

Preparation and Characterization of Spherical Polymer Packings from *N,N*-Dialkylacrylamide for Reversed-Phase Liquid Chromatography

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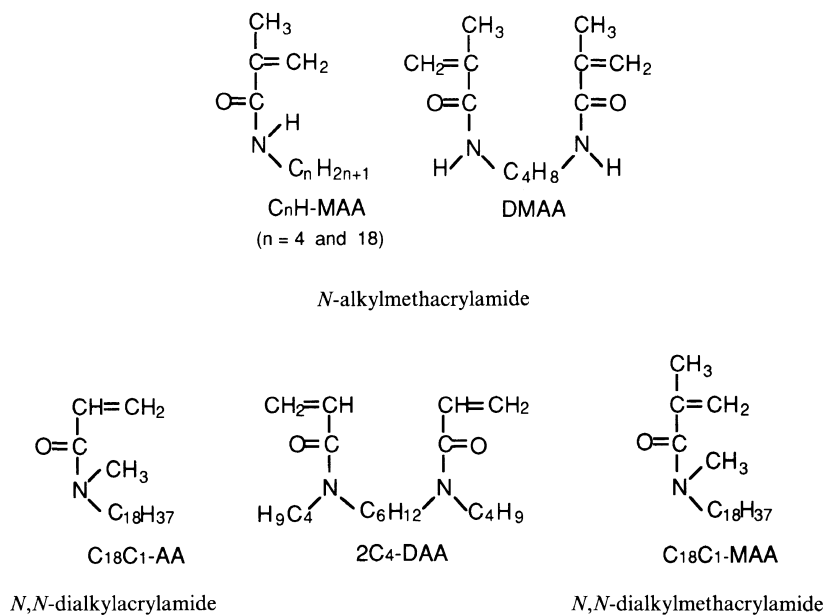
ABSTRACT: Chemically stable, totally organic polymer packings were prepared using newly developed *N,N*-dialkylacrylamides and their chromatographic properties were examined. The polymer packings were composed of *N*-methyl *N*-octadecylacrylamide as a hydrophobic moiety and *N,N'*-diacrylo-*N,N'*-dibutyl-1,6-diaminohexane as a crosslinking monomer. The melting points of both monomers were much lower than those of conventional acrylamide and methacrylamide monomers *e.g.*, *N*-octadecylmethacrylamide, *N,N'*-butylenebis(methacrylamide) and *N,N'*-methylenebis(acrylamide). This property increased miscibility among the monomers and made possible suspension copolymerization without use of a third solvent, which would have lowered the mechanical strength of the polymer obtained. The hydrophobicity of the particles prepared by suspension copolymerization was controlled by the monomer ratio in the polymerization. In the liquid chromatography, the column with polymer particles showed normal reversed-phase liquid chromatographic separations for alkanes, alcohols and aromatic hydrocarbons. In addition, the packings did not show abnormal adsorption for ionic substances because they were ion free, and showed no peak tailing for carbonyl compounds because of their lack of amide protons.

KEY WORDS Acrylamide / Suspension Polymerization / Porous Spherical Particle / Liquid Chromatography /

Totally organic stationary phases should be better than as reversed-phase liquid chromatography (RPLC) packings alkylated silica gels with abnormal adsorption¹⁻⁴ for ionic compounds due to residual silanol groups and metal impurities. However, conventional polymer packings still show abnormal adsorptions for solutes. This undesirable chromatographic behavior is derived from the aromaticity and basic property of the crosslinking monomers used, *e.g.*, divinylbenzene^{5,6} and triallyl isocyanurate.⁷ On the other hand, ethylene glycol dimethacrylate and *N,N'*-methylenebis(acrylamide)⁸⁻¹³ are also useful as crosslinking monomers. However, these monomers show some defects, for example, instability in an alkaline solution and less

solubility in the polymerization process, respectively. Therefore, it is necessary to develop a new crosslinking monomer for RPLC packings. In this study, we newly synthesized *N,N*-dialkylacrylamide derivatives showing some advantageous properties: (1) the monomers show lower melting points compared with corresponding *N*-monoalkyl derivatives. This property increases miscibility among monomers. (2) The monomers have no amide proton which causes undesirable bonding interactions for solutes. (3) The monomers show good alkali-resistance. In addition, the polymer particles obtained from these monomers did not show either abnormal retention or peak tailing for ionic compounds, because they had no ionic groups. In this paper, we

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Scheme 1. Structures of the monomers used.

describe the preparation and characterization of *N,N*-dialkylacrylamides and their spherical polymers as RPLC packings.

EXPERIMENTAL

Synthesis of Monomers

N-Methyl-*N*-octadecylacrylamide ($C_{18}C_1$ -AA). *N*-Methyl-*N*-octadecylamine (10 g, 35.4 mmol) and triethylamine (4.4 g, 43.4 mmol) were dissolved to dry tetrahydrofuran (100 ml). Acryloyl chloride (3.5 g, 38.7 mmol) in dry tetrahydrofuran (100 ml) was added dropwise to the solution, stirring at 0°C. After stirring for 12 h, *N,N*-diethylamino-propylamine (5.1 g, 39.2 mmol) was added to the solution, the precipitates were removed by filtration. The filtrate was washed with 1*N* HCl, 1*N* NaOH and water. After being dried over Na_2SO_4 , the solution was concentrated *in vacuo*. The yield was 77%, 1H NMR δ ($CDCl_3$, ppm): 0.85 (m, 3H, CH_3), 1.25 (m, 30H, $15CH_2$), 1.55 (s, 2H, CH_2), 2.9—3.1 (d, 3H, CH_3), 5.65 (d, 1H, =CH), 6.22—6.64 (m, 2H, = CH_2).

Elemental analysis (%): Found: C=76.7, H=12.5, N=3.9; Calcd. for $C_{22}H_{43}NO$: C=78.3, H=12.7, N=4.2.

N-Methyl *N*-Octadecylmethacrylamide ($C_{18}C_1$ -MAA). $C_{18}C_1$ -MAA was synthesized using *N*-methyl-*N*-octadecylamine and methacryloyl chloride according to a procedure similar to that for the synthesis of $C_{18}C_1$ -AA. The yield was 70%. 1H NMR ($CDCl_3$, δ ppm): 0.85 (m, 3H, CH_3), 1.25 (m, 30H, $15CH_2$), 1.55 (s, 2H, CH_2), 1.95 (s, 3H, CH_3), 2.90—2.95 (d, 3H, CH_3), 3.30—3.40 (d, 2H, CH_2), 5.00—5.20 (m, 2H, = CH_2). *Elemental analysis* (%): Found: C=78.6, H=12.8, N=4.0; Calcd. for $C_{23}H_{45}NO$: C=78.0, H=12.8, N=4.0.

N-Octadecylmethacrylamide ($C_{18}H$ -MAA) and *N*-Butylmethacrylamide (C_4H -MAA). $C_{18}H$ -MAA and C_4H -MAA were obtained from Kojin Co., Ltd. and used after recrystallization from methanol.

N,N'-Diacryloyl *N,N'*-dibutyl 1,6-diaminohexane ($2C_4$ -DAA), *N,N'*-Diacryloyl *N,N'*-dibutyl 1,6-diaminohexane was prepared by reaction

between 1-aminobutane and 1,6-dibromohexane. 2C₄-DAA was synthesized using *N,N'*-dibutyl-1,6-diaminohexane and acryloyl chloride according to a procedure similar to that for the synthesis of C₁₈C₁-AA. The yield was 32.0% ¹H NMR (CDCl₃, δ ppm): 0.85 (m, 3H, 2CH₃), 1.25–1.40 (m, 8H, 4CH₂), 1.40–1.60 (8H, m, 4CH₂), 5.65 (d, 2H, =CH), 6.3–6.6 (m, 4H, 2=CH). *Elemental analysis* (%): Found: C = 70.5, H = 10.8, N = 8.1; Calcd. for C₂₂H₄₀O₂: C = 71.4, H = 10.7, N = 8.3.

N,N'-Butylenebis(methacrylamide) (DMAA). *N,N'*-Butylenebis(methacrylamide) (DMAA) was obtained from Kojin Co., Ltd. and used after recrystallization from methanol.

Preparation of Polymer Particles

C₁₈C₁-AA, 2C₄-DAA, diethylbenzene, and 2,2'-azobis(isobutyronitrile) (AIBN) were mixed at room temperature. The mixture was added to an aqueous solution containing 2 wt% poly(vinyl alcohol) (GH-20, Gohsenol) and suspended by stirring. The suspension was heated at 80°C for 24 h under N₂ gas atmosphere. The copolymer particles obtained were washed successively with water, hot water, methanol and acetone. Similarly, spherical polymer particles were obtained using *N*-octadecylmethacrylamide (C₁₈H-MAA), *N*-butylmethacrylamide (C₄H-MAA), and ethylene glycol dimethacrylate (EGDM, Nippon Oil & Fats Co., Ltd.). Particles with diameters of 5–15 μm were obtained by adjusting the stirring speed.

Chromatography

A slurry of 2.5 g of the beads in 25 ml of methanol was packed into a stainless steel column (150 × 4.6 mm i.d.). As a reference column, ODS (Unisil Pack QC18 (250 × 4.6 mm i.d.), 12500 theoretical plate numbers and Inertsil (150 × 4.6 mm i.d.)) were purchased from GL Science Co., Ltd. Asahipak ODP and C₄P as alkyl bonded polymer packings were also purchased. The chromatograph included a JASCO 880-PU pump, an UV-VIS

Shimadzu photodiode array SPD-M6A and a Shodex refracto monitor SE-51. A 5 μl portion of the sample dissolved in an eluent (1.0 mg ml⁻¹) was injected through a Rheodine Model 7125 injector. All chromatography was carried out at 25°C.

Other Measurements

Differential scanning calorimetry (DSC) of the monomers prepared was carried out using a heating rate of 5°C min⁻¹ with a Seiko I & E SSC-580 with a DSC-10 instrument. The polymerization of monomers was followed by ¹H NMR spectroscopy using a JEOL GX-400. Surface area analysis of the particles was carried out by Brunau-Emmet-Teller (BET) method using a Microtac Model 4200. Scanning electron micrographs of the particles were obtained using a JEOL JSM-840, and IR spectroscopies of the particles were carried out using a Perkin-Elmer 1640 Spectroscopy.

RESULTS AND DISCUSSION

Characterization of Monomers

Table I shows the melting points of monomers (peak-top temperature measured by DSC). *N,N*-Dialkylated acryloyl and methacryloyl monomers showed much lower melting points than those of corresponding *N*-monoalkylated monomers. This is favorable for making spherical particles, because a higher melting point often lowers solubility and miscibility among monomers in suspension copolymerization. When an additional solvent is used in the polymerization process to dissolve monomers, the more the amount used, the softer are the particles produced.

The polymerization properties of the monomers were examined by solution polymerization using AIBN. Monomers were dissolved in diethylbenzene, and heated at 80°C. The polymerization procedure was followed by ¹H NMR spectroscopy. C₁₈H-MAA and C₁₈C₁-AA showed rapid decrease of CH₂=CH protons with increase of CH₂-CH

Table I. Preparation of polymer particles

Packings	Type	Molar ratio				
		C ₁₈ C ₁ -AA	2C ₄ -DAA	C ₁₈ H-MAA	C ₄ H-MAA	EGDM
DAA-1	<i>N,N</i> -Dialkyl	0	100	0	0	0
DAA-2	<i>N,N</i> -Dialkyl	30	70	0	0	0
DAA-3	<i>N,N</i> -Dialkyl	40	60	0	0	0
DAA-4	<i>N,N</i> -Dialkyl	50	50	0	0	0
AMA-1	<i>N</i> -Alkyl	0	0	30	20	50

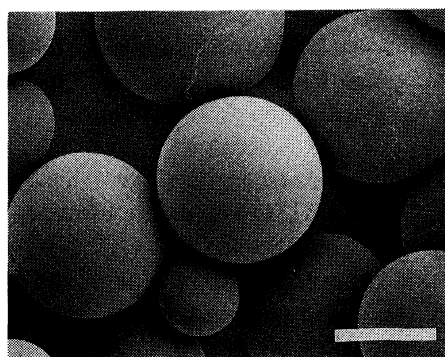
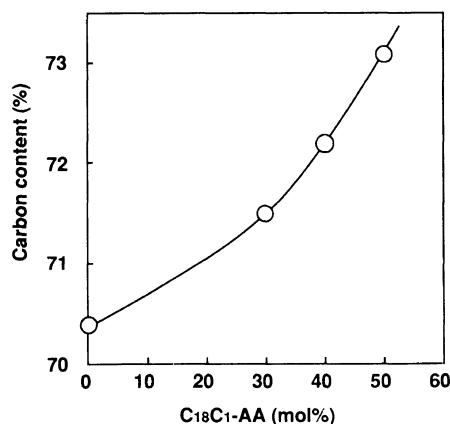
Table II. Melting points of monomers

Type	Monomer	mp/°C
<i>N,N</i> -Dialkyl	2C ₄ -DAA	< -100
	C ₁₈ C ₁ -MAA	29—30
	C ₁₈ C ₁ -AA	28—30
<i>N</i> -Alkyl	DMAA	128—129
	C ₁₈ H-MAA	66—67

protons. C₁₈C₁-MAA showed no decrease of CH₂=C(CH₃) protons, and the polymer was not obtained. *N,N*-Dialkylated methacryloyl monomers are much crowded around CH₂=C(CH₃) according to their CPK models. Therefore, acryl derivatives were examined further in this study.

Preparation of Polymer Particles

Polymer particles, abbreviated in Table II, were prepared by suspension polymerization under the conditions specified. Figure 1 shows a typical electron micrograph of polymer particles prepared from DAA-1 with diameters of 5–15 μm. The particles are perfectly spherical. The IR spectra showed absorptions due to ν_{CH₂} and ν_{C=O} at 2920 cm⁻¹ and 1638 cm⁻¹, respectively. Figure 2 shows the relationship between the molar ratio of C₁₈C₁-AA in the suspension and the carbon content of the polymer particles obtained. The carbon content increased with the molar ratio of C₁₈C₁-AA. This indicates that the hydrophobicity of the packings is easily controllable by the molar ratio in suspension polymeriza-

**Figure 1.** Scanning electron micrographs of particles (DAA-1). A scale bar presents 10 μm.**Figure 2.** Relationship between molar ratios of C₁₈C₁-AA monomer and carbon contents of particles.

tion, as the hydrophobicity of packings is directly related to the retention ability in the liquid chromatography process.

The pore size of *N,N*-dialkyl type polymer

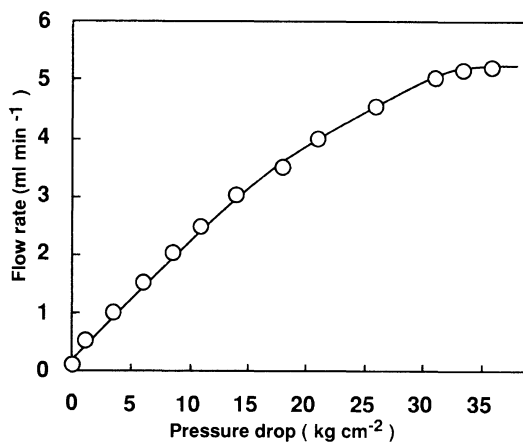


Figure 3. Relationship between flow rate and pressure drop in acetonitrile-water (60:40).

particles was estimated by the size exclusion chromatography method in tetrahydrofuran. The porosity and exclusion molecular weight of the DAA-1 and DAA-4 particles are 38% and 54% and 1400 and 7800 (for polystyrene), respectively. The surface area of the particles were determined by BET method: 0.43, 0.21, 0.46, and 0.28 (m²g⁻¹) in the DAA-1, DAA-2, DAA-3, and DAA-4, respectively.

Physical Stability of Particles

Figure 3 shows flow rate resistance in the liquid chromatography process estimated in terms of flow rate and pressure drop. The DAA-1 columns showed good linear correlation up to about 3 ml min⁻¹ at the flow rate (i.d. 4.6 mm, eluent: acetonitrile-water (60:40)). This value is satisfactory in HPLC. This good high-flow rate resistance is related to the relatively small swelling degree of the DAA-1 particles. Figure 4 shows the specific swelling degree (*S_d*) of DAA-1 particles and Asahipak ODP (most widely used as a totally organic RPLC packing) in benzene, tetrahydrofuran, acetonitrile and methanol. A smaller change of *S_d* value was observed in the DAA-1 particles.

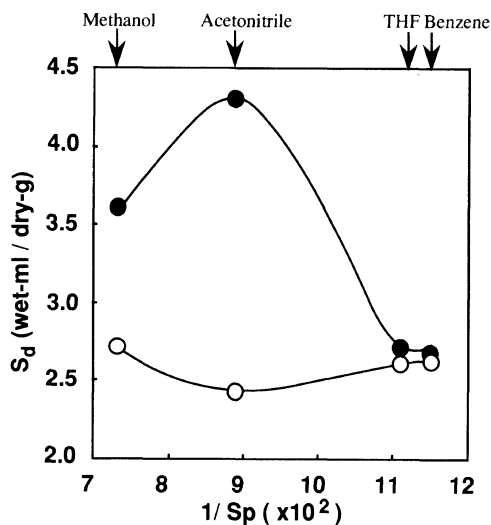


Figure 4. Specific swelling degree (*S_d*) of DAA-1 (—○—) and Asahipak ODP (—●—) particles.

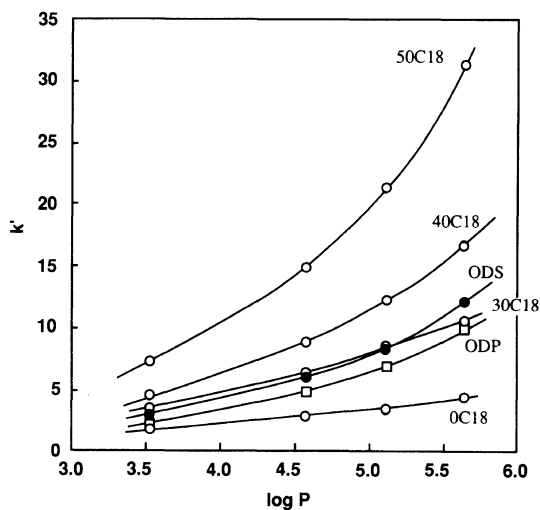


Figure 5. Relationship between capacity factor and log *P* for alkanes.

Chromatographic Property

Retention Capacity (k') of *N,N*-Dialkylacrylamide Polymer Particles. *k'* values were calculated as $k' = (V_r - V_0)/V_0$, where *V_r* is retention volume of the analyte and *V₀* is average of acetonitrile and glycerine. As shown in Figure 5, a close correlation between the *k'* value and hydrophobic parameter

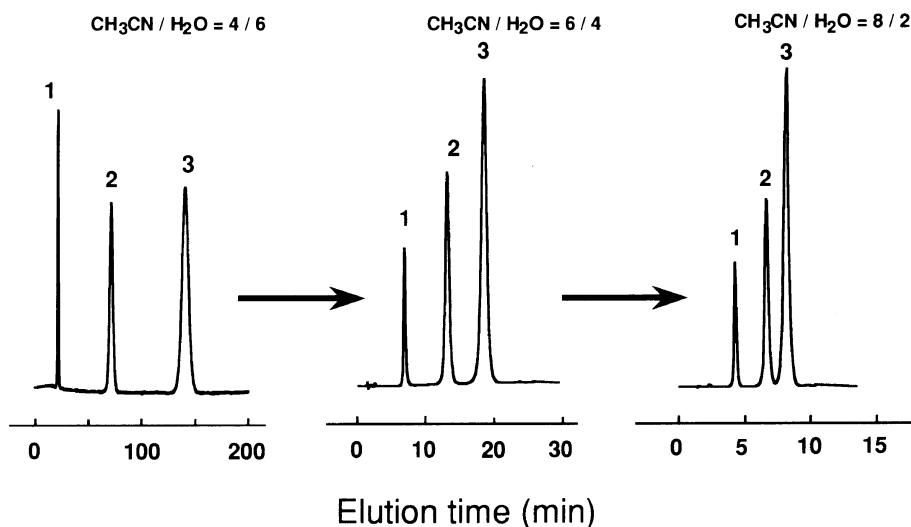


Figure 6. Chromatograms for aromatic compounds in acetonitrile-water: 1 = benzene; 2 = naphthalene; 3 = fluorene.

($\log P$), calculated according to the method of Rekker,¹⁴ was observed for synthetic packings as well as for commercial packings, octadecylated silica gels (ODS) and Asahipak ODP. k' value also increased with increasing molar ratio of $C_{18}C_1$ -AA in the suspension. This shows that the hydrophobicity of the packings is directly related to $C_{18}C_1$ -AA content and the retention ability is easily controllable by adjusting the monomer ratio of the suspension.

Figure 6 shows chromatograms for aromatic compounds in acetonitrile-water. It is evident that increasing the organic content of the mobile phase reduces retention: analysis time is lowered by raising the proportion of acetonitrile in the eluent. In addition, no significant change in peak shape or column efficiency was observed, regardless of change in mobile phase composition. This is related to the small change in swelling degree in various solvents.

RPLC of Carbonyl and Aromatic Compounds

Figure 7 shows examples of the relationship between k' measured in acetonitrile-water and $\log P$ of aromatic compounds. Higher retention strength was observed for the N,N -

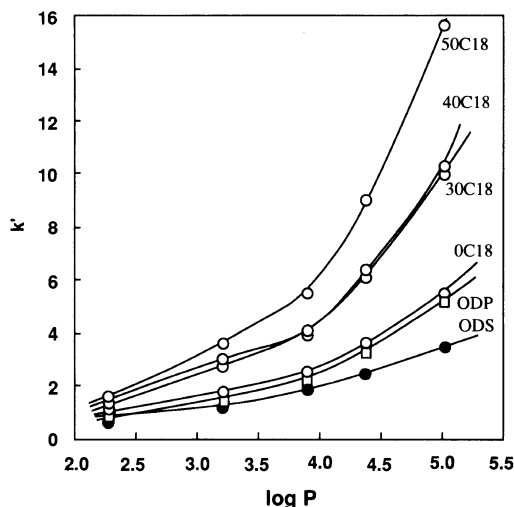


Figure 7. Relationship between capacity factor and carbon number for aromatic compounds. Solutes: benzene, naphthalene, fluorene anthracene, and pyrene.

dialkyl type particles than for ODS packings, although the elution order obeyed an RPLC mode. These results show that N,N -dialkyl type packings have weak affinity including interactions for aromatic molecules as well as hydrophobic interactions. Such affinity is often related to the rigidity of sample mole-

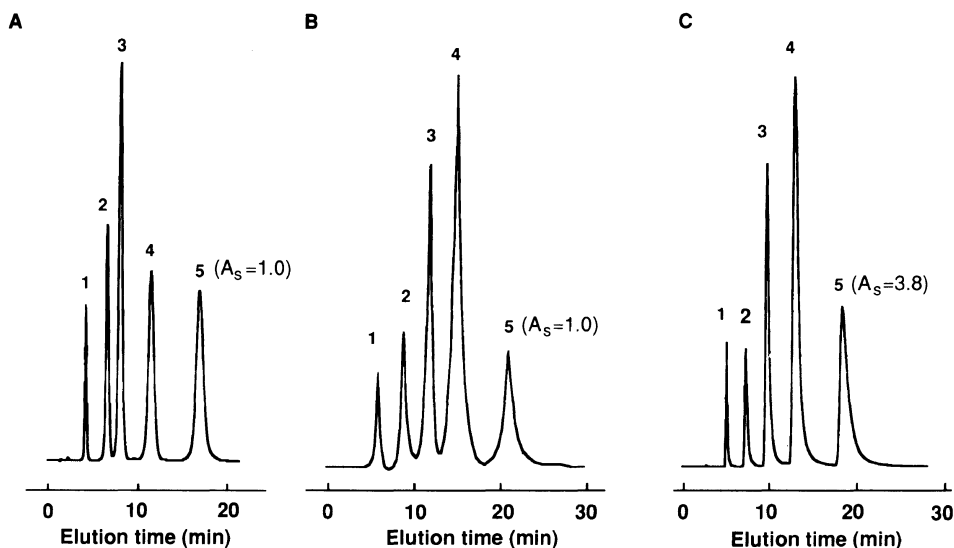


Figure 8. Separation of alkylbenzoates with DAA-1 (A), AMA-1 (B), and Asahipak ODP (C) columns: 1 = benzene; 2 = naphtharene; 3 = fluorene; 4 = anthracene; 5 = pyrene.

cules. It is known that polymer packings with micropores show more excessive retention for rigid and compact molecules such as anthracene and pyrene than for flexible molecules such as alkanes.¹⁵ Therefore, peak shapes for aromatic compounds were compared among polymer packings. Figure 8 shows the separations of some aromatic compounds with the *N*-alkyl type, *N,N*-dialkyl type and Asahipak ODP packings. Asymmetry factors (A_s)¹⁶ measured for pyrene at 10% of the peak height were 1.0, 1.0, and 3.8, respectively: Asahipak ODP showed typical peak tailing. This undesirable peak tailing of Asahipak ODP packings is derived from aromaticity of triallyl isocyanurate used as a crosslinking agent. Both *N*-alkyl and *N,N*-dialkyl type packings showed good symmetrical peaks as both packings have no aromatic rings of solutes.

Figure 9 shows chromatograms for alkyl benzoates using *N*-alkyl and *N,N*-dialkyl type packings. Both elution orders also obeyed an RPLC mode. However, *N*-alkyl type packings showed typical peak tailing. In contrast, *N,N*-dialkyl type packings showed no peak

tailing for carbonyl compounds. The asymmetry factors (A_s) measured for hexyl benzoate at 10% peak height were 1.0 and 2.0 in *N,N*-dialkyl type and *N*-alkyl type, respectively. The peaks in the *N,N*-dialkyl type packings corresponded to the efficiencies of those for aromatic compounds. These results strongly suggest that there are no amide protons in the *N,N*-dialkyl type packings resulting in the lack of hydrogen bonding interactions for carbonyl compounds.

Retention Capacity of Basic Substances

The limited use of alkyl bonded silica gels for basic compounds is due to the residual silanol groups and it is very important to check if *N,N*-dialkyl type packings are applicable to RPLC of basic compounds. Figure 10 compares the peak shapes of basic substances from the ODS and the DAA-1 packings. Pyridine was eluted from DAA-1 with symmetrical peak packings, although it was eluted with peak-tailing from the ODS column. Such peak tailing is often observed for comparably strong basic substances in the case of silica bonded packings. Table III summa-

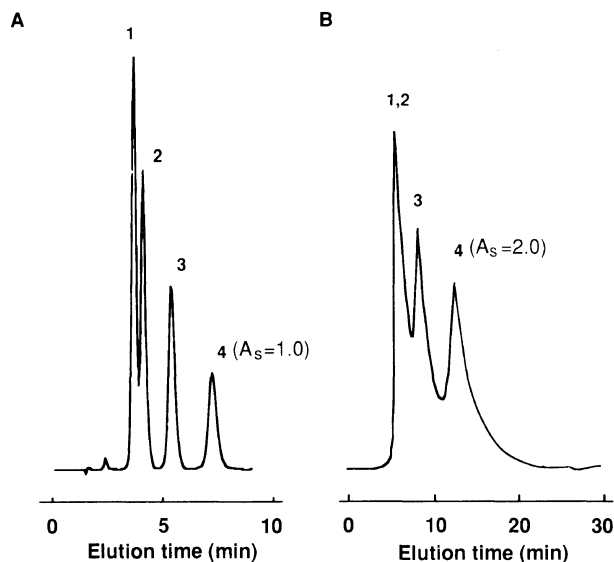


Figure 9. Separation of alkylbenzoates with DAA-1 (A) and AMA-1 (B) columns: 1 = methylbenzoate; 2 = ethylbenzoate; 3 = butylbenzoate; 4 = hexylbenzoate. Eluent, acetonitrile-water (80:20); flow rate, 0.5 ml min^{-1} .

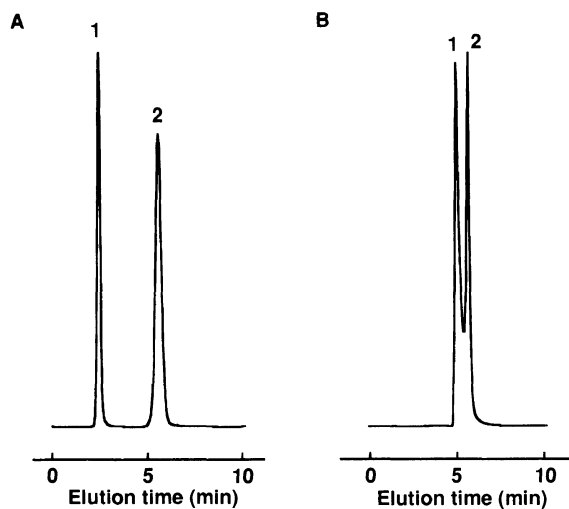


Figure 10. Separation of basic compounds with DAA-1 (A) and ODS (B) columns: 1 = aniline; 2 = pyridine. Eluent, acetonitrile-water (60:40, v/v); flow rate, 0.5 ml min^{-1} .

rizes a comparison of elution behavior for basic compounds. The ODS and octylated silica gels (OS) packings showed peak tailing for quinoline and α -naphthoquinoline, and adsorbed benzyl amine. The excellent properties

of the *N,N*-dialkyl type packings are due to the totally organic polymers prepared using nonionic monomers. On the other hand, Asahipak ODP is also prepared using nonionic monomers. However, it is known that

Table III. Retention behavior of substances

Solute	<i>k'</i>					
	ODS	ODS-TMS	ODP	30C18	40C18	50C18
Pyridine	1.37(t) ^a	-0.06(t)	0.20(t)	0.21	0.21	0.29
Quinoline	1.16(t)	-0.03	0.45(t)	0.53	0.61	0.7
α -Naphthoquinoline	1.81	0.35	1.69(t)	2.4	2.88	3.55
Benzylamine	Not eluted	0.33(t)	1.01(t)	0.16	0.41	0.37

^a (t): peak tailing.

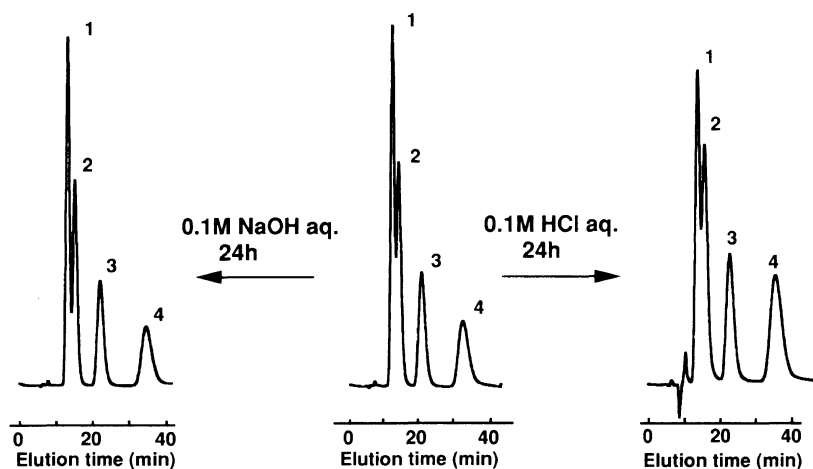


Figure 11. Chromatograms obtained with the DAA-1 columns before and after exposure of alkaline and acidic solutions. Eluent, acetonitrile-water (80:20); flow rate, 0.2 ml min⁻¹.

Table IV. HETP of the columns

Solute	HETP/ × 10 ³		
	ODS	DAA-1	ODP
Pyrene	4.61	6.68	9.24
Hexylbenzoate	5.38	5.63	6.75
Pyridine	270	180	470

Asahipak ODP shows peak tailing for basic compounds. This is due to the carboxylic groups by-produced in the process of introduction of octadecyl group.^{17,18} Dawkins *et al.* reported octadecylated acrylamide polymer particles for RPLC.^{8,9} The bonded phase was introduced by forming strongly nucleophilic amide anions on the surface of treatment with *N*-octadecyl bromide. There-

fore, the strong base most likely creates a risk of partial hydrolysis of CONH moieties to produce undesirable anionic groups.

Comparison of HETP

HETP,¹⁹ a reciprocal of the theoretical plate number, is useful to compare the resolution ability of columns. Table IV summarizes HETP values of the DAA-1, ODS, and Asahipak ODP columns determined using pyrene, hexyl benzoate and pyridine as solutes and acetonitrile-water (60:40) as an eluent. The DAA-1 column showed good HETP values for all samples.

Acid and Alkaline Resistances

Conventional alkyl-bonded silica gels and alkyl-bonded polymer particles are generally

known to decrease rapidly in efficiency in solutions of high and low pH.²⁰⁻²³ Therefore, the acid and alkaline resistances of the *N,N*-dialkyl type packings were examined with aqueous eluents having high and low pH. Figure 11 shows chromatograms obtained for methyl, ethyl, butyl and hexyl benzoates with *N,N*-dialkyl type packings before and after their exposures to 0.1 M NaOH and 0.1 M HCl aqueous solutions, respectively. No significant change in peak shape and column efficiency occurred in either instance. These results indicate that the *N,N*-dialkyl type packings withstand use at pH 1–13 and extend operating pH in RPLC.

CONCLUSIONS

The following conclusions can be drawn on the nature and characterization of *N,N*-dialkylacrylamide monomers and their polymer particles: (1) the melting point of *N,N*-dialkylacrylamide (or methacrylamide) is much lower than the conventional *N*-monoalkylacrylamide (or methacrylamide). This property is advantageous for aqueous suspension copolymerization. (2) *N,N*-Dialkylacrylamide is polymerizable, although the corresponding methacrylamide derivatives are inactive because of steric hindrance around the double bonding. (3) The spherical particles are made by one-step copolymerization of *N,N*-dialkylacrylamide. (4) The hydrophobicity of the particles is easily adjusted by changing the molar ratio of the monomers. (5) The column with the *N,N*-dialkylacrylamide particles showed normal RPLC separation for ionic, carbonyl and aromatic compounds. These excellent chromatographic properties of the packing derive from the totally organic nature of the monomers, in that they do not have amide proton, ionic or aromatic groups. (6) C–C and C–N bondings in *N,N*-dialkylacrylamide polymers are more stable in acid and alkaline solutions than Si–O–C or CO–O

bondings in conventional packings.

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