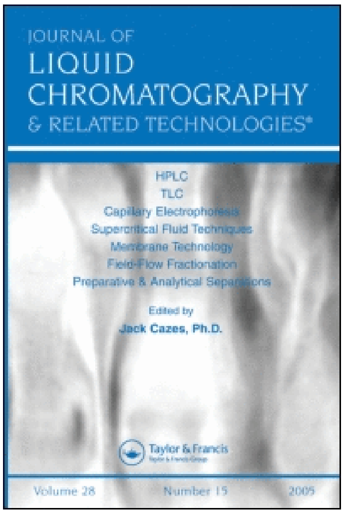

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CHARACTERIZATION OF SPHERICAL POLYMER PACKINGS FROM *N,N*-DIALKYL ACRYLAMIDE FOR REVERSED-PHASE LIQUID CHROMATOGRAPHY

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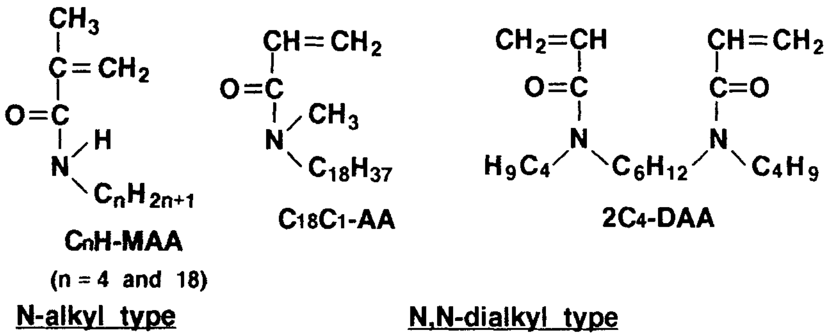
ABSTRACT

Chemically stable and totally organic polymer packings were prepared using *N,N*-dialkyl acrylamide monomers and their chromatographic properties were investigated. The hydrophobic parts of polymer packings were composed of *N*-methyl *N*-octadecyl acrylamide and *N,N'*-dibutylhexane *N,N'*-diacrylamide was used as a crosslinking monomer. The hydrophobicity of the spherical particles was easily controlled varying by the monomer ratio in the suspension polymerization. As for RPLC behavior, the packings had no abnormal adsorption for ionic substances and showed no excessive retention and no peak tailing for carbonyl compounds, because the packings had no ionic groups or amide protons. In addition, the packings show better alkal and acid-resistances than conventional polymer packings int which alkyl groups were introduced through ester bondings.

INTRODUCTION

Many alkyl bonded polymer particles have been use as effective reversed-phase HPLC packing materials over wide range of pH values. For example, Asahipak ARP serie (Asahi Chemical Industry Co., Ltd.) and TSK gels (Toso Corporation) in which alkyl chains were introduced throug ester bondings (1-3), polyacrylamide-based particles b Dawkins et al. (4,5) and alkylated polystyrene-base packings (e.g., Polysher RP18 (E. Merck)) (6) and alk amine-substitute polystyrene gels by Howang et al are we known (7). However, they are not totally satisfactory in the liquid chromatographic properties: the ester bonding contained in the Asahipak ARP series and TSK gels ar unstable in alkaline solution (8,9). Asahipak ARP series an polystyrene gels show peak tailing and low asymmetr factors (A_S) values for aromatic compounds, since triall isocyanurate or styrene units interact with solute molecule due to π-electron of the aromatic rings (9-13). Th polyacrylamide-based packing (4,5) and alkylate polystyrene (7) include NH groups which can cause hydroge bondings interaction between solutes and packings.

In this work, porous polymer packings without th above-mentioned disadvantages were newly synthesize using *N*-methyl *N*-octadecyl acrylamide as hydrophobic pa and *N,N'*-dibutylhexane *N,N'*-diacrylamide as a crosslinkin monomer (SCHEME 1). The HPLC properties are als described.



SCHEME 1 Structures of monomers used

EXPERIMENTAL

Preparation of polymer particles

N-Methyl *N*-octadecyl acrylamide ($C_{18}C_1$-AA) was prepared by reaction of *N*-methyl *N*-octadecylamine with acryloyl chloride. $2C_4$-DAA also were obtained using *N,N'*-dibutyl 1,6-diaminohexane.

$C_{18}C_1$-AA, $2C_4$-DAA, diethylbenzene (DEB, Nakarai Tesque Co., Ltd.) as diluent and 2,2'-azobis(isobutyronitrile) (Nakarai Tesque Co., Ltd.) were mixed at room temperature. The mixture was added to an aqueous solution containing 2 wt% poly(vinyl alcohol) and suspended by stirring. The suspension was heated at 80 °C for 24 hours under N_2 gas atmosphere. The copolymer particles obtained were washed successively with water, hot water, methanol and acetone. Similarly, spherical polymer particles were obtained using *N*-octadecyl or *N*-butyl methacrylamide (C_{18}H-MAA and C_4H-MAA, manufactured by Kojin Co., Ltd.) and ethylene glycol dimethacrylate (EGDM, Nippon Oil & Fats Co., Ltd.). Particles with diameters 5 ~ 10 μm were obtained by adjusting the stirring speed and sieving, and were packed into a stainless column. Their liquid chromatographic properties were examined using a JASCO 880-PU pump, UV-VIS Shimadzu photo diode array SPD-M6A and Shodex refracto monitor SE-51. As a reference column, ODS (Inertsil, 150 x 4.6 mm I. D., GL Science Co., Ltd) was used. All chromatography was carried out at 25 °C.

RESULTS AND DISCUSSION

Characterization of polymer particles

Polymer particles, shown abbreviated in TABLE 1, were prepared by suspension polymerization under the conditions specified. The polymer particles obtained were perfectly spherical and the diameter (5 ~ 10 μm) was adjusted by stirring speed in the polymerization and sieving. The IR spectra showed adsorptions due to octadecyl group and amide bondings (2925, 1659 cm^{-1} in the *N*-alkyl type polymer and 2925, 1638 cm^{-1} in the *N*,*N*-dialkyl type polymer). Their elemental analysis almost agreed with the molar ratio in the suspension polymerization process.

TABLE 1 Preparation of polymer particles

Packings	Type	Molar ratio				
		$C_{18}C_1$-AA	$2C_4$-DAA	C_{18}H-MAA	C_4H-MAA	EGDM
DAA-1	*N*,*N*-dialkyl	40	60	0	0	0
DAA-2	*N*,*N*-dialkyl	0	100	0	0	0
AMA-1	*N*-alkyl	0	0	30	20	50

The surface area of the particles were determined by Brunau-Emmet-Teller method: 0.43 and 0.46 $m^2\ g^{-1}$ in the DAA-1 and DAA-2 particles, respectively. All polymer particles showed similar specific swelling degree S_d = 2.7 wet-ml dry^{-1} in benzene, tetrahydrofuran, acetonitrile and methanol. The flow rate resistance in the liquid chromatography process was estimated in terms of the flow rate and pressure drop (14). The DAA-1 and DAA-2 columns showed good linear correlation up to about 4 ml min^{-1} in the flow rate (I.D.: 4.6 mm, eluent: acetonitrile-water (60 : 40)).

Chromatographic properties

Chromatograms for aromatic compounds using *N*-alkyl type and *N,N*-dialkyl type packings are shown in FIGURE 1. Derived values for k' for benzene, naphthalene, fluorene, anthracene and pyrene using these two kinds of polymer packings and commercial ODS packings are given in TABLE 2. The aromatic compounds were eluted with symmetrical peaks from the *N*-alkyl and *N,N*-dialkyl type packings. These results indicate that neither packing type shows abnormal retention caused by interaction with solute molecules through the π-electron of the aromatic rings of solutes. Close correlations between the k' and the carbon number in aromatic compounds were observed for *N*-alkyl and *N,N*-dialkyl type packings as well as for ODS packings. The k' value increased with increasing molar ratio of $C_{18}C_1$-AA monomer in the suspension polymerization process.

Chromatograms for alkyl benzoates using *N*-alkyl and *N,N*-dialkyl type packings are shown in FIGURE 2. *N*-Alkyl type packings showed typical peak tailing and a lower asymmetry factor (A_s) measured for hexyl benzoate at 10% peak height (FIGURE 2-B). It is assumed that the undesirable chromatographic property is caused by hydrogen

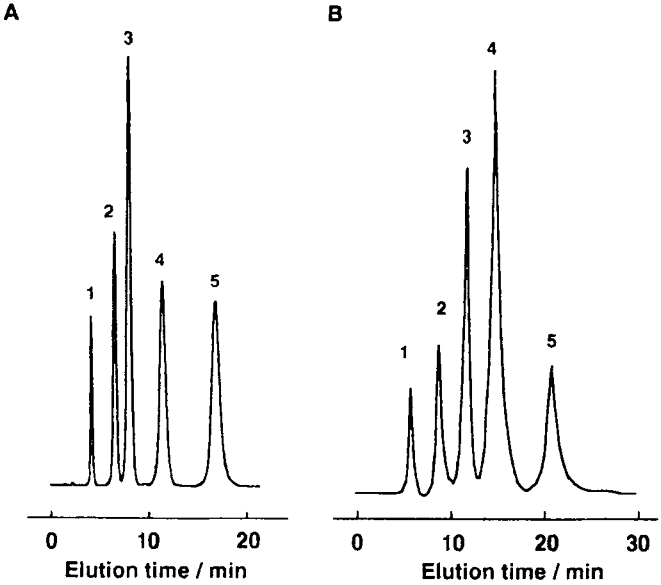


FIGURE 1 Separation of aromatic compounds with DAA-2 (A) and AMA-1 (B) columns: 1=benzene 2=naphthalene 3=fluorene 4=anthracene 5=pyrene. Eluent: acetonitrile-water (80 : 20, v/v); flow rate: 0.5 ml min^{-1}.

TABLE 2 Capacity factor of aromatic compounds with acetonitrile-water (80 : 20, v/v)

Solute	k'			
	DAA-1	DAA-2	AMA-1	ODS
Benzene	1.27	0.89	1.72	0.71
Naphthalene	2.78	1.75	3.27	1.20
Fluorene	4.03	2.36	4.63	1.83
Anthracene	6.35	3.57	6.44	2.40
Pyrene	10.24	5.51	9.72	3.48

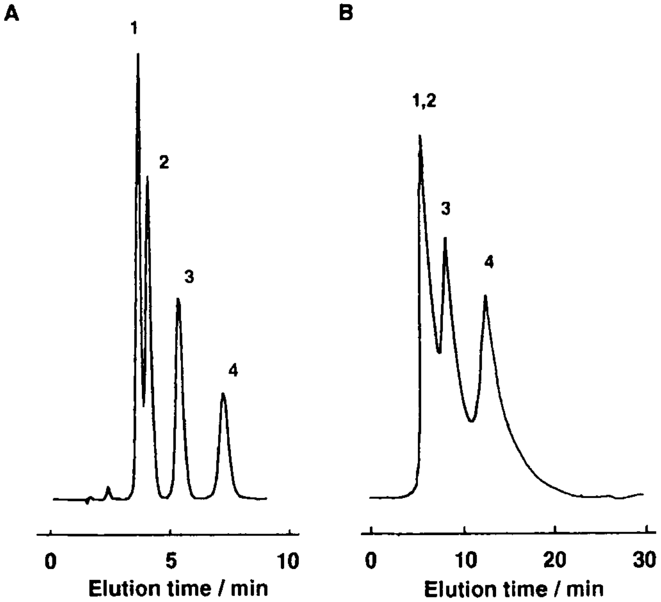


FIGURE 2 Separation of alkyl benzoates with DAA-2 (A) and AMA-1 (B) columns: 1=methylbenzoate 2=ethylbenzoate 3=butylbenzoate 4=hexylbenzoate. Eluent: acetonitrile-water (80 : 20); flow rate: 0.5 ml min^{-1}.

bonding interaction between the carbonyl group in the solute and the amide bondings in the packings. In contrast, *N,N*-dialkyl type packings provided no peak tailing for carbonyl compounds (FIGURE 2-A). Their peaks corresponded to efficiencies of the peaks for aromatic compounds.

This strongly suggests the fact that there were no amide protons in the packings resulted in the lack of abnormal retention for carbonyl compounds. Polyacrylamide-based packing containing *N*-octadecylamide groups prepared by Dawkins and co-workers includes amide bondings (CONH) equal to those of the *N*-alkyl type packings, and accordingly seems to show peak tailing for carbonyl compounds.

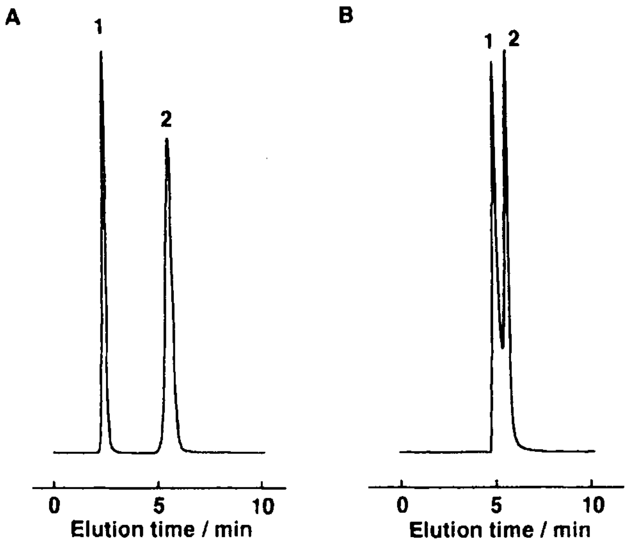


FIGURE 3 Separation of basic compounds with DAA-2 (A) and ODS (B) columns: 1=aniline 2=pyridine. Eluent: acetonitrile-water (60 : 40, v/v); flow rate: 0.5 ml min^{-1}.

Chromatograms for basic compounds using *N,N*-dialkyl type packings are shown in FIGURE 3. In general, reversed-phase silica packings can undergo broad tailing peaks and reversal in elution order, even when various improvements have been made (5,15-17).

Chromatograms for ODS packings show poor resolution of pyridine and aniline compared with *N,N*-dialkyl type packings. The broadening and tailing of the peak for pyridine and aniline with the ODS packings must arise from interaction with residual surface silanol groups. In contrast, *N,N*-dialkyl type packings show symmetrical peaks for those compounds. This shows that the separation mechanism with *N,N*-dialkyl type packings is simpler than the mixed-mode separation occurring with bonded phase silica gels. In addition, irreversible adsorption is often observed for more strongly basic compounds, e.g., benzylamine with bonded silica packings (9,18). However such undesirable adsorption behaviors were not observed for such compounds with the *N,N*-dialkyl type packings. The excellent properties of the *N,N*-dialkyl type packings are derived from the totally organic nature of the polymer prepared using nonionic monomers.

Comparison of HETP

HETP values of the columns were determined using pyrene, hexyl benzoate and pyridine as solutes and acetonitrile-water (60 : 40) as an eluent. As summarized in TABLE 3, the HETP values of the DAA-2 column for pyrene and hexylbenzoate compare to those of the ODP and ODS columns. The higher values in the ODS and ODP columns for pyridine are attributed to undesirable ionic interaction due to residual silanol and isocyanurate groups.

Chemical stability of *N,N*-dialkyl type packings

Conventional alkyl-bonded silica and alkyl-bonded polymer gels are generally known to decrease rapidly in

TABLE 3 HETP values of the columns

Solute	HETP / x 10^3		
	ODS	DAA-2	ODP
Pyrene	4.61	6.68	9.24
Hexylbenzoate	5.38	5.63	6.75
Pyridine	270	180	470

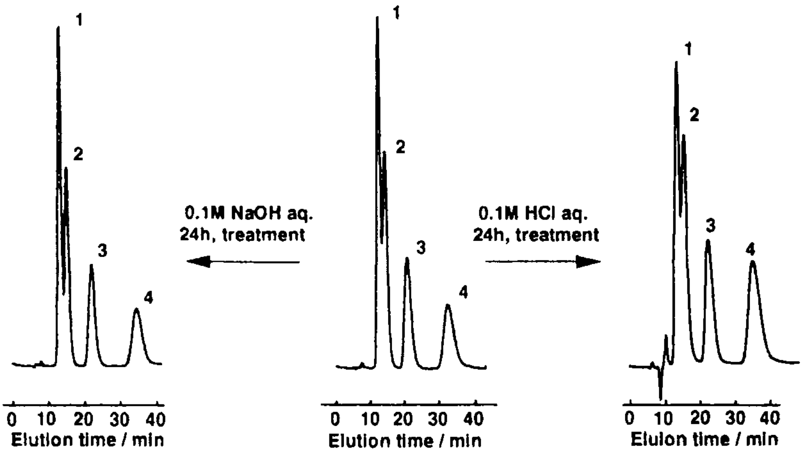


FIGURE 4 Chromatograms obtained with DAA-1 packings before and after exposure of alkaline and acidic solutions. Eluent: acetonitrile-water (80 : 20, v/v); flow rate: 0.2 ml min^{-1}.

efficiency in solutions of high and low pH (7,8,19,20). Therefore, the acidic and alkaline resistances of the *N,N*-dialkyl type packings were examined with aqueous eluent having high and low pH. FIGURE 4 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 packings can withstand use at pH 1~13. Therefore, the *N,N*-dialkyl type column significantly extends the operating pH range in RP-HPLC.

CONCLUSION

We have reported on the chromatographic properties of two kinds of amide polymer packings. *N,N*-Dialkyl type and *N*-alkyl type packings prepared in this work were provided results similar to conventional silica-based packings for retention for aromatic compounds and are particularly advantageous for basic compounds such as aniline and pyridine. However, *N*-alkyl type packings showed peak tailing for carbonyl compounds caused by hydrogen bonding interaction between solutes and the stationary phase. Similar polymer packings containing amide moieties (CONH) have been prepared (4,5). Although the packings show excellent chromatographic properties, they also seem to show peak tailing for carbonyl compounds. In addition, the bonded phase was introduced by forming strongly nucleophilic amide anions on the surface of treatment with *n*-octadecyl bromide. Therefore, the strong base used in this procedure most likely creates a risk of partial hydrolysis of CONH moieties to produce undesirable anionic groups.

The *N,N*-alkyl type packings have the following advantages: (1) they are prepared by one-step polymerization, (2) they show no abnormal retention and no peak tailing for ionic or carbonyl compounds, (3) C-C and C-N bondings in the packings are more stable in acidic and alkaline solutions than Si-O-C and CO-O bondings.

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