Synthesis of Polymer Gel Beads and Application to High Performance Liquid Chromatography

(高分子ゲルビーズの合成と高速液体クロマトグラフィーへの応用)

岡野 貴司

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荻野　賢司

Preface

The present thesis is the collection of studies which have been carried out under the direction of Professor Hisaya Sato at Tokyo University of Agriculture and Technology during 1986-1994. The studies are concerned with the synthesis, the characterization in a swollen state, and the application of polymer gel beads to high performance liquid chromatography of polymeric compounds.

The author express his sincere gratitude to Professor Kazuyuki Horie who has offered the author valuable advice and continuous encouragement throughout the work. Grateful acknowledge is made to Professor Hisaya Sato for his constant guidance and many helpful suggestions and discussions during the work. The author would like to express the heartful gratitude to Professor Hiroyuki Matsui, Professor Kazuhiko Saigo, Associate Professor Takuzo Aida, and Lecturer Toshi Yamashita for their stimulative advice.

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Kenji OGINO

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Part I

General Introduction

I-1. Back ground

I-1-1 Polymer gel

Polymer gel is defined as polymer which is insoluble in any kind of solvents and has three dimensional network or one in a swollen state. A cross-linked polymer which has three dimensional structure shows finite swelling phenomenon. The cross-linked structure is formed via either chemical bond or secondary physical bond such as hydrogen bond and hydrophobic bond between polymer chains.

Polymer gels resulting from physical bonds are referred to as physical gel. It is well-known that the aqueous solutions of various types of naturally occurring polymers such as dextran and gelatin are subject to gelation by cooling the solutions. Gelation of some polymer gels is attributed to the physical transition of polymer chains, i.e., some parts of polymer chain undergoes helix-coil transition, and cross-linking domains are formed by resulting helix. It is reported that some synthetic polymers also form this types of gel. For example, poly(vinyl alcohol) solutions forms gel by the repetitive freezing and thawing.[1] The resulting hydrogel is highly elastic, and has been investigated as a possible candidate for artificial muscle or gel actuator, because it has enough toughness and reasonable response rate of swelling and/or deswelling.[2-4] It is said that polymer chains are partially crystallized to produce the physical cross-link points.

Chemically cross-linked polymer is prepared by polymer reactions in the presence of cross-linking agent or by the copolymerization of difunctional monomers and monomers having three or more functional groups. For example, poly(chloromethyl styrene) is crosslinked by intermolecular Friedel-Crafts type of reaction in using Lewis acid such as $AlCl_3$.[5] Polyelectrolytes such as poly(acrylic acid) forms cross-linking structure by reacting with calcium ion. Hydrophilic polymers having hydroxyl groups such as polysaccharide and poly(vinyl alcohol) are crosslinked by glutaraldehyde, epichlorohydrin, and ethylene glycol glycidyl ether. Chemically cross-linked polymer can also be prepared by the copolymerization of vinyl monomer with di- or polyvinyl monomer. This method provides styrene-divinylbenzene gel bead for packings of high performance liquid chromatography, acrylamide-N,N'-methylene bis(acrylamide) gel for

the stationary phase of electrophoresis, and 2-hydroxyethyl methacrylate gel for soft-contact lens.

In general when a good solvent is added to a cross-linked polymer network, it becomes highly expanded isotropically and extremely porous. If degree of cross-linking is low, the polymer network swells well and swollen network consists largely of solvent with a small fraction of polymer. With the increase of degree of cross-linking, the swelling ability decreases. With non-solvating solvent, cross-linked network shows little tendency to expand. The mechanical stability of polymer networks increases with the cross-linking density.

Spherical shape of polymer gel is extremely convenient in the applications of polymer gel to packings of liquid chromatography, and polymer supports. The most useful technique for synthesizing polymer gel beads is suspension copolymerization of vinyl monomer and divinyl monomer. This usually involves suspending droplets of a mixture of water-insoluble monomer, divinyl monomer, and radical initiator in an aqueous media containing suspension stabilizer such as poly(vinyl alcohol) and poly(vinyl pyrrolidone). In the case of water soluble monomers such as acrylamide and N,N'-methylene bis(acrylamide), inverse suspension polymerization is necessary, where a mixture of monomers and water-soluble initiator is dispersed in a water immiscible medium such as paraffin oil. In both cases, polymerization starts in the monomer droplets as the initiator thermally dissociates.

The author briefly reviews styrene-divinylbenzene gel beads, which have been most commonly used in various fields.

When a mixture comprising only of styrene and divinylbenzene is polymerized, so-called gel-type of polystyrene gel is obtained. In the early stage of polymerization, resulting polymer network are solvated by the unreacted monomers, because both monomers are good solvents for polymer network. As polymerization proceeds, unreacted monomers decrease and finally disappear resulting in the hard polymer gel. Although the polymer gel has negligible pore volume in a dry state or in a poor solvent, it swell with the absorption of a good solvent and the porosity increases. The swelling

ratio or the amount of absorbed solvent decreases with the increase of cross-linking density, or the content of divinylbenzene. The mechanical strength in a swollen state increases with cross-linking density, with the significant reduction of rate of penetrant transfer. Therefore, the content of divinylbenzene of gel-type resin does not usually exceed 10% when the beads are applied to the polymer supports of ion-exchange resins or polymeric catalysts.

Highly porous polymer gel with good mechanical strength can be prepared by the compolymerization of styrene with large amount of divinylbenzene in the presence of inert solvents or diluents which is referred to as porogens.[6-12] Although the diluent must be miscible with the mixture of monomers, it can be either a good solvent or a precipitant for resulting polymer. When the diluent is removed, the pore remains as the image of the diluent, because the polymer does not shrink or swell due to highly cross-linked structure. This type of resin is referred to as macroporous resin. The macroporous structure provides large surface area and allows the facile penetrant transfer and acceptance of non-solvents such as methanol, heptane and acetonitrile as well as good solvents even if crosslinking density is high. Due to these properties, macroporous polymer beads have been applied to the packing materials of liquid chromatography and polymer supports for ion-exchange resins and polymeric catalysts.

When a good solvent such as toluene and diethyl benzene is used as a diluent, the growing chain tends to remain solvated even if the monomers are completely consumed. Because solvated polymer chains have little tendency toward phase separation, the size of resulting pore is small. When a poor solvent such as dodecan or isoamyl alcohol is used as the diluent, growing polymer chains precipitate in the droplet and tend to aggregate to yield permanent large pores containing only the diluent. This macroporous resins is specially referred to as macroleticular resins. Thus, by selecting appropriate diluent, pore size can be controlled almost independently of the crosslinking density, or the mechanical strength.

To use styrene-divinylbenzene gel beads as the polymer support, it is sometimes necessary to modify the polymer chains by various chemical reactions.

Chloromethylation is one of the most important process because of high reactivity of chloromethyl group toward nucleophilic species. Chloromethyl groups can be incorporated in the styrene gel by the reaction with chloromethyl methyl ether in the presence of Lewis acid such as stannic chloride.[13-16] After chloromethylation, a variety of nucleophilic reactions have been conducted to further modify the gel. For example, the chloromethylated resins react with tertiary amine to incorporate quaternary ammonium groups.[17-19] This process is the major route to strong anion-exchange resins. Strong cation-exchange resins are manufactured by sulphonation of styrene gels.[20,21] The other versatile route in chemical modification of styrene resin is lithiation of aromatic rings. Styrene-divinylbenzene copolymers have been lithiated directly using n-BuLi/tetramethylethylenediamine[22,23], and indirectly lithium-bromine exchange[23]. Further chemical modification of lithiated resins is conducted through the electrophilic reaction. Carboxylic acid,[23] thiol,[24] thioether,[25] phosphine,[26,27] groups have been introduced.

I-1-2 Polymer gel beads as packing materials for liquid chromatography

High performance liquid chromatography (HPLC) including size exclusion chromatography (SEC) has been widely applied to the separation, purification, and characterization of low molecular weight compounds as well as high polymers. Polymer gel beads have been applied to HPLC as one of the most important stationary phase. One of the first polymer packing materials was diethylaminoethyl derivatives of polysaccharide for the adsorption and desorption of proteins by changing the ionic strength of eluent.[28] Porath applied cross-linked polydextrane gel to the separation of water soluble biological compounds depending on molecular weight in 1959.[29,30] These types of polymer packings are lightly cross-linked polymer gels. In 1964, More synthesized macroporous styrene-divinylbenzene copolymer beads by suspension polymerization in the presence of porogens and applied them to SEC of organic polymers.[8] One of the characteristics of these gel beads is the fact that the pore size can be controlled almost independently of the crosslinking density, which has enabled preparation of porous beads with high mechanical strength.

In the application of polymer gel beads to packing materials for HPLC, polymer gel beads needs to have special characteristics such as sufficient mechanical strength, porous structure, and facility of penetrant transfer in gel beads. In these points, macroporous type of gel beads are suitable for packings of HPLC. As further important properties, gel beads must have controlled pore size and its distribution, and appropriate diameter with high uniformity.

After the pioneer studies of Porath and Moore, a number of studies have been done on the preparation of polymer gel beads for SEC. Various types of polymer gel beads have been prepared such as styrene-divinylbenzene, methyl methacrylate-ethylene dimethacrylate[31], and vinylacetate-divinyl ether[32] copolymer beads for the packings of SEC. However, styrene-divinylbenzene gel beads are most commonly used and are commercially available in a wide range of pore sizes and particle sizes. Pore size and its distribution of macroporous polymer gel can be controlled by the amount and kind of diluent used as mentioned in a previous section.

In the case of SEC, packing materials must interact with samples sterically only. As is often the case with aqueous SEC, other interactions, such as ionic, hydrophobic, charge transfer interactions, may not become negligible. In such a case, it is difficult to determine the molecular volume of the sample. Therefore, packings used in aqueous SEC should be highly hydrophilic and should not have significant charges besides the factors mentioned above. Cross-linked dextran gels have been still used as the packings because of their hydrophilicity and neutral characteristics, however these gel beads are too soft and shows too low pressure proof to be used under the high pressure. Highly cross-linked poly(2-hydroxyethyl methacrylate)[33-36] gel and poly(vinyl alcohol)[37,38] gel which is prepared by the hydrolysis of poly(vinyl acetate) gel beads are generally used.

In the application of polymer gels to packings of adsorption or partition mode-HPLC, chemical structure of polymer gel beads is also important as well as the factors as mentioned above. Polymer gel beads such as styrene-divinylbenzene[39-42] and octadecyl methacrylate-ethylene dimethacrylate[43] gels can be used as the packing materials of reversed-phase HPLC, and the latter has superior retention ability to octadecyl silica gel

(ODS), which is the most popular stationary phase used in reversed-phase HPLC. In the case of styrene gels, however, the specific interaction with aromatic compounds causes the peak tailing. This problem has been solved by the alkylamination of styrene gels.[44] In order to obtain the properties equal to ODS, the hydrophilic polymer gels such as polyacrylamide[45] or poly(vinyl alcohol)[46] based gel beads have been alkylated.

Finally, particle size and its distributions are important factors when the polymer beads are applied to packings for HPLC. As mentioned above, polymer gel beads for packing materials of HPLC have been usually prepared by conventional or inverse suspension polymerization. However, these methods provide particles having relatively broad size distribution, which can not be used directly for chromatography, because small particles cause serious pressure drop and big ones decrease the column efficiency. Therefore, a time consuming size classification is necessary to obtain a fraction with narrower distribution in particle size which are used as packing materials, while the remaining fractions are cast as wastes. Although the obtained fraction has a reduced polydispersity of size, it is not completely uniform. It is of importance to synthesize the monodisperse packing materials in order to improve productivity and column efficiency.

In order to synthesize monodisperse polymer beads, Ugelstad developed a technique which he termed the "activated multi-step swelling and polymerization" method.[47] This method has been applied to the preparation of monodisperse porous styrene-divinylbenzene copolymer beads for SEC, reversed-phase and ion-exchange chromatography.[48-51] In the first step the seed particles are activated by the absorption of water insoluble molecule, such as 1-chlorododecane and in some case the accelerator such as acetone, and in the next step styrene, divinylbenzene and diluent is absorbed followed by polymerization process.

I-1-3 Characterization of polymer gel beads

The chemical structure, mobility of polymer chains and the morphological characteristics are all important factors which determine the functionality of polymer gel beads. Therefore, the characterization must be done taking thsse factors into consideration. Because polymer gel beads are usually used in a swollen state in various

applications, it is important to characterize polymer gel in a swollen state as well as in a dry state.

Solid state nuclear magnetic resonance (NMR) spectroscopy was revealed to be a powerful tool for the characterization of solid polymer by Schaefer[52-54]. It has been applied to styrene-divinylbenzene gel in a dry state using cross polarization and magic angle spinning (CP-MAS) technique.[55,56] Chemical structure can be determined by this technique. For example, the amount of unreacted vinyl groups is determined by ^{13}C-NMR spectroscopy for styrene-divinylbenzene copolymers.[55] In the case of swollen state, Ford reported[57] that high resolution NMR (for solutions) can not fully cope with the characteristics of solvent swollen styrene-divinylbenzene gels because of great dipole broadening. Cross polarization magic angle spinning (CP/MAS) method is useful for the characterization of solid polymers, however, it is of limited value for gel state samples, because a rapid local segmental motion of polymer chains of gels may reduce the dipole coupling required for efficient cross polarization from proton to carbon nuclei. The method with direct polarization and magic angle spinning (DP/MAS) seems successful as pointed by Stöver[58]; however, this method can be applied for lightly cross-linked materials. Up to now, the method has not been found to characterize the swollen highly cross-linked polymer network structure.

On the other hand, small molecules in cross-linked polymer give high resolution NMR spectra even if crosslinking density is high. A number of studies have been conducted by NMR concerning small molecules in polymer network. For example, Ford reported that each carbon atom in toluene molecule in cross-linked polystyrene gel beads provided doublet peaks in ^{13}C-NMR spectra[59-61], and assigned these signals to toluene inside and outside of polymer gel beads. The exchange rate of two sites were estimated from the apparent values of spin-lattice relaxation times of each signal. The mobility of toluene inside of polymer beads was evaluated by measuring the self-diffusion coefficients of toluene with the pulsed-gradient spin echo NMR method, and experimental results showed the self-diffusion coefficient of toluene inside of cross-linked polystyrene beads was equal to one in toluene solution of linear polystyrene

containing the same weight fraction polymer as in the gel.[62] Luminescence spectroscopy using the triplet probe also revealed that the diffusion of small molecules in semidilute polymer solution is not affected by the crosslink formation between polymer chains.[63,64]

Separate resonances of water or ions inside and outside of ion-exchange resins have also been reported in ^{1}H-NMR, when ion-exchange resins are immersed in aqueous solution.[65-74] This phenomenon has been used for the evaluation of the properties of ion-exchange resins[65-69,71-73] and for the determination of the exchange rate of two sites[70].

As the morphological characterization, the evaluation of pore size and its distribution has been carried out by classical mercury porometry in a dry state. This method is based on the fact that the necessary pressure for the intrusion of mercury into the capillary is the function of the diameter of the capillary.[75] Surface areas are usually measured by nitrogen adsorption-desorption isotherms using the BET method.[76,77] However, these methods can not be applied to the solvent swollen samples.

It is well known that water in a capillary or in a small space of silica and other inorganic materials sometimes remains unfrozen below the freezing point since the thermodynamic nature is different from that in the bulk state.[78-80] Some organic solvents also remain unfrozen below their freezing point in the presence of polymer matrices such as polystyrene network[81] and cross-linked rubber[82,83]. Using this phenomenon, the polymer network structure can be characterized indirectly, since freezing point depression depends on the nature of the network. Brun proposed the method to determine the pore size of styrene-divinylbenzene copolymer beads in a swollen state by monitoring the freezing point depression of benzene with differential scanning calorimetry (DSC).[84] The following relationships was given between the pore diameter (D) and the freezing point depression (ΔT).

$$D/nm = -131.6 / \Delta T - 0.79$$

I-1-4 Application of HPLC to analysis of synthetic polymers

Synthetic polymers have various intermolecular heterogeneity, such as in molecular weight, chemical composition of copolymers, stereo regularity, and isomeric

structure of diene polymers. This fact indicates that various types of distribution exist in prepared polymers. The separation methodology is necessary for the determination of distribution , and chromatographic analysis is suitable for this purpose.

For determination of average molecular weight and its distribution, SEC has been widely used, and polymer gel, typically styrene-divinylbenzene gel beads are the most commonly used packing materials. Analysis of organic polymeric compound by HPLC is predominantly performed according to the size exclusion mechanism. The size exclusion is entropy-controlled process, ideally without any enthalpic interactions between the samples and packing materials. The sample is separated depending on hydrodynamic volume, $M[\eta]$, where M and $[\eta]$ are molecular weight and intrinsic viscosity, respectively. If $[\eta]$ is known, molecular weight and its distribution have been determined by SEC.

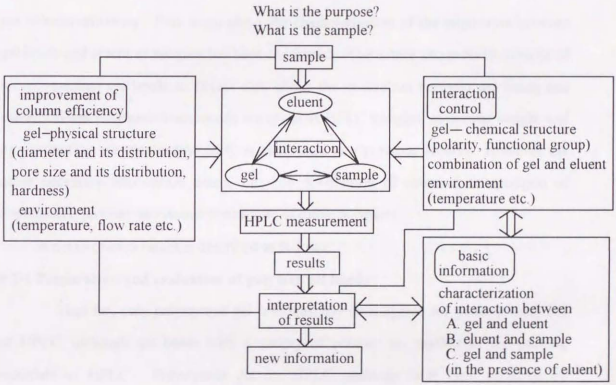
Copolymers have chemical composition distribution (CCD) as well as molecular weight distribution. In order to determine CCD, it is necessary to separate the copolymers dependent on the chemical composition.

If the polymer sample interacts with the packing material strongly, it will not elute out unless the eluent composition is changed. In 1979, Teramachi et al.[85] separated copolymers of styrene and methyl acrylate according to copolymer composition using the adsorption-desorption mechanism and the solvent-gradient method with silica-based packing materials. This separation was attributed to the difference of adsorption ability of each copolymer to the packing materials. A number of studies[86-113] about the separation of copolymers with adsorption HPLC have been done using silica and modified silica gel as the packing materials.

Sato et al. have demonstrated that the polymer gel bead is a superior stationary phase than silica gel for separating polymers by the adsorption mechanism, because of the good reproducibility and proportionality between the peak area and sample amount due to the smaller amount of irreversible adsorption.[114-117] Moreover, utilizing superior characteristics of polymer gel, Sato monitored the elution of poly(styrene-stat-methyl methacrylate) by ultraviolet detector (UV, 254nm, sensitive to styrene) and infrared

detector (IR, 1730cm^{-1}, sensitive to methyl methacrylate) using acrylonitrile gel as packing material.[116] From the peak height ratio of UV/IR, the actual composition of the copolymer could be derived to make the calibration between styrene content in copolymer and elution volume.

I-2. The aim of this study



Scheme I-1. Ideal HPLC experiment

Scheme I-1 shows the ideal guide of HPLC experiments.

In order to obtain new information from HPLC results, experiments must be conducted under well-controlled proper conditions and results must be interpreted completely. The ultimate aim of author's study is to create the complete HPLC system and understand HPLC elution behaviors well based on the basic data about the interaction between gel beads used as packing materials and samples in the presence of eluent.

The author considers this thesis as the first step to fulfill the ultimate aim. This

study is focused on three targets concerning polymer gel beads.

1) Preparation and evaluation of polymer gel beads

2) NMR analysis of the interaction between the gel beads and solvent

3) Application of gel beads to adsorption mode HPLC of polymeric compounds

The control of the chemical and physical structure of gel beads is one of the most important methods to control the interaction between gel beads and samples and improve the column efficiency. Few study about the characterization of the interaction between gel beads and eluent or samples has been conducted. The author chose NMR analysis of solvent-swollen gel beads to obtain data about the interaction between gel beads and eluent. When polymeric compounds are given as HPLC samples, molecular weight and its distribution are obtained by SEC as mentioned in a previous section. However the other necessary information exists, i.e., the distribution of chemical composition of copolymers, tacticity and isomeric structure of diene polymers.

A detail of each target is described as follows.

I-2-1 Preparation and evaluation of polymer gel beads

Thus far, only polystyrene gel is extensively investigated for packing materials of HPLC, although gel beads with a variety of polarity are needed as the packing materials of HPLC. Polystyrene gel for HPLC packings have been prepared by conventional suspension polymerization. The study have started on the preparation and characterization of more hydrophilic polymer gel such as acrylonitrile and 2-hydroxyethyl methacrylate gels, which are difficult to be prepared by conventional suspension polymerization, since the monomers are soluble in water.

More hydrophilic monomers or cross-linking agent, such as acylamide and methylene-bis(acrylamide), cannot be polymerized by suspension polymerization. Inverse suspension polymerization seems suitable for the preparation of polymer gels from these monomers, because polymerization proceeds in water-droplets containing monomers and initiator. Althogh this process has been used for the preparation of lightly cross-linked water-absorbent based on poly(sodium acrylate), few study has been done on the preparation of highly cross-linked polymer gel beads which is suitable for

HPLC.[118,119] One of the aims of this study is to provide the preparation method of hydrophilic packing materials and their characterization.

As mentioned in section 1-2, Ugelstad et al. developed the seed polymerization method using seed prepared by emulsifier-free emulsion polymerization and with multiple swelling technique. However, their method is complicated and only little is known for the control of pore size and its distribution. The author aims to develop the new method for the preparation of monodisperse macroporous styrene-divinylbenzene gels beads, which includes only single-step swelling process and make it possible to control the particle size and pore size. This method will be a valuable one from the industrial and practical points.

The seed polymerization have been applied to the preparation of monodisperse styrene-divinylbenzene gel beads, and only little is known for other monomers.[120,121] The author also aims at the preparation of monosized hydrophilic polymer gel beads by seed polymerization from hydrophilic monomers. Prepared hydrophilic gel beads will be able to be used as the packing materials of aqueous SEC with the improved column efficiency.

I-2-2 NMR Characterization

It is thought that NMR of solvent in swollen polymer gel gives valuable information about the chemical environment and the mobility of the solvent corresponding to chemical shift, and relaxation times, respectively. A few studies have been done on the NMR parameters in relation to the properties of gel beads. Pickup[62] have discussed the relationship between the solvent mobility and crosslinking density using gel beads which were gel-type and had low crosslinking density and reported that the mobility of toluene in cross-linked polystyrene beads is equal to that in linear polystyrene solutions containing the same weight fraction. As pointed by Pickup, it is considered that the equivalence no longer exists due to their highly heterogeneity when the gel beads with higher crosslinking density and macroporous structure are used. As macroporous gel beads with the high crosslinking density are preferably used as packings for HPLC and polymer supports, it is important to evaluate the chemical and physical

interactions between the polymer gel beads and solvent. The author would like to measure ^{1}H and ^{13}C-NMR spectra of solvent in the presence of macroporous styrene-divinylbenzene gel beads and investigate the relationships between the NMR parameters and the characteristics of gel beads.

Pore size in a swollen state have been discussed using freezing point depression of benzene by Brun. It is thought that their methodology is not complete because the effect of crosslinking density and swelling ratio are not taken into consideration in the determination of pore size. Furthermore, the freezing process of organic solvent in polymer matrices has not been investigated using NMR. It is an important target to investigate the freezing process of solvent in styrene-divinylbenzene gel beads by NMR, and discuss the freezing point depression in relation to the characteristics of beads.

I-2-3 Application to HPLC of polymeric compounds

The author also aims at the separation of polymeric samples with similar polarity, e.g., copolymers which consist of monomers with similar polarity, homopolymers with a different stereo regularity, and diene polymers with a different isomeric structure. The separations of these types of polymers are necessary for determination of intermolecular heterogeneity of stereo regularity and of isomeric structure as well as of chemical composition of copolymers.

Polarity is reported to be an important factor to determine the elution behavior of copolymers. In the case of copolymers which consist of monomers with similar polarity, e.g., poly(styrene-co-butyl methacrylate), other specific interactions such as hydrogen bond or π electron interaction may play an important role in the adsorption - desorption processes.

As it is well-known, polymerization of methyl methacrylate leads to polymers having different tacticity dependent on polymerization condition. The effect of tacticity on the elution behavior through adsorption mode HPLC has not been investigated. It is well known that stereo complex is formed between isotactic and syndiotactic poly(methyl methacrylate)s in some specific solvents.[122] It is interesting to explore the elution behaviors of poly(methyl methacrylate)s in relation to the formation of stereo complex.

Butadiene is polymerized to yield three isomeric units of cis-1,4, trans-1,4, and 1,2 units depending on the catalyst and other polymerization conditions. The content of these isomeric units affects the properties of polybutadienes. Polybutadiene has no functional groups which interact with the packing materials except for olefinic groups. Therefore, it is considered difficult to recognize the difference of adsorption ability between the various types of polybutadiene compared with the separation of copolymers such as poly(styrene-co-methyl methacrylate).

I-3. Constitution of this thesis

The present thesis consists of four parts summarized as followed.

In Part I, the author describes the general introduction about the synthesis, the characterization, and the application of the polymer gel beads.

In Part II, the author describes the synthesis of polymer gel beads for HPLC.

In Chapter 1, the author investigates the preparation of hydrophilic polymer gels for aqueous HPLC from the variety of hydrophilic monomers and crosslinking agents via conventional and inverse suspension polymerization. Prepared gel beads are packed into stainless steel columns to obtain HPLC columns and evaluated with respect to the pressure resistance, pore size and its distribution. The control of pore size is conducted for acrylamide-N,N'-methylene-bis(acrylamide) gels. Hydrophilicity is evaluated from the elution volume of normal alcohols. Elution behaviors of various types of amino acids are also explored.

In Chapter 2, the synthesis of the monodisperse styrene-divinylbenzene copolymer beads via seed polymerization with only a single step swelling process is described, in which monodisperse seed particles are prepared by dispersion polymerization of styrene in ethanol or aqueous ethanol. The size uniformity and surface morphology of beads are observed by the scanning electron microscope. The prepared gel beads are evaluated as the packing materials of SEC using tetrahydrofuran as an eluent. The relationship between the preparation conditions and pore size is discussed. The author also investigates the effect of molecular weight and its distribution of seed

particles on the pore size and its distribution.

In Chapter 3, the author describes the synthesis of the monodisperse hydrophilic polymer gel beads with the modified method described in Chapter 2. As hydrophilic monomers, crosslinkable three types of oligo(ethylene glycol) dimethacrylates, in which the degree of oligomerization of oxyethylene units is 2, 3, or 3-4. The applications of prepared gel beads to aqueous SEC are discussed. Hydrophilicity of gel beads is evaluated by the method described in Chapter 1.

In Part III, the NMR characterizations of styrene-divinylbenzene copolymer beads in a swollen state are described.

In Chapter 4, ^{1}H-NMR spectra of chloroform are measured in the presence of styrene-divinylbenzene gel beads. Various types of styrene-divinylbenzene gel beads are prepared by conventional suspension polymerization. NMR parameters such as line shape and chemical shift are discussed in relation to the characteristics of the gel beads such as swelling ratio, diameter, pore size, and crosslinking density, which are all important parameters in the applications of beads.

In Chapter 5, ^{13}C-NMR spectra of various types of solvents are measured in the presence of styrene-divinylbenzene gel beads. The interactions between styrene-divinylbenzene gel beads and small molecules are investigated. The values of chemical shift are discussed by the higher magnetic field shift induced by the aromatic solvent. Solvent mobility is evaluated by the measurements of spin-lattice and spin-spin relaxation times and discussed in relation to the types of solvent, and crosslinking density of gel beads.

In Chapter 6, ^{1}H-NMR spectra of benzene in the presence of styrene-divinylbenzene gel beads are measured in the temperature range from 30 to -80°C. The ^{1}H-NMR signal of benzene can be detected below its freezing point. The freezing point depression of benzene is discussed in relation to the structure of gel beads such as pore size and crosslinking density.

In Part IV, the author describes the applications of polymer beads to the packing materials for HPLC which focuses on the adsorption mode HPLC of polymers.

In Chapter 7, using the solvent-gradient method, the separation of styrene-methyl methacrylate copolymers are conducted dependent on the chemical composition with normal and reversed-phase HPLC using different types of polymer gel beads as packing materials such as acrylonitrile, methyl methacrylate, styrene, and octadecyl methacrylate gels. The effect of molecular weight of samples and pore size of gel beads on the resolution are investigated. The separation of styrene-butyl methacrylate copolymers is also investigated and the importance of specific interactions are discussed on the separation of copolymers which consists of monomers with similar polarity.

In Chapter 8, the separation of poly(methyl methacrylate)s depending on tacticity are investigated using the method described in Chapter 7. The effects of the stereo complex formation from isotactic and syndiotactic poly(methyl methacrylate)s are also discussed.

In Chapter 9, the separations of polybutadienes depending on isomeric structure are investigated using the method described in Chapter 7. Because the adosorption ability of polybutadienes is low, the combination of packing materials and eluents is explored to separate polybutadienes with adsorption-desorption mechanism.

In Part V, the author describes the concluding remarks of this thesis.

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Part II

Synthesis of Polymer Gel Beads

ABSTRACT

In order to prepare polymer packing materials for aqueous HPLC columns, various types of hydrophilic vinyl monomers were copolymerized with crosslinking agents. The copolymerization was carried out by conventional suspension or inverse suspension polymerization in the presence of a diluent. The obtained gels were packed into stainless steal columns. The columns were evaluated in terms of pressure resistance, exclusion limit, and hydrophilicity. It is found that hard gels having pressure limits higher than 110 kg/cm^2 can be obtained if more than 33 or 50 % of crosslinking agent was employed. The hydrophilicity of gels evaluated from the elution volume of normal alcohols showed good relationship with solubility parameters. Gels prepared by the inverse suspension polymerization were more hydrophilic than those prepared by the conventional suspension polymerization. Acrylamide (AA)-N,N'-methylene bis-(acrylamide) (BA) gel was most hydrophilic among all the gels examined. The exclusion limit of the AA-BA column could be changed from $2x10^3$ to $3x10^6$ by changing the ratio of the diluent to monomer, or by addition of poly(ethylene glycol) to the monomer phase. Elution of aminoacids which have aromatic rings was significantly retarded by some specific interactions as well as hydrophobic interaction.

Monodisperse styrene-divinylbenzene gel beads in the range of 4.1~7.5μm were prepared via a single step swelling and polymerization method. The seed particles prepared by dispersion polymerization exhibited a good absorption ability of the monomer phase. The resulting gel beads were evaluated as the packing materials for gel permeation chromatography columns. The pressure resistance of gel beads prepared with the volume ratio of diluent to monomers of unity was higher than 120kg/cm^2, and it decreased with the relative amount of diluent. The pore size tended to decrease with the decrease in the swelling ratio of the seed polymer. The median pore size in a swollen state was controllable in the range from 30 to 550 Å.

Three types of oligo(ethylene glycol) dimethacrylate (degree of oligomerization: 2, 3, and 3-4, which are abbreviated to 2G, 3G, or 4G, respectively) were polymerized by seed polymerization. The gel beads so prepared had narrow size distribution and

were macroporous. These beads were evaluated as the packing materials for aqueous high performance liquid chromatography. The columns packed with gel beads were suitable for aqueous size exclusion chromatography for polysaccharides, proteins and other hydrophilic polymers. The hydrophilicity of 4G gel beads was almost equal to that of 2-hydroxyethyl methacrylate-ethylene dimethacrylate copolymer beads which are usually used for aqueous size exclusion chromatography.

Chapter 1

Preparation and Evaluation of Hydrophilic Polymer Gels for Aqueous HPLC

II-1-1 INTRODUCTION

Aqueous high performance liquid chromatography, (Aqueous HPLC) has become important with the progress of biotechnology, because this is one of the best method for effective separation and purification of water soluble proteins, peptides, and other biologically active compounds.[1-15] Two types of stationary phases are commonly available for HPLC packings, i.e., silica and porous polymer gels. Silica gels have several disadvantages, such as a short lifetime, a strong irreversible adsorption and a limitation of mobile phases to pH lower than 8. For some high molecular weight samples such as proteins and polypeptides, recovery becomes low due to the irreversible adsorption.[4,5] On the other hand, polymer gels is expected to have longer lifetime because of higher chemical resistance compared to silica gel. Soft Dextran[6] have been used as hydrophilic polymer packings for aqueous liquid chromatography, especially for aqueous size exclusion chromatography (SEC). However, these gels have only low cross-linking density leading to low pressure resistance of less than 10-20 kg/cm^2. Recently, several types of hard polymer gels have been commercially available, e.g., poly(2-hydroxyethyl methacrylate)[7-10] and poly(vinyl alcohol) gels[11-13]. However, hydrophilicity of these gel is inferior to the classical dextran, and it causes the unexpected hydrophobic interaction between samples and packings in size-exclusion separation. Therefore, it is necessary to synthesize more hydrophilic polymer gel with enough mechanical strength from highly hydrophilic monomers such as acrylamide, and N,N'-methylene-bis(acrylamide). Inverse suspension polymerization is applied to prepare polymer gel beads from these water soluble monomers. A few studies have been reported about the application of inverse suspension polymerization to the preparation of polymer gels suitable for packing materials of HPLC.[14,15]

In this chapter, the author prepared various types of hydrophilic polymer gels by conventional or inverse suspension polymerization and evaluated columns packed with the obtained gels from the view point of pressure resistance, molecular weight of exclusion limit, hydrophilicity and elution behavior of aminoacids. These basic data give us the principle for designing packing materials for aqueous HPLC from the chemical view point.

II-1-2 EXPERIMENTAL

II-1-2-1 Preparation

All monomers and crosslinking agents used in this study were obtained from Tokyo Kasei Co., Ltd. Acrylamide(AA) and N,N'-methylene-bis(acrylamide) (BA) were used without further purification. The other monomers and crosslinking agents were purified by distillation under reduced pressure before use.

Polymer gels were prepared by a conventional suspension or an inverse suspension polymerization method. The conventional suspension polymerization was conducted using poly(vinyl alcohol) (Kurarray PVA-224) as a suspension stabilizer and isoamylalcohol (IAA) as a diluent (porogen), which is necessary for the formation of the pore in polymer gels. The inverse suspension polymerization was carried out in xylene as dispersing agent and using a mixture of Bentone 27 and 34 (NL Industries, Inc) as a stabilizer and a mixture of water and dimethylformamide (DMF) as a diluent. The obtained copolymer beads were washed 2-4 times with hot water, acetone, DMF and chloroform in order to remove diluent, unreacted monomer, and linear polymer. Particles with diameter of 3-15 μm were collected by a decantation in acetone.

II-1-2-2 HPLC

A slurry of the beads in distilled water (ca. 10%) was packed into stainless steel column of 4.6mm inner diameter and 25cm in length to prepare a HPLC column. HPLC measurements were carried out at room temperature using Jasco 880-PU high pressure pump at 0.5ml/min and Jasco 830-RI refractive index detector to monitor the column effluent. A 5μl portion of a sample solution (10mg/ml) was injected through a Rheodyne

7125 injector. The eluent was distilled water, 50mM phosphate buffer (pH 7), or 0.9 wt.% of sodium chloride solution.

Capacity factor (k') was calculated from the elution volume of n-alcohol (v) and ethylene glycol (v_0) according to the following equation.

$$k' = (v-v_0)/v_0$$

Hydrophobic parameter (log P) was calculated according to the method of Leo[16], which is based on the following equation.

$$\log P = \Sigma N_i f_i$$

where f_i represents a fragment factor (f_{CH_3} = 0.89, f_{CH_2} =0.54, f_{OH} = -1.64) and N_i represents the number of i-fragment. For example, the hydrophobic parameter of 1-hexanol was calculated as follows.

$$\log P \text{ (1-hexanol)} = f_{CH_3} + f_{CH_2} + f_{OH}$$
$$= 0.89 + 5*0.54 + (-1.64)$$
$$= 1.95$$

II-1-3 RESULTS AND DISCUSSION

II-1-3-1 Preparation of hydrophilic polymer gel beads

For preparing polymer gels, two methods can be used, i.e., the conventional and inverse suspension polymerization methods. The conventional suspension polymerization is carried out in an dispersed oil phase containing a monomer, a crosslinking agent, a diluent, and an initiator in water. On the other hand, in the inverse suspension polymerization, monomers are polymerized in a droplet consisting of an initiator and a diluent, which is dispersed in an oil phase.

Since acrylamide (AA) and N,N'-methylene-bis(acrylamide) (BA) have only slight solubility in a water-insoluble organic solvent and high solubility in water, gels containing AA or BA can not be prepared with the conventional suspension polymerization. Therefore, the gel containing AA and/or BA was obtained by the inverse suspension polymerization. On the other hand, because ethylene dimethacrylate (EDMA) and triallylcyanurate(TC) are soluble in the organic solvent and almost insoluble in water, gels containing these crosslinking agents were prepared with the conventional suspension

polymerization. The gels containing the other monomers, 2-hydroxyethyl-methacrylate (HEMA), acrylonitrile (AN), N-vinyl-2-pyrrolidone (VP) and N,N-dimethylacrylamide (DMAA) which are soluble in both IAA and water were prepared with both methods.

II-1-3-2 Properties of columns packed with synthesized gel beads

Table 1-1 shows the polymerization conditions of gels and characteristics of HPLC columns. Column packing was conducted by the slurry method.[17] The flow rate was increased stepwise to attain a constant pressure for each step. The packing was finished just before the pressure starts to increase exponentially. The max pressure represents the final pressure in packing the gel. The column can be used under this pressure. These polymer gels containing 33 or 50% crosslinking agent to total monomer had pressure resistance higher than 110kg/cm^2.

When Pullulan or poly(ethylene glycol) was used as a sample and distilled water as an eluent, the sample eluted from higher molecular weight before the solvent. These results indicate that each column showed size exclusion separation. Therefore, it is considered that if the sample is very hydrophilic, the interaction between the sample and these gels is negligible, when water is used as eluent.

In the case of styrene-divinylbenzene copolymer gels, it is reported that the pore size of the gel which determines the exclusion limit of a column can be controlled by the properties and relative amount of the diluent used.[18] If good solvents of the gel are used as a diluent, the pore size becomes small, while a poor solvent makes the pore size large. By decreasing the amount of diluent, the pore size become small. It is also reported that the addition of inert polymer such as polystyrene to the monomer phase increases the pore size.[19]

The pore size of AA-BA gel was controlled by the amount of diluent and monomers or by the addition of poly(ethylene glycol). The calibration curves of the four AA-BA columns which are listed on Table 1-1 are shown in Figure 1-1. Gel No.1 prepared from 5g of AA and 5g of BA in the presence of 10ml H_2O and 10ml DMF as a diluent provided the exclusion limit of $5x10^4$. By decreasing the amount of DMF from 10ml to 5ml(gel No.2), the exclusion limit decreased to $2x10^4$. Furthermore, changing

Table 1-1. Preparation condition of polymer gels and Characteristics of HPLC columns

Gel	Monomer	CA[a]	Diluent (ml)	Diameter (μm)	Max Pressure (kg/cm^2)	EL[b] (x103)	V_i/V_0[c]	m[d] (ml^{-1})	NTP[e] (plates)	SP[f] ($cal^{0.5}/cm^{1.5}$)
			H_2O+DMF							
AA-BA(1)	5g	5g	10+10	5-10	140	50	1.07	2.68	5000	13.1
AA-BA(2)	5g	5g	10+ 5	5-10	140	20	1.14	1.52	2500	13.1
AA-BA(3)	6g	4g	10+ 5	5-10	140	2	0.80	1.56	3000	13.2
AA-BA(4)	5g	5g	10+10[g]	5-10	280	3000	1.16	3.12	2500	13.1
DMAA-BA	5g	5g	10+10	3-10	250	50	0.87	2.24	2000	12.1
HEMA-BA	5g	5g	10+10	3-10	420	40	0.89	2.22	2500	11.8
AN-BA	5g	5g	10+10	3-10	450	125	1.01	1.60	2000	12.4
VP-BA	5g	5g	10+10	3-10	350	150	0.85	1.84	1200	12.0
			IAA							
DMAA-EDMA	14ml	6ml	20	3-10	120	10	1.05	1.14	1500	10.3
HEMA-EDMA	14ml	6ml	20	5-10	150	70	0.66	2.72	2600	10.5
AN-EDNA	14ml	6ml	20	3- 5	300	1000	0.97	1.60	950	10.9
VP-TC	10ml	10ml	20	3-10	110	3	0.87	0.96	5000	10.3

a);crosslinking agent, b);molecular weight of exclusion limit, c);V_i:pore volume, V_0:void volume, d);the slope of the linear portion of the size exclusion calibration curve, e);Number of theoretical plates per 25cm determined for ethylene glycol, f);Solubility Parameter calculated with the method of Hoy, g);containing 0.4g of poly(ethylene glycol)

Abbreviation; AA:acrylamide, DMAA:N,N-dimethylacrylamide, HEMA:2-hydroxyethyl methacrylate, AN:acrylonitrile, VP:N-vinyl-2-pyrrolidone, BA:N,N'-methylene-bis-(acrylamide), EDMA:ethylene-dimethacrylate, TC:triallylcyanurate, DMF:N,N-dimethylformamide, IAA:isoamyl alcohol

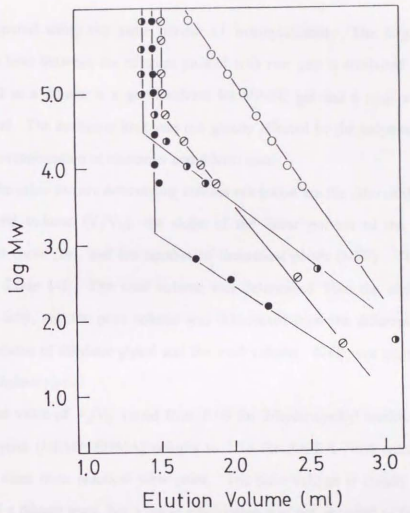

Figure 1-1 Calibration curves of aqueous columns packed with acrylamide gels.
(⊘): No.1, (◑): No.2, (●): No.3, (○): No.4
Eluent: distilled water, Sample: Pullulan

the weight ratio of AA to BA from 5:5 to 6:4 (gel No.3) decreased the exclusion limit to $2x10^3$. The exclusion limit of the column packed with gel No.4 prepared in the presence of poly(ethylene glycol) increased to $3x10^6$. It is considered that the phase separation of added polymer caused the increase of the pore size. Thus, the exclusion limit of acrylamide columns can be controlled from $2x10^3$ to $3x10^6$ by changing the polymerization conditions.

In the case of other gels, the molecular weight of exclusion limit varied from $3x10^3$ for N-vinyl-2-pyrrolidone-triallylcyanurate (VP-TC) column to $1x10^6$ for acrylonitrile-ethylene dimethacrylate (AN-EDMA) column. VP-TC and AN-EDMA gels

were prepared using the same diluent of isoamylalcohol. The large difference in exclusion limit between the columns packed with two gels is attributed to the fact that IAA used as a diluent is a good solvent for VP-TC gel and a poor solvent for AN-EDMA gel. The exclusion limit was not greatly affected by the polymerization method but by the combination of monomer and diluent used.

The other factors determining column resolution are the ratio of the pore volume to the void volume (V_i/V_0), the slope of the linear portion of the size exclusion calibration curve (**m**), and the number of theoretical plates (NTP). These values are listed on Table 1-1. The void volume was determined from the elution volume at exclusion limit, and the pore volume was determined from the difference between the elution volume of ethylene glycol and the void volume. NTP was estimated from the peak of ethylene glycol.

The value of V_i/V_0 varied from 0.66 for 2-hydroxyethyl methacrylate-ethylene dimethacrylate (HEMA-EDMA) column to 1.16 for AA-BA No.4 column, which are permitted ones from practical view point. The pore volume is closely related to the amount of a diluent used, but a linear relationship was not obtained in this study. It is considered that the combination of a monomer (and crosslinking agent) and a diluent also had an effect on the formation of pore.

The slope (**m**) of the linear portion of the size exclusion calibration curve is defined by the following equation:

$$\log M = b - \mathbf{m} (E.V.)$$

where M and E.V. are the molecular weight and elution volume of sample, respectively, and b is constant. The slope (**m**) is dependent on the pore size distribution (PSD) of gel, and influences the resolving ability of column. The gel with the broad PSD produces a calibration curve with a large slope (**m**). On the other hand, a column with small m (sharp PSD) exhibits high resolution. The **m** value of columns examined in this study was dependent on the type of gel and polymerization conditions (AA-BA column). VP-TC and N,N-dimethylacrylamide-ethylene-dimethacrylate(DMAA-EDMA) columns with small pore size (low exclusion limit) exhibited small **m** values (0.96, 1.14 respectively),

which indicates that these columns have inherent high resolving properties for low molecular weight samples. On the other hand, for high molecular weight samples, AN-EDMA column is considered suitable because of high exclusion limit and relatively small **m** value.

The NTP values varied from 950 for AN-EDMA column to 5000 for AA-BA No 1 column. This value is determined by several factors such as a gel beads diameter, its distribution, packing procedures, HPLC conditions, and so on. The obtained values for AA-BA columns overwhelm the results of Dawkins et. al.[14] (1500 estimated by matching their conditions to ours), although no careful attention was paid to increase NTP. This fact suggests the possibility to obtain more efficient column by improving our method.

II-1-3-3 Hydrophilicity of gel beads

The hydrophobicity of gels was evaluated from the elution volume of normal alcohols; ethanol, 1- propanol, 1-butanol and 1-hexanol using water as an eluent. The elution volume of these alcohols increased with the increase of molecular weight, or the length of alkyl chain. This elution order is opposite to those for Pullulan and poly(ethylene glycol). Therefore, it can be considered that the separation mode of the alcohols was not size exclusion mechanism but adsorption or partition mechanism.

In Figure 1-2, the logarithm of capacity factor (log k') is plotted against hydrophobic parameter (log P). The positive linear relationships were obtained between log k' and log P for all columns examined, which indicates that the elution was mainly governed by the hydrophobic interaction between the gel and alcohols. Figure 1-2 demonstrates that the columns can be classified into two groups, one prepared by the conventional suspension polymerization using EDMA or TC as a crosslinking agent (open symbols) and the other by the inverse suspension polymerization using BA as a crosslinking agent (filled symbols). The former had a larger k' value showing stronger interaction between the gel and sample than the latter.

Figure 1-3 shows chromatograms of aliphatic alcohols using two types of HEMA columns, i.e., 2-hydroxyethylmethacrylate-N,N'-methylene bis-(acrylamide) (HEMA-

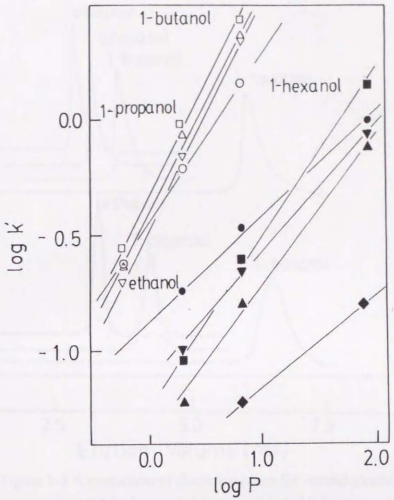

Figure 1-2 Relationship between capacity factors and hydrophobic parameters of normal alcohols.
(◇):acrylamide, (▽):N,N-dimethyacrylamide, (○):2-hydroxyethyl-methacrylate, (△):acrylonitrile, (□):N-vinyl-2-pyrrolidone
Open symbols represent ethylenedimethacrylate or triallylcyanurate and filled ones N,N'-methylenebis(acrylamide)

BA) and HEMA-EDMA columns. When HEMA-EDMA was used, the elution of 1-hexanol was so retarded that the peak became very broad compared with the case of HEMA-BA column. The AN, DMAA and VP columns also exhibited almost the same retardation by changing the polymerization mode from the inverse suspension to the conventional suspension. It is considered that this change is due to the difference of the properties of the crosslinking agents among the two methods; BA is very hydrophilic and shows low solubility in usual organic solvent, while EDMA or TC is not so hydrophilic.

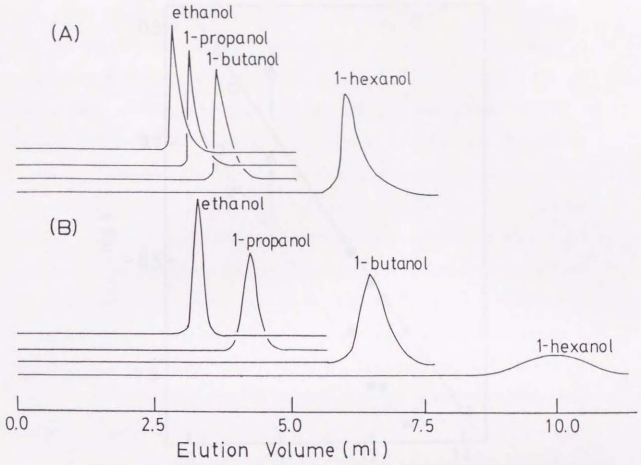

Figure 1-3 Comparison of chromatograms for normal alcohols on (A)2-hydroxyethylmethacrylate-N,N'-methylene bis (acrylamide)stationary phase and (B)2-hydroxyethyl methacrylate-ethylene-dimethacrylate stationary phase.

In all the gel prepared, AA-BA gel was most hydrophilic. It is considered that hydrophilicity of AA and BA was reflected to the properties of polymer gel.

The solubility parameter (SP) of each gels is calculated according to the method of Hoy[20]. The calculation method is based on the following equation.

$$SP = \rho \Sigma F_i / M$$

where ρ and M represent the density of gel and the molecular weight of the repeating unit, respectively, and F_i represents the molar attraction constant of each group. In Figure 1-4, the log k' value of 1-butanol or 1-propanol is plotted against the SP value of the gel. The liner relationship with a negative slope is obtained except for AN-EDMA column. This indicates that hydrophobic interaction became stronger with the decrease

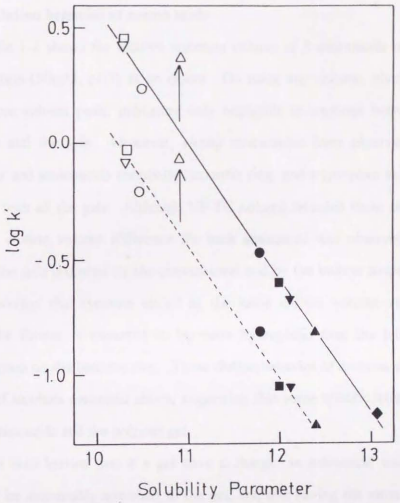

Figure 1-4 Relationship between capacity factor of 1-propanol (broken line) or 1-butanol (solid line) and solubility parameter. (◇): acrylamide, (▽): N,N-dimethyacrylamide, (○): 2-hydroxyethyl-methacrylate, (△): acrylonitrile, (□): N-vinyl-2-pyrrolidone Open symbols represent ethylenedimethacrylate or triallylcyanurate and filled ones N,N'-methylenebis(acrylamide)

of solubility parameter of the gel. In other words, the hydrophilicity of a gel can be evaluated qualitatively from the calculated SP value. AN-EDMA gel has large SP value owing to the polar cyano group. However, AN does not have protic hydrogen such as one in hydroxy or amide group, hence, hydrophilicity of AN-EDMA is considered not so high as expected from the high SP value. This consideration explains the stronger hydrophobic interaction of AN-EDMA gel and the small deviation from the straight lines in Figure 1-4.

II-1-3-4 Elution behavior of amino acids

Table 1-2 shows the relative retention volume of 5 aminoacids using phosphate buffer solution (50mM, pH7) as an eluent. On using any column, glycine and leucine eluted before solvent peak, indicating only negligible interactions between these two aminoacids and the gels. However, strong interactions were observed between the polymer gel and aminoacids containing aromatic ring, and tryptophan had the strongest interaction with all the gels. Although VP-TC column retarded these aminoacids most, no distinct elution volume difference for each aminoacid was observed between two groups of the gels prepared by the conventional and by the inverse suspension methods. It is noteworthy that tyrosine eluted at the same elution volume as phenylalanine, although the former is expected to be more hydrophilic than the latter because of hydroxyl group on the benzene ring. These elution behavior of aminoacids was different from that of alcohols discussed above, suggesting that some specific interactions existed between aminoacids and the polymer gel.

It is well known that if a gel have a charge, an aminoacid with the opposite charge will be irreversibly adsorbed to the gel, and one having the same charge will be repelled from the gel and eluted at the exclusion volume, using distilled water as an eluent.[11,13] By increasing ionic strength of the eluent, these ionic interactions between the gel and aminoacid are masked, and the solute elute at the elution volume expected for the gel without charge. The relative retention volumes of two ionic amino acids, arginine (positive charge at pH=7) and aspartic acid (negative charge at pH=7) were examined as shown in Table 1-3. Both aminoacids eluted out at almost the same relative retention volume of 0.63-0.90 using distilled water as an eluent. Therefore, these gels contained only negligible ionic site. The elution volume increased by 10 to 20% by increasing the ionic strength (sodium chloride solution). This slight elution volume change may be attributed to the slight increase of hydrophobic interaction.

Table 1-2. Relative retention volume of aminoacids

column	Gly	Leu	Tyr	Phe	Trp
AA-BA	0.96	0.96	1.29	1.11	1.67
DMAA-BA	0.89	0.91	1.20	1.10	1.42
HEMA-BA	0.87	0.94	1.26	1.14	1.93
AN-BA	0.91	0.96	1.36	1.17	2.18
VP-BA	0.96	1.00	1.32	1.16	2.17
DMAA-EDMA	0.81	0.86	1.00	1.00	1.61
HEMA-EDMA	0.87	0.94	1.09	1.11	1.81
AN-EDMA	0.89	0.93	1.00	0.99	1.30
VP-TC	0.78	0.90	2.53	1.62	8.64

Eluent:50mM phosphate buffer (0.5ml/min)
Abbreviation; AA:acrylamide, DMAA:N,N-dimethylacrylamide, HEMA:2-hydroxyethylmethacrylate, AN:acrylonitrile, VP:N-vinyl-2-pyrrolidone, BA:N,N'-methylenebis(acrylamide), EDMA:ethylenedimethacrylate, TC:triallylcyanurate, Gly:glycine, Leu:leucine, Tyr:tyrosine, Phe:phenylalanine Trp:tryptophane

Table 1-3. Relative retention volume of ionic aminoacids.

column	Arg		Asp	
	A	B	A	B
AA-BA	0.70	0.92	0.63	0.81
DMAA-BA	0.90	0.97	0.68	0.80
HEMA-BA	0.83	0.97	0.74	0.85
DMAA-EDMA	0.78	0.85	0.66	0.73
HEMA-EDMA	0.85	0.92	0.80	0.85

Eluent A: distilled water, B: NaCl(0.9wt%) solution
Abbreviation; AA:acrylamide, DMAA:N,N-dimethylacrylamide, HEMA:2-hydroxyethylmethacrylate, BA:N,N'-methylenebis(acrylamide), EDMA:ethylenedimethacrylate, TC:triallylcyanurate, Arg:arginine, Asp:asparagine

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Chapter 2

Synthesis of Monodisperse Macroporous Styrene-Divinylbenzene Gel Particle by Single Step Swelling and Polymerization Method

II-2-1 INTRODUCTION

Monodisperse macroporous styrene-divinylbenzene (St-DVB) copolymer beads having a micron-order diameter are demanded strongly in wide range of industrial fields, such as for size exclusion chromatography (SEC) column packing materials, precursors for ion exchange resins, and solid polymeric catalysts.

Ugelstad et al. developed a technique for the preparation of uniformly sized beads which he termed "activated multi-step swelling and polymerization".[1] This method has been applied to the preparation of monodisperse porous styrene-divinylbenzene copolymer beads for the packing materials of SEC, reversed-phase and ion-exchange chromatography.[2-5] This method is an excellent one, but seems rather complex, since at least two-steps are needed in swelling process, i.e., the first step for the seed particles to be activated by the absorption of a water insoluble compound, such as 1-chlorododecane and in some cases, by the initial absorption of an accelerator such as acetone, and a subsequent step for the absorption of styrene, divinylbenzene and diluent.

This chapter describes the preparation of monodisperse macroporous St-DVB beads by a seed polymerization involving only a single step swelling process. The beads thus prepared were packed into chromatographic columns and their properties were assessed. The sizes of the particles and pores are discussed in relation to the preparation conditions.

II-2-2 EXPERIMENTAL

II-2-2-1 Materials

Regent grade styrene (St) and divinylbenzene (DVB), containing ca.45% of ethylvinyl benzene were obtained commercially and were used after distillation under reduced pressure. Other materials were used without further purification.

II-2-2-2 Dispersion polymerization

Monodisperse polystyrene seed particles were prepared by dispersion polymerization of styrene in ethanol or a mixture of ethanol and water under a nitrogen atmosphere using azobis(isobutyronitrile) as an initiator, poly(vinylpyrrolidone) and bis(2-ethylhexyl) ester of sodium sulfosuccinic acid as stabilizers according to the reported method by Paine[6]. After a centrifugal purification, the purified seed particles were dispersed in water containing poly(vinyl alcohol) (PVA) (0.6%) with the particle content 0.1g/ml. Table 2-1 lists the preparation conditions and characteristics of seed particles.

Table 2-1 Preparation conditions and characteristics of seed particles

	styrene (g)	ethanol (ml)	H_2O (ml)	diameter (μm)	$M_n/10^4$	$M_w/10^4$	M_w/M_n
seed-1	27	180	20	1.7	3.0	27.9	9.2
seed-2	27	200	0	3.0	2.6	9.2	3.5

Polym.Temp.;70°C, Polym. Time ; 20 hours
AIBN; 0.27g (1wt% based on styrene)

II-2-2-3 Seed polymerization

Into a 500ml flask fitted with a mechanical stirrer were added varying amount of seed dispersion and 50 ml of water containing 0.6wt% of PVA, and the mixture was stirred slowly. 30 g of a mixture of St, DVB, toluene containing 5wt %(based on the organic phase) of isoamyl alcohol, and 2wt% (based on the total monomer) of 2,2'-azobis(2,4-dimethylvaleronitrile) initiator were emulsified in 300 ml water containing 0.25wt% of sodium n-dodecyl sulfate, and 0.6wt% of PVA with an ultrasonic disrupter (TOMY Co., UD-200) till the particle size of oil drops became at most 0.5μm. The weight ratio of St to DVB was 3/7, and the ratio of toluene to total monomer varied from 1.0 to 2.0. The weight ratio of the organic phase to the seed particles varied from 5 to 100. Then, one third portion of the emulsion was added dropwise to the dispersion of the seed particles, with stirring, over 15 minutes. The second and third one-third

portions of the emulsion were added during 1 hour interval in the same way. The mixture was stirred for 24-48 hours at room temperature so that all the emulsified organic phase was absorbed by the polymer seeds. The temperature was then increased to 70°C, and the polymerization was carried out for 10 hours. The resulting gel beads were washed successively with hot water, acetone and tetrahydrofuran (THF) 2-4 times, followed by drying in vacuo.

II-2-2-4 Characterization of gel beads

Eight grams of gel beads dispersed in 50ml of THF was packed into a stainless steel column (7.6mm i.d. x 30cm length) by a slurry method to obtain a SEC column. The SEC calibration curve of polystyrene standards (Shodex) was obtained using THF as an eluent (0.5ml/min) with JASCO PU 800 pump and JASCO 880 UV detector (254nm). The number of theoretical plates was determined from a benzene peak. Standard sample concentration was 0.1wt% and 5μl portion of sample was injected through a Rheodyne 7125 loop injector. Particle size and surface morphology of beads were investigated by scanning electron microscopy (JEOL JSM-35 CF).

II-2-3 RESULTS AND DISCUSSION

II-2-3-1 Preparation of Monodisperse Beads

St-DVB beads were prepared by seed polymerization using polystyrene seed obtained by dispersion polymerization. Table 2-2 lists the preparation conditions and characteristics of the beads. The swelling ratio was changed from 5 to 200 times of the original seed particles. Figure 2-1(a) and (b) show the scanning electron micrographs of gels 1-1 and 1-2, respectively, which indicate that the irregularly shaped beads were obtained when the swelling ratio was less than 10. With a swelling ratio of 5, the beads became hollow with collapsing. Bead with a swelling ratio of 10 became spheres with navels. It is likely that there was some heterogeneity in the swollen droplets due to the high polystyrene concentration (20% or 10%) which caused the irregularity of beads in the polymerization process. Above 20-fold, regular spherical beads were obtained and their size was essentially monodisperse. Figure 2-1(c) and (d) show the scanning

Table 2-2 Preparation conditions of styrene-divinylbenzene copolymer beads and characteristics of SEC columns

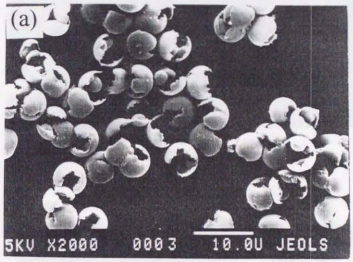
gel	seed		oil/seed	diameter	F.R.[a]	M.P.[b]	NTP[c]	Vp/Vt[d]	D_{50}[e]
	weight (g)	type		(μm)	(ml/min)	(kg/cm^2)	($/10^3$)		(Å)
toluene/momomers=1.0									
1-1	6.0	2	5	n.d.[f]	—	—	—	—	—
1-2	3.0	2	10	n.d.[f]	—	—	—	—	—
1-3	1.5	2	20	6.4	4.5	125	8.2	0.44	552
1-4	1.2	2	25	7.0	10.0	125	8.8	0.41	179
1-5	1.0	2	30	7.3	6.0	122	11.9	0.43	112
1-6	0.75	2	40	7.4	8.0	128	11.6	0.42	57
1-7-2	0.60	2	50	7.5	7.6	124	10.7	0.41	46
1-7-1	0.60	1	50	4.2	1.6	131	8.8	0.41	45
1-8	0.30	1	100	4.6	3.3	130	15.8	0.39	30
1-9	0.15	1	200	5.0	4.6	123	12.7	0.38	32
toluene/monomers=1.5									
2-1	0.60	1	50	4.1	2.0	54	15.0	0.46	65
2-2	0.30	1	100	4.5	1.5	76	12.0	0.46	42
toluene/monomers=2.0									
3-1	0.60	1	50	4.1	0.9	25	9.4	0.52	91
3-2	0.30	1	100	4.4	0.8	20	8.8	0.53	72

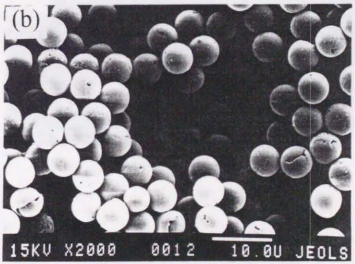
Polym.Temp.; 70°C, Polym. Time; 10hours,. Total amount of organic phase ; 30g, Styrene/divinylbenzene ; 3/7, Initiator ; ADVN (2wt% based on monomers)
a); final flow rate in packing procedure, b); maximum pressure in packing procedure, c); number of theoretical plates (in 30cm), d); ratio of pore volume to total volume of column, e); median pore size of gel in a swollen state, f); not determined

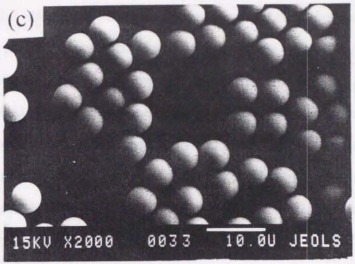
electron micrographs of a gel with swelling ratio of 200. These figures indicate that the beads were uniform in size and had macroporous structure.

It is noteworthy that the swelling ratio could be increased up to 200 times in this experiment. By contrast, Šmigol et al. reported[7] that the swelling ability of "non-activated" latex particles did not exceed 70 fold, when emulsifier-free emulsion latex was used as the seed. The difference in swelling ability can be attributed to the preparation method of seed particles. It has been reported that particles prepared by dispersion polymerization contain a small amount of grafted polymer on the polymeric stabilizer, in our case poly(vinylpyrrolidone).[8] The presence of the graft polymer can be considered

to increase the absorption ability without "activating " the seed particles. Polymer beads prepared by dispersion polymerization have additional advantage that the diameter of the beads can be easily controlled in the range of 1 to 10 μm.[6,8-10]







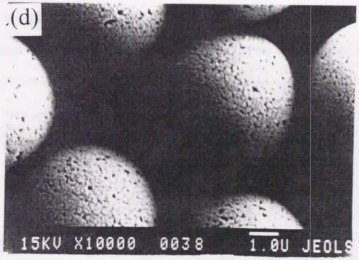


Figure 2-1 Scanning electron micrographs of styrene-divinylbenzene gel beads (a), gel 1-1 (x2000), (b), gel 1-2 (x2000), (c), gel 1-9 (x2000), (d), gel 1-9 (x10000)

II-2-3-2 Characteristics of SEC column

Table 2-2 also shows the packing conditions and characteristics of SEC columns. The maximum pressure represents the final pressure in the packing procedure. When the amount of toluene was equal to that of monomers, each gel had pressure resistance higher than 120 kg/cm^2. On the other hand, gels prepared using toluene 1.5 and 2 times of the monomer had pressure limits around 60 and 20kg/cm^2,respectively. Thus, the pressure limit decreased with an increase in the relative amount of diluent. It appears that the preferable ratio of toluene to the monomer is 1 to 1.5 with respect to pressure resistance. The number of theoretical plates (NTP) was more than 8000 for 30cm length.

Figure 2-2 shows the calibration curves of columns packed with the prepared gel beads. It is clear from the calibration curve that the exclusion limit decreased as swelling ratio increased, although the value of the exclusion limit could not determined because the molecular weight of the polystyrene standard was less than 3.04×10^6. There was a shift in the region of linearity in the fractionation curves, from higher to lower molecular

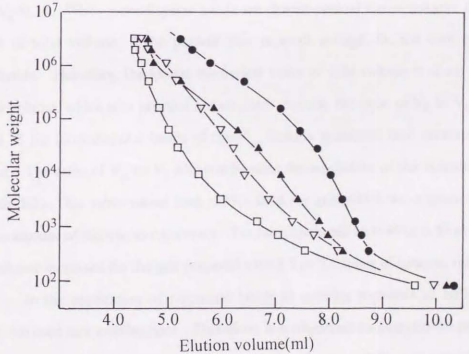


Figure 2-2 Calibration curves of columns packed with prepared styrene-divinylbenzene gel beads
(●), gel 1-3, (▲), gel 1-5,
(▽), gel 1-7-1, (□), gel 1-8
column, 7.6mm i.d. x 30cm, eluent; THF (0.5ml/min)

weight. This correlated with increasing swelling ratio and decreasing pore diameter (Table II). Together with the exclusion limit, this indicates that the gel with low swelling ratio is suitable for SEC of high molecular weight compounds and one with high swelling ratio for SEC of low molecular weight compounds.

In the case of SEC column, total column volume (V_t, in this case, 13.6 ml) is the sum of the volume of three parts, i.e., the interstitial volume (void volume, V_0), the pore volume (V_p), and the volume of polymer matrix (V_s). V_0 is experimentally estimated from the elution volume at the exclusion limit, and it is expected that the ratio V_0/V_t is small because V_0 is useless in conducting SEC. V_p is defined as the difference between the elution volume of the smallest elute (in this case, benzene) and V_0. The large V_p affords the column with the inherent high resolving property.

As shown in Figure 2-2, clear exclusion limits could not be determined due to the lack of standards. However, V_0 could be estimated by extrapolating the curves in Figure 2-2, which generally became steep around 4.4-4.5 ml. The author estimated the ratio V_0/V_t=0.33. When monodisperse beads are closest-packed into a column, they occupy 0.74 of total volume, if the particle size is small enough for the wall effect to be negligible. Therefore, the lowest theoretical value of void volume is about 0.26 of the total volume, which is in practice not attained. Indeed, the ratio of V_0 to V_t is reported to 0.35 for monodisperse beads of 5μm[2]. Results presented here correspond to this value. The ratio of V_p to V_t which influences the resolution of the column is listed in Table 2-2. This value varied from 0.39 - 0.44 for gels which were prepared with the same amount of toluene as monomers. The ratio increased to 0.46 or 0.52 as the amount of toluene increased for the gels prepared with 1.5 or 2.0 times of toluene, respectively.

In the application of polymeric beads as packing materials of HPLC columns, they are used in a swollen state. Therefore, it is important to evaluate the pore size and its distribution in a swollen state. They are often determined from the calibration curve of SEC using the empirical relationship between the dimension and molecular weight of polystyrene in an eluent (THF)[11]

$$D = 0.62(MW)^{0.59}\ \text{Å}$$

where MW is the molecular weight of polystyrene. The median pore size (D_{50}) was determined from Figure 2-2. Elution volume at the center between V_0 and the elution volume benzene was determined and then the corresponding molecular weight of polystyrene was used for MW. The values of D_{50} are listed in the last column of Table 2-2. The D_{50} value also decreased with the increase of the toluene fraction in the organic phase.

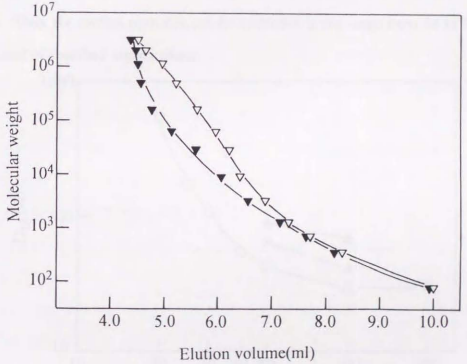


Figure 2-3 Effect of types of seeds on calibration curves
(▽), gel 1-7-1, (▼), gel 1-7-2
column; 7.6mm i.d. x 30cm, eluent; THF (0.5ml/min)

The effect of type of seed particles was observed on the shape of calibration curves as shown in Figure 2-3. The difference between the calibration curves of columns packed with gels 1-7-1 and 1-7-2 lies in the high molecular weight region, which indicates the large pore distributions of both types of gels are different. Both gel beads were prepared at the swelling ratio of 50. The difference decreased with the increase of swelling ratio. As shown in Table I, the weight average molecular weight of seed-1 is much higher than that of seed-2, and its molecular weight distribution is wider. It is likely that the difference in pore structure is due to this difference of molecular weight and its distribution for the seed particles. Cheng also reported[12] that the average pore

size increases with increasing of molecular weight of the seed polymer, and broad pore size distribution is obtained using a seed polymer with broad molecular weight distribution.

In Figure 2-4, the median pore size is plotted against swelling ratio. The pore size decreased with increasing swelling ratio, and it became almost constant when the swelling ratio was 100. Moreover, it slightly increased with the relative amount of a diluent. Thus, the median pore size can be controlled in the range from 30 to 550 Å by the amount of absorbed organic phase.

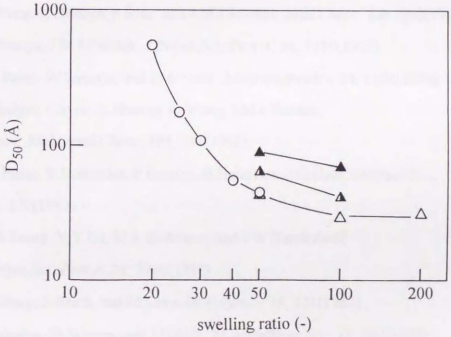


Figure 2-4 Relationship between the pore size and the swelling ratio (○), seed 2, (△)seed 1
toluene/monomers; 1.0 (open), 1.5 (half filled), 2.0 (filled)

II-2-4 CONCLUSION

A single step swelling and polymerization method provided monodisperse macroporous styrene-divinylbenzene gel beads even if the amount of absorbed organic phase was 200 relative to the seed particles. This is due to the high absorption ability of seed polymers prepared by a dispersion polymerization. The SEC columns packed with prepared gel beads showed good properties, e.g. low void volume and relatively high number of theoretical plate. The median pore size ranged from 30 to 550 Å, which was dependent on the ratio of absorbed organic phase to seed particles. Packing materials

which are suitable for polymer samples with a variety of molecular weights can be prepared using this method.

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Chapter 3

Preparation and Characterization of Monodisperse Oligo(ethylene glycol) Dimethacrylate Polymer Beads for Aqueous High Performance Liquid Chromatography.

II-3-1 INTRODUCTION

As described in Chapter 1, aqueous high performance liquid chromatography (HPLC) has been a suitable tool for the separation and analysis of natural polymers such as proteins or polysaccharides, and polymeric packing materials used in aqueous HPLC are found to be superior stationary phase compared to silica-based packing materials, in respect of the chemical resistance and the reproducibility. Polymer beads used as the packing materials are currently prepared by a conventional suspension polymerization. The resulting beads have broad size distributions, and can not be used for chromatography without the time-consuming size fractionation. Therefore, monodisperse packing materials are needed in order to eliminate the need for the size fractionation.

Monodisperse styrene-divinylbenzene copolymer beads were prepared by seed polymerization with a multi-step swelling process.[1-4] It was reported that these beads are superior as stationary phases to those prepared by a conventional suspension polymerization.[1,2] Only a few other monomers have been polymerized by seed polymerization.[5]

The author prepared monosized styrene-divinylbenzene gel by seed polymerization with single-step swelling and found that the gel was a good packing material for gel permeation chromatography as described in Chapter 2. This chapter describes the preparation of monodisperse hydrophilic polymer gel beads by seed polymerization, and the evaluation of beads for aqueous HPLC from the view point of pore size, and hydrophilicity.

II-3-2 EXPERIMENTAL

II-3-2-1 Synthesis

Three types of oligo(ethylene glycol) dimethacrylate (degree of oligomerization: 2, 3, and 3-4: abbreviated to 2G, 3G, and 4G, respectively) were provided by Shin Nakamura Chemical Co. (Wakayama, Japan), and used as received. Styrene was obtained commercially and used after distillation under reduced pressure. All other materials were also used without further purification.

Monodisperse polystyrene seed particles with a diameter of 1.7μm were prepared by the dispersion polymerization of styrene in aqueous ethanol by the method described in Chapter 2.

Into a 500ml flask fitted with a mechanical stirrer was added the seed dispersion (0.1g/ml) and 50 ml of water containing 0.5wt% of poly(vinyl alcohol), and the mixture was stirred slowly. The mixture of 15g of monomer, 15g of butyl acetate (porogen), and 0.3g of 2,2'-azo-bis(2,4-dimethylvaleronitrile) initiator was emulsified in 300ml of 9.0wt% NaCl aqueous solution containing 0.5wt% of PVA with an ultrasonic disrupter (TOMY Co., UD-200) till the particle size of the oil drops became smaller than 0.5μm. The swelling ratio was changed from 50 to 200 by using varying amount of seed particles. Then, one third portion of the emulsion was added dropwise to the dispersion of the seed particles, with stirring, over 15 minutes. The second and third one-third portions of the emulsion were added after 1 hour intervals in the same way. The mixture was stirred for 24-48 hours at room temperature so that all the emulsified organic phase transferred into the polymer seed. The temperature was then increased to 80°C, and the polymerization was carried out for 10 hours. The resulting gel beads were washed successively with hot water, acetone and tetrahydrofuran 2-4 times, followed by drying in vacuo.

II-3-2-2 Characterization of gel beads.

Ten grams of gel beads dispersed in 50ml of distilled water was packed into a stainless steel HPLC column (7.6mm i.d. x 30cm) by a slurry method. SEC calibrations of Pullulan (Shodex) were obtained using distilled water as an eluent (0.5ml/min) with JASCO PU800-pump and JASCO 830-RI detector. Standard sample concentration was 1wt% and 10μl portions of samples were injected through a Rheodyne 7125 loop injector. In the case of proteins (Albumin(Bovine), Ovalbumin, Myoglobin, and

Cytochrome-c ; 0.1wt%), phosphate buffer (1/15 mol/l, pH;7) eluent and JASCO 880 UV detector were used. The hydrophilicity of the gel beads were evaluated from the elution times of normal alcohols (ethanol, 1-propanol, and 1-butanol).

Surface morphology of the beads was analyzed by scanning electron microscopy (JEOL JSM-35CF).

II-3-3 RESULTS AND DISCUSSION

II-3-3-1 Preparation of monodisperse beads

Hydrophilic polymer beads were prepared by seed polymerization of oligo(ethylene glycol) dimethacrylate using polystyrene seed, which was obtained by dispersion polymerization. Table 3-1 represents the preparation conditions and size uniformity of the gel beads. All the gels had fairly narrow size distribution, with the variation coefficient smaller than 17%. The size distribution of the gel tended to become broader with the increase of oxyethylene units in the monomer. A possible explanation is that the solubility of monomer in the aqueous phase increases with the number of oxyethylene units, which decreases the dissolution speed of the monomer to the seed particle. In the case of the 3G gel, the variation coefficient increased as the increase of swelling ratio and decreased as the increase of swelling time.

Table 3-1 Preparation conditions and size uniformity of gel beads

gel	swelling ratio[a]	absorp. time(hr.)	V.C(%)[b]	diameter (μm) [obs.][c]	[cal.]
2G	50	24	5.8	5.5	6.3
3G-1	50	24	7.9	5.8	6.3
3G-2	100	24	15.4	8.0	7.9
3G-3	100	48	6.2	7.5	7.9
3G-4	200	48	17.2	9.7	10.0
4G	50	24	16.1	5.5	6.3

a), ratio of organic phase to seed
b), variation coefficient, c), weight average diameter measured by coulter multisizer

The diameter of the gel particle (d_g) can be calculated from the diameter of seed particle (d_s) and the swelling ratio (S),

$$d_g = d_s \times S^{1/3}$$

The observed and calculated gel diameter are listed in Table 3-1. Although observed values were a little smaller than calculated ones, particle size can be controlled by the initial seed diameter and the swelling ratio. The difference between the observed and calculated values can be explained by shrinkage of the beads accompanying the polymerization and drying processes.

Šmigol et al. reported that the absorbed quantity does not exceed 65-fold volume of the polymer particles even in the best case, which are prepared using seed particles obtained by an emulsifier free emulsion polymerization.[6] As described in Chapter 2, the polystyrene seed particles prepared by dispersion polymerization have a superior ability to absorb the organic phase compared to ones prepared by an emulsifier free emulsion polymerization.

Figure 3-1 shows the scanning electron micrographs of 3G-1. This figure indicates the uniformity in size and macroporous structure of the gel beads.

II-3-3-2 Characteristics of columns

The prepared gel beads were packed into stainless steel columns by a slurry method. Table 3-2 surveys the characteristics of the columns. Each column beads had pressure resistance higher than 150kg/cm^2. The calibration curves of the columns packed with 2G, 3G, or 4G gel beads are shown in Figure 3-2. When Pullulan was used as a sample and distilled water as an eluent, the samples eluted in order of decreasing molecular weight before the solvent. This fact indicates that each column showed size exclusion separation, and that if the sample is hydrophilic, the interaction between the sample and gel beads is negligible when distilled water is used as an eluent. Each column has the molecular weight of exclusion limit over 1×10^6, which indicates that columns can be used for the analysis of water soluble high molecular weight species. From the shape of calibration curves, it is found that the increase of oxyethylene units slightly decreased

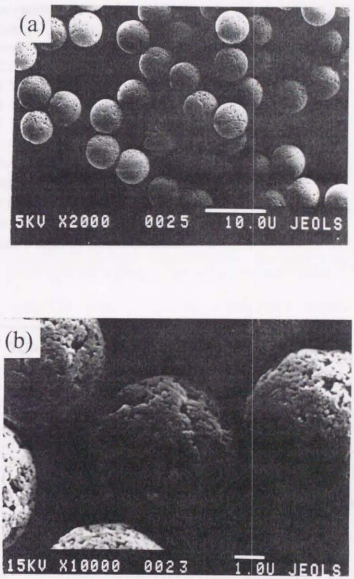

Figure 3-1 Scanning Electron Microscope photographs of 3G-1 beads
(a), ×2000, (b), ×10000

Table 3-2 Characteristics of HPLC column

gel	Flow rate[a] (ml/min)	Pressure[b] (kg/cm^2)	NTP[c]	Ex.L[d] ($x10^4$)
2G	2.0	153	8700	>100
3G-1	1.5	160	16000	>100
3G-3	1.4	150	7500	>100
4G	1.3	155	5000	>100

a);final flow rate in packing procedure.
b);final pressure
c);number of theoretical plates determined by ethylene glycol (flow rate 0.5 ml/min).
d);molecular weight of exclusion limit.

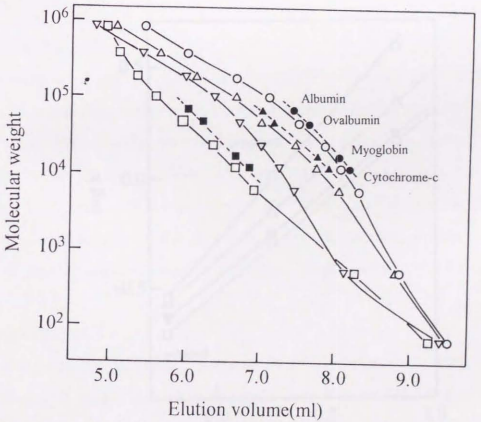

Figure 3-2 Calibration curves of columns packed with prepared gel beads (○),2G-1, (△),3G-1, (▽),3G-3, (□),4G-1
open ; Pullulan (water), filled ; Proteins (phosphate buffer)
Column; 7.6mm i.d.x30cm, Flow rate 0.5ml/min

the pore size. In the case of 3G gel, a slight difference of the shape of the calibration curve was observed between the gels prepared with the swelling ratio of 50 and 100.

The elution behavior of water soluble proteins were investigated, and the relationships between the molecular weight and the elution volume are shown in Figure 3-2. They also eluted in order of decreasing molecular weight, although the elution volume is slightly larger than the Pullulan of the same molecular weight. It is clear that their elutions were governed mainly by the size exclusion mechanism.

II-3-3-3 Hydrophilicity of gel beads

Hydrophilicity of the gel beads was evaluated from the elution volumes of ethanol, 1-propanol, and 1-butanol using water as an eluent. The elution volume of these alcohols increased with the increase of molecular weight or the length of alkyl chains. Therefore, the elution behavior is determined not by a size exclusion mechanism but by

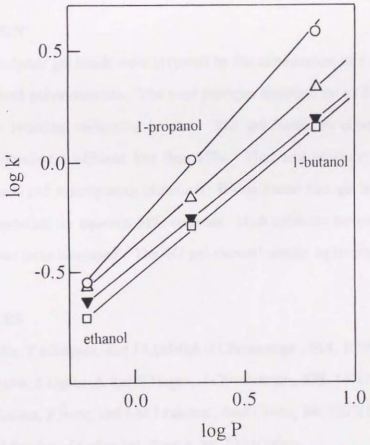

Figure 3-3 Relationship between capacity factor and hydrophobic parameter (○),2G, (△),3G, (□),4G, (▼),HEMA-EDMA

an adsorption or a partition mechanism. In Figure 3-3, the logarithm of capacity factors are plotted against the hydrophobic parameter (log P) calculated according to the method of Leo[7] (see P26, section II-1-2-2). Positive linear relationships were obtained between log k' and log P for all columns examined, which indicates that the elution was governed by hydrophobic interaction between the gel beads and alcohols. With the increase of the oxyethylene units, the position of the straight line shifted toward downside, and the slope of the line became shallow. This fact indicates that hydrophilicity of the gels increases in the order 2G, 3G, and 4G. For comparison, the results are also plotted for the column packed with 2-hydroxyethyl methacrylate-ethylene dimethacrylate (HEMA-EDMA) gel, which was prepared by the method described in Chapter 1. It is widely used as an aqueous SEC packing material.[8] As shown in Figure 3-3, the hydrophilicity of 4G beads is found to be almost equal to that of HEMA-EDMA gel.

II-3-4 CONCLUSION

Hydrophilic polymer gel beads were prepared by the combination of a dispersion polymerization and seed polymerization. The seed particles absorbed up to 200-fold of organic phase while retaining uniformity in size. The gel beads so obtained were monodisperse with variation coefficient less than 17%. They had pressure resistance higher than 150kg/cm^2, and macroporous structure. It was found that gel beads were suitable as packing materials for aqueous SEC columns. Hydrophilicity increased as the number of oxyethylene units increased. The 4G gel showed similar hydrophilicity to a HEMA-EDMA gel.

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Part III

NMR Characterization of Polymer Gel Beads

ABSTRACT

Styrene-divinylbenzene copolymer gel beads in a swollen state were characterized with a proton nuclear magnetic resonance spectroscopy (^{1}H-NMR) using chloroform as a probe. The signal of chloroform was observed as doublet peaks and the peak at higher magnetic field was assigned to chloroform inside of gel beads and the other at lower magnetic field to one outside of gel beads. NMR parameters such as signal chemical shift, intensity, line-width and shape were investigated in relation to the characteristics of gel beads such as the swelling ratio, the diameter, pore size, and crosslinking density. The relative intensity of the signal due to chloroform inside of gel beads increased with the amount of diluent used in preparation of gel beads. With the decrease of the diameter of beads, two signals became closer, because the rate of exchange between the solvent molecules inside and outside increased. The pore size also influenced the shape of doublet peaks. As the pore size increased, the two peaks overlapped due to the decrease of the portion of chloroform which interacts with the polymer matrix. The crosslinking density did not influenced the peak shape although the dynamics of chloroform was affected by the crosslinking density.

The interaction of small molecules such as cyclohexane, tetrahydrofuran and acetonitrile with styrene-divinylbenzene copolymer gel beads were investigated by ^{13}C-NMR spectroscopy. When the gel content was 0.1-0.3g/ml, the ^{13}C-NMR spectrum of the solvent displayed two peaks. A sharp peak at lower magnetic field was assigned to the free solvent, and a broad one to the solvent affected by the gel. This signal splitting is attributed to the upfield shift caused by aromatic rings of styrene units in the polymer chain. The nitrile carbon of acetonitrile showed the largest upfield shift. The mobility of small molecules in gel beads was also investigated using the nuclear magnetic relaxation method. In the case of good solvents for gel beads, the mobility was affected by the crosslinking density, while poor solvents exhibited little dependence of the crosslinking density.

Freezing processes of benzene in the presence of styrene-divinylbenzene gel beads were investigated by proton nuclear magnetic resonance spectroscopy. Some portion of benzene in the pore did not freeze below its freezing point, which was detected until -80°C. Temperature dependence of the amount of unfrozen benzene was discussed in relation to the pore size and the crosslinking density of gel beads. The small pore size and high crosslinking density increased the amount of unfrozen benzene, while the polymer matrix concentration showed little effect on the freezing processes.

Chapter 4

NMR Characterization of Styrene-Divinylbenzene Gel Beads in Swollen State using Chloroform as Probe

III-4-1 INTRODUCTION

Styrene-divinylbenzene (St-DVB) copolymer beads are widely used as the packing materials for size exclusion chromatography[1] and reversed-phase chromatography[2], and the supports for ion-exchange resins and polymeric catalysts[3]. Since the beads are usually used in a solvent swollen state, it is of importance to characterize the beads in a swollen state.

It is difficult to characterize solvent-swollen crosslinked polymers by using NMR spectroscopy, because the gel state is intermediate between the solid and liquid state. As reported by Ford et al., high resolution NMR (for solutions) can not fully cope with the peculiar characteristics of solvent swollen St-DVB gels because of their great dipole broadening[4]. Cross polarization magic angle spinning (CP/MAS) method is useful for the characterization of solid polymers, however, it is of limited value for gel state samples, because a rapid local segmental motion of polymer chains of gels may eliminate the dipole coupling required for efficient cross polarization from proton to carbon. The most successful method is direct polarization magic angle spinning (DP/MAS) method reported by Stöver et al[5]. However, this method can be applied only for lightly crosslinked polymer.

In contrast, solvents in crosslinked polymer give high resolution NMR spectra even if crosslinking density is high. When polymer beads such as St-DVB gel and ion-exchange resins are immersed in organic solvents or water, separate resonances for solvents or ions inside and outside of polymer beads have been reported earlier in both ^{1}H and ^{13}C NMR spectra.[6-20] This phenomenon has been used to evaluate the dynamics of solvent molecules by the determination of the rate constants for exchange between two sites[11,16,17] and the properties of ion-exchange resins[6-10,12-14].

In this chapter, the author investigated ^{1}H-NMR spectra of chloroform in the presence of St-DVB gel beads. NMR parameters such as line shape, and chemical shift were discussed in relation to the characteristics of the gel beads such as swelling ratio, diameter, pore size, and crosslinking density, which are all important parameters in the applications of beads.

III-4-2 EXPERIMENTAL

III-4-2-1 Gel beads preparation

Styrene (St) and divinylbenzene (DVB), containing ca. 45% of ethyl vinyl benzene, were obtained from Tokyo Kasei Co., Ltd., and were distilled under reduced pressure before use. St-DVB copolymer beads were prepared by a conventional suspension polymerization at 80°C in the presence of different amount of a porogenic agent (inert diluent). Swelling ratio, and pore size were controlled by changing polymerization conditions such as DVB content, the type and the amount of a diluent. The obtained gel beads were washed 2-4 times successively with hot water, acetone, and chloroform to remove the suspension stabilizer, unreacted monomers, and the linear polymers, followed by drying in vacuo. The fine beads were removed by a decantation in acetone. In preparation of Gel 4', a suspension with a wide range of diameter of the oil phase droplet (5-200μm) was prepared with Labo-disperser (MRK Co.) to obtain the beads with the wide distribution of size, which were separated into 5 fractions with sieves based on the size. The polymer matrix concentrations, which represent the swelling ratio, were determined by placing the weighted dry gel beads (typically 1.0g) in a 10ml-graduated cylinder with excess chloroform followed by measuring the final apparent volume of gel beads (swollen beads plus interstitial volume) after fully swelling. Table 4-1 represents the preparation conditions and characteristics of the gel beads used.

III-4-2-2 Pore size determination

The pore size of prepared gel beads was evaluated by size exclusion chromatography (SEC). The gel beads were packed into stainless steel column (7.6mmϕ x 30cm) to obtain SEC column with a slurry method. The calibration curves for

Table 4-1 Preparation conditions and characteristics of styrene-divinylbenzene gel beads

Gel	St/DVB[a]	M/DI[b]	PMC[c] (g/ml)	diameter (μm)	Ex L[d] ($x10^3$)
1	80/20	50/50[e]	0.15	50-150	4
2	80/20	67/33[e]	0.24	50-150	3
3	80/20	75/25[e]	0.29	50-150	0.4
4	80/20	100/0	0.36	50-150	0.2
4'-1	80/20	100/0	n.d.[h]	100-200	n.d.
4'-2	80/20	100/0	n.d.	60-100	n.d.
4'-3	80/20	100/0	n.d.	30-60	n.d.
4'-4	80/20	100/0	n.d.	20-30	n.d.
4'-5	80/20	100/0	n.d.	10-20	n.d.
5	70/30	60/40[e]	0.27	50-150	3
6	50/50	50/50[e]	0.26	50-150	3
7	50/50	50/50[f]	0.24	50-150	50
8	50/50	50/50[g]	0.22	50-150	300

Polym. Temp.;80°C, Polym. Time; 10hrs.
a); volume ratio of styrene(St) and divinylbenzene(DVB)
b);volume ratio of monomer(M) and diluent(DI)
c); polymer matrix concentration swollen in chloroform
d); molecular weight of exclusion limit
Diluent is e):toluene, f):toluene+2wt% of polystyrene (for oil phase), and f):isoamyl alcohol.
h); not determined

polystyrene standards (Shodex) were obtained using chloroform as an eluent at room temperature (ca. 25 °C)

III-4-2-3 NMR sample preparation and measurements

Reagent grade chloroform purified by the distillation under nitrogen atmosphere was used as an NMR probe. The NMR samples were prepared in 10-mm NMR tubes by placing the dry gel beads and excess amount of chloroform. After complete swelling, a Teflon plug was inserted to minimize the amount of the interstitial chloroform, and squeezed chloroform was removed by a syringe.

All ^{1}H-NMR measurements were made with a JEOL-FX200 NMR spectrometer operating at 200 MHz for protons. A 4000 Hz frequency range (20ppm), 16K data points, 10-20 s pulse delay, and 16-32 acquisitions were used. Spin-lattice relaxation

time (T_1) measurements were conducted using a conventional inversion recovery method and spin-spin relaxation time (T_2) using the CPMG pulse sequence.

III-4-3 RESULTS AND DISCUSSION

III-4-3-1 ^{1}H-NMR spectrum of chloroform in the presence of St-DVB gel beads

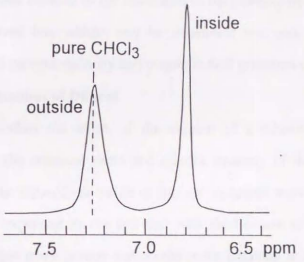

Figure 4-1 ^{1}H-NMR spectrum of chloroform in the presence of Gel 4 (30°C)

Figure 4-1 shows ^{1}H-NMR spectrum of chloroform in the presence of St-DVB gel beads (gel-4, gel type resin) at 30°C, where two peaks are observed. The chemical shift of the signal at lower magnetic field was almost equal to that of chloroform in the absence of gel beads indicated by the broken line in Figure 1. Therefore, the signals were assigned to chloroform outside and inside of the gel beads in the order of increasing magnetic field. No signal of polymer matrix was observed because of high restriction of mobility. It is well-known that ^{1}H-NMR signal of chloroform in an aromatic solvent is shifted toward higher magnetic field due to solvent effect.[21] Therefore, chloroform in polymer matrix is considered to be subjected to pseudo-solvent effect by the aromatic rings of the matrix, which resulted in the appearance of two peaks.

In the case of ion exchange resins, it is also reported that ^{1}H-NMR signals of water or counterions are observed as doublets.[6-15] For example, the signal of water inside of sulfonated polystyrene resins appears at lower magnetic field compared to the external signal[6-13], and this result is contrary to ours. The shift direction of inside

solvents is considered to be determined by the combination of the types of polymer matrix and solvents.

The line width of outside and inside signals at half intensity were 19 and 10 Hz, respectively. If the line width depends only on relaxation times, the inside signal must be broader than outside one because of the restriction of the mobility by the polymer matrix. Therefore, the observed line widths may be dependent not only on relaxation and exchange rate but also on heterogeneity and magnetic field gradients within the sample.

III-4-3-2 Effect of Amount of Diluent

Figure 4-2 exhibits the effect of the amount of a diluent (toluene) used in preparing the gel on the chemical shifts and relative intensity of the two peaks. The relative intensity of the chloroform inside of the gel increased with the amount of the diluent. This can be explained by the fact that with the increase of the amount of the diluent the gel becomes more porous and swells more resulting in the increase of the

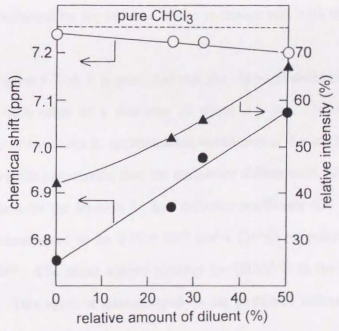

Figure 4-2 The effect of the amount of diluent on the chemical shift and the signal intensity
(○); chemical shift(open; outside, filled; inside)
(▲); relative intensity of inside signal

chloroform inside of the gel. Figure 4-2 also shows that with the increase of the diluent, the inside signal shifted toward the lower magnetic field, which is attributed to the decrease of polymer matrix concentration inside the gel beads. The outside signal slightly shifted toward the higher magnetic field with the increase of the diluent, which may be due to the increase of exchange rate between two types of chloroform.

III-4-3-3 Effect of Size of Gel Beads.

In order to examine the effect of the size of the beads, beads with the wide size distribution was prepared, i.e., Gel 4' (DVB20%, no diluent), and were separated into 5 fractions based on the diameter. Figure 4-3(a) represents the relationship between the chemical shifts of the two peaks and the diameter of gel beads. The gel with the smallest diameter (fraction 5) shows the overlapped peaks due to the high exchange rate of two types of solvent (the exchange rate is determined by the surface area of beads per unit of volume). As the diameter of beads increases, the inside signal and outside one shifted toward the higher magnetic field and lower magnetic field, respectively. These phenomenon can be explained by the increase of the exchange rate with the decrease of the bead size.

As shown in Figure 4-3(a), it is predicted that the chemical shifts of two types of chloroform coincide each other at a diameter of about 3.5 μm. When two peaks coalesce, the life time of two sites is approximately estimated at 4.5×10^{-3} s from the general equation[23] with the assumption that the frequency difference is 100Hz, which is based on the observation for the fraction 1. Self diffusion coefficient (D) of chloroform in the gel beads was determined to be 0.30×10^{-5} cm^2/s (30°C) by pulse gradient spin echo (PGSE) method[24]. The mean square distance ($x=(2Dt)^{1/2}$) in the life time was calculated to 1.6μm. This value is almost equal to the predicted radius of the beads (1.75μm).

In Figure 4-3(b), line widths are plotted against the diameter of the beads. As the diameter decreased, line widths of both types of signals increased. This fact also indicates that the exchange rate increases with the decrease of the diameter.

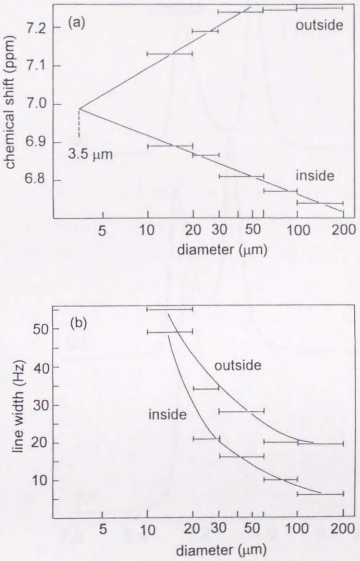

Figure 4-3 The relationship between NMR parameters and diameter of the beads.
(a),chemical shift, (b),line width

III-4-3-4 Effect of Pore Size

The pore size of gel beads in a swollen state is dependent on the type and the amount of the diluent used, the crosslinking density, and so forth. The three types of gel beads (Gels 6,7, and 8) were prepared with the different types of diluent, where the DVB content and the amount of diluent were constant. The SEC columns packed with Gels 6,7, and 8 showed the molecular weight of exclusion limit of $3x10^3$, $5x10^5$, and $3x10^6$ (for polystyrene standards), respectively. Figure 4-4 shows ^{1}H-NMR spectra of chloroform in the presence of these three types of gel beads. Samples in the presence of

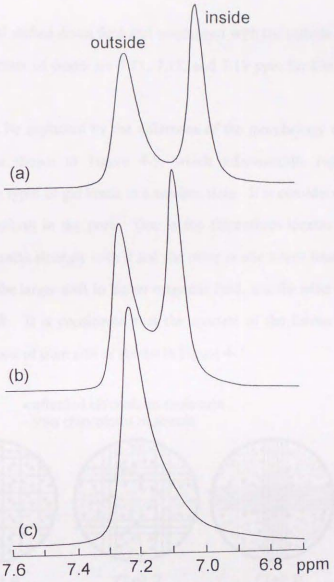

Figure 4-4 The effect of the pore size on ^{1}H-NMR line shapes. The molecular weight of exclusion limit is (a); $3x10^3$ (Gel 6), (b); $5x10^5$ (Gel 7), and (c); $3x10^6$ (Gel 8)

Gels 6 and 7 showed two chloroform peaks of inside and outside of the gel. Chloroform with Gel 7 showed the smaller chemical shift difference of doublet peaks than one with Gel 6. The signal intensity ratio of the two peaks was almost 1 for the both samples, which was the same as the in and out solvent ratio estimated from the SEC curves. Gel 8 showed only one peak with a tailing toward higher magnetic field. The total signal intensity was almost equal among the three samples. Therefore, it is expected that almost all the chloroform inside of the Gel 8 also showed the signals. Thus, it can be

said that the inside signal shifted down field and overlapped with the outside signal. The chemical shifts at the center of peaks are 7.11, 7.17, and 7.19 ppm for Gel 6, 7, and 8, respectively.

This finding can be explained by the difference of the morphology among three types of gel beads as shown in Figure 4-5, which schematically represents the morphology of the three types of gel beads in a swollen state. It is considered that there are two types of chloroform in the pore. One is the chloroform locates near to the polymer matrix and interacts strongly with it and the other is one which locates far from it. The former exhibits the larger shift to higher magnetic field, and the latter only a small high magnetic field shift. It is considered that the amount of the former chloroform decreases with the increase of pore size as shown in Figure 4-5.

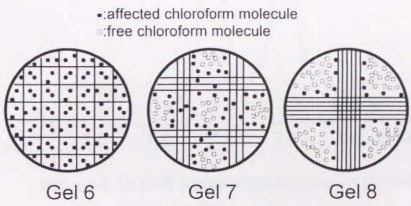

Figure 4-5 Schematic representations of the swollen networks

III-4-3-5 Effect of Crosslinking Density

Polymer gels with almost the same swelling ratio and different crosslinking density (Gels 2, 5, and 6) were prepared by controlling the amount of diluent (toluene) and crosslinking agent (DVB). It is reported that these gel beads have almost the same pore size distribution in a swollen state[22]. Therefore, these gels are suitable to evaluate the effect of the crosslinking density on NMR parameters. Figure 4-6 shows ^{1}H-NMR spectra in the presence of these gel beads at 30°C. The chemical shifts and the shape of the two peaks are almost the same among the three samples.

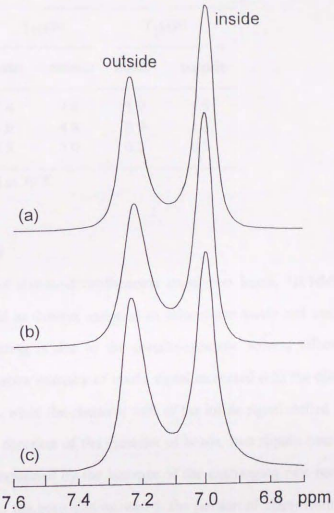

Figure 4-6 ^{1}H-NMR spectra of chloroform in the presence of Gels 2, 5, and 6
(a); Gel 2, (b); Gel 5, (c); Gel 6

Spin-lattice and spin-spin relaxation times of chloroform in the presence of three types of gel beads are tabulated in Table 4-2. As the crosslinking density increased, the relaxation times of inside and outside signals decreased. These values are 'apparent' ones because of the exchange between two sites. However, it is suggested that solvent dynamics such as the relaxation and exchange rates are affected by the crosslinking density. The fact that no change of spectra was observed among the gel beads indicates that the line shape depends not on the dynamics but on the polymer structures such as pore size and its distribution as well as the bead size.

Table 4-2 Relaxation data of chloroform

Gel	T_1(s)[a]		T_2(s)[a]	
	inside	outside	inside	outside
2	7.4	7.6	1.9	1.9
5	3.6	4.8	0.9	1.3
6	2.5	3.0	0.2	0.5

a)determined at 30°C

III-4-4 CONCLUSION

In the presence of styrene-divinylbenzene copolymer beads, ^{1}H-NMR signal of chloroform was observed as doublet assigned to chloroform inside and outside the gel beads. This signal splitting is due to the pseudo-aromatic solvent effect caused by polymer chains. The relative intensity of inside signal increased with the diluent used in preparation of gel beads, while the chemical shift of the inside signal shifted towards the outside signal. With the decrease of the diameter of beads, two signals overlapped with each other, which was explained by the increase of the exchanging rate between inside and outside solvents. As the pore size increased, the portion of chloroform in the pore which interacts with the polymer matrix decreased, which resulted in the signal overlapping. As the crosslinking density increased, relaxation times of inside and outside signals decreased, with only negligible change in the line shape.

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Chapter 5

NMR Analysis of Interaction between Styrene-Divinylbenzene Gel Beads and Small Molecules

III-5-1 INTRODUCTION

Small molecules in polymer gels can be studied by Nuclear Magnetic Resonance (NMR) spectroscopy that gives useful information such as chemical shifts and relaxation times. ^{13}C-NMR is particularly useful because it gives high resolution spectra compared with ^{1}H-NMR, and ^{13}C relaxation is mainly determined by the direct dipole-dipole mechanism[1]. Therefore it is easy to interpret the experimental results.

When styrene gel beads with the degree of crosslinking below 10% is swollen in a good solvent such as $CDCl_3$, polymer chains provide high resolution ^{13}C-NMR spectra[2,3]. Line width, spin-lattice relaxation time, and NOE factor are dependent on the extent of crosslinking, i.e., these parameters are influenced by the mobility of polymer chains. However, it is impossible to characterize highly crosslinked polymer gels directly by ^{13}C-NMR, because the polymer gels provide no high resolution spectra even if the polymers are swollen in a good solvent. The highly crosslinked gels in a dry state can be characterized using solid state NMR with magic angle spinning (MAS) and cross polarization (CP) techniques[4-6]. On the other hand, small molecules in crosslinked polymers exhibit high resolution ^{13}C-NMR spectra and give useful information concerning properties of the gels.

Ford et al. reported that each carbon atom in toluene molecules in crosslinked polystyrene gels provided doublet peaks in ^{13}C-NMR spectra[7-9], and the mobility of solvent has been investigated by monitoring a self diffusion coefficient using pulsed-gradient spin echo method[10] or spin-lattice relaxation times[7-9]. Their interpretation of the diffusion coefficients was based on the weight fraction of polymer in the system when polymer gel beads with low crosslinking density was swollen in toluene, i.e., the self diffusion coefficient in polymer gels was equal to that of a polymer solution containing

the same weight fraction of polymer. However, in highly crosslinked gels, this interpretation is no longer true for the gel-solvent systems owing to heterogeneity.

In this chapter, the author investigated the interactions of small molecules and St-DVB gel by ^{13}C-NMR spectroscopy. The mobility of small molecules in the gel was also investigated using spin-lattice and spin-spin relaxation times of ^{13}C nuclei.

III-5-2 EXPERIMENTAL

III-5-2-1 Preparation of gel beads

Styrene-divinylbenzene gel beads were prepared by the method described in Chapter 4. Preparation conditions and swelling ratios of gel beads are tabulated in Table 5-1. The gel beads with a diameter of 5-15μm were collected by decantation in acetone.

Table 5-1 Preparation conditions of styrene-divinylbenzene gel beads

gel	St/DVB[a)]	M/DI[b)]	PMC[c)] (g/ml)
1	90/10	50/50	0.05
2-1	80/20	50/50	0.14
2-2	80/20	67/33	0.24
3-1	70/30	50/50	0.17
3-2	70/30	60/40	0.25
4	50/50	50/50	0.26
5	100/0	50/50	0.26

a);ratio of styrene (St) and divinylbenzene (DVB)
b);ratio of monomer (M) and diluent (DI)
c);polymer matrix concentration
in swollen state in benzene

III-5-2-2 NMR sample preparation and measurements

Reagent grade solvents were used as NMR probes without further purification for NMR measurement. NMR samples were prepared with two methods. In the first method, 0.10-0.35g of dry polymer gel beads were placed in an 8-mm NMR tube, and 1-ml of solvent was added. The gels were allowed to swell by the treatment of ultra sonic waves generated by Sine Sonic 100 (Kokusai Denki Co., Ltd. :65W) which also

degassed the samples. This tube was inserted into a 10-mm tube containing $CDCl_3$, which provided the spectrometer frequency lock signal and served as the external reference (77.03 ppm)(Method I). In the second method, polymer gels were placed in a 10-mm tube directly, and the excess amount of solvent was added. After the ultrasonic treatment and nitrogen bubbling, a plug was inserted to the level of polymer gels, which assured to minimize the amount of interstitial solvent (Method II). Resolution adjustment was conducted by monitoring proton free induction decay signals since the sample prepared in this method had no deuterated solvent for resolution adjustment.

All ^{13}C-NMR spectra were obtained with a JEOL FX-200 NMR spectrometer which operated at a frequency of 50.1MHz. The temperature was varied in the range from 25°C to 70°C. The chemical shift and signal ratio were obtained by gated decoupler measurements. The measurements of spin-lattice relaxation time (T_1) and spin-spin relaxation time (T_2) were conducted by a conventional inversion recovery (IR) and Carr-Purcell-Meiboom-Gill (CPMG) methods respectively. Exponential functions were fitted to the relaxation decays. The 90° pulse width was checked before measurements, ca. 14μsec.

III-5-3 RESULTS AND DISCUSSION

III-5-3-1 Chemical shifts of small molecules in the presence of gel beads

Figure 5-1 shows ^{13}C-NMR spectra of cyclohexane in the presence of gel 4 (DVB50%, gel content: 0.20-0.34g/ml). Cyclohexane displayed two ^{13}C-NMR peaks, a sharp peak at lower magnetic field and broad one at higher magnetic field. The former had the same chemical shift as one in the absence of gel and its relative intensity decreased with the increase of the gel content. The T_1 value of this signal was longer than that of broad signal, which indicates that mobility of the former is higher than that of the latter. Therefore, the signal at the lower magnetic field was assigned to the free solvent that was unaffected by gel beads and the broad one to the solvent affected by gel beads. As shown in Figure 5-1, the sharp peak disappeared when the gel content became 0.34g/ml. At this content, there remained interstitial solvent enough for gel beads to stay afloat in cyclohexane, because gel content in a closely packed state is about 0.45g/ml

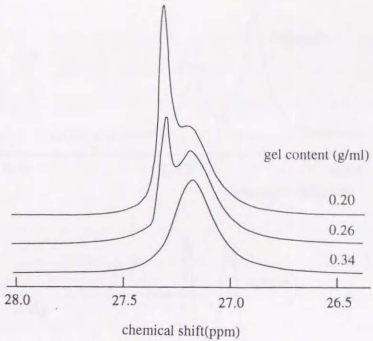

Figure 5-1 ^{13}C-NMR spectra of cyclohexane in the presence of styrene-divinylbenzene gel (gel 4 in Table 5-1).

estimated from swelling ratio. This fact indicates that there are two types of interstitial solvent in the system that gives two peaks. Some part of interstitial solvent and solvent inside gel beads exchange each other on the time scale of NMR signal detection, and the other part does not exchange. The former and solvent uptaken by gel beads give broad signal at higher magnetic field, and the latter gives sharp one.

The ^{13}C-NMR spectrum of acetonitrile in the presence of the gel is shown in Figure 5-2. Resolution of doublet peaks was higher than that of cyclohexane because of the larger chemical shift difference between the signals of free and affected solvents. Assignment of each signal was the same as that of cyclohexane. The nitrile carbon signal of free solvent was broader than methyl carbon signal, which was ascribed to magnetic quadrupole moment of nitrogen atom. Table 5-2 lists the chemical shifts and the signal intensity ratio of affected peak in the presence of 0.20g/ml gel. The ratio of the affected signal increased with the gel content, while the chemical shift was only slightly affected by the gel content. The nitrile carbon of acetonitrile showed the largest upfield shift, 0.34 ppm, and the other compounds, 0.1 to 0.2 ppm. Ford *et al.* studied ^{13}C-NMR spectra of toluene in crosslinked polystyrene gel beads having 16-18 mole% of benzyltri-

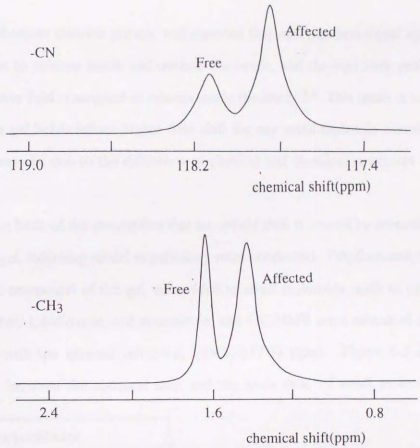

Figure 5-2 ^{13}C-NMR spectrum of acetonitrile in the presence of styrene-divinylbenzene gel (gel 4, 0.28g/ml).

Table 5-2 ^{13}C-NMR chemical shift of small molecules in the presence of styrene-divinyl benzene gel beads[a].

solvent	chemical shift(ppm)			ratio[b]
	free	affected	dif.	
cyclohexane	27.29	27.17	0.12	0.45
THF[c] (α)	67.55	67.46	0.09	0.54
(β)	25.73	25.62	0.11	0.54
1,4-dioxane	67.22	67.09	0.13	0.58
MeCN[d](CH_3)	1.64	1.43	0.21	0.52
(CN)	118.19	117.76	0.43	0.52
methanol	49.34	49.16	0.18	0.44

a);Gel content is 0.20g/ml in all cases.
b); The relative intensity of affected solvent peak.
c);tetrahydrofurane, d);acetonitrile

n-butylphosphonium chloride groups, and reported that every carbon signal appeared as a doublet due to toluene inside and outside the beads, and the narrower peak of each doublet at lower field is assigned to toluene inside the beads.[7,8] This result is contrary to ours, i.e., the gel beads induce higher field shift for any small molecule examined. The discrepancy may be due to the difference of physical and chemical structures of the gel beads used.

On the basis of the assumption that an upfield shift is caused by aromatic rings of the polymer gel, following model experiments were conducted. Ethylbenzene, which can be the model compound of the gel, was added to small molecules, such as cyclohexane, tetrahydrofuran, 1,4-dioxane, and acetonitrile, and ^{13}C-NMR were measured at ambient temperature with the external reference, $CDCl_3$ (77.03 ppm). Figure 5-3 shows the relationships between the chemical shift and the mole ratio of small molecules. The

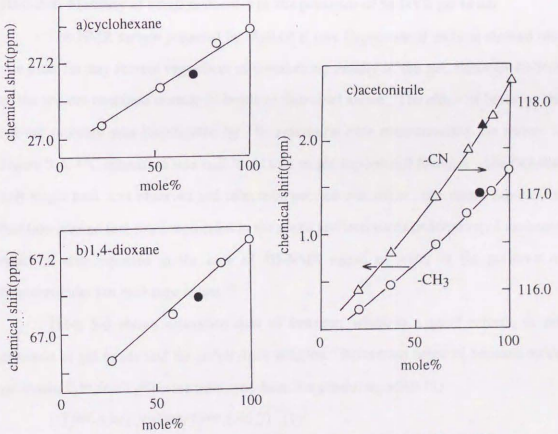

Figure 5-3 Chemical shift of small molecules diluted with ethylbenzene.
(a),cyclohexane, (b) tetrahydrofuran, (c);1,4-dioxane, (d);acetonitrile
filled symbols; chemical shift of small molecules affected by gel beads
(Mole ratio was calculated from signal intensity and gel content.)

chemical shift of any small molecule is shifted to upfield as the content of ethylbenzene increased. In this case, upfield shift of acetonitrile was also the largest of the three kinds of samples. The chemical shifts of signals due to the solvents affected by gel beads are plotted against molar ratio of solvent, which is calculated from the signal ratio (Table 5-2) and styrene unit concentration (filled symbols). These results are closely correlated with the upfield shift caused by ethylbenzene. This effect of aromatic solvent on chemical shift was applied to the heterogeneous systems consisted of St-DVB gel and small molecules, which leads the doublet peaks for carbon signals. Therefore, small molecules near gel beads are placed in the quasi-aromatic environment. The upfield shift in ^{1}H-NMR spectroscopy is well-known as "aromatic solvent-induced shifts", while this effect has been little taken into consideration for ^{13}C-NMR owing to difficulties in interpretation[11].

III-5-3-2 Mobility of small molecules in the presence of St-DVB gel beads

The NMR sample prepared by Method II (see Experimental section) showed only one peak for any solvent regardless of crosslinking density of the gel, although 40-50% of the solvent remained outside of beads as described above. The effect of beads on the solvent mobility was investigated by ^{13}C relaxation time measurements. As shown in Figure 5-4, ^{13}C relaxation was well fitted to a single exponential function. The fact that only single peak was observed and relaxation process was subject to a single exponential function implies that small molecules in the pores and interstices undergo rapid exchange, which is also reported in the case of ^{1}H-NMR signal of water in the presence of macroreticular ion exchange resins.[12]

Table 5-3 shows relaxation data of benzene, which is a good solvent, in the presence of gel beads and for polystyrene solution. Relaxation times of benzene inside gel beads(T_1^{in} and T_2^{in}) were estimated from the general equation (1)

$$1/T_i^{obs}=F^{in}/T_i^{in}+F^{out}/T_i^{out} \quad (i=1,2) \quad (1)$$

, where F^{in} and F^{out} are the volume fraction of solvent inside and outside gel beads, respectively. T_i^{out}s were determined independently for neat benzene(T_1^{out}=24.5s, T_2^{out}=18.0s)

Table 5-3 Relaxation data of benzene in the presence of styrene-divinylbenzene gel beads and for polystyrene solutions

gel	T_1^{obs}[a] (s)	T_2^{obs}[a] (s)	Fin[b]	T_1^{in}[c] (s)	T_2^{in}[c] (s)	T_1^{sol}(g/ml)[d] (s)	T_2^{sol}[d] (s)
1	19.0	9.2	0.63	16.8	7.2	20.2(0.09)	10.0
2-1	14.0	5.6	0.60	11.0	3.8	18.5(0.30)	7.7
2-2	11.6	2.8	0.51	7.6	1.7	13.5(0.73)	2.4
3-1	12.0	2.0	0.55	8.5	1.2	15.8(0.35)	3.9
3-2	9.2	1.1	0.50	5.7	0.6	13.5(0.73)	2.4
4	9.5	0.4	0.50	5.5	0.2	13.5(0.73)	2.4
5	9.5	0.4	0.50	5.5	0.2	13.5(0.73)	2.4

a);Observed relaxation times.
b);The volume fraction of benzene inside gel beads, which were estimated from swelling ratio.
c);These values were estimated from the equation (1).
d);These values were determined for polystyrene solutions. The values in the parentheses represent the polymer concentrations.

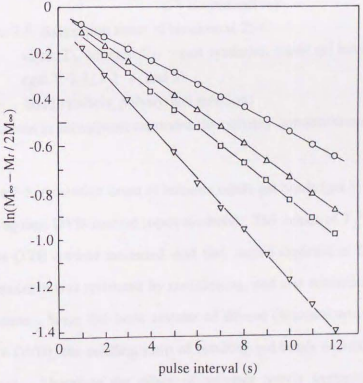

Figure 5-4 Signal intensity profiles in measurements of spin-lattice relaxation times of benzene in the presence of styrene-divinylbenzene gel beads.
(○), gel 1, (△);gel 2-1, (□),gel 3-1, (▽),gel 4

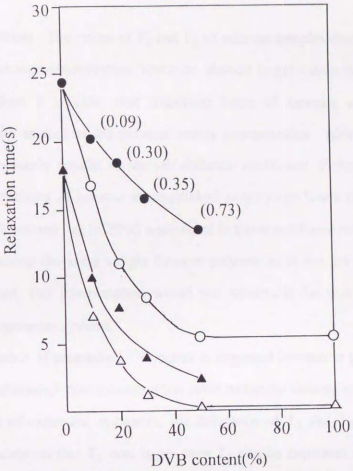

Figure 5-5 Relaxation times of benzene at 25°C
circle;T_1, triangle;T_2, open symboles; inside gel beads
(gel 1, 2-1, 3-1, 4, and 5)
filled symbols; polystyrene solutions
The values in parentheses represent the polymer concentration (g/ml).

In Figure 5-5, relaxation times of benzene inside gel beads (gel 1, 2-1, 3-1, 4, and 5) were plotted against DVB content (open symbols). The values of T_1^{in} and T_2^{in} were decreased as the DVB content increased, and they stayed constant at DVB 50%. The polymer chain mobility was restricted by crosslinking, and this restriction decreased the mobility of benzene. Since the same amount of diluent (toluene) was used as that of monomers (St + DVB), the swelling ratio of resulting gel beads decreased as the DVB content increased. Therefore the effect of polymer matrix concentration was also investigated by measuring the relaxation times of benzene in the linear polystyrene solution. The polymer concentration was adjusted to the polymermatrix concentration inside gel beads in each DVB content of gel sample. The results were shown in Figure

5-5 with filled symbols. The values of T_1 and T_2 of solution samples also decreased with the increase of polymer concentration, however, showed larger values than those of gel samples. Therefore it is clear that relaxation times of benzene are affected by crosslinking density as well as the polymer matrix concentration. Although relaxation times are not necessarily parallel to the self diffusion coefficient, Pickup reported that self-diffusion coefficients of toluene in crosslinked polystyrene beads (gel type resins) with various DVB content (up to 20%) were equal to those in toluene solutions of linear polystyrene containing the same weight fraction polymer as in the gel samples.[10] As Pickup pointed out, this interpretation would not necessarily be true in very highly crosslinked heterogeneous systems.

The relaxation of protonated ^{13}C nuclei in degassed benzene is governed by the direct-dipolar mechanism.[1] Since benzene has small molecular volume and its mobility is isotropic because of molecular symmetry, the difference of T_1 and T_2 value must be small. The phenomenon that T_1 was larger than T_2 can be explained by the principal case reported by Woessner[13,14]. A large T_1/T_2 ratio can be explained by the low molecular mobility, which is characterized by long correlation time τ_c ($\omega_0\tau_c >> 1$, where ω_0 is the Larmour frequency). In Figure 5-6, the T_1^{in}/T_2^{in} values were plotted against the DVB content with the T_1/T_2 for the solution samples. The T_1/T_2 value increased with the DVB content in both cases, and gel samples showed larger values than solution ones. Furthermore, the differences between both T_1/T_2 values were small up to 20% of DVB, while it increased with the DVB content over 30%.

In order to further elucidate the effect of crosslinking, the gel beads with the same swelling ratio and with different DVB content were prepared by changing the ratio of diluent to monomer (gel 2-2, 3-2, and 4) and T_1 and T_2 values of benzene in the presence of these gel beads were measured. Table 5-3 and Figure 5-7 show relaxation data of benzene for the polystyrene solution (0.73g/ml) and in the presenceof gel beads. T_1^{in} and T_2^{in} values also decreased with the DVB content. When the DVB content was 20%, the T_1^{in}/T_2^{in} value was a little smaller than that of the solution, while it increased

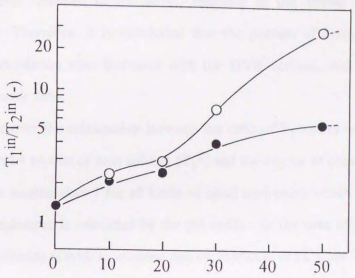

Figure 5-6 The ratio of T_1 to T_2 of benzene.
(○); inside gel beads(gel 1, 2-1, 3-1, 4, and 5)
(●); polystyrene solutions

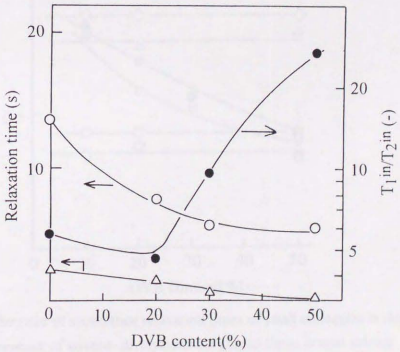

Figure 5-7 Relaxation data of benzene in the same polymer matrix concentration at 25°C.
circle;T_1^{in}, triangle;T_2^{in}, filled circle; T_1^{in}/T_2^{in}

with the DVB content over 30%. Some amount of benzene molecules is adsorbed to polymer chain and/or confined in the pores, resulting in the strong restriction of molecular motion. Therefore, it is concluded that the portion of benzene with low mobility or long correlation time increases with the DVB content, and this effect is manifest when it is over 30%.

Figure 5-8 shows the relationship between the ratio of T_1 values in the presence of gel 1, 2-1, 3-1 and 4 to that of neat solvent (T_1^0) and the degree of crosslinking. The ratio of T_1/T_1^0 was smaller than 1 for all kinds of small molecules, which indicates that mobility of small molecules is restricted by the gel beads. In the case of poor solvents such as dimethylsulfoxide (DMSO), acetone and cyclohexane (25°C), the T_1/T_1^0 values were almost independent of the degree of crosslinking. This result can be explained by the fact that the motion of polymer chains is restricted, and constant, regardless of the degree of crosslinking. The T_1/T_1^0 values of methyl carbons of DMSO and acetone were larger than those of other carbons of poor solvents. It is considered that the

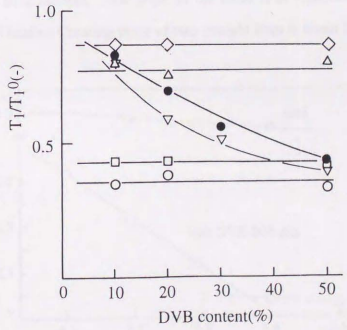

Figure 5-8 The ratio of spin-lattice relaxation times of small molecules in the presence of styrene- divinylbenzene gels to those in neat solvent (○); cyclohexane (open; 25°C, filled 70°C), (▽);benzene (△); dimethylsulfoxide, (□);acetone(C=O), (◇);acetone(CH_3) T_1^0; T_1 of neat solvent

contribution of spin rotation to methyl carbon relaxation was great and spin rotation mechanism might not be much affected by the gel beads.

It is well-known that cyclohexane is a θ solvent for polystyrene at 35°C, a poor solvent below 35°C and a good solvent above it.[15] As shown in Figure 5-8, T_1 of cyclohexane is independent of the degree of crosslinking like acetone and DMSO at 25°C (open circle), whereas T_1 is dependent on the degree of crosslinking like benzene at 70°C (filled symbol). The change of mobility of polymer chains in the gel beads was detectable indirectly by monitoring the mobility of small molecules which coexisted with the gel beads. Figure 5-9 shows the temperature dependence of T_1 of cyclohexane in the presence of gel 2-1 (circle) and that of neat cyclohexane (triangle). Although the T_1 behavior of neat cyclohexane is explained by a single Arrhenius type function in the temperature range examined, T_1 data for cyclohexane in the presence of gel beads can be described by two straight lines, one with a slope of 2.1×10^3 K which represents an activation energy (E_a) of 17.0kJ/mol and the other with a slope of 3.0×10^2 K, which represents E_a of 2.5kJ/mol. The slope of the latter is in accordance with that in the absence of gel beads. Crossing point of two straight lines is about 25°C, and represents

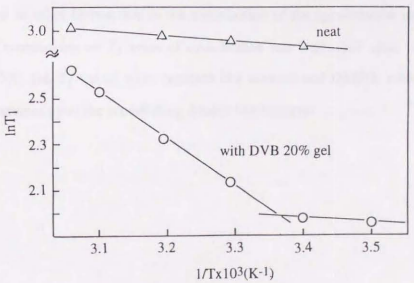

Figure 5-9 Temperature dependence of spin-lattice relaxation time of cyclohexane
circle; in the presence of gel 2-1.
triangle; neat cyclohexane

the temperature at which the mobility of polymer matrix changes. This temperature is 10°C lower than θ temperature, which is explained by the fact that the phase separation temperature of polystyrene-cyclohexane system is lower than θ temperature when the volume fraction of polymer is below unity.[16]

III-5-4 CONCLUSION

In the presence of 0.1-0.3g/ml of St-DVB gel beads, ^{13}C-NMR spectra of small molecules such as cyclohexane, tetrahydrofurane, acetonitrile displayed doublet peaks. A sharp peak at lower magnetic field was assigned to the free solvent, and a broad one was assigned to the solvent affected by gel beads. From the results of model experiments with low molecular weight compounds, signal splitting is attributed to the upfield shift caused by aromatic rings of styrene units possessed by gel beads.

The mobility of benzene in the presence of gel beads was dependent upon the crosslinking density, while it was not parallel to that of polystyrene solution due to heterogeneity. When the DVB content was over 30%, the T_1/T_2 value increased, which indicated that the portion of benzene with low mobility increases with the DVB content. In the case of acetone and DMSO, the T_1 values remained almost constant with the change of the DVB content. The T_1 values of methyl carbons were not as much influenced as other carbon due to the contribution of the spin-rotation interaction. The effect of crosslinking on T_1 value of cyclohexane was dependent upon the temperature, i.e., at 25°C, the T_1 values were constant like acetone and DMSO, while at 70°C they were dependent upon the crosslinking density like benzene.

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Chapter 6

NMR Characterization of Styrene-divinylbenzene gel beads Using Freezing Point Depression of Benzene

III-6-1 INTRODUCTION

The elucidation of the physical and chemical properties of small molecules in styrene-divinylbenzene (St-DVB) gel beads is of importance in practical aspect. The structure and morphology of a polymer network of gel beads in a swollen state also play important roles in various applications, but their direct characterization is rather difficult.

It is well-known that water in a capillary or in a small space of silica and other inorganic materials sometimes remains unfrozen below the freezing point since the thermodynamic nature is different from that in the bulk state.[1-3] This phenomenon has also been observed for water in polymer gels such as crosslinked dextrans[4,5], poly(acrylic acid)/poly(vinyl alcohol) gels[6], and poly(methyl methacrylate) stereo complex gels[7,8] through thermal analyses, and polyacrylamide gels[9,10] through ^{1}H-NMR. Some organic solvents also remain unfrozen below their freezing point in the presence of polymer matrices such as polystyrene network[11] and crosslinked rubber[12,13]. Using this phenomenon, the polymer network structure can be characterized indirectly, since freezing point depression depends on the nature of network. Brun *et al.* proposed the method to determine the pore size of styrene-divinylbenzene copolymer beads in a swollen state by monitoring the freezing point depression of benzene with differential scanning calorimetry (DSC)[14]. However, the properties of polymer beads are determined not only by the pore size but also by the crosslinking density and the swelling ratio. The freezing point depression of benzene in styrene-divinylbenzene gel beads has not been investigated in relation to the crosslinking density or the swelling ratio.

In this chapter the author measured the ^{1}H-NMR spectra of benzene in the presence of styrene-divinylbenzene gel beads and its freezing behavior was discussed in relation to the structure of gel beads such as the pore size and crosslinking density.

Benzene is a suitable solvent for the characterization of gel beads since it is a good solvent for gel beads and shows only one singlet peak for ^{1}H-NMR and its freezing point is relatively high (5°C).

III-6-2 EXPERIMENTAL

III-6-2-1 Preparation of St-DVB gel beads

Styrene-divinylbenzene gel beads were prepared by the method described in Chapter 4. Preparation conditions and swelling ratios of gel beads are tabulated in Table 5-1. The diameter of gel beads was in the range of 50-150 μm. Table 6-1 represents the preparation conditions of gel beads used.

Table 6-1 Preparation conditions of St-DVB gel beads

gel	St/DVB[a)]	M/DI[b)]	PMC[c)] (g/ml)	V_0/V_p[d)]
1	80/20	50/50[e)]	0.13	0.63
2	80/20	67/33[e)]	0.24	0.67
3	80/20	75/25[e)]	0.29	0.75
4	80/20	100/0	0.34	1.00
5	70/30	60/40[e)]	0.25	0.72
6	50/50	50/50[e)]	0.26	0.74
7	50/50	50/50[f)]	0.23	0.73
8	50/50	50/50[g)]	0.22	0.66

Polym. Temp.;80°C, Polym. Time; 10hrs.
a): volume ratio of styrene(St) and divinylbenzene(DVB), b): volume ratio of monomer(M) and diluent(DI), c): polymer matrix concentration in a swollen state in benzene, d): ratio of void volume (V_0) to pore volume (V_p)
Diluent is e): toluene, f): toluene+2wt% of polystyrene (for oil phase), and g): isoamyl alcohol.

III-6-2-2 Evaluation of pore size

The pore size and its distribution of gel beads swollen in benzene were evaluated by size exclusion chromatography (SEC). The gel beads were packed into stainless

steel columns (7.6mm ϕ x 30cm or 4.6mm ϕ x 25cm) to obtain SEC columns with a slurry method. The calibration curves for polystyrene standard (Shodex) and styrene oligomers prepared with an anionic method were made using benzene as an eluent and refractive index detector (Jasco 830-RI) at room temperature (ca. 20°C).

III-6-2-3 Sample preparation and NMR measurement

Reagent grade benzene used as an NMR probe was purified by distillation over sodium under nitrogen atmosphere. The NMR sample was prepared in a 10-mm tube by placing the gel beads in an excess amount of benzene. An ultrasonic wave generated by Sine Sonic 100 (Kokusai Denki Co., Ltd. :65W) was used to remove air bubbles from the samples. After nitrogen bubbling a plug was inserted to the level of polymer gels which assured to minimize the amount of the interstitial solvent. The squeezed benzene was removed by a syringe.

All ^{1}H-NMR measurements were conducted with a JEOL FX-200 NMR spectrometer operating at a proton frequency of 200 MHz. A 4000 Hz frequency range (20ppm), 16K data points, 10-20s pulse delay, and 32-64 acquisitions were used. Samples were spun at 10-15 Hz. The amount of unfrozen benzene was calculated from its signal area compared to that of the signal at 30°C. The sample was maintained at a given temperature for 30 min. to assure the equilibrated state before NMR measurements.

The measurements of ^{13}C spin-lattice relaxation time (T_1) and spin-spin relaxation time (T_2) were conducted by a conventional inversion recovery (IR) and Carr-Purcell-Meiboom-Gill (CPMG) methods respectively. 100s pulse delay and 8-16 acquisitions were used. For T_2 measurements, the pulse interval between 90° and 180° pulses was 1ms. Exponential functions were fitted to the relaxation decays. The 90° pulse width was checked before measurements, ca. 14μsec.

III-6-3 RESULTS AND DISCUSSION

III-6-3-1 ^{1}H-NMR of unfrozen benzene

Figure 6-1 shows ^{1}H-NMR spectra of benzene in the presence of St-DVB gel beads (gel 4). At 30°C a sharp peak of benzene appeared at 7.2 ppm as shown in Figure 6-1(a), and no signal due to the polymer chains was observed because of their low

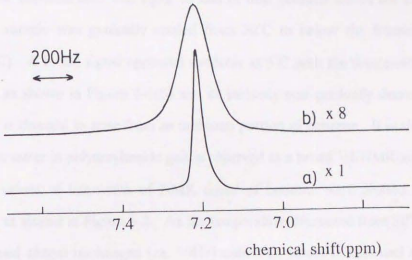

Figure 6-1 ^{1}H-NMR spectra of benzene in the presence of St-DVB gel beads (gel 4)
a)30°C, b)2°C.

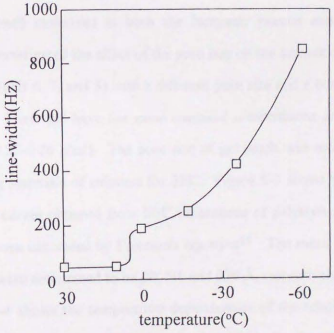

Figure 6-2 Temperature dependencies of the line-width of benzene in the presence of St-DVB gel beads (gel 4).

mobility. The chemical shift was equal to that of neat benzene within the experimental error. The sample was gradually cooled from 30°C to below the freezing point of benzene (5°C). A broad signal appeared suddenly at 5°C with the disappearance of the sharp signal as shown in Figure 6-1(b) and its intensity was gradually decreased. This broad signal is thought to arise from an unfrozen portion of benzene. It is also reported that unfrozen water in polyacrylamide gels is observed as a broad ^{1}H-NMR signal.[9,10]

The values of line-width of NMR signal of benzene were plotted against the temperature as shown in Figure 6-2. As the temperature decreased from 30°C, the line-width remained almost unchanged (ca. 50Hz) until 5°C, where it increased suddenly to 200 Hz with the appearance of frozen benzene. Below 5°C the line-width increased continuously as the temperature decreased. The similar temperature profile was obtained for other St-DVB gel beads. It is considered that line-broadening was caused by a heterogeneity of the magnetic field and a decrease of molecular mobility of benzene.

III-6-3-2 Effect of pore size

It is reported that the pore dimension and its distribution affect the freezing point depression of small molecules in both the inorganic porous materials and polymer networks. We investigated the effect of the pore size on the amount of unfrozen benzene using gel beads (gels 6, 7, and 8) with a different pore size and a constant DVB content (50% in feed). These gel have the same chemical compositions and narrow range of swelling ratio (0.22-0.26 g/ml). The pore size of gel beads was estimated by using gel beads as packing materials of columns for SEC. Figure 6-3 shows the cumulative pore size distribution curves obtained from SEC calibrations of polystyrene. Molecular sizes of polystyrene were calculated by Freeman's equation[15]. The mean pore sizes (D_{50}) of gels 6, 7, and 8 were determined to be 20, 50, and 630 Å, respectively.

Figure 6-4 shows the temperature dependencies of the relative intensity of ^{1}H-NMR signal of benzene in the presence of gel 6, 7, or 8. In all cases, the relative intensities decreased abruptly to 10-60 % at ca. 5°C. This resulted from freezing of the interstitial benzene and some part of benzene inside the gel beads. The intensity decreased almost linearly with a decrease of temperature from 5 to -80°C. This result

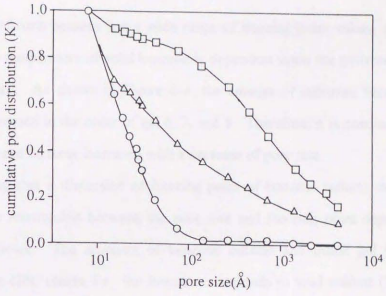

Figure 6-3 Cumulative pore distribution curves obtained from GPC of polystyrene standards and oligostyrenes in benzene (○), gel 6, (△), gel 7, (□), gel 8.

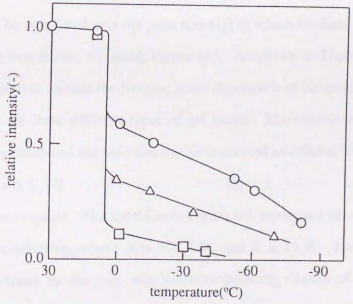

Figure 6-4 Temperature dependencies of the relative intensity of benzene in the presence of St-DVB gel beads. (○), gel 6, (△), gel 7, (□), gel 8.

indicates that unfrozen benzene has a wide range of freezing point values, and that the phase transition temperature of solid benzene is dependent upon the environment where benzene is placed. As shown in Figure 6-4, the amount of unfrozen benzene at any temperature increased in the order of gel 6, 7, and 8. Therefore, it is concluded that the amount of unfrozen benzene increased with a decrease of pore size.

Assuming that a dispersion of freezing point of benzene reflects the pore size distribution, the relationship between the pore size and freezing point depression was obtained as follows. The amounts of benzene outside and inside gel beads were determined from GPC charts, i.e., the former corresponds to void volume (V_0) and the latter to pore volume (V_p). The values of V_0/V_p are tabulated in Table I. From this ratio and the relative intensity of unfrozen benzene, it is possible to calculate the unfrozen portion (UP) of benzene inside gel beads according to equation (1),

$$UP=(V_0/V_p+1) \cdot RI \qquad (1)$$

where RI represents the relative intensity of NMR signal obtained from Figure 6-4. This portion (UP) can be converted into the pore size (D) at which confined benzene shows the freezing point depression, ΔT using Figure 6-3. As shown in Figure 6-5, the pore size values were plotted against the freezing point depression of benzene. The plots fell on a single curve for three different types of gel beads. The relationship between the freezing point depression and the pore size can be expressed as follows.[8,14]

$$D= A / \Delta T + B \qquad (2)$$

where A and B are constant. The solid line in Figure 6-5 represents the optimum fitting curve using this relationship, where A is 555 Å/K, and B is 10 Å. The freezing point depression is governed by the pore size when crosslinking density of beads and the swelling ratio are equal.

As a result of DSC experiments for benzene in crosslinked polystyrene beads, Brun et al. proposed 1316 (Å/K) and -7.9 (Å) for A and B values, respectively.[14] As described below, A values increased with the crosslinking density. The larger A value of Brun may be attributed to the high crosslinking density. In the case of B value, however, it is considered that B is physically interpreted as the threshold value of the pore size

below which confined benzene is "inherently" unfrozen. Therefore, it is unreasonable that the B value proposed by Brun is negative.

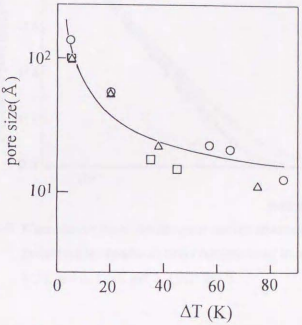

Figure 6-5 Relationship between the freezing point depression and the pore size. (○), gel 6, (△), gel 7, (□), gel 8. Solid line represents the optimum fitting curve (D(Å)=555/ΔT + 10).

III-6-3-3 Effect of crosslinking density

In order to investigate the effect of crosslinking density, the author prepared a series of St-DVB gel beads having different DVB content and with almost the same swelling ratio (0.24-0.26 g/ml). As shown in Figure 6-6, these gel beads showed almost the same cumulative pore size distribution curves.

Figure 6-7 represents the temperature dependencies of the relative intensity of benzene signal. The amount of unfrozen benzene at any temperature increased with the DVB content, although the polymer matrix concentration and pore size were nearly equal in these systems. As shown in Figure 6-8 the relationships between the pore size and the freezing point depression were also obtained for these three gel beads by the method described above. The pore size at any temperature increased with the DVB content. Therefore it is found that crosslinking density is also important for the

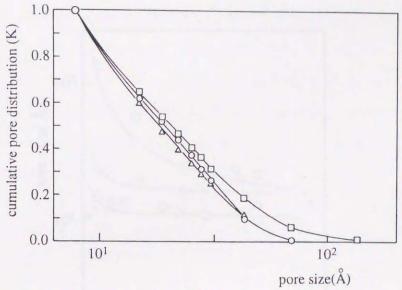

Figure 6-6 Cumulative pore distribution curves obtained from GPC of polystyrene standards and oligostyrenes in benzene. (○), gel 2, (△), gel 5, (□), gel 6.

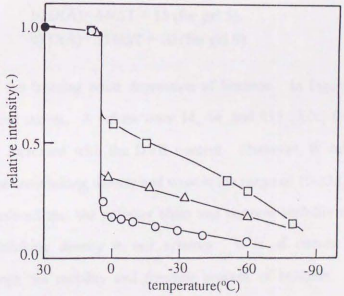

Figure 6-7 Temperature dependencies of the relative intensity of benzene in the presence of St-DVB gel beads. (○); gel 2, (△); gel 5, (□), gel 6.

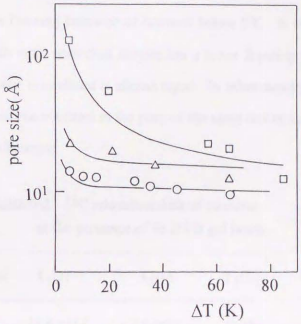

Figure 6-8 Relationships between the freezing point depression and the pore size.
(○); gel 2, (△); gel 5, (□); gel 6.
Solid lines represent the optimum fitting curves;
a) D(Å)= 14/ΔT + 10 (for gel 2),
b) D(Å)= 44/ΔT + 15 (for gel 5),
c) D(Å)= 555/ΔT + 10 (for gel 6).

determination of the freezing point depression of benzene. In Figure 6-8, solid lines represent the fitting curves. A values were 14, 44, and 555 (Å/K) for gel 2, 5 ,and 6, respectively, and increased with the DVB content. However, B values were almost independent of the crosslinking density and were in the range of 10-15 (Å).

It is considered that the polymer chain and benzene mobility decrease with the increase of crosslinking density in our systems. It is of interest to evaluate the correlation between the mobility and freezing process of benzene. The mobility of benzene in the presence of these three types of gel beads was investigated by ^{13}C-NMR relaxation time measurements. Table 6-2 shows the survey of ^{13}C relaxation data of benzene at 25°C. Compared with T_1, T_2 was more dependent upon the DVB content, and the T_2/T_1 value decreased with the increase of the DVB content. This fact indicates that the portion of benzene with low mobility increases as the DVB content, since long

correlation time affects T_2 more effectively than T_1. This result shows a good correlation with the freezing behavior of benzene below 5°C. It is possible to conclude that the portion with more restricted motion has a lower freezing point, even if the size of pore where benzene is confined is almost equal. In other words, the difference of the freezing point of benzene confined in the pore of the same size results from the difference of "stiffness" of confinement.

Table 6-2 ^{13}C relaxation data of benzene in the presence of St-DVB gel beads

gel	T_1(s)	T_2(s)	T_2/T_1
2	11.6±0.1	2.8±0.1	0.24
5	9.2±0.1	1.1±0.1	0.11
6	9.2±0.1	0.40±0.05	0.04

Measurement temperature: 25°C

III-6-3-4 Effect of polymer matrix concentration

In the case of linear polymer solutions, interactions between polymer chain and solvent cause the depression of freezing point, which is dependent on volume ratio of polymer matrix. The effect of polymer matrix concentration on the freezing processes of benzene in the presence of St-DVB gel beads was investigated. Gels 1-4 were prepared using the same amount of DVB and different amount of toluene as a diluent. The resulting gel beads have a different swelling ratio or a different polymer matrix concentration in the range from 0.13 (gel 1) to 0.34 (gel 4) g/ml when they are fully swollen in benzene. The change of pore size is inevitable with the change of polymer matrix concentration when crosslinking density is equal. In fact, the mean pore size of gel beads in a swollen state increased with the decrease of the polymer matrix concentration as shown in Figure 6-9.

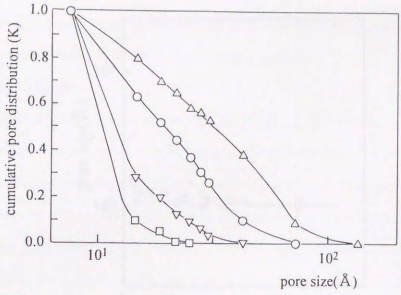

Figure 6-9 Cumulative pore distribution curves obtained from GPC of polystyrene standards and oligostyrenes in benzene. (△), gel 1, (○), gel 2, (▽), gel 3, (□), gel 4

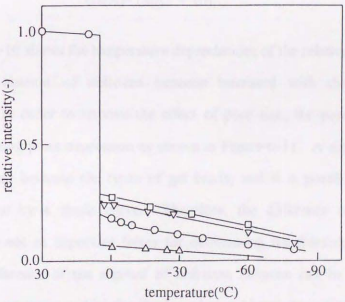

Figure 6-10 Temperature dependencies of the relative intensity of benzene in the presence of St-DVB gel beads. (△), gel 1, (○), gel 2, (▽), gel 3, (□), gel 4

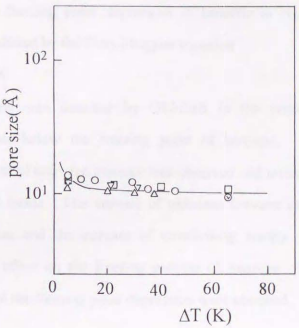

Figure 6-11 Relationships between the freezing point depression and the pore size.
(△), gel 1, (○), gel 2, (▽), gel 3, (□), gel 4
Solid line represents the optimum fitting curve
(D(Å)=14/ΔT + 10).

Figure 6-10 shows the temperature dependencies of the relative intensity of NMR signals. The amount of unfrozen benzene increased with the polymer matrix concentration. In order to remove the effect of pore size, the pore size was plotted against the freezing point depression as shown in Figure 6-11. A significant difference was not observed between the types of gel beads, and it is possible to describe the freezing behavior by a single curve. Therefore, the difference of polymer matrix concentration is not an important factor for determining the freezing point depression. Further, the difference of the amount of unfrozen benzene can be explained by the difference of the pore size and its distribution when gel beads have the same crosslinking density. For linear polystyrene solutions, the freezing point depression can be calculated by the Flory-Huggins equation[16]. If all polymer chains of gel 4 showing the highest polymer matrix concentration contribute to the freezing point depression, the calculated depression value of benzene inside gel beads is 7K. This value is too small to explain our

results. Therefore the freezing point depression of benzene in crosslinked polymer networks can not be predicted by the Flory-Huggins equation.

III-6-4 CONCLUSION

Unfrozen benzene was detected by ^{1}H-NMR in the presence of styrene-divinylbenzene gel beads below the freezing point of benzene. The temperature dependence of the amount of unfrozen benzene was observed and investigated in relation to the properties of gel beads. The amount of unfrozen benzene increased with the decrease of the pore size and the increase of crosslinking density. Polymer matrix concentration had little effect on the freezing process of benzene. The relationships between the pore size and the freezing point depression were obtained.

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Part IV

Application of Polymer Gel Beads to HPLC of Polymeric Compounds

ABSTRACT

The separation of styrene-methacrylate copolymers by chemical composition was studied using high-performance liquid chromatography (HPLC). With the combination of acrylonitrile (polar) gel and non-polar eluent or styrene (non-polar) gel and polar eluent, poly(styrene-*co*-methyl methacrylate) was separated by the adsorption mechanism. The former is designated as normal and the latter as reversed phase. With other combinations, the copolymer was separated mainly by fractional dissolution mechanism. the sample eluted slightly earlier as molecular weight decreased. The molecular weight effect on the reversed phase HPLC was smaller than that on the normal phase. A gel with as exclusion limit of $3x10^3$ exhibited greater molecular weight dependence and worse resolution than a gel with an exclusion limit of $50x10^4$. Poly(styrene-*co*-n-butyl methacrylate) also was separated on the basis of chemical composition by normal and reversed phase HPLC. When octadecyl methacrylate gel was used instead of styrene gel in reversed-phase HPLC, a good separation was not obtained. This indicates a specific interaction between the phenyl group of the styrene gel and the sample.

Poly(methyl methacrylate) (PMMA) was separated by stereoisomerism using HPLC. Using a crosslinked polyacrylonitrile gel as the stationary phase and a mixture of dichloromethane and hexane, ethyl ether, or benzene as the eluent, isotactic PMMA eluted faster than syndiotactic PMMA. With a crosslinked polystyrene gel and a mixture of dichloromethane and nitromethane, syndiotactic PMMA eluted earlier. With some eluent forming stereocomplex of PMMA after injecting a mixture of isotactic and syndiotactic polymers, the sample did not elute or eluted at a different elution time from that expected from an experiment of a separate injection. From the cloud point it was found that the separation was done by an adsorption mechanism rather than phase separation.

Polybutadiene was also separated by isomeric structure. With reversed phase mode using a crosslinked styrene gel as a stationary phase and a mixture of acetonitrile

and dichloromethane as an eluent, the polymer was separated by phase separation mechanism. With a crosslinked acrylamide gel and a mixture of hexane and dichloromethane (normal phase), the samples were eluted in order of 1,2(vinyl), *cis*-1,4, and *trans*-1,4 structure by adsorption mechanism. The resolution by adsorption mechanism was better than one by phase separation mechanism. If acrylonitrile gel was used as stationary phase in the normal phase, samples were adsorbed only weakly to the gel and resolution was not good.

Chapter 7

Separation of Styrene-Methacrylate Copolymers by Composition Using Normal and Reversed-Phase High-Performance Liquid Chromatography

IV-7-1 INTRODUCTION

Analysis of polymeric compounds by high-performance liquid chromatography (HPLC) is predominantly effected by the size exclusion mechanism, in which the interaction between the sample and the packing materials is negligible. If the polymer sample interacts with the packing material, it will not be eluted unless the eluent composition is changed. In 1979, Teramachi et al.[1] separated copolymers of styrene and methyl acrylate according to copolymer composition using the adsorption-desorption mechanism and the solvent-gradient method. Since then, the gradient elution method has been applied to separate several types of copolymers[2-21] depending on the composition. It has been found that the molecular weight effect is negligible when an adsorption mechanism is responsible for the separation. In these experiments, silica and modified silica gels are used for packing materials.

Sato have demonstrated the polymer gel to be a superior stationary phase for separating polymers by the adsorption mechanism, because of the good reproducibility and proportionality between the peak area and the sample amount due to the small amount of irreversible adsorption.[22-25] In this chapter the separation of methacrylate copolymers depending on the composition was studied with normal and reversed-phase HPLC using different types of polymer packing materials. The molecular weight effect of the sample and the effect of pore size of packing materials on the resolution are also discussed.

IV-7-2 EXPERIMENTAL

IV-7-2-1 Samples

Copolymers of methacrylates were prepared using benzoyl peroxide as an initiator in bulk. The conversion was regulated to be less than 10% in order to obtain

samples with a narrow chemical composition distribution. A sample of poly(methyl methacrylate-*co*-styrene) was separated by molecular weight using preparative size exclusion chromatography (SEC). Alternative copolymer of styrene and methyl methacrylate was prepared by ultraviolet (UV) irradiation of a monomer solution in the presence of dichloroethylboron.[26] The composition was determined by ^1H nuclear magnetic resonance (NMR). Average molecular weights, Mn and Mw, were determined

Table 7-1 Characterization data for copolymers

Sample	St content (mol%)	Yield (%)	$M_n/10^4$	$M_w/10^4$	M_w/M_n
St-MMA-1	78	5.0	9.0	13.9	1.5
2	71	6.9	8.7	12.4	1.4
3	60	6.1	8.8	11.9	1.4
4	43	7.6	10.4	13.5	1.3
5	31	7.6	13.7	24.3	1.8
St-MMA-A*	49	—	18.1	24.7	1.4
B*	49	—	7.1	8.1	1.1
C*	49	—	4.8	5.4	1.1
D*	49	—	2.1	2.7	1.3
E*	49	—	1.1	1.3	1.2
St-*alt*-MMA	50	—	5.3	19.8	3.7
St-*n*-BMA-1	77	3.7	9.8	17.6	1.8
2	64	3.8	10.0	19.0	1.9
3	52	4.5	9.2	19.3	2.1
4	33	5.3	15.4	23.1	1.5
5	21	7.3	19.5	27.3	1.4
St-*t*-BMA-1	72	2.6	7.0	14.7	2.1
2	57	3.0	7.5	15.0	2.0
3	46	3.7	7.9	15.8	2.0
4	26	4.6	8.7	17.4	2.0
5	13	4.9	15.7	22.0	1.4

Abbreviation : St;styrene, MMA;methyl methacrylate, *n*-BMA; normal-butyl methacrylate, *t*-BMA;tertially-butyl methacrylate, alt;alternating copolymer
*,SEC fraction

by SEC. The calibration curve for poly(styrene-*co*-methacrylate) was obtained by drawing a curve between those for standard polystyrene and poly(methyl methacrylate) as a position equal to a ratio of methacrylate to styrene content of the copolymer. Table 7-1 gives a survey of the samples used.

Table 7-2 Packing materials used for HPLC

Gel	M/D[a)]	Divinyl monomer	Pressure (kg/cm^2)	Flow rate (ml/min)	Ex L[b)] ($x10^3$)	NTP[c)]
AA	50/50	BA	200	3.5	6000	2000
AN	67/33	EDMA	450	4.5	500	1800
AN-S	67/33	EDMA	87	10.0	3	1200
St	64/36	DVB	300	6.4	500	2000
St-S	64/36	DVB	135	2.4	3	4000
MMA	50/50	EDMA	170	6.0	500	2100
ODMA	50/50	TMPTMA	90	2.0	500	4000

a);Volume ratio of monomer and divinylmonomer
b);Molecular weight at exclusion limit
c);Number of theoretical plates/25cm
Abbreviation: AA;acrylamide, BA;N,N'-methylene-bis(acrylamide)
AN;acrylonitrile, EDMA;ethylene dimethacrylate, DVB;
divinyl benzene,ODMA;octadecyl methacrylate,
TMPTMA;trimethylolpropane trimethacrylate, -S; small pore size

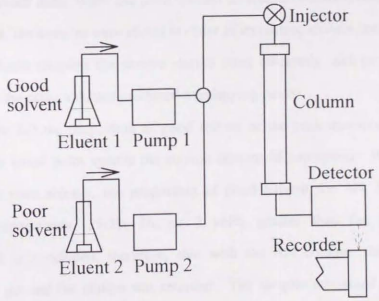
IV-7-2-2 HPLC columns

Cross-linked acrylonitrile (AN) and octadecyl methacrylate (ODM) gels were prepared by a suspension copolymerization of the monomer and ethylene dimetharylate using 2,2'-azobis(2,4-dimethylvaleronitrile) as an initiator and poly(vinyl alcohol) as a suspensifier. Cross-linked styrene (St gel) was prepared by the smilar suspension copolymerization of styrene and divinylbenzene. The resulting copolymers were successively washed with hot water, methanol, N,N-dimethylformamide (DMF), and chloroform. Cross-linked acrylamide gel (AA gel) was prepared by an inverse suspension copolymerization of acrylamide and bisacrylamide using 4,4'-azobis(4-cyanopentanoic acid) as a initiator and a mixture of DMF, water, and poly(ethylene

glycol) as a diluent, and benton 27 and 34 (NL industries, inc.) as disperse reagents. The resulting copolymer was successively washed with hot water, acetone, DMF, and chloroform. The copolymer beads having diameter of 3-10μm were collected by decantation in acetone. Each gel was packed into a 4.6mm i.d. x 25cm stainless steel column by the slurry method. Table 7-2 shows the survey of HPLC columns.

IV-7-2-3 HPLC

HPLC was carried out at room temperature (ca. 25°C) using two Jasco 880-PU pumps, one for the poor solvent and the other for the good solvent. The two solvents were mixed downstream from the pump and were filtered into the injector at a flow rate of 0.5 ml/min, the proportion of good solvent was linearly increased over 25 min. The solvent compositions at the start and end of the gradient are shown in the respective figure captions. A 10 μl portion of a dichloromethane solution of the sample (10mg/ml) was injected through a Rheodyne 7125 injector. The column effluent was monitored with an evaporative mass detector (Applied Chromatography Systems Co. Ltd., United Kingdom, Model 750/14).



Scheme Sovent gradient system

IV-7-2-4 Cloud point

The cloud point was visually determined by adding a poor solvent into a 1.0 mg/ml solution of polymer in dichloromethane at 25°C.

IV-7-3 RESULTS AND DISCUSSION

IV-7-3-1 Separation mechanism for Poly(styrene-*co*-methyl methacrylate)

Mixtures of 3 or 5 poly(styrene-co-methyl methacrylate)s (St-MMA) were separated by HPLC using three columns packed with crosslinked acrylonitrile (AN), methyl methacrylate (MMA), and styrene (St) gels and with a gradient elution to increase the portion of good solvent (dichloromethane). When the non-polar solvent of dichloromethane/hexane was used as the eluent, the samples were eluted from any column with retention increasing with decreasing styrene content (Fig. 7-1a). The elution time for each polymer changed with the type of the column, from smaller elution time St < MMA < AN columns. The peak width became smaller with increasing of elution time. The AN column provided almost a baseline separation, while the other columns gave chromatographs with overlapping peaks.

On the other hand, when the polar solvent of dichloromethane/acetonitrile was used as the eluent, the samples were eluted in order of increasing styrene content (Fig. 7-1b). The St column retarded the sample elution most efficiently, and provided good resolution, while the other columns produced overlapping peaks.

In Figure 7-2 the proportion of good solvent at the peak maximum is plotted together with the cloud point against the styrene content of copolymer. When hexane was used as the poor solvent, the proportion of good solvent for AN, MMA or St columns was, respectively, 25-20, 10, or 2 vol% greater than the cloud point composition. It is concluded, therefore, that with the AN column, the copolymer adsorbed on the gel and the elution was retarded. The samples interacted only weakly with the MMA column. With the St gel there was negligible interaction, and separation was governed chiefly by the fractional dissolution mechanism. It also can be said that the more polar the gel, the stronger adsorption becomes in the normal phase mode.

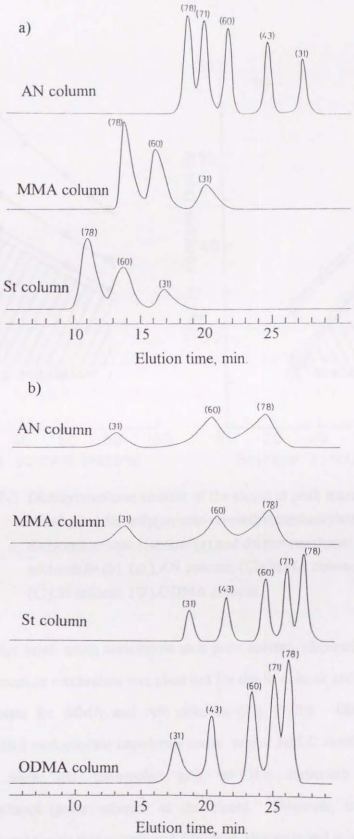


Figure 7-1 Separation of poly(styrene-*co*-methyl methacrylate)s using dichloromethane / hexane (20/80-80/20) (a) and dichloromethane / acetonitrile (0/100-60/40) (b) as an eluent. Values in the parentheses indicate styrene content of the copolymer.

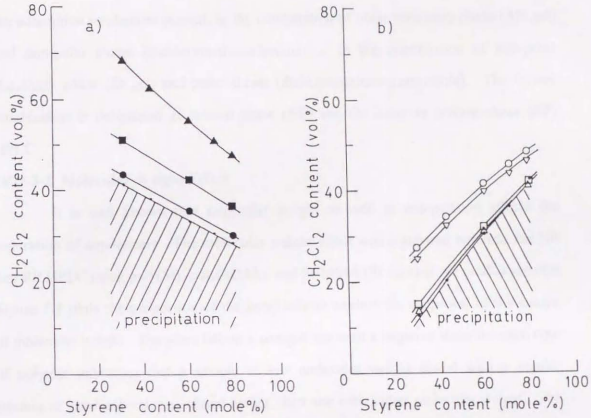


Figure 7-2 Dichloromethane content of the eluent at peak maximum and cloud point for poly(styrene-*co*-methyl methacrylate)s with dichloromethane / hexane (a) and dichloromethane/ acetonitrile (b). (△),AN column, (□),MMA column, (○),St column, (▽),ODMA column

On the other hand, using acetonitrile as a poor solvent, obtained the opposite results, i.e., the adsorption mechanism was observed for the St column and the fractional dissolution mechanism for MMA and AN columns (Fig. 7-2b). Glöckner[19] also separated styrene-ethyl methacrylate copolymer under similar HPLC conditions, namely using octadecyl silica gel (non-polar gel) as the stationary phase and tetrahydrofuran/methanol (polar solvent) as the eluent. However, only negligible adsorption was observed with this system and Glöckner characterized it in the terms of solvophobic retention. Therefore, we concluded that our system using polymer gel differs fro that of Glöckner.

When the polarities of the gel and the eluent are considered, it can be said that

the adsorption mechanism prevails in the combination of polar stationary phase (AN gel) and non-polar eluent (dichloromethane/hexane) or in the combination of non-polar stationary phase (St gel) and polar eluent (dichloromethane/acetonitrile). The former combination is designated as normal phase (NP) and the latter as reverse-phase (RP) HPLC.

IV-7-3-2 Molecular Weight Effect

It is well known that molecular weight as well as composition affects the separation of copolymers. The molecular weight effect was compared between the NP and RP HPLC using poly(St), poly(MMA), and St-MMA (St content: 49 mol%) samples. Figure 7-3 plots the proportion of the good solvent against the reciprocal of the square of molecular weight. The plots fall on a straight line with a negative slope for each type of polymer indicating that a sample of low molecular weight eluted with a smaller amount of good solvent, i.e., eluted earlier, than one with higher molecular weight. The slope for the AN column is 2-5 times steeper than that for the St column. Assuming that the good solvent content difference between poly(St) and poly(MMA) is almost the same for St and AN columns, we concluded that St column (RP) had a smaller molecular weight effect than the AN column (NP) for the separation of St-MMA. We have also reported[24] that NP HPLC (AN gel and hexane/chloroform) has a much smaller molecular weight effect than HPLC with the fractional dissolution mechanism (St gel and hexane/chloroform).

The molecular weight dependences of the two types of HPLC were also compared using an alternating copolymer of St and MMA. The composition difference among the molecules in this polymer is negligible, while the molecular weight difference is large. As shown in Figure 7-4, RP-HPLC showed a narrower peak than NP-HPLC, indicating the smaller molecular weight effect on the former HPLC. It is also noteworthy that the alternating copolymer eluted at almost the same elution volume as a radically prepared random copolymer having the same composition. Considering that the elution volume increased as the styrene sequence length in case of styrene-butadiene

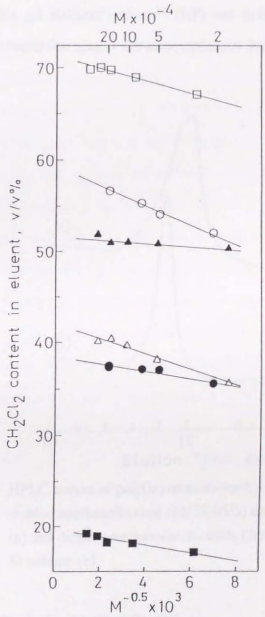


Figure 7-3 Molecular weight effect on dichloromethane content of the eluent for poly(methyl methacrylate)(square), poly(styrene-*co*-methyl methacrylate) (circle), and polystyrene(triangle). Open symbols represent the acrylonitrile column and filled ones the styrene column.

copolymer[23], the same elution volumes of the alternating and random St-MMA may indicate that the radical copolymer has an alternating character, which is expected from the small value of the product of the reactivity ratios ($r_1 x\, r_2$=0.2-0.3).[27]

Glöckner et al. studied the molecular weight effect for styrene-ethyl acrylate copolymer using silica gel stationary phase[16] (NP) and octadecyl silica gel stationary phase (RP)[17]. They found that almost the same molecular weight effects for NP and RP separations.

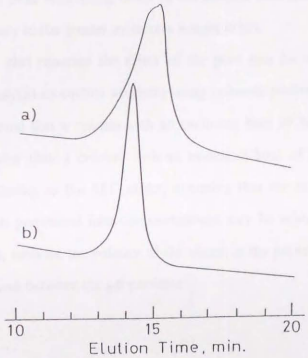


Figure 7-4 HPLC curves of poly(styrene-*alt*-methyl methacrylate) using dichloromethane/hexane (35/65-95/5) eluent and AN column (a) and dichloromethane/acetonitrile (20/80-80/20) eluent and St column (b).

IV-7-3-3 Effect of Exclusion Limit of the Gel

In order to examine the effect of the exclusion limit on the separation, HPLC of St-MMA copolymer was carried out with AN and St columns with different exclusion limits. An AN column having an exclusion limit of $3x10^3$ (AN-S) displayed broader peaks at almost the same elution time, resulting in lower resolution than one having an exclusion limit of $5x10^5$ (AN) for the mixture of copolymers (Fig. 7-5a). The similar effect of the exclusion limit was also observed for St columns, as shown in Figure 7-5b.

The peak width of the sample for columns with an exclusion limit of $3x10^3$ was

almost the same when the sample concentration decreased from 10 to 0.5mg/ml. Therefore, the poor resolution is not due to a smaller surface area of the gel with smaller exclusion limit. However, it is found that the molecular weight effect for the AN column increased by 1.2-1.4 times on decreasing the exclusion limit and that for the St column by 2-3 times. Therefore, the peak broadening effect of the column with smaller exclusion limit can be attributed mainly to the greater molecular weight effect.

Teramachi et al. also reported the effect of the pore size for modified silica gel.[18] They separated poly(St-co-methyl acrylate) using columns packed with cyano-modified silica gel and found that a column with an exclusion limit of $3x10^5$ exhibited peaks several times broader than a column with as exclusion limit of $5x10^4$. They attributed the peak broadening to the SEC effect, assuming that the lower molecular weight components which permeated into the micropores may be adsorbed onto the inner surface of the pores, because the polarity of the eluent in the pores changes more slowly than that of the eluent between the gel particles.

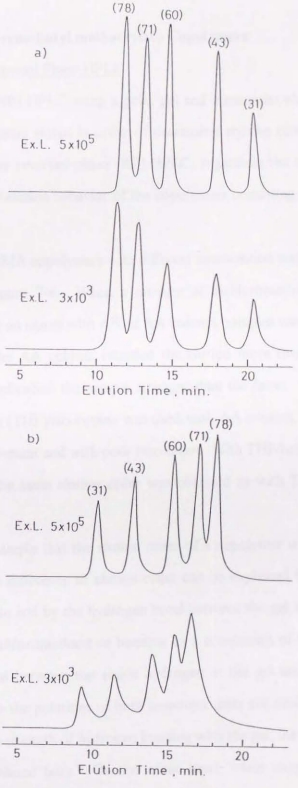


Figure 7-5 Exclusion limit effect on the separation of poly(styrene-*co*-methyl methacrylate)s using dichloromethane/hexane (20/80-80/20) eluent and AN column (a) and dichloromethane / acetonitrile (0/100-60/40) eluent and St column (b). Values in the parentheses indicate styrene content of the copolymer.

IV-7-3-4 Separation of styrene-butyl methacrylate Copolymers

IV-7-3-4-1 Separation by Normal Phase HPLC

By normal phase (NP) HPLC using a polar gel and a nonpolar eluent, styrene-methyl methacrylate copolymers eluted in order of decreasing styrene content, while in the opposite elution order by reversed phase (RP) HPLC, regardless the type of eluent. It is interesting to know the elution behavior of the copolymers consisting of monomers with similar polarities.

A mixture of St-nBMA copolymers with different composition was separated by NP HPLC as shown in Figure 7-6. When a mixture of dichloromethane/hexane or benzene/hexane was used as an eluent with AN or AA column, samples were eluted from higher styrene content. The AA column retarded the elution more than AN column, indicating that the former adsorbed the sample stronger than the latter. On the other hand, when tetrahydrofuran (THF)/iso-octane was used with AA column, samples were eluted from lower styrene content and with poor resolution. With THF/hexane or methyl acetate/iso-octane eluent, the same elution order was obtained as with THF/iso-octane eluent.

This is the first example that the elution order of a copolymer is dependent on the type of an eluent. The difference of elution order can be explained by the polarity difference among the sample and by the hydrogen bond between the gel and the sample or the eluent. By using dichloromethane or benzene as a component of the eluent, the hydrogen bond was formed between the amide hydrogen in the gel and the carbonyl group in the sample. Since the polarities of both monomer units are similar, copolymer was separated based on the strength of hydrogen bonding with the gel, the sample having higher methacrylate unit eluted later. On the other hand, when oxygen containing solvent was used in the eluent, the hydrogen bond was formed between the gel and the eluent interrupting or reducing the hydrogen bond between the gel and the sample. Therefore, the sample is separated by the difference of the polarity of the sample or by the strength of the interaction between the sample and the eluent.

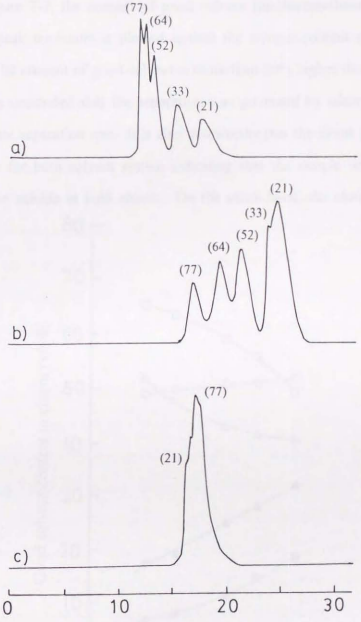


Figure 7-6 Separation of poly(styrene-*co*-n-butyl methacrylate)s using
(a) AN column and dichloromethane/hexane eluent,
(b) AA column and dichloromethane/hexane eluent,
(c) AA column and THF/iso-octane eluent.
The content of dichloromethane or THF increased
from 30 to 80 vol.% in 25minites
Values in the parentheses indicate styrene content of the copolymer.

In Figure 7-7, the content of good solvent (dichloromethane or THF) in the eluent at the peak maximum is plotted against the styrene content together with the cloud point. The content of good solvent is more than 20% higher than the cloud point. Therefore, it is concluded that the separation was governed by adsorption mechanism and not by phase separation one. It is also noteworthy that the cloud point curves have positive slopes for both solvent system indicating that the sample with lower styrene content is more soluble in both eluent. On the other hand, the elution curve for the

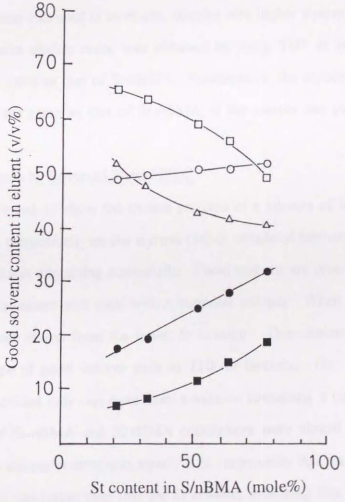


Figure 7-7 Good solvent content of the eluent at peak maximum (open symbol) and cloud point (filled symbol) for poly(styrene-*co*-n-butyl methacrylate)
(open) square: AA column with dichloromethane/hexane eluent,
triangle: AN column with dichloromethane/hexane eluent,
circle: AA column with THF/iso-octane eluent.
(filled) square: dichloromethane/hexane, and circle: THF/iso-octane

eluent containing THF has a positive slope, while the slope of the curve for the dichloromethane eluent was negative. Teramachi et al [29] estimated that the resolution of copolymer would be improved when the slope of elution curve agree with that of cloud point. However, their estimation is not valid in our experiment. It can be concluded that the phase separation, or the solubility does not play signigicant role in the separation using adsorption mechanism.

Figure 7-8 shows the separation of St-tBMA copolymer using AA column. When dichloromethane was used in an eluent, samples with higher styrene content eluted earlier, while opposite elution order was obtained by using THF in an eluent. This elution order is the same as that of St-nBMA. Furthermore, the elution volume of St-tBMA was almost the same as that of St-nBMA, if the sample had the same styrene content.

IV-7-3-4-2 Separation by Reversed Phase HPLC

Figures 7-9 and 10 show the elution patterns of a mixture of S-nBMA and S-tBMA copolymers, respectively, on the styrene (St) or octadecyl methacrylate (ODMA) column using the eluent containing acetonitrile. These systems are reversed-phase (RP) HPLC, since a polar eluent was used with a nonpolar column. When St column was used, the copolymers eluted from the lower St content. This elution order was not changed by the type of good solvent such as THF or benzene. On the other hand, ODMA column provided only one peak from a mixture containing 5 copolymers. The elution volumes of St-nBMA and St-tBMA copolymers were almost equal for both columns, when the styrene content was equal. It is noteworthy that the elution volume for ODMA column was larger than that for St column, indicating that the sample was adsorbed on the ODMA gel stronger than on the St gel.

Glöckner and Müller also found that retention of St-tBMA copolymer increased with styrene content, when THF/iso-octane was used with the phenyl silica column.[21] The elution behavior for the styrene column can be explained by the specific interaction between the phenyl groups of the gel and the copolymer. Thus, the sample was eluted in

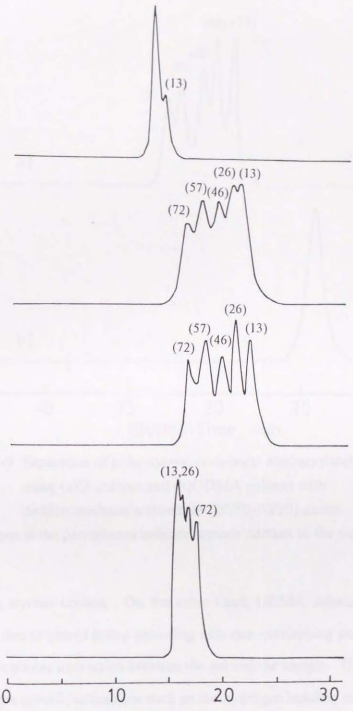


Figure 7-8 Separation of poly(styrene-*co*-t-butyl methacrylate)s using
(a) AN column and dichloromethane / hexane eluent,
(b) AA column and dichloromethane / hexane eluent,
(c) AA column and dichloromethane / hexane,
(d) AA column and THF / iso-octane eluent.
The content of dichloromethane or THF increased
from 30 to 80 vol.% in 25minites
Values in the parentheses indicate styrene content of the copolymer.

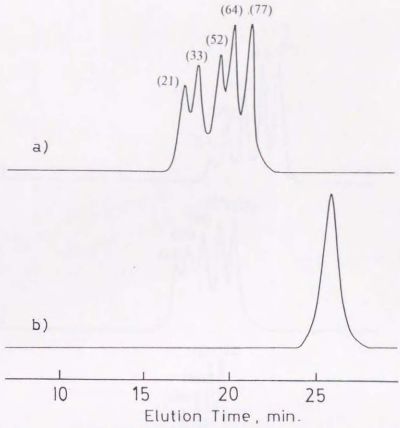


Figure 7-9 Separation of poly(styrene-*co*-n-butyl methacrylate)s using (a)St column and (b)ODMA column with dichloromethane/acetonitrile (20/80-70/30) eluent. Values in the parentheses indicate styrene content of the copolymer.

order of increasing styrene content. On the other hand, ODMA column exhibited no specific interaction due to phenyl group providing only one overlapping peak, although it had a stronger hydrophobic interaction between the gel and the sample. Therefore, it can be concluded that the specific interaction such as the hydrogen bonding in NP HPLC or phenyl-phenyl interaction in RP HPLC is necessary to effectively separate the copolymer consisting of monomers with similar polarity.

The content of good solvent in the eluent at the peak maximum is plotted against the styrene content together with the cloud point in Figure 7-11. The cloud point curve is lower than the good solvent content curve suggesting that the separation for the RP HPLC was also governed by the adsorption mechanism.

The cloud point curve for St-BMA copolymers possessed a positive slope for

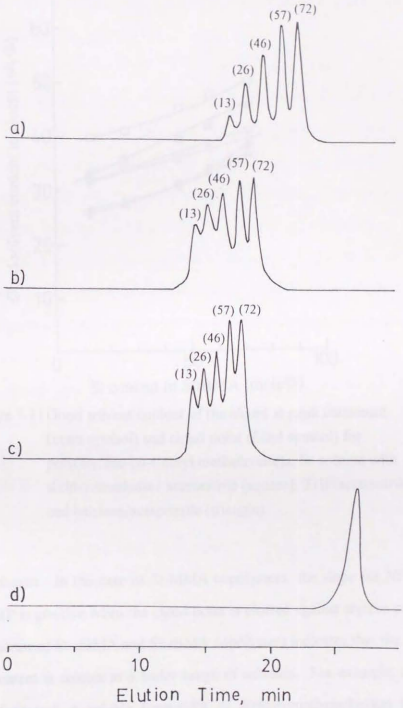


Figure 7-10 Separation of poly(styrene-*co*-t-butyl methacrylate)s using St column (a)dichloromethane/acetonitrile, (b) THF/acetonitrile, and (c) benzene/acetonitrile eluent, and (d) ODMA column and dichloromethane/acetonitrile eluent.

The content of acetonitrile decreased from 80 to 30 vol.% in 25 minites. Values in the parentheses indicate styrene content of the copolymer.

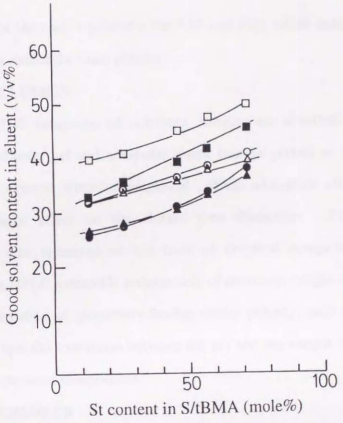


Figure 7-11 Good solvent content of the eluent at peak maximum (open symbol) and cloud point (filled symbol) for poly(styrene-*co*-t-butyl methacrylate)s; St column with dichloromethane / acetonitrile (square), THF/acetonitrile (circle) and benzene/acetonitrile (triangle)

NP and RP eluents. In the case of St-MMA copolymers, the slope for NP is negative and that for RP is positive when the cloud point is plotted against styrene content. The solubility behavior of St-nBMA and St-tBMA copolymers indicates that the sample with a lower St content is soluble in a wider range of solvents. For example, a copolymer with 20% of St unit is soluble from 6/94 of dichloromethane/hexane to 33/67 of dichloromethane/acetonitrile and one with 80% of St unit from 18/82 of dichloromethane /hexane to 46/54 of dichloromethane/acetonitrile. Since the solubility parameter (SP) values of hexane, dichloromethane, and acetonitrile are 7.3, 9.6, and 11.8, respectively[33], the former copolymer is soluble in a soluvent with SP value between 7.4 and 11.8, and the latter between 7.7 and 10.8. The SP values of the center of the nonpolar and polar

cloud points for the two copolymers are 9.30 and 9.25, which indicates that the St and BMA units has almost the same polarity.

IV-7-4 CONCLUSION

In HPLC separation of polymers, samples are adsorbed on the gel when a combination of polar gel and non-polar eluent (normal phase) or of non-polar gel and polar eluent (reversed phase) is employed. When adsorption effect is exercised, the molecular weight effect on the elution time diminishes. Poly(styrene-*co*-methyl methacrylate) was separated on the basis of chemical composition by normal and reversed phase HPLC essentially independent of molecular weight effect. In the case of copolymer consisting of monomers having similar polarity, such as styrene and butyl methacrylate, specific interaction between the gel and the sample was necessary for the separation by chemical composition.

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Chapter 8

Separation of Stereoisomers of Poly(methyl methacrylate) by High Performance Liquid Chromatography

IV-8-1 INTRODUCTION

In chapter 7, poly(styrene-*co*-butyl methacrylate)s can be separated depending on the styrene content by a normal and reversed-phase high performance liquid chromatography (HPLC) using a crosslinked polymer gel as a stationary phase, nevertheless they consist of monomers with the similar polarity. It is found that adsorption mode HPLC can recognize the small difference of properties between the samples.

Vinyl polymers have intermolecular heterogeneity of tacticity as well as molecular weight. Thus, in order to determine the distribution of tacticity of polymers, it is necessary to separate the polymers depending on tacticity. This chapter describes the separation of poly(methyl methacrylate) (PMMA) depending on tacticity using NP and RP-HPLC. The effects of the stereo complex formation are also discussed.

IV-8-2 PMMA samples and HPLC

Isotactic (i) and syndiotactic (s) PMMA samples were prepared using a Grignard reagent as an initiator and atactic (a) PMMA using benzoyl peroxide in solution. Triad tacticity was determined from ^{1}H-NMR spectra using a methyl proton signal around 1ppm. The molecular weight was determined with SEC using standard PMMA as the calibration standard. The polymerization conditions and properties of the samples are listed in Table 8-1. Atactic PMMA samples having different molecular weights were obtained from the General Science Corporation.

Table 8-1 Preparation and properties of PMMA

Sample	Catalyst	Solvent	Temperature (°C)	Triad tacticity[a]			M_n[b] ($x10^3$)
				mm	*mr*	*rr*	
i-PMMA	PhMgBr	toluene	25	94	5	1	43
s-PMMA	*t*-BuMgBr	THF	-78	1	8	91	22
a-PMMA	BPO	toluene	60	2	41	57	19

a),determined by ^{1}H-NMR, b),determined by SEC

Packing materials of the HPLC columns were prepared by suspension polymerization as described in Chapter 7.

HPLC was carried out at room temperature (ca. 20°C) using two Jasco 880-PU pumps, one for providing poor solvent and the other, a good solvent. The two solvents were mixed together after pumping and were delivered to the injector through a filter. The flow rate was set at 0.5ml/min and the proportion of good solvent was increased in 25 min. A 10 µl portion of a dichloromethane solution of the sample (10mg/ml) was injected through a Rheodyne 7125 injector. The column effluent was monitored with a JAC evaporative mass detector.

The cloud point was determined adding a poor solvent into a 1.0mg/ml solution of polymer in a good solvent at 25°C.

IV-8-3 Separation of PMMA depending on tacticity

The elution volume of PMMA was examined using crosslinked acrylonitrile (NP mode) or a crosslinked styrene gel (RP mode) as the stationary phase and with various types of eluents (Table 8-2).

In the case of the acrylonitrile gel, hexane, benzene, or ethyl ether was used as the non-polar poor solvent and the amount of good solvent was increased gradually to elute the polymer. When the i-PMMA and s-PMMA were injected separately, i-PMMA eluted earlier than s-PMMA with all the eluent. If a mixture of i-PMMA and s-PMMA

Table 8-2 Separation of PMMA by tacticity

Stationary phase	Eluent (good solvent/ poor solvent)	Content of good solvent in eluent/%		Elution time/min				Resolution
				Separate injection		Mixed injection		
		Initial	Final	i-PMMA	s-PMMA	i-PMMA	s-PMMA	x[a]
Acrylonitrile								
	CH_2Cl_2/hexane	40	100	17.9	19.6	17.9	19.6	1.5
	$CHCl_3$/hexane	40	100	20.2	23.7	20	23	0.5
	CH_3COOCH_3/hexane	40	100	19.8	22.7	—b)	—b)	2.1c)
	$CH_3COOC_2H_5$/hexane	40	100	26.0	28.4	—b)	—b)	1.9c)
	THF/hexane	40	100	22.3	24.4	—b)	—b)	1.9c)
	$CH_2Cl_2/(C_2H_5)_2O$	40	100	16.4	18.2	16.4	18.2	0.8
	CH_2Cl_2/C_6H_6	20	80	15.0	18.3	14.8	18.3	1.1
Styrene								
	CH_2Cl_2/CH_3NO_2	5	30	16.7	14.3	16.7	14.3	1.1
	CH_3COOCH_3/CH_3NO_2	0	40	40	17.6	16.0	17.6 16.0	0.9c)
	CH_2Cl_2/CH_3CN	0	40	18.0	16.6	18.1	—b) 28	1.0c)

a),Peak at a position other than i-PMMA or s-PMMA.
b)),Peak failed to appear near the elution time of i-PMMA or s-PMMA.
c),Estimated from the experiment of separate injection.

was injected, dichloromethane/hexane eluent displayed two peaks at the same elution time as the separate injection as shown in Figure 8-1. Similarly, by using chloroform/hexane or dichloromethane/benzene as the eluent, the mixture provided two peaks. Therefore, it is concluded that with these eluents, PMMA can be separated depending on the tacticity.

On the other hand, when tetrahydrofuran, methyl acetate, or ethyl acetate was used as a good solvent, no peak was observed around the retention time expected from the experiment of separate injection. This unexpected elution behavior can be attributed to a stereocomplex formation. Challa[1] reported that i-PMMA and s-PMMA make a stereocomplex in tetrahydrofuran. We also found that methyl or ethyl acetate are also stereocomplex forming solvents from precipitation formation by mixing 10mg/ml methyl

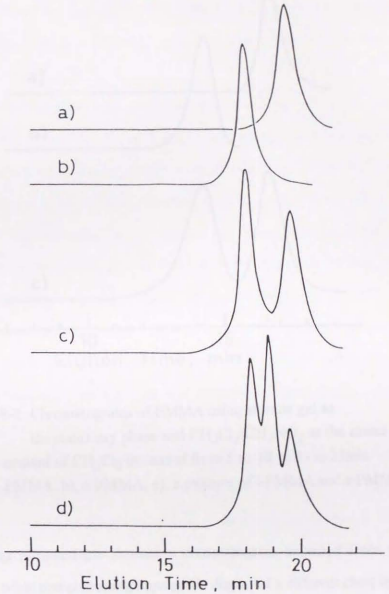

Figure 8-1 Chromatograms of PMMA using acrylonitrile gel as the stationary phase and CH_2Cl_2/hexane as the eluent.
The content of CH_2Cl_2 increased from 40 to 100 vol.% in 25 min.
a);s-PMMA, b);i-PMMA, c) a mixture of i-PMMA and s-PMMA,
d);a mixture of i-PMMA, s-PMMA, and a-PMMA.

or ethyl acetate solutions of i-PMMA and s-PMMA.

When styrene gel was used as the stationary phase, s-PMMA eluted earlier than i-PMMA in the separate injection. This elution order is opposite to that with the acrylonitrile gel (Figure 8-2). When a mixture of i-PMMA and s-PMMA was injected,

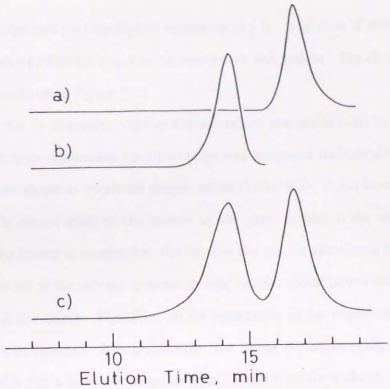

Figure 8-2 Chromatograms of PMMA using styrene gel as the stationary phase and CH_2Cl_2/CH_3NO_2 as the eluent. The content of CH_2Cl_2 increased from 5 to 30 vol% in 25min. a), i-PMMA, b), s-PMMA, c), a mixture of i-PMMA and s-PMMA.

an eluent containing nitromethane showed a chromatogram expected from the two separate injections, while one containing acetonitrile displayed a different chart indicating the stereocomplex formation. It is noteworthy that methyl acetate/nitromethane eluent provided a chromatogram of a simple addition of the two components, although methyl acetate is a complex forming solvent. It was confirmed that with any solvent composition used for eluent, no precipitation was produced by mixing the two polymers. The resolution of i-PMMA and s-PMMA was determined using the elution volumes (EV) and the widths of two peaks (W) using the following equation,

$$R=(EVi-EVs)/[(Wi+Ws)/2]$$

The system using the acrylonitrile gel as the stationary phase and dichloromethane

/hexane as the eluent provided the highest resolution of 1.5. A mixture of three polymers of i-, s-, and atactic(a)-PMMA was also measured with this system. The three polymers were separated as shown in Figure 8-1d.

In order to determine whether the separation was carried out by adsorption or by phase separation mechanism, the cloud point was compared with the content of the good solvent in the eluent at which the sample eluted (Table 8-3). It has been found that the cloud point is almost equal to the content of the good solvent in the case of phase separation and the former is smaller than the latter in the case of adsorption mechanism.[2] It was found that all of the solvent systems showed smaller cloud points than the good solvent content in the eluent. Therefore, all the separations in this experiment occurred by an adsorption mechanism. It is noteworthy that in the NP mode (with acrylonitrile column), s-PMMA has a lower cloud point than i-PMMA, while it eluted later than i-PMMA. In the case of separation of poly(styrene-*co*-methyl methacrylate), the lower the cloud point a sample has, the earlier it elutes.

The molecular weight dependence on the elution time was measured using two atactic PMMA samples having molecular weights of 80000 and 20000. The elution time of the lower molecular weight sample was smaller than that of the higher molecular weight one by 0.8min for NP and 0.3 min for RP conditions. Hence, it can be said that the molecular weight effect is smaller than that of the effect of tacticity, although the molecular weights of samples used in this experiment differed by a factor of 2.

Inagaki et al[3,4] separated i-PMMA and s-PMMA by thin layer chromatography using a silica gel as the stationary phase and with chloroform, ethyl acetate, 2-butanone, or acetone as the mobile phase. Their chromatographic conditions are thought to be the NP mode, because the stationary phase is more polar than the mobile phase. They reported that neither i-PMMA nor s-PMMA developed by chloroform due to adsorption of the polymer on the silica gel, while with the acrylonitrile gel, neither polymers adsorbed onto the gel. This indicates that acrylonitrile has weaker adsorption ability than silica gel. They reported that ethyl acetate developed s-PMMA faster than i-PMMA, and

Table 8-3 Cloud point and content of the good solvent in eluent

Solvent system	Cloud point/%		Good solvent in eluent/%	
	i-PMMA	s-PMMA	i-PMMA	s-PMMA
CH_2Cl_2/hexane	43-47	39-41	66	70
$CHCl_3$/hexane	36-38	31-34	72	80
CH_3COOCH_3/hexane	nd[a)]	nd[a)]	71	91
$CH_3COOC_2H_5$/hexane	nd[a)]	nd[a)]	86	91
THF/hexane	nd[a)]	nd[a)]	77	82
$CH_2Cl_2/(C_2H_5)_2O$	24-27	11-12	63	67
CH_2Cl_2/C_6H_6	——b)	——b)	39	47
CH_2Cl_2/CH_3NO_2	——b)	——b)	15	12
CH_3COOCH_3/CH_3NO_2	——b)	——b)	17	14
CH_2Cl_2/CH_3CN	——b)	——b)	18	15

a),not determined.
b),Soluble at any solvent composition

a-PMMA developed fastest. This elution order is different from our observations under the NP mode, where i-PMMA eluted first and followed a-PMMA and s-PMMA. Further, it is noteworthy that a mixture of i-PMMA and s-PMMA was separated into its component by developing with ethyl acetate, although ethyl acetate is a stereo-complex forming solvent. This may be due to a special interaction between the PMMA sample and the silica gel or a difference of polymer concentration in the mobile phase.

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Chapter 9

Separation of Polybutadiene Depending on Isomeric Structures by High Performance Liquid Chromatography

IV-9-1 INTRODUCTION

Butadiene is polymerized to yield three isomeric units; cis-1,4, trans-1,4, and 1,2 units depending on the polymerization conditions. It is well known that the physical properties of polybutadiene are governed by the amount and the sequence distribution of three isomeric units. It may be also expected that the intermolecular heterogeneity of the distribution of the isomeric units changes the properties of polybutadienes.

This chapter describes the separation of polybutadiene depending on isomeric structure using NP and RP HPLC. This polymer is interesting, because it is less polar than gels normally used for RP column, *e.g.*, polystyrene or poly(octadecyl methacrylate) gels, and also the non-polar eluent used in HPLC is not a poor solvent of it. And also the polarity difference among the samples is very small.

IV-9-2 Polybutadiene samples and HPLC

Polybutadienes were obtained from Japan Synthetic Rubber Co.,Ltd. The characteristics of the samples are listed in Table 9-1. Packing materials for the HPLC columns prepared by suspension or inverse suspension polymerization as described in Chapter 7. The obtained gels were packed into stainless steel column of 4.6mm i.d. and 25 cm in length by a slurry method.

HPLC was carried out at room temperature (*ca.*25°C) using two Jasco 880-PU pumps, for providing a good solvent such as dichloromethane, 1-chloropentane, or carbon tetrachloride and the other, poor solvent such as acetonitrile and hexane. The two solvents were mixed together after pumping and were delivered to a Rheodyne 7125 injector through a filter. The flow rate was set at 0.5ml/min and the proportion of a good solvent was increased linearly in 25min. A 10 μl portion of dichloromethane solution of sample (10mg/ml) was injected. The column effluent was monitored using an

Table 9-1 Characteristics of polybutadiene

Sample	Isomeric units/%			$M_n/10^4$	$M_w/10^4$	M_w/M_n
	cis	*trans*	1,2			
trans	7	89	4	5.6	14	2.5
cis	95	2	3	8.0	29	3.6
V-1	41	51	8	25	28	1.1
V-2	25	30	45	20	22	1.1
V-3	7	8	85	25	27	1.1

evaporative mass detector (Applied Chromatography Systems Co., Model 750/14) at neblizer gas pressure of 1.4kg/cm^2 and at set temperature of 30°C. Each peak of a mixture was assigned from the elution volume of an individual sample injection.

The cloud point was visually determined by adding a poor solvent into a 1.0mg/ml solution of polymer in dichloromethane at 25°C.

IV-9-3 RESULTS AND DISCUSSION

IV-9-3-1 Separation by reversed phase HPLC

Polybutadienes having different isomeric units were separated by RP HPLC using crosslinked styrene or octadecyl methacrylate gel as a stationary phase and a mixture of dichloromethane and acetonitrile as an eluent. As shown in Figure 9-1a, a mixture of *cis*-1,4 and *trans*-1,4polybutadienes showed two broad peaks at ca.16 and 22 min, respectively. Figure 9-1b shows a HPLC curve of polybutadienes having different amount of 1,2 units, which were prepared by butyl lithium catalyst. The sample eluted in the order of decreasing amount of 1,2 unit in the elution range from 18 to 23 min. The peaks of 1,2-polybutadienes were narrower than those of 1,4-polybutadienes, which may be due to the fact that the former has narrower molecular weight distribution than the latter and to the larger molecular weight dependence of elution volume. As mentioned

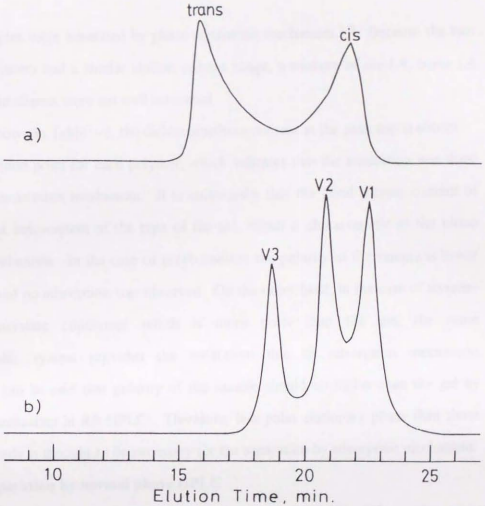

Figure 9-1 HPLC curves of mixtures of polybutadienes using styrene column and dichloromethane/acetonitrile (30:70 to 100:0) eluent.

Table 9-2 Cloud point and CH_2Cl_2 content in eluent

Sample	Cloud point/%	CH_2Cl_2 content in eluent/%	
		St column	ODMA column
trans	69-75	67	69
cis	76-80	75	74
V-1	76-79	75	73
V-2	72-76	71	69
V-3	68-71	67	67

later, the samples were separated by phase separation mechanism.[1-3] Because the two groups of polymers had a similar elution volume range, a mixture of *cis*-1,4, *trans*-1,4, and 1,2-polybutadienes were not well separated.

As shown in Table 9-2, the dichloromethane content at the peak top is almost equal to the cloud point for each polymer, which indicates that the separation was done by the phase separation mechanism. It is noteworthy that the good solvent content of the eluent was independent of the type of the gel, which is characteristic to the phase separation mechanism. In the case of polybutadiene the polarity of the sample is lower than the gel, and no adsorption was observed. On the other hand, in the case of styrene-methyl methacrylate copolymer which is more polar than the gel, the same chromatographic system provides the separation due to adsorption mechanism. Therefore, it can be said that polarity of the sample should be higher than the gel by adsorption mechanism in RP HPLC. Therefore, less polar stationary phase than those used in this study is thought to be necessary for the separation by adsorption mechanism.

IV-9-3-2 Separation by normal phase HPLC

Separation of isomeric polybutadienes was tried by NP mode using dichloromethane/hexane eluent. When polyacrylonitrile (AN) gel was used as a stationary phase, polymers were eluted as the elution range of 12 to 15 min with the elution order of 1,2, *cis*, and *trans* isomers. However, when a mixture of the five polymers was injected, only two peaks were observed; one overlapping peak due to 1,2 and *cis* polymers and the other due to *trans* polymer as shown in Figure 9-2. The poor resolution of the mixture may be attributed to the weak adsorption in this HPLC system. Therefore, it is considered that less polar eluent than hexane is necessary to separate polybutadienes by adsorption mechanism, if AN gel is used as a stationary phase.

It is found that the adsorption became stronger when a more polar gel was used in NP mode.[4,5] Polyacrylamide (AA) gel was found to be more polar than AN gel,[6] since a polymer having a higher solubility parameter (SP) value is generally more polar and SP value of the former is $13 cal^{1/2} cm^{-3/2}$ and that of the latter $11 cal^{1/2} cm^{-3/2}$. AA

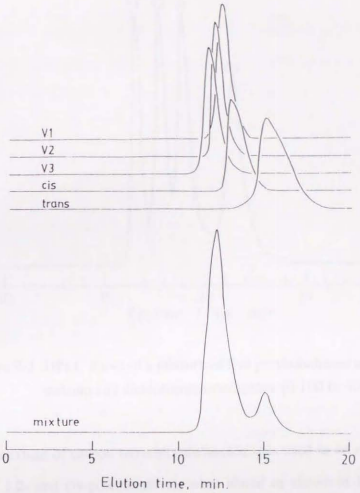

Figure 9-2 HPLC curves of polybutadienes using acrylonitrile column and dichloromethane/hexane (0:100 to 100:0) eluent.

gel was used instead of AN gel with the same eluent. As shown in Figure 9-3, the three types of polymers were separated with the elution order of 1,2, *cis*, and *trans*-polybutadienes. This elution order is same as that of butadiene oligomers separated by NP mode using styrene gel column and 2,2,4-trimethylpentane eluent.[7] The peak width of *cis* and *trans* polymers were narrower compared to RP (Figure 9-1a). The narrower peaks in NP may arise from the smaller molecular weight effect, which is usually observed for the separation by adsorption mechanism. It is clear that the samples were separated by adsorption mechanism, since the samples were soluble in any composition of hexane and dichloromethane.

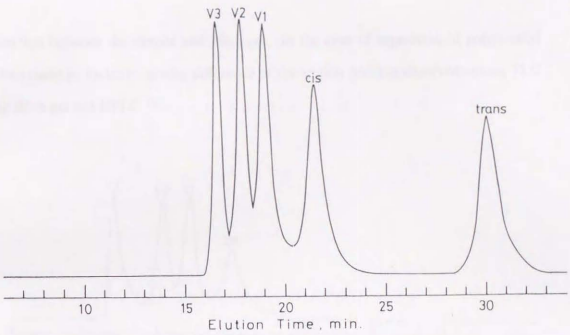

Figure 9-3 HPLC curve of a mixture of five polybutadienes using acrylamide column and dichloromethane/hexane (0:100 to 40:60) eluent.

When a mixture of carbon tetrachloride/hexane was used as an eluent with AA gel column, only 1,2- and *cis*-polybutadienes were eluted as shown in Figure 9-4 and *trans* polymer was not eluted, even pure carbon tetrachloride was used as an eluent. The difference of elution behavior of the two types of eluent is attributed to the fact that carbon tetrachloride has lower SP value (8.6) and weaker desorption ability in NP HPLC than dichloromethane (SP=9.3).[8] By using 1-chloropentane/hexane eluent, the same elution order was obtained as one using dichloromethane/hexane eluent (Figure 9-5). Thus, by using AA column, the elution order did not change by the change of the eluent, although the content of hexane at the peak position differed with the type of solvent.

Inagaki el al.[9] separated isomeric polybutadienes by thin layer chromatography (TLC) using silica gel and various types of eluent. In their experiment the developing order using carbon tetrachloride as eluent is 1,2, *trans*-, and *cis*-polymer, while using 1-chloropentane 1,2- and *cis*-polymers developed to the solvent front and *trans*- polymer remained at the starting point. The difference of HPLC and TLC experiments may be due to the difference of the sample concentration in the mobile phase or the special

interaction between the sample and silica gel. In the case of separation of poly(methyl methacrylate) by tacticity, similar difference of the elution order is observed among TLC using silica gel and HPLC.[10]

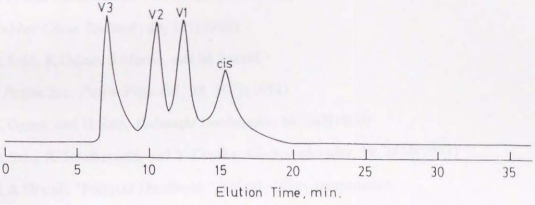

Figure 9-4 HPLC curve of a mixture of five polybutadienes using acrylamide column and carbon tetrachloride/hexane (40:60 to 100:0) eluent. (*trans* polymer did not elute out)

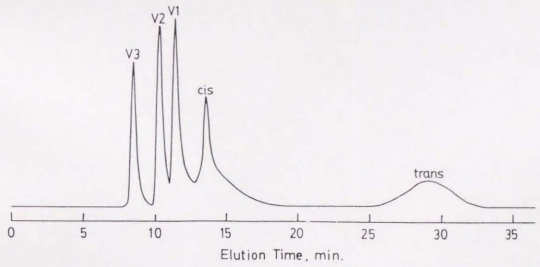

Figure 9-5 HPLC curve of a mixture of five polybutadienes using acrylamide column and 1-chloropentane/hexane (20:80 to 100:0) eluent.

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Part V

Concluding remarks

V-1 General conclusions

This thesis deals with synthesis of new types of polymer gel beads, characterization using nuclear magnetic resonance spectroscopy , and application of polymer gel beads to packing materials for high performance liquid chromatography of polymeric compounds.

The hydrophilic polymer gel beads for packings of aqueous high performance liquid chromatography (HPLC) were synthesized by conventional or inverse suspension polymerization in the presence of a diluent. Gels synthesized by inverse suspension polymerization were more hydrophilic than those synthesized by conventional suspension polymerization. Acrylamide-N,N'-methylene bis(acrylamide) (AA-BA) gel was most hydrophilic among all the gel prepared. Pore size of AA-BA gel could be controlled by changing the polymerization conditions.

A single step swelling and polymerization method provided monodisperse styrene-divinylbenzene (St-DVB) gel and hydrophilic oligo(ethylene glycol) dimethacrylate (OEGMA) gel even if the relative amount of absorbed organic phase was 200 to the seed particles. This is due to the high absorption ability of seed polymer prepared by a dispersion polymerization. In the case of St-DVB gel, the median pore size in a swollen state ranged from 30 to 550 Å, which was dependent on the ratio of absorbed organic phase to seed particles. Packing materials which are suitable for polymer samples with a variety of molecular weight can be synthesized using this method. In the case of OEGMA gel, it is found that gel beads are suitable for the packing materials of aqueous size exclusion chromatography (SEC) columns. Hydrophilicity increased as the number of oxyethylene units increased.

In the presence of St-DVB gel beads, ^{1}H-NMR signal of chloroform was observed as doublet assigned to chloroform inside and outside the gel beads. This signal splitting is due to the pseudo-aromatic solvent effect caused by polymer chains. The relative intensity of inside signal increased with the diluent used in preparation of gel beads, while the chemical shift of the inside signal shifted towards the outside signal. With the decrease of the diameter of beads, two signals overlapped with each other,

which was explained by the increase of the exchanging rate between inside and outside solvents. As the pore size increased, the portion of chloroform in the pore which interacts with the polymer matrix decreased, which resulted in the signal overlapping. As the cross linking density increased, relaxation times of inside and outside signals decreased, with only negligible change in the line shape. ^{13}C-NMR spectra of small molecules such as cyclohexane, tetrahydrofurane, acetonitrile also displayed doublet peaks in the presence of St-DVB gel beads.

The mobility of benzene in the presence of gel beads was dependent on the cross linking density, while it was not parallel to that of polystyrene solution due to heterogeneity. When the DVB content was over 30%, the T_1/T_2 value increased, which indicated that the portion of benzene with low mobility increases with the DVB content. In the case of acetone and DMSO, the T_1 values remained almost constant with the change of the DVB content. The T_1 values of methyl carbons were not so much influenced as other carbons due to the contribution of the spin-rotation interaction.

Unfrozen benzene was detected by ^{1}H-NMR in the presence of St-DVB gel beads below the freezing point of benzene. The temperature dependence of the amount of unfrozen benzene was observed and investigated in relation to the properties of gel beads. The amount of unfrozen benzene increased with the decrease of the pore size and the increase of cross linking density. Polymer matrix concentration had little effect on the freezing process of benzene. The relationships between the pore size and the freezing point depression were obtained.

In HPLC separation of polymers, samples were adsorbed on the gel when a combination of polar gel and non-polar eluent (normal phase) or of non-polar gel and polar eluent (reversed phase) was employed. When adsorption effect was exercised, the molecular weight effect on the elution time diminished. Poly(styrene-co-methyl methacrylate) was separated on the basis of chemical composition by normal and reversed phase HPLC essentially independent of molecular weight effect. In the case of copolymer consisting of monomers having similar polarity, such as styrene and butyl methacrylate, specific interaction between the gel and the sample was necessary for the

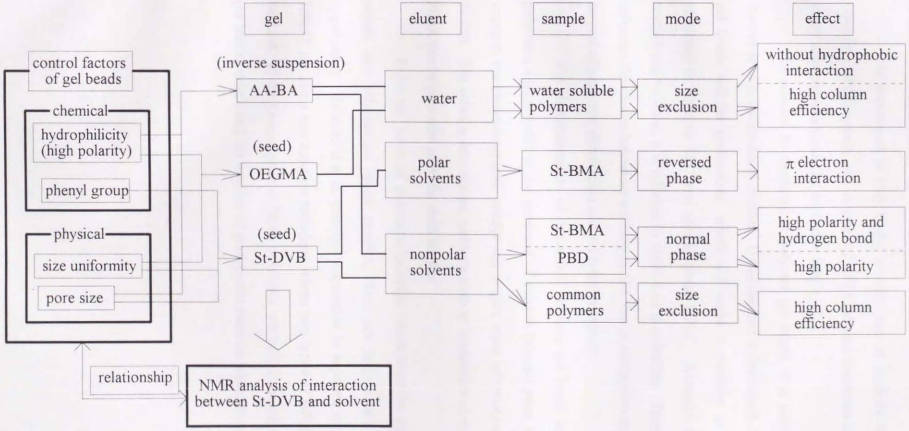
separation by chemical composition.

Poly(methyl methacrylate) (PMMA) was separated by stereoisomerism using HPLC. Using normal phase HPLC, isotactic PMMA eluted faster than syndiotactic PMMA. With reversed phase HPLC, syndiotactic PMMA eluted earlier. With some eluent forming stereocomplex of PMMA after injecting a mixture of isotactic and syndiotactic polymers, the sample did not elute or eluted at a different elution time from that expected from an experiment of a separate injection.

Polybutadiene was separated by isomeric structure using HPLC. With reversed phase mode, the polymer was separated by phase separation mechanism. Using a cross linked acrylamide gel as a stationary phase (normal phase), the samples were eluted in order of 1,2 (vinyl), cis-1,4, and trans-1,4 structure by adsorption mechanism. The resolution by adsorption mechanism was better than one by phase separation mechanism. When a cross linked acrylonitrile gel was used as a stationary phase in the normal phase, samples were adsorbed only weakly to the gel and resolution was not good.

Scheme V-1 shows the overview of this thesis. The author developed the new methods for the preparation of polymer gel beads in order to control the chemical and physical structure of gel beads. The hydrophilic polymer gels synthesized by inverse suspension polymerization can be used as packing materials for aqueous SEC, where unexpected hydrophobic interaction is suppressed, and normal phase HPLC. Synthesis of monodisperse polymer gel beads makes it possible to improve the productivity because time-consuming size fractionation process can be omitted and wasted beads are not produced. Monodisperse packing materials can also improve the column efficiency. The author measured NMR spectra of some solvents in the presence of St-DVB gel beads. NMR parameters such as the intensity and shape of the signals and relaxation times were discussed in relation to the several factors of gel beads such as diameter, swelling ratio, pore size, and crosslinking density, which all affect functions of gel beads. Some information about the interaction between gel beads and solvents were obtained. The method presented here is suitable for the characterization of polymer gel beads in a swollen state. Gel beads were applied to packing materials for HPLC of polymeric

compounds using the solvent-gradient method. The control of the interaction between gel and polymeric compounds made it possible to separate copolymers consisting of monomers with similar polarity, homopolymers with different tacticity and diene polymers with different isomeric structure. The methodology of the separation of polymeric compounds based on the chemical compositions, stereoregularity, and isomeric structure can be utilized for the characterization of the intermolecular heterogeneity of polymers.



Scheme V-1. Overview of this thesis

abbreviation: AA-BA; acrylamide-N,N'-methylene bis(acrylamide) gel
OEGMA; oligo(ethylene glycol) dimethacrylate gel
St-DVB; styrene-divinylbenzene gel
St-BMA; styrene-butyl methacrylate copolymer
PBD; polybutadiene

V-2 Subjects in future

As mentioned in Part I, the ultimate aim of author's study is to create the complete HPLC system and understand HPLC elution behaviors completely. There are many subjects which must be studied in future.

In relation to synthesis of polymer gel beads, it is necessary to develop the inverse seed polymerization method. If this method is developed, monodisperse AA-BA gel beads will be synthesized, which will make it possible to improve the column efficiency in aqueous SEC and normal phase HPLC. Although the average pore size control can be done, it is difficult to control its distribution. Pore size distribution may influence the elution behaviors not only in SEC but in adsorption mode HPLC. Thus, the control of pore size distribution is considered important.

The characterization of interaction between gel beads and samples (including polymeric compounds) in the presence of solvent (eluent) must be started in order to interpret the chromatographic results and to give some information to design the HPLC system. The other spectroscopic methods such as luminescence spectroscopy as well as NMR can be powerful tools for such studies.

From the point of polymeric samples which must be separated by HPLC to obtain new information about samples, there are terpolymers and graft or block copolymers. Because it is in principle impossible to separate terpolymers by a single HPLC, the cross fractionation technique where two types of HPLC are combined must be used. In the case of graft or block polymers, not only chemical composition but graft (or block) length and the number of graft points must be taken into consideration.

List of Publications

a) original paper

1. 親水性ポリマーゲルの合成と評価
   荻野賢司、佐藤寿弥、高分子論文集、**46**, 667-671(1989) (Chapter 1)

2. Separation of Stereoisomers of Poly(methyl methacrylate) by
   High Performance Liquid Chromatography
   Hisaya Sato, Masato Sasaki, and Kenji Ogino,
   Polym.J., **21**, 965-969(1989) (Chapter 8)

3. Separation of Polybutadiene Depending on Isomeric Structures by
   High Performance Liquid Chromatography
   Hisaya Sato, Masato Sasaki, and Kenji Ogino,
   Polym.J., **23**, 23-27(1991) (Chapter 9)

4. Separation of Styrene-Methacrylate Copolymers by Composition Using
   Normal and Reversed-Phase High-Performance Liquid Chromatography
   Hisaya Sato, Kenji Ogino, Susumu Maruo, and Masato Sasaki
   J.Polym.Sci. :Polym. Phys., **29**, 1073-1080(1991) (Chapter 7)

5. 順相及び逆相高速液体クロマトグラフィーによるスチレン－
   メタクリル酸エステル共重合体の組成分離
   荻野賢司、丸尾亨、佐々木雅人、佐藤寿弥
   高分子論文集、**49**, 317-325(1992) (Chapter 7)

6. Separation of Styrene-Butyl Methacrylate Copolymers by
   High Performance Liquid Chromatography
   Kenji Ogino, Susumu Maruo, and Hisaya Sato,
   J.Liq.Chromatogr., **17**, 3025-3041(1994) (Chapter 7)

7. NMR Analysis of interaction Between Styrene-Divinylbenzene Gel Beads and Small Molecules
Kenji Ogino, and Hisaya Sato,
J.Polym.Sci.:Polym.Phys., **33**, 198-195(1995) (Chapter 5)

8. NMR Characterization of Styrene-Divinylbenzene Gel Bead Using Freezing Point Depression of Benzene
Kenji Ogino, and Hisaya Sato,
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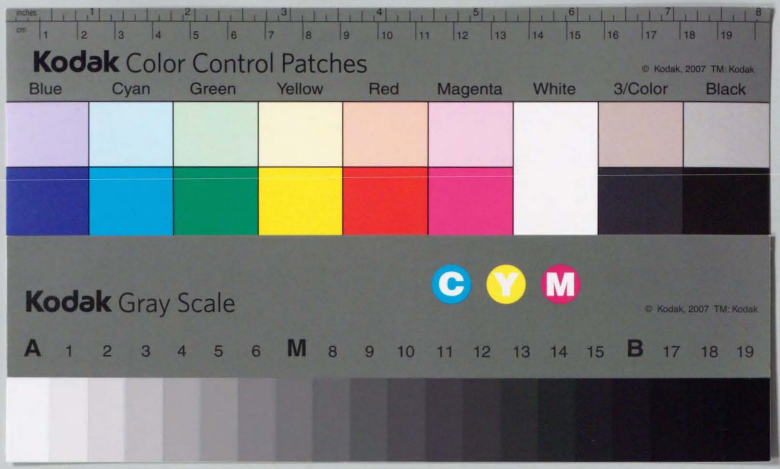
9. NMR Characterization of Styrene-Divinylbenzene Gel Beads Using Chloroform as Probe
Kenji Ogino, and Hisaya Sato, *J.Appl.Polym.Sci.*, submitted (Chapter 4)

10. Synthesis of Monodisperse Macroleticular Styrene-Divinylbenzene Gel Particle and Control of its Pore Size
Kenji Ogino, Hisaya Sato, Kaoru Tsuchiya,
Hiroshi Suzuki, and Soyao Moriguchi, *J.Chromatogr.*, in press (Chapter 2)

11. Preparation and Characterization of Monodisperse Hydrophilic Polymer Beads by Seed Polymerization for Aqueous High Performance Liquid Chromatography
Kenji Ogino, Hisaya Sato, Yuichi Aihara,
Hiroshi Suziki, and Soyao Moriguchi, *J.Chromatogr.*, in press (Chapter 3)

b) review

1. GPC, HPLC, 佐藤寿弥、荻野賢司、高分子、43巻(2月号)(1994)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