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Controlled-Release and Antibacterial Studies of Levofloxacin-Loaded (Gelatin-Poly Acrylamide) Hydrogel IPNs

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الملخص

تم تحضير ستة بوليمرات شبكية التداخل هلامية من الجيلاتين والاكريل امايد والبولي اثيلين كلايكول واستخدم الكلوتيرالديهايد البس اكريل امايد كعوامل تشابك. حملت التراكيب البوليمرية بدواء الليفوفلوكساسين ومن ثم تمت متابعة اطلاقه في كل من محلولي المعدة والامعاء الافتراضيين وفي الماء المقطر باستخدام المطيافية فوق البنفسجية بدرجة حرارة 37 درجة مئوية .كذلك تم تعيين نسب الانتفاخ لهذه التراكيب البوليمريه عند نفس المحاليل والماء المقطر.وجد ان هنالك تاثير للدالة الحامضية على كل من نسب الانتفاخ ومعدل اطلاق دواء الليفوفلوكساسين من التراكيب البوليمرية المحضرة اذ اعطت نسب انتفاخ ومعدل سرعة اطلاق للدواء اعلى في كل من محلولي المعدة والامعاء الافتراضيين مقارنة مع الماء المقطر. درست سمية التراكيب البوليمرية اظهرت النتائج ان التراكيب البوليمرية لا تمتلك اي سمية. درست الفعالية البايولوجية للدواء المطلق من التراكيب البوليمرية بطريقتين ضد نوعين من البكتريا واظهرت النتائج فعالية ممتازة لمدة أربع ايام.

Abstract

Interpenetrating polymer network Hydrogels (J1-J6) were synthesized from gelatin, acrylamide and polyethylene glycol by chemical crosslinking using Gluteraldehyde and bisacrylamide, the hydrogels were loaded with levofloxacin. The slow release of levofloxacin was studied by using UV spectroscopy at temperature 37˚C and with different pH (distilled water pH=6.8, SGF pH=1.2 and in SIF pH=8.2), The swelling of polymers in these solutions was also studied. The results exhibit that the pH has great effect on swelling ratio of the IPN hydrogels and release rate of levofloxacin, the hydrogels were showed high swelling ratio and fast release rate with low and high values of pH (SGF and SIF) compared with neutral solution. The toxicity of hydrogels was studied which is shown that the prepared hydrogels are nontoxic. The hydrogels – levofloxacin delivery were followed as antibacterial activity by Disk Diffusion Testing on Mueller-Hinton Agar and Broth microdilution methods over a period of four consecutive days Against two types of bacteria (Streptococcus mitis and Escherichia coli). The results showed the continuous release of the Levofloxacin from the polymers with effectiveness as an antibacterial for four consecutive days.

1. Introduction

Conventional drug delivery involves the repeated administration of an active compound to maintain therapeutic levels in the body; this compromises patient compliance, efficacy and can lead to side effects due to high doses. Research in drug delivery has therefore focused efforts on achieving controlled and local drug delivery, using nanostructured systems, such as liposomes, nanoparticles, membranes and hydrogels [(1)].

Over the last few years, hydrogels as a biomaterial show the advantages in many ways for their good biodegradability and biocompatibility[(2,3)], in addition to their physiochemistry almost the same as the native (outside of a cell) matrix which can act as supporting material for drug delivery system[(4, 5)]. An example of the use of hydrogel in drug delivery is Bacterial cellulose and gelatin successfully used to develop a hydrogel composite material. Hydrogel was synthesized by copolymerization between bacterial cellulose and gelatin[(6)], also hydrogel polymers displays highly attractive features for use in tissue engineering [(7, 8)].

Interpenetrating polymer networks (IPN) are distinctive “alloys” of crosslinked polymers that can Leastways one network is manufactured and/or crosslinked If the other is present Without any covalent bonds [(9)]. IPN It is considered a mixture of synthetic and natural polymers. IPNs are often established for adding the defining features of one of the components while preserving the critical attributes of another, and Sometimes completely new features are visible that are not observed in either network. IPNs divided into two types according to the method of manufacture [(10)].

Gelrite was used as a gelling agent in combination with hydroxypropylmethyl cellulose to create a levofloxacin in situ hydrogel formulation. The in vitro drug release investigation revealed that the drug might be released for up to 12 hours. This levofloxacin in situ gel formulation was shown to be promising and safe for use as an eye administration system[(11)] The drug has also been used in Chasing bacteria within the cells using levofloxacin-loaded hyaluronic acid Nano hydrogels an innovative approach based on the delivery of levofloxacin from polysaccharide Nano hydrogels for the treatment of bacterial intracellular infections is described the increase in antibacterial efficacy of LVF-NHs with respect to that of free LVF was evidenced. The obtained results allow to conclude that this new approach can be considered as really promising method for intracellular infection treatments[(12)].

In this study, Levofloxacin (Levaquin®) is a fluoroquinolone antibacterial agent with a broad spectrum of activity against Gram-positive and Gram-negative bacteria and atypical respiratory pathogens. It is active against both penicillin-susceptible and penicillin-resistant Streptococcus pneumonia [13, 14], was loaded with prepared IPN hydrogels of gelatin and acrylamide (J1-J6) and study their swelling ratio, Levofloxacin release rate and antibacterial activity.

2. Materials and Methods

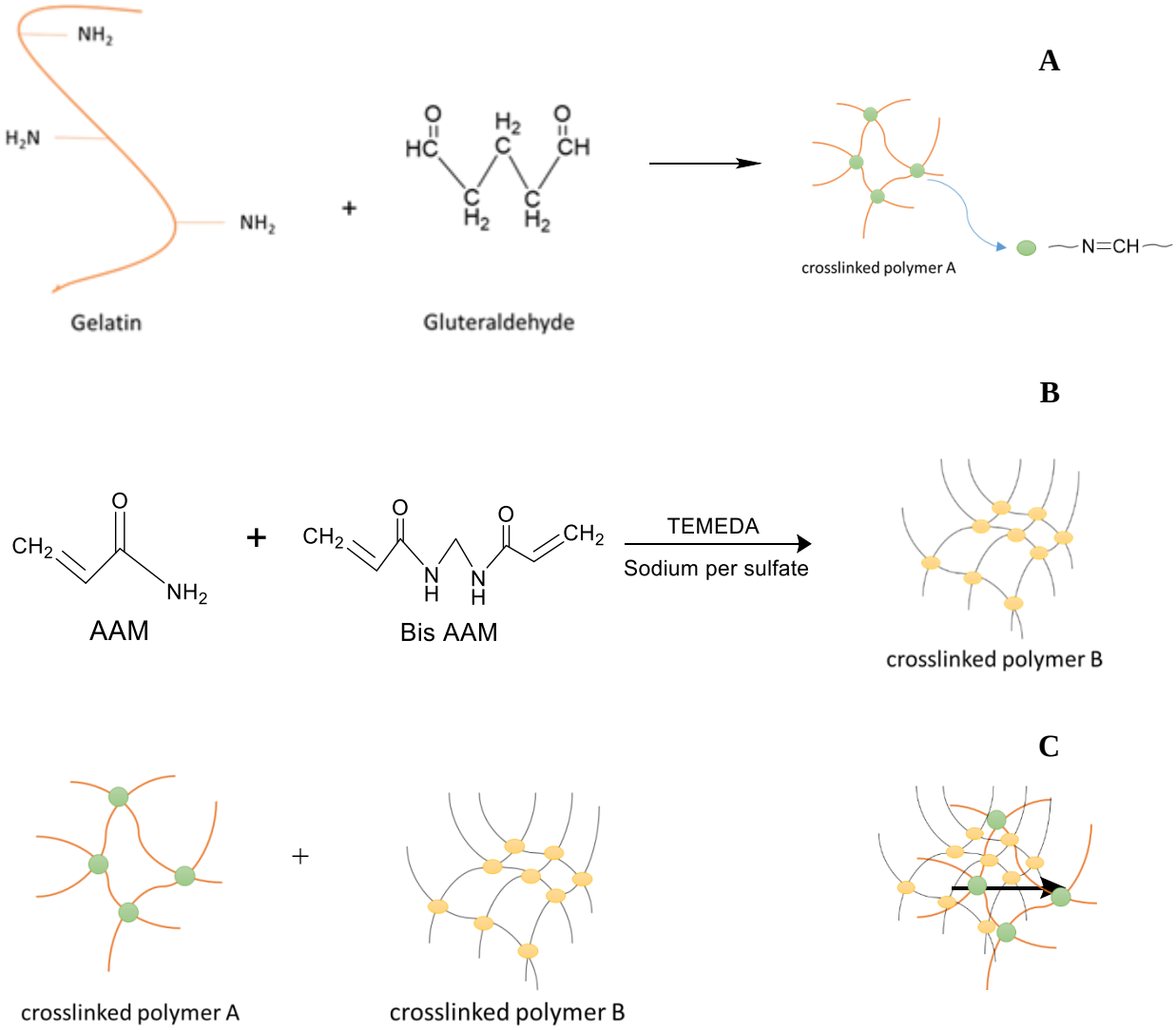
2.1 Chemicals

Chemicals used in this study were obtained from different sources. Levofloxacin was supplied from TEFIC BIOTECH CO., LIMITED. Gelatin powder was supplied from B.D.H. N,N,N',N'-Tetramethylethylenediamine, Sodium per sulfate, Potassium dihydrogen phosphate were supplied from Fluka. Acryl amide, Sodium hydroxide were supplied from Alpha Chemika. N, N'-Methylenebisacrylamide, Gluteraldehyde (% 25) were supplied from Sigma Aldrich. Sodium chloride, Polyethylene glycol 1000 were supplied from Merck. Sulfuric acid was supplied from CHEM-LAB. Hydrochloric acid (%36) was supplied from R.D.H.

2.2 Preparation of hydrogel polymers J1-J6

2.2.1 Preparation of hydrogel polymers J1-J3

Hydrogel Polymer J1 was prepared by gradually dissolving (5 g) of Gelatin powder in (50 ml) distilled water with heating at a temperature less than 50 °C then (1 g) of levofloxacin is added and stirred to obtained the homogeneous solution, 1 ml of glutaraldehyde and three drops of H_2SO_4 were added and heating at 60 ^oC for 30 minutes. The solution from (5 g) of Acrylamide and (0.7 g) of N,N'-Methylenebisacrylamide in (20 ml) distilled water was added with stirring at (35-30°C) then (1 ml) of sodium persulfate (10% W/V) as the initiator for the Acryl amide polymerization process and (0.7 ml) of N,N,N',N'-Tetramethylethylenediamine (TMED) as an accelerating agent for initiator dissociation were added with good mixing for three minutes to completed the polymerization and crosslinking reactions. The resulting gel is cut into pieces close to the weight, rinsed once with water, and dried at 30 °C before being stored in an incubator at 20 °C until used. Schemes 1 were showed the polymerization and crosslinking reactions.



Scheme 1. (A) Crosslinking reaction of Gelatin with glutaraldehyde; (B) Polymerization and crosslinking reaction of Acrylamide with bisacrylamide and (C) Preparation of Interpenetrating polymer networks (IPN Hydrogel)

The IPN hydrogels J2 and J3 were prepared at the same protocol with different monomers ratio, as shown in table 1. Figure 1 shows the composition used in the preparation of IPN hydrogels J1-J3.

Table 1. The composition of the prepared IPN hydrogels J1-J3

Sample	Gelatin (g)	GA (mL)	Drug (g)	AAm (g)	Bis AAm (g)	Ratio Gelatin:AAM
J1	5	1	1	5	0.7	1:1

J2	**5**	**1**	**1**	**10**	**1.4**	**1:2**
J3	**5**	**1**	**1**	**15**	**2.1**	**1:3**

Figure 1. Hydrogel polymers (J1-J3).

2.2.1 Preparation of hydrogel polymers J4-J6

The method established in paragraph (2.2.1) was used to prepare the IPN (J4-J6) and using the same components except that polyethylene glycol was added to the first compound with gelatin. Table (2) shows the composition used in the preparation of IPN hydrogels (J4-J6). Scheme (4) shows the reactions that take place to prepare gel models of this type, while Figure (2) represents these prepared polymeric structures.

Table 2. The composition of the prepared IPNs hydrogels J4-J6

Sample	Gelatin (g)	GA (g)	PEG (ml)	Drug (g)	AAm (g)	Bis AAm (g)	Ratio Gelatin:AAM
J4	5	1	5	1	5	0.7	**1:1:1**
J5	5	1	5	1	10	1.4	**1:1:2**
J6	5	1	5	1	15	2.1	**1:1:3**

Figure 2. Hydrogel polymers (J4-J6).

2.3 Preparation of Simulated Gastric Fluid and Simulated Intestine Fluid

The Simulated Gastric Fluid (SGF) and Simulated Intestinal Fluid (SIF) were prepared. According to the American Medicines Encyclopedia [15].

2.4 Swelling and release studies of prepared hydrogels

The swelling ratio and release rate of the copolymeric hydrogels was studied in different pH at 37°C. The hydrogels species were immersed in distilled water (pH=6.9), SGF (pH=2.1) and SIF (pH=8.2) solutions and at different time intervals. The swelling ratio was calculated according to the following equation [16-18].

$$Swelling\ ratio = \frac{Ws - Wd}{Wd} * 100\%$$

Where W_d = dry weight of hydrogel and Ws = the weight of the swollen hydrogels. The release of the phenolic extract was followed using a UV-vis spectrophotometer with time intervals.

Piece of hydrogel (0.5-0.75 g) were immersed into solutions of SGF, SIF and distilled water. The rate of the Levofloxacin release was followed by UV spectrophotometer (T 80 + Spectrophotometer. PG Instrument Ltd.) at 288 nm in distilled water and 293 nm in SGF solution and 287 nm SIF solution as a function of time.

2.5 Short-toxicity study on Albino rat

2.5.1 Experimental animals

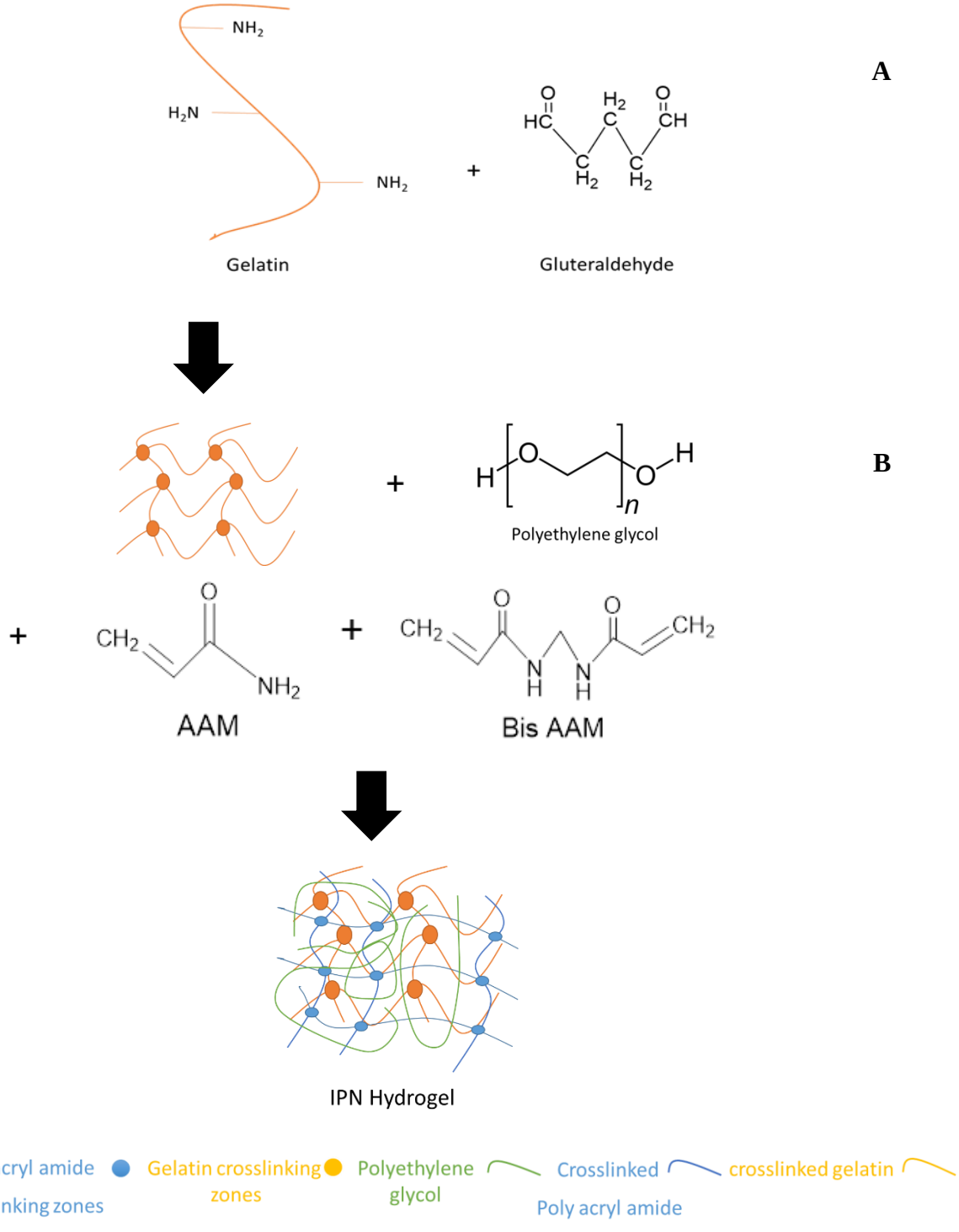
A group of laboratory rats, all males, weighing (150-200) g, and aged about six months, were used for a short-term toxicity study. The animals were housed in polypropylene cages under a cycle (12h light, 12h dark) and under standard laboratory conditions (temperature 27±20C and humidity 55±5%). The rats were permitting unrestricted access to commercial rat food and water while adhering to ethical guidelines and left for a week for acclimatization.

2.5.2 Acute-toxicity study on Albino rat

Six groups of laboratory rats were tested. Each group consisted of three rats (all males) and administered with different doses of hydrogel polymers (J1-J6) loaded with levofloxacin at a rate of (16,18,20) g/kg orally using a metal tube. The seventh group (six rats) was administered with hydrogel polymers (J1-J6) without levofloxacin, and the number of deaths was recorded within (72) hours, while noting the behavioral changes between the groups and the control group.

2.6 Biological activity

The tested for antibacterial activity against two types of bacteria, *Streptococcus mitis*[19] and *Escherichia coli*[20], and the release of levofloxacin was monitored during a period of four consecutive days, and the extent of its effectiveness against this type of bacteria. Disk Diffusion Testing on Mueller-Hinton Agar and Broth microdilution methods were used [21].



Scheme 2. (A) Crosslinking reaction of Gelatin with glutaraldehyde;

(B) Polymerization and crosslinking reaction of Acrylamide with bisacrylamide with Poly ethylene glycol and (C) Preparation of Interpenetrating polymer networks (IPN Hydrogel).

3. Results and discussion

3.1 Drug delivery system

The swelling ratio of the prepared hydrogels was determined as a function of time in distilled water, SFG and SIF at 37°C for 24 hours. The pH of the swelling medium is one of the crucial factors affecting a hydrogel's swelling behavior [15]. The swelling ratio results of hydrogels J1-J3 and J4-J6 are shown in Figure 3.

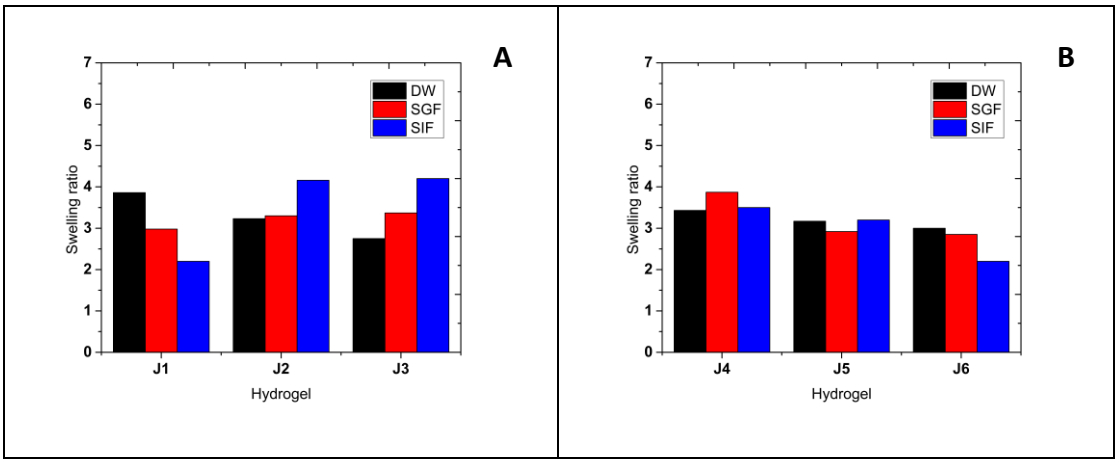


Fig.3 Swelling ratio of hydrogels A) J1-J3 and B) J4-J6 in DW, SGF and SIF

Figure three shows that the swelling ratio increases with the increase in the amount of acrylamide in the polymeric composition due to the ability of acrylamide to absorb large amounts of water. Also, the increase in its swelling ratio in the basic environment due to the partial hydrolysis of the amide groups under the basic conditions and leads to the formation of carboxylate ions that are more amenable to bonding with water.

The swelling ratio was clearly reduced when polyethylene glycol was introduced into the polymeric composition due to the overlap of the polyethylene glycol chains within the crosslinked chains of gelatin and acrylamide, which reduces the free volume between the polymer chains. Also, the formation of hydrogen bonds between the polyethylene glycol chains with gelatin and acrylamide leads to an increase in the physical crosslinking between the polymeric chains, thus reducing the swelling ratio[20]. In SGF, a protonation process of the amine groups of the gelatin will take place, thus increasing the number of the protonation amine groups along the crosslinked gelatin chains, which leads to an increase in the osmotic pressure inside the hydrogel network, which leads

to the swelling of the polymer. In SIF, the carboxylic groups of the gelatin converted to carboxylate groups leading to a decrease in the hydrogen bonding between the polyethylene glycol chains with gelatin and give rise to the swelling ratio increases, as shown in Figure 3 B [21, 22].

3.1.2 Morphology of the prepared hydrogels

The hydrogels (J2) and (J5) were examined by scanning electron microscopy [23]. Figure 4 (A) represents the dry state of the polymeric hydrogel structure (Xerogel) of J2. The figure 4 shows that it has a rough, not smooth, compact surface due to the density of crosslinking, and at the same time, it has nano-clustered structures on its surface due to the presence of gelatin [24]. At the swelling state, many pores appear on its surface, but with relatively small sizes. It seems that some of these pores are formed through top of the nano-clustered structures, while others are formed on the surface of the hydrogel structure as shown in Figure 4 (B). The increased of gelatin within the hydrogel composition compared with acrylamide led to reduces the size of the pores with the swelling state [25,26].

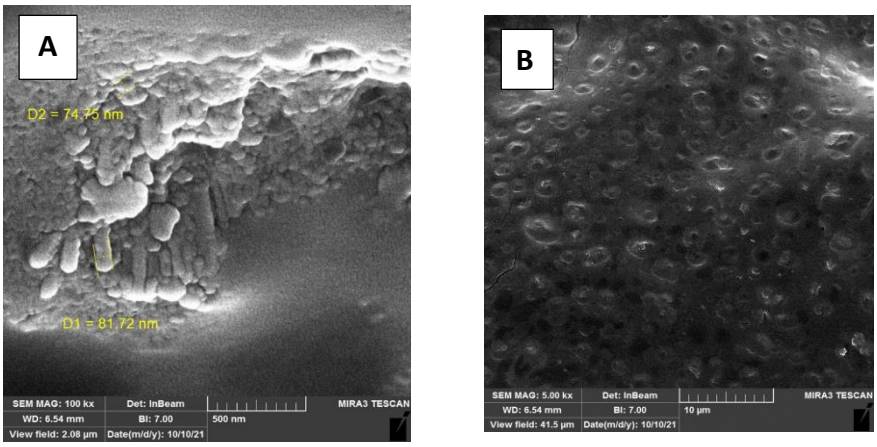


Figure 4. SEM of (A)The dry state (Xerogel), (B) swelling state of the hydrogel J2

When polyethylene glycol was introduced into the hydrogel formula, find that the hydrogel retained the surface roughness, the Nano-clustered structures disappeared and the size of the pores increased significantly, as shown in the Figure (5) for the hydrogel (J5).

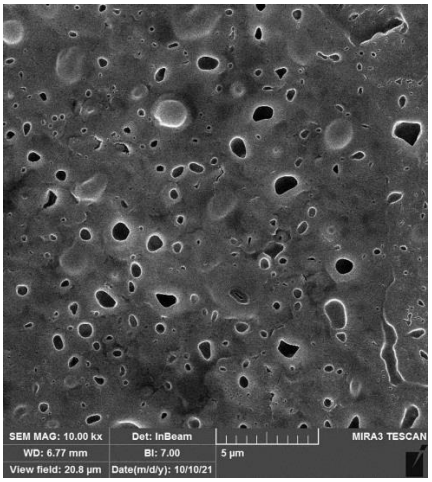


Figure 5. SEM of the swollen state of the hydrogel (J5)

Figure 6 (a-f) shows the release of Levofloxacin at 37 C^o in SGF, SIF and distilled water as a function of time. The obtained results showed that the Levofloxacin release proceeds more efficiently with increasing of acrylamide in the hydrogel. The amide groups do not hydrolyze in the acidic environment and therefore the networks remain in a folded state in the solution and thus the rate of release remains slower, but in the basic medium some groups of amides ($-CONH_2$) will partial hydrolysis to carboxylate ions ($-COO^-$) in the acrylamide chains and lead to electrical repulsion between carboxylate ions leads to a lengthening in the formation of polymeric chains, and therefore more solution will spread between the chains, and accordingly, a higher release rate of the drug will occur[27], for this reason, J1 has the lowest release rate compared with J2 and J3.

The rate of Levofloxacin release from the hydrogels (J4-J6) is somewhat lower compared to the hydrogels (J1-J3) due to the presence of polyethylene glycol and led to increase in the crosslinking density.

The Higuchi release equation is the simplest mathematical equations. It was used to theoretically determine the rate released of levofloxacin as a function of time. Higuchi equation can be represented in the form [28,29]:

$$Q(t) = KH \times t_{1/2}$$

Q(t) is the cumulative percentage levofloxacin release, and KH is the Higuchi dissolution constant. Table 6 shows the Higuchi results with high value of correlation coefficient R^2 which confirmed the release mechanism follows a diffusion control release mechanism [30].

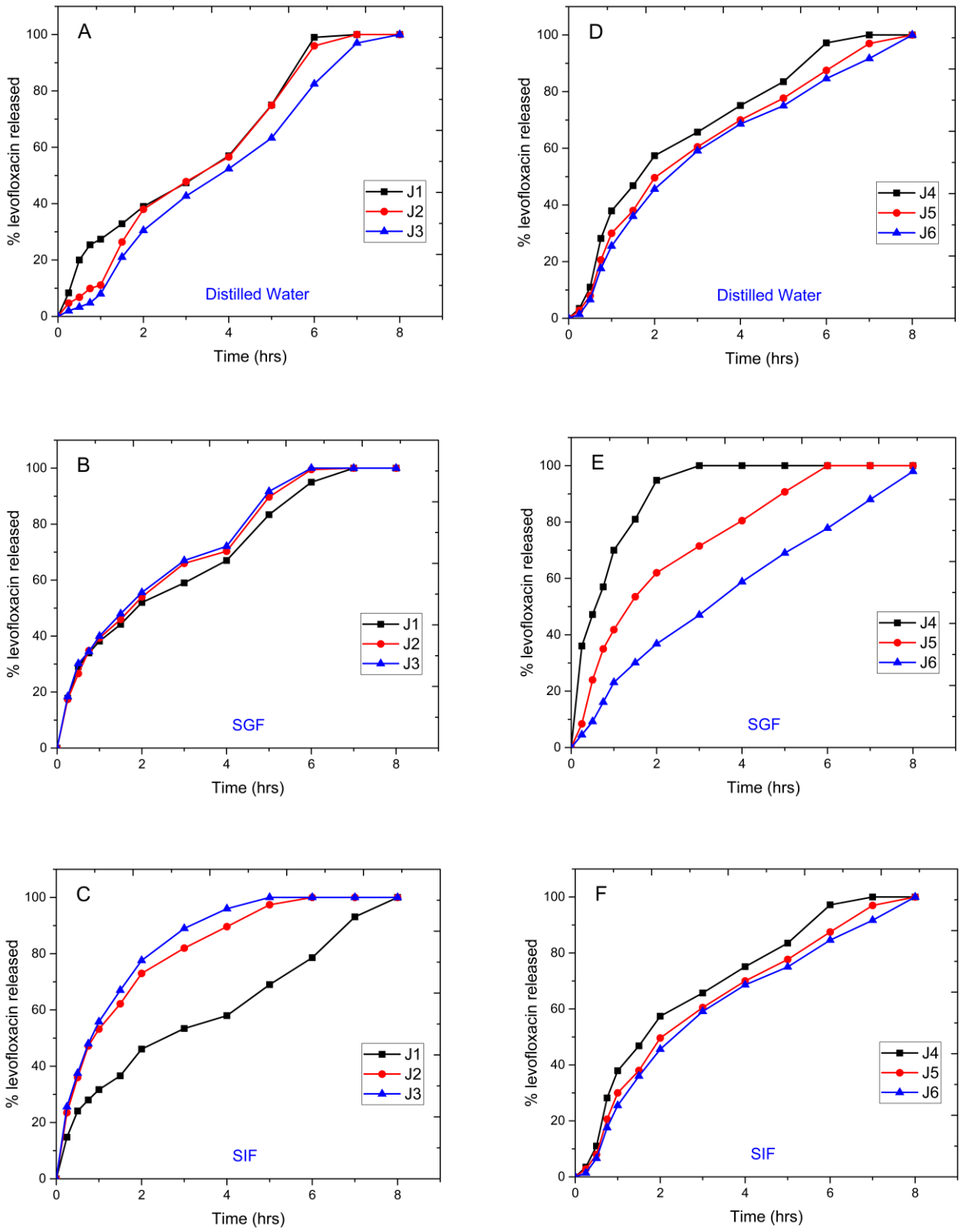


Figure 6. The rate release of levofloxacin from hydrogel J1-J3 (A-C) in distilled water, SGF, and SIF and J4-J6 (D-F) water, SGF, and SIF respectively.

Table 3. Higuchi dissolution constant and correlation coefficient (R2) for released phenolic extract salts from hydrogels (J1-J6) in distilled water, SGF and SIF.

Hydrogel	Distilled water		SGF		SIF	
	K_H	R^2	K_H	R^2	K_H	R^2
J1	38.82872	0.97444	36.54393	0.98953	34.12162	0.9818
J2	42.11291	0.9669	39.10898	0.99008	41.59016	0.96914
J3	40.66996	0.96707	39.93928	0.98947	46.87908	0.97937
J4	42.05222	0.94951	61.33813	0.97925	42.1091	0.97135
J5	40.7316	0.94755	43.09513	0.98188	40.15295	0.97931
J6	40.17952	0.94026	36.98347	0.97674	39.71324	0.97695

Results of Acute-toxicity study on Albino rat

One male rat died during giving a dose of hydrogel polymer J1. At autopsy the lung, appeared hemorrhagic and there was an excess of fluid in the thoracic cavity. These findings are consistent with accidental dosing by the intratracheal route. Apart from this one rat, no deaths or abnormalities in behavior occurred during the study. Rats were weighed over four weeks; there were no statistically significant reductions in body-weight levels in rat. There was obvious fatigue on the rats immediately after giving them the dose and their loss of appetite for four hours and after that, the rats returned to their normal activity. There were no differences or observations between treated and control rats. This indicates that the prepared hydrogel polymers are non-toxic, based on the toxicity classification scheme, which considered the material non-toxic in the absence of deaths in the animals dosed with a concentration (>15 g/kg)[31]. On the other hand, the gelatin and polyacrylamide hydrogels that were used in preparing the compounds are non-toxic and biodegradable[32, 33].

Results of Biological activity

The bacterial activity was studied for four days, Tables 4 and 5 shows the results. It is clear that the release of the Levofloxacin continued for four consecutive days due to the inhibition against bacteria of the two types (*Escherichia coli*) and (*Streptococcus mitis*) was continuous and then gradually decreased. On the other hand, it can be observed from Tables (4) and (5) that the areas of inhibition in general in (*Escherichia coli*) bacteria, began to decrease during the four days compared to

(*Streptococcus mitis*) the reason for this is that the antibacterial targets the bacterial cell wall, and as The wall of bacteria (*Streptococcus mitis*) contains a greater amount of protein, which consists of units called (peptide glycone) and a less amount of (lipoprotein), and thus leads to the ability of levofloxacin to penetrate the cell wall and inhibit bacterial growth, while bacteria (*Escherichia coli*) contains more lipids and less peptide glycone and thus is more resistant to the passage of levofloxacin into the microorganism[34]. Figure 7 and 8 shows the inhibition zone diameter of hydrogels J2 and J6 against *Streptococcus mitis* and *Escherichia coli*.

Table 4. The inhibition zone diameter of hydrogels J1-J3 *against Streptococcus mitis* and *Escherichia coli*

days	J1		J2		J3	
	Mueller Hinton Broth 21g/l Mueller Hinton Agar 38g/l					
	Zone Inhibition (mm)		Zone Inhibition (mm)		Zone Inhibition (mm)	
	E.coli	S.mitis	E.coli	S.mitis	E.coli	S.mitis
First day	30	35	30	40	20	40
Second day	25	45	27	45	20	42
Third day	22	50	25	50	19	43
Fourth day	22	35	20	30	19	29

Table 5. The inhibition zone diameter of hydrogels J4-J6 against *Streptococcus mitis* and Escherichia coli

days	J4		J5		J6	
	Mueller Hinton Broth 21g/l Mueller Hinton Agar 38g/l					
	Zone Inhibition (mm)		Zone Inhibition (mm)		Zone Inhibition (mm)	
	E.coli	S.mitis	E.coli	S.mitis	E.coli	S.mitis
First day	28	45	26	35	25	35
Second day	25	55	24	48	22	50
Third day	23	55	22	47	20	52
Fourth day	23	24	21	30	19	28

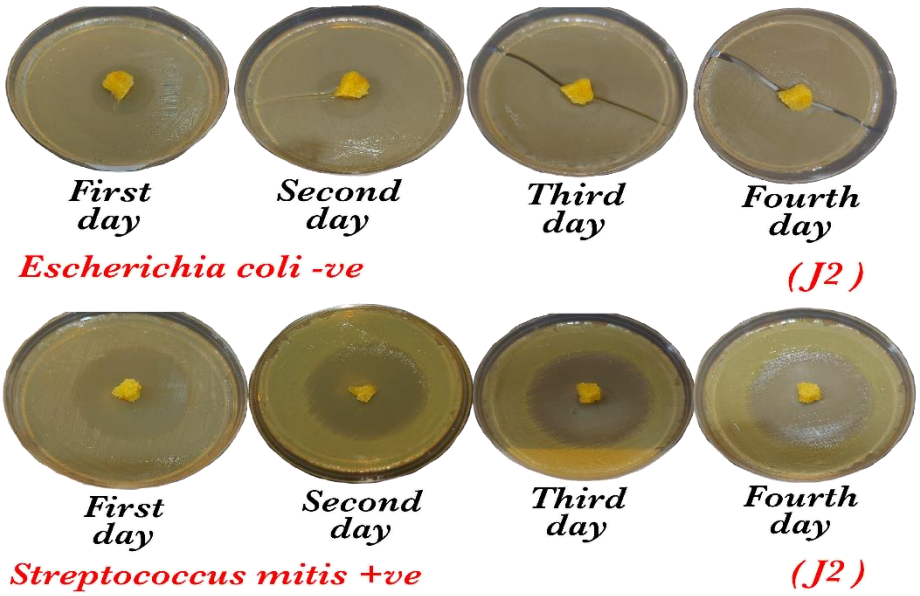


Figure 7. The inhibition zone diameter of hydrogels J2 against *Streptococcus mitis* and *Escherichia coli*

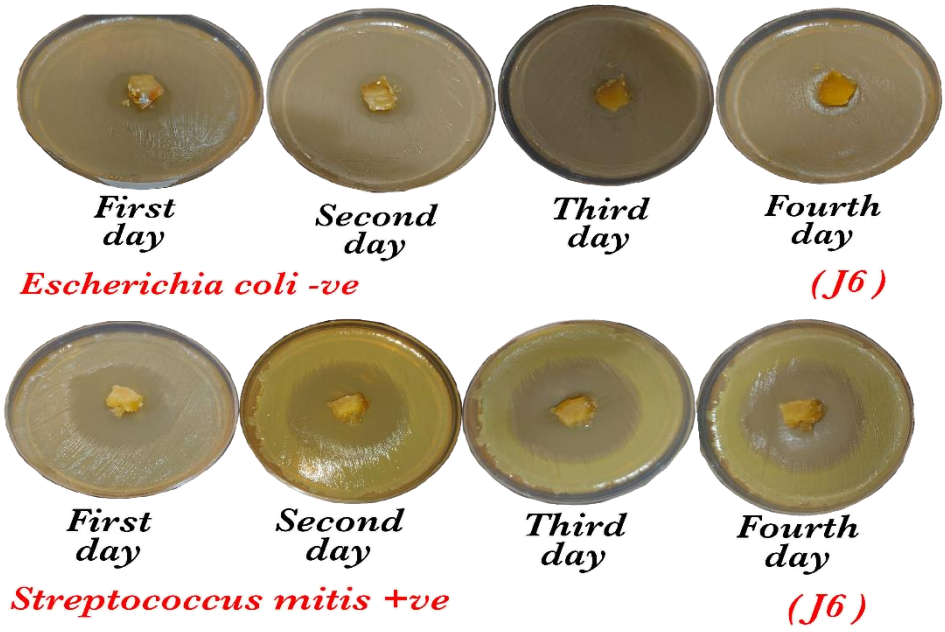


Figure 8. The inhibition zone diameter of hydrogels J6 against *Streptococcus mitis* and *Escherichia coli*

The release and effectiveness of the Levofloxacin released as a function of time were also monitored using the Broth microdilution method by spectrophotometric (turbid metric) analysis. The quantitative of bacterial populations was calculated as mentioned in the literature [19] at 600 nm according to the ratio law and proportionality the numbers of bacteria were extracted with different concentrations as shown in the tables 6 and 7.

Table 6. Numbers of *Escherichia coli* bacteria with different concentrations of the prepared hydrogel polymers

Concentration	Absorbance	Numbers of bacteria CFU
Control	**1.343**	**290**
Stock	**0.003**	**0.648**
1×10^2 dilution	**0.002**	**0.432**
1×10^3 dilution	**0.001**	**0.216**

Control: 10 mL broth solution at constant 1 x 10^6 CFU,

Stock: 0.5 g hydrogel / 10 mL broth solution

Table 7. Numbers of *Streptococcus mitis* bacteria with different concentrations of the prepared hydrogel polymers.

Concentration	Absorbence	Numbers of bacteria CFU
Control	**0.358**	**77**
Stock	**0.023**	**5**
1×10^2 dilution	**0.01**	**2**
1×10^3 dilution	**0.008**	**1.7**

Control: 10 mL broth solution at constant 1 x 10^6 CFU,

Stock: 0.5 g hydrogel / 10 mL broth solution

Figures 9 and 10 show the behavior of the polymer loaded with the drug (Stock) compared with the standard solution (Control) that contains bacteria without hydrogel. The solutions that contain the hydrogel - levofloxacin delivery remain clear compared to the standard solutions which became turbid, indicating the effectiveness of the polymer as an antibacterial for four consecutive days. The effectiveness of the polymer increased by releasing the Levofloxacin because the solutions became more transparent with time periods up to four days.

Conclusion

The swelling behavior of the prepared hydrogel IPNs showed a little effect. Levofloxacin was released with different pH values and distilled water by up to 90% within eight hours. The release of levofloxacin was sufficient to give high activity against two types of bacteria (***Escherichia coli*** **and** ***Streptococcus mitis***) for four days.

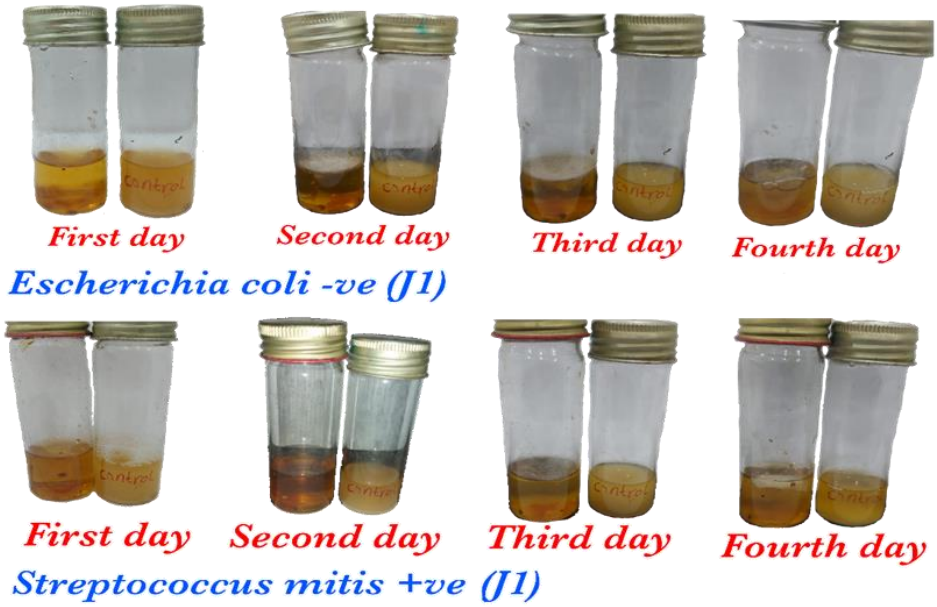


Figure 9. Effect of releasing levofloxacin from hydrogel polymer (J1) as an antibacterial (Broth microdilution)

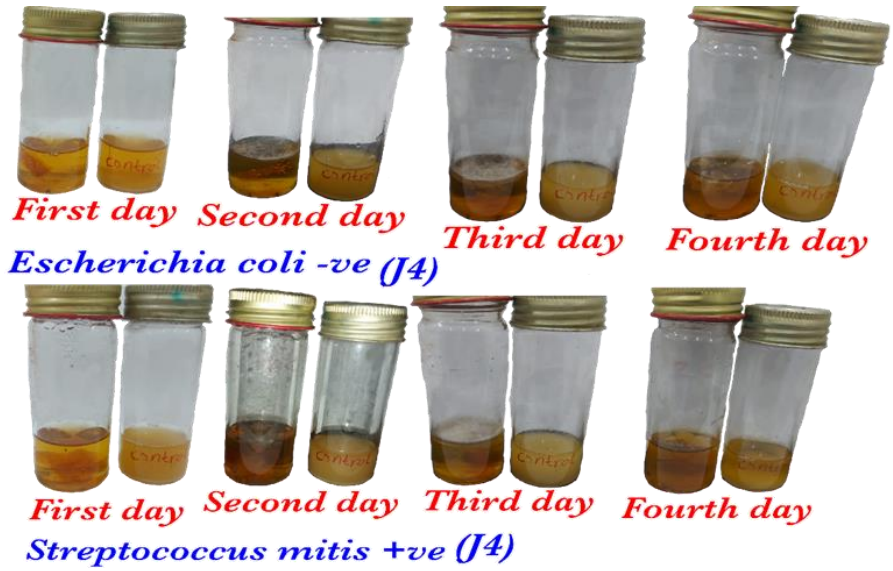


Figure 14. Effect of releasing levofloxacin from hydrogel polymer (J4) as an antibacterial (Broth microdilution).

References

1 Vashist A, Vashist A, Gupta YK and Ahmad S. [2014]: Recent advances in hydrogel based drug delivery systems for the human body. J. of Materials Chemistry B. 2 (10), p 147-166.

2 Manuscript A. [2013]: Polymer Chemistry. ROYAL SOCIENT OF CHEMISTRY. 1 (12), p 1-20.

3 Guo SB and Ma PX. [2015]: Single component thermo-gelling electroactive hydrogels from poly(caprolactone)–poly(ethylene glycol)–poly(caprolactone)-graft-aniline tetramer amphiphilic copolymers. J. Materials Chemistry B. 3 (9), p 8459-8468.

4 Daniela A, Gregor H, Martina T, Eva B, Gibson S and Georg SN. [2016]: Chitosan hydrogel formation using laccase activated phenolics as cross-linkers. J. Carbohydrate Polymers. 2 (6), p 1-27.

5 Bhattarai N, Gunn J and Zhang M. [2010]: Chitosan-based hydrogels for controlled , localized drug delivery. J. Advanced Drug Delivery Reviews. 62 (9), p 83-99.

6 Treesuppharat W, Rojanapanthu P, Siangsanoh C, Manuspiya H and Ummartyotin S. [2017]: Synthesis and characterization of bacterial cellulose and gelatin-based hydrogel composites for drug-delivery systems. J. Biotechnol Reports. 1 (7), p 1-30.

7 Pertici V, Pin-barre C, Rivera C, Pellegrino C, Laurin J, Gigmes D, Trimaille T, Pertici V, Pin-barre C, et al. [2018]: Degradable and injectable hydrogel for drug delivery in soft tissues. J. Biomacromolecules. 1 (12), p 1-34.

8 Hwang CM, Sant S and Masaeli M. [2010]: Fabrication of three-dimensional porous cell-laden hydrogel for tissue engineering. J. Biofabrication. 2 (7), p 1-13.

9 Pesut D, Descotes J, Qadri MF, Malviya R and Sharma PK. [2019]: Biomedical Applications of Interpenetrating Polymer Network System. J. Open Pharmaceutical Sciences. 6 (7), p 21-30.

10 Myung D, Waters D, Wiseman M, Duhamel P, Noolandi J, Ta CN and Frank CW. [2008]: Progress in the development of interpenetrating polymer network hydrogels. J. POLYMERS FOR ADVANCED TECHNOLOGIES. 19 (4), p 647-657.

11 Yu F, Li Y, Huang G, Yang C, Chen C and Zhou T. [2020]: Adsorption behavior of the antibiotic levofloxacin on microplastics in the presence of different heavy metals in an aqueous solution. J. Chemosphere. 260 (6), p 1-11.

12 Gupta H, Aqil M, Khar RK, Ali A, Bhatnagar A and Mittal G. [2011]: Biodegradable levofloxacin nanoparticles for sustained ocular drug delivery. J. drug targeting Cite. 19 (6), p 409-417.

13 Rizwan M, Yahya R, Hassan A, Yar M, Azzahari AD, Selvanathan V, Sonsudin F and Abouloula CN. [2017]: pH sensitive hydrogels in drug

delivery: Brief history, properties, swelling, and release mechanism, material selection and applications. J. Polymers. 9 (4), p 1-37.

14 César C, Cabral LM, Cristina T, Marucci A and Alhaique F. [2003]: Evaluation of new polysaccharides networks for extended-release purposes : mesquite seed gum (MSG), xanthan gum and chitosan. J. Pharmaceutical Sciences. 39 (7), p 274-288.

15 Mitchell J. [2017]: Streptococcus mitis : walking the line between commensalism and pathogenesis. J. molecular oral microbiology. 26 (11), p 89-98.

16 Kaper JB, Nataro JP and Mobley HLT. [2004]: Escherichia coli. J. FEBRUARY. 2 (2), p 123-140.

17 Ula Ali Sadoun, Zainab Shakir Al-Ali and Athir Mahmood Haddad. [2021]: Extraction of phenolic compounds from Iraqi Coriandrum Sativum L. and loaded on copolymeric hydrogels and examine there as drug delivery system and antioxidant. J. Physics: Conference Series. 2063 (1), p 1-17.

18 Abdulwahid, A. A., Alwattar, A. A., Haddad, A., Alshareef, M., Moore, J., Yeates, S. G., & Quayle, P. [2021]: An efficient reusable perylene hydrogel for removing some toxic dyes from contaminated water. J. Polymer International. 70 (1), p 1-12.

19 Morello, Josephine A., Paul A. Granato and HEM. [2004]: Laboratory manual and workbook in microbiology. Elsevier B.V. 1(2), p 1-30.

20 López JE and Peppas NA. [2004]: Effect of poly (ethylene glycol) molecular weight and microparticle size on oral insulin delivery from P(MAA-g-EG) microparticles. J. Drug Development and Industrial Pharmacy. 30 (5), p 497-504.

21 Ooi SY, Ahmad I and Amin MCIM. [2016]: Cellulose nanocrystals extracted from rice husks as a reinforcing material in gelatin hydrogels for use in controlled drug delivery systems. J. Ind Crops Prod. 93 (11), p 227-234.

22 Vishal Gupta N, Satish CS and Shivakumar HG. [2007]: Preparation and characterization of gelatin-poly(methacrylic acid) interpenetrating polymeric network hydrogels as a pH-sensitive delivery system for glipizide. J. Indian Pharmaceutical Sciences. 69(1), p 64-68.

23 Sweah, Zainab J., Hadi S. Al-Lami, and Athir M. Haddad. [2016]: Preparation and Swelling Behavior of L-Lactide Interpenetrating Networks. J. Organic Polymer Materials. 6 (4), p 120-133.

24 Kavoosi G, Bordbar Z and Dadfar SM. [2017]: Preparation and characterization of a novel gelatin–poly(vinyl alcohol) hydrogel film loaded with Zataria multiflora essential oil for antibacterial–antioxidant wound-dressing applications. J. Applied Polymer Science. 134 (10), p 1-9.

25 Torres-garcía R, Flores-estrada J, Cauich-rodríguez J V, Flores-merino M V, Flores-estrada J and Cauich-rodríguez J V. [2020]: Design of a polyacrylamide and gelatin hydrogel as a synthetic extracellular matrix. J. Polymeric Materials and Polymeric Biomaterials. 1 (10), p 1-12.

26 Alwattar, A. A., Haddad, A., Moore, J., Alshareef, M., Bartlam, C., Woodward, A. W., ... & Quayle, P. [2021]: Heavy metal sensors and sequestrating agents based on polyaromatic copolymers and hydrogels. J. Polymer International. 70 (1), p 59-72.

27 Bharti S and Mishra S. [2018]: Controlled drug release behavior of 5- aminosalicylic acid using polyacrylamide grafted oatmeal (OAT- g- PAM): a pH- sensitive drug carrier. J. Polymer Bulletin. 12 (6), p 1-12.

28 Siepmann J and Peppas NA. [2011]: Higuchi equation: Derivation, applications, use and misuse. J. Pharmaceutics. 418 (3), p 6-12.

29 Zaid, A. A., and M. H. Athir. [2018]: Preparation of Polyelectrolyte Hydrogels and Study Their Controlled Release of Gabapentin. J. Drug Designing & Intellectual Properties International. 1 (2), p 1-9.

30 Paul DR. [2011]: Elaborations on the Higuchi model for drug delivery. J. Pharmaceutics. 418 (10), p 13-17.

31 Curtis D. Klaassen. [2008]: CASARETT AND DOULL'S TOXICOLOGYTHE BASIC SCIENCE OF POISONS. Seventh Edition. 2 (1), p 1-13.

32 Curcio M, Altimari I, Gianfranco U, Cirillo G, Vittorio O, Puoci F, Picci N and Iemma F. [2013]: Biodegradable gelatin-based nanospheres as pH-responsive drug delivery systems. J. Nanopart Res 15 (11), p 113-1581.

33 Rai N, Bal T and Swain S. [2020]: In vitro Evaluations of Biodegradable Polyacrylamide Grafted Moringa Bark Gum Graft Copolymer (MOG-g- PAAM) as Biomedical and Controlled Drug Delivery Device Synthesized by Microwave Accelerated free Radical Synthesis. J. Pharmaceutical Education and Research. 54 (2), p 385-396.

34 FidelmaBoyd E. [2012]: Bacteriophage-Encoded Bacterial Virulence Factors and Phage–Pathogenicity Island Interactions. J. Medicinal Chemistry. 82 (6), p 91-118.