ORIGINAL PAPER

Polyaniline–DNA microsphere formation by simple electropolymerization

Jun Yano · Takeaki Kohno · Akira Kitani

Abstract Polyaniline (PANI) microspheres were prepared by electrochemical polymerization. To obtain PANI having novel micro- and nanostructures, by the potential scan technique, aniline was electropolymerized in the presence of DNA using four polymerizing solutions containing different acids: H_2SO_4, $C_6H_5SO_3H$, $HClO_4$, and CF_3COOH. The growth rate of the PANI film on the electrode surface decreased by the presence of DNA, suggesting that DNA interacted with the growing PANI molecules during the electropolymerization. The growth rate also depended on the type of acid, i.e., the anion, in the polymerizing solution and was in the order of $SO_4^{2-} > C_6H_5SO_3^- > ClO_4^- > CF_3COO^-$, which significantly coincided with the reverse order of the Hofmeister series representing the lyophilicity of the anion. When aniline was electropolymerized in the CF_3COOH polymerizing solution containing DNA, PANI microspheres were obtained without any templates. This PANI showed a sufficient redox activity in the less acidic solution in which the ordinary PANI has a slight redox activity. On the other hand, the electronic state of the PANI differed from the ordinary ones; a new absorption band was evident at 620 nm. The difference in the redox activity and electronic state suggested that the DNA molecules were incorporated in the PANI and electronically interacted with the PANI molecules.

J. Yano (✉) · T. Kohno · A. Kitani
Department of Engineering Science,
Niihama National College of Technology,
Yagumocho 7-1,
Niihama, Ehime 792-8580, Japan
e-mail: yano@sci.niihama-nct.ac.jp

Introduction

Polyaniline (PANI) is expected to be one of the best conductive polymers for practical use because of not only its easy preparation and excellent environmental stability, but also its electrical, optical, and photoelectrochemical properties [1] based on the oxidized state, reversible anion doping/dedoping, and acid/base equilibriums [2–4]. Some of these properties have already been utilized in lightweight secondary batteries, electrochromic displays, electromagnetic shielding devices, anticorrosion coatings, etc., while others have also attempted to examine it in order to make them practicable [2–4].

The morphology control of PANI, in particular, the control of micro- and nanostructures, would be crucial to utilize new and favorable functions from PANI as well as to improve its useful properties. For example, the nanofibrillar morphology improves the performance of PANI in most conventional applications involving polymer interactions with its environment [5]. This leads to much faster and more responsive chemical sensors, new inorganic PANI nanocomposites, and ultrafast nonvolatile memory devices [5]. Various micro- and nanostructures (rods, wires, fibers, and tubes) have been obtained using chemical and physical methods. Compared with the physical methods such as electrospinning [6, 7] and mechanical stretching [8], the chemical methods are attractive because they may enable the molecular design of PANI involving molecule structures, molecular weight, molecule rearrangements, intra- and intermolecule linkings, etc. The chemical methods have involved templated syntheses, which have been carried out both electrochemically and chemically by polymerizing aniline with the aid of various templates [9–15]. In spite of the variety of current templated syntheses, there is a need for practical synthetic methods capable of preparing pure,

uniform, and template-free PANI micro- and nanostructures in bulk.

One of the most attractive templates is deoxyribonucleic acids (DNAs) because of their helical molecular structures with negatively charged sites, and several successful examples have been reported [16–21]. In the present study, however, we attempted to employ DNA not as a template, but as an incorporating component with the PANI molecules. It can be expected that, during the oxidative polymerization of aniline, the cationic monomers and oligomers interact with the negatively charged DNA molecules, and a structurally controlled PANI is obtained. The electrochemical polymerization of aniline in the presence of DNA was performed as a preliminary step to synthesize a structurally controlled PANI/DNA composite material. The obtained PANI showed several novel properties vs. the ordinary PANI. The PANI was composed of microspheres having a redox activity even in a less acidic aqueous solution and a different absorption band in the visible wavelength range. The PANI microspheres were obtained by a simple electropolymerization.

Experimental details

Chemicals

Water was purified by an Auto-Still WD-22 system (Yamato) and the aqueous solutions were prepared using the purified water. Reagent grade aniline (Sigma-Aldrich) was distilled under reduced pressure prior to use. Deoxyribonucleic acid (DNA) was purchased from Tokyo Chemical Industry. All other chemicals were of analytical grade and were used as received. The aqueous solutions were prepared using distilled and deionized water. The polymerizing solution for PANI was an aqueous solution containing 0.5 M (1 M=1 mol dm^{-3}) acid and 50 mM aniline. The volume of the polymerizing solution was 50 ml.

Electrochemical measurements

The standard three-electrode cell was composed of a working electrode, a commercial Ag/AgCl electrode, and a Pt plate counter electrode with an electrode area of about 8 cm^2. The Ag/AgCl electrode was connected to the cell by means of a salt bridge filled with a saturated KCl aqueous solution. The solution level in the Ag/AgCl compartment was lower than the level in the working cell, so that contamination by chloride ions was prevented. Three working electrodes were used for each purpose: platinum wire electrodes with an electrode area of 0.296 cm^2 for the cyclic voltammogram (CV) and chronoamperogram measurements; platinum plate electrodes for the morphology observations of the obtained PANI films; and optically

transparent electrodes (ITO) for the measurements of the absorption spectra of the PANI films. Prior to use, the platinum wire and plate electrodes were treated with aqua regia for 30 s and then polarized by repeated potential cycling between −0.2 and 1.2 V vs. Ag/AgCl in 0.1 M H$_2$SO$_4$ until the voltammogram showed features associated with hydrogen adsorption/desorption and oxide formation/removal [22].

The electrochemical experiments were carried out using a Hokuto Denko HSV-100 potentiostat connected to a personal computer.

It is known that DNA possesses a reduced stability at a low pH. In order to reduce this risk, the electropolymerization was carried out immediately after the preparation of the polymerizing solutions.

Morphology observations

The morphology of the obtained PANI was measured using a JEOL JSM-6320F field-emission scanning electron microscope (SEM). The PANI samples were dried under vacuum for 24 h at room temperature after adequate rinsing with water. A 5-nm Pt layer was sputtered on the samples prior to the SEM measurements.

Results and discussion

### Electrochemical behavior during the electropolymerization

It is well known that PANI is easily prepared on an electrode surface by electropolymerizing aniline in any acidic solution. Figure 1 shows typical CVs measured during the oxidative electropolymerization of aniline. In the first and second positive potential scans, the anodic current at about 0.97 V was assigned to the oxidation of aniline to its cation radical. The two-redox current peaks are due to the redox reactions of the PANI film growing on the electrode surface. The yield of the growing PANI film is reflected in the increased current peaks and the first anodic peak (i_{pa}) at about 0.2 V was taken as the criterion of the growth rate of the PANI film. As can readily be seen in the inset of Fig. 1, i_{pa} increases with the potential scan (N). The increase was suppressed by the presence of DNA in the polymerizing solutions (Fig. 2), suggesting that DNA interacts with the growing PANI molecules during the polymerization.

It is widely accepted that the growth rate is apparently dependent on the type of acid, i.e., anion, in the polymerizing solution, although the reason for this has not yet been elucidated [22–25]. The growth rate is almost of the same order as H$_2$SO$_4$ ≫ HCl ≈ HNO$_3$ ≫ HClO$_4$ > HBF$_4$ ≈ CF$_3$COOH as determined by Zotti et al. [23], H$_2$SO$_4$ ≫
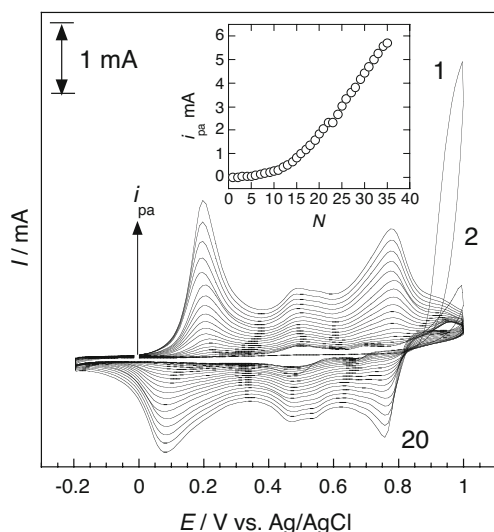
Fig. 1 Cyclic voltammograms at 100 mV s^{-1} during the electro-polymerization of aniline in 0.5 M H_2SO_4 solution containing 0.5 M aniline. (*Inset*) Dependence of the first anodic current (i_{pa}) on the repeated potential scan number (N)

HCl $\approx$ HNO$_3$ $\gg$ HClO$_4$ by Nunziante and Pistoia [24] and Duic et al. [25] and H_2SO_4 $\gg$ HCl>HNO$_3$ $\gg$ HClO$_4$ $\approx$ CF$_3$COOH by Desilvestro and Scheifele [26]. To confirm whether the suppression of the growth rate by DNA is seen regardless of the type of anion, the i_{pa}–N relationship was examined using four polymerizing solutions containing different acids: H_2SO_4, $C_6H_5SO_3H$, HClO$_4$, and CF$_3$COOH (Fig. 3). The suppression is evident in all cases and the suppression effect becomes much smaller as the growth rate is lowered. According to our previous paper [27], the growth rate dependence on the type of anion is strongly related to the stability of the complexes formed between the anions and the cationic species of the monomer through the polymer
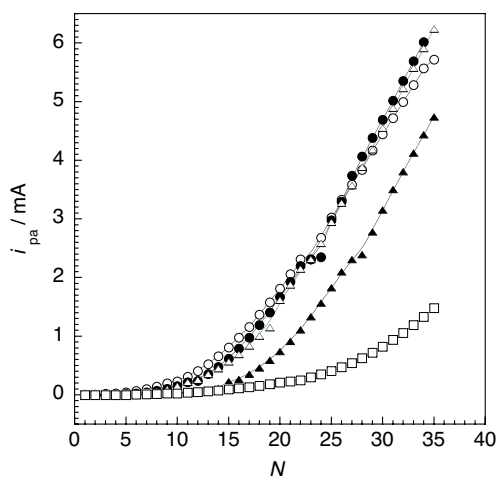
Fig. 2 Effect of the presence of DNA in the H_2SO_4 polymerizing solution on i_{pa}. Added amount of DNA: 0 (*empty circle*), 0.1 (*filled circle*), 1.0 (*empty triangle*), 3.0 (*filled triangle*), and 5.0 g/l (*empty square*)
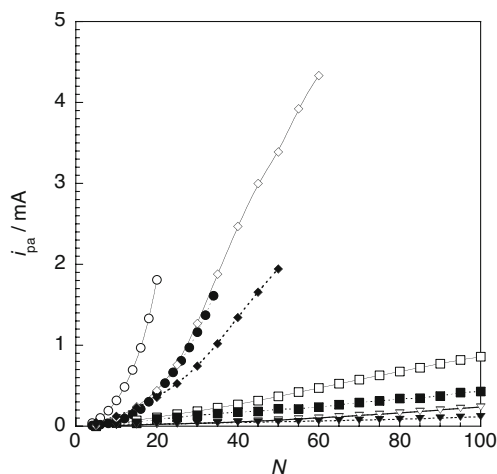

Fig. 3 Effect of the type of acid in the polymerizing solution on i_{pa}. The acids used for the polymerizing solution were the following four acids: H_2SO_4 (*empty circle*, *filled circle*), HClO$_4$ (*empty square*, *filled square*), $C_6H_5SO_3H$ (*empty diamond*, *filled diamond*) and CF$_3$COOH (*empty triangle*, *filled triangle*). To confirm the effect of the presence of DNA, 16.0 g/l of DNA was added to the corresponding polymerizing solution: H_2SO_4 (*filled circle*), HClO$_4$ (*filled square*), $C_6H_5SO_3H$ (*filled diamond*) and CF$_3$COOH (*filled triangle*)

generated during the oxidative electropolymerization. The polymerization requires that these complexes temporally decompose to release the anions. This implies that the lower the stability of the complexes, the higher the growth rate. The order for the stability is in accordance with the Hofmeister series that represents the lyophilicity of the anion: CF$_3$COO$^-$ > ClO$_4^-$ > $C_6H_5SO_3^-$ > SO$_4^{2-}$.

Formation of PANI microspheres

As previously described, the polymerization rate of PANI strongly depends on the type of the anion in the polymerizing solution. The type of the anion also varies the morphology of PANI although the reason for this is not known. For example, some reports described that fibril structures were formed in the ClO$_4^-$ or BF$_4^-$ polymerizing solution, while granular structures in the SO$_4^{2-}$ or NO$_3^-$ polymerizing solution [26, 28, 29]. Others say that the granular structures are seen for the PANI prepared from the BF$_4^-$ polymerizing solution, while the fibril structures for the ones prepared from the SO$_4^{2-}$ or NO$_3^-$ polymerizing solution [30, 31].

This morphology dependence on the type of anion suggests that there is a correlation between the morphology and the polymerization rate. Because the presence of DNA in the polymerizing solution forces the polymerization rate to decrease, it is expected to change the morphology. Several SEM images of the morphological change are shown in Figs. 4, 5, and 6. Figure 4 is the case for the H_2SO_4 polymerizing solution in which the polymerization
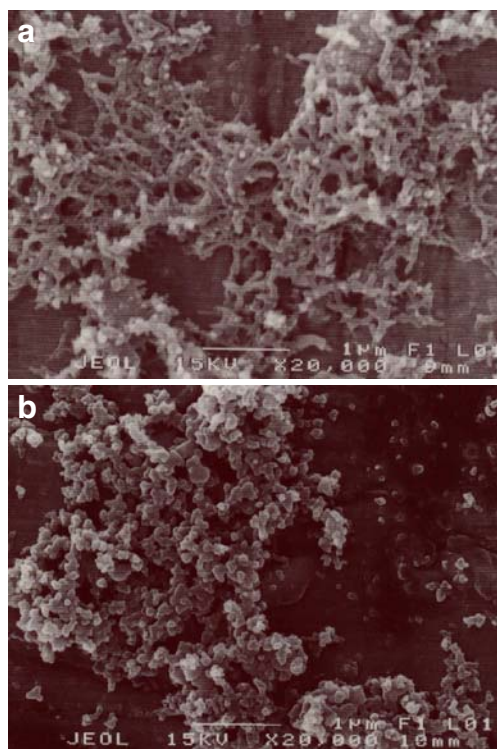
Fig. 4 SEM images of the PANI films prepared from the H$_2$SO$_4$ polymerizing solution **a** without and **b** with DNA

rate is the highest. The presence of DNA in the polymerizing solution causes only a slight morphological change from a coral-like structure (Fig. 4a) to a granular structure (Fig. 4b). Compared to this change, the presence of DNA produces a drastic morphological change in the CF$_3$COOH polymerizing solution (Fig. 5), in which the polymerization rate is the lowest. The fibril structure (Fig. 5a) is transformed into a microsphere structure (Fig. 5b,c). Microspheres with a smooth surface were first successfully obtained for the PANI electrodeposited from a CF$_3$COOH polymerizing solution containing DNA. The microspheres were also obtained from the HClO$_4$ polymerizing solution containing DNA (Fig. 6), in which the polymerization rate is almost the same as that in the CF$_3$COOH polymerizing solution. A variety of PANI morphologies have been reported for various dopant anions [27–30]. Although the formation of microspheres has also been observed, the surface was coarse [31–33]. The PANI microspheres would be important materials for practical use in various fields, for example, conductive paints, antistatic agents, and conductive fillers in transparent conductive thin films.

It can be seen that some of the microspheres partly stick together (Figs. 5b,c and 6b). These phenomena are avoidable by shortening the polymerization time, that is, the number (N) of repeated potential scans. Figure 7 shows two SEM images of the microspheres formed at N=120 and 500. In Fig. 7a, each microsphere does not stick together at

all. The sticking phenomena begin appearing when N exceeds about 400. Another practically important thing is that the diameter (d) of the microspheres can be controlled by altering N. For example, in Fig. 7, d is from 0.4 to 1.0 μm at N=120 and from 1.0 to 3.0 μm at N=500.

Probable factors of the microspheres formation

There is no doubt that the presence of DNA is crucial for the microsphere formation because no morphology except for fibril structures are observable for the PANI prepared from the CF$_3$COOH and HClO$_4$ polymerizing solutions without DNA. Although little is known about the mechanism of the microsphere formation, we now propose a suggested mechanism. The chemical polymerization of aniline proceeds by the oxidation of aniline followed by a
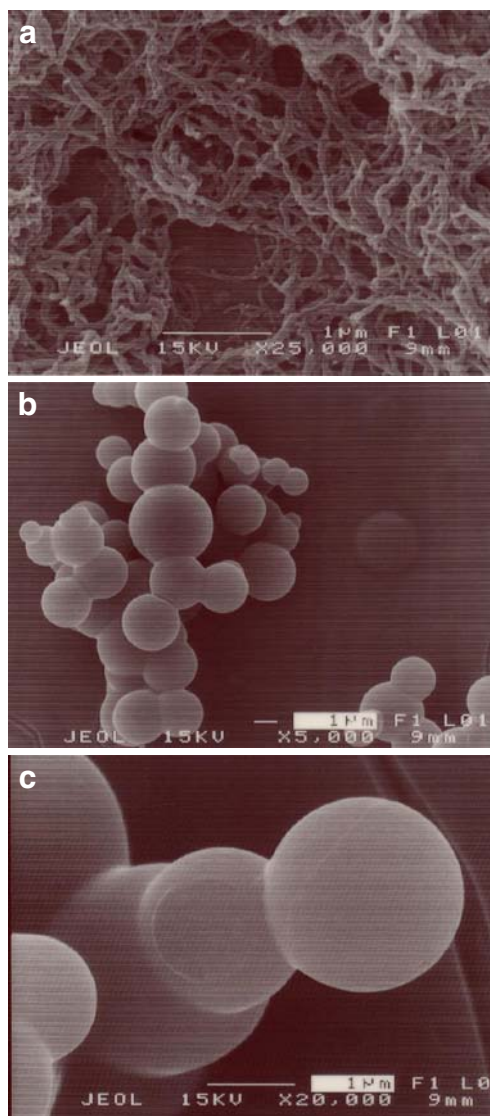
Fig. 5 SEM images of the PANI microspheres obtained from the CF$_3$COOH polymerizing solution **a** without and **b**, **c** with DNA
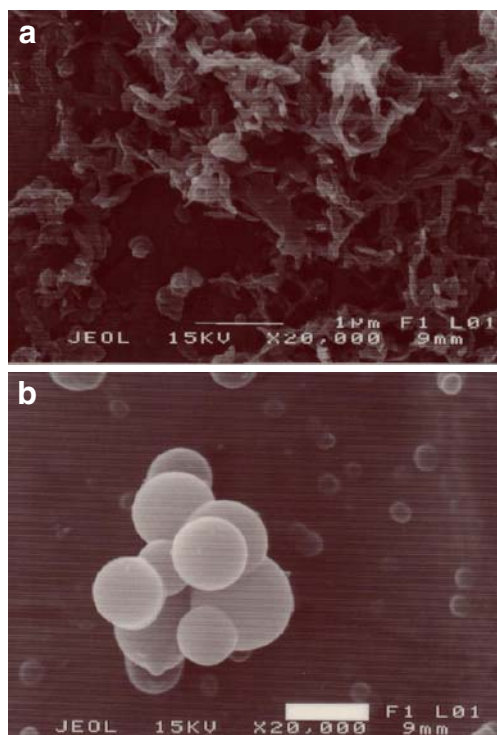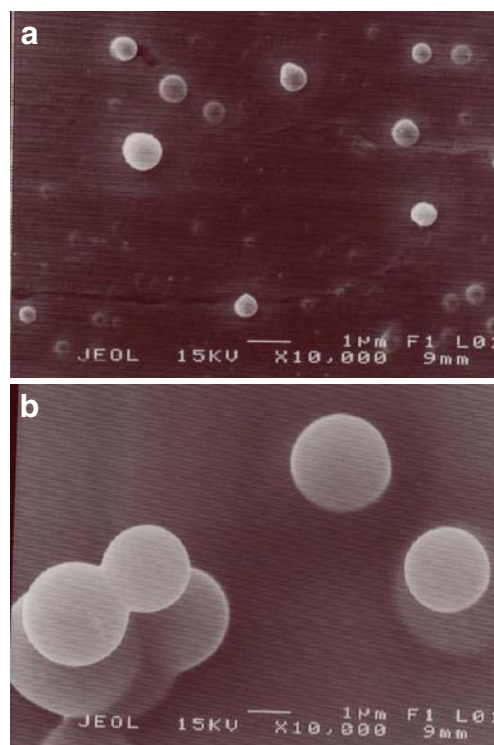
Fig. 6 SEM images of the PANI films prepared from the HClO$_4$ polymerizing solution **a** without and **b** with DNA

coupling of the resulting intermediate species with the aniline. Successive couplings of the oligomeric species occur, and a polymer chain reaches a critical length required for precipitation. In this polymerization, we believe that two factors are strongly related to the microsphere formation: (1) low solubility of the polymer–acid complexes, and (2) uniformity of the polymer chains. These factors are considered to contribute to one another. Due to the oxidative polymerization in the acidic polymerizing solution, the polymer chain possesses cationic sites at the amine or imine groups. The cationic polymer forms a polymer–acid (PANI–acid) complex. As pointed out by Tang and his co-workers [27], the PANI–acid complex has the most stable form when the anion is the most lyophilic. As a result, the PANI–CF$_3$COOH and PANI–HClO$_4$ complexes are the most difficult to dissociate, and their solubility is the lowest. For this reason, the PANI–CF$_3$COOH and PANI–HClO$_4$ complexes precipitate in the stable precipitation form. On the other hand, there is obviously a tendency that the microspheres are formed when the polymerization rate is low. This low polymerization rate enables the polymer chains to rearrange and assemble in the most favorable and stable precipitation form. Besides, the polymerization rate is low enough for the PANI molecules to effectively incorporate bulky DNA molecules. This incorporation results in uniform polymer chains as the precipitant and this uniformity significantly contributes to the microsphere formation.

Electronic interaction between the PANI molecules and DNA

The fact that the PANI microspheres incorporate DNA molecules was strongly supported by two experimental results: a new absorption band at 620 nm and redox activity in a neutral aqueous solution. The PANI film with the microspheres had a color different from the ordinary PANI films. The PANI film without DNA looked yellowish-green, while the PANI film with the microspheres was a bright dark green. This is due to the absorption spectra (Fig. 8). The ordinary PANI films show a broad absorption peak at around 800 nm when they are polarized at 0.6 V [34–36]. The same peak is seen at 0.6 V for the PANI film prepared from the CF$_3$COOH polymerizing solution without DNA (dotted line) and for the PANI film prepared from the H$_2$SO$_4$ polymerizing solution containing DNA (dashed line). Compared with these PANI films, the PANI film prepared from the CF$_3$COOH polymerizing solution containing DNA shows a broad absorption at around 620 nm, which has never been seen in the ordinary PANI film polarized at 0.6 V, showing that there is some electronic interaction between the incorporated DNA and the PANI molecules. In addition, the fact that the PANI film prepared from the H$_2$SO$_4$ polymerizing solution containing DNA has almost the same absorption as the ordinary PANI film suggests that few DNA molecules are incorporated into the

Fig. 7 SEM images of the PANI microspheres obtained from the CF$_3$COOH polymerizing solution at $N=120$ (**a**) and 400 (**b**)
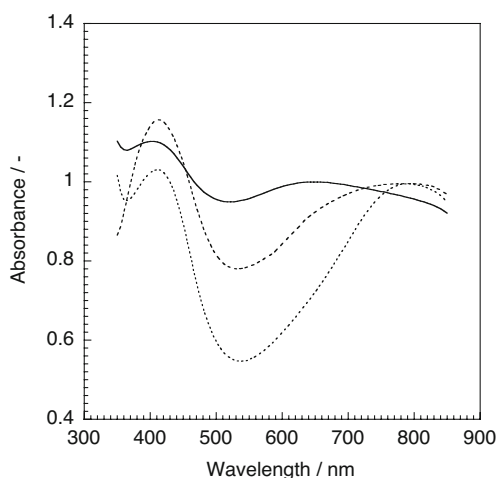
Fig. 8 Absorption spectra of the PANI film prepared from the CF$_3$COOH polymerizing solution without DNA (*dotted line*), with DNA (*solid line*) and the PANI film obtained from the H$_2$SO$_4$ polymerizing solution with DNA (*dashed line*)

PANI film. This suggests that the incorporation effectively takes place when the growth rate of PANI is adequately low. The low growth rate probably enables the PANI molecules to be rearranged to become favorable for the incorporation. In Fig. 3, the presence of DNA decreases i_{pa} most frequently in the H$_2$SO$_4$ polymerizing solution. In the case, nevertheless, the growth rate must be too high for the effective incorporation. As a result, both the PANI and DNA molecules barely stick together, and little electronic interaction occurs.

Improvement of the redox activity in the less acidic solution

The PANI film with the microspheres also had a different redox activity in the less acidic media. It is widely accepted that PANI gradually loses its redox charge as the solution pH increases. The charge suddenly drops at around pH 4 and PANI has only a slight redox charge when the pH>5. Figure 9 shows the relationship between the solution pH and the redox charge of the PANIs. Each PANI film electrode was transferred in six Clark-Lubs' buffer solutions of different pH values except for pH=0, where 1.0 M HCl was used. Then, each CV was individually measured for three sample electrodes prepared in the same manner. The charge was determined by integrating the CVs. The charge (Q) was normalized by the charge (Q_0) at pH=0. As shown in Fig. 9, the PANI film without DNA has the same tendency. Interestingly, however, the PANI film with DNA still has an adequate redox charge at pH 5, about 62% of the redox charge measured in the pH 1 solution. This adequate redox activity in the less acidic solution seems practically advantageous. This is probably due to the enrichment of H$^+$ in the PANI that originated from the

anionic sites (the phosphate part) of the incorporated DNA. This behavior in the redox activity implies that the DNA molecules are effectively incorporated into the PANI molecular chains. In addition, the PANI film prepared from the H$_2$SO$_4$ polymerizing solution containing DNA has only a slight adequate redox activity at pH=5. This also suggests that few DNA molecules are incorporated into the PANI film.

Conclusions

PANI microspheres were obtained by the simple electropolymerization of aniline. It is well known that the PANI film is obtained on the electrode surface by electropolymerizing aniline in any acid solution. The growth rate of the PANI film depended on the type of the acid, i.e., anion, in the polymerizing solution and was in the order of SO$_4^{2-}$ > C$_6$H$_5$SO$_3^-$ > ClO$_4^-$ > CF$_3$COO$^-$, which significantly coincided with the reverse order of the Hofmeister series representing the lyophilicity of the anion. The growth rate further decreased by the presence of DNA in the polymerizing solution. When aniline was electropolymerized in the CF$_3$COOH polymerizing solution containing DNA in which the growth rate was the lowest, the PANI microspheres were obtained without any templates. Altering the electropolymerization time could control the average diameter of the microspheres. This PANI had a sufficient redox activity in the less acidic solution in which ordinary PANI has a slight redox activity. The PANI microspheres are expected to be important materials for practical use in various fields.
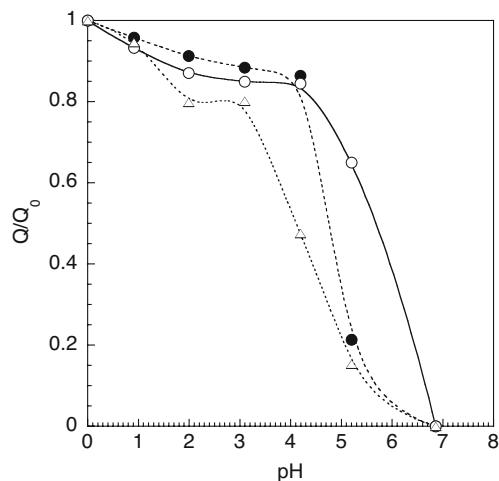
Fig. 9 Relationship between the solution pH and the redox charge (Q) of the PANI obtained from the CF$_3$COOH polymerizing solution with DNA (*empty circle*), without DNA (*filled circle*) and of the PANI obtained from the H$_2$SO$_4$ polymerizing solution with DNA (*empty square*). Q_0: the redox charge measured in an aqueous solution of pH=0

References

1. Bitao PS, Shixiong M, Yongchun T, Jie B (2007) Front Chem China 2:123, doi:10.1007/s11458-007-0025-5
2. Salaneck WR, Lundström I, Rånby B (1993) Conjugated polymers and related. Oxford Science Publications, Oxford, UK
3. Skotheim TA, Elsenbaumer RL, Reynolds JR (1998) Handbook of conducting polymers. Marcel Dekker, New York, USA
4. Chandrasekhar P (1999) Conducting polymers, fundamentals and applications. Kluwer Academic, London, UK
5. Skotheim TA, Reynolds JR (2007) Handbook of conducting polymers, 3rd edn. Marcel Dekker, New York, USA, 7:1
6. Reneker DH, Chun I (1996) Nanotechnology 7:216, doi:10.1088/0957-4484/7/3/009
7. MacDiarmid AG, Jones WE, Norris ID, Gao J, Johnson AT, Pinto NJ, Hone J, Han B, Ko FK, Okuzaki H, Llaguno M (2001) Synth Met 119:27, doi:10.1016/S0379-6779(00)00597-X
8. He HX, Li CZ, Tao NJ (2001) Appl Phys Lett 78:811, doi:10.1063/1.1335551
9. Huang LM, Wang ZB, Wang HT, Cheng XL, Mitra A, Yan YX (2002) J Mater Chem 12:388, doi:10.1039/b107499g
10. Wei ZX, Zhang ZM, Wan MX (2002) Langmuir 18:917, doi:10.1021/la0155799
11. Wang CW, Wang Z, Li MK, Li HL (2001) Chem Phys Lett 341:431, doi:10.1016/S0009-2614(01)00509-7
12. Choi SJ, Park SM (2000) Adv Mater 12:1547, doi:10.1002/1521-4095(200010)12:20<1547::AID-ADMA1547>3.0.CO;2-1
13. Martin CR (1996) Chem Mater 8:1739, doi:10.1021/cm960166s
14. Wu CG, Bein T (1994) Science 264:1757, doi:10.1126/science.264.5166.1757
15. Michaelson JC, McEvoy AJ (1994) Chem Commun 25:79
16. Ma Y, Ali SR, Dodoo AS, He H (2006) J Phys Chem B 110:16359, doi:10.1021/jp0614897
17. Shi L, Xiao Y, Willner I (2004) Electrochem Commun 6:1057, doi:10.1016/j.elecom.2004.08.004
18. Ma Y, Zhang J, Zhang G, He H (2004) J Am Chem Soc 126:7097, doi:10.1021/ja039621t
19. Sergeev VG, Lokshin NA, Golubev VB, Zezin AB, Levon K, Kabanov AVA (2003) Phys Chem 390:118
20. Shao Y, Jin Y, Dong S (2002) Electrochem Commun 4:773, doi:10.1016/S1388-2481(02)00442-3
21. Nagarajan R, Liu W, Kumar J, Tripathy SK, Bruno FF, Samuelson LA (2001) Macromolecules 34:3921, doi:10.1021/ma0021287
22. Breiter M, Böld W (1961) Electrochim Acta 5:145, doi:10.1016/0013-4686(61)87010-2
23. Zotti G, Catterin S, Comisso N (1988) J Electroanal Chem 239:387, doi:10.1016/0022-0728(88)80293-6
24. Nunziante P, Pistoia G (1989) Electrochim Acta 34:223, doi:10.1016/0013-4686(89)87089-6
25. Duic L, Mandic Z, Kovacicek F (1994) J Polym Sci Polym Chem 32:105, doi:10.1002/pola.1994.080320112
26. Desilvestro J, Scheifele W (1993) J Mater Chem 3:263, doi:10.1039/jm9930300263
27. Tang H, Kitani A, Shiotani M (1996) Electrochim Acta 41:1561, doi:10.1016/0013-4686(95)00408-4
28. Yoshikawa K, Yoshioka K, Kitani A, Sasaki K (1989) J Electroanal Chem 270:421, doi:10.1016/0022-0728(89)85055-7
29. Kitani A, Kaya M, Tsujioka S, Sasaki K (1988) J Polym Sci Part Polym Chem 26:1531, doi:10.1002/pola.1988.080260604
30. Huang W, Humphrey BD, MacDiarmid AG (1986) J Chem Soc, Faraday Trans I 82:2385, doi:10.1039/f19868202385
31. Duic L, Mandi Z (1992) J Electroanal Chem 335:207, doi:10.1016/0022-0728(92)80243-W
32. Sharma M, Kaushik D, Singh RR, Pandey RK (2006) J Mater Sci Mater Electron 17:537, doi:10.1007/s10854-006-8238-9
33. Zhou C, Han J, Song G, Guo R (2008) Eur Polym J 44:2850, doi:10.1016/j.eurpolymj.2008.01.025
34. Choi S, Park S (2002) J Electrochem Soc 149:E26, doi:10.1149/1.1432675
35. Kitani A, Yano J, Sasaki K (1986) J Electroanal Chem 209:227, doi:10.1016/0022-0728(86)80200-5
36. Kobayashi T, Yoneyama H, Tamura HK (1984) J Electroanal Chem 161:419, doi:10.1016/S0022-0728(84)80201-6