pH Responsive PAlAm-*g*-PIPA Microspheres: Preparation and Drug Release

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SYNOPSIS

Poly(*N*-isopropylacrylamide) (PIPA) was synthesized by radical polymerization with 2,2'-azobisisobutyronitrile (AIBN) as an initiator and 3-mercaptopropionic acid (MPA) as a chain-transfer reagent in methanol (MeOH) at 70°C for 7 h. The resultant PIPA was grafted to polyallylamine hydrochloride (PAlAm · HCl) by amide formation under the influence of water-soluble carbodiimide 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC). The graft polymer was made into microspheres (MS) by chemical crosslinking. The pH-responsive drug release of the graft polymer microspheres was examined by releasing phenobarbital natrium (PN), which was carried on the microspheres by physical adsorption. A dynamic dialysis technique was used in the drug-release experiment and the drug-release-rate constants reflecting the drug release characteristic of polymer microspheres were obtained by constituting a mathematical model. The results indicated that the homopolymer PAlAm microspheres and the copolymer PAlAm-*g*-PIPA microspheres are both pH responsive to release PN and that in the neutral pH condition the release rate is the slowest. © 1995 John Wiley & Sons, Inc.

INTRODUCTION

The external signal-responsive drug-delivery system has been paid more and more attention. The obvious advantage of this system is the "on–off" switching control release of a drug from a drug carrier by surrounding signals such as heat, chemical compounds, electric field, and pH. Many polymers such as polyacrylamide,[1] poly(*N*-acryloylpyrrolidine),[2] poly(*N*-alkyl-substituted acrylamides),[3] and some liposomes[4] can be used to achieve this "on–off" drug-control release as a drug-carrier martrix. Among these polymers, poly(*N*-isopropylacrylamide)[5] (PIPA) has demonstrated noticeable thermosensitivity in terms of water swelling. Okahata et al.[6] even used a large nylon capsule membrane with a surface-grafted poly(*N*-isopropylacrylamide) to regulate reversily the permeation of NaCl and dyes by ambient temperature change. Recently, Schild[7] reported a detailed review of the specificity, synthesis, and application of PIPA. Changes in the swelling states of PIPA gels can influence the diffusion of solutes from within the gels to the outside aqueous media. The changes were mainly thermosensitive. However, the review reported also a novel extension[8,9] of the PIPA system in which acrylic acid as a comonomer was introduced; consequently, the copolymer possessed not only a thermal response but also pH sensitivity. The lower critical solution temperature (LCST) shifts to higher temperature at higher pH due to repulsion between the ionized groups. Yan[10] also utilized PIPA to obtain controllable catalytic activity of the enzyme immobilized by the medium temperature. According to Yan's work, the phase transition of the graft copolymer polyallylamine hydrochloride (PAlAm)-*g*-PIPA was influenced not only by the temperature but also by the pH of the dissolution medium. Extensively, we carried out drug-release experiments with PAlAm-*g*-PIPA as a drug carrier at various pH's. The pH-responsive drug release of the homopolymer PAlAm was also carried out for comparing and discussing the mechanism of the pH-responsive drug release of PAlAm-*g*-PIPA. A mathematical model proposed by Gupta et al.[11] is quoted to quantitate the drug release of the polymer microspheres in this article.

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EXPERIMENTAL

Materials

N-Isopropylacrylamide (Tokyo Kasei, Tokyo, Japan) was purified by recrystallization from acetone–ether before use. 2,2′-Azobisisobutyronitrile (AIBN) (Shanghai Fourth Factory of Reagents, Shanghai, China) was recrystallized from methanol. 3-Mercaptopropionic acid (MPA) was purchased from Nacalai Tesque, Kyoto, Japan. Other reagents were commercially available. All water used in the experiments was double-distilled.

Synthesis of Poly(*N*-isopropylacrylamide) (PIPA)

Polymerization was carried out with AIBN as an initiator and MPA as a chain-transfer reagent in methanol at 70°C for 7 h. The reaction was protected with N_2. The residual solvent was removed by evaporation. The resultant polymer was dissolved in acetone and then precipitated with ether. Polymers with higher molecular weight were removed with an ultrafiltration membrane (Shanghai Ruili XHP-1, exclusion limit 10,000). The oligomer with a molecular weight of 3053 detected by conductometric titration with a 0.01*N* NaOH solution was collected.

Preparation of Graft Polymer

Both PAlAm · HCl and PIPA were dissolved in water. Soluble carbodiimide EDC was added into the solution. The graft reaction was carried out under stirring at 10°C overnight and the products were purified by dialysis against distilled water. The structures of the final products were confirmed by an infrared spectrum (Nicolet, MX-1, Nicolet Co., England) and the graft ratio was determined by elemental analysis.

Preparation of the Microspheres

The graft copolymer PAlAm-*g*-PIPA, 0.2 g, was dissolved in 0.8 mL H_2O. The copolymer aqueous solution was then added dropwise into the mixture of 1 g span 80, 5 mL toluene, and 5 mL chloroform. The water/oil mixture was emulsified with a vortex mixer for 5 min, then transferred into a triangle-shaped bottle with a 60 mL mixture of toluene/chloroform (3/1) and a 4 g span 80. A glutaraldehyde (25%)-saturated toluene solution (2 mL) was added into the emulsion. The chemical crosslink reaction was carried out under stirring for 5 h followed by adding 1 mL ethanolamine into the reaction mixture to close the aldehyde groups and the reaction continued for 1 h. The resulting microspheres (MS) were washed by a centrifuge with organic solvent and distilled water. The size of the MS was detected with a photomicroscope. The resulting MS were incubated with a dimethylamine–borane solution (7%) for 12 h to reduce the Schiff base in the MS, then washed and lyophilized for storage. MS were also prepared with PAlAm without graft chains for comparison.

Drug Carrying of the Microspheres

Dried MS accurately weighted were added into the phenobarbital natrium solution with a concentration of 10 mg/mL. The MS suspension was stirred at an ambient condition for 24 h and incubated in warm water of 45°C for 1 h. Drug-carrying MS were washed with water. The washing supernatant was determined with a ultraviolet spectrophotometer (Shimadzu, UV-120-02) at 239 nm for the phenobarbital natrium (PN) content. Drug-carrying MS were lyophilized for later experiments. The drug content of drug-carrying MS was calculated by the following equation:

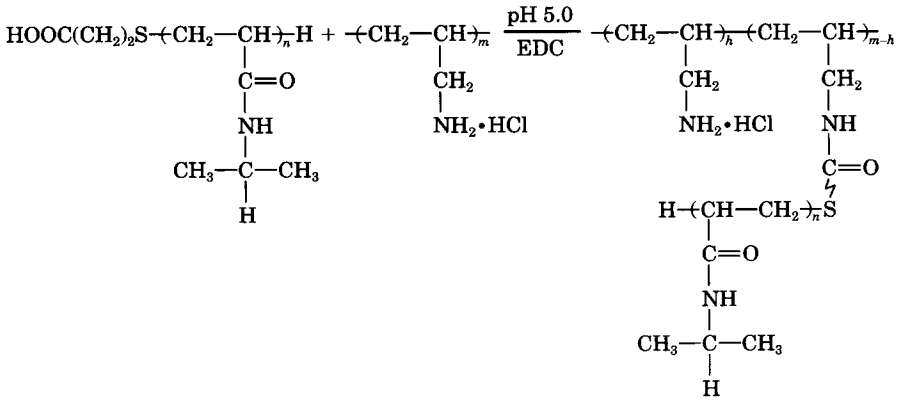
$$Cm = \frac{A_0 - A_1}{W} \ (\mu g/mg \text{ MS})$$

where Cm is the drug content in the MS; A_0, the total amount of the drug added initially; A_1, the drug amount of the supernatant; and W, the total amount of MS. The drug contents of various drug-carrying MS are as follows: PAlAm-*g*-PIPA–MS (graft ratio 10%) 156 μg/mg; PAlAm-*g*-PIPA–MS (graft ratio 5%) 118.7 μg/mg; PAlAm–MS (graft ratio 0) 193 μg/mg.

Release of PN

Calibration curves of PN were done with glycine buffer as the drug-release medium at pH 3, 5, 7, 9, and 11, respectively, and coincident with the UV absorbance at every content of PN at 230 nm.

Drug-carrying MS of both PAlAm and the PAlAm-*g*-PIPA matrix were used for studying drug-release behavior. Thirty milligrams of drug-carrying polymer MS suspended in 5 mL glycine buffer (GB, 0.05*M*) was placed in a dialysis bag. The MS suspension was then dialyzed against 100 mL of GB at 37°C under an oscillating condition. Three milliliters of the outer dialysis medium was taken at the regular time of 20 min over 8 h. Then, the sample was taken once per hour. The samples were immediately as-



Scheme A

sayed for the drug content with a UV spectrophotometer (Shimadzu UV-120-02). The drug release of the free drug was carried out at the same condition as above to estimate the drug permeability constant of the dialysis membrane.

RESULTS AND DISCUSSION

Synthesis and Characterization of the Graft Copolymer Microspheres

Polymerization of PIPA with a low molecular weight was carried out by a radical reaction with lipophilic AIBN as an initiator in MeOH. The molecular weight of the oligomer was controlled by adding the chain-transfer reagent MPA into the system. Because MPA joined in the reaction, the PIPA chain was carried in end carboxyl groups, the amount of which can be determined by conductive titration and which allows the estimation of the molecular weight of the oligomer to be determined. In addition, it was the end carboxyl groups COOH of the PIPA that made the PIPA be grafted onto the main chain of the PAlAm · HCl by amide formation with the amine groups—NH_2 of the PAlAm · HCl when water-soluble EDC was added in the reaction system (Scheme A).

The graft reaction above was confirmed by infrared spectrum analysis (Fig. 1). By comparing the infrared spectra of the three polymers, PAlAm · HCl, PIPA, and PAlAm-*g*-PIPA, it can be seen that the IR spectrum of PAlAm-*g*-PIPA has not only the special absorbance of PAlAm but also the special absorbance of PIPA. Especially, on the spectrum of the graft copolymer PAlAm-*g*-PIPA, the absorbance peak of carboxyl groups—COOH (1719 cm^{-1}) of the PIPA spectrum disappeared and the stretching vibration absorbance of the hydrogen chloride (HCl) bond (720 cm^{-1}) of PAlAm · HCl weakened. This fact confirmed clearly that a part of amino groups of the PAlAm and the carboxyl groups of the PIPA had formed into amide groups. The graft product PAlAm-*g*-PIPA was obtained.

The graft percent of the graft polymer was controlled by the molar ratio of the PIPA and the PAlAm · HCl used in the reaction. The actual graft percent of the graft polymer was estimated by elemental analysis. The experimental data in Table I

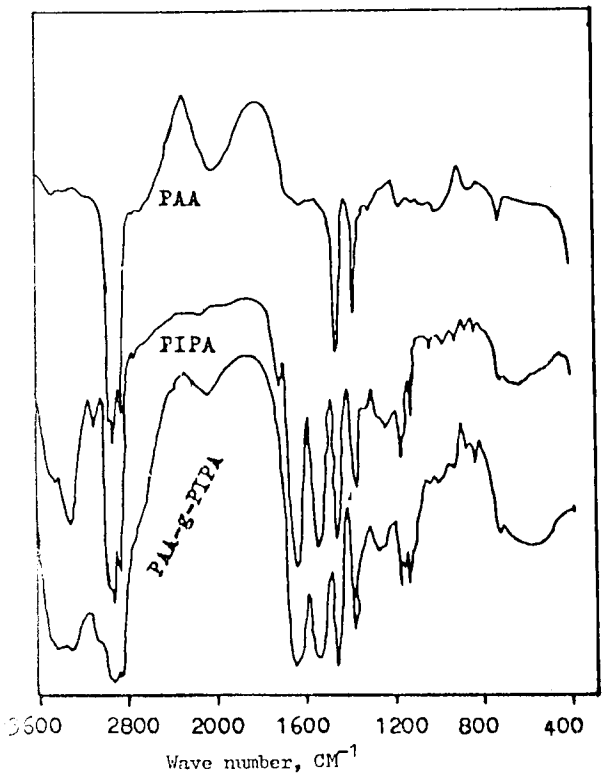


Figure 1 IR spectra of various polymers.

Table I Graft Ratio of the PA1Am-*g*-PIPA from Elemental Analysis and Theory

	PIPA (g)	PA1Am (g)	Elemental Analysis			Graft Ratio	
			C%	N%	H%	Theoretical	Experimental
I	4	2.450	57.38	12.56	9.56	5	5.30
II	4	1.225	52.08	11.71	10.26	10	9.1

M_w: PIPA, 3053, determined by conductometric titration; A1Am, 93.5.

indicates that the actual graft percent of the graft copolymer was almost equal to the predeterminate value of the graft ratio.

The graft copolymer PAlAm-*g*-PIPA was made into MS with a particle size of 10–20 μm in the W/O emulsion system. Glutaraldehyde was added into the system to stabilize the MS by a chemical crosslink reaction. The degree of the crosslink of the MS was influenced by the amount of glutaraldehyde used in the reaction according to Goldberg et al.[12] The Schiff base produced from the aldehyde–amine reaction was reduced by dimethylamine–borane to stabilize the crosslinking bonds.

The Mathematical Model of the Drug-release Process

To estimate exactly the drug-release process, we quote Gupta's mathematical model to quantify the drug-release rate of the drug-carrying MS. As Scheme B shows, Q_m is the total amount of drug associated with MS at time t, K_m is the release rate constant of the drug from the carrier, K_{21} is the drug permeability constant of the dialysis membrane, and C_2 and C_1 are the concentrations of the drug in the dissolution medium inside and outside the dialysis bag, respectively. Generally, the release rate constant K_m can be evaluated only if a permeability constant of the dialysis membrane is known. The permeability constant can be determined by adding a known quantity of the drug inside the dialysis bag and then monitoring the drug concentration in the outer compartment (C_1) as a function of time. According to Fick's law,

$$\frac{dQ_1}{dt} = \frac{K_{21}A(C_2 - C_1)}{\delta} \tag{1}$$

where Q_1 is the amount of drug in the outer compartment at time t, and A and δ are the surface area and the thickness of the membrane, respectively. Simplifying eq. (1) and adding a time-modification item, the following equation can be obtained:

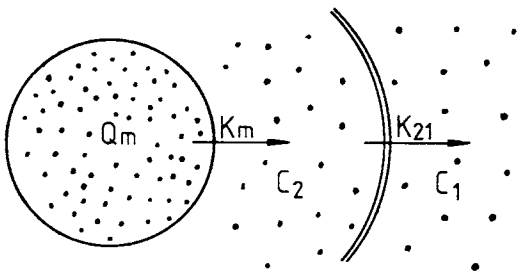
$$In\left(1 - \frac{C_1}{a}\right) = A_0 - K_{cv}t + a_1 \cdot t^2 \tag{2}$$

where $a = Q_s^0(V_1 + V_2)$, Q_s^0 is the total amount of drug present in the system; V_1 and V_2 are the volumes of the dissolution medium outside and inside the dialysis bag, respectively; $A_0 = In(1 - C_1^0/a)$ is a constant related to the concentration of the drug outside the bag at time $0(C_1^0)$; $K_{cv} = K_{21}A(V_1 + V_2)/\delta V_1 V_2$, which is a constant (i.e., independent of the drug concentrations inside the dialysis bag, C_2); and a_1 is a coefficient of the time-modification item introduced to reduce the error of the K_{cv} with time. In eq. (2), Q_s^0, V_1, and V_2 are known, and C_1 and t can be assayed, so the K_{cv}, A_0, and a_1 can be estimated by the least-square method with a computer. The results of the calculations are listed in Table II.

When the drug release from the MS follows a first-order kinetic characteristic, then

$$Q_m = Q_m^0 e^{-K_m t} \tag{3}$$

where Q_m^0 is the total amount of the drug associated with the MS at time 0. Assuming that $K_{cv} \gg K_m$, solving eq. (3) and simplifying it leads to



Scheme B

Table II K_{ev} of the Dialysis Membrane at Various pH

pH		No. 1	2	3	4	5	6	7	$K_{cv} \times 10^2$
3	t	5	21	41	61	81	101	127	2.241
	C_1	5.14	12.07	15.18	17.83	19.00	20.43	20.43	
5	t	5	27	48	68	87	107	127	2.563
	C_1	4.46	13.30	16.15	17.92	18.23	17.15	19.10	
7	t	6	29	48	68	88	108	128	2.102
	C_1	2.85	9.57	13.97	15.92	18.23	19.25	20.19	
9	t	5	25	45	65	85	105	125	2.065
	C_1	4.00	11.51	13.30	15.95	19.25	19.25	20.90	
11	t	6	22	42	62	82	102	122	2.337
	C_1	6.36	12.13	16.97	19.87	21.39	22.23	22.46	

t: time (min); C_1: concentration of drug outside the dialysis bag (μg/mL). Equilibrium concentration: 24.67 μg/mL.

$$In\left(C_1 - \frac{C_2^0 V_2}{V_t} - \frac{Q_m^0}{V_t}\right) = -K_m t + In \frac{K_{cv} Q_m^0}{(K_m - K_{cv}) V_1 V_2} \quad (4)$$

where $V_t = V_1 + V_2$. C_2^0 is the concentration of the free drug inside the dialysis bag at $t = 0$. Hence, a plot of $In(C_1 - C_2^0 V_2/V_t - Q_m^0/V_t)$ vs. time would give a slope equal to K_m, the first-order release rate constant.

Drug-release Experiments of the Microspheres at Various pH

Drug-release experiments were carried out by the dynamic dialysis technique referencing Gupta with some modification. Experimental results are illustrated in Figures 2 and 3. According to the results, the drug delivering of the MS was the first-order kinetic characteristic, and the drug-release behavior of both PAlAm MS and PAlAm-g-PIPA MS was affected by the pH of the dissolution medium.

Figure 4 shows representative plots of $In(C_1 - C_2^0 V_2/V_t - Q_m^0/V_t)$ vs. t of the drug-release process of the drug-carrying MS. From the plots, the process was a biphasic release of the drug and has two constants: the initial release rate constant K_{m1} and the terminal release rate constant K_{m2}.

Figures 5 and 6 are the plots of the initial K_{m1} and terminal K_{m2}, respectively, of the MS at different pH. The plots show clearly that the drug-release rate of the grafted MS and the not-grafted MS are both

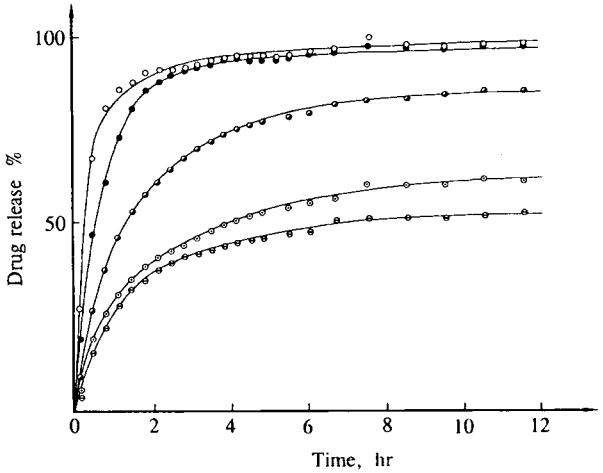


Figure 2 The plots of the percent of PN released from the PA1Am–MS vs. dialysis time at different pH at 37°C: (○) pH3; (⊙) pH 5; (⊘) pH 7; (◕) pH 9; (●) pH 11.

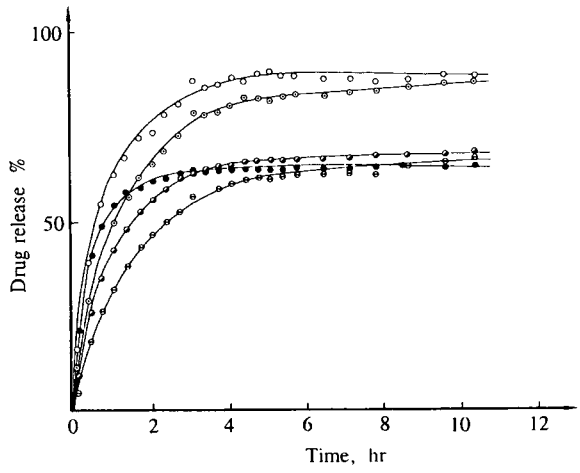


Figure 3 The plots of the percent of PN released from the PA1Am-g-PIPA MS vs. dialysis time at different pH at 37°C: (○) pH3; (⊙) pH 5; (⊘) pH 7; (◕) pH 9; (●) pH 11.

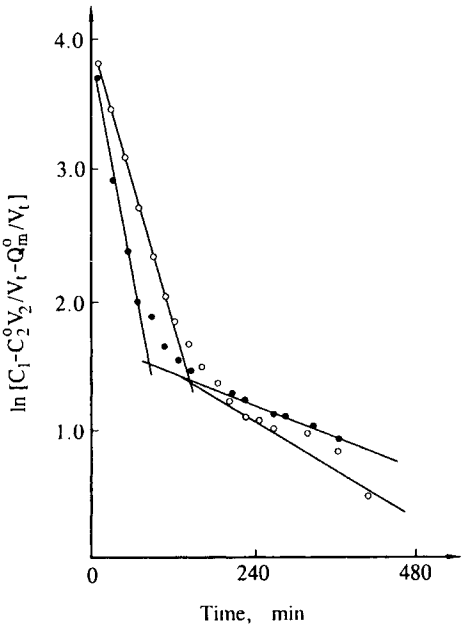


Figure 4 Typical plot of logarithm $In(C_1 - C_1^0V_2/V_t - Q_m^0/V_t)$ vs. dialysis time t.

affected by the pH of the dissolution medium. When the dissolution was neutral, the drug-release rate would be the slowest. With increasing the acidity or

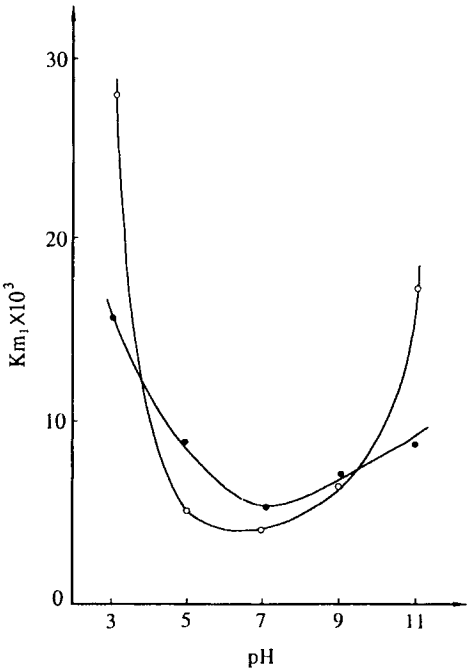


Figure 5 Initial drug release rate constants K_{m1} of the PN-carrying MS vs. pH: (○) PAlAm MS; (●) PAlAm-*g*-PIPA MS.

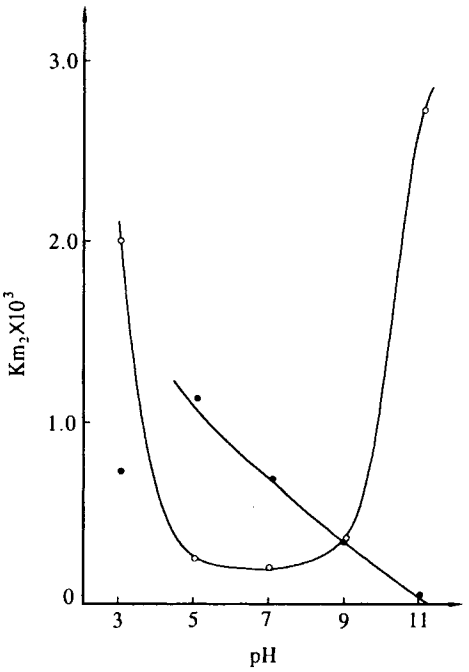


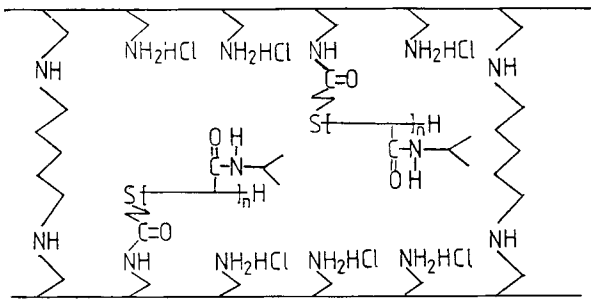
Figure 6 Terminal drug-release rate constants K_{m2} of the pH-carrying MS vs pH: (○) PAlAm MS; (●) PAlAm-*g*-PIPA MS.

the alkalinity of the dissolution, the release rate would increase. However, the release rate of the grafted MS decreased with increase of the alkalinity of the dissolution in the later period of the drug release.

Analysis of the pH-responsive Drug Release

Much literature[6,8,13–15] has contributed hydrogen-bonding and hydrophobic effects to the driving force for the phase transmission of the polymer gel, especially in the PIPA heat-responsive phase transmission. However, in the pH responsive phase transmission of the polymer, the ionic groups on the polymer were thought to be the main reason for the phase transmission. PAlAm·HCl has a large amount of NH_2H^+, the ionicity of which could change from weak to strong with the pH change from high to low in the dissolution. After grafting PIPA chains and crosslinking, the main chain PAlAm of the graft copolymer still has many residual NH_2H^+ groups, as Scheme C shows. So the hydrophility and the phase state of the copolymer are still influenced by the pH in the dissolution. The crosslinked polymer network should swell or shrink when the dissolution was acidic or alkaline.

Because of having a $pK_{a_1} = 7$ and a $pK_{a_2} = 9.5$,



Scheme C

the carried drug PN would change from hydrophilic to hydrophobic with the change of pH in solution. When pH was in the acidic region, the PN was only slightly charged and the pores of the polymer network were open because of swelling, so the drug release rate is fast. When pH was in the alkaline region, though the polymer matrix network shrunk and the pores of the polymer network contracted, the release rate of the PN was still fast due to its negative charge and excellent hydrophility. In addition, the process suggested by Hoffman et al.[3] may be the cause for the pressure generated during the polymer network collapse to squeeze out the drug. In a neutral solution, the polymeric matrix would be partially positively charged and the drugs should be partially negatively charged. The strongest ionic interaction between PN and the polymer matrix would occur in this case, leading to the slowest release rate.

From Figure 6, the terminal drug release rate of the graft copolymer MS decreased with increase of pH in the dissolution. The reason for this phenomenon now is unclear because of many factors affecting the drug release in the later period of drug release. Usually, the initial release behavior is the most representative for the drug-release process.

CONCLUSION

When polyallylamine hydrochloride was introduced into the poly(*N*-isopropylacrylamide) system as a copolymerization component, the copolymer would be pH-responsive on controlled drug release. In the case of a strong-base–weak-acid salt such as phenobarbital natrium as the carried drug, the release rate of the drug from the polymer matrix would increase with the acidity or alkalinity of the dissolution medium. In the neutral solution, the drug-release rate was the slowest.

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