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Non-aqueous capillary zone electrophoresis using polyethylene glycol as a matrix agent

Yukihiro Esaka^{a,*}, Kuninori Yoshimura^a, Masashi Goto^a, Kenji Kano^b

^a*Gifu Pharmaceutical University, 5-6-1 Mitahora-higashi, Gifu 502-8585, Japan*

^b*Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan*

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Abstract

Non-aqueous capillary zone electrophoresis in acetonitrile containing polyethylene glycol as a matrix agent was investigated. Separation of benzoate anions as model samples were optimized by a control of the concentration and the selection of the chain length of polyethylene glycol. Base line resolution was attained in the presence of 15% (w/v) polyethylene glycol 4000 for the benzoates used. In acetonitrile, polyethylene glycol works as a hydrogen-bonding donor and acceptor; the terminal hydroxyl groups serve as a donor toward the dissociated carboxyl groups of the benzoates, while the ether oxygen atoms serve as an acceptor toward the hydrogen-donating substituents of the benzoates. This property of polyethylene glycol is in marked contrast with that observed in aqueous phase, where polyethylene glycol works predominantly as just a hydrogen acceptor. Quantitative analysis of the interaction is presented. © 1998 Elsevier Science B.V. All rights reserved.

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1. Introduction

One of the most interesting features of capillary electrophoresis (CE) is the homogeneous separation system, where the migration behavior reflects directly the interactions between analytes and running solution components involving matrix agents (or modifiers). Furthermore, several kinds of interactions can be incorporated into the CE systems to improve the separation just by dissolving appropriate matrix agents to running solutions. No expensive and specific columns are required and the magnitude of interactions can be controlled simply by changing the concentration of agents. Use of more than two kinds

of agents will be allowed in many cases. This means several different modes of interactions work simultaneously in the separation systems. Thus CE will be a very convenient separation method and researchers can design “made-to-order” CE systems for analyses of their analytes, of interest.

Development of separation methods would provide several advances in life science researches. Most biological molecules possess various hydrogen-bonding active functional groups, which often contribute to the molecular recognition and activation in organisms. Therefore, it would be valuable to introduce separation modes based on the hydrogen-bonding interaction to CE. The hydrogen-bonding interaction with chiral additives such as cyclodextrins, crown ethers, polysaccharides, macrocyclic antibiot-

*Corresponding author.

ics, surfactants and proteins has been employed in chiral separations of amino acids and pharmaceuticals [1–9], and the hydrogen-bonding interaction with additives contributes to the separation of achiral substances [10,11].

In our previous papers, hydrogen-bonding mode CE for separation of general organic analytes using polyethers as matrix agents was proposed and the significance of these separation systems for analyses of biological samples was demonstrated [12–15]. The polyether oxygen atoms of polyethylene glycol (PEG) serve as a hydrogen acceptor to form hydrogen-bonding complexes with hydrogen-donating analytes during the capillary zone electrophoretic (CZE) separations. The separation characteristics can be controlled in part by the PEG concentration [12,13]. Incorporation of non-ionic surfactants possessing polyether moieties as hydrophilic groups (Brij 35 and Tween 20) into an sodium dodecyl sulfate (SDS) system in micellar electrokinetic chromatography, (MEKC) was also found to cause hydrogen-bonding interaction between hydrogen-donating analytes and the polyether moieties, resulting in novel complexes selectivity controlled by the mixing ratio of SDS/Brij 35 or SDS/Tween 20 [14,15]. In those aqueous systems, the hydrogen-bonding interaction would work effectively with the assistance of the hydrophobicity of polyethers and/or micelles. In principle, however, the relative stabilization energy of the hydrogen-bonding interaction is larger in non-aqueous media than in aqueous media.

Recently, CE in non-aqueous systems has been investigated intensively and proved its high-performance [16–37]. The most interesting feature of non-aqueous CE will be such interactions of analytes with the solvents, electrolyte ions or matrix agents as those not encountered in aqueous solutions. Just a replacement of solvents causes a drastic change in the order migration of several inorganic anions [18] and also ammonium ions [19]. Ion-pairs formation in non-aqueous systems, especially in aprotic solvents like acetonitrile, is much stronger than in aqueous systems, resulting in remarkable change in the migration and separation behavior of inorganic ions and ionic surfactants [18,22,23]. Polycyclic aromatic hydrocarbons (PAHs) have been successfully separated in acetonitrile by electrokinetic chroma-

tography using two kinds of planar organic cations as carriers, where the charge transfer interaction between PAHs and the carrier cations is reported to contribute to the separation [31]. Non-ionic crown ethers and linear polyethers have been separated in methanol by the inclusion complex formation activity of the analytes with alkyl metal cations and ammonium ions as electrolytes and carriers [21]. Substituted phenols, benzoic acids and aryl alcohols have been separated in acetonitrile based on the heteroconjugated anion formation between the hydrogen-donating analytes and electrolyte anions [30]. The separation of anionic porphyrins has been achieved using a methanol running solution containing Brij 35 as a matrix agent, where dipole-dipole interactions between the carboxyl groups of the porphyrins and the polyether moiety of Brij 35 is proposed to be responsible for the separation [24,32]. Generally speaking, such electrostatic interaction effects would play important role in non-aqueous CE compared with aqueous systems.

In this work, we attempted to separate substituted benzoate anions using aprotic acetonitrile systems containing PEG. The migration behavior of the analytes in the acetonitrile systems will be compared with the aqueous systems reported previously. Furthermore, qualitative analysis of the interactions between the analytes and PEG in the separation systems will be detailed.

2. Experimental

2.1. Apparatus

All separations were performed on a laboratory-made system consisted of a Matsusada HCZE-30 PNO high-voltage power supply (Siga, Japan), a Jasco CE-970 detector (Tokyo, Japan) and a Shimadzu C-R6A Chromatopac integrator. Uncoated capillaries were obtained from GL Science (Tokyo, Japan), while polydimethylsiloxane-coated capillaries (μ -Sil DB-1) were obtained from J and W Science (CA, U.S.A). The capillaries have an internal diameter of 50 μ m and an outer diameter of 365 μ m. The total length of the capillaries was 50 cm, while the

effective length was 30 cm and 25 cm for the uncoated and coated ones, respectively.

2.2. Reagents and chemicals

Spectrum-grade acetonitrile was obtained from Nacalai Tesque (Kyoto, Japan). Three kinds of PEG with mean molecular masses of 400, 4000 and 20 000 (PEG 400, PEG 4000, PEG 20 000) were obtained from Kishida Chemical (Osaka, Japan). 4-Acetamidobenzoic acid (4CH₃CONH-BA), 4-aminobenzoic acid (4NH₂-BA), 4-hydroxybenzoic acid (4OH-BA), 4-acetoxybenzoic acid (4CH₃COO-BA), 4-toluic acid (4CH₃-BA), terephthalaldehydic acid (4CHO-BA), 4-dimethylaminobenzoic acid (4(CH₃)₂N-BA), 4-nitrobenzoic acid (4NO₂-BA), 2-phthalaldehydic acid (2CHO-BA), salicylic acid (2OH-BA), and benzoic acid (BA) were purchased from Nacalai Tesque. Tetrabutylammonium bromide (Bu₄NBr), tetrapropylammonium bromide (Pr₄NBr), tetrabutylammonium hydroxide (Bu₄NOH) and tetrapropylammonium hydroxide (Pr₄NOH) were obtained from Tokyo Chemical Industry (Tokyo, Japan). Tetrabutylammonium perchlorate (Bu₄NClO₄) and tetrapropylammonium perchlorate (Pr₄NClO₄) were precipitated by the addition of HClO₄ into aqueous solutions of Bu₄NBr and Pr₄NBr, respectively. The precipitants were recrystallized from appropriate solvents and used as electrolytes. All other chemicals were of analytical grade.

2.3. Procedure

The concentrations of the analytes in sample solutions were usually 0.02 mM each. At the beginning of daily experiments for non-aqueous systems, the uncoated capillary was rinsed using an aspirator with 0.1 M NaOH for 10 min, distilled water for 10 min and then acetonitrile for 10 min, while the coated capillary was rinsed with acetonitrile alone (or methanol for methanol systems) for 10 min. These capillaries were further rinsed for 1 min with running electrolyte solutions to be used before each run. All samples were injected on the anodic side for the non-coated capillary and the cathodic side for the coated capillary at a height of 10 cm for 10–15 s.

The applied voltage was fixed at 15 kV (unless otherwise specified), where the current was 3–7 μA for acetonitrile systems and 1–3 μA for methanol systems depending on the concentration of PEG. As non-aqueous running solutions, acetonitrile containing 5.7 mM Bu₄NClO₄+0.16 mM Bu₄NOH or methanol containing 5.7 mM Pr₄NClO₄+0.16 mM Pr₄NOH were used, while 10 mM phosphate buffer (pH=7.9) was used as an aqueous running solution. Appropriate amount of PEG was added to these running solutions.

3. Results and discussion

3.1. CZE separation of benzoates in acetonitrile solution

Fig. 1 shows electropherograms of nine benzoic acids obtained in (A) pH=7.9 aqueous buffer and (B) basic acetonitrile solution. Better separation was achieved in acetonitrile solution. This is in part ascribed to longer separation time in the acetonitrile solution compared with the aqueous solutions. In the aqueous solution, the carboxyl groups of all the benzoic acids are completely dissociated and they have an univalent negative charge. In the basic acetonitrile solution also, it is concluded that the carboxyl groups of all the benzoic acids are completely dissociated, since values of the electrophoretic mobility (m_{ep}) of the analytes was practically independent of the Bu₄NOH concentration in the range from 0.08 mM to 0.64 mM. With respect to 2OH-BA which gave the largest migration time among the benzoates used (Fig. 2), undissociation of the phenolic OH group in the basic acetonitrile solutions was also verified spectroscopically; no absorption peak at the longer wavelength than 300 nm characteristics of the dissociated phenolic OH group was observed on the addition of at least up to 8 mM (50 times excess) of Bu₄NOH. Thus the difference in the degree of the dissociation of the analytes is not responsible for the improvement of their migration behavior in the acetonitrile solution. Table 1 summarizes m_{ep} values of the benzoates in the aqueous and acetonitrile solutions. The $|m_{ep}|$

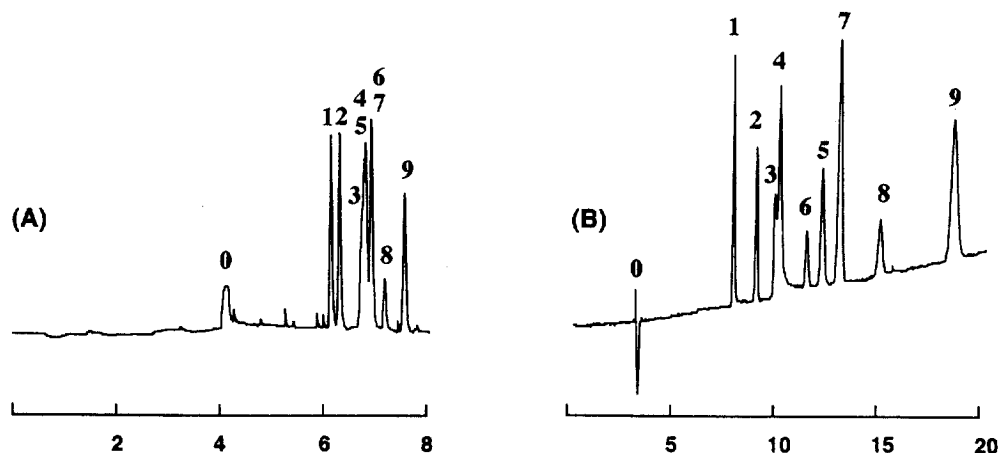


Fig. 1. CZE separations of benzoates in (A) 10 mM phosphate buffer (pH=7.9) and (B) acetonitrile solutions containing 5.7 mM $(n\text{-Bu})_4\text{NClO}_4$ and 0.16 mM $(n\text{-Bu})_4\text{NOH}$. Applied voltage [operating current]: (A) 14 kV [7 μA] and (B) 15 kV [4 μA]. Capillary (uncoated): 50 cm (effective length 30 cm) \times 50 μm I.D. Peaks: 0=mesityl oxide (a marker of electrophoretic flow); 1=4CH₃CONH-BA; 2=4CH₃COO-BA; 3=4OH-BA; 4=4NH₂-BA; 5=4CH₃-BA; 6=4CHO-BA; 7=2CHO-BA; 8= BA; 9=2OH-BA. time scale in min.

values in the acetonitrile solution were more than two times as large as those in the aqueous solution. This is reasonably ascribed to a lower viscosity (η) of acetonitrile than that of water (ratio of η between

water and acetonitrile being 2.7 at 25°C [39]), as expected from Eq. (1) [38]:

$$m_{ep} = q/6\pi\eta R \quad (1)$$

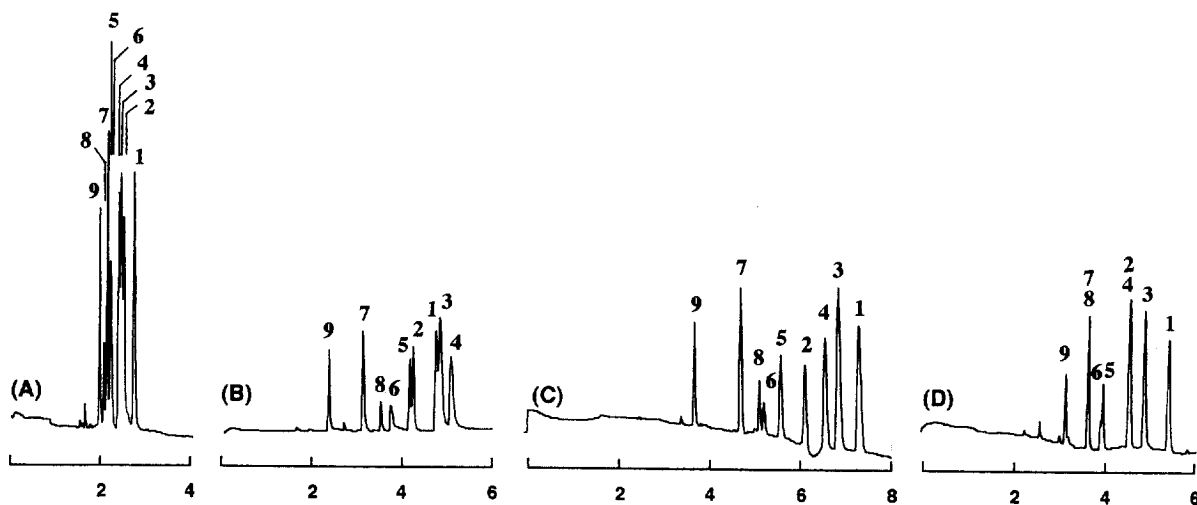


Fig. 2. Electropherogram of benzoates (A) in the absence of and the presence of (B) PEG 400, (C) PEG 4000 and (D) PEG 20 000 in acetonitrile solution. Solution compositions: (A) 5.7 mM $(n\text{-Bu})_4\text{NClO}_4$ + 0.16 mM $(n\text{-Bu})_4\text{NOH}$; (B) 5.5 mM $(n\text{-Bu})_4\text{NClO}_4$ + 0.15 mM $(n\text{-Bu})_4\text{NOH}$ + 3% (w/v) PEG 400; (C) 4.8 mM $(n\text{-Bu})_4\text{NClO}_4$ + 0.14 mM $(n\text{-Bu})_4\text{NOH}$ + 15% (w/v) PEG 4000; (D) 4.8 mM $(n\text{-Bu})_4\text{NClO}_4$ + 0.14 mM $(n\text{-Bu})_4\text{NOH}$ + 15% (w/v) PEG 20 000. Capillary (coated; $\mu\text{-sil DB-1}$): 50 cm (effective length 25 cm) \times 50 μm I.D. Applied voltage = 15 kV. Current = (A) 4 μA ; (B) 4 μA ; (C) 3 μA ; (D) 2 μA . Injection: on the cathodic side. Peaks: 1=4CH₃CONH-BA; 2=4CH₃COO-BA; 3=4OH-BA; 4=4NH₂-BA; 5=4CH₃-BA; 6=4CHO-BA; 7=2CHO-BA; 8= BA; 9=2OH-BA

Table 1

Electrophoretic mobilities (m_{ep}) of analytes in the aqueous solution and the acetonitrile solution.

	$m_{ep}/\text{cm}^2 \text{ kV}^{-1} \text{ min}^{-1}$	
	In the aqueous solution	In the acetonitrile solutions
4CH ₃ CONH-BA	-12.20	-25.87
4CH ₃ COO-BA	-12.78	-28.05
4OH-BA	-14.20	-29.44
4NH ₂ -BA	-14.48	-29.85
4CH ₃ -BA	-14.53	-32.32
4CHO-BA	-14.85	-31.49
2CHO-BA	-14.85	-33.12
benzoic acid (BA)	-15.66	-34.53
2OH-BA	-16.72	-35.87

where q and R represent the charge and the hydrodynamic radius of analytes, respectively.

3.2. Separation of benzoates in acetonitrile solution containing PEG

In view of further improvements of CZE separation in acetonitrile, we attempted to use PEG as a modifier. However, serious interference was observed due to the adsorption of PEG 4000 or PEG 20 000 on the inner wall of the uncoated capillary, though PEG 400 could be used at the concentration at least up to 20% (w/v). In order to avoid such adsorptive interference, we employed a capillary coated with hydrophobic polydimethylsiloxane in the following experiments. Fig. 2 represents electropherograms of nine benzoate anions (A) in the absence and (B) presence of 3% (w/v) PEG 400, (C) 15% (w/v) PEG 4000 and (D) 15% (w/v) PEG 20 000. In the absence of PEG, the anionic analytes migrated at large net-velocity toward the anodic side because of the large m_{ep} (to the anodic side) and the extremely small rate of the electroosmotic flow (to the cathodic side) in the coated capillary (Fig. 2A). Under the conditions, the analysis time was reduced, but the resolution was somewhat sacrificed, as judged by comparison to the electropherogram using the uncoated capillary (Fig. 1B). The separation was greatly improved by the addition of PEG, especially PEG 4000, where a baseline separation was almost attained within 8 min for the benzoates used.

One of the advantages of the use of the acetonitrile system is a higher solubility of PEG; for PEG

20 000, a limiting concentration was as high as 30% (w/v) in acetonitrile system, while it was 5% (w/v) in aqueous system. Such a wide range of the available concentration of PEG would make the system optimization easy. Mixing of two kinds of PEGs might be also useful in the acetonitrile system. For example, by using 2% (w/v) PEG 400 and 15% (w/v) PEG 20 000 as modifiers, electropherograms with such resolution as in Fig. 2C were obtained (data not shown).

The change of the migration behavior on the addition of PEG is ascribed to the interaction of the analytes with PEG as well as the change in the viscosity. PEG migrates at a rate of the electroosmotic flow even in acetonitrile, as evidenced by the result that PEG 400 can be detected (at 195 nm) at the time of the electroosmotic peak in experiments using a uncoated capillary (data not shown). This means that no significant interaction occurs between PEG and the electrolytes in acetonitrile and suggests that in the coated capillary the migration rate of PEG is very close to zero. Therefore, the interaction of PEG with the analytes causes a decrease in the electrophoretic mobility. From such a point of view, it is interesting that 2OH-BA (peak 9) migrates first among the benzoates with hydrogen-donating groups, but the other ones [4CH₃CONH-BA (peak 1), 4OH-BA (peak 3) and 4NH₂-BA (peak 4)] migrate much slowly (Fig. 2B-D). That is, the interaction between 2OH-BA and PEG is very weak. The behavior is in marked contrast with that observed in aqueous phase, where 2OH-BA interacts strongly with PEG as well as the other hydrogen-

donating analytes [12,13]. In the case of 2OH-BA, a strong intramolecular hydrogen-bonding formation is expected between the C(2)-OH and C(1)-COO⁻ groups in non-aqueous phase. For the other *para*-substituted benzoates, such an intramolecular hydrogen-bonding formation is not expected. This seems to be the reason of the weak interaction of 2OH-BA with PEG in acetonitrile.

3.3. Analysis of the interactions between the benzoates and PEG

As shown in Fig. 2, the kind of PEG used is also a factor to determine the migration order of the benzoates. Thus we attempted to evaluate the strength of the interaction between PEGs and analytes as a complex formation constant (K). Assuming a stoichiometric interaction between PEG and the analytes, the electrophoretic mobility (m_{ep}) is expressed as a function of the concentration of PEG [12]:

$$m_{ep} = m_{ep,f} + K(m_{ep,c} - m_{ep,f})[\text{PEG}] \quad (2)$$

where the subscripts c and f denote complexed and free analytes, respectively. In order to eliminate the effect on m_{ep} arising from the change in the viscosity, we will define $m_{ep}/m_{ep,o}$ as a relative value of m_{ep} against the electrophoretic mobility of a reference compound with $K \cong 0$ ($m_{ep,o}$). Thus Eq. (1) can be rewritten as:

$$\begin{aligned} m_{ep}/m_{ep,o} = m_{ep,f}/m_{ep,o} \\ + K[(m_{ep,c} - m_{ep,f})/m_{ep,o}][\text{PEG}] \quad (3) \end{aligned}$$

In the present work, 2OH-BA was chosen as a reference compound because the interaction with PEG in the acetonitrile systems was very weak compared with the other benzoates, as described above.

Fig. 3 shows the dependence of $m_{ep}/m_{ep,o}$ on the concentration of PEG 4000. From this result, it can be concluded that the optimum concentration of PEG 4000 is in the range from 10 to 20% (w/v) for the separation. An example of the electropherograms under such conditions is given in Fig. 2C. As predicted from Eq. (3), the $m_{ep}/m_{ep,o}$ values decreased linearly with the PEG concentration up to ca. 10% (w/v). Thus K values were evaluated from the

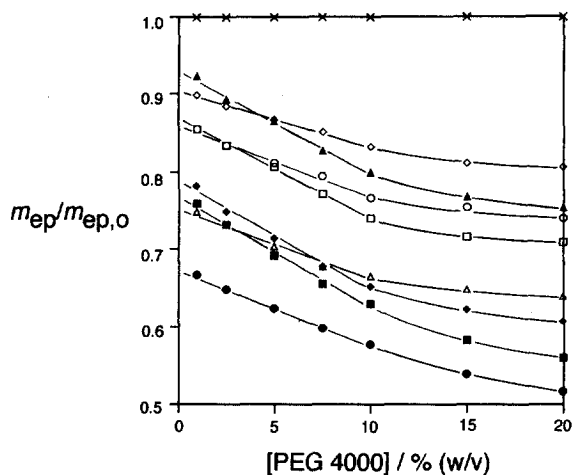


Fig. 3. Relative electrophoretic mobility ($m_{ep}/m_{ep,o}$) as a function of the concentration of PEG 4000. Symbols: \times = 2OH-BA, \blacktriangle = BA, \diamond = 2CHO-BA, \circ = 4CHO-BA, \square = 4CH₃-BA, \triangle = 4CH₃COO-BA, \blacklozenge = 4NH₂-BA, \blacksquare = 4OH-BA, \bullet = 4CH₃CONH-BA. See text for the definition of $m_{ep}/m_{ep,o}$.

slopes of the linear part of the $m_{ep}/m_{ep,o}$ versus the PEG concentration profiles by assuming that $m_{ep,c} - m_{ep,f} \cong -m_{ep,f}$; this assumption would be reasonable for neutral PEG with sufficiently large hydrodynamic size.

Table 2 summarizes the K values of ten benzoates for PEG 400, PEG 4000 and PEG 20 000 in the acetonitrile solutions as well as PEG 400 in aqueous solution. As discussed in previous papers [12,13], the order of magnitude of the K values in aqueous solutions is dominated by the hydrogen-bonding acidity of substituents and also in part the hydrophobicity of the benzoates. Similar tendency of the interaction was observed for PEG 20 000 in the acetonitrile system, except 2OH-BA. This result can be fundamentally interpreted in terms of the hydrogen-bonding interaction between the substituents of the analytes (as donor) and the ether oxygen atoms of PEG (as acceptor).

With regard to the PEG 400-containing acetonitrile system, however, benzoates with substituents of very weak hydrogen-bonding acidity such as 4(CH₃)₂N-BA, 4CH₃-BA and BA gave K values comparable to those of 4NH₂-BA, 4OH-BA, and 4CH₃CONH-BA. The order of magnitude of the K values in the PEG 400-containing acetonitrile system is consistent with the order of basicity of the

Table 2
Estimated values of complex formation constants (K)

Samples	$K \times 10^3 / [\text{additives}]^a$					
	In aq. solution ^b	In acetonitrile solution ^c				In methanol solution ^c
	PEG 400	PEG 400	PEG 4000	PEG 20 000	<i>n</i> -Nonanol	PEG 400
4OH-BA	10.0	118	18.9	13.0	29.3	18.8
2OH-BA	9.7	0 ^c	0 ^c	0 ^c	0 ^c	0 ^c
4CH ₃ CONH-BA	9.5	103	14.6	13.0	28.0	12.6
4NH ₂ -BA	6.6	127	18.1	9.8	31.9	31.7
4CH ₃ -BA	7.0	113	14.6	6.0	30.5	9.5
4CH ₃ COO-BA	4.8	91	9.8	6.8	24.1	7.6
4CHO-BA	4.5	67	11.0	6.0	22.9	-0.2
benzoate anion (BA)	2.7	99	14.5	5.9	27.6	8.0
2CHO-BA	0 ^b	54	8.0	4.2	18.7	4.2
4(CH ₃) ₂ N-BA	–	127	17.3	6.8	33.2	30.1
4NO ₂ -BA	–	57	7.5	5.1	20.1	1.1

^a [additives] in % (w/v).

^{b,c} These values were estimated using 2CHO-BA and 2OH-BA, respectively.

benzoates. In order to verify the point, the logarithmic values of K in the PEG 400 containing the acetonitrile system were plotted against the Hammett σ values of the benzoic acids [40,41] in Fig. 4 (symbol ●). The regression result was: $\log K = -0.162\sigma + 2.00$ with a correlation coefficient of 0.911. In contrast, such a linear relation was not observed in the aqueous system (Fig. 4, symbol ▲).

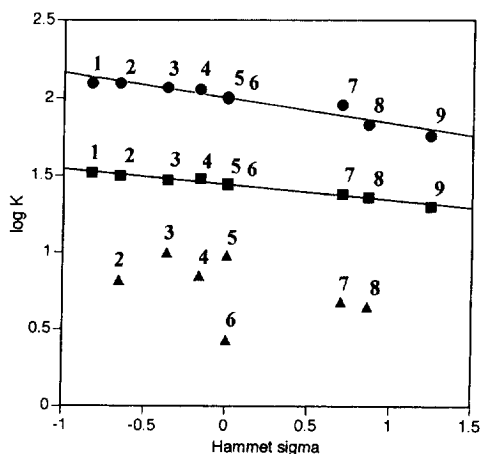


Fig. 4. Correlation between $\log K$ and Hammett σ in (●) the PEG 400 containing acetonitrile system, (■) the *n*-nonanol containing acetonitrile system, and (▲) the PEG 400 containing aqueous system. Compound: 1=4(CH₃)₂N-BA; 2=4NH₂-BA; 3=4OH-BA; 4=4CH₃-BA; 5=4CH₃CONH-BA; 6=BA; 7=4CHO-BA; 8=4CH₃COO-BA; 9=4NO₂-BA.

These results strongly suggest that the terminal OH groups of PEG serve as a hydrogen donor to interact with the COO⁻ groups of the benzoates in acetonitrile. In aqueous phase, the anionic charge of benzoates is sufficiently neutralized by the strong solvation, and then such an effect would not be observed. Similar electrostatic interaction has been reported in CZE separation of phenols, benzoic acids and alcohols in acetonitrile [30], where the neutral analytes interact with inorganic anions (even perchlorate ions) in the running solution to form heteroconjugated anions.

For further insight into the function of the terminal OH groups of PEG, *n*-nonanol was used in place of PEG in the acetonitrile system, since *n*-nonanol has no polyether moiety. Values of K for the interaction between *n*-nonanol and the benzoates were also evaluated as described above (Table 2), though there might exist a systematic underestimation arising from the assumption that $m_{ep,c} - m_{ep,f} \cong -m_{ep,f}$; $m_{ep,c}$ for *n*-nonanol system could not be assumed to be zero due to a relatively small hydrodynamic size. The logarithmic values of the evaluated K were well correlated with the Hammett σ values, as in the case of PEG 400 (Fig. 4, symbol ■). The regression was: $\log K = -0.101\sigma + 1.44$ with a correlation coefficient of 0.974. This result well supports our proposal concerning the interaction.

PEG 400 has relatively large amount of the

terminal OH groups per unit mass compared with highly polymerized PEGs; PEG 400 contains 50 times larger amount of the terminal OH groups per unit mass than PEG 20 000. Thus such a property of PEG as a hydrogen donor is predominantly observed for the PEG 400-containing acetonitrile system. In the case of PEG 20 000, the property of the polyether moiety as a hydrogen acceptor becomes predominant, as in the aqueous system.

It is also noteworthy that PEG 400 gave about ten times larger values of K than PEG 20 000 in the acetonitrile systems (Table 2). This can be qualitatively interpreted as follows. The dissociated "anionic" carboxyl groups is much stronger hydrogen acceptor than the "non-anionic" ether oxygen atoms of PEG. Then it is reasonably expected the hydrogen-bonding interaction between the COO^- group of benzoates and the terminal OH group of PEG is stronger than that between the ether oxygen atoms of PEG and the hydrogen-donating substituent of benzoates.

We also examined methanol in place of acetonitrile to investigate the effect of solvent; methanol is a protic non-aqueous solvent and methanol molecules are expected to work as a hydrogen donor and acceptor like water molecules. The results are summarized in Table 2 as K values for the interaction between PEG 400 and the benzoates. The K values in the methanol system are much smaller than those in the acetonitrile system. This indicates that the hydrogen-bonding interaction between the COO^- groups of the benzoates and the terminal OH groups of PEG is weakened remarkably by methanol molecules, although it would not be completely suppressed. This result also supports the significance of the hydrogen-bonding interaction in non-polar solvent.

As a result, the interaction between PEG and benzoates may be understood as follows using 4OH-BA as a model analyte. Dissociated 4OH-BA is unstable in non-polar acetonitrile and then the COO^- group interacts relatively strongly with the terminal OH group of PEG. In addition, the phenolic OH group of 4OH-BA can also interact with the ether oxygen atoms of PEG at a weaker strength. In other words, PEG can work as hydrogen donor and acceptor in acetonitrile. The overall function of PEG would depend on the degree of the polymerization of PEG as well as the characteristics of analytes.

In the aqueous systems, both of the COO^- group of 4OH-BA and the terminal OH groups of PEG is solvated by water molecules, a strong hydrogen donor and acceptor. Then they would scarcely interact with each other. However, the phenolic OH group of 4OH-BA would interact weakly with the ether oxygen atoms of PEG in terms of the hydrogen-bonding, presumably because of the assist of the hydrophobic interaction between the analyte and PEG [12,13]. Anyway, PEG serves as a hydrogen-bonding acceptor in aqueous system.

In conclusion, this report demonstrates the significance of the use of PEG as a modifier in non-aqueous (acetonitrile) separation systems. The degree of the polymerization, the concentration, and also the mixing ratio of PEGs are important parameters for the system optimization to control the strength of the interaction. The separation systems would be applied to analyses of several organic compounds in foods and biological samples. Related study is in progress.

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