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In vitro dynamic swelling behaviors of polyhydroxamic acid hydrogels in the simulated physiological body fluids

Dursun Saraydin*, Yasemin Çaldiran

Cumhuriyet University, Department of Chemistry, 58140 Sivas, Turkey
e-mail: saraydin@cumhuriyet.edu.tr

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Summary

Influence of some simulated physiological body fluids on the dynamic swelling behaviour of polyelectrolytic hydroxamic acid hydrogels (PHA) was investigated at 37 °C *in vitro*. The simulated physiological body fluids are distilled water, human sera, physiological saline (0.89 % NaCl), isoosmotic phosphate buffer at pH 7.4, gastric fluid at pH 1. 1, (gylicine-HCl buffer), urea (0-3 mol L^{-1}), and the aquatic solutions of K_2HPO_4 and KNO_3 (the sources of K^+). The values of equilibrium swelling of PHA hydrogels varied in the range of 130-4625%, while the values of equilibrium fluid content of the hydrogels varied in the range of 57-97%. The initial rate of swelling, diffusional exponent, and, diffusion coefficient were calculated using swelling kinetics data. Diffusion of the fluids into the hydrogel was found to be *non-Fickian* character. The diffusion coefficients of the hydrogel varied between $0.6x10^{-6}$ - $8.1x10^{-6}$ cm^2 s^{-1}.

Introduction

Polyelectrolyte hydrogels are highly swelling polymer networks. They possess many interesting properties. They may swell hundreds of times their initial volume or drastically reduce their size under the influence of external forces or in response to the changes in the surrounding media. This makes them a very interesting scientific object of observation and useful material for practical applications (1).

Hydrogels have been extensively studied and used for a large number of applications in the medical field as implants, controlled drug release devices, for enzyme and cell immobilization, blood-contacting applications and other uses. A hydrogel can be defined as a polymeric material that is characterized by its capacity to absorb water, other solvents and biological fluids. The utility of hydrogels as biomaterials lies in the similarity of their physical properties with those of living tissues. This resemblance is based on their water content, soft and rubbery consistency and low interfacial tension with water and other biological fluids. So, from this viewpoint more hydrophilic hydrogels are better as implants, as long as their mechanical properties are acceptable (2).

* Corresponding author

The hydrogels of unmodified and modified acrylamide exhibit a very high capability to absorb water, and permeable to oxygen and posses good biocompatibility. Alternatively, hydroxamic acid exhibits similarity with the acrylamide and its derivatives. And even, hydroxamic groups can be obtained from polyacrylamide as pendant from main chain which make the network more hydrophilic than the original amide groups.

In our previous works, radiation induced acrylamide based hydrogels (3-7) had been studied in adsorption of protein (8,9), and biocompatibility with human sera (10,11), and biocompatibility with subcutaneous tissue of rats (12) and the influence of amino acids on the swelling behaviour (13-14). The aim of the work is the investigation of the influence of some simulated physiological body fluids (15) on the dynamic swelling behaviour of hydrogels based on poly(hydroxarnic acid) (PHA) from crosslinked polyacrylamide (PAAm) (16), with the high water absorption capacity. PAAm hydrogel is non-ionogenic in nature, while hydroxamic acid hydrogel is ionic in character.

Experimental

Acrylamide (Merck, Darmstadt, Germany) as monomer, N,N'-methylenebisacrylamide (N) and ethylene glycol dimethacrylate (E) (Sigma, St. Louis, US) as crosslinkers, ammonium persulfate (Merck, Darmstadt, Germany) as initiator, N,N,N',N'-tetramethylethylenediamine (Sigma, St. Louis, US) as catalyst, hydroxylamine hydrochloride (Sigma, St. Louis, US) and sodium hydroxide (Sigma, St. Louis, US) as modifiers were used as received. The samples of human sera were obtained from The Blood Bank in Cumhuriyet University, Sivas, Turkey.

Preparation of the hydrogels

An aqueous solution of acrylamide and N or E (95:5 molar ratio in 0.4 mol water) with 0.01 mmol ammonium persulfate and 2.5 mmol N, N, N', N'-tetramethylethylenediamine were mixed and placed in PVC straws of 3 mm diameter. Acrylamide hydrogels having hydrophilic crosslinkers were prepared in a thermostated water bath at +4 °C. A gel formed after 20 min of reaction. After 24 h the hydrogel rods were cut into pieces 4-5 mm in length and washed with distilled water and dried in air and vacuum, and stored. The polymers were named as N-0 and E-0 for N and E, respectively.

For preparation of H-form of poly(hydroxamic acid), a solution of hydroxylamine hydrochloride (3 M, 100 mL) was added to 20 g crosslinked poly(acrylamide) in 300 mL distilled water. The resulting mixture was stirred for 2 hours at ambient temperature. The hydrogels were washed with distilled water and dried in air and vacuum, and stored. The polymers were named as N-1 and E-1.

A solution of NaOH (7.5 M 50 mL) was added to 20 g H-form of poly(hydroxamic acid) in 300 mL distilled water and stirred for 24 h for preparation of Na-form of poly(hydroxamic acid). The polymers were named as N-2 and E-2.

***In vitro* swelling studies**

The swelling nature of PAAm and PHA hydrogels in distilled water, human sera, physiological saline (0.89 % NaCl), isoosmotic phosphate buffer at pH 7.4, simulated gastric

fluid at pH 1.1, (gylicine-HCl buffer), urea (0.3 mol L^{-1}), and the aquatic solutions of K_2HPO_4 (0-2 mol L^{-1}) and KNO_3 (0.2 mol L^{-1}) was studied at 37 °C to determine the parameter swelling, initial swelling rate and diffusion. Swollen gels removed from the water-thermostated bath at regular intervals were dried superficially with filter paper, weighed, and placed in the same bath. The radii of cylindrical gels were measured by a micrometer.

Results And Discussion

***In vitro* swelling**

A fundamental relationship exists between the swelling of a polymer in a fluid and the nature of the polymer and the fluid. Dried hydrogels were placed in beakers containing certain amount of physiological fluids above left to swell in a water bath at 37±0.1°C. Swollen gels removed from the water bath at regular time intervals and dried with filter paper, weighed and placed in the same bath. The percentage swelling, S% is calculated from the following relation (4-16):

$$S\% = [(m_t - m_o) / m_o] \times 100 \qquad 1$$

Where m_o is the mass of the dry gel at time 0 and m_t is the mass of the swollen gel at time t.

The phosphate buffer at pH 7.4 (pH of cell fluid, plasma, edema fluid, synovial fluid, cerebrospinal fluid, aqueous humour, tears, gastric mucus, and jejunal fluid), glycine-HCl buffer at pH 1.1 (pH of gastric juice), human sera, physiological saline (17) and distilled water intake of initially dry hydrogels were followed for long enough to reach a plateau region. A representative swelling curves of N-2 and E-2 hydrogels in the physiological saline solution are shown in Fig. 1a. The percentage swelling (S%) of the hydrogels are presented in Table 1a-b.

Fig. 1a and Table 1a-b show that the swellings of E-type hydrogels are higher than N-type hydrogels in the simulated physiological fluids. Due to the structural and flexibility differences of crosslinkers in hydrogels, these swelling results are conceivable. Crosslinker E is flexible, while crosslinker N is semi-flexible. Thus, flexible crosslinker E can be retain the fluids more than the semi flexible crosslinker N. On the other hand, the swelling of the hydrogels in the all simulated physiological fluids is generally following order; type-0 <type-1 <type-2 hydrogel.

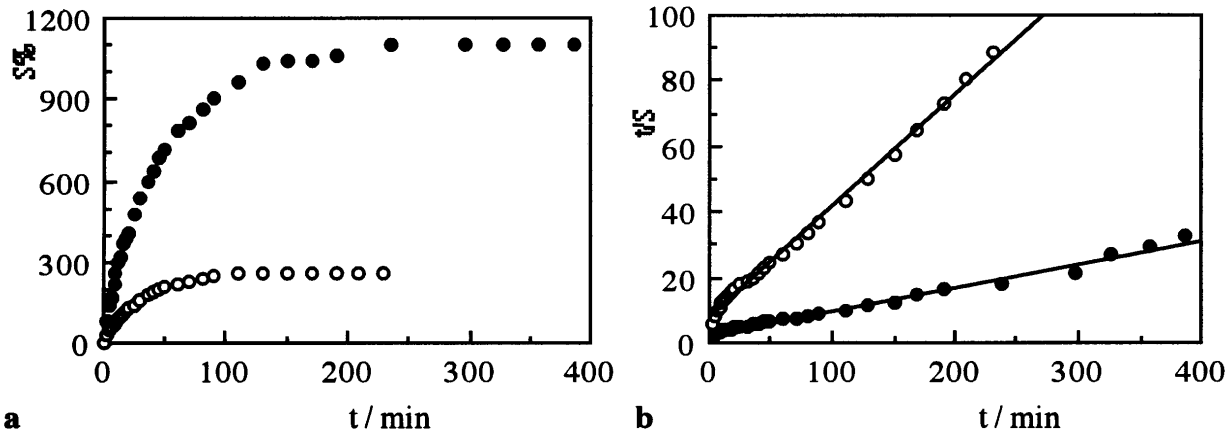

Fig 1. (a) Swelling (b) swelling kinetics of PHA in the physiological saline, o; N-2, •; E-2.

Table 1a. The values of S_{eq}% and EFC% of the hydrogels in the simulated physiological fluids

Hydrogel	N-0		N-1		N-2	
Simulated fluid	S_{eq}%	EFC%	S_{eq}%	EFC%	S_{eq}%	EFC%
Human sera	130	57.0	195	66.1	235	70.1
Glycine-HCl buffer	150	59.8	180	64.3	220	68.9
Phosphate buffer	160	61.1	200	66.7	245	70.9
Distilled water	185	64.8	200	67.0	325	76.4
Urea	240	70.6	190	65.7	305	75.3
NaCl	410	80.3	200	66.8	260	72.3
K_2HPO_4	425	80.9	195	66.0	265	72.4
KNO_3	460	82.1	210	67.4	290	74.2

Table 1b. The values of S_{eq}% and EFC% of the hydrogels in the simulated physiological fluids

Hydrogel	E-0		E-1		E-2	
Simulated fluid	S_{eq}%	EFC%	S_{eq}%	EFC%	S_{eq}%	EFC%
Human sera	280	73.7	800	88.8	935	90.3
Glycine-HCl buffer	175	63.5	510	83.6	545	84.5
Phosphate buffer	410	70.9	415	80.5	1230	92.5
Distilled water	940	90.4	1345	93.1	4625	97.9
Urea	1150	92.0	850	89.4	3745	97.4
NaCl	435	81.3	790	88.7	1100	91.7
K_2HPO_4	415	80.6	945	90.4	1300	92.8
KNO_3	435	81.3	560	84.9	1190	92.2

Since type-2 is the sodium salt form of hydroxamic acid, it is the most ionogenic form of all other types. Thus, type-1 (poly(hydroxamic acid)) contains as only as the ionizing degree of PHA while polyacrylamide has non-ionogenic groups. It is evident that, the more the ionogenic content of a hydrogel the more the swelling degree gets. It can be expected that the polyelectrolytic hydrogels would provide a broad range of swelling degrees for the medical uses of materials in comparison with the non-ionogenic nature of the PAAm hydrogel.

The fluid absorbed by the gel network is quantitatively represented by the EFC, equilibrium fluid content (18,19),

EFC% =[(mass of fluid in the gel / mass of hydrogel] x 100 2

EFCs of the hydrogels for all physiological fluids were calculated. The values of EFC% of the hydrogels are tabulated in Table la-b. All EFC values of the hydrogels were generally greater than the percent water content values of human body which is about 60%. Thus, the PAAm and PHA hydrogels were exhibit similarity of the fluid contents with those of living tissues.

For extensive swelling of polymers, it can be written following relation (20, 21);

$$\frac{t}{S} = A + Bt \qquad 3$$

here B= 1/Seq is the inverse of the equilibrium swelling, $A=1/(dS/dt)_0$, is the reciprocal of the initial swelling rate (r_0) of the gel. The relation represents second order kinetics (20).

A representative swelling kinetics curves of N-2 and E-2 hydrogels in the physiological saline solution are shown in Fig. 1b. The initial swelling rate and the values of theoretical equilibrium swelling of the hydrogels are calculated from the slope an intersection of the lines and, are presented in Table 2a-b.

Tables 2a-b show that the values of theoretical equilibrium swelling of the hydrogels are parallel the results of swelling of the gels. Swelling processes of PHA hydrogels are faster than the swelling rate of PAAm hydrogels in the body fluids.

Table 2a. The values of initial swelling rate and theoretical equilibrium swelling of the PHA crosslinked with N hydrogels

Hydrogel	N-0		N-1		N-2	
Simulated fluid	S_{eq}	r_0x10^2	S_{eq}	r_0x10^2	S_{eq}	r_0x10^2
Human sera	1.45	14.3	1.75	7.9	1.82	15.0
Glycine-HCl buffer	1.54	31.7	2.10	7.2	2.44	13.2
Phosphate buffer	1.64	21.5	2.31	8.2	2.76	12.0
Distilled water	2.00	18.2	2.55	6.9	3.68	18.5
Urea	2.64	21.7	2.28	7.2	3.55	16.0
NaCl	3.28	26.1	1.73	11.7	2.01	15.6
K_2HPO_4	3.27	21.8	1.64	9.8	2.04	13.4
KNO_3	3.70	28.9	1.92	8.8	2.53	16.1

Table 2. The values of initial swelling rate and theoretical equilibrium swelling of the PHA crosslinked with E hydrogels

Hydrogel	E-0		E-1		E-2	
Simulated fluid	S_{eq}	r_0x10^2	S_{eq}	r_0x10^2	S_{eq}	r_0x10^2
Human sera	2.35	20.0	9.60	13.9	8.47	28.6
Glycine-HCl buffer	1.81	14.8	6.27	7.2	6.12	15.5
Phosphate buffer	4.58	23.2	6.44	6.9	14.73	44.5
Distilled water	8.96	35.9	18.75	18.9	56.38	108.7
Urea	12.71	55.5	11.91	15.8	42.88	123.0
NaCl	3.59	22.6	8.48	12.2	10.72	35.4
K_2HPO_4	3.25	17.6	9.50	15.6	12.03	31.6
KNO_3	3.60	36.1	5.93	10.0	10.29	55.0

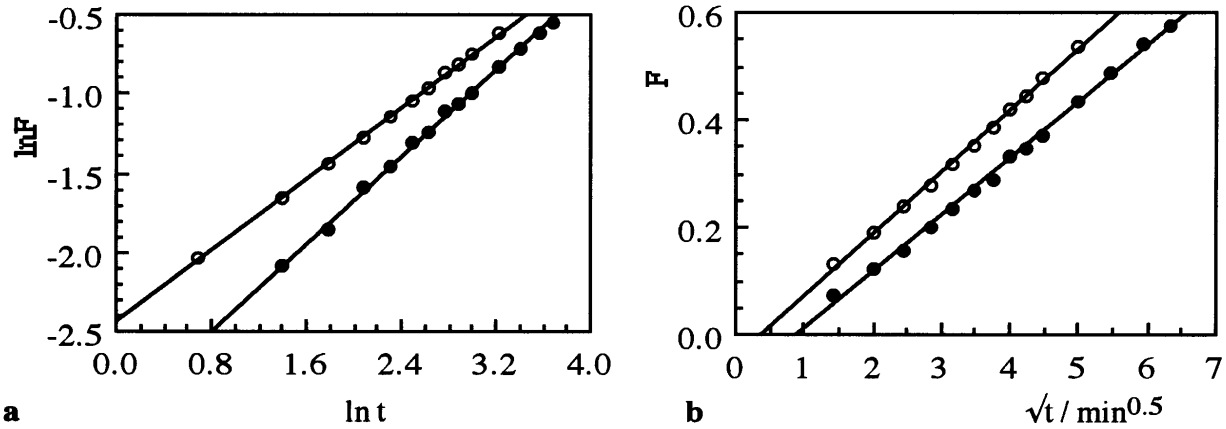

Fig 2. (a) ln F - ln t (b) F – √t graphs of PHA in the physiological saline, o; N-2, •; E-2.

Diffusion of simulated physiological fluids

The following equation was used to determine the nature of diffusion of water and fluids into hydrogels (22)

$$F = kt^{n} \qquad 4$$

Where F denotes the amount of solvent fraction at time t, k is a constant related to the structure of the network and the exponential n is a number indicative of the type of diffusion.

This equation is applied to the initial stages of swelling and plots of 1n(F) versus 1n(t) were applied. A representative curves of N-2 and E-2 hydrogels in the physiological saline solution are shown in Fig. 2a. The exponents are calculated from the slope of the lines and, are presented in Table 3.

Table 3 shows the values of diffusional exponent range between 0.50 and 0.83. For the hydrogels studied here the n values indicating the type of diffusion is found to be over 1/2. Hence, the diffusion of the physiological fluids into the hydrogels was taken to be a non-Fickian in character. This is generally explained as a consequence of slow relaxation rate of the polymer matrix.

Table 3. The values of diffusional exponent of hydrogels

Simulated fluid	N-0	N-1	N-2	E-0	E-1	E-2
Human sera	0.68	0.65	0.52	0.59	0.78	0.64
Glycine-HCl buffer	0.50	0.65	0.56	0.53	0.72	0.58
Phosphate buffer	0.54	0.61	0.59	0.59	0.82	0.64
Distilled water	0.50	0.58	0.52	0.68	0.83	0.77
Urea	0.54	0.58	0.55	0.68	0.82	0.73
NaCl	0.54	0.59	0.57	0.57	0.76	0.69
K_2HPO_4	0.54	0.55	0.58	0.54	0.73	0.68
KNO_3	0.55	0.64	0.61	0.55	0.75	0.59

Table 4. Diffusion coefficient ($cm^2\ s^{-1}$) of the hydrogels

Simulated fluid	N-0	N-1	N-2	E-0	E-1	E-2
Human sera	$8.1x10^{-6}$	$1.4x10^{-6}$	$1.9x10^{-6}$	$2.4x10^{-6}$	$1.3x10^{-6}$	$1.7x10^{-6}$
Glycine-HCl buffer	$4.0x10^{-6}$	$1.7x10^{-6}$	$1.7x10^{-6}$	$2.2x10^{-6}$	$0.6x10^{-6}$	$1.0x10^{-6}$
Phosphate buffer	$3.6x10^{-6}$	$1.3x10^{-6}$	$1.7x10^{-6}$	$3.3x10^{-6}$	$0.8x10^{-6}$	$3.0x10^{-6}$
Distilled water	$2.6x10^{-6}$	$1.0x10^{-6}$	$1.9x10^{-6}$	$3.8x10^{-6}$	$1.7x10^{-6}$	$4.9x10^{-6}$
Urea	$2.7x10^{-6}$	$1.0x10^{-6}$	$1.6x10^{-6}$	$4.3x10^{-6}$	$1.8x10^{-6}$	$5.6x10^{-6}$
NaCl	$1.9x10^{-6}$	$1.4x10^{-6}$	$1.8x10^{-6}$	$2.3x10^{-6}$	$0.9x10^{-6}$	$3.3x10^{-6}$
K_2HPO_4	$1.9x10^{-6}$	$1.9x10^{-6}$	$2.0x10^{-6}$	$1.7x10^{-6}$	$1.2x10^{-6}$	$1.8x10^{-6}$
KNO_3	$2.3x10^{-6}$	$1.4x10^{-6}$	$1.9x10^{-6}$	$3.2x10^{-6}$	$0.7x10^{-6}$	$3.1x10^{-6}$

The study of diffusion phenomena in hydrogels and fluids is of value in that it clarifies polymer behavior. The short time approximation method is used for calculation of diffusion coefficients of PHA hydrogels (23). The short time approximation is valid for the first 60% of the swelling.

The diffusion coefficients (D) of the cylindrical PAAm and PHA hydrogels are calculated from the following relations;

$$F = \frac{M_t}{M_\infty} = 4\left[\frac{Dt}{\pi r^2}\right]^{1/2} - \pi\left[\frac{Dt}{\pi r^2}\right] - \frac{\pi}{3}\left[\frac{Dt}{\pi r^2}\right]^{3/2} + \ldots\ldots \qquad 5$$

here D is in $cm^2\ s^{-1}$, t is in sec and r is the radius of cylindrical polymer sample. A graphical comparations of eqs. 4 and 5 shows the semi-empirical eq. 4 with n=0.5 and $k=4(Dt/\pi r^2)^{1/2}$.

For the hydrogels, F versus $t^{1/2}$ plots were plotted. A representative curves of N-2 and E-2 hydrogels in the physiological saline solution are shown in Fig. 2b. The diffusion coefficients were calculated from the slope of the lines. The values of diffusion coefficient determined for the hydrogels are listed in Table 4.

If Table 4 is examined, it is shown that the values of the diffusion coefficient of the hydrogels varied from $0.6x10^{-6}$ $cm^2\ s^{-1}$ to $8.1x10^{-6}$ $cm^2\ s^{-1}$.

Conclusion

In this study, *in vitro* dynamic swelling behavior and diffusional properties of polyelectrolytic poly(hydroxamic acid) crosslinked with N and E were investigated. Poly (hydroxamic acid) crosslinked with E were swelled among 175-4625 %, while PHA crosslinked with N were swelled among 130-460 %. The EFC% values of the hydrogels were around the percent water content values of human body (about 60%). The fluid diffusion in the hydrogels was *non-Fickian*..

To sum up, the ionogenity of a hydrogel together with the nature of crosslinker have a great effect on swelling degree of hydrogels in the physiological fluids. It is possible to check the swelling ratio of a hydrogel by controlling the amount of ionogenic groups on the network.

These type of biocompatibility preliminary in vitro studies in simulated physiological fluids have a great importance on the application of biomaterials. The resemblance of PHA hydrogels with those of biomaterials that used in bioapplications shows that these type of materials have potent applications in this field.

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