pH-Dependent Coordination of Metal–Lisinopril Complex Investigated by Attenuated Total Reflection/Fourier Transform Infrared Spectroscopy

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In order to simulate the *in vivo* binding behavior of angiotensin-converting enzyme (ACE) inhibitors to the zinc-containing active center of ACE, the *in vitro* interaction between lisinopril and zinc or nickel ions was investigated in aqueous solutions of different pH by using attenuated total reflection (ATR)/Fourier transform infrared (FT-IR) spectroscopy with second-derivative IR spectral analysis. The results indicated that the lisinopril dissociation process occurred in a stepwise fashion during increase in pH. The IR peaks at 1642 cm^{-1} (carbonyl stretching of tertiary amide) and at 1582 cm^{-1} (asymmetric COO^- stretching) for lisinopril in solution at pH 3.5 shifted to 1606 and 1586 cm^{-1} after addition of Ni^{2+} ions, respectively, but there was no marked changes in IR spectra of lisinopril after addition of Zn^{2+} ions. When the Zn^{2+} ions were added to lisinopril solution at pH 5.0, the peak at 1642 cm^{-1} also shifted to 1604 cm^{-1} and the peak at 1582 cm^{-1} shifted to 1586 cm^{-1}, similar to the changes at pH 3.5 after adding Ni^{2+} ions. However, the peaks at 1582 and 1642 cm^{-1} both shifted to 1599 cm^{-1} after addition of Ni^{2+} ions at pH 5.0 or at pH 7.3. The peak at 1576 cm^{-1} also shifted to 1599 cm^{-1} after addition of Zn^{2+} ions to lisinopril solution at pH 7.3. Different coordination sites or types (chelating, bridging or pseudo-unidentate complex) between lisinopril and Zn^{2+} or Ni^{2+} ions were proposed, based on the separation value between ν_{as} (COO^-) and ν_s (COO^-), and the shifting of carbonyl groups. Coordination of the secondary amine in lisinopril to metal ions was also evidenced.

Key words lisinopril; metal ions; ATR/FT-IR; coordinating complex formation; pH

Angiotensin-converting enzyme (ACE), producing the potent vasopressor peptide angiotensin II, plays an important role in circulatory homeostasis.[1] ACE inhibitors act through competitive binding to the active site of the ACE molecule and thereby inhibit enzyme activity, leading to decrease in circulating angiotensin II levels, and reduction in blood pressure and peripheral vascular resistance without an increase in heart rate.[2,3] ACE inhibitors thus have an important role as therapeutic agents in the treatment of hypertension and congestive heart failure.[4,5] Since ACE is a zinc-metallopeptidase activated by chloride, the mechanism of action of ACE inhibitors results mainly from competition with the natural substrate by binding to the zinc ion in the active site on the ACE molecule. The chemistry of nickel-containing enzymes has been recognized to perform several biochemical properties such as urealysis, biocarbonylation, hydrogenation in body.[6] Hydrogen bonding and hydrophobic interactions also influence their pharmacological activity.[3,7,8] The ACE inhibitors used in clinical therapy can be classified into three different structural types according to the ligand of the zinc ion of ACE, involving either a sulfhydryl, carboxyl or phosphorus moiety.[9,10] Due to the high affinity of inhibitors for ACE, they also provide powerful tools for studying the structure, function, and tissue distribution of ACE.[11]

In a series of structure–activity studies, the importance of the terminal carboxyl, the amide carbonyl, and a zinc-binding group for the interaction of ACE inhibitors with the active site of ACE has been emphasized.[3,9] Visible absorption and magnetic circular dichroic spectropolarimetry have been used to examine the interaction between Co–ACE and captopril, and these techniques have provided physical evidence for the direct ligation of the thiolgroup (SH) to the active site metal.[12] The result of fast-atom bombardment tandem mass spectroscopy suggests that the metal binding preferentially occurred at the amine nitrogen in the gas phase for enalaprilat and lisinopril.[13] On the other hand, the crystal structure of copper–lisinopril complex determined by X-ray crystallography shows the co-ordination site for metal ions involves the amino nitrogen, carboxylate oxygen, and the amide oxygen atoms.[14] Although the potentiometric titration and ^{13}C-NMR had been used to study the binding of metal ions to lisinopril, the detailed evidence of binding mechanism should still be investigated.[15,16] The pK_a values of lisinopril at 25 °C are 2.5, 4.0, 6.7 and 10.1.[17] These were assigned as follows: 10.1 to the lysyl $^+NH_3$, 6.7 to the secondary $^+NH_2$ group, which is more acidic than the lysyl $^+NH_3$ due to the proximity of the electron-withdrawing amide group, 4.0 to the prolyl COOH and 2.5 to the central COOH, which is more acidic than the prolyl COOH due to the proximity of the $^+NH_2$ group.[14]

From physiological and pharmacological considerations, the interaction between ACE and its inhibitor should occur in solution after administration of the ACE inhibitor, rather than in the solid state. However, there is scanty information on the interaction between them in solution at different pH values. Spectral studies have not yet addressed the structures of metal chelates formed from ACE inhibitors in aqueous solution. We have previously studied the pH-dependent conformational changes of proteins in solution by using attenuated total reflection (ATR)/Fourier transform infrared (FT-IR) spectroscopy.[18,19] In this study we use this technique to investigate the co-ordination of metal–ACE inhibitor complexes in aqueous solutions of different pH. Lisinopril was used as a model ACE inhibitor, and the chelating ions Zn^{2+} and Ni^{2+} were used.

Materials and Methods

Materials Lisinopril dihydrate of pharmaceutical grade was purchased from Chemical Works Gedeon Richter Ltd., Hungary. $Ni(NO_3)_2$ and $ZnSO_4$ were of analytical reagent grade, purchased from Nakalai Tesque, Kyoto,

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Japan.

Preparation of Different Lisinopril Solutions Five percent (w/v) solutions of lisinopril in double distilled water were adjusted to pH values of 1.2, 3.5, 5.0 and 7.3, using 12 N HCl or 12 N NaOH as required.

Preparation of Metal–Lisinopril Solutions Lisinopril and solid $Ni(NO_3)_2$ or $ZnSO_4$ were dissolved together in double distilled water to give concentrations of 5% (w/v) of each component. The pH of these solutions were also adjusted by using 12 N HCl and 12 N NaOH to give pH values of 3.5, 5.0 and 7.3.

ATR/FT-IR Spectroscopic Determination Each sample solution was analysed in an ATR/FT-IR spectrophotometer (FT/IR-620, Jasco, Tokyo, Japan) equipped with a Duterated L-Alanine Triglycine Sulphate (DLATGS) detector detector. The sample solution was placed in a horizontal ATR accessory (Pike Tech., WI, U.S.A.) with a zinc selenide prism for the analysis. All spectra were carried out at 200 scans and a resolution of 4 cm^{-1} at ambient temperature (25 °C). Solvent spectra were also examined in the same accessory and with the same instrument conditions. Difference spectra were obtained by digitally subtracting solvent spectra from the corresponding sample spectra. Three batches of each sample solution were analysed. The individual spectrum of three analyses of each sample was obtained and averaged to produce a single spectrum for subsequent data processing. Spectral Manager for Windows software (Jasco, Tokyo, Japan) was used for data acquisition and handling. Second-derivative spectral analysis was applied to locate positions and assign them to different functional groups.

Results

Figure 1 shows the original and second-derivative IR spectra of lisinopril in solutions of different pH between 1800 and 1200 cm^{-1}. Four distinct peaks and two shoulders were observed in the spectra of lisinopril at pH 1.2: the peak at 1724 cm^{-1} was assigned to the carbonyl stretching of COOH; the peak at 1642 cm^{-1} corresponded to the carbonyl stretching of tertiary amide; the peak at 1453 cm^{-1} was due to the scissoring band of CH_2; the peak at 1237 cm^{-1} was assigned to the C–O stretching of COOH; the shoulder peaks at 1490 and 1285 cm^{-1} were due to the aromatic ring mode and the contribution of C–O and O–H in plane deformation, respectively. The appearance of the peak at 1724 cm^{-1} reveals that the dissociation of lisinopril did not occur in pH 1.2 solution. With the increase of pH from 1.2 to 7.3, the peak at 1724 cm^{-1} shifted to 1717 cm^{-1} and finally disappeared. The peaks at 1582 and 1576 cm^{-1} assigned to the asymmetric COO^- stretching are markedly and gradually observed with the increase of pH. Moreover, the appearance of the peak at 1395 cm^{-1} due to the symmetric COO^- stretching and the disappearance of the peak at 1237 cm^{-1} assigned to the C–O stretching of COOH strongly indicated that the process of lisinopril dissociation occurred in a stepwise manner as the pH increased (Fig. 2). Lisinopril was mostly dissociated in pH 7.3 solution.

In order to simulate the *in vivo* binding behavior of inhibitor to the zinc-containing ACE, the *in vitro* interaction between lisinopril and zinc or nickel ions was investigated using second-derivative IR spectra of lisinopril in different pH solutions. Figure 3A indicates the second-derivative IR spectra of lisinopril before and after addition of Ni^{2+} or Zn^{2+} ions in pH 3.5 solution. It is evident that there was no significant difference between the spectra except in the 1700—1500 cm^{-1} range. Two main peaks at 1642 (C=O of tertiary amide) and 1582 (asymmetric COO^-) cm^{-1}, and one shoulder at 1625 cm^{-1} were clearly observed for lisinopril in the absence of any metal ion. By addition of Ni^{2+} ions, the peak at 1642 cm^{-1} shifted to 1606 cm^{-1} and the peak 1582 cm^{-1} shifted to 1586 cm^{-1}. However, there was no marked difference after addition of Zn^{2+} ions to the lisinopril solution except a new peak at 1615 cm^{-1}. The second-derivative IR spectra shown in Fig. 3B were obtained before and after addition of Zn^{2+} or Ni^{2+} ions to lisinopril solution at pH 5.0. There was no significant difference except in the 1700—1500 cm^{-1} range, as was observed at pH 3.5. The two main peaks at 1642 and 1582 cm^{-1} were still present but a slight shoulder at 1625 cm^{-1} was observed for lisinopril without any addition. Once the Zn^{2+} ions were added into the pH 5.0 lisinopril solution, the peak at 1642 cm^{-1} shifted to 1604 cm^{-1} and the peak at 1582 cm^{-1} shifted to 1586 cm^{-1}, similar to the changes observed in the pH 3.5 lisinopril solution after addition of Ni^{2+} ions. On the other hand, the peaks at 1582 and 1642 cm^{-1} both shifted to 1599 cm^{-1} after addition of Ni^{2+} ions to lisinopril solution at pH 5.0. Metal ion-in-

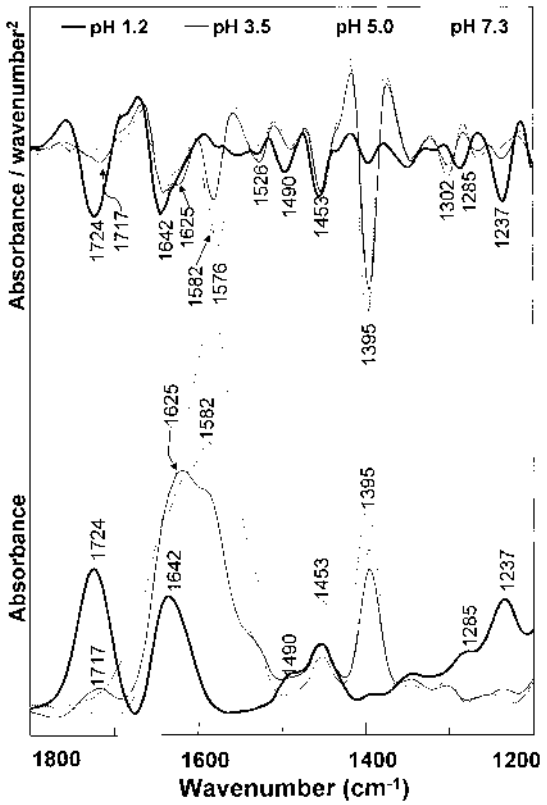

Fig. 1. Original and Second-Derivative IR Spectra of Lisinopril in Aqueous Solutions of Different pH between 1800 and 1200 cm^{-1}

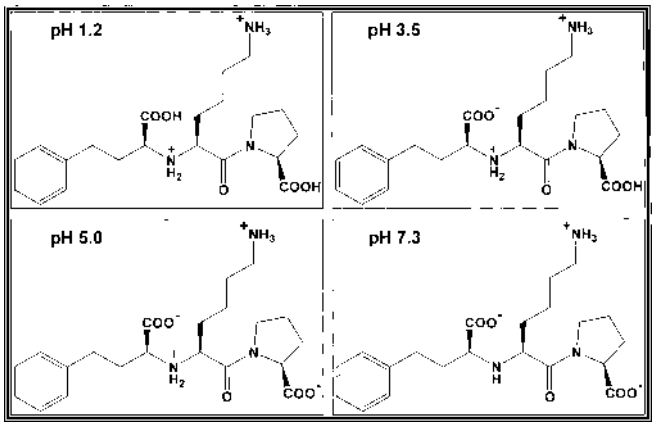

Fig. 2. The pH-Dependent Dissociation of Lisinopril in Aqueous Solution

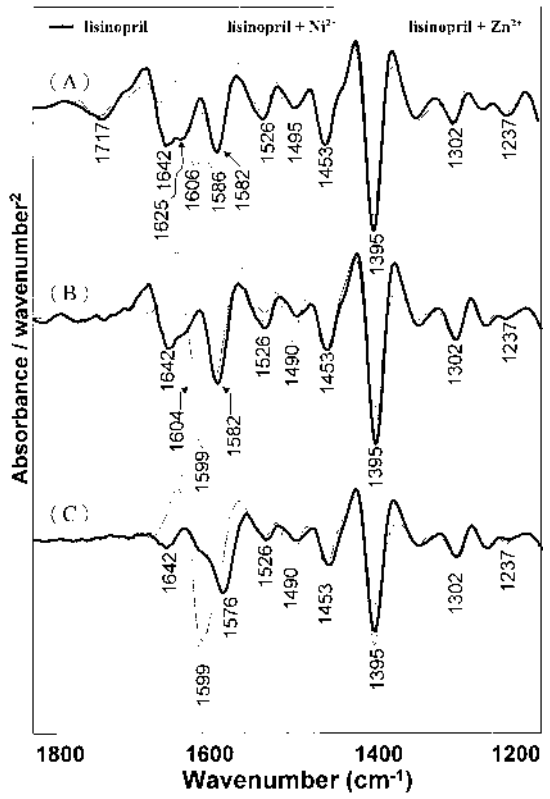

Fig. 3. Second-Derivative IR Spectral Changes of Lisinopril before and after Addition of Ni^{2+} or Zn^{2+} Ions in Aqueous Solutions of pH 3.5 (A), 5.0 (B) and 7.3 (C)

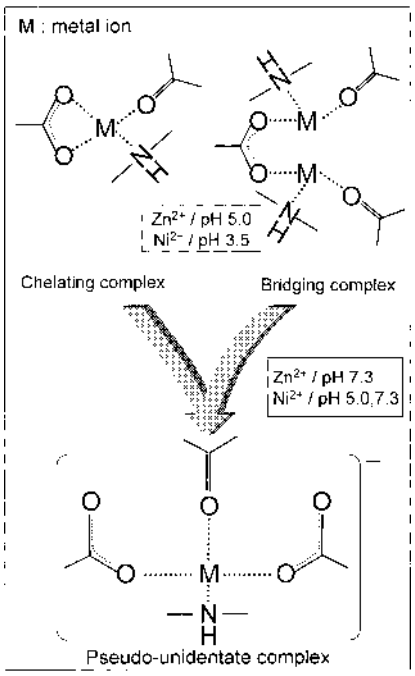

Chart 1. The Coordinating Complex Formation Proposed between Lisinopril and Metal Ions in Aqueous Solutions of Different pH

duced changes at pH 7.3 are shown in Fig. 3C. The spectral behavior of lisinopril after addition of the Ni^{2+} ions to solutions at pH 5.0 and 7.3 was found to be similar. Moreover, the peak at 1576 cm^{-1} also shifted to 1599 cm^{-1} after addition of Zn^{2+} ions at pH 7.3.

Discussion

Early analyses of molecular structure of compounds *via* IR absorption were restricted to samples in the solid state or in D_2O solution because of strong overlapping water absorptions. The advent of computer-controlled IR spectroscopy has permitted digital subtraction of overlapping water absorptions from the spectra of aqueous samples, enabling water to be used routinely as an IR solvent. In the present study, the interaction of metal ions with lisinopril was studied *in vitro* by FT-IR spectroscopy to simulate the *in vivo* binding of ACE inhibitors to zinc ions located in the active site of the ACE molecule.

Lisinopril in the pH 1.2 solution (below pK_a 2.5) exhibited the IR peak at 1724 cm^{-1} due to non-dissociation of the COOH group. As the pH of the solution increased, IR peaks at both 1582 and 1395 cm^{-1}, corresponding to the asymmetric and symmetric COO^- stretching gradually became more prominent. When the pH of the solution reached 7.3, the structural change due to the dissociation of the secondary amine group resulted in a frequency shift on the asymmetric COO^- from 1582 to 1576 cm^{-1}. Since the shift of the frequency of the ν_s COO^- at 1395 cm^{-1} was less sensitive than ν_{as} COO^-, the coordinative relation between carboxylate and metal was mainly dependent on the ν_{as} COO^-, leading to less shifting of ν_s COO^- at 1395 cm^{-1}.[20] Moreover, the peak at 1237 cm^{-1} assigned to the C–O stretching of COOH also disappeared stepwise.

It has been reported that the carbonyl or carboxylate groups of compounds are capable of forming a complex with metal ions in aqueous solution.[21] After formation of acetate complexes, the IR frequency of the carbonyl group might shift to a lower frequency which was dependent on the metal ions involved. On the other hand, the coordination between carboxylate ion and metal ion could be obtained by a empirical criteria,[22,23] which was also evidenced by theoretic calculation by *ab initio* molecular orbital calculation and normal-coordinate treatment.[24,25] The separation (Δ) values between ν_{as} (COO^-) and ν_s (COO^-) are defined as follows: Δ values$\geq$ 200 cm^{-1} indicate unidentate coordination; Δ values$\leq$105 cm^{-1} are associated with chelating coordination; Δ values$>$ 105 cm^{-1} and close to the ionic values correspond to chelating and/or bridging coordination.[22,23] In fact it is obviously difficult to draw structural conclusions when Δ value is near ionic values. Since the structure of lisinopril has the above two functional groups, it easily chelates with metals in aqueous solution. Thus simple mixing of metal ions with lisinopril solutions at different pHs will result in formation of the metal–lisinopril complex.

Because the second-derivative IR spectra of lisinopril after addition of Zn^{2+} ions are almost identical to the original spectra of lisinopril, there appears to be little interaction between lisinopril and Zn^{2+} ions in pH 3.5 solution (Fig. 3). However, the second-derivative IR spectra of lisinopril after addition of Ni^{2+} ions showed changes from 1642 to 1606 cm^{-1} and from 1582 to 1586 cm^{-1}, implying interaction occurred between them. The Δ value between ν_{as} (COO^-) of 1586 cm^{-1} and ν_s (COO^-) of 1395 cm^{-1} was about 191 cm^{-1} and close to ionic value (187 cm^{-1}), suggesting that chelating and/or bridging coordination was formed between lisinopril

and Ni^{2+} ions.[21,22] The IR peak shifting from 1642 to 1606 cm^{-1} was also confirmatory evidence of carbonyl group coordination. When the pH was changed from 3.5 to 5.0, similar interaction was formed between lisinopril and Zn^{2+} ions due to the peaks shifted from 1642 to 1606 cm^{-1} and from 1582 to 1586 cm^{-1}. In this pH 5.0 solution, the interaction of lisinopril and Ni^{2+} ions seemed to be much stronger, since both peaks at 1606 and 1586 cm^{-1} significantly shifted to 1599 cm^{-1}. In addition, the Δ value between ν_{as} (COO^-) of 1599 cm^{-1} and ν_s (COO^-) of 1395 cm^{-1} is about 204 cm^{-1}, suggesting the complicated coordination like unidentate could be occurred between carboxylate group of lisinopril and Ni^{2+} ions. When the pH changed from 3.5 to 5.0, the more basic medium favored the dissociation of the prolyl COOH of lisinopril. The greater the degree of dissociation of lisinopril, the more the electron density will induce complex formation with metal. Consequently, the carbonyl group absorption shifted to lower frequency (from 1606 cm^{-1} in pH 3.5 to 1599 cm^{-1} in pH 5.0). In pH 7.3 solution, a marked IR spectral shifting was found for lisinopril after addition of Ni^{2+} or Zn^{2+} ions, from 1642 or 1576 cm^{-1} to 1599 cm^{-1}. The Δ value between ν_{as} (COO^-) of 1599 cm^{-1} and ν_s (COO^-) of 1395 cm^{-1} was 204 cm^{-1}, higher than 200 cm^{-1} and its ionic value (181 cm^{-1}), implying the interaction between lisinopril and Ni^{2+} or Zn^{2+} ions was also a complicated coordination like unidentate in pH 7.3 solution.

Lisinopril has two carboxyl groups including prolyl COOH and central COOH, which can interact with metal ions. In pH 3.5 solution, only central COOH was dissociated to carboxylate ion and own a high electronic density to easily interact with metal ions than the undissociated prolyl COOH. Above pH 5.0 solution, both COOH groups were in a dissociation form. In the present study, the ionic value for lisinopril is 187 cm^{-1} in pH 3.5 and 5.0 solutions, but is 181 cm^{-1} in pH 7.3 solution. However, the Δ value for lisinopril–Ni^{2+} complex is 191 cm^{-1} in pH 3.5 solution, and is 204 cm^{-1} in pH 5.0 and 7.3 solutions; for lisinopril–Zn^{2+} complex is 187 cm^{-1} in pH 5.0 solution, and is 204 cm^{-1} in pH 7.3 solutions, respectively. The shift of Δ value for Ni^{2+} ions from pH 3.5 to 5.0 and for Zn^{2+} ions from pH 5.0 to 7.3 might possibly arise from two reasons. First, it may be more favorable coordination at the either COOH group. In pH 3.5 solution of this study, because the central COOH of lisinopril could dissociate, so we regard only this central COOH could coordinate with Ni^{2+} ions. Moreover, the Δ value of lisinopril–Ni^{2+} complex was also found to near its ionic value of lisinopril. According to the ^{13}C-NMR study in pH 5.0 solution,[16] Zn^{2+} ions had been proven to only interact with central COOH group of lisinopril. In this study, the Δ value of lisinopril–Zn^{2+} complex was also close to its ionic value of lisinopril in pH 5.0 solution. From the results of ^{13}C-NMR and our present studies in pH 3.5 and 5.0 solutions, we suppose that when only central COOH of lisinopril interacted with metal ions, it may cause the Δ value close to the ionic value and result in a chelating or bridging coordination between lisinopril and metal ions.

On the other hand, Ni^{2+} ions had also been found to coordinate with both COOH groups of lisinopril in pH 5.0 solution from ^{13}C-NMR study.[16] In this study, moreover, the Δ value of lisinopril–Ni^{2+} complex was also determined to larger than its ionic value of lisinopril. Thus it is reasonable to presume that both COOH groups of lisinopril interacted with metal ions will produce the larger Δ value. This is the reason of Δ value shift for Ni^{2+} ions from pH 3.5 to 5.0 and for Zn^{2+} ions from pH 5.0 to 7.3. The second possibility for the shift of Δ value may come from different binding types. When pH value increases, the coordinating type between carboxyl group and metal ions will shift from chelating and/or bridging coordination to "pseudo-unidentate" coodination,[22] as indicated in Chart 1.

Since the solid lisinopril–Cu^{2+} complex has been evidenced as an unidentate coordination by X-ray crystallographic data,[14] but in the ^{13}C-NMR and present study, the complex with metal in carboxyl group is chelating, bridging or pseudo-unidentate coordination in solution. This suggests that the metal ion will interact with central COOH group or both COOH groups or form various coordinating types in different pH solution of lisinopril. Because Ni^{2+} ions could interact with lisinopril in pH 3.5 than Zn^{2+} ions in pH 5.0, thus we suppose the coordinating order of metal ions to the active site of lisinopril was $Ni^{2+} > Zn^{2+}$.

In pure lisinopril solution, a pH change from 5.0 to 7.3 would cause the shift of the ν_{as} (COO^-) group from 1582 to 1576 cm^{-1}. This change originated from the disappearance of positive charge on the secondary amine group after dissociation. In this study, the IR spectral shift for the lisinopril–Ni^{2+} complex is quite similar at pH 5.0 and 7.3, indicating the similar electronic condition in both pH solutions. In pure lisinopril solution, it was known that the secondary amine group would dissociate during change from pH 5.0 to 7.3, but there was no change for lisinopril–Ni^{2+} complex between pH 5.0 and 7.3 solutions, implying the Ni^{2+} will also coordinate with the secondary amine group. From the above result, the pH-dependent coordination of the metal–lisinopril complex is proposed in Chart 1. The co-ordination between lisinopril and metal ions might occur through the carboxylate oxygen, amino nitrogen and carbonyl oxygen atoms.

In conclusion, the pH-dependent coordination of the metal–lisinopril complex is evidenced. The coordination interaction between lisinopril and metal ions might occur through the carboxylate oxygen, secondary amine group and carbonyl oxygen atoms. The chelating and/or bridging, or pseudo-unidentate coordination can be formed by the interaction between carboxylate group of lisinopril and metal ions.

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