Recent Advancements in Spectrophotometric pKa Determinations: A Review

Job Herman Berkhout¹, Aswatha Ram HN²*,

¹Faculty of Science, Radboud University, Nijmegen, NETHERLANDS.
²Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal Academy of Higher Education, Manipal, Karnataka, INDIA.

ABSTRACT

The pKa value is a key feature in Absorption, Distribution, Metabolism and Excretion (ADME) of drugs, thus knowing this value is critical in drug development. In this review, pKa values determined in the past decade, using UV-Vis spectrometry, are discussed. To determine the pKa, four methods were applied: Henderson-Hasselbach, Albert-Serjeant, Bates-Schwarzenbach and Spectrometric titrations. This review will show the value of this aged but well-established technique in the past decade, due to its simplicity, accuracy, cost efficiency and reproducibility.

Key words: Albert-Serjeant, Bates-Schwarzenbach, Henderson-Hasselbach, pKa, Spectroscopy, Titrations.

INTRODUCTION

The acid dissociation constant (pKa) indicates the ionization state of a compound at a given pH. Since most drug molecules are ionizable,¹ the ionization state is of utmost importance in Absorption, Distribution, Metabolism and Excretion (ADME) of drugs.²,³ Therefore, the pKa value of a compound impacts psychochemical properties like: pH dependent aqueous solubility, protein interaction and membrane permeability.² For this reason compounds with a different pKa are absorbed in different compartments of the digestive tract, since the different compartments contain a different pH (e.g. stomach pH 1-3.5, colon pH 5.5-8, intestine pH 5.5-8 and blood pH 7.4).⁴ Hence it is crucial to properly understand and analyze the pKa value of the compound during early drug development. Although the pKa is referred to as a constant, it is influenced by the temperature, ionic strength, and the solvent dielectric constant.⁵ To produce accurate results, these factors need to remain constant throughout the experiments. Since the pH of a solution is also influenced by these factors,⁶ pH meters should be calibrated under the same conditions. Temperature (T), ionic strength (IoStr) and solvent heavily impact the pKa value of a compound. Therefore, reports of pKa values in literature should contain these exact details.

There are many methods suitable for pKa determination, which have been comprehensively evaluated by Reijenga et al.⁵ Therefore, these methods will not be evaluated here as it is beyond the scope of this review. As novel pKa values are estimated throughout the years, this article aims to evaluate the spectroscopic determinations of the past decade.

UV-Vis spectrometry

UV-Vis spectrometry is traditionally one of the most used methods to determine the pKa of a compound. To this day it is still commonly used due to its availability, accuracy, simplicity and reproducibility.⁶ To use UV-Vis spectrometry for pKa determination, it is required that a chromophore is present close to the ionization site of
the compound. If this is fulfilled, a distinction in the spectrum of the dissociated and the non-dissociated form of the molecule can be observed. The absorption is then plotted against the pH, from which a sigmoid curve is obtained and the pKa can be estimated from the inflection point.\(^5\) Beside curve fitting, there are multiple different approaches with regard to pKa determination, which will be discussed in this review.

**Henderson-Hasselbach method**

The most known pKa determination method is to this day is using the Henderson-Hasselbalch equation (1), established in 1916.\(^7\)

\[
(1) \quad \text{pH} = \text{pKa} + \log 10 \left( \frac{[A^-]}{[HA]} \right)
\]

It relates pH and pKa to the equilibrium concentration of dissociated [A-] and non-dissociated [HA] acids. The pKa value is often experimentally determined by plotting a certain parameter as a function of pH. This results in a sigmoid curve, where the inflection point indicates the pKa. This method was applied by Bartistela et al. to quantify the pKa values of xanthenes at 30°C and the IoStr [NaCl] 0.1M. Xanthenes are a class of widely used dyes, which often present three acid-base groups: one carboxylic site and two phenolic sites.\(^8\) Because of close pKa values in combination with a strong UV-Vis spectral overlap, multivariate analysis was applied. The determined pKa values of the dye azafluorescein are: pKa\(_{\text{OH1}} = 2.81,\) pKa\(_{\text{COOH}} = 3.88,\) pKa\(_{\text{OH2}} = 5.62\) and pKa\(_{\text{OH2}} = 6.23.\) For eosin Y the estimated pKa values are: pKa\(_{\text{OH2}} = 2.02\) and pKa\(_{\text{COOH}} = 3.8.\) To assure the pKa the attribution, an eosin methyl-ester derivative was synthesized, which contains only the phenol-acid-base group. A pKa of 2.11 was established. This confirms that the pKa 2.02 of eosin is related to the phenolic center. The pKa values of rose Bengal B were estimated to be 1.89 and 3.93, attributed to the carboxylic and OH group of the xanthene ring respectively. The pKa values of erythrosine B were established at 2.35 and 3.79. These values are attributed to the carboxylic and the phenolic groups respectively. pKa\(_{\text{OH2}} = 3.79\) was found to be lower than stated by previous studies, potential due to different experimental conditions. The erythrosin methyl ester was synthesized and a pKa of 3.74 was quantified. This corresponds to the phenol group and thus supports that the pKa values of 3.93 for the rose bengal B and 3.79 for erythrosine B. At last the pKa(s) of tetrani trofluorescein were determined. Chemometric analysis showed that three pKa(s) were present in the molecule of which two: pKa 0.38 and pKa 2.48 were concluded, while the lowest pKa \(\text{pH} < 0\) was not determined.\(^8\) FIA/UV-vis, a novel method proven to contain a higher throughput, high sensitivity and has a lower sample consumption, compared to the standard UV-vis methods was developed by Musel et al. FIA/UV-vis was used to establish the pKa of oxime based acetylcholinesterase reactivators. All pKa values were estimated in a range of 7.00-8.35 (Figure 1A).\(^9\) In Hossain et al. UV-spectroscopy was compared to potiometry and RP-HPLC in determination of antimalarial drug lead; Cyclen. Here UV-spectroscopy was the most accurate method, since it does not require co-solvents, among others and thus eliminating potential interference. The pKa value(s) of Cyclen are: 5.9, 6.6 and 8.7.\(^10\)

**Albert and Serjeant method**

Another method is to calculate the pKa of a compound is the Albert-Sergeant method,\(^11\) which uses the following equations:

\[
(2a) \quad \text{pKa} = \text{pH} + \log 10 \left( \frac{\text{A} - \text{A}}{\text{A} - \text{AM}} \right)
\]

\[
(2b) \quad \text{pKa} = \text{pH} + \log 10 \left( \frac{\text{AM} - \text{A}}{\text{A} - \text{Al}} \right)
\]

In this method the compound is considered to be either a weak acid (2a) or a weak base (2b). Here, the pH is the value recorded on the pH meter, D is the absorbance of the compound in the selected buffer, AM and Al indicate the absorbance of the unionized and ionized compound respectively. Using this, a rough estimate of pKa is required upon which one acidic, one basic and 7 buffer solutions are prepared (with a pH of the estimated pka value, ± 0.2, 0.4 and 0.6). Using this method, the pKa’s of Felodipine: 5.07 at IoStr 0.02M,\(^12\) Resperidone: 8.62, IoStr 0.02M\(^13\) and Brimonidine Tartrate: 7.22, at 25°C IoStr 0.3M\(^14\) were determined using this equation. This method of pKa calculation was compared to linear regression in two different studies, which both resulted in a similar pKa value. In the first study, the pKa of PPB (1,4-bis(3-(2-pyridyl)pyrazol-1- ylmethyl)benzene) in the solvent mixtures: EtOH – H\(_2\)O and THF -H\(_2\)O, was considered to be: 10.77 and 11.14 respectively.\(^15\) In the latter, the pKa(s) of Nilutamide: approximately 10 and 14 were determined (Figure 1B).\(^16\) Retention time and electrophoresis mobility are dependent on the pKa of the analyte and the pH of the mobile phase or analytical medium. Therefore, the pKa of the analyte is determined before HPLC or electrophoresis experiments.\(^17\) Kuntworb et al. revisited the pKa of cryptolepine: 10.99 at 20°C and Celebier et al. the pKa of phenazopyridine hydrochloride: 5.17 at 22-23°C.\(^18\) Both studies corrected for the ionic strength.

**Bates-Schwarzenbach method**

The Bates-Schwarzenbach method\(^19\) uses the following equation:
Where \( p(aHcCl) \) is an acidity function, \( DHA, DA \) and \( D \) are the absorbance value in acid, base and buffer, respectively. For this method, there is only one buffer used, where the \( pH \) of the buffer depends on the estimated \( pKa \) of the compound. Domańska et al. and Pobudkowska et al. performed many \( pKa \) studies on an array of compounds in different solvents using the Bates-Schwarzenbach method, which is in their opinion the most accurate method. They revisited the compounds: Atropine \( pKa \) 10.3, ibuprofen \( pKa \) 5.38, promethazine hydrochloride \( pKa \) 6.47 and flurbiprofen \( pKa \) 4.50. These values were estimated higher compared to previous publications. In another study, the \( pKa \) values of Cimetidine 6.84, Phenylbutazone 5.03, Fenbufen 4.33, Nitrofurantoin 6.67 and Triamterene 7.16, were revisited (Figure 1C). The determined \( pKa \) values were similar to the values previously described in literature. Domanska et al. also revisited the \( pKa \) values for the compounds: chlorpromazine hydrochloride: 9.15, trifluoperazine dihydrochloride: 8.87, fluphenazine dihydrochloride: 10.01, thioridazine hydrochloride: 8.89, promazine hydrochloride: 9.37 and trifluopromazine hydrochloride: 9.03. Here, Fluphenazine dihydrochloride was estimated higher compared to previous studies. Also the \( pKa \) values of Niflumic acid, Flufenamic acid and diclofenac sodium were determined to be 4.42, 4.62 and 5.70 respectively. Again, each value was considered to be significantly higher compared to previous studies. Pobudowska and Domářka compared the \( pKa \) values of five compounds at a temperature of 298.1 or 310.2 Kelvin. The results are: flufenamic
acid: 4.62 and 5.23, mephenamic acid 3.88 and 4.33, niflu- 
mec acid 4.42 and 4.60, diclofenac sodium 5.70 and 4.51 and meclofenamic acid 4.39 and 3.99 re- spectively.24 Displaying the influence of temperature on the pKa value. Furthermore, the pKa(s) of nadolol: 9.30, nimesulide: 7.34, bifonazole: 5.85 and mephenamic acid: 3.88.25 At last, Pobudkowska estimated the pKa of multiple other compounds at a temperature of 298.15 and 310.15. Pka values of Octopamine·HCl, Theophylline, Theobromine, Aminophylline, Lobeline hydrochloride, Perphenazine and Indomethacin and Theobromine at 298.15 K were determined to be 9.38, 9.74, 10.35, 5.1, 8.9, 7.3 and 4.5 respectively. At 310.15 K the pKa(s) of the compounds: Octopamine·Hcl, Theobromine, aminophylline, Lobeline hydrochloride, Perphenazine and Indomethacin are: 9.85, 8.66, 5.8, 9.0, 7.0 and 3.4 respectively.26-28 In the studies without a given tempera- ture or ionic strength; the temperature was 298.5 K and the ionic strength 0.020M.19

**Spectrophotometric titrations**

In pKa determination by spectrophotometric titrations, the absorption is measured against an increasing pH. Thereafter, (sigmoidal) curve fitting can be applied and the inflection point calculated from the second deriva- tive. Sanli et al. displayed the pKa values of leucovorin, 5-fluorouracil and irinotecan as a function of solvent composition at 25°C and IoStr 0.1M, since the pKa differs depending on the mole fraction of acetoni trile present.29 It is assumed that water causes preferen- tial solvation of the charged particles. This could then result in a monotonic dependence of the acidity constants of studied compounds on the solvent composition. In water the pKa value(s) of leucovorin are 3.12, 4.60 and 10.0, of 5-fluorouracil 8.05 and of irinotecan 8.71 (Figure 1D).29 Vidal-Salgado et al. compared four different methods (two graphical and two mathematical) on pKa determination of the universal pH indicator Carlo Ebra 1-11. The combined average pKa of the four methods was 8.277.20 Ribeiro and Smith examined the pKa of Cefapirin and Cefotiofur using spectrophotometric titra- tions and a computational model.31 The authors also revised 14 already determined Cephalosporins. The computational models used were Marvin and ACD/ Percepta, which results were compared to experimental obtained data. For cefotiofur, the experimentally deter- mined pKa was 2.68, associated to the carboxylic acid group deprotonation. Two values were determined for cefapirin: 2.74, carboxylic acid group deprotonation and 5.13, associated to pyridinium ring deprotonation. The in silico predicted data agreed with the experimental val- ues, however for cephalosporins having imine and ami- nothiazole groups structurally close, Marin presented problems in pKa prediction.31 Ibrutinib contains four ionizable centers, each with a pKa of: 3.22, 4.17, 6.77 and 9.82 at 25°C.32 The five thermodynamic dissociation constants from Eltrombag were estimated, depend- ing on ionic strength at a temperature of 25°C. Which are 2.69, 6.97, 7.13, 7.65 and 8.30.33 It is suggested that, at acidic pH melatonin is unstable when interacting with the environment, thus provoking changes in spectral behavior. Therefore, it is assumed that the currently known pKa is not valid. Zafra-Roldán et al. managed to correctly estimate the pKa of melatonin, by protecting it from light and oxygen, which resulted in pKa values of 5.77 and 10.20. They used spectrometry accompa- nied by the SQUAD software in their procedure.34

**CONCLUSION**

The fact that spectroscopy is often used in pKa deter- mination, proves the value of this aged, but well-pre- served technique. The many potential analysis methods allows flexibility of spectroscopy to quantify the pKa of many different compound types. With the novel computer modeling however, pKa determination becomes quicker and easier over the years. However, due to limits in computer modeling, still the simplicity, accuracy, low maintenance costs and reproducibility of spectroscopy makes it excel over other methods and thus keeps it uses in future research.

**ACKNOWLEDGEMENT**

The authors sincerely thank Manipal College of Pharmaceuti- cal Sciences, Manipal Academy of Higher Educa- tion, Manipal, India and Radboud University, the Netherlands for providing necessary facilities to carry out this work.

**CONFLICT OF INTEREST**

The authors declare that there is no conflict of interest.

**ABBREVIATIONS**


**REFERENCES**


In the drug development, determination of pKa is utmost important in the aspect of ADME. Even though, many techniques and methods are available for their determination, UV-Vis spectrophotometry has gained its importance in this recent trend. Therefore, the various spectrophotometric methods used in the determination of pKa were discussed in this review. Because of the simplicity, accuracy and low maintenance costs, this method is still advantageous in the determination of pKa.
Cite this article: Berkhout JH, Ram AHN. Recent Advancements in Spectrophotometric pKa Determinations: A Review. Indian J of Pharmaceutical Education and Research. 2019;53(4s):s475-s480.