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Preparation of a reticulated polyurethane foam grafted with poly(acrylic acid) through atmospheric pressure plasma treatment and its lysozyme immobilization

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We successfully introduced peroxide groups onto the surface of PU(Polyurethane) foam(10 PPI) through one atmospheric pressure plasma treatment and sequentially grafted PAAc(poly(acrylic acid)) on the surface of PU through radical copolymerization. The plasma treatment can generate large amount of peroxides on the surface of PU foam and the peroxide groups act as initiators for further grafting of PAAc in the monomer solution. To introduce large amount of peroxides on the surface of PU foam, we studied the effect of plasma rf-power and treatment time on the maximum grafting of PAAc. Through this study, we found that the optimum plasma treatment condition was the rf-power of 100 W and the treatment time of 100 s. On the other hand, we also studied the effect of graft reaction conditions such as temperature, monomer concentration and reaction time on the change of grafting degree (GD). The GD increased with increasing temperature and increased with reaction time before it leveled off at 3 h after reaction started. At low concentration of AAc, the GD was very low but it showed a maximum at the monomer concentration between 60 and 70%. The surface of the modified PU foam was qualitatively and quantitatively analyzed through the use of FT-IR and weight measurement, respectively. We also observed the surface change before and after plasma induced graft co-polymerization through photo and SEM analysis. Finally, we confirmed that the PU foams grafted with PAAc successfully immobilized lysozyme and other proteins from hen egg white.

1. Introduction

Ion exchangers in bead or granular form are widely used in the field of biotechnology but they have a problem due to high pressure drop. Using a macro-porous form ion exchange resins can allow us to achieve high through-put of bio-macromolecules like proteins and enzymes. Polyurethane(PU), which shows wide mechanical properties from rubber to plastic and good resistance to both weak acidic and alkaline solution, is widely used in industrial application. Based on our knowledge, few researchers used PU as ion exchangers. Braun *et al*. [1] first made ion exchangers in the form of foam by grafting or foaming cation exchanger powder with PU precursors. Several patents mentioned forming an ion exchange material—PU interpenetrating network [2–6].

On the other hand, the modification of polymer surfaces by exposure to glow discharge has become common in recent years. The major virtues of these techniques are that they involve clean reaction, which takes only seconds to achieve the required results, and are therefore ideally suited to flow systems, and whilst producing profound changes in the surface properties of the polymer, the properties of the bulk material, for which it was originally chosen, remain essentially unchanged [7]. The thickness of the modified layer may be as much as several micrometers, depending on the parameters of the plasma (pressure, power, gas, flow rate, treatment time, etc.); however, the surface characteristics of the material are determined solely by the composition of the outermost few mono-layers [8, 9].

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Polymeric surfaces are important as biomedical materials or devices that would not adsorb or deposit proteins and other biological molecules. Proteins and cells were widely exploited for selection of appropriate non-adsorbent. Lysozyme, bovine serum albumin, α-lactalbumin, and β-globulin are such examples [10–12].

This research was devoted to the synthesis and characterization of carboxylic groups grafting on the PU foam surface for the purpose of developing a weak acid cation exchanger of carboxylic acid type. PU foam surfaces were treated by one atmospheric pressure plasma and exposed to air to generate peroxides. Peroxides work as initiators when PU reacts with AAc to produce PU-g-PAAc. Carboxyl groups, which are chemically introduced to the surfaces, will work as ion exchangers. Lysozyme, an enzymatic protein (hydrolase) will be tested as a biological adsorbate for adsorption onto the PU foam samples.

2. Experimental

2.1. Materials

The reticulated PU foams used in this study were kindly offered by Urecel Technology Co., Korea. They are the ester type 10 PPI (pores per inch) foams with the size of 1 × 1 × 1 cm. Acrylic acid (AAc) with the purity of 99% was purchased from Junsei Chemical Co., Japan. Ethyl alcohol with the purity of 99.8% produced by Duksan Chemical Co., Korea was used for Soxhlet extraction. Other reagents used were of analytical grade.

2.2. Surface modification of PU foams by plasma discharge

A schematic diagram of the apparatus used in this study was represented in Fig. 1. This apparatus equipped with a flat-type electrode was a Model ATMOS, manufactured by Plasmart Inc., Daejeon, Korea. This is a dielectric barrier discharge and low-temperature apparatus with the frequency of 13.56 MHz and the output range from 30 to 500 W. This is a multi-purpose apparatus, which can generate plasma with only using Ar gas and can automatically control the treatment time and rate. In this apparatus, other gas such as oxygen can be mixed until 5%. The gas flow rate was measured by a mass flow controller (Brooks, Model 5850E, Japan). The stepping motor equipped in this apparatus can control the moving speed of sample holder and can eventually control the treatment time and rate. After cleaning PU foam with methanol and drying it, we adjusted the position of sample holder while putting sample under glow discharge and then set up the speed and the time of stepping motor by using digital sensor. The flow rate of Ar gas was maintained at 5 ℓ/min. Samples were treated at the range of treatment time from 30 to 180 s and of rf-power from 60 to 200 W. After treatment, the treated PU foam was taken out from the sample die and was exposed to air for 5 min to generate peroxide.

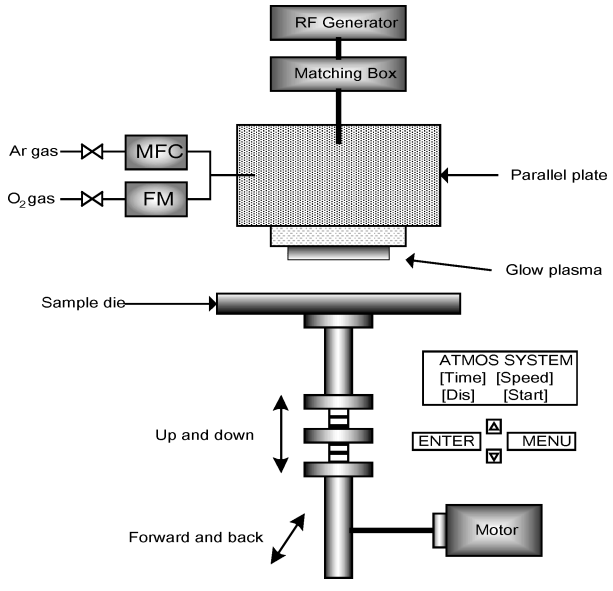

Figure 1 A schematic diagram of one atmospheric pressure plasma apparatus.

2.3. Graft copolymerization of PAAc on the surface of PU foam

The foam exposed to air after plasma treatment was immersed in the aqueous solution of AAc with a proper concentration in a flask without any additives. After vigorous degassing, the flask was kept at constant reaction temperature under nitrogen atmosphere for constant reaction time to prepare PU-g-PAAc foam (Fig. 2). To remove the monomer and possible homopolymer on the surface, sample was taken out and washed twice with warm deionized water, and then extracted the remaining homopolymers with using ethanol as a solvent for 24 h in a soxhlet extractor. Finally, they were rinsed with de-ionized water several times, dried under reduced pressure for 24 h at room temperature, and stored in a desiccator. The grafting degree was calculated by measuring the weight difference before and after graft co-polymerization as follows;

$$\mathrm{GD}(\%) = [(W - W_\mathrm{O})/W_\mathrm{O}] \times 100 \qquad (1)$$

where W is the weight of PU foam after grafting and W_O is the weight before grafting.

2.4. Lysozyme immobilization

Lysozyme(muramidase) hydrolyzes preferentially the β-1,4-glucosidic linkage between N-acetylmuramic acid and N-acetylglucosamine which occurs in the mucopeptide cell wall structure of certain microorganisms, such as *Micrococcus lysodeikticus*. A somewhat more limited activity is exhibited towards chitin oligomers [13]. Materials used obtained from hen egg white. The hen egg white was diluted 10 times in distilled water and the insoluble materials were separated in a centrifuge with 8000 rpm for 30 min. The phosphate buffer solution was composed of mono-basic (Duksan, Co., Ltd., Korea) and di-basic (Sigma, USA) with the concentration of 0.02 M and the pH of 7.0. For the activity measurement, *M.luteus* (Sigma, USA) was used. After

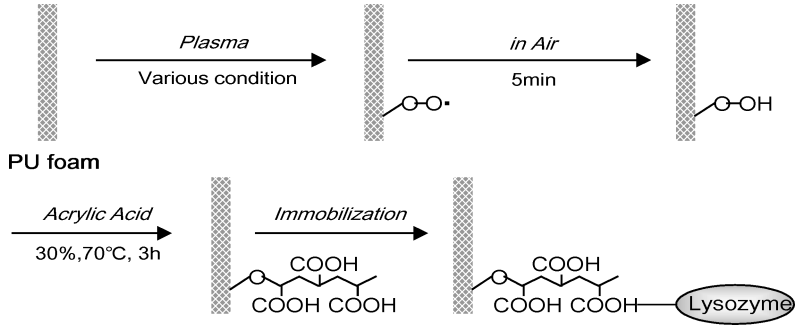

Figure 2 Overall reaction mechanism including plasma treatment, graft polymerization and immobilization of biomolecules.

all PU-g-PAAc foams were put into the Falcon tube and the phosphate buffer solution of 25 ml was injected, let it reach equilibrium for 30 min. Then, the buffer solution was removed. After injecting hen egg white solution, let it sufficiently exchange ions for 30 min while shaking it from time to time. Then, the injected solution was removed. To further remove proteins entangled in the pore of PU foams without forming ionic bond, we shook it from time to time for 30 min after injecting the phosphate buffer solution of 25 ml. Finally, we desorbed all proteins and enzymes, which formed ionic bond, from PU-g-PAAc foams in a 2 M NaCl solution dissolved at 0.02 M phosphate buffer solution.

2.5. Protein assay and SDS-PAGE

We used Lowry method for protein assay and measured absorbance at the wavelength of 750 nm. This method is widely used because it has high sensitivity and good consistency even in the presence of urea or ammonium sulfate. We made 2% Na_2CO_3 solution after dissolved Na_2CO_3 in 0.1 N NaOH solution. Then, we also made 0.5% $CuSO_4 \cdot 5H_2O$ after dissolved it in 1% $Na_3C_6H_5O_7 \cdot 2H_2O$. After mixing the former reagent of 50 ml and the latter reagent of 1 ml just before use, let it be the reagent A. After mixing Folin-Ciocalteu phenol reagent and distilled water with equal volume, let it be the reagent B. After mixing the sample solution of 0.5 ml, which contains proteins and enzymes, and the reagent A of 2.5 ml, preserve it at room temperature for 10 h. Then, we measured absorbance at 20 or 30 min after quickly adding the reagent B of 0.25 ml and mixing it. Finally, we measured the concentration of sample obtained through desorption from the calibration curve pre-prepared from various BSA concentrations. We used 15% acrylamide SDS-PAGE to qualitatively investigate whether the lysozyme is included in the sample solution obtained [14].

2.6. Activity measurement

The activity was measured through Li-Chan's method with using *M.luteus*. When we used 0.066 M phosphate buffer solution of pH 6.24 as a standard, we made the suspension of the prepared buffer solution and *M.luteus* have the initial absorbance between 0.6 and 0.7. After putting the sample solution of 0.1 ml with the suspension of 2.5 ml, we measured the change of absorbance for 2 min. One unit of activity was defined as the absorbance change of 0.001 per min. The recovery of Lysozyme was obtained as follows;

$$\text{Recovery } (\%) = [A/A_O] \times 100 \qquad (2)$$

where A is the total activity of sample and A_O is the total activity of hen egg white.

3. Results and discussions

3.1. Effect of plasma treatment conditions on the PAAc grafting degree

In this section, we investigated the optimum plasma treatment condition for the maximum grafting of PAAc on PU foam surface. First, we investigated the effect of plasma treatment time on the grafting degree of PAAc. Each sample was prepared under the treatment condition of 100 W rf-power and the graft reaction conditions of 70 °C, 3 h, 30%. As shown in Fig. 3, the GD slightly increased from 30 s, showed a maximum at around 100 s, and then decreased. This is because the long time plasma exposure cannot help generating more peroxides and rather partly convert produced peroxides into inactive species, which can not yield radicals. It is well explained in our previous paper [15, 16]. Similar results were also reported by other groups [17, 18].

The effect of changing rf-power on the GD of PAAc is shown in Fig. 4. The GD rapidly increased until 100 W and then decreased before asymptotically approaching

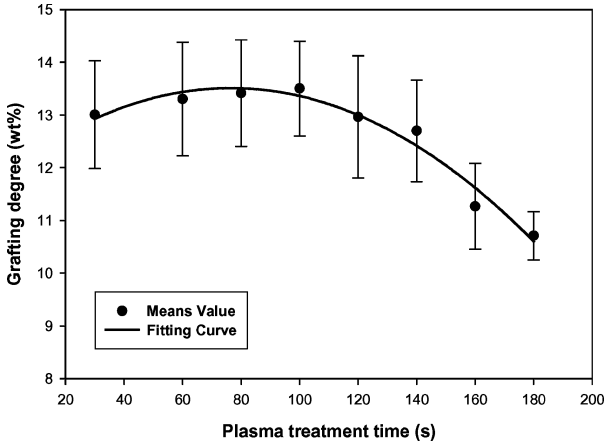

Figure 3 Effect of one atmospheric pressure plasma discharge time on the grafting degree of AAc onto PU foams (plasma discharge power: 100 W, reaction time: 3 h, reaction temperature: 70 °C, AAc concentration: 30%).

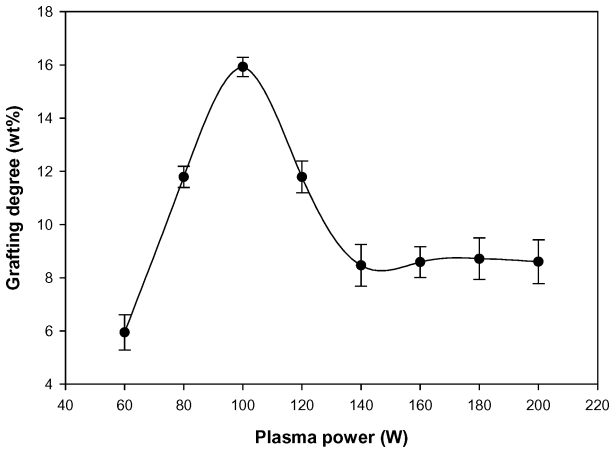

Figure 4 Effect of one atmospheric pressure plasma discharge power on the grafting degree of AAc onto PU foams (plasma discharge time: 100 s, reaction time: 3 h, reaction temperature: 70 °C, AAc concentration: 30%).

a constant value from 140 W. An increase in discharge power at a fixed flow rate means an increased rate of excitation or an increased rate of initiation of growth reactions. The change was most drastic when the discharge power became insufficient to sustain the full glow. This figure also shows the independence of discharge power on the GD at a sufficient power. This was well explained by Yasuda [19]. In our previous study [15, 16] done with PU film, the optimum plasma treatment conditions for the maximum generation of peroxides were the treatment time of 30 s and the rf-power of 100 W.

3.2. Effect of graft copolymerization conditions on the PAAc grafting degree

To control the graft copolymerization conditions is very important for proper modification of PU surface since homopolymerization competitively proceeds. In this study, we changed three variables such as reaction temperature, time and concentration. As shown in Table I,

TABLE I Effect of copolymerization conditions on the grafting degree (%) of AAc onto the surface of PU foam

Reaction time, hr	Monomer concentration (%)	Reaction temperature (°C)			
		30	50	70	80
1	10	0.450	1.076	1.347	1.409
	30	0.469	1.140	4.586	6.706
	50	0.476	1.304	7.697	9.128
	70	0.754	1.890	7.360	9.023
1.5	10	1.053	1.119	1.573	1.640
	30	1.060	1.286	6.981	7.814
	50	1.412	1.647	8.777	9.502
	70	1.516	1.568	8.241	9.372
3	10	1.068	1.146	1.633	1.881
	30	1.096	1.458	13.442	15.252
	50	1.448	10.428	19.207	24.486
	70	1.558	9.734	18.701	23.954
5	10	1.072	1.296	1.649	1.967
	30	1.256	1.577	13.675	15.703
	50	1.518	11.436	19.869	25.885
	70	1.419	10.968	19.158	24.487

GD mostly increases with increasing reaction temperature [20], although the reaction temperature does not affect the increase of GD at the concentration below 30% AAc. This is because the chemical reaction rate increases with increasing free radicals formed due to the thermal decomposition of peroxides on the surface of PU foam and the diffusion rate of monomers from bulk solution to PU foam surface increases as the reaction temperature increases. At the concentration above 50% AAc and the reaction time over 3 h, GD considerably increases with increasing reaction temperature. In this case, a lot of homopolymers are also formed in the solution and the increase of viscosity due to this makes it difficult for us to get precise GD data [21, 22].

GD shows a maximum at the monomer concentration of 60% and it rather decreases at the concentration over 70%. We expected that the polymerization rate generally decreased because monomers and initiators were used as copolymerization proceeded. The copolymerization rate rapidly increases with increasing the concentration of monomers because termination becomes difficult due to the increase of viscosity, the decrease of collision frequency and the increase of entanglement. This was called as the effect of Trommsdorff [23, 24]. This phenomenon was frequently observed when the concentration of monomers was high at the liquid-phase graft copolymerization in a normal solvent. When the concentration of monomers are low, the growth reaction from active sites becomes more dominant than the formation of homopolymer. However, when the concentration of monomers are high, the formation of homopolymer becomes faster than the growth reaction from limited number of peroxides. That is to say, GD becomes rather low at over certain concentration of monomers due to the dominant reaction between monomers. This phenomenon was also observed at other researches [17, 25, 26].

As shown in Table I, GD increases with increasing reaction time until 3 h and levels off. Until 3 h, the graft copolymerization actively proceeds through the thermal decomposition of hydroperoxides formed on the surface of PU foam. After 3 h, termination occurs. In addition, higher GD was obtained and more homopolymers were observed as the concentration of monomers and the reaction temperature increase. Lei *et al.* [17] reported that GD increased until the reaction time of 2.5 h and leveled off and Bunyakan *et al.* [27] also reported the same. Thus, 3 h grafting reaction is most efficient for obtaining maximum GD in our experimental system. In addition, over 3 h reaction results in the weakening of the mechanical property of PU and the swelling of our samples.

3.3. FTIR analysis

Fig. 5 shows the FT-IR spectrum of PU foam. To obtain this spectrum, we destroyed PU foams in liquid nitrogen and analyzed their chemical components. The surface modified PU foam shows strong absorption peak at the wavelength of 1,635 cm^{-1}. This is due to the contribution of carboxyl groups from acrylic acid grafted on the surface of PU foam. Lei *et al.* [17] also confirmed

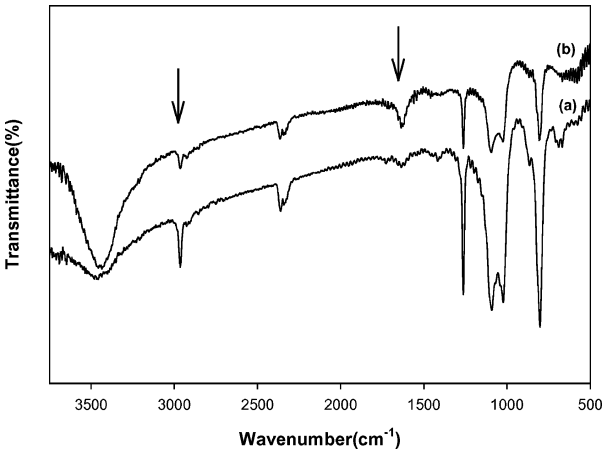

Figure 5 FT-IR spectra of (a) Virgin PU foam, (b) PU-g-PAAc foam. Arrows indicate two typical peaks for comparison.

the carboxyl groups on the surface of LDPE film after grafting of acrylic acid and showed the increase of peak intensity with increasing reaction time. Ghosh *et al.* [28] also qualitatively analyzed the carboxyl functional groups formed on the surface of LDPE through the analysis of IR peak.

Table II shows the peak area ratio calculated from the absorption peaks of C—O (1635) and C—H (2964). From this result, we can know that the band ratio of modified PU foam is almost 10 times higher than untreated PU foam. This can be a strong evidence for the successful introduction of large amount of carboxyl groups on the surface of PU foam.

TABLE II IR band ratios in virgin PU foam and PU-g-PAAc foam

	Virgin PU foam	PU-g-PAAc foam
IR band ratio C═O(1635)/C—H(2964)	0.184	1.722

( ) denotes wavenumber.

TABLE III Protein assay for modified PU samples

Sample	1	2	3	4	5	6	7	8
Total protein (mg)	28.4	30.2	31.4	36.6	33.0	29.4	0.1	0.1
Recovery (%)	8.9	9.3	9.9	11.5	10.2	9.3	0.05	0.05

3.4. SEM analysis

Fig. 6 shows SEM photographs which represent PU foams before and after acrylic acid grafting. While the surface of untreated PU foam does not show anything particular, the modified PU foam shows wavelike structure at the edge of the foam. This wavelike structure is expected to come from the result of drying polymer brush. We can also observe that the surface of modified PU foam becomes smooth due to the etching of untreated PU foam by plasma and the subsequent grafting

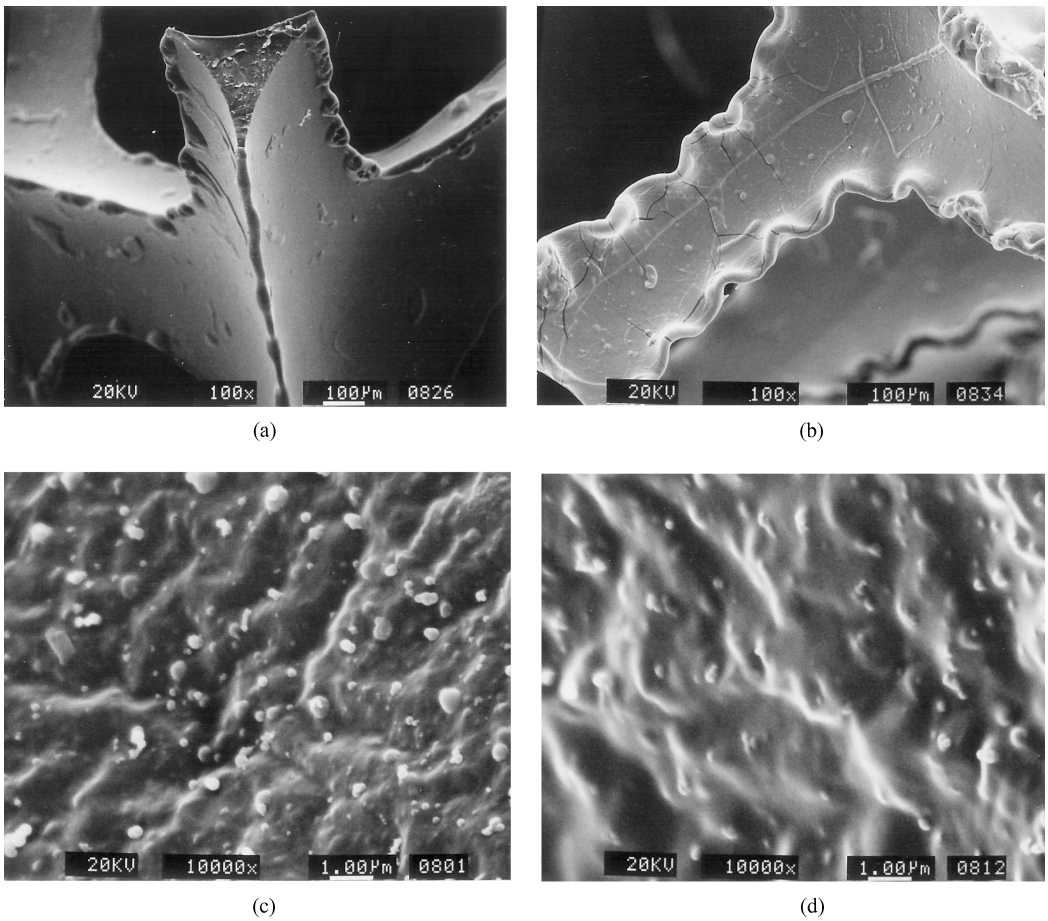

Figure 6 SEM analysis of PU foam surface; (a) Virgin PU foam (100×), (b) PAAc grafted PU foam (100×), (c) Virgin PU foam (10,000×), (d) PAAc grafted PU foam (10,000×).

TABLE IV Activity and recovery of lysozyme for modified PU samples

Sample	1	2	3	4	5	6	7	8
Total protein (U)	31875	34405	40625	35750	36125	35250	6500	8375
Specific activity (U/ml)	1275	1329	1625	1430	1445	1410	235	297
Recovery(%)	55.3	57.7	70.5	62.0	62.7	61.2	10.2	12.9

of acylic acid. When we put this modified PU foam into liquid solvent like water or alcohol, we could observe polymer brushes formed on the surface of PU foam due to the swelling of grafted poly(acrylic acid) into liquid phase. Kang *et al.* [29] showed SEM photos of functional group grafted polymers by way of plasma treatment and Xu *et al.* [22] also reported SEM photos of grafted acrylic acid on the surface of polypropylene [30].

3.5. Lysozyme immobilization characteristics of modified PU foam

We used an electrophoresis method in order to identify proteins and enzymes like lysozyme adsorbed on the surface of our modified PU foam [31, 32]. As shown in Fig. 7, BSA(66.2 kDa), ovalbumin (45 kDa) and lysozyme (14.4 kDa) were sequentially separated after the adsorption experiment of egg white. BSA that has high molecular weight appears first, showing large amount. The band of ovalbumin comes next around the top side whereas that of lysozyme at the bottom. This result shows that our modified PU foam can successfully immobilize relatively large amount of proteins and lysozyme. There are a couple of reports asserting that the PU foam modified by newly formed hydrophilic groups–carboxylic groups-immobilizes proteins and lysozyme through a mechanism of ion exchange [33, 34]. Electrostatic interaction between the negatively charged polymer surface (-COO^-) and lysozyme applies since the enzyme has net positive surface charges attributed to 33 charged residues at neutral pH. The interaction could be substantial because the experimental pH was far from the isoelectric point of the protein. Probably this interaction explains the mechanism of ion exchange in part. On the other hand, protein adsorption onto the polymeric surfaces seems to be governed by water hydration effect in aqueous surroundings [35, 36]. That is, a certain water-structure is transferred to that of proteins, and sequentially causes protein adsorption and its denaturation on the polymer surface [36]. Comparing PU foam with PU film, the adsorption capacity of PU foam was much larger than that of PU film, which means the structural superiority of PU foam such as larger surface area per unit volume and hence larger surface coverage of carboxylic groups. However, we cannot simply say that the grooves found in virgin and modified samples (Fig. 6) play an important role in the adsorption of lysozyme since the protein molecules are as small as nano-scale ($4.5 \times 3.0 \times 3.0\,nm^3$) and therefore have no preference to the surface morphology as frequently stated in case of cell adsorption (micron-scale). In summary, protein adsorption on the modified PU foams enhances via water hydration (hydrophilicity) as well as an electrostatic attraction or ionic bond.

We also quantitatively analyze the protein adsorption capacity of modified PU foam through Lowry method and Li-Chan's method [37]. The total amount of adsorbed proteins was measured by Lowry method (Table III). The modified PU foams treated by high rf-power plasma show high yield. At low power, the increase of treatment time enhances the yield of protein. At high power, however, the increase of treatment time does not enhance the yield of protein. This result can be explained from the existence of optimum plasma power for the modification of PU surface, as shown in Table II. Table IV shows the activity of lysozyme measured by Li-Chan's method. Pure lysozyme adsorption is more efficient at the PU foam prepared at low power and for long treatment. Marolia *et al.* [38] measured the activity of lysozyme after immobilizing lysozyme on the cross-linked egg white foam and Kacar *et al.* [39] investigated the activity of lysozyme through the adsorption and desorption of lysozyme on the pHEMA film.

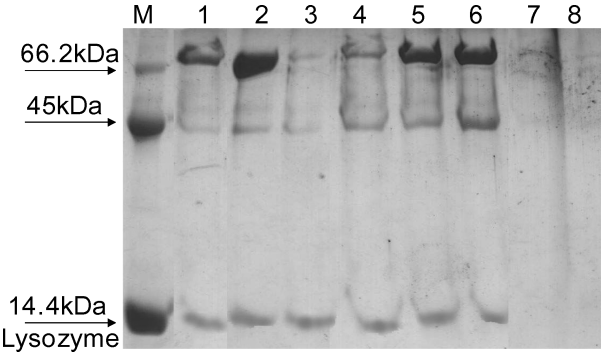

Figure 7 15% SDS-PAGE of the PU foams (sample 1–6) and PU films (sample 7, 8) [Sample treatment conditions: (1) 60 W, 60 s, 70 °C, 30%; (2) 60 W, 90 s, 70 °C, 30%; (3) 60 W, 120 s, 70 °C, 30%; (4) 80 W, 60 s, 70 °C, 30%; (5) 80 W, 90 s, 70 °C, 30%; (6) 80 W, 120 s, 70 °C, 30%; (7, 8) PU film].

4. Conclusions

In this study, we successfully modified the surface of reticulated PU foam through one atmospheric plasma treatment and subsequent liquid grafting of acylic acid. The modified PU foam was able to have large amount of carboxylic groups. This also shows that the one atmospheric pressure plasma can successfully modify the surface of 3-dimensionally structural materials like the reticulated PU foam.

The optimum treatment conditions of the one atmospheric pressure plasma were 100 W and 100 s for maximum introduction of acylic acid. The optimum conditions of liquid-phase grafting reaction were the reaction time of 3 h, the monomer concentration of 60% AAc, and the reaction temperature over 70 °C. As a

result of FT-IR peak analysis, modified PU foam showed strong peak at around 1635 cm^{-1} and this confirmed the successful introduction of carboxyl groups through plasma-induced grafting of AAc.

From the adsorption experiment of lysozyme from egg-white, we confirmed that the modified PU foam had a possibility to be used as an ion-exchanger in which electrostatic attraction or ionic bond could be formed and was generally able to immobilize large amount of lysozyme as GD increased.

Acknowledgments

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