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Radiation Preparation of Smart Hydrogel Has Antimicrobial Properties for Controlled Release of Ciprofloxacin in Drug Delivery Systems

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ABSTRACT

The objective of the present work was to synthesize co polymer hydrogel composed of (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 induce 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 (FTIR) spectroscopy, The morphological structure using X–ray diffraction analysis and scanning electron microscopy (SEM) 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 19000 % for (PAAc) hydrogel and 10000% for (PAAc/pectin) hydrogels after 24 h. Drug loading measurements using ciprofloxacin drug at pH_7 for (PAAc) hydrogel after 24 h and at pH_{11} for (PAAc/pectin) hydrogels. Studies of drug releasing of ciprofloxacin 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 co polymeric 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.

Introduction

Hydrogels are three-dimensional cross-linked polymeric networks capable of absorbing and retaining large amounts of water and physiological fluids while remaining insoluble in aqueous solutions. Typically 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 [(1,2,3)]. 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 three-dimensional network structures in the hydrogel [(4,5,6)]. 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)].

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Pectin is a naturally occurring biopolymer that is finding increasing applications in the pharmaceutical. Also has 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 development of implantable [23,24,25] .

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 poly acrylic 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.

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

Materials and methods

Materials

Pure Acrylic Acid (AAc) from (Aldrich) of purity 99% was used as received ($C_3H_6O_2$) , Polysaccharide pectin (pec) M.wt 30000-10000 PKd.by oxford laboratory, ciprofloxacin(CPFX)500ml/g from European Egyptian Pharma international . Buffer Solutions of pH range (2 to 13). Other chemicals were used as received without further purification.

Preparation of hydrogel

The preparative method were 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 N2 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% gm pectin dissolve in 100 ml distilled water and was allowed to stir for 10 min, 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/tri sodium citrate) and (Sodium di hydrogen phosphate/ disodium hydrogen phosphate) were used to prepare buffer solutions of pH values ranged from 3-5 and 6-7, respectively [29,30].

Swelling measurements

Swelling experiments were performed by placing the prepared polymer discs in buffer solutions of varying pH of (2 to 12) at 37°C and measuring Sample weight given as a function of time. The discs 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):

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

Drug Loading efficiency and drug Release

Ciprofloxacin (CPFX) was used a model drug. The disc samples (PAAc) hydrogels (1 g ± 0.0001), were accurately weighted and immersed in solution of ciprofloxacin (CPFX , 0.54 g dissolved in 50 mL of buffer solution) at 37°C for 24 h. The loading amount of drug in the hydrogels was calculated from the decrease in the concentration of the CPFX solution which was determined using 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 buffer solution of at Different pH's 37°C for 24 h. 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 400-4000cm^{-1} by KBr pellet method using FTIR spectrophotometer.

X-ray diffraction measurements

X-ray diffraction (XRD) measurements were made using an X-ray diffract meter. The diffract grams were measured at 2 θ , 5-50^0.

Ultraviolet (UV) measurements

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

Scanning electron microscopy (SEM)

The dry sample, spread on a double sided conducting adhesive tape pasted on a metallic stub, was coated 100 micron

with gold in a sputter coating unit 2 min 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 tophi (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 10^7cfu/ml for yeasts. The bacterial suspension was adjusted with sterile saline to an optical OD (optical density) of 0.2-0.3.

Antibacterial activity

Disk diffusion test. *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia, Salmonella typhi and Escherichia 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 (optical density) 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 1gm laboratory lemco, 2gm yeast, 5gm peptone, 5gm sodium chloride, 20gm agar-agar and Distal water 1 liter, Oxoid, were cooked and sterilized at 1 Bar for 20 min . in addition to Muller Hinton broth (lack agar-agar) those are used for bacterial growth. Malt agar medium composed of 30 g Malt extract, 5gm Mycological peptone, 20gm agar-agar and Distal water 1 liter, Oxoid , were cooked and sterilized at 1 Bar for 20 min . 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 el fraction percent

Figure (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 cross linking by monomer concentrations which transforms a Linear polymer then produces a three-dimensional 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 Figure(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].

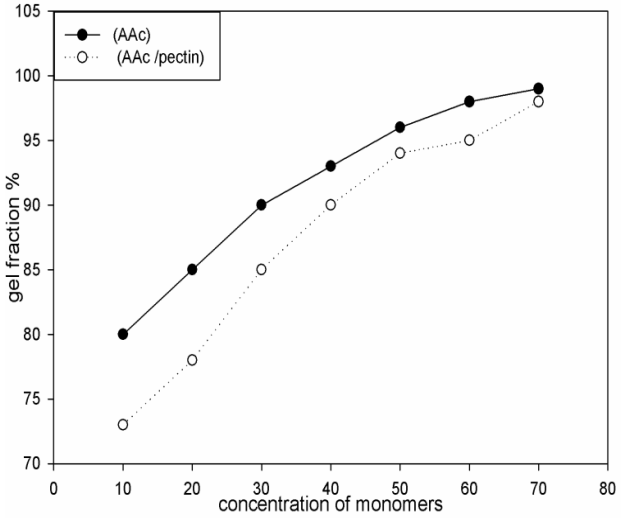


Fig 1. Effect of monomer concentrations onto gel fraction percent of (PAAc) and copolymer (PAAc/pectin) hydrogel

Effect of the swelling ratio of different concentrations:

Figure (2) shown the swelling behavior of the (PAAc) and copolymer (PAAc / pectin) hydrogel at PH_7. It was observed that the polymer swelling decreased with increase of the monomer concentration of hydrogels.

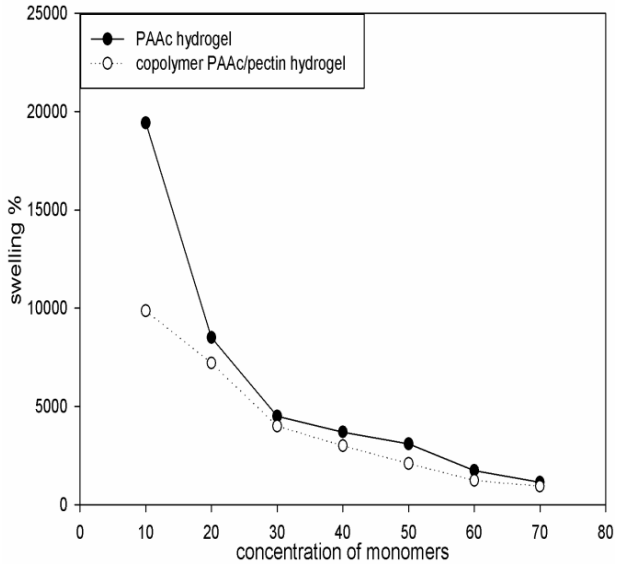


Fig 2. Effect of monomer concentrations onto the swelling ratio after 24 h of (PAAc) and copolymer (PAAc/pectin) hydrogels

This is attributable to the three-dimensional 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 19000 % after 24 for the case of monomer concentration of30 wt% AAc and 5 wt% pectin (PAAc /pectin) hydrogel reached to 10000% after 24 h. These prove 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 [31,32 ,33]. Generally, 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 -9]. Figure (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 three-dimensional 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].

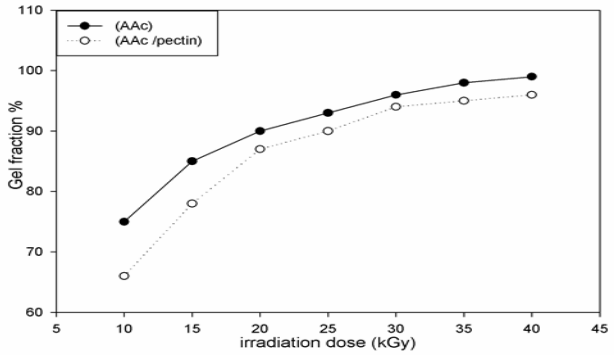


Fig 3. effect of different irradiation dose (kgy) onto the gel fraction percent of prepared (paac) hydrogel and copolymer (paac/pectin) hydrogels

Kinetic study prepared high swelling hydrogel

Figure (4) study the kinetic swelling of copolymer hydrogel at different time intervals it is clear that the initial increase occurred by the ratio 6000 percent after 24 h the high swelling occurred a by the ratio 19000 percent however in case of AAc /pectin the initial swelling occurred by the ratio 3000 percent after 40 min and the highest ratio occurred after 24 h by the ratio 9000 percent it is attributed to the presence of (OH) groups in pectin structure beside (COOH) groups in AAc structure which make electro static force act as force deriving for OH and COOH group which may restriction the swelling [52].

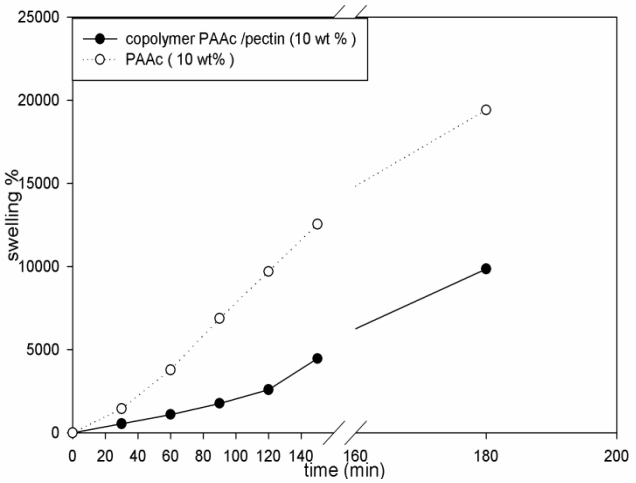


Fig 4. Effect of time (min) onto the swelling percent of PAAc hydrogel and copolymer(PAAc/pectin) hydrogels at 30wt% of AAc and 5wt%of pectin and 20kGy

Study the effect of Different pH on welling ratio of hydrogel

Figure (5): The effect of different pH on welling ratio of PAAc hydrogel and copolymer (PAAc / pectin) was investigated and shown in fig (3). 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 $\leq$ 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] . Also in figure (5) shows the pH dependent swelling behavior of(PAAc / pectin) hydrogels.

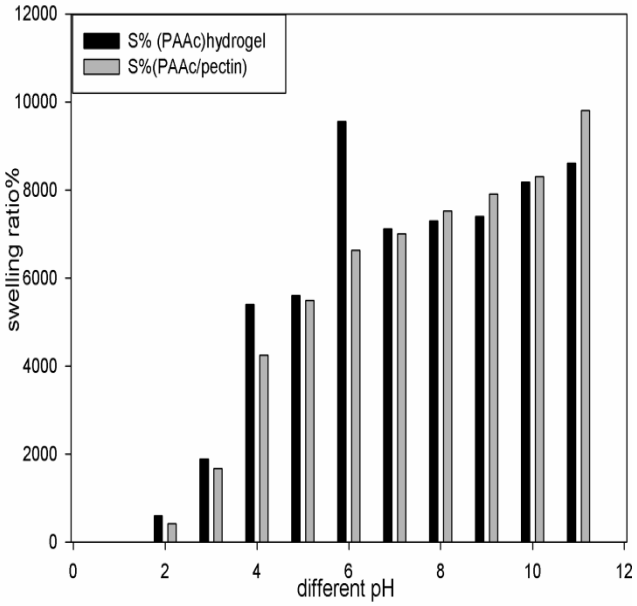


Fig 5. Effect of different PH onto swelling percent of (PAAc) hydrogel copolymer (PAAc/pectin) hydrogels after 24 h and 20 kGy

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] .

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

Figure (6): The study of the drug ciprofloxacin loaded onto (PAAc) hydrogel and copolymer (PAAc /pectin) hydrogel were investigated. The concentration of ciprofloxacin 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 on to the hydrogels with high degrees of drug loading $\geq$ 100 % at about time 200 min and after this time loading of drug decrease. Were prepared by the swelling – diffusion method [22,34] .

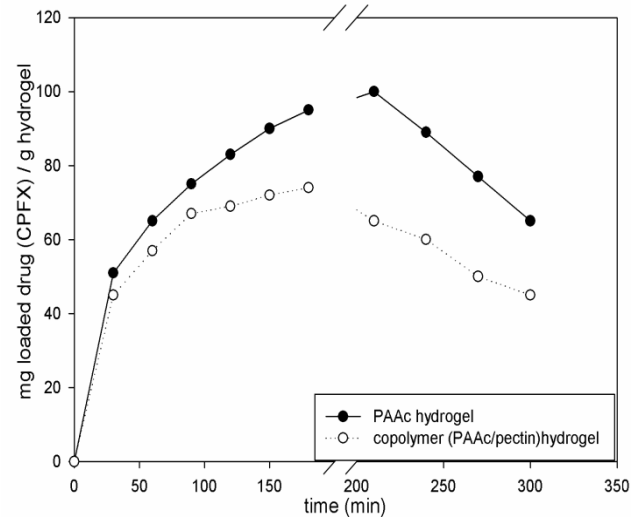


Fig 6. The dependency of the drug loading amount to time (min) (PAAc) hydrogel and Copolymer (PAAc/pectin) hydrogels in PH$_7$

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 min and then begins decreased [22]. The problem reasons for the copolymer (PAAc / pectin) , (PAAc) superabsorbent hydrogel were loaded by diffusion through the three- dimensional 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 Ciprofloxacin

Figure (7) **:** it was found the effect of Different pH onto the releasing of drug ciprofloxacin has been studied. The concentration of ciprofloxacin released at selected time 24 h. Intervals was determined by (UV) spectrophotometer [22 ,34] . The drug ciprofloxacin loaded copolymer (PAAc / pectin) hydrogels shown in figure (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 ciprofloxacin 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 ciprofloxacin and it is application as drug delivery system [23, 24]. At low pH values, electrostatic repulsion between the carboxylic acid groups of backbone is low, thus decreases gel swelling and minimizes release of ciprofloxacin 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 Ciprofloxacin was increased [22,34, 35]. The drug ciprofloxacin loaded polymer (PAAc) hydrogels it was shown in figure (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].

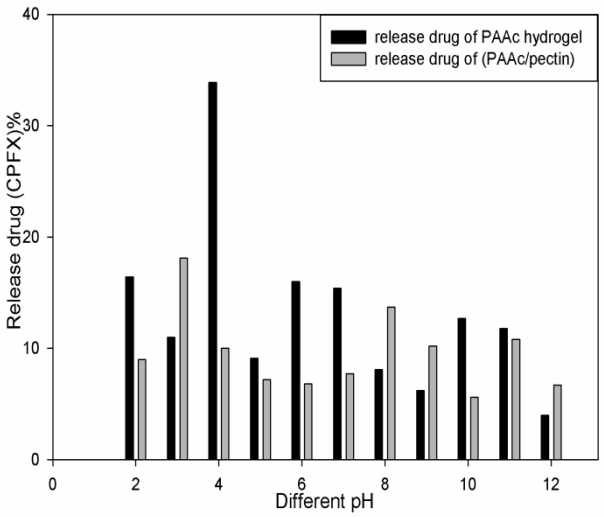


Fig 7. Effect the different PH on the drug released of (PAAc) hydrogel and copolymer (PAAc/pectin) hydrogels After 24 h

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 Ciprofloxacin was increased [22,34, 35]. The drug ciprofloxacin loaded polymer (PAAc) hydrogels it was shown in figure (5) 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].

The effect Release of ciprofloxacin from hydrogel carrier as a function of time

According to the results of figure (7) it was found that the high value pH3 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 figure (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 h .However, the release content was comparatively high at up to 250 min and then increased slightly. The probable reason for the CPFX drug release from hydrogels by diffusion through the three-dimensional 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.

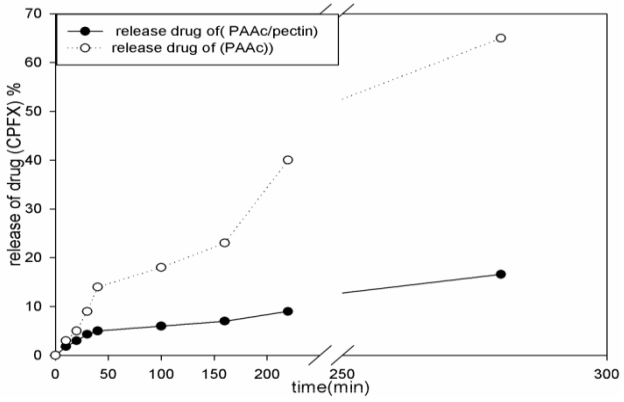


Fig 8. Effect the drug release of (PAAc) hydrogel at pH$_4$ and copolymer (PAAc/pectin) hydrogels at PH$_3$ At different time (min)loaded with ciprofloxacin (CPFX)

Fourier Transform Infrared Spectroscopy

Figure (7) : represents the FTIR spectra of pectin , (PAAc / pectin) hydrogels ,and PAAc hydrogels . The spectrum of pectin in Figure 7 (a) indicates peak at 3400 cm-1 due to stretching of -OH groups. The peaks at 2913 cm-1 indicate C-H stretching vibration. The peaks at 1556 cm-1 indicate C=O stretching vibrations due to the presence of COOCH3 group. The peaks at 1441 cm-1 and 1342 cm-1 could be attributed to CH2 scissoring and -OH bending vibration, respectively. The peak at 1150 cm-1 suggested the presence of CH-OH group. The main peaks in FTIR spectrum of pure poly (acrylic acid) in Figure 7 spectrum (b) are –OH stretch at 3380 cm-1, -CH stretch at 2922 cm-1 and - C=O stretch at 1718.5 cm-1[38,39]. However, FTIR spectrum of (PAAc/pectin) hydrogels in (c) indicate that the characteristics -OH stretching vibration peak of pectin at 3400 cm-1 is shifted to lower frequency. This lowering in 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 acrylic acid [22,37] . The disappear of peak in pectin onto (PAAc /pectin) copolymer hydrogel which may be make good blending of (PAAc /pectin)copolymer hydrogels.

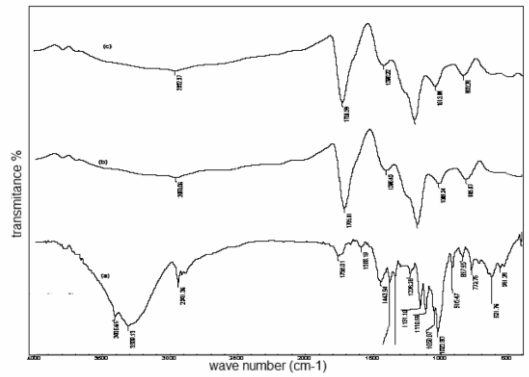


Figure 9. FTIR spectrum of (a) pectin powder, (b) PAAc hydrogel and (c) copolymer hydrogels (AAc / pectin)

XRD (x-rays diffraction)

Fig (10"a") X- Ray Diffraction (XRD) is a fundamental technique in determining the crystal and amorphous of the grafted and un grafted film. XRD technique was performed to clarify the changes in morphological structure caused in polymeric substrates. Pectin exhibited well defined peaks at 6◦, 15◦and 25◦, related to its crystallinity [40]. (b) 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].(c) from figure it seen that These results The XRD pattern of the prepared hydrogel did not show any characteristic peaks which indicates that the structure is complete amorphous[42,43,44] and the crystillinity 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.

Also In Fig (10 "b") The X-ray diffraction (XRD) patterns of ciprofloxacin and the copolymer hydrogels are depicted. Ciprofloxacin is a crystalline material having salient peaks centered at 10_, 22_ and 30_ suggesting its crystalline nature. Poly (AAc) is an amorphous polymer showing a broad halo diffraction pattern. The absence of any diffraction peak of crystalline ciprofloxacin in the XRD pattern of poly (AAc) ciprofloxacin indicated that the ciprofloxacin molecules were distributed in the without forming any crystalline aggregates [45,46,47] . Or, these peaks are not found in Ciprofloxacin loaded, indicating that the drug is dispersed at a molecular level in the polymer matrix [48] .

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

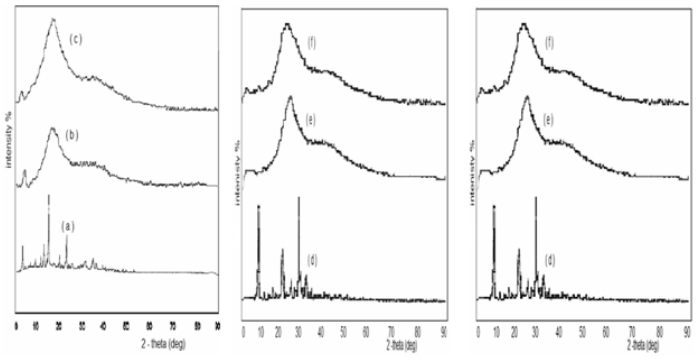


Fig 10. XRD patterns of (a) : pectin powder . (b) : paac hydrogel and (c) : copolymer (paac/ pectin) hydrogels . (d) : drug ciprofloxacin . (e) : paac hydrogel loading drug . (f) : paac hydrogel release drug . (g) : copolymer (aac/ pectin) hydrogel loading drug . (k) : copolymer (aac/ pectin) hydrogel release drug

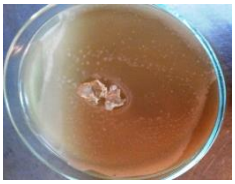
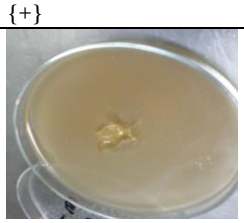
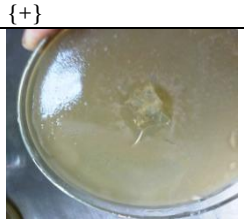
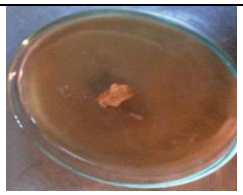
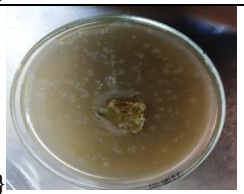
Application of prepared copolymer hydrogel as Antimicrobial active polymer

The synthesized polymeric compounds, Acrylic acid (AAc) and Acrylic acid/pectin, are tested for their antibacterial and antifungal activities. The synthesized polymeric compounds, were screened against eight selected microbial isolates, the yeasts, one Gm + ve bacteria and four Gm -ve bacteria. The results of the disk diffusion tests showed different degrees of growth inhibition. No antimicrobial activity against yeast strain under this study *Candida albicans, Saccharomyces cerevisiae and rhodotorula glutinis* is observed. While Acrylic acid has antibacterial activity against the Gm +ve bacteria and Gm -ve bacteria isolates selected. Addition of picten to acrylic acid reduce this property except against Salmonella tophi. On the other side, Combination of Pectin to the acrylic acid moiety keep the antimicrobial activity against the selected bacterial isolate except against *Klebsiella 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 Candida albicans, Saccharomyces cerevisiae and rhodotorula glutinis. This may be referred to the concentration of Acrylic acid used, or the combination of them which effect on the active group of acrylic acid that has antibacterial activity. On the other hand, Acrylic acid, has activity against all bacterial isolates used by different degree. While addition of pectin to acrylic acid exhibit bactericidal effect against Staphylococcus aurous , salmonella tophi, Pseudomonas aeruginosa, and Escherichia coli, while Klebsiella pneumonia has strong resistant to this mixture of polymeric compound this may referred to the change in the stereochemistry of the new molecules.

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

Polymeric Compound / Name of Isolates	Poly Acrylic acid(AAc) hydrogel	(Pectin /poly Acrylic acid) (PAAc/pec) copolymer hydrogel
Staphylococcus aureus	 {+}	 {+}
Escherichia coli	 {+}	 {+}
Klebsiella pneumonia	 {+}	{-}
Pseudomonas aeruginosa	 {+}	 {+}
Salmonella typhi	 {+}	{+}
Candida albicans,	{-}	{-}
Saccharomyces cerevisiae	{-}	 {-}
rhodotorula glutinis	{-}	{-}

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 . And 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 19000 wt% and copolymer (PAAc / pectin) 10000wt% 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 ciprofloxacin 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 $\geq$ 100 % at about time 200 min .And also of copolymer (PAAc /pectin) hydrogels the drug loaded increase gradually until reach to 74% at about time 185 min. Study the effect of Different pH onto the releasing of drug cpfx after 24 h. 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 h from the(PAAc) hydrogel at pH 4 and could reach 68 % and copolymer (PAAc /pectin) hydrogel at pH_3 reached also 17 % . And also the synthesized polymeric compounds, were screened against eight selected microbial isolates, three yeasts, one Gm +ve bacteria and four Gm -ve bacteria. The results of the disk diffusion tests showed different degrees of growth inhibition. No antimicrobial activity against yeast strain under this study Candida albicans, Saccharomyces cerevisiae and rhodotorula glutinis is observed. While Acrylic acid has antibacterial activity against the Gm + ve bacteria and Gm - ve bacteria isolates selected. On the other side, Combination of Pectin to the acrylic acid moiety keep the antimicrobial activity against the selected bacterial isolate except against Klebsiella pneumonia.

References

[1] Peppas, N. A. Bures, P., Leobandung,W., Ichikawa, H., Eur. J.Pharma. Biopharm.Vol 50,Pp27, (2000).
[2] Saraydin,D., Karadadag ,E,Guven ,O. Sep Sci Techonol.Vol.30,Pp32-91,(1995).
[3] A. Thakur, R. K. Wanchoo, And P. Singha…., Chem. Biochem.Eng. Q. 25 (2) 181–194 (2011).
[4] KOPECEK, J., Biomaterials,Vol. 7, Pp 44-53, (2007).
[5] PARK, K., SHALABY, S.W., And PARK, H., :"Biodegradable Hydrogels For Drug Delivery", Technomic Publishing Co. Inc., Lancaster,Vol. 6-Pp21, (1993).
[6] D. Darwis. / Atom Indonesia Vol. 35 No. 2,Pp 85 – 104, (2009).
[7] Muhammad Hanif1, Nazar Muhammad Ranjha, Muhammad Harris Shoaib1, Jahanzaib Mudasser, Rabia Ismail Yousuf1, Ahmad Khan , And Muhammad Zia-Ul-Haq , Pak. J. Pharm. Sci., Vol.24, No.4, Pp503-511, (2011).
[8] Ranade, V. V.; Hollinger, M. A. Drug Delivery Systems; CRC Press: Boca Raton, FL, (1995).
[9] Huixia Yang1,2, Wenbo Wang1, Junping Zhang1, And Aiqin Wang1 International Journal Of Polymeric Materials And Polymeric Biomaterials,vol. 62 , Pp369–376 ,(2012).
[10] Sullad, A. G.; Manjeshwar, L. S.; Aminabhavi, T. M. Ind. Eng. Chem. Res, vol.49, Pp7323 , (2010).
[11] Namazi, H.; Jafarirad, S. Int. J. Polym. Mater ,vol.60,Pp 603, (2011) .
[12] Liu, L.; Sheardown, H. Biomaterials,vol. 26, Pp233 , (2005).
[13] Zhang, X. Z.; Wu, D. Q.; Chu, C. C. Biomaterials,vol. 25,Pp 3793 , (2004).
[14] Vandammetf,Lenourrya,Charrueayc,Chaumeil J-C. The Use Of Polysaccharides To Target Drugs To Colon. Carbohydrpolym , vol.48,Pp219–31, (2002).
[15] Liu LS,Fishmanml,Hicks KB. An In Vitro Study Of Mucoadhesive Properties Of Pectins. Abstracts Of The 7th US–Japan Symposium On Drug Delivery Systems,vol.16, Pp14–19 , (2003).
[16] Griset, A. P.; Walpole, J.; Liu, R.; Gaffey, A.; Colson, Y. L.; Grinstaff, M. W. J. Am. Chem. Soc, vol.131, Pp2469 , (2009).
[17] Yang, S. P.; Fu, S. Y.; Zhou, Y. M.; Xie, C. L.; Li, X. Y. Int. J. Polym. Mater. Vol.60-Pp52 , (2010).
[18] George, M.; Abraham, T. E. Int. J. Pharm. Vol.335, Pp123 , (2007).
[19] Maji, T. K.; Devi, N. Int. J. Polym. Mater, vol. 59, Pp828 ,(2010) .
[20] F. L. Buchholz and A. T. Graham "Modern Superabsorbent Polymer Technology," Wiley, New York, (1997).
[21] Mohammad Sadeghi ,Journal Of Biomaterials And Nanobiotechnology ,vol 2, Pp36-40 , (2011).
[22] R. K. Mishra1, A. K. Banthia2 And A. B. A. Majeed1 ,Asian Journal Of Pharmaceutical And Clinical Research , vol. 5 , Pp974 – 2441 ,(2012) .
[23] Juan Carlos Cabrera, Pierre Cambier And Pierre Van Cutsem International Journal Of Pharmacy And Pharmaceutical Sciences Vol 3 ,Pp0975-1491, (2011) .
[24] Linshuliua,_,Marshall L. Fishmana,Kevin B. Hicksa,Meirkendeb , Biomaterials ,vol.26 Pp5907–5916 , (2005).
[25] Zhou, X., Weng, L., Chen, Q., Zhang, J., Shen, D., Li, Z., Shao, M., Xu, J., Polym. Intern.52 1153, (2003).
[26] Katime, I., Novoa, R., De Apodaca, E., Mendizabal, E., Puig, J., J. Polm. Testing vol.18 , Pp559 , (1999).
[27]Isik, B., Kis ., M., J. Appl.Polym.Sci, vol.94,Pp15 - 26,(2004).
[28] R.Cruickshank, J. P. Duguid, B. P. Marmion, R. H. A. Swain, , Churchill, L. Medical microbiology: (The Practice Of Medical Microbiology), Vol. II, Edinburgh And New York , (1975).
[29] Yahya H. F. Al-Qudah1, Arab Journal Of Nuclear Sciences And Applications, vol.45 (2),Pp179-185, (2012).
[30] Pappas, N.A.; Sahlin, J.J. Hydrogel As Mucoadhesive And Bioadhesive Materials: A Review Biomaterials, vol.17, Pp1553–1561 , (1996).
[31] Peppas, N.A.; Klier, J. Controlled Release By Using Poly(Methacrylic Acid-G-Ethyleneglycol) Hydrogels. J. Control. Release, vol.16,Pp 203–214 ,(1991).
[32] Young-Chang Nho , Jong-Seok Park , And Youn-Mooklim , Polymers , Vol 6 , Pp890 -898 ,(2014).

[33] Mohammad Sadeghi, Behrouzheidari, Korushmontazeri , World Academy Of Science, Engineering And Technology, vol52,Pp421-423,(2011) .
[34] Pourjavadi, A., Sadeghi, M. And Hosseinzadeh, H. Polym. Adv. Technol. 15 , 645–653 (2004).
[35] Shin, B.K.; Baek, E.J.; Choi, S.G.; Davaa, E.; Nho, Y.C.; Lim, Y.C.; Park, J.S.; Huh, K.M.;Park, J.S. Preparation And Irradiation Of Pluronic F127-Based Thermoreversible And Mucoadhesive Hydrogel For Local Delivery Of Naproxen. Drug Dev. Ind. Pharm. Vol.39, Pp1874–1880,(2013).
[36] Choi, S.G.; Baek, E.J.; Davaa, E.; Nho, Y.C.; Lim, Y.C.; Park, J.S.; Gwon, H.J.; Huh, K.M.; Park, J.S. Topical Treatment Of The Buccal Mucosa And Wounded Skin In Rats With A Triamcinolone Acetonide-Loaded Hydrogel Prepared Using An Electron Beam. Int. J. Pharm, vol.447, Pp102–108,(2013).
[37] Nazar M Ranjha, Jahanzebmudassir, And Zuhairzubair Sheikh, Iranian Polymer Journal,vol.20(2), Pp147-159 ,(2011) .
[38] Ranjha, N.M.; Mudassir, J.; Sheikh, Z.Z. Synthesis And Characterization Of Ph-Sensitive Pectin/Acrylic Acid Hydrogels For Verapamil Release Study. Iranian Polym. J., V.20, N.2, P.147-159, 2011.
[39]Kashif Sohail1, Ikram Ullah Khan,Yasser Shahzad4, Talib Hussain, Nazar Muhammad Ranjha1 Le Brazilian Journal Of Pharmaceutical Sciences Vol. 50, N. 1,Pp 173-184 Jan./Mar., 2014
[40]Khan, I.U.; Serra, C.A.; Anton, N.; Vandamme, T. Continuous-Flow Encapsulation Of Ketoprofen In Copolymer Microbeads Via Co-Axial Microfluidic Device: Influence Of Operating And Material Parameters On Drug Carrier Properties. Int. J. Pharm.,Vol.441, N.1-2, Pp.809-817, (2013).
[41]Andreia Bagliotti Meneguina, Beatriz Stringhetti Ferreira Curyb, Raul Cesar Evangelistab, Carbohydrate Polymers ,vol.99 ,Pp140– 149, (2014).
[42]Anup Das A,B,C, Sourabh Ghosh C, Alok R. Ray , Polymer 52 (2011) 3800e3810. [43]Yonghyun Kim, V. Ramesh Babu, Daniel T. Thangadurai, K.S.V. Krishna Rao, Hyeongrae Cha,Changdae Kim,§ Woohong Joo And Yong-Ill Lee, Bull. Korean Chem. Soc. Vol. 32, No. 2 Pp553 ,(2011).
[43] A. El-Hag Ali,Journal Of Macromolecular Science, Part A: Pure And Applied Chemistry vol.49, Pp7–14 (2012).
[44]Soumendra Sahoo , Rashmireka Sahoo , Yogesh Panditrao Palve And Padmalochan Nayak ,Journal Of Pharmaceutical And Biomedical Sciences ,Vol.22 No. 15 Pp2230-7885 ,(2012).
[45] G. Verreck, I. Chun, J. Rosenblatt, J. Peeters, A. Van Dijck, J. Mensch, M. Noppe And M. E. Brewster, J. Controlled Release, vol.92, Pp349–360 ,(2003).
[46] A. Toncheva, D. Paneva, N. Manolova And I. Rashkov, Macromol. Res., vol. 19,Pp 1310–1319 ,(2011).
[47] Serkan Demirci,Ac Asli Celebioglu,Ab Zeynep Aytacab And Tamer Uyar,Abcite This: Polym. Chem.,vol. 5, Pp2050, (2014).
[48] Michael Rajeshp ,, Shreya A Bhattp, G Venkata Ramanap ,Harshad Brahmbhatt, Khushboo Gajjar , C B Tripathi ., Pritpal Singh Anand, Kiritkumar Mangaldas Popat International Journal Of Pharmacy And Pharmaceutical Sciences IssnVol 6, Pp - 0975-1491,(2014).
[49] B. Mallikarjuna, K. Madhu Sudhana Rao, C.V. Prasad, K.Chowdoji Rao, K.S.V. Krishana Rao And M.C.S. Subha Journal Of Applied Pharmaceutical Science vol.01 (06); Pp171-177, (2011).
[50] Yahya H. F. Al-Qudah, Arab Journal Of Nuclear Sciences And Applications, vol.45 (2) , Pp179-185, (2012).
[51] Hudson, Sarah P. Et Al , Biomaterials , , Vol. 31.N O (6),Pp 1444- 1452, (2010).