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Article

Cyclotriphosphazene-based Stimuli-responsive Semi-IPN Hydrogels: Synthesis, Diffusion, and Anti-cancer Drug Release Characteristics

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ABSTRACT

In the recent years, there has been an emerging focus in the designing of various therapeutic devices for biomedical and biological applications. A variety of chemical compositions, structures, morphology, desired functional properties, thermodynamic properties, stimuli response, and chemical and physical interactions have shown significant interest in the use of natural, synthetic, and biohybrid polymeric hydrogels for drug delivery. In the last few decades, researchers have been mainly focused on organically modified poly(phosphazenes) (OPZs) to develop hybrid polymeric and organic-inorganic compounds. By understanding these points, here in the present investigation, we developed stimuli sensitive Hexachlorocyclotriphosphazene (HCP) based semi-IPN hydrogels as potential devices for controlled delivery of 5-fluorouracil (5-FU), a chemotherapeutic agent. At first, we synthesized a new vinyl monomer, that is, mono(ethacryloyl-2-ethoxy)-pentakis(N^1,N^1-dimethylpropane-1,3-diamino)-cyclotriphosphazene using HCP, 2-hydroxy ethylacrylate and propyl amine. Then the synthesized monomer was involved in the designing of semi-IPNs through free radical polymerization using poly(vinyl alcohol) (PVA) as a supporting polymer backbone. The synthesized monomer was confirmed by Fourier transform infrared (FTIR), ^{1}H, and ^{13}C NMR. The structural, morphological, thermal properties, and polymer-drug interactions of the networks were investigated by FTIR, scanning electron microscope (SEM), thermo gravimetric analysis, and X-ray diffraction, respectively. From the SEM images, it was observed that the network is compact and dense. The pH and temperature responsiveness of semi-IPNs was investigated by performing the diffusion studies in physiological solutions ranging from pH = 1.0–10.0 and at temperatures 25°C and 37°C. The % swelling ratio is maximum for high polymer-monomer ratio, minimum cross-linker concentration, low pH, and at 25°C. Different network parameters $(\overline{M_c}, \chi, \upsilon_e \text{ and } \xi)$ were investigated. The *in vitro* release of 5-FU was conducted in pH = 1.2 and pH = 7.4 at 25°C and 37°C, and noticed that the release was affected by both the pH and temperature and also controlled by the composition of HCP monomer. Different kinetic models were approached to drug release profiles and finally, it was observed that non-Fickian diffusion was involved in the release mechanism and the data were well fitted with Higuchi square root model.

Key words: Cyclotriphosphazene, Poly(vinyl alcohol), Semi-IPN hydrogel, 5-fluorouracil, Network parameters, Controlled drug release.

1. INTRODUCTION

Now a day, cancer is one of the well-known multifaceted diseases which is leading to common causes of high mortality rate. A wide range of anti-cancer drugs has been developed and employed for the treatment of cancer, but their activity is seriously limited due to adverse side effects on normal cells. To address these problems, controlled release (CR) formulations have been introduced and developed for improving the therapeutic efficacy that has limited by toxicity, uneven distribution of drug and its stability [1]. The concept of these CR formulations also focuses at control delivery of active pharmaceutical ingredient (API) at the target site to avoid possibility toxicity toward normal cells, thus enhancing therapeutic index and patient compliance. Therefore, CR formulations are best promising approaches for a variety of APIs to extend the release time for minimization of potential side effects.

Over past few decades, the development of macromolecular therapeutics has been envisioned for CR formulations where an API is embedded into the polymeric network. Recently, a wide range of natural and synthetic polymers have been utilized for CR formulations. However, the difficulty is to gain desirable characteristics such as permeability, stimuli response, surface active functional groups, biocompatibility, and biodegradability. To produce all these tunable properties, hydrogels have shown a novel route. Hydrogels are three dimensional, hydrophilic, and high molecular weight polymeric networks crosslinked either by physical or chemical interactions [2]. When hydrogels immersed in aqueous media, the solvent penetrates into the network, results considerable swelling, but the presence of crosslinks between the polymeric chains holds their structure intact by preventing dissolving of three dimensional network [3]. The

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high water retention capacity of hydrogels attributed to provide soft tissue-like consistency [4]. In addition, the hydrogels have offered more advantageous properties including non-toxicity, excellent biocompatibility and biodegradability, all of these make them as suitable biomaterials for various biomedical [5,6], pharmaceutical [7,8], and industrial [9,10] applications. Of these, hydrogel-based drug delivery has become a leading application in recent research activities. Semi-interpenetrating polymer networks (semi-IPNs) are one class of network polymers, where one of the two polymers is cross-linked in the presence of another through non-covalent interactions between the two polymers [11]. The combination of two polymers mimics their excellent features such as good mechanical strength and divergent sensitivity [12]. These unique properties are superior over either of the two individual polymers. In the literature, various semi-IPN hydrogels have been explored to remarkable biomedical application [13-20].

The responsive behavior of hydrogel networks has a key concept for developing advanced polymeric systems with enhanced properties. The responsive behavior is majorly aroused from non-covalent bonding interactions such as hydrogen bonding, hydrophilic, π-π stacking, and electrostatic interactions [16]. Hydrogels when exposed to external environmental conditions, they do exhibit dramatic alterations in the network due to phase transition with respect to such conditions including pH, temperature, light, ionic strength, electric and magnetic stimuli, analyte composition are defined as "stimuli-responsive" or "smart" or "intelligent hydrogels" [17,18]. In most cases, the phase change in the network is caused by the construction or destruction of non-covalent bonding forces, simple acid-base reactions of pendant moieties in response to specific stimuli. On other hand, the external stimuli can also transform the network properties by causing reversible or irreversible cleavage of polymeric bonds or pendant crosslinking bonds [19]. A variety of stimuli-responsive hydrogels have been envisioned as attractive materials in the areas of water treatment, sensing, and biomedical applications [20-22]. Among all stimuli, pH and temperature have been well-studied in the development of stimuli-sensitive polymeric matrices. pH-sensitivity of hydrogel networks come from the structural properties of the network such as pendant acidic and basic groups that are responsible for phase changes through protonation or deprotonation. In general, the acidic groups can deprotonate at high pH, while the basic groups can protonate at low pH. Accordingly, hydrogels undergo association, dissociation as well binding of various ions to the network, all of these lead to swelling and shrinking processes of hydrogels [23,24]. Thermosensitive polymers, a new class of stimuli sensitive materials have created new platform for various bio-related applications including drug delivery, membranes, cell encapsulation, separation, tissue engineering, and enzyme activity control [1,23]. The phenomenon of LCST has been involved in the phase changes of water soluble polymers with increase in the temperature.

Poly(vinyl alcohol) (PVA) is a well-known synthetic, hydrophilic and water soluble polymer has acquired properties including excellent hydrophilicity, biocompatibility, good transparency, high chemical stability, easy preparation, desirable mechanical strength, non-immunogenecity, and non-carcinogenecity [15-19]. All of these properties could be potentially helpful in divergent environmental, industrial, pharmaceutical, and biomedical applications [15-19]. Since PVA is highly hydrophilic in nature, it is less stable in aqueous media and it can be prevented by known techniques such as blending, copolymerization, crosslinking of networks, and polymer modification. Lack of stimuli sensitivity of traditional cross-linked PVA hydrogels limits its tendency toward CR studies.

Recently researchers have been mainly focused on organically modified poly(phosphazenes) (OPZs) to develop hybrid polymeric or organic-inorganic compounds. Phosphazenes, a new class of inorganic species, exist with repeating units of phosphorous and nitrogen atoms in linear or cyclic form [23]. Hexachloro cyclotriphosphazene (HCP), a small cyclic phosphazene, has been in the history since 1832 and has so many reports in the literature, as it is easily functionalized by nucleophilic substitutions of chlorine atoms with retention of P-N structure. The fascinating properties of HCPs are ring opening ability, regio and streo chemical control mainly emphasize the functionalization [25]. A broad range of HCP derivatives have been attracted for numerous applications, including flame retardants, hydraulic fluids, lubricants, insecticides, pesticides, fertilizers, and catalyst supports. Interesting physicochemical properties of HCP derivatives such as biocompatibility, biodegradability, thermo-sensitivity and define tumor targeting ability permit them as ideal materials for controlled drug release studies [26]. Therefore, there is a necessity to introduce stimuli-responsive moieties or functionalities in to the hydrogel network. Hence, in the present investigation, we have induced the sensitivity by introducing pH and temperature responsive HCP derivative and the responsiveness was also evaluated, which plays a key role in controlled drug delivery.

Among various chemotherapeutic drugs, 5-fluorouracil (5-FU) has been the most popular drug for the treatment of solid tumors such as colon, breast, and pancreas [27]. 5-FU is a sparingly water soluble and slightly alcohol soluble pyrimidine antimetabolite, has very short biological half-life of 10-20 min. The clinical administration of 5-FU was significantly limited because of short half-life, rapid metabolism, low bioavailability, severe side effects, and non-specific activity against healthy human cells [27-30]. Due to non-selective action against normal body cells, 5-FU causes problematic side effects including hair loss, fatigue, birth effects, ulcers, liver disease, and temporary failure of bone marrow. Therefore, the targeting ability and therapeutic index of 5-FU can be improved by developing 5-FU entrapped polymeric devices, which provide an effective and safe therapy with reducing adverse side effect [30].

2. EXPERIMENTAL

2.1. Materials

PVA, acryal amide, HCP, and 2-hydroxy ethylacrylate were purchased from Aldrich Chemicals (USA). 5-FU was received from N,N-methylene bisacrylamide (MBA) and ammonium persulfate (APS) were purchased from S.D. Fine Chemicals (Mumbai, India). H_3PO_4, KH_2PO_4, K_2HPO_4, HCl, and NaOH were received from Merck (India). All the other chemicals were analytical grade and used without further purification. Double-distilled water was used throughout the experiment.

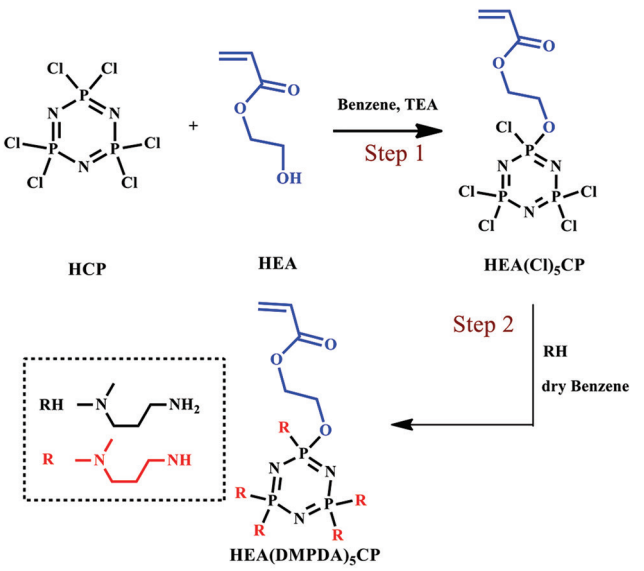
2.2. Synthesis of Mono(ethacryloyl-2-ethoxy)-pentakis(N^1,N^1-dimethylpropane-1,3-diamino)-Cyclotriphosphazene (HEA(DMPDA)$_5$CP) Monomer

In the first step of synthetic procedure, mono(ethacryloyl-2-ethoxy)-pentaclorocyclotriphosphazene (HEMA(Cl)$_5$CP) was prepared by the reaction of HCP with 2-hydroxy ethylacrylate (HEA) in dry benzen. In a 250 mL round bottom flask, a solution of 6.9 g (5 mM) of HCP and 2.32 g (5 mM) of HEA was prepared in 30 mL of dry benzene which after being stirred for 1 h at room temperature. After stirring, a solution of 1.25 mL of triethylamine (TEA) was added to the above solution and stirring is further continued for 4 h. The amine hydrochloride formed in this step was filtered and the residue was collected by removing the solvent under reduced pressure (Rotavapor, Buchi, R215, Singapore), which was then subjected to column chromatography (silica and hexane) and recrystallized from hexane.

In the second step, the product obtained in step-1 ($HEA(Cl)_5CP$) was first dissolved in 30 mL of dry benzene charged in a 250 mL round bottom flask, to which a solution of DMAPA (14.17 g) and TEA (6.2 g) in dry benzene (30 mL) was added drop wise and stirring is allowed for 6 h at room temperature. The resulting mixture was filtered and the solvent was distilled off under reduced pressure (Rotavapor, Buchi, R215, Singapore). The residue was then dissolved in ether followed by washing with DDW and the solvents were removed under reduced pressure again. Finally, the obtained crude was purified by subjecting it to column chromatography (silica, chloroform-methanol [5:1]) to get brown color oil of EDP. The schematic representation of synthesis of monomer is shown in Scheme 1.

2.3. Synthesis of PAEDP Hydrogels

Poly(vinylalcohol)/poly(acrylamide)/mono(ethacryloyl-2-ethoxy)-pentakis(N^1,N^1-dimethylpropane-1,3-diamino)-cyclotriphosphazene (PAEDP) hydrogels were synthesized via free radical polymerization. Different feed ratios [Table 1] of aqueous solutions of PVA, Am, EDP and MBA were taken in a beaker and homogenized for 1 h under constant stirring maintained on an ice-bath. Before the addition of initiator, nitrogen was purged through the homogenized mixture for about 30 min to remove the dissolved oxygen. After purging, the aqueous solution of APS was added as an initiator and the polymerization was allowed to proceed for 12 h at 10°C. On completion of gelation, the obtained hydrogels were placed in DDW for about 4 days at room temperature by changing the water with fresh one per twice a day to remove unreacted chemical fractions of monomers and initiator. Thereafter, the hydrogels were dried at room temperature for approximately 2 days and then dried in hot air oven at 60°C until reach a constant dry weight. The schematic representation of the hydrogel network is shown in **Scheme 2**.



Scheme 1: Schematic representation of $HEA(DMPDA)_5CP$ monomer.

2.4. Characterization

All characterizations and other procedures were followed as per our published procedures [9].

3. RESULTS AND DISCUSSION

3.1. Characterization

3.1.1. Fourier transform infrared (FTIR) and nuclear magnetic resonance (NMR) spectroscopy

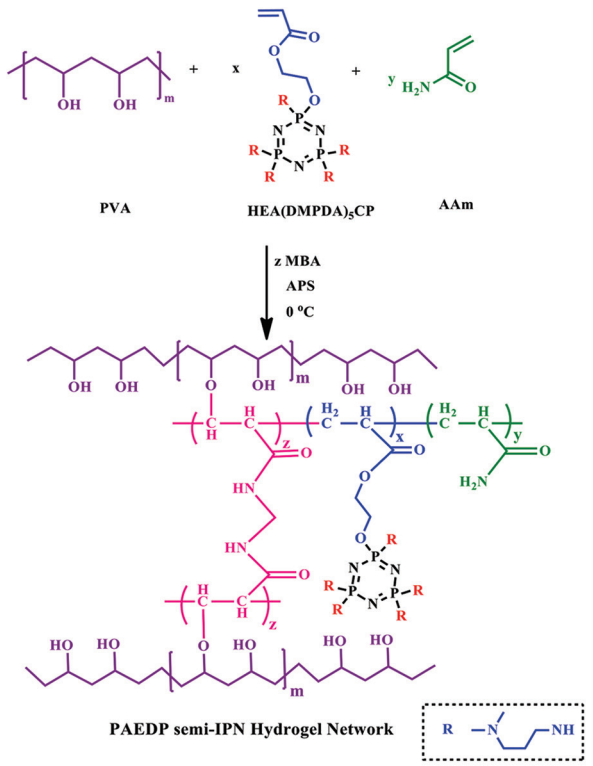
The characteristics of synthesized monomer were determined from FTIR and ^{1}H NMR studies. For EDP monomer, the principal IR bands are found [Figure 1] at 3420 cm^{-1} (N-H str.), 2964 cm^{-1} (=C-H str.), 2862 cm^{-1} (-C-H str.), 2147 cm^{-1} (C=C asymmetric str.), 1706 cm^{-1} (C=O str. of α, β-unsaturated ester), 1648 cm^{-1} (C=C str.), 1470 cm^{-1} (alkane C-H bend.), 1358 cm^{-1} (geminal dimethyl C-H bend.), 1220 cm^{-1} (C-N str.), 1095 cm^{-1} (C-O str.), 958 cm^{-1} (C=C bend.), 722 cm^{-1} (-CH_2 rocking), while the prominent P=N, P-N and P-O str. vibrations are observed at 1273 cm^{-1}, 1162 $cm^{-1}$and 1037 cm^{-1} P=N, P-N and P-O str. vibrations are observed at 1273 cm^{-1}, 1162 $cm^{-1}$and 1037 cm^{-1} respectively. Figures 3 and 4 represent the ^{1}H NMR and ^{13}C NMR spectra of synthesized EDP monomer. The ^{1}H-NMR spectrum of EDP monomer [Figure 2] gives the respective proton signals at δ (ppm): 6.4(5P), 6.1(1P), 5.5(2P), 4.2(2P), 3.4(2P), 3.0(10P), 2.5(10P), 2.3(30P), and 1.7(10p). ^{13}C NMR chemical shifts values were found at δ (ppm): 168.7(1C), 132.2(1C), 125.6(1C), 65.5(1C), 58.1(5C), 45.0(10C), 54.0(1C), 39.0(5C), and 27.0(5C).

FTIR spectra of plain PAEDP hydrogel, 5-FU loaded PAEDP hydrogel, and pristine 5-FU are shown in Figure 4. The plain PAEDP hydrogel showed stretching vibrations of O-H, N-H, C-H correspondingly at 3414 cm^{-1}, 3194 cm^{-1}, and 2915 cm^{-1}, respectively. Peaks at 1664 cm^{-1}, 1320 cm^{-1}, and 1099 cm^{-1} are related to C=O, C-N, and C-O stretching vibrations, respectively. However, the spectra of 5-FU loaded PAEDP hydrogel exhibited sharp peaks at 1210 cm^{-1} and 808 cm^{-1} might be attributed to the C-H in-plane and C-H out-of plane deformations, while the peak at 750 cm^{-1} corresponds to C-F stretching vibrations of

Table 1: Feed formulation compositions of different PAEDP hydrogels, % equilibrium swelling (%ESR), and % encapsulation efficiency (%EE) results.

Code	PVA (5%) (mL)	Am (g)	(HEA $(DMPDA)_5CP$) (g)	MBA (2%) (mL)	APS (10%) (mL)	%ESR	%EE±S.D
PAEDP-1	1.0	1.0	0	1.0	1.0	1036.57	42.23
PAEDP-2	1.0	1.0	0.25	1.0	1.0	1065.89	44.37
PAEDP-3	1.0	1.0	0.5	1.0	1.0	1073.99	56.43
PAEDP-4	1.0	1.0	0.75	1.0	1.0	1123.56	68.69
PAEDP-5	1.0	1.0	0.5	0.5	1.0	1471.82	71.69
PAEDP-6	1.0	1.0	0.5	2.0	1.0	784.23	20.83

SD: Standard deviation, MBA: N, N-methylene bisacrylamide, PAEDP: Poly (vinylalcohol)/poly (acrylamide)/mono (ethacryloyl-2-ethoxy)-pentakis (N1, N1-dimethylpropane-1,3-diamino)-cyclotriphosphazene hydrogels, ESR: Equilibrium swelling ratio, EE: Encapsulation efficiency, PVA: Poly (vinyl alcohol), (HEA $(DMPDA)_5CP$): Mono (ethacryloyl-2-ethoxy)-pentaclorocyclotriphosphazene, APS: Ammonium persulfate



Scheme 2: Schematic representation of PAEDP semi-IPN hydrogel network.

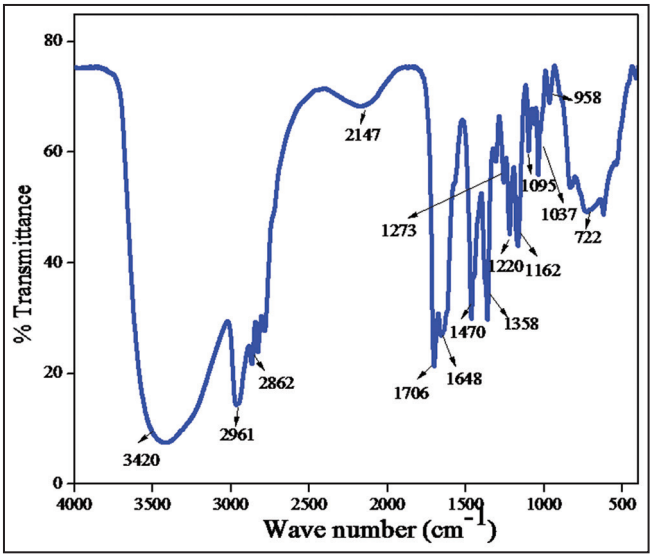


Figure 1: Fourier transform infrared spectra of HEA(DMADP)$_5$CP monomer.

5-FU. The appearance of these new peaks confirmed the presence of 5-FU in the drug loaded hydrogel networks.

3.1.2. Thermo gravimetric analysis (TGA)

To evaluate the thermal stability of drug loaded hydrogels, plain PAEDP hydrogels, 5-FU-loaded PAEDP hydrogels, and pristine 5-FU were characterized by TGA and the TG curves are displayed in Figure 5. The figure shows TG curve of 5-FU with sharp decomposition curve at 281°C attributed to its melting. The TG curve of plain PAEDP hydrogel shows three events of weight losses, wherein the first step is observed at 32°C that moved down to 86°C with a weight loss of 12%, indicating the loss of moisture. The second event of weight loss of 53%, corresponding to decomposition of PVA, took place at 315°C. The third event occurred at 430°C, with a gradual weight loss of 17% due to decomposition of phosphazene moieties of the network. However, 5-FU-loaded hydrogel matrix has followed similar behavior, but the incorporation of 5-FU into the network alter the temperatures of events to a slight extension due to high melting point of 5-FU and no premature degradation is observed that strongly suggests the stabilization of 5-FU within the hydrogel network.

3.1.3. Scanning electron microscopy (SEM)

The morphological features of PAEDP hydrogels were examined through SEM micrographs which are represented in Figure 6. From the SEM micrographs, it has been observed that the surface of PAEDP hydrogel is compact, dense, and rough in existence.

3.1.4. X-ray diffraction (XRD)

X-ray diffractograms of plain PAEDP hydrogel, 5-FU-loaded PAEDP hydrogel and pristine 5-FU are shown in Figure 7. In the case of placebo hydrogels, one broad and another small intense peak were observed at 2θ of 21° and 26°, respectively. Pristine 5-FU shows its characteristic peak at 2θ of 28° attributed to its diffraction pattern of (001) plane of corresponding crystal structure. However, less intense characteristic peaks of 5-FU were observed in drug loaded PAEDP hydrogels, suggesting the molecular dispersion of 5-FU in the PAEDP hydrogel matrix as well the adherence of small amount of drug on the surface of the hydrogel matrix.

3.2. Swelling Studies

Drug release from the hydrogel matrix is greatly evidenced from the swelling of cross-linked network. The swelling behavior of PAEDP hydrogels was first demonstrated in DD water at room temperature. As shown in Figure 8a, the values of % ESR are increased with respect to monomer content in the feed mixtures of the hydrogels. The hydrophilic functionalities present in the matrix might facilitate the interactions with more liquid molecules. Therefore, for PAEDP-1, the % ESR was found as 1036.57, which is significantly increased up to 1123.56. On the other hand, the % ESR decreased with increase in cross-linker (MBA) content, due to restricting the network to take large amount of aqueous media by forming more denser and rigid network. As represented in Table 2 and Figure 8a, lower swelling (784.23) was found for PAEDP-6 because of maximum cross-linker concentration (2.0 mL), while formulation PAEDP-5, exhibits higher equilibrium swelling suggesting that high water absorptivity (1471.82) is due to lesser extent of crosslinking density (0.5 mL). Similar to swelling trends of PAEDP hydrogels the deswelling studies were also evaluated and the results are represented in Figure 8b.

The swelling studies were also illustrated in terms of pH and temperature. Therefore, the studies were demonstrated for formulation PAEDP-4 by varying pH of the swelling media as well as temperature. The pH-responsive behavior of PAEDP hydrogels [Figure 9a] has been resulted from the pendant 2° amino groups of EDP monomer. By varying the pH of swelling media ranging from pH = 2.0 to pH = 10.0, the %ESR values were taken, from which it is observed that as the pH of the media increased from low to high, the %ESR slightly moved down, which might be attributed due to the protonation/deprotonation of 2° amino groups. Under acidic environment, amino groups were ionized by protonation, which readily interact with the aqueous media through electrostatic interactions, results high water uptake capacity for PAEDP hydrogels. However, at alkaline conditions, we found that amino groups involved in deprotonation rather than protonation; hence, electrostatic repulsions were produced between the network

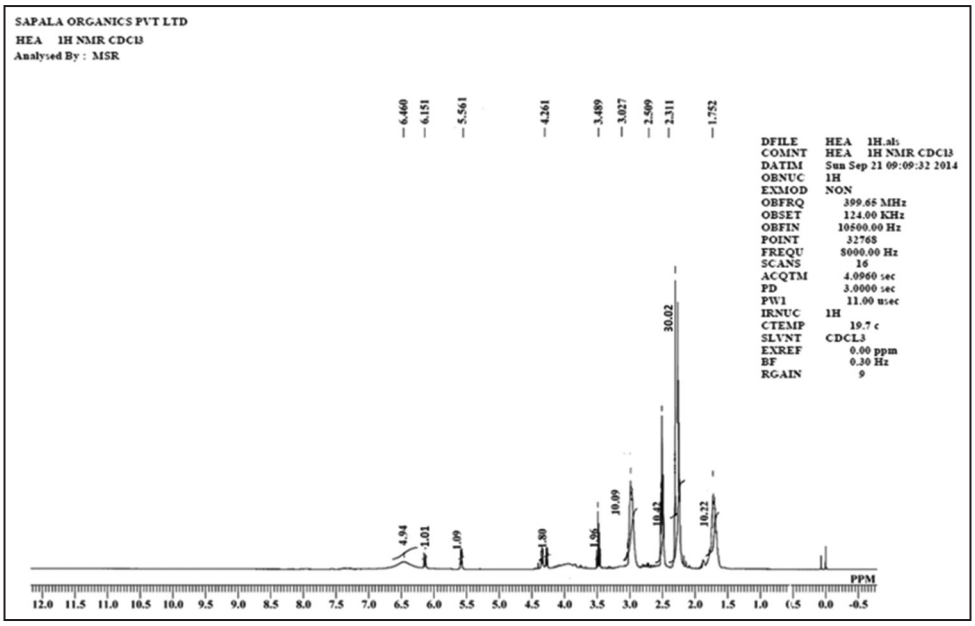


Figure 2: ^{1}H NMR spectra of HEA(DMADP)$_5$CP monomer.

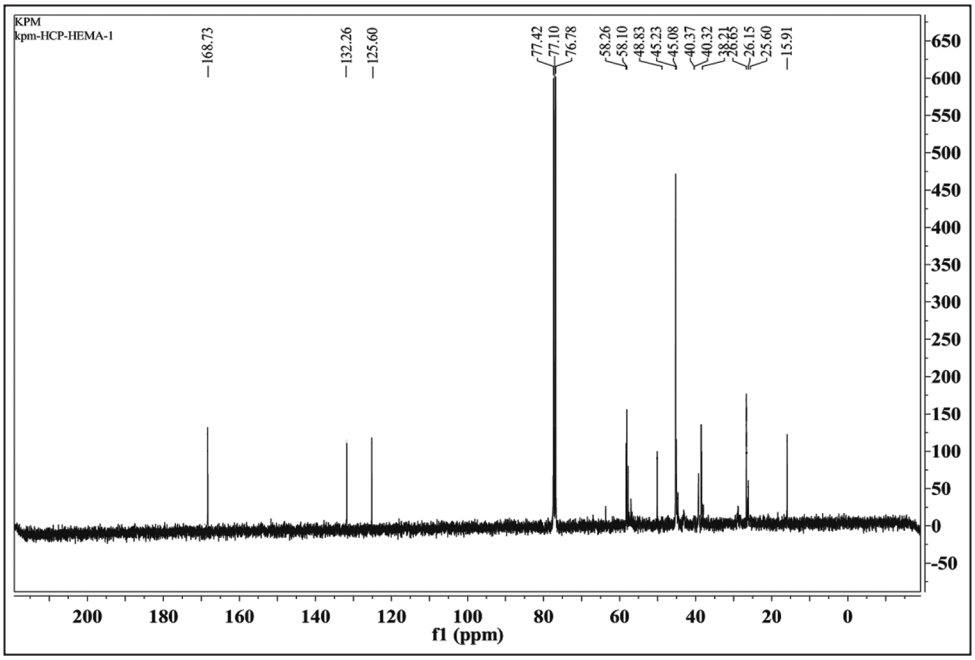


Figure 3: ^{13}C NMR spectra of HEA(DMADP)$_5$CP monomer.

and surrounding water molecules led to poor water uptake and less swelling.

The temperature dependent swelling was investigated by performing the swelling experiments under pH = 2, 7 and 10 at temperatures 25 and 37°C [Figure 9b]. The results supported that at three pH conditions, the maximum equilibrium swelling was appeared at a temperature of 25°C compared to 37°C. This could be explained by the fact that the hydrophobic 3° amines of EDP monomer undergo phase transition with respect to changes in the external temperature. At low temperatures (25°C), the network shows good capability to hold water molecules through electrostatic interactions, so maximum swelling is experienced. As the temperature increased from 25°C to 37°C, the hydrophobic behavior of polymeric network predominates, the gel may shrink or may collapse in to lumps, due to this the absorbed water leached out forcefully, lead to decrease in the equilibrium swelling.

3.2.1. Evaluation of network parameters

The prominent structural parameters of PAEDP hydrogel networks such as average molecular weight of polymeric fraction between two adjacent crosslinking centers ($\overline{M_c}$), the effective crosslinking density of network (υ_e), mesh or mean pore size (ξ), and Flory-Huggins polymer-solvent interaction parameter (χ) of present hydrogel system were evaluated from respective equations and are noted in Table 1. In the present study, the ($\overline{M_c}$) values followed the swelling behavior of hydrogels. With increase in monomer concentration, ($\overline{M_c}$) values are also increased from 4458 to 10308. However, the higher cross-linker concentration leads to decrease the ($\overline{M_c}$) value (2655), due to increase in number of cross-links results consequent reduction in the polymer chain between two consecutive crosslinking points. The maximum value of ($\overline{M_c}$) (12466) was observed for formulation PAEDP-5, having lower cross-linker concentration. Experimental values revealed

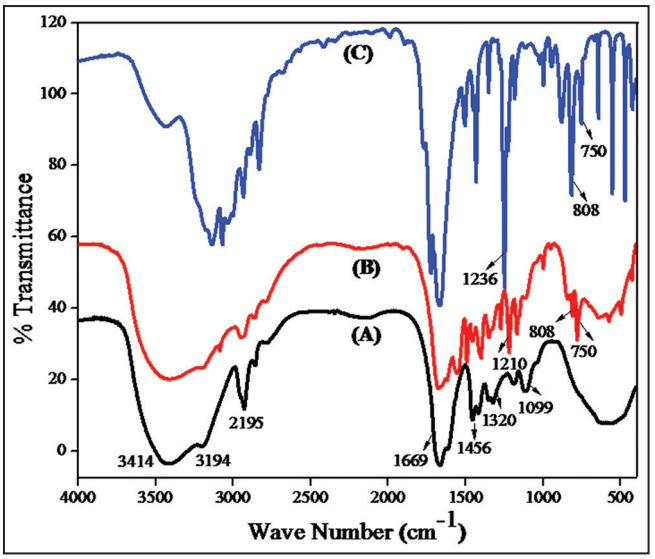


Figure 4: Fourier transform infrared spectra of (A) plain PAEDP hydrogels, (B) 5-FU loaded PAEDP hydrogels and (C) pristine 5-FU.

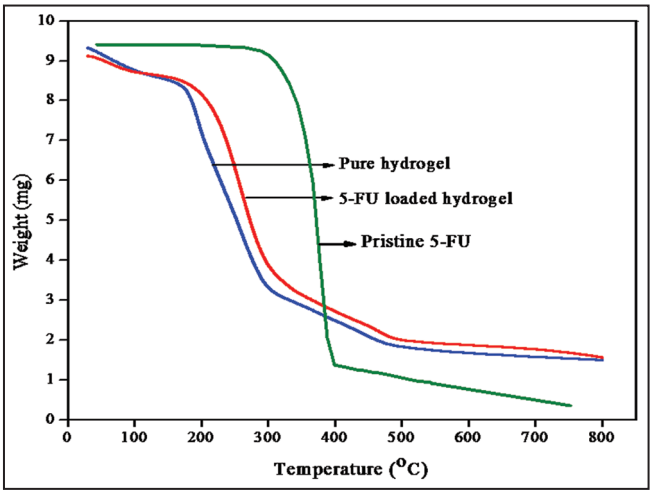


Figure 5: Thermo gravimetric analysis analysis of PAEDP hydrogels.

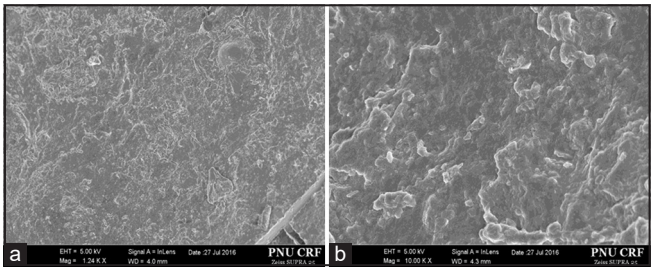


Figure 6: Scanning electron microscopic images of PAEDP hydrogel at magnifications (a) 1.24 KX and (b) 10.00 KX.

that the effective crosslinking densities (υ_e) of various formulations are found in the range of 0.189-0.614 mol/dm^3. The higher value of υ_e is observed for the sample prepared with high crosslinking agent (PAEDP-6). Hydrogel mesh size or mean pore size (ξ) is one of the important hydrogel parameters which defines the available space between two neighboring crosslinking points, predominantly influence the solute, and solvent transport as well drug encapsulation

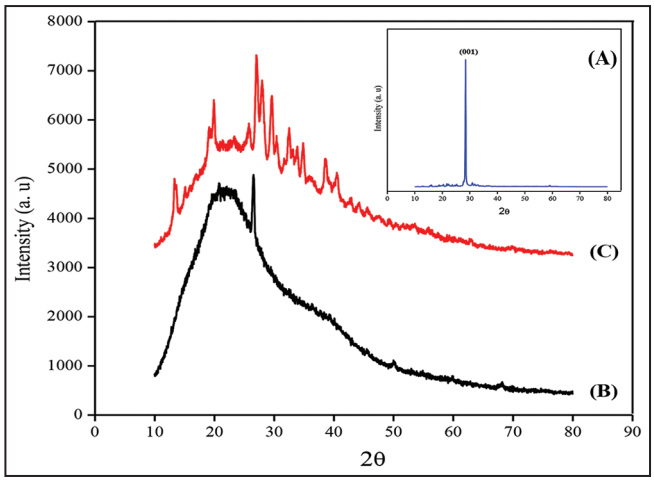


Figure 7: X-ray diffraction patterns of (A) pristine 5-FU, (B) plain PAEDP hydrogels and (C) 5-FU loaded PAEDP hydrogels.

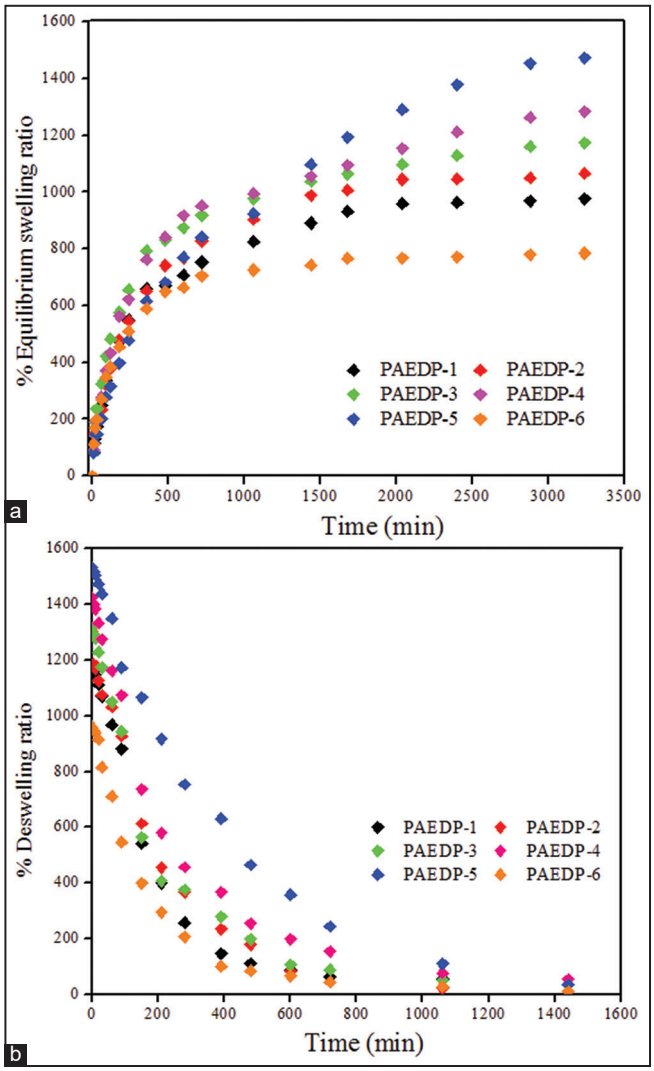


Figure 8: (a) Swelling and (b) deswelling behavior of PAEDP hydrogels in double distilled water (at room temperature).

and release phenomenon. The present study achieved the pore size of hydrogels ranged from 8.06 to 17.56 nm. The pore size of the network is increased with increase in monomer concentration and is decreased

Table 2: Curve fitting results of 5-FU release kinetics of PAEDP hydrogels for various kinetic models.

Sample code	Zero order		First order		Higuchi		Hixson-Crowell		Koresmeyer-Peppas		
	R^2	K_0	R^2	K_1	R^2	K_h	R^2	K_c	R^2	K_p	n
PAEDP-1	0.952	0.246	0.942	0.018	0.986	0.0696	0.997	0.016	0.993	0.392	0.941
PAEDP-2	0.975	0.273	0.809	0.019	0.987	0.0757	0.955	0.019	0.979	0.384	0.812
PAEDP-3	0.98	0.348	0.815	0.017	0.989	0.432	0.945	0.019	0.981	0.462	0.762
PAEDP-4	0.981	0.157	0.755	0.021	0.985	0.171	0.936	0.009	0.986	0.382	0.905
PAEDP-5	0.948	0.354	0.714	0.011	0.976	0.292	0.892	0.035	0.987	0.403	0.876
PAEDP-6	0.956	0.173	0.846	0.019	0.994	0.033	0.894	0.013	0.982	0.361	0.956

PAEDP: Poly (vinylalcohol)/poly (acrylamide)/mono (ethacryloyl-2-ethoxy)-pentakis (N1, N1-dimethylpropane-1,3-diamino)-cyclotriphosphazene hydrogels

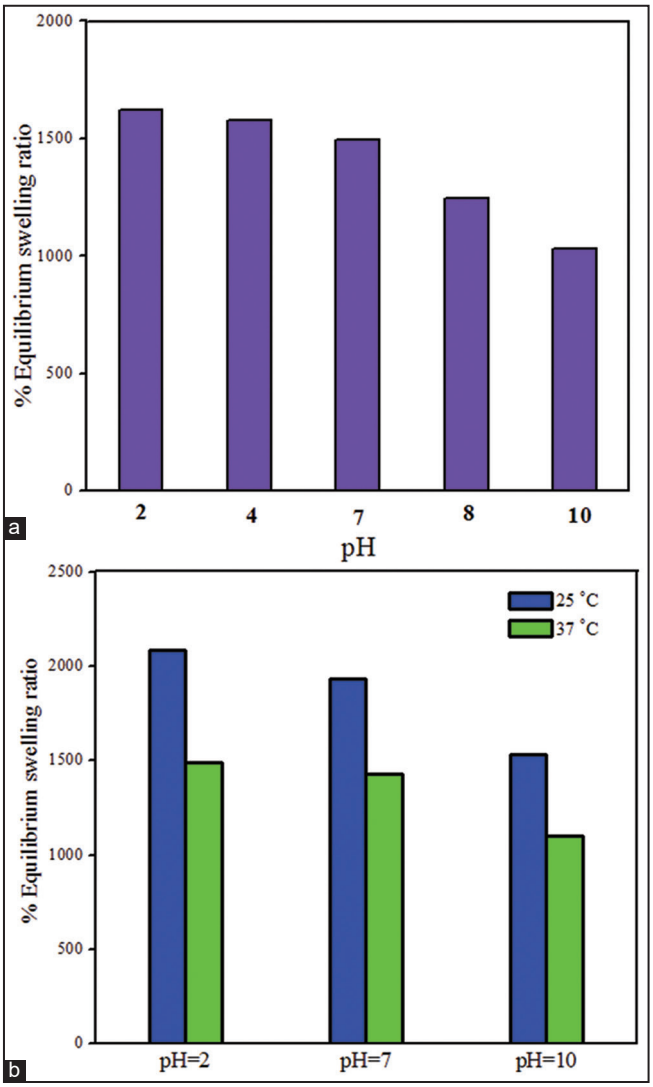


Figure 9: (a) pH and (b) temperature dependent equilibrium swelling behavior of PAEDP hydrogels.

with increase in crosslinking agent. The values of Flory-Huggins Polymer-solvent interaction parameter (χ) were found around 0.6 for all the formulations, described good polymer-solvent interaction during the swelling studies.

Diffusion constants (D) of PAEDP hydrogels are listed in Table 3. Based on diffusion parameters, we could predict the supporting mechanism for the transport of solvents and solutes through the hydrogel network.

Table 3: Various network and diffusion parameters of PAEDP hydrogels.

Sample code	Diffusion parameters		Network parameters				
	n	D	$\overline{M_c}$	χ	ξ	ϕ	ve
PAEDP-1	0.941	2.669	4458	0.588	10.74	0.088	0.568
PAEDP-2	0.812	3.302	5607	0.591	13.72	0.079	0.481
PAEDP-3	0.762	4.578	8101	0.620	14.64	0.082	0.401
PAEDP-4	0.905	10.168	10308	0.637	17.17	0.071	0.189
PAEDP-5	0.876	36.167	12466	0.646	17.56	0.061	0.327
PAEDP-6	0.956	2.661	2655	0.571	8.06	0.055	0.614

PAEDP: Poly (vinylalcohol)/poly (acrylamide)/ mono (ethacryloyl-2-ethoxy)-pentakis (N1, N1-dimethylpropane-1,3-diamino)-cyclotriphosphazene hydrogels

In the present investigation, values of n are ranged in between 0.762 and 0.956, indicates non-Fickian type transport mechanism, which involves relaxation of polymer chains.

3.3. Encapsulation Efficiency

Swelling behavior of polymeric networks plays a key role during drug encapsulation and release phenomenon. The % encapsulation efficiency (%EE) of 5-FU in the PAEDP hydrogels is tabulated (Table 1) and is ranged between 20.83 and 71.69, depends on the feed composition of network. Hydrogels prepared with high concentration of EDP monomer were able to load a maximum amount of 5-FU, due to their high degree of swelling. For instance, the PAEDP-4 (0.75 g of EDP) formulation has higher EE value of 68.69% than do PAEDP-3 (0.5 g EDP), PAEDP-2 (0.25 g EDP) and PAEDP-1 (0.0 g EDP) formulations, which showed %EE of 56.43, 44.37 and 42.23, respectively. Apart from this, %EE was also influenced by the crosslinking density. As the cross-linker amount increased from 0.5 to 2.0 mL (PAEDP-3, PAEDP-5 and PAEDP-6), the % EE values decreased from 71.69 to 20.83, this could be due to the formation of compact and denser network of hydrogel at higher concentration of cross-linking agent.

3.4. In Vitro 5-FU Release

PAEDP hydrogels loaded with 5-FU were employed for *in vitro* release experiments in gastric media (pH = 1.2) and intestinal media (pH = 7.4) at 37°C. The percentage 5-FU release was plotted with respect to time and the graphs are displayed in Figure 10a and b. As evidenced from the swelling data, the CR studies indicate that the maximum and controlled delivery of 5-FU was achieved in pH = 1.2 at

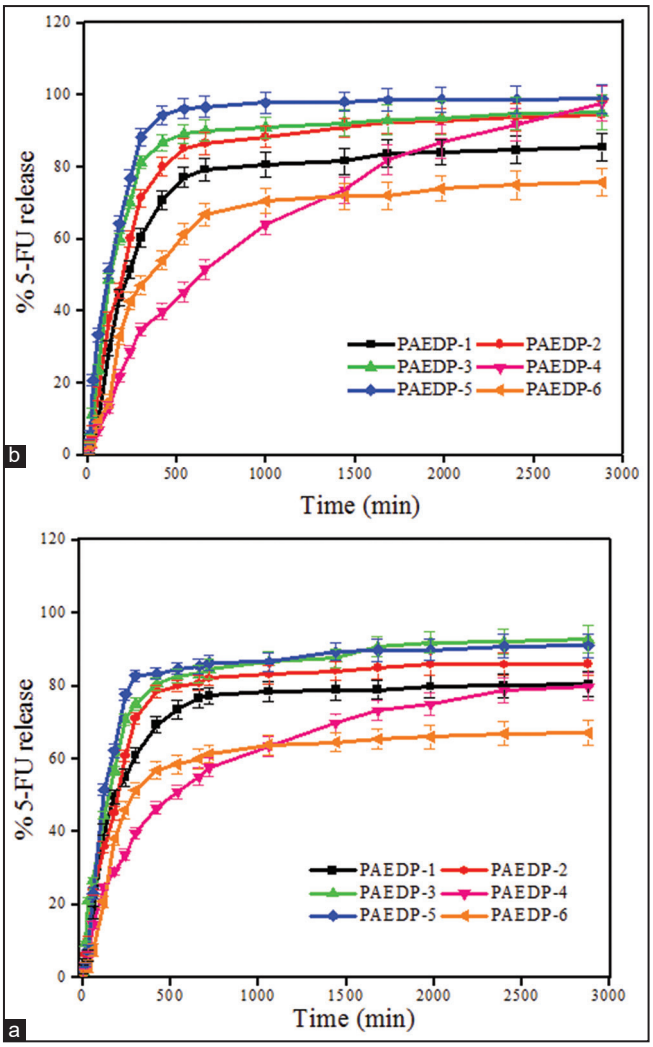


Figure 10: % 5-fluorouracil release from PAEDP hydrogels at (a) pH = 1.2 and (b) pH = 7.4.

37°C. However, the lower release rates were observed in pH = 7.4 due to less swelling of hydrogel network. The release experiments were also performed in pH = 1.2 at 25°C, to know the effect of temperature on 5-FU release. In the following section, the release studies were discussed interns of monomer content, extent of crosslinking density, and temperature.

3.4.1. Effect of (HEA(DMPDA)$_5$CP) monomer content

As seen from Figure 10a, significant difference is observed in release rate of 5-FU by varying monomer content from PAEDP-1 to PAEDP-4. The release rate of 5-FU increased with increasing EDP monomer content in the feed ratio. The hydrophilic groups of EDP monomer facilitate the swelling of hydrogel network, consequently the release of drug molecules. Furthermore, the greater amount of EDP monomer might hold the drug molecules tightly, hence released the molecules in a controlled manner even though the swelling is high. In the present experiment, CR of 5-FU was achieved for formulation PAEDP-4, it exhibits ~90% of 5-FU release up to 48 h. However, PAEDP-1, PAEDP-2, and PAEDP-3 showed a fast release in initial 5 h followed by a slow release, this might be caused by the poorly entrapped drug molecules on the surface of the hydrogels.

3.4.2. Effect of crosslinking density

As displayed in Figure 10a, a fast release behavior was observed for PAEDP-5, which contains a lower concentration of MBA, that is, only 0.25 mL permits larger pore size thereby rapid swelling. Noticed that

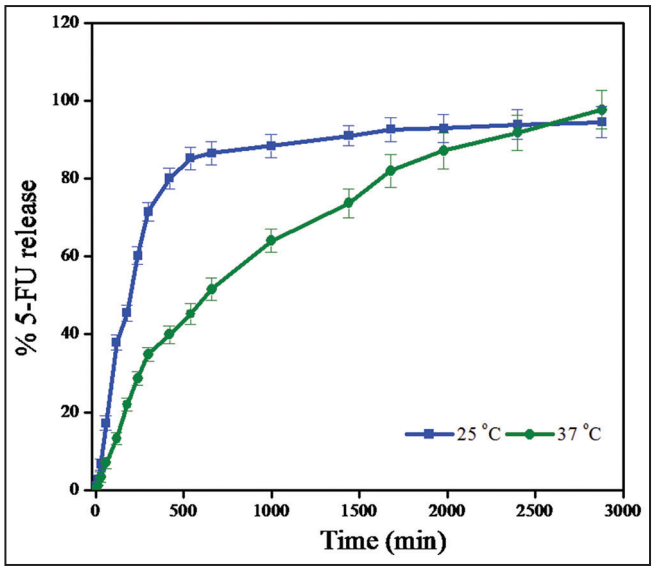


Figure 11: % 5-fluorouracil release from PAEDP hydrogels at two different temperatures 25 and 37°C.

lower % of 5-FU release for PAEDP-6, since it has higher crosslinking density (2.0 mL of MBA) and it acquired denser network that results small pore size. About 76% of 5-FU released in 5 h for PAEDP-5, whereas 42% release is observed for PAEDP-6. A moderate release, that is, nearly 69 % in 5 h was shown by PAEDP-3.

3.4.3. Effect of temperature

Figure 11 represents the effect of temperature on % release of 5-FU, confirms that a burst release was achieved at 25°C (below LCST), but at 37°C (above LCST) the release was controlled. At 25°C, about 72 % of 5-FU was released within 5 h in a burst manner followed by slow release, whereas at 37°C, the drug is leached out from the network in a controlled way. This could be explained on the basis that at low temperatures, the hydrogels are readily hydrated and are highly swollen, therefore exhibits higher tendency to uptake buffer solution, subsequently the diffusion rate of drug is enhanced. However, at higher temperatures (37°C), the collapsed network has no such tendency towards uptake of buffer and hence the diffusion rate is decreased.

3.4.4. Analysis of drug release mechanism

Five different empirical kinetic models were explored to analyze the release mechanism of 5-FU from the PAEDP hydrogels. The results are tabulated in Table 3, from that we could noticed that the regression coefficient (R^2) of 0.997 was observed for Hixson-Crowell equation for formulation PAEDP-1, indicates the drug release follows cube root of time relationship. For remaining formulations (PAEDP-2 to PAEDP-6), the high R^2 values were found for Higuchi and Koresmeyer-Peppas equations, indicating that the drug release from respective formulations depends on square root of time and the process is diffusion controlled. From Koresmeyer-Peppas equation, we could know that the n values are found in between 0.762 and 0.956 ($n > 0.5$), suggesting that the release phenomenon follows non-Fickian type transport.

4. CONCLUSION

A stimuli responsive monomer (HEA(DMPDA)$_5$CP) was synthesized from cyclotriphosphazene and was utilized for the preparation of semi-IPN hydrogels in combination with PVA, by a simple free radical polymerization. These hydrogels were used to investigate the CR of 5-FU with respect to pH and temperature and %EE was varied from 20.83 to 71.69. The synthesized monomer, hydrogels, and drug encapsulation were confirmed by ^{1}H NMR, FTIR, TGA, and XRD. The

hydrogels showed maximum swelling percentage at low pH (pH = 1.2), low temperature (25°C), and also influenced by the composition. The network parameters were evaluated to understand the hydrogel structure. The release of 5-FU from PAEDP hydrogels occurred through non-Fickian diffusion mechanism. The release data have obeyed different kinetic models and were well fitted for Higuchi square root equation with high R^2 values. The excellent biocompatibility, targeting ability, and dual responsive behavior of PAEDP hydrogels make them as potential site specific drug delivery carriers for cancer treatment.

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