Controlled drug release of levofloxacin from poly (acrylamide) hydrogel

Madhusudana T.[1], Mamatha G. P.[*2], Demappa T.[3], Satyanarayan N. D.[1]

[1]Department of Pharmaceutical Chemistry, Kuvempu University, P.G. Centre, Kadur-577548, Karnataka, India

[2]Department of Chemistry, Davangere University, Shivagangotri, Davangere-577002, Karnataka, India

[3]Department of Polymer Science, University of Mysore, Sir M.V.P.G. Centre, Mandya-571402, Karnataka, India

ABSTRACT

Hydrogels are 3D polymer networks capable to absorb and release water or biological fluids. They are stimuli-responsive materials, which can show rapid volume changes with response to small changes in environmental parameters such as ionic strength, pH, and temperature. In this work, we performed a synthesis of Poly(acrylamide) hydrogel and tested for controlled release of levofloxacin hemihydrate as a model drug. We used sodium metabisulfite and potassium persulphate as free radical initiators to prepare hydrogel with methylenebisacrylamide as a crosslinker. Characterization of hydrogel was performed by TGA, SEM, and FT-IR. Swelling study and drug release were performed at pH 1.2 and 7.4 solutions, identical to the gastrointestinal fluid at 37°C (human body temperature) to examine possible site-specific drug delivery. UV-Visible spectrophotometer was used to measure the concentration of drug release. Results exhibited the pH and temperature-dependent drug release. The amount of drug release was found to be 17% and 99% in acidic and alkaline pH of 1.2 and 7.4, respectively, after 6 hours.

*Corresponding Author

Name: Mamatha G. P.
Phone: +918197112206
Email: mamatha_gp2005@rediffmail.com

INTRODUCTION

The polymers are continuous repeating monomer units. The general biomedical uses of polymers are in drug delivery systems, pharmaceutical adhesives, coating material, and emulsifying agents for dosage forms in site-specific and controlled drug delivery systems. Polymer molecules are linear or branched or may be crosslinked. The chemical response of polymers depends on the monomer unit present in the polymer chain. The homopolymers are having identical monomeric units and copolymers are formed from more than one monomer (Kamboj and Verma, 2015; Florence and Attwood, 1998; Moghimi and Hunter, 2000).

The first polymer gel was prepared in 1949 by Katchalsky. This gel responds to the surrounding environment solution by swelling or gathering from a network of water-soluble polyelectrolytes (Katchalsky and Gillis, 1949). In 1950 medical applications and its importance of hydrogels were revealed and using 2-hydroxyethyl methacrylate gel, soft contact lenses are manufactured. The smart hydrogel, like temperature-sensitive hydrogels, is focused till the mid-1980 (Kim *et al.*, 2013). In the drug delivery systems, pH-sensitive hydrogels are the best materials for drug release to the target

site of the body (Hamidi *et al.*, 2008; Huynh *et al.*, 2011).

Poly(acrylamide) hydrogel polymer backbone containing functional group like amine is sensitive to charge by either release or accept protons in the aqueous media. The electrostatic repulsion in the polymer backbone network promotes swelling and then water diffusion. These hydrogels are more sensitive to slight changes in environmental factors. Those are called smart hydrogels (Wan *et al.*, 2016; Wu *et al.*, 2016; Katchalsky and Gillis, 1949).
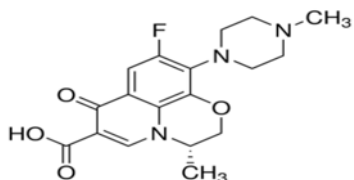

Figure 1: Molecular structure of levofloxacin

Levofloxacin, molecular formula $C_{18}H_{20}FN_3O_4$ and Figure 1 show its molecular structure. Levofloxacin is a fluoroquinolone antibacterial drug with an active L-isomer of ofloxacin (Hurst *et al.*, 2002; Chen *et al.*, 2003). Levofloxacin is used to cure the disease of gram-negative and gram-positive bacteria like keratitis, bacterial conjunctivitis, and other eye infections by inhibiting topoisomerase IV and DNA gyrase enzymes. These enzymes are important to DNA replication, recombination, transcription, and repair (Noel, 2009; Hooper, 1999).

In the present work, we evaluate the drug release from poly(acrylamide) hydrogel using different temperature and pH solutions. Crosslinker methylenebisacrylamide used to control the network characteristic and model drug levofloxacin hemihydrate was used for drug release studies.

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

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 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) were 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 $50^{o}C$ in the oven for 24 hours.

Characterization

FT-IR analysis

Levofloxacin and levofloxacin loaded poly(acrylamide) hydrogel spectra were recorded using an FT-IR spectrometer (Shimadzu ATR) in the range of 400 to 4000 cm^{-1} to determining 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 $20^{o}C$.

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

The swelling study of synthesized hydrogels was determined using dry samples in acidic buffer pH 1.2 and phosphate buffer pH 7.4 solutions. The preweighed hydrogel samples were immersed in solutions at $37^{o}C$ 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 then weighed. The swelling ratio of hydrogel was determined from Equation (1). Similarly, the swelling ratio was observed at $27^{o}C$ in pH 7.4 solution with time intervals.

$$Swelling\ ratio\ (\%) = \frac{W_b - W_a}{W_a} \times 100 \qquad (1)$$

Where, W_a and W_b is the dry and swollen gel weight.

Preparation of calibration curves

To construct a calibration curve, a stock solution 1000 mg/l of Levofloxacin drug solution was prepared using 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 absorption maximum was recorded to construct a calibration curve.

Drug loading and drug release studies

Levofloxacin hemihydrate was selected as a model drug. The loading of the drug was conducted by 1 mg/ml concentration solution using water as a solvent. Place 0.1 g dry hydrogel to 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 100 ml of acidic buffer pH 1.2 and phosphate buffer pH 7.4 solution at 37°C and withdrawn 1 ml 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, note down the λ_{max} absorbance. The percentage of released levofloxacin was calculated and its corresponding drug release graph was plotted. Similarly, temperature effects on drug release will study by setting temperatures at 27 and 37°C in pH 7.4 solution.

Kinetic model drug release

The kinetic models of drug release will determine using Zero-order, first-order, Hixson-Crowell, higuchi and Korsmeyer-Peppas models (Baishya et al., 2017; Kadam et al., 2019).

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 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 Poly(acrylamide) hydrogel preparation steps are shown in Figure 2. After the gel preparation, the swelling study was performed (Anbarasan et al., 2003; Isık and Kıs, 2004; Teo et al., 2008).

FT-IR analysis

The FT-IR spectra of levofloxacin, poly(acrylamide) hydrogel and levofloxacin loaded poly(acrylamide) hydrogel were recorded are shown in Figure 3. The characteristic peaks of levofloxacin (Figure 3a) were found at 3243 cm^{-1} and 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 (Figure 3b) shows the peaks at 3348 cm^{-1} (-NH stretching of hydrogel), 1648 cm^{-1} (C=O stretching). The levofloxacin loaded poly(acrylamide) hydrogel spectra (Figure 3c) shows 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 confirm the drug loading in acrylamide hydrogel (Mouzam et al., 2011; Sagdinc and Bayari, 2004).

TGA analysis

The thermogram of hydrogel was shown in Figure 4. The first stage of weight loss, consider as loss of moisture present in the hydrogel, was observed at 169ºC with mass loss of 3.4%, then degradation occurred at 179ºC with weight loss of 9.6%, and maximum weight loss occurred at 381ºC with mass loss of 24.4% due to cleavage of the polymer chain in hydrogel (Ebrahimi and Salavaty, 2018).

Morphological examination

The surface morphology of synthesized hydrogel was studied by SEM. The micrograph Figure 5(a) and Figure 5(b) reveal that the surface is uniform and smooth in nature (Chen et al., 2009; Aouada et al., 2009).

Swelling study

The swelling study of the synthesized hydrogel was performed in buffer solutions. The swelling study of poly(acrylamide) hydrogels in pH 1.2 and 7.4 solutions similar to gastrointestinal fluids at 37°C are shown in Figure 6. From these results, the higher swelling rate observed at pH 7.4 when compared to pH 1.2 solution. In an acidic medium of pH 1.2, the ammonium groups (NH^{3+}) are formed by protonation but due to the presence of chloride (Cl$^-$), counterions drastically decreased its swelling (Wu et al., 2001; Pourjavadi and Mahdavinia, 2006). However, at pH 7.4, the (-CONH$_2$) and (-CONH-) groups are deprotonated and the presence of sodium (Na$^+$) ions in the solution will produce high osmotic swelling pressure hence shows maximum swelling. Similarly, the swelling was observed at temperature 27 and 37°C (Figure 7) for temperature sensitivity. The results show when the temperature increases swelling ratio also increases.

Drug selection

Drug selection for the loading and release is most important because it should not react with hydrogel and solvents. This helps to avoid the λ_{max} shift. Levofloxacin drug has good material because no change was observed in the λ_{max} over time. Using a UV-Visible spectrophotometer scan, the solutions

---

Table 1: Results of different models in terms of correlation coefficient (R^2), slope, and intercept

Name of the kinetic model	R^2	Slope	Intercept
Zero-order	0.996	21.11	3.428
First-order	0.950	-0.199	2.064
Higuchi	0.943	0.021	0.308
Korsmeyer-Peppas	0.442	1.335	1.142
Hixson-Crowell	0.982	0.535	-0.091
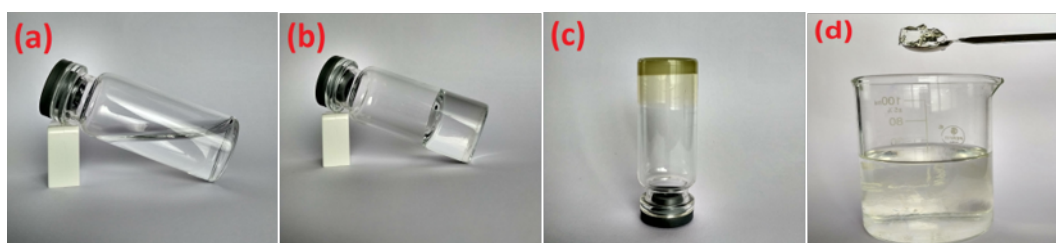
Figure 2: Hydrogel preparation steps (a) initial solution, (b) after gel formation, (c) drying, and (d) swelling
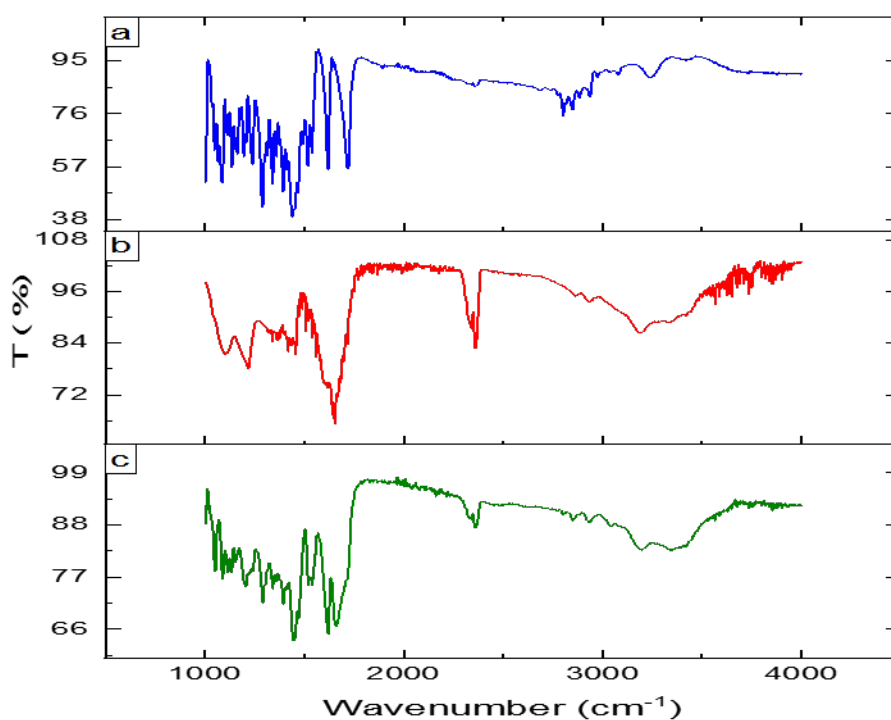
Figure 3: FTIR spectra of (a) levofloxacin, (b) poly (acrylamide) hydrogel, (c) levofloxacin loaded poly (acrylamide) hydrogel

between 200 to 400 nm absorption maximum was recorded at λ_{max} 286 nm. From the calibration curve, the slope and intercept are 0.144 and 0.017, respectively, and the correlation coefficient (R^2) is 0.999 (Figure 8, Figure 9 and Figure 10).
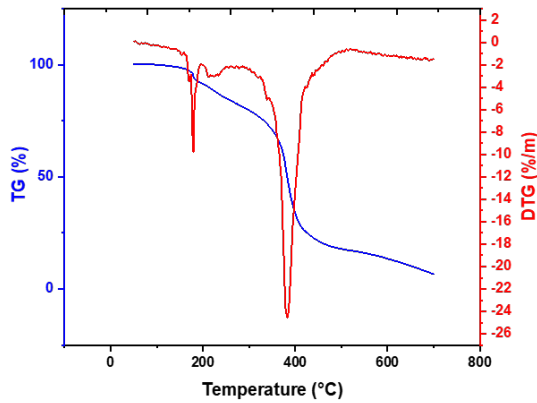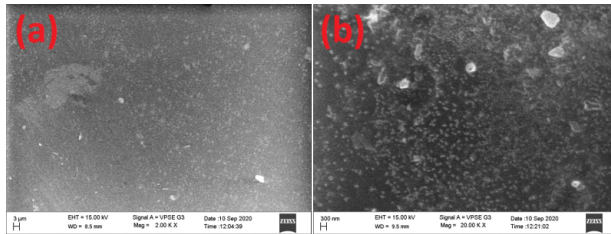

Figure 4: TGA graph of poly (acrylamide) hydrogel
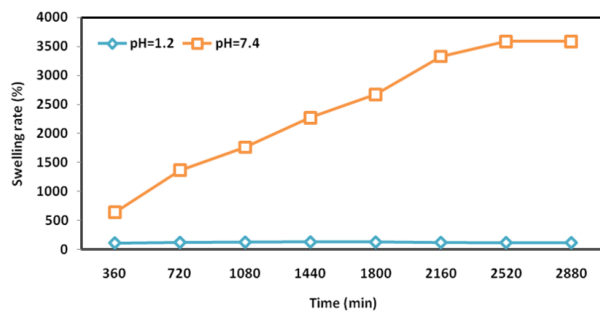

Figure 5: SEM images of poly (acrylamide) hydrogel

Figure 6: Swelling ratio of the hydrogel at pH 1.2 and pH 7.4

Drug release study

Drug release with time

Based on the swelling studies of the synthesized hydrogel in pH 1.2 and pH 7.4 solutions. The result shows more swelling at pH 7.4 than in pH 1.2 solutions. Similarly, the drug-loaded hydrogels were placed in pH 1.2 and pH 7.4 solutions identical to gastrointestinal fluids. The percentage of levofloxacin drug release is more at pH 7.4 than in
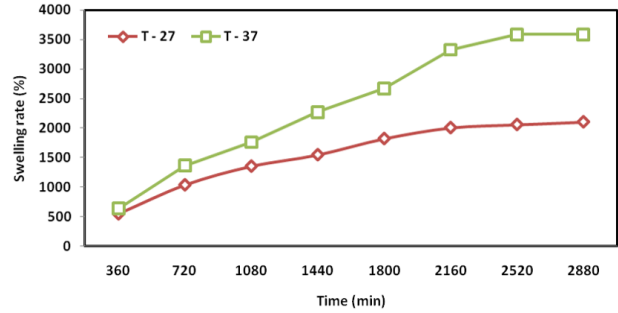

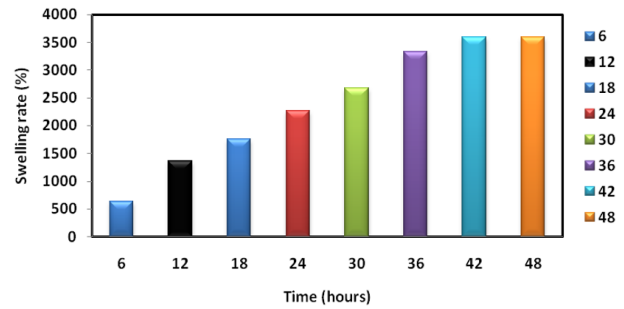**Figure 7: Swelling ratio at 27 and 37°C temperature in pH 7.4 solution**

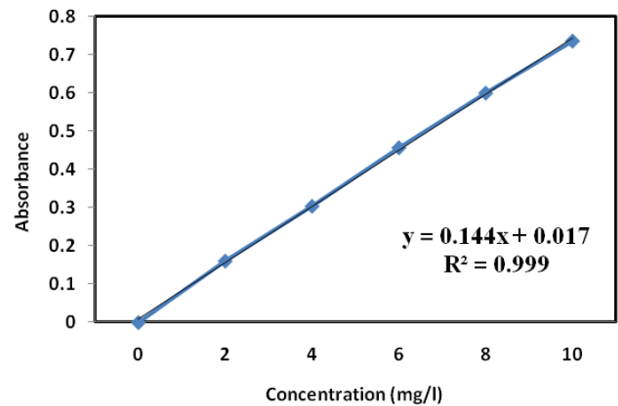**Figure 8: Swelling ratio changes over time**

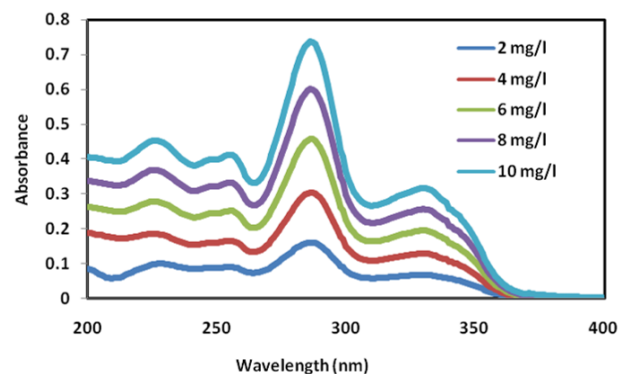**Figure 9: Calibration curve of Levofloxacin (Absorbance v/s Concentration)**

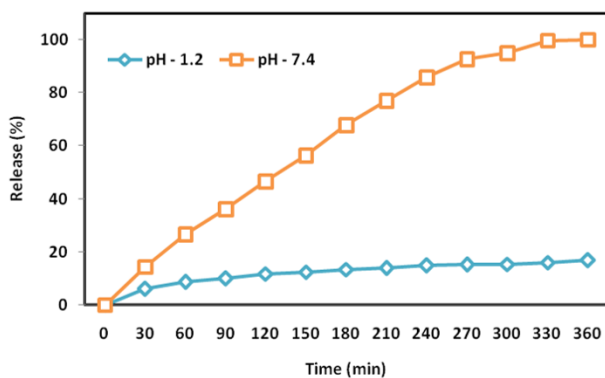**Figure 10: Spectral graph of the calibration curve**

Figure 11: Levofloxacin release in pH 1.2 and pH 7.4 solutions with time
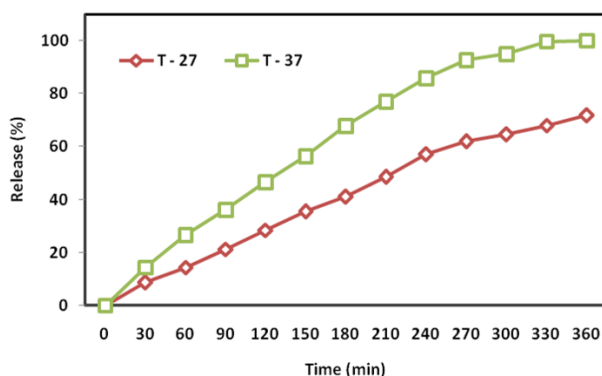

Figure 12: Levofloxacin release at 27 and 37°C temperature with time

pH 1.2 solutions. Hence, we conclude that drug release depends on the pH of the solution because the swelling ratio is more in pH 7.4 than in the pH 1.2 solution. The drug release from hydrogel into solution depends on swelling and the controlled release of levofloxacin was observed up to 6 hours (Figure 11).

Drug release with temperature

The temperature effect on drug release was evaluated and studied at temperatures 27 and 37°C with time (Figure 12). When the temperature increases, drug release also increased, and at 37°C we observed the maximum drug release. When the temperature increases, the hydrogel network flexibility also increases. Hence, the more amount of buffer solution enters into hydrogel promotes more amount of drug is released.

Kinetic model drug release

The kinetic model of drug release will study by using various mathematical models. The obtained results are given in Table 1. It helps to promote an ideal kinetic model to illustrate in vitro drug release data in terms of relevant parameters. From the obtained results, the best correlation coefficient (R^2) is 0.996 in Zero-order kinetics; hence synthe-

sized poly(acrylamide) hydrogel follows the zero-order kinetic model.

CONCLUSION

Poly(acrylamide) hydrogel cross-linked with methylenebisacrylamide 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 solutions at 37°C and levofloxacin drug release studies were carried out in the same conditions. The drug released amount from the hydrogel was more at alkaline pH 7.4 than in the acidic pH 1.2 solution. Because at pH 7.4 solution, the amide groups are de-protonated and the presence of sodium (Na^+) ions in the solution 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 and the hydrogel follows a zero-order kinetic model; hence these hydrogels can be used in controlled drug release and biomedical applications due to good swelling properties.

REFERENCES

Anbarasan, R., Jayaseharan, J., Sudha, M., Gopalan, A. 2003. Sonochemical polymerization of acrylic acid and acrylamide in the presence of a new redox system? A comparative study. *Journal of Applied Polymer Science*, 89(13):3685–3692.

Aouada, F. A., Chiou, B.-S., Orts, W. J., Mattoso, L. H. 2009. Physicochemical and morphological properties of poly(acrylamide) and methylcellulose hydrogels: Effects of monomer, crosslinker and polysaccharide compositions. *Polymer Engineering & Science*, 49(12):2467–2474.

Baishya, H., *et al.* 2017. Application of Mathematical Models in Drug Release Kinetics of Carbidopa and Levodopa ER Tablets. *Journal of Developing Drugs*, 6(2):1–8.

Chen, J., Liu, M., Liu, H., Ma, L. 2009. Synthesis, swelling and drug release behavior of poly (N,

N-diethylacrylamide-co-N-hydroxymethyl acrylamide) hydrogel. *Mater Sci Eng C*, 29(7):2116–2123.

Chen, T., Embree, H. D., Brown, E. M., Taylor, M. M., Payne, G. F. 2003. Enzyme-catalyzed gel formation of gelatin and chitosan: potential for in situ applications. *Biomaterials*, 24(17):2831–2841.

Ebrahimi, R., Salavaty, M. 2018. Controlled drug delivery of ciprofloxacin from ultrasonic hydrogel. *e-Polymers*, 18(2):187–195.

Florence, A. T., Attwood, D. 1998. Physicochemical Principles of Pharmacy, sixth edition. London. Macmillan Press. ISBN 9780857111746.

Hamidi, M., Azadi, A., Rafiei, P. 2008. Hydrogel nanoparticles in drug delivery. *Advanced Drug Delivery Reviews*, 60(15):1638–1649.

Hooper, D. C. 1999. Mode of Action of Fluoroquinolones. *Drugs*, 58(Supplement 2):6–10.

Hurst, M., Lamb, H. M., Scott, L. J., Figgitt, D. P. 2002. Levofloxacin: an updated review of its use in the treatment of bacterial infections. *Drugs*, 62(14):2127–2167.

Huynh, C. T., Nguyen, M. K., Lee, D. S. 2011. Biodegradable pH/temperature sensitive oligo (β-amino ester urethane) hydrogels for controlled release of doxorubicin. *Acta Biomat*, 7(8):3123–3130.

Isık, B., Kıs, M. 2004. Preparation and determination of swelling behavior of poly(acrylamide-co-acrylic acid) hydrogels in water. *Appl Polym Sci*, 94(4):1526–1531.

Kadam, S., Dattatray, A., Landge, D. 2019. Formulation and evaluation of colon targeted matrix tablet of azathioprine. *Indo American Journal of Pharmaceutical Research*, 9(7):3157–3168.

Kamboj, V. K., Verma, P. K. 2015. Poloxamers based nanocarriers for drug delivery system. *Der Pharmacia Lettre*, 7(2):264–269.

Katchalsky, A., Gillis, J. 1949. Theory of the potentiometric titration of polymeric acids. *Recueil des Travaux Chimiques des Pays-Bas*, 68(9):879–897.

Kim, J. K., Basavaraja, C., Yamaguchi, T., Huh, D. S. 2013. Preparation and characterization of smart hydrogel nanocomposites sensitive to oxidation–reduction. *Polymer Bulletin*, 70(1):207–220.

Moghimi, S. M., Hunter, A. C. 2000. Poloxamers and poloxamines in nanoparticle engineering and experimental medicine. *Trends in Biotechnology*, 18(10):412–420.

Mouzam, M. I., Dehghan, M. H. G., Asif, S., Sahuji, T., Chudiwal, P. 2011. Preparation of a novel floating ring capsule-type dosage form for stom-ach specific delivery. *Saudi Pharmaceutical Journal*, 19(2):85–93.

Noel, G. J. 2009. A Review of Levofloxacin for the Treatment of Bacterial Infections. *Clinical Medicine Insights*, 1:433–458.

Pourjavadi, A., Mahdavinia, G. R. 2006. Super absorbency, pH-Sensitivity and Swelling Kinetics of Partially Hydrolyzed Chitosan-g-poly(Acrylamide) Hydrogels. *Turkish Journal of Chemistry*, 30(5):595–608.

Sagdinc, S., Bayari, S. 2004. Theoretical study of ofloxacin: geometrical parameters and vibrational wavenumbers. *Journal of Molecular Structure: Theochem*, 668(2-3):93–99.

Teo, B. M., Prescott, S. W., Ashokkumar, M., Grieser, F. 2008. Ultrasound initiated miniemulsion polymerization of methacrylate monomers. *Ultrasonics Sonochemistry*, 15(1):89–94.

Wan, T., Xiong, J., Zhao, Q. 2016. Crosslinker effects on swelling and gel properties of pH- and temperature-responsive poly (NIPAM/IA/AM) hydrogels. *Polym Bull*, 73:1447–1458.

Wu, C., Wang, D., Wu, H., Dan, Y. 2016. Synthesis and characterization of macroporous sodium alginate-g-poly(AA-co-DMAPMA) hydrogel. *Polym Bull*, 73:3255–3269.

Wu, J., Lin, J., Li, G., Wei, C. 2001. Influence of the COOH and COONa groups and crosslink density of poly(acrylic acid)/montmorillonite superabsorbent composite on water absorbency. *Polymer International*, 50(9):1050–1053.