

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

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

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

46

47 **1. Introduction**

48 Since the first report of capillary electrophoresis (CE) [1,2], several separation modes of CE
49 have been developed for the separation of a large variety of ions and molecules. The separation
50 modes include zone electrophoresis for inorganic and organic ions, gel and sieving electrophoresis
51 for biomolecules including DNA and proteins, micellar electrokinetic chromatography (MEKC) for
52 molecules and ions, and isoelectric focusing for proteins. An advantage of CE beyond the other
53 chromatographic techniques is the use of a replaceable separation medium, e. g., zone
54 electrophoresis is carried out in a free buffer solution [1,2], micellar electrokinetic chromatography
55 permits the separation of electrically neutral molecules by adding a charged surfactant at a
56 concentration above the critical micellar concentration [3], sieving electrophoresis employs a
57 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

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

82

83 **2. Experimental**

84 *2.1 Materials*

85 Bare fused-silica capillaries with an i.d. of 50 μm and an o.d. of 375 μm were purchased from
86 GL sciences (Tokyo, Japan). All reagents were of analytical grade and were used without further
87 purification. Guanosine-5'-monophosphate disodium salt, D,L-tryptophan, 1-naphthol, 2-naphthol,
88 4-ethylresorcinol, hydroquinone, potassium dihydrogenphosphate, dipotassium hydrogenphosphate,
89 sodium hydroxide, ethanol, adenine (Ade), guanine (Gua), cytosine (Cyt), and thymine (Thy) were
90 obtained from Wako Pure Chemicals (Osaka, Japan). D,L-Phenylalanine was purchased from
91 Kishida Chemical (Tokyo, Japan). D,L-Tyrosine, 4-n-dodecylresorcinol and
92 2,6-dihydroxynaphthalene were from Aldrich (MO, USA). 4-n-Hexylresorcinol and
93 2,3-dihydroxynaphthalene were obtained from Tokyo Chemical Industry (Tokyo, Japan).
94 Pyrocatechol and 1,5-dihydroxynaphthalene were bought from Nacalai tesque (Kyoto, Japan).
95 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,

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

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

123

124 **3 Results and discussion**

125 *3.1 A model for the determination of association constants*

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

130

$$131 \mu_{ep} = \frac{1}{1 + K_{as} [G]} \mu_A + \frac{K_{as} [G]}{1 + K_{as} [G]} \mu_{AG} \quad (1)$$

132

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

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

137

$$138 \quad K_{ass} = \frac{[AG]}{[G][A]} \quad (2)$$

139

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

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

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

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

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

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

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

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

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

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

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

191 = 5–40 mM) of the analytes. Assuming that the μ_{AG} ranged from -2.50×10^{-4} to $-1.50 \times 10^{-4} \text{ cm}^2$
192 $\text{s}^{-1} \text{ V}^{-1}$, the K_{ass} and the error sum of the squares was obtained from the regression curves calculated
193 using a μ_A measured without G-gel and with different μ_{AG} values. ~~In Fig. 2, the obtained K_{ass}~~
194 ~~values of some representative analytes (pyrocatechol, L-tryptophan, and 2,3-dihydroxynaphthalene)~~
195 ~~were plotted against the assumed μ_{AG} . The results suggested that the relative magnitude of the K_{ass}~~
196 ~~values was independent of μ_{AG} while the absolute values of K_{ass} increased as the absolute value of~~
197 ~~μ_{AG} was reduced. In other words, any μ_{AG} value that is close to the electrophoretic mobility of~~
198 ~~free GMP can be used if one needs only the relative order of K_{ass} or semi-quantitative values.~~

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

204

205 3.2 Association constants of analytes

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

210 4, ~~the regression curves showed good correlation~~ the calculated mobilities are in good agreement
211 with the experimental data ($\mu_{\text{calc}} = 1.0094 \mu_{\text{exp}} + 0.0051$, $R^2 = 0.9863$ for all). In Fig. 4, only
212 cytosine and thymidine (white and gray circles) showed small deviations from the calculated
213 mobilities ($\mu_{\text{calc}} = 0.9972 \mu_{\text{exp}} - 0.0002$, $R^2 = 0.996$ except for cytosine and thymidine), although the
214 reason is still unclear. As Table 1 shows, the K_{ass} of the analytes with a benzene ring were around
215 3~5 except for 4-n-dodecylresorcinol, while the molecules with a naphthalene ring had a K_{ass} of
216 roughly 10~16. Tryptophan consisting of a heterocyclic ring showed approximately 7, which
217 corresponded to the intermediate value between benzene and naphthalene derivatives. This
218 indicates that the planar structure is preferable to binding with G-gel and extended π -conjugated
219 molecules have a stronger interaction with G-gel, taking into account the order of naphthalene ring
220 > tryptophan > benzene ring. Therefore, the interaction could be attributed to the intercalation of
221 the planer analytes into stacked guanine tetramers in G-gel.

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

229 affinity with G-gel.

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

235 Nucleobases also had intermediate K_{ass} values between benzene and naphthalene derivatives:
236 12.9 for Ade, 9.13 for Gua, 6.05 for Cyt, and 6.14 for Thy. The electropherograms of four
237 nucleobases in the absence and presence of G-gel are also shown in Fig. 5. As expected from their
238 basic skeletons, Thy and Ade co-migrated with Cyt and Gua in a migration buffer without G-gel,
239 respectively. However, the addition of G-gel to the buffer at a concentration of 30 mM permitted
240 the separation of four nucleobases on the order of Cyt < Thy < Ade < Gua. **The interaction**
241 **energies of nucleobases are calculated to be -26.3 kcal mol⁻¹ for Gua-Cyt and -16.0 for Gua-Thy**
242 **[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**
243 **Gua-Thy. So, if H hydrogen bonding, is significant, as it is with DNA, Cyt must have a much**
244 **larger K_{ass} than the other bases. Therefore, the interaction of nucleobases with G-gel is different**
245 **from hydrogen bonding in double-stranded DNA.**

246 The affinity between G-gel and nucleobases is expected to be due to stacking and hydrophobic
247 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 [2129]. Also, a migration order of Cyt < Thy < Ade < Gua has been reported at pH 10 [2230],
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 [2328], 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

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

281

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286 **References**

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323

324 **Figure Legends**

325 Figure 1. Structures of analytes used in this study.

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

329 ~~Circle; pyrocatechol, square; L-tryptophan, triangle; 2,3-dihydroxynaphthalene.~~ Conditions of
330 electrophoresis: capillary; i.d., 50 μm , effective and total lengths, 56 and 64.5 cm; migration buffer,
331 25 mM phosphate (pH 7) containing different concentrations of GMP; applied voltage, 20 kV; and,
332 temperature, 25 $^{\circ}\text{C}$.

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

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

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

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

342 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.

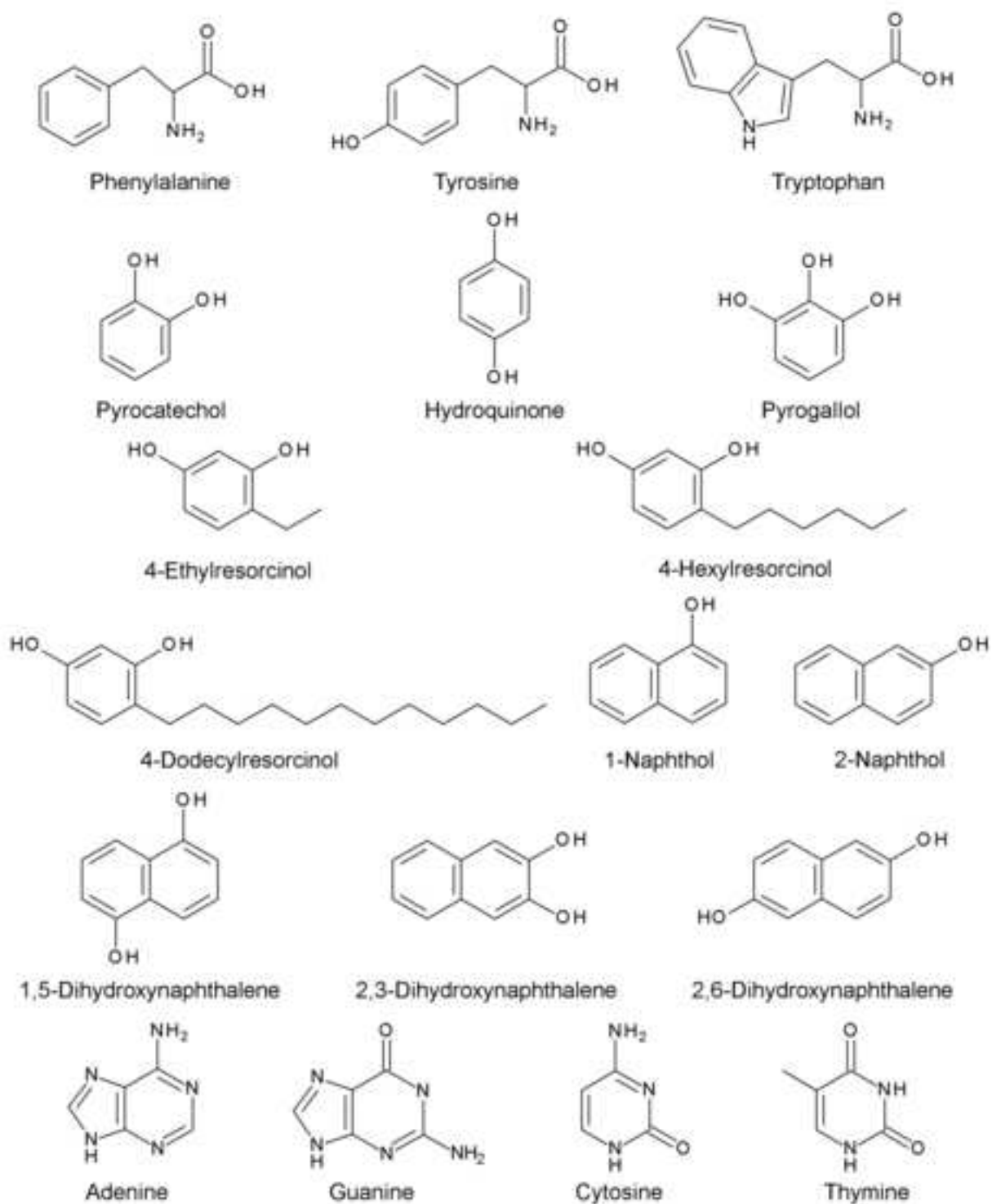


Fig. 1 Yamaguchi et al.

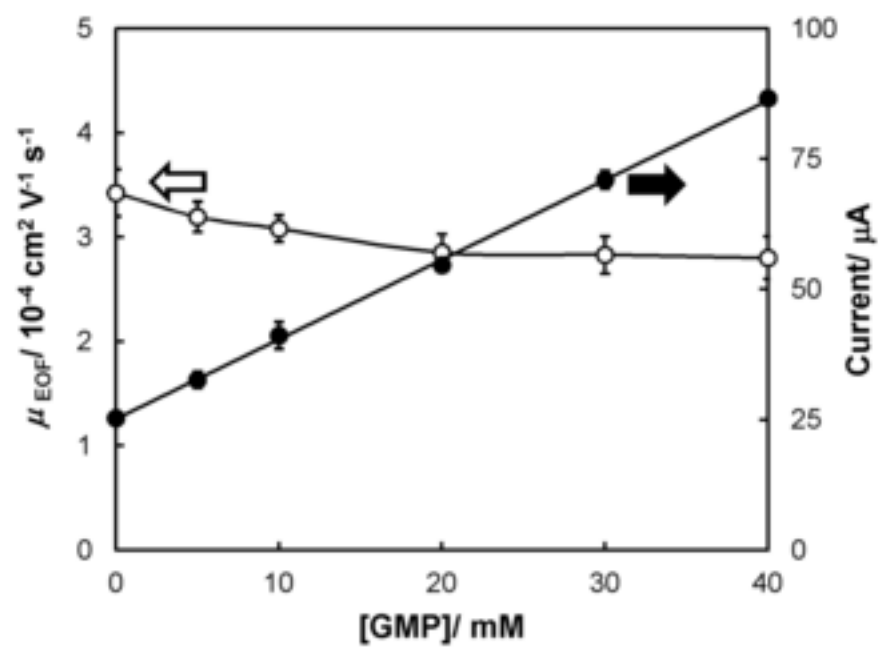


Fig. 2 Yamaguchi et al.

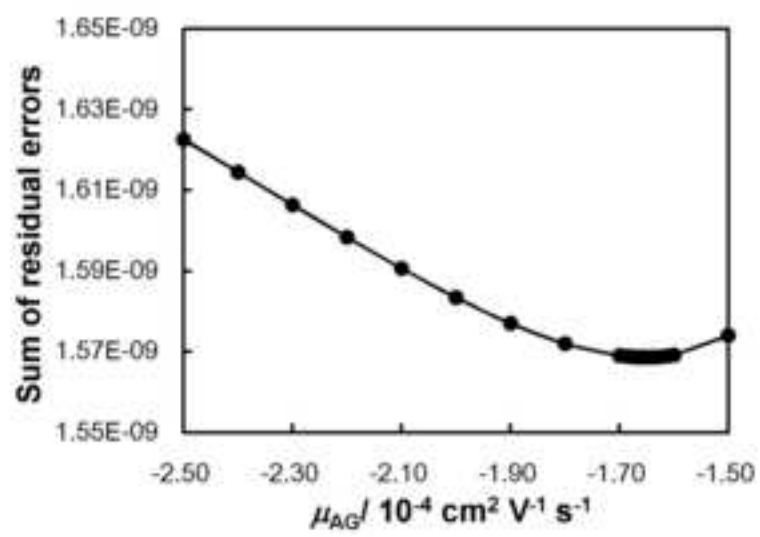


Fig. 3 Yamaguchi et al.

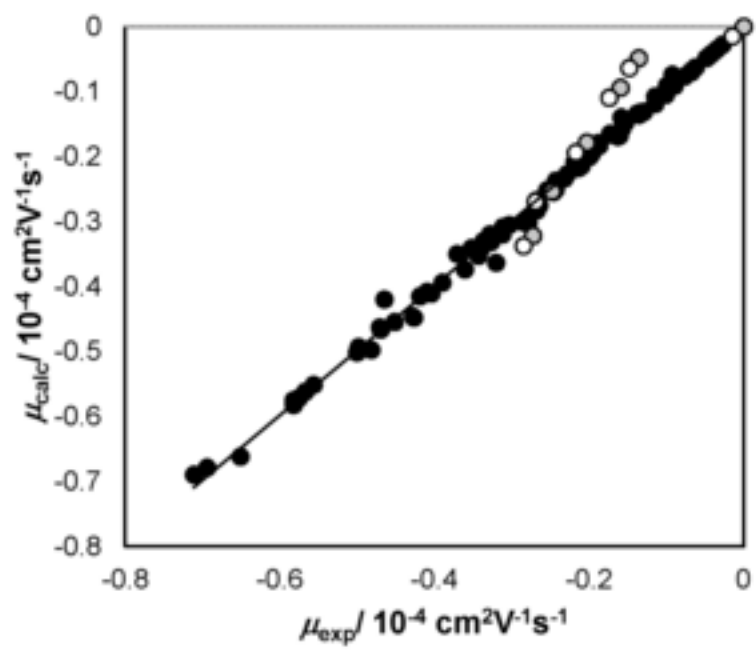


Fig. 4 Yamaguchi et al.

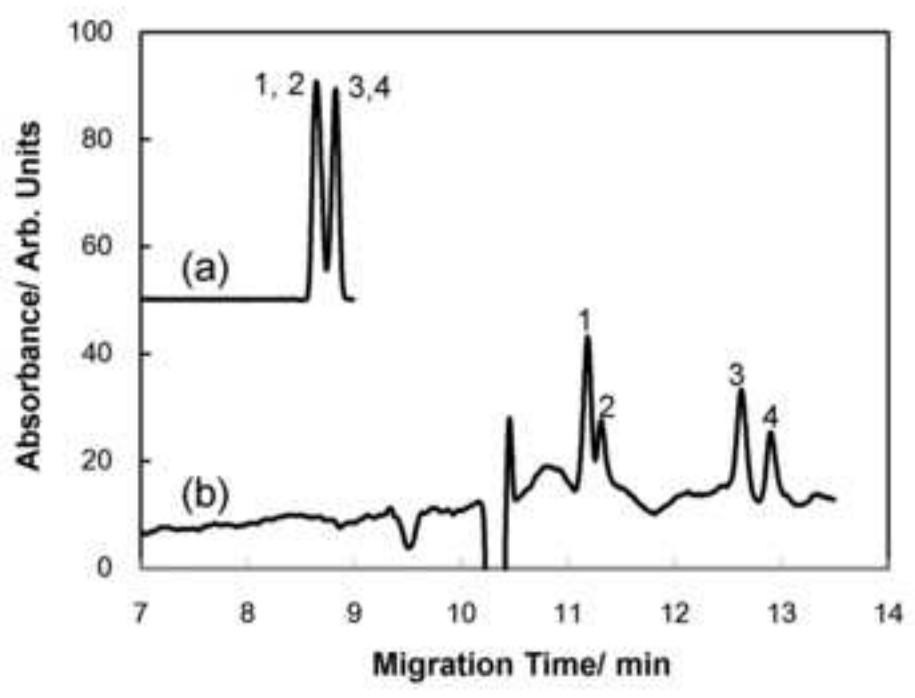


Fig. 5 Yamaguchi et al.

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