Assemblies of stimulus sensitive gel particles and composite to DNA-dye complexes

TAKASHI NISHIYAMA

Doctoral Program in Advanced Materials Science and Technology Graduate School of Science and Technology
Niigata University

CONTENTS

Chapter 1

General introduction

1.1 Functional gel in bio-medical and sensor applications

A polymer gel consists of a three dimensional polymer network and a solvent filling the internal spaces of the network. Polymer gels are wet and soft material and are possible to alter their volume. The characteristics are quite difference from most industrial materials such as metals and plastics. The structure of the polymer gel is likely to living tissue. Cell, muscle, cartilage, internal organ and many other living tissues are formed from three dimensional polymer network structures containing solvents. This morphology enables the tissue to transport ions and molecules more easily. Stimulus sensitive polymer gels change their volume in response to the external environmental stimuli, such as pH, temperature, light, electric fields, and biomolecules[1-7]. For example, a poly acrylic acid gel alters its volume in response to pH. The carboxylic acid groups of poly acrylic acid cause an electrostatic repulsion of ions along the polymer chain and an expansion of the polymer network. The ability of stimulus sensitive polymer gels to undergo swelling and shrinking as a function of their environment is one of the most remarkable properties of these materials. The characteristic of stimulus sensitive polymer gel, which can be induced by pH, temperature, light, electric fields, are expected to be developed for use in applications such as sensor, actuators and drug carriers[8-14].

However, stimulus sensitive gels are necessary to improve their some problems for developing to industrial application. First, improvement of their mechanical strength is the important challenges for industrial application. Recently, there have been reported novel polymer gels exhibiting excellent mechanical properties as compared with previous gel. Gong et al. have reported a double network (DN) gel, which is formed interpenetrating polymer networks (IPN) structure consists of robust poly (2-acrylamido-2-methylpropanesulfonic acid) (PAMPS) gel as a first network and flexible poly (acrylamide) (PAAm) as a second network[15]. The PAMPS/PAAm DN gel exhibits the fracture strength as high as a few to several tens MPa and shows high wear resistance due to their extremely low coefficient of friction. In other, Nanocomposite gel and topological gel have been reported as novel functional gel exhibiting excellent physical properties[16-19].

Second, a response time of typical stimulus sensitive polymer gels is not sufficiently fast

to make them widely industrial use. Tanaka et al. have reported the swelling kinetics is determined by the relaxation of the polymer network, which is proportional to the square of the gel size[20-21]. There have been efforts to improve the response kinetics by using sophisticated techniques that incorporate porosity into the hydrogel[22-24] or achieve either grafting of dangling chains[25-26] or hybridizing nanoparticles[27-28] within the polymer network, and there have been investigated to fabricate a novel stimulus sensitive polymer gel with a fast response time on macroscopic length scales.

1.2 Design of polymer gel particle assemblies

In nature, living things form in various shape of assembly in which the organization is optimally assembled for the expression of various functions. As an example, the honeycomb structure has excellent physical properties and is often applied to artificial materials[29-32]. Recently, assemblies of colloid polymer particles, which have excellent properties such as high responsiveness and colloidal photonic crystals, have been reported[33-35]. The concept of organization and assembly on the micro or nano-scale could provide various functions to smart materials.

Self-assembly is the autonomous organization of various components formed into various pattern or structure without intervention[36-37]. The concept of self-assembly is great attention in various fields and is used increasingly in many processes. In living things, many kinds of living tissue are formed by self-organization. Living cell, proteins and nucleic acids control precisely their complicated and higher-ordered structures by self-organization which occurs by various weak interactions such as hydrogen bond, electrostatic interaction and hydrophobic interaction. DNA has a double strand structure which is formed by complementary base pairing of adenine-thymine and guanine-cytosine of polynucleotides with hydrogen bonds. The inter layers of base pairs exhibits aromaticity and are intercalated planer aromatic compounds by $\pi-\pi$ stacking[38-46]. The weak interactions make it possible to form structures dynamically and reversibility, and the material design utilized such weak interactions can accomplish to novel function in various fields.

Hydrogels have been attracted much attention as a smart materials. The hydrogels are flexible, elastic and wet material which is consisting of three dimensional network

structures containing aqueous solution. Hydrogels can transport the solvents or matters through the three dimensional network and stimulus sensitive hydrogels are altered their volume in response to external stimuli such as pH, temperature, electric field and biomolecules.[1-7]. These structure and characteristics are similar to living tissues. Cell, muscle, cartilage, internal organ and many other living tissues are formed from three dimensional polymer network structures containing solvents. The hydrogel materials are expected to be developed for use in biomedical applications such as biosensor, drug delivery systems and artificial muscle [8-14]. However, stimulus sensitive gels are necessary to increase the response speed for applications in novel sensor devices. The relaxation time of a network of gels is proportional to the square of the diameter in equilibrium swelling[20-21].

Consequently, we investigated the organization of stimulus sensitive gel particles by inducing a crosslinking reaction between the gel particles. An assembly of stimulus sensitive gel particles forms interparticle spaces. These microspaces are expected to reduce the relaxation time and diffuse solutions through the networks easily. Each gel particle composing the assembly responds to stimuli at nearly the same time as a result of the external solution diffusing through the interparticle spaces. That is, a microscopic alteration of the gel particles quickly induces a macroscopic alteration. The assembly of stimulus sensitive gel particles could achieve a high response speed, similar to that of the gel particles. In addition, we also focused on the self-organization of stimulus sensitive gel particles by week interaction between the gel particles. An assembly of the stimulus-sensitive gel particles by week interactions could dynamically disperse and aggregate in response to external environment in the same manner as living tissues, and this bio-inspired material might be expression to novel functionalities. Furthermore, the assembly of stimulus sensitive gel particles by interactions would also possess an excellent responsiveness as compared with typical macro gels because a porous structure is fabricated by the formation of interparticle spaces. The assembly of stimulus sensitive gel particles by crosslink or interaction could be contributed to gel application in various fields.

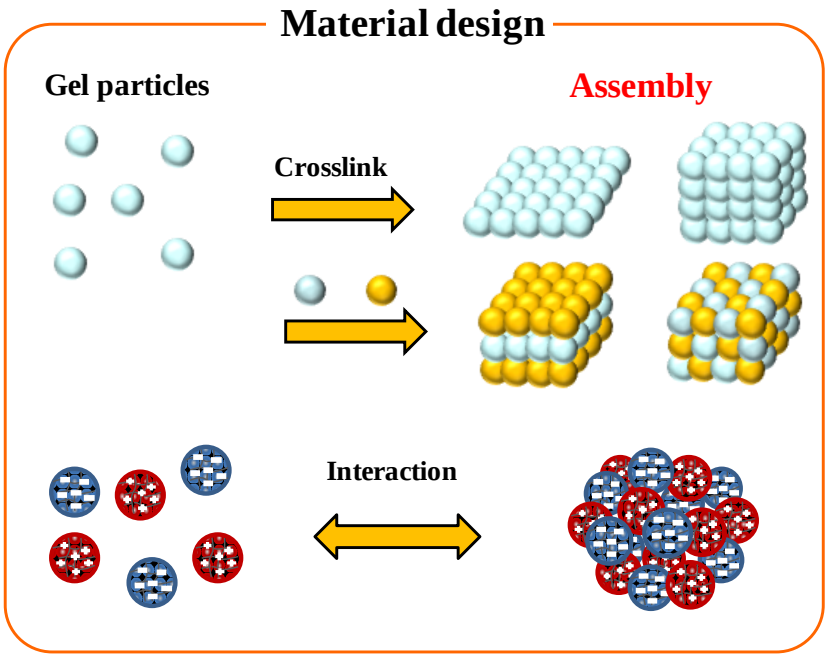

Figure 1-1 Design of assembly of stimulus sensitive polymer gel particles by crosslink and interaction between the gel particles

1.3 DNA as functional materials

DNA is well known as one of naturally occurring polymers and has a double strand structure. The double strand structure of DNA was revealed by Watson, Crick, and Wilkinson. One of the important characteristics of DNA that makes DNA both attractive and successful for designing a wide variety of structures and devices is its molecular recognition capabilities[47-56]. Molecular recognition properties of DNA have proven to be extremely useful in the design of biosensors[57-61] and diagnostic tools[62-70]. DNA-programmed assemblies[71-77] are recent development that utilize the assembly of biomolecules and may find applications in nanoscale bioanalysis and nano-materials construction.

On the other hands, Okahata et al. have reported that the conversion of water-soluble Na^+-DNA^- into organic-soluble DNA-alkyl quarternary ammonium complexes, Q^+-DNA^- [78-79]. Natural DNA is soluble in water and buffer solutions, whereas Q^+-DNA^- salts or complexes are soluble in organic solvents. Q^+-DNA^- salts of alkyl ammonium ions are

water-soluble. The DNA-quaternary ammonium complexes can be soluble and forms a double strand structure in organic solutions. A self-standing DNA films could be easily prepared by casting method, and DNA strands were easily aligned in the film by stretching it in one direction. This report sparked the most recent research on the material science of DNA. In many researches, DNA-hexadecylcetyltrimethylammonium (DNA-CTMA) is often used.

In particular, Optical properties of DNA are also attracted to many attentions in device applications. The fluorescence property of DNA intercalated with fluorescence dye is enhanced as compared with only a fluorescence dye. It has been reported that certain kinds of dye can be intercalated in double-stranded structure of DNA[80-87]. Intercalated dye is immobilized in the double strand structure of DNA, and dye is not diffused to regions external to the DNA. Intercalated dye is increased the fluorescence intensity because of inhibiting concentration quenching and vibrational deactivation[44-45]. DNA-CTMA complexes intercalated with a fluorescence dye, 4-(4-dimethylaminostyryl)-1-dodecylpyridinium bromide (DMASDPB) and the composite film achieved the excellent fluorescence intensity[44-45]. Ogata et al. observed an amplified spontaneous emission of DNA-CTMA doped dye, a lasing action without cavities, when the DNA-CTMA complex was doped with a Rhodamine 6G at 1.36 wt% to make films of 5-7 mm thickness[46]. Both the emission line narrowing and the linear dependence of emitted light intensity occur at the same excitation energy. This result indicates that the DNA-CTMA doped fluorescence dye would be applied to laser sources. Grote et al. reported that the DNA-CTMA/europium complexes films showed a strong amplification of fluorescence emission at 614 nm when irradiated with UV light[44]. The DNA-CTMA film exhibits excellent transmissivity over a range of wavelengths from 300 to 1600 nm and a refractive index ranging from 1.540 at 500 nm to 1.526 at from 630 to 1600 nm. From these results, DNA-CTMA films doped fluorescence dye may be applied in optical devices such as optical waveguides.

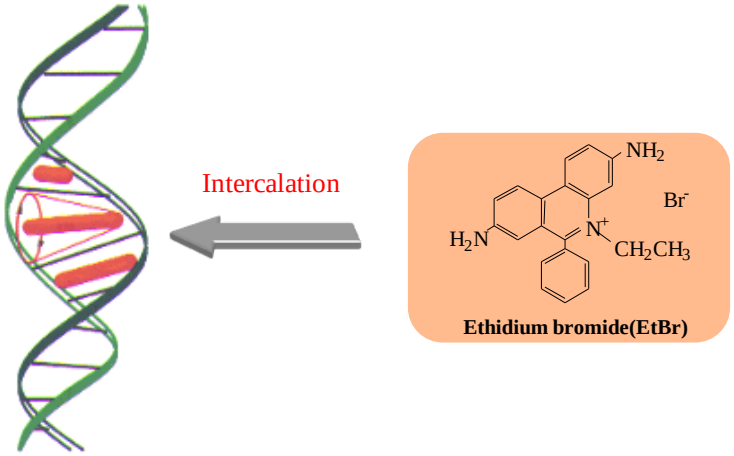

Figure 1-2 The intercalation of aromatic compounds in base-pairs of DNA

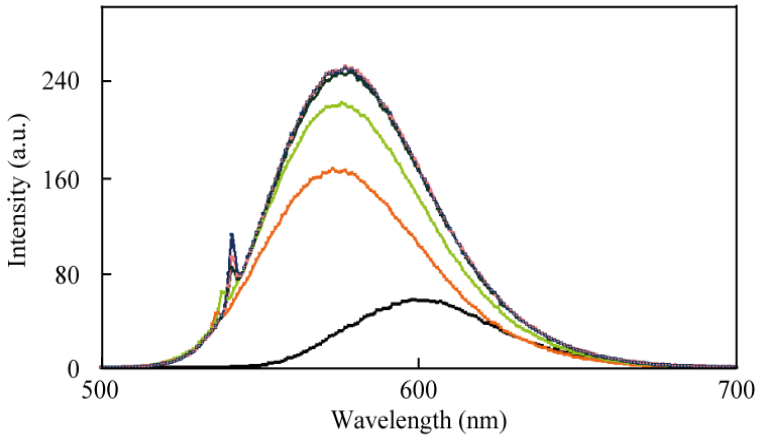

Figure 1-3 Fluorescence intensity of DNA-DBASMPI at various molar ratios

1.4 A composite material of DNA and stimulus sensitive gel

The optical properties of DNA characteristics, intercalation of fluorescence dye and its fluorescence intensity enhancement have been attracted much attention. Then, we utilized DNA as functional materials, and a composite of DNA and stimulus sensitive gel was investigated. In contrast, the DNA from salmon milt was often used and most salmon milt is wasted in Japan. The utilization of DNA from salmon milt as a functional material is important and useful in terms of environmental programs and regional contributions. Stimulus sensitive polymer gels are altered their swelling ratio in response to external environments and the stimulus sensitive gels containing dye are applied to optical sensor

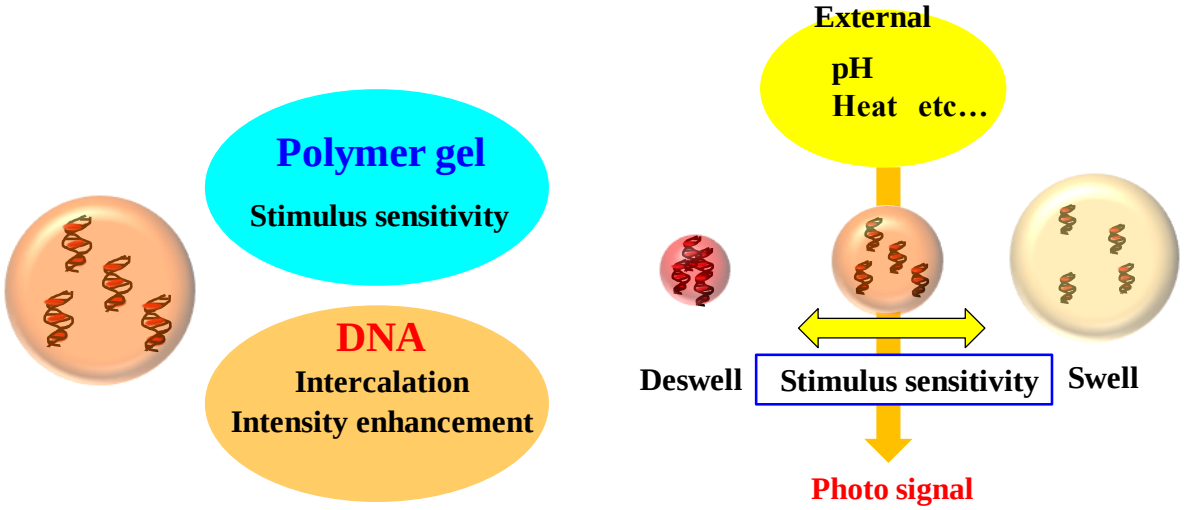

Figure 1-4 Optical sensing mechanism of composite material of stimulus-sensitive gel and DNA

devices to detect fluorescence intensity resulting from volume changes in response to environmental stimuli. However, most of dye in the gels is eluted to an external solution through the three-dimensional polymer networks. Consequently, DNA acts as a role of dye entrapment into stimulus sensitive polymer gels. The optical sensing mechanism of the composites containing stimulus sensitive gels and DNA-dye complexes is that the volume of the composite will alter in response to external stimuli. The volume alteration of the gels causes a change in the concentration of the dye because the dye is intercalated into DNA that is immobilized in the gel particles. When the concentration of the dye is varied by altering the volume of the gels, the amount of dye that is excited by laser illumination is changed. Therefore, the resulting fluorescence intensity of the gels is increased or decreased in response to external stimuli. The composite of stimulus sensitive polymer gel and DNA would be expected to application for bio sensor devices.

1.5 Objectives of this study

Yamauchi and his co-workers have investigated biomimetic and bio-inspired materials for application in sensor, artificial muscle (actuator), drug delivery system and numbers of bio-medical and bio-mechanical devices by utilizing functional polymers containing

stimulus sensitive polymer gels and conducting polymers with hybridization of organic and inorganic biomaterials, structural modification and any other methods. In nature, there are a great number of interested phenomena such as self-assembly and biomineralization. The material design utilizing these phenomena makes it possible to create a novel material having excellent functionalities as a manner of nature and to perform with a small amount of energy.

From the points of view described above, the present study has been carried out for investigating assemblies of stimulus sensitive polymer gel particles by crosslink or interaction between the gel particles which possess excellent stimulus sensitivities and dynamically changed their morphology as a manner of nature, and a composite of stimulus sensitive gel and DNA to apply as an optical-detected bio sensor by utilizing DNA-fluorescence dye complexes.

In Chapter 2, preparation of stimulus sensitive gel particles with DNA-dye complexes were discussed. An ability of the dye entrapment in the gel particles by intercalation of DNA, swelling ratio and fluorescence intensity of the gel particles in response to external stimuli were investigated.

In Chapter 3, assembly of stimulus sensitive gel particles with DNA-dye complexes by crosslink were discussed. The gel particles obtained in Chapter 2 was used. An ability of the dye entrapment in the assembly, the relationship between mechanical property and swelling ratio of the assembly and the swelling kinetics of the assembly as compared with the gel particles which consisted of the assembly and typical bulk gel which had the same volume and chemical composition as the assembly were investigated. Swelling ratio and fluorescence intensity of the assembly in response to external stimuli were also investigated.

In Chapter 4, assembly of stimulus sensitive gel particles by interaction were discussed. Anionic gel particles and cationic gel particles were prepared and their pH- and thermo-sensitivities were investigated. Morphology of the assembly by electrostatic interaction in various pH values and swelling behavior of the assembly in response to temperature were also investigated.

The conclusions are presented in Chapter 5.

References

1. Gümüşderelioğlu, M. & Topal, U. I., *Radiat. Phys. Chem.*, **73**, 272–279 (2005)

2. Osada Y. & Gong P. J., *Adv. Mater.*, **10**, 827–837(1998)

3. Miyata, T., Asami, N. & Uragami, T., *Nature*, **399**, 766–769, (1999)

4. Miyata, T., Jikihara, A., Nakamae, K. & Hoffman, A. S., *J. biomater. Sci. Polym. Edn.*, **15**, 1085–1098 (2004)

5. Miyata, T., Asami, N., and Uragami, T., *Macromolecules*, **32**, 2082–2084 (1999)

6. Dowding J. P, Vincent B., & Williams E., *J. Colloid and Interface Science*, **221**, 268–272 (2000)

7. Panayiotou M, Pohner C., Vandevyver C., Wandrey C., Hilbrig F. & Freitag R., *Reactive & Functional Polymers*, **67**, 807–819, (2007)

8. Tabata O., Hirasawa H., Aoki S., Yoshida R. & Kokufuta E., *Sensors and Actuators A*, **95**, 234–238 (2002)

9. Maeda S., Hara Y., Yoshida R. & Hashimoto S., *Advanced Robotics*, **22**, 1329–1342 (2008)

10. Akashi R., Tsutsui H. & Komura A., *Adv. Mater.*, **14**, 1808–1811 (2002)

11. Oh K. J., Drumright R., Siegwart J. D. & Matyjaszewski K., *Prog. Polym. Sci.*, **33,** 448–477 (2008)

12. Kato Y. & Tokuyama H., *Colloids and Surfaces B: Biointerfaces*, **67**, 92–98 (2008)

13. Trinh, T. Q., Gerlach, G., Sorber, J. & Arndt, K.-F., *Sens. Act. B*, **117**, 17–26 (2006)

14. Guenther, M., Gerlach, G., Corten, C., Kuckling, D., Sorber, J., Arndt, K.-F., *Sens. Act. B*, **132**, 471–476 (2008)

15. Gong J. P., Katsuyama Y., Kurokawa T. & Osada Y., *Adv. Mater.*, **15**, 1155–1158 (2003)

16. Haraguchi K. & Takehisa K., *Adv. Mater.*, **14**, 1120–1124 (2002)

17. Haraguchi K. & Li H. -J., *Angew. Chem. Int. Ed.*, **44**, 6500–6504 (2005)

18. Okumura Y. & Ito K., *Adv. Mater.*, **13**, 485–487 (2001)

19. Ito K., *Polym. J.*, **39** 488–500 (2007)

20. Tanaka T. & Filmore D. J., *J. Chem. Phys.*, **70**, 1214–1218 (1979)

21. Li Y. & Tanaka T., *J. Chem. Phys.*, **92**, 1365–1371 (1990)

22. Dong L. C. & Hoffman, A. S., *J. Controlled Release*, **13**, 12–31 (1990)

23. Zhang X. Z., Yang, Y. Y., Chung, T. S. & Ma, K. X., *Langmuir*, **17**, 6094–6099 (2001)

24. Kuang M., Wang D., Gao M., Hartmann J. & Mohwald H., *Chem. Mater.*, **17**, 656–660 (2005)

25. Yoshida R., Uchida K., Kaneko Y., Sakai K., Kikuchi A., Sakurai Y. & Okano T., *Nature*, **374**, 240–242 (1995)

26. Kaneko Y., Nakamura S., Sakai K., Aoyagi T., Kikuchi A., Sakurai Y. & Okano T., *Macromolecules*, **31**, 6099–6105 (1998)

27. van Durme K., van Mele B., Loos W. & du Prez F. E., *Polymer*, **46**, 9851–9862 (2007)

28. Petit L., Bouteiller L., Brulet A., Lafuma F. & Hourdet D., *Langmuir*, **23**, 147–158 (2007)

29. Yabu H., Matsuo Y., Ijiro K., Nishino F., Takaki T., Kuwahara M., & Shimomura M., *Appl. Mater. Inter.*, **2**, 23–27 (2010)

30. Yabu H., Hirai Y., Kojima M. & Shimomura M., *Chem. Mater.*, **21**, 1787–1789 (2009)

31. Yabu H., Jia R., Matsuo Y., Ijiro K., Yamamoto S., Nishino F., Takaki T., Kuwahara M. & Shimomura M., *Adv. Mater.*, **20**, 4200–4204 (2008)

32. Kojima M., Nakanishi T., Hirai Y., Yabu H. & Shimomura M., *Chem. Commun.*, **46**, 3970–3972 (2010)

33. Hu Z., Lu X. and Gao J., *Adv. Mater.*, **13**, 1708–1712 (2001)

34. Zhou B., Gao J. and Hu Z., *Polymer*, **48**, 2874–2881 (2007)

35. Suzuki D. & Yoshida R., *Macromolecules*, **41**, 5830–5838 (2008)

36. Whitesides G. M. & Grzybowski B., *Science*, **295**, 2418–2421 (2002)

37. Philip D. & Stoddart J. F., *Angew. Chem. Int. Ed.*, **35**, 1154–1196 (1996)

38. Waring M. J., *J. Mol. Biol.*, **13**, 269–282 (1965)

39. LePecq J. B. & Paoletti C., *J. Mol. Biol.*, **27**, 87–106 (1967)

40. Burns V. W. F., *Arch. Biochem. Biophys.*, **133**, 420–424 (1969)

41. Hudson B. & Jacobs R., *Biopolymers*, **14**, 1309–1312 (1975)

42. Waleh A., Hudson B. & Loew G., *Biopolymers*, **15**, 1637–1640 (1976)

43. Olmsted J. & Kearns D. R., *Biochemistry*, **16**, 3647–3654 (1977)

44. Grote J. G., Hagen J. A., Zetts J. S., Nelson R. L., Diggs D. E., Stone M. O., Yaney P. P.,

Heckman E., Zhang C., Steier W. H., Jen A. K. Y., Dalton L. R., Ogata N., Curley M. J., Clarson S. J. & Hopkins F. K., *J. Phys. Chem. B*, **108**, 8584–8591 (2004)

45. Grote J. G., Diggs D. E., Nelson R. L., Zetts J. S., Hopkins F. K., Ogata N., Hagen J. A., Heckman E., Yaney P. P., Stone M. O. & Dalton L. R., *Mol. Cryst. Liq. Cryst.*, **426**, 3–17 (2005)

46. Kawabe Y., Wang L., Horinouchi S. & Ogata N., *Adv. Mater.*, **12**, 1281–1283 (2000)

47. Winfree E., Liu F., Wenzler L. A. & N. C. Seeman, *Nature*, **394**, 539–544 (1998)

48. Seeman N. C., *Annu. Rev. Biophys. Biomol. Struct.*, **27**, 225–248 (1998)

49. Seeman N. C., *Nature*, **421**, 427–431 (2003)

50. Mao C. D., Sun W. Q. & Seeman N. C., *J. Am. Chem. Soc.*, **121**, 5437–5443 (1999)

51. Malo J., Mitchell J. Venien-Bryan C., C., Harris J. R., Wille H., Sherratt D. J. & Turberfield A. J., *Angew. Chem. Int. Ed.*, **44**, 3057–3061 (2005)

52. Rothemund P. W. K., *Nature*, **440**, 297–302 (2006)

53. Shih W. M., Quispe J. D. & G. Joyce F., *Nature*, **427**, 618–621 (2004)

54. X. J. Li, X. P. Yang, J. Qi & Seeman N. C., *J. Am. Chem. Soc.*, **118**, 6131–6140 (1996)

55. Seeman N. C., *Nano Lett.*, **1**, 22–26 (2001)

56. Fu T. J. & Seeman N. C., *Biochemistry*, **32**, 3211–3220 (1993)

57. Nutiu R. & Li Y. F., *Angew. Chem. Int. Ed.*, **44**, 5464–5467 (2005)

58. Beyer S. & Simmel F. C., *Nucleic Acids Res.*, **34**, 1581–1587 (2006)

59. Stojanovic M. N., de Prada P. & Landry D. W., *ChemBioChem*, **2**, 411–415 (2001)

60. Weizmann Y., Beissenhirtz M. K., Cheglakov Z., Nowarski R., Kotler M. & Willner I., *Angew. Chem. Int. Ed.*, **45**, 7384–7388 (2006)

61. Liedl T., Sobey T. L. & Simmel F. C., *Nano Today*, **2**, 36–41 (2007)

62. Lueking A., Horn M., Eickhoff H., Bussow K., Lehrach H. & Walter G., *Anal. Biochem.*, **270**, 103–111 (1999)

63. de Wildt R. M. T., Mundy C. R., Gorick B. D. & Tomlinson I. M., *Nat. Biotechnol.*, **18**, 989–994 (2000)

64. Sano T., Smith C. L. & Cantor C. R., *Science*, **258**, 120–122 (1992)

65. Adler M., Wacker R., Booltink E., Manz B. & Niemeyer C. M., *Nat. Methods*, **2**, 147–149 (2005)

66. Boozer C., Ladd J., Chen S. F. & Jiang S. Y., *Anal. Chem.*, **78**, 1515–1519 (2006)

67. Niemeyer C. M., *Nano Today*, **2**, 42–52 (2007)

68. Albrecht C., Blank K., Lalic-Multhaler M., Hirler S., Mai T., Gilbert I., Schiffmann S., Bayer T., Clausen-Schaumann H. & Gaub H. E., *Science*, **301**, 367–370 (2003)

69. Strother T., Cai W., Zhao X. S., Hamers R. J. & Smith L. M., *J. Am. Chem. Soc.*, **122**, 1205–1209 (2000)

70. Lu M. C., Knickerbocker T., Cai W., Yang W. S., Hamers R. J. & Smith L. M., *Biopolymers*, **73**, 606–613. (2004)

71. Niemeyer C. M., Burger W. & Peplies J., *Angew. Chem. Int. Ed*., **37**, 2265–2268 (1998)

72. Niemeyer C. M., *Angew. Chem. Int. Ed.*, **40**, 4128–4158 (2001)

73. Niemeyer C. M., Adler M., Lenhert S., Gao S., Fuchs H. & Chi L. F., *ChemBioChem*, **2**, 260–264 (2001)

74. Yan H., Park S. H., Finkelstein G., Reif J. H. & LaBean T. H., *Science*, **301**, 1882–1884 (2003)

75. Liu Y., Ke Y. G. & Yan H., *J. Am. Chem. Soc.*, **127**, 17140–17141 (2005)

76. Liu Y., Lin C. X., Li H. Y. & Yan H., *Angew. Chem. Int. Ed.*, **44**, 4333–4338 (2005)

77. Lund K., Liu Y., Lindsay S. & Yan H., *J. Am. Chem. Soc.*, **127**, 17606–17607 (2005)

78. Tanaka K. & Okahata Y., *J. Am. Chem. Soc.*, **118**, 10679–10683 (1996)

79. Wang L. L., Yoshida J. & Ogata N., *Chem. Mater.*, **13**, 1273–1281 (2001)

80. Douthart, J. R., Burnet, P. J., Beasley W. F., & Frank, H. B., *Biochemistry*, **12**, 214–220 (1973)

81. LePecq B. J. & Paoletti, C., *J. Mol. Biol.*, **27**, 87–106 (1967)

82. Byrne, D. C., & de Mello, J. A., *Biophys. Chem.*, **70**, 173–184 (1998)

83. Tsuboi, M., Benevides, M. J., & Thomas Jr, J. G., *Biophys. J.*, **92**, 928–934 (2007)

84. Lawrence B. Hendry, Virendra B. Mahesh, Edwin D. Bransome Jr., Douglas E. Ewing, *Mutat. Res.*, **623**, 53–71 (2007)

85. Garbett, C. N., Hammond, B. N., & Graves, E. D., *Biophys. J.*, **87**, 3974–3981 (2004)

86. Hudson B. & Jacobs R., *Biopolymers*, **14**, 1309–1312 (1975)

87. Waleh A., Hudson B. and Loew G., Biopolymers, 15, 1637–1640 (1976)

Chapter 2

Preparation of stimulus sensitive gel particles with DNA-dye complexes

2.1 Abstract

In this chapter, stimulus sensitive gel particles with DNA-dye complexes were prepared. This composite has potential use in optical micro-sensors because it can change its dye concentration in response to stimuli by intercalating dye into DNA. Poly (acrylic acid) (PAAc) gel particles containing DNA were synthesized by emulsion polymerization and immersed in ethidium bromide (EtBr) solution to form DNA-EtBr complexes by intercalating dyes in the double stranded structure. The pH response characteristics were measured in buffer solution with an optical and fluorescence microscope, and the dye entrapment of the composite gel particles was evaluated with a UV-Visible spectrophotometer. As a result, the EtBr was immobilized in PAAc gel particles containing DNA, and its volume could be varied by changing the pH in buffer solution. Furthermore, by forming the gel in a particle shape, the PAAc gel reached equilibrium swelling in approximately 60 seconds. The fluorescence intensity of PAAc gel particles embedded with DNA-EtBr complexes displayed high-sensitivity and was found to exponentially decrease as a function of pH.

2.2 Introduction

Stimulus sensitive polymer gels change their volume in response to environmental stimuli such as pH, temperature, light, electric field, and biomolecules[1-5]. These soft and smart materials are expected to be developed for use in sensor devices in various fields[6-7]. However, it is necessary to improve the stimulus response speed of stimulus sensitive gels for application in novel sensor devices. It is well known that the relaxation time of various polymer gels is dependent on the volume [8-9]. For sensor applications, the improvement of stimulus response speed is one of the important challenges. Micro gel particles have a low volume and a high specific surface area[10]. These properties make it possible to improve the stimulus response speed using a stimulus sensitive gel formed into micro- or nano-particles.

Stimulus sensitive gels containing dye are applied to optical sensor devices to detect fluorescence intensity resulting from volume changes in response to environmental stimuli. However, most of the dye in the gels is eluted to an external solution through three-dimensional polymer networks. Consequently, we focused on dye entrapment of DNA into stimulus sensitive gels to develop a new type of optical sensor device. It has been previously reported that certain kinds of dye can be intercalated in double-stranded structure of DNA[11-16]. Intercalated dye is immobilized in the double-stranded structure of DNA, and dye is not diffused to regions external to the DNA. Additionally, intercalated dye is increased the fluorescence intensity because of inhibited concentration quenching and vibrational deactivation[17-18]. Such characteristics of DNA would be useful for applications in optical devices[19-21]. The optical sensing mechanism of composites containing stimulus sensitive gels and a DNA-dye complex is that the volume of the composite will change in response to external stimuli. The volume alteration of the gel particles causes a change in the concentration of the dye because the dye is intercalated into DNA that is immobilized in the gel particles. When the concentration of the dye is varied by altering the volume of the gel particles, the amount of dye that is excited by laser illumination is changed. Therefore, the resulting fluorescence intensity of the gel particles is increased or decreased in response to external stimuli.

In this chapter, we investigated the preparation of poly (acrylic acid) (PAAc) gel particles with DNA-dye complexes and their pH response characteristics. PAAc gel is a soft material

that can swell or contract in response to variations in pH. Thus, PAAc gel particles with DNA-dye complexes could be used to detect local pH information in micro-regions as a photo-signal. This unique characteristic is expected to be applied in optical micro-pH sensors in fields such as microbiology, cell studies and tissue engineering.

2.3 Experimental procedure

2.3.1 Materials and reagents

The monomer, acrylic acid (AAc) was purchased from Kanto Chemical Co., Inc. AAc was purified by reduced-pressure distillation. *N,N'*-methylene-bis-acrylamide (MBAA), which was used as a crosslinking agent, was purchased from Wako Pure Chemical Industries, Ltd. The initiator, potassium persulfate (KPS) was purchased from Kanto Chemical Co., Inc. MBAA and KPS were used without further purification. DNA from salmon milt was supplied by Ogata Material Science Institute Corp. The molecular weight was approximately 6.0×10^7, the purity was 91.55% and the total amounts of N and P were 14.46% and 8.29%, respectively. Span 80, which was used as a surfactant, and cyclohexane were purchased from Kanto Chemical Co., Inc. Ethidium bromide (EtBr), which was used as a fluorescent dye, was purchased from Wako Pure Chemical Industries, Ltd.

Table 2-1 Properties of DNA from salmon milt

	Molecular weight	Purify (%)	Contents (%)	
			N	P
DNA from salmon milt	6.0×10^7	91.55	14.46	8.29

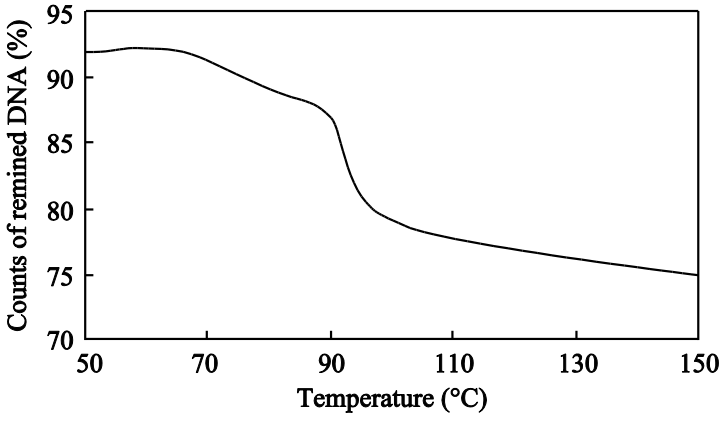

Figure 2-1 Thermal decomposition of DNA from salmon milt

2.3.2 Preparation of PAAc gel particles with the DNA-EtBr complex

PAAc gel particles with DNA were prepared by emulsion polymerization. In this process, 0.721 g of AAc (the molarity of AAc was adjusted to 1 mol/l) and 0.031 g of MBAA (2 mol% relative to AAc) were first dissolved in distilled water. Subsequently, DNA (the molarity of the DNA was adjusted to 1 mmol/l) was dissolved in distilled water, and each solution was mixed thoroughly. This solution was dropped into 300 ml of cyclohexane, while simultaneously adding 2.1 g of Span 80, which is higher than the critical micelle concentration, and the resulting mixuture was stirred at 300 rpm under a nitrogen atmosphere. Then, 0.054 g of KPS, which was deoxidized for 30 minutes, was added, and the polymerization was performed at 60 °C for 6 hours. The obtained PAAc gel particles with DNA were micro-filtered and washed repeatedly in methanol with centrifugation (15,000 rpm, 10 °C, 30 min). The PAAc gel particles with DNA were finally immersed in distilled water for 24 hours to remove the unreacted reagents.

Ethidium bromide (EtBr) was used as a fluorescent dye. A suitable amount of EtBr was dissolved in distilled water. PAAc gel particles with DNA-EtBr complexes were prepared by immersing the PAAc gel particles containing the DNA into the EtBr solution. The PAAc gel particles with DNA-EtBr complexes were then immersed in distilled water until equilibrium swelling was reached.

2.3.3 Evaluation of immobilized DNA in PAAc gel particles

Confirmation of immobilized DNA within the PAAc gel particles was performed using a UV-Visible spectrophotometer (Shimazu UV-1600). The PAAc gel particles embedded with DNA were kept immersed in distilled water until equilibrium swelling was reached, and then they were measured over the UV-Visible spectrum. The distilled water, in which the PAAc gel particles embedded with DNA were immersed, was also measured using UV-Visible spectrum to evaluate the elution of DNA from the PAAc gel particles. Circular dichroism (CD) spectra of PAAc gel particles with or without DNA were also measured.

2.3.4 Elution test of EtBr from PAAc gel particles with DNA

The PAAc gel particles containing DNA-EtBr complexes were immersed for 24 hours in

distilled water and Tris (hydroxymethyl amino methane) hydrochloric acid (Tris-HCl) buffer solution, which was prepared at a concentration of 0.1 mol/l and pH of 7.4. PAAc gel particles containing DNA in Tris-HCl buffer solution were separated from the solution. The solution was then measured by a UV-Visible spectrophotometer to evaluate the elution of EtBr from gel particles containing DNA. PAAc gel particles without DNA were similarly measured.

2.3.5 pH-sensitivity of PAAc gel particles with DNA-EtBr complexes

The evaluation of pH response speed was performed using a 0.1 mol/l of Tris-HCl buffer solution, at a pH of 7.4. The PAAc gel particles containing the DNA-EtBr complex were immersed in the buffer solution, and then were examined by optical microscope (Shimazu STZ-168-TL). The time response of the swelling ratio was measured with the image analysis system of the optical microscope.

The measurement of the swelling ratio of PAAc gel particles with DNA-EtBr complexes was performed using the image analysis system of the optical microscope, with several types of buffer solutions (pH 2 ~ 12). The gel particles were kept immersed in distilled water, until equilibrium swelling was reached. The distilled water was then removed, and the gel particles were immersed in each buffer solution for 10 minutes. The volume of the PAAc gel particles with DNA-EtBr complexes was measured using the image analysis system of the optical microscope. The swelling ratio of PAAc gel particles with DNA-EtBr complexes was calculated by evaluating the volumes of the particles before and after immersion in each of the buffer solutions.

2.3.6 Fluorescence property of PAAc gel particles with DNA-EtBr complexes

The observation of fluorescence from PAAc gel particles with DNA-EtBr complexes was performed with a fluorescence microscope (Olympus, BX-51). The objective lenses used were as follows: UPlanI 10x (NA=0.30, WD=9.5 mm), SLCPlanFI 40x (NA=0.55, WD=2.6 mm) and UMPlanFI 50x (NA=0.80, WD=2.1 mm).

The fluorescence intensity of PAAc gel particles with DNA-EtBr complexes was measured with a microspectrophotometer, which consists of a microscope, lasers, ND

filters, detector and monitor. The PAAc gel particles containing DNA-EtBr complexes were kept immersed in distilled water, until equilibrium swelling was reached. After the distilled water was removed, the PAAc gel particles with DNA-EtBr complexes were kept immersed in each of the buffer solutions for 5 minutes. The fluorescence intensity of the gel particles was measured in the buffer solutions.

2.4 Results and discussion

2.4.1 Preparation of PAAc gel particles with DNA-EtBr complexes

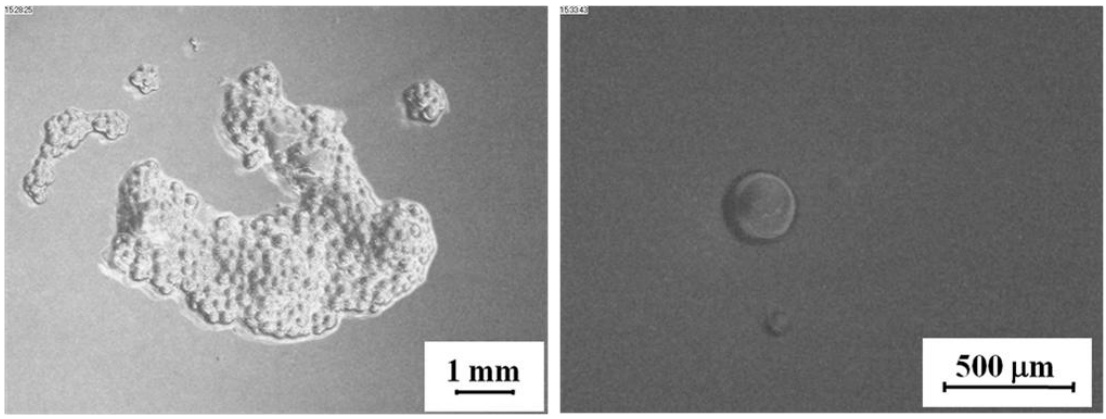

Figure 2-2 Optical micrographs of PAAc gel particles with DNA

Table 2-2 Properties of PAAc gel particles with DNA

	Average diameter (μm)	Standard deviation (μm)	CV (%)
PAAc gel particle with DNA	222.5	40.4	18.7

PAAc gel particles with DNA were synthesized by emulsion polymerization as shown in Figure 2-2 and the properties were shown in Table 2-2. The average diameter of the gel particles was about 250 μm, calculated from 100 measurements using the image analysis system of the optical microscope. The critical micelle concentration of the surfactant, Span 80 was defined as the concentration at which surface-active agents form micelles. Therefore, PAAc gel particles with DNA were synthesized at surfactant concentrations higher than the critical micelle concentration.

2.4.2 Evaluation of immobilized DNA into PAAc gel particles

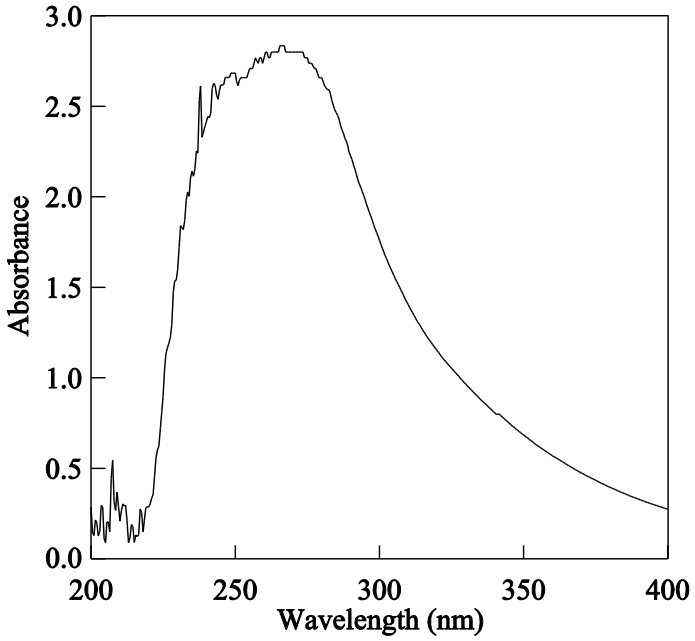

Figure 2-3 UV-Vis spectrum of PAAc gel particles with DNA, which is a differential spectrum based on PAAc gel particles without DNA

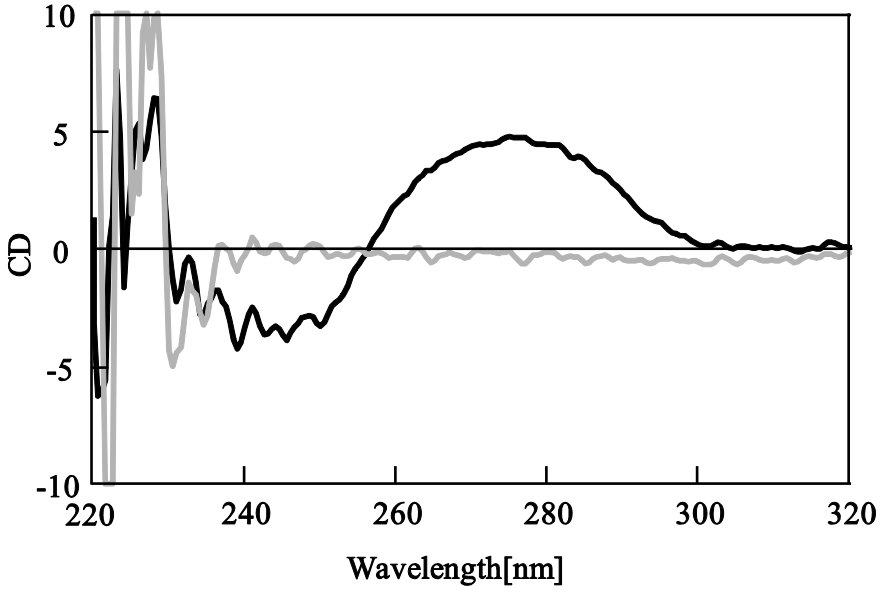

Figure 2-4 CD spectra of PAAc gel particles with or without DNA. The black line indicates PAAc gel particles with DNA and the gray line indicates the gel particles without DNA

Figure 2-3 shows the UV-Vis spectrum of PAAc gel particles with DNA synthesized by emulsion polymerization. The signal from DNA is observed at the absorption peak from the nucleobases (adenine, guanine, cytosine, and thymine) at 260 nm[22-23]. The absorption peak of PAAc gel particles with DNA was also observed at 260 nm. Figre 2-4 shows the CD spectra of PAAc gel particles with DNA. The CD spectrum of PAAc gel particles with DNA exhibited the positive cotton effect from 300 to 260 nm and negative cotton effect from 260 to 230 nm. The cotton effects is from right handed strand structure of DNA[24]. In contrast, the CD spectrum of PAAc gel particles without DNA was not comfirmed a cotton effect. These results indicated that DNA was included in the PAAc gel particles by this synthesis method. The amount of the DNA incorporated in the gel particles was measured by UV-Vis spectra. Using this synthesis process, the content of DNA in the gel particles was found to be 0.11 mmol g^{-1}. A small amount of DNA was incorporated due to swelling of the gel particles while washing and immersing in distilled water. The solution in which the PAAc gel particles with DNA were immersed for 24 hours was not observed the absorption peak from DNA at 260 nm. DNA was not eluted from the PAAc gel particles because DNA entangles in the three-dimentional network structure of the gel particles.

2.4.3 Elution of EtBr from PAAc gel particles containing DNA

The PAAc gel particles containing the DNA-EtBr complexes were prepared by immersing PAAc gel particles with DNA in an EtBr solution. After one hour of immersion in the solution, EtBr was incorporated in the PAAc gel particles with DNA. EtBr has an absorption peak at 490 nm. The PAAc gel particles with DNA-EtBr complexes were evaluated to measure the elution of EtBr from the gel particles to external solution by using a UV-Visible spectrophotometer, as shown in Figure 2-5. In the case of the PAAc gel particles without DNA, the absorption peak of EtBr in the external solution was observed at 490 nm. However, the absorption peak at 490 nm was not observed for the external solution of the PAAc gel particles with DNA. These results indicate that EtBr was immobilized into PAAc gel particles and did not diffuse into the external solution because it was intercalated into the double-stranded structure of the DNA.

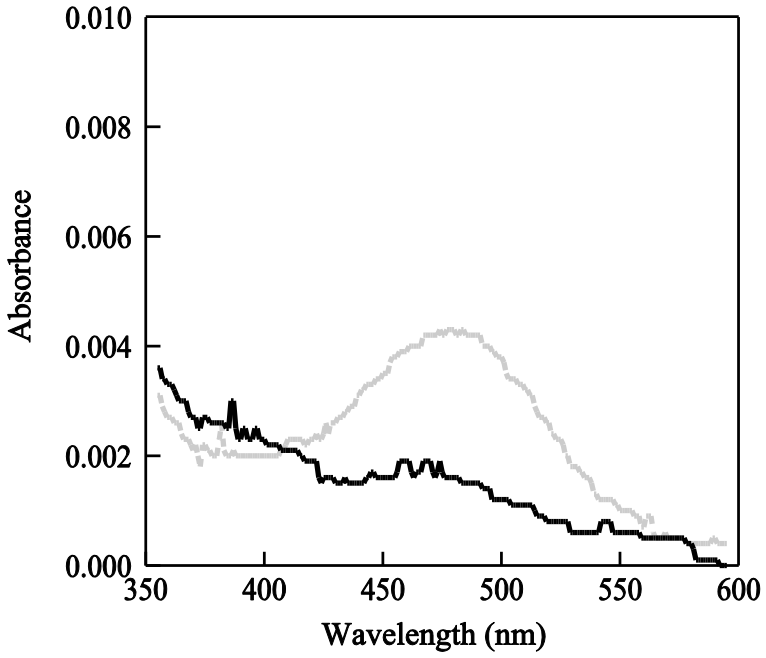

Figure 2-5 UV-Vis spectra of the buffer solution in which the PAAc gel particles containing EtBr and PAAc gel particles with DNA-EtBr complexes were immersed. The gray line indicates the UV-Vis spectrum of the solution of PAAc gel particles containing EtBr, and the black line indicates that of PAAc gel particles with DNA-EtBr complexes.

2.4.4 pH-sensitivity of PAAc gel particles with DNA-EtBr complexes

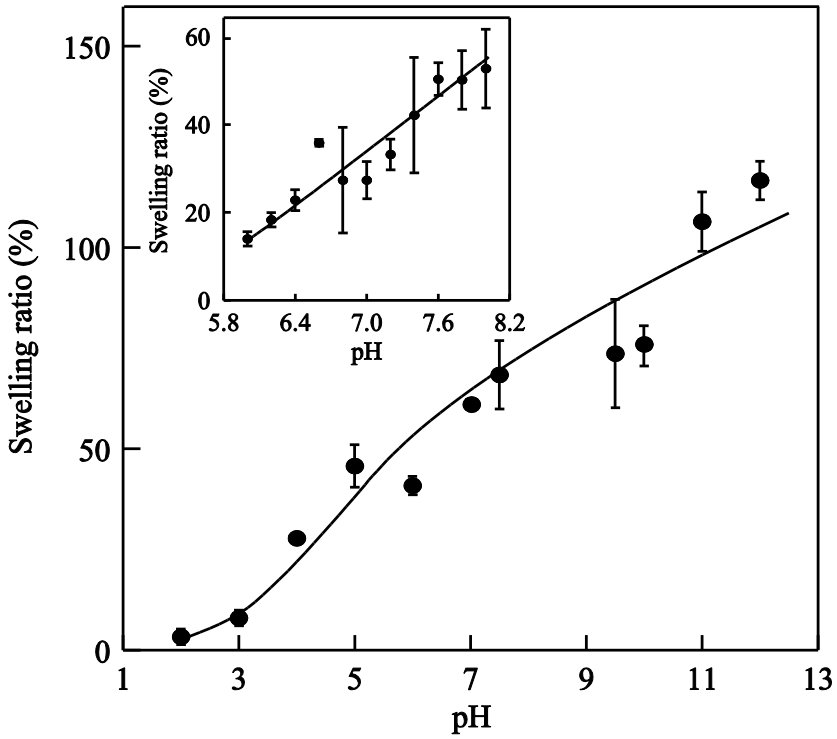

Figure 2-6 Swelling ratio of PAAc gel particles with DNA-EtBr complexes at constant ionic strength buffer solutions in various pH (ionic strength is 0.1). The inset data show the swelling ratio of the gel particles at constant ionic strength buffer solutions in the neutral pH region (ionic strength is 1.0).

PAAc gel is a material that swells and shrinks in response to changes in the pH of the external environment. Figure 2-6 shows the swelling ratio of PAAc gel particles with DNA-EtBr complexes in the pH range from 2 to 12. The swelling ratio of PAAc gel particles with DNA-EtBr complexes increased with pH in constant ionic strength buffers. The inset data in Figure 2-6 show the swelling ratio of PAAc gel particles with DNA-EtBr complexes in the neutral pH region. The swelling ratio of PAAc gel particles with DNA-EtBr complexes in the neutral pH region was closely measured. The pH buffer solutions in the neutral pH region were finely adjusted for pH value. Similar to the results

shown in Figure 2-6, the swelling ratio in the neutral pH region increased linearly with pH.

The effect of ionic strength on the swelling behavior was investigated by using different ionic strength buffers. The ionic strength for the data in Figure 2-6 and the corresponding inset data was at 0.1 and 1.0, respectively. The swelling ratio for an ionic strength at 1.0 reflected more swelling behavior than that for an ionic strength at 0.1. This result suggests that the solvent enhanced the release of ionic solution in terms of osmotic pressure.

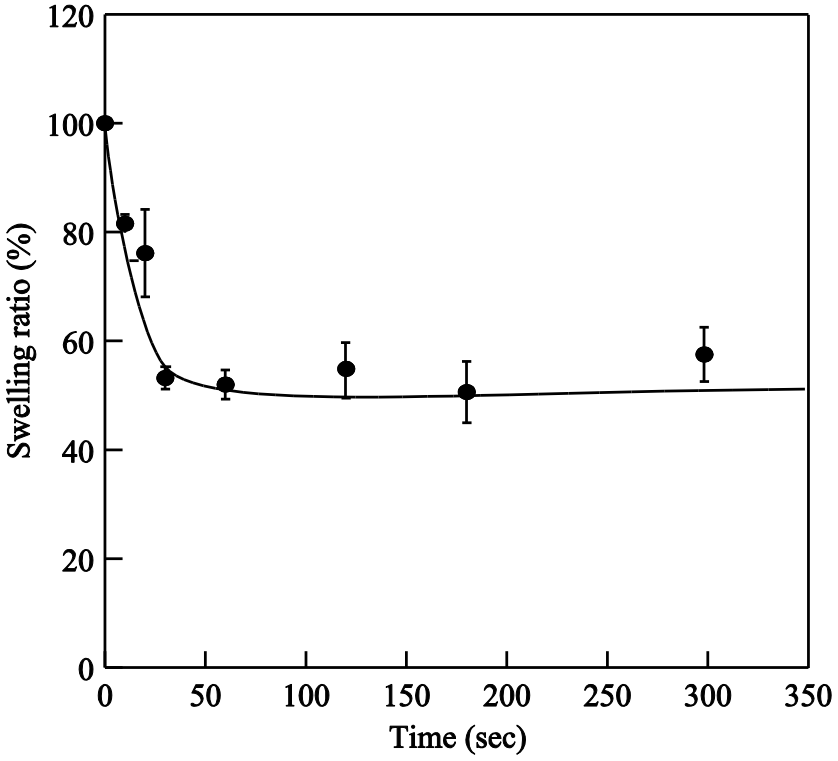

Figure 2-7 Time course of the swelling ratio of PAAc gel particles with DNA-EtBr complexes in pH 7.4 buffer solution

Figure 2-7 shows the time course of the swelling ratio of PAAc gel particles with DNA-EtBr complexes at a pH of 7.4. In this buffer solution, PAAc gel particles with DNA-EtBr complexes shrunk rapidly, and the volume was constant after approximately 60 seconds, which implies that equilibrium swelling was reached in approximately 60 seconds.

A PAAc block gel, which forms in a cylindrical shape and has the same chemical composition as the gel particles, was also synthesized, and the pH response speeds of the block gel and the gel particles were compared. The PAAc block gel with DNA (with a volume of approximately 10 cm^3) reached equilibrium swelling after approximately 10 hours. It was observed that the pH response speed of PAAc gel particles was approximately 600 times faster than that of the PAAc block gel. Additionally, the color of PAAc gel particles with DNA-EtBr complexes changed as a function of the volume alteration of the particles. The concentration of dye in the gel particles increased with the shrinkage of the gel particles and decreased with the swelling of the gel particles. This result indicates that pH-sensing could be accomplished by photo analyzing micro-regions with the gel particles.

2.4.5 Fluorescence property of PAAc gel particles with DNA-EtBr complexes

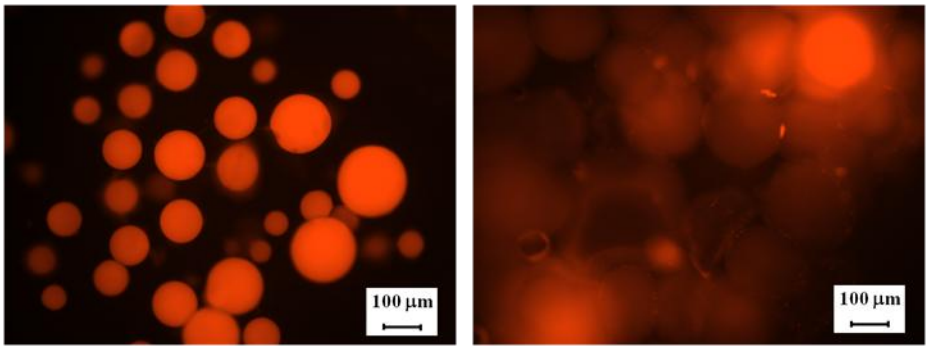

Figure 2-8 Fluorescence micrographs of PAAc gel particles with DNA-EtBr complexes. The left image indicates the gel particles in pH 2.1 buffer solution and the right indicates that of pH 11.0.

Figure 2-8 shows fluorescence micrographs of PAAc gel particles with DNA-EtBr complexes in buffer solutions at pH 2.1 and 11.0. In the pH 2.1 buffer solution, fluorescence emission was observed to be strong in PAAc gel particles with DNA-EtBr complexes by irradiating with green laser light at a wavelength of 532 nm. Limited fluorescence emission was observed at pH 11.0. The fluorescence intensity was measured

for one of the PAAc gel particles with DNA-EtBr complexes in buffer solution at pH values of 2.1, 5.0, 7.4, 9.0, 11.0, and 13.0, shown in Figure 2-9. The fluorescence intensity was exponentially decreased with increasing pH, and the intensity could be resolved with high-sensitivity. The fluorescence intensity was low at pH values of 11.0 and 13.0. In the case of pH 11.0, the fluorescence peak was only observed at 625 nm, but no peak was observed at pH 13.0.

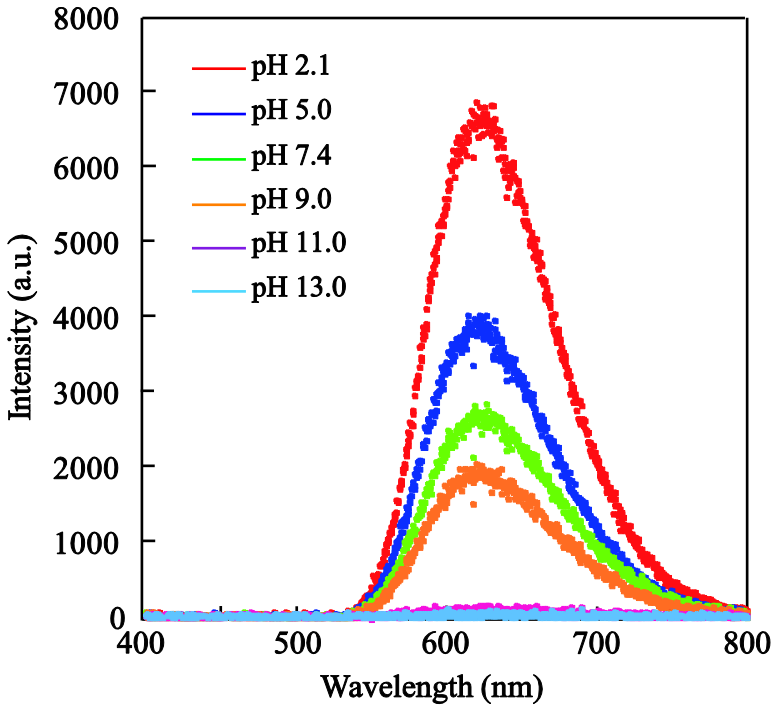

Figure 2-9 Fluorescence spectra of PAAc gel particles with DNA-EtBr complexes in various pH buffer solutions

Figure 2-10 shows the relationship between fluorescence intensity and the pH of the PAAc gel particles with DNA-EtBr complexes at wavelength 625 nm. The pH changes of the gel particles could be detected with high-sensitivity by the fluorescence intensity. The fluorescence intensity was exponentially decreased from pH 2.1 to pH 9.0. Fluorescence intensity at pH 11.0 and 13.0 was sharply decreased. In pH 11.0 and 13.0 buffer solutions, a small amount of EtBr was eluted from the PAAc gel particles with DNA-EtBr complexes

because the double-stranded structure of DNA was unwound by the strong alkalinity. However, almost all of the EtBr was immobilized in the DNA which was embedded in the gel particles. Therefore, slight fluorescence intensity at pH value over 11 was observed due to swelling of the gel particles. From these results, it is clear that PAAc gel particles with DNA-EtBr complexes can be used to detect and measure the pH in the surrounding environment by fluorescence intensity and that this approach could be used to create optical micro-pH sensors.

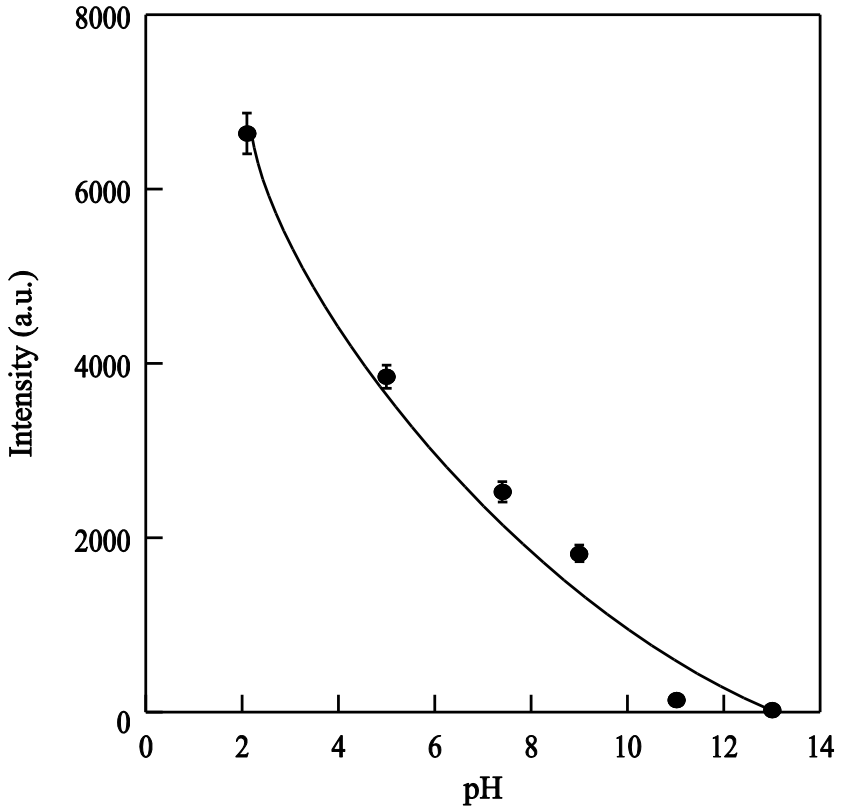

Figure 2-10 Relationship between pH and florescence intensity of PAAc gel particles with DNA-EtBr complexes

2.5 Conclusions

PAAc gel particles with DNA-EtBr complexes were synthesized by emulsion polymerization. DNA was entrapped in PAAc gel particles, and it was not eluted from the gel particles. EtBr was immobilized in PAAc gel particles by intercalating it into the double-stranded structure of DNA, and EtBr did not diffuse into the external solution. The swelling ratio of PAAc gel particles with DNA-EtBr complexes increased with pH. The PAAc gel particles with DNA-EtBr complexes reached equilibrium after one minute. The pH response speed of the PAAc gel particles was about 600 times faster than that of a PAAc block gel (with a volume of about 10 cm^3). The fluorescence intensity of PAAc gel particle with DNA-EtBr complexes provided high-sensitivity and exponentially decreased relative to pH. Consequently, PAAc gel particles with DNA-EtBr complexes could be useful in the development of optical micro-pH sensor devices.

References

1. Gümüşderelioğlu, M. & Topal, U. I., *Radiat. Phys. Chem.*, **73**, 272–279 (2005)

2. Li X., Wu, W. & Liu, W., *Carbohydr. Polym.*, **71**, 394–402 (2008)

3. Miyata, T., Asami, N. & Uragami, T., *Nature*, **399**, 766–769, (1999)

4. Miyata, T., Jikihara, A., Nakamae, K. & Hoffman, A. S., *J. biomater. Sci. Polym. Edn.*, **15**, 1085–1098 (2004)

5. Miyata, T., Asami, N., and Uragami, T., *Macromolecules*, **32**, 2082–2084 (1999).

6. Trinh, T. Q., Gerlach, G., Sorber, J. & Arndt, K.-F., *Sens. Act. B*, **117**, 17–26 (2006)

7. Guenther, M., Gerlach, G., Corten, C., Kuckling, D., Sorber, J., Arndt, K.-F., *Sens. Act. B*, **132**, 471–476 (2008)

8. Tanaka T. & Filmore D. J., *J. Chem. Phys.*, **70**, 1214–1218 (1979)

9. Li, Y. & Tanaka T., *J. Chem. Phys.*, **92**, 1365–1371 (1979)

10. Kishi, R., & Osada, Y., *J. Chem. Soc., Faraday Trans.1*, **85**, 655–662 (1989)

11. Douthart, J. R., Burnet, P. J., Beasley W. F., & Frank, H. B., *Biochemistry*, **12**, 214–220 (1973)

12. LePecq B. J. & Paoletti, C., *J. Mol. Biol.*, **27**, 87–106 (1967)

13. Byrne, D. C., & de Mello, J. A., *Biophys. Chem.*, **70**, 173–184 (1998)

14. Tsuboi, M., Benevides, M. J., & Thomas Jr, J. G., *Biophys. J.*, **92**, 928–934 (2007)

15. Lawrence B. Hendry, Virendra B. Mahesh, Edwin D. Bransome Jr., Douglas E. Ewing, *Mutat. Res.*, **623**, 53–71 (2007)

16. Garbett, C. N., Hammond, B. N., & Graves, E. D., *Biophys. J.*, **87**, 3974–3981 (2004)

17. Grote J. G., Hagen J. A., Zetts J. S., Nelson R. L., Diggs D. E., Stone M. O., Yaney P. P., Heckman E., Zhang C., Steier W. H., Jen A. K. Y., Dalton L. R., Ogata N., Curley M. J., Clarson S. J. & Hopkins F. K., *J. Phys. Chem. B*, **108**, 8584–8591(2004)

18. Kawabe Y., Wang L., Horinouchi S. & Ogata N., *Adv. Mater.*, **12**, 1281–1283 (2000)

19. Ito, Y., Yagi, T., Ohnishi, Y., Kikuchi, K. & Utikawa, Y., *Int. J. Appl. Electromagn. Mech.*, **14**, 347–352 (2001/2002)

20. Kanda, H., Morimoto, T., Fujikado, T., Tano, Y., Fukuda, Y. & Sawai1, H., *Investig. Ophthalmol. Vis. Sci.*, **45**, 560–566 (2004)

21. Veraart, C., Wanet-Defalque, M.-C., Gérard, B., Vanlierde, A. & Delbeke, J., Pattern *Artif. Organs*, **27**, 996–1004 (2003)

22. Johnson W. C., eds. Berova N., Nakanishi K. & Woody R. W., *John Wiley & Sons, Inc, New York*, pp. 703–718 (2000)

23. Wang L. L., Yoshida J. & N. Ogata, *Chem. Mater*., **13**, 1273–1281 (2001)

24. Tanaka K. & Okahata Y., *J. Am. Chem. Soc.*, **118**, 10679–10683 (1996)

Chapter 3

Assembly of stimulus sensitive gel particles with DNA-dye complexes

3.1 Abstract

In this chapter, assembly of stimulus sensitive gel particles with DNA-dye complexes was investigated for application in optical-sensing devices. The composite material of stimulus sensitive gels and DNA detects external stimuli as photo-signal. Assembly of the composite gel particles with DNA-dye complexes would be expected to achieve high responsiveness and to quickly detect environmental information as a photo-signal because the each composite gel particles consisting of the assembly is responded in external environment at the same time and the porous structure which is fabricated from interparticle spaces is decreased with relaxation time of the three dimensional networks structure. These characteristics could be useful for application in novel optical-sensor devices.

Poly (acrylic acid) (PAAc) gel particles with DNA were prepared by emulsion polymerization and the gel particles were assembled by crosslinking reaction between the gel particles utilizing glutaraldehyde. The assembly of PAAc gel particles with DNA was possible to prepare in sheet and block shapes by utilizing each shaped forms in this synthesis process. Swelling ratio of the assembly was increased with pH, and the assembly reached equilibrium swelling about 180 seconds. The response speed of the assembly was improved more 30 times faster than that of typical PAAc block gel. Furthermore, the assembly of PAAc gel particles with DNA-ethidium bromide complexes detected pH information in external solution as an optical signal.

3.2 Introduction

Stimulus sensitive gels change their volume in response to external environmental stimuli, such as pH, temperature, light, electric fields, and biomolecules[1-7]. By measuring the volume alteration quantitatively, the stimulus sensitive gels can be used to detect a significant amount of environmental information and unique elements. These smart and soft materials are expected to be developed for use in applications such as sensor devices, actuators and drug carriers[8-14]. However, stimulus sensitive gels are necessary to increase the response speed for applications in novel sensor devices. The relaxation time of a network of gels is proportional to the square of the diameter in equilibrium swelling[15-16].

Recently, assemblies of polymer gel particles, which have excellent properties such as high stimulus responsiveness and colloidal photonic crystals, have been reported[17-19]. In nature, living things form in various shape of assembly in which the basic unit is optimally self-organized for the expression of various functions. The honeycomb structure has excellent physical properties and is often applied to artificial materials[20-23]. The concept of organization and assembly on the microscale could provide various functions to smart materials. Consequently, we investigated the organization of the stimulus sensitive gel particles by inducing a crosslinking reaction between the gel particles. An assembly of the stimulus sensitive gel particles forms interparticle spaces. These microspaces are expected to reduce the relaxation time and diffuse solutions through the networks. Each gel particle composing the assembly responds to pH changes at nearly the same time as a result of the buffer solution diffusing through the interparticle spaces. That is, a microscopic change of the gel particles quickly induces a macroscopic change. The assembly of the stimulus sensitive gel particles can achieve a high response speed, similar to that of the gel particles.

Furthermore, DNA was utilized as a dye-entrapped agent in assembly of stimulus sensitive gels particles. The supplied DNA was obtained from salmon milt; most salmon milt is wasted in Japan. The utilization of DNA from salmon milt as a functional material is important and useful in terms of environmental programs and regional contributions. It has been reported that aromatic compounds such as dyes, harmful materials and carcinogens can be intercalated into double strands of DNA by molecular attraction because base-pairs of DNA exhibit aromaticity[24-29]. The double strand structure inhibits the dye aggregation in

the gel. The concentration quenching of dye does not occur, and effective fluorescence emission can be expected. When the concentration of the dye is varied by altering the volume of the assembly of stimulus-sensitive gel particles, the fluorescence intensity of the assembly is increased or decreased in response to external stimuli because the amount of dye that is excited by laser illumination is changed.

Additionally, the assembly of stimulus sensitive gel particles with DNA could not be used only as a sensor material, but also as an absorbent material for harmful substances in the external environment. DNA can immobilize harmful materials, such as carcinogens. The assembly has a highly porous structure, and its high specific surface area is an advantage for absorbent material applications. The assembly of stimulus sensitive gel particles with DNA would be expected to be used for sensing and absorbing materials.

In this chapter, an assembly of poly (acrylic acid) (PAAc) particles with DNA-ethidium bromide (EtBr) complexes was prepared, and the pH response characteristics were evaluated. The assembly of PAAc gel particles with DNA-EtBr complexes can be used to quickly detect pH information as a photo-signal.

3.3 Experimental procedure

3.3.1 Materials and reagents

Acrylic acid (AAc) and allylamine hydrochloride (AA) were purchased from Kanto Chemical Co., Inc. AAc was purified by reduced-pressure distillation. *N,N'*-methylene-bis-acrylamide (MBAA), which was used as a crosslinking agent, was purchased from Wako Pure Chemical Industries, Ltd. The initiator, potassium persulfate (KPS), was purchased from Kanto Chemical Co., Inc. The MBAA and KPS were used without further purification. DNA from salmon milt was supplied by Ogata Material Science Institute Corp. The molecular weight was approximately 6.0×10^7, the purity was 91.55% and the total amounts of N and P were 14.46% and 8.29%, respectively. Span 80, which was used as a surfactant, and cyclohexane were purchased from Kanto Chemical Co., Inc. Ethidium bromide (EtBr), which was used as a fluorescent dye was purchased from Wako Pure Chemical Industries, Ltd. Glutaraldehyde was purchased from Kanto Chemical Co., Inc.

3.3.2 Preparation of PAAc gel particles with DNA

PAAc gel particles with DNA were prepared by emulsion polymerization. First, 0.721 g of AAc (the molarity of AAc was adjusted to 1 mol/l), 0.096 g of AA (10 mol% relative to AAc) and 0.031 g of MBAA (2 mol% relative to AAc) were dissolved in distilled water. DNA (the molarity of the DNA was adjusted to 1 mmol/l) was dissolved in distilled water, and each solution was mixed. This solution was dropped into 300 ml of cyclohexane, while simultaneously adding 2.1 g of Span 80, and the resulting mixture was stirred at 300 rpm under a nitrogen atmosphere. Then, 0.005 g of KPS, which was deoxidized for 30 minutes, was added, and the polymerization was performed at 60 °C for 6 hours. The obtained PAAc gel particles with DNA were microfiltered and washed repeatedly in methanol with centrifugation. The PAAc gel particles with DNA were finally immersed in distilled water for 24 hours to remove the unreacted reagents.

3.3.3 Preparation of assembly of PAAc gel particles with DNA-EtBr complexes

The assembly of PAAc gel particles with DNA was prepared by a crosslinking reaction

of the interparticles with glutaraldehyde. A suitable amount of the gel particles was put in a form which was composed of a silicone rubber sheet (1 mm in thickness) sandwiched between two glass plates (100 mm in height, 100 mm width, and 2 mm thickness). Glutaraldehyde was dissolved in distilled water, and the solution was poured into the form. Subsequently, the form was set in a water bath, and the crosslinking reaction was performed at 40 °C for 12 hours. The obtained assembly of PAAc gel particles with DNA was repeatedly washed and immersed in distilled water to remove the unreacted reagents.

Ethidium bromide (EtBr) was used as a fluorescent dye. 10 ml of EtBr aqueous solution (1 mmol/l) was prepared by dissolved EtBr in distilled water. The assembly of PAAc gel particles with DNA-EtBr complexes was prepared by immersing 0.5 g of the assembly of PAAc gel particles containing DNA in the EtBr solution. The molar ratio of base pair vs. EtBr was approximately 5.5 : 1. The obtained assembly of PAAc gel particles with DNA-EtBr complexes was then immersed in distilled water until equilibrium swelling was reached.

The assembly of PAAc gel particles with DNA-EtBr complexes was immersed for 24 hours in distilled water and 0.01 N HCl aqueous solutions. The assembly of PAAc gel particles containing DNA-EtBr complexes in HCl aqueous solution was separated from the solution. The solution was measured by a UV-Visible spectrophotometer (Shimadzu UV-1600) to evaluate the elution of EtBr from the assembly containing DNA. The assembly of PAAc gel particles without DNA was similarly measured.

3.3.4 Mechanical property of assembly of PAAc gel particles with DNA-EtBr complexes

Young's modulus of the assembly of PAAc gel particles with DNA-EtBr complexes was measured by thermomechanical analysis (Shimadzu TMA-60). The assembly of PAAc gel particles with the DNA-EtBr complex was fabricated with a 10 mm height, width and thickness and kept immersed in distilled water until equilibrium swelling. The assembly was then set on the sample stage and a load was applied (0.01 N/min of load rate and 0.1 N of object load) by a 25 mm^2 of a circle indenter at room temperature. Young's modulus was calculated from the stress-strain curves. Young's modulus of PAAc block gel was similarly

measured and compared with the assembly. Furthermore, the relationship between Young's modulus and the swelling ratio of the assembly of PAAc gel particles with DNA was investigated.

3.3.5 pH-sensitivity of assembly of PAAc gel particles with DNA-EtBr complexes

The measurement of the swelling ratio in response to the pH of the assembly of PAAc gel particles with DNA-EtBr complexes was performed using the image analysis system of an optical microscope (Shimadzu STZ-168-TL) with several types of buffer solutions with pH values ranging from 2.0 to 12.0. The assembly was kept immersed in distilled water until equilibrium swelling was reached. The distilled water was then removed, and the assembly was immersed in each buffer solution for 1 hour. The volume of the assembly was measured using the image analysis system of the optical microscope. The swelling ratio of the assembly was calculated by evaluating the volumes of the assembly before and after immersion in each of the buffer solutions.

The evaluation of the relaxation time was performed by measuring the swelling ratio of the assembly with time. The assembly of PAAc gel particles with DNA-EtBr complexes was immersed in a buffer solution at pH 2.0 and then examined by the optical microscope. The time response of the swelling ratio was measured with the image analysis system of the optical microscope. Furthermore, the time response of the gel particle and the block-shaped gel, which had the same volume and chemical composition relative to the assembly, were similarly measured and compared with that of the assembly. From the results of swelling ratio as function of time, relaxation time, τ was calculated from the following equation (1).

$$(\alpha(t) - \alpha_\infty) / (\alpha_0 - \alpha_\infty) = \exp(-t/\tau) \qquad (1)$$

Where α_∞ is the equilibrium swelling ratio, α_0 is the initial swelling ratio and t is time. The resulting relaxation time was compared with each other.

3.3.6 Optical property of assembly of PAAc gel particles with DNA-EtBr complexes

The optical properties of the assembly of PAAc gel particles with DNA-EtBr complexes were evaluated to consider the application of the assembly in optical sensor devices. The absorbance of the assembly was measured with the UV-Visible spectrometer. The assembly was kept immersed in each buffer solution for 1 hour. The assembly was set in the center of a quartz cell, and the absorbance of the assembly was measured. The fluorescence intensity of the assembly was evaluated by using an image analysis system. The assembly of PAAc gel particles with DNA-EtBr complexes was kept immersed in each of the buffer solutions for 10 minutes. The assembly was irradiated with ultraviolet light at a wavelength of 265 nm and then observed with the optical microscope in the dark. The intensity of the assembly at each pH was calculated relatively by using the image analysis system.

3.4 Results and discussion

3.4.1 Preparation of PAAc gel particles with DNA-EtBr complexes

PAAc gel particles with DNA could be prepared by emulsion polymerization (Figure 3-1). The average diameter of the gel particles was approximately 330 μm, calculated from 100 measurements using the image analysis system of the optical microscope (Table 3-1). For the PAAc gel particles with DNA, it was confirmed that DNA was included in the particles from the absorption peak at 260 nm, which was measured using the UV-Vis spectrophotometer.

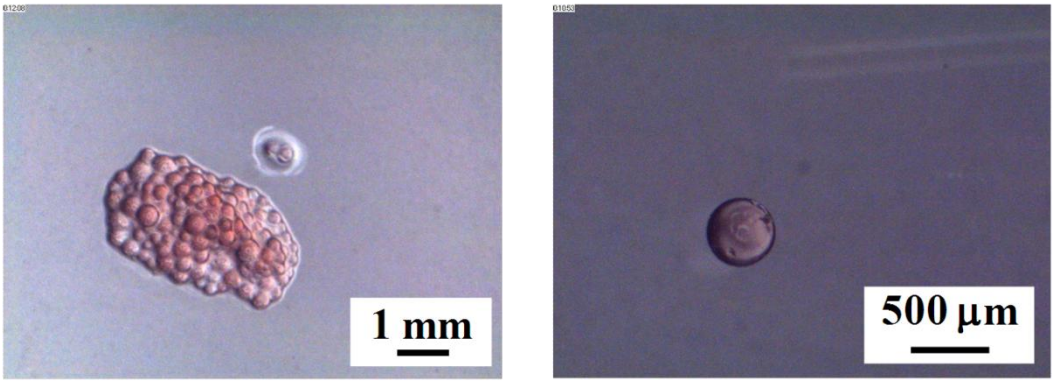

Figure 3-1 Optical micrographs of PAAc gel particles with DNA-EtBr complexes

Table 3-1 Properties of PAAc gel particles with DNA

	Average diameter (μm)	Standard deviation (μm)	CV (%)
PAAc gel particle with DNA-EtBr complexes	334	63	19

3.4.2 Preparation of assembly of PAAc gel particles with DNA-EtBr complexes

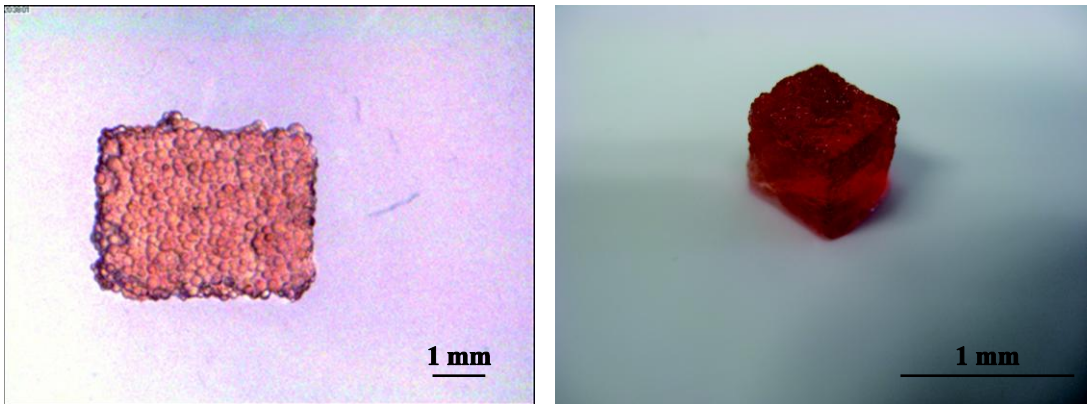

Figure 3-2 Optical micrograph and photograph of assembly of PAAc gel particles with DNA-EtBr complexes. The left side indicates the sheet-shaped assembly and the right side indicates the block-shaped assembly.

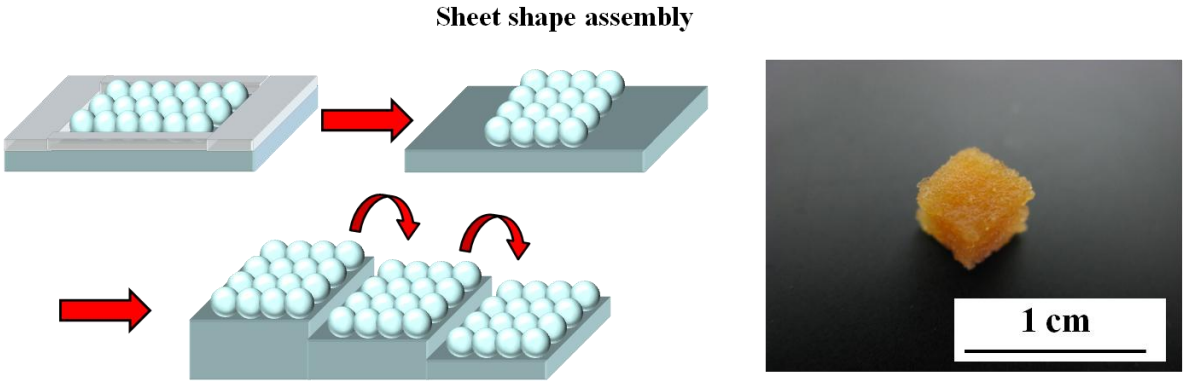

Figure 3-3 Block shaped assembly of PAAc gel particles with DNA prepared by stepwise crosslinking reaction between the sheets shaped assembly

The assembly of PAAc gel particles with DNA was obtained by a crosslinking reaction of the interparticles with glutaraldehyde. The assembly was formed into a sheet shape according to the shape of the form, which was 100 mm in height, 100 mm width, and 1 mm thickness (Figure 3-2, left side). Using a form that was 50 mm in height and 10 mm in width and thickness, a block-shaped assembly could be prepared (Figure 3-2, right side).

The assembly of PAAc gel particles with DNA could be prepared in various shapes by utilizing various forms in this process. Furthermore, the sheet shaped assembly was stacked three layers and block shaped assembly was prepared by stepwise crosslinking reaction (Figure 3-3).

The assembly of PAAc gel particles with DNA-EtBr complexes was prepared by immersing the assembly of PAAc gel particles containing DNA in an EtBr solution. After 1 hour of immersion in the solution, the EtBr was incorporated in the assembly. The EtBr has an absorption peak at 490 nm. The assembly of PAAc gel particles with DNA-EtBr complexes was evaluated to measure the elution of EtBr from the assembly to the external solution by using a UV-Visible spectrophotometer, as shown in Figure 3-4. In the case of the assembly without DNA, the absorption peak of EtBr in the external solution was observed at 490 nm. However, the external solution surrounding the assembly with DNA did not exhibit the absorption peak at 490 nm. These results indicate that EtBr was immobilized in the assembly with DNA and did not diffuse into the external solution because it was intercalated into the double strands of the DNA.

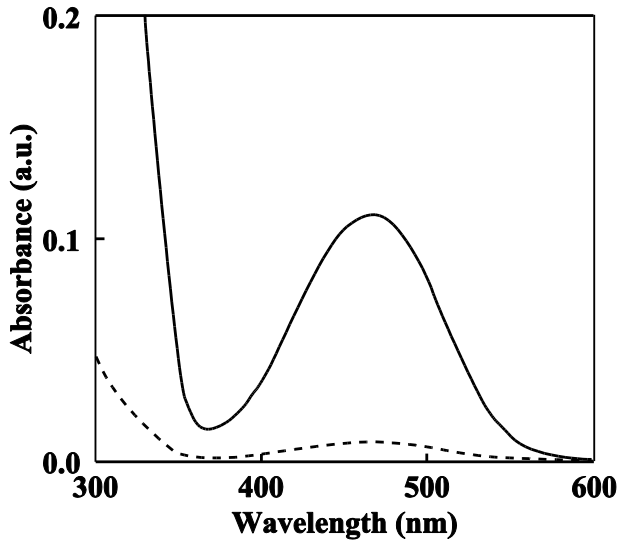

Figure 3-4 UV-Vis spectra of the solution in which assembly of PAAc gel particles with or without DNA containing EtBr was immersed. Solid line indicates UV-Vis spectrum of the solution of the assembly without DNA. Broken line indicates that of the assembly with DNA.

3.4.3 Mechanical property of assembly of PAAc gel particles with DNA-EtBr complexes

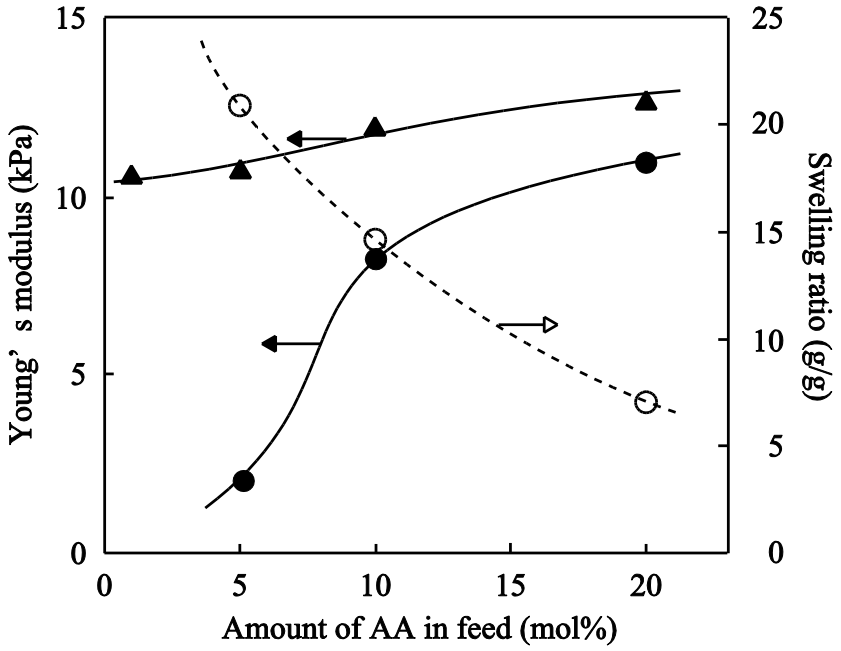

Figure 3-5 Relationship between of young's modulus (filled circle) and swelling ratio (open circle) of assembly of PAAc gel particles with DNA-EtBr complexes. Young's modulus of the PAAc block gel which was the same volume and chemical composition with the assembly (filled triangle) shows by comparison to the assembly.

The Young's modulus of the assemblies of the PAAc gel particles with DNA that were prepared at different AA molar ratios was evaluated by calculations based on the stress-strain curve. The Young's modulus of the assembly exponentially increased with the AA molar ratio of the assembly, and the Young's modulus of the assembly that was prepared at 20 mol% of the AA molar ratio was approximately 10 kPa (Figure 3-5). It was found that the Young's modulus of the assembly was almost the same as that of the typical PAAc block gel. Additionally, the swelling ratio of the assembly of PAAc particles with DNA was measured. The swelling ratio of the assembly decreased exponentially with the AA molar ratio of the assembly. It was assumed that the assembly formed a highly porous

structure that was composed of the gel particles, and the stimulus responsiveness of the assembly was controllable by the amount of crosslinking points between the gel particles. The formation of the interparticle spacing increased the swelling speed and made it possible to include and release micromaterials, such as drugs, biopolymers, and other functional particles. Based on the relationship between Young's modulus and the swelling ratio, the assembly that was prepared with a 10 mol% AA molar ratio was used.

3.4.4 pH-sensitivity of PAAc gel particles with DNA-EtBr complexes

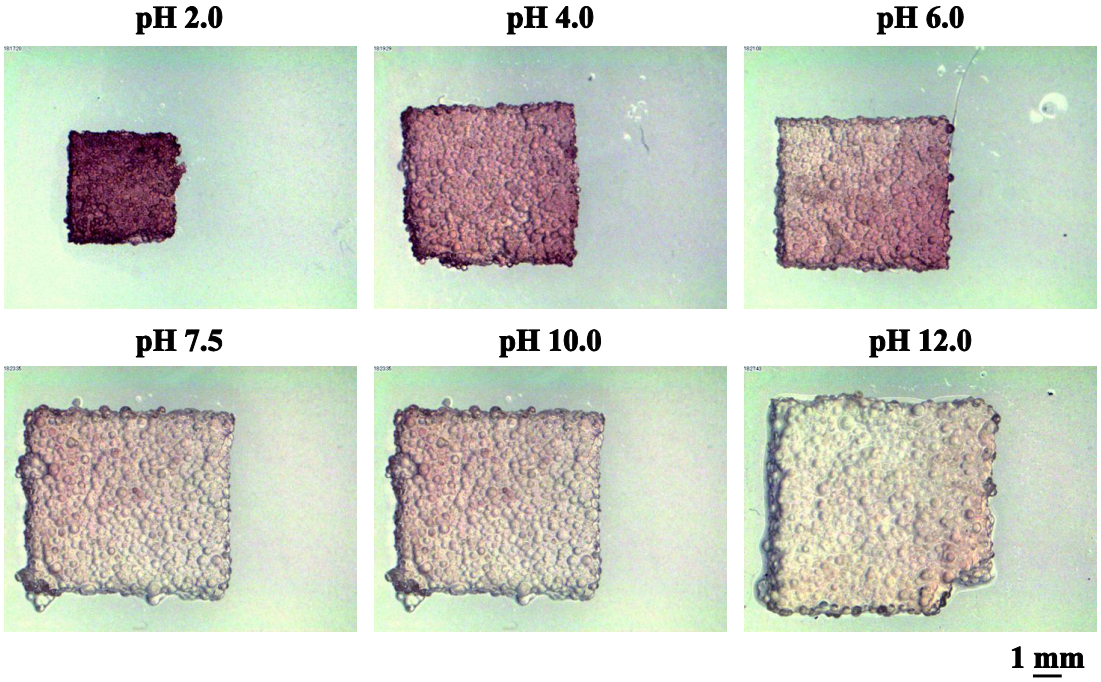

Figure 3-6 Optical micrographs of assembly of PAAc gel particles with DNA-EtBr complexes in various buffer solutions at the pH value of 2.0, 4.0, 6.0, 7.5, 10.0 and 12.0.

Figure 3-6 shows the optical micrographs of the assembly of the PAAc gel particles with DNA-EtBr complexes in various pH buffer solutions. The assembly changed its volume in response to pH. Furthermore, a dye density change of the assembly caused by the volume alteration was also observed. The dye density in the assembly increased with the shrinkage

of the assembly. Additionally, the dye density of the assembly changed repeatedly with the volume alteration. These results indicate that pH-sensing could be accomplished by optical analysis of the assembly. Figure 3-7 shows the swelling ratio of the assembly in the pH range from 2.0 to 12.0. The swelling ratio of the assembly of PAAc gel particles with DNA-EtBr complexes regularly increased with the pH in constant ionic strength buffers.

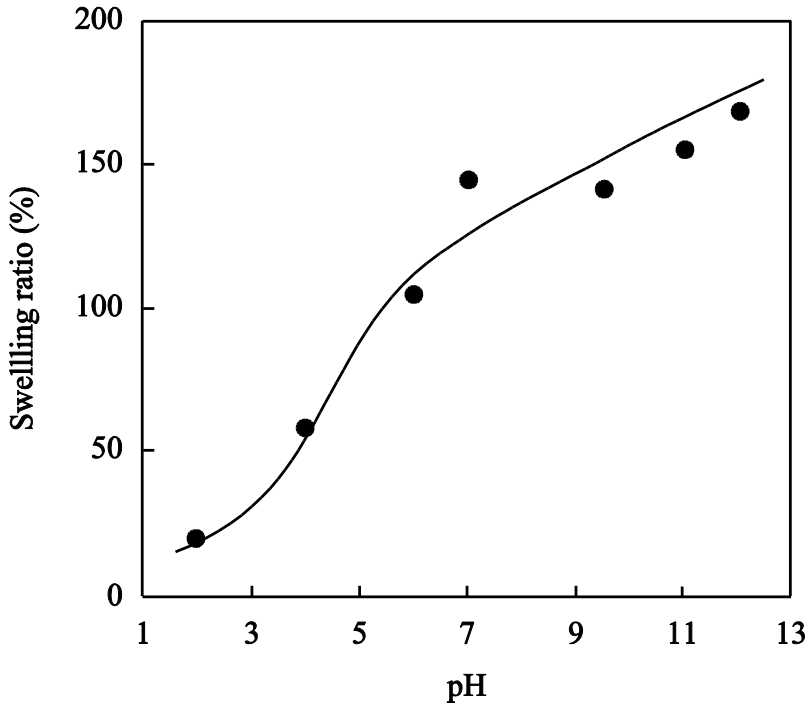

Figure 3-7 Swelling ratio of assembly of PAAc gel particles with DNA-EtBr complexes in various buffer solutions at the pH value of 2.0, 4.0, 6.0, 7.0, 9.5, 11.0 and 12.0.

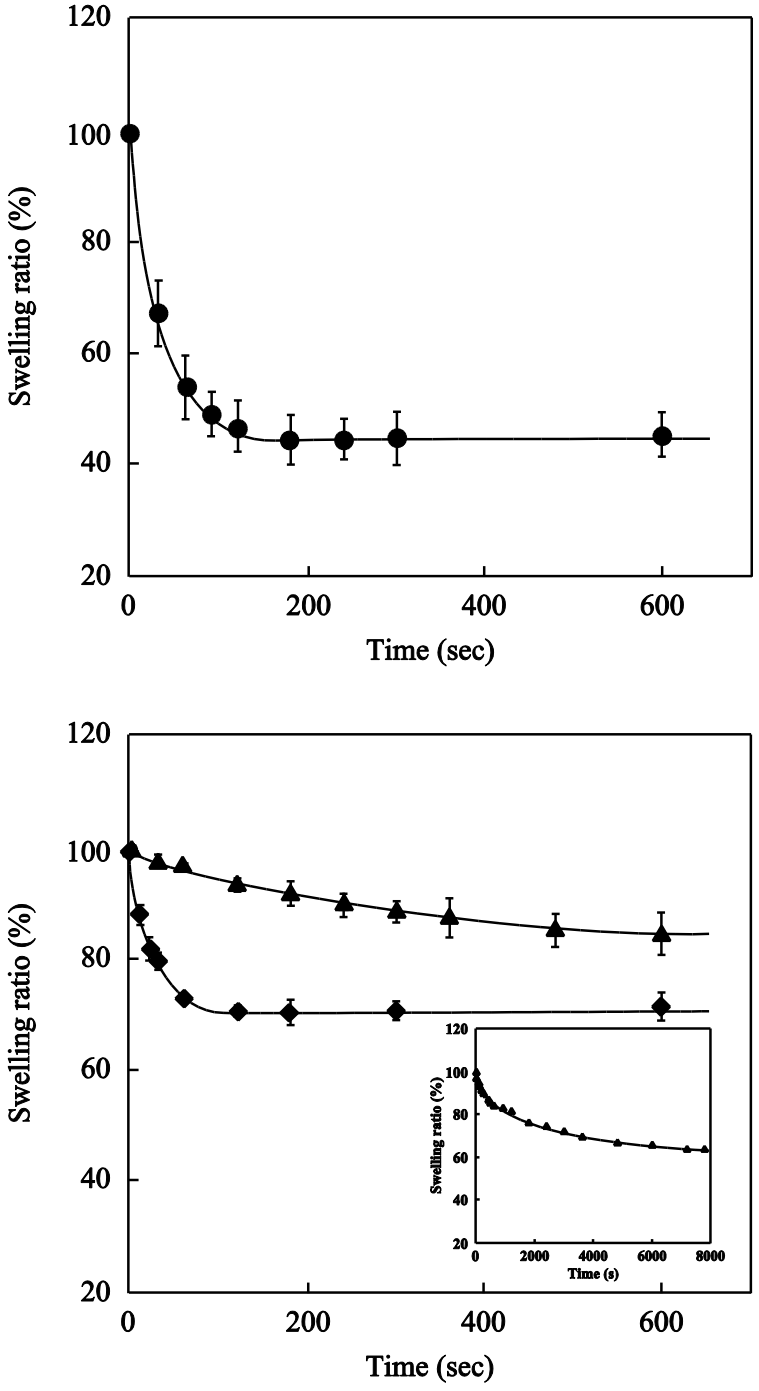

Figure 3-8 Time course of the swelling ratio of the assembly of PAAc gel particles (circle), the PAAc gel particle (diamond) and the PAAc block gel (triangle) in pH 2.0 buffer solution.

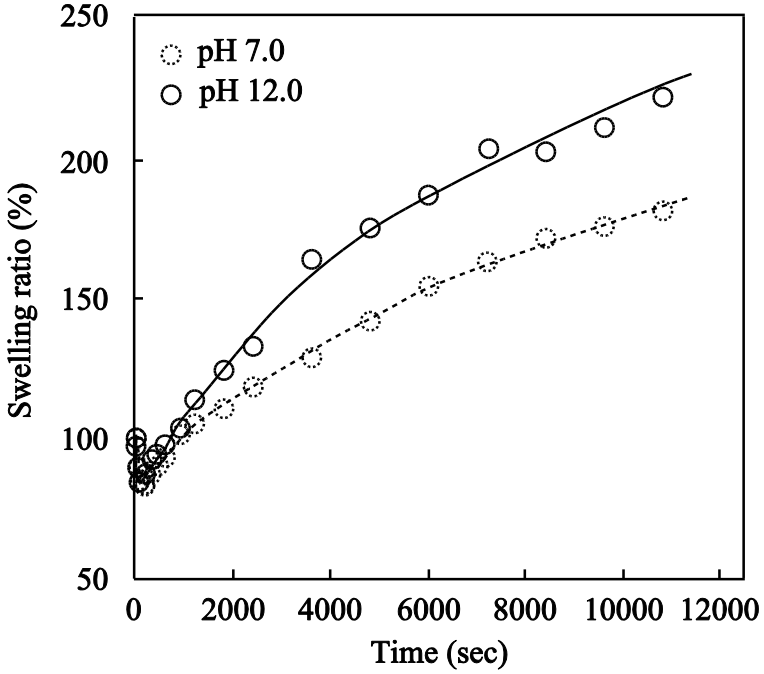

Figure 3-9 Time course of the swelling ratio of the assembly of PAAc gel particles in pH 7.0 (dash line) and 12.0 (solid line) buffer solution.

Table 3-2 Relaxation times of the assembly of PAAc gel particles with DNA-EtBr complexes, the gel particles and the block gel

	Relaxation time, τ (sec)
The gel particle	8.65×10^{1}
The block gel	2.50×10^{3}
The assembly	1.60×10^{2}

Fig. 3-8 shows the time course of the swelling ratio of the assembly of PAAc gel particles with DNA-EtBr complexes, the gel particles and the block gel at a pH of 2.0, and Fig. 3-9 shows the time course of swelling ratio of the assembly of PAAc gel particles with DNA-EtBr complexes at pH of 7.0 and 12.0. The assembly shrunk rapidly, and the volume was constant after 180 seconds, which implies that equilibrium swelling was reached in approximately 180 seconds. Furthermore, this value of the assembly was approximately the same as the value of the gel particle, although their volumes were quite different. In contrast, the block gel, which had the same volume and chemical composition as the assembly, was also synthesized, and the pH response speeds of the block gel and the assembly were compared. The block gel with DNA shrunk slowly, and the volume was constant at approximately 6000 seconds. The swelling kinetics of the assembly was evaluated with the relaxation time, τ, by measuring the swelling ratio of the assembly as a function of time at pH of 2.0, and compared with the gel particles and the bulk gel which had the same chemical composition and volume of the assembly (Table 3-2). The $\tau_{assembly}$ was approximately 1.6×10^2 sec, and it was nearly equal to the $\tau_{particle}$, 8.7 x 10 sec and was approximately 15 times faster than that of bulk gel, τ_{bulk} 2.5×10^3. The swelling kinetics of the assembly is probably occurred by particles size effect and the formation of porous structure (Fig. 3-10). The assembly is consisted of the gel particles, and the relaxation time, τ is proportional to the square of the final diameter of a gel, thus the volume of a gel. The micro stimulus sensitive domains are independently existed in the assembly by crosslink with non-stimulus sensitive spacer molecules, and each micro domain is responded to external stimuli at approximately the same time. Furthermore, the porous structure of the assembly decreased with the relaxation time of the three-dimensional network structure and external solution easily diffuse into the network of the assembly. As the results above, the relaxation time of the assembly could be achieved a great improvement as compared with the typical bulk gel. Therefore, the particles making up the assembly responded to external stimuli at approximately the same time.

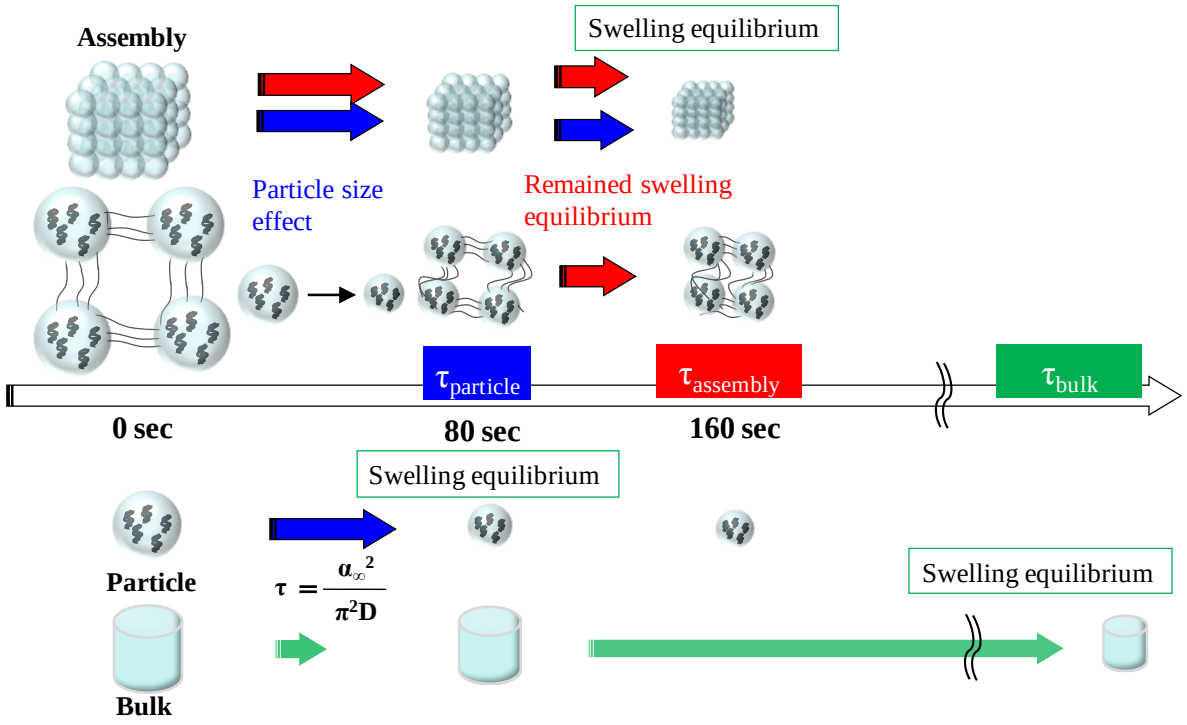

Figure 3-10 Swelling kinetics of assembly of PAAc gel particles with DNA.

3.4.5 Optical property of assembly of PAAc gel particles with DNA-EtBr complexes

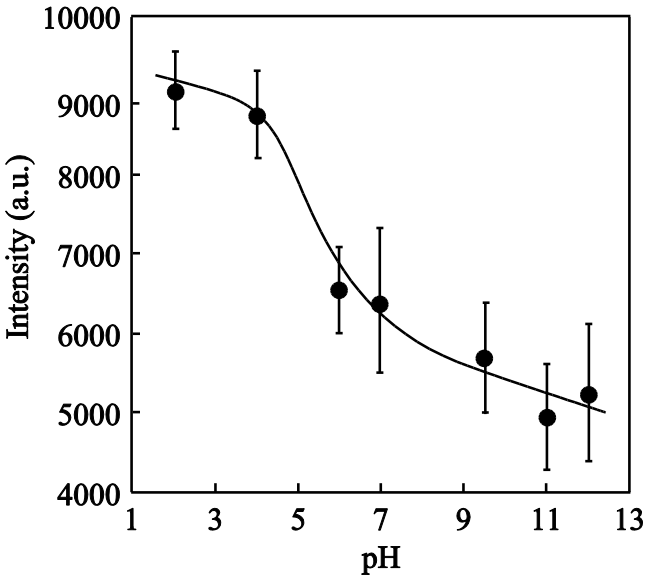

Figure 3-11 The intensity of assembly of PAAc gel particles with DNA-EtBr complexes in various pH buffer solutions at the pH value of 2.0, 4.0, 6.0, 7.0, 9.5, 11.0 and 12.0

The dye density of the assembly could be changed by volume alteration in response to pH. The evaluation of the optical properties of the assembly is important for optical sensor device applications. The absorbance of the assembly at a wavelength of 500 nm decreased exponentially with the pH value. Fig. 3-11 shows the relationship between fluorescence intensity and the pH of the assembly. The fluorescence intensity of the assembly was measured relatively with the image analysis system by irradiating UV light at a wavelength of 265 nm in buffer solution at pH 2.0, 4.0, 6.0, 7.0, 9.5, 11.0, and 12.0. The intensity of the assembly exponentially decreased with pH. In particular, the intensity of the pH values from 2.0 to the neutral region was clearly detected. In contrast, the fluorescence intensity at pH values over 11.0 was slight. Therefore, the color of the assembly of PAAc gel particles with DNA-EtBr complexes changed several times in response to pH, and it was confirmed that the EtBr was hardly eluted resulting from the absorbance spectra of DNA-EtBr in HCl and NaOH aqueous solutions. These results assume that the fluorescence intensity of the assembly of PAAc gel particles with DNA-EtBr complexes corresponded with the swelling

behavior of the assembly. It is clear that the assembly of PAAc gel particles with DNA-EtBr complexes can be used to detect and quickly measure the pH in the surrounding environment via the fluorescence intensity and that this approach could be used to create optical sensor devices.

3.5 Conclusions

In this chapter, we demonstrated the preparation of an assembly of PAAc gel particles with DNA-EtBr complexes and evaluated its pH sensitivity for application in optical detection devices. PAAc gel particles with DNA-EtBr complexes were prepared by emulsion polymerization, and the assembly was prepared by a crosslinking reaction between the gel particles that utilized glutaraldehyde. DNA was trapped in the PAAc gel particles, and it was not eluted from the gel particles. EtBr could be immobilized into the assembly by intercalation with DNA, and EtBr did not diffuse in the external solution. The assembly of PAAc gel particles with DNA-EtBr complexes could be fabricated in various shapes by utilizing various forms. The Young's modulus of the assembly was almost the same as that of the typical block gel, and it was controlled by the AA molar ratio in feed. The swelling ratio of the assembly of PAAc gel particles with DNA increased regularly with pH, and the response speed of the assembly was approximately 180 seconds. The relaxation time was approximately 15 times faster than that of the block gel, which had the same volume and chemical composition as the assembly. The fluorescence intensity of the assembly of PAAc gel particles with DNA-EtBr complexes was measured with an image analysis system, and it exponentially decreased with pH. The assembly of PAAc gel particles with DNA-EtBr complexes can quickly detect the pH in the surrounding environment as an optical signal. The high stimulus-sensitivity of the assembly of stimulus-sensitive gel particles with DNA is due to its highly porous structure, and the gel particles that formed the assembly responded to external stimuli at the same time. The porous structure is an advantage for absorbent materials. DNA can immobilize not only dyes but also harmful materials, such as carcinogens. It has been reported that gas sensor devices using DNA could detect harmful materials. The assembly might be utilized as an absorbent filter for harmful materials because the assembly has a high specific surface area and can be fabricated in various shapes based on the mold used. The assembly would also be able to detect the surrounding environmental conditions as an optical signal at the same time. Consequently, the utilization of DNA is a novel and useful approach for functional materials and the assembly of gel particles with DNA is expected to be useful as a novel optical sensing material.

References

1. Gümüşderelioğlu, M. & Topal, U. I., *Radiat. Phys. Chem.*, **73**, 272–279 (2005)

2. Osada Y. & Gong P. J., *Adv. Mater.*, **10**, 827–837(1998)

3. Miyata, T., Asami, N. & Uragami, T., *Nature*, **399**, 766–769, (1999)

4. Miyata, T., Jikihara, A., Nakamae, K. & Hoffman, A. S., *J. biomater. Sci. Polym. Edn.*, **15**, 1085–1098 (2004)

5. Miyata, T., Asami, N., and Uragami, T., *Macromolecules*, **32**, 2082–2084 (1999)

6. Dowding J. P, Vincent B., & Williams E., *J. Colloid and Interface Science*, **221**, 268–272 (2000)

7. Panayiotou M, Pohner C., Vandevyver C., Wandrey C., Hilbrig F. & Freitag R., *Reactive & Functional Polymers*, **67**, 807–819, (2007)

8. Tabata O., Hirasawa H., Aoki S., Yoshida R. & Kokufuta E., *Sensors and Actuators A*, **95**, 234–238 (2002)

9. Maeda S., Hara Y., Yoshida R. & Hashimoto S., *Advanced Robotics*, **22**, 1329–1342 (2008)

10. Akashi R., Tsutsui H. & Komura A., *Adv. Mater.*, **14**, 1808–1811 (2002)

11. Oh K. J., Drumright R., Siegwart J. D. & Matyjaszewski K., *Prog. Polym. Sci.*, **33,** 448–477 (2008)

12. Kato Y. & Tokuyama H., *Colloids and Surfaces B: Biointerfaces*, **67**, 92–98 (2008)

13. Trinh, T. Q., Gerlach, G., Sorber, J. & Arndt, K.-F., *Sens. Act. B*, **117**, 17–26 (2006)

14. Guenther, M., Gerlach, G., Corten, C., Kuckling, D., Sorber, J., Arndt, K.-F., *Sens. Act. B*, **132**, 471–476 (2008)

15. Tanaka T. & Filmore D. J., *J. Chem. Phys.*, **70**, 1214–1218 (1979)

16. Li, Y. & Tanaka T., *J. Chem. Phys.*, **92**, 1365–1371 (1990)

17. Hu Z., Lu X. and Gao J., *Adv. Mater.*, **13**, 1708–1712 (2001)

18. Zhou B., Gao J. and Hu Z., *Polymer*, **48**, 2874–2881 (2007)

19. Suzuki D. & Yoshida R., *Macromolecules*, **41**, 5830–5838 (2008)

20. Yabu H., Matsuo Y., Ijiro K., Nishino F., Takaki T., Kuwahara M., & Shimomura M., *Appl. Mater. Inter.*, **2**, 23–27 (2010)

21. Yabu H., Hirai Y., Kojima M. & Shimomura M., *Chem. Mater.*, **21**, 1787–1789 (2009)

22. Yabu H., Jia R., Matsuo Y., Ijiro K., Yamamoto S., Nishino F., Takaki T., Kuwahara M. & Shimomura M., *Adv. Mater.*, **20**, 4200–4204 (2008)

23. Kojima M., Nakanishi T., Hirai Y., Yabu H. & Shimomura M., *Chem. Commun.*, **46**, 3970–3972 (2010)

24. Douthart, J. R., Burnet, P. J., Beasley W. F., & Frank, H. B., *Biochemistry*, **12**, 214–220 (1973)

25. LePecq B. J. & Paoletti, C., *J. Mol. Biol.*, **27**, 87–106 (1967)

26. Byrne D. C., & de Mello J. A., *Biophys. Chem.*, **70**, 173–184 (1998)

27. Tsuboi M., Benevides M. J., & Thomas Jr J. G., *Biophys. J.*, **92**, 928–934 (2007)

28. Lawrence B. Hendry, Virendra B. Mahesh, Edwin D. Bransome Jr., Douglas E. Ewing, *Mutat. Res.*, **623**, 53–71 (2007)

29. Garbett, C. N., Hammond, B. N., & Graves, E. D., *Biophys. J.*, **87**, 3974–3981 (2004)

Chapter 4

Assembly of stimulus sensitive gel particles by electrostatic interaction

4. 1 Abstract

In nature, biopolymers such as proteins and nucleic acids control their complicated and higher-ordered structures precisely by self-organization which occurs by various weak interactions such as hydrogen bond, electrostatic interaction and hydrophobic interaction. These interactions make it possible to form structures dynamically and reversibility, and the material design utilized such interactions could accomplish to novel function in various fields. In this chapter, an assembly of anionic- and cationic-charged stimulus sensitive gel particles was investigated. The stimulus sensitive gels are well known as functional materials, altering their volume in response to external stimuli such as pH, temperature, electric field and bio molecules. These excellent functions are possible to be useful to bio-medical and bio-sensing application. An assembly of the stimulus sensitive gel particles formed by electrostatic interaction would be controllable to the morphology of dispersion and aggregation states by pH conditions and would achieve high responsiveness as a macro gel.

Poly (*N*-isopropylacrylamide) (PNIPAM) was copolymerized with acrylic acid (AAc) as an anionic monomer and *N*-(3-Dimethylaminopropyl) methacrylamide (DMAPMA) as a cationic monomer, respectively. Poly (NIPAM-*co*-AAc) and poly (NIPAM-*co*-DMAPMA) gel particles were prepared by soap-free emulsion polymerization. Swelling ratio of anionic and cationic gel particles was altered by dissociation of each ionic group in response to pH and zeta potential of anionic and cationic gel particles was drastically increased around the dissociation constant of each ionic groups. Anionic and cationic gel particles possessed the thermo-sensitivity from NIPAM and the volume transition temperatures were shifted toward a higher temperature by positive- and negative-charged. Binary mixtures were finely dispersed in HCl and NaOH solutions, and could assemble by electrostatic interaction in ion-exchanged water. The assembly of anionic and cationic gel particles was swollen and shrunken in response to temperature and the swelling ratio was approximately 34% compared with before heating.

4.2 Introduction

Self-assembly is the autonomous organization of various components formed into various pattern or structure without intervention[1-2]. The concept of self-assembly is great attention in various fields and is used increasingly in many processes. In living thing, many kinds of living tissue are formed by self-organization. Living cell, proteins and nucleic acids control precisely their complicated and higher-ordered structures by self-organization which occurs by various weak interactions such as hydrogen bond, electrostatic interaction and hydrophobic interaction. DNA has a double strand structure which is formed by complementary base pairing of adenine-thymine and guanine-cytosine of polynucleotides with hydrogen bonds. The inter layers of base pairs exhibits aromaticity and are intercalated planer aromatic compounds by π–π stacking[3-11]. The weak interactions make it possible to form structures dynamically and reversibility, and the material design utilized such weak interactions could accomplish to novel function in various fields.

On the other hands, stimulus sensitive gels have been attracted much attention as a smart material. The stimulus sensitive gels are flexible, elastic and wet material which is consisting of three dimensional network structures containing aqueous solution. Stimulus sensitive gels can transport the solvents or matters through the three dimensional network and stimulus sensitive gels are altered their volume in response to external stimuli such as pH, temperature, electric field and biomolecules[12-18]. These structure and characteristics are similar to living tissues. Cell, muscle, cartilage, internal organ and many other living tissues are formed from three dimensional polymer network structures containing solvents. The stimulus sensitive gels materials are expected to be developed for use in biomedical applications such as biosensor, drug delivery systems and artificial muscle [19-25].

Then, we focus on the self-organization of the stimulus sensitive gel particles by week interaction between the gel particles. An assembly of the stimulus sensitive gel particles by week interactions could dynamically disperse and aggregate in response to external environment in the same manner as the living tissues, and the bio-inspired material might be expression to novel functionalities. Furthermore, the assembly of stimulus sensitive gel particles would possess an excellent responsiveness as compared with typical macro gels because a porous structure is fabricated by the formation of interparticle spaces. These

microspaces are expected to reduce the polymer network relaxation time and easily diffuse solutions through the networks.

In this chapter, an assembly of anionic and cationic poly (*N*-isopropylacrylamide) (PNIPAM) gel particles by electrostatic interaction was investigated. PNIPAM is a representative thermo-sensitive polymer exhibiting a lower critical solution temperature at approximately 30 °C. PNIPAM gel particles were copolymerized with acrylic acid (AAc) as an anionic monomer and *N*-(3-Dimethylaminopropyl) methacrylamide (DMAPMA) as a cationic monomer. The assembly of anionic and cationic PNIPAM gel particles by electrostatic interaction would be controllable to their morphology in pH and sharply response to temperature as a macro gel.

4.3 Experimental procedure

4.3.1 Materials and reagents

N-isopropylacrylamide (NIPAM) and acrylic acid (AAc) were purchased from Kanto Chemical Co., Inc. NIPAM was purified by recrystallinezation in hexane. AAc was purified by reduced-pressure distillation. *N*-(3-Dimethylaminopropyl) methacrylamide (DMAPMA) was purchased from Tokyo Chemical Industry Co., Ltd. DMAPMA was purified by reduced-pressure distillation. *N,N'*-methylene-bis-acrylamide (MBAA), which was used as a crosslinking agent, was purchased from Wako Pure Chemical Industries, Ltd. The initiator, potassium persulfate (KPS), was purchased from Kanto Chemical Co., Inc. and 2,2'-Azobis(2-amidinopropane) dihydrochloride (V-50) was purchased from Wako Pure Chemical Industries, Ltd. The MBAA, KPS and V-50 were used without further purification.

4.3.2 Preparation of poly (NIPAM-*co*-AAc) gel particles

Poly (NIPAM-*co*-AAc) gel particles were prepared by soap-free emulsion polymerization. First, 3.1 g of NIPAM and 0.17 g of KPS were dissolved in 200 ml of ion-exchanged water. The solution was deoxidized for 30 minutes by a nitrogen atmosphere. The solution was set in four neck round-bottom flask, was stirred at 300 rpm and tuned up the temperature at 70 °C under a nitrogen atmosphere. Then, 0.17 g of AAc (molar ratio of NIPAM:AAc was 93:7) and 0.43 g of MBAA were dissolved in 10 ml of ion-exchanged water, and the solution was dropped into the flask. The resulting mixture was stirred at 300 rpm under a nitrogen atmosphere, and the polymerization was performed at 70 °C for 4 hours. The obtained poly (NIPAM-*co*-AAc) gel particles were microfiltered and washed repeatedly in ion-exchanged water with centrifugation. The poly (NIPAM-*co*-AAc) gel particles were finally immersed in ion-exchanged water.

4.3.3 Preparation of poly (NIPAM-*co*-DMAPMA) gel particles

Poly (NIPAM-*co*-DMAPMA) gel particles were prepared by soap-free emulsion polymerization. First, 3.1 g of NIPAM, 0.25 g of DMAPMA (molar ratio of NIPAM: DMAPMA was 95:5), 0.13 g of MBAA and 0.16 g of V-50 were dissolved in 210 ml of

ion-exchanged water. The mixture solution was deoxidized for 30 minutes by bubbling nitrogen. The mixture solution was set in four neck round-bottom flask, was stirred at 120 rpm and tuned up the temperature at 70 °C under a nitrogen atmosphere. The polymerization was performed at 70 °C for 24 hours. The obtained poly (NIPAM-*co*-DMAPMA) gel particles were microfiltered and washed repeatedly in ion-exchanged water with centrifugation. The poly (NIPAM-*co*-AAc) gel particles were finally immersed in ion-exchanged water.

4.3.4 pH- and thermo-sensitivity of poly (NIPAM-*co*-AAc) gel particles

The measurement of the particle size distribution of the anionic gel particles in response to the pH was performed using SALD-7100 (Shimadzu Corporation, Kyoto) with several types of buffer solutions in pH values ranging from 2.0 to 12.0. The gel particles were kept immersed in distilled water until equilibrium swelling was reached. The distilled water was then removed, and the gel particles were immersed in each buffer solution for 1 hour. The average diameter of the gel particles was measured using the particle size distribution measurement. The zeta potential of the anionic gel particles was measured by zeta potential measurement (ELSZ-2, Otsuka Electronics Co. Ltd., Osaka). The gel particles were kept immersed in several types of buffer solutions with pH values ranging from 2.0 to 12.0, until equilibrium swelling was reached. Then, the zeta potential of the gel particles was measured by ELSZ-2.

The transmittance of the anionic gel particles was performed by UV-Visible spectrophotometer (UV-1800, Shimadzu corporation, Kyoto) with several types of buffer solutions with pH values ranging from 4.0 to 8.0. 0.1 g of the gel particles were immersed in 10 ml of each buffer solution for 1 hour. The transmittance wavelength at 500 nm of the gel particles were measured by UV-1800. The thermo-sensitivity of the anionic gel particles were evaluated with the particle size distribution alteration in response to the temperature. 0.1 g of the gel particles were immersed in 10 ml of each buffer solution until equilibrium swelling was reached. Then, the average diameter of the gel particles was measured by ELSZ-2 (Otsuka Electronics Co. Ltd., Osaka), while changing the temperature range from 20 to 60 °C. The particle diameter alteration ratio was calculated from before and after

average diameter in response to temperature.

4.3.5 pH- and thermo-sensitivity of poly (NIPAM-*co*-DMAPMA) gel particles

The measurement of the particle size distribution of the cationic gel particles in response to the pH was performed using ELSZ-2 (Otsuka Electronics Co. Ltd., Osaka) with several types of buffer solutions with pH values ranging from 2.0 to 12.0. The gel particles were kept immersed in distilled water until equilibrium swelling was reached. The distilled water was then removed, and the gel particles were immersed in each buffer solution for 1 hour. The average diameter of the gel particles was measured using the particle size distribution measurement. The zeta potential of the cationic gel particles was measured by zeta potential measurement (ELSZ-2, Otsuka Electronics Co. Ltd., Osaka). The gel particles were kept immersed in several types of buffer solutions with pH values ranging from 2.0 to 12.0, until equilibrium swelling was reached. Then, the zeta potential of the gel particles was measured by ELSZ-2.

The transmittance of the cationic gel particles was performed by UV-Visible spectrophotometer (UV-1800, Shimadzu corporation, Kyoto) with several types of buffer solutions with pH values ranging from 4.0 to 12.0. 0.1 g of the gel particles were immersed in 10 ml of each buffer solution for 1 hour. The transmittance wavelength at 500 nm of the gel particles were measured by UV-1800. The thermo-sensitivity of the cationic gel particles were evaluated with the particle size distribution alteration in response to the temperature. 0.1 g of the gel particles were immersed in 10 ml of each buffer solution until equilibrium swelling was reached. Then, the average diameter of the gel particles was measured by ELSZ-2 (Otsuka Electronics Co. Ltd., Osaka), while changing the temperature range from 20 to 60 °C. The particle diameter alteration ratio was calculated from before and after average diameter in response to temperature.

4.3.6 Assembly of poly (NIPAM-*co*-AAc) and poly (NIPAM-*co*-DMAPMA) gel particles by electrostatic interaction

Assembly of the anionic and cationic gel particles by electrostatic interaction was observed by visual and microscopy, and measured by particle size distribution measurement

(SALD-7100, Shimadzu Corporation, Kyoto). 0.5 g of anionic and cationic gel particles were mixed in 5 ml of ion-changed water, 0.01 of HCl and NaOH aqueous solution, respectively. The mixture solution was observed in visual and measured particle size distribution by SALD-7100. Furthermore, the temperature of the mixture solution was tuned up at 60 °C, and the above measurements were similarly performed.

Swelling ratio of the assembly of the anionic and cationic gel particles was measured by image analysis system of microscope (STZ-168-TL, Shimadzu Corporation, Kyoto). The assembly was immersed in ion-changed water, and the area was measured by the image analysis system. Then, the assembly was set on a hot stage and tuned up the temperature at 60 °C. The area after heating was similarly measured and swelling ratio of the assembly was calculated from the area before and after heating. The reversibility was evaluated by repeatedly heating and cooling.

4.4 Results and discussion

4.4.1 Preparation of poly (NIPAM-*co*-AAc) and poly (NIPAM-*co*-DMAPMA) gel particles

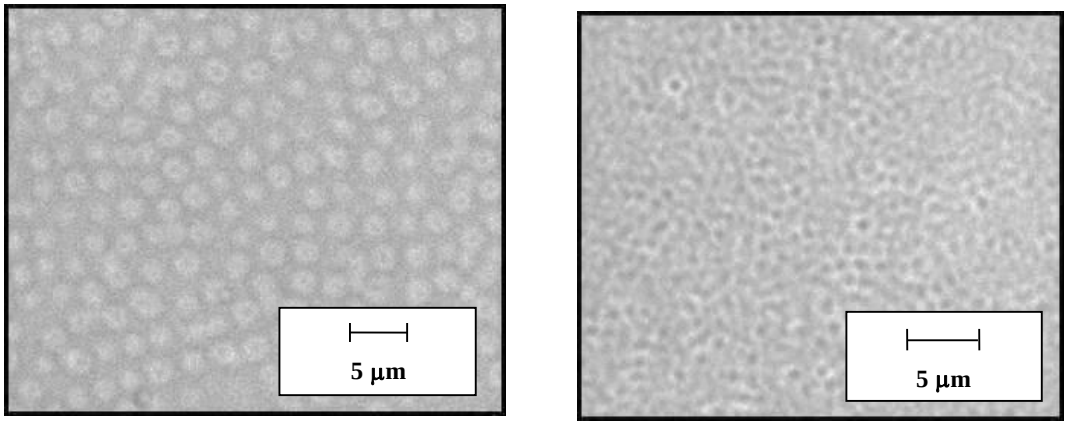

Figure 4-1 Optical micrographs of poly (NIPAM-*co*-AAc) and (NIPAM-*co*-DMAPMA) gel particles

Table 4-1 Properties of poly (NIPAM-*co*-AAc) and (NIPAM-*co*-DMAPMA) gel particles

	Poly (NIPAM-*co*-AAc) gel particle	Poly (NIPAM-*co*-DMAPMA) gel particles
Average diameter (μm)	1.688	0.615
Standard deviation (μm)	0.123	0.114
CV (%)	7.3	18.5

Figure 4-1 shows the optical micrographs of the obtained poly (NIPAM-*co*-AAc) and (NIPAM-*co*-DMAPMA) gel particles, and table 4-1 shows their properties. Poly (NIPAM-*co*-AAc) gel particles could be prepared by soap-free emulsion polymerization. The average diameter of the gel particles in pH 7.0 buffer solution was approximately 1.70

μm, and the coefficient of valance (CV) was approximately 7.1%, which was calculated from the equation: standard division / average diameter x 100 (%). The obtained gel particles were high monodispersity. FT-IR spectra of poly (NIPAM-*co*-AAc) was confirmed the characteristic absorptions at 1650 cm^{-1} and 1550 cm^{-1} from amido bond, 1370 cm^{-1} from isopropyl group and 1715 cm^{-1} form carboxyl group. Poly (NIPAM-*co*-AAc) gel particles were complementary prepared.

Poly (NIPAM-*co*-DMAPMA) gel particles could be prepared by soap-free emulsion polymerization. The average diameter of the gel particles in pH 7.0 buffer solution was approximately 0.62 μm, and the coefficient of valance (CV) was approximately 18%, which was calculated from the equation: standard division / average diameter x 100 (%). The obtained gel particles were nearly monodispersity.

4.4.2 pH-sensitivity of poly (NIPAM-*co*-AAc) and poly (NIPAM-*co*-DMAPMA) gel particles

The average diameter of the poly (NIPAM-*co*-AAc) gel particles in response to the pH was measured in pH buffer solution with pH value ranging from 2.0 to 12.0 shown in Figure 4-2 (left). The average diameter at pH 2 was approximately 1.3 μm. The average diameter was drastically changed with pH value ranging from 4.0 to 7.0. The dissociation constant of AAc is approximately 4.25, and PAAc network was swollen by electrostatic repulsion between carboxylate anions above pH value of 4. Over pH value of 7.0, the average diameter was almost constant value. The zeta potential of the poly (NIPAM-*co*-AAc) gel particles in response to the pH was similarly measured in pH buffer solution with pH value ranging from 2.0 to 12.0 (Figure 4-2, right). The gel particles were charged negatively and the maximum value was approximately -11 mV. The zeta potential was drastically changed with pH value ranging from 4.0 to 7.0 because the dissociation constant of AAc was approximately 4.25. From these results, the obtained poly (NIPAM-*co*-AAc) gel particles were possessed pH-sensitivity.

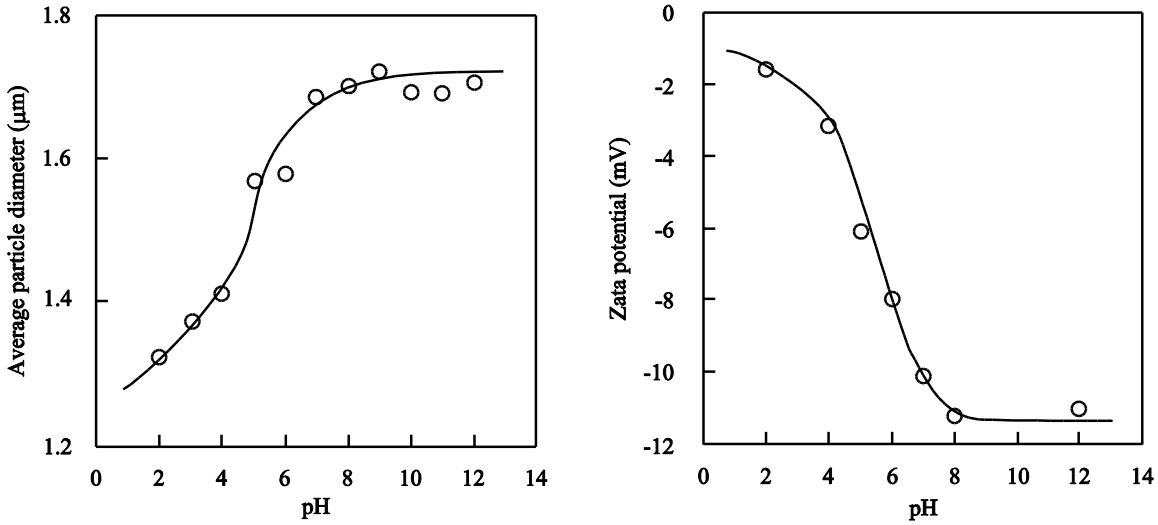

Figure 4-2 pH-sensitivity of poly (NIPAM-*co*-AAc) gel particles at pH value ranging from 2.0 to 12.0. The left side indicates the average particle diameter of the anionic gel particles in various pH values measuring with particle size distribution analysis. The right side indicates the zeta potential of the anionic gel particles in various pH values.

The average diameter of the poly (NIPAM-*co*-DMAPMA) gel particles in response to the pH was measured in pH buffer solution with pH value ranging from 2.0 to 12.0 (Figure 4-3, left). The average diameter at pH 2 was approximately 0.61 μm. The average diameter was drastically changed with pH value ranging from 7.0 to 10.0. The dissociation constant of DMAPMA is approximately 9.2, and the DMAPMA network was swollen by electrostatic repulsion below pH value of 10. Over pH value of 10.0, the average diameter was approximately 0.55 μm, almost constant value. The zeta potential of the poly (NIPAM-*co*-DMAPMA) gel particles in response to the pH was similarly measured in pH buffer solution with pH value ranging from 2.0 to 12.0 (Figure 4-3, right). The gel particles were charged positively and the maximum value was approximately -5 mV. The zeta potential was drastically changed with pH value ranging from 7.0 to 10.0 because the dissociation constant of DMAPMA was approximately 9.2. From these results, the obtained poly (NIPAM-*co*-DMAPMA) gel particles were possessed pH-sensitivity.

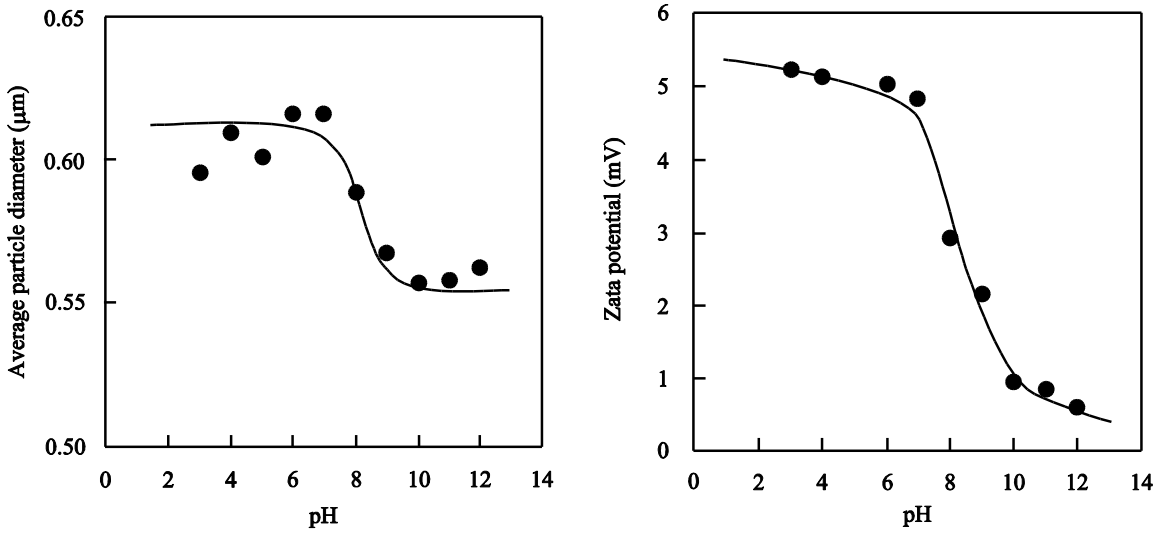

Figure 4-3 pH-sensitivity of poly (NIPAM-*co*-DMAPMA) gel particles at pH value ranging from 2.0 to 12.0. The left side indicates the average particle diameter of the cationic gel particles in various pH values measuring with particle size distribution analysis. The right side indicates the zeta potential of the cationic gel particles in various pH values

4.4.3 Thermo-sensitivity of poly (NIPAM-*co*-AAc) and poly (NIPAM-*co*-DMAPMA) gel particles

The transmittance of poly (NIPAM-*co*-AAc) gel particles was measured by UV-Visible spectrophotometer with pH buffer solutions at pH values of 4.0, 6.0 and 8.0 (Figure 4-4, left). PNIPAM has a lower solution critical temperature (LCST) at approximately 30 °C. In buffer solution at pH value of 4.0, the transmittance of the gel particles wavelength at 500 nm was drastically decreased from the solution temperature at 30 °C. This result was similar to the behavior of PNIPAM gel. In contrast, the transmittances of the gel particles at pH value of 6.0 and 8.0 were not decreased at 30 °C. In buffer solution at pH value of 6.0, the transmittance was drastically decreased from the solution temperature at 50 °C, and the transmittance of the gel particles at pH value of 8.0 was not decreased above the solution temperature at 50 °C. The LCST shift toward a higher temperature is attributed to the electrostatic repulsion between the gel network and increase of hydrophilic property by

AAc dissociation. The results of particle size distillation of the poly (NIPAM-*co*-AAc) gel particles in response to temperature were similar to the results of the transmittance (Figure 4-4, right). In buffer solution at pH value of 4.0, the gel particles were rapidly shrunken at 30 °C and the diameter change modulus was approximately 50% compared with before heating. From the results of the particle diameter change modulus at several pH values, the temperature of volume phase transition was shifted toward a higher temperature with the increasing pH value, thus increasing AAc dissociation.

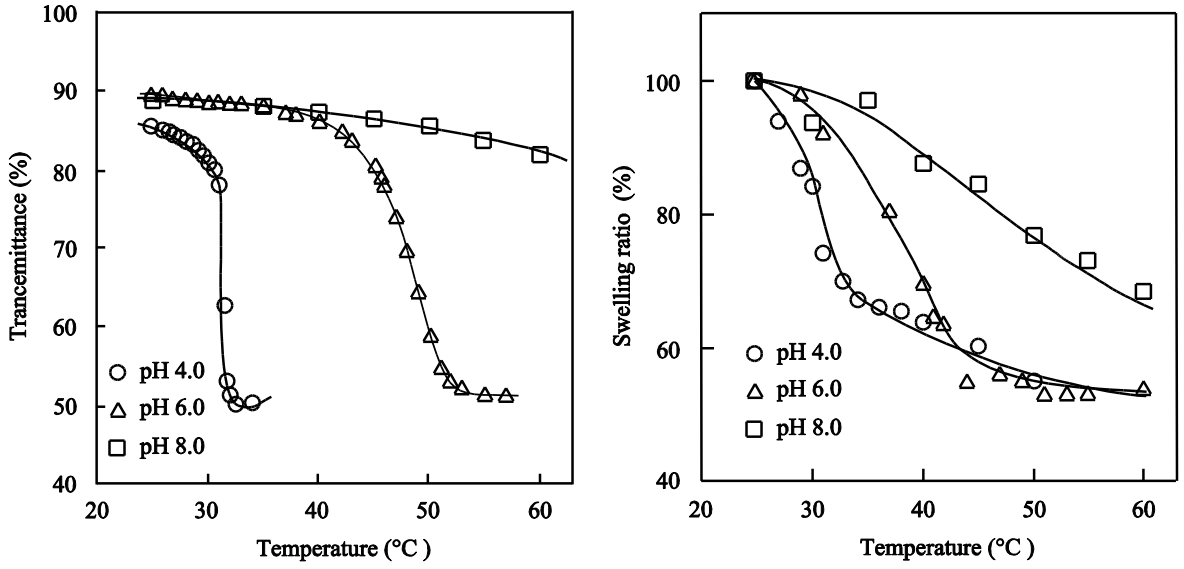

Figure 4-4 Thermo-sensitivity of the poly (NIPAM-*co*-AAc) gel particles with temperature ranging from 20 to 60 °C in pH values of 4.0, 6.0 and 8.0. The left side indicates the transmittance of the anionic gel particles wavelength at 500 nm measuring with UV-Vis spectrometry. The right side indicates the swelling ratio of the anionic gel particles.

The transmittance of poly (NIPAM-*co*-DMAPMA) gel particles was measured by UV-Visible spectrophotometer with pH buffer solutions at pH values of 4.0, 9.0 and 12.0 (Figure 4-5, left). In buffer solution at pH value of 12.0, the transmittance of the gel particles wavelength at 500 nm was drastically decreased from the solution temperature at 31 °C. This result was similar to the behavior of PNIPAM gel. In buffer solution at pH

value of 9.0, the transmittance was drastically decreased from the solution temperature at 41 °C, and the transmittance of the gel particles at pH value of 4.0 was not decreased above the solution temperature at 50 °C. The LCST shift toward a higher temperature is attributed to the electrostatic repulsion between the gel network and increase of hydrophilic property by positively charged DMAPMA. The results of particle size distillation of the poly (NIPAM-*co*-DMAPMA) gel particles in response to temperature were shown in Figure 4-5, right. In buffer solution at pH value of 4.0, the gel particles were rapidly shrunken at 31 °C and the diameter change modulus was approximately 55% compared with before heating. From the results of the particle diameter change modulus at several pH values, the temperature of volume phase transition was shifted toward a higher temperature with the decreasing pH value, thus increasing positively-charged DMAPMA. It was confirmed that Both the poly (NIPAM-*co*-AAc) and the poly (NIPAM-*co*-DMAPMA) gel particles possessed thermo-sensitivity and the thermo-sensitive behavior was altered to pH conditions.

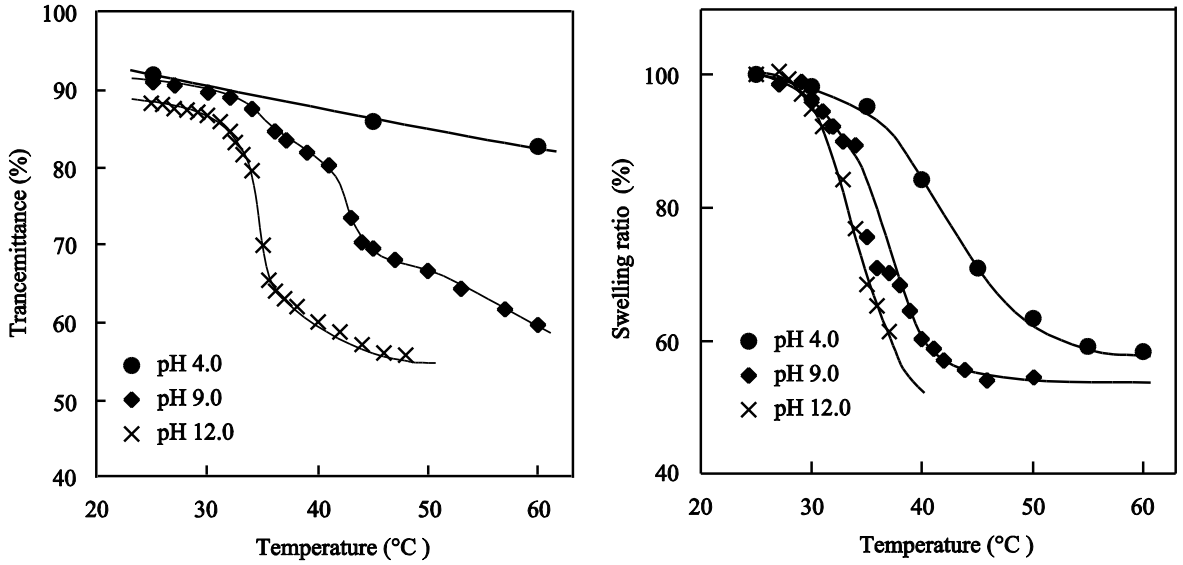

Figure 4-5 Thermo-sensitivity of the poly (NIPAM-*co*-DMAPMA) gel particles with temperature ranging from 20 to 60 °C in pH values of 4.0, 9.0 and 12.0. The left side indicates the transmittance of the cationic gel particles wavelength at 500 nm measuring with UV-Vis spectrometry. The right side indicates the swelling ratio of the cationic gel particles.

4.4.4 Assembly of poly (NIPAM-*co*-AAc) and poly (NIPAM-*co*-DMAPMA) gel particles by electrostatic interaction

Table 4-2 Observation in visual of binary mixtures of anionic and cationic gel particles in ion exchanged water, 0.01 mol/l solutions of HCl and NaOH, at 25 and 60 °C

	Mixture solutions of the anionic and cationic gel particles		
	0.01 M HCl pH 12.0	Ion exchanged water pH 6.4	0.01 M NaOH pH 12.0
25 °C			
60 °C			

Assembly of the poly (NIPAM-*co*-AAc) and poly (NIPAM-*co*-DMAPMA) gel particles by electrostatic interaction was observed in visual by immersing both gel particles in ion-exchanged water (pH 6.4) and 0.01 N of HCl and NaOH aqueous solutions (Table 4-2). Both anionic and cationic gel particles were finely dispersed in HCl and NaOH solutions. From the above results, only the cationic gel particles were charged in HCl solution, and only the anionic gel particles were charged in NaOH solution. On the other hand, in ion-exchanged water, the aggregates consist from both the gel particles were observed and settled out to the bottom of sample tube. This result indicated that the cationic and anionic gel particles could assemble by electrostatic interaction because both the gel particles were well charged in ion-exchanged water (pH 6.4) from zeta potential results. These behaviors

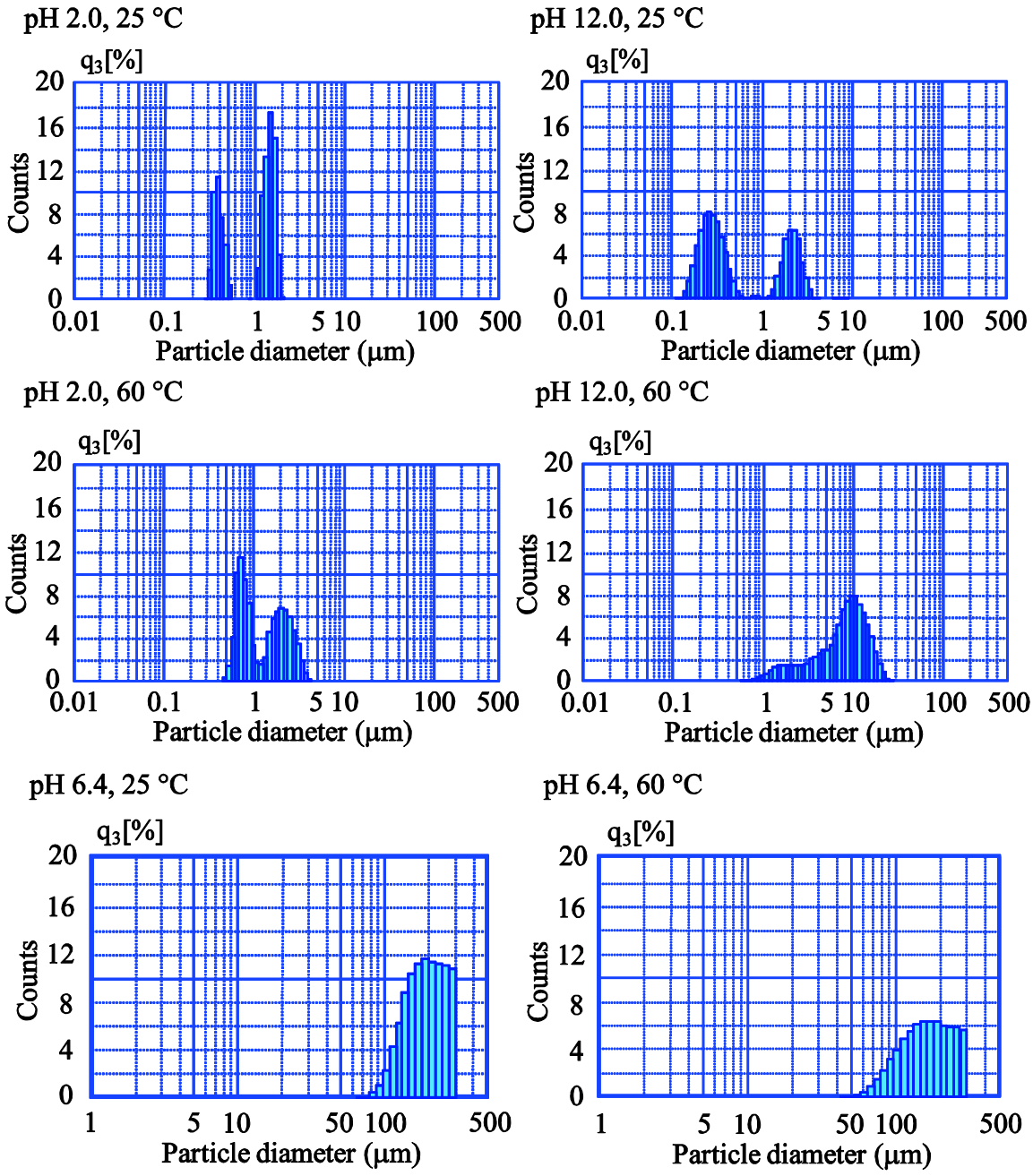

Figure 4-6 Particle size distributions of binary mixtures of anionic and cationic gel particles in ion-exchanged water, HCl and NaOH solutions at the temperature of 25 and 60 °C.

were also investigated by using particle size distribution measurement (Figure 4-6). In HCl and NaOH solutions, each particle size distribution peak of the cationic and anionic gel particles was observed in several. In contract, the particle size distribution peak of the gel particles in ion-exchanged water was observed only a single distribution peak and shifted to a few hundred micrometers. Consequently, the cationic and anionic gel particles were well dispersed in the mixture solution while only the either particle charging and assembled while both the particles well-charging.

Therefore, the above behaviors were also observed at 60 °C. In HCl and NaOH, the mixture solutions got a white turbidity, being tuned up the temperature at 60 °C. The peaks particle size distribution were only a few overlapped with each other. This result considered that the charge density of the gel particles was enhanced by shrinking the volume in response to temperature and the hydrophobic interaction between the gel particles was occurred by tuned up the temperature above LCST. In ion-exchanged water, the assembly of the gel particles was shrunken rapidly in visual. The particles size distribution peak in ion-exchanged water was shifted to a lower particle diameter. The assembly of anionic and cationic gel particles was swollen and shrunken in response to temperature and could be visually workable as a ‘macro gel’. Then, swelling ratio of the assembly of cationic and anionic gel particles was measured by image analysis system of microscope shown in Figure 4-7. Swelling ratio of the assembly of cationic and anionic gel particles was calculated from 10 measurement of the area change modulus of the assembly before and after heating. The assembly was shrunken rapidly by tuned up the temperature at 60 °C, and the swelling ratio was approximately 34% compared with before heating. The swelling ratio of the assembly was much larger than that of anionic and cationic gel particles, 58% and 50% respectively. This result suggests that the assembly has a high porous structure. The interparticle spaces enhance the stimulus-sensitivity due to decrease of polymer network relaxation time and high solution diffusion rate. Furthermore, the assembly could be repeatedly swollen and shrunken within 5% of volume fluctuation, with 5 cycles of heating and cooling (Figure 4-8). The assembly of anionic and cationic gel particles could be controlled dispersion and assembly states by pH condition and could achieve high sensitivity and reversibility. The assembly of gel particles by electrostatic interaction was

expected to application for biomedical and sensor devices such as drug carriers.

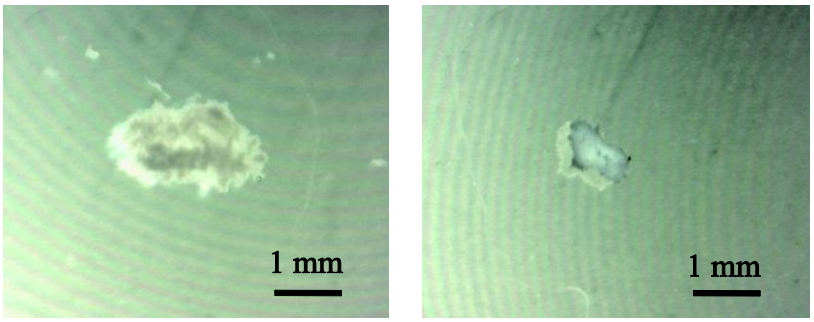

Figure 4-7 Optical micrographs of assembly of anionic and cationic gel particles in ion-exchanged water. The left side shows the assembly at temperature of 25 and the right side shows that of 60 °C.

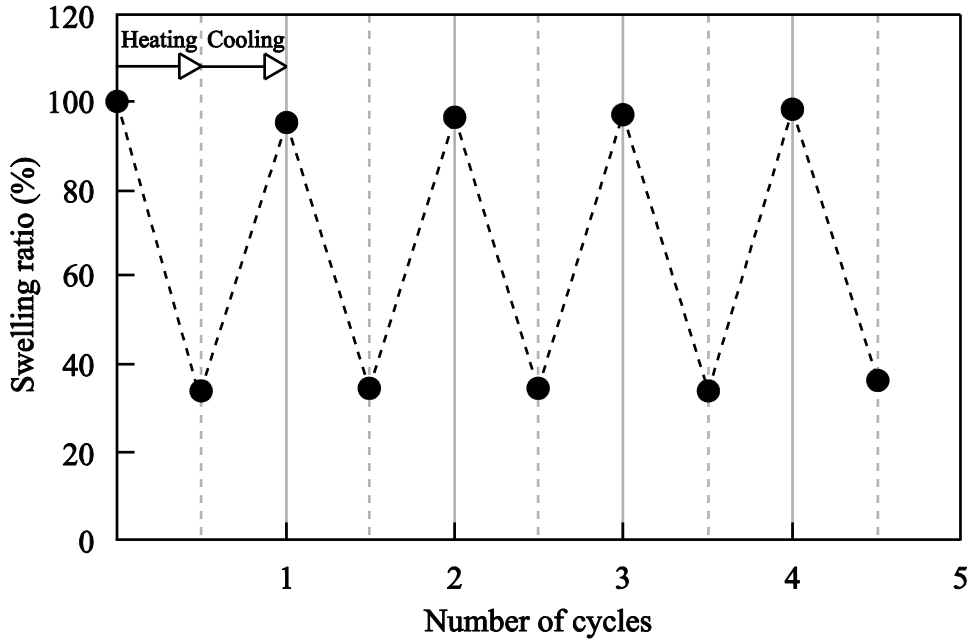

Figure 4-8 Swelling ratio of assembly of anionic and cationic gel particles in ion-exchanged water at 5 cycles of heating and cooling.

4.5 Conclusions

In this chapter, we demonstrated the preparation of an assembly of poly (NIPAM-*co*-AAc) and poly (NIPAM-*co*-DMAPMA) gel particles by electrostatic interaction and evaluated its characteristics. Poly (NIPAM-*co*-AAc) gel particles as an anionic gel particle and poly (NIPAM-*co*-DMAPMA) gel particles as a cationic gel particle could be prepared by soap–free emulsion polymerization. Swelling ratio of anionic and cationic gel particles was altered by dissociation of each ionic group in response to pH and zeta potential of anionic and cationic gel particles was drastically increased around the dissociation constant of each ionic groups. Anionic and cationic gel particles possessed the thermo-sensitivity from NIPAM characteristics and the volume transition temperatures were shifted toward a higher temperature by positive- and negative-charged. Both anionic and cationic gel particles were finely dispersed in HCl and NaOH solutions, and in ion-exchanged water, cationic and anionic gel particles could assemble by electrostatic interaction because both the gel particles were well-charged in ion-exchanged water. The assembly of anionic and cationic gel particles was swollen and shrunken in response to temperature and the swelling ratio was approximately 34% compared with before heating. The swelling ratio of the assembly was much larger than that of anionic and cationic gel particles. Furthermore, the assembly could be repeatedly swollen and shrunken within 5% of volume fluctuation. The assembly of anionic and cationic gel particles is controllable to dispersion and assembly states by pH conditions and could be visually workable as a one of a ‘macro gel’, having a excellent properties.

References

1. Whitesides G. M. & Grzybowski B., *Science*, **295**, 2418–2421 (2002)

2. Philip D. & Stoddart J. F., *Angew. Chem. Int. Ed*., **35**, 1154–1196 (1996)

3. Waring M. J., *J. Mol. Biol.*, **13**, 269–282 (1965)

4. LePecq J. B. & Paoletti C., *J. Mol. Biol*., **27**, 87–106 (1967)

5. Burns V. W. F., *Arch. Biochem. Biophys.*, **133**, 420–424 (1969)

6. Hudson B. & Jacobs R., *Biopolymers*, **14**, 1309–1312 (1975)

7. Waleh A., Hudson B. & Loew G., *Biopolymers*, **15**, 1637–1640 (1976)

8. Olmsted J. & Kearns D. R., *Biochemistry*, **16**, 3647–3654 (1977)

9. Grote J. G., Hagen J. A., Zetts J. S., Nelson R. L., Diggs D. E., Stone M. O., Yaney P. P., Heckman E., Zhang C., Steier W. H., Jen A. K. Y., Dalton L. R., Ogata N., Curley M. J., Clarson S. J. & Hopkins F. K., *J. Phys. Chem. B*, **108**, 8584–8591 (2004)

10. Grote J. G., Diggs D. E., Nelson R. L., Zetts J. S., Hopkins F. K., Ogata N., Hagen J. A., Heckman E., Yaney P. P., Stone M. O. & Dalton L. R., *Mol. Cryst. Liq. Cryst.*, **426**, 3–17 (2005)

11. Kawabe Y., Wang L., Horinouchi S. & Ogata N., *Adv. Mater.*, **12**, 1281–1283 (2000)

12. Gümüşderelioğlu M. & Topal U. I., *Radiat. Phys. Chem.*, **73**, 272–279 (2005).

13. Osada Y. & Gong P. J., *Adv. Mater.*, **10**, 827–837(1998)

14. Miyata T., Asami N. & Uragami T., *Nature*, **399**, 766–769, (1999).

15. Miyata T., Jikihara A., Nakamae K. & Hoffman A. S., *J. biomater. Sci. Polym. Edn.*, **15**, 1085–1098 (2004)

16. Miyata T., Asami N., and Uragami T., *Macromolecules*, **32**, 2082–2084 (1999)

17. Dowding J. P, Vincent B., & Williams E., *J. Colloid and Interface Science*, **221**, 268–272 (2000)

18. Panayiotou M, Pohner C., Vandevyver C., Wandrey C., Hilbrig F. & Freitag R., *Reactive & Functional Polymers*, **67**, 807–819, (2007)

19. Tabata O., Hirasawa H., Aoki S., Yoshida R. & Kokufuta E., *Sensors and Actuators A*, **95**, 234–238 (2002)

20. Maeda S., Hara Y., Yoshida R. & Hashimoto S., *Advanced Robotics*, **22**, 1329–1342 (2008)

21. Akashi R., Tsutsui H. & Komura A., *Adv. Mater.*, **14**, 1808–1811 (2002)

22. Oh K. J., Drumright R., Siegwart J. D. & Matyjaszewski K., *Prog. Polym. Sci.*, **33,** 448–477 (2008)

23. Kato Y. & Tokuyama H., *Colloids and Surfaces B: Biointerfaces*, **67**, 92-98 (2008)

24. Trinh T. Q., Gerlach G., Sorber J. & Arndt K.-F., *Sens. Act. B*, **117**, 17–26 (2006)

25. Guenther M., Gerlach G., Corten C., Kuckling D., Sorber J., Arndt K.-F., *Sens. Act. B*, **132**, 471–476 (2008)

Chapter 5

Concluding remarks

Assemblies of stimulus sensitive gel particles by crosslink with spacer molecules and interaction between the gel particles were designed as a novel stimulus sensitive gel material, which possessed excellent stimulus sensitivities and dynamically changed their morphology in aqueous solution, and a composite of stimulus sensitive gel and DNA was prepared to apply as an optical-detected bio sensor by utilizing DNA intercalation. The results and conclusion are followings.

(1) Poly (acrylic acid) (PAAc) gel particles with DNA could prepare by emulsion polymerization and PAAc gel particles with DNA-ethidium bromide (EtBr) complexes were prepared with only immersion the gel particles in EtBr solution. PAAc gel particles with DNA-EtBr complexes could immobilize EtBr in the gel particles by intercalation of DNA, and the EtBr was not diffuse to external solution from the gel particles. The swelling ratio of the gel particles was regularly altered with pH and the fluorescence intensity of the gel particles was exponentially decreased with pH. The gel particles could detect external pH information as a photo signal by the alteration of their swelling ratio.

(2) An assembly of PAAc gel particles with DNA-EtBr complexes was prepared by condensation reaction between amino groups of the gel particles surface with glutaraldehyde. The assembly could from various shapes such as sheet and block-like with various shaped molds. The Young's modulus of the assembly was increased with the molar ratio of incorporated allylamine (AA) in feed. The maximum value was approximately 10 kPa in 20 mol% of AA in feed and was nearly equal to that of the same chemical composition and volume of bulk gel. The swelling kinetics of the assembly was evaluated with the relaxation time, τ, which was calculated from the results of swelling ratio of the assembly as a function of time, and compared with the gel particles and the bulk gel which had the same chemical composition and volume of the assembly. The $\tau_{assembly}$ was approximately 1.6×10^2 sec, and it was nearly equal to the $\tau_{particle}$, 8.7 x 10 sec and was approximately 15 times faster than that of bulk gel, τ_{bulk} 2.5×10^3. The swelling kinetics of the assembly is probably occurred by particles size effect and the formation of porous structure. The assembly is consisted of the gel particles, and relaxation time, τ is proportional to the square of the final diameter of a gel, thus the volume of a gel. These micro stimulus sensitive domains in the assembly are independently existed by crosslink

with non-stimulus sensitive spacer molecules, and each micro domain is responded to external stimuli at approximately the same time. Furthermore, porous structure of the assembly is decreased with the relaxation time of the three-dimensional network structure and external solution diffuses easily into the network of the assembly. As the described above, the relaxation time of the assembly could be achieved a great improvement as compared with the typical bulk gel. The swelling ratio of the assembly was regularly increased with pH and the fluorescence intensity was regularly decreased with the increase of the swelling ratio. The assembly of PAAc gel particles with DNA-EtBr complexes was exhibited an excellent stimulus sensitivities and could be detected to external environments as a photo signal.

(3) An assembly of anionic and cationic gel particles by electrostatic interaction was prepared and evaluated its characteristics. AAc as an anionic monomer and *N*-(3-dimethylaminopropyl) methacrylamide (DMAPMA) as a cationic monomer were copolymerized with *N*-isoproprylacrylamide (NIPAM). Poly (NIPAM-*co*-AAc) gel particles and poly (NIPAM-*co*-DMAPMA) gel particles could be prepared by soap–free emulsion polymerization. The swelling ratio of anionic and cationic gel particles was altered by dissociation of each ionic group in response to pH, and the zeta potential of anionic and cationic gel particles was drastically increased around the dissociation constant of each ionic groups. The anionic and cationic gel particles possessed the thermo-sensitivity from NIPAM characteristics and the volume transition temperatures were shifted toward a higher temperature by positively and negatively charged. Binary mixture of anionic and cationic gel particles was finely dispersed in HCl and NaOH solutions, and in ion-exchanged water, the anionic and cationic gel particles could assemble by electrostatic interaction because both the gel particles were well-charged in ion-exchanged water. The assembly of anionic and cationic gel particles was swollen and shrunken in response to temperature and the swelling ratio was approximately 34% compared with before heating. The swelling ratio of the assembly was much larger than that of the anionic and cationic gel particles. Furthermore, the assembly could be repeatedly swollen and shrunken within 5% of volume fluctuation. The assembly of the anionic and cationic gel particles is controllable to dispersion and assembly states by pH conditions and could be visually workable as a one of

a ‘macro gel’, having a excellent properties.

As described above, DNA hybridization in stimulus sensitive gel is possible to create a photo detection property and the composite material of stimulus sensitive gel and DNA would be expected to application in optical bio sensors. The assembly of stimulus sensitive gel particles can provide an excellent stimulus response time and the assembly by electrostatic interaction can be dynamically changed their morphology in response to pH. The novel gel material design would contribute to gel application in various field.

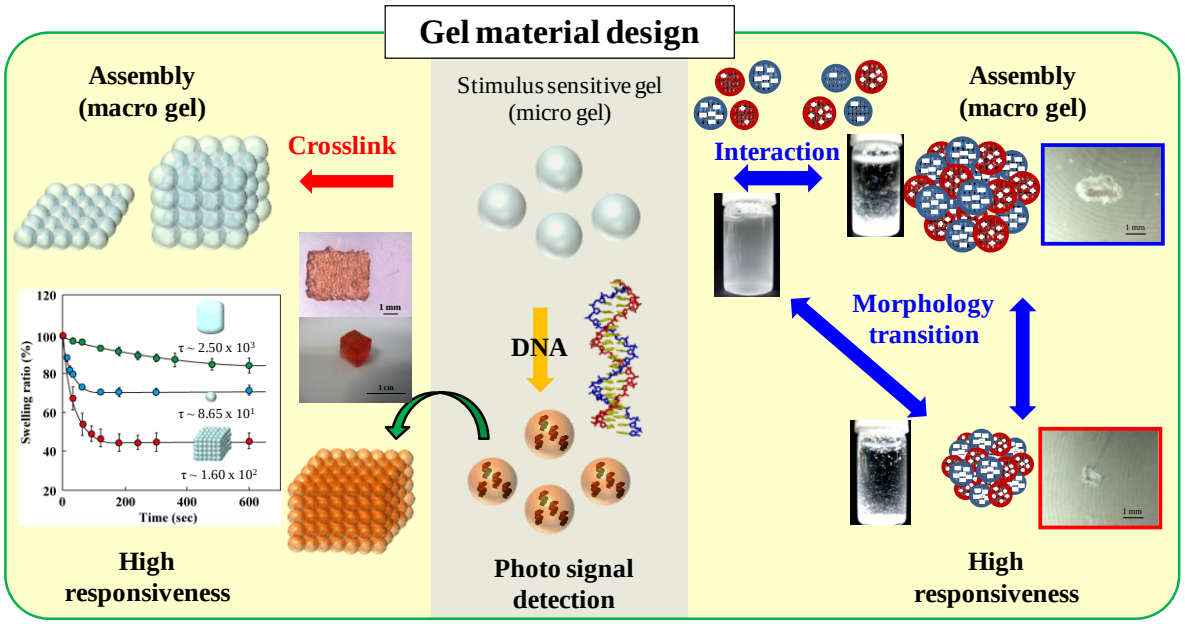

Figure 5-1 Designs of stimulus sensitive gel material in this study

List of publications

1. “Preparation of stimulus-sensitive gel particles with a DNA-dye complex and their pH sensitivity”, Takashi Nishiyama, Yoshiharu Kagami, Takeshi Yamauchi and Norio Tsubokawa, *Polym. J.*, **44**, 396-400 (2012) (**Chapter 2**)

2. “Assembly of stimulus-sensitive gel particle with DNA-dye complexes”, Takashi Nishiyama, Yoshiharu Kagami, Takeshi Yamauchi and Norio Tsubokawa, *Polym. J.*, in press **(Chapter 3)**

3. “Assembly of stimulus sensitive gel particles by electrostatic interaction”, Takashi Nishiyama, Tomohiro Hirano, Takeshi Yamauchi and Norio Tsubokawa, in preparation (**Chapter 4**)

4. “pH Responsive characteristics of micro gel with DNA-dye complex”, Takashi Nishiyama, Takeshi Yamauchi, Norio Tsubokawa and Yoshiharu Kagami, SAMPE 2008, Technical Conference Proceedings, CD-ROM-7 pp. (**Chapter 2**)

Acknowledgements

This dissertation has been carried out during 2009-2012 as a doctorate thesis under the direction of Professor Dr. Takeshi Yamauchi at the Graduate school of Science and Technology, Niigata University.

The author would like to express his deepest gratitude to Professor Dr. Takeshi Yamauchi, Faculty of Engineering, Niigata University for his constant guidance and kind suggestions throughout this study.

The author is also deeply thankful to Professor Dr. Norio Tsubokawa, Faculty of Engineering, Niigata University for his reviewing this thesis, helpful advice and discussions.

The author is indebted to Professor Dr. Masayuki Yagi, Faculty of Engineering, Niigata University, Professor Dr. Toshiki Aoki, Faculty of Engineering, Niigata University, Professor Dr. Takashi Kaneko, Graduate school of Science and Technology, Niigata University, for their reviewing this thesis.

The author is deeply grateful to Dr. Yoshiharu Kagami for his helpful advices and suggestions. The author could not have been accomplished the results of this study, without his help.

The author wishes to thank Mr. Tomohiro Hirano for his collaboration in this study, and also appreciate Mrs. Kumi Satoh, Mr. Hiroshi Saitoh and all current and former colleagues of Tsubokawa and Yamauchi laboratory for their help, discussion and many suggestions.

Finally, the author would like to express my hearty gratitude to my family for their encouragement and many supports throughout my fulfillment of this study.

Doctoral Program in Advanced Materials Science and Technology,
Graduate School of Science and Technology,
Niigata University,
March, 2013.