Structural Attributes of Organic Compounds for UV-Spectrophotometric Determination of Dissociation Constant-A Systematic Review

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ABSTRACT

UV-spectrophotometric determination of dissociation constant (pK_a) is used routinely in various research fields. This review highlights the structural attributes of organic compounds that exhibit distinct pH-sensitive UV-absorbance for ionized and unionized species qualifying for pK_a measurement. Organic compounds must possess a double bond, the chromophore adjacent to the ionizing functional group. Compounds bearing up to five sigma bonds between the chromophore and ionizing group are eligible for UV-spectrophotometric determination of pK_a. This review serves as a quick guide for knowledge about structural requirements expediting pK_a determination by UV-spectrophotometry. Besides, the study also identified the gap in research on pK_a in drug discovery and food chemistry, revealing the necessity of determining pK_a at the early stages of drug and food research to enhance the success rate in their development.

Keywords: Chromophore, Dissociation constant, Functional group, Ionization, pH, UV-spectrophotometry.

INTRODUCTION

The dissociation constant (pK_a) is the pH at which there exists an equal proportion of ionized and unionized species of a compound or a drug. Knowledge about pK_a values of chemical compounds is indispensable in organic chemistry, medicinal chemistry, analytical chemistry, biochemistry, food chemistry, apart from their applications in allied biological sciences. UV-spectrophotometric measurement of pK_a affords efficient, simple, sensitive, and reliable pK_a measurement at concentrations lower than micromoles. The choice of UV-spectrophotometry for pK_a assessment depends on the chemical structures of compounds, especially an ionizing functional group adjacent to an organic moiety capable of UV absorbance known as the chromophore. Compounds display characteristic UV absorbance for unionized and ionized species on structural compliance. The ionization ratio depends on the pH of the solvent or medium used in the UV study. A plot of UV absorbance over the pH yields...
a sigmoidal curve usually, from which pK_a could be calculated. Compounds possessing multiple ionizing groups can also display pH-related shift of UV-spectrum at the distinct \( \lambda_{\text{max}} \) region enabling pK_a determination, provided the ionizing groups exhibit significant variation in their molar absorptivity. Hence, substantial knowledge of the structural attributes of chemical compounds contributing to pK_a determination by UV-spectrophotometry is essential to make it a time-effective method. Literature reviews that explored the theoretical background, significance, analytical methods, applications, factors affecting pK_a, and its measurement are available. No thought is public that delineates the structural attributes of organic compounds for UV-spectrophotometric determination of pK_a. Therefore, this systematic review aimed to analyze the structural characteristics of organic compounds for UV-spectrophotometric determination of pK_a.

**METHOD**

The literature review was conducted by customized search in “Google Scholar” using the search terms “pK_a determination by UV spectrophotometry.” A literature search was also achieved through “ScienceDirect” and “PubMed.” Relevant data were retrieved from literature published during the years 2011-2021. Fifty-six articles were available, out of which 27 were excluded. The exclusion criteria included 1 book chapter, 4 review articles, 8 pK_a determination in non-aqueous solvents, and 14 analyses of inorganic compounds. Thus, data of experimental determination of pK_a of organic compounds in aqueous buffers by UV spectrophotometry extracted from a total of 29 publications were utilized for systematic review. The flow chart for the systematic review method is shown in Figure 1.

**RESULTS AND DISCUSSION**

**Dissociation Constant: A Pharmaceutical Perspective**

There exists a strong correlation between pK_a and Lipinski’s molecular descriptors. Almost all drug discovery and design studies quote the rule of five and report predicted molecular descriptors without focusing on pK_a. The drug’s ionization status will decide the concentration of the polar form of the drug, which in turn will affect the partition coefficient and the solubility. pK_a values can explain the extent of drug ionization and the nature of the contributing ion. Knowledge about experimental or predicted pK_a is desirable at earlier stages of drug discovery to determine the rate of absorption, distribution, metabolism, and excretion (ADME) of lead/drug. This would enable scientists to optimize the chemical structure to exhibit better pharmacokinetic behaviour at changing pH of body compartments. Early determination assures the success of leading to a better drug after oral administration. It is more prudent to use pK_a combined with other molecular properties to discover and optimize lead and formulate suitable dosage forms of drugs. One of the methods to improve the water solubility of the
drug is to prepare their salt forms. The dissociation constant is the prime factor that decides whether the salt form will be energetically favorable in the acidic and basic pH of the stomach and intestine, respectively. The pKa values of the parent drug and the salt derivative should be different such that there exist at least 2 pH units difference between the two forms. The majority of drugs are either weak acids or bases. World drug index has 63% of drugs capable of ionization at the pH of 2 to 12 prevailing in various human body compartments. Among these ionizable drugs, 71.9% are monobasic or dibasic compounds, 14.6% are monoprotic or diprotic acids, whereas 7.5% are ampholytes with an acid and a basic function.

Acidic drugs with pKa less than 4 and basic drugs with pK_a more than 12 may not enter the central nervous system due to their hydrophilic character. It is imperative to determine the pK_a of drugs as early as possible in medicinal chemistry research. It is the critical determinant of absorption and partition of drugs across the blood-brain barrier and other biological compartments in the body. Knowledge of pK_a of active pharmaceutical ingredients is essential in biopharmaceutics. Its application has been extended to assess the environmental contamination by pharmaceuticals, which has emerged as a potential contaminant from drug manufacturing industries. Apart from these known applications of pK_a, it is also helpful in separating and analysing acid-base drugs to standardize procedures like liquid chromatography and capillary electrophoresis for detection, isolation, and quantification of polarizable moieties.

Categorizing the Field of Application

Analysis of published works on pK_a revealed that 50% of them was carried out as a part of drug discovery and development research in which UV method was used to determine the dissociation constant of new lead molecules, 18% of studies were aimed at determination of pKa by UV technique for existing drugs or natural products or dyes in the field of pharmaceutical analysis. In comparison, 29% of reports dealt with the ionization behaviour of organic compounds at different pH using the UV method, which we have classified under the field of applied analytical chemistry, and 3% of data was from the experiments related to food analysis. Table 1 shows information gained from the literature about the nature of compounds analyzed, ionizing groups present, and the field of application of pK_a. The results delineate the structural attributes of organic compounds essential for UV-spectrophotometric determination of pK_a.

Structural Attributes of Organic Compounds

Most organic compounds possess more than one ionizable functional group and may contain acid and base moieties. An organic compound may exist in different ionization forms based on the strength of the acid/base functional group and pH to which it is exposed. The extent of acid-base behaviour of the chemical is denoted by K_a, the ionization constant, synonymous with an acid dissociation constant or protonation constant. The exponential values of K_a are challenging to handle; hence it is usual to convert to its negative log and written as:

\[ K_a = - \log pK_a \] (1)

If protonation status is considered, then K_a is as follows:

\[ K_a = 1/K_b \] (2)

pK_a = log K_a

These predictions depend on the nature and quality of data available and the number of similar compounds that software uses to identify possible ionization centers in the chemical structure of compounds under investigation. UV spectrophotometry remains a viable option for pK_a measurement.
| Name/Number of compounds studied (Reference) | Ionizing groups present | Field of application |
|-------------------------------------------|------------------------|----------------------|
| Xanthene dyes, 11 derivatives\(^{21}\)   | Di/triprotic acids: 2-phenolic OH and 1-COOH | Pharmaceutical analysis |
| Aromatic hydrazones\(^{30}\)               | Monoprotic base: -C=N | Drug discovery, design & development |
| 1,2,4-triazole-5-thiones, 4 derivatives\(^{11}\) | Monoprotic bases: -C=S | Drug discovery, design & development |
| 6-acylbenzothiazolones as drug precursors, 12 derivatives\(^{42}\) | Monoprotic bases: -C=O | Drug discovery, design & development |
| Oligopeptides, 2 derivatives\(^{46}\)     | Multiple ionizing groups: -NH\(_2\) and -COOH | Pharmaceutical analysis |
| 2,4-dinitrophenol\(^{47}\)               | Monoprotic acid: -O.H. | Applied analytical chemistry |
| Bis-(pyridinium) quaternary salts, 11 derivatives\(^{52}\) | Monoprotic acids: -CH\(_2\)-C=O | Drug discovery, design & development |
| Thiourea organocatalysts, 6 catalysts\(^{53}\) | Diprotic acids: -(NH)\(_2\)-C=S | Applied analytical chemistry |
| Anti-trypanosomal imino imidazolines, 11 derivatives\(^{24}\) | Diprotic bases: Ar-N=C of Imidazoline -NH- of imidazoline | Drug discovery, design & development |
| Felodipine\(^{25}\)                      | Monoprotic base: -NH- | Pharmaceutical analysis |
| Naringenin\(^{46}\)                      | Triprotic acid: -O.H. | Applied analytical chemistry |
| Carboxylic acids, 3 compounds\(^{35}\)   | Monoprotic acids: -COOH | Applied analytical chemistry |
| Cardiolipin (Phospholipid)\(^{55}\)      | Diprotic acid: (O.H.)\(_2\)-P=O | Drug discovery, design & development |
| Universal pH indicator\(^{56}\)           | Composition unknown; microspecies/ionizing groups not reported | Applied analytical chemistry |
| Humic acid fractions, 4 fractions\(^{26}\) | Polyprotic acids: poly phenolic -OH and -COOH | Drug discovery, design & development |
| Cyclen Bisquinoline\(^{54}\)             | Polyprotic bases: -N= | Pharmaceutical analysis |
| Anti-cancer drugs, 4 drugs\(^{59}\)      | Monoprotic acid/diprotic/triprotic acids: phenolic -OH | Drug discovery, design & development |
| Sulfonphthaleine dyes, 5 compounds\(^{27}\) | Monoprotic acid: penolic -OH | Applied analytical chemistry |
| Coumarin-dihydropyrimidinone dyad\(^{28}\) | Monoprotic bases: alkyl/aryl/aralkyl amines | Drug discovery, design & development |
| Range of acids and bases, 20 compounds\(^{9}\) | Monoprotic acids: -COOH | Applied analytical chemistry |
| Amphoteric: -NH; -COOH, phenolic -OH. | Diprotic acid: phenolic (-OH)\(_2\) | Drug discovery, design & development |
| Acetylcholinesterase reactivators containing oximes, 10 compounds\(^{39}\) | Diprotic acid: (-C=N-OH)\(_2\) | Drug discovery, design & development |
| Range of acids and bases, 10 compounds\(^{37}\) | Monoprotic bases: alkyl/aryl/aralkyl amines | Drug discovery, design & development |
| Amphoteric: -NH; -COOH, phenolic -OH. | Monoprotic acids: -COOH | Drug discovery, design & development |
| Aporphine alkaloids, 3 compounds\(^{35}\) | Diprotic acid: phenolic (-OH)\(_2\) | Applied analytical chemistry |
| Aryl guanidine and 2-(aryl/imo) imidazolines, 45 compounds\(^{31}\) | Triprotic bases: -N= | Drug discovery, design & development |
| Hydrazinyldiene-chroman-2,4-diones, 8 compounds\(^{30}\) | Monoprotic base: -N=N-HR | Drug discovery, design & development |
| Risperidone\(^{43}\)                      | Monoprotic bases: -N=N | Drug discovery, design & development |
| p-Rosolic acid and Bromoxylenol blue\(^{38}\) | Diprotic acids: phenolic (-OH)\(_2\) | Applied analytical chemistry |
| Allura red\(^{39}\)                      | Monoprotic acid: -SO\(_3\)H | Food analysis |
| Paracetamol\(^{40}\)                     | Monoprotic acid: phenolic -OH. | Pharmaceutical analysis |
Drugs, organic compounds, or dyes are of interest to medicinal chemists and analytical chemists. These compounds may contain acidic functional groups like carboxylic acid, phenolic -OH, enolic -OH, sulfonic acid, sulphonamide, lactam, tetrazole ring, and basic functional groups like aromatic/aliphatic primary, secondary and tertiary amines. Acidic/basic groups present in drugs are ionizable at different pH and would determine the concentration of drug absorbed and distributed through lipophilic membranes. pKa values of drugs lie in the range of 0 to 14. Lower the pKa, stronger will be the acidic property and vice-versa.

Organic compounds require chromophores in their molecular structure to exhibit UV absorption. Predominant structural characteristic for the determination of pKa by UV spectrophotometry is the presence of an ionizable functional group adjacent to the chromophore, usually a double bond or conjugated system in drugs. Successful establishment of pKa value depends on the distance between the chromophore and the ionizing group.

Figure 2 shows the chemical structures of xanthene dyes containing acidic groups near conjugated double bonds of the aromatic ring. Reported values of dissociation constants determined by UV-spectrophotometry of these xanthene dyes correlate to an ionizable phenolic hydroxyl group and a carboxylic acid group. The presence of electron-withdrawing atoms or groups like -Br or -Cl on the xanthene ring increased the acidity of the phenolic -OH group. The acidity of the carboxylic acid group was not much affected by the nature of the substituents. Structures in Fig. 3, successfully analysed for pK_a values by UV method, include bis-(pyridinium)diquaternary salts, thiourea organocatalysts, imino imidazolines, Felodipine, Naringenin, Sulphonphthaleine dyes, Coumarin dihydropyrimidinone dyad, Oximes, phenolic aporphine alkaloids, aryl guanidine, and arylimidazoline. All the abovementioned structures also possess chromophores adjacent to the ionizing group.

Acetic acid, Allura red, Aromatic hydrazones, Bromoxynol blue, Cardiolipin, Doxorubicin, Humic acid, Paracetamol, Propionic acid, Rosolic acid, Thioguanine, and Vincristine, have the usual structural features of acids/bases near the chromophore.

A protonation pattern deduced for 5-substituted derivatives of 4-phenyl-1,2,4-triazoline-3-thione is shown in Fig. 4, suggesting that protonation occurs at sulfur atom at position 3. This study indicates that pK_a of compounds possessing ionizable group two sigma bonds away from chromophore would also be sensitive to pH changes enabling its determination by UV spectrophotometry.
benzothiazol-2-one represented in Fig. 5. This study revealed that the accurate evaluation of acid dissociation constants of compounds with protonation site three sigma bonds distal to conjugated double bonds is possible using the UV method. The oxygen atom of the 3-carboxamide functional group in its keto form underwent protonation at acidic pH. UV spectrometric determination of pKₐ for risperidone in Fig. 5 also indicates that absorbance of structures with an ionizable group located three sigma bonds distal to chromophore would respond to small changes in pH.

Fig. 5. Structures with three sigma bonds distance between the ionizing group and chromophore

3-phenyl-1-propylamine, 3-phenylpropanoic acid, and buspirone were compounds whose dissociation constants were established by the UV method, suggesting that structures containing four sigma bond distance between ionizing group and chromophore would be suitable for the UV method of pKₐ determination. The dissociation constant of Cyclen bisquinoline, a lead molecule for anti-malarial drugs, has four sigma bonds between an ionizable secondary amine and the double bond chromophore has been reported. Structures of buspirone and cyclen bisquinoline as representative molecules for this class are in Figure 6.

Fig. 6. Compounds having four sigma bonds between the ionizable group and chromophore

The above study has also proved that UV spectrophotometry is relevant for determining acidity/dissociation constants of molecules possessing a five-sigma bond distance between the ionizing group and chromophore. Drugs like propranolol and diphenhydramine in Fig. 7 exemplify the abovementioned group.

Fig. 7. Drugs possessing ionizable group at a distance of five sigma bonds from the chromophore

Valsartan, an angiotensin II receptor blocker used for hypertension therapy, exhibits two pKₐ values determined by UV-spectrophotometry. Valsartan structure in Fig. 8 has an ionizable carboxylic acid group at a distance of four sigma bonds and an ionizable secondary amine in tetrazole ring two sigma bonds far from the chromophore.

Fig. 8. Drug structure with multiple ionization sites

Improvisation of UV-spectrophotometry for high throughput screening of pKₐ by hyphenation with microtiter plates enables quick determination.

CONCLUSION

Determination of the dissociation constant of organic compounds in different fields of chemical research is essential but tedious. The small number of research articles on pKₐ published in the past decade reveals the declined interest and focus on the critical physicochemical parameter of organic compounds. Extensive studies on pKₐ values of drugs and food at the initial stages of development are necessary for an enhanced success rate. UV-spectrophotometry is a reliable and sensitive method for determining pKₐ. A detailed understanding of the structural attributes of organic compounds for UV-spectrophotometric determination of dissociation constant can expedite the process. Organic compounds possessing ionizing functional groups at a distance of a maximum of five sigma bonds from the chromophore are sensitive to pH changes. Their pKₐ can be measured accurately using UV-spectrophotometry. Compounds with multiple ionization sites exhibiting multiple pKₐ values also follow the same structural rule.
This review report emphasizes the essential knowledge of structural characteristics of organic drugs, pharmaceuticals, and food products to determine the dissociation constant by the UV-spectrophotometric method. Future research must focus on describing the structural features of organic compounds concerning the UV-spectrophotometric determination of dissociation constant. The steer to analyze dissociation constants by UV-spectrophotometry of organic compounds having chromophores farther from the ionizing groups is set forth by this study.

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Conflicts of Interest

None, declared.

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