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Article

Dual Responsive Poly(vinyl alcohol)-2-(dimethylamino)ethyl Methacrylate Hydrogels: Effect of Crosslinking Agent on Swelling and Anti-cancer Drug Release Properties

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ABSTRACT

In this manuscript, dual responsive semi-interpenetrating polymer networks hydrogels (polyvinyl alcohol/poly(acrylamide-co-(2-dimethylamino)ethyl methacrylate) [PVPAD]) fabricated from (2-dimethylamino)ethyl methacrylate, PVP, and acrylamide for anti-cancer drug (5-fluorouracil [5-FU]) delivery application. PVPAD hydrogels are successfully prepared by a simple free radical polymerization using N,N[1]-methylene-bis-acrylamide, and bis[2-methacryloyloxy] ethyl phosphate as a cross-linkers. The developed hydrogels are characterized by Fourier transform infrared spectrometer, X-ray diffraction, thermogravimetry, and scanning electron microscopy analysis to confirm the formation, structural interactions, thermal, and morphological properties. This study aimed to investigate the effect of crosslinker on swelling behavior and delayed release of 5-FU from PVPAD polymeric network. The studied parameters included: Monomer concentration, crosslinking agent type and concentration, and their overall effect on swelling of the hydrogels, drug loading efficiency, diffusion, and *in vitro* release characteristics of 5-FU from PVPAD hydrogels.

Key words: Hydrogel, Poly(vinyl alcohol), (2-Dimethylamino)ethyl methacrylate, Stimuli responsive, Drug delivery, 5-Fluorouracil.

1. INTRODUCTION

Hydrogels are three-dimensional polymer networks; they are physically and chemically cross-linked networks and hydrophilic in nature. Hydrogels swell in an aqueous environment but do not dissolve. Due to the potential features of hydrogels [1-6], they are favored in a broad range of biomedical applications such as sensors, artificial organs, wound dressings, and drug carriers [4-10]. Among all types of hydrogels, semi-interpenetrating polymer networks are emphasized for pharmaceutical applications due to tuneable physicochemical and mechanical properties along with the reversible volume change in response to the external stimuli, thatis, temperature, ionic strength of the solution, pH, and electric field [6]. In particular, hydrogels are designed to get the therapeutic result in the required target, as well as sustained drug release with minimum adverse effects.

The widely used synthetic polymer for biomedical application is poly(vinyl alcohol) (PVA); it is continuously scoring points in all fields of biomedical research mainly on biomedical devices in various forms of the multifunctional biomaterials. The prime properties of PVA are: It is linear, hydrophilic, and good mechanical strength. Due to these properties, it plays a key role in the fabrication of biomaterials, especially in drug delivery. In addition to this, it is a biocompatible and biodegradable polymer. Due to the above features, it is widely using for a verity of applications such as sensors, pervaporation, toxic metal ion removal, artificial organs, wound dressings, and drug delivery systems these applications [2,4,5,9-12]. However, PVA alone cannot be used as drug delivery device because of the lack of stimuli-responsive behavior. To add stimuli-responsive behavior PVA has been modified, blended and copolymerized with other monomers and polymers [10-12]. Hence, to meet the desired properties, it has to functionalize via blending and graft/copolymerization.

The copolymerization of (2-dimethylamino)ethyl methacrylate, a pH, and temperature responsive monomer of wide applications in the biomedical field it is due to easy copolymerization with other hydrophilic monomers [13-16]. Furthermore, it has a critical phase transition (pKa = 7.6) and lower critical solution temperature (38–50°C), which is nearer to the physiological range [13,14]. Hence, (2-dimethylamino)ethyl methacrylate enhances the mechanical properties and hydration degree of the hydrogel network in physiological conditions. Copolymerization of acryl amide and (2-dimethylamino)ethyl methacrylate has been assayed with PVA to obtain a hydrogel network that maintains a high degree of hydration, which helps in modulation of the 5-fluorouracil (5-FU) release to the medium by variation of the crosslinker and the monomer concentration.

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Safety and biocompatibility are the essential properties to fabricate a drug delivery system for delayed-release and therapeutic application. The impact of crosslinker type on swelling, 5-FU encapsulation, and *in vitro* drug release was studied by two types of crosslinkers. In the present investigation, authors have attempted to fabricate a dual responsive biocompatible hydrogel to achieve the control release of anti-cancer drug. PVA/poly(acrylamide-co-(2-dimethylamino) ethyl methacrylate) (PVPAD) based polymeric hydrogels that were fabricated by a simple free radical polymerization using potassium persulfate. 5-FU a chemotherapeutic agent successfully encapsulated into the hydrogels, and the *in vitro* release profiles at pH 1.2 and 7.4 were investigated.

2. MATERIALS AND EXPERIMENTAL DETAILS

2.1. Materials

(2-dimethylamino)ethyl methacrylate (the varying monomer), PVA, N,N[1]-methylene-bis-acrylamide, and bis[2-methacryloyloxy] ethyl phosphate were received from the Sigma-Aldrich Chemicals. Acrylamide, potassium persulfate, potassium dihydrogen phosphate, sodium hydroxide, hydrochloric acid, and 5-FU were received from s.d. fine chemical, Mumbai, India. All the chemicals used as received. Double-distilled water is used throughout the experiment.

2.2. Development of PVP/PVPAD Hydrogels

Two different series formulations of PVPAD hydrogels were synthesized using the free-radical polymerization method, using potassium persulfate as an initiator. To the 50 mL beaker, 5 mL of 4% PVA solution, required amounts of acryl amide, (2-dimethylamino) ethyl methacrylate, and crosslinker were added and stirred well until to get a homogeneous solution (Table 1). Nitrogen gas was purged through the reaction mixture for 5 min, and the mixture was subjected to the ultrasonication for the removal of gas bubbles. Finally, 1.0 mL of 10% aqueous initiator solution is added, and the beaker was transferred to the water bath, and the temperature is maintained at 55 ± 2°C. After 5h a soft hydrogel is formed; these were removed from the beaker and transferred to 100 mL of double-distilled water to remove any unreacted traces. At last, these swollen hydrogels were removed from the water and dried at room temperature, then followed by 38 ± 2°C in an electronically controlled oven. These hydrogels are named as PVPAD hydrogels, but N,N[1]-methylene-bis-acrylamide cross-linked hydrogels named as PVPAD-M and bis[2-methacryloyloxy] ethyl phosphate cross-linked hydrogels named as PVPAD-P. Plausible schematic representation of hydrogels is drawn based on the Fourier transform infrared (FTIR) spectrometer, results (Figure 1).

Swelling performance and the drug release studies of PVPAD hydrogels were performed by our laboratory's usual procedures [15].

2.3. Characterization

Structural characterization PVPAD hydrogels was performed by the FTIR (Perkin Elmer, Model: Spectrum 2). Morphology and drug distribution throughout the hydrogel matrix were shown by the X-ray diffractometer (Rigaku, Model: mini flex 600, JAPAN). Thermogravimetry analysis of the PVPAD hydrogels was recorded by the TA instruments (STA, Model: Q600, USA), surface morphology, of PVPAD hydrogels was performed by the scanning electron microscopy (SEM) (JOEL Model: IT500A, JAPAN).

3. RESULTS AND DISCUSSION

3.1. FTIR Spectra

FTIR spectra of plain PVPAD hydrogels, drug-loaded PVPAD hydrogels and pure drug 5-FU are platted intensity verses wavenumber (Figure 2). From the FTIR data [12,16] of PVPAD-MP and PVPAD-PP hydrogels, a broad peak was observed at 3424 cm^{-1}, which corresponds to –OH stretching vibrations of PVP. Furthermore, stretching peaks at 1665 cm^{-1}, 1125 cm^{-1}, and 1321 and 1255 cm^{-1} observed for –NH of 1° amide, C-O of ester group, and C-N of 2° amide, respectively; for monomers [17].

However, in the case of phosphate crosslinker based hydrogels, 1126 and 1050 cm^{-1} stretching peaks are observed for the P-O and P=O, respectively. In the case of 5-FU loaded hydrogels, in addition to above-mentioned characteristic peaks of pristine hydrogel, the other peaks are found at 1263, 814, and 780 cm^{-1} for C-F, C-H out of plane deformation, and C-H in-plane deformation, respectively. These results demonstrate the formation of PVPAD hydrogel networks as well as the molecular level distribution of 5-FU molecules in the PVPAD hydrogel networks.

3.2. X-ray Diffraction (XRD) Studies

XRD patterns of plain PVPAD hydrogels; 5-FU loaded PVPAD hydrogels, and pure 5-FU (Figure 2A-E), are platted intensity versus 2 theta (Figure 3). XRD patterns of pure 5-FU showed a sharp crystalline and characteristic peak at 2θ of 28.7° and week crystalline peak at 2θ 16.3°, which provides the crystallinity behavior of 5-FU [18].

Table 1: Formulation compositions, % encapsulation efficiency (% EE) and kinetic parameters of PGAGA hydrogels.

PVPAD-M	PVA (4%, mL)	Am (g)	DMA EMA (mL)	MBA (2%) (mL)	% EE	pH=1.2			pH=7.4		
						r^2	*n*	*k*	r^2	*n*	*k*
PVPAD-M 1	5	1.0	0.5	2	62.28±1.15	0.960	0.72	12.05	0.965	0.37	36.31
PVPAD-M 2	5	1.0	1.0	2	31.86±1.36	0.938	0.54	21.48	0.927	0.20	55.72
PVPAD-M 3	5	1.0	2.0	2	32.88±0.87	0.961	0.39	41.78	0.973	0.43	36.64
PVPAD-M 4	5	1.0	1.0	4	22.16±1.80	0.910	0.41	26.92	0.969	0.34	34.12
PVPAD-M 5	5	1.0	1.0	1	29.40±1.05	0.979	0.52	26.18	0.947	0.35	51.17
PVPAD-P	**PVA (4%, mL)**	**Am (g)**	**DMA EMA (mL)**	**BMEP (10%,mL)**	**% EE**	r^2	*n*	*k*	r^2	*n*	*k*
PVPAD-P 1	5	1.0	0.5	0.5	58.30±0.75	0.944	0.62	25.29	0.941	0.71	13.90
PVPAD-P 2	5	1.0	1.0	0.5	68.30±0.56	0.975	0.47	33.50	0.888	0.73	11.54
PVPAD-P 3	5	1.0	2.0	0.5	76.37±0.95	0.968	0.43	37.76	0.911	0.77	12.74
PVPAD-P 4	5	1.0	1.0	1.0	78.92±1.62	0.978	0.80	17.99	0.772	0.93	22.39
PVPAD-P 5	5	1.0	1.0	0.25	79.74±0.95	0.968	0.74	20.94	0.949	0.90	12.05

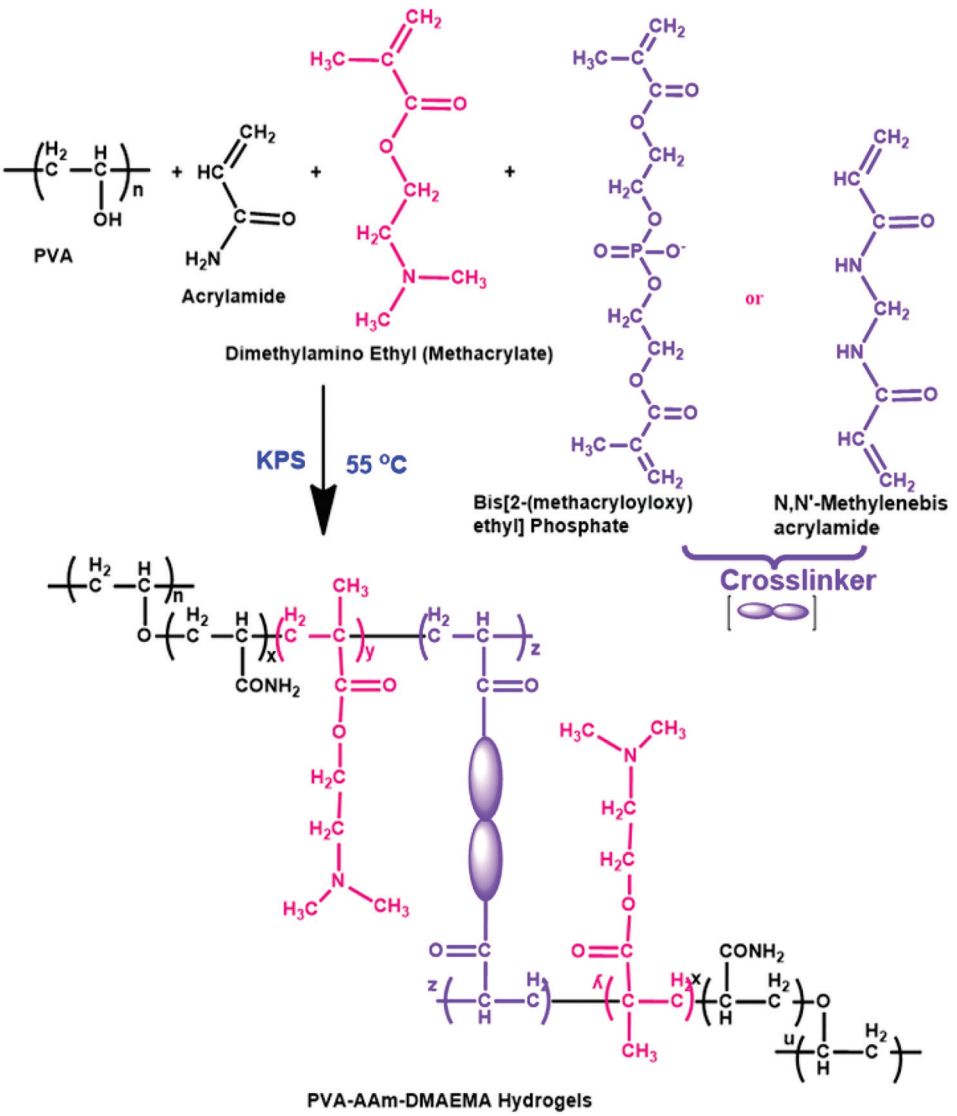

Figure 1: Plausible structural representation of poly(acrylamide-co-(2-dimethylamino)ethyl methacrylate)-P hydrogel.

However, characteristic peaks 5-FU is not much intensive in the case of the 5-FU loaded PVPAD hydrogel, which indicates the molecular level dispersion of the drug in the PVPAD hydrogels network.

3.3. Thermogravimetry Analysis

Thermogravimetry analysis was performed to evaluate the thermal properties and the confirmation of presence of drug in the PVPAD gels (Figure 4). The thermogram of 5-FU exhibits the weight loss between 220 and 320°C, it is due to vaporization and structural decomposition of 5-FU [19,20]. When we compare the thermograms of pure hydrogels and the drug-loaded hydrogels, we can notice a remarkable difference in thermal degradation behavior. Thermograms of PVPAD hydrogels were exhibited a multi-step degradation process (Table 2). The overall temperature range for the first stage was up to 200°C, the second stage from 200 to 320°C, and the third stage from 320°C up to 450°C. The amount of weight loss and the temperature range for each stage are listed in Table 2. According to thermogravimetry analysis results (Figure 3), the thermal stability has decreased for drug-loaded PVPAD-P as degradation temperature of 205°C is increased to 450°C, and weight loss of 67.0% drops to 53.5% with a lower rate of degradation compared to pure gels. However, for the PVPAD-M gels, the same decreased from 77.5% to 67% after loading the drug into the pure gels. This phenomenon may be explained based on the thermal stability and morphological characteristics of PAPAD hydrogels due to the disordered state of polymer chains at the time of drug loading in the polymeric network.

3.4. SEM

To understand the surface morphology of pristine and drug-loaded PVPAD hydrogels, SEM analysis performed, the images indicates that the surface of pristine PVPAD-M and PVPAD-P hydrogels are smooth (Figure 5). In contrast, the 5-FU loaded hydrogels show some aggregated microcrystals of 5-FU. These results demonstrate that the 5-FU present in the hydrogels not only distributed the molecular level in the hydrogel network but also on the surface.

3.5. Swelling Studies

To understand the diffusion mechanism and mass transport through polymeric networks, PVPAD hydrogels were evaluated for swelling properties in double-distilled water, various pH (pH 1–11), and temperatures (25–40°C) (Figures 6-8). In the present work, pH and temperature-responsive hydrogels were developed based on two crosslinkers. Here, N,N[1]-methylene-bis-acrylamide, a standard/routine cross-linker and bis[2-methacryloyloxy] ethyl phosphate as

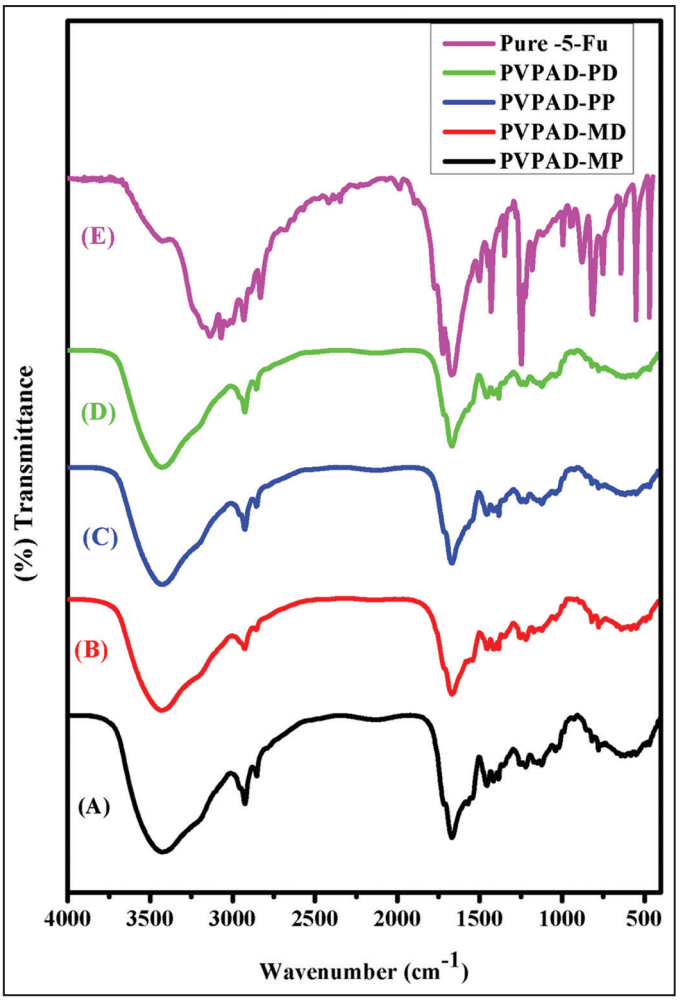

Figure 2: (A-E) Fourier transform infrared spectra of poly(acrylamide-co-(2-dimethylamino)ethyl methacrylate) (PVPAD)-M and PVPAD-P pure and drug loaded gels, (E) pure 5-fluorouracil.

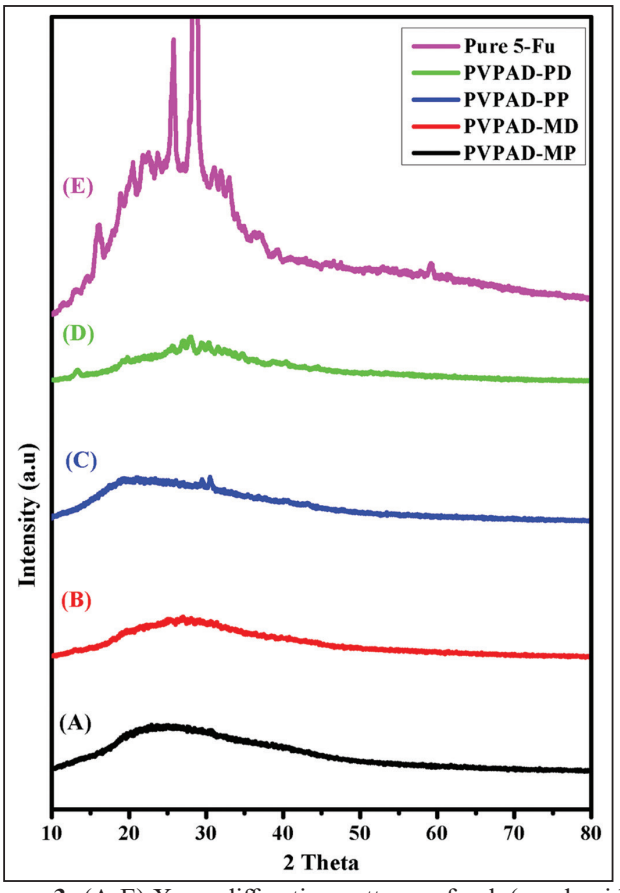

Figure 3: (A-E) X-ray diffraction patterns of poly(acrylamide-co-(2-dimethylamino)ethyl methacrylate) (PVPAD)-M and PVPAD-P pure and drug loaded gels (E) pure 5-fluorouracil.

Table 2: Thermogravimetry analysis results of PVPAD gel with drug and without drug.

S. No.	Sample code	Temperature range (°C)	Weight loss (%)	Thermal stability (205–450)
1	PVPAD-P	Below 200 200–320 320–450	83.5 62.0 17.0	53.5
2	PVPAD-PD	Below 200 200–320 320–450	87.8 66.0 23.5	67
3	PVPAD-M	Below 200 200–320 320–450	85.0 66.0 6.0	77.5
4	PVPAD-MD	Below 200 200–320 320–450	87.0 63.5 18.0	67

phosphorous-containing cross-linker. At first, swelling kinetics and equilibrium swelling ratio (ESR) studies of PVPAD hydrogels were performed in double-distilled water at room temperature (Figure 6). It was observed that the equilibrium swelling of all formulations attained in around 24 h. However, ESR of each formulation is dependent on the individual feed composition of monomers. Hence, ESR increased with the increase of (2-dimethylamino) ethyl methacrylate in each series of hydrogel network; this may be due to the hydrophilicity of the varying monomer. The high ESR observed for formulation 3 and least was observed for formulation 1 because the prier one is having more amounts of hydrophilic (2-dimethylamino)ethyl methacrylate, while 1 is having the least amount. Furthermore, ESR increased with a decreasing amount of crosslinker; this may be due to low crosslinking density of the network with non-rigidity of network and hence diffusion of more water molecules into the hydrogel network [5,9-11].

Based the chemical structure and functionalities such as hydroxyl of PVA, the amide of arcrylamide and (2-dimethylamino)ethyl methacrylate, and phosphate of bis[2-methacryloyloxy] ethyl phosphate [-OH, -(C=O)-NH-, -O-(O^--P=O)-O-] present the hydrogel, it forms a network based on simple complexation of polymer chains using large number of hydrogen bonds. This hydrogen bond driven complexation shrinks the hydrogel network at low pH conditions. However, at lower pH conditions, the hydrophobic tertiary amine groups of (2-dimethylamino)ethyl methacrylate can convert into hydrophilic nature. In general, the pKa value of monomer (DMAEMA pKa is 7.3–7.5) is lower than the pH, which results the termination of hydrogen bonds, and charge repulsion leads to the high swelling [15].

However, in various studies of (2-dimethylamino)ethyl methacrylate based hydrogels in the combination of chitosan results are reverse [21-24]. It indicates that the swelling behavior entirely depends on the comonomer/copolymer present in the hydrogel network. The results of the swelling ratio of hydrogels slowly increase with increasing

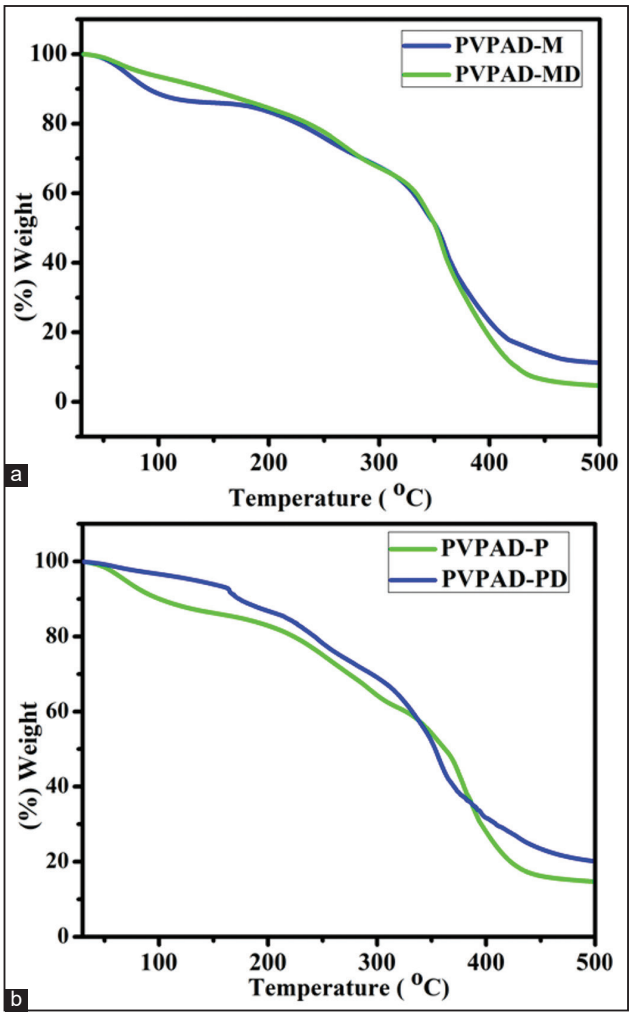

Figure 4: Thermogravimetric analysis curves of (a) poly(acrylamide-co-(2-dimethylamino)ethyl methacrylate) (PVPAD)-M pure and drug loaded gels and (b) PVPAD-P pure and drug loaded gels.

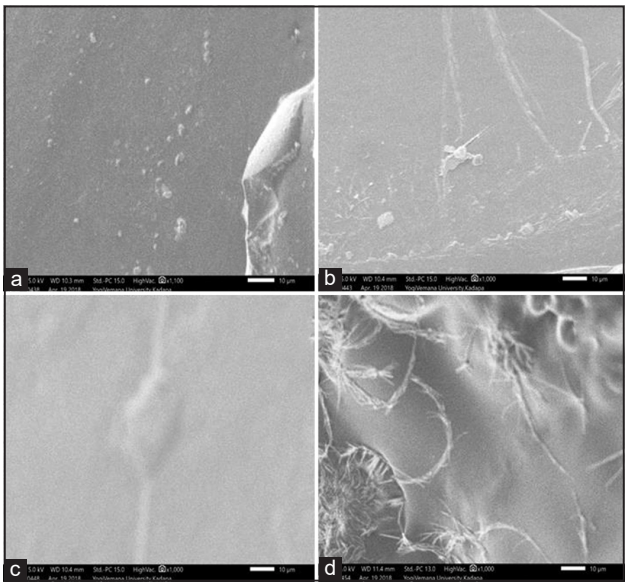

Figure 5: Scanning electron microscopes images of (a) poly(acrylamide-co-(2-dimethylamino)ethyl methacrylate) (PVPAD)-M, (b) PVPAD-M drug loaded (c) PVPAD-P (d) PVPAD-P drug loaded hydrogels.

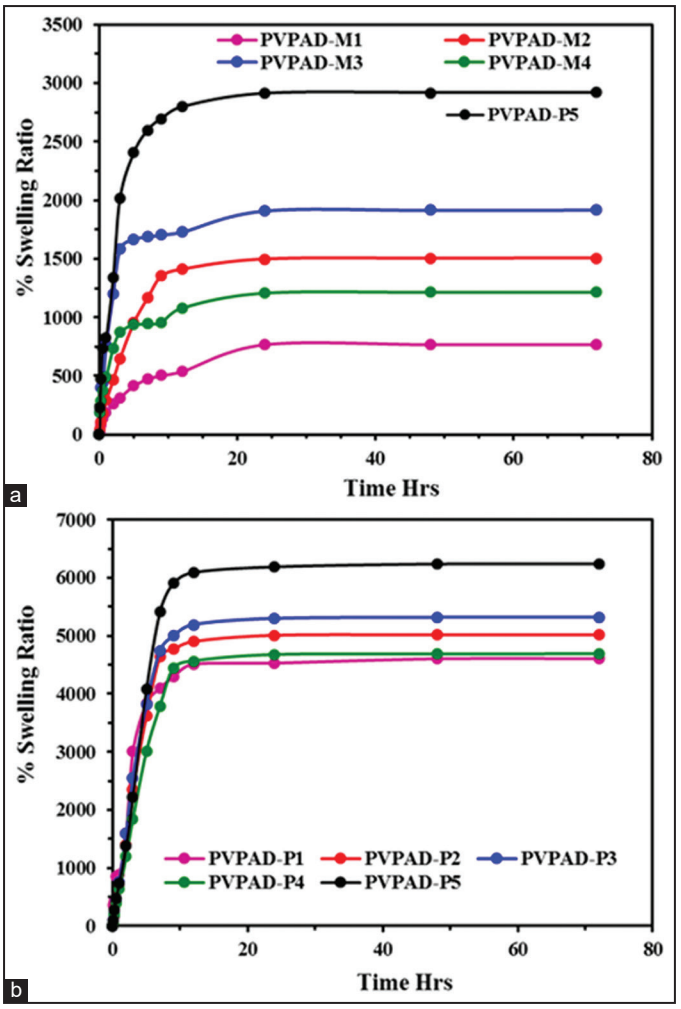

Figure 6: Swelling studies of different formulations of (a): Poly(acrylamide-co-(2-dimethylamino)ethyl methacrylate) (PVPAD-M) hydrogels, (b): PVPAD-P hydrogels.

pH up to 5 after that there was no significant change in swelling ratio observed for all formulations (Figure 7). The pH and temperature responsive nature mainly attributed to the varying monomer. Further, it is evidenced that, in the case of phosphate crosslinker based hydrogels (PVPAD-P) increased swelling in higher pH is responsible by phosphate groups, which are hydrolyzed easily in alkaline conditions. Due to the hydrolyzing nature of the phosphate cross-linker in alkaline medium, the polymers containing phosphorous groups are the most promising platform for biomedical applications such as regenerative medicine and drug delivery [12,13].

The temperature dependency of ESR is plotted for formulation 3 of each series (Figure 8). Poly((2-dimethylamino)ethyl methacrylate) contains a hydrophilic group amide [(-C=O)-NH-)] and hydrophobic N-dimethyl ethyl group (-$(CH_2)_2$ NMe_2) present in the linear polymer chain. Hence, it can able to forms the hydrogen bond [23] with a hydroxyl group of the PVA. Therefore, at low temperature (below the gel transition temperature) the hydrophilic group in the polymer structure will form an intermolecular hydrogen bond with surrounding water; therefore, from 25 to 40°C swelling is increased gradually. However, above lower critical solution temperature, the hydrophobic interactions become more dominant and so hydrogen bonds are broken, and the water molecules are expelled from the polymer. Both formulations PVPAD-M3 and PVPAD-P3 have increased ESR up to 40°C drastically.

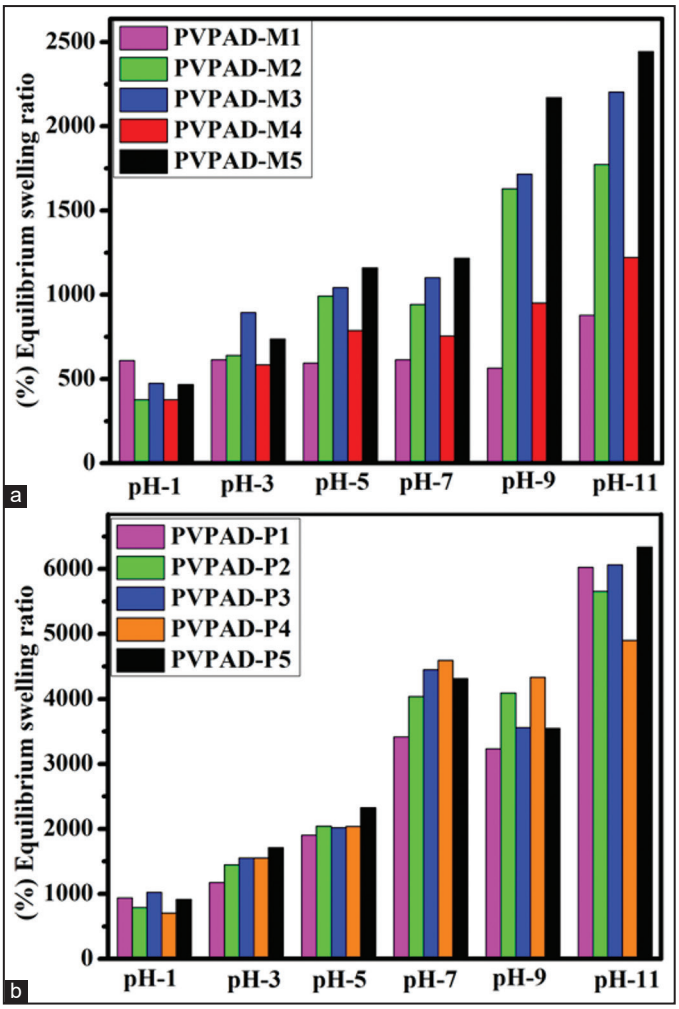

Figure 7: Equilibrium swelling studies of different formulations at various pH solutions (a): Poly(acrylamide-co-(2-dimethylamino)ethyl methacrylate) (PVPAD-M) hydrogels, (b): PVPAD-P hydrogels.

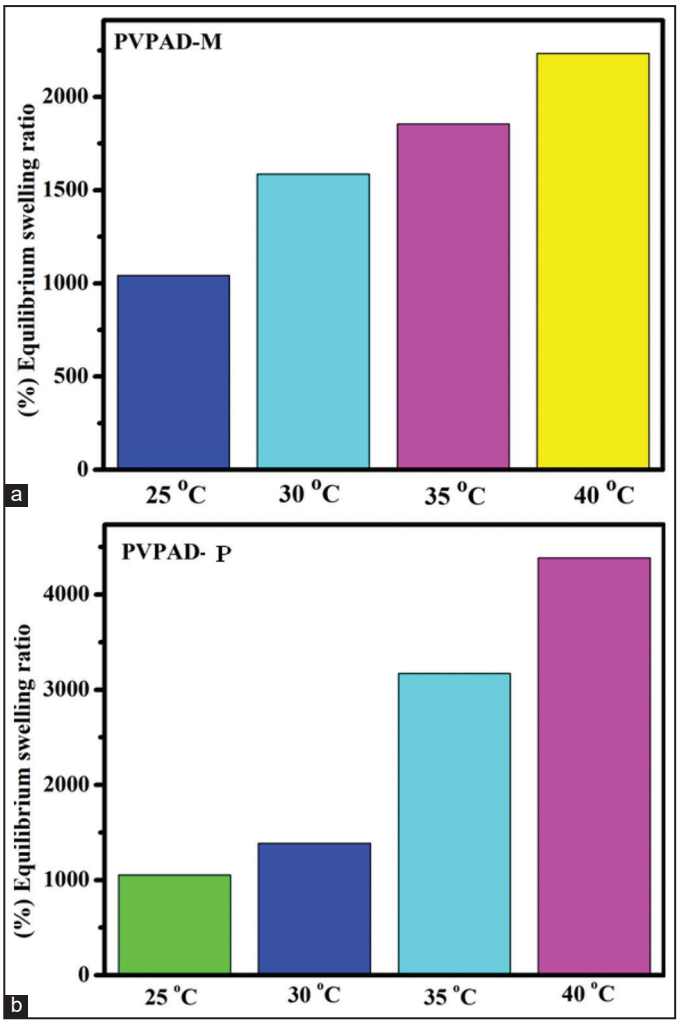

Figure 8: Temperature effect of (a) poly(acrylamide-co-(2-dimethylamino)ethyl methacrylate) (PVPAD-M) and (b) PVPAD-P hydrogels.

3.6. In-vitro Drug Release Kinetics

The *in vitro* 5-FU release profile from drug-loaded hydrogels is plotted (Figure 9). The Korsmeyer-Peppas model fitting values were presented in Table 2. Both the series of hydrogels displayed distinct 5-FU release profiles with different characteristics. The N,N[1]-methylene-bis-acrylamide cross-linked PVPAD-M series of hydrogels released 5-FU at relatively faster rates at both pH 1.2 and 7.4 phosphate buffer media. In contrary to this, bis[2-methacryloyloxy] ethyl phosphate cross-linked series show a slower release of 5-FU. In both series, the formulations varied with different extent of varying monomer in feed ratio and cross-linker and thus resulted in a controlled and sustained 5-FU release profile. In both cases, two distinct phenomena determine the drug release kinetics, one being the extent of solvent penetration (Fickian diffusion [$n < 0.5$]) and the other being rate of polymer relaxation, that is, anomalous release ($n > 0.5$). These two phenomena are supposed to take place at two distinct fronts, that is, solvent diffusion front and polymer relaxation front, which travel through the polymer matrix with the incubation time until equilibrium.

Figure 9a and b shows the 5-FU release profiles of MBA cross-linked PVAPAD hydrogels at pH 1.2 and 7.4, respectively, at 37°C. Figure 9c shows the 5-FU release profiles of MBA cross-linked PVAPAD-M3 hydrogel at pH 7.4 an, at 25 and 37°C. Figure 9d and e shows the 5-FU release profiles of BMEP cross-linked PVAPAD hydrogels at pH 1.2 and 7.4, respectively, at 37°C. Figure 9c shows the 5-FU release profiles of BMEP cross-linked PVAPAD-P3 hydrogel at pH 7.4 an, at 25 and 37°C. The release of 5-FU depends on the swelling property of each formulation that means the release of 5-FU assisted by the diffusion of solvent molecules into hydrogel networks. Because of diffusion of solvent into the gel, it expands on the network of the hydrogel; meanwhile, drug molecules could release into the media. The release of drug molecules depends on the external pH media (pH 1.2 and 7.4) and temperature (25 and 37°C). 5-FU release also supported by the swelling properties of PVPAD hydrogels that maximum drug is released in pH 7.4 and 37°C

4. SUMMARY

In this work, PVA based pH and temperature responsive hydrogels with tuneable swelling and *in vitro* drug release properties have been prepared by simple free-radical polymerization. Primarily, the ESR of hydrogels was performed in distilled water, various pH, and temperatures. FTIR has investigated the chemistry of structural changes of PVA based hydrogels. In addition, crystallinity, thermal stability, and surface morphology of hydrogels were investigated by the X-ray direction studies, thermogravimetry analysis, and scanning electron microscopy studies, respectively; these results demonstrate that the drug is encapsulated at a molecular level. The swelling property of hydrogels depends on the pH and temperature, which is directly related

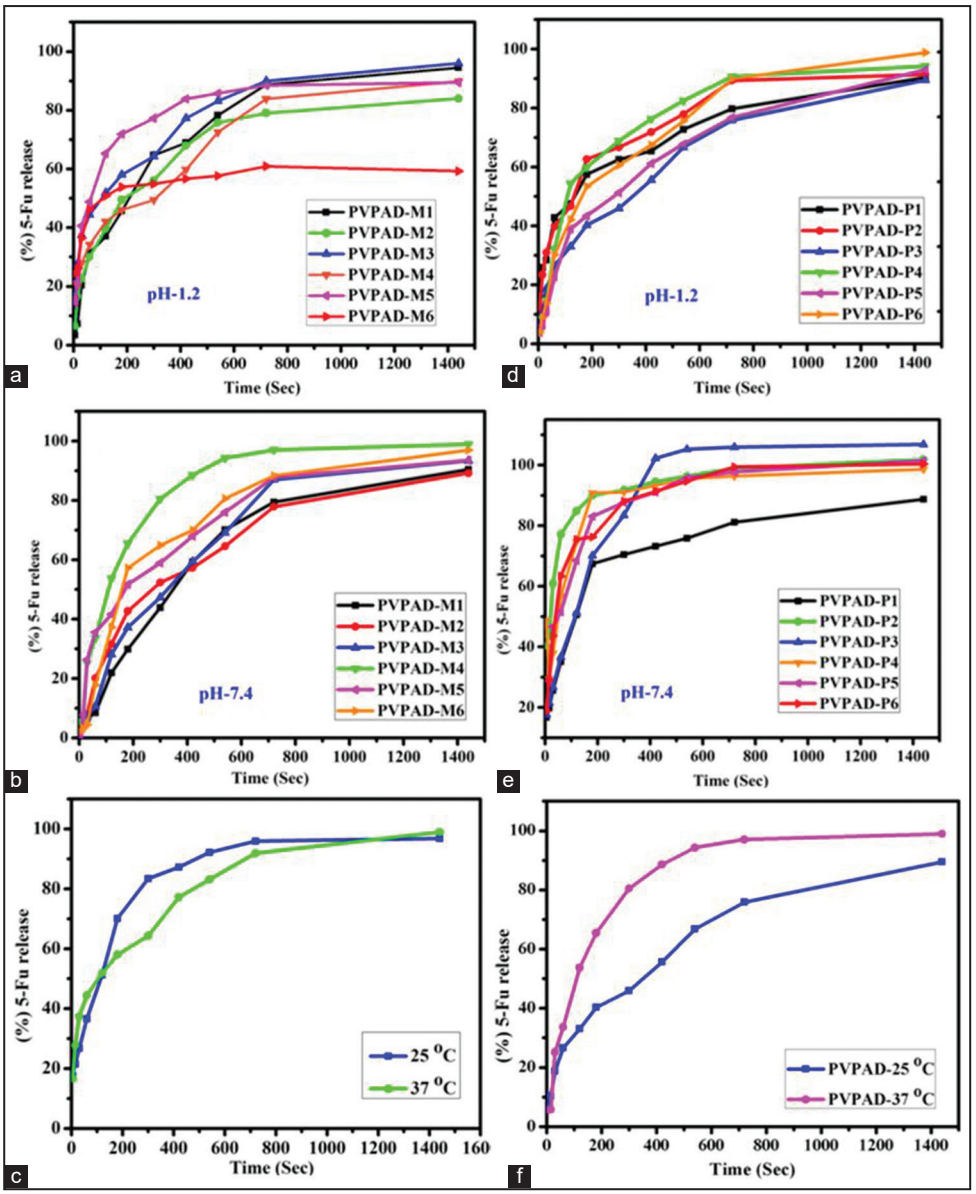

Figure 9: (a-g) % of drug release from poly(acrylamide-co-(2-dimethylamino)ethyl methacrylate) (PVPAD)-M at pH 1.2 and 7.4 and at different temperatures at 25 and 37°C.

to the hydrogen bonding present in the polymer network. ESR of all formulations is increased with increased pH and temperature. *In vitro,* 5-FU release results also followed the same trend as swelling results. However, the hydrogels cross-linked with bis[2-methacryloyloxy] ethyl phosphate showed a significantly higher in the ESR than the N,N[1]-methylene-bis-acrylamide. The drug release behavior of the hydrogels was found to be the same as ESR. Finally, this study not only provides a comparative study about the effect of crosslinker on swelling and *in vitro* release kinetics of PVPAD hydrogels but also provides the foundation for further research on (2-dimethylamino)ethyl methacrylate and other biodegradable or biocompatible polymers.

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