

Molecular Recognition Systems Designed from Novel Functional Polymer Materials

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Yasumasa Kanekiyo

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Chapter 1 Introduction to the field of molecular recognition

1.1 Polymer materials as a platform for molecular recognition	12
1.2 Introduction	14
1.3 Properties of novel polymer films	15
1.4 Synthesis and characterization of films	16
1.5 Self-assembly of functional polymer materials	17
1.6 Construction of novel molecular recognition systems	18
1.7 Effect of polymer structure on molecular recognition	20
1.8 Conclusion	21
1.9 Introduction	22
1.10 References and notes	23

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Chapter 2 Molecular recognition in polymer complexes

2.1 Introduction	26
2.2 Design of polymer complexes	27
2.3 Synthesis of ACP-templated polymer complexes	40
2.4 Binding of ACP to polymer complexes	42
2.5 Sensing function of ACP-templated polymer complexes	44
2.6 Self-assembly of binding and sensing	46
2.7 Binding response to analytes	47

Yasumasa Kanekiyo

CONTENTS

Chapter 1	General introduction	1
Chapter 2	Facile construction of a novel metal-imprinted polymer surface without a polymerization process	14
2.1	Introduction	14
2.2	Preparation of metal-imprinted films	15
2.3	Spectroscopic characterization of films	18
2.4	Stoichiometry of imprinted metal complexes	23
2.5	Evaluation of metal re-binding ability	25
2.6	Effect of poly(propylene glycol) blending	27
2.7	Conclusion	33
2.8	Experimental	33
2.9	References and notes	35
Chapter 3	Molecular imprinting in polyion complexes	38
3.1	Introduction	38
3.2	Preparation of polyion complexes	43
3.3	Removal of AMP from a polyion complex	46
3.4	Re-binding of AMP to polyion complexes	46
3.5	Shrinking kinetics of AMP-imprinted polyion complex	54
3.6	pH-dependence of binding and shrinking	56
3.7	Shrinking response to nucleosides	61

3.8	Conclusion	64
3.9	Experimental	64
3.10	References	67

Chapter 4 Application of AMP-imprinted polyion complexes

	as a sensing element on a QCM system	70
4.1	Introduction	70
4.2	Preparation of a QCM resonator surface depositing multi-layered polyion complexes	73
4.3	Frequency change in AMP-imprinted QCM systems	79
4.4	Frequency change in non-imprinted QCM systems	84
4.5	Conclusion	84
4.6	Experimental	87
4.7	References	88

Chapter 5 Nucleotide-responsive hydrogels designed from

	copolymers of boronic acid and cationic units	92
5.1	Introduction	92
5.2	Preparation of copolymers	96
5.3	Swelling and shrinking phenomena	96
5.4	Nucleotide sensing using a QCM resonator	107
5.5	Conclusion	113
5.6	Experimental	113
5.7	References	116

Chapter 6 Summary and concluding remarks

119

Acknowledgements

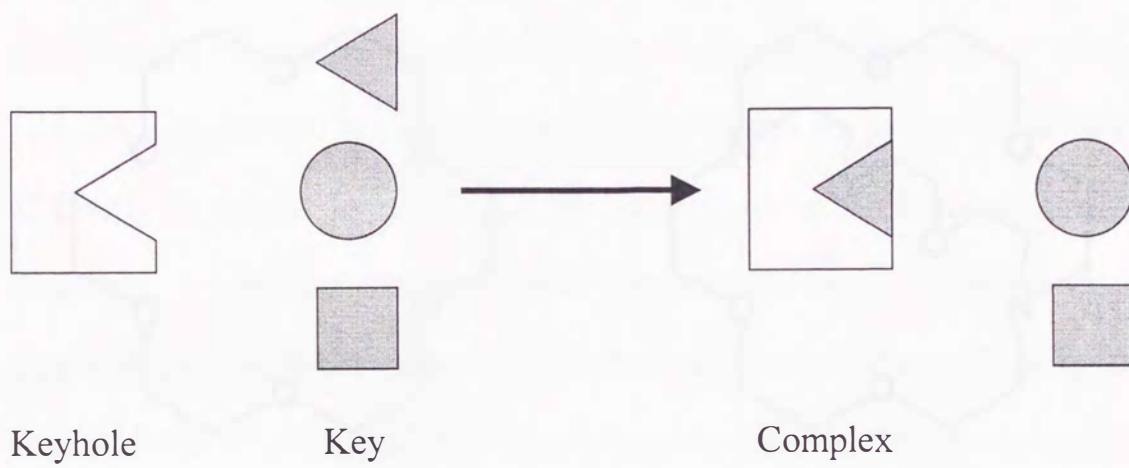
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Chapter 1 General introduction

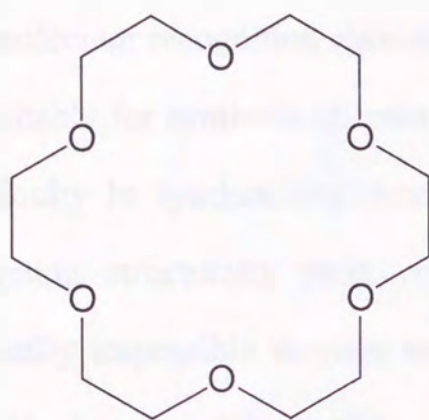
Molecular recognition is the fundamental process for chemical reactions in human body, *e.g.*, enzyme reaction, information transmission, material transportation, olfactory sense, gustatory system, *etc.* Among those, the substrate specificity in the enzyme reaction is understood by the “key-keyhole” concept (Scheme 1.1). Enzyme is a polypeptide whose molecular weight ranges from 10,000 – 1,000,000. The polypeptide chain takes the specific higher-order structure defining the shape of the “keyhole” around the active center of the enzyme reaction. Thus, the enzyme can selectively bind with the substrate molecule and subsequently the specific reaction takes place.

In the history of molecular recognition chemistry, many chemists have dreamed to synthesize an artificial receptor molecule that has extremely high binding selectivity like enzymes. In 1967, Pedersen inadvertently found a macrocyclic compound “crown ether” (Scheme 1.2), which has ability to bind metal ion selectively.^{1,2} It is believed that the binding selectivity of crown ether arises from the size fitness between the hole surrounded by six oxygen atoms in crown ether molecule and a metal ion. This is the first example of an artificial molecular recognition molecule. Subsequently, “cryptand” (Scheme 1.2) which has a three-dimensional structure was reported by Lehn³⁻⁵ in 1969. Cryptand shows ion selectivity

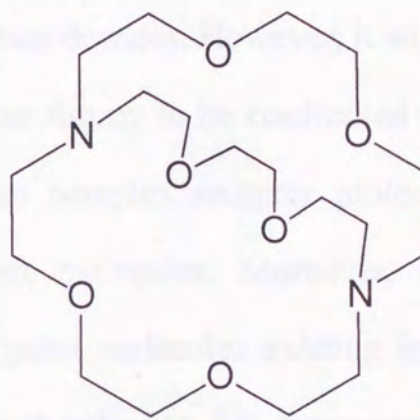
Scheme 1.1



Scheme 1.2



Crown ether

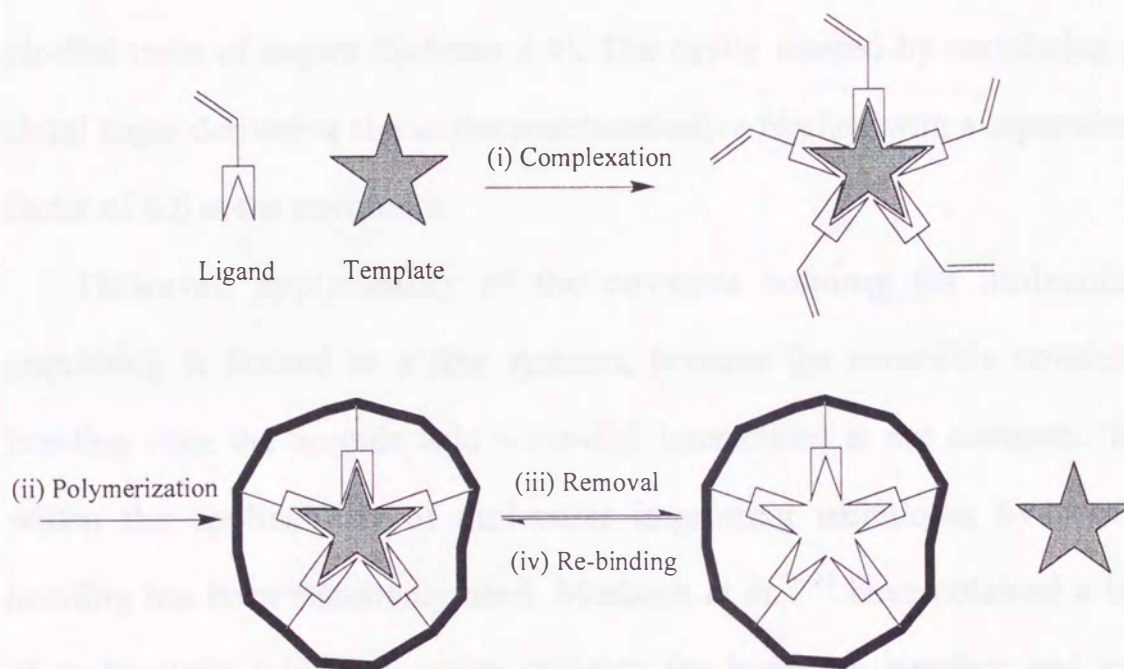


Cryptand

superior to that of crown ether, because it has a highly preorganized metal binding site. These studies pioneered the way to more sophisticated molecular recognition systems, and a lot of attempts have been made to synthesize receptors having high binding selectivity, targeting various kinds of guest molecules. As the results, remarkable progress has been achieved in molecular recognition chemistry in past two decades. However, it will be inevitable for synthetic chemists, in the near future, to be confronted with difficulty in synthesizing structurally more complex receptor molecules targeting structurally more complex guest molecules. Moreover, it is virtually impossible to cope with all the guest molecules existing in the world, because the number of target molecules to be recognized is continuously getting longer and longer.

An alternative way toward the construction of artificial molecular recognition systems is "molecular imprinting" that was arisen in 1970's by chemists who were interested in molecular recognition in relation to biomimetic chemistry.⁶⁻¹⁰ Molecular imprinting technique basically consists of four steps as illustrated in Scheme 1.3: (i) complex formation between functional vinyl monomers with a target template, (ii) radical polymerization of the vinyl monomers with crosslinking reagents in the presence of the template and (iii) removal of the template from the crosslinked copolymer resin, and finally (iv) the imprinted resin binds the guest molecule with high affinity.

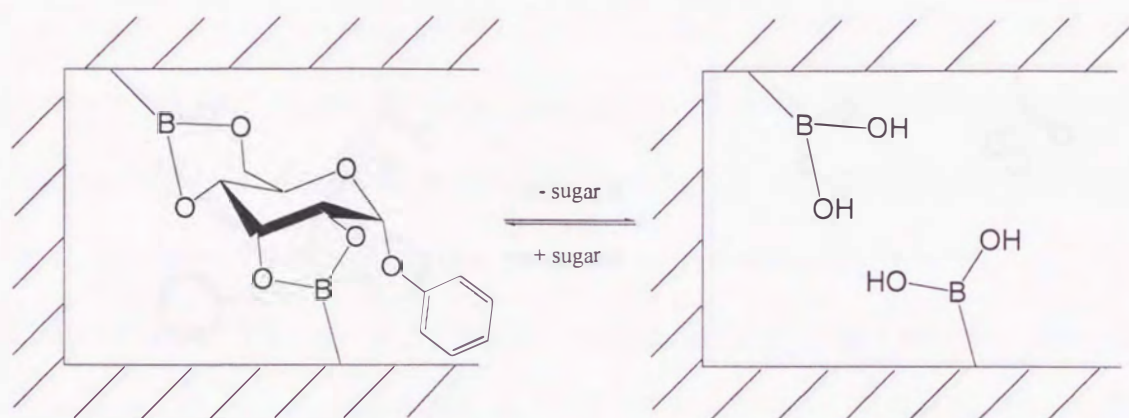
Scheme 1.3



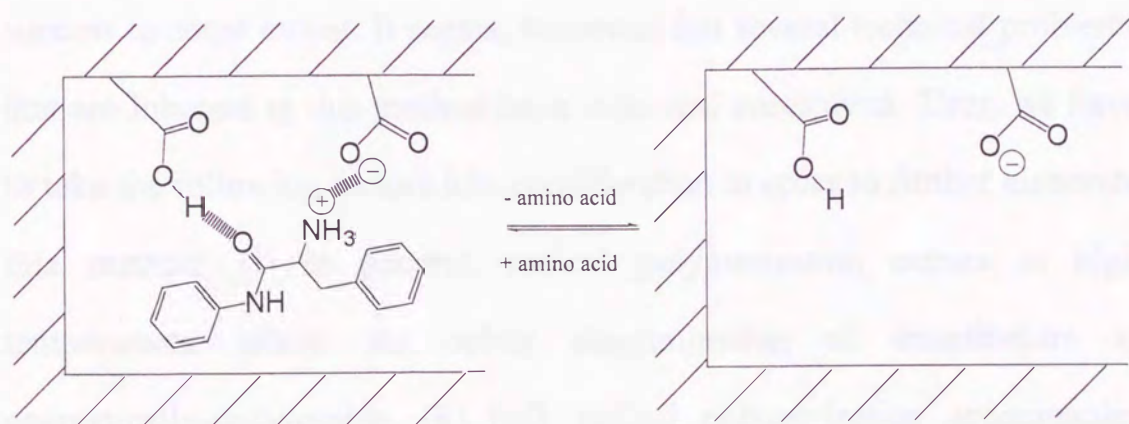
The complex formation between a template and ligands is driven by both covalent and non-covalent bonding. The non-covalent bonding includes hydrogen bonding, electrostatic interaction, coordination bonding, *etc.* Firstly, Wulff *et al.*¹¹⁻¹³ designed the sugar-imprinted resins using a boronic acid monomer that can form a reversible covalent bond with *cis*-diol units of sugars (Scheme 1.4). The cavity created by templating a chiral sugar derivative shows the enantioselective binding with a separation factor of 6.0 at the maximum.

However, applicability of the covalent bonding for molecular imprinting is limited to a few systems, because the reversible covalent bonding (like the boronic acid – *cis*-diol interaction) is not common. To widen the applicability of molecular imprinting technique, hydrogen bonding has been commonly used. Mosbach *et al.*^{14,15} have obtained a lot of molecularly imprinted resins utilizing the hydrogen bonding and the electrostatic interaction. Scheme 1.5 shows an example of the molecularly imprinted system created by them. This technique achieved high enantioselectivity that is similar to those obtained with the covalent bonding techniques by Wulff *et al.*¹¹⁻¹³ Using the similar strategy, Takeuchi *et al.*¹⁶⁻¹⁸ have applied the molecularly imprinted polymers as a stationary phases for liquid chromatography and achieved excellent peak separations between template molecules and others. It is worth paying attention to the works done by Arnold *et al.*¹⁹⁻²¹ They developed the molecularly imprinted

Scheme 1.4



Scheme 1.5

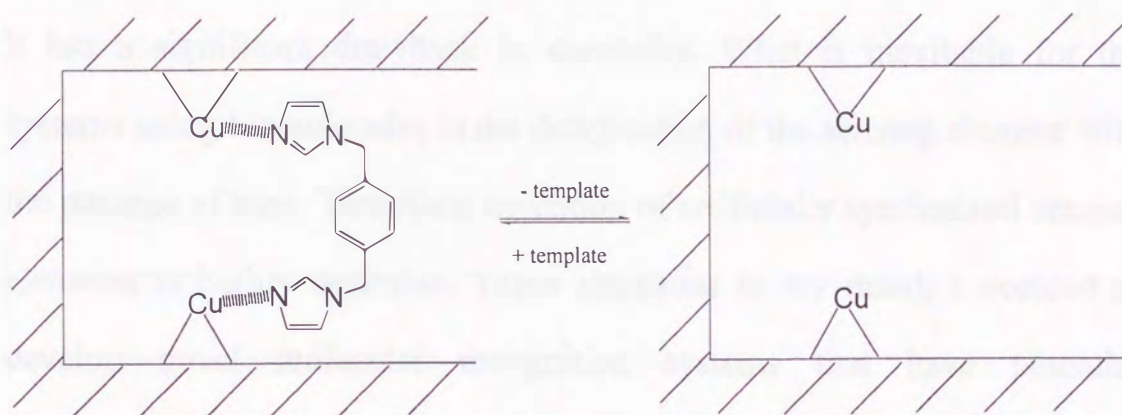


polymers utilizing the metal-ligand coordination bonds (Scheme 1.6). They first formed the assembly between metal-chelating monomers and a template, then polymerized them with cross-linkers. The resultant cavity shows high selectivity for the template molecule.

As stated above, the molecular imprinting technique has achieved a success to some extent. It seems, however, that several technical problems that are inherent to this method have remained unresolved. Thus, we have to take the following factors into consideration in order to further elaborate this method: (i) in general, radical polymerization occurs at high temperatures where the subtle discrimination of enantiomers is energetically-unfavorable, (ii) bulk radical polymerization accompanies shrinkage of the volume: under such a condition the structure of template molecules may be deformed, which would make the retention of the nanometer-sized memory for template molecules difficult, (iii) the reversible binding capacity is relatively low because most of the recognition sites are formed deeply inside the polymer resin where outer solution cannot contact, and (iv) spectroscopic characterization of the imprinted binding-site is difficult. It is desirable to invent some novel molecular imprinting technique to solve these problems.

When we turn our eyes into practical fields relating to molecular recognition chemistry, there is a great demand for sensing devices that can detect a target molecule more selectively and sensitively. Biosensors²²

Scheme 1.6



could be one of the candidates meeting these requirements. This technique utilizes biomolecule (enzyme, microbial, or antibody) as a sensing element. Since these biomolecules have high molecular recognition ability in nature, all we have to do is just to combine the receptor with a sensing device. Although this method has succeeded in preparing various kinds of sensors, it has a significant drawback in durability. What is inevitable for the systems using biomolecules is the deactivation of the sensing element with the passage of time. Therefore, invention of artificially synthesized sensing elements is highly desirable. These situations in my mind, I decided to develop novel molecular recognition systems that have potential application to sensing elements.

This thesis is composed of six chapters. Chapter 2 describes the creation of metal-imprinted films. In Chapter 3 a polyion complex formation process was applied to molecular imprinting of adenosine monophosphate (AMP). The resultant AMP-imprinted polyion complex was used as a sensing element on a QCM (quartz crystal microbalance) system in Chapter 4. Chapter 5 describes the nucleotide-responsive hydrogels consist of crosslinked copolymer.

I hope that you could spend a pleasant time with reading this thesis.

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Chapter 2 Facile construction of a novel metal-imprinted polymer surface without a polymerization process

Metal-imprinted films have been prepared by casting a THF/water mixed solution of poly(vinyl chloride-co-acrylic acid). When metal(II) ion was imprinted in the casting process, the film created metal binding sites. After removal of the template metal(II) ion, the film re-bound the metal(II) ion with high binding affinity. The imprinting processes could be thoroughly monitored by FT-IR spectroscopy.

2.1 Introduction

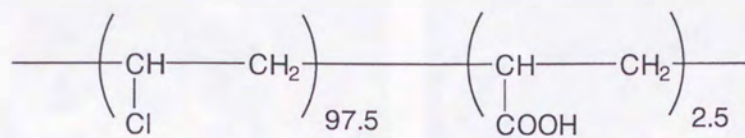
The molecular imprinting technique has been actively studied since 1970's and has achieved a success to some extent.¹⁻¹⁰ However, there are some drawbacks in this method as mentioned in Chapter 1. The molecularly imprinted polymer resin is usually synthesized by a radical polymerization of functional vinyl monomers in the presence of a template molecule. It seems to me that the most serious disadvantage of the resin prepared by this method is low binding capacity and slow binding rate. To widen the applicability of the molecularly imprinted polymers, it is necessary to solve these problems. It occurred to me that if the molecular motion of a polymer bearing functional groups can be quickly "frozen" as a porous thin film in the presence of template molecules,¹¹ most of these

problems would be solved. Because of the wide surface area in the film system, template molecules can easily be removed from and re-bind with the film. In addition, the film is easier to characterize the imprinting and re-binding process by various spectroscopic methods.

I have succeeded in the preparation of porous thin films by casting a specific THF/water solution of poly(vinyl chloride-*co*-acrylic acid) [Poly(VC-*co*-AA), Figure 2.1] and in the spectrophotometric characterization of the metal imprinting and re-binding processes. The results have clearly disclosed how the memory is imprinted into and how the re-binding takes place onto the film surface.

2.2 Preparation of metal-imprinted films

To prepare porous films by which one can acquire the wide surface area, a THF/water mixed solvent was chosen. When the Poly(VC-*co*-AA) solution was cast on a flat polyethylene (PE) plate and uniformly spread with a glass bar, THF (good solvent) volatilized first to produce a film structure and remaining water (poor solvent) in the film created pores. By this casting method I obtained the films with 10 μm thickness. Scanning electron microscope (SEM) photographs of the air side and the PE plate side of the resulting films are shown in Figure 2.2. This figure shows that the film surface of the PE plate side is relatively flat, whereas the surface of the air side has plenty of pores (0.5 - 5 μm). These cavities should have



Poly(VC-co-AA)

Figure 2.1 Structure of the functional polymer.

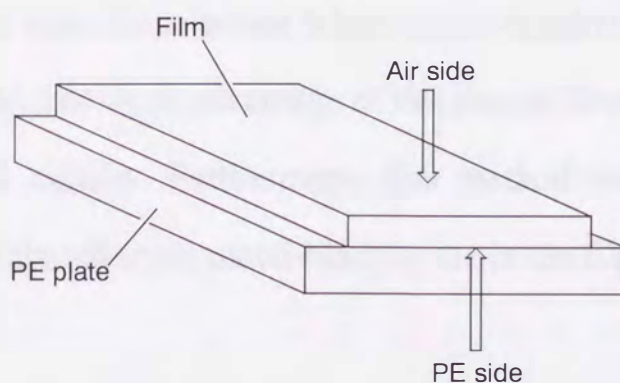
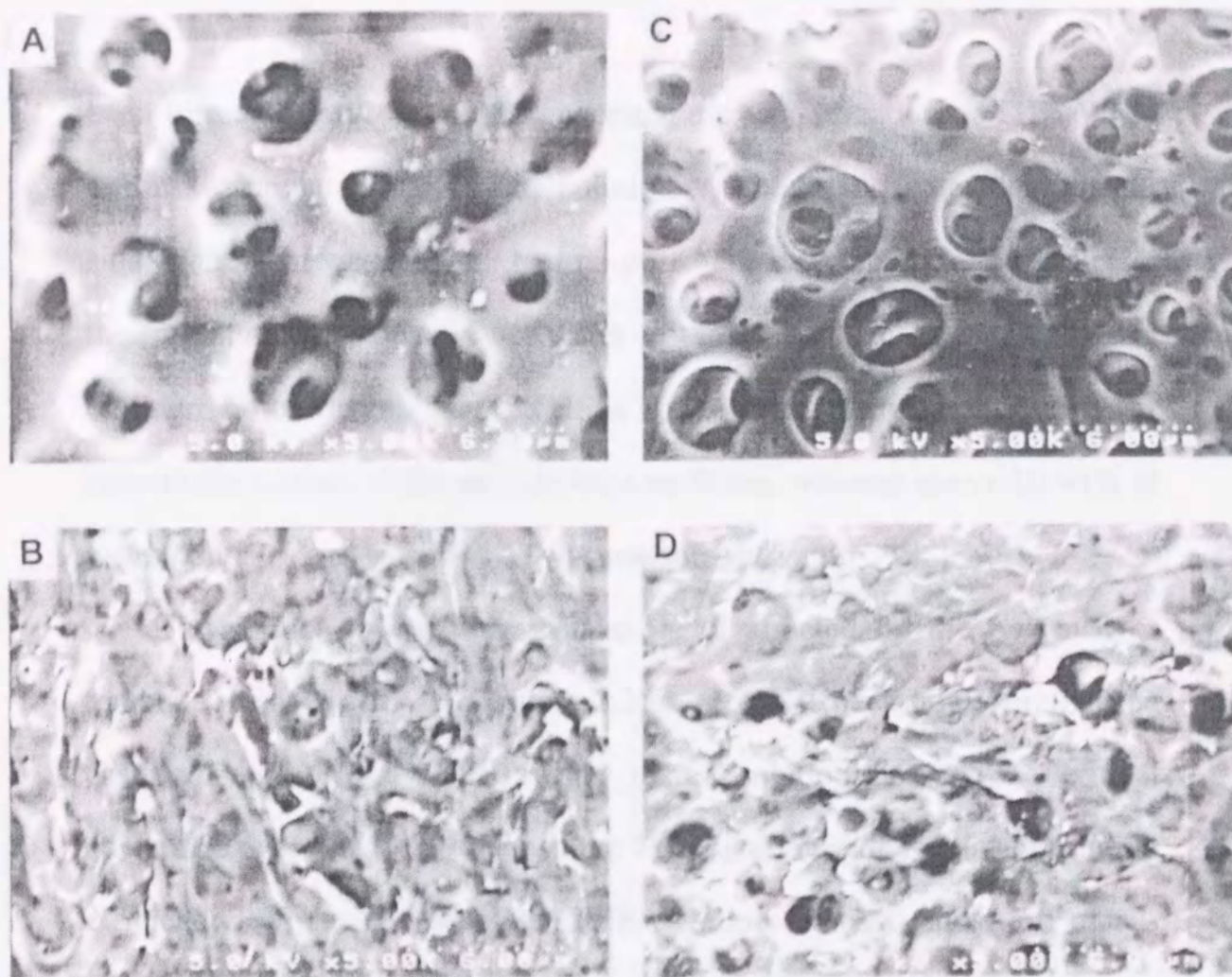


Figure 2.2 SEM photographs of the Cu^{2+} -imprinted (A, B) and unimprinted (C, D) films: upper (A) and (C) are the air side whereas lower (B) and (D) are the PE plate side.

been formed by water droplets generated after vaporization of THF. When a film was prepared from a THF solution not containing water, even the air side of the film was flat and had no pore. The difference clearly indicates the importance of concomitant water in the cast solvent. I found that the optimum ratio of water versus THF is 10:90 wt/wt: below 10 wt% of water content the surface of the air side became flatter, whereas above 10 wt% of water content Poly(VC-co-AA) was not soluble.

The surface morphology was scarcely affected by the presence of template metal cations (see Figure 2.2). One can expect, however, that the casting method from a THF/water mixed solution possesses a critical advantage over that from the absolute THF solution in the metal-imprinting efficiency. When the absolute THF solution is cast, most of metal-complexed sites would be trapped inside the film. In contrast, when the THF/water mixed solution is cast, the hydrophilic metal-complexed sites would be placed onto the interface where water droplets are trapped. This difference should lead to an advantage of the porous film in the removal of imprinted metal cations. Furthermore, this method would increase the concentration of the effective metal-binding site in the film.

2.3 Spectroscopic characterization of films

The metal-imprinted films were prepared from a neutral (without NaOH) or a basic (with NaOH) solution. The distribution state of

carboxylic acid groups was characterized by FT-IR spectroscopy. The film prepared from a neutral solution in the absence of a template metal ion gave two carbonyl peaks at 1747 cm^{-1} and 1710 cm^{-1} , which are assigned to monomeric and dimeric carboxylic acids, respectively¹² (solid line in Figure 2.3.A). When this film was washed with a methanolic 0.10 mol dm^{-3} HNO_3 solution, the two carbonyl peaks were scarcely affected (solid line in Figure 2.3.B). The film prepared from a neutral solution in the presence of $\text{Cu}(\text{NO}_3)_2$ gave a new peak appeared at 1620 cm^{-1} which is assignable to $\text{COO}^- \cdot \text{Cu}^{2+}$ (dotted line in Figure 2.3.A).¹² This peak nearly disappeared by washing the film with a methanolic HNO_3 solution, indicating that most of Cu^{2+} can be extracted out of the film (dotted line in Figure 2.3.B).

The film prepared from a basic solution in the absence of template metal ions gave a broad carbonyl peak around 1570 cm^{-1} for $\text{COO}^- \cdot \text{Na}^+$ (solid line in Figure 2.4.A).¹² A shoulder at 1770 cm^{-1} is ascribed to an ester carbonyl group. Presumably, this was yielded by the intramolecular nucleophilic attack of the COO^- group. When this film was washed with a methanolic 0.10 mol dm^{-3} HNO_3 solution, the broad peak disappeared while a peak at 1747 cm^{-1} that is attributable to monomeric carboxylic acid became stronger (solid line in Figure 2.4.B). The peak for dimeric carboxylic acid (1710 cm^{-1}) was slightly observable as a shoulder in the 1747 cm^{-1} peak. The comparison with Figure 2.3.B indicates the following facts: (i) the COOH group tends to dimerize owing to the hydrogen-

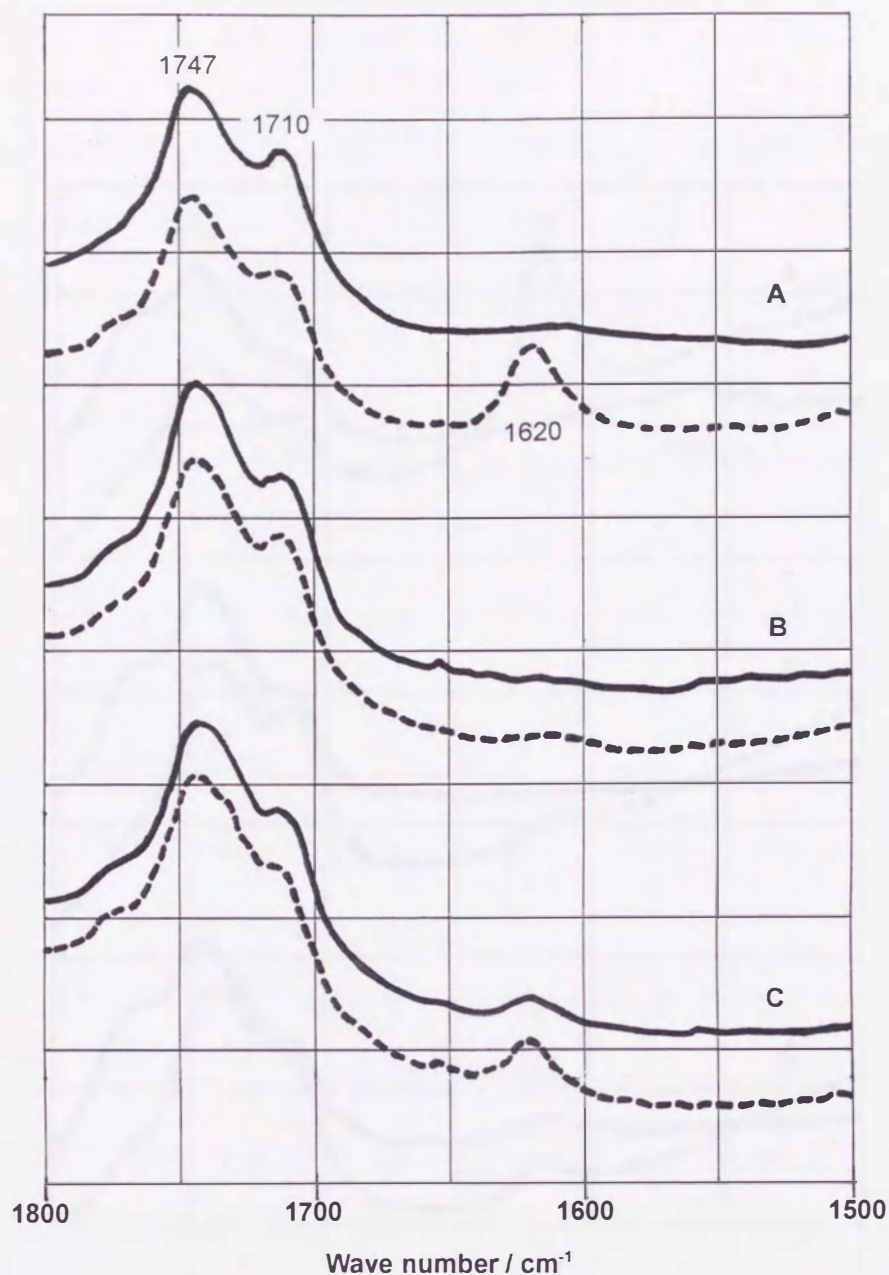


Figure 2.3 Partial FT-IR spectra of the films prepared from the neutral solution. The solid lines denote the films in the absence of template $\text{Cu}(\text{NO}_3)_2$ whereas the dotted lines denote the films in the presence of template $\text{Cu}(\text{NO}_3)_2$: (A) cast from the NaOH-containing water-THF solution and washed with a MeOH/water (9:1 wt/wt) mixed solvent for 10 h, (B) after washing with a methanolic $0.10 \text{ mol dm}^{-3} \text{ HNO}_3$ solution for 10 h and (C) after immersing in a methanolic $1.0 \text{ mmol dm}^{-3} \text{ Cu}(\text{NO}_3)_2$ solution for 10 h.

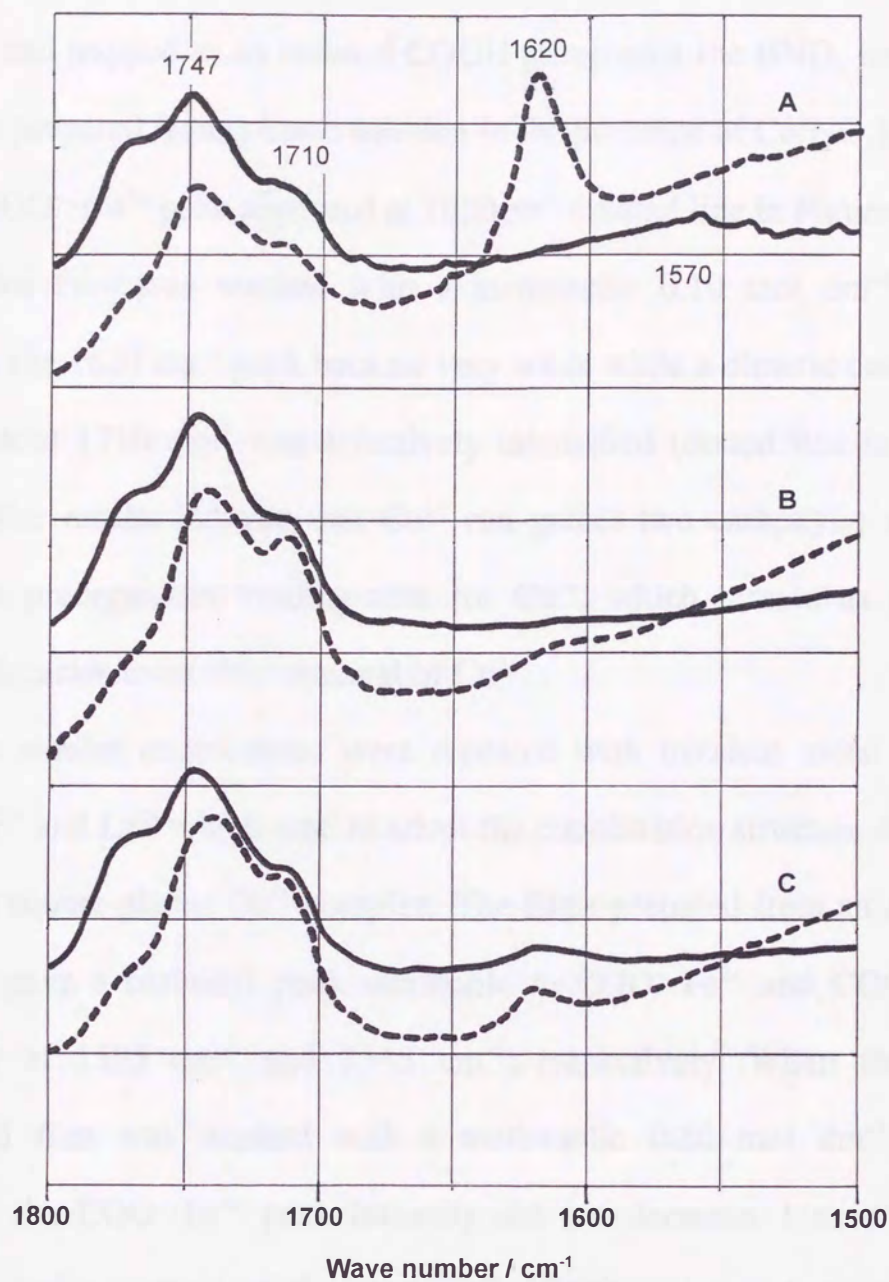


Figure 2.4 Partial FT-IR spectra of the films prepared from the alkaline solution. The explanations are recorded in a caption to Figure 2.3.

bonding interaction and (ii) the $\text{COO}^- \cdot \text{Na}^+$ group is discretely dispersed in the film and trapped as an isolated COOH group after the HNO_3 treatment. The film prepared from a basic solution in the presence of $\text{Cu}(\text{NO}_3)_2$ gave a strong $\text{COO}^- \cdot \text{Cu}^{2+}$ peak appeared at 1620cm^{-1} (dotted line in Figure 2.4.A). When this film was washed with a methanolic 0.10 mol dm^{-3} HNO_3 solution, the 1620 cm^{-1} peak became very weak while a dimeric carboxylic acid peak at 1710 cm^{-1} was selectively intensified (dotted line in Figure 2.4.B). The results indicate that Cu^{2+} can gather two carboxylic acids to construct preorganized binding-sites for Cu^{2+} , which remain as dimeric carboxylic acids even after removal of Cu^{2+} .

The similar experiments were repeated with trivalent metal ions. I chose Fe^{3+} and La^{3+} which tend to adopt the coordination structure different from the square-planar Cu^{2+} complex. The films prepared from an alkaline solution gave a carbonyl peak ascribable to $\text{COO}^- \cdot \text{Fe}^{3+}$ and $\text{COO}^- \cdot \text{La}^{3+}$ appeared at 1595 cm^{-1} and 1545 cm^{-1} , respectively. When the Fe^{3+} -imprinted film was washed with a methanolic 0.10 mol dm^{-3} HNO_3 solution, the $\text{COO}^- \cdot \text{Fe}^{3+}$ peak intensity did not decrease. I used neutral EDTA solution instead of the methanolic HNO_3 solution but could not extract Fe^{3+} out of the film. On the other hand, when the La^{3+} -imprinted film was subjected to the similar HNO_3 treatment, the $\text{COO}^- \cdot \text{La}^{3+}$ peak totally disappeared and a new peak ascribable to dimeric carboxylic acid appeared at 1710 cm^{-1} . The difficulty in the extraction of imprinted Fe^{3+}

may be rationalized by two different explanations. (i) The metal complex is trapped deeply inside the film or (ii) The stability constants are too large to remove the metal ion by solid (film) - liquid extraction. In the present system, the second rationale is more likely because the order of the stability constants¹³ for the *bis*-acetato complexes in aqueous solution is $\text{Fe}^{3+} \gg \text{Cu}^{2+} > \text{La}^{3+}$.

The foregoing results indicate that the metal ion useful for the metal imprinting must have the “moderate” binding affinity for ligands: it should be large enough to allow the efficient deposition in the metal-imprinting process whereas it should be small enough to allow the efficient extraction in the de-metalation process.

2.4 Stoichiometry of imprinted metal complexes

To obtain a stoichiometrical insight into the metal-imprinting process, I measured the carbonyl peak intensity of the $\text{COO}^- \cdot \text{M}^{n+}$ ($\text{M}^{n+} = \text{Cu}^{2+}$, Fe^{3+} and La^{3+}) in the FT-IR spectra as a function of the metal concentration in the cast solution. As shown in Figure 2.5.A, the peak intensity was saturated at $[\text{Cu}(\text{NO}_3)_2]/[\text{COO}^-] = 0.5$. This ratio indicates that the imprinted Cu^{2+} undergoes the coordination of two COO^- groups and the complex has the stoichiometry of $(\text{COO}^-)_2 \cdot \text{Cu}^{2+}$. Surprisingly, the similar saturation curve with a break-point at $[\text{Fe}(\text{NO}_3)_3]/[\text{NaOH}] = 0.5$ was also obtained from the $\text{Fe}(\text{NO}_3)_3$ system (Figure 2.5.B). The result suggests that

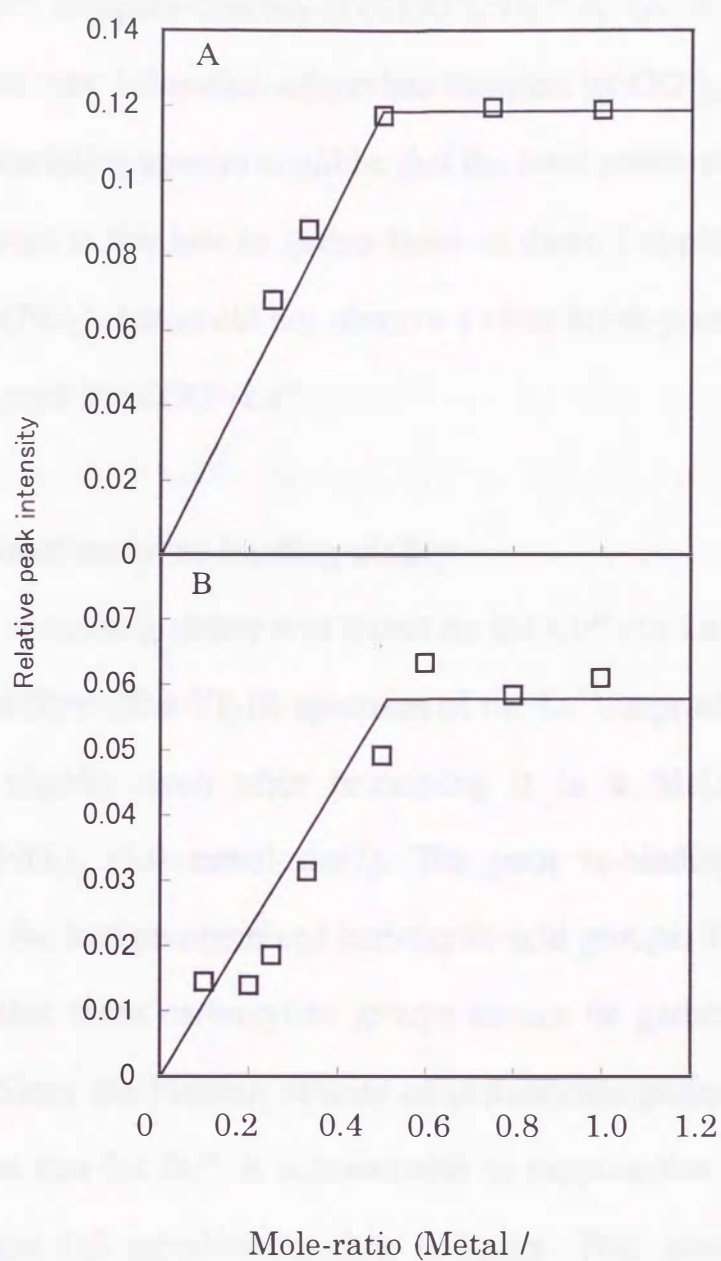


Figure 2.5 Relative peak intensity of the metal-coordinated carboxylate group plotted against the imprinted metal concentration: (A) $\text{Cu}(\text{NO}_3)_2$, (B) $\text{Fe}(\text{NO}_3)_3$. The absorption peak of a C-H deformation band ($1400 - 1470 \text{ cm}^{-1}$) was used as a standard reference.

the imprinted Fe^{3+} complex consists of $(\text{COO}^-)_2 \cdot \text{Fe}^{3+} \cdot \text{X}^-$ ($\text{X}^- = \text{NO}_3^-$ or OH^-). The reason why 1:3 metal/carboxylate complex $[(\text{COO}^-)_3 \cdot \text{Fe}^{3+}]$ is not formed as an imprinting species could be that the local concentration of the carboxylate groups is too low to gather three of them. I applied the same technique to $\text{La}(\text{NO}_3)_3$ but could not observe a clear break-point due to the broad carbonyl peak for $\text{COO}^- \cdot \text{La}^{3+}$.

2.5 Evaluation of metal re-binding ability

The metal re-binding ability was tested for the Cu^{2+} - or La^{3+} -imprinted and unimprinted films. The FT-IR spectrum of the La^{3+} -imprinted film was changed only slightly even after immersing it in a MeOH solution containing $\text{La}(\text{NO}_3)_3$ (1.0 mmol dm^{-3}). The poor re-binding ability is associated with the less preorganized carboxylic acid groups. The previous section shows that three carboxylate groups cannot be gathered to form $(\text{COO}^-)_3 \cdot \text{Fe}^{3+}$. Since the binding affinity of carboxylate group for La^{3+} is much lower than that for Fe^{3+} , it is reasonable to suppose that La^{3+} is also not able to form 1:3 metal/carboxylate complex. This means that the cationic charge of La^{3+} is not completely neutralized by the binding with anionic carboxylate groups and should greatly reduce the metal binding ability in the solid-liquid extraction system. I thus decided to evaluate the “*memory effect*” by comparing the Cu^{2+} -imprinted film with the unimprinted film.

To quantitatively estimate the Cu^{2+} concentration present in the film phase by FT-IR spectroscopy, I prepared a calibration curve from cast films containing the known amount of $\text{Cu}(\text{NO}_3)_2$. I have found that the peak area (1620 cm^{-1} for $\text{COO}^- \cdot \text{Cu}^{2+}$) is linearly proportional to the $\text{Cu}(\text{NO}_3)_2$ concentration in the range of $0\text{-}30\text{ }\mu\text{mol g}^{-1}$.

In the Cu^{2+} -imprinted film prepared from the alkaline solution, the Cu^{2+} concentration after the HNO_3 treatment was $2.9\text{ }\mu\text{mol g}^{-1}$ (dotted line in Figure 2.4.B). It increased up to $8.6\text{ }\mu\text{mol g}^{-1}$ after immersing in the $\text{Cu}(\text{NO}_3)_2$ solution (1.0 mmol dm^{-3} ; dotted line in Figure 2.4.C). Thus, the re-binding capacity maintaining the memory for original template Cu^{2+} was estimated to be $5.7\text{ }\mu\text{mol g}^{-1}$. On the other hand, the re-binding capacity for the unimprinted film was estimated to be $4.6\text{ }\mu\text{mol g}^{-1}$ (solid line in Figure 2.4.C). The difference ($1.1\text{ }\mu\text{mol g}^{-1}$) is ascribable to "metal imprinting effect".

In the Cu^{2+} -imprinted film prepared from the neutral solution, the re-binding capacity maintaining the memory for original template Cu^{2+} was estimated to be $12.2\text{ }\mu\text{mol g}^{-1}$ (dotted line in Figure 2.3.C). In contrast, the re-binding capacity for the unimprinted film was estimated to be $6.5\text{ }\mu\text{mol g}^{-1}$ (solid line in Figure 2.3.C). Provided that all carboxylic acid groups form the $(\text{COO}^-)_2 \cdot \text{Cu}^{2+}$ complex, 6.0 % of carboxylic acid groups are used for the reversible binding Cu^{2+} in the case of Cu^{2+} -imprinted film. One can thus regard that the difference of the re-binding capacity ($5.7\text{ }\mu\text{mol g}^{-1}$) is

created by metal imprinting effect.

2.6 Effect of poly(propylene glycol) blending

I have proved that the metal-imprinted films have high affinity for the imprinted metal ions dissolved in methanol. However, the imprinted films scarcely showed re-binding ability toward metal ions in water. The inferior binding ability in water is probably due to (i) low hydrophilicity of the film because the film is composed of hydrophobic Poly(VC) having small amount of COOH groups, so that aqueous solution cannot enter inside the film where the metal binding sites exist and (ii) low stability of metal-carboxylate complexes at the film-water interface, because metal ion is more strongly solvated by water than by methanol. To overcome these drawbacks, I attempted to blend poly(propylene glycol) [Poly(PG)] to the film matrix. In this system, Poly(VC-co-AA) acts as the metal-binding site whereas Poly(PG) is effective in (i) enhancement of hydrophilicity of the film due to the introduction of etheral oxygen that have higher hydrogen bonding ability compared with Poly(VC) and (ii) stabilization of the metal complexes by the interaction between metal ion and ethereal oxygen. I used Poly(PG) of higher molecular weight, which is insoluble in water.

Preparation method of the metal-imprinted film was similar to the case of the non-blended film. A Poly(VC-co-AA)/Poly(PG) (10:1 wt/wt) blend solution containing metal salts was cast on a flat polyethylene plate and

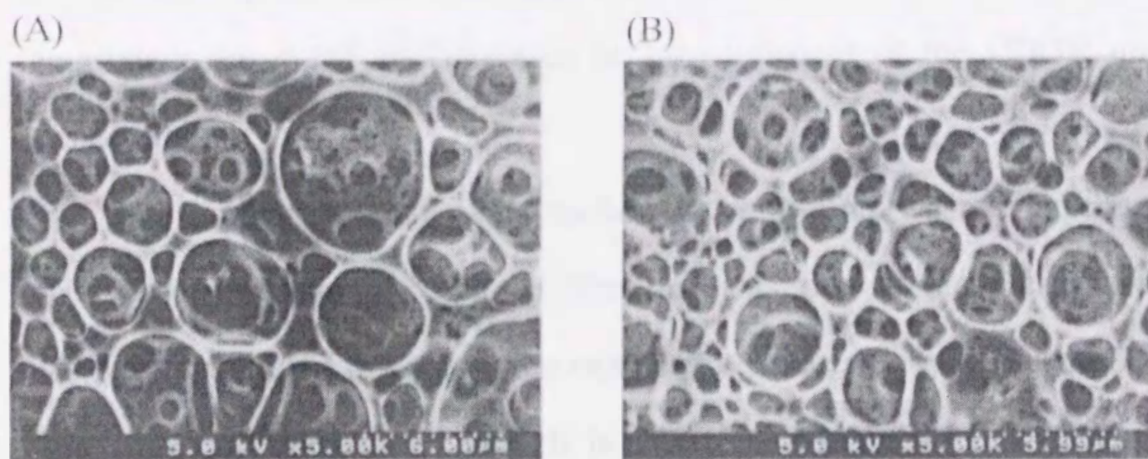


Figure 2.6 SEM pictures of the Poly(PG)-blended films: (A) Cu^{2+} -imprinted, (B) Li^{+} -imprinted.

uniformly spread with a glass bar. Thus, I obtained the films with 10 μm thickness. SEM pictures show that the surface of the film has a plenty of pores (Figure 2.6). The surface morphology is scarcely affected by changing template metal ions (Figure 2.6.A: Cu^{2+} -imprinted, 2.6.B: Li^{+} -imprinted: the metal concentration is 1.0 equivalent of the COOH unit concentration).

The distribution state of the carboxylic acid group was characterized by FT-IR spectroscopy. For the film that was cast in the presence of $\text{Cu}(\text{NO}_3)_2$ [$\text{COOH}:\text{Cu}^{2+} = 2:1$], one can observe two carbonyl peaks at 1732 and 1624 cm^{-1} (Figure 2.7.A-a). It is shown in Section 2.3 that the non-blended film has two carbonyl peaks at 1747 and 1710 cm^{-1} , which are assigned to monomeric and dimeric carboxylic acids, respectively. Hence, the 1732 cm^{-1} peak observed in the blend film is assignable to the carboxylic acid group hydrogen-bonding with ethereal oxygen in Poly(PG) [$\text{COOH}\dots\text{O}$]. Thus, it is clear that Poly(PG) is mixed with Poly(VC-co-AA) at the molecular level. The 1624 cm^{-1} peak is assignable to the $(\text{COO}^-)_2\cdot\text{Cu}^{2+}$ complex.¹²

When this film was immersed in a 0.10 mol dm^{-3} aqueous HNO_3 solution for 60 h at room temperature, the 1624 cm^{-1} peak was markedly weakened while the 1732 cm^{-1} peak became stronger (Figure 2.7.A-b). This spectral change indicates that Cu^{2+} ion was mostly extracted out of the film. Then, after the HNO_3 -treated film was immersed in a 0.10 mmol dm^{-3}

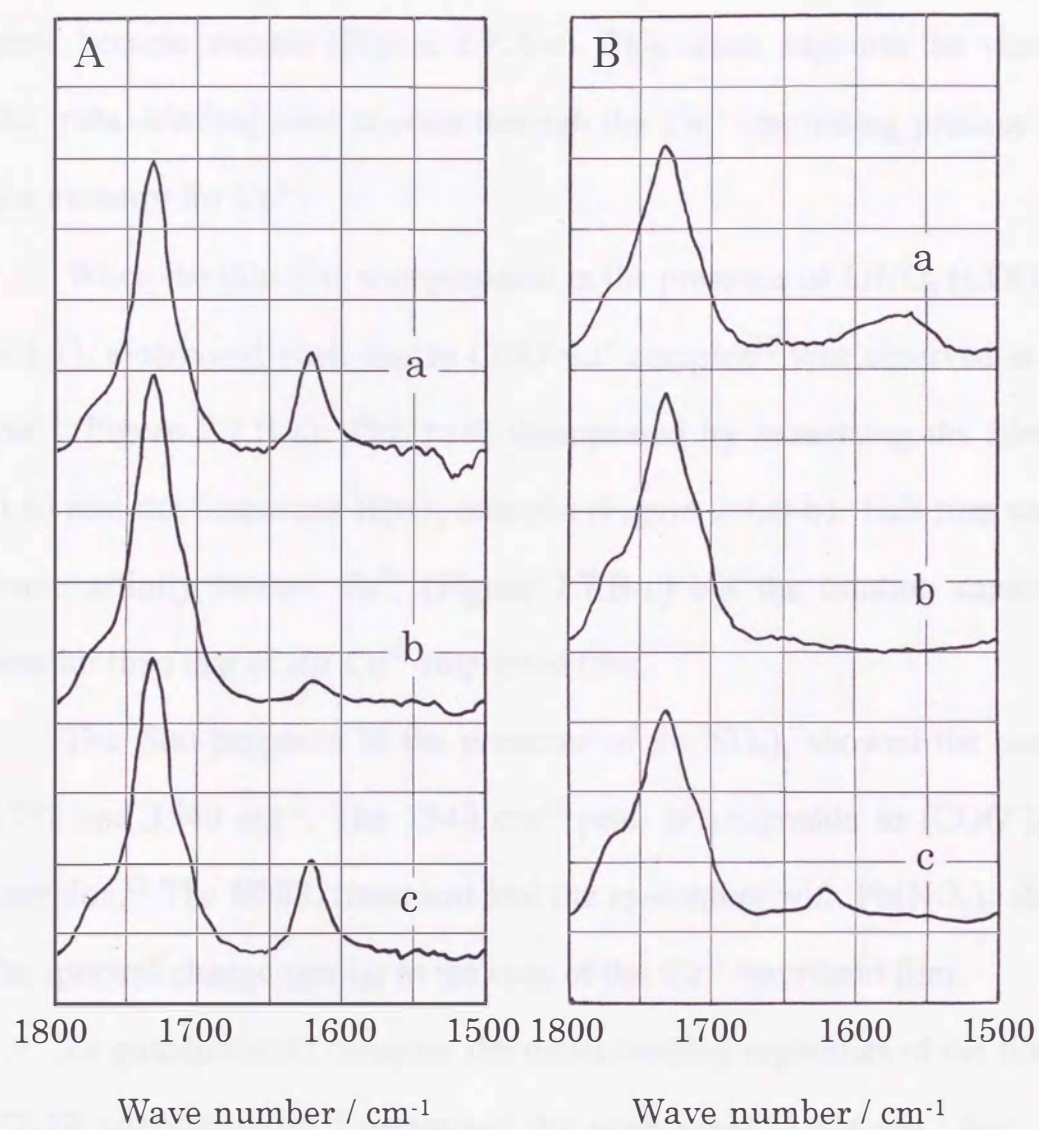


Figure 2.7 FT-IR spectra of the Poly(PG)-blended films: (A) Cu^{2+} -imprinted, (B) Li^{+} -imprinted.

$\text{Cu}(\text{NO}_3)_2$ solution for 60 h at room temperature, the 1624 cm^{-1} peak came back as strongly as that in the Cu^{2+} -imprinted film while the 1732 cm^{-1} peak became weaker (Figure 2.7.A-c). This result supports the view that the metal-binding sites created through the Cu^{2+} -imprinting process retain the memory for Cu^{2+} .

When the thin film was prepared in the presence of LiNO_3 ($\text{COOH}:\text{Li}^+ = 1:1$), a carbonyl peak due to $\text{COO}^-\cdot\text{Li}^+$ complex¹² was observed at 1570 cm^{-1} (Figure 2.7.B-a). This peak disappeared by immersing the film into 0.10 mol dm^{-3} aqueous HNO_3 solution (Figure 2.7.B-b). This film showed some affinity toward Cu^{2+} (Figure 2.7.B-c) but the binding capacity is smaller than that of the Cu^{2+} -imprinted film.

The film prepared in the presence of $\text{Pb}(\text{NO}_3)_2$ showed the peaks at 1732 and 1540 cm^{-1} . The 1540 cm^{-1} peak is assignable to $(\text{COO}^-)_2\cdot\text{Pb}^{2+}$ complex.¹² The HNO_3 treatment and the re-binding with $\text{Pb}(\text{NO}_3)_2$ showed the spectral change similar to the case of the Cu^{2+} -imprinted film.

To quantitatively compare the metal-binding capacities of the films by FT-IR spectroscopy, I measured the peak areas [1624 cm^{-1} for $(\text{COO}^-)_2\cdot\text{Cu}^{2+}$ and 1540 cm^{-1} for $(\text{COO}^-)_2\cdot\text{Pb}^{2+}$]. As shown in Figures 2.8.A and 2.8.B, the Cu^{2+} -binding capacity for the Cu^{2+} -imprinted film ($12\text{ }\mu\text{mol g}^{-1}$)¹⁴ and the Pb^{2+} -binding capacity for the Pb^{2+} -imprinted film ($14\text{ }\mu\text{mol g}^{-1}$)¹⁴ were 2.4 times and 1.5 times higher than those for the Li^+ -imprinted film, respectively. These differences are ascribable to the metal

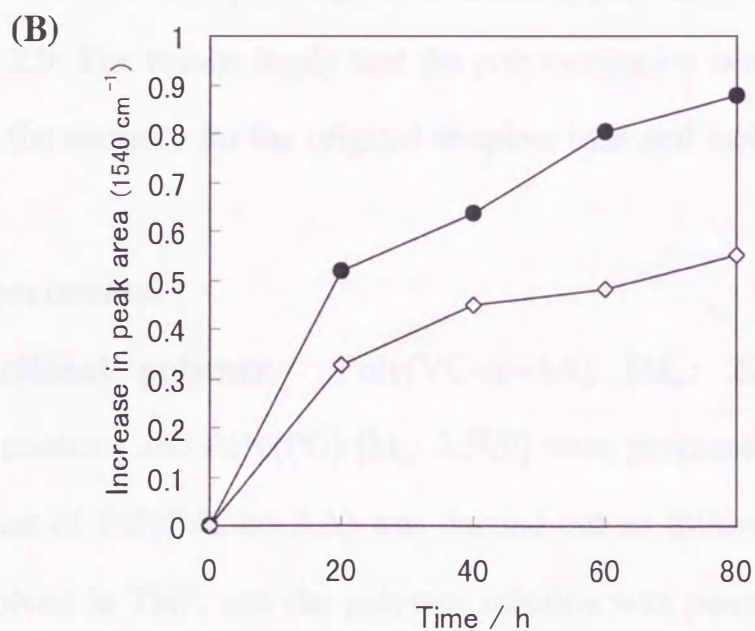
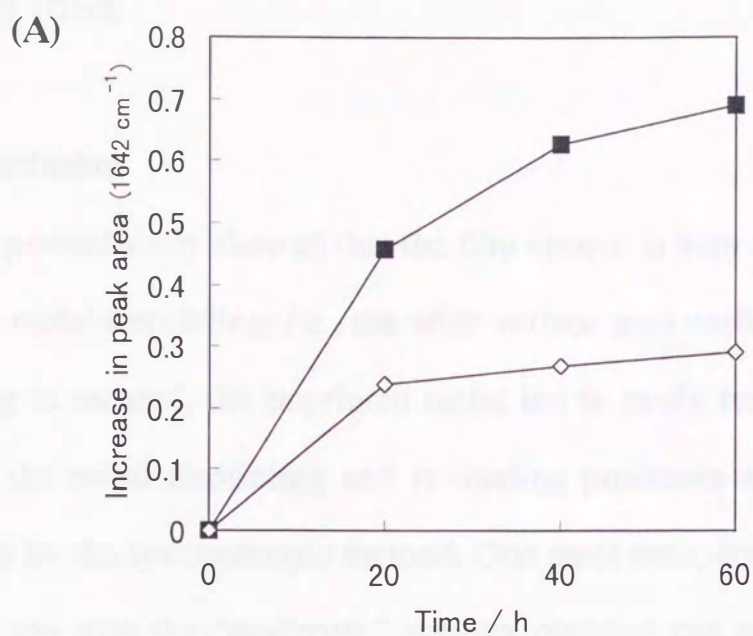


Figure 2.8 Binding abilities of the metal-imprinted films. (A): binding of Cu^{2+} with Cu-imprinted (■) and Li-imprinted (◇) films. (B): binding of Pb^{2+} with Pb-imprinted (●) and Li-imprinted (◇) films.

imprinting effect.

2.7 Conclusion

The present study showed that the film system is very advantageous to the facile metal-imprinting: *i.e.*, the wide surface area useful for the metal-imprinting is secured, the imprinted metal ion is easily removed from the film and the metal imprinting and re-binding processes are conveniently monitored by the spectroscopic method. One must note, however, that only the metal ion with the “*moderate*” stability constant can be loaded on this method. These metal imprinting and re-binding processes are illustrated as in Figure 2.9. The results imply that the polymerization is not obligatory to construct the memory for the original template ions and molecules.

2.8 Experimental

Functional polymer. Poly(VC-*co*-AA) [M_w : 220,000, 1.8 % carboxyl content] and Poly(PG) [M_n : 3,500] were purchased from Aldrich. Purification of Poly(VC-*co*-AA) was carried out as follows. The polymer was dissolved in THF, and the polymer solution was poured into acetone. The resultant precipitate was recovered by centrifugation. This process was repeated, and then the purified polymer was dried under vacuum. Poly(PG) was used without purification.

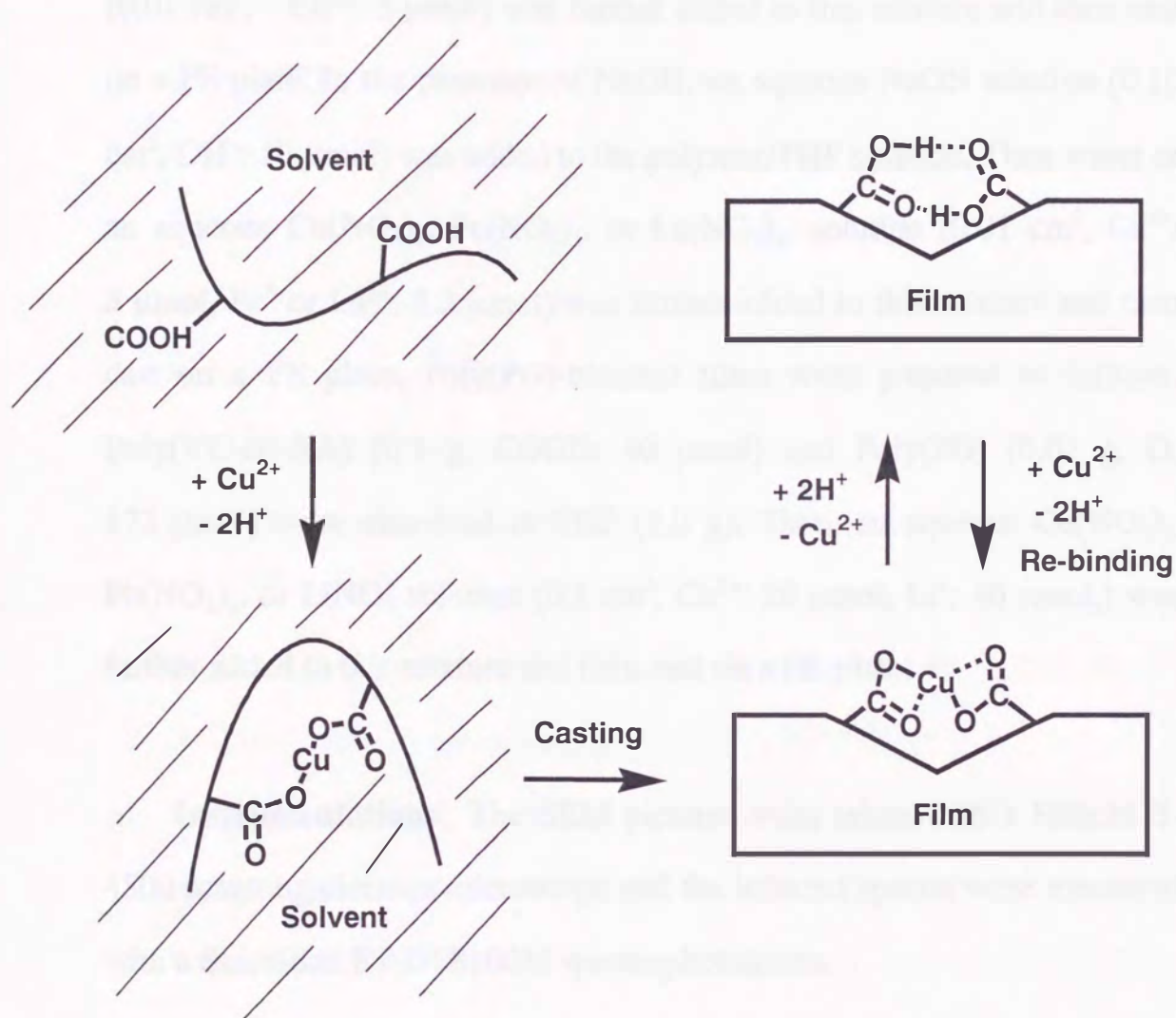


Figure 2.9 Schematic representation of the metal imprinting and re-binding processes.

Metal-imprinted film. Poly(VC-co-AA) (0.10 g, COOH: 40 μmol) was dissolved in THF (1.0 g). In the absence of NaOH, water (0.10 cm^3) was added to this solution. Then, water or an aqueous $\text{Cu}(\text{NO}_3)_2$ solution (0.01 cm^3 , Cu^{2+} : 5 μmol) was further added to this mixture and then cast on a PE plate. In the presence of NaOH, an aqueous NaOH solution (0.10 cm^3 , OH^- : 10 μmol) was added to the polymer/THF solution. Then water or an aqueous $\text{Cu}(\text{NO}_3)_2$, $\text{Fe}(\text{NO}_3)_3$, or $\text{La}(\text{NO}_3)_3$ solution (0.01 cm^3 , Cu^{2+} : 5 μmol , Fe^{3+} or La^{3+} : 3.3 μmol) was further added to this mixture and then cast on a PE plate. Poly(PG)-blended films were prepared as follows. Poly(VC-co-AA) (0.1 g, COOH: 40 μmol) and Poly(PG) (0.01 g, O: 172 μmol) were dissolved in THF (1.0 g). Then, an aqueous $\text{Cu}(\text{NO}_3)_2$, $\text{Pb}(\text{NO}_3)_2$, or LiNO_3 solution (0.1 cm^3 , Cu^{2+} : 20 μmol , Li^+ : 40 μmol ,) was further added to this mixture and then cast on a PE plate.

Instrumentation. The SEM pictures were taken with a Hitachi S-4500 scanning electron microscope and the infrared spectra were measured with a Shimadzu FT-IR 8100M spectrophotometer.

2.9 References and notes

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- 14 The absolute values of the metal-binding capacities were estimated from the decrease in the peak areas for the free carboxylic acid group (1732 cm^{-1}).

3.1 Introduction

During recent years, the polymer-solvent system has been the focus of considerable interest. The polymer-solvent system has been the subject of many studies, and the results have been used to explain the behavior of polymers in solution. The polymer-solvent system has been the subject of many studies, and the results have been used to explain the behavior of polymers in solution. The polymer-solvent system has been the subject of many studies, and the results have been used to explain the behavior of polymers in solution.

Here, I present an attempt to explain the behavior of polymers in solution.

Chapter 3 Molecular imprinting in polyion complexes

Polyion complexes were applied to molecular imprinting. Polyanion containing boronic acid units can sustain adenosine monophosphate (AMP) by a boronate – cis-diol interaction. This polyanion formed a polyion complex with a polycation according to 1:1 cation/anion stoichiometry. In the resultant polyion complex, the phosphate anionic charges of AMP were also neutralized by the polycation. Thus, after removal of AMP from the polyion complex the memory for the AMP template was imprinted. The AMP-imprinted polyion complex showed high binding affinity with AMP. I also found that the removal and re-binding processes for AMP coincide with swelling and shrinking phenomena of the polyion complex.

3.1 Introduction

Mixing aqueous polycation and polyanion solutions results in the formation of charge-neutralized polyion complex precipitates.¹⁻⁵ Because polyion complexes have unique physicochemical properties with high biocompatibility, attention has been devoted to their applications to ecology, biotechnology, and medicines.⁶⁻⁸ However, no one has noticed that this unique material can be a platform for molecular imprinting.

Here, I present the novel strategy for the preparation of molecularly

imprinted materials utilizing the polyion complex formation. If the polycation or polyanion has functional groups (for example, boronic acid group) by which charged guest molecules are bound, the guest molecules can be imprinted in the polyion complex. The basic concept for this methodology is illustrated in Figure 3.1: that is, (i) the polyion complex formation between polyanion containing boronic acid groups and a polycation in the presence of an anionic guest molecule (template) which can be bound by the boronic acid groups and (ii) removal of the anionic template by extensive extraction from the precipitate. The molecularly imprinted polyion complex thus created should show a “memory” for the original template molecule.

To test this working hypothesis I considered AMP (Figure 3.3) as a template molecule and a polyanion functionalized with boronic acid groups. The ribose moiety of AMP has a *cis*-diol function that is bound to the boronic acid group under basic pH conditions.⁹⁻¹¹ The binding of AMP results in the introduction of the phosphate negative charges onto the polyanion. Hence, this pendant phosphate anion site in addition to the boronate anion site and the carboxylate anion site in polyanion **1** (Figure 3.2) were counted for the formation of a charge-neutralized polyion complex with polycation **2** (Figure 3.2). After removal of AMP under acidic pH conditions, a “memory” which possesses a boronic acid group and cationic charges was created. Then, binding affinities of the

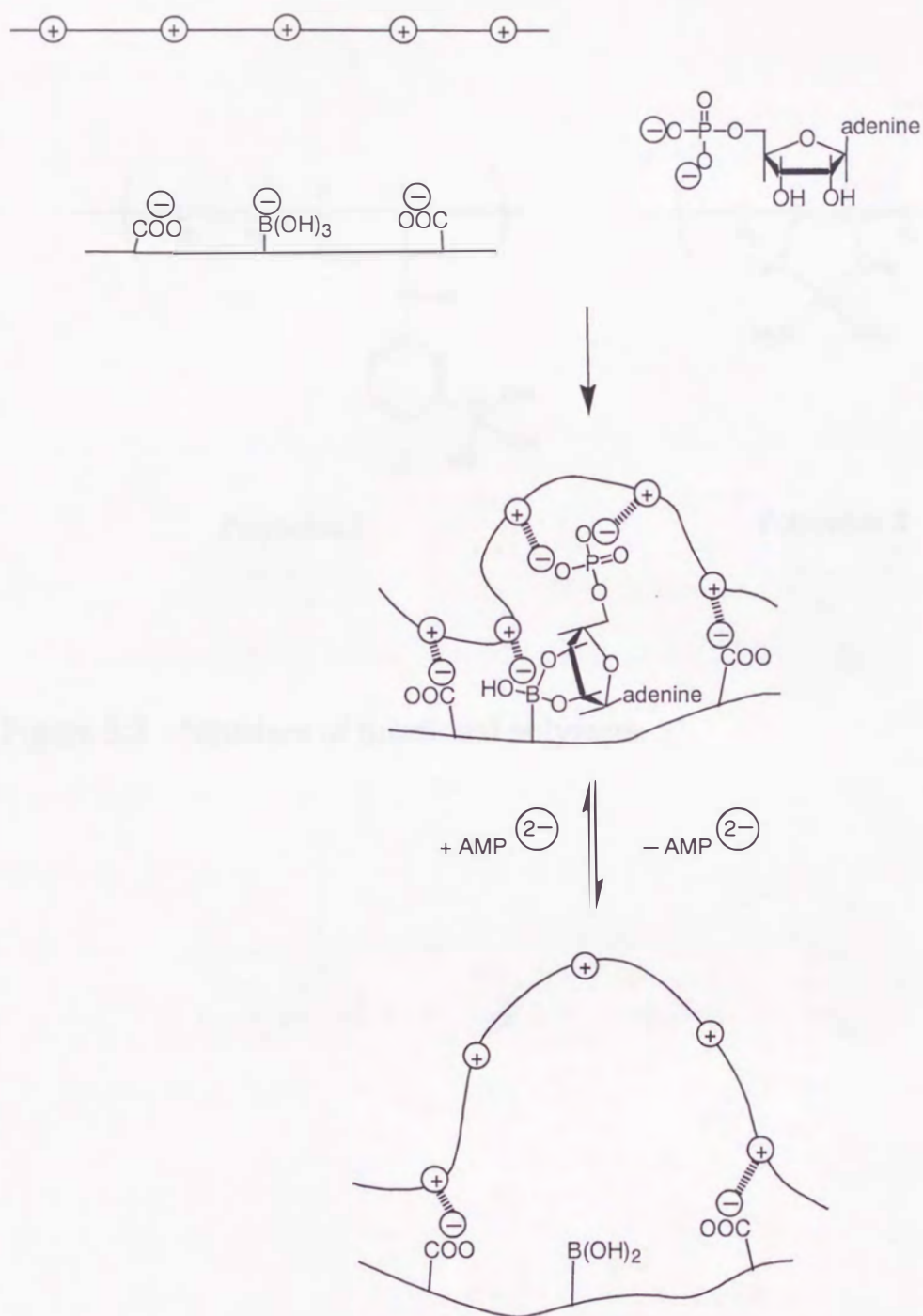
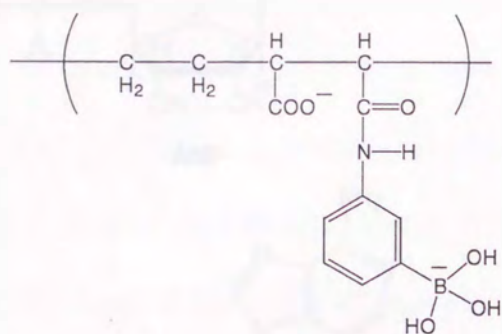
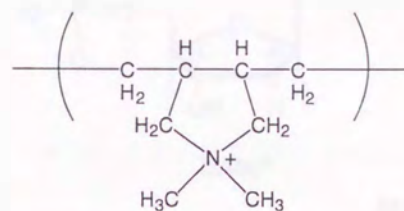


Figure 3.1 Schematic representation of the AMP-imprinted polyion complex.



Polyanion 1



Polycation 2

Figure 3.2 Structure of functional polymers.

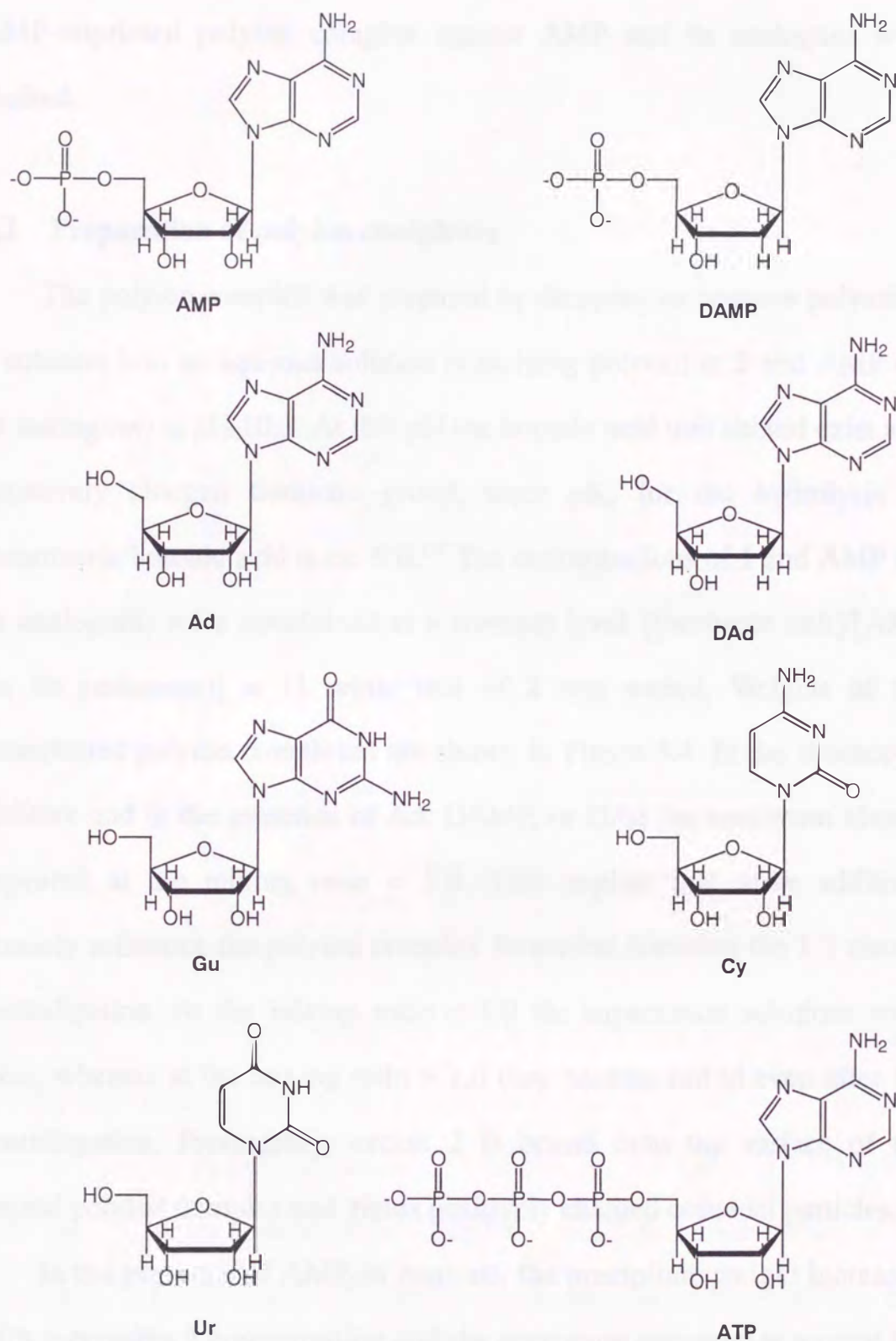


Figure 3.3 Structure of AMP and its derivatives.

AMP-imprinted polyion complex against AMP and its analogues were studied.

3.2 Preparation of polyion complexes

The polyion complex was prepared by dropping an aqueous polyanion **1** solution into an aqueous solution containing polycation **2** and AMP (or its analogues) at pH 10.3. At this pH the boronic acid unit should exist as a negatively charged boronate group, since pK_a for the hydrolysis of monomeric boronic acid is *ca.* 9.0.¹⁻³ The concentrations of **1** and AMP (or its analogues) were maintained at a constant level $\{[\text{boronate unit}]/[\text{AMP (or its analogues)}] = 1\}$ while that of **2** was varied. Weights of the precipitated polyion complexes are shown in Figure 3.4. In the absence of additive and in the presence of Ad, DAMP, or DAd the maximum always appeared at the mixing ratio = 1.0. This implies that these additives scarcely influence the polyion complex formation featuring the 1:1 charge neutralization. At the mixing ratio < 1.0 the supernatant solutions were clear, whereas at the mixing ratio > 1.0 they became turbid even after the centrifugation. Presumably, excess **2** is bound onto the surface of the neutral polyion complex and yields positively charged colloidal particles.

In the presence of AMP, in contrast, the precipitate weight increased with increasing **2** concentration and the maximum appeared at around the mixing ratio = 2.0. The supernatant solutions were clear up to the mixing

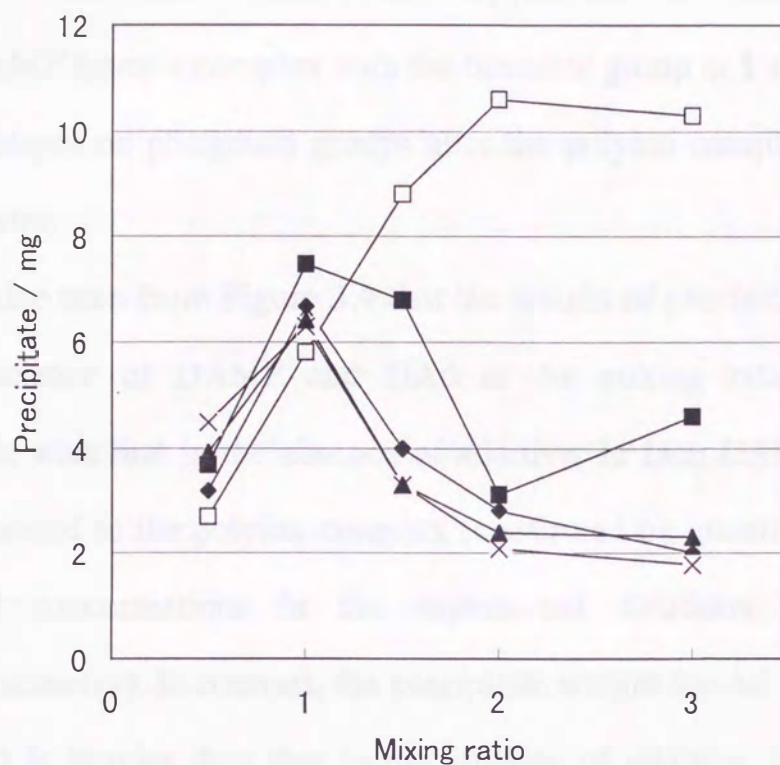


Figure 3.4 Plots of the precipitate (polyion complex) weight against the mixing ratio ($[\text{cation unit of } \mathbf{2}] / [\text{anion unit of } \mathbf{1}]$). The polyion complexes were prepared in the presence of AMP (□), Ad (■), DAMP (◆), DAd (▲), or in the absence of additive (×). Here, the concentration of the anion unit of **1** is defined as the total concentration of carboxylate unit and boronate unit.

ratio = 2.0 and then became turbid above the mixing ratio = 2.0. It was confirmed by a UV spectrophotometry of the supernatant solution that the amount of AMP bound by the polyion complex corresponds to 53 mol% of the total boronate units. These results support the view that the *cis*-diol group in AMP forms a complex with the boronate group in **1** and additional anionic charges on phosphate groups alter the polyion complex formation stoichiometry.

It is also seen from Figure 3.4 that the weight of precipitates prepared in the presence of DAMP and DAd at the mixing ratio = 1.0 are comparable with that in the absence of additive. In fact, DAMP and DAd were not bound to the polyion complex (confirmed by quantifying DAMP and DAd concentrations in the supernatant solutions by the UV spectrophotometry). In contrast, the precipitate weight for Ad at the mixing ratio = 1.0 is heavier than that in the absence of additive. It was shown from the UV spectrophotometry of the supernatant solution that the amount of Ad bound to the polyion complex corresponds to 59 mol% of the total boronate units. This must be reflected in the heavier precipitate weight.

These results clearly indicate the importance of the *cis*-diol group and the anionic phosphate group to alter the polyion complex formation behavior: that is, DAMP and DAd without *cis*-diol group are scarcely bound to the polyion complex, whereas Ad having *cis*-diol group is significantly bound to **1** but does not influence the polyion complex

formation because of the lack of the negatively charged phosphate group. Only AMP, which has both *cis*-diol and phosphate groups, has influence on the polyion complex formation.

3.3 Removal of AMP from a polyion complex

AMP bound to the polyion complex prepared at the mixing ratio = 2.0 was extracted out of the polyion complex precipitate by immersing the precipitate in an acidic aqueous solution (pH 5.5 with 100 mmol dm⁻³ sodium acetate buffer) at 25 °C. The acidic condition is useful for the extraction, because at pH 5.5 the boronate – *cis*-diol interaction tends to be dissociated. In addition, the phosphate group of AMP is protonated and the electrostatic interaction between the polyion complex and AMP should be weakened. The amount of AMP extracted from the polyion complex precipitate by this treatment was estimated by a UV spectrophotometry. The result shown in Figure 3.5 indicates that the extraction rate gradually decreased with extraction time and was saturated at around 40 h. After 41 h more than 90 % of AMP was extracted out of the polyion complex precipitate. This AMP-imprinted polyion complex was used for the subsequent re-binding experiment.

3.4 Re-binding of AMP to polyion complexes

The binding ability of the AMP-imprinted polyion complex was

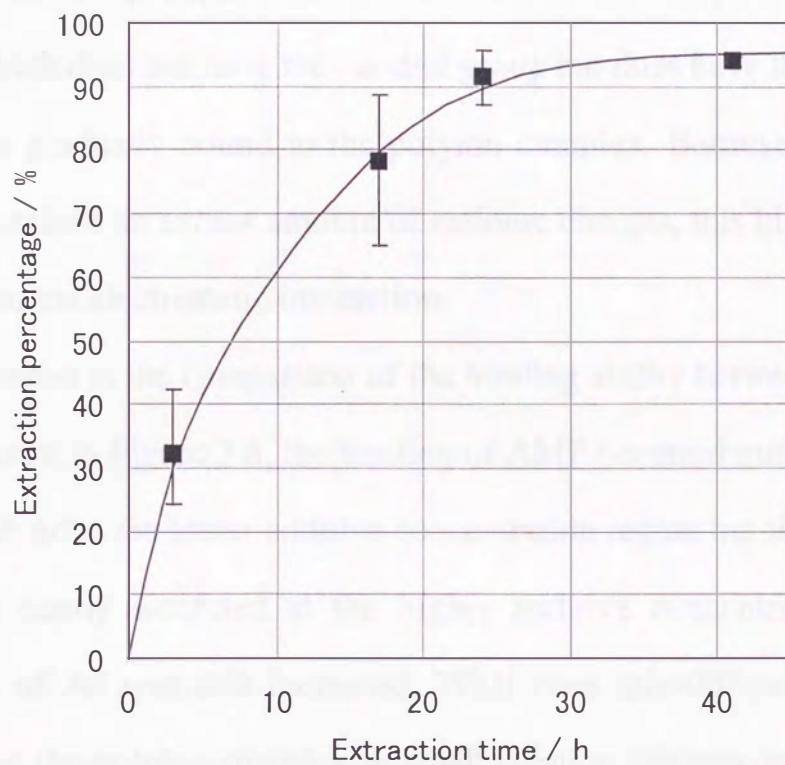


Figure 3.5 Removal of AMP from the polyion complex prepared at the mixing ratio = 2.0.

estimated in a basic aqueous solution (pH 9.3 with 10 mmol dm⁻³ ammonium buffer) at 25 °C. I plotted the equilibrium binding values against the additive concentration (Figure 3.6). DAd having neither the *cis*-diol group nor the phosphate group was not bound to the polyion complex. DAMP, which does not have the *cis*-diol group but does have the phosphate group, was gradually bound to the polyion complex. Because the polyion complex retained an excess amount of cationic charges, this binding can be attributed to the electrostatic interaction.

Of interest is the comparison of the binding ability between AMP and Ad. As shown in Figure 3.6, the binding of AMP occurred more efficiently than that of Ad at the lower additive concentration region but the binding of AMP was nearly saturated at the higher additive concentration region, while that of Ad was still increased. What does this difference mean? I noticed that the polyion complex in AMP solution changes its appearance with the binding time. Before the binding with AMP the polyion complex was swollen by adsorbing water molecules (Figure 3.7, left), and after the addition of AMP the polyion complex changed to the shrunken state (Figure 3.7, right). These phenomena are related to the charge balance of the polyion complex. The driving force for the swelling of polyelectrolyte gels is explained by these terms¹²⁻¹⁶: (i) mixing entropy of polymer chains and counter ions with water and (ii) electrostatic repulsion between the charges on the polymer chains. The AMP-imprinted polyion complex

swells like a cross-linked polycation gel because it has excess cationic charges. Then, the re-binding with AMP neutralizes the polyion complex gel, so that the driving force for the swelling has been lost. Figure 3.8 shows the equilibrium swelling ratios of the AMP-imprinted polyion complex. Here, the swelling ratio is defined as equation (3.1):

$$\text{Swelling ratio} = \frac{W_{\text{swollen}} - W_{\text{dry}}}{W_{\text{dry}}} \quad (3.1)$$

where W_{swollen} and W_{dry} are the weight of swollen and dried gel, respectively.

The size of the polyion complex was scarcely affected by the addition of DAd and only to a smaller extent by Ad and DAMP. In contrast, the same polyion complex shrunk remarkably with increasing AMP concentration. The findings imply, therefore, that the shrinking of the polyion complex suppresses further binding of AMP at higher concentration. Ad, which does not carry such anionic charge, does not induce the shrinking of the polyion complex. Hence, further binding of Ad at higher concentrations is still possible.

As a reference system, the same experiment was repeated using the non-imprinted polyion complex, which had been prepared in the absence of

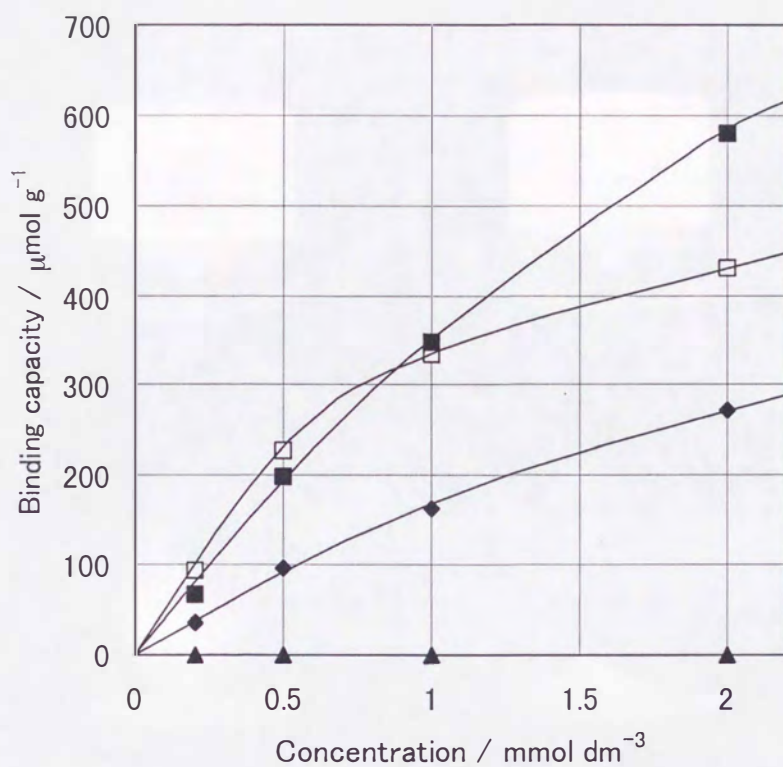


Figure 3.6 Equilibrium binding capacities of the AMP-imprinted polyion complex in a basic aqueous solution (pH 9.3): $\square$ AMP, $\blacksquare$ Ad, $\blacklozenge$ DAMP, $\blacktriangle$ DAd.

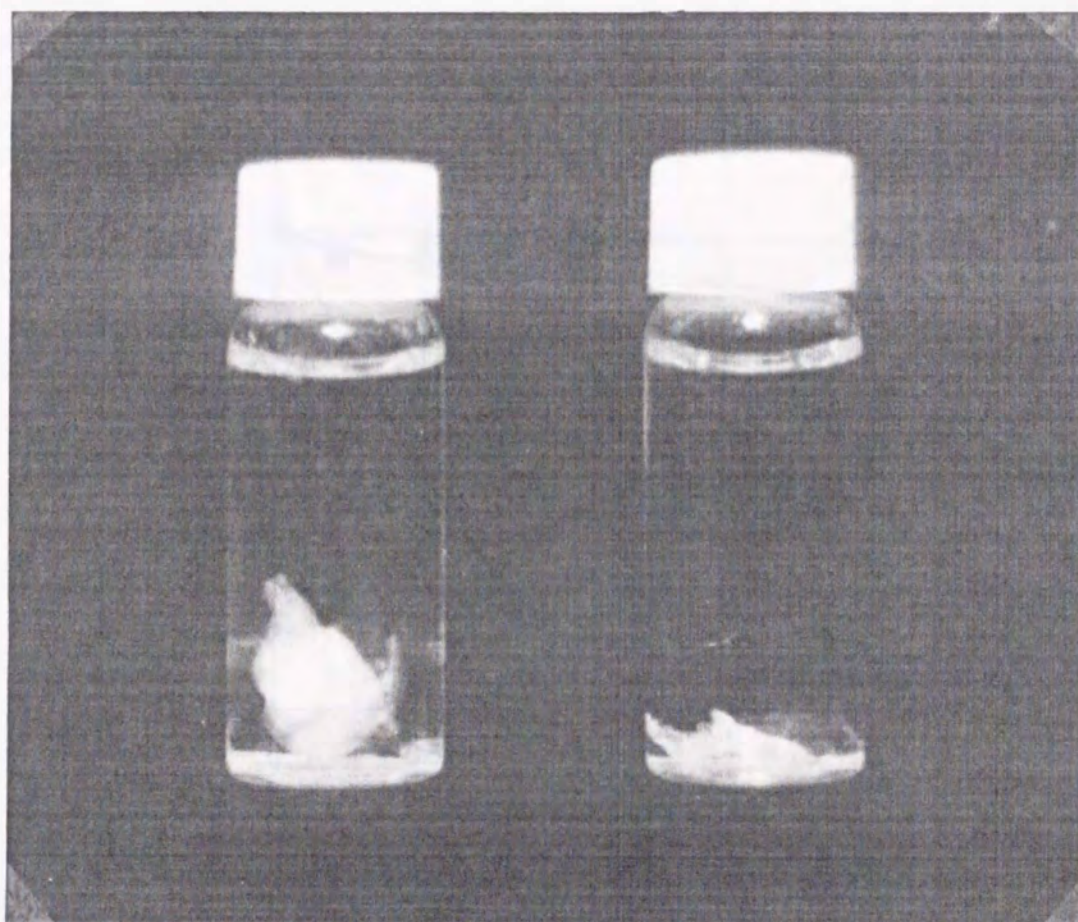


Figure 3.7 Picture of the swollen (left) and shrunken (right) polyion complexes.

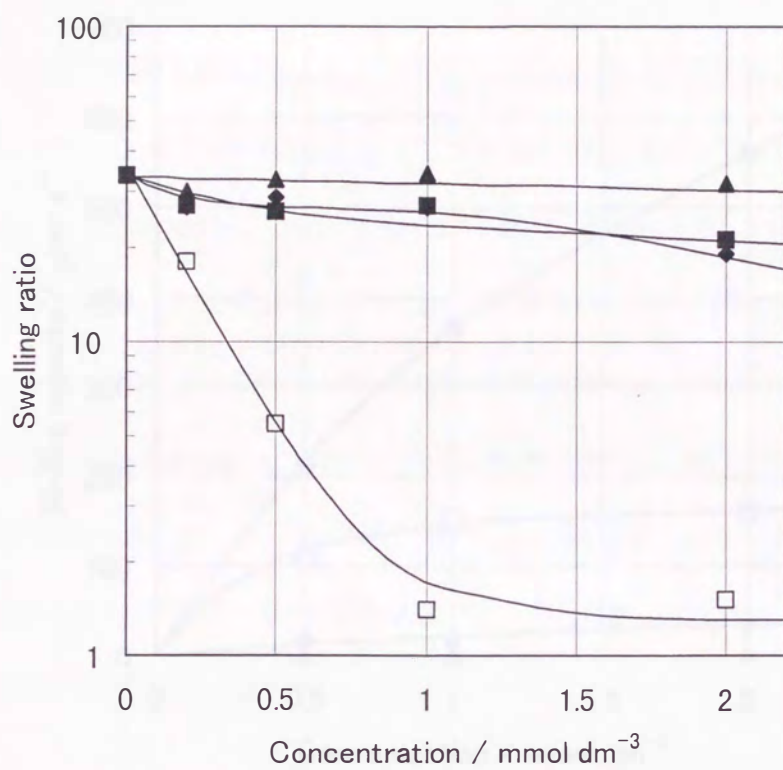


Figure 3.8 Plots of the equilibrium swelling ratio of the AMP-imprinted polyion complex vs. the additive concentration: □ AMP, ■ Ad, ◆ DAMP, ▲ DAd.

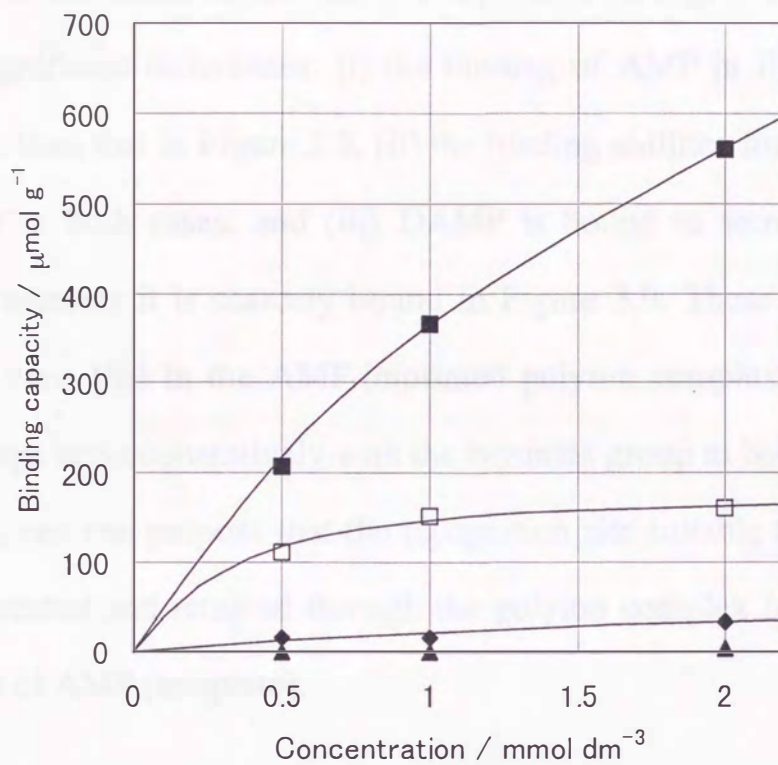


Figure 3.9 Equilibrium binding capacities of the non-imprinted polyion complex in a basic aqueous solution (pH 9.3): $\square$ AMP, $\blacksquare$ Ad, $\blacklozenge$ DAMP, $\blacktriangle$ DAd.

AMP (and its analogues). This charge-neutralized polyion complex kept its shrunken state during this experiment. Figure 3.9 shows the binding ability of the non-imprinted polyion complex. It appears in the order of Ad > AMP > DAMP > DAd. When Figure 3.9 is compared with Figure 3.6, one can see some significant differences: (i) the binding of AMP in Figure 3.6 is much higher than that in Figure 3.9, (ii) the binding abilities for Ad are the same extent in both cases, and (iii) DAMP is bound to some extent in Figure 3.6, whereas it is scarcely bound in Figure 3.9. These differences support the view that in the AMP-imprinted polyion complex the excess cationic charge acts cooperatively with the boronate group to bond AMP. In other words, one can propose that the recognition site suitable to the AMP binding is created and retained through the polyion complex formation in the presence of AMP (template).

3.5 Shrinking kinetics of AMP-imprinted polyion complex

Figure 3.10 shows the shrinking kinetics of the AMP-imprinted polyion complex. Initially, it was equilibrated in ammonium buffer solution (pH 9.3) and then AMP was added to the solution. After 1 h, the swelling ratio became $1/2 - 1/3$ of the initial state and then it shrunk to $1/6$ after 3 h. After that, the polyion complex reduced its size more slowly.

Recently a thermo-responsive poly(*N*-isopropylacrylamide) cross-linked gel has received attention because it shows unique swelling-

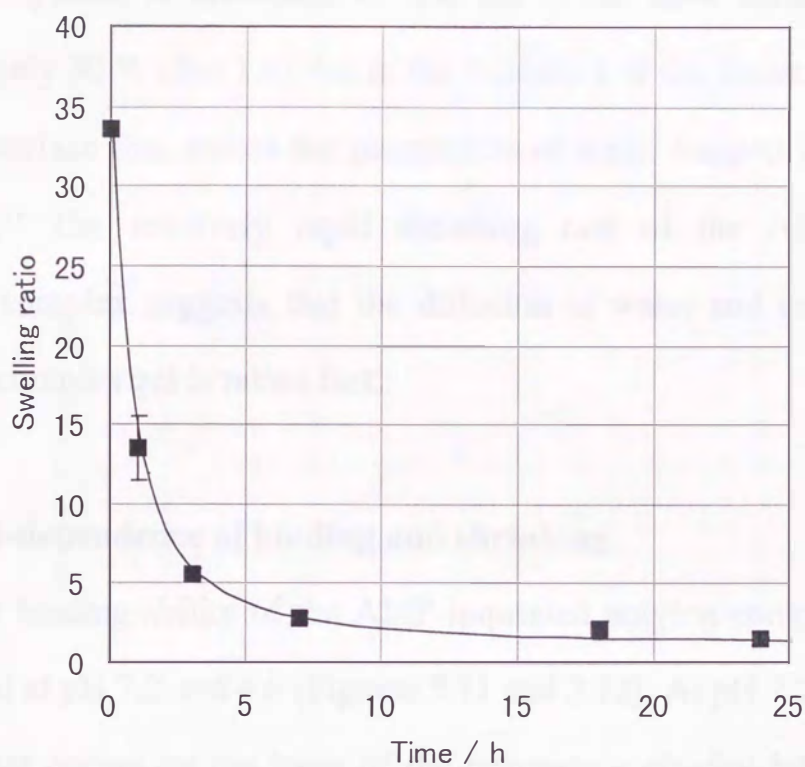


Figure 3.10 Shrinking kinetics of the AMP-imprinted polyion complex. The polyion complex was first swelled in a basic aqueous solution (pH 9.3), and then aqueous AMP solution (pH 9.3) was added to the solution to make the final AMP concentration at 2 mmol dm^{-3} .

shrinking transition phenomenon at LCST (lower critical solution temperature) and has potential applications in many fields, *e.g.*, drug delivery system. A drawback of this gel is the slow shrinking rate (it shrinks only 30 % after 1 h) due to the formation of the dense skin layer on the gel surface that makes the permeation of water trapped inside the gel difficult.¹⁷ The relatively rapid shrinking rate of the AMP-imprinted polyion complex suggests that the diffusion of water and ions inside the polyion complex gel is rather fast.

3.6 pH-dependence of binding and shrinking

The binding ability of the AMP-imprinted polyion complex was also estimated at pH 7.2 and 4.6 (Figures 3.11 and 3.12). At pH 7.2, the binding of Ad that occurs on the basis of the boronate – *cis*-diol interaction was markedly decreased. The binding of AMP was comparable with that of DAMP. It is well-known that the lower the pH of the solution, the weaker the stability of the boronate – *cis*-diol complex.⁹⁻¹¹ Since AMP and DAMP behaved similarly, one can consider that the major driving force for the binding is an electrostatic interaction between the anionic phosphate group and the positively-charged polyion complex. At pH 4.6, Ad was scarcely bound, while AMP was still bound although weakly owing to the residual electrostatic interaction.

The shrinking behavior of the polyion complex is shown in Figures

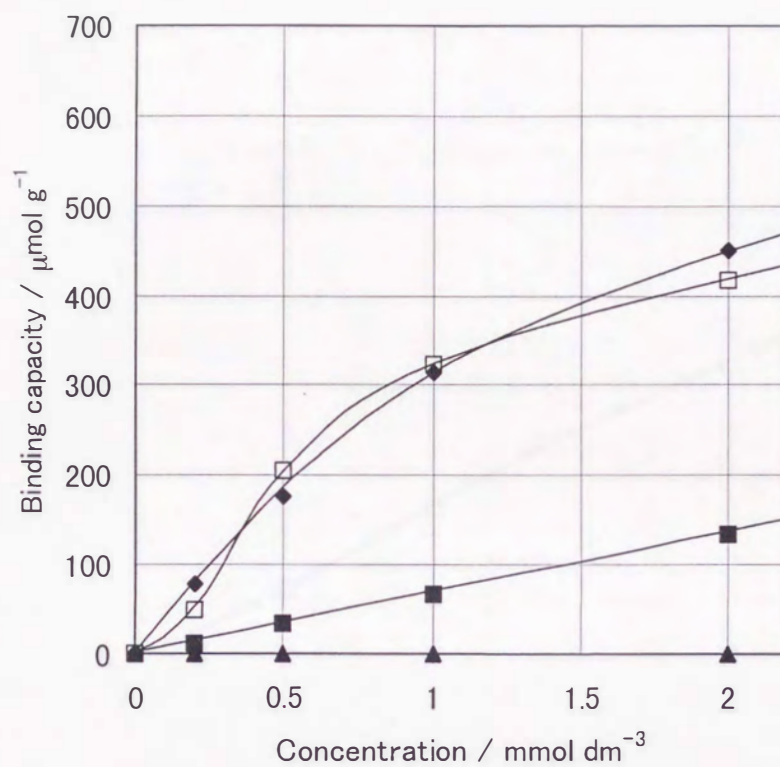


Figure 3.11 Equilibrium binding capacities of the AMP-imprinted polyion complex at pH 7.2: $\square$ AMP, $\blacksquare$ Ad, $\blacklozenge$ DAMP, $\blacktriangle$ DAd.

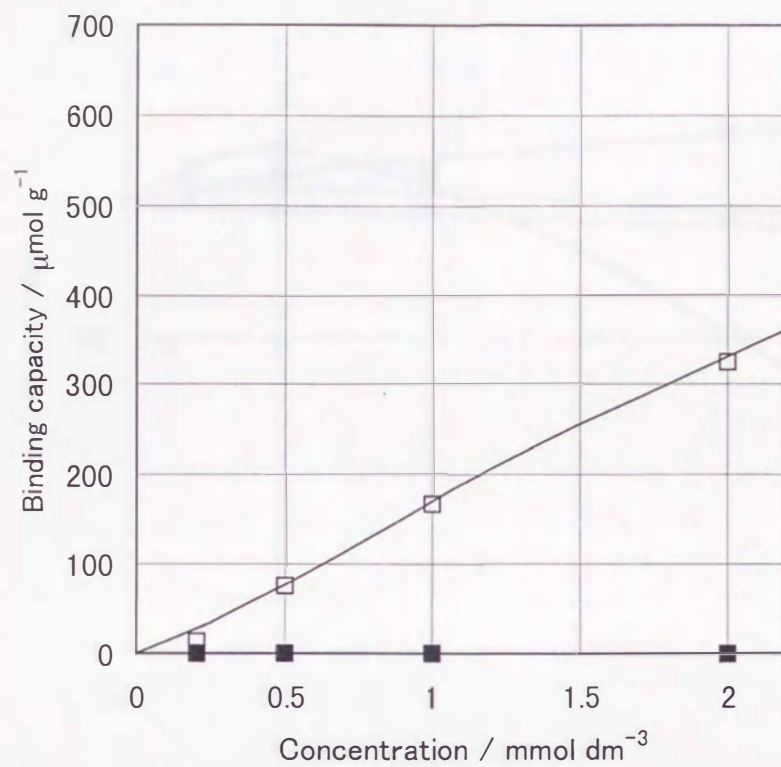


Figure 3.12 Equilibrium binding capacities of the AMP-imprinted polyion complex at pH 4.6: $\square$ AMP, $\blacksquare$ Ad.

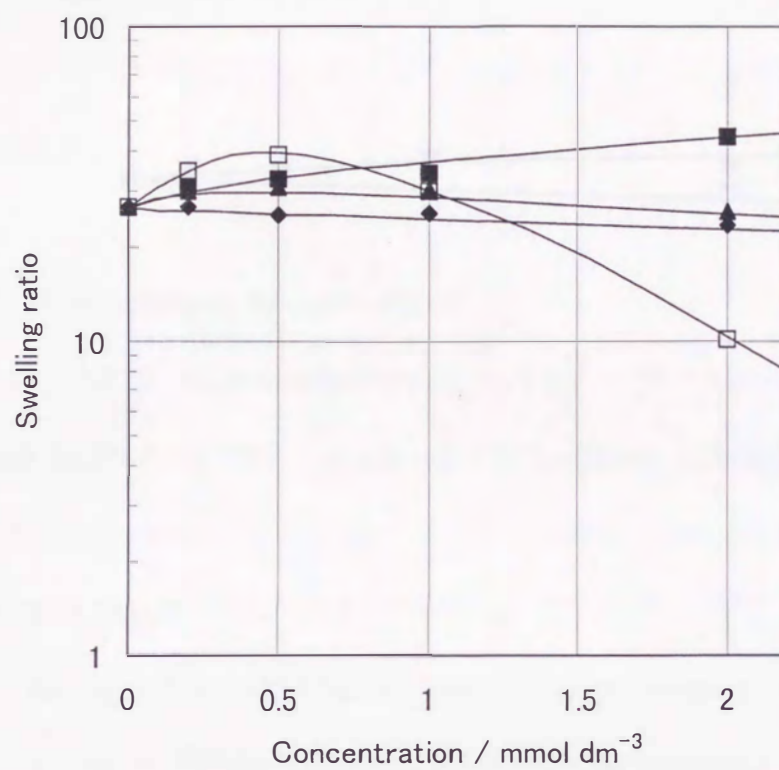


Figure 3.13 Plots of the equilibrium swelling ratio of the AMP-imprinted polyion complex vs. additive concentration at pH 7.2: □ AMP, ■ Ad, ◆ DAMP, ▲ DAd.

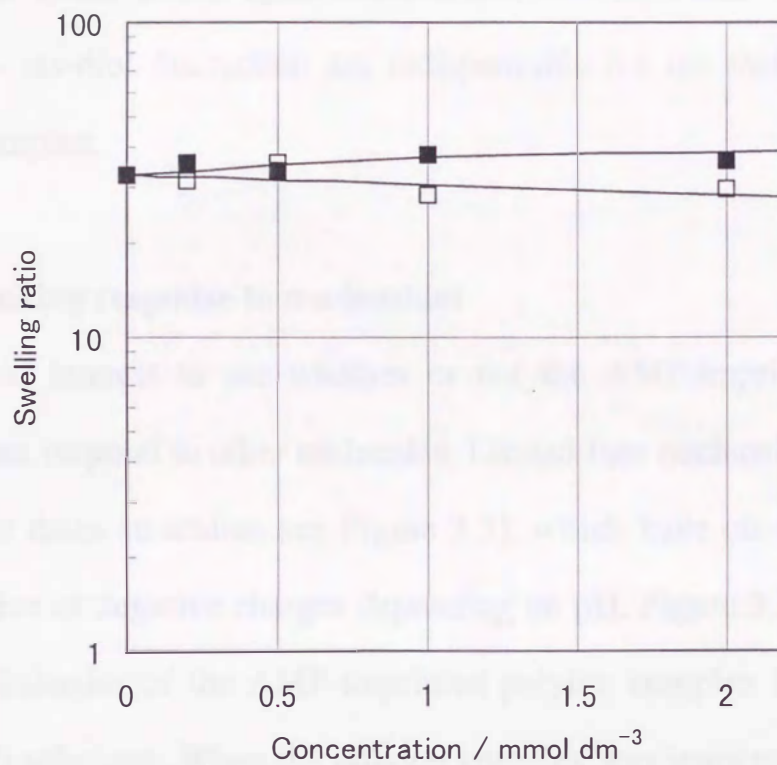


Figure 3.14 Plots of the equilibrium swelling ratio of the AMP-imprinted polyion complex vs. additive concentration: □ AMP, ■ Ad.

3.13 and 3.14. At pH 7.2, the polyion complex did not shrink in the solution containing lower AMP concentration but did in the solution containing higher AMP concentration. At pH 4.6, the shrinking did not take place at all. These results again indicate that both the electrostatic and the boronate – *cis*-diol interaction are indispensable for the shrinking of the polyion complex.

3.7 Shrinking response to nucleosides

It is of interest to see whether or not the AMP-imprinted polyion complex can respond to other molecules. I tested four nucleosides (Ad, Gu, Cy, Ur: for these structures see Figure 3.3), which have *cis*-diol unit and have positive or negative charges depending on pH. Figure 3.15 shows the shrinking behavior of the AMP-imprinted polyion complex in nucleoside (and AMP) solutions. When the polyion complex was immersed in Gu and Ur solutions at pH 9.3, it gradually shrunk with increasing additive concentrations, although the degree of shrinking were not so significant as that for AMP. The polyion complex was, however, only slightly shrunk in Ad and Cy solutions.

This selectivity can be explained by the charge difference of the nucleosides. Due to the dissociation of proton from guanine and uracyl units (Figure 3.16), Gu and Ur exist as mono-anionic species at pH 9.3. On the other hand, Ad and Cy do not have such deprotonation equilibria at pH

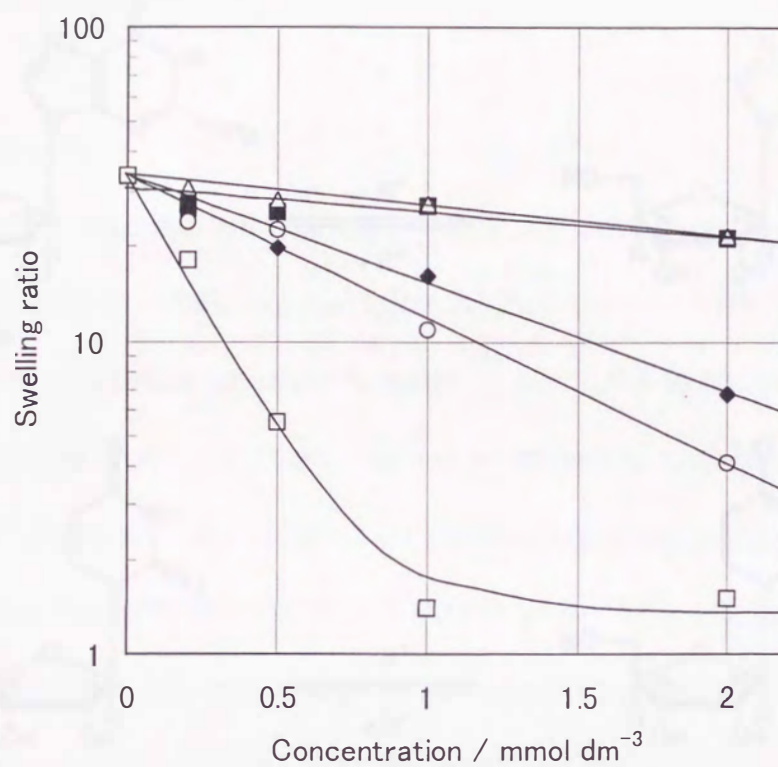


Figure 3.15 Plots of the equilibrium swelling ratio of the AMP-imprinted polyion complex vs. additive concentration at pH 9.3: ■ Ad, ◆ Ur, ○ Gu, △ Cy, □ AMP.

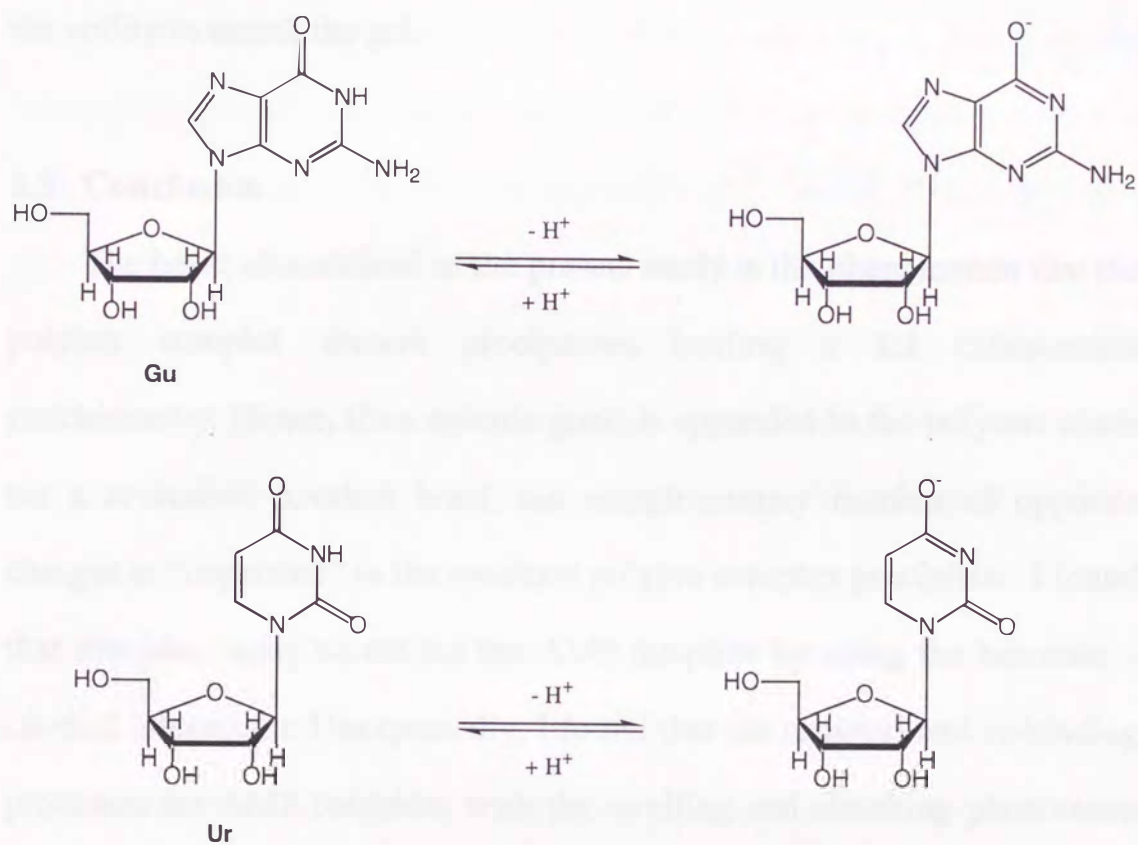


Figure 3.16 Proton dissociation equilibria.

9.3 and exist as neutral species. Therefore, I conclude that guest molecules having both *cis*-diol unit and negative charge can shrink the AMP-imprinted polyion complex, and the greater the negative charge, the greater the ability to shrink the gel.

3.8 Conclusion

The basic idea utilized in the present study is the phenomenon that the polyion complex always precipitates holding a 1:1 cation/anion stoichiometry. Hence, if an anionic guest is appended to the polymer chain *via* a reversible covalent bond, the complementary number of opposite charges is “imprinted” in the resultant polyion complex precipitate. I found that this idea really works for the AMP template by using the boronate – *cis*-diol interaction. Unexpectedly, I found that the removal and re-binding processes for AMP coincides with the swelling and shrinking phenomena of this polyion complex gel. These findings provide a new possibility that the polyion complex can be applied to a novel chemomechanical system, drug delivery system, sensing system, *etc.* The application of the AMP-imprinted polyion complex to a sensing system is described in Chapter 4.

3.9 Experimental

Preparation of polymers. Polyanion **1** was synthesized by reacting poly(ethylene-*alt*-maleic anhydride) (Aldrich, M_w : 100,000-500,000) with

3-aminophenylboronic acid (Tokyo Kasei) in DMF at 70 °C for 20 h. Formation of polyanion **1** was confirmed by FT-IR and ^1H NMR spectra. Poly(ethylene-*alt*-maleic anhydride) showed a $\nu_{\text{C=O}}$ band at 1790 cm^{-1} due to the acid anhydride group. This peak disappeared after the reaction and new peaks assignable to the carboxylic acid and the amide group appeared at 1710 cm^{-1} and 1670 cm^{-1} , respectively. In the ^1H NMR spectrum polyanion **1** showed broad signals at around 7.5 (which consists of four peaks), 2.7, and 1.7 ppm which were assignable to aromatic protons, CH proton, and CH_2 protons, respectively. The integral ratio was 4:2:4, which supports the view that all acid anhydride groups had reacted with 3-aminophenylboronic acid. Polycation **2** ([poly(diallyldimethylammonium chloride), M_w : 400,000-500,000] was purchased from Aldrich.

Preparation of polyion complexes. An aqueous solution (1.0 cm^3 , pH 10.3 with $100\text{ mmol dm}^{-3}\text{ Na}_2\text{CO}_3/\text{NaHCO}_3$) containing AMP or its analogues (10 mmol dm^{-3}) and an aqueous solution ($0.5 - 3.0\text{ cm}^3$, pH 10.3 with $100\text{ mmol dm}^{-3}\text{ Na}_2\text{CO}_3/\text{NaHCO}_3$) containing polycation **2** (20 monomer unit mmol dm^{-3}) were mixed and the solution was stirred at 25 °C for 1 h. To this solution was added dropwise an aqueous solution (1.0 cm^3 , pH 10.3 with $100\text{ mmol dm}^{-3}\text{ Na}_2\text{CO}_3/\text{NaHCO}_3$) containing polyanion **1** ([carboxylate unit] = [boronate unit] = 10 mmol dm^{-3}) [thus the ratio of AMP (or its analogue) vs. boronate unit was maintained as 1:1] and then

the mixture was stirred at 25 °C for 1 h. The precipitate formed by this reaction was collected by centrifugation and dried under vacuum. To estimate the amount of bound AMP or its analogues, UV spectra of the supernatant solutions were measured (adenine group has an adsorption maximum at 260 nm) and the concentrations of the unbound nucleotides were determined.

Removal of AMP from polyion complex. The AMP-imprinted polyion complex prepared at the mixing ratio ([cation unit] / [boronate unit]) = 2.0 was immersed in 100 mmol dm⁻³ acetate buffer (pH 5.5) at 25 °C for 41 h. The amount of extracted AMP was determined by measuring UV absorption spectra of the solution phase. After that, the polyion complex was dried under vacuum.

Re-binding of AMP to polyion complexes. After the removal of AMP from the AMP-imprinted polyion complex, it was immersed in aqueous solutions (pH 9.3 with 10 mmol dm⁻³ NH₃/NH₄Cl, pH 7.2 with 10 mmol dm⁻³ MOPS buffer, pH 4.6 with 10 mmol dm⁻³ CH₃COOH/CH₃COONa) of AMP or its analogues at 25 °C for 24 h with occasional stirring. The polyion complex was taken out of the solution and weighed after removing excess water from the polyion complex surface. The amount of bound AMP or its analogues was determined by a UV

measurement of the solution.

Shrinking kinetics of polyion complex. The AMP-imprinted polyion complex was immersed in 10 mmol dm⁻³ NH₃/NH₄Cl buffer (3.0 cm³, pH 9.3) at 25 °C for 24 h. To this solution was added an aqueous AMP solution (5 mmol dm⁻³, 2.0 cm³, pH 9.3 with 10 mmol dm⁻³ NH₃/NH₄Cl). Thus, the AMP concentration in the solution became 2.0 mmol dm⁻³ after the mixing. The weight of the polyion complex was measured following the time course.

Instrumentation. UV spectra were recorded on Jasco V-570 spectrophotometer using a quartz cell with 10 mm path length. ¹H NMR and FT-IR spectra were measured with Bruker AC-250P and Shimadzu FT-IR 8100 spectrometers, respectively.

3.10 References

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