0273-2289/99/82/0115/$12.75

Influence of Some Amino Acids on the Dynamic Swelling Behavior of Radiation-Induced Acrylamide Hydrogel

Dursun Saraydin,*[,1] H. Nursevin Öztop,[1] Erdener Karadağ,[2] Yasemin Çaldıran,[1] and Olgun Güven[3]

[1]Department of Chemistry, Cumhuriyet University, 58140 Sivas, Turkey, E-mail: saraydin@cumhuriyet.edu.tr; [2]Adnan Menderes University, Department of Chemistry, 09010 Aydın, Turkey; and [3]Hacettepe University, Department of Chemistry, 06532 Beytepe, Ankara, Turkey

Received April 27, 1999; Revised September 8, 1999; Accepted October 15, 1999

Abstract

The influence of some amino acids—alanine, glycine, valine, glutamine, histidine, phenylalanine, and tryptophan—on the swelling behavior of acrylamide (AAm) hydrogel prepared by γ-radiation was investigated. Swelling experiments of AAm hydrogel were made in the universal buffer solutions and the amino acid solutions at certain pHs at 37°C. These selected pH values were pK_1, pK_2 and isoelectric point (p*I*) values such as ionization of α-carboxyl groups, ionization of α-amino groups, and the p*I*s of the amino acids, respectively. The swelling of AAm hydrogel increased when pH values of solutions were increased. The value of equilibrium swelling of AAm hydrogel in the solution of universal buffer was 880% at pH 10.0, whereas it was 670% at pH 2.0. The values of equilibrium swelling of AAm hydrogel in amino acid solutions were between 830 and 965% at pH 10.0, whereas they were between 635 and 775% at pH 2.0. The rate constant of swelling, initial swelling rate, theoretical maximum swelling, diffusional exponent, network parameter, and diffusion coefficient were calculated by swelling kinetics. Diffusions of the amino acid solutions into the hydrogel were generally found as non-Fickian in character. The diffusion coefficients of the hydrogel were between 0.91×10^{-6} and 2.41×10^{-6} cm^2/s.

Index Entries: Amino acid; hydrogel; acrylamide; swelling; diffusion.

*Author to whom all correspondence and reprint requests should be addressed.

Introduction

Hydrogels consist of a three-dimensional polymer network swollen in water and represent an important class of materials in biomedicine, biotechnology, and medicine. This is reflected by the large number of biomedical applications of hydrogels in the field of synthetic hydrogel chemistry. Since the late 1960s, a great variety of hydrogels differing in structure, composition, and properties have been developed. The recognition of the usefulness of hydrogels for biomedical applications opened a new field in polymer science by bringing polymer chemistry to biology and medicine *(1–3)*.

The unique character of hydrogels, owing to the ability of the polymer network, is to imbibe and retain large quantities of water. This characteristic determines, to a large extent, the transport properties that allow the water-soluble molecules that are free of low molar mass to pass through porous hydrogels *(1)*.

Ionizing radiation provides "a very clean method" for the production of hydrogels. No chemicals or catalysts must be added to the reaction matrix. Crosslinking is achieved by free radicals created in the materials by radiation. Thus, no chemicals or catalysts remain in the material at the end of the process. It is not surprising, therefore, that a number of useful biomaterials have been produced using radiation processes *(4,5)*.

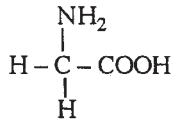
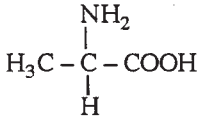
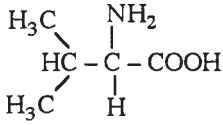
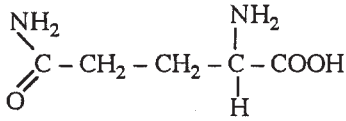
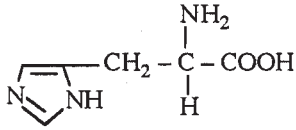
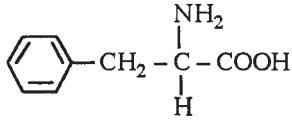
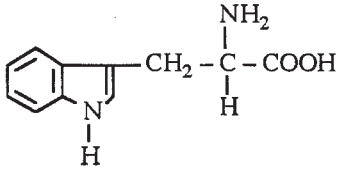
In our previous studies, we investigated the adsorption of proteins such as bovine serum albumin *(5,6)*, the biocompatibility of some biochemical parameters of human sera *(7,8)*, the interaction of nicotine *(9)*, with acrylamide-based hydrogels, and the influence of some aromatic acids on the swelling behavior of acrylamide/maleic acid hydrogel *(10)*. The swelling of acrylamide and acrylamide-based hydrogels is important to the study of column techniques and protein adsorptions.

The present study was aimed at investigating the influence of some aliphatic amino acids (alanine, valine, and glycine), nonionic polar amino acids (glutamine), and aromatic amino acids (histidine, phenylalanine, and tryptophan) on the swelling behavior of acrylamide (AAm) hydrogel. These amino acids were selected for aliphatic side chains (such as the H group in glycine, the methyl group in alanine, and the isopropyl group in valine) and aromatic rings (such as the imidazol ring in histidine, the phenyl ring in phenylalanine, and the indole ring in tryptophan). In addition, phenylalanine and tryptophan contain nonionic polar side chains, whereas histidine contains ionic side chains. Glutamine contains the γ-amide group of glutamic acids. Glycine is unique in that it does not possess an R group. Alanine and valine have nonpolar aliphatic side chains.

Materials and Methods

AAm monomer was obtained from BDH (Poole, UK). Amino acids were purchased from Merck (Darmstadt, Germany). Table 1 gives some of the properties of these amino acids *(11)*.

Table 1
Some Properties of Amino Acids *(11)*

Name and abbreviation	Chemical structure	Molar mass (g/mol)	pK_1 α-COOH	pK_2 α-NH_3^+	p*I*
Glycine (Gly, G)		75.07	2.34	9.60	5.97
Alanine (Ala, A)		89.09	2.34	9.69	6.02
Valine (Val, V)		117.15	2.32	9.62	5.96
Glutamine (Gln, Q)		146.15	2.17	9.13	5.65
Histidine (His, H)		155.2	1.82	9.17	7.59
Phenylalanine (Phe, F)		165.2	1.83	9.13	5.48
Tryptophan (Trp, W)		204.2	2.83	9.39	5.89

One gram of AAm was dissolved in 1 mL of the aqueous solutions and placed in 3-mm diameter polyvinyl chloride straws and irradiated. A dose of 5.20 kGy in air at ambient temperature in a Gammacell 220 type γ irradiator was applied at a fixed rate of 0.72 kGy/h for 433 min. An irradiation dose of 2 kGy is sufficient for the 100% gelation of AAm *(12)*. AAm hydrogel obtained in long cylindrical shapes was cut and dried first in air and then in a vacuum. The preparation and characterization of AAm hydrogels were reported in our previous studies *(12)*.

To measure the parameters of diffusion and swelling, AAm hydrogel was accurately weighed. Then it was transferred into the universal buffer solutions (the buffer solutions were prepared with a mixture of phosphoric,

acetic, and boric acid [0.04 *M*], respectively, and the desired amount of 0.2 *M* NaOH solution was added to the mixture until reaching the required pH value) *(13)* or aromatic amino acid solutions at a concentration of 10 g/L at various pHs at 37°C in a beaker. These selected pH values were pK_1, pK_2, and isoelectric (p*I*) values such as ionization of α-carboxyl groups, ionization of α-amino groups, and the p*I*s of the amino acids, respectively. Solution uptake with respect to time was obtained by periodically removing a sample from the solution, quickly blot drying, and reweighing. The measurements were conducted at 37 ± 0.1°C in a water bath.

Results and Discussion

Analysis of the mechanisms of diffusion in swellable polymeric systems has received considerable attention in recent years, because of important applications of swellable polymers in biomedical, pharmaceutical, environmental, and agricultural engineering.

The swelling of AAm hydrogel in the universal buffer solutions and the amino acid solutions at a concentration of 10 g/L at 37°C was calculated at different pH values by using the following equation *(14,15)*:

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

in which m_t is the mass of swollen gel at time t; and m_o is the mass of the dry gel at time 0.

Swelling curves of the hydrogel in the universal buffer solutions and amino acid solutions were plotted. Figure 1 shows the representative swelling curves for AAm hydrogel in the solutions of valine (Fig. 1A) and phenylalanine (Fig. 1B) at certain pHs.

Figure 1 shows that swelling increases with time but reaches a constant value after a certain point. This value of swelling may be called equilibrium swelling. Figure 2 gives the values of equilibrium swelling of AAm hydrogel in amino acid solutions at different pH values.

To investigate the influence of pH on the equilibrium swelling of AAm hydrogels in the buffer solutions, the values of the equilibrium swelling of AAm hydrogels vs pH values were plotted (*see* Fig. 3).

The swelling of AAm hydrogel in the universal buffer solution increased with pH (Fig. 3). In general, the swelling process was quicker at higher pH values. This behavior could be explained by dipole-dipole and hydrogen-bonding specific interactions between groups in the water and amide group of acrylamide. These interactions become stronger as the concentration of the hydroxyl group of the medium increases, favoring the swelling of AAm hydrogel *(16)*. On the other hand, the presence of $-NH_2$ groups in the hydrogels affected the equilibrium swelling behavior. At a pH lower than 5.0, the increase in equilibrium swelling is mainly owing to the presence of protonated amino groups (NH_3^+), whereas at pH values higher than 5.0, the dominant charges in the hydrogels are the unprotonated amino groups *(17)*.

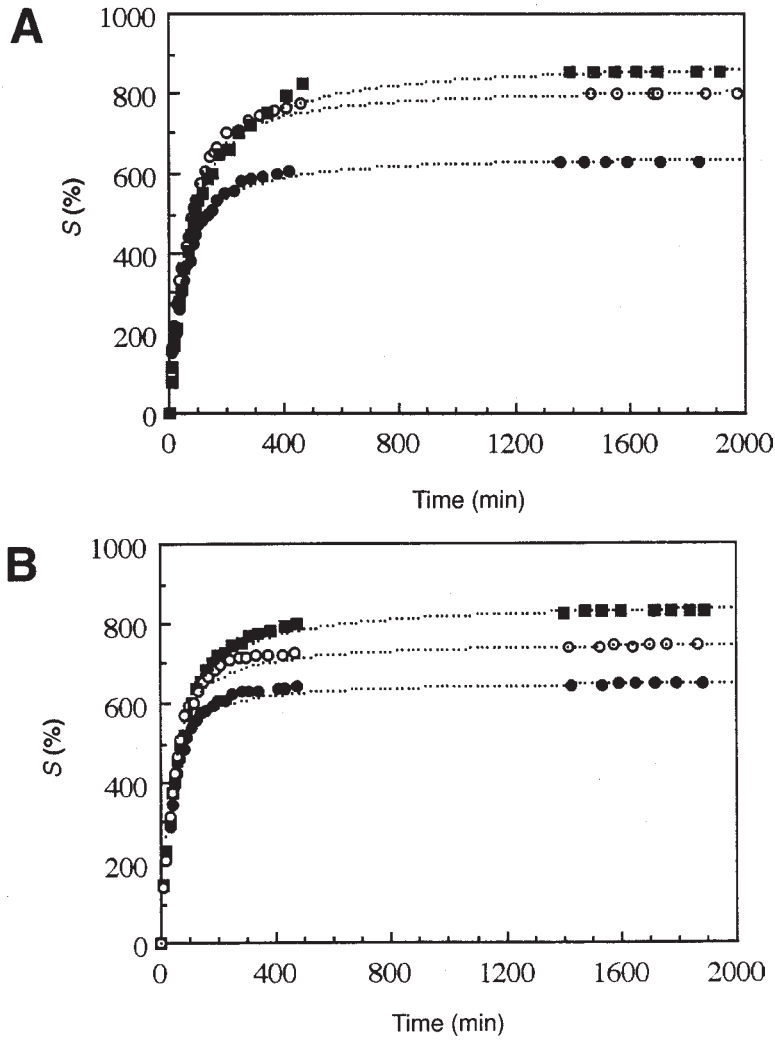


Fig. 1. **(A)** Influence of pH on the swelling behavior of AAm hydrogel in a solution of valine. ●, pH 2.0; ○, p*I*; ■, pH 10.0; (· · · ·), theoretical curves obtained using Eq. 2. **(B)** Influence of pH on the swelling behavior of AAm hydrogel in a solution of phenylalanine. ●, pH 2.0; ○, p*I*; ■, pH 10.0; (· · · ·), theoretical curves obtained using Eq. 2.

All the amino acids in this study contain positively charged amino groups at pH 2.0 *(18)*. These amino acids contain negatively charged carboxyl groups at pH 10.0. Thus, carboxyl groups of amino acids and hydrogel repel each other. For these reasons, the swelling of hydrogel at pH 10.0 in all the solutions of amino acids was higher than the swelling at pH 2.0, as shown in Figs. 1–3 and Table 2.

For extensive swelling of polymers, following second-order kinetics, the following equation can be used *(16,19,20)*:

$$(t/S) = A + Bt \tag{2}$$

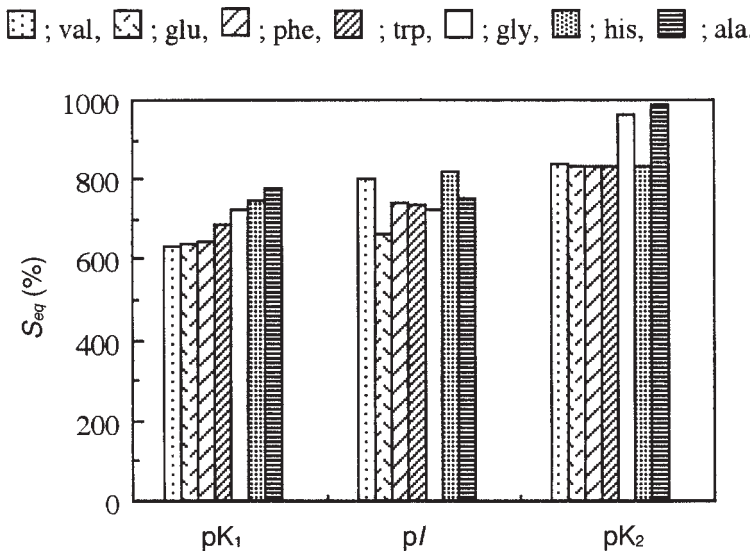


Fig. 2. The equilibrium swelling values of AAm hydrogel in the solutions of amino acids.

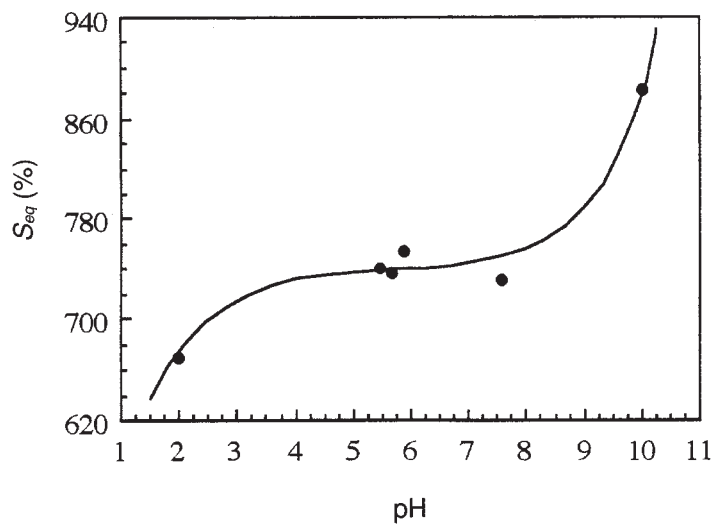


Fig. 3. Change of equilibrium swelling of AAm hydrogel with pH in the buffer solution.

in which $B = 1/S_{eq}$ is the inverse of the maximum or equilibrium swelling; $A = 1/k_S S_{eq}^2$ is the reciprocal of the initial swelling rate $[(dS/dt)_0]$ of the hydrogel; and k_S is the swelling rate constant.

Figure 4 shows the linear regression of the swelling curves obtained by means of Eq. 2 for the AAm hydrogel in phenylalanine solutions at certain pH values. The initial swelling rate, the swelling rate constant, and the values of theoretical equilibrium swelling of the hydrogel are calculated

Table 2
Swelling Rate Parameters of AAm Hydrogel in the Solutions at Various pHs

	pH								
	2.00	5.48	5.65	5.89	5.96	5.97	6.02	7.59	10.0
	$(dS/dt)_0$/gsolution/ggel·min								
Gly	0.16	—	—	—	—	0.16	—	—	0.17
Ala	0.16	—	—	—	—	—	0.19	—	0.15
Val	0.16	—	—	—	0.20	—	—	—	0.13
Gln	0.31	—	0.28	—	—	—	—	—	0.11
His	0.24	—	—	—	—	—	—	0.17	0.16
Trp	0.29	—	—	0.24	—	—	—	—	0.29
Phe	0.28	0.27	—	—	—	—	—	—	0.20
Buffer	0.14	0.24	0.31	0.16	0.16	0.16	0.16	0.21	0.13
	$k_S \times 10^3$/ggel/gsolution·min								
Gly	2.90	—	—	—	—	2.86	—	—	1.68
Ala	2.48	—	—	—	—	—	3.13	—	1.55
Val	3.38	—	—	—	2.70	—	—	—	1.68
Gln	7.43	—	6.11	—	—	—	—	—	1.55
His	4.13	—	—	—	—	—	—	2.33	2.07
Trp	5.87	—	—	4.17	—	—	—	—	5.83
Phe	6.42	4.65	—	—	—	—	—	—	2.66
Buffer	2.85	4.19	5.59	2.72	2.72	2.72	2.72	3.79	1.51
	S_{max}/gsolution/ggel								
Gly	7.39	—	—	—	—	7.46	—	—	9.91
Ala	7.99	—	—	—	—	—	7.74	—	10.2
Val	6.50	—	—	—	8.20	—	—	—	8.89
Gln	6.46	—	6.72	—	—	—	—	—	8.58
His	7.63	—	—	—	—	—	—	8.43	8.63
Trp	6.98	—	—	7.52	—	—	—	—	6.98
Phe	6.56	7.56	—	—	—	—	—	—	8.54
Buffer	6.90	7.55	7.48	7.77	7.77	7.77	7.77	7.47	9.19

from the slope and the intersection of the lines, respectively. Table 2 presents the results.

Table 2 shows that the values of theoretical equilibrium swelling of the hydrogels are parallel with the results of swelling of the gels. The swelling processes of AAm hydrogel at pH 10.0 are quicker than the swelling rate of AAm hydrogels at pH 2.0.

The relations between the swelling of the hydrogels and the molar mass of the amino acids were not found. The swelling of AAm hydrogel is affected with ionization of amino acids and pH.

Equation 3 was used to determine the nature of diffusion of the universal buffer solutions and the solutions of amino acids into hydrogels *(14,15)*:

$$F = kt^n \tag{3}$$

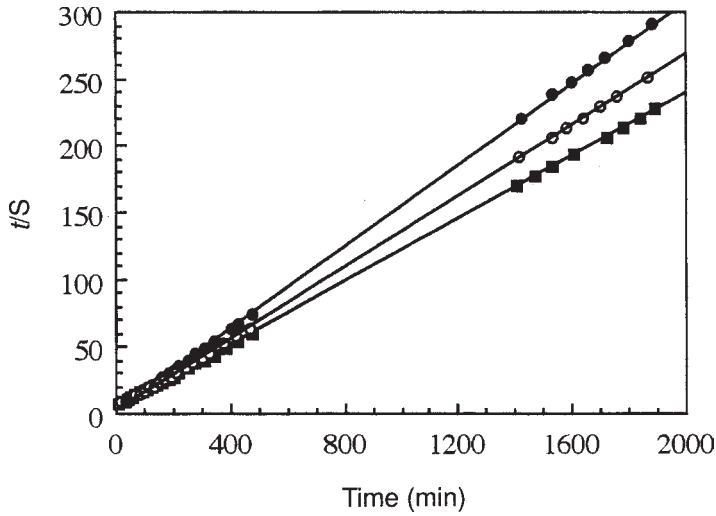


Fig. 4. Swelling rate curves of AAm hydrogel in phenylalanine solutions at various pHs. ●, pH 2.0; ○, p*I*; ■, pH 10.0.

in which F is the amount of solvent fraction at time t; k is a constant incorporating the characteristic of the macromolecular network system and the penetrant; and n is the diffusional exponent, which is the indicative of the transport mechanism. Equation 3 is applied to the initial stages of swelling, and plots of ln F vs ln t are given in Fig. 5. The values of the exponents n and k were calculated from the slope and intercept of the lines, respectively, and are presented in Table 3.

In the experiments, the n values were generally found higher than 0.50 except in the solution at pH 5.89. Hence, the diffusion of penetrants into AAm hydrogels was taken to have a non-Fickian character *(15)*. This is generally explained as a consequence of the slow relaxation rate of the hydrogel matrix. The k values of the systems at pH 2.0 were higher than the k values at pH 10.0.

Diffusion coefficients of hydrogels can be calculated by various methods *(21–25)*. One of these methods is *the short time approximation method*. This method is generally used to calculate diffusion coefficients of AAm hydrogel *(22)*. The short time approximation is valid only for the first 60% of the swelling.

The diffusion coefficients of the cylindrical AAm hydrogel are calculated from the following relations:

$$F = 4\left(\frac{Dt}{\pi r^2}\right)^{1/2} - \pi\left(\frac{Dt}{\pi r^2}\right) - \frac{\pi}{3}\left(\frac{Dt}{\pi r^2}\right)^{3/2} + \cdots \qquad (4)$$

in which D is in cm^2/s; t is in s; and r is the radius of the cylindrical polymer sample. A graphical comparison of Eqs. 3 and 4 shows the semiempirical Eq. 3 with $n = 0.5$ and $k = 4(D/\pi r^2)^{1/2}$.

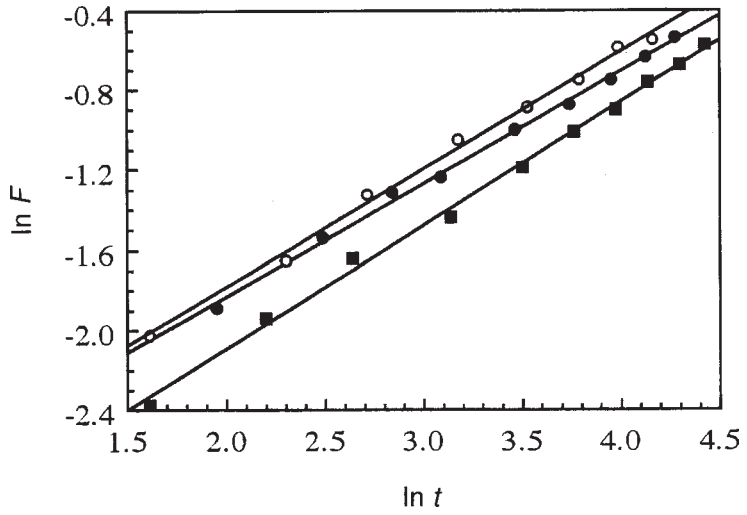


Fig. 5. Swelling kinetics curves of AAm hydrogel in alanine solutions at various pHs. ●, pH 2.0; ○, p*I*; ■, pH 10.0.

Table 3
Effect of pH changes on the *n* and *k* Values
of AAm Hydrogel with Different Amino Acid Solutions

	pH								
	2.00	5.48	5.65	5.89	5.96	5.97	6.02	7.59	10.0
					n				
Gly	0.60	—	—	—	—	0.58	—	—	0.62
Ala	0.56	—	—	—	—	—	0.59	—	0.61
Val	0.55	—	—	—	0.58	—	—	—	0.63
Gln	0.68	—	0.49	—	—	—	—	—	0.61
His	0.61	—	—	—	—	—	—	0.64	0.63
Trp	0.62	—	—	0.73	—	—	—	—	0.62
Phe	0.64	0.72	—	—	—	—	—	—	0.63
Buffer	0.61	0.63	0.46	0.67	0.67	0.67	0.67	0.56	0.66
					$k \times 10^2$				
Gly	4.86	—	—	—	—	4.95	—	—	3.73
Ala	5.22	—	—	—	—	—	5.18	—	3.63
Val	5.99	—	—	—	5.33	—	—	—	3.48
Gln	4.82	—	8.17	—	—	—	—	—	3.50
His	5.16	—	—	—	—	—	—	3.74	3.74
Trp	5.24	—	—	3.63	—	—	—	—	3.98
Phe	5.00	3.47	—	—	—	—	—	—	4.22
Buffer	4.37	4.94	10.41	3.54	3.54	3.54	3.54	6.15	2.89

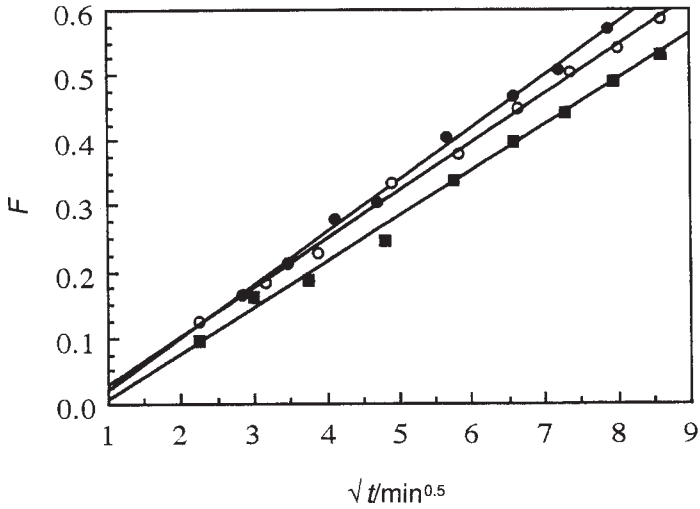


Fig. 6. Diffusion curves of AAm hydrogel in a solution of glycine at various pHs. ●, pH 2.0; ○, p*I*; ■, pH 10.0.

Table 4
Diffusion coefficients ($D \times 10^6$/cm^2/s)
of AAm Hydrogel/Amino Acid Systems at Various pHs

	pH								
	2.00	5.48	5.65	5.89	5.96	5.97	6.02	7.59	10.0
Gly	1.38	—	—	—	—	1.37	—	—	1.34
Ala	1.29	—	—	—	—	—	1.19	—	1.30
Val	1.53	—	—	—	1.45	—	—	—	1.43
Gln	1.95	—	1.74	—	—	—	—	—	0.91
His	1.75	—	—	—	—	—	—	1.52	1.41
Trp	2.08	—	—	2.41	—	—	—	—	1.20
Phe	2.00	2.28	—	—	—	—	—	—	1.60
Buffer	1.51	1.60	1.78	1.54	1.54	1.54	1.54	1.65	1.34

For the hydrogels, F vs $t^{1/2}$ plots were plotted; Figure 6 shows some representative results. The diffusion coefficients were calculated from the slope of the lines. Table 4 gives the values of diffusion coefficient determined for the hydrogels.

Table 4 shows that the values of the diffusion coefficient of the hydrogels varied from 0.91×10^{-6} cm^2/s to 2.41×10^{-6} cm^2/s. The values of the diffusion coefficient of the AAm hydrogel in the solution of amino acids at pH 2.0 were higher than the values of the diffusion coefficient at pH 10.0. Thus, diffusion of the penetrants to the AAm hydrogel at pH 2.0 is faster than at pH 10.0.

In conclusion, the aliphatic amino acids were affected by the swelling behavior of AAm hydrogel. It is important to know the behavior of AAm

hydrogel in amino acid solutions at various pH values used as biomaterials. In addition, in the protein structure, amino acids have no free carboxyl and amino groups, so the hydrogel should also be used in the study of column techniques.

Acknowledgments

We thank the members of the Polymer Group in the Department of Chemistry, Hacettepe University, for assistance in the preparation of hydrogels.

References

1. Woerly, S. (1993), *Biomaterials* **14,** 1056–1058.
2. Hsiau, L. T., Lee, W. C., and Wang, F. S. (1997), *Appl. Biochem. Biotechnol.* **62,** 303–315.
3. Park, T. G. and Hoffman, A. S. (1988), *Appl. Biochem. Biotechnol.* **19,** 1–9.
4. Gopinathan, C. (1989), *Bull. Mat. Sci.* **12,** 49–52.
5. Saraydın, D., Karadağ, E., Öztop, H. N., and Güven, O. (1994), *Biomaterials* **15,** 917–920.
6. Karadağ, E., Saraydın, D., Öztop, H. N., and Güven, O. (1994), *Polym. Adv. Technol.* **5,** 664–668.
7. Karadağ, E., Saraydın, D., Çetinkaya, S., and Güven, O. (1996), *Biomaterials* **17,** 67–70.
8. Saraydın, D., Karadağ, E., Çetinkaya, S., and Güven, O. (1995), *Radiat. Phys. Chem.* **46,** 1049–1052.
9. Karadağ, E., Saraydın, D., and Güven, O. (1997), *J. Appl. Polym. Sci.* **66,** 733–739.
10. Oztop, H. N., Saraydın, D., Karadağ, E., Çaldıran, Y., and Güven, O. (1998), *Polym. Bull.* **40,** 575–581.
11. Smith, E. L., Hill, R. L., Lehran, I. R., Lefkowitz, R. J., Handler, P., and White, A. (1985), *Principles of Biochemistry*, 7th ed., McGraw-Hill, Auckland.
12. Saraydın, D., Karadağ, E., and Güven, O. (1995), *Polym. Adv. Technol.* **6,** 719–726.
13. Lurie, J. (1975), *Handbook of Analytical Chemistry*, Mir Publ., Moscow.
14. Saraydın, D., Karadağ, E., and Güven, O. (1997), *Polym. J.* **29,** 631–636.
15. Peppas, N. A. and Franson, N. M. (1983), *J. Polym. Sci.: Polym. Phys. Ed.* **21,** 983–997.
16. Katime, I., Velada, J. L., Novoa, R., and de Apodaca, E. D. (1996), *Polym. Int.* **40,** 281–286.
17. Duran, S., Solpan, D., and Güven, O. (1999), *NIMB Nuc. Instr. Met. Phys. Res. B* **151,** 196–199.
18. Roskoski, R., Jr. (1996), *Biochemistry*, WB Saunders, Philadelphia.
19. Peniche, C., Cohen, M. A., Vasquez, B., and Roman, J. S. (1997), *Polymer* **38,** 5977–5982.
20. Saraydın, D., Karadağ, E., and Güven, O. (1998), *Polym. Bull.* **41,** 577–584.
21. Buckley, D. J., Berger, M., and Poller, D. (1962), *J. Polym. Sci.* **56,** 163–174.
22. Ritger, P. L. and Peppas, N. A. (1987), *J. Cont. Release* **5,** 23–36.
23. Crank, J. and Park, G. S. (1968), *Diffusion in Polymers*, Academic, New York.
24. Shankar, V. (1979), *Polymer* **20,** 254–256.
25. Li, Y. and Tanaka, T. (1990), *J. Chem. Phys.* **92,** 1371–1376.