Equilibrium Adsorption Isotherm and Controlled Release of Antibiotic Drug Chloroamphenicol from Poly(2-vinyl pyridine/acrylic acid) Hydrogels Prepared by Gamma Radiation

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ABSTRACT: High water sorption of 2-vinyl pyridine (2-VP)/acrylic acid (AAc) hydrogel were prepared by free-radical polymerization in aqueous solution of 2-VP with AAc as comonomer. The amount of ionic monomer (AAc), the irradiation dose of prepared hydrogel, the pH, and the concentration of drug play an important factor on loading, adsorption, and releasing of water-soluble chloroamphenicol drug. As a result of dynamic swilling tests, the effect of relative content of AAc on the swelling showed that it allowed a non-Fickian type of water diffusion. The adsorption of the drug onto (2-VP/AAc) hydrogels was studied by Freundlish adsorption isotherm. The drug concentrations showed an influence on the adsorption of drug which increased with increasing AAc content. From Freundlish adsorption isotherm, the empirical constants, k and n, can be evaluated and showed the ability of hydrogel to be loaded by the drug and the affinity of the drug to be uptake onto the hydrogel respectively. FTIR, TGA, and SEM techniques were used to study the characterization of hydrogel (2-VP/AAc). Additionally, the release of the drug loaded from hydrogel discs was studied microbiologically to show that hydrophilic structure of the hydrogel has an antimicrobial effect as a dehydration of cytoplasm and unbalance of the cell wall functions. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 111: 1369–1380, 2009

Key words: hydrogels; radiation; swelling; adsorption; release; microbiological; *Staphylococcus*; *auries*

INTRODUCTION

Hydrogels are three-dimensional crosslinked polymeric structure which are able to swell in the aqueous environment. These materials are of great interest due to their promising applications such as sensors, separation membranes, adsorbents, and materials in medicine and pharmacy as drug-delivery systems. In solving some ecological problems as well as in modern technologies, super absorbent polymers can swell up to thousand times of their weight in aqueous media. Swelling behavior of super absorbent polymers may be characterized by water separation.[1–3] Gel properties may be programmed by the choice of the main polymer that forms network and of either the comonomer content or irradiation dose.[4–8] The inherent advantages of using high-energy radiation in the synthesis of hydrogels for biomedical applications have been reviewed.[9] The preparation of hydrogels by radiation treatment of aqueous solutions of hydrophilic monomers carries some advantages over the conventional techniques. It does not require initiators, crosslinkers and can be used practically with any vinyl monomer. Responsive behavior of hydrogels make them also very attractive materials for some specific applications in adsorption and the sites of the hydrogel showing selectivity of bimolecular such as the drugs which can be easily incorporated into network structure prepared by radiation-induced polymerization.[10] The success of hydrogels, prepared by radiation, as biomaterials lies in their resemblance to living tissue because of their relatively high-water content, which minimizes the frictional irradiation of surrounding tissue.[11,12] Additional advantages of hydrogels are their nontoxicity, nonantigencity, nonirritability, and chemical stability.[13]

The high-solute permeability of hydrogels made them ideal materials of choice as devices for the controlled release of drugs and other active agents. Many researches in the field of medical hydrogels have been focused on the application in controlled drug delivery.[14] (2-VP/AAc) Hydrogel discs loaded by chloramphenicol antibiotic drug can be used as

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antimicrobial activity discs against Gram-positive microorganisms[15] such as *Staphylococcus auries* (*S. auries*). *S. auries* is a bacterium that can cause a variety of diseases and infections such as: skin infections, pneumonia, meningitis, septicemia, and toxic shock syndrome. This bacterium is frequently present on the skin or in the nose of healthy people, and many staphylococcal infections occur every year in hospitals around the world (each year $\sim$500,000 cases in US hospitals alone.

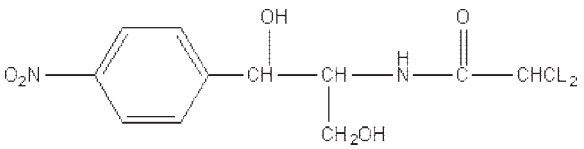
In the modern medicine,[16] polymeric materials have been used for a wide range of applications because much of them are biocompatible with blood, tissues, cells, i.e., in the human body. In the last decades, chemically and physically diverse hydrogels have became standard materials for scaffolds for the regeneration of new skin, encapsulation of cells, and regeneration of tendons and cartilage,[17–20] corneal implants, contact lenses and intelligent controlled-drug release devices for site-specific drug delivery.[21–23]

The aim of this work is to study the swelling behavior of P(2-VP/AAc) hydrogel. Hydrogels were prepared by radiation free-radical copolymerization in aqueous solution and were evaluated as adsorbed materials loaded by chloroamphenicol antibiotic drug. The study of its controlled release in both water and the microbiological media (agar–agar) was discussed as antimicrobial activity against Gram-positive pathogen microorganisms such as *S. auries* microbe.

EXPERIMENTAL

Chemicals

The two monomers used in this study, namely 2-vinylpyridine (2-VP) and acrylic acid (AAc) were obtained from Aldrich. Chloroamphenicol obtained from its commercial drug form Lamisil of Novartis Company. The chemical formula of chloroamphenicol is shown in Scheme 1.



Scheme 1 Chloroamphenicol.

Preparation of hydrogels

The mixture of 2 mL of 2-VP and 2, 5, 10, 15, 18, and 20 mL of AAc in 1 : 1 methanol/water solvent was prepared. The mixture of monomers in aqueous solutions were placed into a number of PVC tubs of 4 mm diameter and irradiated up to 60 kGy at a fixed dose rate of 1.7 Gy/s at ambient temperature in Gamma cell 220 type Gamma irradiator of Co^{60} gamma radiation. The copolymer P(2-VP/AAc) was obtained in a different compositions, the copolymer gel was subjected to soxlet extraction apparatus with methanol/water as a solvent mixture at 80°C for 4 h. After that, the gel yield was taken and dried under vacuum at 50°C up to a constant weight. Uncrosslinked polymer and/or residual monomers were removed from the gel structure by this extraction. The gel fraction yield in the hydrogel was determined from the following equation:

$$\text{Gel } (\%) = (w_e/w_d) \times 100 \quad (1)$$

where w_d and w_e represent the weights of the dry hydrogel and the gelled part after extraction, respectively

Swelling and diffusion studies

Dried hydrogel discs (0.3–0.4 mm thickness, 4 mm diameter) were left to swell in a phosphate buffer solutions of desired pH (2–9) and ionic strength I = 0.1 mol/L at 25°C. Swollen gels were removed from the swelling medium at regular time intervals and dried superficially with filter paper, weighed, and placed in the same bath. The measurements were continued until a constant weight was reached for each sample.

$$\text{Swelling } (\%) = W_s - W_d/W_d \times 100 \quad (2)$$

Where W_s and W_d represent the weights of swollen and dry samples, respectively.

The water-intake process was monitored by the determination of the swelling ratio of the hydrogel at desired time intervals as previously described. For the kinetic analysis of the results, Fick's law[24] was applied.

$$F = W_t/W_\infty = kt^n \quad (3)$$

Therefore,

$$\ln F = \ln k + n \ln t \quad (4)$$

where k is the swelling rate front factor, which is related to the structure of the network, and the exponential n is a number which determine the type of diffusion The constants k and n are obtained from the intercept and the slope of the Fick's straight line relationship. W_t is the water intakes at time t, and W_∞ is the water intake at the time of equilibrium. F denotes the amount of water absorbed at time t.

To study the kinetic of water-sorption mechanism, as well as the type of water diffusion inside the

polymeric network structure, the n value must be determined. For instance, $n = 0.5$ shows Fickian type in which the sorption is diffusion controlled, whereas a value of $n > 0.5$ and 1.0 indicates a non-Fickian type which contributes to the water-sorption process.

Loading of drug onto hydrogel

Adsorption isotherm method by batching experiment were carried out using the bottle-pointed method, where 1 g of dry hydrogel discs of (2 mm thickness and 4 mm diameter) the three different compositions 10/90, 20/80, and 30/70 of P(2-VP/AAc) were used as adsorbent materials. A series of concentrations 0, 10, 20, 40, 60, 80, and 100% of stock drug concentration 250 mg/mL, with constant volumes 20 mL (V) for each bottle containing the adsorbent polymeric hydrogel, were prepared. The samples were shaken well for 24 h and then left to 4 days, to ensure adsorption equilibrium state. The concentrations of drug at equilibrium C_e were determined using UV–visible spectrometer Unicam 1000 model at wavelength 274 nm, where the calibration curve was put through UV-absorption intensity measurements of pure chloroamphenicol drug concentration ranged from 0 to 250 mg/mL. Therefore, q_e values are calculated from the following equation as follows:

$$q_e = x/m = [(C_i - C_e)V/1000]/m \quad (5)$$

where q_e is the weight x in (mg) of drug adsorbed per 1 g of dry hydrogel adsorbent, C_i and C_e are the initial and equilibrium concentrations of drug adsorbate solution in mg/mL, whereas V is the volume of drug solution in (mL) used in the batch adsorption process.

The Freundlish equation was employed to describe the adsorption data for drug compound as follows:

$$q_e = k\ C_e^{1/n} \quad (6)$$

$$\log q_e = \log k + \log C_e^{1/n} \quad (7)$$

where k and n are the Freundlish empirical constants.

Releasing of drug from loaded hydrogel

Release experiments were performed by placing the 2-VP/AAc hydrogels loaded with chloroamphenicol into buffer solution at pH 8 as similar as to the intestine pH organ fluid at 37°C. At first, the loaded drug concentration onto gel as well as 250, 100, and 50 mg/mL were prepared in 25 mL of 0.2M phosphate buffer (pH 8). One milliliter sample was withdrawn on time intervals to follow the release process for 24 h. The concentration of chloroamphenicol was measured using the standard calibration curve at wavelength 274 nm. After the complete releasing, the hydrogels were immersed in pH 3.0 buffer solution and then 0.1M HCl for 2 days to remove any remaining drug in the gel system. The total uncertainty for all experiments ranged from 2 to 3%. The burst release of adsorbed chloroamphenicol as a specific adsorption (physical adsorption) from the hydrogel was at pH 8, whereas the controlled release of nonspecific adsorbed chloroamphenicol (chemical adsorption) took place at other lower pH values. The percentage release of chloroamphenicol drug at pH 8 was calculated from the following equation:

$$\text{Releasing } (\%) = (w/w_{\text{total}}) \times 100 \quad (8)$$

Where w is the weight of released chloroamphenicol drug at pH 8, and w_{total} is the total weight of specific and nonspecific adsorbed chloroamphenicol drug in the gel system.

The culture of *S. auries* and the hydrogel and/or chloroamphenicol antibiotic sensitivity test

Gram-positive cocci as well as *S. auries* microorganism was extracted, isolated, and supplied from bacteriology lab, microbiology department, NCCRT, Atomic energy authority, Cairo, Egypt. Nutrient agar (OXOID) was supplied from Hampshire, England.

It can be prepared by dissolving 1 g in 100 mL of phosphate buffer solution at pH 8 in a clean conical flask and closed it well with a clean stopper. The media was shaken well and heated warmly to ensure that nutrient agar was dissolved. Sterile the nutrient agar by autoclaving it for 30 min at temperature 121°C and pressure 1 mm/Hg.

Leave the agar to be warm at about 60°C, and then pour the nutrient agar into a number of sterile petrel dishes under a UV lamp in a clean and sterile place. Leave the dishes to become cold, and the agar will be gelled.

One colony of the fresh and isolated *S. auries* colonies were taken by a sterile wire lop and transported to a sterile petrel dish. The colony was spread in all directions of nutrient gel agar. A sterile disc (0.01 g) of prepared polymer hydrogels PAAc and P(2-VP/AAc) without loaded and loaded by a chloroamphenicol antibiotic drug (250 mg/mL) were put quietly on the surface. After that, the dishes were incubated at 37°C for 24 h. The microorganism will be grown under the incubation conditions with appearing a clear and transparent circle zone around the discs.

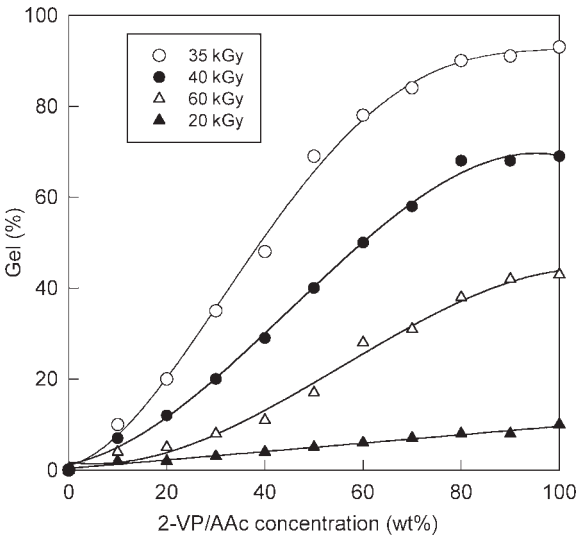


Figure 1 Effect of comonomer concentration on gel percent at different irradiation doses.

The transparent circle zone indicated the inhibition and killing of the microorganism by the antimicrobial active material. The degree of the sensitivity test for polymer and polymer loaded by the drug was determined by taking the average measuring values of the circle diameter (cm) of the inhibited transparent zone.

RESULTS AND DISCUSSION

This study deals with the radiation synthesis of some stimuli response hydrophilic materials, for possible use as drug-delivery system. The formation of intermolecular crosslinks in one of the most important changes brought about radiation, as crosslinking of polymers leads to beneficial changes in some of their properties such as thermal stability, swelling behavior and its adsorption capacity.

Gelation studies

Effect of comonomer concentration on gel content

Results given in Figure 1 showed the effect of comonomer concentration on the gel percent as calculated from eq. (1) at a constant composition ratio 50/50 as 2-VP/AAc. The study takes place at different irradiation dose ranged from 20 to 60 kGy.

Both 2VP and AAc monomers were dissolved in methanol/water mixture by ratio 1 : 1. It is clear that the gel percent increases as the comonomer concentration increases, and the maximum gel percent occurs at 80% comonomer concentration for all the doses studied. These results explained that as the concentration increases, the free radicals become rich. Accordingly, the possible monomer–monomer interaction is higher more than monomer–solvent or solvent–solvent interaction[25] and therefore, the AAc free radicals interacted and crosslinked through the 2-VP chains than the other interactions. So, the obtained hydrogel having a maximum gel fraction yield percent as well as 95% where all the 2-VP chains are completely and saturated crosslinked than the other lower comonomer concentration (lower than 80%).

Effect of the comonomer composition on gel content

The influence of 2-VP/AAc composition on the gel-fraction yield formed in the respective copolymer hydrogel was determined and shown in Figure 2 at constant concentration 80% and dose 35 kGy. It is observed that as the 2-VP content decreases, the gel fraction yield increases. This behavior was observed for 2-VP/AAc hydrogel in which the content of gel is slightly higher for 10/90 comonomer composition than the other compositions. These results indicated that the enhancement of the crosslinking process arises by the monomer-free radical-rich solution and also the enhancement of such free radicals producing high crosslinking percent.

Effect of irradiation dose on gel content

The effect of irradiation dose on gel content of poly 2-VP/AAc copolymer hydrogel prepared at concentration 80% and comonomer composition 10 : 90 wt % was investigated and shown in Figure 1. It is clear that as the irradiation dose increases, the gel content increases to reach a maximum value at a range from 30 to 35 kGy. Also, it was found that higher the dose, the higher the gel fraction yield obtained at a given comonomer composition. After that, it tends to decrease by increasing the irradiation dose. The

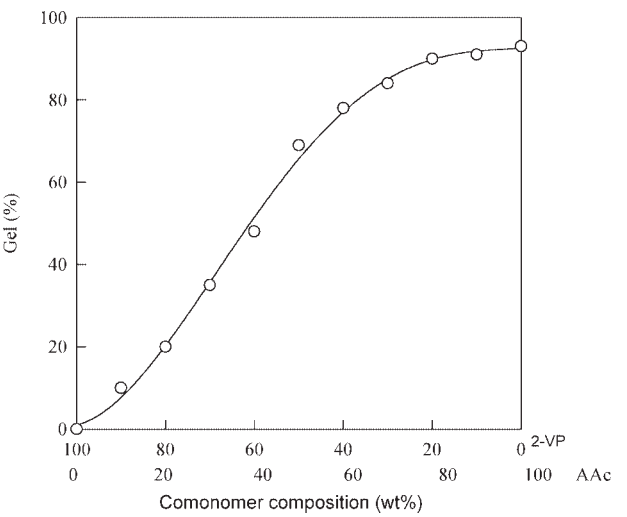


Figure 2 Effect of comonomer composition on gel percent of P(2-VP/AAc) at irradiation dose 35 kGy.

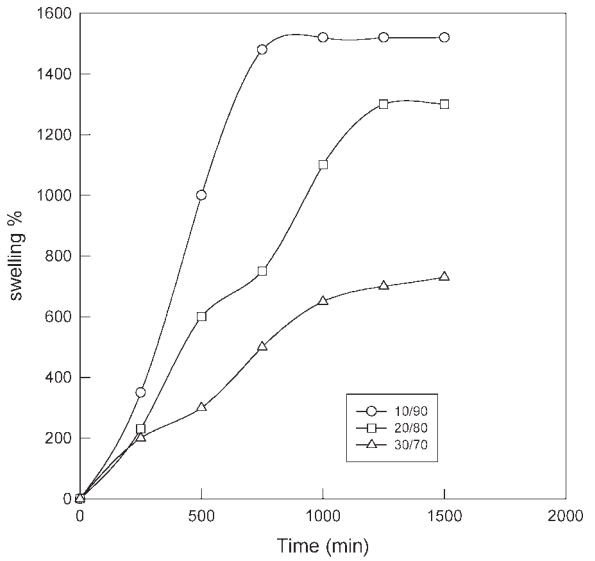


Figure 3 Effect of time on the swelling percent for different compositions of P(2-VP/AAc) hydrogel.

results suggested that the concentration of free radicals formed from the 2-VP and AAc monomers increases with increasing irradiation dose till 35 kGy and, alternatively, increase the gel fraction yield of the hydrogel by increasing the crosslinking density of the network structure. Results also indicated that the crosslinking process in the prepared hydrogel was enhanced at higher doses and at lower 2-VP content as 10% in the comonomer composition where the higher gel fraction yield was obtained.

Swelling studies

The swelling percent (the degree of swelling) of the hydrogel can be calculated from eq. (2) as illustrated earlier. The degree of swelling was influenced by different factors that will be discussed later.

Effect of time on the swelling behavior

Figure 3 shows the swelling percent of 2-VP/AAc copolymer as a function of time immersed in aqueous solution using different copolymer compositions. It is observed that the swelling process increases with increasing immersion time for each composition. The highest percent of swelling was obtained after about 18 h. However, the highest swelling occurs at composition 10/90 than the other two compositions 20/80 and 30/70 of copolymer hydrogel, where the latter compositions having much trick effect due to participate of pyridine rings between the crosslinked chains. This trick effect leads to reduce the volume space in the hydrogel matrix which retards water diffusion and therefore lowering the swelling percent. Although in composition 10/90, the higher crosslinking by AAc free radicals with the vinyl pyridine chains resulted to a matrix characterized by hydrophilic carboxylic groups that required less time to swell with water (about 500 min) and to reach equilibrium state faster than the other two compositions (about 1000–1200 min).

Effect of pH on the swelling behavior

Figure 4 shows the effect of pH medium on the degree of swelling for different compositions at temperature 25°C and in a phosphate buffer solution. It was found that the degree of swelling increases with increasing the pH in all compositions. The maximum extent of equilibrium degree of swelling was ranged between pH 6.5–8 where a complete dissociation of acid groups of AAc occurred. The dissociation of COOH groups can be followed by measuring the pH values after swelling which showed its tendency to be lowered than the starting initial pH values. Moreover, the dissociation of COOH groups led to form a charged group impregnated to the polymeric network chains structure resulted in electrostatic forces which played an important role in water diffusion and increase the swelling properties.[26]

Effect of irradiation dose on the swelling behavior

The irradiation dose has a great effect on the swelling properties of polymer prepared with gamma radiation. It was observed from Figure 5 that as the irradiation dose of the prepared hydrogel increases, its affinity and degree of swelling also increases. The most degree of swelling is 1700% as recorded for the composition 10/90, whereas the lower one was 200%

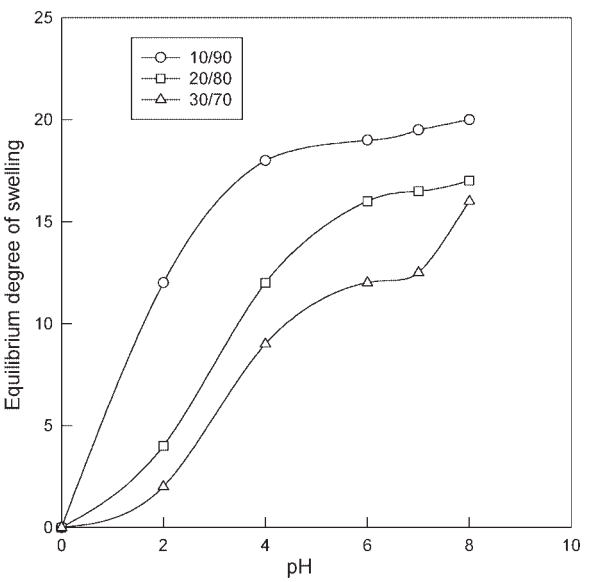


Figure 4 Effect of pH on the equilibrium degree of swelling at different compositions of P(2-VP/AAc).

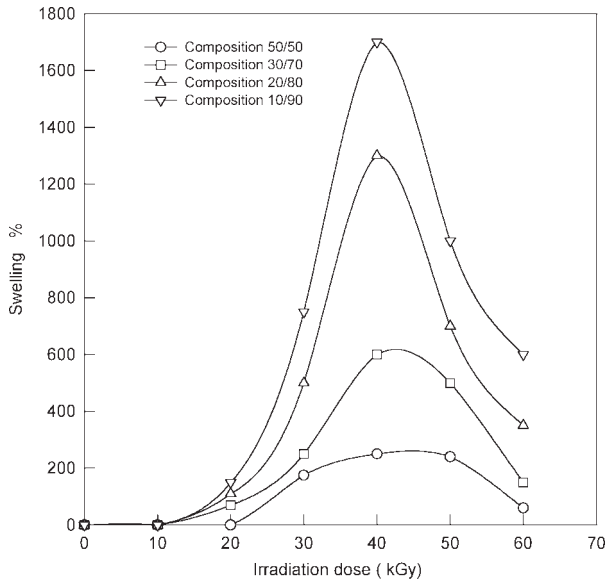


Figure 5 Effect of irradiation dose for preparing P(2-VP/AAc) hydrogel on their swelling % of water at different composition and constant temperture 25°C.

for composition 50/50. This is because the crosslinking density, which increases with the irradiation dose, leads to increase the number of hydrophilic groups that is responsible for water uptake by hydrogen bonding and the electrostatic force effects.

The hydrogel compositions prepared by gamma radiation at doses more than 35 kGy up to 60 kGy showed lower swelling affinity. Therefore, the degree of swelling decreases with increasing the irradiation doses, where the diffusion of water into the matrix becomes difficult. As a result, the degree of swelling decreases for all other compositions forming P(2VP/AAc) hydrogels. The explanation for the observed findings may be that increasing the number of crosslinking in the hydrogel should lower the molecular weights of the chain units between the crosslinks and there by reduces the free volumes between the macromolecular chains.[27] This clearly lowers the degree of swelling of the hydrogel with increasing gamma irradiation dose.

Effect of temperature on the swelling behavior

In this study, the effect of temperature on the swelling percent of the hydrogel P(2-VP/AAc) copolymer was investigated at different temperatures of the swelling medium as 20, 30, 40, and 60°C. The results are represented in Figure 6 and showed that the swelling percentage increases with increasing temperature from 20°C up to 30°C. After 30 to 60°C, a fall down in the swelling % was observed. These results can be explained that, with increasing the temperature of the swelling medium up to 30°C, the network chains of the polymer tends to undergo faster relaxation due to its high energy which facilitate and enhance the water molecules for diffusion process through the hydrogel. A fall down in the swelling % was observed at temperature > 30°C which may be attributed to the degradation of AAc monomer crosslinking with the chain of P(2-VP), and furthermore, the degradation of intermolecular hydrogen bonding between the COOH groups of the parallel chains in the network structure. Therefore, the high temperature leads to decrease in the molecular weights between the crosslinks and accordingly reduces the free-volume space between the chains of the network structure which decreases the chance of water molecules to be penetrated and therefore decreases the swelling of the hydrogel.

Kinetic study of water swelling

Figure 7 represents the effect of contact time on the swelling percent for different compositions. This figure explains the type of water diffusion inside the network structure of polymeric hydrogel according to Fick's law as written in eq. (4).

This equation is applied to the initial stages of swelling as well as the short-time approximation which is valid for the first 60% of the swelling. The results showed that n values ranged a number (0.6–0.7) which reveals to the fact that the composition of P(2VP/AAc) hydrogel was taken as non-Fickian character. It can be said that, for higher swelling values of the hydrogels, the transport of water into the hydrogel matrix where the lower polymer relaxation rate resulted in higher diffusion rate. This is generally explained on a consequence of the relaxation

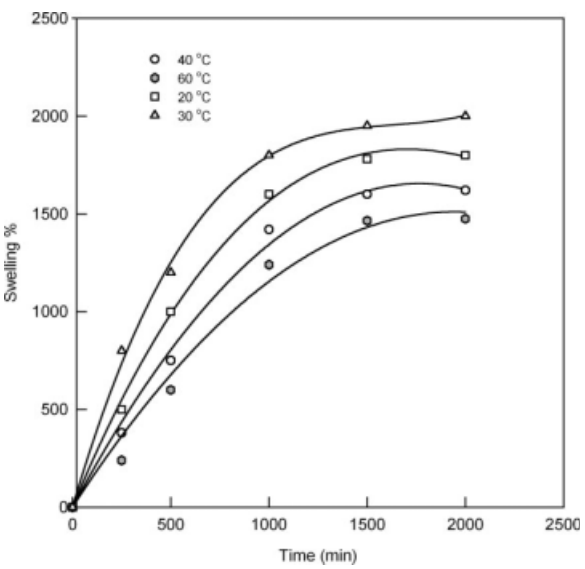


Figure 6 Effect of time on the swelling % at different temperature of the hydrogel P(2-VP/AAc) for composotion 10/90 and concentration 80 wt % and irradiation dose 35 kGy.

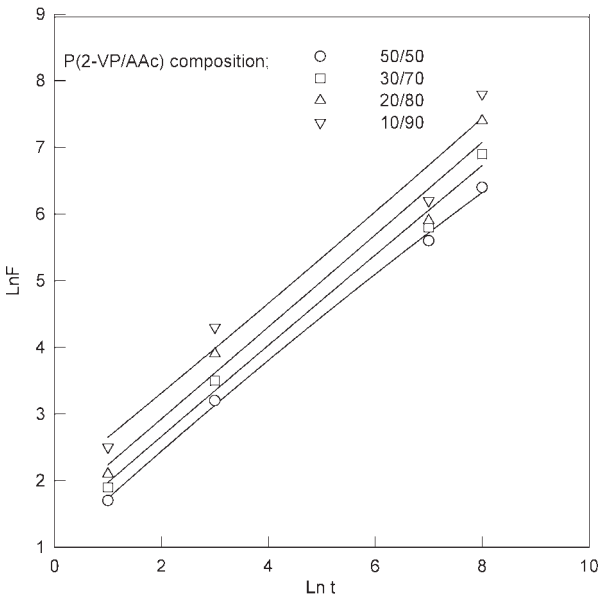


Figure 7 Swelling kinetic curve of P(2VP/AAc) hydrogel as ln F versus ln t.

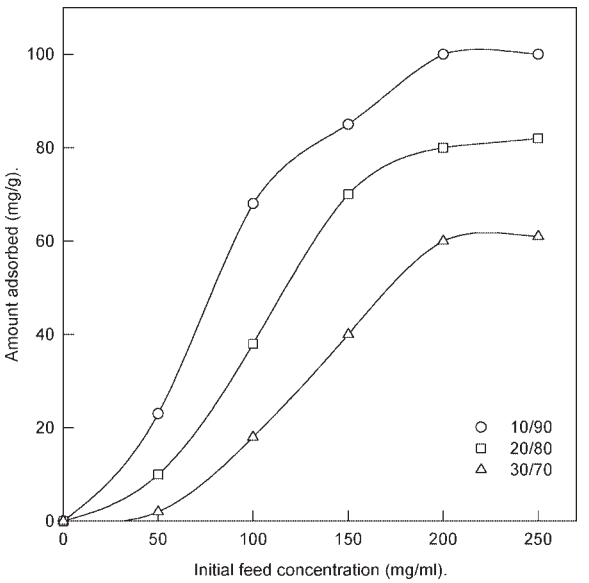


Figure 8 Effect of initial feed concentration of drug on the adsorption capacity of 2VP/AAc hydrogel at different compositions.

rate of the polymer matrix. Also, the equilibrium swelling for P(2-VP/AAc) hydrogels at all the compositions used reached the maximum at pH 8 and the composition 10/90 as 2VP/AAc ratio showed that the equilibrium degree of swelling is the optimum one.

Loading of chloroamphenicol drug on P(2VP/AAc) hydrogel

Effect of drug concentration

Freundlish adsorption isotherm model as represented in eq. (6) was used in the equilibrium adsorption isotherm studies. This equation revealed two important empirical constants k and n. These constants referred to the ability of hydrogel and the affinity of drug, respectively, in the adsorption equilibrium isotherm processing. Figure 8 shows the relationship between the equilibrium concentration C_e (mg/mL) of chloroamphenicol as a remaining concentration after complete adsorption, where the initial feed concentrations 5, 10, 50, 100, 200, and 250 mg/mL drugs were carried out by equilibrium adsorption batching experiment isotherm studies.

It was found that the amount of chloroamphenicol drug adsorbed increased with the hydrogel of composition 10/90 than the other compositions. This means that the adsorption of drug increases with increasing AAc monomer content in the hydrogel. The reason for this increase was attributed to the increase of free-volume space in composition 10/90 with less-strike effects by pyridine rings than the other compositions that have more strike effect with lower volume space available for diffusion and specific bonding of OH groups in the drug with the carboxylic COOH groups as ionized groups in the hydrogel to be interacted.

Figure 8 also shows that an increase of initial drug concentration from 5 to 250 mg/mL in the swelling medium leads to increase the amount of adsorption of drug. The reason for such result was attributed to three different factors. The first factor is the higher acid content of the gel system, and the second factor is the specific bonding of positively charged drug with partially ionized carboxylic groups of the hydrogel, and the third factor is the higher free volume available for diffusion. According to these reasons, the ability of the hydrogel network structure and the affinity of the drug to intake and diffuse into the hydrogel can be insured by the values of the empirical constants k and n, respectively.

To obtain these two constant values, the logarithmic form of eq. (7) and the relationship of log (q_e) versus log (C_e) resulted in a straight line with a slope equal $1/n$ and an intercept log k on the vertical axis of (log q_e) as shown in Table I for different composition ratios of the prepared hydrogels.

TABLE I
Freundlish Empirical Constant n and k of the Hydrogel P(2-VP/AAc) of Different Composition Adsorbed by the Drug of Concentration 250 mg/mL at pH 7

Hydrogel composition	n	k	R^2
10/90	50	1.90	0.994
20/80	52	1.78	0.998
30/70	56	1.68	0.995

TABLE II
Freundlish Empirical Constants *n* and *k* of the Hydrogel P(2-VP/AAc) of Composition 10/90 Ratio Adsorbed by the Drug of Concentration 250 mg/mL at Different pH Values

pH	*n*	*k*	R^2
3	1.04	33	0.789
7	1.9	50	0.994
8	1.98	38	0.988
10	3.06	56	0.987

It was found that the composition ratio of 2-VP/AAc as 10/90 has the higher value of *k* which referred to the high ability of polymeric hydrogel to be loaded by the drugs. This result was due to its higher free volume spacing in their matrix structure which was available for diffusion than the other composition ratio 20/80 and 30/70. Moreover, the higher content of AAc as well as COOH content of the gel play an important role as active charged groups, which allowed the hydrogel on the surface and inside the gel structure as an active polar, and therefore, the surface energy is much active. On the other hand, Table I shows that all the hydrogels of various compositions approximately have the same slope and nearly the same values of *n*. This result is because the swelling medium contains only one component of the drug, and therefore, the affinity of their molecular transportation to the hydrogel has the same behavior. Furthermore, it was observed that the water swelling in presence of drug solution becomes more than that in water only, and this may be due to the difference in ionic strength of their medium.[27]

Effect of pH on the loading of drug

Table II shows the effect of pH on the adsorption of chloroamphenicol drug into poly (2-VP/AAc) hydrogel. The values of *k* and *n* as the empirical constants of Freundlish equation were evaluated from the adsorption isotherm curve of the poly (2-VP/AAc) hydrogel at only composition ratio 10/90 at different pH 2, 7, 8, and 10 at constant temperature 25°C.

It was found that *k* value is highest at alkaline medium pH 10 as 3.06 and a lowest value in acidic medium pH 2 as 1.04. The results explained that, at pH of alkaline solution, the *k* value is the highest value due to the high activity of the carboxylic groups of the hydrogel. Also, in acid medium, adsorption of such hydrogel decreases because there is less protonation of amine groups of the drug and then the migration of drug molecules becomes low. Moreover, the water uptake in the alkaline pH is higher than the drug solute uptake and this informed that the hydrogel cannot keep the solute because the texture structure becomes weak and can explode. These results showed that both acidic and higher alkaline mediums are not suitable for both the adsorption and the expected releasing process of the drug. This can be proved by the lower affinities as a low value of constant *n* for the drug molecules that try to be loaded onto the hydrogel at different pHs lower than 7 and more than 8. The best adsorption result which referred to the best expected releasing process was occurred at pH 7–8, where the affinity of the drug onto the hydrogel and its reversible process as control releasing has a maximum *n* value as 50.

Spectroscopic analysis studies

FTIR spectroscopic analysis

FTIR spectroscopic analysis showed that the vinyl groups located around 1660 cm^{-1} in both AAc and vinyl pyridine (2-VP) are of interest to be studied. As shown in Figure 9, the vinyl stretching vibration band for both monomers is responsible for the free radical formation to copolymerize with AAc free radicals and to produce the expected copolymer hydrogel.

The prepared hydrogel P(2-VP/AAc)showed the disappearance of the vinyl group C=C at wave number 1660 cm^{-1}. This band which indicated the copolymerization of C=C as SP^2 hybridization by gamma radiation through cleavage the π bond and resulting in the formation of free radicals and the complete compensation of vinyl groups. On the other hand, the C—C stretching vibration band

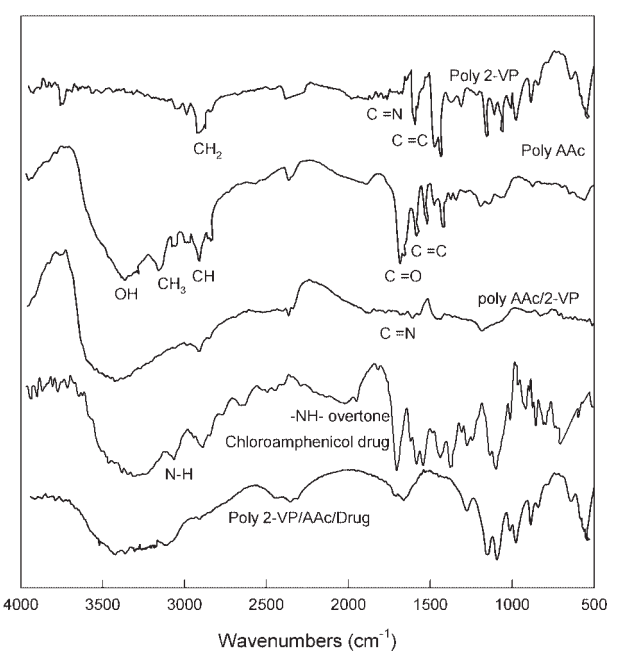


Figure 9 FTIR spectroscopy of poly AAc, poly 2-VP, copolymer 2-VP/AAc, chloroamphenicol drug, and copolymer 2-VP/AAc/drug.

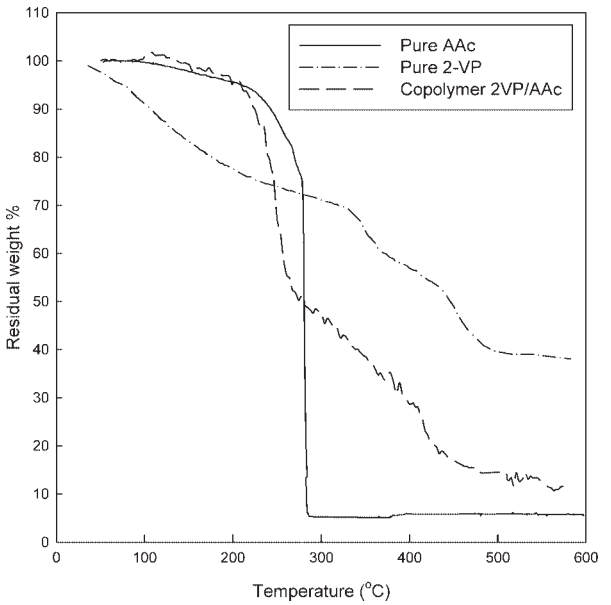


Figure 10 Thermograghmetric analysis of pure AAc, pure2-VP, and copolymer 2VP/AAc.

increases its intensity at 1370 cm^{-1} due to increase in the crosslinking of 2-vinyl pyridine and AAc free radicals in their copolymerization.

The structure of chloroamphenicol drug is confirmed by its characteristic functional groups. The characteristic stretching vibration bands as well as O—H (3500), *N*—H (3300), C—H, CH_2 (3050, 2950), C=O (1710), aromatic C=C (1600), C—C (1350), C—N (1400), C—Cl (1480), and *N*—O (1500) in cm^{-1}. The IR date represented the uptake of drug onto hydrogel 2-VP/AAc between cationic OH, NH groups, and anionic COOH groups of the hydrogel as adsorption interaction. A broad band at 3500 cm^{-1} indicated the intramolecular hydrogen bonding between polymeric hydrogel chains and the drug molecules.

Thermal Gravimetric Analysis (TGA)

From the TGA curves in Figure 10, initial degradation temperature and final degradation temperature were determined. The weight loss of PAAc begins at 23°C and reaches maximum at 275°C. The TGA curve of PAAc homopolyrner indicates one reaction stage. Initial degradation temperature of PAAc that showed the complete degradation as 100% was due to random chain scission.

On the other hand, the P(2-VP) copolymers are little more thermally stable than PAAc homopolymer. Therefore, initial degradation temperatures of P(2-VP) networks were increased slightly from 50 to 330°C with slow decompositions. After 330 up to 420°C, the matrices suffer from a further low degradation due to two opposite effects. The first effect was the thermal stability due to its high-resonance effect along their chains in its matrix, and the degradation of its chains to smaller chains of P(2-VP) due to the trick effect of pyridine rings resulted in collision of pyridine rings present as close to each other through the chain. After 420°C, a complete degradation to small fragments of gases may be CO_2, NO_2, and NH_3 with remaining residual 25% of P(2-VP) as small chains.

As can be seen in P(2-VP/AAc), networks degrade in two stages. This was attributed to anhydride formation which is accompanied by loss of water molecules as in Stage (I) and thermal degradation to small chains as in Stage (II). This behavior showed that thermal degradation reaction mechanism of P(2-VP/AAc) networks is different than PAAc homopolymer. This observation also indicated that the change may correspond to the formation of anhydride by eliminating and loss of water molecules from the carboxylic acid groups in the P(2-VP/AAc) copolymer networks structure.

Surface morphology analysis (SEM) of the hydrogels

The surface morphology of the hydrogels was observed for the hydrogels prepared by gamma radiation. From the results, it was deduced that microporous hydrogels could be successfully prepared by free-radical polymerization using gamma radiation technique. The porosity on the surface depended on irradiation dose and the amount of AAc monomer content of the monomer composition. As shown in Figure 11(a), the poly AAc shows a smooth surface with lower porosity on the surface, whereas Figure 11(b) shows the surface morphological structure as a crystalline shape with some pores in its surface, which resulted in the strike effects of pyridine rings and therefore increases the volume space between the chains in the network structure. Figure 11(c) shows a white and dense agglutination on the surface in a regular crystalline shape and this morphological investigation referred to the crystal shapes of the hydrogel copolymer P(2-VP/AAc). The morphological structure in Figure 11(d) represents a highly dense drug crystal growth in a regular distribution on the surface, referring that the loading of drug on the surface of hydrogel occurred by a physical adsorption as multimolecular layers.

Releasing behavior of drug from the hydrogel

Figure 12 shows the change of pH of water from 2 to 8 through releasing of chloroamphenicol drug as calculated from eq. (8), when the medium start from acidic to alkaline medium as transported from the stomach to the intestine in the digestive system of

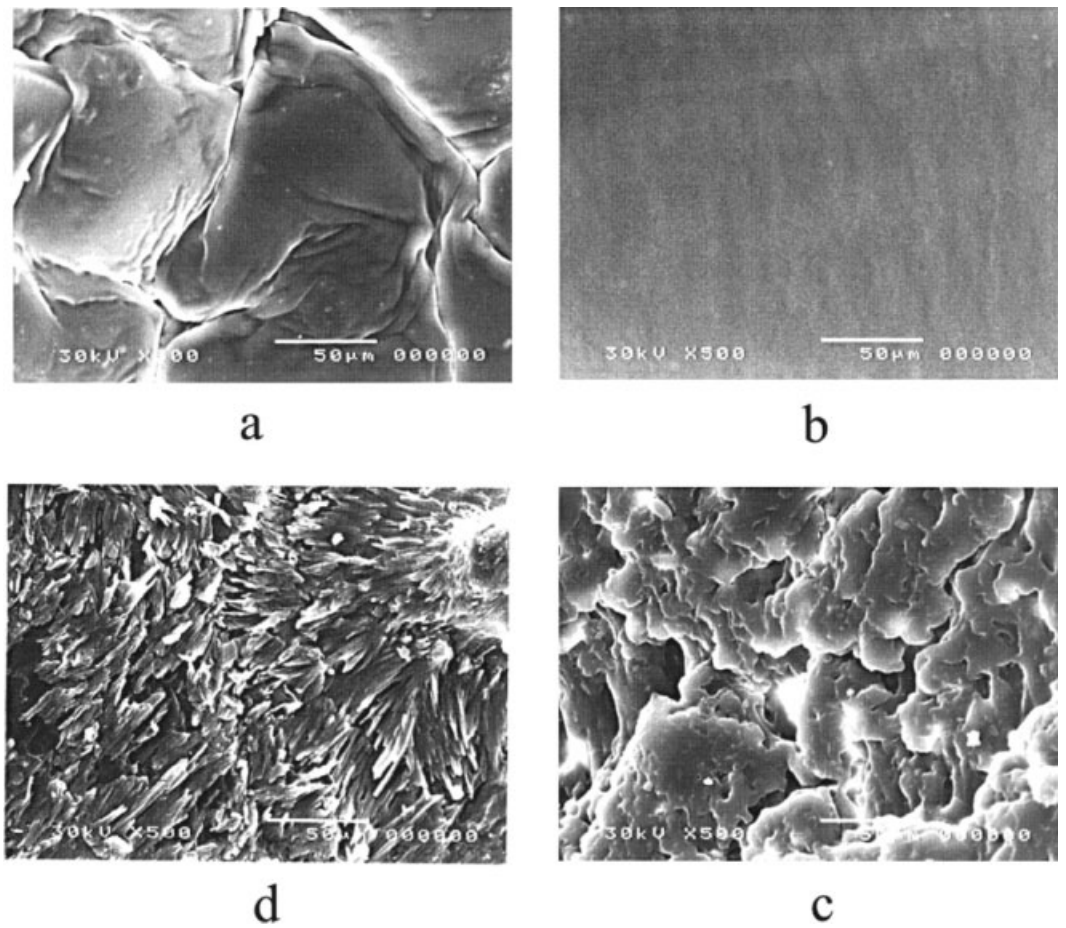


Figure 11 Scanning electron microscope of (a) P(2VP); (b) P(AAc); (c) P(2VP/AAc); (d) P(2VP/AAc/Drug).

human body. It was observed that in acid medium the drug releasing from the hydrogel is remarkably low and then increases suddenly in alkaline medium at pH range 7–8. This behavior indicated the sensor pH effects on operating the drug releasing from the hydrogel. Starting the batch releasing at pH 8 for the adsorbed disc of hydrogel (1 g) loaded by an amount of drug approximately adsorbed as 250 mg/mL and other disc loaded by 100 mg/mL and the lower one loaded by 50 mg/mL as bounding concentration. It was found that the hydrogel which was loaded by higher concentration of drugs, its releasing became higher than the other concentration released 50 and 100 mg/mL. The reason was attributed to the fact that at high concentration, the releasing is a burst releasing because the hydrogel was physically adsorbed and loaded by much more amount of drug on the surface as multi molecular drug layers than the other lower concentrations.

The burst releasing, by the accumulated multi molecular layers on the surface of the hydrogel that formed by physical adsorption, decreases with decreasing the multimolecular layers and with the time consumed. It was found that the amount release was 78%, 56%, and 14% for 250, 100, and 50 mg/mL loaded disc, respectively, for releasing. As the pH of intestine organ of the human body is 8, it takes about 1–2 h to absorb the digestive food and transport it to the blood. So, the chloroamphenicol

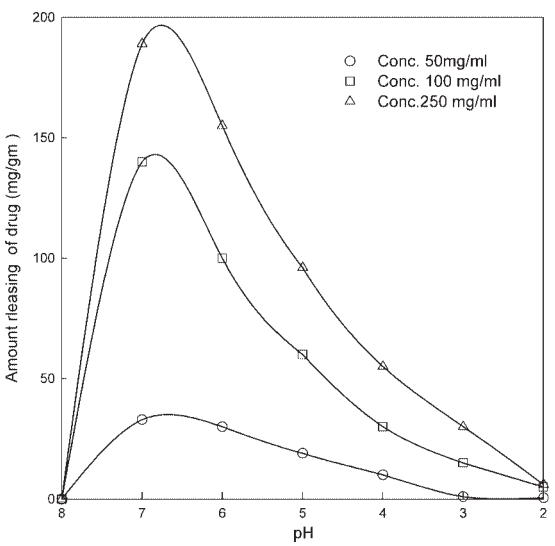


Figure 12 Effect of pH on the amount of drug releaseing (mg/mL) at different concentrations.

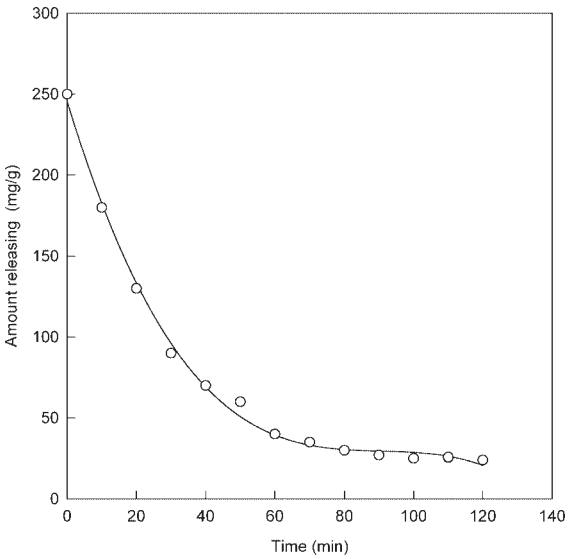


Figure 13 Effect of time on the amount releasing of drug as 250 mg/mL loaded by the hydrogel.

must take a time less than 2 h to make its biological function as antibiotic drug against the pathogenic microorganism *S. auries*. Therefore, the activation of the drug releasing at pH 8–7 increases rapidly from the hydrogel as a burst releasing within 40 min as shown in Figure 13 At this time, the burst releasing was due to the physical adsorption process caused for the drug on the polymer surface by accumulating layers that can be separated and released rapidly with the concentration 250 mg/mL.

After 40 min, the controlled releasing of drug occurred from inside the hydrogel matrix bounded by chemical adsorption to outside under osmotic pressure in a slow releasing through the pores. The controlled releasing takes place slowly by dissociation of ester groups formed between OH of the drug and COOH of the hydrogel. On the other side, a further decomposition of the hydrogen bonding occurred for transportation the drug molecules gradually from inside to outside starting from 40 to120 min with controlled releasing about 19%. Therefore, the total releasing through the 2 h is about 97% of the antibiotic chloroamphenicol drug (250 mg/mL) with shifting the pH value to about 6.8. After this time, traces of drug (about 2–3%) may be bounded with the polymer matrix and/or decomposed to small fragments as its transformation to small organic compounds under the effects of the surrounding conditions.

The antimicrobial activity of polymer and polymer loaded by drug

Basically, a polymer matrix which loaded with antibiotic chloroamphenicol showed a slow release into the environment, where they kill microbes. The release of the antibiotic drug can be free and controlled. In the case of free release, the antibiotic drug burst out of the material immediately, whereas with controlled release the antibiotic drug as slow release either by interactions with the material or by degradation of the matrix. The release is then dependent on the specific environmental conditions.[28]

Matrices loaded by the drug are used by loadings, sustained release of the antimicrobials which is achieved through the use of a hybrid polymer matrix that contains hydrophilic and hydrophobic functional groups in polymer structure. The hydrophilic polymer allows water molecules to diffuse into the matrix so that the antimicrobial can dissolve in water and elute out of the matrix to the surface to protect it from microorganism colonization.[29]

The release is achieved by linkages that may be cleaved under mild conditions.[29] The hydrophobic polymer interacts with the hydrophilic polymer through chain entangling and other intermolecular forces to make sure the hydrophilic polymer remains insoluble in water.[30] The antimicrobials that are released from the loaded hydrogel will kill the bacteria not only in contact with the surface but it also diffuses more through the interior layers of the cell and a dehydration effect of cytoplasm fluid content of cell microorganism occurs.[31] Antimicrobials in this form are not chemically bonded to the matrix and retain their activity. The loading are not intended to produce therapeutic concentrations of the drug in tissues or body fluid but to prevent the development of microcolonies.[32] The drug delivery is a local delivery because the concentration of antimicrobials is very high in the vicinity of the catheter surface and very low at a distance from the surface.

Matrix loading systems are designed to allow for minimal antimicrobial loading and sustained release over a period of time. Because the antimicrobial loading is kept at a minimum, the devices usually do not exhibit observable signs of toxicity. Table III shows that the polymeric matrix hydrogels as well as PAAc and P(2-VP/AAc) have antimicrobial activity toward *S. auries* microorganism. The antimicrobial effects of the hydrophilic polymer matrix are

TABLE III
The Polymer Structure Effects and Their Drug Releasing 250 mg/L on the Inhibition of *Staphylococcus aurius* Microorganism as Zoon Diameter (cm) as Sensitive Test on Nutrient Agar

	Inhibited zoon (cm)	
Copolymer	Without drug	With drug
PAAc	1.6	4.1
P(2-VP/AAc)	2..3	5.1

attributed to its inhibitions of water including cytoplasm of *S. auries* cell. Therefore, the hydrophilic polymer matrix has the dehydration effect by the carboxylic group on the cell composition. So, the inhibited zone was 1.6 cm for PAAc. On the other side, the copolymer P(2-VP/AAc) showed more inhibition due to the degradation of cell wall by the effect of nitrogen atom participated in pyridine ring. The lone pair of electrons on nitrogen atom trapped electrolyte ions which controlled the balance of cell wall. Therefore, the degradation takes place by the uncontrolled balance of the cell wall. According to these reasons the inhibition zone is 2.3 cm for copolymer P(2-VP/AAc) more than PAAc effect. In addition, the copolymers' discs loaded by chloroamphenicol drug releasing showed higher inhibition.

CONCLUSIONS

Poly(2-vinyl pyridine-*co*-AAc) hydrogel was prepared by gamma radiation as a free-radical copolymerization reaction. The optimum condition for the preparation of P(2-VP/AAc) hydrogel at monomer concentration 50 wt % in methanol/water (1 : 1) solvent and monomer composition as 2VP/AAc monomers. At fixed irradiation dose 1.7 Gy/s, the complete gelation was obtained at irradiation dose 35 kGy where a sufficient crosslinking by the monomers free radicals occurred. Swelling behaviors were studied and showed as highly swelling percentage = 1500%, and the result showed that the diffusion of water followed a non-Fickian type as a nomulance, where n value ranged between 0.6 and 0.7. The diffusion of water increased with increasing pH value of the external solution. The loading adsorption of chloroamphenicol antibiotic drug onto the hydrogel P(2-VP/AAc) was studied and showed that the maximum adsorption occurred at pH ranged 7–8, and the amount of drug loaded increased with increasing the concentration up to 250 mg/mL. The anionic carboxylic groups $-COO^-$ play the most important role in the adsorption capacity of the hydrogel for the drug. The ability and the affinity of the adsorption of chloroamphenicol drug were described according to Freundlich adsorption isotherm curve depending on the evaluation of their empirical constants k and n. The drug release behaviors have been investigated and showed that the basic parameter affecting the drug release behavior from P(2-VP/AAc) hydrogels is the pH sensor of the drug solution. The hydrogel was considered as potential carriers for the drug-delivery systems as confirmed by the FTIR spectroscopic and scanning electron microscope. The hydrogel may be used especially as local therapeutic as antibiotic loading and its application as releasing of the antibiotic against *S. auries* microorganism which showed the inhibition of water fluid in the cell content of *S. auries* microorganism at pH 7–8 as in the intestine organ of the human body.

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