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# Influence of some aromatic amino acids on the swelling behavior of acrylamide/maleic acid hydrogel

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**Summary**
Influence of some aromatic amino acids (histidine, phenylalanine and tryptophan) on the swelling behavior of acrylamide/maleic acid hydrogel (AAm/MA) prepared by γ-radiation was investigated. Swelling tests of AAm/MA hydrogel were made in buffer solutions and amino acid solutions at various pH at 37 ºC. The pH values are ionization of α-carboxyl groups ($pK'_1$), α-amino groups ($pK'_2$) and, isoelectric points (pI) of amino acids. The swelling of AAm/MA hydrogel increased when pH values of solutions were increased. The value of equilibrium swelling of AAm/MA hydrogel was 1035% at pH 10 buffer, while it was 880% at pH 2 buffer. The values of equilibrium swelling of AAm/MA hydrogel in phenylalanine, tryptophan and histidine solutions varied among 1130–1245% at pH 10, while they were among 790–975% at pH 2. The rate constant of swelling, diffusional exponent, network parameter and, diffusion and intrinsic diffusion coefficient were calculated by swelling kinetics. Diffusion of the penetrants into the hydrogel was found to be *non-Fickian* character. The diffusion coefficients of the hydrogel varied between $3.33 \times 10^{-6}$ - $7.71 \times 10^{-6}$ cm $^2 s^{-1}$, while the intrinsic diffusion coefficients waried between $4.03 \times 10^{-6}$ -$8.48 \times 10^{-6}$ cm $^2 s^{-1}$.

## Introduction

Hydrogels have found a wide range of biomedical applications, including controlled drug delivery systems, replacement blood vessels, wound dressing, coatings for biosensor, soft tissue substitution, contact lenses and a variety of other related and potential uses. As a family of polymeric materials, synthetic hydrogels are generally well tolerated when implanted *in vivo* and can be tailored to suit the many potential functions of prosthetic in contact with blood or soft tissues. This success of hydrogels as biomaterials lies partially in their superficial resemblance to living tissue, a property attributable to their relatively high

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water content (20–99%), which immediately results in minimal frictional irritation of surrounding tissue. In addition, hydrogels can be non-toxic, chemically stable and (due to their water content) can exhibit a low interfacial tension with aqueous environments (1).

In our previous studies, adsorption of protein such as bovine serum albumin (2,3), biocompatibility of some biochemical parameters of human sera (4,5), and biocompatibility of subcutaneous tissue of rat with acrylamide based hydrogels (6) have been investigated.

The swelling of acrylamide and acrylamide based hydrogels is important about the studying of column techniques and protein adsorptions.

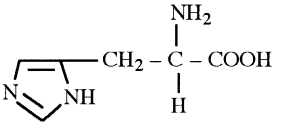
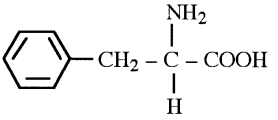
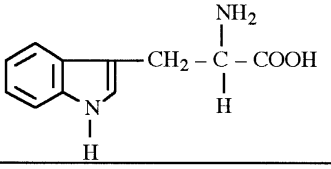
The present paper is aimed to investigate influence of some aromatic amino acids such as histidine (His, H), phenylalanine (Phe, F), and tryptophan (Trp, W) on the swelling behavior of acrylamide/maleic acid (AAm/MA) hydrogel. These amino acids were selected for aromatic rings such as imidazol ring in histidine, phenyl ring in phenylalanine and indole ring in tryptophan. In addition, phenylalanine and tryptophan contain non-ionic polar side chains, while histidine contains ionic side chain.

## Experimental

Acrylamide (AAm) and maleic acid (MA) monomers were obtained from BDH (Poole, UK). Histidine, tryptophan, phenylalanine amino acids were obtained from Merck (Darmstatd, Germany). Some properties of these amino acids (7) are listed in Table 1.

One g of acrylamide was dissolved in 1 mL of the aqueous solutions with 40 mg of maleic acid. These solutions were placed in PVC straws of 3 mm diameter and irradiated. Dose of 5.20 kGy in air at ambient temperature in a Gammacell 220 type γ irradiator were applied at a fixed rate of 0.72 kGy $h^{-1}$. AAm/MA hydrogel obtained in long cylindrical shapes were cut and dried first at air and then in a vacuum. Preparation and characterization of AAm/MA hydrogels were reported in an our previous study (8).

Table 1. Some properties of the aromatic amino acids (7)

| Name and abbrevation | Chemical Sctructure | Molar Mass g $mol^{-1}$ | $pK'_1$ α-COOH | $pK'_2$ α-$NH_3^+$ | pI |
|---|---|---|---|---|---|
| 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 |

To measure the parameters of diffusion and swelling, AAm/MA hydrogel was accurately weighed, and transferred into the universal buffer solutions (8) and aromatic amino acid solutions such as histidine, tryptophan, phenylalanine in the concentration of 10 g $L^{-1}$ at various pH at 37 ºC in a beaker. The pH values are ionization of α-carboxyl groups ($pK'_1$), α-amino groups ($PK'_2$) and, the isoelectric points (pI) of the amino acids. 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±0.1ºC 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/MA hydrogel in the universal buffer solutions and the amino acid solutions such as histidine, phenylalanine, and tryptophan, in the concentration of 10 g $L^{-1}$ at certain pH at 37 ºC was calculated from the following relation (10, 11).

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

Here $m_t$ 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 buffer solutions and amino acids solutions are plotted. Swelling curves of AAm/MA hydrogel in buffer solutions, the solutions of tryptophan at certain pH and in the solution of amino acids at their pI values shown in Figure 1, 2 and 3 respectively.

In Figures 1, 2 and 3 are studied together, it is seen thet swelling increases with time but reaches a constant value after certain point. This value of swelling may be called equilibrium swelling. The values of equilibrium swelling of AAm/MA hydrogel are given in Table 2.

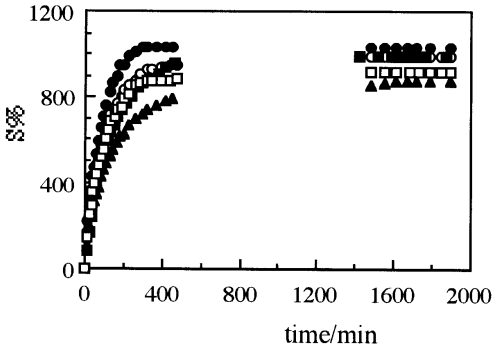



Figure 1. The influence of pH on the swelling behaviour of AAm/MA hydrogel in the buffer solution.
–▲–; pH 2.0, –□–; pH 5.48, –■–; pH 5.89, –○–; pH 7.59, –●–; pH 10.0

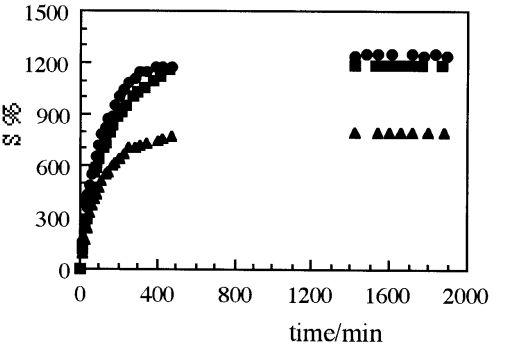



Figure 2. The influence of pH on the swelling behaviour of AAm/MA hydrogel in the solution of tryptophan.
–▲–; pH 2.0, –■–; pH 5.89, –●–; pH 10.0

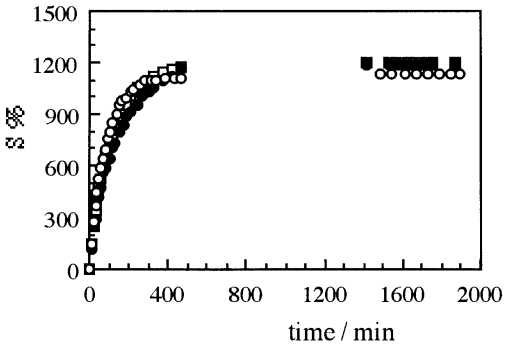



Figure 3. The swelling curves of AAm/MA hydrogel in the buffer solutions at the pI values of amino acids.
–□–; pH 5.48 –■–;pH 5.89 –○–; pH 7.59.

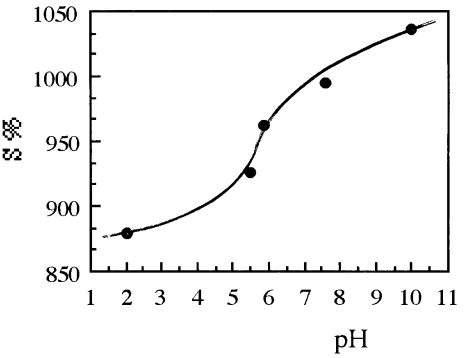



Figure 4. The change of the equilibrium swelling of AAm/MA hydrogel with pH in the buffer solution.

Table 2. The values of the equilibrium swelling of AAm/MA hydrogels in the solutions at various pH.

| pH | 2.0 | 5.48 | 5.89 | 7.59 | 10.0 |
|---|---|---|---|---|---|
| Phenylalanine solution | 845 | 1195 | - | - | 1215 |
| Tryptophan solution | 790 | - | 1180 | - | 1245 |
| Histidine solution | 975 | - | - | 1135 | 1130 |
| Buffer solution | 880 | 925 | 960 | 995 | 1035 |

To determine of swelling rate coefficient, swelling vs. the square root of time were plotted, and curves are shown in Figures 5. Swelling rate coefficients were calculated from the slopes of the straight portion of the curves (12) in Figure 5, and are tabulated in Table 3. Swelling rate coefficients of the hydrogel in the solution are parallel to result of equilibrium swelling as shown in Table 2.

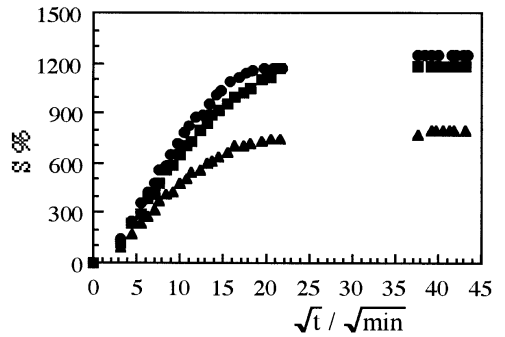



Figure 5. Swelling rate curves of AAm/MA hydrogel in the solution of tryptophan at various pH.
–▲–; pH 2.0, –■–; pH 5.89, –●–; pH 10.0

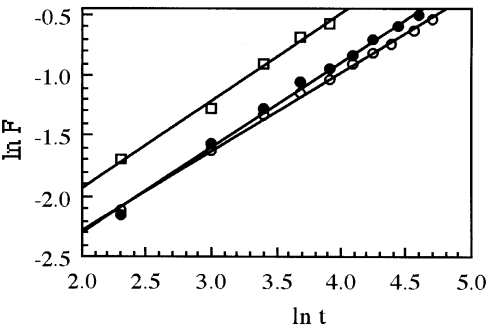



Figure 6. Swelling kinetics curves of AAm/MA hydrogel in the solution of tryptophan at various pH.
–▲–; pH 2.0, –■–; pH 5.89, –●–; pH 10.0

Table 3. The variation of the swelling rate constant of AAm/MA hydrogel in the solutions with pH.

| pH | 2.0 | 5.48 | 5.89 | 7.59 | 10.0 |
|---|---|---|---|---|---|
| Phenylalanine solution | 0.684 | 0.753 | - | - | 0.696 |
| Tryptophan solution | 0.514 | - | 0.690 | - | 0.763 |
| Histidine solution | 0.622 | - | - | 0.800 | 0.723 |
| Buffer solution | 0.478 | 0.594 | 0.593 | 0.644 | 0.737 |

The following equation was used to determine the nature of diffusion of the universal buffer solutions and the solutions of amino acids into hydrogels (10, 11).

$$F = kt^n \tag{2}$$

In this equation, $F$ denotes the amount of solvent fraction at time $t$. The $k$ is a constant incorporating characteristic of the macromolecular network system and the penetrant and $n$ is the diffusional exponent, which is the indicative of the transport mechanism. This equation is applied to the initial stages of swelling, and plots of ln $F$ versus ln $t$ in Figure 6.

The exponents $n$ and $k$ values were calculated from the slope and intercept of the lines, respectively, and presented in Table 4.

Diffusion coefficients are important parameters about penetration of some chemical species into polymeric systems. Diffusion coefficient ($D$) gives a measure diffusion and mass flow of penetrant to the system (bulk diffusion), but, intrinsic diffusion coefficient ($\mathcal{D}$) gives only diffusion (pore diffusion). Diffusion coefficients were calculated from the following relation (13)

$$D = 0.049/(t / 4\, l^2)_{1/2} \tag{3}$$

where $D$ in is $cm^2\ sec^{-1}$, $t$ is the time at which the swelling is one half the equilibrium value ($V/V_o = 1/2$) and $l$ is radius of cylindrical sample. Intrinsic diffusion coefficient may be expressed as

$$\mathcal{D} = D\,(1\text{-}V)^{-3} \tag{4}$$

where $V$ is the volume fraction of solvent penetrating the polymer by the time $t$. Values for parameters of swelling and diffusion, and diffusion coefficients of AAm/MA hydrogels are listed in Table 5.

Table 4. The variation of the n and k values of AAm/MA hydrogel in the solutions with pH.

| Solution | Buffer | | Phenylalanine | | Tryptophan | | Histidine | |
|---|---|---|---|---|---|---|---|---|
| pH | n | k x $10^2$ | n | k x $10^2$ | n | k x $10^2$ | n | k x $10^2$ |
| 2.00 | 0.57 | 3.89 | 0.72 | 3.41 | 0.72 | 2.44 | 0.70 | 2.63 |
| 5.48 | 0.59 | 4.20 | 0.71 | 2.44 | - | - | - | - |
| 5.89 | 0.78 | 1.62 | - | - | 0.70 | 2.27 | - | - |
| 7.59 | 0.59 | 4.18 | - | - | - | - | 0.78 | 2.20 |
| 10.00 | 0.68 | 3.29 | 0.65 | 2.80 | 0.68 | 2.65 | 0.68 | 2.84 |

Table 5. Diffusion (D/cm$^2$ s$^{-1}$) and intrinsic diffusion ($\mathcal{D}$/cm$^2$ s$^{-1}$) coefficients of AAm/MA hydrogel in the solutions at various pH

| Solution | Buffer | | Phenylalanine | | Tryptophan | | Histidine | |
|---|---|---|---|---|---|---|---|---|
| pH | Dx10$^6$ | $\mathcal{D}$x10$^6$ | Dx10$^6$ | $\mathcal{D}$x10$^6$ | Dx10$^6$ | $\mathcal{D}$x10$^6$ | Dx10$^6$ | $\mathcal{D}$x10$^6$ |
| 2.00 | 3.33 | 4.03 | 7.71 | 8.48 | 4.78 | 5.53 | 4.59 | 5.43 |
| 5.48 | 5.02 | 6.32 | 4.93 | 5.86 | - | - | - | - |
| 5.89 | 4.64 | 5.76 | - | - | 4.72 | 5.77 | - | - |
| 7.59 | 4.97 | 5.82 | - | - | - | - | 5.51 | 6.40 |
| 10.00 | 4.92 | 5.75 | 4.14 | 5.16 | 4.91 | 5.98 | 4.42 | 5.23 |

The swelling of AAm/MA hydrogel in the universal buffer solution were increased with pH (Fig. 3). In general, the swelling processes is quicker at higher pH values, This behavior could be explained by dipole-dipole and hydrogen bonding specific interactions between hydroxylic groups in the water and carboxylic group of maleic acid. These interactions become stronger as the hydroxyl group concentration of the medium increases favouring the swelling of AAm/MA hydrogel (14).

In the experiments, the number that determine type of diffusion *n* was found over 0.50. Hence the diffusion of penetrants into AAm/MA hydrogels was taken to have a *non-Fickian* character (11). This is generally explained as being a consequence of the slow relaxation rate of the hydrogel matrix.

If Table 5 is examined, it is shown that the values of the intrinsic diffusion coefficient of the AAm/MA hydrogel in the solution of amino acids are higher than the values of the diffusion coefficient of them.

All of the amino acids in this study contain positively charged amino group at pH 2 (15). Salt bridges (ionic interaction) occur from electrostatic attraction between positively charged groups of the amino acids and negatively charged carboxyl groups of AAm/MA hydrogel. These amino acids contain negatively charged carboxyl groups at pH 10. Thus carboxyl groups of amino acids and hydrogel repuls to each other. For these reasons, the swellings of hydrogel at pH 10 in the solutions of all amino acids are higher than the swelling at pH 2 as shown Figs. 1, 2, 3 and Table 2.

The relations between the molar masses of the amino acids (Table 1) and equilibrium swellings of the hydrogel in the solutions of the amino acids at certain pH (Table 2) are shown in Table 6. The relations between the swelling of the hydrogels and the molar mass of the amino acids were not found. The swelling of AAm/MA hydrogel is affected with ionization of amino acids and pH.

Table 6. The relations between swelling and molar mass of amino acids

| pH | molar mass relation | swelling relation | molar mass-swelling relation |
|---|---|---|---|
| 2 | His < Phe < Trp | His > Phe >Trp | inverse |
| pI | His < Phe < Trp | His < Phe > Trp | no relation |
| 10 | His < Phe < Trp | His < Phe < Trp | true |

It is important that the behaviour of AAm/MA hydrogel in amino acid solutions at various pH values should be known for using as biomaterials. Number of hydrophilic site in the chains of AAm/MA increase with increasing of pH. Increase of equilibrium swelling values are parallel to this situation. On the other hand, in the protein structure amino acids have not free carboxyl and amino groups, so, the hydrogel should be also used in the study of column tecniques.

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