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Review

Micelles for signal enhancement and novel selectivity of dansyl amino acids

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Abstract

Fluorescence intensity of various chemical species is enhanced in the microenvironment provided by micelles. Parameters which affect fluorescence intensities are examined by using dansyl (Dns) amino acids as the probe. The retention behavior of Dns-amino acids in micellar LC is examined by using ion-exchange-induced stationary phases. The type and concentration of micellar agent and modifier ion as well as the concentration of acetonitrile in the mobile phase affect the retention and signal intensity of Dns-amino acids. The order of elution of Dns-amino acids obtained with the micellar mobile phase is very different from that observed in conventional reversed-phase LC. Fluorescence intensities of Dns-amino acids are enhanced by the micellar mobile phase. © 1997 Elsevier Science B.V.

Keywords: Reviews; Fluorescence detection; Amino acids

Contents

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1. Introduction

When a chemical species is positioned in a restricted space provided by cyclodextrins (CDs), micelles, vesicles and other microenvironment donors, its UV–visible absorption, fluorescence, chemiluminescence, phosphorescence, circular dichroism or nuclear magnetic resonance spectra often change. It is known that the fluorescence intensities of dansyl (Dns) amino acids [1] and polynuclear aromatic hydrocarbons (PAHs) [2–4] are enhanced when they are partitioned into micelles. The effects of various micellar systems on the spectrofluorimetric method for the determination of Dns-amino acids, drugs and other compounds have been assessed. Fluorescence enhancements with factors varying from 8 to 20 are achieved for Dns-amino acids compared to that in water alone [1].

The fluorescence intensities of niobium and tantalum complexes with several fluorimetric organic reagents can be enhanced by micelles [5]. Flavone derivatives such as morin and quecetin produce the more intense fluorescing reaction in a cationic micellar medium. Fluorescence determination of ultratraces of Nb (V) and Ta (V) have been demonstrated [5].

Fluorescence enhancement of PAHs is observed when the solution contains taurocholate (NaTC) micelles and metal cations such as Tb^{3+}, Eu^{3+} and Al^{3+} [6]. It is likely that the metal cation is coordinated to the negatively charged NaTC micelles. The resulting increase in structural rigidity and decrease in accessibility of the interior of the micelle to the surrounding aqueous solution should serve to decrease nonradiative deexcitation pathways of the bound PAHs, thereby increasing fluorescence quantum yield.

Room temperature sensitized biacetyl phosphorescence enhanced via molecular organization is observed for many aromatic compounds [7,8]. Micelles composed of sodium dodecyl sulfate (SDS) and a host such as β-CD organize the donor–acceptor pair in a small reaction volume producing a higher effective concentration of the acceptor seen by the donor, thus facilitating the triplet–triplet energy transfer reaction.

Alkyltrimethylammonium hydroxide surfactants have an ability to enhance the integrated light intensity observed from a lucigenin chemiluminescence reaction with biological reductants such as fructose, glucose, ascorbic acid and uric acid [9]. Hydroxide surfactants are superior to chloride surfactants with respect to signal enhancement.

The use of micellar mobile phase in reversed-phase LC (RPLC) has attracted a lot of interests because it generates different selectivities and causes signal enhancement [2,10–15].

Armstrong et al. [2] first demonstrated fluorescence enhancement of PAHs assisted by micellar SDS in RPLC and applied their micellar mobile phase system to phosphorescence detection.

Lopez-Lopez et al. [16] separated nine PAHs with baseline resolution using Brij-35 on octadecylsilica C_{18} and octylsilica columns and obtained better sensitivity than with an isocratic RPLC methanol–water method. The fluorescence enhancement for pyrene was at most 3.5. Hadjmohammadi and Fatemi [17] utilized the greater fluorescence of PAHs in SDS micelles to achieve lower detection limits than in conventional RPLC.

Determination of steroids in urine can be performed by micellar LC with detection by sensitized terbium fluorescence [18]. The SDS micelle provides both proximity between donor and acceptor and protection from quenching of terbium fluorescence by water. Detection limits down to 100 pg have been achieved for steroids with an α,β-unsaturated carbonyl group in the A-ring.

This chapter describes the fluorescence enhancement and novel selectivities of Dns-amino acids generated by using micellar mobile phases in LC. The effects of micelles on fluorescence enhancement and chromatographic behavior of Dns-amino acids have been reported [19,20].

2. Fluorescence enhancement of Dns-amino acids

2.1. Effect of surfactant concentration on fluorescence enhancement

The fluorescence intensity of Dns-amino acids is enhanced when they are placed in the microenvironment provided by micelles. It is therefore expected that the fluorescence intensity increases with increas-

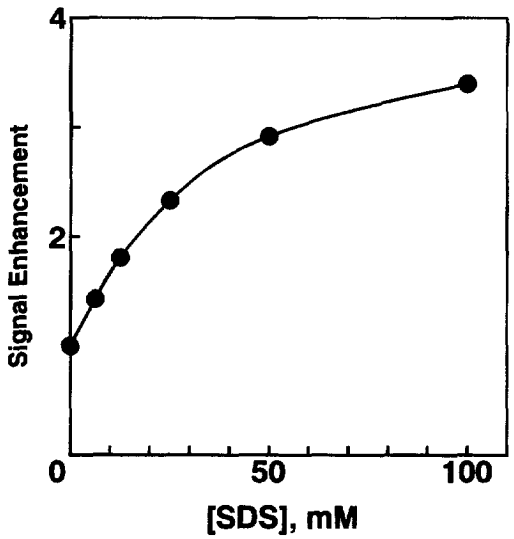

Fig. 1. Signal enhancement as a function of SDS concentration. Solutions, 40 μ*M* Dns-Phe dissolved in 0.8% (v/v) acetonitrile containing 40 m*M* ammonium acetate and SDS as indicated (pH 6.9); excitation wavelength, 335 nm; reproduced from Ref. [19] with permission of Elsevier Science B.V.

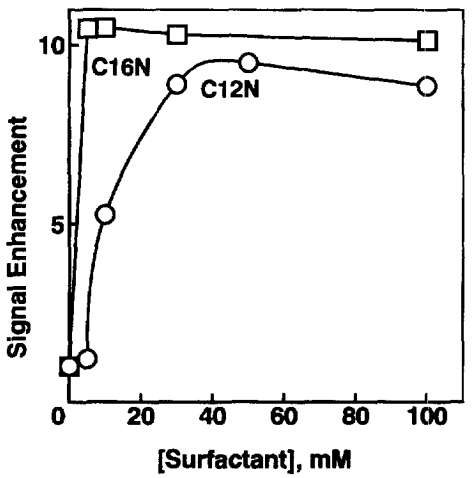

Fig. 2. Signal enhancement as a function of the concentration of cationic surfactants. Solutions, 40 μ*M* Dns-Phe dissolved in 0.8% (v/v) acetonitrile containing 40 m*M* ammonium acetate and $C_{12}N$ or $C_{16}N$ as indicated; excitation wavelength, 335 nm.

ing surfactant concentration. Fig. 1 illustrates the enhancement factor of Dns-Phe as a function of the concentration of anionic surfactant, SDS, at the acetonitrile concentration of 0.8% (v/v) [19]. The enhancement factor is defined here as the ratio of the fluorescence intensity observed in the presence of micelles to that without micelles. It is found that the enhancement factor increases with increasing SDS concentration in Fig. 1. This is because the concentration of micellar SDS increases with increasing SDS concentration over its critical micelle concentration (CMC). It is known that the CMC of SDS in water is around 8 m*M*, and no critical point in the correlation between signal enhancement and the SDS concentration appears around the CMC in Fig. 1. When the SDS concentration is 100 m*M*, the enhancement factor is 3.51 for Dns-Phe.

Cationic surfactants also enhance the fluorescence of Dns-amino acids. Fig. 2 shows the effect of the surfactant concentration on the enhancement factor of Dns-Phe in the case of hexadecyltrimethylammonium bromide ($C_{16}N$) and dodecyltrimethylammonium bromide ($C_{12}N$). The CMC values in water are around 4 m*M* and 1 m*M* for the former and the latter surfactant, respectively. It is seen from Figs. 1 and 2 that the enhancement factors achieved by the cationic surfactants are larger than those achieved by SDS. This may be related with the fact that Dns-Phe is anionic under the conditions in Figs. 1 and 2 and it is likely to undergo repulsion from the anionic surfactant micelle. Electrostatic interaction between analytes and surfactants can affect the partition mechanism and may lead to different profiles observed in Figs. 1 and 2.

2.2. Effect of alkyl chain length on fluorescence enhancement

The alkyl chain length affects the signal enhancement. Fig. 2 reveals that $C_{16}N$ achieves larger signal enhancement than $C_{12}N$. The effect of the alkyl chain length on the signal enhancement for alkyltrimethylammonium bromides is illustrated in Fig. 3. The concentration of each surfactant is 50 m*M*, which is larger than the CMC of each surfactant. It is seen that the enhancement factor increases with increasing chain length of the alkyltrimethylammonium type surfactants. This result may be because the longer the alkyl chain length, the more hydrophobic is the microenvironment provided by the micelle, the less quenching is caused by water.

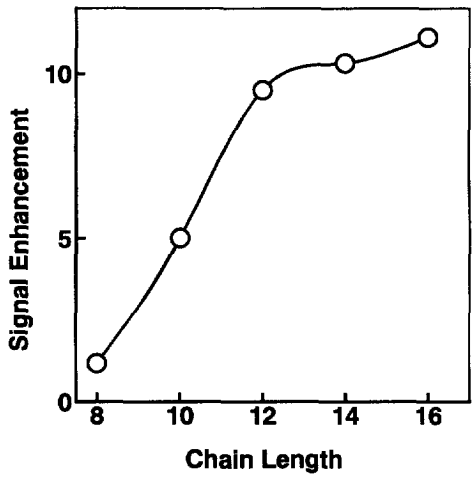

Fig. 3. Effect of the alkyl chain length of cationic surfactants on signal enhancement. Solutions, 40 μ*M* Dns-Phe dissolved in 0.8% (v/v) acetonitrile containing 40 m*M* ammonium acetate and 50 m*M* of each surfactant; excitation wavelength, 335 nm.

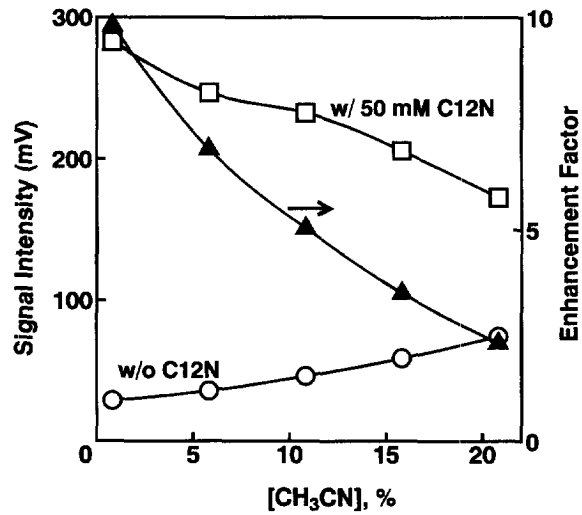

Fig. 4. Effect of acetonitrile concentration on the signal intensity and the signal enhancement. Analyte solutions, 40 μ*M* Dns-Phe dissolved in aqueous acetonitrile in 40 m*M* ammonium acetate with or without 50 m*M* $C_{12}N$; excitation wavelength, 335 nm; (□ and ○) signal intensity observed with or without 50 m*M* $C_{12}N$; (▲) signal enhancement.

2.3. Effect of organic solvent on fluorescence enhancement

The enhancement factor observed for Dns-amino acids is strongly affected by the acetonitrile concentration. The CMC of surfactants increases with increasing organic solvent concentration. In other words, the concentration of micellar surfactant decreases with increasing organic solvent concentration in case the total concentration of the surfactant is constant. It is therefore expected that the enhancement factor decreases with increasing acetonitrile concentration.

Fig. 4 shows the effect of acetonitrile concentration on the signal intensity and the signal enhancement of Dns-Phe. The analyte is dissolved in aqueous acetonitrile containing ammonium acetate in 40 m*M* and $C_{12}N$ as the micelle-forming agent. In the absence of $C_{12}N$, the signal intensity increases with increasing acetonitrile concentration. This phenomenon probably results because water quenches the fluorescence of Dns-amino acids [21]. On the other hand, when $C_{12}N$ micelles are present in the solution, the signal intensity of Dns-Phe decreases with increasing acetonitrile concentration. In this case, there exist two opposite contributions affecting the signal intensity. The increase in acetonitrile concentration increases the signal intensity of Dns-Phe, whereas the signal enhancement due to $C_{12}N$ micelles decreases with increasing acetonitrile concentrations. The latter contribution is larger, and the signal enhancement factor totally decreases with increasing acetonitrile concentration, leading to around 2 at ca. 20% (v/v). Nearly the same phenomenon is observed for SDS micelles [19].

2.4. Fluorescence enhancement of Dns-amino acids

Table 1 compares the enhancement factor achieved by SDS and $C_{14}N$ micelles. The shifted values of the maximum fluorescence wavelength, $\Delta\lambda_{max}$ caused by the presence of each surfactant are also listed in Table 1. The analyte solutions measured are 40 m*M* Dns-amino acids dissolved in 40 m*M* ammonium acetate containing 0.8% acetonitrile with or without each surfactant. The concentrations of SDS and $C_{14}N$ are 100 and 20 m*M*.

In the case of SDS, it is found that the largest enhancement factor is achieved for Dns-Arg, e.g., 3.88. For neutral and basic amino acids enhancement

Table 1
Signal enhancement and the shift of the maximum fluorescence wavelength for Dns-amino acids

Dns-amino acid	SDS		$C_{14}N$	
	$\Delta\lambda_{max}$ (nm)	Enhancement factor	$\Delta\lambda_{max}$ (nm)	Enhancement factor
αAB	8	2.44	9	10.2
Ala	6	2.16	12	11.3
Arg	12	3.88	14	4.5
Asn	8	2.05	10	9.3
Asp	2	1.08	12	10.6
Gln	6	2.08	8	8.9
Glu	0	1.08	14	11.0
Gly	–	–	10	11.3
Hyp	–	–	17	10.4
Ile	10	3.22	14	9.4
Leu	10	3.12	9	8.4
Lys	–	–	9	7.6
Met	9	2.65	10	8.4
Nleu	–	–	12	8.8
NVal	9	3.01	14	9.4
Phe	10	3.51	14	9.6
Pro	9	3.46	13	11.0
Ser	8	2.11	14	10.6
Thr	4	2.03	12	9.8
Trp	9	3.45	15	13.2
Val	8	2.65	11	9.4

Solution, 40 μ*M* each Dns amino acid dissolved in 40 m*M* ammonium acetate and 0.8% (v/v) acetonitrile with or without surfactant; concentrations of SDS and $C_{14}N$, 100 and 20 m*M*; excitation wavelength, 335 nm; adapted from Refs. [19,20].

factors between 2 and 3.5 are observed whereas almost no enhancement is observed for acidic amino acids. The latter result can be explained by the fact that SDS is anionic and the acidic amino acids undergo electrostatic repulsion from the SDS micelle. The maximum fluorescence wavelengths are sifted to shorter wavelengths on addition of SDS. The larger the signal enhancement, the larger wavelength shift is observed. The largest wavelength shift is observed for Dns-Arg, e.g., 12 nm.

On the other hand, in the case of $C_{14}N$, fluorescence enhancement by around 10 times is achieved for most of Dns-amino acids. Dns-Arg gives the lowest enhancement, which is because Dns-Arg with a positive charge may undergo electrostatic repulsion from the cationic micelle. Hypsochromic shifts by 4–17 nm are also observed in the presence of the $C_{14}N$ micelle. The enhancement factors achieved by $C_{14}N$ micelles are larger than those achieved by SDS micelles.

Fluorescence intensities of Dns-amino acids are also enhanced by non-ionic micelles such as Brij-35.

3. Separation of Dns-amino acids in the presence of micelles

3.1. Ion-exchange-induced stationary phase

As discussed above, it is expected that micelles in the mobile phase affect the retention time and the fluorescence intensity of analytes. The retention time is affected by chemical properties of both analytes and micellar agent. In particular, the selectivity strongly depends on the type of micelles when analytes are ionizable [10,12]. Ion-exchange-induced stationary phases are used for the separation of Dns-amino acids because it is difficult to find RPLC conditions using a micellar mobile phase for the separation of Dns-amino acids.

In micellar LC complex interactions between analytes and both the stationary and mobile phases are involved, and a three-phase model has been proposed to predict the retention factor (k) of non-ionic analytes [10,11]. When ionic surfactants are used, it becomes more complex to predict the

retention time of ionizable analytes due to additional electrostatic interactions with the surfactant [10,12]. The fact that monomeric surfactant can adsorb on hydrophobic stationary phases makes the retention behavior in micellar LC much more complex.

It is expected that ionic surfactants undergo repulsion from the stationary phase when their charge is the same as that of the stationary phase. In such a case both the micellar and monomeric surfactants are excluded from the stationary phase, leading to a simplified retention mechanism.

It has been reported that ion-exchange-induced stationary phases work in RPLC [19,22,23]. Separations of PAHs and Dns-amino acids have been demonstrated by using anion-exchangers modified with alkane sulfonate or alkyl sulfate [19,22] as well as by using cation-exchangers modified with alkyltrimethylammonium ions [23].

3.2. Selection of modifier ion and micellar agent [20]

Concentrations of modifier ion, micellar agent, and organic modifier affect the retention of Dns-amino acids. Table 2 lists the combinations of ion-exchanger, modifier ion and micellar agent. The length of the alkyl group of the modifier ion is restricted when ionic micellar agents are employed because of lower solubility of the paired ion formed between the modifier ion and the micellar agent.

The anion-exchange-induced stationary phase systems achieve separation of some Dns-amino acids and provide different retention behavior compared with that observed in common RPLC. On the contrary, the cation-exchange-induced stationary phase systems listed in Table 2 are not successful. When octyltrimethylammonium bromide (C_8N) and SDS are employed as the modifier and micellar agents, Dns-amino acids are not retained on the cation-exchange-induced stationary phase. On the other hand, although the cation-exchanger modified with $C_{12}N$ resolves Dns-Ala, Dns-αAB, Dns-Val and Dns-Leu in this order, the mobile phase containing micellar Brij-35 focuses the four peaks into a single peak, resulting in no resolution of these analytes.

Table 2
Combinations of ion-exchanger, modifier ion and micellar agent

Ion-exchanger	Modifier ion	Micellar agent
Anion-exchanger	hexane sulfonate	$C_{14}N$
Anion-exchanger	octane sulfonate	$C_{14}N$
Anion-exchanger	hexane sulfonate	Brij-35
Cation-exchanger	octyltrimethylammonium	SDS
Cation-exchanger	dodecyltrimethylammonium	Brij-35

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3.3. Effect of modifier ion concentration [20]

Fig. 5 shows the relationships between k of Dns-amino acids and modifier ion concentration for the anion-exchange-induced stationary phase systems. The mobile phase for the cationic micellar

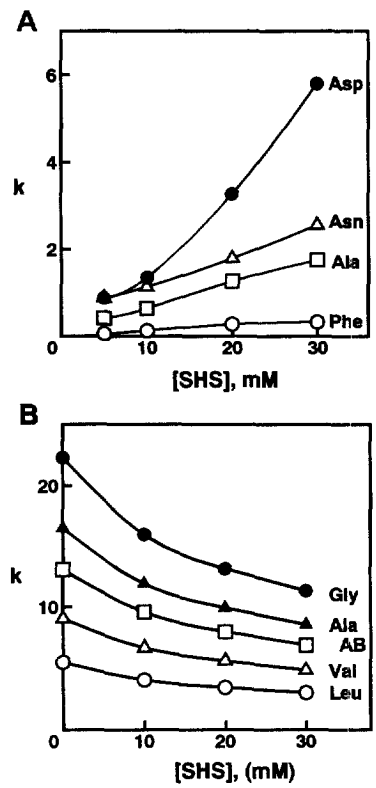

Fig. 5. Retention factor (k) versus SHS concentration in cationic and non-ionic micellar mobile phase system. Column, TSKgel IC-Anion SW (150×0.35 mm I.D.); mobile phases, 5% (v/v) acetonitrile in 30 mM $C_{14}N$, 40 mM ammonium acetate and SHS as indicated for A, 10% (v/v) acetonitrile in 40 mM Brij 35, 40 mM ammonium acetate and SHS as indicated for B; flow-rate, 2.8 μl min^{-1}; injection volume, 0.11 μl; reproduced from Ref. [20] with permission of Vieweg Publishing.

agent system (A) is composed of 5% (v/v) acetonitrile in 30 m*M* $C_{14}N$ and sodium hexane sulfonate (SHS), whereas 10% (v/v) acetonitrile containing Brij-35 in 40 m*M* and SHS is used for the non-ionic micellar agent system (B). It is found that k increases with increasing SHS concentration in the former case, whereas it decreases with increasing SHS concentration in the latter case. The former result is due to the fact that the concentration of the micellar $C_{14}N$ decreases with increasing SHS concentration owing to the formation of ion pairs between them, leading to the decrease in the eluting force. On the other hand, the latter result is due to the fact that hexane sulfonate ion competes with the analytes for the anion-exchange site and SHS does not interact with the non-ionic micellar agent under the conditions in Fig. 5B.

It is also seen in the Fig. 5A that Dns-Asp and Dns-Asn elute after neutral amino acids such as Dns-Ala and Dns-Phe. The elution order is opposite to that observed in common RPLC. In addition, the retention factors of Dns-amino acids achieved with sodium octane sulfonate as the modifier agent are larger than those achieved with SHS. This result indicates that the analytes also interact with the alkyl group of the modifier agent.

In the cationic micellar system, the peak areas of the Dns-amino acids slightly decrease with increasing SHS concentration, which is because the concentration of micellar $C_{14}N$ decreases with increasing SHS concentration. On the other hand, the dependence of the SHS concentration on the signal intensities is not recognized in the non-ionic micellar system, which can also be explained if we can assume that the concentration of micellar Brij-35 is not influenced by the SHS concentration.

In ion-pair chromatography or dynamic ion-exchange chromatography, the charge of the ion-pairing agent is opposite to that of analytes. On the contrary, when Brij-35 is used as the micellar agent (Fig. 5B), the charge of the modifier ion is the same as that of the analytes, i.e., both are negative and no ion pairing is involved. It is expected that the selectivity is therefore dominated by partitioning into Brij-35 micelles, and the elution order of the Dns-amino acids is opposite to that observed in reversed-phase LC. In the case of micellar $C_{14}N$ (Fig. 5A), it can also be concluded that partitioning of analyte into the micelle dominates the selectivity. The analytes may be able to form ion pairs with the monomeric cationic surfactant, but the hydrophobic interaction between the paired ions and the modified stationary phase with hexane sulfonate is not significant.

In addition, under the conditions in Fig. 5B in a non-ionic micellar mobile phase system dansyl derivatives of acidic amino acids can not be eluted from the column, whereas those of basic amino acids can be eluted. On the other hand, dansyl derivatives of acidic amino acids can also be eluted after basic and neutral amino acids under the conditions in Fig. 5A in cationic micellar mobile phase system. These results indicate that electrostatic interaction between acidic amino acids and anion-exchange sites are stronger than that for neutral and basic amino acids. It is also indicated that the charge of the surfactant affects the partitioning of the analytes into its micelle.

3.4. Effect of micellar agent concentration on the retention [20]

It is expected that the retention time of Dns-amino acids decreases with increasing micellar agent concentration when partitioning of the analytes into the micelle is a dominant factor in the retention. The retention factor is plotted as a function of $C_{14}N$ concentration in Fig. 6, where $C_{14}N$ dissolved in 5% (v/v) acetonitrile containing SHS in 20 m*M* and ammonium acetate in 40 m*M* is used as the mobile phase. As expected, k of the analytes decreases with increasing $C_{14}N$ concentration.

3.5. Effect of acetonitrile concentration [20]

Acetonitrile concentration affects the retention of Dns-amino acids in a complex manner. It is expected that the eluting force increases with increasing acetonitrile in RPLC. On the contrary, the concentration of micellar agent decreases with increasing acetonitrile concentration because the CMC of the surfactant increases with increasing acetonitrile concentration, which in turn reduces eluting force of the mobile phase. The above two opposite contributions which affect the retention of Dns-amino acids in a complex manner.

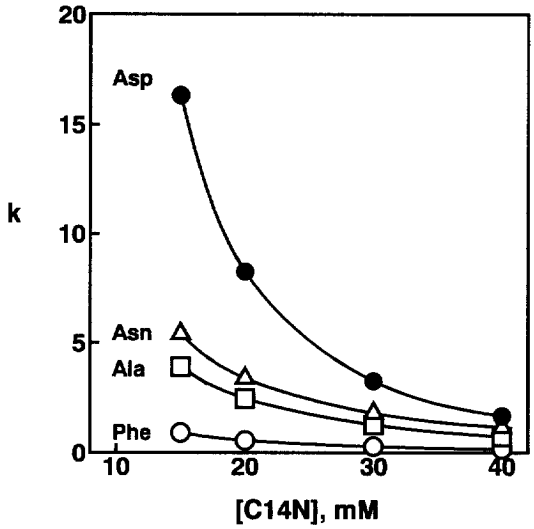

Fig. 6. k versus $C_{14}N$ concentration in cationic micellar mobile phase system. Mobile phase, aqueous acetonitrile (5%, v/v) in 20 mM SHS, 40 mM ammonium acetate and $C_{14}N$ as indicated; other operating conditions as in Fig. 5; reproduced from Ref. [20] with permission of Vieweg Publishing.

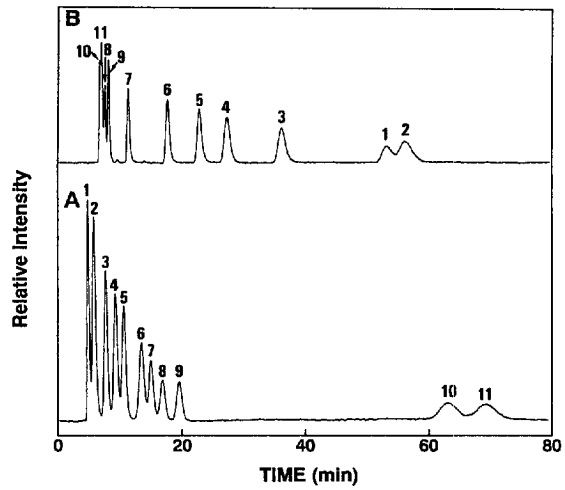

Fig. 7. Separation of 11 Dns-AAs in cationic micellar LC (A) and in the reversed-phase mode (B). Columns, 150×0.35 mm packed with TSKgel IC-Anion SW (A) and Develosil ODS-5 (B); mobile phases, 5% (v/v) acetonitrile in 30 mM SHS,15 mM $C_{14}N$ and 40 mM ammonium acetate (pH 6.9) for A, 28% (v/v) acetonitrile in 40 mM ammonium acetate (pH 7.2) for B; flow-rate: 2.8 μl min^{-1}; excitation wavelength, 335 nm; emission wavelengths, 520 nm (A) and 528 nm (B); analytes, 1=Dns-Trp, 2=Dns-Phe, 3=Dns-Ile, 4=Dns-NVal, 5=Dns-Val, 6=Dns-aAB, 7=Dns-Thr, 8=Dns-Hyp, 9=Dns-Gln, 10=Dns-Asp and 11=Dns-Glu; reproduced from Ref. [20] with permission of Vieweg Publishing.

In addition, the peak areas of the analytes slightly decrease with increasing acetonitrile concentration.

3.6. Separation of Dns-amino acids [20]

Fig. 7A demonstrates the isocratic separation of eleven Dns-amino acids using an anion-exchange-induced stationary phase and a micellar mobile phase. The chromatogram obtained in the reversed-phase mode using C_{18} as the stationary phase is also demonstrated in Fig. 7B for the comparison. The former separation is achieved by using 5% (v/v) acetonitrile in 15 mM $C_{14}N$, 20 mM SHS and 40 mM ammonium acetate (pH 6.9) is used as the mobile phase, whereas the latter is achieved by using 28% (v/v) acetonitrile in 40 mM ammonium acetate (pH 7.2) is used as the mobile phase. The emission wavelengths are 520 and 528 nm in the former and the latter separation, respectively. Different orders of elution are observed between the chromatograms in Fig. 7. Signal enhancement assisted by micellar $C_{14}N$ is not very remarkable under the conditions in Fig. 7, and the enhancement factor is at most 3.0 for Dns-Trp. The enhancement factor (3.0) achieved for Dns-Phe in Fig. 7 is much smaller than expected value (13.2) in Table 1. This is because higher acetonitrile and SHS concentrations as well as lower $C_{14}N$ concentration are selected as the conditions in Fig. 7 in comparison with those in Table 1, leading to the decrease in the enhancement factor in Fig. 7.

In the case of the $C_{14}N$ micellar system (Fig. 7A), the retention times of acidic amino acids such as Dns-Asp and Dns-Glu are much larger than those of other type of Dns-amino acids. This is due to electrostatic interaction between the acidic amino acids and the anion-exchanger. Basic amino acids such as Dns-Asn and Dns-Gln also give relatively larger retention times, whereas basic amino acids such as Dns-Arg and Dns-Lys give smaller retention times than Dns-Asn and Dns-Gln. This may be because the isoelectric point values of Dns-Arg and Dns-Lys are higher than those of Dns-Asn and Dns-Gln, and the former analytes undergo more repulsion from the anion-exchange site of the stationary phase, leading to the decrease in the retention time. As for neutral Dns-amino acids, the elution order observed

in the present micellar mobile phase system is generally opposite to that observed in the reversed-phase mode. It is clear that the selectivity for the neutral amino acids is dominated by partitioning into the $C_{14}N$ micelle.

In addition, when the mobile phase contained a modifier surfactant only, the elution order of the Dns-amino acids investigated was nearly the same as that observed in RPLC [19,23].

Isocratic separation of dansyl derivatives of neutral amino acids can be performed when the anion-exchanger is used as the stationary phase and 20% acetonitrile in 15 m*M* SHS, 50 m*M* Brij 35 and 40 m*M* ammonium acetate (pH 4.4) as the mobile phase. The elution order of dansyl derivatives of neutral amino acids achieved under this conditions is also opposite to that achieved in RPLC. On the contrary, dansyl derivatives of acidic amino acids are not eluted in a reasonable time, whereas those of basic amino acids can be eluted from the column.

3.7. Retention mechanism

Considering the above results, it can be concluded that Dns-amino acids are retained on the anion-exchanger dynamically modified with SHS by electrostatic interaction between the anion-exchange sites and the carboxylic group(s) of Dns-amino acids as well as by hydrophobic interaction between the alkyl group of the modifier agent and the analytes. Since the former interaction is stronger for dansyl derivatives of acidic amino acids than for other Dns-amino acids, longer retention times are observed for the former analytes. Selectivities of Dns-amino acids are dominated by partitioning into micelles. The partitioning into the micelle is supported by the fact that the fluorescence intensity is increased on addition of micellar agents in the mobile phase.

4. Abbreviations and symbols

C_8N	octyltrimethylammonium bromide
$C_{12}N$	dodecyltrimethylammonium bromide
$C_{14}N$	tetradecyltrimethylammonium bromide
$C_{16}N$	hexadecyltrimethylammonium bromide
C_{18}	octadecylsilica
CD	cyclodextrin
CMC	critical micelle concentration
Dns	dansyl
Dns-αAB	Dns-L-α-amino-*n*-butyric acid
Dns-Ala	Dns-L-alanine
Dns-Arg	Dns-L-arginine
Dns-Asn	Dns-L-asparagine
Dns-Asp	Dns-L-aspartic acid
Dns-Gln	Dns-L-glutamine
Dns-Glu	Dns-L-glutamic acid
Dns-Gly	Dns-glycine
Dns-Hyp	Dns-trans 4-hydroxy-L-proline
Dns-Ile	Dns-L-isoleucine
Dns-Leu	Dns-L-leucine
Dns-Lys	N^{ϵ}-Dns-L-lysine
Dns-Met	Dns-L-methionine
Dns-NLeu	Dns-norleucine (racemate)
Dns-NVal	Dns-L-norvaline
Dns-Phe	Dns-L-phenylalanine
Dns-Pro	Dns-L-proline
Dns-Ser	Dns-L-serine
Dns-Thr	Dns-L-threonine
Dns-Trp	N^{α}-Dns-L-tryptophan
Dns-Val	Dns-L-valine
NaTC	sodium taurocholate
PAHs	polynuclear aromatic hydrocarbons
RPLC	reversed-phase LC
SDS	sodium dodecyl sulfate
SHS	sodium hexane sulfonate
k	retention factor
λ_{max}	maximum fluorescence wavelength
$\Delta\lambda_{max}$	shift of maximum fluorescence wavelength

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