Determination of association constants between 5'-guanosine monophosphate gel and aromatic compounds by capillary electrophoresis

Kaori Yamaguchi, Nobuyuki Takeyasu, and Takashi Kaneta*

Department of Chemistry, Division of Earth, Life, and Molecular Sciences, Graduate School of Natural Science and Technology, Okayama University, Tsushimanaka, Okayama 700-8530, Japan

Keywords:

Capillary electrophoresis

5'-Guanosine monophosphate (GMP)

G-gel

Association constant

∗Corresponding author. Tel.: +81 86 2517847; fax: +81 86 2517847.

E-mail address: kaneta@okayama-u.ac.jp (T. Kaneta).

Hydro gel formed by 5'-guanosine monophosphate (GMP) in the presence of a potassium ion is expected to exhibit interesting selectivity in capillary electrophoretic separations. Here, we estimated the conditional association constants between the hydro gel (G-gel) and aromatic compounds by capillary electrophoresis in order to investigate the separation selectivity that is induced by the G-gel. Several aromatic compounds ~~molecules including amino acid enantiomers, benzene and naphthalene derivatives, and nucleobases~~ were separated in a solution containing GMP and potassium ion at different concentrations. The association constants were calculated by correlating the electrophoretic mobilities of the analytes obtained experimentally using a concentration of G-gel. ~~The G-gel showed different selectivities to planer aromatic molecules such as benzene, naphthalene, and heterocyclic compounds.~~ During semi-quantitative estimation, naphthalene derivatives had larger association constants (K_{ass} = 10.3~16.8) compared with those of benzene derivatives (K_{ass} = 3.91~5.31), which means ~~would imply~~ that the binding sites of G-gel match better to a naphthalene ring than to a benzene ring. A hydrophobic interaction was also found when the association constants for alkyl resorcinol were compared with those of different hydrocarbon chains~~, although short alkyl chains like ethyl and n-hexyl groups showed no difference in affinity~~. The association constants of nucleobases and tryptophan ranged from 6.05~12.6, which approximated the intermediate values between benzene and naphthalene derivatives. ~~According to those results, the interaction was attributed mainly to an intercalation into the G-gel rather than to hydrogen bonding. Small differences between pyrimidine (cytosine and thymine)~~

~~and purine bases (adenine and guanine) were attributed to steric hindrance and/or hydrogen bonding that differs from that in a DNA duplex since no significant difference was observed in the selectivity between cytosine and thymine.~~ Consequently, the selective interaction between G-gel and aromatic compounds was classified as one of three types: (1) an intercalation into stacked planar GMP tetramers; (2) a hydrophobic interaction with a long alkyl chain; or, (3) a small contribution of steric hindrance and/or hydrogen bonding with functional groups such as amino and hydroxyl groups.

1. Introduction

Since the first report of capillary electrophoresis (CE) [1,2], several separation modes of CE have been developed for the separation of a large variety of ions and molecules. The separation modes include zone electrophoresis for inorganic and organic ions, gel and sieving electrophoresis for biomolecules including DNA and proteins, micellar electrokinetic chromatography (MEKC) for molecules and ions, and isoelectric focusing for proteins. An advantage of CE beyond the other chromatographic techniques is the use of a replaceable separation medium, e. g., zone electrophoresis is carried out in a free buffer solution [1,2], micellar electrokinetic chromatography permits the separation of electrically neutral molecules by adding a charged surfactant at a concentration above the critical micellar concentration [3], sieving electrophoresis employs a replaceable polymer solution [4,5] that is a substitute for cross-linked gel formed in a capillary [6,7],

58 and isoelectric focusing is achieved in an aqueous carrier ampholyte solution [8].

59 This advantage leads to the use of several additives to control the separation selectivity of CE.
60 In particular, the separation of enantiomers is an important field in CE since high resolution of
61 enantiomers was achieved only by adding a chiral selector into a migration buffer at the appropriate
62 concentration. Several chiral selectors have been attempted in CE separations such as metal
63 chelate [9], cyclodextrin [10], chiral surfactant [11], crown ether [12], and protein [13], which
64 permits the separation of drug and amino acid enantiomers. Recently, hydrogel of 5'-guanosine
65 monophosphate (GMP), called G-gel, was also used as an additive to separate the enantiomers of
66 some aromatic compounds [14,15].

67 The hydrogel is compatible with CE separations since it is easily prepared by adding potassium
68 ion to a GMP solution—GMP tetramers are formed by the surrounding potassium ions and are
69 stacked upon each other [16]. In addition, G-gel is easily injected into a small-bore capillary
70 because of its low viscosity. In fact, MacGown's group has demonstrated the utility of G-gel as an
71 additive for the CE separation of enantiomers [14,15] and DNA with different sequences [17,18].
72 While their research is focused on enantiomeric and DNA separations, G-gel is expected to lead to
73 interesting selectivity to other molecules, resulting in an improvement in the separation.

74 Herein, we describe the process we used to determine the association constants between G-gel
75 and some aromatic compounds, which include benzene and naphthalene derivatives, with some
76 hydroxyl groups, amino acid enantiomers, and nucleobases. The association constants were

semi-quantitatively estimated by a curve-fitting method based on change in the electrophoretic mobilities of analytes by varying the concentration of G-gel. The electrophoretic mobility of G-gel was predicted by minimizing the errors of regression curves for all the analytes used in the present study. According to the results of the determined association constants, ~~the mechanism of~~ the possible interactions with G-gel ~~was~~ were discussed.

2. Experimental

2.1 Materials

Bare fused-silica capillaries with an i.d. of 50 μm and an o.d. of 375 μm were purchased from GL sciences (Tokyo, Japan). All reagents were of analytical grade and were used without further purification. Guanosine-5'-monophosphate disodium salt, D,L-tryptophan, 1-naphthol, 2-naphthol, 4-ethylresorcinol, hydroquinone, potassium dihydrogenphosphate, dipotassium hydrogenphosphate, sodium hydroxide, ethanol, adenine (Ade), guanine (Gua), cytosine (Cyt), and thymine (Thy) were obtained from Wako Pure Chemicals (Osaka, Japan). D,L-Phenylalanine was purchased from Kishida Chemical (Tokyo, Japan). D,L-Tyrosine, 4-n-dodecylresorcinol and 2,6-dihydroxynaphthalene were from Aldrich (MO, USA). 4-n-Hexylresorcinol and 2,3-dihydroxynaphthalene were obtained from Tokyo Chemical Industry (Tokyo, Japan). Pyrocatechol and 1,5-dihydroxynaphthalene were bought from Nacalai tesque (Kyoto, Japan). Pyrogallol was purchased from Kanto Chemical (Tokyo, Japan). Water used in all experiments

96 was purified by means of an ultrapure Milli-Q system (Millipore, Molsheim, France). The
97 chemical structures of the analytes used in this study are shown in Fig. 1.
98 Solutions of G-gel were prepared by dissolving GMP and KCl in 25 mM potassium phosphate
99 buffer (pH 7.0) at various concentrations as the molar ratio of GMP and KCl was kept at 1:1. The
100 concentrations of 5, 10, 20, 30, and 40 mM were used for the measurement of the electrophoretic
101 mobilities for the analytes. Prior to use, G-gels were let stand overnight at room temperature,
102 according to procedures from previous studies found in the literature [15].
103
104 *2.2 CE separations*
105 Capillary electrophoresis was carried out using an Agilent Technologies ^{3D}CE system equipped
106 with an absorbance detector. The total and effective lengths of a capillary were 64.5 cm and 56 cm,
107 respectively. The capillary was held in a cartridge in which the temperature was controlled at 25
108 ˚C throughout the experiments. Electropherograms were monitored at wavelengths of 210~254
109 nm depending on the absorption maxima of the analytes.
110 At the beginning of the experiments, the capillary was conditioned by rinsing at high pressure
111 with 0.1 M NaOH for 5 min, deionized water for 5 min, and the run buffer for 10 min. Between
112 runs, the capillary was flushed with 0.1 M NaOH for 5 min, deionized water for 5 min, and the run
113 buffer for 2 min in a high-pressure mode. Samples were injected for 5 s at 3.55 kPa. After the
114 experiments, the capillary was washed with 0.1 M NaOH for 10 min, deionized water for 10 min,

filled with water, and stored by immersing both ends in water. The electrophoretic runs were repeated more than three times at each concentration of GMP to obtain the mean value of the electrophoretic mobility for each analyte.

The electrophoretic mobilities were calculated using the migration times of analytes and the electroosmotic flow determined by ethanol as a marker. Throughout the study, the electrophoretic mobility was defined as the direction to the cathode is positive. Using a C program written by our group, the K_{ass} values and error sums of the squares for the analytes were obtained on the basis of least-squares approximation.

3 Results and discussion

3.1 A model for the determination of association constants

The association constants, K_{ass}, between G-gel and the aromatic compounds were determined by measuring their electrophoretic mobilities at different concentrations of GMP. Based on a well-known model [19,20], the observed mobility for an analyte can be expressed by the following relationship,

$$\mu_{ep} = \frac{1}{1+K_{ass}[\mathrm{G}]}\mu_A + \frac{K_{ass}[\mathrm{G}]}{1+K_{ass}[\mathrm{G}]}\mu_{AG} \qquad (1)$$

where μ_{ep} is the observed electrophoretic mobility of the analyte, μ_A is the electrophoretic mobility

of the free analyte, μ_{AG} is the electrophoretic mobility of the analyte bound with G-gel, [G] is the concentration of G-gel, and K_{ass} is the association constant of the analyte. In equation (1), K_{ass} is defined by

$$K_{ass} = \frac{[AG]}{[G][A]} \qquad (2)$$

where [AG] is the concentration of the analyte bound with G-gel. In this study, the K_{ass} was defined according to the model for the binding to micelle in which the binding capacity of the micelle is assumed to be "infinity", that is, the micelle can incorporate any number of solute molecules [21].

The similar model was successfully applied to MEKC studies in which equation (1) was also rewritten by a linear equation [22-25]. Rundlett and Armstrong have reported that a linear regression and nonlinear regression showed no difference in the results [24]. So, we employed the nonlinear regression in this study since it is more convenient to compare the errors of the experimental mobilities with the regression curve directly.

In the measurement of the electrophoretic mobilities for the analytes, we may need to take into account influences of G-gel on viscosity, the electroosmotic flow, and p*K*a values of the analytes. The dependences of the electric current and electroosmotic mobility on the concentration of GMP in the running buffer are shown in Fig. 2. The electric current was proportional to the concentration

of GMP (I = 1527.4[GMP] + 25.188, R^2 = 0.9993). In polymer solutions, viscosity is not proportional to the concentration of the polymer [26]. So, if viscosity, which influences the electric conductivity of a running buffer, changes significantly, the electric current is not proportional to the concentration of GMP. Thus, the linear dependence of the electric current indicates that the increase of viscosity is negligible at the concentration of GMP up to 40 mM. Conversely, the electroosmotic mobility gradually reduced with increasing the concentration of GMP. The decreased electroosmotic mobility would be explained by increase of the ion concentration in the running buffer [27]. The p*K*a values of the analytes used in this study were more than 9.2 (to be anionic species), so all analytes should be almost electrically neutral. So, we assumed that influence on the degree of dissociation was also negligible.

To calculate K_{ass}, we needed two constants, μ_A and μ_{AG}, which must be obtained either experimentally or computationally. The value of μ_A was obtained experimentally by measuring the migration time of the analyte in the absence of G-gel. However, it ~~is~~ was difficult to ~~determine~~ measure μ_{AG} experimentally~~, since μ_{AG} must be measured~~ under conditions where no free analyte exists, since the signals of the analytes were not detectable at a high concentration of GMP due to increase of the background signal. Therefore, we attempted to predict a reasonable μ_{AG} value from the results of the curve fittings using experimental data.

To predict the μ_{AG} value, we proposed the following hypotheses.

(1) The absolute value of μ_{AG} is smaller than the absolute value of the electrophoretic mobility of

the GMP monomer although the values are relatively close to one another. This would be reasonable since potassium ions are incorporated in G-gel ~~located at the center of the GMP tetramer in the gel~~, resulting in a reduction in the negative charge per each GMP molecule.

(2) The concentration of G-gel is approximately equal to the concentration of GMP monomer added to a migration buffer, i. e., all GMP molecules are supposed to contribute to the formation of G-gel. ~~since the critical concentration of a G-gel formation has not been reported in contrast to the critical micellar concentration of surfactants.~~ In the preliminary study, we attempted to find a critical concentration for the formation of G-gel by spectrophotometry and capillary electrophoresis where we measured the absorption spectra and electrophoretic mobility of GMP as an analyte at different concentrations (0.5-20 mM). However, we found no difference in the spectra and electrophoretic mobility. So, we assumed that all GMP molecules contributed to the formation of G-gel or the critical concentration was much smaller than the concentration used in this study.

(3) The μ_{AG} is constant for all analytes used in this study since the absolute values of μ_A would be much smaller than the absolute value of the electrophoretic mobility of G-gel, μ_G, i. e., μ_{AG} is assumed to be equal to μ_G. This assumption would be reasonable since a similar approximation was proposed in the original study of MEKC where the migration velocity of the analyte that was completely incorporated into micelles was equal to that of the micelle [3].

The electrophoretic mobility of the free GMP was measured at -2.22×10^{-4} cm^2 s^{-1} V^{-1} for pH 7 when a GMP solution was injected as a sample. We also determined the μ_A ([G] = 0) and μ_{ep} ([G]

= 5–40 mM) of the analytes. Assuming that the μ_{AG} ranged from -2.50 x 10^{-4} to -1.50 x 10^{-4} cm^2 s^{-1} V^{-1}, the K_{ass} and the error sum of the squares was obtained from the regression curves calculated using a μ_A measured without G-gel and with different μ_{AG} values. ~~In Fig. 2, the obtained K_{ass} values of some representative analytes (pyrocatechol, L-tryptophan, and 2,3-dihydroxynaphthalene) were plotted against the assumed μ_{AG}. The results suggested that the relative magnitude of the K_{ass} values was independent of μ_{AG} while the absolute values of K_{ass} increased as the absolute value of μ_{AG} was reduced. In other words, any μ_{AG} value that is close to the electrophoretic mobility of free GMP can be used if one needs only the relative order of K_{ass} or semi-quantitative values.~~

To find an appropriate μ_{AG} value, we added the error sums of the squares for all analytes at a given μ_{AG} and plotted the values against the corresponding μ_{AG}, as shown in Fig. 3. The summation of the error sum of squares was minimized at -1.65 x 10^{-4} cm^2 s^{-1} V^{-1}, which led to a minimum error. Consequently, the value of -1.65 x 10^{-4} cm^2 s^{-1} V^{-1} was employed for the μ_{AG} in calculating the association constants for all analytes.

3.2 Association constants of analytes

The association constants of the analytes were determined by curve fitting when the μ_{AG} was set to -1.65 x 10^{-4} cm^2 s^{-1} V^{-1}, and the results are listed in Table 1. ~~As examples, the results of the curve fitting for pyrocatechol, L-tryptophane, and 2,3-dihydroxynaphthalene are~~ The relationship between the experimental mobility and calculated mobility is also shown in Fig. 4. As seen in Fig.

4, ~~the regression curves showed good correlation~~ the calculated mobilities are in good agreement with the experimental data (μ_{calc} = 1.0094 μ_{exp} + 0.0051, R^2 = 0.9863 for all). In Fig. 4, only cytosine and thymidine (white and gray circles) showed small deviations from the calculated mobilities (μ_{calc} = 0.9972 μ_{exp} - 0.0002, R^2 = 0.996 except for cytosine and thymidine), although the reason is still unclear. As Table 1 shows, the K_{ass} of the analytes with a benzene ring were around 3~5 except for 4-n-dodecylresorcinol, while the molecules with a naphthalene ring had a K_{ass} of roughly 10~16. Tryptophan consisting of a heterocyclic ring showed approximately 7, which corresponded to the intermediate value between benzene and naphthalene derivatives. This indicates that the planar structure is preferable to binding with G-gel and extended π-conjugated molecules have a stronger interaction with G-gel, taking into account the order of naphthalene ring > tryptophan > benzene ring. Therefore, the interaction could be attributed to the intercalation of the planer analytes into stacked guanine tetramers in G-gel.

As seen in the different K_{ass} values between analogues, G-gel recognized positional isomers, e. g., between benzene or naphthalene derivatives with hydroxyl groups. Since dihydroxynaphthalene isomers had a larger K_{ass} than naphthol isomers, hydrogen bonding, rather than steric hindrance, contributed to the binding with G-gel in the case of naphthalene derivatives. It is interesting that naphthalene derivatives with a hydroxyl group at the 2-position had a larger K_{ass} compared with the others, i. e., 2-naphthol > 1-naphthol and 2,6- > 2,3- > 1,5-dihydroxynaphthalene. These results ~~imply~~ mean that the hydroxyl group at the 2-position of the naphthalene ring slightly enhanced the

affinity with G-gel.

Of the three resorcinol derivatives, the K_{ass} of 4-n-dodecylresorcinol was much larger than either ethyl or 4-n-hexylresorcinol, although ethylresorcinol and 4-n-hexylresorcinol had the same K_{ass}, which resulted in no separation. The results suggested that G-gel could interact with a relatively long hydrocarbon chain, although it cannot discriminate short chains like ethyl and n-hexyl groups. So, G-gel showed a weak hydrophobic interaction, although the selectivity was relatively poor.

Nucleobases also had intermediate K_{ass} values between benzene and naphthalene derivatives: 12.9 for Ade, 9.13 for Gua, 6.05 for Cyt, and 6.14 for Thy. The electropherograms of four nucleobases in the absence and presence of G-gel are also shown in Fig. 5. As expected from their basic skeletons, Thy and Ade co-migrated with Cyt and Gua in a migration buffer without G-gel, respectively. However, the addition of G-gel to the buffer at a concentration of 30 mM permitted the separation of four nucleobases on the order of Cyt < Thy < Ade < Gua. The interaction energies of nucleobases are calculated to be -26.3 kcal mol^{-1} for Gua-Cyt and -16.0 for Gua-Thy [28], i. e., the binding constant for Gua-Cyt is estimated to be $10^{4.47}$ ($e^{26.3}/e^{16.0}$)-fold of that for Gua-Thy. So, if ~~If~~ hydrogen bonding, is significant, as it is with DNA, Cyt must have a much larger K_{ass} than the other bases. Therefore, the interaction of nucleobases with G-gel is different from hydrogen bonding in double-stranded DNA.

The affinity between G-gel and nucleobases is expected to be due to stacking and hydrophobic interactions. The results obtained in the present study showed that the order of K_{ass} was Cyt < Thy

248 < Gua < Ade. Conversely, we can speculate as to the order of hydrophobic interactions for
249 nucleobases from the results obtained by MEKC where the order of the distribution coefficients was
250 Cyt < Thy < Ade when using a migration buffer (pH 7) containing 0.1 M sodium dodecylsulfate
251 [~~21~~29]. Also, a migration order of Cyt < Thy < Ade < Gua has been reported at pH 10 [~~22~~30],
252 although the pH was different in the present study. The stacking interactions between nucleobases
253 were also calculated based on their geometric overlapping and were increased on the order of
254 Cyt-Gua < Ura (uracil)-Gua < Ade-Gua < Gua-Gua [~~23~~28], which was similar to the order of
255 hydrophobic interactions. This means that the interaction between G-gel and nucleobases can be
256 attributed to the stacking affinity and/or hydrophobicity, although the order of Gua < Ade was
257 inconsistent with the results of the hydrophobic and stacking interactions of Ade < Gua.
258 Obviously, the difference between pyrimidine and purine bases can be attributed to the stacking and
259 hydrophobic interactions, as reported in the results of the MEKC and computational calculations.
260 Therefore, the largest association constant for Ade among nucleobases may be due to additional
261 interactions such as the hydrogen bonding between Ade and G-gel or the steric hindrance of Gua to
262 G-gel.
263
264 **4. Conclusions**
265 The interaction between G-gel and aromatic compounds was semi-quantitatively estimated with
266 a curve-fitting method using least-squares approximation. Hydro gel formed by GMP showed

interesting selectivity for benzene and naphthalene derivatives in CE separations. Naphthalene derivatives had larger K_{ass} values (larger than 10 M^{-1}) than benzene derivatives (around 4 M^{-1}) and different affinities were also observed depending on the functional groups. The interaction between G-gel and aromatic compounds can mainly be attributed to an intercalation into stacked GMP tetramers and to the intercalation site fit to naphthalene or heterocyclic rings such as tryptophan and nucleobases rather than to the benzene ring. For nucleobases, the interaction cannot be explained only by hydrophobic and stacking effects since the order of Ade and Gua is against their hydrophobicity and stacking affinity to Gua. These results imply that hydrogen bonding and/or steric hindrance somewhat contribute to the interaction with G-gel. This interaction, however, is not specific as with hydrogen bonding in double-stranded DNA since they showed a similar K_{ass} to Cyt, which should be specific to Gua. Nevertheless, G-gel is a useful medium for the sequence-dependent separation of DNA because of different affinities for the four nucleobases. Consequently, G-gel would be a good separation medium not only for enantiomers and DNA, but also for positional isomers and several analogues.

Acknowledgment

This research was supported by Grants-in-Aid for Scientific Research, Scientific Research (B) (No. 22350036).

References

[1] F. E. P. Mikkers, F. M. Everaerts, Th. P. E. M. Verheggen, J. Chromatogr. 169 (1979) 11–20

[2] J. W. Jorgenson, K. D. Lukacs, Anal. Chem. 53 (1981) 1298–1302.

[3] S. Terabe, K. Otsuka, K. Ichikawa, A. Tsuchiya, T. Ando, Anal. Chem. 56 (1984) 111–113.

[4] D. N. Heiger, A. S.Cohen, B. L. Karger, J. Chromatogr. 516 (1990) 33–48.

[5] A. Widhalm, C. Schwer, D. Blaas, E. Kenndler, J. Chromatogr. 549 (1991) 446–451.

[6] A. S. Cohen, D. R. Najarian A. Paulus, A. Guttman, J. A. Smith, B. L. Karger, Proc. Natl. Acad. Sci. USA 85 (1988) 9660–9663.

[7] A. S. Cohen, B. L. Karger, J. Chromatogr. 397 (1987) 409–417.

[8] S. Hjertén and M.-d. Zhu, J. Chromatogr. 346 (1985) 265–270.

[9] P. Gozel , E. Gassmann , H. Michelsen , R. N. Zare, Anal. Chem. 59 (1987) 44–49.

[10] T. Ueda, F. Kitamura, R. Mitchell, T. Metcalf, T. Kuwana, A. Nakamoto, Anal. Chem. 63 (1991) 2979–2981.

[11] A. Dobashi, M. Hamada, Y. Dobashi, J. Yamaguchi, Anal. Chem., 67 (1995) 3011–3017.

[12] R. Kuhn, F. Erni, T. Bereuter, J. Häusler, Anal. Chem. 64 (1992) 2815–2820.

[13] G. E. Barker, P. Russo, R. A. Hartwick, Anal. Chem. 64 (1992) 3024–3028.

[14] J. T. Davis, *Angew. Chem*., Int. Ed. 43 (2004) 668–698.

[15] V. A. Dowling, J. A. M. Charles, E. Nwakpuda, L. B. McGown, Anal. Chem. 76 (2004) 4558–4563

[16] D. Dong and L. B. McGown, Electrophoresis 32 (2011) 1735–1741.

[17] W. B. Case, K. D. Glinert, S. LaBarge, L. B. McGown, Electrophoresis 28 (2007) 3008–3016.

[18] Y. Dong, L. B. McGown, Electrophoresis 32 (2011) 1209–1216.

[19] R. A. Alberty and E. L. King, J. Am. Chem. Soc., 73 (1951) 517.

[20] S. Honda, A. Taga, K. Suzuki, S. Suzuki, K. Kakehi, J. Chromatogr. 597 (1992) 377–382.

[21] L. Sepulveda, E. Lissi, F. Quina, Adv. Colloid Interface Sci. 25 (1986) l-57.

[22] M. G. Khaledi, S. C. Smith, J. K. Strasters, Anal. Chem. 63 (1991)1820-1830.

[23] S. C. Smith, . G. Khaledi, J. Chromatogr. 632 (1993) 177-184.

[24] K. L. Rundlett, D. W. Armstrong, J. Chromatogr. A 721 (1996) 173-186.

[25] P. G. Muijselaar, H. A. Claessens, C. A. Cramers, J. Chromatogr. A 765 (1997) 295-306.

[26] S. N. Chinai, W.C. Schneider, J. Polymer Sci., A. 3, (1965) 1359-1371.

[27] R. Kuhn, S. Hoffstetter-Kuhn, Capillary Electrophoresis: Principles and Practice,

Springer-Verlag, Berlin, 1993, pp.22-29.

[~~23~~28] J. Šponer, J. Leszczynski, P. Hobza, Biopolymers 61 (2002) 3–31.

[~~21~~29] A. S. Cohen, S. Terabe, J. A. Smith, B. L. Karger, Anal. Chem. 59 (1987) 1021–1027.

[~~22~~30] G. Chen, X. Han, L. Zhang, J. Ye, J. Chromatogr. A 954 (2002) 267–276.

Figure Legends

Figure 1. Structures of analytes used in this study.

Figure 2. Dependence of ~~association constants on the assumed electrophoretic mobility of the analytes bound with G-gel~~ the electric current and electroosmotic mobility on the concentration of GMP.

~~Circle; pyrocatechol, square; L-tryptophan, triangle; 2,3-dihydroxynaphthalene.~~ Conditions of electrophoresis: capillary; i.d., 50 μm, effective and total lengths, 56 and 64.5 cm; migration buffer, 25 mM phosphate (pH 7) containing different concentrations of GMP; applied voltage, 20 kV; and, temperature, 25 °C.

Figure 3. Relationship between the assumed electrophoretic mobilities of the analytes bound with G-gel and summation of residual errors.

Residual errors for all analytes obtained using an assumed μ_{AG} were summed. The conditions for electrophoresis were similar to those in Fig. 2.

Figure 4. ~~Fitting curves for representative analytes.~~ Relationship between the experimental mobility and calculated mobility. The mobilities at the concentrations of 5, 10, 20, 30, and 40 mM GMP were plotted. White circle, thymine; gray circle, cytosine; and, black circle, other molecules. The conditions for electrophoresis were similar to those in Fig. 2.

~~Symbols and the experimental conditions were similar to those of Fig. 2.~~

Figure 5. Electropherograms of nucleobases.

343 Migration buffer, 25 mM phosphate (pH 7) containing (a) without GMP, (b) 30 mM GMP. 1,

344 Cyto; 2, Thy; 3, Gua; and, 4, Ade. Other conditions were the same as Fig. 2.

Figures1.TIF
Click here to download high resolution image

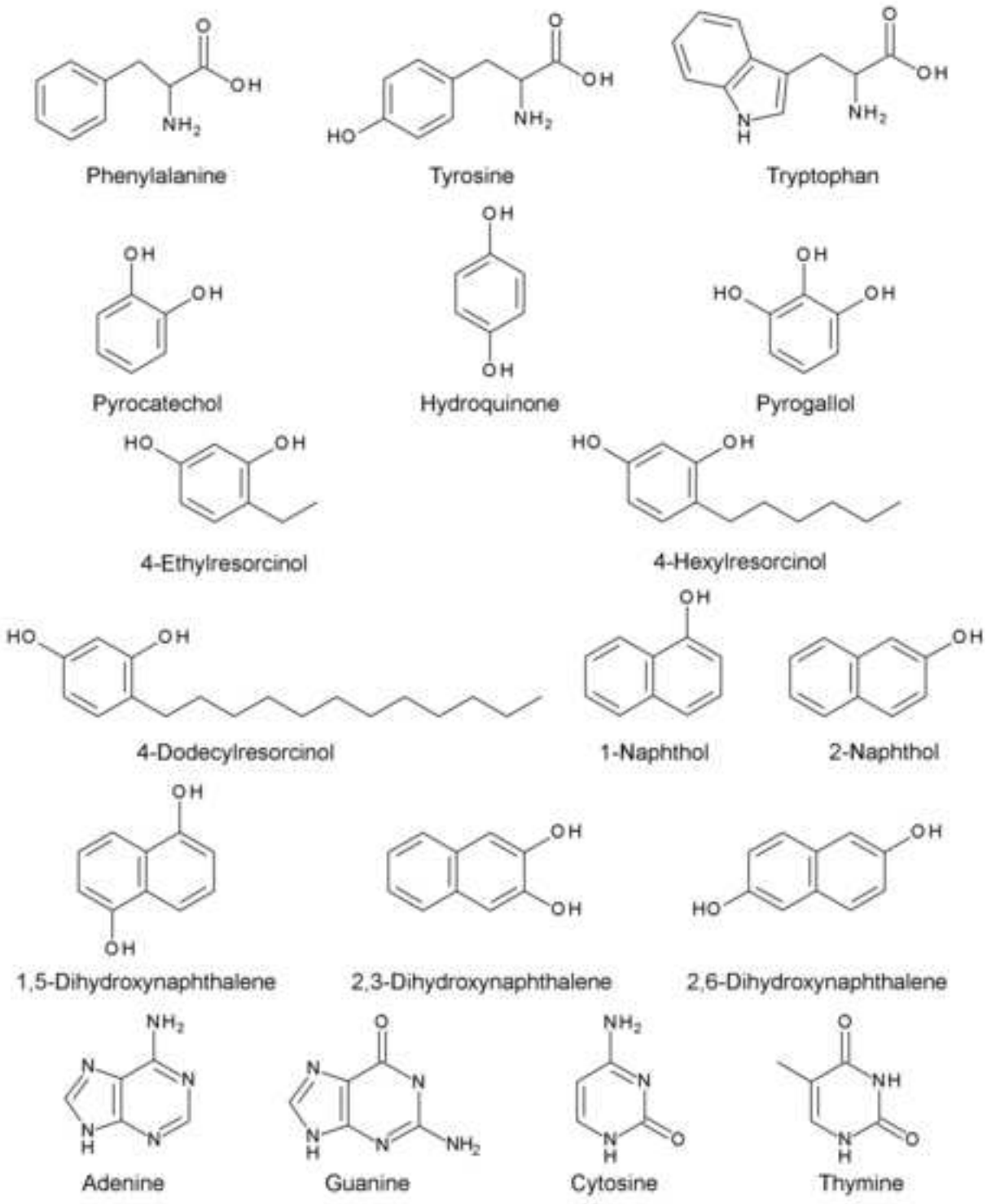

Fig. 1 Yamaguchi et al.

Figures2.TIF
Click here to download high resolution image

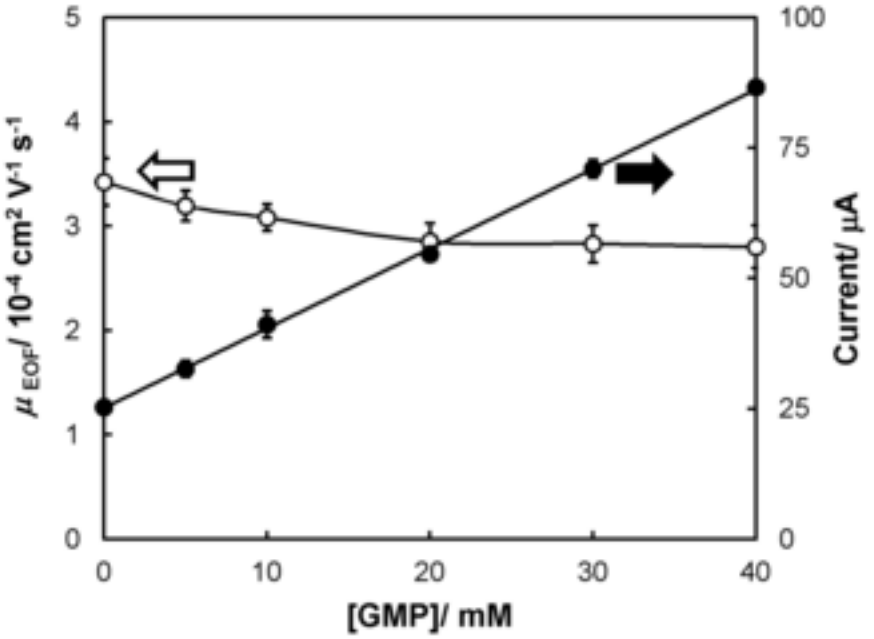

Fig. 2 Yamaguchi et al.

Figures3.TIF
Click here to download high resolution image

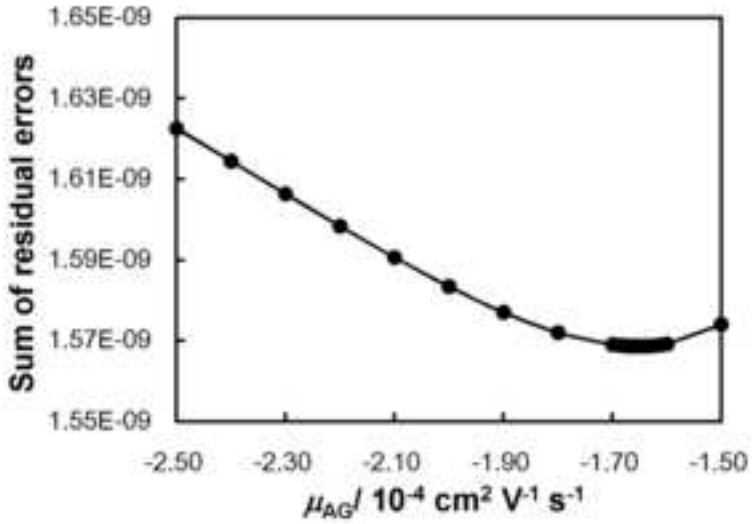

Fig. 3 Yamaguchi et al.

Figures4.TIF
Click here to download high resolution image

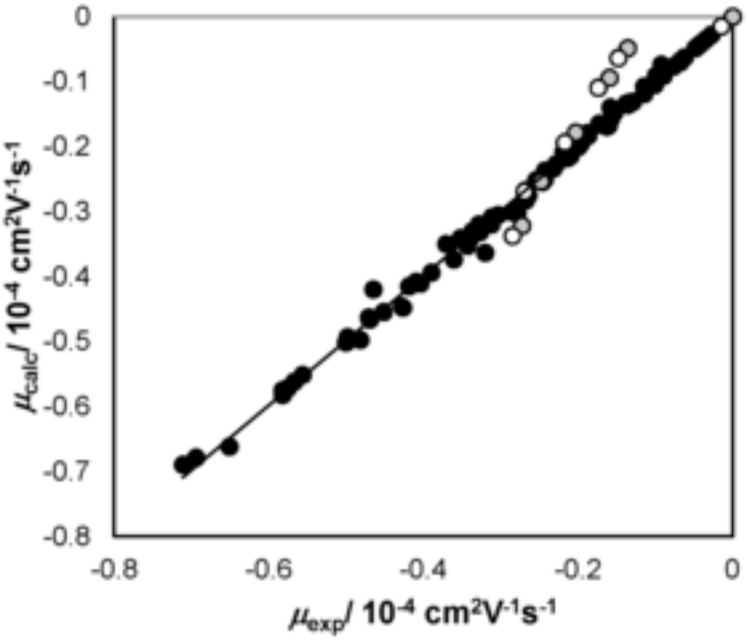

Fig. 4 Yamaguchi et al.

Figures5.TIF
Click here to download high resolution image

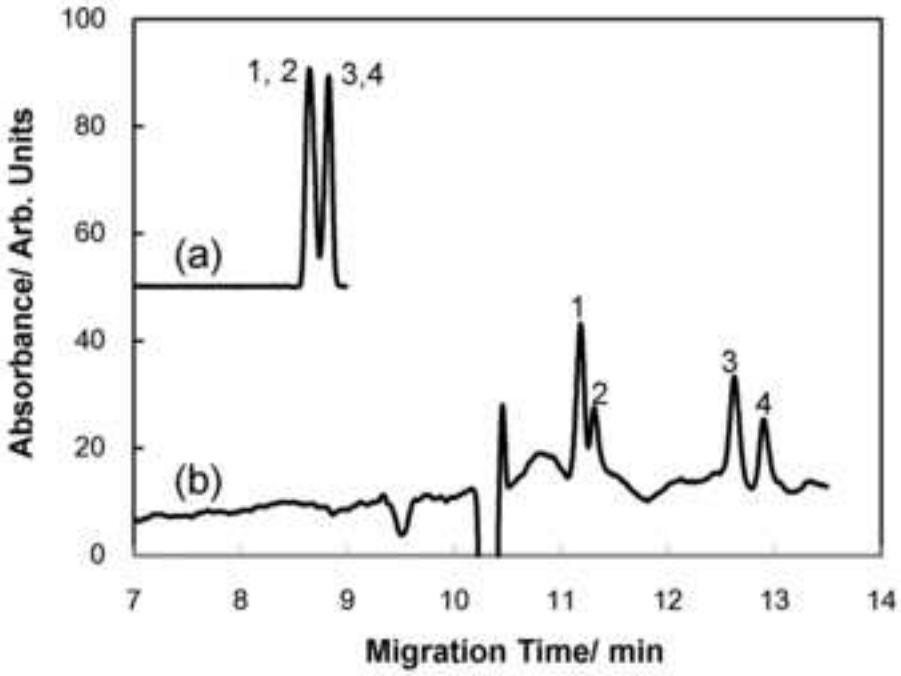

Fig. 5 Yamaguchi et al.

Table 1. Association constants of analytes used in this study

Types	Analyte	Association constant/ M^{-1}
Benzene ring	Pyrocatechol	3.91
	Hydroquinone	4.11
	Pyrogallol	5.31
	Ethylresolcinol	4.09
	Hexylresolcinol	4.09
	Dodecylresolcinol	13.0
Amino acid	D,L-Phenylalanine	2.72
	D,L-Tyrosine	4.58
	D-Tryptophan	7.14
	L-Tryptophan	7.50
Naphthalene ring	1-Naphthol	10.3
	2-Naphthol	11.9
	2,3-Dihydroxynaphthalene	15.7
	2,6-Dihydroxynaphthalene	16.8
	1,5-Dihydroxynaphthalene	11.9
Nucleobase	Adenine	12.6
	Guanine	9.13
	Cytosine	6.05
	Thymine	6.14