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RESEARCH ARTICLE

Synthesis and Characterization of Poly(2-Hydroxyethylethylmethacrylate-Co-Acrylamide) Hydrogel for Intestinal Drug Delivery

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ABSTRACT

Hydrogels, the swellable polymeric materials have been used widely as a carrier for drug delivery systems and have gained attention owing to their peculiar characteristics like swelling in aqueous medium, pH or temperature sensitivity or sensitivity towards external stimuli. Hydrogels being biocompatible due to their high water content and low interfacial tension with the biological fluids have been helpful as targetable carriers for bioactive drugs with tissue specificity. The purpose of research is to provide the targeted drug release in the intestine for a prolong period of time. pH sensitive hydrogel, 2-Hydroxyethylmethacrylate-co-acrylamide was prepared by polymerization in aqueous solution from 2-Hydroxyethlmethacrylate(2-HEMA) and acrylamide monomers using N,N-Methylenebis(acrylamide) as a cross linker. It was shown that the swelling behavior of 2-HEMA-co-acrylamide can be controlled by changing the molar concentration of acrylamide. The hydrogel was characterized by FT-IR, SEM, tests to assess swellability, drug loading and dissolution techniques.

KEYWORDS

Hydrogel; 2-Hydroxyethylmethacrylate-co-acrylamide; N,N-Methylenebis (acrylamide); pH sensitive.

INTRODUCTION

Hydrogels are the controlled release drug delivery systems i.e. deliver the drug at a predetermined rate either systemically or locally for a specified period of time[1]. Their network structure can be nonporous (10-100^{0}A) where the drug release is by diffusion only; macro porous (0.1-1.0µm) where the drug release is by molecular diffusion and convection; micro porous (100-1000^{0}A) where the drug release is by partition coefficient[2].

Hydrogels, the polymeric chain networks when kept in contact with water, aqueous or biological fluids; the hydrogels imbibe the aqueous medium and swell due to diffusion which involves migration of water into pre-existing or dynamically formed spaces among the hydrogel chains[3]. Hydrogels are classified depending upon their mechanism of drug release such as pH/ion sensitive hydrogel, temperature sensitive hydrogel, and external stimuli sensitive hydrogel in which the degradation of the biomolecule causes the drug release[4]. Some of the examples of external stimuli sensitive hydrogels are mentioned in Table 1[5].

2-Hydroxyethylmethacrylate-co-acrylamide is a pH sensitive hydrogel and it forms swollen hydrogels of cross linked species due to the presence of both hydrophilic amide groups and hydrophobic methacrylate groups in its side chains[6].

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Table 1: Examples of external stimuli sensitive hydrogels

Stimuli	Polymer	Drug
Magnetic field	Ethylene-co-vinyl acetate(EVA's)	Insulin
Ultrasonic radiation	EVA's, Ethylene-co-vinyl alcohol	Zinc bovine insulin, Insulin
Electric field	Poly(2-Hydroxyethyl methacrylate)	Propranolol hydrochloride
Antibody	Ethylene-co-vinyl acetate	Naltrexone, Ethynil estradiol

pH sensitive hydrogels are useful for applications as drug delivery systems[7], wound healing, colon and intestinal specific targeting, cosmetology, tissue engineering and immobilization of enzymes[8]. In these applications both mechanical strength and swelling behavior are important, although the later has attracted greater attention than the former. The swelling degree of hydrogels depends not only on the nature of the hydrogel and the swelling medium but also on the cross linker density. Furthermore, in general, hydrogels have poor mechanical strength and durability for some applications such as drug delivery matrix, and hence investigating and possibly enhancing these properties will make hydrogels acceptable for many future applications.

The present study focuses on the synthesis of poly(2-Hydroxyethylmethacrylate-co-acryl amide) hydrogels having a range of acrylamide contents and subsequent attention to effects of cross linker. To prepare pH sensitive hydrogels 2-Hydroxyethylmethacrylate (2-HEMA) and acrylamide monomers were chosen because (1) 2-HEMA swells in water and is a typical pH sensitive hydrogel that exhibits a typical volume phase transition in response to pH changes at around pH 7.4 (2) On the other hand, acrylamide is a versatile hydrophilic monomer but its homopolymer does not show volume phase transition pH in water. Introduction of acrylamide component improves mechanical strength of hydrogels as in this case poly(2-HEMA-co-acrylamide) hydrogels should have both good mechanical strength and pH sensitivity[9].

MATERIALS AND METHODS

Reagents and Chemicals

2-Hydroxyethylmethacrylate-co-acrylamide hydrogel was synthesized by cross linking polymerization mechanism. Thus, the synthesis requires the use of a cross linker namely N, N-Methylene bis acrylamide (BIS). The monomers 2-Hydroxyethylmethacrylate (2-HEMA) and acrylamide were cross linked and polymeric chains were synthesized. The copolymerization reaction was initiated with the help of the initiator Ammonium persulphate. Also, the reaction was accelerated using the accelerator such as N, N, N', N'- Tetramethyethylene diamine (TEMED). The polymerizing solvents are distilled water and acetone in the ratio of 1:1. The drug sample of antibiotic amoxicillin sodium was obtained from Cipla, Research and Development Centre. The monomers, cross linker and other reagents were obtained from SD Fine Chemicals Ltd. The hydrogels synthesized with different monomer ratios as mentioned in Table 2.

Table 2: Samples of the hydrogels synthesized along with their monomer ratio

Sr. No.	Sample	2-HEMA:Acrylamide
1.	HG1	1:1
2.	HG2	1:2
3.	HG3	1:3
4.	HG4	1:4
5.	HG5	1:5

Method of Preparation and Instrumentation

2-HEMA-co-acrylamide was synthesized by copolymerization reaction as mentioned in figure 1 which requires a cross linker for the reaction to take place. It is a process of reacting the monomer molecules in a chemical reaction to form polymer chains or three dimensional networks. Here the reaction takes place due to intermolecular hydrogen bonding between 2-HEMA and acrylamide.

The reaction is as shown in figure 1:

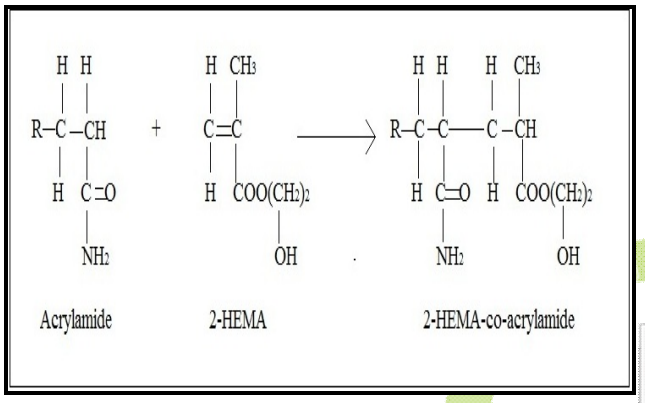

Figure 1: Copolymerization reaction

The hydrogel was synthesized by free radical copolymerization reaction.

The monomers namely 2-HEMA and acrylamide along with the cross linker N, N-Methylene bis acrylamide (BIS) and the initiator Ammonium persulphate (APS=0.056M) were weighed. These compounds were then dissolved in the polymerizing solvent that is distilled water and acetone in the ratio of 1:1. The above solution was ultrasonicated for about 10 minutes till a clear solution was obtained i.e. till all the ingredients had completely dissolved in the polymerizing solvent. The accelerator TEMED (0.5ml) was added to the above solution and then poured in the metallic straws and polymerization reaction was allowed to take place. The time required for polymerization for the different ratios of monomers was different.

These hydrogels were then kept at 20^0C for 24 hours and then removed and cut into cylindrical spheres. These hydrogels were immersed in large excess of distilled water to remove any unreacted compound for 15 days and the water was replaced every day. The hydrogels samples were then dried at 50^0C to constant weight and were then stored at room temperature.

The time required for polymerization for hydrogels with different ratios of the monomers is as mentioned in Table 3.

Table 3: Time for polymerization for specific batches

Sr. No.	BATCH	TIME (min.)
1.	HG1	75
2.	HG2	60
3.	HG3	45
4.	HG4	45
5.	HG5	60

Test to Assess Swellability

The equilibrium degree of swellability was assessed by immersing the dried hydrogels in aqueous buffer of HCl of pH 1.2 and phosphate buffer of pH 7.4. for 24 hours. 0.1 gram of dried hydrogel was immersed in the aqueous buffer solution for 60, 120, 180, 240, 300, 360 minutes and 24 hours. At these time intervals the surface of the hydrogel was wiped with filter paper and was weighed.

Drug Loading Technique

The antibiotic drug was then entrapped in the dried hydrogels by swelling the hydrogel in the drug solution of known concentration and is known as drug loading technique. The dried hydrogels were soaked in the aqueous drug solution of 10mg/ml for 24 hours. The hydrogels were then separated from the drug solution by filtration and dried till constant weight was obtained. The amount of drug loaded or entrapped was determined by ultraviolet spectrophotometer.

Dissolution

For the dissolution studies, transfer the drug loaded hydrogels in a muslin bag and attach it to the paddle of the USP Test Apparatus II. Introduce the paddle to the Dissolution Test Apparatus, TDT06T USP and adjust the speed of the paddle at 100 rpm and maintain the temperature at 37^0C. Withdraw the samples of 5ml each at intervals of 30 minutes, 1hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours and 24 hours. Replace the same amount of buffer to maintain the sink conditions. Dilute 5ml to 10ml of the test solution with the buffer solution and measure the absorbance at 227 nm. The first two hours of the dissolution studies were carried out in the acidic medium using HCl buffer of pH 1.2 and note down the absorbance at 227 nm.

RESULTS AND DISCUSSION

Analytical Method Development and Validation

The antibiotic Amoxicillin sodium is freely soluble in water. Also, the hydrogel should not release the drug in the acidic medium of pH 1.2 but should release the drug in the basic medium of pH 7.4 as the drug should be released in intestine. For this reason, the analytical method development was carried out in HCl buffer of pH 1.2, phosphate buffer of pH 7.4 and in distilled water. The calibration curve was plotted and the R^2 square values calculated.

Peak Curve of Amoxicillin Sodium in Distilled Water

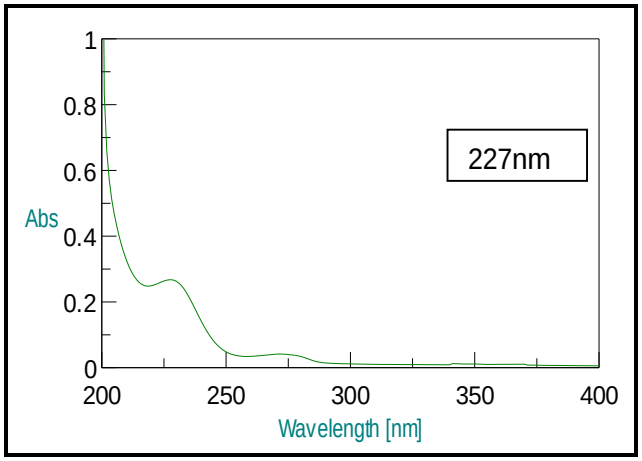

Figure 2: Peak of amoxicillin sodium in water

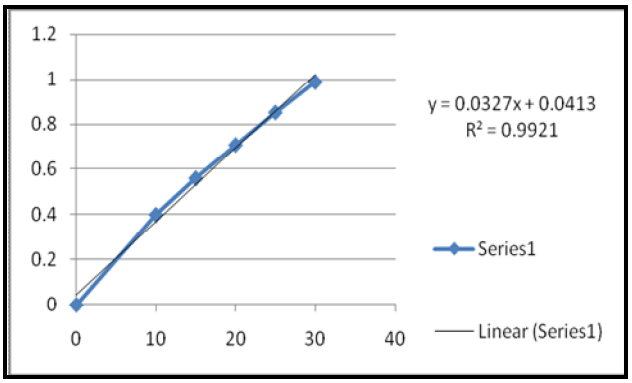

Figure 3: Calibration curve for distilled water

The absorbance of the antibiotic in distilled water is as mentioned in Table 4.

Table 4: Absorbance of antibiotic in distilled water

Sr. No.	Conc.(ppm)	Absorbance
1	10	0.4011
2	20	0.5612
3	30	0.7090
4	40	0.8535
5	50	0.9889

Peak Curve of Amoxicillin Sodium in Phosphate Buffer pH 7.4

Place 50 ml of 0.2 M KH_2PO_4 in a 200ml volumetric flask. Take 39.1ml of 0.2M NaOH and make up the volume with distilled water to prepare phosphate buffer of pH 7.4, the peak is as shown in Figure 4.

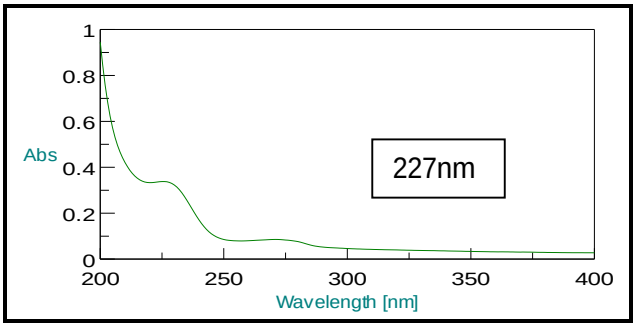

Figure 4: Peak of amoxicillin sodium in phosphate buffer

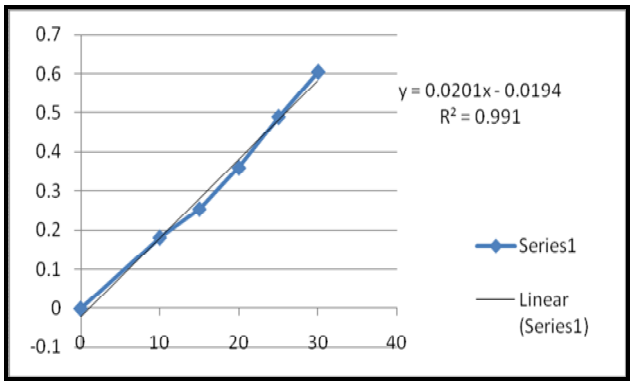

Figure 5: Calibration curve of phosphate buffer pH 7.4

The absorbance of amoxicillin sodium in phosphate buffer is as mentioned in Table 5.

Table 5: Absorbance of antibiotic in phosphate buffer pH 7.4

Sr. No.	Conc.(ppm)	Absorbance
1	10	0.1815
2	15	0.2542
3	20	0.3595
4	25	0.4894
5	30	0.6040

Peak of Amoxicillin Sodium in HCl Buffer of pH 1.2

Place 50ml of 0.2M KCl in a volumetric flask and add 85ml of 0.2M HCl and make up the volume with distilled water to prepare the HCl buffer of pH 1.2. The peak curve is as shown

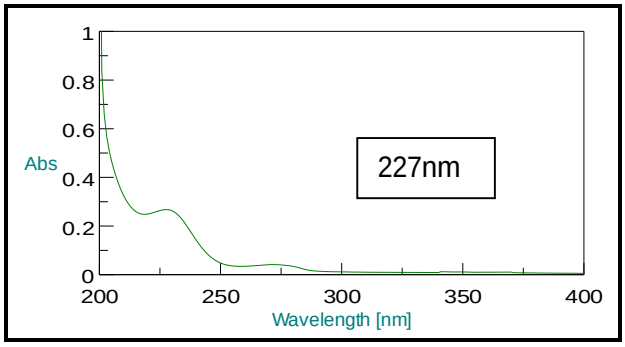

Figure 6: Peak of amoxicillin sodium in HCl buffer

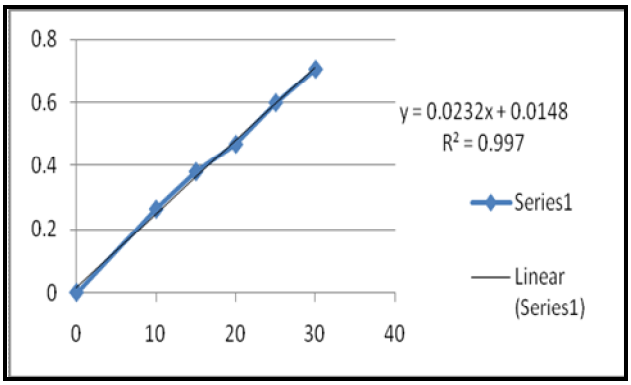

Figure7: Calibration curve of HCl buffer pH 1.2

The absorbance of amoxicillin sodium in HCl buffer is as shown in Table 6.

Table 6: Absorbance of antibiotic in HCl buffer of pH 1.2

Sr. No.	Conc.(ppm)	Absorbance
1	10	0.2629
2	15	0.3795
3	20	0.4666
4	25	0.5981
5	30	0.7050

Test to Assess Swellability

The dynamic swelling experiment was conducted in hydrogels by measuring the humid weight of the hydrogels which were immersed in the aqueous buffer solutions. The equilibrium degree of swellability of these hydrogels was noted by the following formula:

$$EDS = \frac{W_I - W_O}{W_O}$$

Where,

EDS: - Equilibrium degree of swellability

W_I : - Weight of hydrogel immersed in buffer in grams.

W_o : - Weight of the original hydrogel without immersion in aqueous buffer in grams.

For pH 1.2

The EDS values of the hydrogel along with W_I for acidic pH 1.2 are as mentioned in Table 7.

Table 7: EDS values for pH 1.2

Time (hrs.)	W_I (grams)	EDS
1	0.2310	1.31
2	0.2541	1.541
3	0.2873	1.873
4	0.3231	2.231
5	0.3574	2.574
6	0.3986	2.986
24	0.4054	3.054

For pH 7.4

The EDS values of the hydrogel along with W_I for acidic pH 7.4 are as mentioned in Table 8.

Table 8: EDS values for pH 7.4

Time (hrs.)	W_I (grams)	EDS
1	0.4494	3.494
2	0.5192	4.192
3	0.5585	4.585
4	0.5609	4.609
5	0.5664	4.664
6	0.5776	4.776
24	0.5934	4.934

Scanning Electron Microscopy (SEM)

The SEM studies were carried out to obtain the surface imaging of the hydrogels. The hydrogels were immersed in the aqueous buffers of HCl of pH 1.2 and phosphate buffer of pH 7.4. These hydrogels were dried and then observed for SEM imaging. The objective was to observe any pores formation in the basic pH[10]. The reason being 2-HEMA-co-acrylamide is a pH sensitive polymer as it swells well and releases the drug in the basic pH and not in the acidic pH. The surface images of acidic and basic pH are as shown in figure 8 and figure 9 respectively:

SEM image of 2-HEMA-co-acrylamide hydrogel at acidic pH 1.2

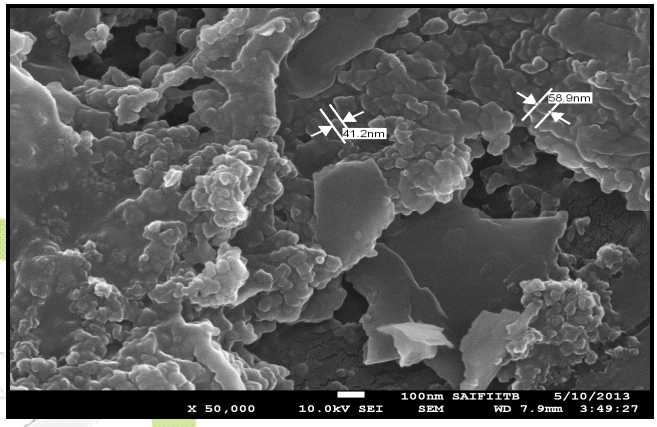

Figure 8: SEM image at pH 1.2

The above Figure 8 describes that 2-HEMA-co-acrylamide polymer does not swell in the acidic pH and thus its surface imaging does not show any pores. Hence the drug release cannot be obtained in the acidic pH. Thus drug release to the specific tissue is possible.

SEM image of 2-HEMA-co-acrylamide hydrogel at basic pH 7.4

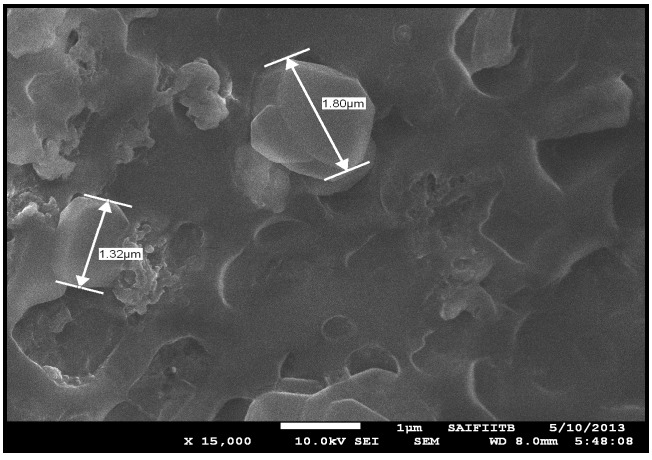

Figure 9: SEM image at basic pH 7.4

The above Figure 9 describes that 2-HEMA-co-acrylamide polymer when immersed in the basic pH 7.4 it swells. Since the polymer swells at the basic pH the drug release takes place. Thus drug release at the specific tissue can be achieved.

Infrared Spectroscopy

IR spectroscopy is the one which deals with the infrared region of the electromagnetic spectrum that is the light which has a lower frequency and a longer wavelength as compared to the visible light. A laboratory instrument that uses this technique is the Fourier Transform infrared (FTIR) Spectroscopy[11].

The IR studies thus help to identify the functional groups which are present in the sample. It does not give the structure of the synthesized compound but gives the various functional groups present in the hydrogel. Thus it also states that the polymerization reaction has taken place and thus the synthesized hydrogel has the functional groups of both the monomers which are being used for synthesis[12]. The IR spectra of the 2-HEMA-co-acrylamide hydrogel are as shown in the spectra and based on the spectra the IR interpretation of the various functional groups present is as mentioned in Figure 10.

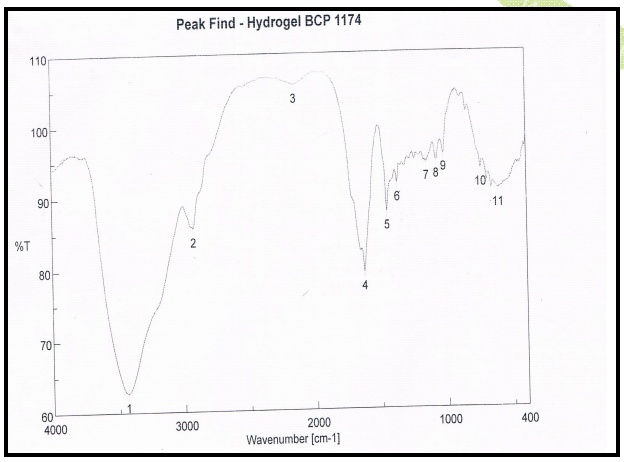

Figure 10: IR spectra of hydrogel

IR interpretation:

The functional groups present in the hydrogel along with their absorption range are mentioned in Table 9.

Table 9: Interpretation of the IR spectra

Absorption (cm^{-1})	Stretching	Functional groups
3436.53	N-H stretching	Amide group ($-NH_2$) may be present.
2992.34	O-H stretching	Alcoholic group (-OH) may be present.
1656.96	C-C=C symmetric stretching	Alkenes group (-C-C=C) may be present.
1750.49	C=O stretching	Esters group (-C=O) may be present.

Drug Loading in the Hydrogels

Amount of drug loaded in the hydrogels by drug loading technique was determined by the following formula:

$$EE\% = \frac{\text{Total amount of drug} - \text{Free drug}}{\text{Total amount of drug}} \times 100$$

Where,

EE = Entrapment efficiency.

The entrapment efficiency for amoxicillin sodium was observed to be 78%.

Dissolution Studies

The drug release or the dissolution studies were carried out to observe the rate of the release of the drug. The drug release should not occur in the acidic pH but should occur in the basic pH. A minimum drug release was observed in the acidic medium. Then the dissolution studies were continued in the basic medium using phosphate buffer of pH 7.4 by maintaining the sink conditions and the absorbance were measured at 227 nm and the dissolution studies were carried out. 85% drug release was obtained in the basic medium.

CONCLUSION

Drug delivery has undergone advancement in the past few years. There are a number of evidences in which the potentiality of the drug delivery systems can be used to release the drug at the desired target site of action[13]. These hydrogels being biocompatible and biodegradable in nature have been used in the production of the nano biotechnology products and have a vast number of applications in the field of controlled drug delivery systems. In present scenario, the main consideration in synthesizing the hydrogel is their mechanical strength and response time in physiological environment[14]. Thus, these hydrogels have gained importance as intelligent carriers in drug delivery systems. Moreover a high level of in vivo-in vitro correlation will determine the future success of the hydrogels[15].

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