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Solvent Extraction of Dansyl-Amino Acid Anions by Dicationic Extractants

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The extraction of dansyl-amino acid anions from an alkaline aqueous medium was studied by the use of dicationic anion-exchange extractants, polymethylenebis(trioctylphosphonium)s (abbreviated as C_nBPs; n=2—12). As for the extraction of dianionic species, such as dansyl-aspartic acid anion (Dns-Asp^{2-}) and dansyl-glutamic acid anion (Dns-Glu^{2-}), the dicationic extractants were greatly superior to a monocationic extractant, butyltrioctylphosphonium (BuMP). However, regarding the extraction of monoanionic species, such as dansyl-serine anion, dansyl-phenylalanine anion, and dansyl-valine anion, the extraction abilities of the dicationic extractants and the monocationic extractant were similar to one another. The extraction ability of dicationic extractants was remarkably dependent on the length of their bridge-chain, which connects the two ionic centers. For extractants having short bridge-chain (C_2BP—C_4BP), Dns-Asp^{2-} was more extracted than Dns-Glu^{2-}. On the contrary, for extractants with long bridge-chain (C_8BP—C_{12}BP), Dns-Glu^{2-} was more extracted than Dns-Asp^{2-}. The solvent effect on the extraction of Dns-Asp^{2-} and Dns-Glu^{2-} was also studied.

Solvent extraction using anion-exchange extractants (liquid anion-exchangers) is widely used in separation processes of analytical chemistry.[1] Not only the extraction of metal complex anions, but also that of organic anions, have been studied by many researchers. However, as for the extraction of organic anions, 1 : 1 ion-pair formation between an extractant and an extracted anion has been most extensively discussed. Few studies have been concerned with multi-point ion-pair formation within a complex. Most of the liquid anion-exchange extractants hitherto reported are monoonium salts, i.e., long-chain quaternary ammonium ions and protonated long-chain tertiary amines have been used.

In recent years, we have developed dicationic anion-exchange extractants, which are lipophilic diphosphonium ions bearing two cationic centers within a molecule.[2–5] In our previous paper,[6] the extraction of phthalate isomer dianions (phthalate, isophthalate, and terephthalate) from an alkaline aqueous medium was studied by the use of such dicationic extractants, polymethylenebis(trioctylphosphonium)s (abbreviated as C_nBPs; n=2—12; Fig. 1). Two-point ion-pair formation within a complex between the dicationic extractant and the dianionic species caused some peculiar phenomena.

In the present study we investigated the extraction of dansyl-amino acid anions by use of the dicationic extractants (C_nBPs) and a monocationic extractant, butyltrioctylphosphonium (BuMP). Dansyl-amino acids have frequently been used in the field of chromatography. However, the extraction of dansyl-amino acids from alkaline media has not yet been sufficiently studied in detail. Especially, regarding the extraction of dicarboxylates, Dns-Asp^{2-} and Dns-Glu^{2-} (Fig. 1), two-point ion-pair formation within a complex has not yet been studied.

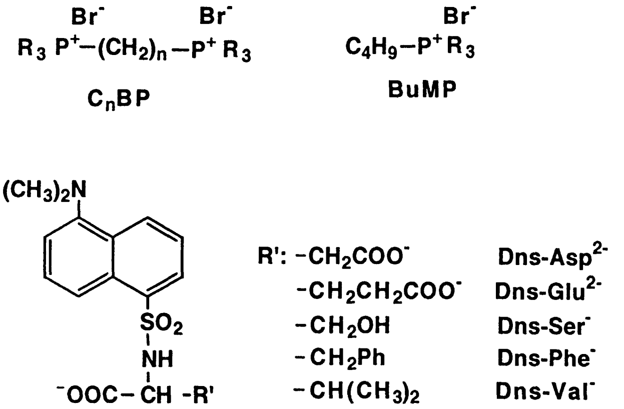

Fig. 1. Anion-exchange extractants, C_nBPs and BuMP (R=octyl; n=2—12), and dansyl-amino acid anions. The all carboxyl groups of dansyl-amino acids act as carboxylate anions under the relevant pH conditions (pH 10). Therefore, dansyl-aspartic acid, dansyl-glutamic acid, dansyl-serine, dansyl-phenylalanine, and dansyl-valine are abbreviated as Dns-Asp^{2-}, Dns-Glu^{2-}, Dns-Ser$^-$, Dns-Phe$^-$, and Dns-Val$^-$, respectively.

The effect of extracting solvent is one of the important factors in anion-exchange extractions. However, this effect has not yet been studied regarding the extractions using dicationic extractants. We found and analyzed a peculiar solvent effect when the dicationic extractants were used.

Experimental

Reagents. The synthesis of C_nBPs and BuMP was described in our previous paper.[5] Dansyl-amino acids (Sigma) and other chemicals were of reagent grade and used without further purification.

Extraction Procedure. An aqueous solution (10 ml) containing a 0.1 mM (1 M=1 mol dm^{-3}) dansyl-L-amino acid, sodium bromide, and 10 mM $Na_2B_4O_7$–NaOH buffer (pH 10) was shaken with an organic solution (10 ml) containing a 0.2 mM C_nBP (or 0.4 mM BuMP) in a stoppered centrifuge tube at 25 °C for 30 min. After phase separation, the concentration of the dansyl-amino acid anion remaining in the aqueous phase was determined by spectrophotometry using a Hitachi 228 instrument (λ_{max}=328 nm). The amount of the dansyl-amino acid anion extracted into the organic phase was taken as the difference between the amount initially added and that remaining in the aqueous phase after equilibrium. In some runs, the material balance on the distribution of dansyl-amino acid anions between the aqueous and organic phases was confirmed by back-extraction experiments.[2)]

Results and Discussion

Extraction of Dansyl-Amino Acids. The extraction of dansyl-amino acids by various anion-exchange extractants is summarized in Table 1. The concentration of the monocationic extractant (BuMP) initially added to the organic phase was twice that of the dicationic extractants (C_nBPs). Therefore, the cationic charge concentration in the aqueous phase was the same for both the monocationic and dicationic extractants.

It is reasonable to consider that the dansyl-derivatives of neutral amino acids (Dns-Ser$^-$, Dns-Phe$^-$, and Dns-Val$^-$) serve as monoanions under the relevant pH conditions (pH 10). When these dansyl-amino acids were extracted, the extraction abilities of the monocationic extractant (BuMP) and the dicationic extractants (C_4BP and C_{10}BP) were similar to each other.

However, when the dansyl-derivatives of acidic amino acids (Dns-Asp^{2-} and Dns-Glu^{2-}) were extracted, which serve as dianions, the extraction ability of the dicationic extractants greatly surpassed that of the monocationic extractant. These results suggest that the compatibility of the charge number between the extractant and the extracted anion greatly affects the extractability. We observed such a concept in the extraction of dianionic species including anionic metal complexes[2,4,5)] and phthalates;[6)] we call this concept "multiple charge compatibility."

A commercially available monocationic extractant, Capriquat (methyltrioctylammonium), showed a similar extraction behavior to that of BuMP in the extraction of the monoanionic dansyl-amino acids, as well as in the extraction of the dianionic dansyl-amino acids.

Table 1. % Extraction of Dansyl-Amino Acids[a)]

Dansyl-amino acid	[NaBr][b)] mM	Extractant BuMP	Extractant C_4BP	Extractant C_{10}BP
Dns-Asp^{2-}	1.2	3.9	57.3	26.2
Dns-Glu^{2-}	1.2	2.7	41.8	30.2
Dns-Ser$^-$	50	30.0	25.7	27.3
Dns-Phe$^-$	200	79.6	76.3	79.6
Dns-Val$^-$	200	44.3	46.7	39.1

a) Extracting Solvent: 1,2-dichloroethane. b) Sodium bromide was initially added in the aqueous phase.

Extraction Equilibrium of Dianionic Dansyl-Amino Acids. The extraction of dianionic dansyl-amino acids, Dns-Asp^{2-} and Dns-Glu^{2-}, was investigated in detail. One can assume that the extraction of the dianion (A^{2-}) by the dicationic extractant (C_nBP) involves the formation of a 1 : 1 ion-pair complex between C_nBP and A^{2-}. The reaction and the extraction constant (K_{ex}) can be defined as follows:

$$(Q\cdot Br_2)_o+(A^{2-})_w \rightleftarrows (Q\cdot A)_o+2(Br^-)_w,$$

and

$$K_{ex}=[Q\cdot A]_o[Br^-]_w^2/[Q\cdot Br_2]_o[A^{2-}]_w, \qquad (1)$$

where $Q\cdot Br_2$ represents C_nBP, and subscripts w and o denote the aqueous and organic phases, respectively. It can also be expressed logarithmically:

$$\log[Q\cdot A]_o/[Q\cdot Br_2]_o[A^{2-}]_w= \log K_{ex}-2\log[Br^-]_w. \qquad (2)$$

The $Q\cdot Br_2$ and the complex, $Q\cdot A$, are highly lipophilic and do not appreciably dissolve in the aqueous phase. Thus, the values of $\log[Q\cdot A]_o/[Q\cdot Br_2]_o[A^{2-}]_w$ and $\log[Br^-]_w$ in Eq. 2 can be easily obtained experimentally.

With the monocationic extractant (BuMP) calibrations were carried out in such a way that $[Q\cdot Br_2]_o$ and $[Q\cdot A]_o$ in Eq. 2 were taken as $2\times[BuMP^+\cdot Br^-]_o$ and $[(BuMP^+)_2\cdot A^{2-}]_o$, respectively.

Figure 2 shows a plot of $\log[Q\cdot A]_o/[Q\cdot Br_2]_o[A^{2-}]_w$

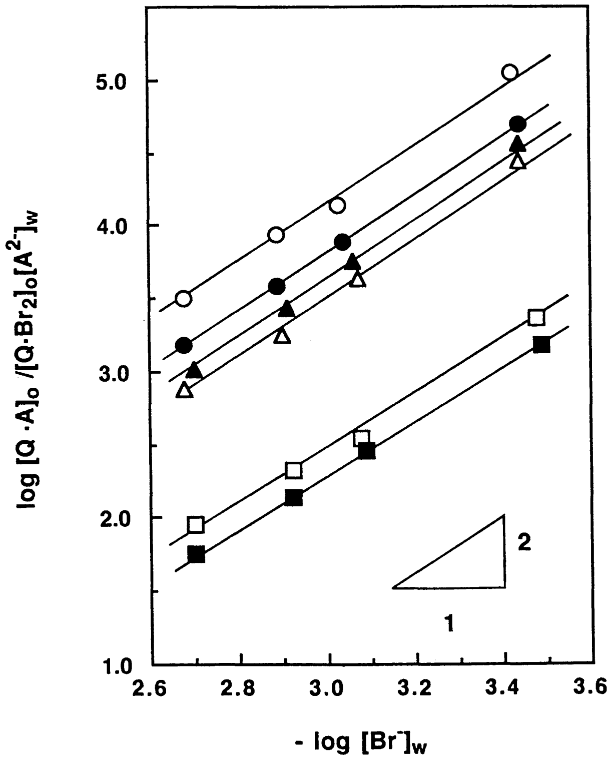

Fig. 2. Extraction of Dns-Asp^{2-} (○, C_4BP; △, C_{10}BP; □, BuMP) and Dns-Glu^{2-} (●, C_4BP; ▲, C_{10}BP; ■, BuMP) (extracting solvent: 1,2-dichloroethane).

against $-\log[\mathrm{Br}^-]_\mathrm{w}$ in the extractions of Dns-Asp^{2-} and Dns-Glu^{2-}; the extractants (C_4BP, C_{10}BP, and BuMP) were used and 1,2-dichloroethane was used as an extracting solvent (diluent). All of the plots in Fig. 2 are straight lines with a slope of 2. These results suggest that the extraction reaction proceeds via the formation of a 1 : 1 ion-pair complex between C_nBP^{2+} and A^{2-} (or a 2 : 1 complex between BuMP$^+$ and A^{2-}). These results again indicate that the dansyl-amino acids serve as dianions, so that the dissociation of the sulfonamide proton in dansyl-amino acids is negligibly small under the present conditions.

Figure 3 (a and b) shows graphical representations of the extraction constants ($\log K_\mathrm{ex}$) as a function of the length of "bridge-chain" of C_nBPs; the "bridge-chain" is defined as the bridging chain connecting the two ionic centers of the extractant (or extracted anion). The bridge-chain length greatly affects the extraction ability of C_nBPs, and the plots possess maximum values at n=4 for the Dns-Asp^{2-} extraction and around n=4—6 for the Dns-Glu^{2-} extraction. Therefore, such a extraction behavior cannot be explained simply in terms of the change in the lipophilicity of C_nBPs via the change in the length of the methylene unit. We have proposed a concept called "geometrical charge distribution compatibility" regarding the extraction of phthalates; the compatibility between the ionic center distance of the dicationic extractants and that of the dianionic phthalates has fundamental significance regarding the extractability of the phthalates.[6] Obviously, the concept is at work as well concerning the present dansyl-amino acid extractions.

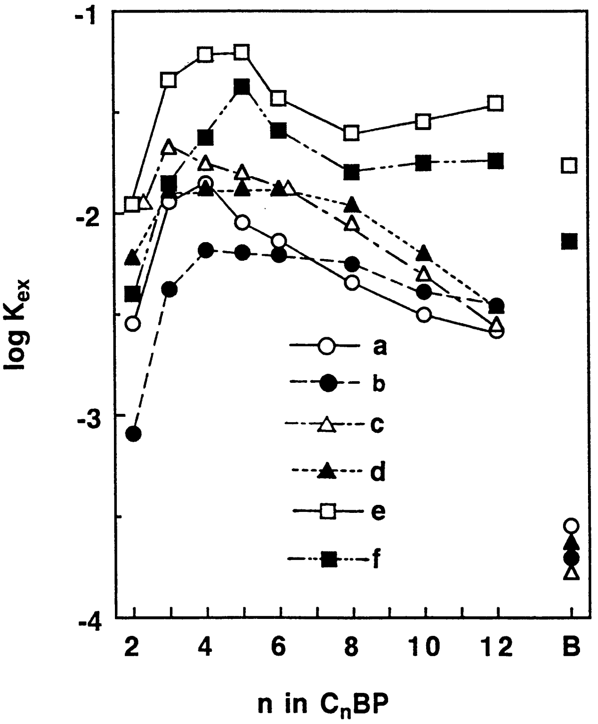

Fig. 3. Extraction constants ($\log K_\mathrm{ex}$) obtained in the cases of 1,2-dichloroethane (a, Dns-Asp^{2-}; b, Dns-Glu^{2-}), 1-pentanol (c, Dns-Asp^{2-}; d, Dns-Glu^{2-}), and toluene (e, Dns-Asp^{2-}; f, Dns-Glu^{2-}). B represents BuMP.

It is noted that Dns-Asp^{2-} was more extracted than Dns-Glu^{2-} in the cases of C_2BP—C_6BP, whereas conversely Dns-Glu^{2-} was more extracted in the cases of C_8BP—C_{12}BP. The bridge-chain of Dns-Glu^{2-} is longer by one methylene unit than that of Dns-Asp^{2-}. Therefore, the short bridge-chain extractants (the C_nBPs possessing short bridge-chain) prefer the Dns-Asp^{2-} extraction, whereas the long bridge-chain extractants prefer the Dns-Glu^{2-} extraction.

Extranction of Dianionic Dansyl-Amino Acids with Various Extracting Solvents. 1-Pentanol and toluene were used as extracting solvents instead of 1,2-dichloroethane. Figure 3 again indicates plots of $\log K_\mathrm{ex}$ vs n in C_nBP by the use of these solvents (Fig. 3, c—f). In all cases, the extraction equilibrium, Eq. 1, was confirmed to hold.

When 1-pentanol was used (Fig. 3, c and d), the order of the extractabilities of Dns-Asp^{2-} and Dns-Glu^{2-} was reversed at n=6; the short bridge-chain extractants preferred Dns-Asp^{2-} and the long bridge-chain extractants preferred Dns-Glu^{2-}. When 1-octanol was used, the extraction ability of the extractants was systematically larger than that in the extraction using 1-pentanol (see Table 2). However, the

Table 2. Extraction Constants ($\log K_\mathrm{ex}$) by Use of Various Extracting Solvents

Extracting solvent	ε[a]	Dns-Asp^{2-}			Dns-Glu^{2-}		
		BuMP	C_4BP	C_{10}BP	BuMP	C_4BP	C_{10}BP
Toluene	2.4	−1.76	−1.22	−1.54	−2.14	−1.62	−1.75
Chlorobenzene	5.6	−2.78	−1.51	−1.67	−3.09	−2.06	−1.78
o-Dichlorobenzene	9.9	−3.06	−1.49	−2.01	−3.45	−2.07	−2.15
1-Octanol	10.3	−3.06	−1.23	−1.45	−2.96	−1.37	−1.33
1,2-Dichloroethane	10.4	−3.55	−1.85	−2.51	−3.71	−2.17	−2.38
1-Pentanol	13.9	−3.78	−1.75	−2.29	−3.63	−1.88	−2.19
$\Delta \log K_\mathrm{ex}$[b]		2.02	0.53	0.75	1.49	0.26	0.44

a) Dielectric constant. b) $\Delta \log K_\mathrm{ex}$ is defined as the difference between the $\log K_\mathrm{ex}$ in the case of toluene and that in the case of 1-pentanol (see text).

extractability order of the dansyl-amino acids was similarly reversed at $n=6$.

When toluene was used (Fig. 3, e and f), the extractability of Dns-Asp^{2-} was greater than that of Dns-Glu^{2-} in all cases concerning the extractants. The uses of chlorobenzene and *o*-dichlorobenzene as extracting solvents resulted in a similar behavior regarding the extractability order of Dns-Asp^{2-} and Dns-Glu^{2-}; Dns-Asp^{2-} was more extracted than Dns-Glu^{2-}.

The extraction constants ($\log K_{ex}$), in terms of the use of various extracting solvents, are summarized in Table 2. The extraction ability of BuMP was remarkably dependent on the polarity of the extracting solvents. Low polar solvents, such as toluene, promote both the extractions of Dns-Asp^{2-} and Dns-Glu^{2-}. It is noted that when C$_4$BP was used as an extractant, the dependence of $\log K_{ex}$ on the polarity of the solvents became relatively small compared to the extractions by BuMP.

The value of $\Delta \log K_{ex}$ is defined as the difference between $\log K_{ex}$ in the case of toluene and that in the case of 1-pentanol; the former is the least polar solvent of all indicated in Table 2, while the latter is the most polar solvent. As shown in Table 2, the value of $\Delta \log K_{ex}$ decreased in the order BuMP$\gg$C$_{10}$BP$>$C$_4$BP in both Dns-Asp^{2-} and Dns-Glu^{2-} extractions.

"Multiple charge compatibility" can be at work when the dicationic extractants are used, and "geometrical charge distribution compatibility" can be more effectively at work in the case of C$_4$BP than in the case of C$_{10}$BP. Therefore, especially in the case of C$_4$BP, two-point ion-pair formation can be smoothly and compatibly carried out, so that the ion-pair is considered to be very tight. Thus, the instabilization for the ion-pair by polar solvents may be relaxed to a considerable extent, since the insertion of the solvent molecule between the cation and anion composing the complex is retarded. Such a situation leads to a lowering of the dependence of $\log K_{ex}$ on the polarity of the solvents in the case of C$_4$BP. The polarity and/or other solvent properties may complicatedly affect the stability of the ion-pair and, thus, the solvent effects cannot be elucidated sufficiently.

In conclusion, two-point ion-pair formation within a complex causes some unique phenomena in the extraction of Dns-Asp^{2-} and Dns-Glu^{2-}. The concepts presented in this study can be applied to chromatographic separation systems including ion-pair chromatography, and may offer a new possibility in the separation among dansyl-amino acids.

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