Polymer Journal

REGULAR ARTICLE

Characterization of Insulin Adsorption Behavior on Amphoteric Charged Membranes

*By Shaoling ZHANG, Keiichiro SAITO, Hidetoshi MATSUMOTO, Mie MINAGAWA, and Akihiko TANIOKA**

With view to develop an amphoteric charged membrane for drug delivery, we have studied the adsorption of insulin on it. In the present study, the amphoteric charged membranes were prepared by pore-surface modification of porous poly(acrylonitrile) (PAN) membranes by grafting with acrylic acid (AAc) and/or *N*,*N*-(dimethylamino)propyl acrylamide (DMAPAA). Their surface charge properties and the insulin adsorption behaviors were investigated by zeta potential measurement and UV spectrophotoscopy, respectively, at different pHs. The equilibrium adsorbed amount of insulin correlates well with the charge properties of the membranes and insulin, which indicates that electrostatic interaction played an important role in the insulin adsorption. In addition, adsorption kinetics changed from a Fickian mode to a non-Fickian one when adsorbed amount increased to very high values.

KEY WORDS: Insulin / Poly(acrylonitrile) / Amphoteric Charged Membrane / Zeta Potential / Adsorption /

Insulin is a polypeptide hormone produced by the pancreatic β cells, and regulates carbohydrate homeostasis. Currently, the administration of insulin through parenteral injection (*e.g.*, subcutaneous injection) is the commonest therapy of diabetes. Since pain and risk for infection cannot be completely avoided in this method, novel non-invasive insulin delivery techniques such as iontophoresis have been required.[1–5]

The adsorption of proteins on biomaterial surfaces has been widely studied,[6–10] and it has been identified to be very complex and can be influenced by a lot of factors such as electrostatic interactions and hydrophilicity/hydrophobicity. Protein adsorption on membranes not only could change the pore size and charge property of the membrane but also could give valuable information for the study of the protein transport mechanism within the membrane.[6,11,12] There have been, however, few reports on the relationship between insulin adsorption on and transport through a membrane for the drug delivery. It is of great interest to study the insulin adsorption behavior on the charged membranes for the administration of insulin release.

Recently, amphoteric charged membranes have attracted considerable interests due to their pH-responsibility, anti-fouling property, flexibility in the design of charge structure, and they are expected to be applied in various fields such as proteins separation and drug delivery systems.[11,13–16] In the present study, a series of amphoteric charged membranes with different ratios of acidic to basic groups were prepared, and the insulin adsorption on the prepared membranes was investigated at different pHs. The aim of this study is to investigate the relationship between the insulin adsorption behavior and the surface charge property of the amphoteric charged membranes for drug delivery.

EXPERIMENTAL

Materials

Poly(acrylonitrile) (PAN, M_w 410,000 Da) and poly(vinyl-pyrrolidone) (PVP, M_w 40,000 Da) were provided by Mitsubishi Rayon, Japan and Wako Pure Chemical Industries, Japan, respectively. Recombinant human insulin ($M_w \sim$ 6000 Da, isoelectric point (IEP) $\sim$ 5.4) was obtained from Wako Pure Chemical Industries, Japan. *N*,*N*-dimethylform-amide (DMF), Acrylic acid (AAc, pK_a = 4.26), *N*,*N*-(dimethyl-amino)propyl acrylamide (DMAPAA, pK_a = 10.35), ferrous sulfate, hydrogen peroxide, sodium bisulfite, ammonium persulfate (APS), *N*,*N*,*N*′,*N*′-tetramethyl-ethylenediamine (TMEDA), glycerol were purchased from Wako Pure Chemical Industries, Japan. These reagents were of extra-pure grade and were used without further purification.

Membrane Preparation

Porous PAN membranes were prepared by phase inversion method, and then modified by grafting with AAc and/or DMAPAA as described in our previous papers.[13,17] Single-charged membranes were prepared by graft polymerization with the initiation of redox systems—Fe^{2+}-H_2O_2 for AAc and $NaHSO_3$-$(NH_4)_2S_2O_8$ for DMAPAA. The amphoteric charged membranes were prepared by a radical reaction with APS as initiator and TMAEDA as additive. After grafting, the membranes were washed sufficiently with 1 mM HCl and 0.1 mM NaOH $\sim$ NaOH, respectively. The degree of graft polymerization (DG) was estimated using eq 1.

$$DG = \frac{W_2 - W_1}{W_1} \quad (1)$$

Department of Organic and Polymeric Materials, Tokyo Institute of Technology, 2-12-1 Ookayama, Meguro-ku, Tokyo 152-8552, Japan
*To whom correspondence should be addressed (Tel: +81-3-5734-2426, Fax: +81-3-5734-2876, E-mail: tanioka.a.aa@m.titech.ac.jp).

Table I. Physicochemical properties of the prepared membranes

Sample membrane	Monomer feed ratio[a]	DG (wt %)	Contact angle (°)[b]	Hydraulic permeability (mL/m^2·h·cm H_2O)	Pore size[d] (nm)	Zeta potential (mV)[e] pH 3.3	pH 7.4
AAc-grafted	100:0	0.53	52	695	12.7	−4.4	−6.1
Amphoteric-1	80:20	0.63	53	884	13.5	0.3	−5.1
Amphoteric-2	55:45	0.90	52	758	13.0	4.8	−0.8
Amphoteric-3	30:70	1.16	57	616	12.3	7.2	1.7
DMAPAA-grafted	0:100	0.97	59	584	12.1	8.0	4.4
Unmodified PAN	—	—	56	1895	16.3[c]	−1.8	−5.1

[a]Molar ratio of AAc to DMAPAA. [b]Measured with deionized water at pH 5.6 with the dynamic sessile drop method. [c]Measured by the capillary condensation method. [d]Estimated according to the Hagen-Poisseuille equation. [e]Obtained from zeta potential measurement.

where W_1 and W_2 are the weights of the dried membranes before and after graft polymerization, respectively.

Characterization of the Prepared Membranes

Hydraulic Permeability Measurement. The hydraulic permeability of the membranes was calculated from the flow rate of deionized water at 25 °C, with a constant pressure difference 0.1 MPa across the membrane. The membrane area exposed to the flow was 3.8 cm^2. Pore sizes of the membranes can be estimated from the hydraulic permeability according to the Hagen-Poiseuille equation.[5,15] In our case, by assuming that the pore number and thickness of the membrane did not change after grafting, the pore sizes of the grafted membranes were estimated by eq 2.

$$\frac{J}{J_0} = \left(\frac{r}{r_0}\right)^4 \tag{2}$$

where J_0 and r_0 are the hydraulic permeability and pore size of the unmodified PAN membrane, respectively. r_0 was measured by the capillary condensation method as 16.3 nm.

Water Contact Angle Measurement. The contact angles of the water droplets were measured by the sessile drop method using a contact angle measurement system (DropMaster 500, Kyowa Interface Science, Japan). The weight of the water droplets for the measurements was 2 mg. All measurements were carried out at five or more different points for each sample at 25 ± 1 °C and were reproducible within 3°.

Zeta Potential Measurement. The streaming potential measurement was done using an electrokinetic analyzer (SurPASS, Anton Paar GmbH, Austria) equipped with a Clamping Cell.[18] A pair of the prepared membranes having an area of 25 × 55 mm^2 were placed in the measuring cell. The membranes were separated by a spacer that forms a streaming channel. The streaming potential was detected by the Ag/AgCl electrodes. A background electrolyte of 1 mM KCl solution was used and the pH was adjusted with 0.1 M HCl and 0.1 M NaOH. The zeta potential was obtained from the streaming potential using eq 3.

$$\zeta = \frac{dU_{str}}{dp}\frac{\eta\kappa}{\varepsilon_0\varepsilon_r} \tag{3}$$

where U_{str} is the streaming potential, p is the pressure drop across the streaming channel, ε_0 is the vacuum permittivity (8.854×10^{-12} J^{-1} C^2 m^{-1}), ε_r is the dielectric constant of the solution (78.3), η is the solution viscosity (0.8902 mPas), and κ is the electrical conductivity of the bulk solution.

Adsorption Measurement. The freeze-dried test membrane was first immersed in a buffer solution for one day and then soaked in 10 mL of 0.22 mg/mL insulin solution. The UV absorption intensity of the insulin solution was measured by a UV-Vis spectrophotometer (U-2000, Hitachi, Japan) at 276 nm. The concentration of insulin in the solution was determined by the calibration curves. The adsorbed amount of insulin was estimated from the decrease of insulin amount in the solution. The adsorbed insulin onto the vessel was deduced as a blank. All adsorption experiments were carried out at 37 °C.

RESULTS AND DISCUSSION

Physicochemical Properties of the Prepared Membranes

Physicochemical properties of the prepared membranes were summarized in Table I. After grafting, hydraulic permeability of the membranes decreased, and the grafted membranes have smaller pore sizes than the unmodified PAN membrane. This suggests that the functional monomers were grafted onto the membrane pore surface. However, all of the grafted membranes have a similar water contact angle with the unmodified PAN membrane. As functional monomers, both AAc and DMAPAA have hydrophilic charge groups, and there are reports that the water contact angle of the membrane decreased with degree of grafting until a constant value.[19–22] Therefore, the similar water contact angle before and after modification is possibly due to the relatively low degree of grafting. Another possible reason in our case is that the water contact angle of the unmodified PAN membrane is in the same range as that of the reported AAc and DMAPAA grafted membranes, so the effect of graft polymerization on the water contact angle of the membranes is not remarkable.

The zeta potential of the membranes grafted with AAc and/or DMAPAA varied with pH. The AAc-grafted membrane that had functional anionic groups showed negative charge at both pH 3.3 and 7.4, and the zeta potential was more negative at higher pH due to the dissociation of the carboxylic groups. In the case of the DMAPAA-grafted membrane that had functional cationic charge groups, the zeta potential was always positive, and larger values appeared at lower pH due to the

existence of amine groups. For the amphoteric membranes, their zeta potential depended on the ratio of AAc to DMAPAA, and it can be seen that the zeta potential increased with the DMAPAA ratio in the grafting feed solution. This demonstrates that the surface charge of the amphoteric charged membranes can be designed by controlling the ratio of AAc to DMAPAA. Here we should note that besides the ratio of AAc to DMAPAA, the conformation of the grafted polyelectrolyte chains and the degree of graft can also influence the zeta potential of the membranes.[13,15] Additionally, the zeta potential of the unmodified PAN membrane was also measured and it turned to be negative at both pHs, although this membrane has no charge groups. Similar phenomena have been found, and it is ascribed to the specific adsorption of electrolyte anions onto the membrane surface.[13,23]

Equilibrium Adsorbed Amount

The adsorption measurements were conducted at different pHs. Since the IEP of insulin is about 5.4, two kinds of pH conditions, pH 3.3 (pH lower than the IEP, 10 mM acetic acid buffer) and pH 7.4 (pH higher than the IEP and the physiological pH, 10 mM Tris-HCl buffer), were used (We cannot prepare insulin solution near pH 5.4 due to the aggregation of insulin). The reported zeta potential values of insulin at these two pHs are about 33 mV and −65 mV, respectively.[24] Figure 1 shows the effect of pH on the insulin equilibrium adsorbed amount onto the prepared membranes.

At pH 3.3 (Figure 1(a)), the AAc-grafted membrane was negatively charged and the other grafted membranes and insulin were positively charged. Insulin adsorbed on the oppositely (negatively) charged AAc-grafted membrane and the Amphoteric-1 membrane with almost neutral charge. At pH 7.4 (Figure 1(b)), the physiological pH condition, on the other hand, the DMAPAA-grafted membrane and the Amphoteric-3 membrane were positively charged and the other grafted membranes and insulin were negatively charged. Adsorbed amount of insulin on the grafted membranes decreased with the zeta potential value from +4.4 to −6.1 mV. These results show that the electrostatic effect (attractive or repulsive) is an important factor for insulin adsorption, and higher equilibrium adsorption amount appeared when insulin and the membrane carried opposite charge.

Besides electrostatic interaction, other factors like the hydrophobic interaction between the membrane and insulin may also contribute to the insulin adsorption.[8,25] For the unmodified PAN membrane, because it had no charge groups, its insulin adsorption amount was less dependent on pH, and the adsorption of insulin was mainly due to the hydrophobic interaction. Also, for the membranes with almost neutral charge (*i.e.*, Amphoteric-1 membrane at pH 3.3 and the Amphoteric-2 membrane at pH 7.4), the insulin adsorption could be ascribed to the contribution of hydrophobic interaction. In addition, the insulin adsorption on the AAc-grafted membrane at pH 7.4 seems to contradict the electrostatic interaction; even though both the insulin and the AAc-grafted membrane were negatively charged at pH 7.4, there was still

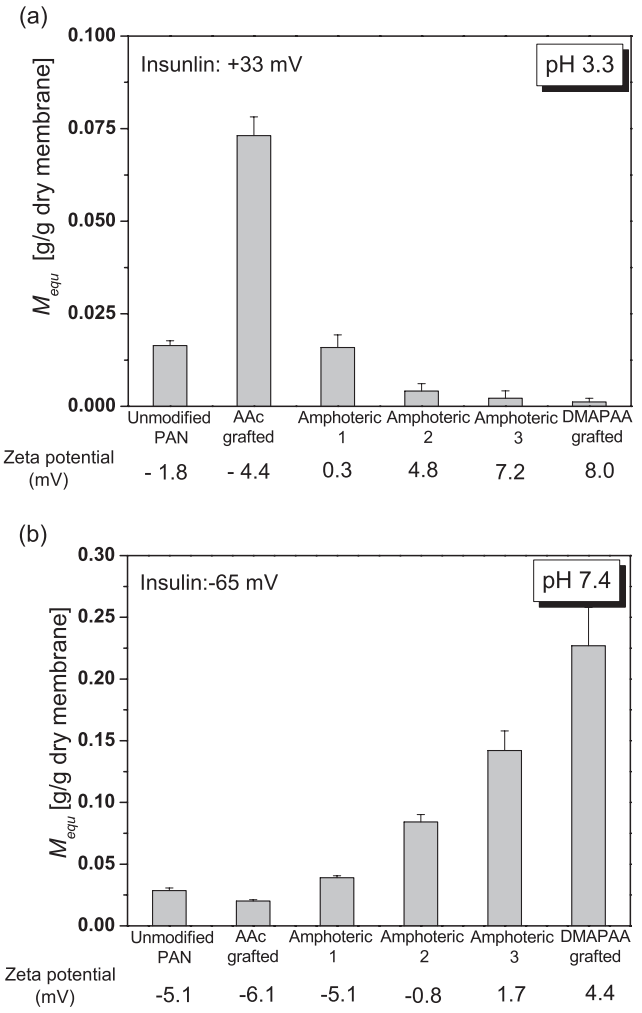

Figure 1. Insulin equilibrium adsorbed amount on the prepared membranes in (a) 10 mM acetate buffer (pH 3.3) and (b) 10 mM Tris-HCl buffer (pH 7.4).

some adsorption of insulin on the membrane. This may be due to the inhomogeniety of the grafted layer on the membrane, and the hydrophobic interaction may also play a role to some degree. Here we should note that the association state of insulin, which can be affected by buffer and pH,[26–29] also, might influence the insulin adsorption.[6]

Adsorption Kinetics

All the adsorption measurements showed similar time dependence; the adsorbed amount increased with time until reached the equilibrium. According to the equation for membrane sorption,[30] insulin adsorption was analyzed by using eq 4.

$$\frac{M_t}{M_\infty} = 4\left(\frac{Dt}{\pi l^2}\right)^{\frac{1}{2}} \tag{4}$$

where M_t/M_∞ is the fraction of insulin adsorption, D is the diffusion coefficient, t is time and l is the membrane thickness. Figure 2 presents the kinetics of insulin adsorption plotted in coordinates of the sorption equation. At pH 3.3, only curves

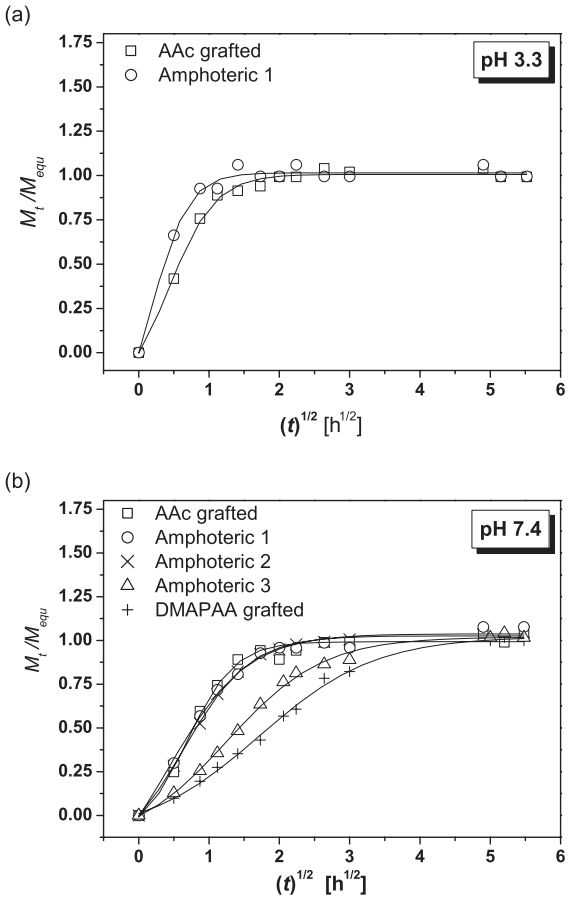

Figure 2. Insulin adsorption kinetics (a) at pH 3.3 (10 mM acetate buffer) and (b) at pH 7.4 (10 mM Tris-HCl buffer).

of AAc-grafted and Amphoteric-1 membranes are shown (Figure 2(a)), because insulin adsorbed amounts on Amphoteric-2, Amphoteric-3 and DMAPAA-grafted membranes were very low at this pH (as shown in Figure 1(a)), and the adsorption reached the equilibrium in a very short time, so that it was difficult to get a relationship between the adsorption fraction and time.

In the cases of AAc-grafted and Amphoteric-1 membranes at both pHs and Ampohteric-2 at pH 7.4, insulin adsorption kinetics belongs to Fickian diffusion: the fractional adsorption M_t/M_∞ was proportional to the square root of time at the early adsorption stage. However, the curves of Amphoteric-3 and DMAPAA-grafted membranes at pH 7.4 (Figure 2(b)) were sigmoid in shape, indicating an anomalous behavior. Under such conditions, the adsorption behavior seems to need more parameters to describe besides diffusion coefficient. One possible reason for the non-Fickian behavior is the reaction between insulin and the charge groups of the grafted polyelectrolyte chains when insulin and the membrane carried opposite charge. Such reaction changed the structure of the interface between the membrane and solution[31] or the charge properties of the membranes, which makes the interfacial diffusion of insulin from the solution to the membrane become important in the insulin adsorption. As a consequence, insulin adsorption behavior changed with the adsorbed amount. Moreover, it seems that this effect is more remarkable when the adsorbed amount increased.

CONCLUSIONS

We focus on the amphoteric charged membranes as a material for insulin delivery system. A series of amphoteric charged membranes were prepared by grafting AAc and/or DMAPAA onto porous PAN membranes. By controlling the ratio of AAc to DMAPAA, the surface charge property of the amphoteric membranes can be designed. The adsorbed amount of insulin correlated well with the charge properties of the membranes and insulin. This result showed that the contribution of electrostatic interaction was dominant for insulin adsorbed amount. In addition, it revealed that the adsorption kinetics showed non-Fickian behavior when the insulin adsorbed amount increased to very high values. These results provided the fundamental information for amphoteric charged membrane-based controlled release of insulin. Further studies on transport of insulin through the amphoteric charged membranes are now in progress and will be published soon.

Acknowledgment. We express our great appreciation to Mr. Jun Okumura (Mitsubishi Rayon Co., Ltd., Japan) for kindly providing poly(acrylonitrile) powder.

Received: April 15, 2008
Accepted: May 23, 2008
Published: July 16, 2008

REFERENCES

1. S. Tokumoto, N. Higo, and K. Sugibayashi, *Int. J. Pharm.*, **326**, 13 (2006).
2. A. C. Sintov and U. Wormser, *J. Controlled Release*, **118**, 185 (2007).
3. O. Pillai, S. D. Borkute, N. Sivaprasad, and R. Panchagnula, *Int. J. Pharm.*, **254**, 271 (2003).
4. Y. N. Kalia, A. Naik, J. Garrison, and R. H. Guy, *Adv. Drug Delivery Rev.*, **56**, 619 (2004).
5. L. Chu, Y. Li, J. Zhu, H. Wang, and Y. Liang, *J. Controlled Release*, **97**, 43 (2004).
6. S. H. Mollmann, J. T. Bukrinsky, S. Frokjaer, and U. Elofsson, *J. Colloid Interface Sci.*, **286**, 28 (2005).
7. A. Nakajima and Y. Hata, *Polym. J.*, **19**, 493 (1987).
8. H. Matsumoto, Y. Koyama, and A. Tanioka, *J. Colloid Interface Sci.*, **264**, 82 (2003).
9. S. H. Mollmann, L. Jorgensen, J. T. Bukrinsky, U. Elofsson, W. Norde, and S. Frokjaer, *Eur. J. Pharm. Sci.*, **27**, 194 (2006).
10. T. Serizawa, K. Yamashita, and M. Akashi, *Polym. J.*, **38**, 503 (2006).
11. T. Jimbo, P. Ramirez, A. Tanioka, S. Mafe, and N. Minoura, *J. Colloid Interface Sci.*, **225**, 447 (2000).
12. P. M. Biesheuvel, P. Stroeve, and P. A. Barneveld, *J. Phys. Chem. B*, **108**, 17660 (2004).
13. T. Jimbo, A. Tanioka, and N. Minoura, *Langmuir*, **14**, 7112 (1998).
14. T. Xu, *J. Membr. Sci.*, **263**, 1 (2005).
15. H. Matsumoto, Y. Koyama, and A. Tanioka, *Langmuir*, **17**, 3375 (2001).
16. P. Ramírez, A. Alcaraz, and S. Mafé, *J. Electroanal. Chem.*, **436**, 119 (1997).
17. T. Jimbo, M. Higa, N. Minoura, and A. Tanioka, *Macromolecules*, **31**,

1277 (1998).
18. T. Luxbacher, *Desalination*, **199**, 376 (2006).
19. J. H. Lee, G. Khang, J. W. Lee, and H. B. Lee, *J. Biomed. Mater. Res.*, **40**, 180 (1998).
20. S. Lee, G. Hsiue, P. Chang, and C. Kao, *Biomaterials*, **17**, 1599 (1996).
21. Y. Wada, H. Mitomo, K. Kasuya, N. Nagasawa, N. Seko, A. Katakai, and M. Tamada, *J. Appl. Polym. Sci.*, **101**, 3856 (2006).
22. C. Wang and J. Chen, *Appl. Surf. Sci.*, **253**, 4599 (2007).
23. E. Uchida, Y. Uyama, and Y. Ikada, *Langmuir*, **10**, 1193 (1994).
24. K. Waizumi and S. Nawata, *Journal of the Japanese Association for Crystal Growth*, **33**, 148 (2006).
25. S. Koutsopoulos, K. Patzsch, W. Bosker, and W. Norde, *Langmuir*, **23**, 2000 (2007).
26. T. Nylander, in "Biopolymers at Interfaces," 2nd ed., M. Malsten Ed. Marcel Dekker, New York, 2003, Chapter 10.
27. Y. Pocker and S. B. Biswas, *Biochemistry*, **20**, 4354 (1981).
28. V. Sluzky, J. A. Tamada, A. M. Klibanov, and R. Langer, *Proc. Nat. Acad. Sci. U.S.A.*, **88**, 9377 (1991).
29. A. Ahmad, V. N. Uversky, D. Hong, and A. L. Fink, *J. Biol. Chem.*, **280**, 42669 (2005).
30. J. Crank and G. S. Park, in "Diffusion in Polymers," Academic Press, London and New York, 1968, Chapter 1.
31. V. A. Kabanov, V. B. Kkobeleva, V. B. Rogacheva, and A. B. Zezin, *J. Phys. Chem. B*, **108**, 1485 (2004).