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Experimental investigation and modeling of the anti-cancer drug delivery from poly(N-isopropylacrylamide-co Acrylic acid) copolymeric hydrogels

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Abstract

The diffusion mechanism of a model anti-cancer drug in cross-linked poly(N-isopropylacrylamide/acrylic acid) (P(NIPAAm/AA) copolymeric hydrogels was studied. The crosslinking ratiowas constant but acrylic acid ratio ranged from 10:1 to 10:3. P(NIPAAm/AA) copolymeric hydrogels were synthesized by redox-initiated free radical polymerization in water at room temperature The presence of poly(ethyleneglycol) in the hydrogel formulation resulted the higher mechanical strength. Use of acrylic acid resulted in higher hydrogel swelling. Drug size was also found to be a significant factor. 5-FU is used as amodel anti-cancer drug. The effect of 5-FU solution on swelling characteristics P(NIPAAm/AA) copolymeric hydrogels have also been studied. The percent swelling, equilibrium swelling, diffusion constant values are evaluated for P(NIPAAm/AA) copolymeric hydrogels at 1.5% of 5-FU solution at room temperature Based on the release kinetic of the 5-FU drug, the hydrogels displayed a non-Fickian diffusion mechanism. According the diffusion kinetic data in hydrogels became clear that diffusion kinetic data were best described by Peppas model. Permeation from P(NIPAAm/AA) copolymeric hydrogels followed a Super Case II transport mechanism, most likely driven by macro molecular chain relaxation and swelling of hydrophilic polymers.

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Introduction

Hydrogels are three-dimensional polymeric networks capable of sequential adsorption and desorption of water and physiological fluids can be in a variety of application s as smart materials in engineering science and medicine (Wei and cai, 2009).This is due to the presence of hydrophilic chains in the gel structure is as sociated with the ionic group son the chains, can become super-absorbent hydrogels with the ability to change in many different situations large volumes different environmental conditions. So with changing pH, ionic strength and temperature and the electric field can be used in a variety of super absorbent. Among the applications of nano-gels are sensitive to environmental stimuli can pointed out to separated proteins, biomolecular detection, separation of heavy ions in water, and rheology control of pharmaceutical and health care products, and transport of drugs, scaffolds for tissue engineering applications, cell culture and tissue fillers(Ma *et al*., 2010). Smart or intelligent polymer networks show sharp changes in response to physical stimuli like pH, temperature, ionic strength, electric or magnetic field (Baumann *et al*., 2009). Among many intelligent polymers poly (N-isopropylacrylamide) and demonstrate a lower critical solution temperature (LCST) of about 32 ℃ in aqueous medium. It undergoes a sharp coil to globule transition in water above the lower critical solution temperature (LCST = 32 ℃) i.e. changing from a hydrophilic state to a hydrophobic state above LCST .The main mechanism of phase separation is thermally induced release of water molecule bound to isopropyl side groups, resulting in increased inter- and intramolecular hydrophobic interactions between isopropyl groups above LCST. This unique property is widely used in drug delivery, tissue engineering and biotechnology (Manna and Patil, 2009; Qiu and Bae, 2007).These hydrogels are rendered pH-sensitive by copolymerizing NIPAAm with acidic or basic comonomers, often at low degree of substitution (Alli *et al*., 2012). Changes in pH affect the ionization state and hydrophilic/hydrophobic balance in these hydrogels, shifting the LCT above or below ambient temperature. A detailed review of the transition behaviour can be found in the literature (Minkova *et al*., 1989; Xia *et al*., 2008). Preparation of hydrogels can is formed by various methods: free-radical crosslinking polymerization, chemical crosslinking, γ-irradiation crosslinking (lambov *et al*., 1997) or UV irradiation crosslinking (Rosiak and Ulansky, 1999; Rosiakana Yoshii, 1999) and physical interaction crosslinking. Chemically crosslinked hydrogels were developed in the past as carriers for drugs (Doytcheva *et al*., 2001; Vervoort *et al*., 1998). The controlled drug delivery devices can assure a sustained release and targeted effect (Qiu and Park, 2001). The great advantage of the drug-controlled release from the hydrogels is a possibility for improvement of patient compliance (Park, 1993). In recent years, polyacrylic acid (PAA) and its copolymers have often been used as carriers in drug release systems, because of their multifunctional nature, unique properties and good biocompatibility (Devine *et al*., 2006; Dittgen *et al*., 1997).

N-isopropylacrylamide/acrylicacid hydrogels were synthesized by redox-initiated free radical polymerization in water at room temperature for 24 hours by using N-isopropylacrylamide) particles and acrylic acid as monomer ,polyethyleneglycol as macromonomer, N,N'-methylene bisacrylamide(BIS) as crosslinking agent, APS as free-radical initiator and TEMED as accelerator. The dependence of swelling and release properties on percent of acrylic acid and BIS were examined. In this study, 5-Fluorouracil (5-FU) was chosen as a model anticancer drug for the investigation of drug release behavior of the P (NIPAAm/AA) copolymeric hydrogels. (5-FU) 5 - Fluorouracil one of the antitumor agents most frequently used for treating various types of tumors, including cancers of the colon, breast cancer, pancreatic cancer and gastric cancer. However due to the large number of secondary effects that accompany its conventional administration and due to the short plasma half-life and poor oral absorption of this compound, (5-FU) 5 - Fluorouracil has been selected for inclusion in a controlled release of different systems.(Song *et al*., 2008; Zhang *et al*., 2004).

5-FU release was studied as a function of temperature, disc thickness, disc load and degree of crosslinking of the poly (2-hydroxyethylmethacrylate) hydrogels and poly(acrylamide-co-monomethylitaconate) hydrogels (Garcia *et al.*, 2000). In recent years, synthesis of hydrogels was studied based on poly(N-isopropylacrylamide/maleicacid) P(NIPAAm/MA) and poly(N-isopropylacrylamide/itaconicacid) P(NIPAAm/IA) copolymeric via radiation-induced (Tasdelen *et al.*, 2004). In other works, synthesis of poly (N-isopropylacrylamide) hydrogels and their application was studied for the control release of anticancer drugs (Castro *et al.*, 2012). The 5-FU was trapped in the gels by its inclusion in the polymerization mixture. To incorporate the 5-FU to the feed mixture of polymerization, water solutions of 5-FU were used. However, in this work, we have studied drug release behavior of P (NIPAAm/AA) copolymeric hydrogels. The effect of 5-FU solution on swelling characteristics P (NIPAAm/AA) copolymeric hydrogels have also been investigated. The controlled release profiles were followed by UV-Vis spectroscopy and the mechanisms of drug release by diffusion were modeled. These measurements are made with the purpose of characterizing these hydrogels as drug delivery systems.

Materials and methods

Materials and instruments

N-isopropylacrylamide (NIPAAm,monomer: Sigma-Aldrich, $C_6H_{11}NO$,M = 113.16 g/mol). Acrylic acid (AA, monomer: Merk Chemical Company, $C_3H_4O_2$, M=73.06g/mol). N,N'-methylenebisacrylamide (BIS, cross link : Sigma-Aldrich). Polyethylene glycol (M = 1000 g/mole) (macromonomer, Sigma-Aldrich). Ammonium per sulphate (APS, initiator: Merck chemical Company, M=228.18g/mol, $(NH_4)_2S_2O_8$). *N,N',N'',N'''*-tetramethylethylenediamine (TEMED, accelerator: Sigma-Aldrich, $C_7H_{10}N_2O_2$, M =116.2) 154.17 g/mol)5-fU($C_4H_3N_2O_2$, M= 130.1 g/mol) was supplied by Roche Laboratories as a crystalline powder. Deionized water was used in all experiments. UV-visible absorption spectra were recorded on HP8251 spectrophotometer.

Preparation of copolemeric hydrogels

Copolymerization of Poly(N-isopropylacrylamide/acrylic acid) hydrogels with PEG was carried out in deionized water at room temperature using BIS as cross-linker and APS as initiator by redox-initiated free radical polymerization.

The samples were prepared by varying amounts of AA in the initial feed ranging from 5 to 20% w/w AA and 10% w/w for BIS. Temperature of polymerization increased with increasing AA. In order to eliminate any unreacted monomer, oligomer and non-cross-linked polymer chains, each sample was washed in excess water for 3 days. Extracted gels were dried in vacuum. Copolymer gels have been designated as NPA followed by numerical suffix indicating ml AA in the initial feed. In tables1 was shown the detail of sample designation of various hydrogels prepared by redox free-radical polymerization.

Characterization

Swelling measurements

First, blank hydrogels (1cm length, 2.5mm diameter) were freshly made and then dried in an incubator for 2 days at 30°C. The swelling studies were carried out in triplicate by placing of blank gels in 50 mL water and 5-FU solution (1.5 ml drug in 20ml water) at room temperature. In time intervals, the gels were removed, gently dried with a kim-wipe and weighed, and then returned to the vials with 1 mL of fresh water or solution. The swelling percent (%ESR) was estimated by comparing the ratio of the wet hydrogel weight (M_{wet}), which was measured at the various time intervals, to the initial dry hydrogel weight (M_{dry}), which was measured before the swelling study began (Brahim *et al.*, 2003):

$$\%ESR = ((M_{wet} - M_{dry}) / M_{dry}) \times 100\ \% \quad (1)$$

Drug loading and release experiments

The dry hydrogels were equilibrated in a of 5-FU solution (1.5 ml drug in 20ml water) at room temperature for 1 week in a dark environment (in order to avoid degradation). Drug loaded were dried

in an incubator for several days at 30°C to remove residual water. For drug release experiments, previously incubated drug gels were placed in a vessel containing 50 mL of water at a constant temperature (37 ± 0.1 °C) with a constant shaking rate.

The amount of released drug was determined by UV-Vis spectrophotometer (HP8251) with a quartz cuvette at an absorbance wave length of 266 nm. In order to determine the concentration of 5-FU, absorbance 5-FU solutions were prepared in phosphate buffer with different concentrations and concentration(y, in 10−3 g/mL) versus absorbance values (x) at 266nm curves were platted. The amount of release drugs at any time was calculated from the 5-FU standard calibration line y=0.3833x + 0.4 with a linear regression coefficient of $R2$ = 0.9998. For examining the release of 5-FU from P(NIPAAm/AA) hydrogels, percentage release of the drug was calculated from the following equation(Song *et al.*, 2008; Zhang *et al.*, 2004):

$$\%Release = (W_t / W_{total}) \times 100 \quad (2)$$

Where W_t is the weight of released drug in water at any time and W_{total} is the initial total weight of the loaded drug by the gel system.

Results and discussion

Swelling properties

A fundamental relationship exists between nature of the polymer and the solvent and swelling of a polymer in a solvent.

Table 1. Details of feed composition and sample designation for copolymers prepared using redox free-radical polymerization and water.

Sample designation	NIPAAm(wt%)	PEG(wt%)	AA(wt%)	BIS (wt%)	WATER(wt%)
NPA-1	3.69	3.69	0.0	0.369	92.2
NPA-2	3.68	3.68	0.18	0.368	92
NPA-3	3.67	3.67	0.367	0.367	91.9
NPA-4	3.66	3.66	0.55	0.366	91.7
NPA-5	3.65	3.65	0.732	0.365	91.5

Table 2. The equilibrium swelling percentages of PNIPAAm and P(NIPAAm/AA) hydrogels in deionized water and 5-FU solutions at room temperature.

Gel name	Equilibrium mass swelling (%) in distilled water	Equilibrium mass swelling (%)in 5-FU	EWC
NPA-1	339	1988.57	0.95
NPA-2	372.6	2086.4	0.79
NPA-3	486.4	2516.6	0.83
NPA-4	510.9	3068.5	0.84
NPA-5	873	3269.4	0.89

Swelling curves in water with change of AA content are shown in Fig.1, swelling curves in water with change of BIS content are shown in Fig.3swelling curves in 5-FU solution (1.5 ml drug in 20ml water) with change of AA content are shown in Fig.2.As can be seen from the figure, the swelling capabilities of the hydrogels are increased by time, reaching constant swelling (equilibrium swelling) after a certain period of time. It is indicate that the equilibrium percentage mass swelling of NIPAAm/AA copolymeric hydrogels in water increased from 372.6 to 873 and In the presence of 5-FU increased from 2086.4to 3269.4 as AA content increased from 5 to 20%(w/w)and in the presence of 5-FU, these

percentages are further than in water because the porosity of the hydrogel increased with increasing AA content, hence ,molecules of 5-FU can diffuse into gel pores easily and weight of swelling hydrogel in 5-FU is bigger than same hydrogel in water because of the molecular weight of the 5-FU is larger than the molecular weight of water (Tasdelen *et al.*, 2004).The equilibrium swelling percentages of PNIPAAm and P(NIPAAm/AA) hydrogels in deionized water and 5-FU solution, are given in Table2. The incorporation of AA into the polymer network with higher AA content will lead to an increase in electrostatic repulsive force between charge sites on carboxylate ions upon their complete dissociation and enhance a more extended configuration. The extended structure with high-AA content might cause a higher swelling ratio of the hydrogel in the drug solutions. (Castro *et al.*, 2012; Song *et al.*, 2008).

Table 3. Pameters of diffusion of 5-FU into the P(NIPAAm/AA)hydrogels.

GEL SAMPLE	n	C	R^2	$D\times 10^5 (cm^2/s)$
NPA_2	0.857	3.807	0.981	2.9
NPA_3	0.867	3.952	0.988	3.2
NPA_4	0.886	3.966	0.998	3.5
NPA_5	0.321	2.336	0.989	0.22
NPA_4 (5)	0.466	2.306	0.999	2.3
NPA_4 (10)	0.886	3.966	0.990	3.5
NPA_4 (15)	0.406	2.231	0.993	1.1

Release mechanism

Hydrogel matrixes are considered swelling-controlled systems, because the drug release is controlled by the inward flux of solvent (Pepas *et al.*, 2000). These swelling-controlled systems are often analyzed with Fickian and non-Fickian diffusional behavior kinetics. Equation (3) displays the simplified expression for Fickian and non-Fickian diffusion that the 5-FU Release data can be fitted against (Pepas *et al.*, 2000; Pepas *et al.*, 1980):

$$\text{Ln } E = \ln (M_t / M_\infty) = n \ln t + C \qquad (3)$$

Here E is the culmulative fraction of drug release, M_t / M_∞ ,where M_t is the amount of diffusional absorbed at time t, M_∞ is the maximum amount absorbed; C is the rate constant characteristic of the system, and n is the diffusional exponent (Brahim *et al.*, 2003). Eq.(3) can only be applied to the first 60% of drug release. The diffusional exponent (n) is calculated as the slope and the rate constant (C) is calculated as the intercept of linear regression lines fitted to the $\ln E$ versus $\ln t$ plots. A calculated n equal to 0.5 represents Fickian diffusion, when the rate of diffusion is slower than the relaxation one, so we have a diffusion-controlled drug release, while a calculated n greater than 0.5 represents non-Fickian diffusion occurs, when the diffusion and relaxation rates are comparable. In this case, the drug release behaviour can be regarded as the superposition of both phenomena (Wei and Cai, 2009). Therefore, using the calculated n value, the diffusional behavior of the hydrogel release can be determined. The plots of E versus t for the series of P(NIPAAm/AA) copolymeric hydrogels in 5-FU solution are shown in Fig.4and 5, respectively and the exponents n and C values were calculated from the slope and intercept of the lines, respectively.. The data were collected in Table 3.

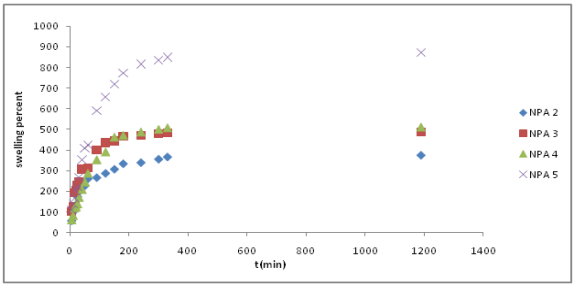

Fig. 1. The equilibrium swelling percentages of P(NIPAAm/AA)copolymeric hydrogelsin deionized water at room temperature at different percent of AA.

It is clear from the analysis that as the AA content in the gel structure increases from 5%(w/w) to 15%(w/w), the diffusional release kinetic exponent n

increases from 0.857 to 0.886 for P(NIPAAm/AA) hydrogels but if AA content very icreased n decreases because the hydrogel toughness increases with increasing acid and release rate decreases. n values are specified that diffusion of 5-FU solutions into P(NIPAAm/AA) hydrogels was assumed to be non-Fickian character. Diffusion coefficients are important permeation parameters of some chemical species to polymeric systems.

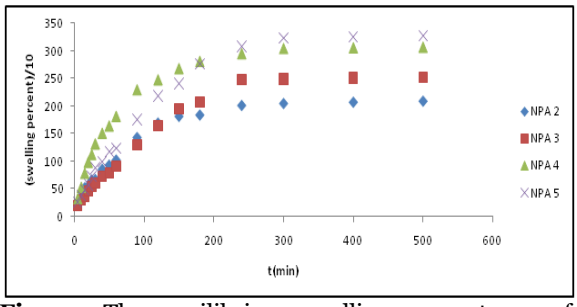

Fig. 2. The equilibrium swelling percentages of P(NIPAAm/AA)copolymeric hydrogels in 5-FU solution at room temperature at different percent of AA.

Using "n" and "C", the diffusion coefficient (D) of solvent in the matrix could be calculated using the following equation (Brahim *et al.*, 2003):

$$e^{C} = 4[\ D / \pi r^2\ ]^n \qquad (4)$$

Where "D" is the diffusion coefficient and "r"is radius of gel disc. Diffusion coefficients of hydrogels in solutions of 5-FU are also listed in Table 3. As expected, the diffusion coefficients increases with an increase in equilibrium mass swelling of the present hydrogel in the solutions.

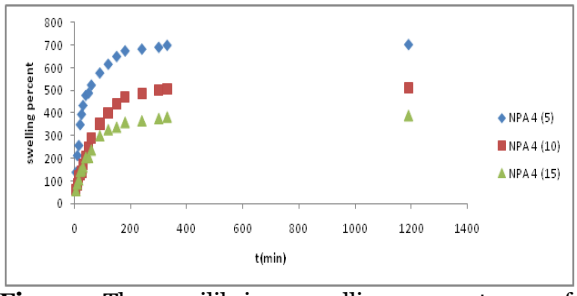

Fig. 3. The equilibrium swelling percentages of P(NIPAAm/AA)copolymeric hydrogels by 5wt% ,10wt% and 15wt% of BIS in deionized water at room temperature.

Equilibrium water Content

Equation (5) displays the water absorbed by PNIPAAm and P(NIPAAm/AA) copolymeric hydrogels is quantitatively represented by the equilibrium water content (EWC) (Wei and Cai, 2009):

$$EWC = (\ W_{eq} - W_o\ ) / W_{eq} \qquad (5)$$

Where W_{eq} is the weight of the swollen gel at time t (equilibrium) and W_o is the weight of the dry gel at time0. The EWC values of the hydrogels were calculated and tabulated in Table 2. All EWC values of the hydrogels (0.79–0.89) were greater than the percent values of body about 0.6. Thus, the PNIPAAm and P(NIPAAm/AA) copolymeric hydrogels exhibited fluid contents similar to those of living tissues. Table 2.

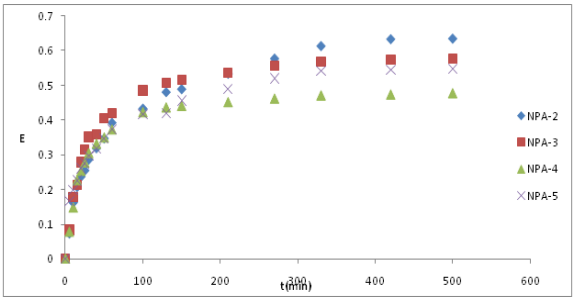

Fig. 4. In vitro release profiles of 5-FU in P(NIPAAm/AA)copolymeric hydrogels with different percentages of AA in deionized water at 37℃.

Release behavior of hydrogels

The release profiles of 5-FU in P(NIPAAmAA) copolymeric hydrogels in water at 37℃ are shown in Fig. 4.show the fractional 5-FU release, expressed as M_t / M_∞ where M_t and M_∞ are the amounts of drug released at the times t and infinite, respectively, as a function of time for the hydrogels. In this figure, the drug release during the first stage couldbe influenced for the relaxation of polymer chains. Thus the values for n, or the slope of the linear regression lines fitted to the ln E versus ln t plots, all resulted in values greater than 0.5 (Table 3), suggesting non-Fickian diffusion. Non-Fickian diffusion is desirable as it indicates that the media penetration rate is in the same range as drug diffusion(Tasdelen *et al.*, 2005). One of the most attractive features of PNIPAAm based hydrogels as drug carriers is their intelligent

property to external temperature changes. Thus the effect of temperature is important factor to the hydrogel's drug release.

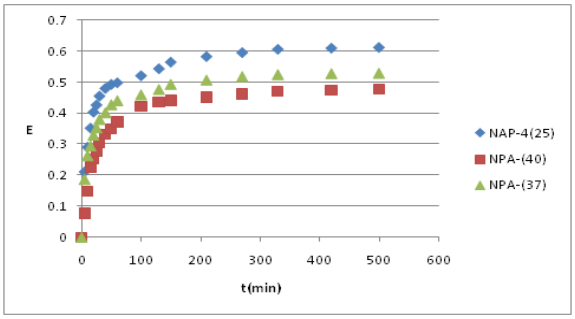

Fig. 5. In vitro release profiles of 5-FU in P(NIPAAm/AA)copolymeric hydrogels with different percentages of AA in deionized water at three different temperatures (25,37and 40℃).

Optimum release should occur around 37 ℃ for the hydrogel to be effective inside the body's conditions. It is important and practical to examine the drug release data from those P(NIPAAm/AA) hydrogels at a temperature LCST like the body temperature (37 ℃). Fig. 5.shows the 5-FU release behavior from P (NIPAAm/AA) copolymeric hydrogels in water at three different temperatures (25, 37 and 40℃). Release of the adsorbed 5-FU at high temperature were lower than those at low temperature because of the collapse nature of PNIPAAm structure at a temperature greater than its LCST (Aoshima and Kanaoka, 2008).

Conclusion

This research is a first step towards finding an efficient drug delivery system for anticancer drugs. The experiments explored the use of hydrogels for 5-FU as amodel anticancer drug release by analyzing the synthesisand temprature-responsive characteristics of the P(NIPAAm/AA) copolymeric hydrogels the ability to promote 5-FU release into the surrounding environment, and the significant factors involved in optimal drug release. The results from these experiments support the following conclusions:

1) The equilibrium percentage swelling of the P(NIPAAm/AA) hydrogels in 5-FU solutions increased from 1988.57 to 3269.4 as AA content increased from 5 to20%(W/W).

This has been explained due to the incorporation of more specific acidic groups into the Network and consequent higher swelling capacity of the gels.

2) In the diffusion transport mechanism study, Using the 5-FU release data at various temperatures, and acrylic acid ratios and fitting it to the Fickian and non-Fickian diffusion Eq. (3), the values for n is found to be over 0.5 for the hydrogels. This implies that the swelling transport mechanism is a non-Fickian transport. The fractional cumulative release of the drug from the hydrogels have showed an initial non-Fiction

behavior, probably indicating a comparable rates of Fiction diffusion and polymer relaxation. This finding is confirmed by similarities between our swelling ratio and 5-FU release profiles.

3) When loading and releasing the drugs, pore size of the hydrogel decreased and increased, respectively, without reaching the initial pore size of the hydrogel. The result show that the greater concentration of drug loaded into the hydrogel, the greater reduction in pore size.

4) P(NIPAAm/AA) copolymeric hydrogels easily absorbed and released 5-FU, and its release was temperature-responsive.

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