

Capillary Electrophoretic Behaviors of Cobalt(II) and Copper(II) with Pyridinedicarboxylates and 2-Pyridinecarboxylate by On-column Complexation

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Capillary electrophoretic species of Co(II) and Cu(II) with 2-pyridinecarboxylate (2-PC) and pyridinedicarboxylates (PDCs) by on-column complexation were determined. These migration orders were Co(II) < Cu(II) for 2,3- and 2,5-PDC, Cu(II) < Co(II) for 2-PC and 2,4-PDC, Co(II) = Cu(II) for 2,6-PDC on a 20 mM sodium tetraborate buffer with a 1 mM 2-PC or PDC at pH 9.2. Separation voltage and electric current were 20 kV and 15 mA, respectively. These retention orders were also Co(II) < Cu(II) for 2-PC, Cu(II) < Co(II) for 2,3- and 2,5-PDC, and Cu(II) \approx Co(II) for 2,4- and 2,6-PDC on the buffer in HPLC using silica gels as a packing material. It was concluded that Co(II) formed $[\text{Co}(2\text{-PC})_3]^-$ with 2-PC, $[\text{Co}(2,6\text{-PDC})_2]^{2-}$ with 2,6-PDC, $[\text{Co}(\text{PDC})_2(\text{H}_2\text{O})_2]^{2-}$ with 2,3- and 2,5-PDC, and $[\text{Co}(2,4\text{-PDC})_3(\text{H}_2\text{O})_2]^+$ with 2,4-PDC, and that Cu(II) formed $[\text{Cu}(2\text{-PC})_2]$ with 2-PC, $[\text{Cu}(2,6\text{-PDC})_2]^{2-}$ with 2,6-PDC, and $[\text{Cu}(\text{PDC})_2(\text{H}_2\text{O})]^{2-}$ with 2,3-, 2,4- and 2,5-PDC in the buffer. Co(II) formed a specific anionic complex with 2,4-PDC. These anionic Co(II)-PDC complexes could be formed only by the on-column complexation.

1. Introduction

A separation of transition metal ions with some 1,10-phenanthroline (phen) derivatives and Alizarin complexone (ALC) as chelating reagents has been developed by capillary zone electrophoresis (CZE) [1-5]. Since $[\text{Cu}(\text{phen derivative})_2]^{2+}$ has two vacant axial coordination sites, migration behavior in CZE depended on axial coordination equilibria of $[\text{Cu}(\text{phen derivative})_2]^{2+}$ with some aliphatic carboxylate ions [1,2]. Cu(II)-ALC-H₂O ternary complex could substitute its coordinated water molecules into some amines. The substitution equilibria also produced a variation for separation selectivity of copper ion in CZE [3]. CZE separation method using the axial coordination and the substitution equilibria formed the ternary

complexes would be a useful separation mode, because it could give variations for separation selectivity of the metal ions.

Complexation of nickel ion with a bidentate chelating reagent of bathophenanthrolinedisulfonate (BPS) produced three complexes of 1 : 1, 1 : 2, and 1 : 3 of the nickel ion to BPS in CZE [4]. When the bidentate chelating reagent is used in CZE separation of metal ions, it is interesting to investigate migration behaviors of the metal ions, because the unexpected migration behavior could be often obtained as the nickel ion-BPS complexes.

Chelating reagent pyridinedicarboxylate (PDC) forms metal complexes with many metal ions [6]. Separation of some metal ions on a porous silica bound iminodiacetic acid [7] and separation of some

anions, magnesium and calcium ions on an anion exchange resin [8] using tridentate 2,6-PDC chelating reagent as an eluent have been reported by high performance liquid chromatography (HPLC). It has described that complexation and ion-exchange reaction in HPLC were governed by stability constants of the metal ions with 2,6-PDC and the iminodiacetic acid. The metal ion-2,6-PDC species in an effluent have not been obvious. Although a separation of some anions with 2,6-PDC as a background electrolyte has been also reported by CZE [9], it has not been reported for separation of metal ions. A reversed HPLC separation of some metal ions with bidentate 2-pyridinecarboxylate (2-PC) including acetonitrile as an eluent at pH 7.0 by on-column complexation has been reported [10]. CZE separation methods of metal ions with 2,6-PDC including cationic surfactants by on-column [11-13] and pre-column [14] complexation have been also reported. The metal ion-2-PC and 2,6-PDC complexes could directly detect as positive peaks at a UV range, not an indirect detection. In these cases, the separation metal complex species would depend on stability constants of the metal ions with 2-PC and 2,6-PDC. However, systematic investigation for migration behaviors of metal ions with 2-PC, 2,3-, 2,4-, 2,5-, and 2,6-PDC has not been reported yet. Chelating reagents, 2,3-, 2,4-, and 2,5-PDC, generally behave as a bidentate chelating reagent between an oxygen of 2-carboxylate ion and a nitrogen of pyridine ring in PDC [6]. However, 3-, 4-, and 5-carboxylate ions in PDC can behave as even monodentate ligands. Therefore, the 2,3-, 2,4-, 2,5-, 3,4- and 3,5-PDC could form some transition metal ions-PDC polymers in crystals [15-26]. Since the 2,3-, 2,4-, and 2,5-PDC could behave as both of the monodentate and the bidentate ligands, it is interesting to investigate migration behaviors of metal ions with these PDCs in CZE. Especially, the migration behaviors of cobalt(II) and copper(II) with these PDCs in an alkaline solution were specific in some metal ions as samples. Therefore, the migration behaviors of cobalt(II) and copper(II) with these PDCs in CZE by on-column complexation were investigated.

2. Experimental

2.1. Apparatus

Capillary electrophoresis was carried out by 270A-HT (Applied Biosystems, California) at 30°C. A 720 mm long (500 mm to detector cell) 50 μ m i.d. fused silica capillary (GL Science, Tokyo) was used. Sample was introduced into the capillary from anode side in vacuum (-17 kPa) for 5 s. Applied voltage was 20 kV. Detection was performed by directly UV absorption at 270 nm.

Chromatographic system (TOSOH, Tokyo) consisted of a CCPD dual pump, a Rheodyne 7725 injector with 100 μ l sample loop, a UV-8000 UV-detector and a CO-8000 air-oven at 30°C. Detection was performed by directly UV absorption at 270 nm.

2.2. Chemicals

Chelating reagents 2-PC, 2,3-, 2,5-, 2,6-, and 3,5-PDC were purchased from Wako (Osaka, Japan). 2,4- and 3,4-PDC were purchased from Aldrich (Milwaukee, WI). All reagents were used as obtained. Electrophoretic buffers and eluents were prepared from sodium tetraborate, boric acid and the chelating reagents. For CZE, acetophenone was used as a marker of electroosmotic flow (EOF). For HPLC, silica particles of 10 μ m diameter of Wakogel LC-10H (Wako, Osaka, Japan) was loaded onto a 4.6 mm i.d. x 250 mm long stainless steel column. Standard solutions of Co(II) and Cu(II) were prepared from metal nitrate. All solutions were filtered through 0.45 μ m PTFE filter (Advantec, Tokyo).

2.3. Procedure

For CZE by on-column complexation, capillary was first conditioned with electrophoretic buffer (10 min) immediately prior to injection. The capillary was systematically washed with 0.1 M KOH (2 min), then rinsed with water (2 min) between runs.

For CZE by pre-complexation, 1 mM Co(II) and Cu(II) pre-complexed with a 10 mM PDC in a 20 mM sodium tetraborate buffer at pH 9.2 as samples were used. The 20 mM sodium tetraborate buffer at pH 9.2 without PDC as the electrophoretic buffer was used. The other procedure was as CZE by the on-column complexation.

For HPLC by the on-column complexation, since the 20 mM sodium tetraborate eluents including 1 mM PDC at pH 9.2 dissolved silica particles as

packing materials, column was re-loaded by the packing materials every time before use. The flow rate was 1 ml min^{-1} .

3. Results and discussion

3.1. CZE migration behavior

Fig. 1 shows electropherograms of Co(II) and Cu(II) on a 20 mM sodium tetraborate buffer at pH

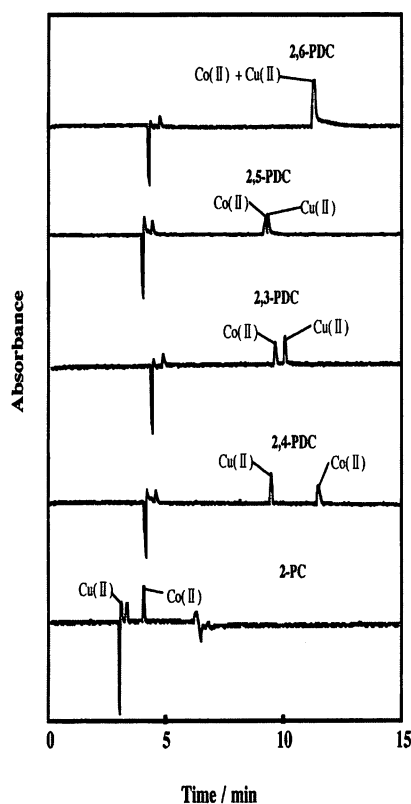


Fig. 1 Electropherograms of $5 \mu\text{g ml}^{-1}$ Co(II) and Cu(II) on 20 mM sodium tetraborate buffer at pH 9.2 in presence of 1 mM 2-PC, 2,3-PDC, 2,4-PDC, 2,5-PDC, and 2,6-PDC.

9.2 in presence of 1 mM 2-PC, 2,3-, 2,4-, 2,5-, and 2,6-PDC, respectively. In case of 2-PC, migration order was EOF \cong Cu(II) < Co(II). Since pK_a of 2-PC is 5.23 (Ionic strength (I) = 0.1) at 20°C [6], the 2-PC exists as an anion of -1 charge in the sodium tetraborate buffer at pH 9.2. Also, since a peak of Cu(II)-2-PC was observed immediately after a peak of EOF (water dip peak), it formed a neutral 1 : 2 complexes of Cu(II) with 2-PC. Stability constant ($\log \beta_2$) of Cu(II) with 2-PC is 14.95 ($I = 0.1$) at 20°C [6]. On the other hand, since a peak of Co(II)-2-PC was observed after the peaks of EOF and Cu(II)-2-PC,

it formed a 1 : 3 charged complex of -1 for Co(II) with 2-PC. The stability constants ($\log \beta_2$ and $\log \beta_3$) of Co(II) with 2-PC at 20°C ($I = 0.1$) are 10.44 and 14.09, respectively [6]. It is reasonable that the 1 : 2 Cu(II)-2-PC and the 1 : 3 Co(II)-2-PC complexes, which have the large $\log \beta_2$ and $\log \beta_3$, stably existed in the electrophoretic buffer at pH 9.2. The 2-PC behaved as a bidentate ligand for Co(II) and Cu(II).

In cases of PDCs, since pK_{a2} s of 2,3-, 2,4-, 2,5-, and 2,6-PDC at 20°C ($I = 0.5$) are 4.63, 4.73, 4.64, and 4.68, respectively [6], these PDCs exist as anions of -2 charge in the sodium tetraborate buffer at pH 9.2. Also, the stability constants ($\log \beta_2$) of Cu(II) with 2,3-, 2,4-, 2,5-, and 2,6-PDC at 20°C ($I = 0.5$) are 14.15, 14.55, 14.35, and 16.52 ($I = 0.1$), respectively [6]. The $\log \beta_2$ value of Co(II) with 2,6-PDC at 20°C ($I = 0.1$) is 12.70 [6]. The $\log \beta_2$ and $\log \beta_3$ values of Co(II) with 2,3-, 2,4-, and 2,5-PDC have not been reported. It could be, however, expected that these $\log \beta_2$ and $\log \beta_3$ values would be nearly the similar to those of Co(II)-2-PC, because these $\log \beta_2$ values of Cu(II)-PDC are nearly the similar to that of Cu(II)-2-PC. Therefore, although it was first expected that analogous Cu(II) and Co(II) complexes of 2-PC for 2,3-, 2,4-, and 2,5-PDC would be formed, these migration orders were Cu(II) \ll Co(II) for 2,4-PDC and Co(II) < Cu(II) for 2,3- and 2,5-PDC. Surprisingly, the migration order for 2,4-PDC was different from those for 2,3- and 2,5-PDC, in spite of only isomers of PDC. This would mean that Cu(II) might form 1 : 2 (= metal ion : PDC) charged complexes of -2 with PDC and that Co(II) might form 1 : 3 charged complex of -4 with 2,4-PDC and 1 : 2 charged complexes of -2 with 2,3- and 2,5-PDC, because peaks of Co(II) were close to those of Cu(II) for 2,3- and 2,5-PDC. Only the Co(II)-2,4-PDC complex was formed at a specific ratio of metal ion to PDC. It is also considered that geometries of the 1 : 2 charged complexes of -2 for Cu(II) with PDC might be different from those for Co(II) with 2,3- and 2,5-PDC, because of no separation between Co(II) and Cu(II) with 2,6-PDC, whose Co(II) and Cu(II) formed the similar geometry of the 1 : 2 charged complex of -2 .

In cases of 3,4- and 3,5-PDC, peaks of Co(II) and Cu(II) on the 20 mM sodium tetraborate buffer at pH

9.2 in presence of 1 mM 3,4- and 3,5-PDC were not observed. Therefore, since it was found that oxygen of 2-carboxylate ion and nitrogen on pyridine ring in PDC were required in order to obtain the peaks of Co(II) and Cu(II), complex formation of Co(II) and Cu(II) with 3,4- and 3,5-PDC as only monodentate ligands would be very weak in the electrophoretic buffer at pH 9.2. For example, the stability constant ($\log \beta_2$) of Cu(II) with 3-PC is only 6.2 ($I = 0.5$) at 20°C [6].

3.2. CZE migration behaviors of Co(II) and Cu(II) pre-complexed with PDC

In cases of CZE separations of Co(II) and Cu(II) pre-complexed with 2,3-, 2,4- and 2,5-PDC in the 20 mM sodium tetraborate buffer at pH 9.2 as samples, peaks of only Cu(II) were observed, because a precipitation of Co(II) with hydroxide ions was produced in the sample. Anionic Co(II)-PDC complexes could exist in the buffer at pH 9.2 only by on-column complexation method. This means that Cu(II) formed more stable complexes with PDC in the sodium tetraborate buffer at pH 9.2 and that Co(II) would form PDC complexes hydrolyzed by the hydroxide ion or water molecules.

3.3. HPLC retention behavior

Fig. 2 shows chromatograms of Co(II) and Cu(II) on a 20 mM sodium tetraborate buffer at pH 9.2 in presence of 1 mM 2-PC, 2,3-, 2,4-, 2,5-, and 2,6-PDC, respectively, under the same buffer conditions as CZE. Retention orders were Cu(II) < Co(II) for 2,3- and 2,5-PDC and Cu(II) \cong Co(II) for 2,4-PDC. Chromatographic behavior of Co(II)-2,4-PDC was apparently different from those of Co(II)-2,3- and 2,5-PDC as CZE migration behaviors. Since silica gels phase has a negative charge on the buffer at pH 9.2 [27], more negatively charged complexes would elute at first. This can be proved by facts that retention time of $[\text{Co}(2\text{-PC})_3]^-$ of a charged complex of -1 was shorter than that of $[\text{Cu}(2\text{-PC})_2]$ of a neutral complex and that peak of $[\text{Cu}(2\text{-PC})_2]$ was broad. Therefore, the Co(II)-2,4-PDC complex has a more negative charge than Co(II)-2,3- and 2,5-PDC. Sensitivity of Co(II)-2,4-PDC was also higher than those of Co(II)-2,3- and 2,5-PDC. This means that the number of PDC in the Co(II)-2,4-PDC complex is larger than those in the Co(II)-2,3- and 2,5-PDC.

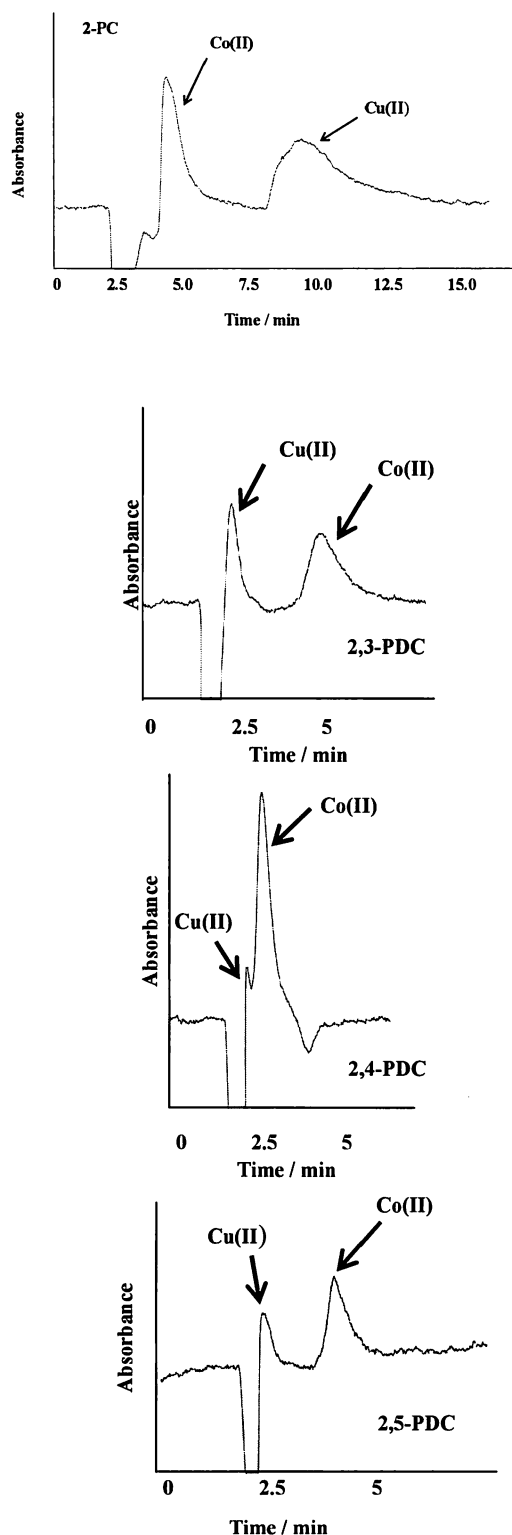


Fig. 2 Chromatograms of Co(II) and Cu(II) on 20 mM sodium tetraborate buffer at pH 9.2 in presence of 1 mM 2-PC, 2,3-PDC, 2,4-PDC, and 2,5-PDC. Flow rate, 1 ml min⁻¹; concentrations ($\mu\text{g ml}^{-1}$) of Co(II) and Cu(II), 6 and 25 for 2-PC; 5 and 5 for 2,3-PDC; 3 and 3 for 2,4-PDC; 10 and 5 for 2,5-PDC.

Co(II) would form a 1 : 3 charged complex of -4 with 2,4-PDC as cases of CZE. However, sensitivity of the Co(II)-2,4-PDC complex was low in CZE. This means the Co(II)-2,4-PDC complex for a long migration was hydrolyzed. The Co(II)-2,3- and 2,5-PDC and the Cu(II)-PDC complexes were 1 : 2 complexes of -2 charge of different geometry as the cases of CZE, because of separation between Co(II) and Cu(II). These HPLC results would support the CZE migration behaviors.

3.4. CZE migration behavior on pH

Dependencies of electrophoretic mobilities (μ_{ep}) on pH are shown in Figs. 3 (a) – (d). Separation currents at pH 7.0 – 8.0 and 8.5 – 9.2 were 16.0 and 15.0 mA, respectively. Therefore, an effect of μ_{ep} values on the ionic strengths would be negligible. In case of 2-PC, the μ_{ep} values of Co(II) and Cu(II) were constant. This means that the hydroxide ions or water molecules could not coordinate to $[\text{Cu}(2\text{-PC})_2]$ and could not substitute 2-PC in $[\text{Co}(2\text{-PC})_3]$.

In cases of PDC, since the μ_{ep} values of Co(II) and Cu(II) decreased with increasing pH, water molecules would be coordinating to Co(II)- and Cu(II)-PDC complexes. Therefore, the dependencies of the μ_{ep} values on pH would be due to a dissociation of a proton in the coordinating water molecules. Although the dependencies of the μ_{ep} values of Co(II) and Cu(II) with 2,3- and 2,5-PDC were almost the same, the μ_{ep} values of Co(II) and Cu(II) with 2,4-PDC suddenly decreased at above pH 8.5. This might mean that geometries of Co(II)- and Cu(II)-2,4-PDC complexes were changed from bidentate to monodentate complexes for the coordinated 2,4-PDC including substitution of water molecules. The detail could not be obvious in this work. However, 3-, 4- and 5-carboxylate ion obviously interrupted coordination of 2-carboxylate ion and nitrogen of pyridine ring. Consequently, coordination of water molecules to Co(II) and Cu(II)-PDC complexes occurred.

3.5. Migration species of Co(II) and Cu(II) with PDC

Chemical structures of Co(II)- and Cu(II)-2,3-, 2,4- and 2,5-PDC have been determined by X-ray crystal structures [15-19]. The chemical structures depended on condition to form these crystals.

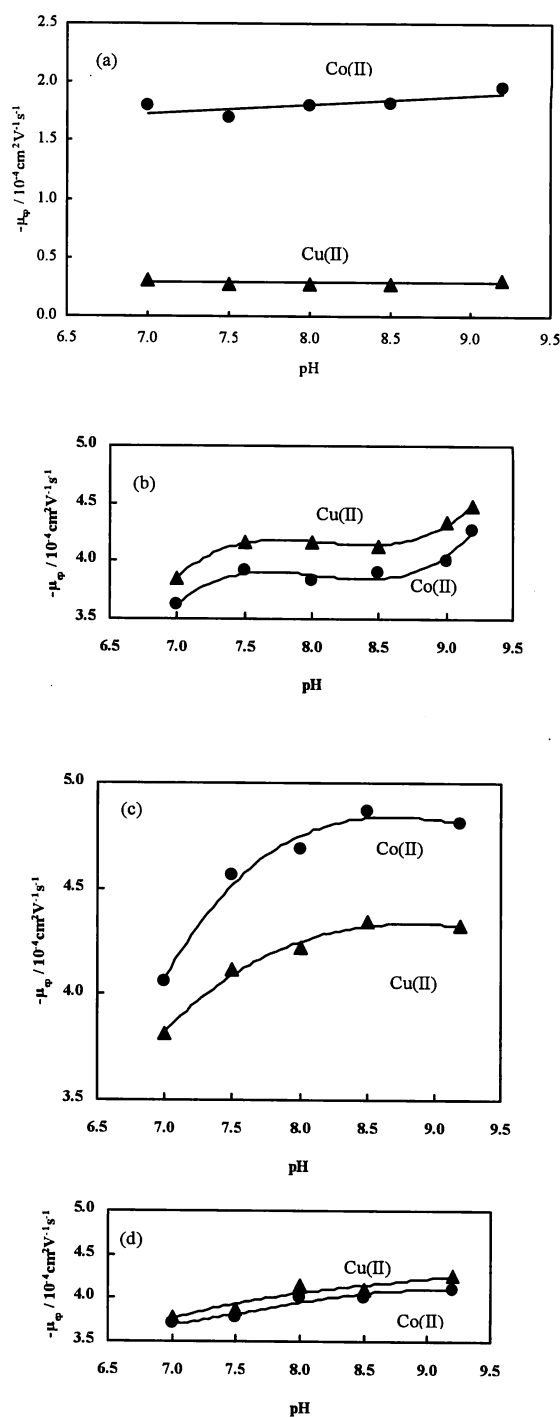


Fig. 3 Dependencies of μ_{ep} of Co(II) and Cu(II) on pH for 2-PC (a), 2,3-PDC (b), 2,4-PDC (c), and 2,5-PDC (d). Co(II), ●; Cu(II), ▲.

Although PDC formed a mononuclear complex with Cu(II) under an acidic condition (pH = 3), it formed a polynuclear complex with Cu(II) under a less acidic condition (pH = 5) [18]. In other words, PDC behaved as only bidentate ligand in case of existing

as its monoanion, and as both monodentate and the bidentate ligands in case of existing as its dianion. Therefore, PDC would behave as both the monodentate and the bidentate ligands under these buffer conditions at pH 7.0 – 9.2. In addition to the X-ray crystal structures from references [15–19], facts that Co(II) formed anionic 1 : 3 complex with 2,4-PDC as described in sections 3.1. – 3.3. and that hydroxide ions and water molecules were coordinating to Co(II)- and Cu(II)-PDC complexes as described in section 3.4. suggest chemical structures of the Co(II) and Cu(II)-PDC complexes as illustrated in Figs. 4 (a) – (c). The anionic Co(II)-PDC complexes could be formed only by the on-column complexation in alkaline solution in presence of PDC. Although the reason why only the 2,4-PDC formed the 1 : 3 complex with Co(II) could not be obvious, the 2,4-PDC might produce a polynuclear complex with Co(II).

4. Conclusions

CZE separation between Co(II) and Cu(II) with 2-PC, 2,3-, 2,4-, and 2,5-PDC was accomplished. Migration species of Co(II) and Cu(II) with 2-PC and PDC were found. Specific separation of Co(II)-2,4-PDC was due to formation of anionic 1 : 3 Co(II)-2,4-PDC complex. Chelating reagents, 2,3-, 2,4-, 2,5-PDC behaved as both of monodentate and bidentate ligands in alkaline solution. Using isomers of PDC in CZE, separation selectivity of metal ions could be changed.

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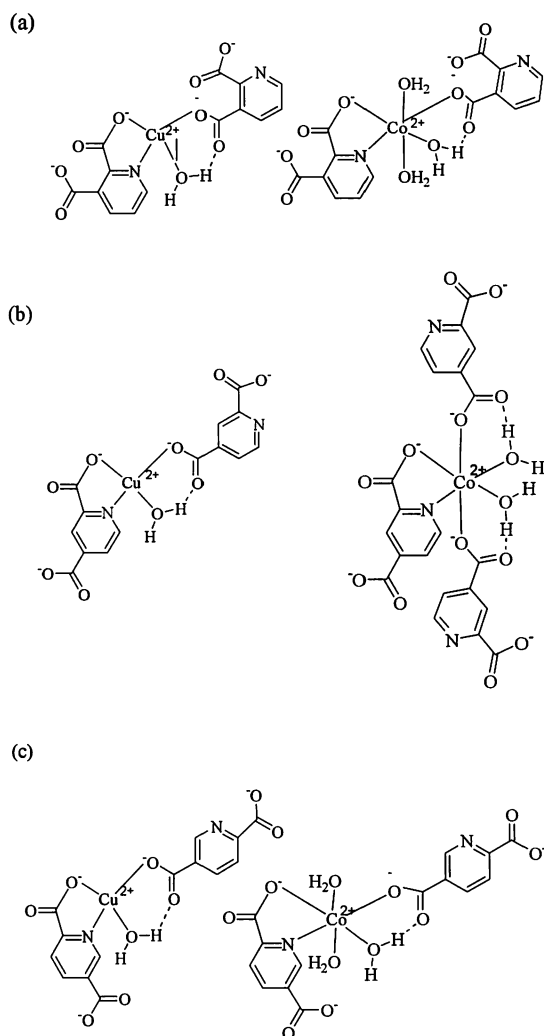


Fig. 4 Chemical structures of migration species for Co(II) and Cu(II) with 2,3- (a), 2,4- (b), and 2,5-PDC (c).

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