Synthesis of polymers and copolymers of c-(N^{ε}–AcrLys–Sar) and their interaction with small molecules in solution

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An acrylamide derivative having a mono-*N*-methylated cyclic dipeptide as a substituent, c-(N^{ε}AcrLys–Sar), was synthesized and polymerized by radical polymerization, giving homopolymers and copolymers with styrene, 4-vinylpyridine, and *N*-dodecylacrylamide. The relative reactivities of these monomers in the radical polymerization decreased in the following order: styrene > 4-vinylpyridine > *N*-dodecylacrylamide ≃ c-(N^{ε}-AcrLys–Sar). Poly[c-(N^{ε}-AcrLys–Sar)] was soluble in water and the usual organic solvents except diethylether and n-hexane. In $CHCl_3$/tetrahydrofuran mixed solvent, poly[c-(N^{ε}-AcrLys–Sar)] interacted most strongly with Ba^{2+} among alkali and alkaline–earth metal cations. A cyclic dipeptide, which is equivalent to the side chain of poly[c-(N^{ε}-AcrLys–Sar)], did not interact with these cations, indicating that the side chain ligand groups of poly[c-(N^{ε}-AcrLys–Sar)] interacts specifically with Ba^{2+} by intramolecular cooperation. However, the copolymer of c-(N^{ε}-AcrLys–Sar) with styrene interacts more strongly with larger alkali cations, and equally well with Ba^{2+} and Ca^{2+}. These results indicate that the styrene units in the copolymer influence its solubility and regulate the intramolecular cooperation of the cyclic dipeptide ligand groups as spacer groups. The copolymer of c-(N^{ε}-AcrLys–Sar) with 4-vinylpyridine interacted very strongly with Ba^{2+} as a result of the intramolecular cooperation of a pyridyl group and a cyclic dipeptide group. The formation constant of the ternary complex was determined to be $10^9\ M^{-3}$, which is larger by 10^2 fold than that by a polymer carrying benzo-15-crown-5 in the side chain. The copolymer of c-(N^{ε}-AcrLys–Sar) with *N*-dodecylacrylamide was found to bind methyl orange in aqueous solution by hydrophobic interaction. The copolymer of c-(N^{ε}-AcrLys–Sar) with styrene was found to extract phenylalanine from an aqueous solution to a n-octanol phase. However, the extraction was not enantiomer-selective.

(Keywords: cyclo(N^{ε}-acryloyl-L-lysylsarcosyl); radical polymerization; copolymerization; metal–ion complex; ion selectivity; intramolecular cooperation)

INTRODUCTION

We have synthesized vinyl compounds carrying a cyclic dipeptide substituent, which has the ability to complex with a metal salt[1]. On the basis of the intramolecular cooperation of side-chain cyclic dipeptides in polymers and copolymers, an efficient and selective complexation with small molecules such as metal salts has been observed.

Firstly, cyclo(N^{ε}-acryloyl-L-lysylglycyl)[c-(N^{ε}-AcryLys–Gly)] was synthesized, and the polymers and copolymers with styrene were synthesized by radical polymerization[2]. The copolymers with styrene were soluble in the usual organic solvents, and their complex formation with barium picrate ($BaPi_2$) or sodium tetraphenylborate ($NaBPh_4$) was found in solution. However, the solubility of metal–salt complexes with the styrene copolymer in organic solvents was too low to perform a quantitative investigation.

Next, by *N*-methylation of three amide bonds of c-(N^{ε}-AcrLys–Gly) and cyclo(N^{ε}-acryloyl,$N^{\alpha,\varepsilon}$-dimethyl-lysylsarcosyl)[c-(N^{ε}-Acr,$N^{\alpha,\varepsilon}$-Me_2Lys–Sar)] was synthesized, and the polymers and copolymers with styrene were synthesized by radical polymerization[3]. With *N*-methylation the homopolymer became very soluble in water as well as usual organic solvents. The ability to form complexes with NaPi or $NaBPh_4$ decreased in the order; homopolymer > styrene copolymer > low-molecular-weight model compound. By these investigations, the intramolecular cooperation of side-chain cyclic dipeptides was demonstrated in the complex formation with metal–salt, and the styrene units in the copolymer were found to act as spacers against ligand groups and reduce the feasibility of intramolecular cooperation of ligand groups. However, in these experiments the elemental analysis of c-(N^{ε}-Acr, $N^{\alpha,\varepsilon}$-Me_2Lys–Sar) was not carried out because of the extreme hygroscopic nature, and a quantitative discussion on the complex formation was not possible.

In the present investigation, cyclo(N^{ε}-acryloyl-L-lysylsarcosyl)[c-(N^{ε}-AcrLys–Sar)] was synthesized in a stepwise fashion by the liquid-phase method, and the polymers and copolymers with various vinyl compounds were synthesized by radical polymerization. The efficient and selective complex formation of these polymers and copolymers with small molecules, such as metal salt, α-amino acid, and dyestuff, was investigated. In particular, with the styrene copolymers the sequential distribution was calculated on the basis of the monomer reactivity ratio in the copolymerization, and its effect on the complex formation with metal–salt was discussed.

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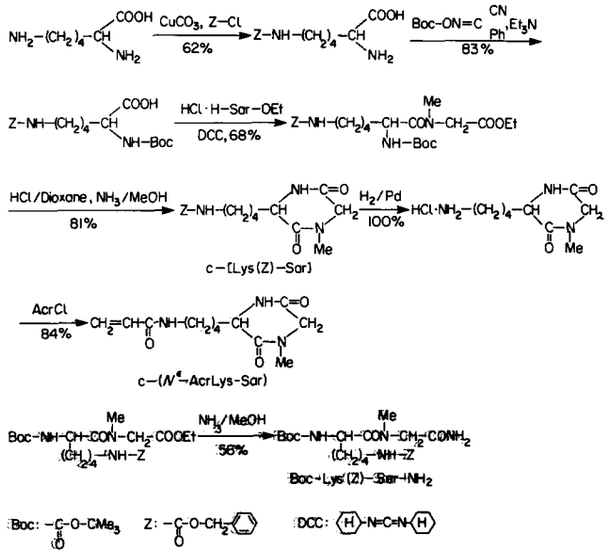

Scheme 1 Synthetic route to Boc-Lys(Z)-Sar-NH_2, c-[Lys(Z)-Sar], and c-(N$^{\varepsilon}$-AcrLys–Sar)

EXPERIMENTAL

Synthesis

c-(N$^{\varepsilon}$-AcrLys–Sar) was synthesized by the method described in *Scheme 1*. The procedures involved in each step were general ones which have been used in liquid-phase peptide synthesis. Boc and Z represent a *t*-butyloxycarbonyl group protecting the α-amino group of the lysyl residue and a benzyloxycarbonyl group protecting the ε-amino group of the lysyl residue, respectively. DCC represents dicyclohexylcarbodiimide which is a condensation agent. The carboxyl group of the sarcosyl residue was protected by an ethyl ester (–OEt). Intermediates containing sarcosyl residues are highly soluble in organic solvents and oily so that the purification was difficult. The purities of the synthetic intermediates were checked by thin-layer chromatography (t.l.c.). The product, c-(N$^{\varepsilon}$-AcrLys–Sar), was extracted with ethylacetate (AcOEt) or methanol (MeOH), dried with Na_2SO_4 overnight, the solvent being distilled off, and dried in a vacuum and it was found to be a hydroscopic oily substance. $R_f = 0.10$ in t.l.c. ($CHCl_3$/MeOH/AcOH, 95:5:3); $R_f = 0.25$ in t.l.c. (BuOH/AcOH/H_2O/pyridine, 30:6:24:20). Elemental analysis, calculated for $C_{12}H_{19}N_3O_3 \cdot 2H_2O$: C, 49.82%; H, 8.01%; N, 14.52%. Found: C, 50.44%; H, 7.84%; N, 14.52%. The structure of c-(N$^{\varepsilon}$-AcrLys–Sar) was identified by the infra-red spectrum: 990 cm^{-1} (δCH_2=CH–), 1540 cm^{-1} (amide II), 1640–1680 cm^{-1} (amide I), 3040 cm^{-1} (νCH_2=CH–), and 3200–3460 cm^{-1} (νNH).

Standard materials for the polymers and copolymers of c-(N$^{\varepsilon}$-AcrLys–Sar), cyclo(N$^{\varepsilon}$-benzyloxycarbonyl-L-lysyl-sarcosyl)[c-[Lys(Z)–Sar]] and N$^{\alpha}$-t-butyloxycarbonyl, N$^{\varepsilon}$-benzyloxycarbonyl-L-lysylsarcosine amide [Boc–Lys(Z)–Sar–NH_2] were used. The synthesis of c-[Lys(Z)–Sar] is described in *Scheme 1*. It was obtained as a needle-like crystal after repeated recrystallizations from ether (Et_2O): m.p. 115°C. $R_f = 0.36$ in t.l.c. ($CHCl_3$/MeOH/AcOH 95:5:3 v/v). $R_f = 0.75$ in t.l.c. (BuOH/AcOH/H_2O/pyridine 30:6:24:20 v/v). Elemental analysis, calculated for $C_{17}H_{23}N_3O_4$: C, 61.25%; H, 6.95%; N, 12.60%. Found: C, 61.42%; H, 6.96%; N, 12.62%. Boc-Lys(Z)–Sar–NH_2 was synthesized by dissolving the synthetic intermediate Boc–Lys(Z)–Sar–OEt in *Scheme 1* into MeOH saturated with NH_3. It was purified by eluting an AcOEt solution through a silica-gel column: m.p. 128°–130°C. Yield 56%. Elemental analysis, calculated for $C_{22}H_{34}N_4O_6$: C, 58.65%; H, 7.61%; N, 12.44%. Found: C, 58.37%; H, 7.65%; N, 12.34%.

The radical polymerization of c-(N$^{\varepsilon}$-AcrLys–Sar) was carried out using azobisisobutyronitrile (AIBN) as an initiator in dimethylformamide ($HCONMe_2$) as a solvent in a vacuum-sealed glass ampoule at 80°C for 42 h. Commercial AIBN was used without further purification and $HCONMe_2$ was distilled twice *in vacuo*. After 42 h polymerization the solution was poured into an excess of acetone (Me_2CO) (20 times the amount of the polymerization solution) to precipitate the polymer. After washing with Me_2CO and MeOH the polymer was dried *in vacuo*. The number-average molecular weight of the polymer was determined by the vapour-pressure osmometry (v.p.o.) in benzene solution.

The radical copolymerization of c-(N$^{\varepsilon}$-AcrLys–Sar) and styrene was carried out under the same conditions as the homopolymerization. The molar ratio of the two monomers in the feed was always 1:1. Styrene was purified in the normal manner. After the required reaction time, the solution was poured into an excess of water (20 times the volume of the copolymerization solution) to precipitate the reaction product. The product was washed with Me_2CO and dried *in vacuo*. The copolymer composition was determined by elemental analysis, and the number-average molecular weight was determined by v.p.o. in benzene solution. The copolymer was dissolved in HCO-NMe_2 and fractionated into three fractions by addition of Et_2O. The composition of each fraction was determined by elemental analysis, and the viscosity of $CHCl_3$ solution was measured at 25°C. To determine the monomer reactivity ratio, the mixtures of monomers (8 mmol) having different compositions were dissolved in HCO-NMe_2 (2 ml) and polymerized with AIBN (3 mg) under nitrogen stream at 80°C for 1 h. The reaction solution was poured into an excess of Me_2CO to precipitate the copolymer, which was then washed with Me_2CO and MeOH and dried *in vacuo*. The conversion was always kept below 10%. The copolymers were dissolved in dimethylsulphoxide-d_6 and subjected to the 90 MHz nuclear magnetic resonance (n.m.r.) measurement, the internal standard being tetramethylsilane. The copolymer composition was determined by the area ratio of the signal at 2.4 ppm due to N–CH_3 protons against the signal at 1–2 ppm due to alkyl protons which are not involved in cyclic dipeptide moieties. Only with the copolymer obtained from the feed containing c-(N$^{\varepsilon}$-AcrLys–Sar) and styrene with the molar ratio 1:9, was the phenyl proton signal used for the determination in place of the N–CH_3 signal.

The radical copolymerization of c-(N$^{\varepsilon}$-AcrLys–Sar) and 4-vinylpyridine was carried out under the same conditions as the homopolymerization of c-(N$^{\varepsilon}$-AcrLys–Sar). The molar composition in the feed was 1:1. 4-Vinylpyridine was purified by vacuum distillation: b.p. 65°C (15 mmHg). After the requisite copolymerization time, the solution was poured into an excess of Et_2O to precipitate the reaction product, which was then washed with MeOH and water and dried *in vacuo*. The copolymer

was a pale green powder and the composition was determined by elemental analysis. The number-average molecular weight of the copolymer was determined by v.p.o. of benzene solution.

The radical copolymerization of c-(*N*$^{\varepsilon}$-AcrLys–Sar) and *N*-dodecylacrylamide was carried out under the same conditions as the homopolymerization of c-(*N*$^{\varepsilon}$-AcrLys–Sar). The molar composition in the feed was 1:1. The synthesis and purification of *N*-dodecylacrylamide were performed as described in the literature[4]. After a requisite copolymerization time, the solution was poured into an excess of Me_2CO to precipitate the reaction product, which was then washed repeatedly with water and ligroin and dried *in vacuo*. The copolymer composition was determined by elemental analysis, and the number-average molecular weight was determined by v.p.o.

Complexation with small molecules

The interaction of the homopolymers and the styrene copolymers of c-(*N*$^{\varepsilon}$-AcrLys–Sar) with metal picrates in solution was investigated by ultra-violet (u.v.) spectroscopy. Picrates of Na^+, K^+, Rb^+, Cs^+, Ca^{2+} and Ba^{2+} were used. Commercial caesium chloride, commercial rubidium carbonate, and other metal hydroxides were heated with commercial picric acid in ethanol (EtOH) for 10 min, and the solution was left at room temperature to allow metal picrates to crystallize out. The metal picrates were recrystallized repeatedly from EtOH. 5×10^{-5} M metal picrate solution in $CHCl_3$/tetrahydrofuran (THF) (1:1 v/v) mixture was prepared, to which the compounds containing the cyclic dipeptide were added up to the molar ratio of cyclic dipeptide/metal picrate being 150. The compounds containing the cyclic dipeptide include poly[c-(*N*$^{\varepsilon}$-AcrLys–Sar)], c-(*N*$^{\varepsilon}$-AcrLys–Sar)/styrene copolymer, and their low-molecular-weight model compounds such as c-[Lys(Z)–Sar] and Boc-Lys(Z)-Sar-NH_2. $CHCl_3$ and THF were distilled just before use from P_2O_5 and CaH_2, respectively. The extent of complex formation between the cyclic dipeptide ligand and metal picrate was estimated by the red shift of the absorption maximum of the ion-pair of metal picrate according to the dissociation by complexation[3,5].

The composition of the complex between the metal picrates and the copolymer of c-(*N*$^{\varepsilon}$-AcrLys–Sar) with styrene or 4-vinylpyridine was determined by variation of the electroconductivity by complexation[6]. The variation of conductivity was measured on the addition of c-[Lys(Z)–Sar] for comparison. A 3×10^{-5} M metal picrate solution in $CHCl_3$/THF (1:1 v/v) mixture was prepared, to which a $CHCl_3$ solution of the compound containing the cyclic dipeptide was added and the variation of the conductivity was followed. The highest molar ratio of cyclic dipeptide unit to metal picrate was 3. The conductivity was measured with Wayne Kerr Universal Bridge B224.

The interaction of c-(*N*$^{\varepsilon}$-AcrLys–Sar)/4-vinylpyridine copolymer with metal picrates was investigated by the partition–equilibrium method. An aqueous solution containing 5×10^{-5} M picric acid and 1×10^{-2} M metal hydroxide was brought into contact with a $CHCl_3$ solution of c-(*N*$^{\varepsilon}$-AcrLys–Sar)/4-vinylpyridine copolymer. The concentration of cyclic dipeptide unit in the $CHCl_3$ solution was 1–5 times as much as that of picric acid in the aqueous solution. The mixture was stirred for 1 h at room temperature, left to stand for 30 min, and the residual metal picrate in aqueous solution was determined by u.v. spectroscopy. Metal ion in aqueous phase (M^+_{aq}), picrate ion in aqueous phase (Pi^-_{aq}), and cyclic peptide ligand in $CHCl_3$ phase (Pep_{org}) are in equilibrium as described by equation (1) with the complex in the $CHCl_3$ phase ($M^+ \cdot Pep \cdot Pi^-_{org}$).

$$M^{n+}_{aq} + mPep_{org} + nPi^-_{aq} \overset{K}{\rightleftharpoons} M^{n+} \cdot mPep \cdot nPi^-_{org} \quad (1)$$

An apparent extraction equilibrium constant K is given by equation (2)[7],

$$K = \frac{1}{\gamma^{M^{n+}} \cdot \gamma^{Pi^-}} \cdot \frac{[M^{n+} \cdot mPep \cdot nPi^-]}{[M^{n+}][Pep]^m[Pi^-]^n} \quad (2)$$

where n represents the charge number of metal ion and m is determined by the composition of the complex as determined by conductivity measurements.

The interaction of polymers containing the cyclic dipeptide ligands with dye stuff and α-amino acid was investigated. To an aqueous solution of methyl orange (5×10^{-5} M), c-(*N*$^{\varepsilon}$-AcrLys–Sar)/*N*-dodecylacrylamide copolymer was added and the change in u.v. spectrum was followed, the cyclic dipeptide unit/dye stuff molar ratio being 25. pH was adjusted at 6.8 by a phosphate buffer. An aqueous solution of D- or L-phenylalanine (5×10^{-3} M) was shaken with a n-octanol solution of varying concentrations of c-(*N*$^{\varepsilon}$-AcrLys–Sar)/styrene copolymer, and the amount of phenylalanine extracted into the n-octanol phase was determined by the absorption intensity at 260 nm.

RESULTS AND DISCUSSION

Polymerization and copolymerization of c-N$^{\varepsilon}$-AcrLys–Sar) and product characterization

C-(*N*$^{\varepsilon}$-AcrLys–Sar) was polymerized or copolymerized with other monomers, and the experimental results are summarized in *Table 1*. The relative reactivity of the monomers decreased in the following order: styrene > 4-vinylpyridine > *N*-dodecylacrylamide ≃ c-(*N*$^{\varepsilon}$-AcrLys–Sar). The degree of polymerization of the c-(*N*$^{\varepsilon}$-AcrLys–Sar) copolymers decreased in the same order with reference to comonomers as above.

The infra-red spectrum of poly[c-(*N*$^{\varepsilon}$-AcrLys–Sar)] did not show the absorption at 990 cm^{-1} due to the double bond of monomer, indicating the formation of a polymer.

In the infra-red spectrum of c-(*N*$^{\varepsilon}$-AcrLys–Sar)/styrene copolymer, the absorptions appearing at 695 and 750 cm^{-1} are ascribable to the out-of plane bending of the CH group involved in a mono-substituted phenyl group, and one at 3050 cm^{-1} to the stretching of the phenyl CH group, indicating the presence of the styrene units in the polymer. However, the amide I absorption at 1650 cm^{-1} and the amide II absorption at 1550 cm^{-1} were observed, indicating the presence of c-(*N*$^{\varepsilon}$-AcrLys–Sar) units in the polymer. These observations, together with the solubility of the polymer, indicate the formation of a true copolymer.

The c-(*N*$^{\varepsilon}$-AcrLys–Sar)/styrene copolymer was fractionated into three fractions (*MW*: 2.2×10^4, 2.0×10^4 and 5.9×10^3), and the compositions of each fraction were similar (c-(*N*$^{\varepsilon}$-AcrLys–Sar) mol%: 38.3, 39.7 and 32.4). Fraction 1 of the copolymer was used for the complex formation experiments, which will be described later.

Table 1 Polymerization and copolymerization of c-(*N*$^{\varepsilon}$-AcrLys–Sar) [a]

Monomer	[M] g/100 ml	[I] g/100 ml	Polymer Yield (%)	Polymer c-(*N*$^{\varepsilon}$-AcrLys–Sar) (mol %)	Polymer *MW* [b]
c-(*N*$^{\varepsilon}$-AcrLys–Sar)	94.5	5.7	64	100	
c-(*N*$^{\varepsilon}$-AcrLys–Sar)/styrene	21.1/51.3	4.3	72	37.6/62.4	2.13×10^4
c-(*N*$^{\varepsilon}$-AcrLys–Sar)/4-vinylpyridine	15.6/6.48	1.3	46	42.0/58.0	1.1×10^4
c-(*N*$^{\varepsilon}$-AcrLys–Sar)/*N*-dodecylacrylamide	12.1/11.5	1.4	62	50.0/50.0	3.3×10^3

[a] Solvent, $HCONMe_2$; initiator, AIBN; temperature, 80°C; time, 42h; in vacuum
[b] Molecular weight determined by v.p.o. in benzene solution

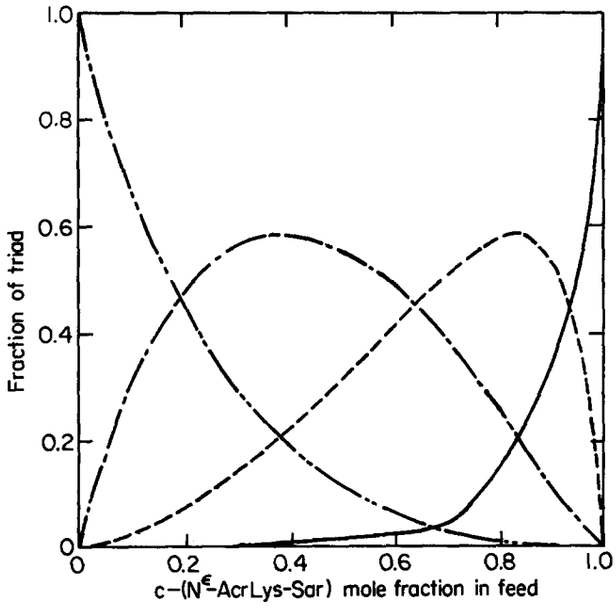

Figure 1 Dependence of triad fractions on the mole fraction of c-(*N*$^{\varepsilon}$-AcrLys–Sar) (M_1) in feed of the copolymerization with styrene(M_2). Triad distinction: (——), $M_1M_1M_1$; (-----), $M_1M_2M_1 + 2\times M_1M_1M_2$; (-·-·-), $2\times M_2M_2M_1 + M_2M_1M_2$; (-··-··-), $M_2M_2M_2$

The monomer reactivity ratios of c-(N$^{\varepsilon}$-AcrLys–Sar) (M_1) and styrene (M_2) in the radical polymerization were determined by the cross-section method to be $r_1 = 0.2$ and $r_2 = 0.6$. Using these values, the fractions of triad sequences of comonomer units in the copolymer were calculated against the molar fractions of c-(*N*$^{\varepsilon}$-AcrLys–Sar) in the monomer feed, and are shown in *Figure 1*. In the present experiments, the complex formation was examined with a styrene copolymer which was the product of a monomer feed containing c-(*N*$^{\varepsilon}$-AcrLys–Sar) of molar fraction 0.5. According to *Figure 1*, a high population of triad sequences consisting of two styrene units and one c-(*N*$^{\varepsilon}$-AcrLys–Sar) unit should be most prevalent in this type of copolymer.

In the infra-red spectrum of c-(*N*$^{\varepsilon}$-AcrLys–Sar)/4-vinylpyridine copolymer, a shoulder at 1590 cm^{-1} is due to the stretching of the C=C and C=N double bonds involved in a pyridine ring, and the absorptions at 1640 cm^{-1} and 1540 cm^{-1} due to the amide I and II absorptions, respectively. These observations together with the solubility of the polymer indicate the formation of a true copolymer.

In the infra-red spectrum of the c-(*N*$^{\varepsilon}$-AcrLys–Sar)/*N*-dodecylacrylamide copolymer, the absorption at 750 cm^{-1} is due to the backbone vibration of a polymethylene chain, indicating the presence of the *N*-dodecylacrylamide units in the polymer. The absorption intensities at 1330 cm^{-1} due to the C–C stretching involving a CH_2 group and those at 2920 and 2850 cm^{-1} due to the C–H stretching of a CH_2 group increased as compared with those in poly[c-(*N*$^{\varepsilon}$-AcrLys–Sar)], indicating the presence of the *N*-dodecylacrylamide units in the polymer, also. However, the absorption at 1450 cm^{-1} is due to a *cis* amide group involved in the c-(*N*$^{\varepsilon}$-AcrLys–Sar) units in the polymer. These observations together with the solubility of the polymer indicate the formation of a true copolymer.

The solubilities of a homopolymer and copolymers of c-(*N*$^{\varepsilon}$-AcrLys–Sar) were investigated and are shown in *Table 2*. Poly[c-(*N*$^{\varepsilon}$-AcrLys–Sar)] is very soluble in water and the usual organic solvents such as halogenated hydrocarbons. Its solubility is comparable with that of poly[c-(*N*$^{\varepsilon}$-Acr, *N*$^{\alpha\varepsilon}$-Me_2Lys–Sar)], in which three amide groups per monomer unit are *N*-methylated[3]. The solubility of poly[c-(*N*$^{\varepsilon}$-AcrLys–Gly)], in which none of the amide groups is substituted, has been reported to be very low[2]. It was therefore revealed that the solubility of poly[c-(*N*$^{\varepsilon}$-AcrLys–Gly)] is very much increased by *N*-methylation of one of three amide bonds in the monomer unit. The solubility of c-(*N*$^{\varepsilon}$-AcrLys–Sar)/styrene copolymer is similar to that for poly[c-(*N*$^{\varepsilon}$-AcrLys–Sar)], but insoluble in water due to the presence of the styrene units. The solubility of c-(*N*$^{\varepsilon}$-AcrLys–Sar)/4-vinylpyridine copolymer in water increased but decreased in Me_2CO and dioxane, as compared with that of c-(*N*$^{\varepsilon}$-AcrLys–Sar)]/styrene copolymer. c-(*N*$^{\varepsilon}$-AcrLys–Sar)/*N*-dodecylacrylamide copolymer was soluble in almost all the usual organic solvents, due to the presence of *N*-dodecylacrylamide units having a long alkyl chain. Using the excellent solubilities of the polymer and copolymers of c-(*N*$^{\varepsilon}$-AcrLys–Sar), we can then investigate their interactions with small molecules under a variety of conditions.

Metal–salt complexation

U.v. spectroscopy investigation. When various types of peptide compounds were added to a $CHCl_3$/THF (1:1 v/v) solution of metal picrate, the absorption maximum for the picrate ion red-shifted. The experimental results are shown in *Figures 2a–d*. Bourgoin *et al.*[5] reported that sodium picrate shows an absorption maximum at 351 nm in nonpolar solvents and the absorption maximum shifts to a longer wavelength with increasing solvent polarity until 380 nm for free ions. Therefore, the red shift shown in

Table 2 Solubility of polymer and copolymers of c-(*N*$^{\epsilon}$-AcrLys–Sar)

Solvent	Homopolymer	Styrene copolymer	4-Vinylpyridine copolymer	*N*-Dodecylacrylamide copolymer
H_2O	HS	I	PS	PS
$HCONMe_2$	HS	HS	HS	S
MeOH	HS	HS	HS	HS
EtOH	HS	HS	HS	HS
Me_2CO	PS	PS	I	HS
THF	PS	PS	PS	HS
$CHCl_3$	HS	HS	HS	HS
CH_2Cl_2	HS	HS	HS	HS
Et_2O	I	I	I	PS
Benzene	S	S	S	HS
Dioxane	S	S	I	HS

HS: highly soluble; I: insoluble; S: soluble; PS: partly soluble

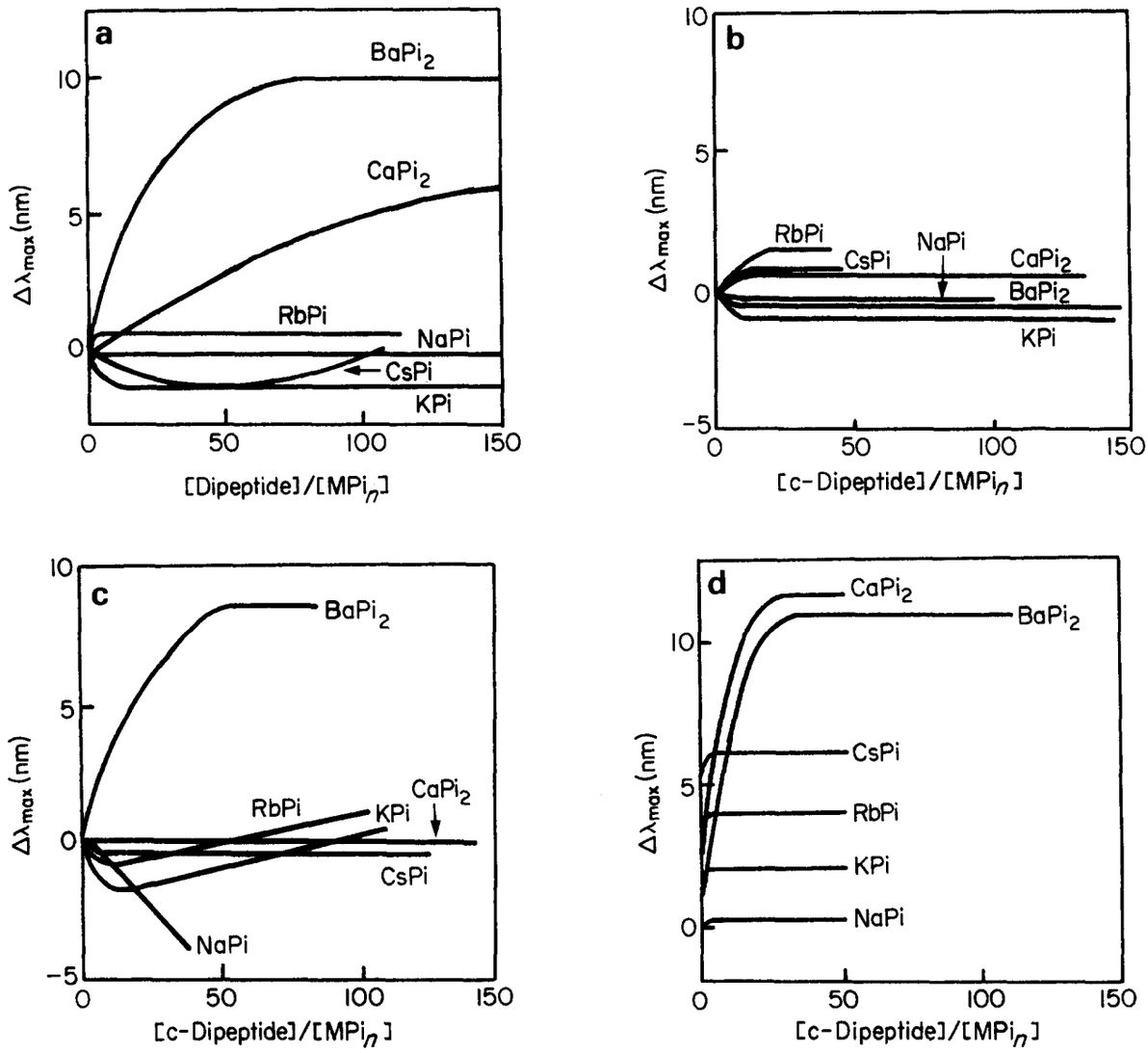

Figure 2 Shift of the adsorption maximum of various metal picrates in $CHC1_3$/THF (1:1 v/v) solution by the addition of peptide ligand. Concentration of metal picrate, $5x10^{-5}$M. (a) Boc-Lys(Z)-Sar-NH_2, (b) c-[Lys(Z)-Sar], (c) poly[c-(*N*$^{\epsilon}$-AcrLys–Sar)], (d) c-(*N*$^{\epsilon}$-AcrLys–Sar)] styrene copolymer

Figure 2 may represent the extent of solvation of the counter cation by the peptide compound added.

As can be seen in *Figure 2a*, the addition of the linear dipeptide, Boc–Lys(Z)–Sar-NH_2 caused a red shift only for $BaPi_2$ and $CaPi_2$. This fact indicates that the linear dipeptide has a certain degree of complexation ability toward alkaline-earth metal ions.

As can be seen in *Figure 2b*, the addition of the cyclic dipeptide c-[Lys(Z)–Sar] did not show a detectable shift of the absorption maximum for the alkali and alkaline-

earth metal picrates investigated. The somewhat flexible linear dipeptides lose their flexibility on cyclization hence the ability of solvation toward picrate ion-pairs.

As can be seen in *Figure 2c*, poly[c-(N^{ε}-AcrLys–Sar)], which possesses cyclic dipeptide units in the side chain, red-shifted the absorption maximum for $BaPi_2$ only. It was considered that some of the cyclic dipeptide units distributed along the flexible chain of the vinyl polymer cooperated intramolecularly to solvate Ba^{2+}. Since the absorption maximum of $CaPi_2$ was not shifted by the same polymer, a selective intramolecular cooperation of cyclic dipeptide units may exist according to the size of the cation. The different degrees of red shift between $BaPi_2$ and KPi, which have nearly the same ionic diameter, may be explained in terms of the stronger solvation of the divalent cation than the monovalent cation or a participation of the picrate anion in the complexation.

In *Figure 2d*, the red shifts of the absorption maximum for metal picrates induced by the c-(N^{ε}-AcrLys–Sar)/styrene copolymer are shown. Among three fractions of the styrene copolymer having different molecular weights, there was no difference in the effect. In *Figure 2d*, the results on fraction 1 are shown. In the case of the copolymer, small degrees of red shift (less than 7 nm) were observed for alkali metal picrate. Furthermore, the extent of the red shift decreased in the order $Cs^+ > Rb^+ > K^+ > Na^+$, indicating that the larger the cation size, the stronger the solvation. Another feature is that the shift of the absorption maximum for alkali metal picrate occurred on adding a very small amount of styrene copolymer. It was shown above that in the present styrene copolymer, a triad sequence consisting of two styrene units and one c-(N^{ε}-AcrLys–Sar) unit is most prevalent. This type of sequence would form an arrangement of ligand groups suitable for the solvation of alkali metal ions and at the same time produce a selectivity for metal ions according to their size. Alternatively, the styrene copolymer solvates $BaPi_2$ and $CaPi_2$ more strongly than alkali metal picrates, but more copolymers were necessary to attain the final value of the red shift of $BaPi_2$ and $CaPi_2$ than the alkali metal picrates. Very little selectivity was observed between $BaPi_2$ and $CaPi_2$. It is plausible that the number of ligand groups necessary to solvate alkaline-earth metal ions is different from that for alkali metal ions and the mechanism of the intramolecular cooperation of ligand groups for solvation differs according to the nature of metal ions.

Comparison of *Figure 2c* with *Figure 2d* indicates the higher solvation ability of the styrene copolymer toward alkali metal picrates than the homopolymer of c-(N^{ε}-AcrLys–Sar). In our previous paper[3], it was reported that the homopolymer of c-(N^{ε}-Acr, $N^{\alpha,\varepsilon}$-Me_2Lys–Sar) is more efficient at solvating sodium picrate than the styrene copolymer. The styrene units in the copolymer enhances the solubility of the copolymer in CH_2Cl_2/THF (1:1 v/v) mixed solvent presumably by the suppression of hydrogen bonding of the ligand groups. The incorporation of styrene units into poly[c-N^{ε}-AcrLys–Sar)] seriously affects the solubility and the hydrogen bonding ability. However, the introduction of styrene units into poly[c-(N^{ε}-Acr, $N^{\alpha,\varepsilon}$-Me_2Lys–Sar)], which is very soluble in organic solvents and free from amide NH, does not have much influence. For this reason, the effect of styrene copolymerization on the intramolecular cooperation of peptide ligand groups might differ between the two types of copolymers.

The addition of ten molar equivalents of c-(N^{ε}-AcrLys–Sar)/4-vinylpyridine copolymer to a CH_2Cl_2/THF (1:1 v/v) solution of metal picrates yielded a precipitate. It was considered that the 4-vinylpyridine copolymer is highly affinitive toward metal salts but the solubility of the complexes in organic solvents is low.

Electric conductivity investigation. Since copolymers of c-(N^{ε}-AcrLys–Sar) with styrene or 4-vinylpyridine were found to form complexes with metal salts easily, the compositions of the complexes were determined by the electric conductivity method. The experimental results are shown in *Figures 3a–c*. The electrical conductivity of CH_2Cl_2/THF (1:1 v/v) solution containing only metal picrate was as low as 1–5 $\mu\,\Omega^{-1}$, which is shown as a zero conductivity in *Figures 3a–c*.

Figure 3a shows the relationship between the electrical conductivity of the CH_2Cl_2/THF (1:1 v/v) solution of metal picrate and the amount of added cyclic dipeptide c-[Lys(Z)–Sar]. With four kinds of alkali picrates, no turning point of the plot was observed until the molar ratio of cyclic peptide/metal salt reached 3. This result indicates that c-[Lys(Z)–Sar] cannot solvate metal picrates, which agrees well with the conclusion reached from u.v. spectroscopy (*Figure 2b*).

Figure 3b shows the relationship between the electrical conductivity of the CH_2Cl_2/THF (1:1 v/v) solution of metal picrate and the amount of c-(N^{ε}-AcrLys–Sar)/styrene copolymer added. A sudden change in the conductivity was observed in the solutions containing KPi, RbPi and CsPi, when two molar equivalent peptide ligand groups were added. It was therefore suggested that a complex having composition of cyclic dipeptide/metal salt in the ratio 2:1 was formed.

With NaPi some unusual behaviour was observed. In this case the formation of two kinds of complexes (1:1 and 2:1) was suggested and a peculiar mechanism of complexation may be operating. However, the small red-shift of the absorption maximum and the low conductivity prevented us from carrying out a detailed study.

Figure 3c shows the relationship between the electrical conductivity of the CH_2Cl_2/THF (1:1 v/v) solution of metal picrate and the amount of c-(N^{ε}-AcrLys–Sar)/4-vinylpyridine copolymer added. With KPi, RbPi and CsPi, the formation of a cyclic peptide/metal picrate (1:1) complex was suggested. The absorption intensity at 260 nm for the 4-vinylpyridine copolymer in CH_2Cl_2/THF (1:1 v/v) solution decreased on the addition of cesium picrate. This indicates the coordination of the pyridyl groups in the copolymer with caesium picrate. Therefore, it was suggested that a cyclic dipeptide/pyridyl group/metal picrate (1:1:1) complex was formed.

With NaPi, the formation of two kinds of complexes (1:1 and 2:1) was suggested, but a more detailed study was impossible, as in the case of the styrene copolymer, because of the low conductivity. In this case too, the participation of the pyridyl group in the complexation is evident, because the hypochromic effect of the pyridyl group was observed on mixing with NaPi.

Extraction investigation. Metal picrates were extracted from an aqueous solution into a chloroform solution by c-(N^{ε}-AcrLys–Sar)/4-vinylpyridine copolymer. The thermodynamic equilibrium constant K for the two-phase extraction was determined according to equation

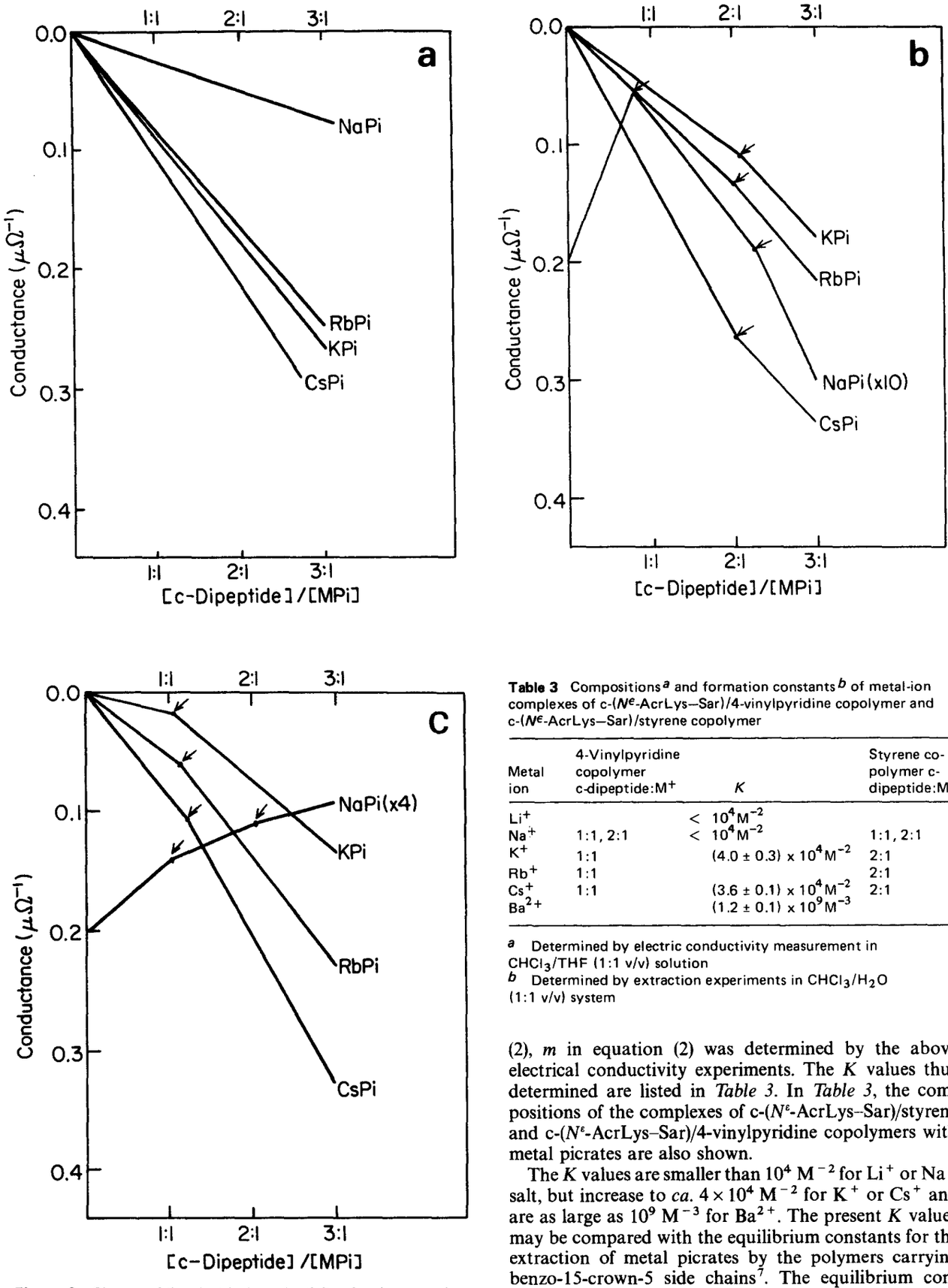

Figure 3 Change of the electrical conductivity of various metal picrates in $CHCl_3$/THF (1:1 v/v) solution by the addition of peptide ligand. Concentration of metal picrate, 3×10^{-5} M. (a) c-[Lys(Z)-Sar], (b) c-(*N*$^{\epsilon}$-AcrLys–Sar) styrene copolymer, (c) c-(*N*$^{\epsilon}$-AcrLys–Sar)/4-vinylpyridine copolymer

Table 3 Compositions[a] and formation constants[b] of metal-ion complexes of c-(*N*$^{\epsilon}$-AcrLys–Sar)/4-vinylpyridine copolymer and c-(*N*$^{\epsilon}$-AcrLys–Sar)/styrene copolymer

Metal ion	4-Vinylpyridine copolymer c-dipeptide:M^+	*K*	Styrene copolymer c-dipeptide:M^+
Li^+		$< 10^4 M^{-2}$	
Na^+	1:1, 2:1	$< 10^4 M^{-2}$	1:1, 2:1
K^+	1:1	$(4.0 \pm 0.3) \times 10^4 M^{-2}$	2:1
Rb^+	1:1		2:1
Cs^+	1:1	$(3.6 \pm 0.1) \times 10^4 M^{-2}$	2:1
Ba^{2+}		$(1.2 \pm 0.1) \times 10^9 M^{-3}$	

[a] Determined by electric conductivity measurement in $CHCl_3$/THF (1:1 v/v) solution
[b] Determined by extraction experiments in $CHCl_3/H_2O$ (1:1 v/v) system

(2), *m* in equation (2) was determined by the above electrical conductivity experiments. The *K* values thus determined are listed in *Table 3*. In *Table 3*, the compositions of the complexes of c-(*N*$^{\epsilon}$-AcrLys–Sar)/styrene and c-(*N*$^{\epsilon}$-AcrLys–Sar)/4-vinylpyridine copolymers with metal picrates are also shown.

The *K* values are smaller than 10^4 M^{-2} for Li^+ or Na^+ salt, but increase to *ca.* 4×10^4 M^{-2} for K^+ or Cs^+ and are as large as 10^9 M^{-3} for Ba^{2+}. The present *K* values may be compared with the equilibrium constants for the extraction of metal picrates by the polymers carrying benzo-15-crown-5 side chains[7]. The equilibrium constants are approximately the same order of magnitude for Li^+, Na^+, K^+ and Cs^+. However, the equilibrium constant of the extraction of Ba^{2+} by c-(*N*$^{\epsilon}$-AcrLys–Sar)/styrene copolymer is larger by *ca.* 10^2 fold than that

by the crown-ether polymer. Poly(4-vinylpyridine) alone does not show such complexation ability with these metal salts.

Complexation with dyestuff and α-amino acid

The interaction of the polymer, carrying cyclic dipeptides in the side chains, with methyl orange was investigated. By the addition of c-(*N*$^{\varepsilon}$-AcrLys–Sar)/*N*-dodecylacrylamide copolymer to the aqueous solution of methyl orange, an absorption maximum at 464 nm for the molecularly dispersed methyl orange[9] decreased and a broad absorption at lower wavelengths than 400 nm increased, where methyl orange molecules in a stacked form absorb[9], giving an isosbestic point at 417 nm. The spectral change indicates the formation of the hydrophobic associate of methyl orange induced by the addition of hydrophobic *N*-dodecylacrylamide copolymers. Such an associate was not formed by the addition of c-[Lys(Z)–Sar] or poly[c-(*N*$^{\varepsilon}$-AcrLys–Sar)].

Phenylalanine was extracted from an aqueous solution by the n-octanol solution of c-(*N*$^{\varepsilon}$-AcrLys–Sar)/styrene copolymer. With increasing concentration of the styrene copolymer in the n-octanol solution, the amount of phenylalanine transported from the aqueous phase to the n-octanol phase increased linearly. The transport of phenlalanine from the aqueous to the organic phase may be driven by the ion-dipole interaction between the zwitterionic α-amino acid and the peptide carbonyl groups of the styrene copolymer. D- and L-phenylalanines were extracted in nearly the same amount, so that an enantiomer-selective extraction by the chiral polymeric ligands was not attained.

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