delivery and other potential biomedical applications. Pectin is known to be rapidly degraded by colonic microorganisms and thus makes it a potential carrier for colon targeted drug delivery. Pectin based formulations have shown promise as novel biomaterials for the development of implantable [23-25].

In recent years, hydrogel-based drug-delivery devices have become a major area of research interest with synthetic hydrogels. One of the important types of synthetic hydrogels are those of polyacrylic acid (PAAc), either based on cross-linked polymers or combined with other co monomer [3]. The development of a drug delivery system requires

the control of the water content within the polymeric structure as it is one of the important factors influencing the solute transport. The permeation rate can be controlled either by changing the cross-linking densities or by preparing hydrogel with co monomer of controlled hydrophilicity. PAAc hydrogels, owing to the existence of hydrophilic – COOH groups, have the capacity to absorb large amounts of water.

Furthermore, due to the presence of hydrophilic carboxylic acid side groups, the swelling behavior of these hydrogels is highly dependent on the pH of the surrounding medium [26].

Hence, PAAc hydrogels have been investigated for use as adsorbents in drug delivery systems [7,27]. AAc is a pH - sensitive, synthetic polymer extensively used in the area of the site-specific drug delivery of the gastrointestinal tract. Because of the presence of carboxylic acid groups, the swelling behavior of the AAc hydrogel is highly dependent on the pH of the surrounding medium. Since pKa of AAc is between 4.5 and 5.0, AAc hydrogels showed significant swelling in the small intestine. However, they do not swell significantly below pH 4 in the stomach. Therefore, one of the major applications of AAc gels is sustained gastrointestinal drug delivery systems [28].

METHODS

Materials

Pure AAc from Aldrich of purity 99% was used as received ($C_3H_6O_2$), polysaccharide pectin (pec) molecular weight 30,000-10,000 Pkd. by Oxford Laboratory, ciprofloxacin (CPFX) 500 ml/g from European Egyptian Pharma International. Buffer solutions of pH range (2-13). Other chemicals were used as received without further purification.

Preparation of hydrogel

The preparative method was used for synthesis of PAAc and PAAc/pectin hydrogels, which obtained by radiation-induced copolymerization of mixture of AAc at different concentration and distilled water. The glass tube was exposure to N_2 gas to remove O_2 and then irradiated at different doses using gamma-rays from a ^{60}Co source located at the National Center for Radiation Research and Technology (NCRRT), Atomic Energy Authority of Egypt. The hydrogel was washed with excess distilled water to remove the unreacted monomer and then dried under vacuum until a constant weight was obtained. On the other hand, the preparative method of copolymer PAAc/pectin hydrogel by the addition of mixture of 5 wt% g pectin dissolve in 100 ml distilled water and was allowed to stir for 10 minutes, after complete dissolution of the pectin to form a homogeneous solution different ratio of pectin solution was added to AAc monomer.

Preparation of buffer solutions of different pH

Citric acid/trisodium citrate and sodium dihydrogen phosphate/ disodium hydrogen phosphate were used to prepare buffer solutions of pH values ranged from 3-5 to 6-7, respectively [29,30].

Swelling measurements

Swelling experiments were performed by placing the prepared polymer disks in buffer solutions of varying pH of 2-12 at 37°C and measuring sample weight given as a function of time. The disks were withdrawn from the buffer solutions and weighed after removal of excess surface water by gentle blotting with a paper tissue.

Percent swelling is expressed as the percent weight ratio of water held in hydrogel to dry hydrogel at any constant time. The swelling ratio was calculated as shown in equation (1):

$$\%\text{Swelling} = \frac{(\text{weight of swelled hydrogel} - \text{weight of dry hydrogel})}{\text{weight of dry hydrogel}} \times 100 \quad (1)$$

Drug loading efficiency and drug release

CPFX was used a model drug. The disc samples PAAc hydrogels (1 g±0.0001), were accurately weighted and immersed in solution of CPFX, 0.54 g dissolved in 50 ml of buffer solution at 37°C for 24 hrs. The loading amount of drug in the hydrogels was calculated from the decrease in the concentration of the CPFX solution, which was determined using ultraviolet (UV) spectrophotometer at 400 nm. The loading efficiency of the PAAc based hydrogels was calculated as the ratio of the final to the initial CPFX concentration.

In drug release of PAAc hydrogel was dipped in CPFX dissolved in a buffer solution of at different pH's 37°C for 24 hrs. and then dried in air and putting in the buffer solution and then At specific time intervals, 4 ml of solution was withdrawn and after suitable dilution the concentration of drug released was measured by UV spectrophotometer at 400 nm. The drug release percent was calculated twice using the following equation (2).

$$\text{Released drug (\%)} = (Rt/L) \times 100 \quad (2)$$

Where L and Rt represent the initial amount of drug loaded and the final amount of drug released at time (t).

Measurements

Fourier transform infrared (FTIR) measurements

The FTIR spectra of the copolymer hydrogels and pectin powder were recorded over the range 400-4000/cm by KBr pellet method using FTIR spectrophotometer.

X-ray diffraction (XRD) measurements

XRD measurements were made using an XRD meter. The diffract grams were measured at 2 θ, 5-50°.

UV measurements

Determination of the loading and release amount of drug CPFX were carried out using JASCO V560 spectrophotometer in the range from 200 to 900 nm. The concentration of CPFX drug were measured at 400 nm.

Scanning electron microscopy

The dry sample, spread on a double sided conducting adhesive tape, pasted on a metallic stub, was coated 100 μ with gold in a sputter coating unit 2 minutes and absorbed in electron microscope at 20 kV.

Microbial strains

The bacterial and fungal strains used in this study were obtained from the Microbiology Laboratory, Department of Microbiology, National Center for Radiation Research and Technology, NCRRT, Atomic Energy Authority, Cairo, Egypt. Tested yeast isolates are *Candida albicans*, *Saccharomyces cerevisiae* and *Rhodotorula glutinis*. Gram-positive bacteria *Staphylococcus aureus* and Gram-negative bacteria are *Pseudomonas aeruginosa*, *Salmonella typhi* non-lactose fermenter and lactose fermenter *Escherichia coli* and *Klebsiella pneumonia*. All the microorganisms used are checked for purity and maintained at 4°C in slants of nutrient agar and malt extract agar for bacteria and yeast, respectively [51].

Preparation of inocula

A loopful of each microbial isolates is transferred from new slant into 25 ml broth medium in 250 ml Erlenmeyer flasks and cultivated on a rotary shaker at 100 rpm for 18 hrs at 37°C for bacteria, and for 48 hrs for yeasts. Inocula were prepared by transferring into (5 ml) 0.9% sterile saline solution to obtain the required working suspensions, 10^8 cfu/ml for bacteria and 10^7 cfu/ml for yeasts. The bacterial suspension was adjusted with sterile saline to an optical density (OD) of 0.2-0.3.

Antibacterial activity

Disk diffusion test. *S. aureus*, *P. aeruginosa*, *K. pneumonia*, *S. typhi* and *E. coli*, were used in order to investigate the antibacterial activity of the synthesized polymeric compounds. The bacterial suspension was adjusted with sterile saline to an optical OD of 0.2-0.3. The inocula were

daily prepared and stored at 4°C until use. Dilutions of the inocula were cultured on solid medium to verify the absence of contamination and to check the validity of the inoculums.

Preparation of plates

Mueller–Hinton agar medium composed of 1 g laboratory lemco, 2 g yeast, 5 g peptone, 5 g sodium chloride, 20 g agar-agar and distal water 1 L, oxoid, were cooked and sterilized at 1 bar for 20 minutes. In addition to Muller–Hinton broth (lack agar-agar) those are used for bacterial growth. Malt agar medium composed of 30 g Malt extract, 5 g mycological peptone, 20 g agar-agar and distal water 1 L, oxoid, were cooked and sterilized at 1 bar for 20 minutes. In addition to Malt broth (lack agar-agar). Those are used for yeast growth.

Antibacterial activity assay of polymeric substance

In-vitro antibacterial activity of the synthesized polymeric compounds was determined by the agar disc diffusion method. 18 ml of sterilized Mueller–Hinton agar medium was taken in each petri dish and then spread with a suspension of the tested micro-organism (average concentration is 10^8 cells/ml). 150 µg of each polymer was placed on the seeded agar plates and then incubated at 37°C for 24 hrs. The antibacterial activity of the test agent was determined by measuring the mean diameter of zone of inhibitions in millimeter.

RESULTS AND DISCUSSION

Study the effect of monomer concentrations on gel fraction percent

Fig. 1 study the effect of monomer concentrations on the gel percent of PAAc hydrogel. It was observed that the gel fraction increased with monomer concentrations increase and also copolymer PAAc/pectin scenes the increase of crosslinking by monomer concentrations which transforms a linear polymer then produces a 3D molecule, resulting in a significant increase with gel percent. Therefore, it is explained that the increase in the gel fraction by concentrations, as shown in Fig. 1, the increase gel percent of pure AAc, which is cross-linkable monomer is higher than in case of copolymer AAc/pectin hydrogels science pectin is degradable polymer, which effect on the crosslinking of the polymer and the crosslink density [33].

Effect of the swelling ratio of different concentrations

Fig. 2 shows the swelling behavior of the PAAc and copolymer PAAc/pectin hydrogel at pH 7. It was observed that the polymer swelling decreased with the increase of the monomer concentration of hydrogels. This is attributable to the 3D network structure in water and the increase of crosslink density through the irradiation processes increasing with the concentration of PAAc and PAAc/pectin hydrogels, resulting in a restriction in the water movement and diffusion through the polymer chains, these leads to a decrease in the swelling of the hydrogel [33]. In case of PAAc hydrogel swelling degree reached to 19,000% after 24 for the case of monomer concentration of 30 wt% AAc and 5 wt% pectin PAAc/pectin hydrogel reached to 10,000% after 24 hrs. This proves that the low concentration facilitate the high swelling ratio of copolymers.

Study the effect of irradiation dose onto gel percent

A considerable number of studies have been performed on the cross-linking of PAAC in aqueous solution by using irradiation [31-33]. In general, the PAAC hydrogels were cross-linked through free radical formation on the polymer chains during the exposure to irradiation. Furthermore, the radiolysis of water molecules generates the formation of micro radicals [33-39]. Fig. 3 study the effect of irradiation dose on the gel percent of 30 wt% AAc concentrations. It was observed that the gel fraction increased with an increase in irradiation dose and also PAAc/pectin increase in crosslinking by irradiation which transforms a linear polymer then increases with increases produces a 3D molecule, resulting in a significant increase with gel percent. Therefore, it is explained that the increase in the gel fraction by irradiation is mostly due to the crosslinking of the polymer and increase of crosslink density [33].

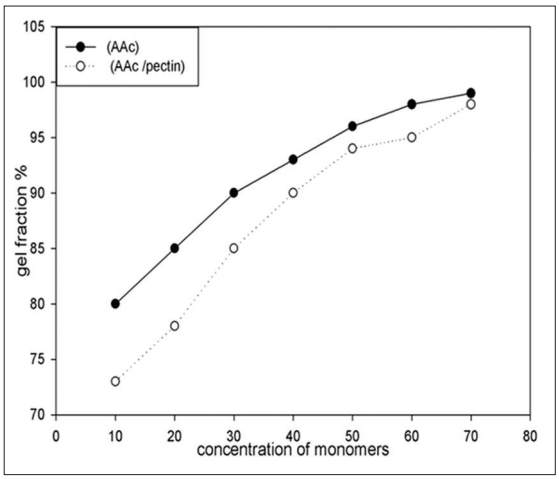


Fig. 1: Effect of monomer concentrations onto gel fraction percent of (poly acrylic acid [PAAc]) and copolymer (PAAc/pectin) hydrogel

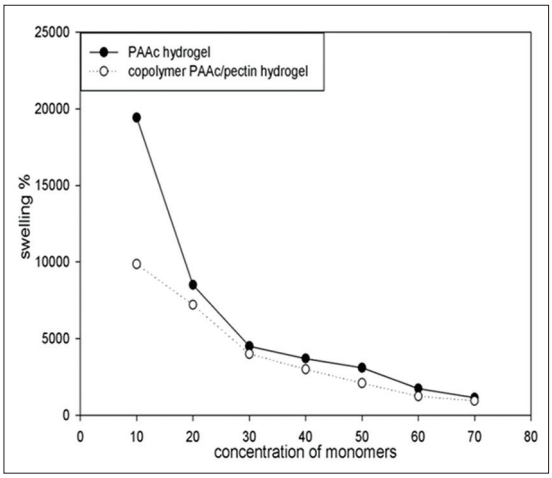


Fig. 2: Effect of monomer concentrations onto the swelling ratio after 24 hrs of (poly acrylic acid [PAAc]) and copolymer (PAAc/pectin) hydrogels

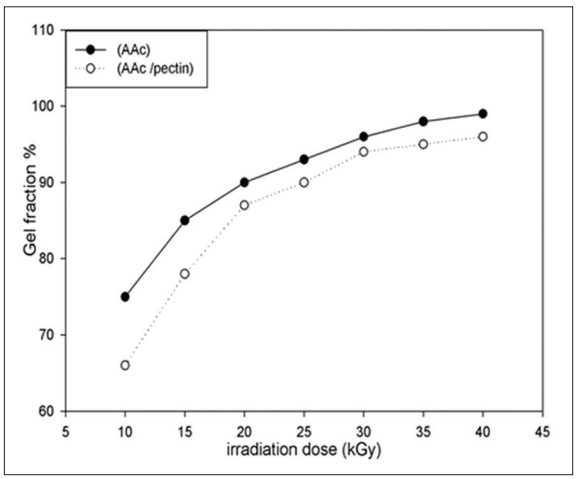


Fig. 3: Effect of different irradiation dose (kGy) onto the gel fraction percent of prepared (poly acrylic acid [PAAc]) hydrogel and copolymer (PAAc/pectin) hydrogels

Kinetic study prepared high swelling hydrogel

Fig. 4 study the kinetic swelling of copolymer hydrogel at different time intervals it is clear that the initial increase occurred by the ratio 6000%

after 24 hrs the high swelling occurred a by the ratio 19,000% however, in case of AAc/pectin the initial swelling occurred by the ratio 3000% after 40 minutes and the highest ratio occurred after 24 hrs by the ratio 9000% it is attributed to the presence of OH groups in pectin structure beside COOH groups in AAc structure, which make electrostatic force act as force deriving for OH and COOH group, which may restriction the swelling [52].

Study the effect of different pH on welling ratio of hydrogel

The effect of different pH on welling ratio of PAAc hydrogel and copolymer PAAc/pectin was investigated and shown in Fig. 5. It was found that the higher swelling ratio at pH 6. It is observed that the swelling ratio of PAAc hydrogel is very small when the pH is lower than pH 5 and then increases with increasing pH 6, but then decreases at pH 7 and after again increasing to pH 11. It's produces by the reason of containing anionic carboxyl and hydroxyl groups in the matrix, the swelling could be controlled by the pH. When pH ≤2 –COO groups convert to –COOH groups and form hydrogen bonding with –OH groups, which is responsible for the small swelling ratio. When the pH of the solution increases gradually to pH 6 most of the –COOH groups change into $–COO^-$ groups and the hydrogen bonding among –COOH and –OH groups dissociates among the –COOH and –OH groups. As a consequence, the electrostatic repulsion within the PAAc hydrogel dramatically swell. However, when pH > 7-12, the swelling ratio increases, which may be attributed to, –COOH converted to $–COO^-$ gradually, intermolecular hydrogen bonding was destroyed too. So which swelling rate of PAAc hydrogel exhibited increasing [9]. Furthermore, the pH dependent swelling behavior of PAAc/pectin hydrogels. It is clear that copolymer PAAc/pectin hydrogels shown they possessed higher swelling degrees at buffer solution of high pH values (pH>4) much higher than that possessed at low pH values (pH<4). This behavior can be explained as follow At pH values lower than pH 4, the contained PAAc chains are associated and forming inter- and intra-molecular hydrogen bonding which acquire its chains and consequently all the sample a relative hydrophobic character and minimum free spaces for water retention resulting in very low swelling rate and capacity [16]. On the other hand, at pH values higher than pH 4, the dissociation of the carboxylic groups of the PAAc into carboxylate is the major driving force for the swelling. The ionized pendant carboxylic groups develop fixed charges on the polymer network not only possess high degree of hydration but also the electrostatic repulsive forces leads to maximize the free spaces within the sample which consequently enlarge the amount of retained water [52].

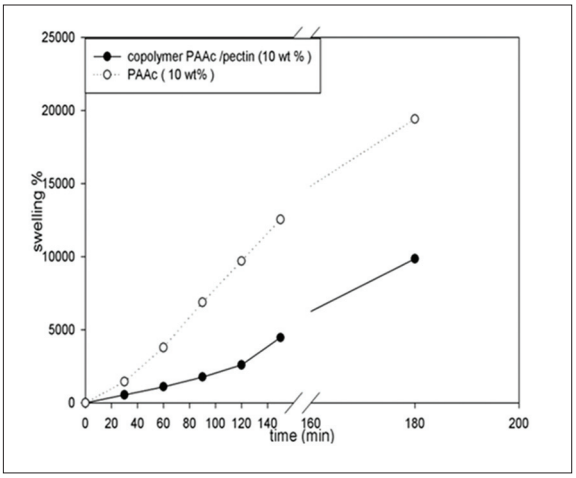


Fig. 4: Effect of time (minutes) onto the swelling percent of poly acrylic acid (PAAc) hydrogel and copolymer (PAAc/pectin) hydrogels at 30 wt% of AAc and 5 wt% of pectin and 20 kGy

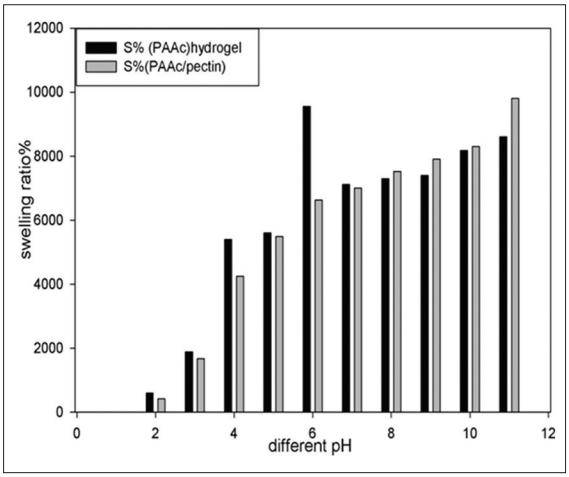


Fig. 5: Effect of different pH onto swelling percent of (poly acrylic acid [PAAc]) hydrogel and copolymer (PAAc/pectin) hydrogels after 24 hrs and 20 kGy

Study the drug loading amount to the loading time (minutes)

The study of the drug CPFX loaded onto PAAc hydrogel and copolymer PAAc/pectin hydrogel were investigated (Fig. 6). The concentration of CPFX drug loaded at selected time intervals was determined by UV spectrophotometer, in PAAc hydrogel at specific pH 7 (distilled water). The drug CPFX was loaded onto the hydrogels with high degrees of drug loading ≥100% at about time 200 minutes and after this time loading of drug decrease. Were prepared by the swelling - diffusion method [22,34]. In case of copolymer PAAc/pectin hydrogels and also at specific (pH 7) the drug loaded increase gradually until reach to 74% at about time 185 minutes and then begins decreased [22]. The problem reasons for the copolymer PAAc/pectin, PAAc superabsorbent hydrogel were loaded by diffusion through the 3D network structure of hydrogels, resulting in the initial burst [33]. Which may causes partial decrease in loading ratio of more drug in both polymers.

The effect of different pH onto the releasing of drug CPFX

It was found the effect of different pH onto the releasing of drug CPFX has been studied. The concentration of CPFX released at selected time 24 hrs. Intervals was determined by UV spectrophotometer [22,34]. The drug CPFX loaded copolymer PAAc/pectin hydrogels shown in Fig. 7 the highest degree of drug release at pH 8, but at pH 3 is very high more than others different pH study, which prepared by the swelling diffusion method. It means that the drug CPFX in pH 3 better release in a medium with a pH much higher than that of the stomach [34,35]. And also in colon area for application as amphoteric copolymer structure used for the application in loading and release for CPFX and it is application as a drug delivery system [23,24]. At low pH values, electrostatic repulsion between the carboxylic acid groups of the backbone is low, thus decreases gel swelling and minimizes the release of CPFX diffusion. However, in alkaline media the presence of $–OH^-$ increases the electrostatic repulsion between carboxylate groups, thus increases the gels swelling degree and so the release of CPFX was increased [22,34,35]. The drug CPFX loaded polymer PAAc hydrogels it was shown in Fig. 7 the initial increase of release at pH 2, and the highest increase at pH 4. This may be attributed to the ability of polymer to swell at this pH so it will swatch on for releasing the drug by high value in pH (2,4) and can be used in stomach atmosphere for application [34]. However, in alkaline media the presence of $–OH^-$ increases the electrostatic repulsion between carboxylate groups, thus increases the gels swelling degree, and hence the release of CPFX was increased [22,34,35]. The drug CPFX loaded polymer PAAc hydrogels it was shown in Fig. 7 the initial increase of release at pH 2, and the highest increase at pH 4. This may be attributed to the ability of polymer to swell at this pH so it will swatch on for releasing the drug by high value in pH (2,4) and can be used in stomach atmosphere for application [34].

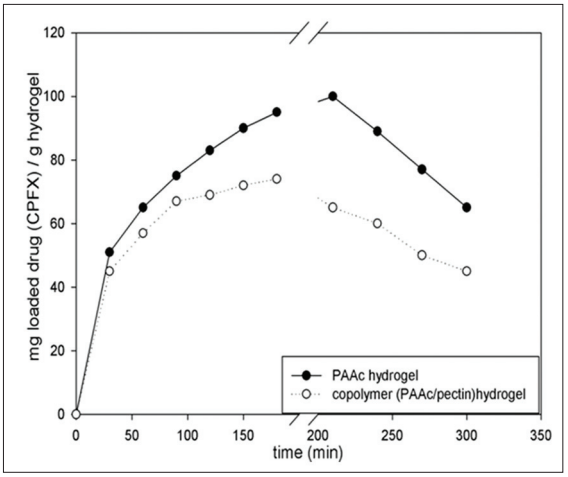


Fig. 6: The dependency of the drug loading amount to time (minutes) of (poly acrylic acid [PAAc]) hydrogel and copolymer (PAAc/pectin) hydrogels in pH 7

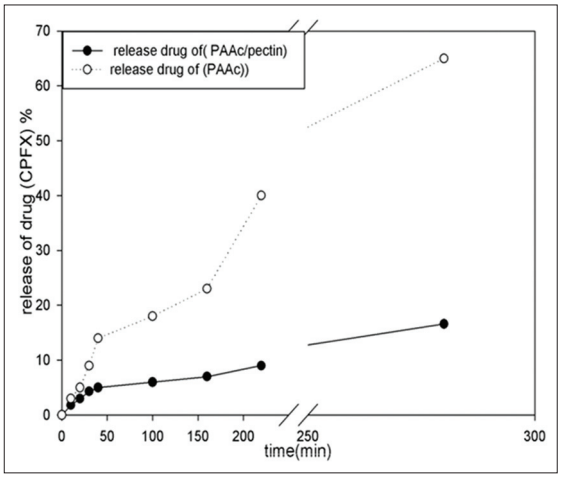


Fig. 8: Effect the drug release of (poly acrylic acid [PAAc]) hydrogel at pH 4 and copolymer (PAAc/pectin) hydrogels at pH 3 at different time (minutes) loaded with ciprofloxacin

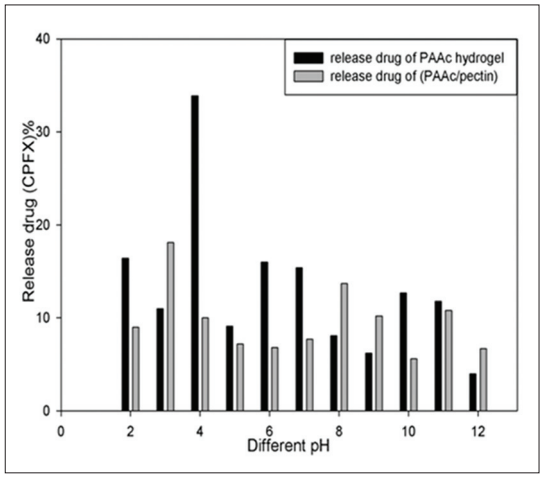


Fig. 7: Effect the different pH on the drug released of (poly acrylic acid [PAAc]) hydrogel and copolymer (PAAc/pectin) hydrogels after 24 hrs

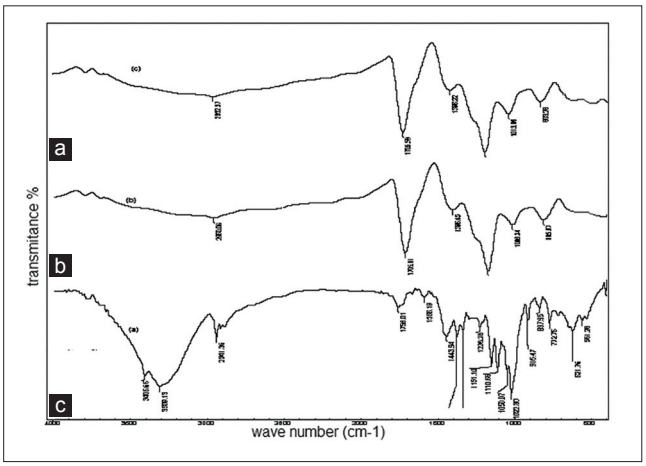


Fig. 9: Fourier-transform infrared spectrum of, (a) pectin powder, (b) poly acrylic acid (AAc) hydrogel, and (c) copolymer hydrogels (AAc/pectin)

The effect release of CPFX from hydrogel carrier as a function of time

According to the results of Fig. 7 it was found that the high value pH 3 in copolymer PAAc/pectin and pH 4 in PAAc hydrogels released drug so studied specific pH 3 and pH 4 in released drug CPFX, it was investigated as shown in Fig. 8 the drug was constantly released from the PAAc hydrogel at pH 4 and could reach 68% and copolymer PAAc/pectin hydrogel at pH 3 reached also 17% at about 24 hrs. However, the release content was comparatively high at up to 250 minutes and then increased slightly. The probable reason for the CPFX drug release from hydrogels by diffusion through the 3D network structure of the PAAc, copolymer PAAc/pectin hydrogels, resulting in the initial burst [33,36,37], which may ceases a dissociation of drug molecules in the media.

FTIR spectroscopy

Fig. 9 represents the FTIR spectra of pectin, PAAc/pectin hydrogels, and PAAc hydrogels. The spectrum of pectin in Fig. 9a shows a peak at 3400/cm due to stretching of –OH groups. The peaks at 2913/cm indicate C-H stretching vibration. The peaks at 1556/cm indicate C=O stretching vibrations due to the presence of $COOCH_3$ group. The peaks at 1441/cm and 1342/cm could be attributed to CH_2 scissoring and –OH bending vibration, respectively. The peak at 1150/cm suggested the presence of CH–OH group. The main peaks in FTIR spectrum of pure poly AAc in Fig. 9b spectrum are –OH stretch at 3380/cm, –CH stretch at 2922/cm and –C=O stretch at 1718.5/cm [38,39]. However, FTIR spectrum of PAAc/pectin hydrogels in Fig. 9c shows that the characteristics –OH stretching vibration peak of pectin at 3400/cm is shifted to lower frequency. This lowering in the frequency of –OH groups indicates the presence of hydrogen bonding in hydrogels. These indications showed –OH groups of pectin have reacted with –COOH groups of AAc [22,37]. The disappear of the peak in pectin onto PAAc/pectin copolymer hydrogel, which may be make good blending of PAAc/pectin copolymer hydrogels.

XRD

Fig. 10a XRD is a fundamental technique in determining the crystal and amorphous of the grafted and ungrafted film. XRD technique was performed to clarify the changes in the morphological structure caused in polymeric substrates. Pectin exhibited well-defined peaks at 6°, 15° and 25°, related to its crystallinity [40], (Fig. 10b) PAAc is known as amorphous polymer. Wide-angle XRD showed similar diffraction pattern with ranging in between 18 and 20, which indicated that the membranes were amorphous [41], from Fig. 10c it seen that these results the XRD pattern of the prepared hydrogel did not show any characteristic peaks, which indicate that the structure is complete

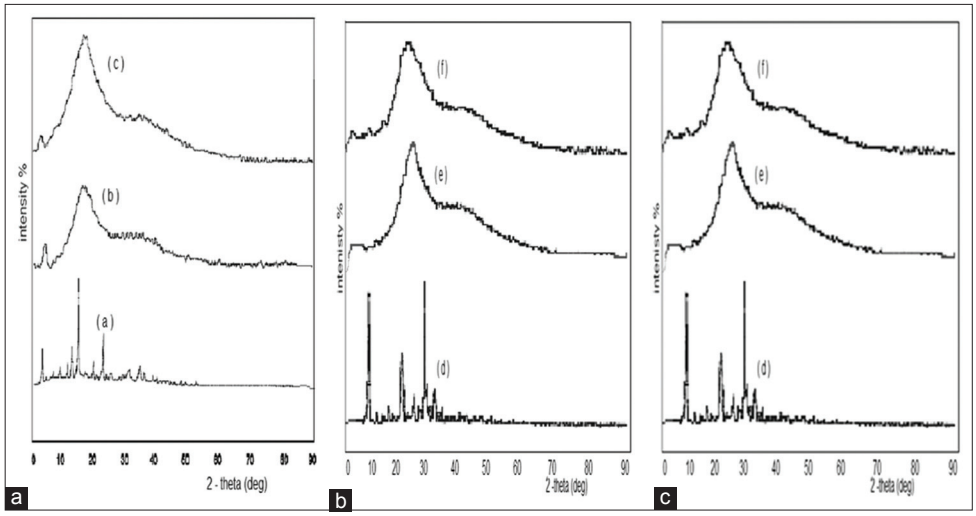


Fig. 10: (A) X-ray diffraction (XRD) patterns of, (a) Pectin powder, (b) poly acrylic acid (PAAc) hydrogel and (c) copolymer (PAAc/pectin) hydrogels; (B) XRD patterns of, (d) drug ciprofloxacin, (e) PAAc hydrogel loading drug, (f) PAAc hydrogel release drug; (C) XRD patterns of, (g) copolymer (AAc/pectin) hydrogel loading drug, (k) copolymer (AAc/pectin) hydrogel release drug

amorphous [42-44] and the crystallinity of pectin was not appear indicated that the pectin molecules were distributed in the without forming any crystalline. However, low intensity of all the peaks ensured its amorphous nature.

Furthermore in Fig. 10b, the XRD patterns of CPFX and the copolymer hydrogels are depicted. CPFX is a crystalline material having salient peaks centered at 10, 22 and 30 suggesting its crystalline nature. PAAc is an amorphous polymer showing a broad halo diffraction pattern. The absence of any diffraction peak of crystalline CPFX in the XRD pattern of PAAc CPFX indicated that the CPFX molecules were distributed in the without forming any crystalline aggregates [45-47]. Or, these peaks are not found in CPFX loaded, indicating that the drug is dispersed at a molecular level in the polymer matrix [48].

And also in Fig. 10c the XRD of copolymer PAAc/pectin hydrogel release drug shows that there are no peaks of CPFX due to complex formation between CPFX and copolymer PAAc/pectin hydrogel, which indicates that crystalline nature of drug has been converted to amorphous form. This finding confirms that the entrapped is CPFX dispersed on matrix copolymer PAAc/pectin hydrogel [49,50].

Application of prepared copolymer hydrogel as antimicrobial active polymer

The synthesized polymeric compounds, AAc and AAc/pectin, are tested for their antibacterial and antifungal activities. The synthesized polymeric compounds, were screened against eight selected microbial isolates, the yeasts, one Gram-positive bacteria and four Gram-negative bacteria. The results of the disk diffusion tests showed different degrees of growth inhibition. No antimicrobial activity against yeast strain under this study *C. albicans*, *S. cerevisiae* and *R. glutinis* is observed. While AAc has antibacterial activity against the Gram-positive bacteria and Gram-negative bacteria isolates selected. Addition of picten to AAc reduce this property except against *S. tophi.* On the other side, a combination of pectin to the AAc moiety keep the antimicrobial activity against the selected bacterial isolate except against *K. pneumonia*.

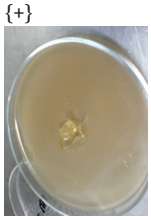
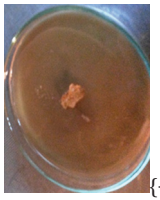
The antimicrobial activity of the synthesized polymeric compounds, under study was evaluated based on the diameters of clear inhibition zone surrounding the polymeric substances. If there is no inhibition zone, it is assumed that there is no antimicrobial activity as shown in Table 1.

The synthesized polymeric compounds, show no antimicrobial activity against yeast isolates under the study *C. albicans*, *S. cerevisiae* and *Rhodotorula clutinis*. This may be referred to the concentration of AAc used, or the combination of them which effect on the active group of AAc that has antibacterial activity. On the other hand, AAc, has activity against all bacterial isolates used by different degree. While addition of pectin to AAc exhibit bactericidal effect against *S. aureus*, *S. typhi*, *P. aeruginosa*, and *E. coli*, while *K. pneumonia* has strong resistant to this mixture of polymeric compound this may referred to the change in the stereochemistry of the new molecules.

CONCLUSION

In this study, the hydrogel of PAAc and copolymer PAAc/pectin hydrogels were synthesized through gamma radiation. The gel percent of PAAc hydrogel and copolymer PAAc/pectin it was observed that the gel fraction increased with monomer concentrations increase. Moreover, the swelling behavior of the PAAc and copolymer PAAc/pectin hydrogels at pH 7. It was observed that the polymer swelling decreased with increase of the monomer concentration of hydrogels the maximum swelling of PAAc hydrogel 19,000 wt% and copolymer PAAc/pectin 10,000 wt% have enable them to find extensive application in drug delivery system and also the gel fraction increased with an increase in irradiation dose. Study the effect of different pH on welling ratio of PAAc hydrogel it was found that the higher swelling ratio at pH 6. Also PAAc/pectin hydrogels they possessed higher swelling degrees at high pH values (pH>4) much higher than that possessed at low pH values (pH<4). Study the concentration of CPFX drug loaded at selected time intervals in PAAc hydrogel at specific pH 7. The drug CPFX was loaded on to the hydrogels with high degrees of drug loading ≥100% at about time 200 minutes. Moreover, also of copolymer PAAc/pectin hydrogels the drug loaded increase gradually until reach to 74% at about time 185 minutes. Study the effect of different pH onto the releasing of drug CPFX after 24 hrs. The drug loaded CPFX in PAAc hydrogel it was found that the initial increase of release at pH 2, and the highest increase at pH 4. This may be attributed to the ability of polymer to swell at this pH so it will swatch on for releasing the drug by high value in pH (2,4) and can be used in stomach atmosphere for application. And also copolymer PAAc/pectin hydrogels the highest degree of drug release at pH 8, but at pH 3 is very high more than others different pH. It means that the drug in pH 3 better release in a medium with a pH much higher than that of the stomach. And also in colon area for application as amphoteric copolymer structure. Study the drug released after 24 hrs from the PAAc hydrogel at pH 4 and could reach 68% and copolymer PAAc/pectin hydrogel at pH 3 also reached 17%. And also the synthesized polymeric compounds, were screened against eight selected microbial isolates, three yeasts, one Gram-positive bacterium and four Gram-negative

Table 1: The inhibition zone by the agar disc diffusion method of the three synthesized polymeric compounds on the selected microorganisms

Name of isolates	Polymeric compound	
	PAAc hydrogel	(Pectin/PAAc) (PAAc/pec) copolymer hydrogel
S. aureus	 {+}	 {+}
E. coli	 {+}	 {+}
K. pneumonia	 {+}	{−}
P. aeruginosa	 {+}	 {+}
S. typhi	 {+}	{+}
C. albicans	{−}	{−}
S. cerevisiae	{−}	 {−}
R. glutinis	{−}	{−}

R. glutinis: Rhodotorula glutinis, S. cerevisiae: Saccharomyces cerevisiae, C. albicans: Candida albicans, S. typhi: Salmonella typhi, P. aeruginosa: Pseudomonas aeruginosa, K. pneumonia: Klebsiella pneumonia, E. coli: Escherichia coli, S. aureus: Staphylococcus aureus, PAAc: Poly acrylic acid

bacteria. The results of the disk diffusion tests showed different degrees of growth inhibition. No antimicrobial activity against yeast strain under this study *C. albicans*, *S. cerevisiae* and *R. glutinis* is observed. While AAc has antibacterial activity against the Gram-positive bacteria and Gram-negative bacteria isolates selected. On the other side, a combination of pectin to the AAc moiety keep the antimicrobial activity against the selected bacterial isolate except against *K. pneumonia*.

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