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Determination of Acid Dissociation Constant of Benzimidazole-Amino Acid Conjugate Ligands by Spectrophotometric and Cyclic Voltammetric Method

Sabithakala Thatituri*, Pushpanjali Kale

Department of Chemistry, Jawaharlal Nehru Technological University Hyderabad-UCEST, Hyderabad- 500085, **INDIA**

Email: sabithakalatatituri@jntuh.ac.in

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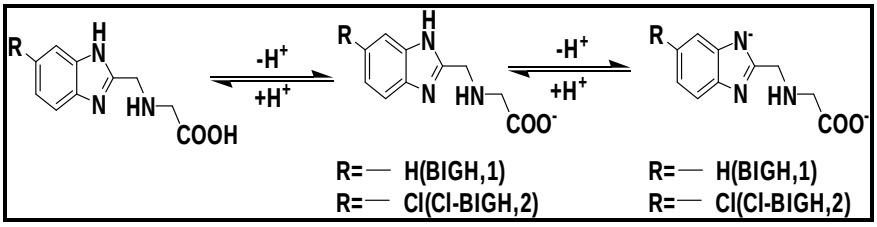
ABSTRACT

*The acid dissociation constant is the most frequently used physicochemical parameter, and its determination is of interest to a wide range of research fields. The acid dissociation constant (pKa) of the of 2-((1H-benzimidazol) methyl amino) acetic acid (**1**) and 2-(((6-chloro-benzimidazol)methyl) amino) acetic acid (**2**) were determined using UV-Visible spectrophotometry and Cyclic voltammetry. Graphical method used to estimate the acid dissociation constant (pKa). In UV-Visible spectrophotometry graph was plotted taking absorbance vs. pH at the λ_{max} (218 and 245nm), pKa was obtained at the point of intersection of these curves. In Cyclic voltammetry the graph was plotted for oxidation peak potential as a function of pH, pKa was determined from the intersection point of the linear segments of peak potential and pH plots. The resulting pKa of compound **1** is 2.45 in spectrophotometric method and 2.48 in cyclic voltammetric method, and for compound **2**, 2.25 in spectrophotometric and 2.24 in Cyclic voltammetric method. Further, at higher pH deprotonation of another hydrogen atom from the nitrogen of benzimadazole ring observed in spectrophotometric method.*

Graphical Abstract:



Oxidation mechanism of Ligand 1 and 2.

Keywords: Buffers, pH, pKa, Synthesis, Electrochemistry, Oxidation potential.

INTRODUCTION

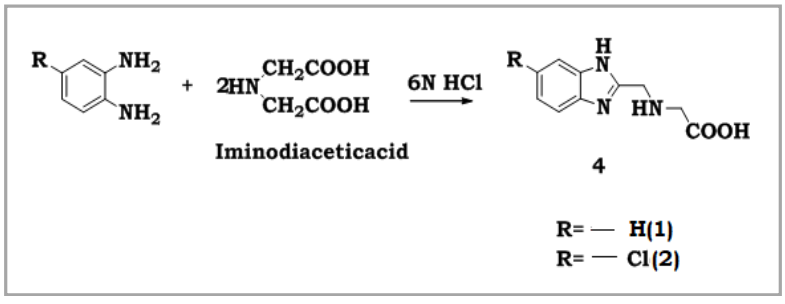
An acid dissociation constant, is a quantitative measure of the strength of an acid in solution. It can be expressed by either K_a or pKa (pKa = $-\log_{10}$ Ka). The larger the pKa value, the smaller the acid dissociation constant. The acid dissociation constant (pKa) is an essential parameter, it indicates the proton dissociation of the compound in solution at different pH values. Both pH and pKa are essential for understanding the behavior of chemical substances in many biological, chemical and physical properties [1-5]. The pK_a of a drug influences solubility, lipophilicity, permeability and protein binding which in turn directly affects pharmacokinetic characteristics such as distribution, absorption, metabolism and excretion [6-11]. The pKa controls metabolism and even transport across the membranes. Hence, the study has importance in biological, chemical and pharmaceutical fields [12-14]. The potentiometry [15], cyclic voltammetry [16-18], spectrophotometry [19] , conductometry [20] and NMR [21] were different techniques for determining the pKa values of the molecules [22]. The spectrophotometric and cyclic voltammetric methods have been used in the present study.

MATERIALS AND METHODS

Chemicals: Methanol (Aldrich), Hydrochloric acid (HCl), Potassium chloride (KCl), Potassium hydrogen phthalate (PHP), Sodium hydroxide (NaOH), Potassium dihydrogen phosphate ($K_2H_2PO_4$), Sodium bicarbonate ($NaHCO_3$) and all the buffer solutions were prepared by mixing the required chemicals by following the reported procedure [23].

pH=1 (50 mL 0.2 M KCl + 134 mL 0.2 M HCl), pH=2 (50 mL 0.2 M KCl + 13 mL 0.2 M HCl), pH=3 (100 mL 0.1 M PHP + 44.6 mL 0.1 M HCl), pH=4 (100 mL 0.1 M PHP + 0.2 mL 0.1 M HCl), pH=5 (100 mL 0.1 M PHP + 45.2 mL 0.1 M NaOH), pH=6 (100 mL 0.1 M $K_2H_2PO_4$ + 11.2 mL 0.1 M NaOH), pH=7 (100 mL 0.1 M $K_2H_2PO_4$ + 58.2 mL 0.1 M NaOH), pH=6 (100 mL 0.1 M $K_2H_2PO_4$ + 93.4 mL 0.1 M NaOH), pH=9 (100 mL 0.025 M $Na_2B_4O_7$ + 9.2 mL 0.1 M HCl) and pH=10(100 mL 0.05 M $NaHCO_3$+21.4 mL 0.1 M NaOH).

Synthesis: 2-((1H-benzimidazol)methylamino) acetic acid(**1**) and 2-(((6-chloro-benzimidazol)methyl) amino) acetic acid (**2**) were synthesized (Scheme 1) by reported literature procedure [24]. To a refluxing solution of iminodiacetic acid (15.98 g, 120.0 mmol) in 6 M HCI (100 mL), a solution of orthophenelynediamine (8.5 g, 60 mmol) in 4 M HCI(40 mL) was added drop wise, 20 mL immediately and 20 mL after 16 h. After 72 h reaction mixture was allowed to cool to room temperature. The precipitate (dimer) was separated by filtration. From the filtrate, most of the HC1 was evaporated whereby a solid separates out which was collected by filtration. The solid was dissolved in 120 ml of water and neutralized with NaOH slowly whereby compound**1** precipitates as white solid and dried in the hot air oven.



Scheme 1. Synthesis of Ligand 1 and 2.

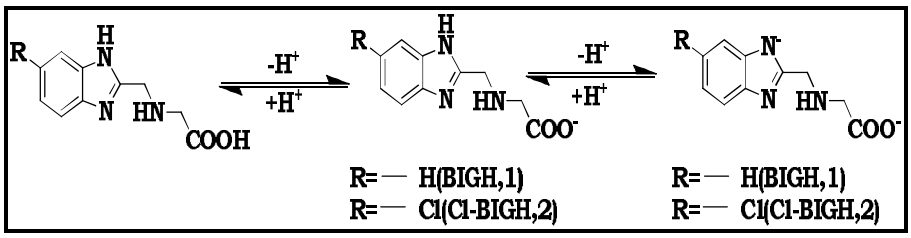
Structures of compound **1** and **2** were confirmed by means of elemental analysis, IR, ^{1}HNMR, ^{13}CNMR and HRMS studies.

2-((1H-benzimidazol)methylamino)acetic acid(1): Yield: 89%; M.P: 209-210°C; ^{1}H NMR (400MHz, DMSO-d_6/TMS, δ) 7.5 (s, 2H, Ar-H), 7.15 (s, 2H, Ar-H), 4.1 (s, 2H, CH_2), 3.3 (s, 2H, CH_2); ^{13}C NMR(100MHz, DMSO-d_6,δ) 45.72, 49.79, 114.15, 121.47, 137.78, 140.15, 171.85; HRMS(ESI): m/z 206.0649 $[M+H]^+$; FTIR(KBr): ν(cm^{-1}) = 3460 (carboxylic OH), 3013 (N-H, imidazole ring), 2781(N-H, aliphatic amine), 1643 (C=O, acid), 1596 (C=N, cyclic azomethine); Anal. calcd. for $C_{10}H_{11}N_3O_2$: C, 58.53; H, 5.40; N,20.48%; found: C, 58.42; H, 5.45; N, 20.36%. ^{1}H NMR, ^{13}C NMR, HRMS and IR spectra of compound **1** are shown in Figures S1-S5(Supporting Information).

2-(((6-chloro-benzimidazol)methyl)amino)acetic acid (2): Yield: 72%; M.P: 234–236°C; ^{1}H NMR (400MHz, DMSO-d_6/TMS,δ) 7.6 (s, 1H, Ar-H), 7.52 (d, 1H, Ar-H), 7.2 (d, 1H, Ar-H), 4.15 (s, 2H, CH_2), 3.69 (s, 2H, CH); ^{13}C NMR(100MHz, DMSO-d_6,δ) 45.6, 49.3, 116.0, 116.9, 125.5, 129.3, 138.1, 140.0, 140.9, 175.0; HRMS: (*m/z*) =240.0395 $[M+H]^+$; FTIR(KBr): ν(cm^{-1}) = 3413 (carboxylic OH), 3014(N-H, imidazole ring), 2851(N-H, aliphatic amine), 1640 (C=O, acid), 1592 (C=N, cyclic azomethine); Anal. calcd. for $C_{10}H_{10}ClN_3O_2$: C, 50.12; H, 4.21; N,17.53%; found: C, 50.53; H, 4.30; N, 17.48%.^{1}H NMR, ^{13}C NMR, HRMS and IR spectra of compound **2** are shown in Figures S5-S8(Supporting Information).

RESULTS AND DISCUSSION

The redox behaviour of **1** and **2** were pH dependent further pH - potential variations show a change in the slope at pH = pKa [25]. Organic molecules whose oxidation potentials are pH dependent undergo deprotonation during oxidation [26]. The carboxylic group in **1** and **2** form carboxylate ion as intermediate via oxidation. Below pH 3 it is apparent that H^+ ion is deprotonated from a molecule of **1** and **2**. Further, in basic environment another proton released from nitrogen present in benzimadazole ring the following oxidation mechanism was proposed in Scheme 2 for **1** and **2**.



Scheme 2. Oxidation mechanism of Ligand 1 and 2.

Spectrophotometric method: Absorbance measurements [27-29] were performed in the wavelength range of 200 - 700 nm. The absorbance spectra of compound 1 and 2 at 1mM concentration presented in figure 1 and the variation in absorbance spectra with pH, illustrated in figure 2. Graphical method was used to determine pKa. The absorption spectra of the **1** and **2** at extreme pH levels (pH = 1.54 and pH = 12.21) are compared and λ_{max} is calculated as illustrated in figure 3. The absorbance spectrum of compound **1** at pH = 1.54 exhibited a λ_{max} at 218 nm while that at pH = 9.83 is 245 nm. Under similar pH conditions the λ_{max} of compound **2** is found to be at 220 nm and 248 nm respectively.

The plot of the absorbance vs. pH is presented in figure 4 at the λ_{max} values. The pKa obtained by point of intersection of these curves as shown in figure 4. Finally from the spectrophotometric method it is confirmed that the compound **1** and **2** are having dissociable proton at pKa = 2.45 and pKa = 2.24 respectively. Further, we clearly observed deprotonation of another proton, of compound **1** at pH 11.5 and compound **2** at pH 10.4 from nitrogen present on benzimadazole ring.

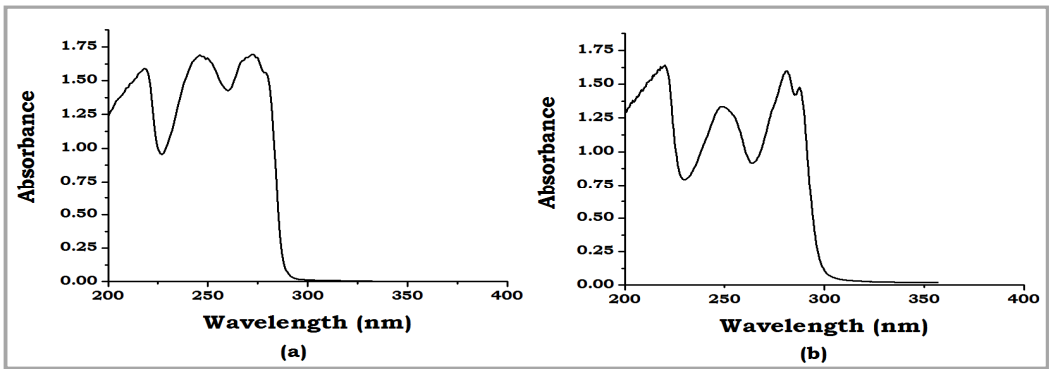


Figure 1. Absorbance spectra of the **1** and **2.**

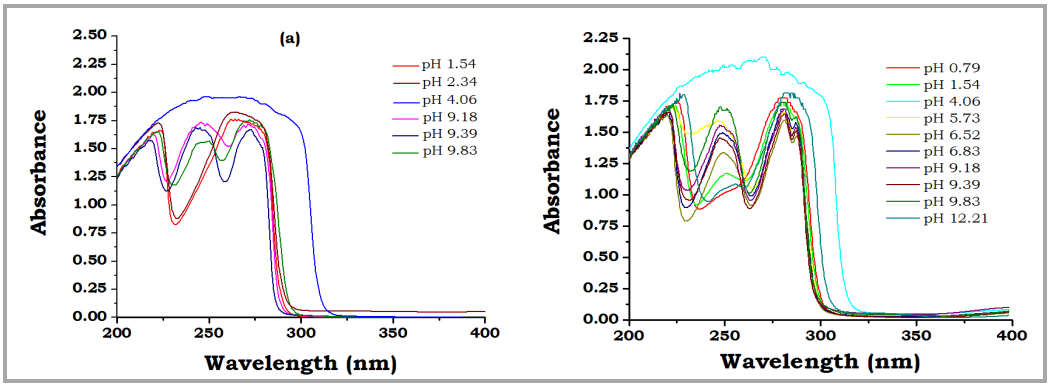


Figure 2. Absorbance spectra of the **1** and **2** at different pH levels.

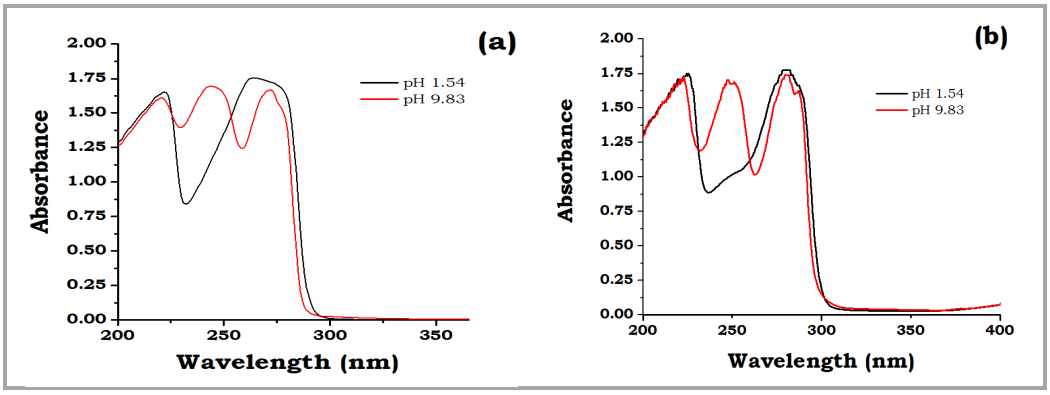


Figure 3. Absorbance spectra of **1** and **2** at extreme pH levels.

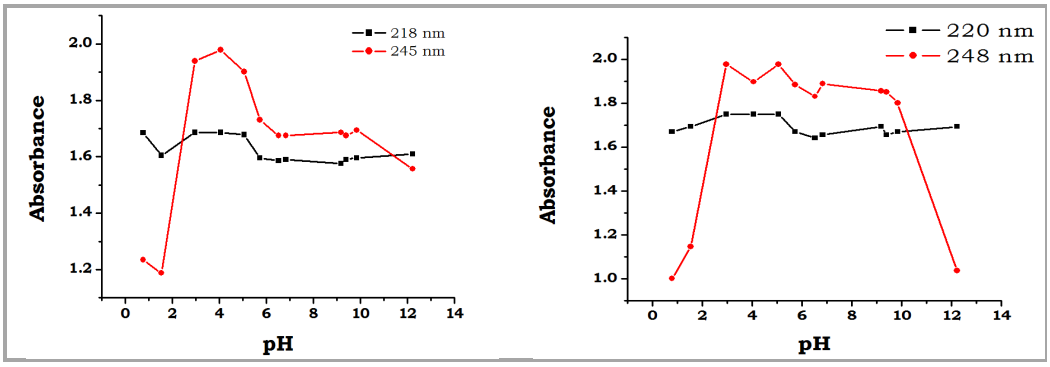


Figure 4. Plot of absorbance vs. pH at λ_{max} of 218 and 245 nm for **1** and 220 and 248 nm for **2**

Cyclic voltammetric method: The influence of pH on the oxidation peak potential [**30**] of the cyclic voltammograms of compound **1** and **2** at 1 mM concentration was investigated over the range of pH 1-10. The shift in the oxidation peak potential as a function of pH studied. When the pH of the buffer increased, the peak shifted to a more positive potential. The figures 5 and 6 show the dependence of the oxidation peak potential on pH in the cyclic voltammetric study. The redox behavior of compound **1** and **2** are pH dependent. Further, pH - potential variations show a change [**25**] in slope at pH = pKa. Organic compounds whose oxidation potentials are pH dependent undergo deprotonation during oxidation [**26**]. The carboxylic acid group of **1** and **2** forms carboxylate ion during oxidation process. Below pH 10.0, it is apparent that, one H^+ ion removed from a molecule of **1** and **2**. The fact that one electron oxidation wave is obtained and one hydrogen ion is involved in the electrode reaction at pH 4, 7 and 10, leads to the proposal of the following oxidation mechanism (Scheme 2) for **1** and **2** at the electrode. Accordingly, the pKa of compound **1** and **2** are determined as 2.48 and 2.25 respectively.

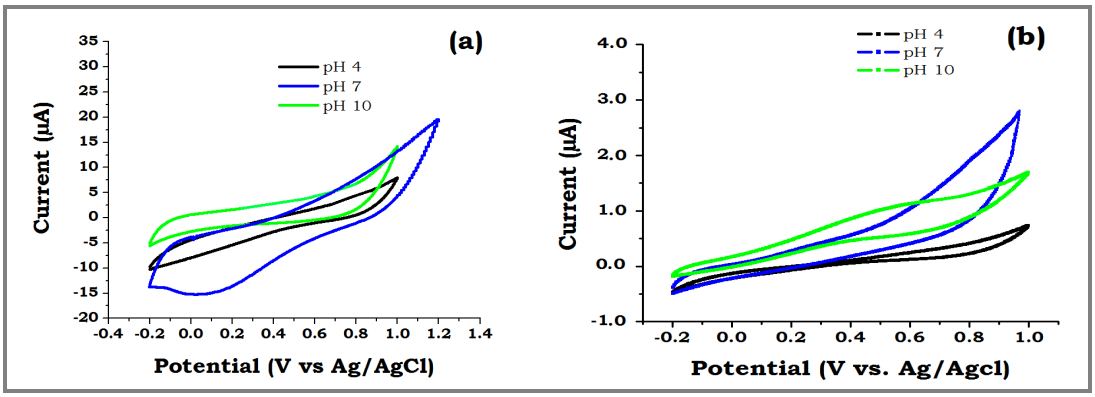


Figure 5. Cyclic voltammograms of 1 mM (a) **1** (b) **2** obtained in acidic, neutral and alkaline conditions at 200 and 100mV/s respectively.

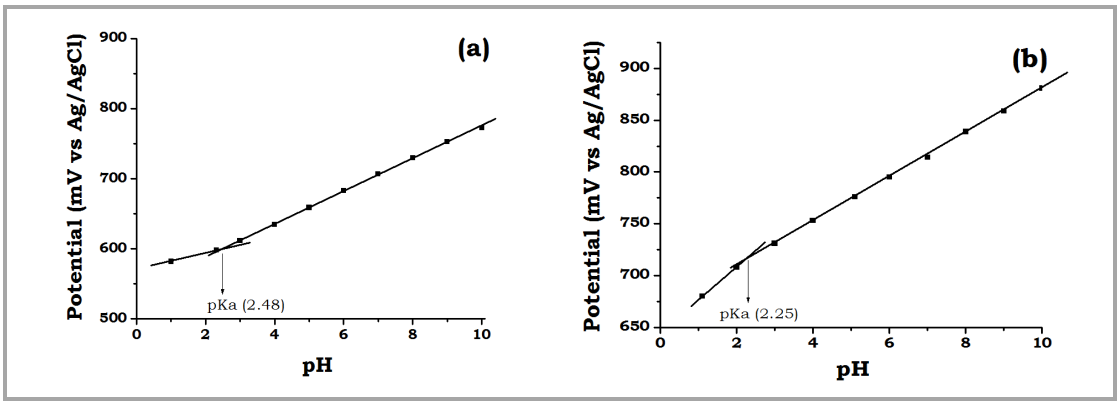


Figure 6. Plot of oxidation peak potential as a function of pH of the 1 mM compound **1**(a) and compound **2** (b) at a scan rate of 200 and 100mV/s respectively.

CONCLUSION

The acid dissociation constant (pKa) of the of 2-((1H-benzimidazol)methylamino)acetic acid (**1**) and 2-(((6-chloro-benzimidazol)methyl)amino)acetic acid (**2**) had been calculated using UV-Visible spectrophotometry and Cyclic voltammetry by graphical method. The carboxylic acid group of compound **1** and **2** forms carboxylate ion during oxidation process and a H^+ ion gets dissociated from compound **1** or **2**. Acording to cyclic voltammetric studies one electron oxidation wave obtained and hence, hydrogen ion is involved in the electrode reaction at pH 4, 7 and 10. Finally, pKa of compound **1**, 2.45 in spectrophotometric method and 2.48 in Cyclic voltammetric method, and for compound **2**, 2.25 in spectrophotometric and 2.24 in Cyclic voltammetric method. The results obtained using

spectrophotometric and cyclic voltammetric methods are complimentary to each other. Further, we clearly observed deprotonation of another proton, of compound **1** at pH 11.5 and compound **2** at pH 10.4 from nitrogen present on benzimadazole ring using spectrophotometric method.

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REFERENCES

[1]. M.N. Martinez, and G.L. Amidon, *J Clin Pharmacol*, **2002**, 42, 620.
[2]. L. Stoll, L. Gentile, *Biochem Biophys Res Commun*, **2005**, 29, 622.
[3]. L. Lin, and H. Wong, *Pharmaceutics*, **2017**, 26, 41.
[4]. Z. Dong, Li J, Wu F, *CPT Pharmacometrics Syst Pharmacol*, **2020**, 9, 456.
[5]. M. Azman, A.H. Sabri, Q.K. Anjani, M.F. Mustaffa, K.A. Hamid, *Pharmaceuticals*, **2022**, 15, 975
[6]. M. S. Alqahtani, M. Kazi, M. A. Alsenaidy and M. Z. Ahmad, *Front. Pharmacol*, **2021**, 12 618411(article number).
[7]. E.H. Kerns, L.Di, *Drug Discov. Today Technol*., 2004, 1, 343.
[8]. A.Avdeef , *Curr. Top. Med. Chem*., **2001**, 1, 277.
[9]. X. Xie, S. H. Steiner, M. H. Bickel, *Drug Metab. Dispos*, **1991**, 19, 15.
[10]. T. Jones, G.Taylor, *Proc. Eur. Congr. Biopharm. Pharmacokinet*., **1987**, 3(2), 181.
[11]. G.M. Mitani, I. Steinberg, E.J. Lien, E.C. Harrison, U.Elkayam, *Clin. Pharmacokinet*, 1987, 12, 253.
[12]. H.Wan, J.Ulander, *Drug Metab Toxicol*., **2006,** 2, 139.
[13]. T. M. David, *Perspect Medicin Chem*., **2007**, 1, 25.
[14]. C. Zhou , Y. Jin ,J.R. Kenseth, M.Stella, K.R. Wehmeyer, *J Pharm. Sci*., **2005**, 94, 576.
[15]. H. Sigel, A.D. Zuberbuhler, and Yamauchi, *Anal Chim Acta*., **1991**, 225, 63.
[16]. W.C. Barette, Jr. H.W. Johnson, and Jr.D.T. Sawyer, *J Am Chem Soc*., **1984**, 56, 1890.
[17]. Kim H-s, T.D. Chung, and H. Kim, *J Electroanalytical Chem*., **2001**, 498, 209.
[18]. N. Gupta, and H. Linschitz, *J Am Chem Soc*., **1997**, 119, 6384.
[19]. R.I. Allen, K.J. Box, J.E. Comer, C. Peake, K.Y.Tam, *J Pharm Biomed Anal.*, **1998**, 17, 699.
[20]. R.I.Gelb, *Anal Chem*., **1971**, 43, (1110).
[21]. K.Popov, H. Rönkkömäki, and L.H.J. Lajunen, (IUPAC Technical Report) *Pure Appl Chem*., **2006**, 78, 663.
[22]. R. Jetse , A.van Hoof, A.van Loon, B.Teunissen, *Anal Chem Insights*, **2013**, 8, 53.
[23]. "Electrolyte solutions" Robinson, R. A., Stokes, R. H., 2nd ed., rev. London, Butterworths, **1968**.
[24]. P. D.Verweij, J. S. N. van der Geest, W.L.Driessen, J.Reedijk, D. C.Sherrington, *Reactive Polymers*, **1992**, 18, 191.
[25]. P. H. Rieger, Electrochemistry, Prentice-Hall International, New Jersey, **1987**.
[26]. E.R. Brown, J. R. Sandifer, Physical Methods of Chemistry, Volume II, Electrochemical Methods, Rossiter, W.; Hamilton, J.F. (Eds.); Wiley-Interscience: New York; **1986**.
[27]. M. Meloun, S. Bordovská, T. Syrový, A. Vrána, *Analytica Chimica Acta*, **2006**, 580, 107.
[28]. M. Meloun, S. Bordovská, T. Syrovy, *Journal of Physical Organic Chemistry*, **2007**, 20, 690.
[29]. L. E. Vidal Salgado, C. Vargas-Hernández, *American Journal of Analytical Chemistry*, **2014**, 5, 1290.
[30]. E. Biçer, C. Arat, *Croat. Chem. Acta*, **2009**, 82, 583.