

Solvent effects on protonation process of clindamycin in mixed solvents

Fatemeh Farrokhmanesh¹, Farhoush Kiani¹,
Ali Farajtabar², Fardad Koohyar^{3,4,*} and
Seyed Hassan Noshirvankolaei¹

¹Department of Chemistry, Faculty of Science,
Ayatollah Amoli Branch, Islamic Azad University, Amol, Iran

²Department of Chemistry, Jouybar Branch, Islamic Azad University,
Jouybar, Iran

³Division of Computational Physics, Institute for Computational
Science, Ton Duc Thang University, Ho Chi Minh City, Vietnam

⁴Faculty of Applied Sciences, Ton Duc Thang University,
Ho Chi Minh City, Vietnam

Clindamycin is used in the treatment of bacterial infections. The protonation constant of this drug was determined in mixed solvents of water and methanol as well as water and ethanol in the volume range 0–50 (% v/v) at 298.15 K and 0.1 M NaClO₄ as the inert electrolyte. The results show that in both mixed solvents, pK_a decreases when the volume fraction of alcohols increases. We considered the effect of the solvent on equilibrium constants using the electrostatic model of Born and the linear solvation energy relationship model developed by Kamlet and Taft. The results indicate that both hydrogen-bonding interactions and electrostatic interactions give rise to the observed changes. Using the derived equations, it is possible to determine pK_a of clindamycin in the desired composition throughout the studied range.

Keywords: Antibiotic drugs, ethanol, methanol, protonation constant, solvent effect.

CLINDAMYCIN is used as an antibiotic drug to treat several bacterial infections¹. It is used in the treatment of acne and malaria in addition to quinine. The drug can be taken orally, or as an injection or applied as a cream on the skin². The common side effects of clindamycin are nausea, diarrhoea, rash and pain at the site of injection³. This drug can be used instead of penicillin for the treatment of dental abscesses⁴. It is also effective in the treatment of various skin and soft tissue infection because the drug is active against streptococci, anaerobes and *Staphylococcus aureus*¹.

It is well known that solvents can considerably affect on the physico-chemical properties of a solute during a chemical process^{5–8}. Therefore, chemists show interest in the study of interactions between solutes and solvents, as well as between solvents molecules. The solvent effects depend on the nature as well as extent of solute–solvent interactions occurring in the solvation shell of the solutes⁹. In recent years, many studies have been done in mixed solvents to examine the effect of solvent–solvent

interactions on the solvation process of solutes. The results of these studies are important in physical chemistry and relevant subjects. Also, this is an important topic to study because the medium can influence the kinetics and thermodynamic properties of most reactions¹⁰. Furthermore, in mixed solvents, interactions between the solute and solvent molecules, as well as between solvent molecules, are more complex than those of the pure solvent because of preferential solvation¹¹. These interactions can result in new mixed solvent units in the solvation shell. The properties and structures of these new mixed solvent units can be distinct and different from those in the pure solvent. In this case, the solute molecules can usually interact at different degrees, with each molecule of the solvent. It is important to note that the combination of solvation layers, around solute molecules (local composition), varies from that of the bulk combination of mixed solvents. Preferential solvation is a special feature that appears in a mixture of solvents^{12,13}.

In the process to determine the physico-chemical properties for a drug, the first step is to dissolve it in a solvent. So, information about the solubility of a drug, in a solvent is important, especially at different temperatures and ionic strengths. These data can provide a thermodynamic description (including enthalpy and entropy changes of the dissolution process) of the system. Most drugs have agents or groups with acidic or basic property. Depending on the pH value of the solution and protonation constant of the drug, various chemical species may be formed in the solution. These species can have cationic, anionic or neutral structures, and usually differ with regard to their solubility, UV absorption, etc. It is clear that the ionized species have more solubility in water, while the neutral form is more lipophilic^{14,15}. Following from previous works^{16,17}, in the present study, the protonation constant of clindamycin has been determined in different aqueous solutions of methanol and ethanol at 298.15 K temperature and atmospheric pressure.

Methanol, ethanol and clindamycin were procured from Sigma-Aldrich Company, USA. All other reagents were purchased from Merck. Stock solutions of NaOH and HCl (0.1 M) were prepared from titrisol ampoules. NaClO₄ was dried in an oven at 180°C under vacuum for 24 h before use. Double-distilled water was used for dilution and preparation of mixed solvents (aqueous solvents of methanol and ethanol). The purity of all chemical was greater than 98%.

The potentiometric measurements were done using a pH-meter (Metrohm model 781, USA). All potentiometric titrations were carried out in an 80 ml double-walled glass vessel which was thermostated at 298.15 K by a circulating water bath.

Next, 25 ml of HCl solution (0.01 M) was titrated using 0.1 M NaOH solution at 298.15 K for potentiometric calibration of each mixed solvent. The process of titration was continued until 2 ml of NaOH solution was

*For correspondence. (e-mail: fardadkoohyar@tdtu.edu.vn)

Table 1. Calibration parameters, E_a (the specific constant of the potentiometric cell in the acidic region) and k (Nernst's slope), also, protonation constant of clindamycin (pK_a) in aqueous solutions of methanol and ethanol at 25°C and 0.1 M NaClO₄

% $\frac{\text{Volume}_{(\text{alcohol})}}{\text{Volume}_{(\text{water})}}$	Water + methanol			Water + ethanol		
	E'_a (mV)	k (mV)	pK_a	E'_a (mV)	k (mV)	pK_a
0	411.33	59.265	7.89	411.33	59.265	7.89
10	406.52	59.144	7.66	408.93	59.143	7.62
20	406.72	59.229	7.46	407.30	58.735	7.47
30	406.46	59.866	7.15	411.56	58.999	7.23
40	401.69	58.764	6.87	406.79	59.197	7.06
50	397.69	58.931	6.75	400.07	59.388	6.95

added at intervals of 0.1 ml. In this step, the recorded potential of the electrode was used to calculate calibration parameters using Gran's method¹⁸⁻²⁰. In the second step, 40 mg of clindamycin was dissolved in mixed solvents of water + methanol and water + ethanol to determine protonation constants of the drug at a given temperature. After completing solubilization, the solution was titrated using NaOH solution up to pH \approx 11. All titrations were repeated at least thrice at 298.15 K and constant ionic strength of 0.1 M NaClO₄.

The potential of the glass electrode, E , for the pH-meter can be represented according to the Nernst equation²¹ as the below

$$E = E^0 + k \log [H^+] + k \log \gamma_{H^+} + E_{LJ}, \quad (1)$$

where E^0 , E_{LJ} , k and γ_{H^+} are the standard potential, liquid junction potential, Nernst's slope and the proton activity coefficient respectively. At constant ionic strength, the values of γ_{H^+} and E_{LJ} will be constant. Therefore, eq. (1) can be rewritten as follows

$$E = E'_a - kp[H^+], \quad (2)$$

where E'_a is the sum of E^0_{cell} , $k \log \gamma_{H^+}$ and E_{LJ} . In the calibration step, the values of E'_a and k can be easily obtained using eq. (2). The graph of E versus $p[H^+]$ is a linear plot. The slope and y -intercept of this linear plot are k and E'_a respectively. Table 1 shows the results of the calibration step.

In the second step to determine the protonation constant, calibration parameters were used for determination of hydrogen ion concentration during the titration process.

In the pH range used in this study, clindamycin reacts as a monoacid. For constant ionic strength, stoichiometric deprotonation constant of a monoacid ($L^- + H^+ \rightleftharpoons HL$) can be expressed as follows

$$K_a = \frac{[HL]}{[L^-][H^+]}. \quad (3)$$

With reference to Bjerrum's method, the fraction of protons bound to ligand, \bar{n} , is obtained using the following equation²²

$$\bar{n} = \frac{C_H - [H^+]}{C_L} = \frac{[HL]}{[L^-] + [HL]}, \quad (4)$$

where C_H and C_L show the total concentration of proton and clindamycin respectively. Equations (3) and (4) can be combined as given below

$$\bar{n} = \frac{K_a[H^+]}{1 + K_a[H^+]} = \frac{10^{pH-pK_a}}{1 + 10^{pH-pK_a}}. \quad (5)$$

On the other hand, during titration, \bar{n} can be calculated according to the electrical neutrality of the solution as follows

$$\bar{n} = \frac{C_L + [ClO_4^-] - [Na^+] - [H^+] + [OH^-]}{C_L}. \quad (6)$$

In eq. (6) the concentration of $[OH^-]$ is calculated using autoprotolysis constant, K_{ap} , of water ($[OH^-] = K_{ap}/[H^+]$). The values of K_{ap} are available in the literature^{23,24}. Finally, protonation constant was determined by fitting experimental data in eqs (5) and (6). In this study, the fitting was done with Microsoft Excel Solver using nonlinear least-squares method²⁵. For clindamycin dissolved in different aqueous solutions of methanol and ethanol, Table 1 shows the measured pK_a values. In some studies, the pK_a values for clindamycin in water have been reported as 7.77 and 7.66 respectively^{26,27}. As it can be seen from Table 1, there is a little difference between these values and our measured pK_a of clindamycin in water at 298.15 K temperature. This can be due to the different methods of determination and experimental conditions.

Table 1 shows that in both mixed solvents, viz. water + methanol and water + ethanol, the pK_a values decrease when the volume fraction of alcohol in the mixed solvents increases. For any reaction, the Gibbs free energy is related to non-specific electrostatic interactions and specific interactions like hydrogen-bonding. The Born model can predict electrostatic interactions^{28,29} and the non-electrostatic term which includes specific solute-solvent interactions. Therefore, a linear relationship can exist

between pK_a and dielectric constant of the medium ϵ , as given below

$$pK_a = A + (B/\epsilon), \quad (7)$$

where A and B are model constants.

The values of dielectric constant are available in the literature. Using these data, the following equations were obtained for mixed solvents of water + methanol and water + ethanol respectively³⁰

$$pK_a = (11.42 \pm 0.41) - \left(\frac{283.10 \pm 27.90}{\epsilon} \right), R^2 = 0.96, \quad (8)$$

$$pK_a = (9.62 \pm 0.32) - \left(\frac{145.98 \pm 20.44}{\epsilon} \right), R^2 = 0.93. \quad (9)$$

Equations (8) and (9) show that the pK_a value of clindamycin, in mixed solvents of water + methanol and water + ethanol, decreases with decrease in the dielectric constant of aqueous solutions of methanol and ethanol (mixed solvents).

To obtain more information on the type and extent of interactions, solvent effect was considered by linear solvation energy relationship model developed by Kamlet and Taft (KAT)^{9,31,32}. Both non-specific and specific solute-solvent interactions are separately considered by the KAT equation in a linear solvation energy relationship framework

$$pK_a = A_0 + a\alpha + b\beta + p\pi^*, \quad (10)$$

where α , β and π^* are the solvent hydrogen-bond donor (HBD) acidity, hydrogen-bond acceptor (HBA) basicity and dipolarity/polarizability respectively. A_0 , a , b and p are values of regression which show the sensitivity of protonation constant to each parameter of the solvent.

The values of α , β and π^* are available in the literature³³. The multiple linear regression can result in the best KAJ equation representing the experimental pK_a values for clindamycin in mixed solvents of water + methanol and water + ethanol³⁴. The results of multiple linear regressions are shown in eqs (11) and (12) below for mixed solvents of water + methanol and water + ethanol respectively

$$pK_a = (21.79 \pm 0.95) - (9.13 \pm 0.37)\beta - (8.84 \pm 0.71)\pi^*, \\ R^2 = 0.96, \quad (11)$$

$$pK_a = (3.98 \pm 0.44) + (4.97 \pm 0.23)\alpha - (1.78 \pm 0.40)\pi^*, \\ R^2 = 0.96. \quad (12)$$

The results show that the pK_a values of clindamycin increase as dipolarity/polarizability of the solvent decreases. Also, the solvent effect is sensitive to specific

hydrogen bonding interactions. In the water-methanol mixed solvent, the pK_a value of clindamycin decreases with an increase in the hydrogen bonding basicity of the solvent. On the other hand, in water-ethanol mixed solvent, the pK_a value of clindamycin decreases with the decrease in hydrogen bonding acidity of the solvent.

The protonation constant of clindamycin was determined in mixed solvents of water + methanol and water + ethanol at 298.15 K temperature. The pK_a value of clindamycin decreases when the volume fraction of alcohols (methanol and ethanol) increases in the mixed solvents. The protonation constant was correlated with dielectric constant and KAT parameters. The electrostatic interactions contribute to variation of the protonation equilibria. Also, the KAT model shows that pK_a of clindamycin is sensitive to the solvent HBD acidity and the HBA basicity in the mixed solvents of water + methanol and water + ethanol. The derived KAT equations allow us to estimate pK_a of clindamycin in the desired composition throughout the 0–50% v/v range at 298.15 K and 0.1 M NaClO₄.

- Smieja, M., Current indications for the use of clindamycin: a critical review. *Can. J. Infect. Dis.*, 1998, **9**, 22–28.
- Daum, R. S., Clinical practice. Skin and soft-tissue infections caused by methicillin-resistant *Staphylococcus aureus*. *N. Engl. J. Med.*, 2007, **357**, 380–390.
- Jemec, G. B. E., Revuz, J. and Leyden, J. J., *Hidradenitis Suppurativa*, Springer, Berlin, Germany, 2006, p. 152.
- Levine, M., Lexchin, J. and Pellizzari, R., *Drugs of Choice: A Formulary for General Practice*, Canadian Medical Association, Canada, Ottawa, 1995.
- Jabbari, M. and Gharib, F., Solute-solvent interaction effects on protonation equilibrium of some water-insoluble flavonoids. *J. Sol. Chem.*, 2011, **40**, 561–574.
- Shamel, A., Jaber, F. and Gharib, F., Protonation constants of adenine and adenosine in different aqueous solutions of methanol and ethanol. *J. Chem. Eng. Data*, 2010, **55**, 5176–5181.
- Jabbari, M. and Gharib, J., Equilibrium studies of triphenyltin(IV) complexes with glycine, glycyl-glycine, and glycyl-glycyl-glycine in different aqueous solutions of ethanol. *Can. J. Chem.*, 2010, **88**, 877–885.
- Jabbari, M. and Gharib, F., Solvent effects on protonation equilibria of some amino acids and peptides in different aqueous solutions of ethanol. *Acta Chim. Slov.*, 2010, **57**, 325–331.
- Reichardt, C., *Solvents and Solvent Effects in Organic Chemistry*, VCH, New York, USA, 2004, 3rd edn.
- Giusti, L. A., Marini, G. and Machado, V. G., Solvatochromic behavior of 1-(*p*-dimethylaminophenyl)-2-nitroethylene in 24 binary solvent mixtures composed of amides and hydroxylic solvents. *J. Mol. Liq.*, 2009, **150**, 9–15.
- Farajtabar, A., Jaber, F. and Gharib, F., Preferential solvation and solvation shell composition of free base and protonated 5, 10, 15, 20-tetrakis(4-sulfonatophenyl)porphyrin in aqueous organic mixed solvents. *Spectrochim. Acta, Part A*, 2011, **83**, 213–220.
- Reichardt, C., Pyridinium *N*-phenolate betaine dyes as empirical indicators of solvent polarity: some new findings. *Pure Appl. Chem.*, 2004, **76**, 1903–1919.
- Marcus, Y., *Solvent Mixtures: Properties and Selective Solvation*, Marcel Dekker, New York, USA, 2002.
- Gracin, S. and Rasmuson, A. C., Solubility of phenylacetic acid, *p*-hydroxyphenylacetic acid, *p*-aminophenylacetic acid, *p*-hydroxybenzoic acid, and ibuprofen in pure solvents. *J. Chem. Eng. Data*, 2002, **47**, 1379–1383.

15. Toes, M. J., Jones, A. L. and Prescott, L., Drug interactions with paracetamol. *Am. J. Ther.*, 2005, **12**, 56–66.
16. Chahiyani, H., Gharib, F. and Farajtabar, A., Thermodynamic studies on solubility and protonation constant of acetaminophen at different ionic strengths and various temperatures. *J. Mol. Liq.*, 2014, **199**, 137–142.
17. Faraji, M., Farajtabar, A. and Gharib, F., Determination of water-ethanol mixtures autoprotolysis constants and solvent effect. *J. Appl. Chem. Res.*, 2009, **9**, 7–12.
18. Farajtabar, A. and Gharib, F., Solvent effect on protonation constants of salicylic acid in mixed aqueous organic solutions of DMSO. *Monatsh. Chem.*, 2010, **141**, 381–386.
19. Farajtabar, A. and Gharib, F., Solvent effect on protonation constants of 5, 10, 15, 20-tetrakis(4-sulfonatophenyl)porphyrin in different aqueous solutions of methanol and ethanol. *J. Sol. Chem.*, 2010, **39**, 231–244.
20. Gran, G., Determination of the equivalence point in potentiometric titrations, Part II. *Analyst*, 1952, **77**, 661–671.
21. Nernst, W., Dielectric constant and chemical equilibrium. *Z. Phys. Chem.*, 1984, **13**, 531–536.
22. Beck, M. T. and Nagypal, I., *Chemistry of Complex Equilibria*, Ellis Harwood, New York, USA, 1990.
23. Kilic, E. and Aslan, N., Determination of autoprotolysis constants of water-organic solvent mixtures by potentiometry. *Microchim. Acta*, 2005, **151**, 89–92.
24. Farajtabar, A., Naderi, F. and Gharib, F., Autoprotolysis in water/methanol/NaCl ternary systems. *J. Serb. Chem. Soc.*, 2013, **78**, 1561–1567.
25. Maleki, N., Haghghi, B. and Safavi, A., Evaluation of formation constants, molar absorptivities of metal complexes, and protonation constants of acids by nonlinear curve fitting using Microsoft Excel Solver and user-defined function. *Microchem. J.*, 1999, **62**, 229–236.
26. ACD/Labs 6.00 C. ACD pKa DB Advanced Chemistry Development Inc., Ontario, Canada.
27. Wan, H., Holmen, A. G., Wang, Y., Lindberg, W., Englund, M., Nagard, M. B. and Thompson, R. A., High-throughput screening of pK_a values of pharmaceuticals by pressure-assisted capillary electrophoresis and mass spectrometry. *Rapid Commun. Mass Spectrom.*, 2003, **17**, 2639–2648.
28. Barbosa, J., Barron, D., Beltran, J. L. and Buti S., On the role of solvent in acid-base equilibria of diuretics in acetonitrile-water mixed solvents. *Talanta*, 1998, **45**, 817–827.
29. Barbosa, J., Toro, I. and Sanz-Nebot, V., Acid-base behaviour of tripeptides in solvents used in liquid chromatography. Correlation between pK values and solvatochromic parameters of acetonitrile-water mixtures. *Anal. Chim. Acta*, 1997, **347**, 295–304.
30. Puranik, S. M., Kumbharkhane, A. C. and Mehrota, S. C., The static permittivity of binary mixtures using an improved brugge-man model. *J. Mol. Liq.*, 1994, **59**, 173–177.
31. Taft, R. W., Abboud, J. L. M and Kamlet, M. J., Linear solvation energy relationships. An analysis of Swain's solvent 'acity' and 'basity' scales. *J. Org. Chem.*, 1984, **49**, 2001–2005.
32. Kamlet, M. J., Abboud, J. L. M., Abraham, M. H. and Taft, R. W., Linear solvation energy relationships. A comprehensive collection of the solvatochromic parameters, π^* , α , and β , and some methods for simplifying the generalized solvatochromic equation. *J. Org. Chem.*, 1983, **48**, 2877–2887.
33. Marcus, Y., The use of chemical probes for the characterization of solvent mixtures. Part 2. Aqueous mixtures. *J. Chem. Soc. Perkin Trans.*, 1994, **2**, 1751–1758.
34. Farajtabar, A. and Gharib, F., Spectral analysis of naringenin deprotonation in aqueous ethanol solutions. *Chem. Pap.*, 2013, **67**, 538–545.

Received 8 September 2018; revised accepted 16 March 2020

doi: 10.18520/cs/v119/i2/374-377

Soil organic carbon prediction using visible–near infrared reflectance spectroscopy employing artificial neural network modelling

Justin George, K. *, Suresh Kumar and R. Arya Raj

Agriculture and Soils Department, Indian Institute of Remote Sensing (ISRO), 4 Kalidas Road, Dehradun 248 001, India

Visible–near infrared (VNIR) spectroscopy is a relatively fast and cost-effective analytical technique for estimating soil organic carbon (SOC). The present study was undertaken for predicting SOC using VNIR reflectance spectroscopy employing artificial neural network (ANN). Surface soil samples (0–15 cm) were collected from 75 georeferenced locations through grid sampling approach in a hilly watershed of Himachal Pradesh, India, and analysed for SOC. The reflectance spectra of soil samples was measured using a spectroradiometer in the wavelength range of 350–2500 nm. Various spectral indices were generated using the sensitive bands in the visible region. The SOC-sensitive spectral indices and reflectance transformations were utilized for predictive modelling of SOC using the ANN model. This model could predict SOC values with R^2 of 0.92 and MSE value of 0.24, indicating that this technique can be used to predict SOC in a spatial domain when coupled with high-resolution hyperspectral satellite/airborne data.

Keywords: Artificial neural network model, reflectance spectroscopy, soil organic carbon, visible and near infrared region.

SOIL organic carbon (SOC) plays a fundamental role in determining the physical, chemical and biological properties of the soil. It is beneficial for maintaining soil productivity, water-holding capacity as well as carbon sequestration for alleviating the ill-effects of greenhouse gases and thus climate change^{1–3}. SOC holds about 4.5 times the amount of the biotic carbon pool and 3.3 times the amount of atmospheric carbon pool⁴. Thus reliable estimation of SOC is vital for understanding the human-induced effects on the global carbon cycle and associated climate change⁵. Conventional methods for SOC determination in soil laboratories are costly, time-consuming and may be environmentally hazardous⁶. Thus there is an urgent need for the development of fast, accurate and non-destructive methods (thus reducing the number of soil chemical analyses) for SOC estimation, which will help in generating high-resolution soil property maps of large areas at modest costs.

*For correspondence. (e-mail: justin@iirs.gov.in)