

Preparation conditions and properties of biodegradable hydrogels prepared by γ -irradiation of poly(aspartic acid)s synthesized by thermal polycondensation

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Poly(aspartic acid) (PAsp) hydrogels have been prepared by the γ -irradiation of PAsp produced by thermal polycondensation reactions with an acid catalyst. The effects of the molecular weight of PAsp, pH, concentration of PAsp in the aqueous solution and dosage of γ -irradiation on the PAsp hydrogel preparation were investigated. PAsp hydrogels were prepared in the case when the PAsp weight-average molecular weight was 95 000, the pH of the solution was 7.5 or higher, the concentration of the solution was 5–10 wt/vol%, the dosage of γ -irradiation was 32 kGy or more and the preparation of PAsp solutions was done under N_2 . On the other hand, PAsp with low-molecular weight ($M_w = 15\,000$) could not form hydrogel by γ -irradiation. The swelling of PAsp hydrogels in deionized water and artificial urine was measured. The maximum swelling by deionized water was 3400 g-water/g-dry hydrogel. Biodegradation of the hydrogel was checked using the activated sludge (Japanese Industrial Standards (JIS) K6950 method) indicating that the hydrogel showed about 50% biodegradation for 28 days. © 1997 Elsevier Science Ltd.

(Keywords: L-aspartic acid; poly(aspartic acid); γ -irradiation)

INTRODUCTION

It is known that commercially used water-absorbent polymeric materials, such as poly(acrylic acid), are applied to dippers and hygienic applications, but do not exhibit biodegradability. Therefore, they are rarely collected and composted. They are stored in nature, which produces a grave environmental issue if they are scattered in nature. In this situation, the biodegradable water-absorbent polymeric materials exert no adverse influence on the environment as actively studied in recent years.

As poly(amino acid) is biodegradable and used as a medical, cosmetic, fabric, and metal absorbent material^{1–3}, modified poly(amino acid)s with free carboxylic acid group or amino group are interesting as biodegradable water-absorbent polymeric materials. As a modification of poly(amino acid), hydrogen preparation by γ -irradiation was studied^{4–6}. It was found that microbial poly(γ -glutamic acid) (PGA) and microbial poly(ϵ -lysine) (PL) were converted to biodegradable transparent hydrogels with a high water sorption ability on γ -irradiation. The properties of the hydrogels depended on the dosage of γ -irradiation and the concentration of the solution. The hydrogel from a microbial PGA and PL mixture solution

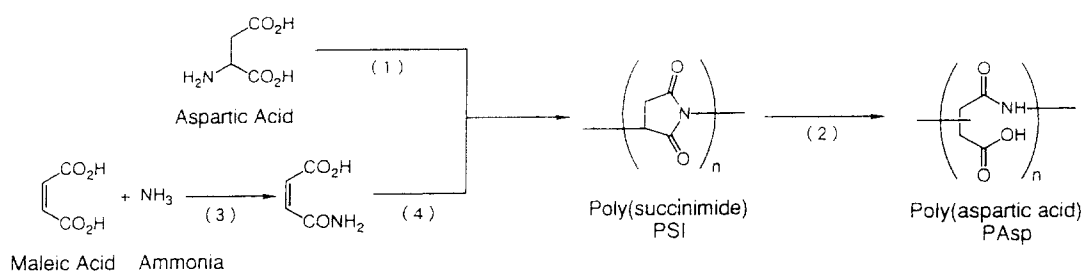
using γ -irradiation was also prepared to control the swelling properties.

Recently the chemical syntheses of poly(aspartic acid) (PAsp) have been investigated, especially the thermal polycondensation of aspartic acid^{7–9} as a monomer that the preparation route is shown in *Scheme 1* (1), or the ammonium salts of maleic acid¹⁰ and malic acid¹⁰ as monomer that the preparation route is shown in *Scheme 1* (3), (4) to form poly(succinimide) (PSI) followed by hydrolysis (*Scheme 1* (2)) is attracted because phosgene or diphosgene is not used in its preparation that differ from NCA (*N*-carboxyanhydride) method. However the molecular weight of PAsp obtained by thermal polycondensation is lower than that by the NCA method.

PAsp obtained by thermal polycondensation that is applied as a detergent builder, antiscalant and moisturizer shows good biodegradability. However it has some irregularities in its structure. For example, it has α - and β -amide linkages of aspartic acid¹¹ and a mixture of L- and D-isomer, even if the monomer is L- or D-aspartic acid. The ratio of L-form/D-form is about 50/50¹².

In this article we report the crosslinking reaction of PAsp obtained by thermal polycondensation by γ -irradiation as a modification. The properties of swelling and biodegradability of PAsp hydrogel are studied.

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Scheme 1 Chemical structure and reaction scheme of poly(aspartic acid)

EXPERIMENTAL

Materials

L-Aspartic acid (L-Asp) was obtained from the Mitsubishi Chemical Corporation (Tokyo, Japan). *o*-Phosphoric acid (85%), mesitylene, sulfolane, *N,N*-dimethylformamide, sodium hydroxide, deionized water, urea, sodium chloride, anhydrous calcium chloride and magnesium sulfate heptahydrate were commercially available and used without further purification.

Preparation of PAsp

Preparation of low-molecular-weight PAsp. L-Asp (0.752 mol) and 85% *o*-phosphoric acid (75 mmol) was put into a blender (Oster (USA) Osterizer) and mixed for 15 min at room temperature.

A 200 ml four-necked round-bottom flask was equipped with a thermometer, a cooler, a mechanical stirrer and an N₂ inlet was charged with the mixture which was made under N₂ atmosphere. The mixture was heated at 200°C for 90 min. The product was washed with methanol (200 ml) and then with water (200 ml) several times until it was neutral. The residue was washed with methanol (200 ml) and dried at 85°C under reduced pressure. This product was PSI as shown in *Scheme 1* (1).

A 100 ml beaker with a stirring bar was cooled by an ice bath, then charged with PSI (3 g) and a solution of sodium hydroxide (1.4 g, equivalents per succinimide residue) and deionized water (20 ml). The mixture was stirred for 1 h. After the reaction, the pH of the solution was adjusted by the addition of 35% aqueous HCl into the solution. The solution was then poured into methanol (300 ml), and the precipitate was filtered and dried at 40°C under reduced pressure. PAsp as shown in *Scheme 1*² was obtained. The molecular weight of the obtained PAsp was estimated in H₂O containing 0.4 mol l⁻¹ NaNO₃ using gel permeation chromatography (g.p.c.) (column: TSKgel G6000PWXL + G3000PWXL, detector: refractive index, standard: polyethylene glycol). The average molecular weight (*M_w*) of the obtained PAsp was 15 000.

Preparation of high-molecular-weight PAsp. A 200 ml four-necked, round-bottom flask equipped with a thermometer, a Dean–Stark trap with a reflux condenser, a mechanical stirrer and an N₂ inlet was charged with L-Asp (0.188 mol), mesitylene (65 ml), sulfolane (19 ml) and 85% *o*-phosphoric acid (9.4 mmol) under an N₂ atmosphere. The mixture was refluxed for 4.5 h. After removing the solvent, the precipitate was washed with methanol (200 ml) and then with water (200 ml) several times until it was neutral. The residue was washed with methanol (200 ml) and dried at 85°C under reduced

pressure. The different point is additions of mesitylene and sulfolane compared with the preparation of PAsp with low-molecular weight. This product was PSI with high molecular weight. Thus, the hydrolysis from PSI to PAsp was conducted in the same way as preparation of low-molecular weight PAsp. The *M_w* of the obtained PAsp was 95 000.

Preparation of PAsp hydrogels

PAsp hydrogels were prepared by γ -irradiation (1.6 kGy h⁻¹) of PAsp aqueous solutions (1–25 wt/vol%) using an irradiation system with a ⁶⁰Co (110 TBq) source. The PAsp solution (2 ml) was contained in a 10 ml glass vial capped under nitrogen and air. The resultant hydrogels were swollen to equilibrium for 1 week at room temperature. During this time, the uncrosslinked PAsp was removed by changing the swelling media daily.

The ratio of gelation was calculated as

$$\text{ratio of gelation(\%)} = (W_1/W_f) \times 100$$

where *W_f* is the weight of PAsp feed in the vial and *W₁* is the dry weight of the PAsp hydrogel after the removal of the uncrosslinked PAsp.

Swelling of PAsp hydrogel

The weight of the wet PAsp hydrogels was measured after equilibrium with deionized water and artificial urine at room temperature using the tea-bag method. The composition of the artificial urine was a mixture of deionized water (970.0 ml), urea (19.4 g), sodium chloride (8.0 g), anhydrous calcium chloride (0.6 g) and magnesium sulfate heptahydrate (2.05 g). The tea-bag method that was performed in this study used a 250 mesh nylon net (NBC Kougyou (Japan), N-255HD, size: 100 mm × 200 mm). The weight of the nylon net was measured (*W_n*). The PAsp hydrogels were placed inside the net and the net was suspended in deionized water or artificial urine at room temperature. After 1 week, the tea bag was hung in the air for 15 min, then the weight of the net and swollen hydrogel was measured (*W_t*). The net and swollen hydrogels were dried at 40°C under reduced pressure, then the weight of the net and dry hydrogel was measured (*W_p*). The specific water content of PAsp hydrogel was calculated as

$$\begin{aligned} \text{specific water content (g-water/g-dry hydrogel)} \\ = (W_t - W_p)/(W_p - W_n) \end{aligned}$$

Biodegradability of PAsp hydrogel

The biodegradability of PAsp hydrogel and uncrosslinked PAsp was measured using a Japanese Industrial Standards (JIS) K6950 in which a sample was placed in

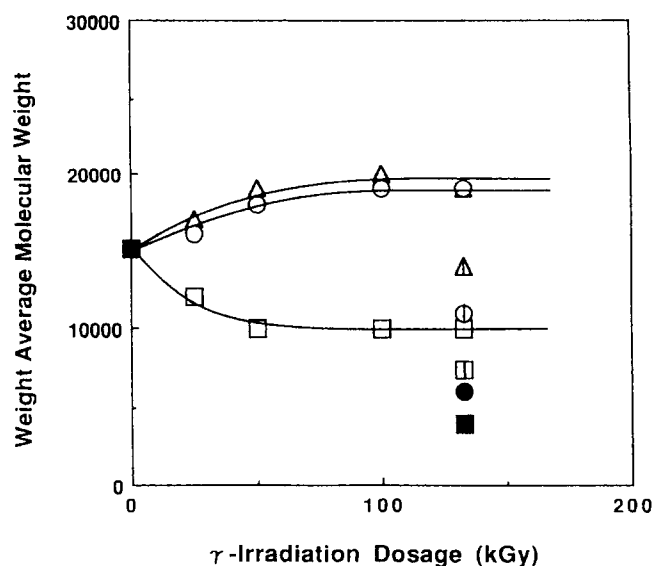


Figure 1 Changes of molecular weight of low-molecular-weight poly(aspartic acid) (PAsp) ($M_w = 15\,000$) depend on the concentration of PAsp in the solution and pH of the solution by γ -irradiation dosage. The molecular weight of PAsp in: 1 (\square), 5 (\circ) or 10 wt/vol% (\triangle) of PAsp concentration in pH 13; 1 (\square), 5 (\circ) or 10 wt/vol% (\triangle) in pH 7.5; and 1 (\blacksquare) or 5 (\blacktriangle) in pH 3.0

the standard activated sludge at $25 \pm 1^\circ\text{C}$ for 28 days. This activated sludge was prepared by the Chemicals Inspection & Testing Institute, Tokyo, Japan. Aniline was used as the standard to check the activity of the standard activated sludge. The biodegradability was evaluated in order to measure the biological oxygen demand (BOD) using an OM3001 coulometer from the Ohkura Electric Co. Ltd, Tokyo, Japan, which detected the consumption of the oxygen during the evaluation. Total organic carbon (TOC) was evaluated using the Total Carbon Analyzer TOC-5000A from the Shimadzu Corporation, Kyoto, Japan. TOC was calculated by comparing the amount before and after the evaluation of the biodegradability.

RESULTS AND DISCUSSION

Effect of γ -irradiation conditions on preparation of PAsp hydrogel

PAsp prepared by thermal polycondensation is a water-soluble and biodegradable polymer. As a modification of PAsp, the preparation of PAsp hydrogel by γ -irradiation was studied.

At first, low-molecular-weight PAsp ($M_w = 15\,000$) was used. Under various pH, concentration of PAsp in the solution, and dosage of γ -irradiation, gel formation was investigated. However, no gel was formed from this PAsp ($M_w = 15\,000$) under any conditions. So the change of molecular weight of irradiated PAsp solution with various pH (3.0, 7.5 and 13) was measured by g.p.c. after γ -irradiation. This change of molecular weight depends on the dosage of γ -irradiation as shown in Figure 1. When pH was 13 and the concentration of PAsp in the solution was 5 and 10 wt/vol%, the molecular weight of the sample increased according to the increase of γ -irradiation dosage. In the case of pH 3, 7.5, or 13 of 1 wt/vol%, the molecular weight decreased.

Figure 1 shows that in the case of pH 13 where the concentration of PAsp in the solution was 5 and 10 wt/vol%,

the crosslinking reaction proceeded and in the case of pH 3, 7.5 where the concentration of PAsp in the solution was 1 wt/vol%, the chain cleavage reaction proceeded. This result reveals PAsp radicals that were induced by γ -irradiation are not stable in pH 3 and 7.5 and low PAsp concentration in the solution. The difference of pH is attributed to the difference of the form of the carboxylic acid group. When the pH is 13 all carboxylic acids form the sodium salts of the carboxylic acids, when the pH is 3 all carboxylic acids remain as carboxylic acid, and when pH is 7.5 both the carboxylic acids and sodium salts exist. It is postulated that the stability of PAsp in the sodium salt form is higher than that of the carboxylic acid form. The difference of the concentration is attributed to the possibility of the radical collision. The higher concentration of PAsp in the solution induces the higher possibility of the collision between PAsp radicals.

In the case of pH 13 and 5 or 10 wt/vol% PAsp concentration, the molecular weights increased from 15 000 to 20 000. However, PAsp solutions did not change to hydrogel. From g.p.c. curves of these samples, there was almost no peak which was more than 40 000. This means that the PAsp molecule has one or zero crosslinking point. This small crosslinking density cannot make the three-dimensional network existing in a hydrogel. On the other hand, the peak which had a smaller molecular weight was observed in PAsp solution after γ -irradiation. It is indicated that PAsp has a cleavage point by γ -irradiation in its polymer chain.

To make a polymer network in PAsp solution by γ -irradiation, high molecular weight PAsp ($M_w = 95\,000$) was selected. This PAsp molecule may have five or more crosslinking points by γ -irradiation due to the higher molecular weight. The result was shown in Table 1. The hydrogel was formed when pH was 7.5 and 13, the concentration of the solution was 5 and 10 wt/vol% and the dosage of γ -irradiation was 32 kGy or more. The tendency is similar to the case of low-molecular-weight PAsp.

The mechanism of PAsp crosslinking and the structure of PAsp hydrogel are postulated and shown in Scheme 2. The γ -ray-induced cleavage of C-H bonds may generate free radicals at the methyne carbons of PAsp as well as poly(acrylate)¹³. However it is postulated that only a few methyne carbons generate free radicals. Then, the recombination reaction of the radicals produces the crosslinking between PAsp.

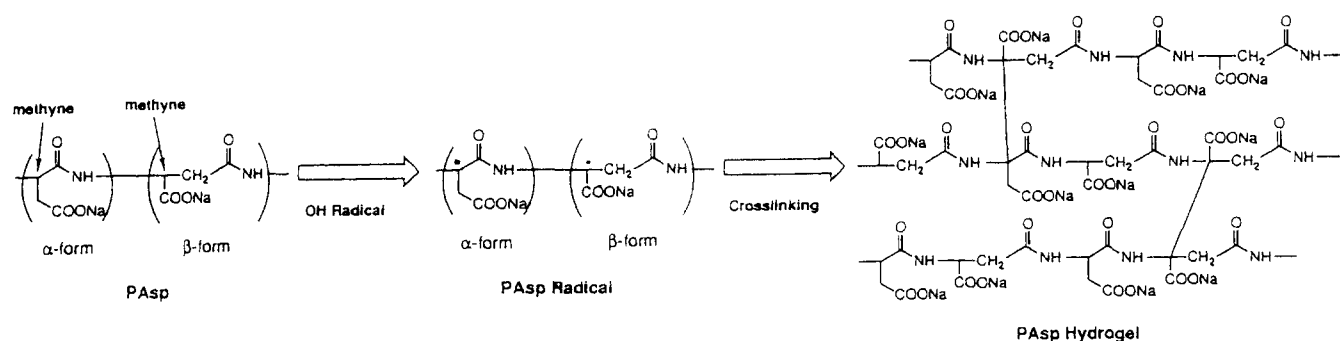
The ratio of gelation of the 5 wt/vol%, pH 7.5, 100 kGy sample was evaluated and its gelation ratio was 92%. It was found that most of the PAsp molecules

Table 1 Specific water contents^a of poly(aspartic acid) (PAsp) hydrogels with the concentration of PAsp in the solution, γ -irradiation dosage and pH of the solution

PAsp conc. (wt/vol%)	γ -Irradiation dosage (kGy)	Specific water content (g g^{-1})		
		pH = 3	pH = 7.5	pH = 13
5	32	NG ^b	NG	1000
5	56	NG	NG	3200
5	63	NG	1100	3100
5	100	NG	820	2500
10	55	NG	NG	NG
10	63	NG	3400	NG

^a Specific water content = (weight of absorbed water/weight of dry hydrogel)

^b NG = no gel formation



Scheme 2 Reaction mechanism and chemical structure of PAsp hydrogel

were connected by crosslinking bonds and were not degraded to a soluble product by γ -irradiation.

The effect of the atmosphere was investigated and the sample that was made in air did not produce a hydrogel even in the case of pH 14 and 10 wt/vol%. It is postulated that oxygen in the solution acted as a radical scavenger. Therefore, the evacuation of oxygen is necessary to prepare PAsp hydrogel.

Swelling of PAsp hydrogel

Swelling in deionized water. The specific water contents (wt. of absorbed water/wt. of dry gel) of all hydrogels prepared in this article were measured. The specific water content was defined in Experimental. Results are shown in Table 1 and Figure 2. Maximum swelling was over 3000 g-water/g-dry hydrogel. Figure 2 also shows the dependency of specific water contents on the dosage of γ -irradiation. The specific water content shows a peak around 60 kGy (pH 7.5).

The maximum swelling of PAsp hydrogels is almost the same as the PGA value studied by Kunioka *et al.*⁴. It is very interesting that the molecular weight of PAsp is much lower than that of the PGA (number average molecular weight = 1250000) used in γ -irradiation experiments, but the PAsp hydrogels showed almost

the same specific water contents. One of the possible reasons is that as the molecular weight of aspartic acid is lower than that of glutamic acid, the density of the carboxylic acid of PAsp is higher than that of PGA. The higher density of the carboxylic acid in PAsp induced almost the same specific water content.

The reason that the specific water content shows a peak depending on the γ -irradiation dosage is postulated that this phenomenon is related to the density of the crosslinking. From 32 to 63 kGy, a hydrogel is formed due to a crosslinking reaction of PAsp molecules with a cleavage reaction by γ -irradiation. It can be presumed that the polymer network of PAsp is formed and then decreased by a scission of polymer chain. As the result of this crosslinking and scission reactions, the hydrogel that is formed in this condition does not have the high ability to maintain deionized water. Cleavage points of PAsp polymer molecule by γ -irradiation may be consumed after 60 kGy, because the molecular weight of PAsp with low molecular weight was constant over 60 kGy during γ -irradiation in the case of pH 13 and 1 wt/vol% PAsp concentration as shown in Figure 1. So a crosslinking reaction may be predominant indicating that the crosslinking density is increased and the specific water content is decreased.

Repetitive swelling of PAsp hydrogel. The repetitive

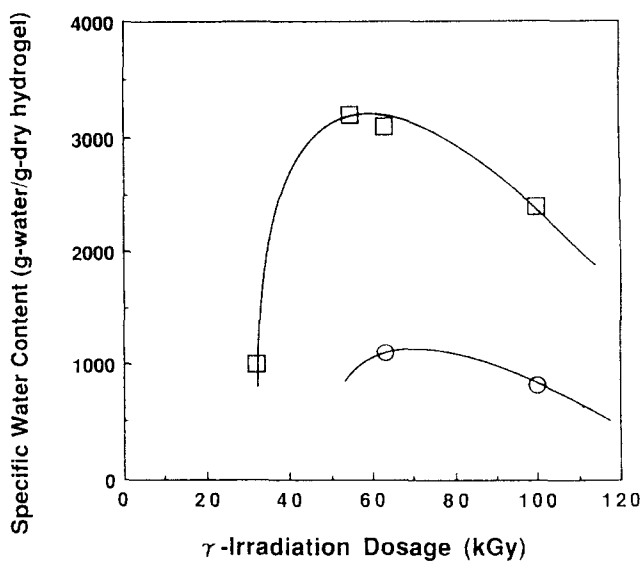


Figure 2 Changes in specific water content in deionized water of PAsp hydrogels during γ -irradiation. PAsp concentration of irradiated solutions was 5 wt/vol% in pH 7.5 (\circ) or 13 (\square)

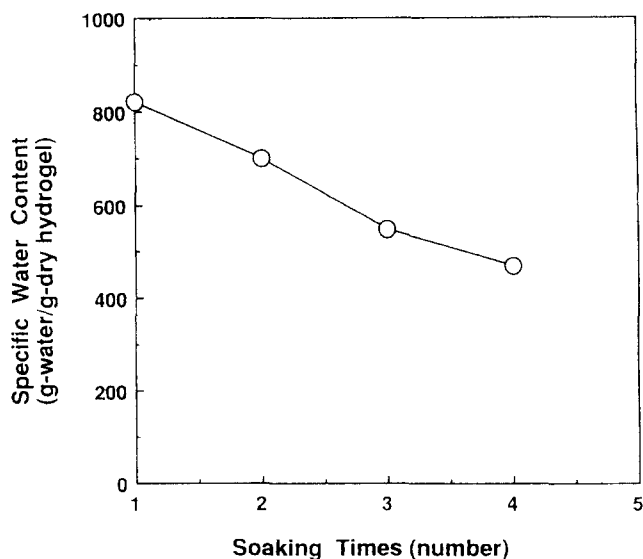


Figure 3 Swelling of PAsp hydrogel by repetitive soaking in deionized water. PAsp hydrogel was formed with 100 kGy of γ -irradiation dose, 5 wt/vol% of PAsp concentration and 7.5 of pH in the solution

Table 2 Results of biodegradability of PAsp and PAsp hydrogel that were evaluated in activated sludge for 28 days

Sample	Biodegradation degree (%)	
	From TOC data	From BOD data
PAsp	51.0	45.2
PAsp hydrogel	44.5	51.0
Aniline	83.2	66.7

PAsp was the raw material of PAsp hydrogel. The weight-average molecular weight of PAsp before γ -irradiation was 95000. The condition prepared PAsp hydrogel was 100 kGy γ -irradiation dose, 5 wt/vol% PAsp concentration in the solution and pH 7.5 of the solution

swelling of the PAsp hydrogel prepared using 5 wt/vol%, pH 7.5, 100 kGy was investigated (Figure 3). The PAsp hydrogel was first swollen in deionized water at room temperature for 1 week, then the specific water content was measured using the tea-bag method. The PAsp hydrogel was freeze-dried and the swelling of the PAsp hydrogel in deionized water was repeated. The specific water content was gradually decreased.

The reason for this decrease of the specific water content is possibly that the aggregation (ex ionic interaction or hydrogen bond) of PAsp polymer molecules is freeze-dried. This aggregation point is maintained in deionized water. This plays the role of a crosslinking point. It means that the crosslinking density of PAsp hydrogel is increased.

Swelling in artificial urine. The specific water content of a 5 wt/vol%, pH 7.5, 100 kGy sample was evaluated in artificial urine where it produced a 27.4 g-artificial urine/g-dry hydrogel. Compared with the value of water, it is about thirty times smaller. Thus, the swelling of PAsp hydrogel may be due to the ionic osmotic pressure generated from mobile counter ions to changed ion in the network, similar to the swelling of the poly(acrylic acid) hydrogel. The effects of the mobile counter ions are decreased by adding salt ions and swellability is decreased dramatically.

Biodegradability of PAsp hydrogel

The biodegradability of uncrosslinked PAsp using raw material ($M_w = 95000$) and PAsp hydrogel (conditions

of γ -irradiation: 100 kGy γ -irradiation dose, 5 wt/vol% PAsp concentration in the solution, pH 7.5 of the solution) was evaluated and the results shown in Table 2. Aniline was used as a standard to check the biodegradable activity of the activated sludge. This activated sludge could degrade 66.7% (from BOD data) and 83.2% (from TOC data) of aniline for 28 days. Almost half of PAsp or PAsp hydrogel was degraded by this activated sludge. The biodegradability of the PAsp hydrogel was almost equal to that of PAsp in spite of its crosslinking. A possible reason for this high biodegradability of the PAsp hydrogel is that the PAsp hydrogel is not stable in the evaluation condition and is easily hydrolysed and that the PAsp polymer backbone has a high biodegradability.

CONCLUSION

PAsp hydrogel using PAsp that was prepared by thermal polycondensation was formed by γ -irradiation. The specific water content of PAsp hydrogel in deionized water was 820–3400 times depending on the preparation conditions. However the swelling of PAsp hydrogels using artificial urine was 27.4 g-water/g-dry hydrogel. The biodegradability was about 50% for 28 days using activated sludge.

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