

Novel surface-modification techniques for polymer-based separation media Stimulus-responsive phenomena based on double polymeric selectors

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Abstract

A pair of polymeric selectors potentially responding to stimulation was introduced onto monosized porous polymer particles to be evaluated as a packing material for HPLC. Possible complexes formed between polyacrylamide (PAAm) and poly(methacrylic acid) (PMAA) were utilized as stimulus responsive polymeric selectors. Uniformly sized base polymer particle was prepared by multi-step swelling and polymerization method, while the introduction of PAAm and PMAA was done by newly invented modification technique. In this technique, a solvent in which both acrylamide (AAm) and methacrylic acid (MAA) monomers are soluble, but PAAm and PMAA are insoluble, was utilized as a modification medium. The polymer particle doubly modified with PAAm and PMAA was utilized as packing material for HPLC and the stimulus responses were evaluated by changing temperature or pH to check change of the slope of a Van't Hoff plot. By using water as a mobile phase, the expected inflection point of the Van't Hoff plot was observed at upper critical solution temperature (UCST) of the polymer complexes and the temperature responsive ability was observed. Moreover, pH responsive ability was studied by using buffer of either pH 4 or 10 as mobile phase. Slope of the plot was changed in buffer of pH 4, but no change of slope was observed in the buffer of pH 10.

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1. Introduction

In biological systems, many phenomena where the property changes reversibly by responding to external field, such as temperature, light, pH, electricity field and chemical material are observed. Change of external field is perceived in molecular level and expression of property is controlled along the change of molecular structure or formation of molecular assembly. If an artificial material having stimulus-responsive ability seen in biological system can be developed, the material is expected to have a lot of applications with good biological adaptability and highly effective and gentle properties to the environment. In addition, if stimulus responsive material is used as a packing material for HPLC, advanced separation will be achieved.

In fact, poly(*N*-isopropylacrylamide) (PNIPAM) is well-known as stimulus-responsive material [1–5], in water, thermo-reversible conformation transfer (from random coil to globule) is observed with increasing temperature on the boundary of lower critical solution temperature (LCST). Hydrogen bonds between PNIPAM and water or between PNIPAMs are important factor to realize the coil–globule type transfer. At lower temperature than the LCST, hydrogen bonding between PNIPAM and water is preferably formed. PNIPAM is swollen by hydration and its property is hydrophilic. At higher temperature than the LCST, hydrogen bond between PNIPAMs selectively forms than those between PNIPAM and water. PNIPAM shrinks by dehydration and its property is turned to hydrophobic.

Packing material for HPLC modified with PNIPAM as a selector was already prepared [6,7]. This packing material can change surface hydrophilicity or hydrophobicity by changing temperature and hydrophobic interaction between

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stationary phase and solutes can be controlled by temperature change in reversed-phase HPLC (RPLC). Kanazawa et al. separated steroids at higher temperature by using this property, while steroids could not be separated at lower temperature [7]. It is also reported that LCST can be changed by copolymerization of *N*-isopropyl acrylamide (NIPAM) with butyl methacrylate [8].

When we separate biomaterials, such as proteins by RPLC, buffer and/or some organic modifier are often used as a mobile phase. In RPLC, proteins tend to be separated by content ratio of hydrophilic amino acids rather than molecular size. Hydrophobic proteins are eluted on high concentration of organic modifier, therefore, proteins are generally separated by gradient elution. Proteins which are eluted very late or not eluted can be eluted faster through gradient elution system. However, there is a problem that the function of proteins is damaged by using the organic modifier. Here, the selectivity for solutes can be changed without using the organic solvent but by using temperature responsive packing material.

Packing material modified with PNIPAM can change its surface property from hydrophilic to hydrophobic with increment of temperature in RPLC. Such temperature responsive ability can work in RPLC with water as a mobile phase. However, in RPLC, retention of solutes usually decreases with increment of temperature and it is a potential problem of the packing material modified with PNIPAM. Then, the packing material with upper critical solution temperature (UCST) can be expected to work more effectively than packing material with LCST like with PNIPAM.

To separate or to collect efficiently the bioproducts which are unstable on heating, this type of temperature responsive polymer is more useful. In fact, poly(*N*-acetylacrylamide) is developed for such purpose and hydrates by becoming enol structure at higher temperature than UCST. At lower temperature, poly(*N*-acetylacrylamide) becomes keto structure by forming hydrogen bond between amides. This polymer is designed to have UCST by utilizing keto–enol equilibrium.

PAAm and PAA [9–11], or PMAA can form complex which also has UCST by hydrogen bonding between amide groups and carboxylic acid groups in water and this complex controls formation and disentanglement of complex by change of external field. This complex is formed by interaction between polymers at lower temperature and its property is hydrophobic, but complex is disentangled at higher temperature and each polymer is hydrated. Moreover, this complex also responds to the change of pH. At lower pH, this complex can be formed, but the complex is disentangled at higher pH because hydrogen bonding cannot form by ionization of carboxylic acid group.

It is thought that packing material modified with two kinds of polymeric selectors can form such complex to work as a stimulus responsive packing material with UCST. This packing material is expected to work more efficiently with

increasing temperature than the packing material modified with PNIPAM. Moreover, carboxylic acid groups of PMAA as ion exchange groups are screened by forming complex but are exposed by disentangling complex, so influence of ion exchange groups of PMAA for retention of solute can be controlled by temperature change. This property is expected to realize advanced separation based on different level of interaction through ion exchange groups.

In this study, we tried to develop new type of stimulus responsive polymer packing material by introducing two kinds of polymers into monosized polymer particle [12]. Here, we have utilized two modification methods. Add method, where monomer is added to polymerization medium of particles in multi-step swelling and polymerization method was first utilized as a modification method of polymeric selector to polymer particle. By using add method, polymeric selector can be modified easily as polymeric form. In our laboratory, PNIPAM as temperature responsive selector [13] and methacrylamide derivatives as chiral selector [14,15] were modified by this method.

When we modify polymeric selector, there is the problem that introduction rate of polymer selector is limited in water, especially if added polymers are water soluble. Additionally, it is expected to be difficult that the introduction of two kinds of polymers which interact each other and are not copolymerized. We investigated novel modification method of PAAm and PMAA to polymer particles and the prepared particles were used as packing material for HPLC. The response of packing material by change of temperature or pH of mobile phase was evaluated by HPLC using a Van't Hoff plot.

2. Experimental

2.1. Materials

Acrylamide (AAm) (Ultra Pure) purchased from Schwarz/Mann Biotech was used as received. Methacrylic acid (MAA) and toluene purchased from Nacalai Tesque (Mukoh, Japan) were distilled to be used. Ethylene dimethacrylate (EDMA) bought from Wako (Osaka, Japan) was distilled before use. Acetone bought from Nacalai Tesque (Mukoh, Japan) was used as received. [3-(Methacryloxy)propyl]trimethoxysilane (3-MOP) was purchased from Chisso (Chiba, Japan).

2.2. Preparation of monosized polymer particle

Uniform size polystyrene seed particles were prepared by a typical emulsifier-free emulsion polymerization method and purified by a centrifugation method. The size of seed particles was around 1 μm in diameter with excellent size monodispersity.

Preparation of uniform size macroporous polymer particle by a multi-step swelling and polymerization method

was carried out as follows. In the first step, 1.53 ml of aqueous dispersion of the purified polystyrene seed particles (8.7×10^{-3} ml/ml) was admixed with microemulsion of dibutyl phthalate (activating solvent), 0.04 g of sodium dodecylsulfate, and 10 ml of distilled water by sonication. This first step swelling was carried out at room temperature while the solution was stirred at 12.57 rad/sec. Completion of the first step swelling was determined by the vanishing point of oil droplets in added microemulsion using an optical microscope.

A dispersion of 30 ml of toluene (porogenic solvent) 0.30 g of benzoyl peroxide and 0.80 g of sodium dodecylsulfate into 180 ml of water containing 2.88 g of poly(vinyl alcohol) (degree of polymerization, DP = 500; saponification value = 86.5–89 mol%) as dispersion stabilizer was added to the dispersion of swollen seed particles. This second step swelling was carried out at room temperature with stirring at 12.57 rad/sec.

After the second step swelling was completed, the other dispersion of 15 ml of ethylene dimethacrylate, 0.40 g of sodium dodecylsulfate into 90 ml of water containing 1.44 g of poly(vinyl alcohol) was added to the dispersion of the swollen particles. This swelling step was carried out for 6 h at room temperature while the solution was stirred at 12.57 rad/sec. For the polymerization of swollen particles, the aqueous dispersion was stirred at 70 °C for 24 h under argon atmosphere. The polymer particles obtained were washed with water, methanol, and tetrahydrofuran to remove the porogenic solvent and other impurities. This is a preparation procedure for the base polymer particles.

2.3. Modification methods with polymeric selectors

2.3.1. "Add method"

After 4 h from the initiation of polymerization step in multi-step swelling and polymerization method as described in the previous section, the prescribed amount of AAm and potassium persulfate as a water soluble radical initiator was added to the aqueous polymerization medium. After 1 h from the addition of AAm, the prescribed amount of MAA and potassium persulfate was added to the aqueous polymerization medium. The polymerization was continued for 24 h (total) and particles obtained were washed with water, methanol, and tetrahydrofuran.

2.3.2. Newly invented method: "dispersion method"

The unmodified polymer particles prepared using the multi-step swelling and polymerization method (2.0 g) (base polymer particles) were dispersed in 25 ml of acetone and in AAm and benzoyl peroxide [BPO, 1% (w/w) of the monomer] were added and polymerized at refluxing temperature of acetone. One hour after the polymerization was started, MAA and benzoyl peroxide were added and polymerized for an additional 3 h. The obtained particles were washed with acetone and water.

2.4. Preparation of 3-MOP silica

We also prepared silica-based modified particles to study modification efficiency. In this case, silica particles (particle diameter, 5 μ m; surface area, 92 m²/g; pore size, 500 Å, produced by Shiseido) (15 g) dried under reduced pressure at room temperature were added to 200 ml of toluene followed by the addition of 3.2 g of 3-MOP and 1.4 g of 2,6-lutidine. The dispersion was kept under reflux for 6 h. The resulting silica particles (3-MOP silica) were washed with methanol and chloroform and dried under vacuum at room temperature.

2.5. Modification of 3-MOP silica with polymer selector

The 3-MOP silica (2.0 g) was dispersed in the 25 ml of acetone, then AAm and BPO were added and polymerized while refluxing. An hour after the polymerization was started, MAA and benzoyl peroxide were added, and polymerized for an additional 3 h. The obtained particles (modified 3-MOP silica) were washed with acetone and water.

2.6. Column packing method

The prepared particles were packed into a polyether ether ketone (PEEK) column (30 mm \times 4.6 mm i.d.) by slurry techniques to evaluate their characters. We mainly utilized mixture of water, isopropanol, and glycerol as packing medium.

2.7. Chromatographic measurement

HPLC measurement was carried out with a Shimadzu LC-10AD or a Jasco 880-PU HPLC pump, a Shimadzu SPD-10Ai UV detector or a Jasco UV-970 UV detector, a Toyo Soda refractive index (RI) detector RI-8. Peak information was recorded on a Shimadzu C-R6A Chromatopak or a C-R4A Chromatopak. We utilized ²H₂O as a void volume maker of each column.

2.8. Pore size measurement

Pore size and pore size distribution as well as surface area measurement were carried out with a Micromeritics Gemini 2375 along with a Micromeritics FlowPrep 060 using a standard BET calculation.

3. Results and discussion

3.1. Evaluation of packing material modified with only PMAA

Before evaluation of the packing material modified with two kinds of polymers of PAAM and PMAA, a packing material modified with only PMAA by add method was used to be evaluated (Fig. 1). The packing material modified only

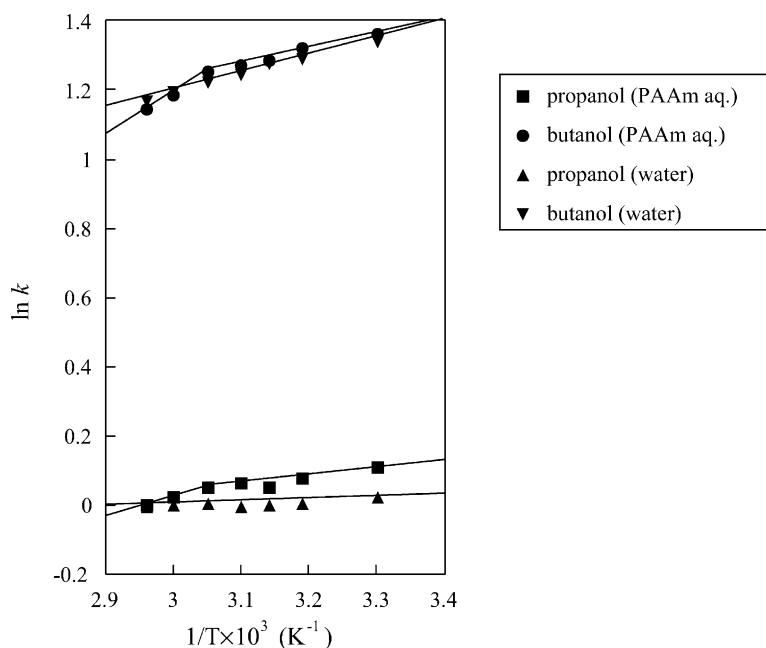


Fig. 1. Evaluation of polymer packing material modified with only PMAA. Chromatographic conditions: mobile phase, water or 5% PAAm aqueous solution; samples, propanol and butanol; flow rate, 1.0 ml/min; detection, RI; column size, 30 mm × 4.6 mm i.d.

with PMAA was evaluated at various temperatures by using water or PAAm aqueous solution as a mobile phase and the influence by temperature change was examined in terms of change of slope of Van't Hoff plot.

When water was used as a mobile phase, the slope of Van't Hoff plot was straight. This means that the property of the stationary phase was not changed by temperature change. On the other hand, when a PAAm aqueous solution was used as a mobile phase instead of water, the slope of the Van't Hoff plot was changed around the expected UCST. This suggests that the characteristics of the stationary phase changed by the temperature change. This change in the Van't Hoff plot was not observed when water was used as a mobile phase, therefore, this temperature responsive phenomenon was due to formation and disentanglement of the complex between PAAm in the mobile phase and PMAA immobilized on the polymer particles. From the property of this complex, PMAA of the stationary phase and PAAm in the mobile phase formed the complex at lower temperature and were disentangled at higher temperature than the UCST.

3.2. Modification of PAAm and PMAA by add method

As described before, response of the packing material modified with PMAA by temperature change was shown in PAAm aqueous solution as a mobile phase, and it is expected that property of polymer complex will work if both the PAAm and PMAA is immobilized onto the stationary phase. Then, PAAm and PMAA were introduced to polymer particles. It is necessary to introduce PAAm and PMAA

into the position where two kinds of introduced polymer selectors can be mutually interactive to afford temperature responsive complexes.

We used to expect that a pair of the polymers can be introduced under the complexed condition if introduction of polymers are carried out at lower temperature than UCST. So, we carried out add method using a redox polymerization at 0 °C. Add method has an advantage that introduction of polymers can be done easily by one step because monomer is just added during the polymerization of the base polymer particles in the multi-step swelling and polymerization method.

3.3. Introduction rate of PAAm on packing material prepared by add method

Table 1 shows the results of elemental analyses of packing materials prepared by add method. The introduction rate of PAAm was calculated based on the content of nitrogen (N) of elemental analyses ($N_{\text{found}}/N_{\text{calculated}}$). As a result, only about 4% of the added amount of PAAm was introduced. This is probably because majority of the glowing PAAm chains tend to be existing in water phase during polymerization process due to the partition characteristics as described later.

The distribution characteristics of AAm, MAA, PAAm and PMAA in two phases system of water and toluene are shown in Table 2. AAm and PAAm exist in water phase, therefore, AAm and PAAm did not react with polymer particles (oil phase). This might result in no complex of the polymers is formed on the polymer particles.

Table 1
The introduction rate of PAAm

Packing material ^a (particle/PAAm/PMAA)	Solvent	C (%; calc.)	H (%; calc.)	N (%; calc.)
1/0.25/0.25	Acetone	57.11 (58.16)	7.14 (7.05)	1.77 (3.29)
1/0.1/0.1	Acetone	58.67 (59.38)	7.17 (7.06)	0.63 (1.64)
1/0.05/0.05	Acetone	58.86 (59.94)	7.08 (7.07)	0.40 (0.90)
1/0.5/0.5	Water	57.97 (56.93)	6.99 (7.05)	0.20 (4.93)

^a Feed ratio (mass ratio).

3.4. Introduction rate of PMAA in packing material prepared by add method

Table 3 shows ion-exchange capacities of packing materials prepared by add method. The amount of the introduced PMAA was also obtained by measuring the ion-exchange capacity of the packing material. The result showed PMAA was introduced by only 20% or less of the added MAA. PMAA showed higher introduction rate than PAAm because MAA slightly dissolves to toluene phase, porogenic solvent as shown in Table 2. However, PMAA cannot dissolve in toluene, so PMAA polymerized in water phase was not introduced to the polymer, and the introduction rate did not increase (only 20% or less). It is indicated that introduction of water soluble polymer is quite difficult in water dispersion polymerization system.

3.5. Evaluation of temperature responsive ability of packing material prepared by add method

The response by the temperature change is tested using a Van't Hoff plot. The result is shown in Fig. 2. The change of slope of the Van't Hoff plot was not observed and packing material did not respond by the temperature change. The amount of the introduction of PAAm and PMAA is too low

Table 2
Partition phenomena of materials

	Water (%)	Toluene (%)
AAm	100	0
MAA	27	73
PAAm	100	0
PMAA	100	0

Table 3
The introduction rate of PMAA

Packing material (particle/PAAm/PMAA)	Solvent	Ion-exchange capacity (m equiv./g)
1/0.25/0.25	Acetone	1.34
1/0.1/0.1	Acetone	0.59
1/0.05/0.05	Acetone	0.29
1/0.5/0.5	Water	0.550

to show the expected temperature response as expected from the introduction rate.

3.6. Modification of PAAm and PMAA in acetone

When packing materials were modified with the polymeric selector by add method, temperature responsive ability was not seen as described in the previous section mainly due to the limited amount of introduced polymers. So, we have to develop new modification technique.

It is thought that PAAm and PMAA can be introduced onto the polymer particles by depositing from polymerization medium during the polymerization process. Then, acetone was used as a polymerization medium. Both the monomers, AAm and MAA, can dissolve in acetone but once they polymerize to become PAAm and PMAA, these polymers are found to deposit from acetone phase.

However, acetone could not be utilized as polymerization medium of multi-step swelling and polymerization method. So, polymer particles were prepared by multi-step swelling and polymerization method, then the polymer particles prepared are re-dispersed in acetone for further modification. Introduction of PAAm and PMAA was achieved with a different timing of the addition of each monomer and copolymerization between AAm and MAA should be minimized by timing each other.

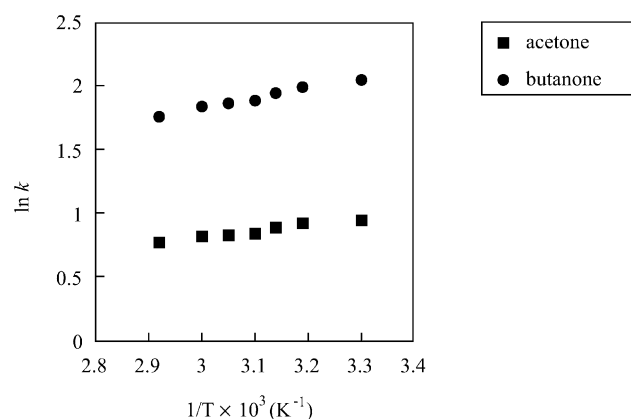


Fig. 2. Evaluation of polymer packing material modified with PAAm and PMAA prepared by add method. Chromatographic conditions: mobile phase, water; samples, ²H₂O as void volume marker, acetone and butanone; flow rate, 1.0 ml/min; detection, UV at 280 or 220 nm; column size, 30 mm × 4.6 mm i.d.

3.7. Introduction rate of PAAm in packing material prepared in acetone

The amount of the introduced PAAm was calculated from the elemental analysis data. Again, Table 1 shows the result. Three kinds of packing materials were prepared using different amount of monomers added. The introduction rate of PAAm was calculated from the ratio of the nitrogen (%), 40–50% of the added PAAm was found to be introduced as expected, and here higher introduction rate was obtained with each packing materials prepared in acetone than packing material prepared by add method previously mentioned.

Then the introduction rate of PAAm could also be increased by using acetone as a reaction medium because PAAm could be introduced into polymer particle by depositing from the polymerization medium. Moreover, almost the same introduction rate among three prepared packing materials was found. These observations suggest that the amount of introduced polymer can be controlled by change of the amount of added monomer.

3.8. Introduction rate of PMAA in packing material prepared in acetone

The amount of PMAA introduced was calculated based on ion exchange capacity of the packing materials prepared. Table 3 shows the result. About 60% of the added monomer was introduced in three packing materials without big differences between the introduction rates. In the case of PMAA, higher introduction rate was also obtained than the add method. Moreover, the amount of the introduction was able to be controlled. By using solvent in which monomer is soluble and polymer is insoluble as polymerization medium, the introduction of polymer selector was done more effectively.

Table 4

Physical properties of polymers

	Total pore volume (cm ³ /g)	Surface area (m ² /g)
Base polymer particle	0.897	534.6
1/0.25/0.25	0.732	438.3
1/0.1/0.1	0.716	405.3
1/0.05/0.05	0.345	107.2

3.9. Measurement of surface area and distribution of pore diameter by the nitrogen adsorption method (BET)

Surface area and total pore volume of the packing material prepared were measured by the nitrogen adsorption method (BET). Surface area was measured by BET method [16] and total pore volume was measured by the BJH method. [17] The results were summarized in Table 4 and Fig. 3. The total pore volume decreases as the amount of the introduction of PAAm and PMAA increased. Moreover, the surface area of the packing material also decreased as the amount of the introduction of polymer selector increased. These findings strongly support that internal surface of the polymer particles was modified with added polymeric selectors.

3.10. Evaluation of packing materials modified with PAAm or PMAA

Fig. 4 shows the result of evaluation of temperature responsive ability of packing materials modified with PAAm or PMAA in acetone. As a result, the change of slope of Van't Hoff plot was not seen for these packing materials. This result suggests that property of stationary phases did not change by the change in temperature and packing materials where only PAAm or PMAA introduction did not respond to the change of temperature.

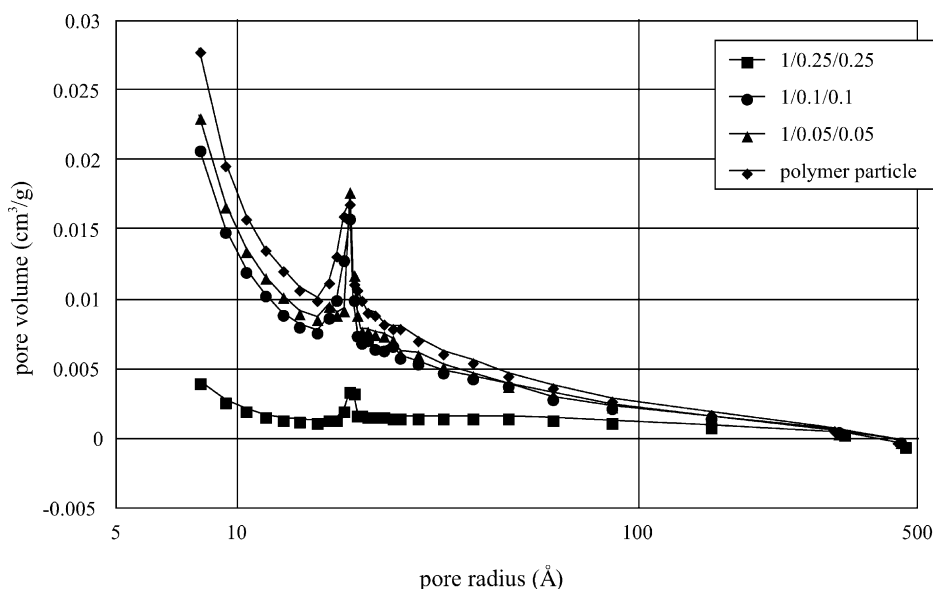


Fig. 3. Distribution of pore diameter measured by nitrogen adsorption method.

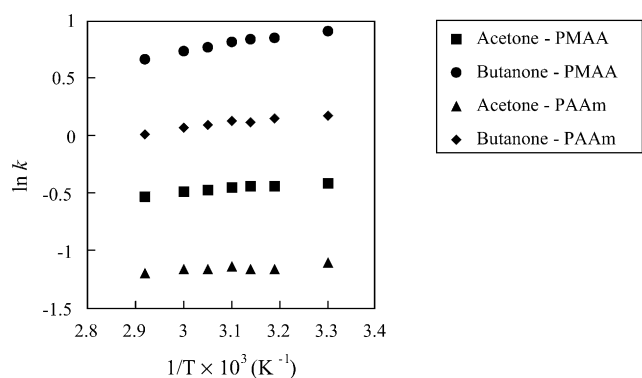


Fig. 4. Evaluation of polymer packing material modified with PAAm or PMAA. Chromatographic conditions: mobile phase, water; samples, $^2\text{H}_2\text{O}$, acetone and butanone; flow rate, 1.0 ml/min; detection, UV at 280 or 220 nm; column size, 30 mm \times 4.6 mm i.d.

3.11. Influence of introduced amount on temperature responsive ability of packing materials modified with PAAm as well as PMAA

Three packing materials which have different amounts of PAAm and PMAA were introduced were evaluated in terms of temperature responsive ability. The result was shown in Fig. 5. The packing material whose rate of polymer particle/PAAm/PMAA was 1/0.25/0.25 afforded relatively clear change of slope on the Van't Hoff plot towards two solutes utilized for evaluation. The changing temperature of slope was almost corresponding to expected UCST for the complex of PAAm and PMAA. Temperature responsive ability of this packing material might be based on property of complex of the polymers.

The other two packing materials that rate of polymer particle/PAAm/PMAA was 1/0.1/0.1 and 1/0.05/0.05 did not afford the change of slope of the plot and property of packing materials were not changed by temperature change. We

thought the reason why these packing materials did not afford the temperature responsive ability is attributed to lack of enough polymeric selectors on the surface. So, it is thought that low introduction density could not form complex effectively to respond to the temperature change.

3.12. Influence of mobile phase on temperature responsive ability of packing material modified with PAAm and PMAA

The packing material which had the highest amount of introduced polymer selectors (1/0.25/0.25) was evaluated by using three kinds of mobile phases and Fig. 6 shows the results. Water, 0.1 M sodium chloride aqueous solution, and 10% acetonitrile aqueous solution were used as mobile phases and the influence by the temperature change was evaluated using Van't Hoff plot. Temperature changed from 30 to 70 °C. When water was used as a mobile phase, a change of the slope of the Van't Hoff plot was observed around the UCST. This is thought that a change of the slope of the Van't Hoff plot was caused by change of property of stationary phase by formation and disentanglement of complexes between PAAm and PMAA on the packing material.

When 0.1 M sodium chloride aqueous solution was used as a mobile phase, a change of the slope of the Van't Hoff plot was not seen. The formation and disentanglement of the complex of PAAm and PMAA were obstructed by addition of sodium chloride to the mobile phase. When 10% acetonitrile aqueous solution was used as a mobile phase, a different slope was shown at lower and higher temperature. However, the clear change of the slope around UCST same as that in water as a mobile phase was not seen. Response based on formation and disentanglement of the complexes around UCST dulled by adding the organic solvent to the mobile phase. Hydrogen bonding between the amide and the carboxylic acid become weak by adding acetonitrile and complex formed unstably in 10% acetonitrile aqueous solution than that in water.

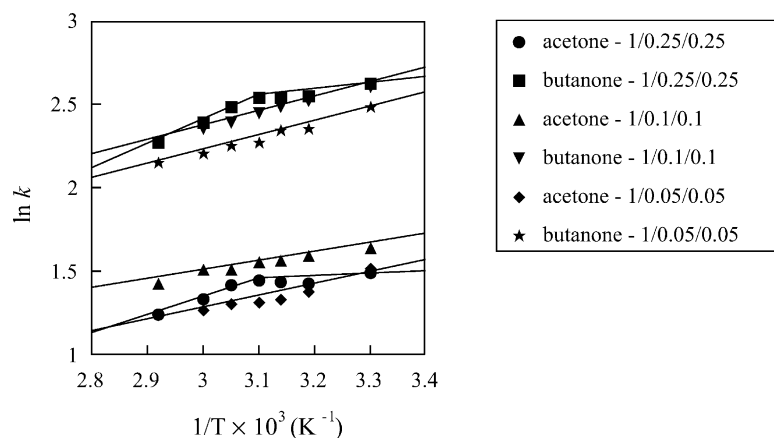


Fig. 5. Evaluation of polymer packing material modified with PAAm and PMAA comparison of different amount of introduction. Chromatographic conditions: mobile phase, water; samples, $^2\text{H}_2\text{O}$, acetone and butanone; flow rate, 1.0 ml/min; detection, UV at 280 or 220 nm; column size, 30 mm \times 4.6 mm i.d.

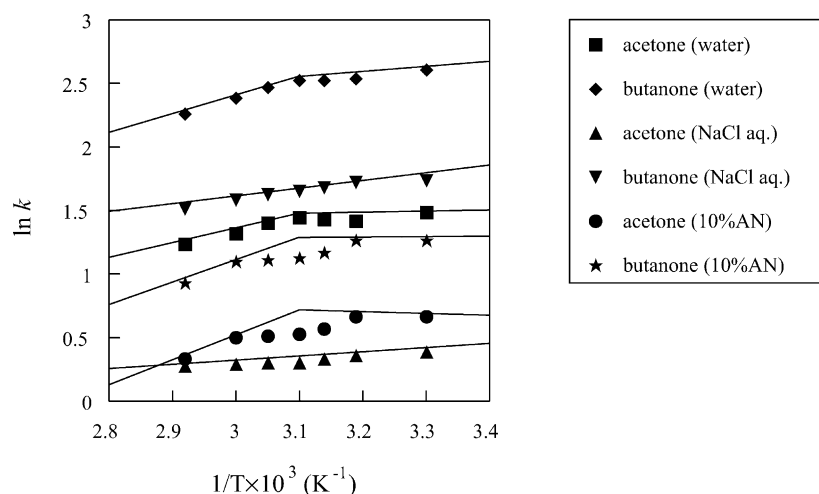


Fig. 6. Evaluation of influence by temperature change in packing material modified with PAAm and PMAA comparison of different mobile phases. Chromatographic conditions: mobile phase, water or 0.1 M sodium chloride aqueous solution ($\text{NaCl}_{(\text{aq.})}$) or 10% acetonitrile aqueous solution (10% AN); samples, $^2\text{H}_2\text{O}$, acetone and butanone; flow rate, 1.0 ml/min; detection, UV at 220 or 280 nm; column size, 30 mm \times 4.6 mm i.d.

3.13. Influence of pH of the mobile phase

Response of the packing material which had the highest amount of introduced polymer selector (1/0.25/0.25) was evaluated through pH change by using buffer of pH 4 or 10 as mobile phase (mobile phase: 0.05 M citric acid; 0.1 M phosphate buffer for pH 4 or 0.1 M carbonate buffer for pH 10) and Fig. 7 shows the results. When pH 4 buffer was used as mobile phase, the complexes based on PAAm and PMAA were able to be formed and the change of slope of the Van't Hoff plot according to the formation and disentanglement of the complex was observed.

However, when pH 10 buffer was used as mobile phase, the hydrogen bonding between polymers was not able to form by dissociation of the carboxyl acid group and the change of the slope of the Van't Hoff plot was not seen. At higher temperature than UCST, polymer complex disentan-

gled and PAAm and PMAA solvated in pH 4 and 10 buffer. At lower temperature than UCST, polymer complex could be formed in pH 4 buffer but could not be formed in pH 10 buffer. Thus, this packing material is found to be responded by the change of pH of mobile phase.

3.14. Evaluation of silica gel modified with PAAm and PMAA

Fig. 8 shows the result of evaluation of silica gel modified with PAAm and PMAA (modified 3-MOP silica). Temperature responsive ability was evaluated by Van't Hoff plot, a change of the slope of plot was not seen and silica packing material did not show response to temperature change. The reason why silica packing material did not show response was that distance between polymer selectors on the silica packing material was shorter than that on the polymer pack-

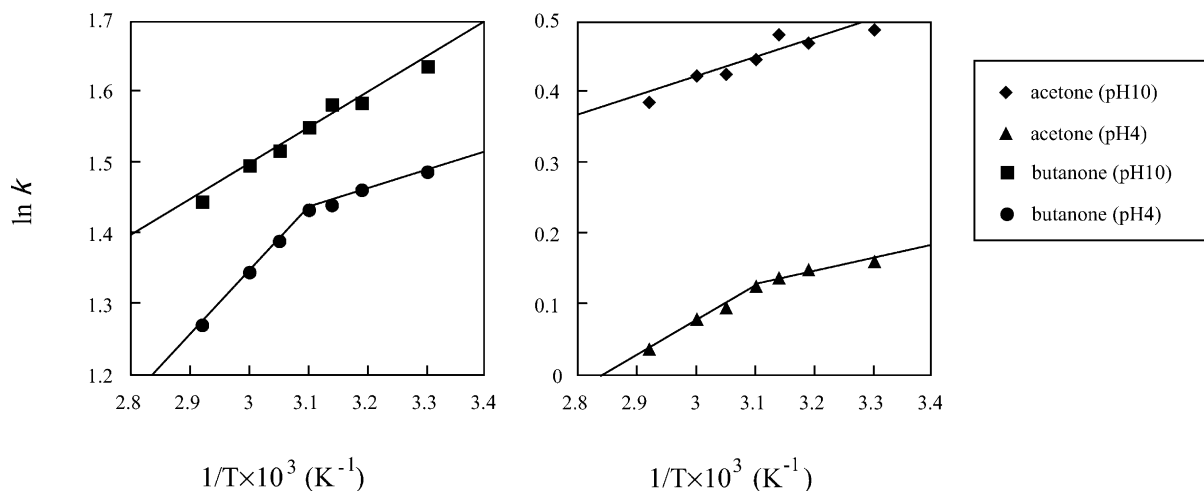


Fig. 7. Evaluation of influence by pH in packing material modified with PAAm and PMAA. Chromatographic conditions: mobile phase, 0.05 M citric acid and 0.1 M phosphate buffer (pH 4) or 0.1 M carbonate buffer (pH 10); samples, acetone and butanone; flow rate, 1.0 ml/min; detection, UV 220 nm or RI; column size, 30 mm \times 4.6 mm i.d.

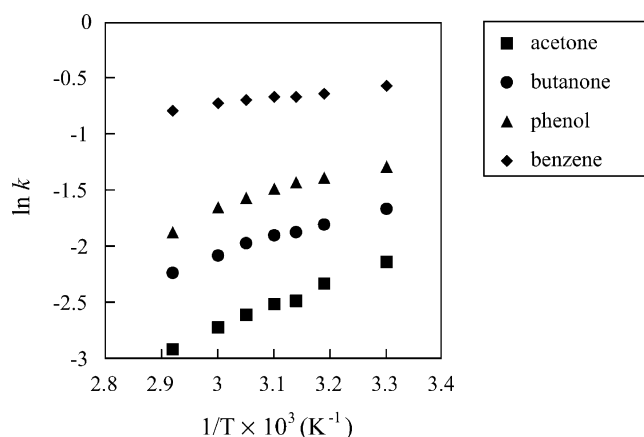


Fig. 8. Evaluation of silica packing material modified with PAAm and PMAA. Chromatographic conditions: mobile phase, water; samples, $^2\text{H}_2\text{O}$, acetone, butanone, phenol and benzene; flow rate, 1.0 ml/min; detection, UV at 280, 220 or 254 nm; column size, 30 mm \times 4.6 mm i.d.

ing material which was responded by temperature change. Then the different amount of introduced PAAm and PMAA in 1.0 g of silica packing material or the polymer packing material which responded by temperature change was obtained. One gram of polymer packing material (1/0.25/0.25) was modified with 0.20 g of polymer selector, silica packing material was modified with 0.30 g of polymer selector. The amount of introduction of polymer selectors was higher on the silica packing material than that on the polymer packing material.

However, surface area of the base silica gel and polymer particle were 92 and 534 m^2/g , respectively. These results indicated introduction density on the silica packing material was higher than that on the polymer packing material. If PAAm and PMAA coexist and form the polymer complex in water with high concentration, many polymer complexes does not disentangle at higher temperature than UCST. On silica gel modified with PAAm and PMAA, PAAm and PMAA introduced with higher density than those on the polymer packing material. It is thought that many polymer complexes did not disentangle on the silica packing material owing to high density of polymer selectors. It is supposed that moderate density of polymer selector is needed to respond by temperature change.

3.15. Evaluation by pyridine as a sample

The polymer packing material with a mass ratio of polymer particle/PAAm/PMAA 1/0.25/0.25 and modified 3-MOP silica packing material were evaluated by pyridine as solute in HPLC. The results are summarized in Table 5. Increment of pressure drop was seen by the injection of pyridine. We investigated only packing material modified with PMAA showed the pressure drop change, thus increment of pressure drop was caused by introduced PMAA. The silica packing material did not respond by temperature change but

Table 5
Pressure drop by the injection of pyridine

	Pressure drop before injection (kg/cm^2)	Pressure drop after injection (kg/cm^2)
3-MOP silica modified with PMAA		
30 °C 0.5 μl	82	91
30 °C 1.0 μl	81	97
30 °C 2.0 μl	83	113
70 °C 0.5 μl	53	62
70 °C 1.0 μl	50	68
70 °C 2.0 μl	54	86
Polymer 1/0.25/0.25		
30 °C 1.0 μl	168	171
70 °C 1.0 μl	114	133

increased pressure drop by pyridine injection at both 30 and 70 °C.

Moreover, when PMAA modified silica packing material was evaluated with differing injection volume of pyridine, increase of pressure drop correlated with the injection volume of pyridine, and degrees of increase of pressure drop were similar between 30 and 70 °C. On the silica packing material, many polymer complexes did not disentangle at higher temperature than UCST and excess PMAA was introduced in comparison with PAAm, silica packing material did not differ so much in increase of pressure drop at 30 and 70 °C. These observations might suggest that the introduced PMAA chains on the silica were interacted with injected pyridine to result in swelling of the polymer chains, which presumably prevents the flow of water as a mobile phase.

Contrary to the silica, polymer packing materials which were responded by temperature change differed in increment of pressure drop at 30 and 70 °C. At 70 °C, the pressure drop was increased. However, very few increment of pressure drop was seen at 30 °C, because PMAA was covered with PAAm by forming complex and could not suffer the influence of pyridine. Thus, degrees of increment of pressure drop at 30 °C differed by forming or disentangling complex on polymer packing material.

4. Conclusion

Introduction of PAAm as hydrophilic polymer by add method was difficult. However, PAAm was introduced more effectively by using polymer particle prepared by multi-step swelling and polymerization method and using acetone as the modification solvent. Polymer selectors were introduced inside the pores was understood by distribution of pore diameters.

Polymer packing material modified with PAAm and PMAA responded to temperature change around UCST of the complex in water as a mobile phase. We prepared packing materials which had different amount of polymer selectors and evaluated these packing material. As a result, it was shown that moderate amount of introduction of

polymer selectors was needed to respond to temperature change.

Polymer packing material which responded to temperature change was evaluated by changing pH of mobile phase. The temperature responsive ability of packing material was seen in pH 4 buffer as a mobile phase but not seen in pH 10 buffer. Thus, this packing material showed different property in each pH of mobile phase.

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