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Synthesis and Characterization of Smart Hydrogel in Drug Delivery System

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Abstract: *Hydrogels are three-dimensional, porous polymeric skeletons with a high hydrophilic structure that enables them to retain large quantities of water or biological fluids while remaining mechanically stable due to cross-linking. This research focuses on the synthesis and characterization of smart poly(acrylamide) hydrogels specifically for the controlled delivery of the highly water-soluble antibiotic Levofloxacin. The hydrogels were synthesized via a free-radical polymerization mechanism using acrylamide as the monomer, N, N-methylene-bis-acrylamide (MBA) as the crosslinker, and a redox initiator pair consisting of potassium persulphate and sodium metabisulfite. Successful synthesis and structural integrity were confirmed through FTIR spectroscopy, SEM morphology analysis, and TGA thermal stability studies, which revealed a uniform and smooth surface structure. Experimental results demonstrate that the swelling capacity of the synthesized hydrogels is highly sensitive to external stimuli, including pH, temperature, and ionic strength. Maximum swelling was observed in alkaline conditions (pH 12.0 and pH 7.4) and at higher temperatures (37°C), while swelling decreased as the concentration of the crosslinking agent increased. Furthermore, the presence of salt solutions (NaCl, $CaCl_2$, and $AlCl_3$) reduced swelling due to the osmotic pressure screening effect.*

In vitro drug release studies using Levofloxacin as a model drug showed significant pH-dependent release profiles: approximately 99.92% of the drug was released in a phosphate buffer at pH 7.4, compared to only 16.82% in an acidic medium of pH 1.2. Kinetic modeling indicated that the release mechanism follows the Higuchi model. Additionally, drug-loaded hydrogels exhibited effective antibacterial activity against both S. aureus and E. Coli. These findings suggest that these smart poly(acrylamide) hydrogels are promising candidates for controlled drug delivery in pharmaceutical and biomedical applications

Keywords: Smart hydrogels, Poly(acrylamide), Levofloxacin, Drug delivery, pH-sensitive, Free-radical polymerization, Higuchi model

I. INTRODUCTION

1.1. DEFINITION:

Hydrogels are a solid-fluid continuum where the solid is a polymeric material, and the fluid is water. They are also referred to as colloidal gels, where polymeric chains and water are considered as the dispersed phase and dispersion medium, respectively. Certain researchers identify hydrogels as water-swollen physically or chemically cross-linked macro-molecules of the same or different monomers (Ahmed, 2015a). Others describe the swelling of hydrogels as a volumetric growth process wherein additional fluid occupies the pores in the hydrogels. According to Buwalda et al. (2014), the term hydrogel was coined by Bemmelen (1894), which represents the colloidal gel of inorganic salts. The first commercial hydrogels, PHEMA (poly(2-hydroxyethyl methacrylate)) gels for soft contact lenses, in the biomedical field were developed by Wichterle and Lim (1960).1,2

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1.1.1. Properties of hydrogels:

Hydrogel's properties are highly dependent on the environmental conditions and their compositions. Here, the properties mentioned are considered at laboratory conditions (room temperature 25◦ C) subject to a certain time period of nearly 24 hours.3

In general, hydrogels possess the following properties

- Hydrogels' three-dimensional porous polymeric skeleton contains a hydrophilic structure, which helps them to hold a large amount of water. They can also release water in a controllable manner.
- Hydrogels are mechanically stable due to the cross-links; thus, they do not dissolve in water.
- Hydrogels are soft, lightweight, and flexible. They can undergo large deformation.
- Hydrogels are responsive to the external environment or stimuli such as pH, light, ions, and electric and magnetic fields.
- Transport or diffusion of water inside the gel differentiates them from the elastomers.
- Hydrogels provide frictionless and moist surfaces.

1.1.2. Characteristics of hydrogels:

The characteristics of hydrogels are as follows:

Water holding capacity

The water holding ability and permeability are the most important characteristic features of hydrogels. The polar hydrophilic groups first hydrated on contact with water and leads to the formation of primary bound water. Thus, the network swells and exposes the hydrophobic groups, capable of interacting with water molecules.

Flexibility

The capacity to retain a large amount of aqueous content and selective diffusion of solute particles makes hydrogel flexible. These properties of hydrogels resemble with biological tissues. Hydrogels are porous in nature. The porosity of the hydrogels permit the loading of drug into gel matrix and subsequent release at predetermined rate and specific time intervals.

Biodegradable

Biodegradable hydrogels contain labile bonds hence, used in different fields such as tissue engineering, wound healing and drug delivery. These bonds are present either in the polymer backbone. The labile bonds are broken either by enzymatically and chemically.

Biocompatible

Biocompatibility is another most important characteristic property of the hydrogels. Hydrogels possess a good biocompatibility and hydrophilic surface has a low interfacial free energy when in contact with body fluids. It results in a low tendency for proteins and cells to adhere the surfaces. Moreover, the soft and rubbery nature of hydrogels minimises the irritation to surrounding tissue. The cross-linking between the different polymer chains results in viscoelastic and elastic behaviour which provide gel its structure (hardness), elasticity and contribute to stickiness. Hydrogels due to their significant water content possess a degree of flexibility similar to natural tissue.

Swelling

Hydrogels are cross-linked polymer networks swollen in a liquid medium. Swelling is the property to absorb and retain water for a relative long time. It is evaluated by measuring the dry weight and swollen state weight and computed either from water uptake or volume of adsorbed solvent. The imbibed liquid serves as a selective filter to allow free diffusion of solute molecules, while the polymer network serves as a matrix to hold the liquid together. Hydrogels swells about 10-20% (an arbitrary lower limit) to thousands of times to their dry weight in water.4,5

1.1.3.1. Classification:

Hydrogels are generally classified into two categories as follows:

1) Permanent or chemical hydrogel: These gels are cross-linked network with covalent bond. They attained an equilibrium swelling state depending on the polymer-water interaction and crosslink density.

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2) Reversible or physical hydrogel: These gels are held together by molecular forces such as ionic, hydrogen bonding or hydrophobic interactions. The dissolution is prevented by physical interactions which exist between different polymer chains. In these hydrogels the interactions are reversible and disrupted by changes in physical conditions.8,9

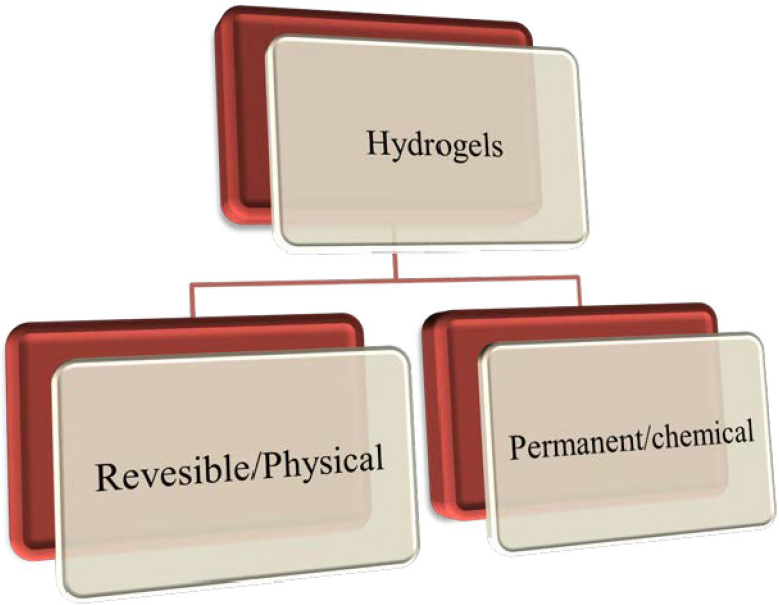

Fig. 1.3: Classification of hydrogels

1.1.3.2. Classification based on cross-linking:

Based on the cross-linking network, hydrogels were classified into two main categories: (a) physically cross-linked hydrogel, (b) chemically cross-linked hydrogel. Different types of hydrogel were prepared from natural/ synthetic polymers for numerous applications in a different field (Table 1). Classification of hydrogel based on the cross-linking type is discussed in detail one by one.10

A. Physically cross-linked hydrogel:

In recent days, hydrogel based on physical cross-linking network attracted more attention because of their easy production and the other factors, which plays a significant role i.e. not using the cross-linking agents during the synthesis of the material. The physically cross-linked hydrogel is generally found insoluble because of the physical interaction between polymeric chains. The selection of hydrocolloid type depends on some factors that include the concentration of polymer/monomer, pH of the medium, which leads to the formation of a broad variety of gel textures. Physically cross-linked hydrogels found a significant role in various applications such as in food industry, pharmaceutical and biomedical applications due to no cross-linking agent was used during the synthesis protocol.11

Physically cross-linked hydrogel involves the various method such as follows:

- Freeze-thawing
- Stereo complex formation
- Ionic interaction
- Hydrogen Bonding

Detail discussion of the methods are as follows:

(a) Freeze-thawing

The freeze-thaw cycle is an important method for the development of physically cross-linked hydrogel. This synthesis involves the formation of the microcrystals in the network because of freezing and thawing. Hydrogels based on

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poly(vinyl alcohol) (PVA) is a popular example of hydrogel which is prepared by the freeze-thawing method. PVA hydrogels are found interconnected because of H-bonding, and exhibiting improved properties such as more porous, spongy, rubbery, and higher elastic properties than the PVA hydrogel that is synthesized by any other method. In the present era, such types of hydrogels were implemented in biotechnology fields especially in molecules (protein, peptides) and whole cell immobilization.

(b) Stereo complex formation

In this type of hydrogel formation grafting of the monomer on the polymeric backbone in aqueous medium taken place. The major advantage of these types of the hydrogel is the easy formation of a hydrogel network that is formed by simply dissolving each product in water and mixed them in solution. One of the best examples of the stereo complex hydrogel formation is the hydrogel of polylactic acid (PLA). The abilities of the PLA hydrogel was explained by Tsuji et al. Hydrogel-based on stereo complex used mainly for drug delivery system but there is a limitation of stereo complex based hydrogel which restrict its application and that have a relatively restricted range of polymer composition, which can be used for the synthesis of the hydrogel.

(c) Ionic interaction

Hydrogel formed via ionic interaction by the addition of di- or trivalent counter ions which allowing the cross-linking between the polymeric chains. This mechanism involves the principle of gelation of polyelectrolyte solution with multivalent ions of opposite charges. The Chitosan-glycerol phosphate salt and poly-[di (carboxylatophenoxy) phosphazene] calcium salt are the examples of hydrogel formed from ionic interaction.

(d) Hydrogen Bonding

Hydrogen bonding plays an important role in the physically cross-linked hydrogels. The various properties of the hydrogel become improved such as swelling ratio, self-healing, and mechanical strength because of the formation of H-bonding. The best example of hydrogel which is formed by H-bonding is the hydrogel based on Carboxymethyl cellulose which is formed by dispersing in 0.1M HCl, during the gelation sodium ions of CMC were replaced by hydrogen in the acid.12

B. Chemically cross-linked hydrogels:

The chemically cross-linked hydrogel is formed through covalent bond existing between polymeric chains. Since the covalent bond is a stable bond and makes a rigid structure which, does not allow to dissolve in any solvent unless the covalent crosslink is cleaved. To design physically cross-linked hydrogel many strategies are followed with many restrictions due to difficulty in decoupling of the variables, like gelation time, porosity, chemical modification and degradation time. While hydrogel formed through chemical cross-linking exhibited the improvement in different properties such as mechanical strength, elastic nature, more stability and have higher degradation time, etc. In the literature, various methods are reported for the synthesis of chemically cross-linked hydrogel which is as follows:

(a) Chemical cross-linking

Hydrogels formed by chemical cross-linking involves the use of cross-linker agents such as glutaraldehyde, epichlorohydrin, adipic acid dihydrazide and polyaldehydes, borax etc. for the various synthetic and natural polysaccharide-based hydrogel formation. Covalent linkage such as Schiff's base formation between the amino group of chitosan and carbonyl group of aldehyde leads the formation of chemically cross-linked hydrogel with good reactivity.

(b) Grafting

Synthesis of the hydrogels based on the grafting method includes the polymerization of the monomeric moiety on the backbone of the polymer in the presence of the initiators; further grafting is classified into two types chemical grafting or radiation grafting.

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(i) Chemical grafting

Polymeric backbone is activated by the chemical reactions, where a chemical reaction takes place during grafting occurred. For example, the grafting of acrylic acid on to the granular guar gum in an aqueous medium in the presence of redox imitator is an example of chemical grafting.

(ii) Radiation Grafting

Radiation grafting involves the initiation of grafting through high-energy radiation like gamma and electron beam. The best example of radiation grafting is the grafting of acrylic acid on to the Carboxymethylcellulose in the presence of electron beam radiation in an aqueous medium.

(c) Radical polymerization

Chemically cross-linked hydrogels can also be synthesized from the low molecular weight monomers in the presence of different cross-linking agent via radical polymerization methodology. This is the methodology which is being used to prepare most of the hydrogels, through the radical polymerization in the presence of an initiator such as ammonium persulfate or potassium peroxide, the formation of the hydrogel takes place in very less time even in mild conditions.

(d) Condensation reaction

When the small molecule is removed from the reaction mixture is known as a condensation reaction. The hydrogel formed after removal of the small molecule is categorized in this type. The hydrogels having the amine/ hydroxyl group with the carboxylic acid and their derivative are utilized to form this type of hydrogels. De Nooy et al. reported the hydrogel via Passerine and Ugi condensation reactions, which is a good example of condensation reaction to form hydrogels. The Passerine condensation reaction involves the formation of hydrogel with ester bond formation in their cross-linking. In the above case, the condensation reaction between carboxylic functional group and carbonyl functional group of the compounds are allowed to condense to produce α-(acryloxy) amide. In Ugi condensation reaction amine is added in the reaction mixture to produce α-(acrylamino) amide this involves the amide bonds in their cross-linking sites.12,13

(e) Enzymatic reaction

The hydrogel can be synthesized by using an enzymatic reaction, for example, Sperinde et al. reported a PEG-based hydrogel by enzymatic reaction. During this process, functionalization of the glutamine group was carried out; and the PEG network was formed after the addition of transglutaminase in the aqueous solution of PEG-Qa and poly (lysine-co phenylalanine). In this type of enzymatic reaction, an amide linkage was formed between the polymer chains due to the reaction between the γ-carboxamide group of the PEG-Qa and the ε-amino group of lysine.

(f) High-energy radiation

The High-energy radiation is used to polymerize the compounds having the double and triple bond by utilizing the high radiations like gamma & electron beam radiations. When the vinyl group is exposed in the gamma radiation & electron beam radiation, it forms radically on the polymeric chain by the hemolytic cleavage. Formation of the micro radicals was taken place due to the facilitation of water molecule by high energy radiations. The developed micro radicals were recombined on to the different polymeric chain and form a covalent bond, which leads to the formation of a cross-linked network. This method was found to be quite efficient because the reaction can be carried out in water under mild conditions such as room temperature and physiological pH in the absence of harmful cross-linking agents. Some best examples of hydrogels synthesized by radiation methodology are as follows: formation of (viny1 methyl ether) (PVME) and poly (N-isopropyl acrylamide) (PNIPAAm) hydrogels.14

II. EXPERIMENTAL METHODS

Controlled drug release of levofloxacin from poly (acrylamide) hydrogel

Materials and Methods

Materials

Sodium metabisulfite was received from Avra Synthesis Pvt. Ltd, Hyderabad, India. Potassium persulphate, methylenebisacrylamide, acrylamide, Potassium dihydrogen orthophosphate and sodium hydroxide were obtained from

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SDFCL, Mumbai, India. Hydrochloric acid, disodium hydrogen phosphate anhydrous were from Merck, Mumbai, India. Levofloxacin hemihydrate was gifted from Micro labs limited, Bangalore, India.

Poly(acrylamide) hydrogel synthesis

Poly(acrylamide) hydrogel was synthesized by a free radical mechanism. Primarily, redox initiators of potassium persulphate (45 mg) and sodium metabisulfite (32 mg) were shifted into a glass vial containing 10 ml of deionized water. Then, add acrylamide (600 mg), allowed to stir for 10 minutes at room temperature after this crosslinker methylenebisacrylamide (06 mg) was added. Then, this composite was kept in a water bath until the gel was formed. The synthesized gel was washed with water to remove unreacted components. Then, the hydrogel was dried at 50o C in the oven for 24 hours.

FT-IR analysis

Levofloxacin, poly(acrylamide) hydrogel and levofloxacin-loaded hydrogel spectra were recorded using an FT-IR spectrometer (Shimadzu ATR) in the range of 400 to 4000 cm−1 to determine their intermolecular interactions and structure.

TGA analysis

To determine the thermal stability of poly(acrylamide) hydrogel was performed using a thermogravimetric analyzer (Perkin Elmer STA 600) by increasing the heating rate to 20oC.

Morphological examination

The morphology of poly(acrylamide) hydrogel structures was determined using SEM (scanning electron microscope). Hydrogel composites were cut to expose their structure and imaged in an (SEM Zeiss, LS15) scanning electron microscope.

Swelling study of poly(acrylamide) hydrogel

The swelling study of synthesized poly(acrylamide) hydrogel was determined using dry samples in acidic buffer pH 1.2 and phosphate buffer pH 7.4. The pre-weighed hydrogel samples were immersed in solutions at 37oC for swelling. At periodic intervals, the swollen samples were taken out from the solution and excess droplets on the surface of the hydrogel were withdrawn by wiping with filter paper and then weighed. The swelling (%) of hydrogel was determined from the following equation. Similarly, the swelling (%) was observed at 27oC in pH 7.4 with time intervals.

$$\textbf{Swelling}\ (\%) = \textbf{Wb} - \textbf{Wa}\ /\textbf{Wa}\ \textbf{x}\ \textbf{100} \ \ldots\ldots\ldots\ldots\ldots\ (\mathbf{4.1})$$

Where,

Wa and Wb are the weight of dry and swollen gel.

Construction of Levofloxacin calibration curve

The stock solution of 1000 mg/l of Levofloxacin drug solution was prepared using distilled water as a solvent, then 2, 4, 6, 8 and 10 mg/l solutions were prepared by dilution of the stock solution. Using a UV-9000A spectrophotometer (Shanghai Metash), scan the solutions between 200 to 400 nm and the absorption maximum was recorded to construct the calibration curve.

Drug loading and drug release studies

Levofloxacin hemihydrate was selected as a model drug. The loading of the drug was conducted in a 1000 mg/l solution using water as a solvent. Place 0.1 g of dry hydrogel in 100 ml levofloxacin solution. The loaded hydrogel was dried and note down the loaded hydrogel weight. The in-vitro release study was conducted by placing drug-loaded hydrogel in acidic buffer pH 1.2 and phosphate buffer pH 7.4 at 37oC and 27oC respectively using the paddle method in the dissolution test apparatus (LabIndia, DS-8000, India). Next withdraw dissolution medium sample at regular time intervals (30 minutes) with stirring and replace fresh solution to maintain constant dissolution media. Using a UV-Visible spectrophotometer, scan the solutions between 200 to 400 nm with suitable dilution and note down the λmax

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absorbance. The percentage of released levofloxacin was calculated and its corresponding drug release graph was plotted. Similarly, effects of temperature on drug release has been studied at 27oC and 37oC respectively in pH 7.4.

Kinetics model drug release

The kinetics models of drug release will determine using Zero-order, firstorder, Hixson-Crowell, Higuchi and Korsmeyer-Peppas models.

Antibacterial study

The synthesized hydrogel and levofloxacin drug-loaded hydrogel were used for the in-vitro antibacterial test by using the agar diffusion method. For this purpose, 5 µg/ml of levofloxacin drug, levofloxacin drug-loaded hydrogel and synthesized hydrogel were prepared in distilled water and each 0.1 ml loaded on nutrient agar plates. After 24 hours of incubation at 37°C the inhibition zones were calculated.

Results and Discussion

Poly(acrylamide) hydrogel was synthesized by a radical polymerization method and its swelling study was performed. Moreover, drug loading and drug release were also performed using levofloxacin as a model drug and the effect of pH, temperature and time of the drug release will also be studied.

Hydrogel preparation

The various steps involved in the preparation of Poly(acrylamide) hydrogel are shown in Fig. 6.2. After the gel preparation, the swelling study was performed.

FT-IR analysis

The FT-IR spectra of levofloxacin, poly(acrylamide) hydrogel and levofloxacin loaded poly(acrylamide) hydrogel were recorded are shown in Fig. 6.3. The characteristic peaks of levofloxacin (Fig. 6.3a) were found at 3243 cm−1and 1439 cm−1(stretching and bending vibrations of the carboxylic acid -OH group), 2849 cm−1 and 1618 cm−1 (C-H stretching of -CH3 and aromatic rings respectively), 1712 cm−1 (C=O stretching of the cyclohexanone), 1289 cm−1 (C-F stretching peak). The poly(acrylamide) hydrogel spectra (Fig. 4.3b) show the peaks at 3348 cm−1 (-NH stretching of hydrogel), 1648 cm−1 (C=O stretching). The levofloxacin-loaded poly(acrylamide) hydrogel spectra (Fig. 4.3c) show the peaks at 3348 cm−1 (-NH stretching of hydrogel), 3196 cm−1 (carboxylic acid -OH stretching of levofloxacin), 1667 cm−1 (C=O stretching), 1616 cm−1 (C=C stretching), 1296 cm−1 (C-F stretching), 1209 cm−1 (C-N stretching). The presence of amide, fluoro and carbonyl groups in loaded hydrogel confirms the drug loading in acrylamide hydrogel.

TGA analysis

The thermogram of poly(acrylamide) hydrogel was shown in Fig. 4.4. The first stage of weight loss, consider as loss of moisture present in the hydrogel was observed at 169ºC with a mass loss of 3.4% then degradation occurred at 179ºC with weight loss of 9.6% for the elimination of ammonia gas from amide groups of poly(acrylamide) chains and maximum weight loss occurred at 381ºC with mass loss of 24.4% due to cleavage of the polymer chain and cross-linked network in hydrogel.

Morphological examination

The surface morphology of synthesized hydrogel was studied by SEM. The micrographs Fig. 6.5(a) and Fig. 6.5(b) reveal that the surface is uniform and smooth in nature.

Swelling study of poly(acrylamide) hydrogel in pH solutions

The swelling study of the synthesized poly(acrylamide) hydrogel was performed in pH 1.2 and 7.4. The swelling study results of pH 7.4 are tabulated in the Table 6.3 and their corresponding graphical representation is showed in Fig. 6.6. The comparison of poly(acrylamide) hydrogel swelling (%) in pH 1.2 and 7.4 at 37o C is showed in Fig. 6.7 and their corresponding statistical data was given in Table 6.1. From these results, a higher swelling rate was observed at pH 7.4 when compared to the pH 1.2. In an acidic medium of pH 1.2, the ammonium groups (NH3+) are formed by protonation but due to the presence of chloride (Cl−) counterions the swelling decreased drastically. However, at pH

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7.4 the (-CONH2) and (-CONH-) groups are deprotonated and the presence of sodium (Na+) ions in the solution will produce high osmotic swelling pressure hence showing maximum swelling. Similarly, for temperature sensitivity the swelling study was conducted at temperatures of 27o C and 37o C respectively. The results are tabulated in the Table 6.2 and their corresponding graphical representation is showed in Fig. 6.8. The results showed that when the temperature increases swelling (%) also increases.

Construction of Levofloxacin calibration curve

Drug selection for the drug loading and drug release is most important because it should not react with hydrogel and solvents. This helps to avoid the λmax shift. Levofloxacin drug is better material because no change was observed in the λmax over time. Using a UV-Visible spectrophotometer, the solutions were scanned between 200 nm to 400 nm. The absorption maximum were recorded at λmax 286 nm. The results are tabulated in the Table 6.4 and their corresponding graphical representation is showed in Fig. 6.9 and 6.10. From the calibration curve, the slope and intercept are found to be 0.144 and 0.017 respectively and the correlation coefficient (R2) is 0.999.

Levofloxacin drug release study from poly(acrylamide) hydrogel

Levofloxacin drug release study in pH 1.2 and 7.4

The swelling studies of the synthesized hydrogel are carried out in the solution of pH 1.2 (acidic buffer) and pH 7.4 (phosphate buffer). The result showed that the maximum swelling % 3590.25 in pH 7.4 and the minimum swelling % 117.50 in pH 1.2. The results are tabulated in the Table 4.1 and their corresponding graphical representation is showed in Fig. 6.7. The drug-loaded hydrogels were placed in the solutions of pH 1.2 and pH 7.4. About 99.92 % of levofloxacin drug was released in the solution of pH 7.4, whereas only 16.82% of drug was released in the solution of pH 1.2. The results are tabulated in the Table 6.5 and their corresponding graphical representation is showed in Fig. 6.11. Hence, we conclude that drug release depends on the pH of the solution because the swelling (%) is more in pH 7.4 than in the pH 1.2. The drug release from hydrogel into solution depends on swelling and the controlled release of levofloxacin was observed for up to 6 hours.

Levofloxacin drug release study at different temperature

The effect of temperature on Levofloxacin drug release was studied at temperatures of 27oC and 37oC respectively. The results are tabulated in the Table 6.6 and their corresponding graphical representation is showed in Fig. 6.12. About 71.69% of drug has released at 27o C and at 37oC 99.92% of drug has been released. This indicates that as the temperature increases, the drug release also increased. When the temperature increases, the hydrogel network flexibility also increases, as a result more amount of buffer solution enters into hydrogel which releases more amount of drug.

Kinetic model for drug release

The kinetic model of drug release has been studied by using various mathematical models like Zero-order, first-order, Hixson-Crowell, Higuchi and Korsmeyer-Peppas models. The obtained results are given in Table 6.7 and their corresponding graphical representation is showed in Fig. 6.13. The observed maximum correlation coefficient (R2) is 0.967 for the Higuchi model; hence the synthesized poly(acrylamide) hydrogel follows the Higuchi model.

Antibacterial activities

The levofloxacin drug-loaded hydrogel was investigated for in-vitro antibacterial effect. Fig. 6.14 illustrated that Levofloxacin (L) pure drug and levofloxacin drug-loaded hydrogel (LLH) had an antibacterial effect on S. aureus and E. coli bacteria and no effect at unloaded hydrogel (H) after 24 hours incubation at 37°C. After measuring the inhibition zone the levofloxacin pure drug exhibited 11mm and 10mm for the levofloxacin drug-loaded hydrogels against S. aureus (Fig 6.14a) and 12mm and 10mm against E. coli, respectively (Fig. 6.14b). As the result showed, levofloxacin pure drug and levofloxacin drug-loaded hydrogel shows antibacterial activity because of the bioactivity of the drug but the unloaded hydrogel will not show a zone of inhibition compared to pure drug and drug-loaded hydrogel. This effect could be due to the presence of levofloxacin in drug-loaded hydrogel.

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Table 6.1. Statistical data of the poly(acrylamide) hydrogel swelling(%) in pH 1.2 and 7.4

Time (min)	pH 1.2 Swelling (%)	pH 7.4 Swelling (%)
0	0.00	0.00
360	113.33	637.01
720	121.66	1367.53
1080	125.83	1760.38
1440	129.16	2272.72
1800	127.50	2673.37
2160	120.83	3331.16
2520	118.33	3588.96
2880	117.50	3590.25

Table 6.2. Statistical data of the poly(acrylamide) hydrogel swelling (%) in a pH 7.4 at 27°C and 37°C.

Time (min)	27°C Swelling (%)	37°C Swelling (%)
0	0.00	0.00
360	547.02	637.01
720	1036.75	1367.53
1080	1353.51	1760.38
1440	1547.02	2272.72
1800	1821.08	2673.37
2160	2006.48	3331.16
2520	2058.91	3588.96
2880	2104.32	3590.25

Table 6.3. Statistical data of the poly(acrylamide) hydrogel swelling (%) changes in pH 7.4over time

Time (hours)	pH 7.4 Swelling(%)
0	0.00
6	637.01
12	1367.53
18	1760.38
24	2272.72
30	2673.37
36	3331.16
42	3588.96
48	3590.25

Table 6.4. Statistical data of the calibration curve for levofloxacin (Absorbance v/s Concentration).

Concentration (mg/l)	Absorbance
0	0.00000
2	0.16078
4	0.30437
6	0.45747
8	0.60067
10	0.73669

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Table 6.5. Statistical data of the levofloxacin drug release (%)overtimeinpH1.2and 7.4 from poly(acrylamide) hydrogel.

Time (min)	pH 1.2 Drug release (%)	pH 7.4 Drug release (%)
0	0.00	0.00
30	6.07	14.45
60	8.72	26.69
90	9.93	36.14
120	11.86	46.59
150	12.46	56.38
180	13.47	67.82
210	13.90	76.94
240	14.81	85.84
270	15.31	92.72
300	15.39	94.99
330	15.98	99.73
360	16.82	99.92

Table 6.6. Statistical data of the levofloxacin drug release (%) over time at 27°C and 37°C in pH 7.4 from poly(acrylamide)hydrogel.

Time (min)	27°C Drug release (%)	37°C Drug release (%)
0	0.00	0.00
30	8.78	14.45
60	14.43	26.69
90	21.27	36.14
120	28.41	46.59
150	35.46	56.38
180	41.03	67.82
210	48.55	76.94
240	57.04	85.84
270	61.92	92.72
300	64.60	94.99
330	67.97	99.73
360	71.69	99.92

Table 6.7. Levofloxacin drug release results in different kinetic models for up to 6hours.

Name of the kinetic model	R^2	Slope	Intercept
Zero order	0.963	17.16	10.08
First order	0.773	-0.444	2.470
Higuchi	0.967	0.02	0.323
Korsmeyer - Peppas	0.541	1.207	1.159
Hixson - Crowell	0.958	0.698	-0.346

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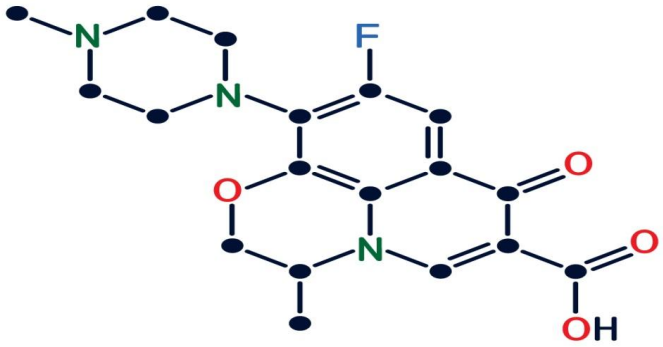

Levofloxacin

Figure 6.1. Molecular structure of levofloxacin.

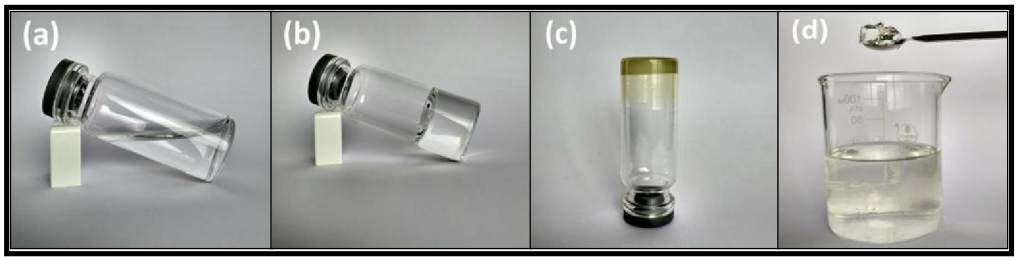

Figure 6.2. Poly(acrylamide) hydrogel preparation steps (a) initial solution, (b) after gel formation, (c) drying and (d) swelling.

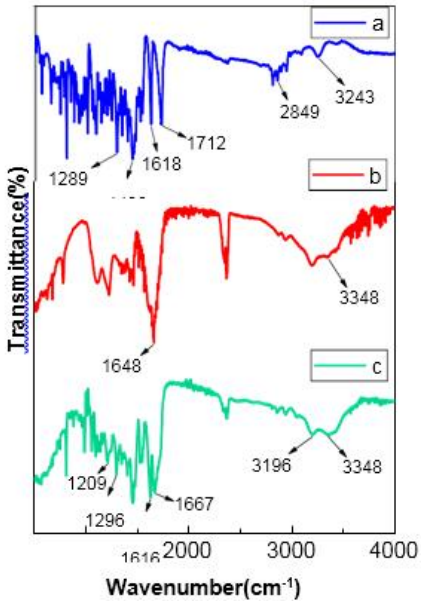

Figure 6.3. FTIR spectra of (a) levofloxacin, (b) poly(acrylamide) hydrogel and (c) levofloxacin loaded poly(acrylamide) hydrogel.

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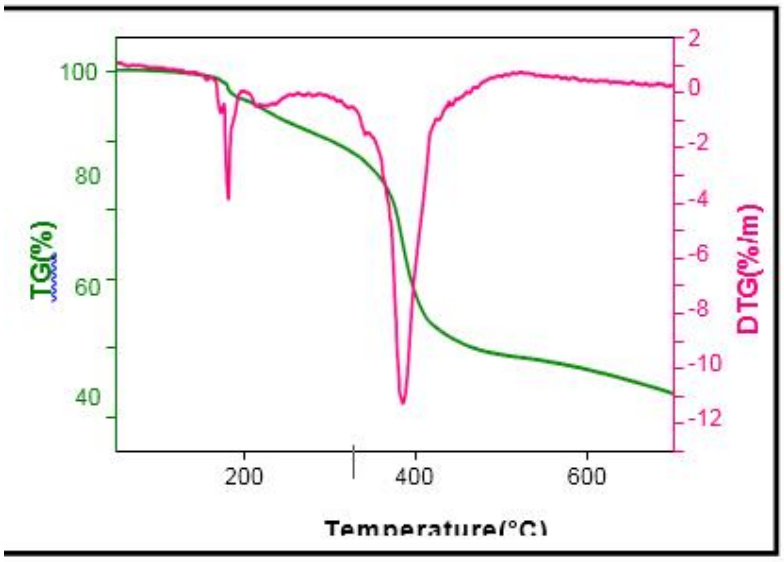

Figure 6.4. TGA graph of poly(acrylamide) hydrogel.

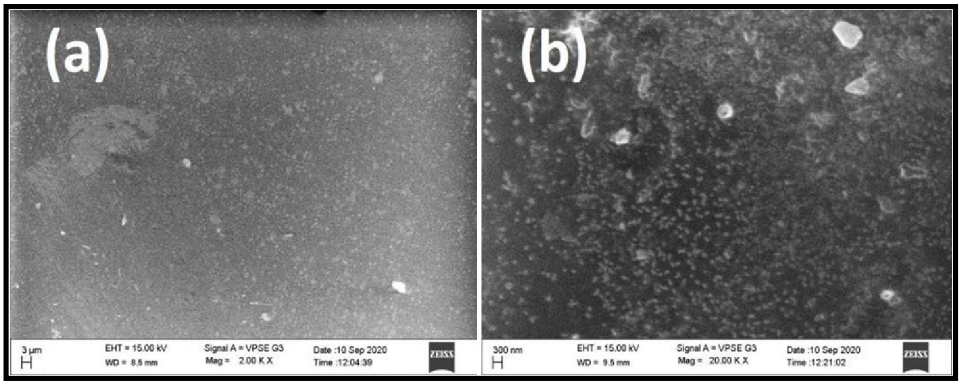

Figure 6.5. SEM images of poly(acrylamide) hydrogel.

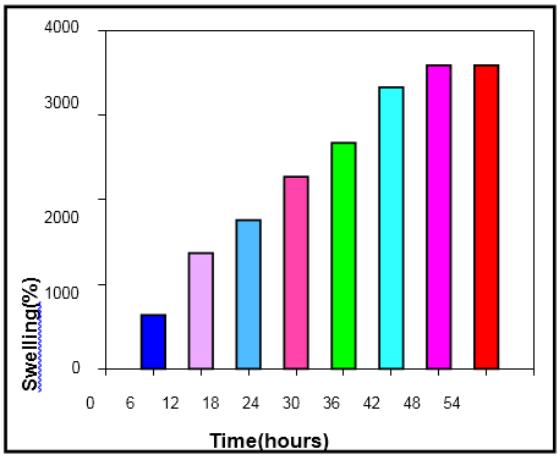

Figure 6.6. Poly(acrylamide) hydrogel swelling (%) changes over time in pH 7.4.

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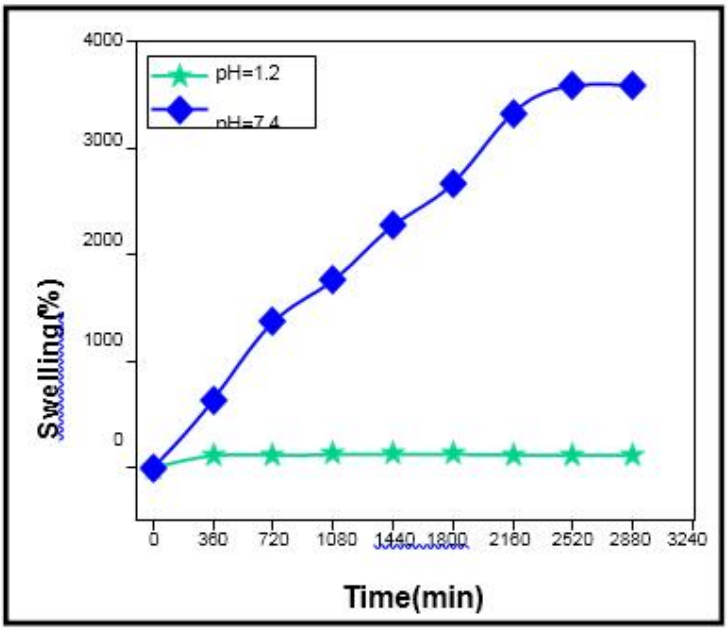

Figure 6.7. Swelling (%) of the poly(acrylamide) hydrogel in pH 1.2 and pH 7.4.

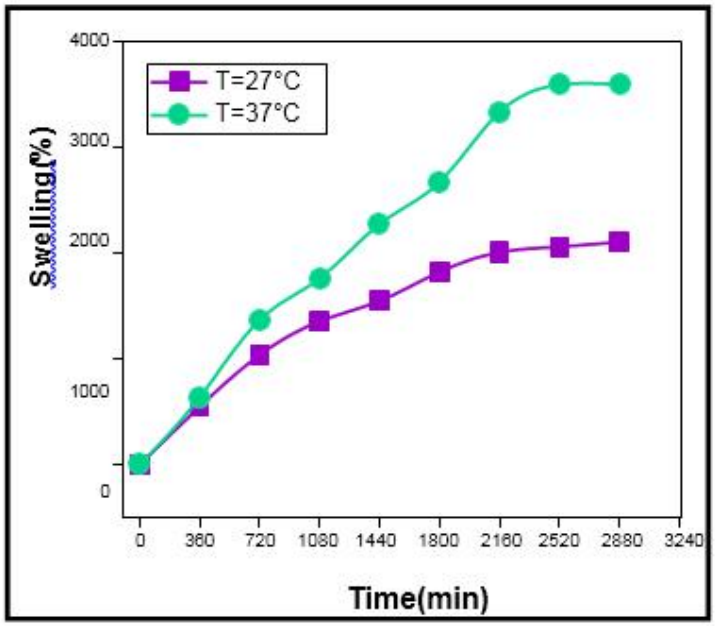

Figure 6.8. Swelling (%) of the poly (acrylamide) hydrogel at 27o C and 37o C temperatures in pH 7.4

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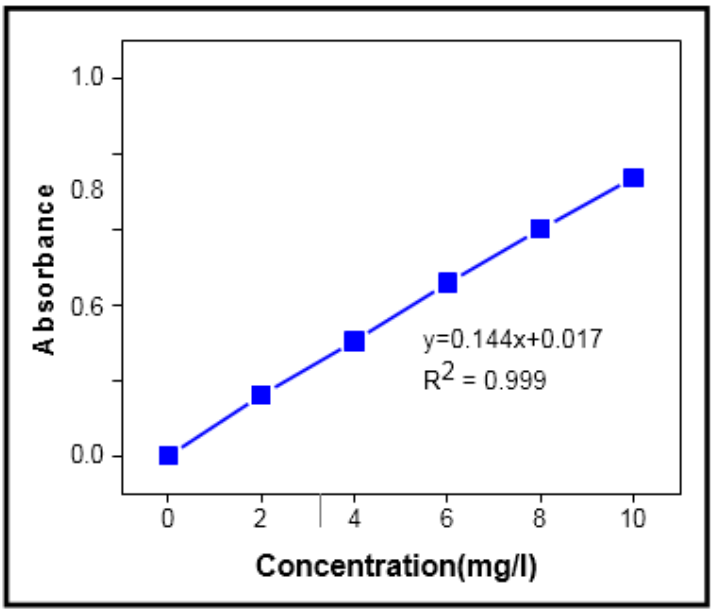

Figure 6.9. Calibration curve of levofloxacin (Absorbance v/s Concentration).

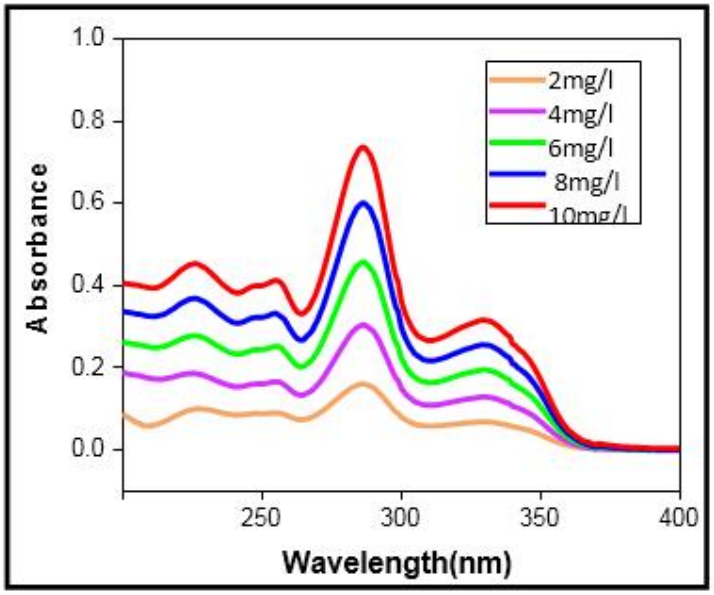

Figure 6.10. Spectral graph of the levofloxacin calibration curve.

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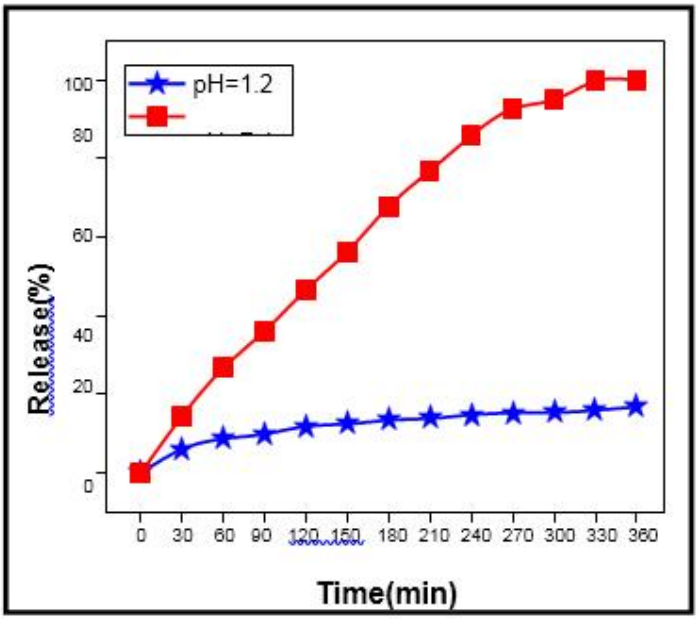

Figure 6.11. Levofloxacin drug release in pH 1.2 and pH 7.4 from poly (acrylamide) hydrogel with time.

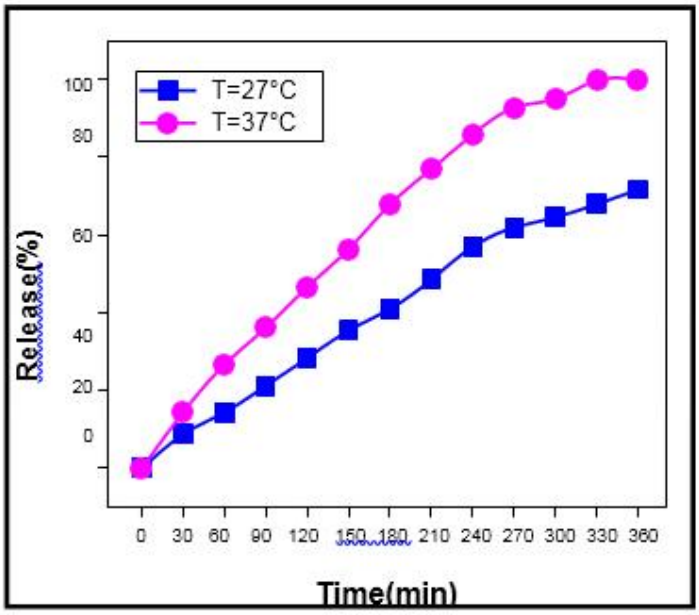

Figure 6.12. Levofloxacin drug release from poly(acrylamide) hydrogel with time at 27o C and 37o C temperature.

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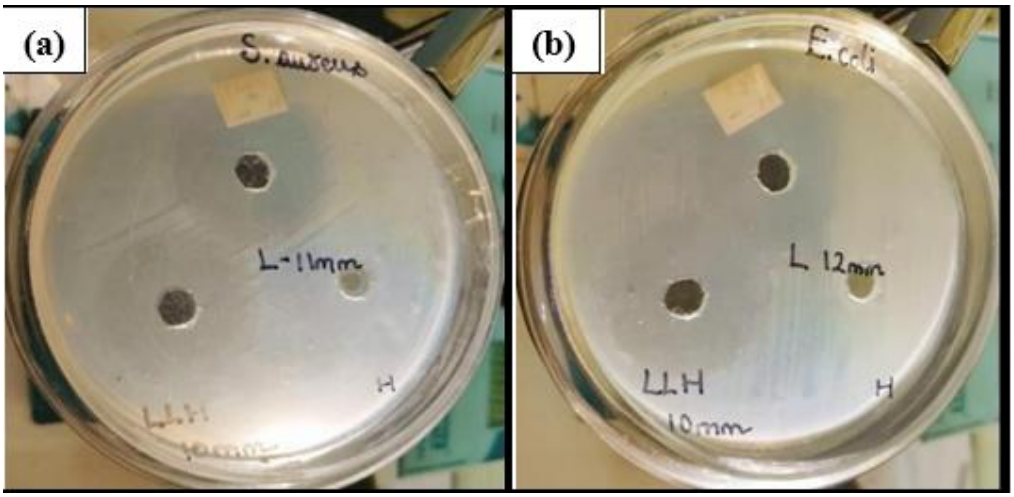

(a) (b)

Figure 6.14. Antibacterial activity of levofloxacin drug-loaded hydrogel on (a) S.aureus(Gram-positivebacteria) and (b) E.Coli (Gram- negative bacteria).

III. CONCLUSION

Poly(acrylamide) hydrogel cross-linked with methylene bisacrylamide was synthesized and studied their swelling and drug release properties. The swelling study of synthesized hydrogel was examined at pH 1.2 and pH 7.4 at 37o C and levofloxacin drug release studies were carried out under the same conditions. The drug released amount from the hydrogel was more at alkaline pH 7.4 than in the acidic pH 1.2. Because of its hydrophilic nature and the capability of hydrogen bonding of acrylamide molecules with water, it will produce high osmotic swelling pressure hence it will swell more and the amount of drug release is more. The temperature and pH effects on drug release were also studied, the hydrogel follows a Higuchi model and drug-loaded hydrogel shows good antibacterial activity; hence these hydrogels can be used in controlled drug release and biomedical applications due to good swelling properties.

The work has projected on the synthesis of polymer-based Smart Hydrogels for drug delivery applications. The poly(acrylamide) hydrogels are synthesized by using N, N′ -methylene-bisacrylamide as a crosslinker and used to study the controlled release of Levofloxacin drug.

The topics of the thesis focus on current ways for creating controlled-release drug delivery systems using polymers and the hydrogel formulations that produced longer-lasting effects. The findings of this research are likely to have a bigger impact on the pharmaceutical industry. However, in order to produce appropriate formulations and maintain their stability, the controlled-release formulations developed in this work will be further detailed in the future. Formulations must also be tested in animal models for intoxication, effectiveness and feasibility before being used in humans.

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