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Exploring pK_a of Peroxycitric Acid Coexisting with Citric Acid in Aqueous Solution with Voltmmetric, Potentiometic and Chromatographic Approaches

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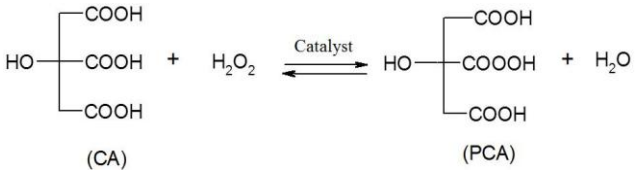
pK_a value of peroxycarboxylic (–COOOH) group of peroxycitric acid (PCA) without separating from the coexisting citric acid (CA) in aqueous solution could be successfully determined with voltammetric, chromatographic and potentiometric titration methods. Effect of solution pH on cyclic voltammetric reduction of –COOOH group of PCA was evaluated. Reversed phase HPLC was employed to recognize the pK_a zones of PCA and the result was found to support the voltammetric determination of the pK_a value. pH-metric titrations of CA and the mixture of PCA and CA were carried out and an independent titration curve of PCA was derived *via* the interpolation method. Titration results were analyzed by Niels Bjerrum graphical method to estimate the three pK_a values for each of PCA and CA. A brief discussion on the pK_a values of PCA is rationally given with the support of *ab initio* molecular orbital calculations on the optimized structures of CA and PCA.

Keywords: Peroxycitric acid; Citric acid; pK_a; Cyclic voltammetry; Niels Bjerrum method

1. INTRODUCTION

Peroxycitric acid (PCA) [1-5] synthesized from citric acid (CA) and H_2O_2 (eq 1) has been aimed to be used as food preservative in the place of the oxidants such as chlorine, ozone and H_2O_2 that are commonly applied in chemical processing and synthesis, bleaching and disinfection purposes

in food and beverage industries [1–14]. Due to their excessive solubility in aqueous solution, PCA and CA could not be isolated, that is, PCA coexists with CA and H_2O_2 [1–5] as in the case of peroxyacetic acid (PAA) [6–8,10,16-18]. Previously, we have successfully determined the molecular structure of PCA without separating from coexisting CA and H_2O_2 with a combined use of potentiometric, chronocoloumetric, reversed-phase high performance liquid chromatographic (RP-HPLC) and electrospray ionization mass spectroscopic measurements [2]. In fact, among three carboxylic (–COOH) groups of CA, the –COOH group adjacent to –OH group (i.e., central –COOH group) is only oxidized by H_2O_2 to form –COOOH group in PCA (eq 1) [2,5].



(1)

The objective of this study is to determine pK_a values of PCA particularly for –COOOH group because the knowledge about pK_a of a compound is very important from the viewpoint of its practical applications. The pK_a value of PAA, an aliphatic peroxyacid, is 8.2, which is higher than that of its parent acetic acid, AA (i.e., 4.7) [20,21]. Aromatic peroxyacid such as mono-peroxyphthalic acid that is a derivative of phthalic acid possessing two –COOH groups with pK_a of 2.9 and 5.4 has two distinct pK_as of 2.9 and 8.2 [22]. In fact, the pK_a of –COOOH group has generally been reported to be higher by ca. 3.5 unit than that of the corresponding parent –COOH group [20].

Table 1. pK_a Values of various peroxycompounds and their parent molecules

Type of parent compounds	Name of compounds	pK_a	References
Water (H–OH)	Water	14.0	-
	Hydrogen peroxide	11.6	20,21
Alcohol (R–OH)	Methanol	16.0	20,21
	Methyl hydroperoxide	11.5	21
	Ethanol	15.9	20,21
	Ethyl hydroperoxide	11.8	20,21
	iso-Propanol	16.5	20,21
	Propyl hydroperoxide	12.1	20,21
	tert-butyl alcohol	16.5	20,21
	tert-butyl hydroperoxide	12.8	20,21
Organic acid (R–COOH)	Formic acid	3.7	20,21
	Peroxyformic acid	7.1	21
	Acetic acid	4.7	20,21
	Peroxyacetic acid	8.2	20,21
	Propionic acid	4.8	21
	Peroxypropionic acid	8.1	21
	n-butyric acid	4.8	21
	Peroxybutyric acid	8.2	21
	CA	3.1, 4.6, 6.4	23,24
		2.9, 4.4, 6.2	This study
	PCA	3.5, 5.4, 8.3	This study

In contrast, as can be seen in Table 1, the pK_a values of organic peroxides are commonly lower than those of their parent alcohols [21]. It is, however, expected that the pK_a values of one –COOOH and two –COOH groups in PCA would be different from those of three –COOH groups in the parent CA. The answer regarding the extent of the change in pK_a of different groups in PCA from those groups in CA [20,21] is not straightforward, since it contains one –OH group that is well-known as electron-withdrawing in nature. Since PCA cannot be isolated from the reaction mixture, the determination of its pK_a values is not an easy task. Here, the dissociation constant for –COOOH in PCA is *arbitrarily* considered as K_{a1} and those for two terminal –COOH groups are K_{a2} and K_{a3}. Similarly, K'_{a1} is the dissociation constant of the central –COOH group and K'_{a2} and K'_{a3} define those of two terminal –COOH groups of CA.

Several methods including potentiometric titration [25–28], HPLC technique [29–31], NMR [32–34] and so on have been frequently employed for the determination of acid dissociation constant of a compound. In the present case, the peroxide (–O–O–) bond in –COOOH group is electroactive [2,3,16–18,20,21], like H_2O_2 [3,16–18,20,21] and hydroperoxides [21], and its electrochemical reduction has been reported to follow a two-electron and two-proton mechanism [3,16,35]. We have previously found that –COOOH group of PCA is also electrochemically active [2,3] and thus the pH-dependent reduction of PCA is expected to essentially provide the information regarding K_{a1}. Moreover, it may be reasonably expected that the dissociation of different functional groups in PCA and CA would also be pH-dependent and correspondingly affects the HPLC response. However, these facts would assist to explore the pK_a values of PCA.

In this study, the cyclic voltammetric reduction of PCA coexisting with excess H_2O_2 and CA was carried out at a gold electrode that has been investigated extensively in our previous study [3]. The effect of solution pH on the characteristics of the reduction of PCA was evaluated. pH dependent RP-HPLC studies of PCA and CA was also carried out to explore the pK_a zones. Finally, a pH-metric titration of PCA coexisting with CA were performed and using Niels Bjerrum graphical method [25,26], pK_a values of CA and PCA were determined. The pK_a values obtained was also discussed with the support of *ab initio* molecular orbital calculation performed by density functional theory (DFT) method [36,37].

2. EXPERIMENTAL

2.1. Reagents

(*Cautionary note!* It has been reported earlier [9] that peroxyacid and H_2O_2 are strong oxidizing agent and their high concentrations in a solution may form explosive mixture). All the chemicals used were of analytical grade including water which was purified to be deionized (Milli-Q, Millipore, Japan). HPLC grade 60% perchloric acid ($HClO_4$), sodium perchlorate ($NaClO_4$) and CA (1,2,3-tricarboxylic-2-hydroxy propane, purity 99.5%) were purchased from Kanto Chemical Co. Inc. (Japan). PCA was synthesized by following the method described elsewhere [1,2].

2.2. Instrumentation and methodology

All electrochemical measurements were carried out with a computer-controlled electrochemical system (Model: ALS / CHI 832A). The electrochemical cell was a two-compartment Pyrex® glass container containing a gold (diameter, $\varphi = 1.6$) working electrode, a spiral platinum wire counter electrode and Ag | AgCl | KCl (sat.) reference electrode. The gold electrode was polished with aqueous slurries of 1 and 0.06 μm alumina powder to mirror finish and was washed to remove the abrasive particles with Milli-Q water under sonication for 10 min. The working electrode was electrochemically pretreated in an N_2-saturated 0.05 M H_2SO_4 solution by repeating the potential scan in the range of −0.2 to 1.5 V until the voltammogram characteristic of the clean gold electrode was obtained. The cyclic voltammetric measurement of PCA was carried out in 0.05 M acetate buffer solutions containing 0.1 M Na_2SO_4 with different pHs. The solution pH was adjusted with NaOH or H_2SO_4 solution. The solution was deaerated by bubbling N_2 gas for 15 min before electrochemical measurements. All of the measurements were carried out at 25 ± 2 ºC.

The conditions of RP-HPLC measurement employed for the analysis of PCA have been reported in our previous papers [2,4]. The RP-HPLC unit consists of a pump (Model 7410, GL Science, USA), a UV detector (Model 7450, GL Science, USA), a column-oven (Model 7432, GL Science, USA), an auto-sampler (Model L-7200, Hitachi, Japan) and a personal computer for data acquisition with software (EZChrome *elite*, Scientific Software, Inc., CA, USA). The column was Intersil C8-3 (5 μm 250 × 4.6 mm I.D. and bonded phase is $-(CH_2)_7CH_3$ group). The flow rate of the mobile phase used for all measurements was maintained to be 0.5 mL / min and the column temperature was 25 °C and the wavelength of the UV detector was fixed at 210 nm. The injected sample volume was 10 μl. Retention factor that is the measure of retention of the sample on the column, k': $k' = (t_r - t_0) / t_0$, where t_r and t_0 are the retention times of unretained and retained samples on the column, respectively. In this work, the t_r of H_2O_2 (6.0 min, that can be seen later in Fig. 2) was assumed as t_0 because no effects of the mobile phase composition, pH and column temperature on t_0 of H_2O_2 were noticed [4]. This RP-HPLC method was also employed to separate PCA or H_2O_2 from the equilibrium mixture, if necessary.

A TOA Electronics ion meter (Model 1M-55G pH meter, Japan) was used to measure the solution pH. The meter was calibrated with standard buffer solutions with pHs of 1.7, 4.1 and 6.8. For pH-metric titration, an adequate amount of solution containing CA or CA and $HClO_4$ or the reaction mixture (PCA and CA) and $HClO_4$ of known concentrations were taken in a beaker and the initial volume of the solution was adjusted to be 40 mL by adding Milli-Q water. In every case, 0.1 M $NaClO_4$ was used to keep the ionic strength of the solution to be constant. pH of the thus-prepared solution was measured and the content of the beaker was titrated with 0.095 M NaOH (standardized) solution. The pH data were plotted against the added volume of NaOH solution and each curve of pH *versus* volume of added NaOH solution was reproduced thrice. The thus-obtained pH *versus* volume of added NaOH solution plots were subjected to further analysis to determine the pK_a values using Niels Bjerrum graphical method [25-27]. In this method, the titration of a weak acid is often carried out in the presence of a strong acid that prevents the initial dissociation of the weak acid and the contribution to the initial pH value of the solution under study. Previously, this approach has been successfully

employed for the determination of pK_a values of formic and glucuronic acids in the presence of HCl [27]. However, in our study, pH-metric titrations of aqueous solution of CA containing $HClO_4$ (blank sample) and the mixture of CA and PCA (run sample) containing $HClO_4$ were carried out separately. The results (i.e., pH *versus* volume of titrant) of the blank sample and the run sample are subjected to interpolation process where the difference of the volume of titrant added at a certain pH of both blank sample and run sample, that is, ΔV (= volume of the titrant for the run sample – volume of the titrant for the blank sample), is derived for each pH value. The pairs of ΔV and pH generated in this way practically correspond to the titration points of PCA.

2.3. Theoretical calculations

Gaussian09 program package was used to perform all the quantum calculations. The calculations for CA and PCA were performed with the Beck's three parameter hybrid functional using the Lee-Yang-Parr correlation functional (B3LYP) [36]. The geometry optimizations were carried out with 3-21G basis set [37] and water was considered as the solvent. Calculations of analytical vibrational frequencies with B3LYP/3-21G optimized structures were done to confirm the true minima [36,37].

3. RESULTS AND DISCUSSION

3.1. Cyclic voltammetric measurement

Fig. 1 (A) represents the CVs recorded for the reaction mixtures of CA, PCA and H_2O_2 at a gold electrode in N_2-saturated 0.1 M acetate buffer solutions containing 0.1 M Na_2SO_4 (pH 4.0). Two well-defined cathodic peaks at 0.35 and –0.50 V *versus* Ag | AgCl | KCl (sat.) were observed, but no anodic peak in the reverse scan of the measured CVs was found.[3] In addition, no anodic peak was also observed (see the inset in Fig. 2) when the CV was measured within the potential range of 0.6 → 0 → 0.6 V. On the successive addition of the reaction mixture in the measured solution, the currents of both cathodic peaks increased gradually, while only the current of the cathodic peak at –0.50 V was found to increase for the continuous addition of H_2O_2 (Fig. 1B-b′ and 1B-c′). CA is not electroactive within the measured potential range and the first and second cathodic peaks have been reasonably assigned to the reductions of PCA and H_2O_2, respectively. Therefore, PCA is reduced at a more positive potential by ca. 0.9 V than the coexisting H_2O_2 [2,3].

Previously, the reduction of PCA has also been characterized to be an irreversible, diffusion-controlled process and from the cathodic peak potential (E_p^c) *versus* pH plots for the reduction of PCA, the pK_a value of –COOOH group of PCA has been roughly estimated to be ca.4.5 [3,16,35]. In this study, similar effect of solution pH on the cyclic voltammetric reduction of PCA was also noticed (Fig. 1C). However, the observed E_p^c *versus* pH plot with two slopes (i.e., ca. 0 and ca. −100 mV/pH) [3] may suggest the slowest steps of the reduction of –COOOH group of PCA as follows:

At solution pH < 4.5

$$Cit–COOOH\ (aq) + 2e^- \rightarrow Cit–COO^-\ (aq) + OH^-\ (aq) \quad (2)$$

which is followed by a fast protonation of the ions produced on the electrode.

At solution pH > 4.5

$$Cit–COOO^-\ (aq) + 2e^- + 3H^+\ (aq) \rightarrow Cit–COOH\ (aq) + H_2O\ (l) \quad (3)$$

The pK_a value of –COOOH group will be further justified with chromatographic measurement and three pK_a values of PCA including that of –OOOH group estimated with pH-metric titration as mentioned below.

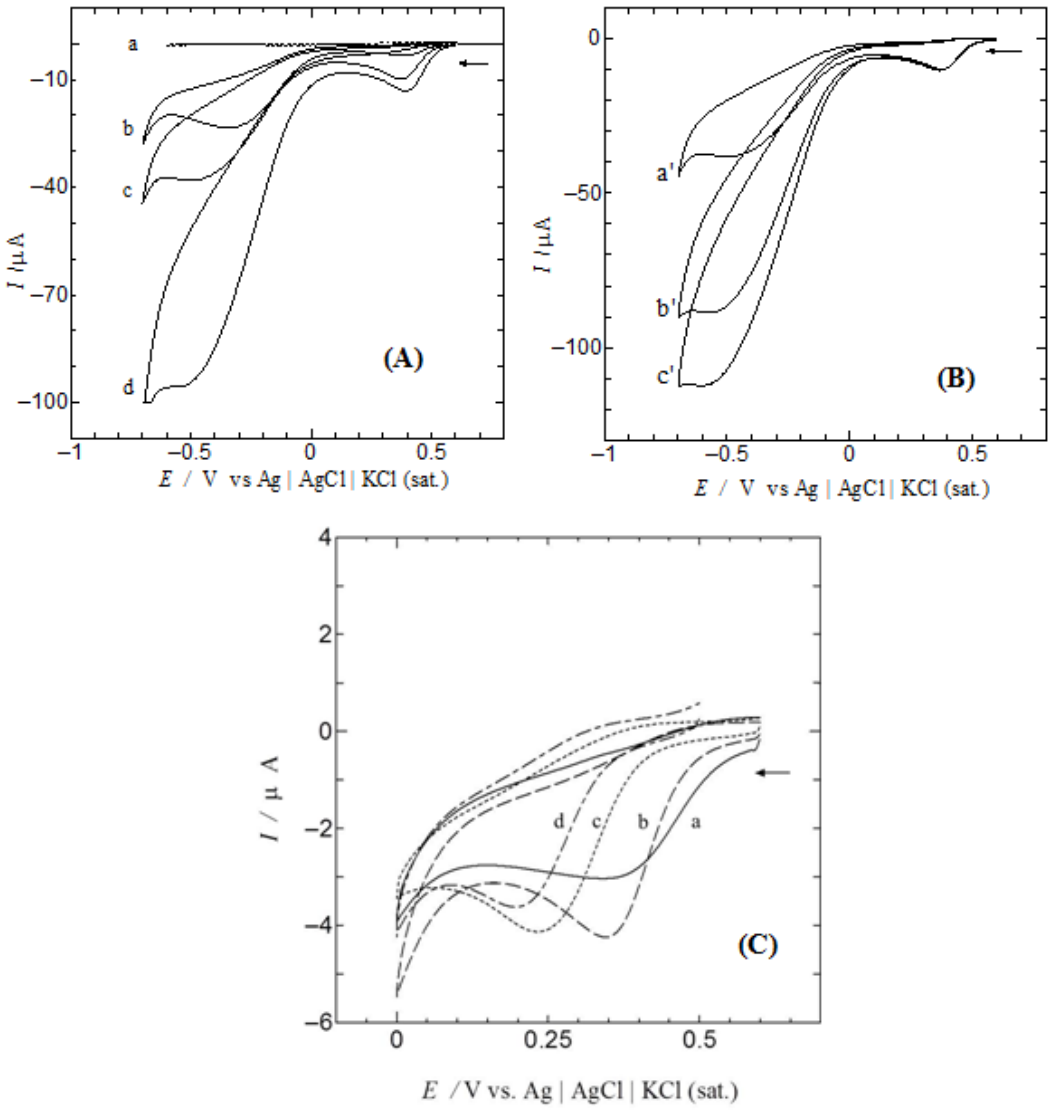
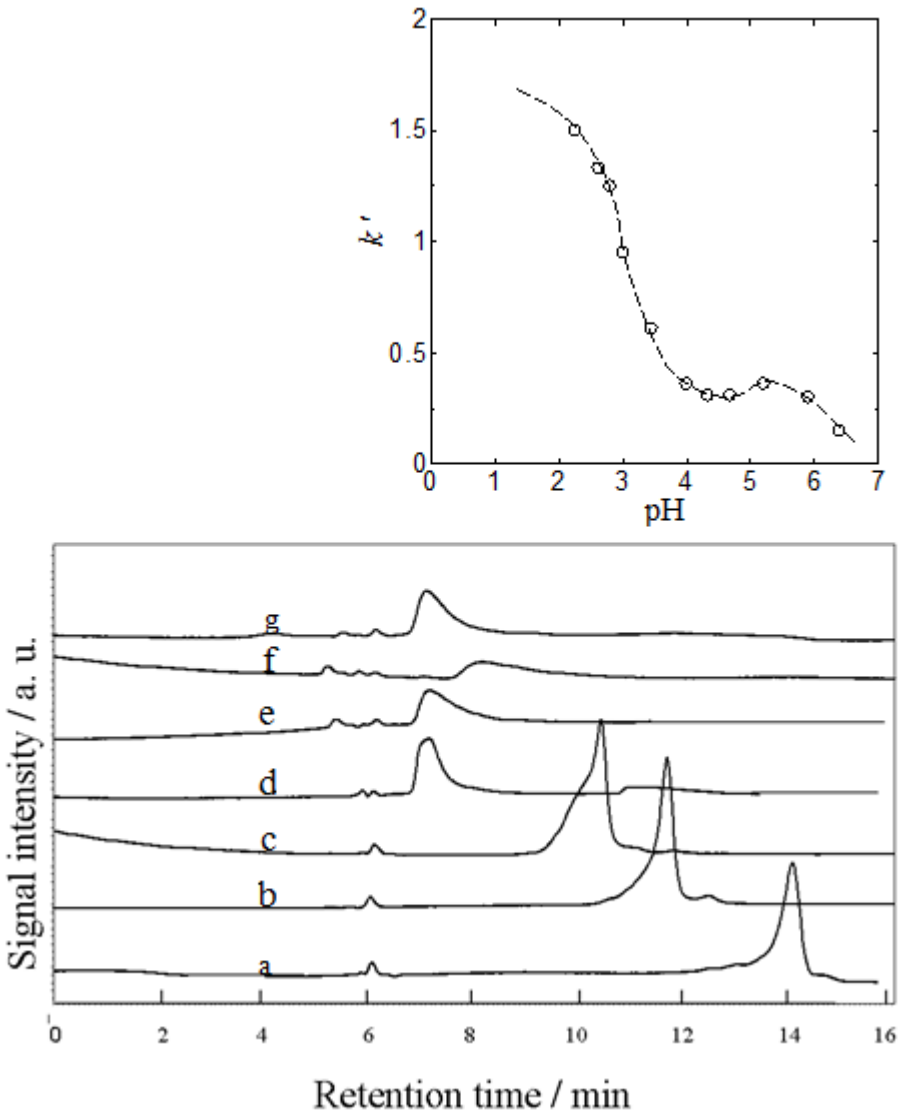


Figure 1. (A) CVs obtained at an gold electrode in N_2–saturated 0.05 M acetate buffer solutions (pH = 4.0) containing 0.1 M Na_2SO_4 in the (a) absence and presence of (b-d) of the reaction mixtures: (b) 0.42 mM PCA + 0.91 mM H_2O_2, (c) 2.0 mM PCA + 1.8 mM H_2O_2 and (d) 2.4 mM PCA + 5.1 mM H_2O_2. (B) CVs obtained for the same buffer solutions containing (a′) 2.4 mM PCA + 1.8 mM H_2O_2, (b′) a′ + 2.8 mM H_2O_2 and (c′) b′ + 2.5 mM H_2O_2. (C) Typical CVs obtained for the reduction of 0.8 mM PCA at a gold electrode in N_2–saturated 0.05 M acetate buffer solutions containing 0.1 M Na_2SO_4 at various pHs of (a) 2.3, (b) 3.5, (c) 4.5 and (d) 5.6. Potential scan rate: 0.1 V s^{-1}.

3.2. Chromatographic measurement

Fig. 2 shows the chromatograms for PCA measured by varying the pH of the mobile phase. It is noted that PCA was separated from the reaction mixture of CA and H_2O_2 using RP-HPLC. The main peak for PCA shifts to lower t_r initially with a peak broadening as the pH was increased while a small peak arisen at t_r of about 6.0 min has been assigned to H_2O_2. However, the values of t_r at pH of 4.3 and 4.7 (chromatograms d and e) remained almost unchanged, while peak intensity was found to significantly decrease compared to those in the chromatograms a-c. Other features may be noted as: (i) the intensity of peak drastically decreased at pH 5.3 (chromatogram f), and once again increased at higher pH of 6.6 (chromatogram g), and (ii) broadening of the peak took place with further increase in pH of the mobile phase. The mentioned features may also indicate the second dissociation of PCA which is further justified below by pH-metric titration. The plot of *k′ versus* pH constructed is sigmoidal in shape, but it does not possess a defined horizontal shoulder that is important for a precise determination of pK_a of a compound [29-31].



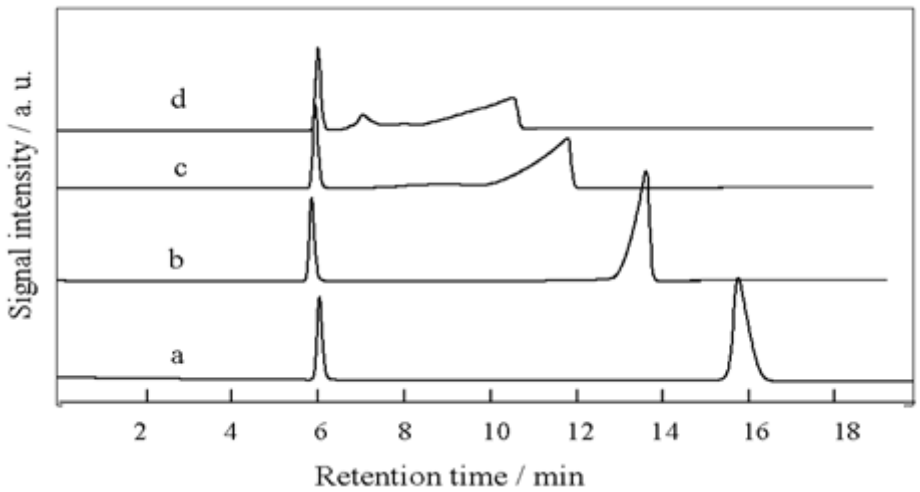


Figure 2. *Upper panel*: Typical chromatograms measured for PCA with mobile phase of 3.0 mM $HClO_4$ at pHs of (a) 2.6, (b) 3.0, (c) 3.3, (d) 4.3, (e) 4.7, (f) 5.3 and (g) 6.6. The inset shows the plot of *k´ versus* pH for PCA. *Lower panel*: Typical chromatograms obtained for CA containing H_2O_2 with pHs of mobile phase of (a) 2.2, (b) 2.9, (c) 3.3 and (d) 4.4.

It has been generally known that the value of t_r decreases with a distortion of chromatographic peak as the pH of the mobile phase becomes close to the pK_a value of a compound [29].

However, from this fact, we may speculate pK_a values of PCA from the characteristic deviations in the shape of peak and t_r at the pH range of 2.5-4.0 (zone-I) and 4.0-6.6 (zone-II). In zone-I, the first dissociation of PCA may be completed as the t_r of PCA remained unchanged (compare chromatograms d and e). For clarifying this result, similar study (see Lower panel in Fig. 2) was performed for CA whose pK_a values are well-known (see Table 1). The peak broadening along with the decrease in peak intensity and the decrease in t_r value could be seen when the pH of the mobile phase becomes close to the first pK_a (i.e., 3.1 [23,24]) of CA (chromatograms b and c). This study with CA essentially assists to recognize the pK_a zones of PCA, indeed. Thus, the pK_a of −COOOH group determined by cyclic voltammetric method (ca. 4.5) can naturally be located in the zone-I identified with RP-HPLC method.

3.3. pH-metric titration

To determine the pK_a values of PCA more precisely, a pH-metric titration of PCA coexisting with CA was performed where Niels Bjerrum graphical method [25,26] was adopted for analysis the titration results. Fig. 3 shows the titration curves CA (curve a), and the mixtures of CA and $HClO_4$ (curve b) and CA, PCA and $HClO_4$ (curve c). The curves of CA measured in the absence and presence of $HClO_4$ are sigmoidal in shape, while a negligible shift of this curve along the axis of added volume of NaOH solution was observed.

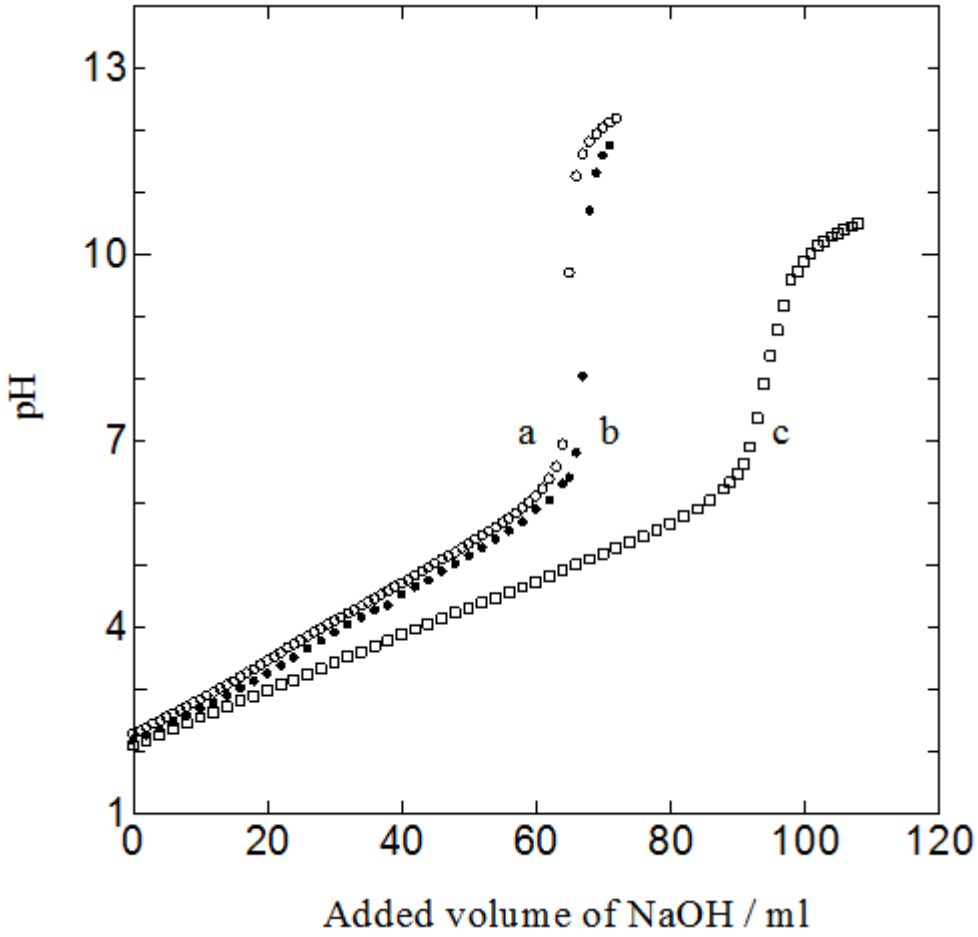


Figure 3. Titration curves for (a) 2.4 mM CA, (b) 2.4 mM CA containing $HClO_4$ and (c) the mixture of 2.4 mM CA, 0.75 mM PCA and $HClO_4$.

On the other hand, a slight increase of pH value for the mixture of CA, $HClO_4$ and PCA was observed initially for the addition of NaOH solution. However, the curve shown in Fig. 3c was compared with that in Fig. 3b to generate the independent titration curve for PCA (Fig. 4). The shape of the constructed curve for PCA is comparable to that of the mixture for CA and $HClO_4$ (Fig. 3b).

The experimental data of CA and PCA were analyzed with Niels Bjerrum graphical method using the following equation given for a three-pK_a system [25,26]:

$$\bar{n}_H = \frac{K_{a1}[H^+] + 2K_{a1}K_{a2}[H^+]^2 + 3K_{a1}K_{a2}K_{a3}[H^+]^3}{1 + K_{a1}[H^+] + K_{a1}K_{a2}[H^+]^2 + K_{a1}K_{a2}Ka_3[H^+]^3} \quad (4)$$

where $\bar{n}_H$ is an average number of dissociable protons bound to the weak acid and

$$\bar{n}_H = n(=3) + \frac{A - C_b v - (10^{-pH} - 10^{-pKw+pH})(V+v)}{L} \quad (5)$$

where A, C_b and L are the concentrations of strong acid (e.g., $HClO_4$), titrant (e.g., NaOH) and weak acid (e.g., CA or PCA) in moles, respectively. V and v are the initial volume of solution and the volume of titrant added in mL, respectively. n represents the number of protic groups, that is, n = 3 in the cases of CA and PCA. The other parameters have their usual meanings. The values of $\bar{n}_H$ were

determined using eq 5 from the experimental results for CA and PCA and $\bar{n}_H$ was plotted against the corresponding pH value and the $\bar{n}_H$ *versus* pH plot is known as "difference plot" or "formation curve".

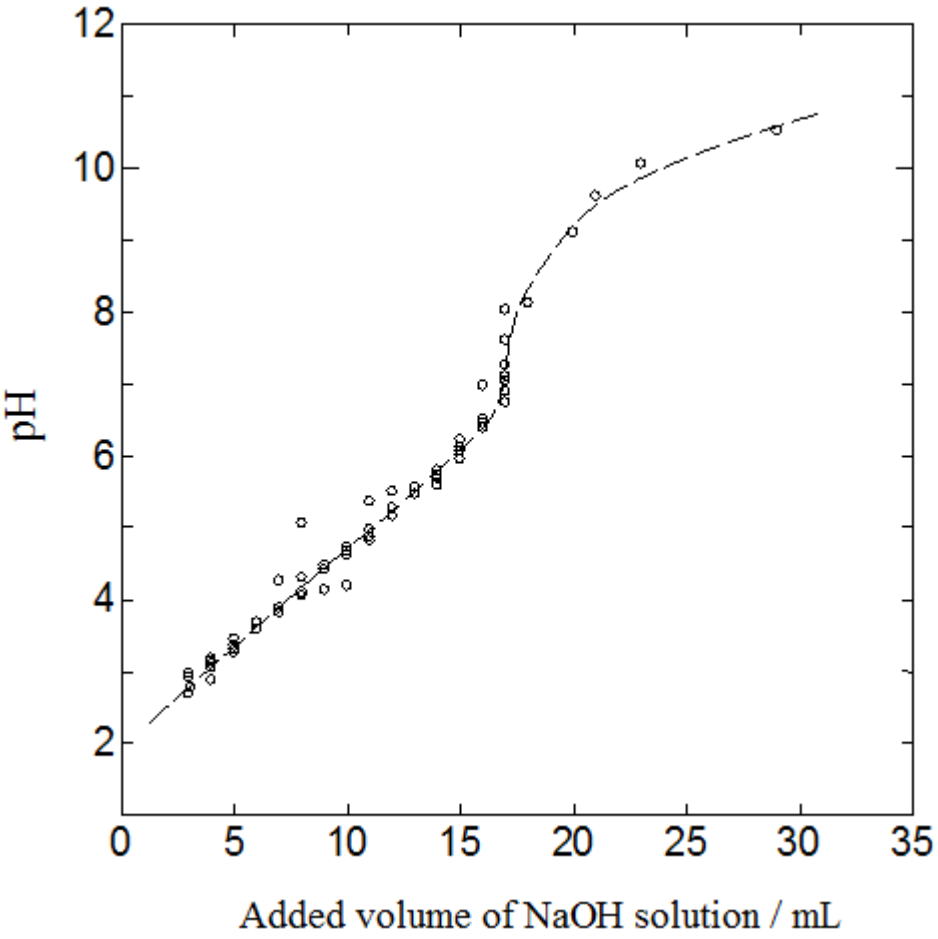


Figure 4. The plot of pH *versus* volume of NaOH added derived from the plots of pH *versus* volume of NaOH added for CA and $HClO_4$ (Fig. 3b) and the mixture of CA, PCA and $HClO_4$ (Fig. 3c) via the subtraction method as described in the text.

Fig. 5 represents the difference plots for CA and PCA in which symbols and solid lines indicate experimental and simulated results, respectively. The simulation was done using eq 4. The plot obtained for CA agreed well, except for the positive deviation at higher pH, with the theoretical curves of a three-pK_a system. Several sources causing this deviation have been known, but such a deviation negligibly affects only the higher pK_a value [25]. From the plot for CA, the pH values that correspond to the $\bar{n}_H$ values of 2.5, 1.5 and 0.5 were estimated to be 2.9, 4.4 and 6.2, respectively. These values are the pK_a of CA and are similar to the values reported, that is, 3.1, 4.6 and 6.4 [23,24]. Thus, the so-called Niels Bjerrum graphical method could be successfully employed in the present case. Similarly, the pK_a values of PCA were estimated to be 3.5, 5.4 and 8.3. All the values determined for CA and PCA are summarized in Table 1. It can be seen that the pK_a value of PCA determined (ca. 4.5) with cyclic voltammetric method [3] is close to the first pK_a obtained with pH-metric titration, and the first two pK_a values (i.e., 3.5 and 5.4) fall in the so-called zone-I and zone-II detected by RP-HPLC method as described above.

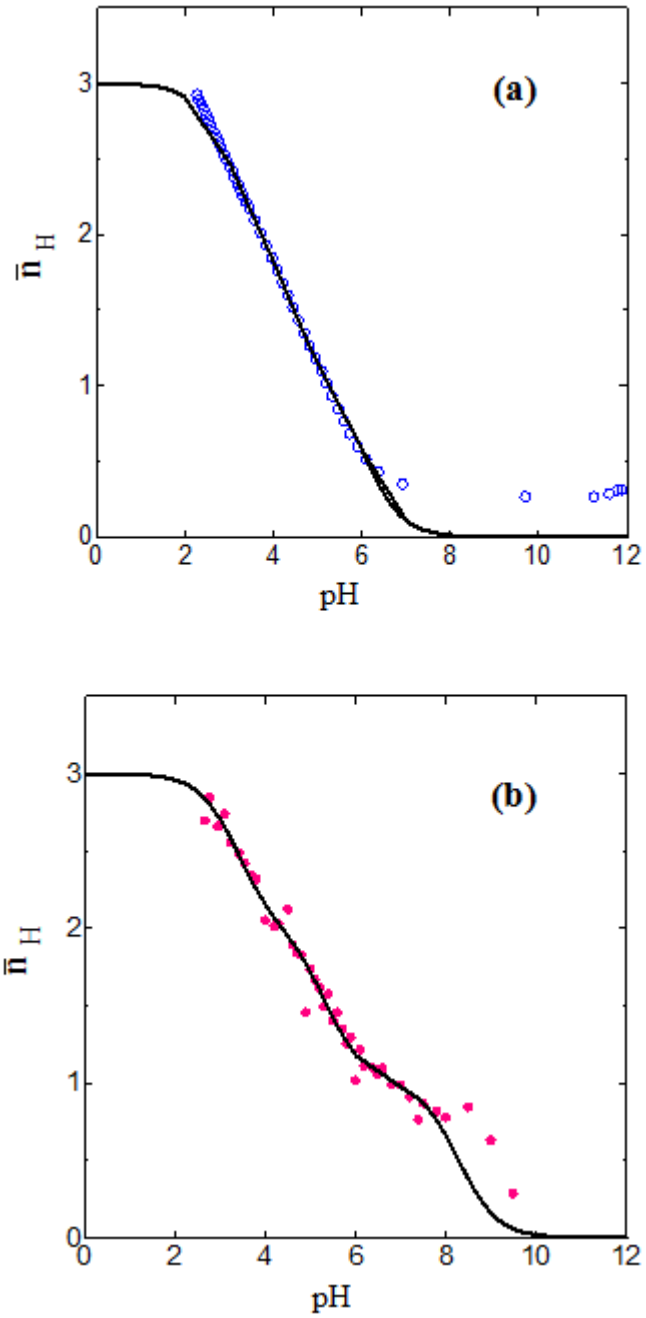


Figure 5. Difference plot of $\bar{n}_H$ *versus* pH for (a) CA and (b) PCA obtained by analyzing the titration curves shown in Figs. 3b and 4. Experimental and simulated results are represented by symbols and solid lines, respectively.

3.4. ab initio calculation and explanation of pK_a values determined

Fig. 6 illustrates the optimized structures of CA and PCA with distribution of charges on all the member atoms of both molecules. In both structures, the carbon atoms of the central –COOH and –COOOH groups attached to the carbon skeleton containing –OH group, which is known to be electron-withdrawing in nature (e.g., the central –COOH group in CA or –COOOH group in PCA), possess the highest positive charges. In addition, the charges on the hydrogen atoms of the central –COOH and –COOOH groups in CA and PCA, respectively, are higher compared to any of the same

in these structures. It has been reported that the pK_a value of –COOH group in CH_3CH_2COOH changes from 4.9 to 3.9, when a –OH group is introduced to its α-position to form $CH_3CH(OH)COOH$, that is, after the introduction of –OH group this compound becomes more acidic [41]. Thus, it is clear that the particular hydrogen atoms considered in CA and PCA may reasonably be considered to be acidic in nature.

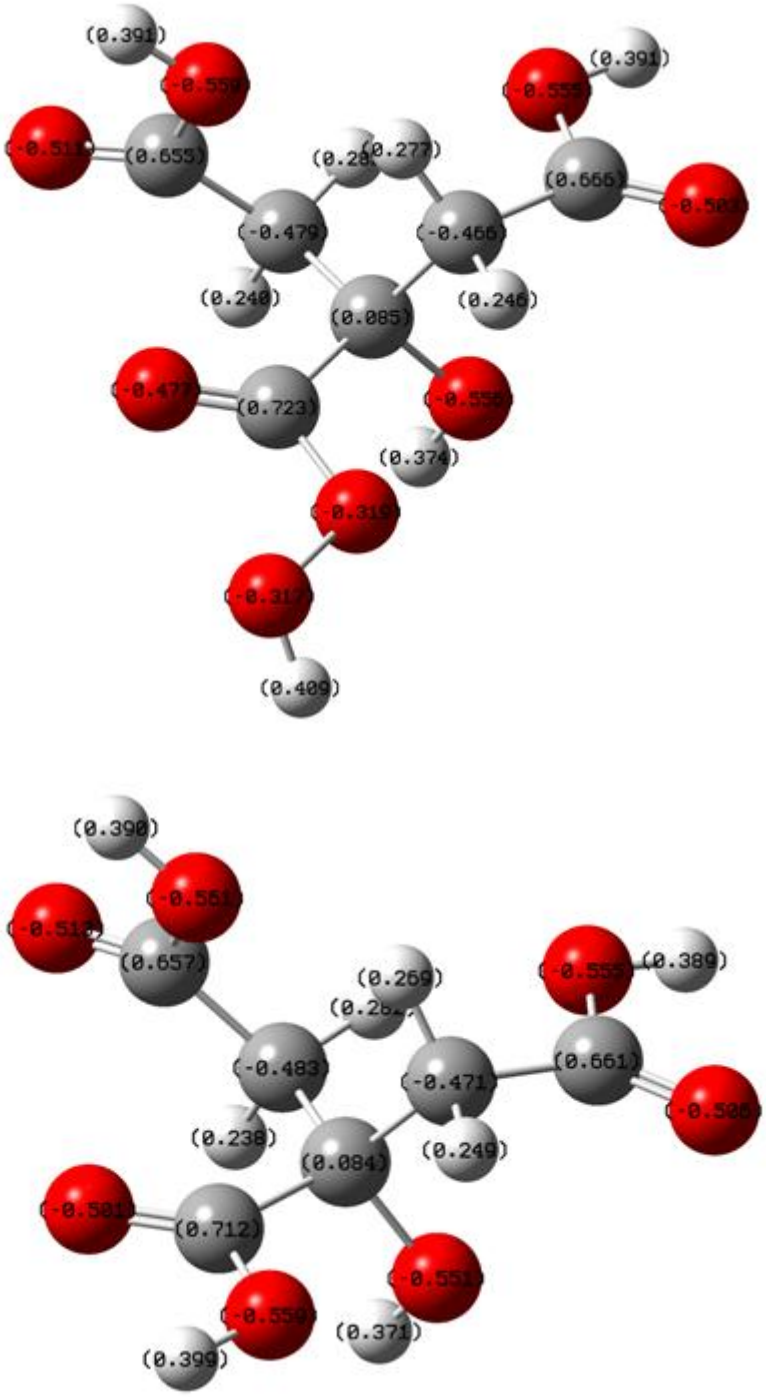


Figure 6. Optimized structures with charge distribution on various atoms of (upper) PCA and (lower) CA; O (red ball), C (gray, big ball) and H (gray, small ball).

Tremendous efforts [33,34,38-40] have been devoted to specify the relative pK_a values of two terminal and one central –COOH groups in CA by ^{1}H NMR, ^{13}C NMR and pH-metric titration techniques. It has been ultimately concluded that two protons in mono-ionized citrate ion are preferentially localized on two terminal –COOH groups, while in the case of di-ionized citrate ion, one proton is bound to a greater extent to the central –COOH group [24,33]. This is mysterious indeed. However, the central –COOH group in CA and the –COOOH group in PCA may be presumed to be de-protonated first. This fact may also be supported by considering the ease of formation of intra-molecular hydrogen bonding between –OH group and mono-ionized citrate ion in CA and mono-ionized peroxycitrate ion in PCA, resulting in the formation of five- and six-membered stable ring structures, respectively. For both mono- and di-ionized citrate ions the gauche confirmation has been postulated [33]. In tri-ionized citrate ion, the electrostatic repulsion among negatively charged moieties is accompanied by a conformational transition (*trans*) and the intra-molecular hydrogen bonding (ring) does not exist [24], resulting in high pK_a value. From above discussion, the values of 2.9, 4.4 and 6.2 determined in this study may be assigned to pK'_{a1}, pK'_{a2} and pK'_{a3}, respectively, that is, the first pK_a of CA may be reasonably presumed to correspond to the central –COOH group. Similarly, pK_a values of 3.5, 5.4 and 8.3 determined for PCA would be reasonably considered to correspond to pK_{a1}, pK_{a2} and pK_{a3}, respectively. Thus, the pK_{a1} determined for the –COOOH group (i.e., 3.5) is higher than that of its parent –COOH group (i.e., 2.9).

4. CONCLUSIONS

We are successful for the first time in determining the pK_a values of two tri-basic weak acids (i.e., CA and PCA) coexisting in aqueous equilibrium mixture. The pK_a value of PCA that is the derivative of CA formed by the reaction with H_2O_2 can be determined with cyclic voltammetric, RP-HPLC and pH-metric titration methods. The concept of interpolation process and Niels Bjerrum graphical method can be employed to analyze the pH-metric titration data obtained for the coexisting CA and PCA as the equilibrium mixture and to explore their pK_a values. The pK_a value of –COOOH group in PCA is greater than that of the corresponding –COOH group in CA. This is rational as discussed with the support of *ab initio* calculations of the optimized structures of CA and PCA and is generally observed for other organic peroxyacids [20,21].

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References

1. B.N. Ferdousi, M.M. Islam, M.I. Awad, T. Okajima, F. Kitamura and T. Ohsaka, *Electrochemistry*, 74 (2006) 606.
2. B.N. Ferdousi, M.M. Islam, T. Okajima and T. Ohsaka, *Talanta*, 74 (2008) 1355.
3. B.N. Ferdousi, M.M. Islam, T. Okajima and T. Ohsaka, *Electrochim. Acta*, 53 (2007) 968.

4. B.N. Ferdousi, M.M. Islam, T. Okajima and T. Ohsaka, *J. Chromatogr. Sci*., 49 (2011) 40.
5. M.M. Islam, B.N. Ferdousi, M.I. Awad and T. Ohsaka, *Peroxycitric Acid: A Potential Derivative of Citric Acid, In Citric Acid: Synthesis, Properties and Applications;* Nova Science Publishers, Inc.: New York, USA, 2011.
6. D. Swern, *In Organic Peroxides*; vol. 1, John Wiley and Sons: New York, USA, 1970.
7. D. Swern, *Chem. Rev*., 45 (1949) 1.
8. W.E. Parker, C. Riciuti, C.L. Ogg and D. Swern, *J. Am. Chem. Soc.*, 77 (1955) 4037.
9. S.S. Block, *Disinfection, Sterilization, and Preservation;* Williams and Wilkins: Philadelphia, PA, USA, 2001.
10. Y. Sawaki and Y. Ogata, *Bull. Chem. Soc. Jpn*., 38 (1965) 2103.
11. T. Luukkonen, T. Heyninck, J. Ramo and U. Lassi, *Water Research*, 5 (2015) 275.
12. A. Vimont, I. Fliss and J. Jean, *Food Environ. Virol*, 7 (2015) 49.
13. S. Antonello and F. Maran, *J. Am. Chem. Soc.*, 121 (1999) 9668.
14. M.R. gen. Klaas, K. Steffens and N. Pattet, *J. Mol. Catals. B: Enzym.*, 19–20 (2002) 499.
15. S. Antonello, M. Musumeci, D.D.M. Wayner and F. Maran, *J. Am. Chem. Soc.*, 119 (1997) 9541.
16. M.I. Awad, A. Denggerile and T. Ohsaka, *J. Electrochem. Soc.*, 151 (2004) E 358.
17. A. Denggerile, M.I. Awad, T. Okajima, C. Harnood and T. Ohsaka, *Electrochim. Acta*, 49 (2004) 4135.
18. M.I. Awad, C. Harnoode, K. Tokuda and T. Ohsaka, *Anal. Chem.*, 73 (2001) 1839.
19. J. Li, W. Tu, J. Lei, S. Tang and H. Ju, *Electrochim. Acta*, 56 (2011) 3159.
20. A.J. Martin, *Anal. Chem.*, 29 (1957) 79.
21. A.J. Everett and G.J. Minkoff, *Trans. Faraday Soc*., 49 (1953), 410.
22. P. Jones, M.L. Haggett, D. Holden and P.J. Robinson *J. Chem. Soc. Perkin Trans.-II*, (1989) 443.
23. R.G. Bates and G.D. Pinching, *J. Am. Chem. Soc*., 71 (1949) 1274.
24. A. Apelblat, *Citric Acid*; Springer: Cham, Switzerland, 2014.
25. A. Kraft, *J. Chem. Edu*., 80 (2003) 554.
26. S.C. Castillo, S.G. Micolta and T.M. Graiales, *J. Chem. Edu*., 61 (1984) 1067.
27. J.M.B.F. Diniz and T.M. Herrington, *J. Chem. Eng. Data*, 38 (1993) 109.
28. C.J. Willis, *J. Chem. Edu*., 58 (1981) 659.
29. R. LoBrutto, A. Jones, Y.V. Kazakevisch and H.M. McNair, *J. Chromatogr. A*, 913 (2001) 173.
30. P. Wiczling, P. Kawczak, A. Nasai and R. Kaliszan, *Anal. Chem*., 78 (2006) 239.
31. J.L. Beltrán, N. Sanli, G. Fonrodona, D. Barrón, G. Özkan and J. Barbosa, *Anal. Chim. Acta*, 484 (2003) 253.
32. A.M.N. Silva, X. Kong and R.C. Hider, *Biometals*, 22(5) (2009) 771.
33. N.N. Tananaeva, E.K. Trunova, N.A. Kostromina and Y.B. Shevchenko, *Theor. Exp. Chem.*, 26 (1990) 660.
34. F. Castiglione, A. Baggioli, A. Citterio, A. Mele and G. Raos, *J. Phys. Chem. A*, 116 (2012) 1814.
35. S. Hayano and N. Shinozuka, *Bull. Chem. Soc. Jpn*., 43 (1970) 2039.
36. C. Lee, W. Yang and R.G. Parr, *Phys. Rev. B*, 37 (1988) 785.
37. P.C. Hariharan and J.A. Pople, *Chem. Phys. Lett.*, 66 (1972) 217.
38. **N.**K. Pearce and K.L. Creamer, *Aust. J. Chem*., 28 (1975) 2409.
39. A. Loewenstein and J.D. Roberts, *J. Am. Chem. Soc.*, 82 (1960) 2705.
40. R.B. Martin, *J. Phys. Chem*., 65 (1961) 2053.
41. H.-B. Burgi and J.D. Dunitz, *Structure Correlation*; vol. 1 VCH: Weinheim, Germany, 1994.