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Determination of the Dissociation Constants of Metformin from a Second Derivative UV Spectrum

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ABSTRACT

An environmentally begnin second derivative spectrometric approach was developed for the estimation of the dissociation constants pKa(s) of metformin, a common anti-diabetic drug. The ultraviolet spectra of the aqueous solution of metformin were measured at different acidities, then the second derivative of each spectrum was graphed. The overlaid second derivative graphs exhibited two isobestic points at 225.5 nm and 244 nm pointing out to the presence of two dissociation constants for metformin pKa1 and pKa2, respectively. The method was validated by evaluating the reproducibility of the acquired results by comparing the estimated values of the dissociation constants of two different strategies that show excellent matching. As well as, the whole procedure was repeated with a new set of standard solutions and buffers for further verification. The average calculated values of pKa1 and pKa2 were found to be 2.72±0.01 and 11.61±0.08, with correlation coefficients (R^2)of 0.9916 and 0.9614, respectively. The established method was fast, affordable, reproducible, and the mean pKa values obtained were accurate and can be applied for the estimation of the dissociation constants of other active pharmaceutical compounds.

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INTRODUCTION

Metformin as a hydrochloride salt (Figure 1) is a white hygroscopic crystalline powder, odorless with a bitter taste, highly soluble in water at room temperature (about 300 mg/ml) (Tilley *et al.*, 2010), also called 1,1-Dimethylbiguanide monohydrochloride (Papadakis *et al.*, 2017), dimethylated biguanide, or Glucophage is the most widely used medicine for the treatment of type II diabetes mellitus since the year of 1957. Its mechanism of action depending mainly on the reduction of glucose secreted by the liver as well as boosting the sensitivity of body cells toward insulin. The World Health Organization has listed metformin as one of the supremely effective and benign drugs in its List of Essential Medicines those necessary for public health (Boeckel *et al.*, 2014). However, recent studies revealed anti-cancer properties for metformin, which make it a good approach for the treatment of leukemia (Rosilio *et al.*, 2014; Pryor and Cabreiro, 2015).

A precise and accurate spectrometric method, which is analogous to the one described in the British Pharmacopoeia, was used routinely for the determination of metformin hydrochloride in tablets. In which, after the analyte getting extracted from its aqueous solution, it was reacted with iodine to form a charge-transfer complex (Brittain, 1998; Ewaid and Al-Ansari, 2019; Ewaid *et al.*, 2019).

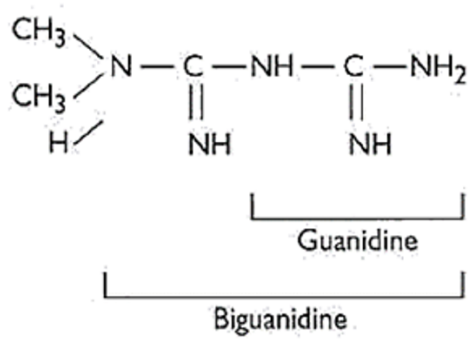

Figure 1: Chemical structure of metformin

The measurement of the dissociation constant (pKa) of a medicinal compound is significant for the design of a new pharmaceutical molecule and for the improvement of the drug delivery mechanism. Such a physiochemical parameter has an influence on the dissolution of the therapeutic active ingredients in body fluids and may affect its biological absorptivity and metabolism in vivo (Ewaid and Abed, 2017).

The orally taken pills are distributed inside the body into either neutral or ionized form depending on their dissociation constant and the acidity of the medium. The acid dissociation constants (pKa) of Metformin are 2.8 and 11.5. Thus more than 99% of Metformin usually found in vivo fluids in the form of the hydrophilic cation, which makes it a medication with very high basicity. Accordingly, the diffusion of metformin into the living cells controlled largely by its lipophilicity (Graham *et al.*, 2011). The lipophilicity of any medication is governed by its pKa value and the pH of the physiological fluid (Troy *et al.*, 2006).

The ionic medicinal molecule can react to a counter-ion at adjusted pH to produce a neutral complex, which is usually more stable with improved solubility and hence better transportability across in vivo cellular membranes. The same issue with the production of drug salt, as it can be developed by the usage of different ionization species of it to produce a diverse series of compounds with better physicochemical properties than those of the parent drug (Babić *et al.*, 2007).

A drug molecule that has more than one ionization form may exhibit different absorption spectrums over a varying pH. This characteristic can be implemented to be the base of a spectrometric method for working out pKa values by direct measurement of the absorption wavelength at diverse acidity (Allen *et al.*, 1998). The literature determines two dissociation constants (pK values) for Metformin hydrochloride at 32°C; those were 2.8 and 11.5 (Brittain, 1998).

The recent work has demonstrated that the metformin molecule can have different absorption spectra corresponding to its various ionization forms over a variable pH range. The study makes use of this correlation by calculating the values of pKa of metformin spectrometrically by the measurement of the absorption spectrum of metformin solution at different pH values. The method was affordable and accurate when the resulting data found to be compatible when compared to pharmacological literature (Graham *et al.*, 2011). The same procedure can be applied successfully to other compounds to determine their dissociation coefficients.

EXPERIMENTAL

Instrumentation

1. The performance of spectrophotometric measurements was made using a UV-Visible Spectrophotometer (T80+ PG Instruments Ltd.).
2. UV-Visible Spectrophotometer (UV-1800 Shimadzu, Japan).
3. A digital pH meter with a combined glass electrode (pH/mV Bench Meter, Hanna Instruments).
4. Micropipette Single-Channel Adjustable 100-1000μl, (Microlit).
5. Analytical Electronic balance (Sartorius, with four decimals).

Material and reagents

Preparation of 1000 μg/ml Metformin stock solutions: a mass of 100 mg of the pure compound weighed accurately then dissolved into a volumetric flask of 100 mL and made up the volume to the mark using an appropriate buffer.

Preparation of working solutions: A set of Metformin standard working solutions were prepared in the concentration of (0.0035 μg/ml) by the dilution of its stock solution that has settled earlier with buffer mediums of different acidities (1.8, 2.1, 3.0, 4.0, 5.0, 6.8, 8.0, 9.1, 9.9, 10.0, 11.9, 12.6). Those solutions were kept refrigerated and safe from direct exposure to sunlight.

Preparation of buffer solutions: Buffers were prepared according to the accredited standard procedure. However, pH adjustment was required, especially for extreme pHs and thus has accomplished by the micro-addition of 0.5 M HCl/ NaOH whenever required, as listed in (Table 1).

Table 1: Chemical composition of some buffer solutions covering the pH range of (2.6 – 10.6)

Buffer pH	The volumes of the buffer components (ml)	
	X ml	Y ml
	(0.1M) Citric acid	(0.2M) Na2HPO4
2.6	89.10	10.90
3.0	79.45	20.55
4.0	61.45	38.55
5.0	48.50	51.50
6.0	36.85	63.15
7.0	17.65	82.35
	(0.2M) Na2HPO4	(0.2M) NaH2PO4
6.0	6.15	43.85
7.0	30.5	19.5
8.0	47.35	2.65
	(0.1M) Na2CO3	(0.1M) Na2HCO3
9.1	20	80
9.9	60	40
10.6	90	10

Table 2: Names, formulas, dissolved masses, and DDW dissolving volumes for the preparation of the buffer solutions.

Compound name	Chemical formula	Conc. (mol/L)	Mass (g)	DDW (ml) added
Citric acid monohydrate	C6H8O7● H2O	0.1	2.101	100
Disodium phosphate dihydrate	Na2HPO4● 2H2O	0.2	3.561	100
Sodium dihydrogen phosphate monohydrate	NaH2PO4● H2O	0.2	2.760	100
Sodium Carbonate Decahydrate	Na2CO3● 10H2O	0.1	2.862	100
Sodium bicarbonate	NaHCO3	0.1	0.840	100

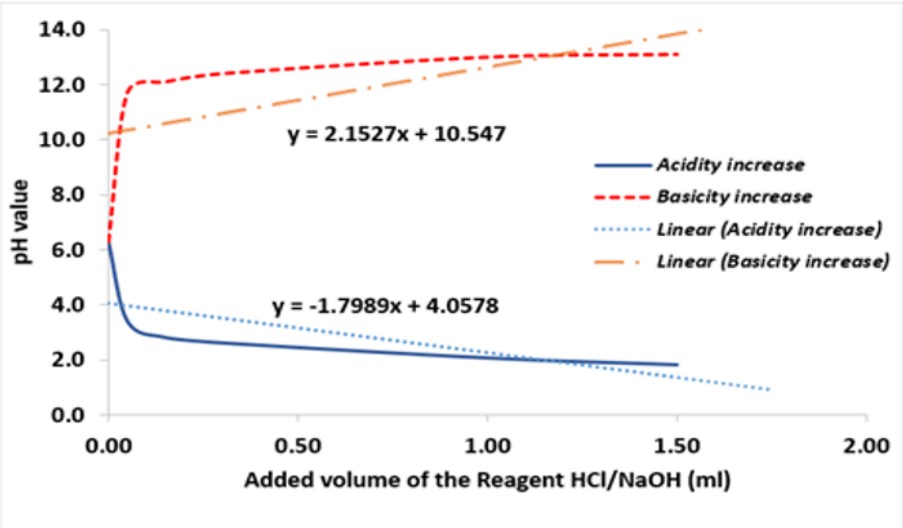

Figure 2: Plotting the micro addition volume of the reagents HCl/NaOH (0.05mol/L) each as a function of metformin hydrochloride solution (0.0035 μg/ml) showing the charge neutralize point at pH= 6.3.

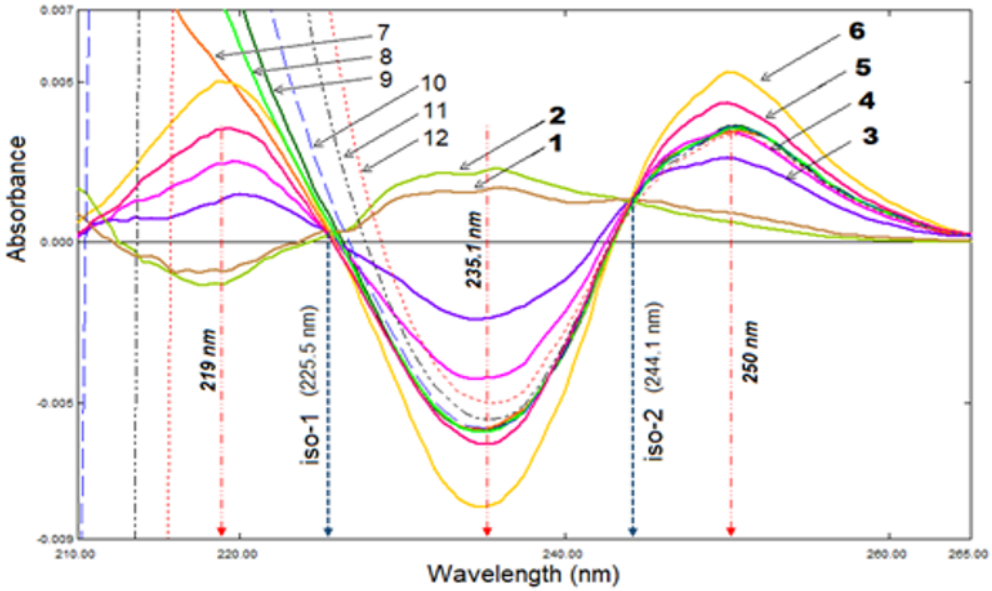

Figure 3: An overlaid of the second derivative absorbance spectra of metformin in the concentration of 0.0035 ppm dissolved in aqueous media of various pH values showing two isobestic points (iso-1 and iso-2). Spectra (1 to 12) corresponds to pH medium (1.8, 2.1, 3.0, 4.0, 5.0, 6.0, 6.8, 8.0, 9.1,9.9, 10.0, 11.9, 12.6) respectively.

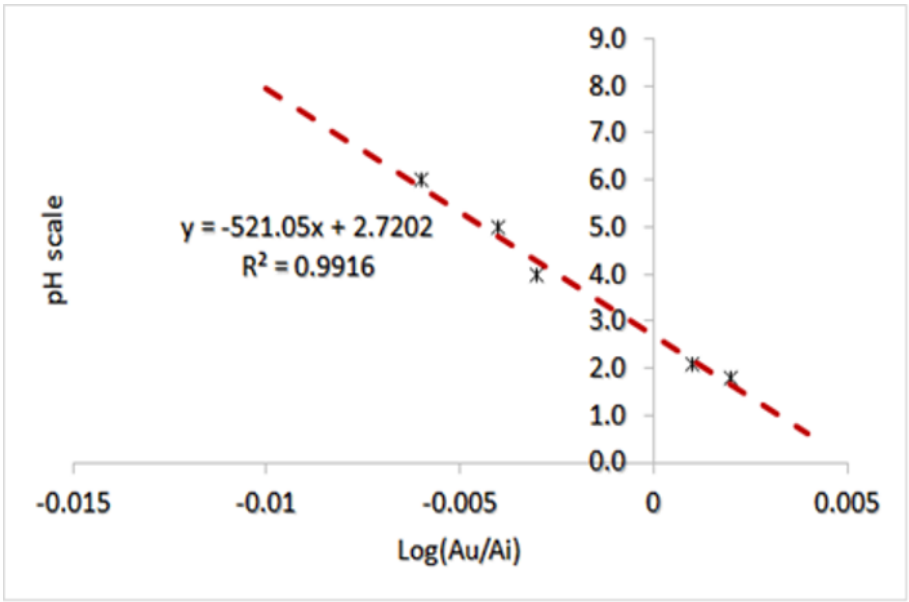

Figure 4: A UV plot at 235.1 nm representing the fraction of the second derivative of the absorbance spectrum of metformin at different acidic pH mediums (1.8, 2.1, 3.0, 4.0, 5.0, 6.0) as a function of the pH of metformin solutions

Preparation of the solution of Citric acid 0.1 mole/L: it has been prepared by transferring a 2.101 g of citric acid monohydrate into a 100 ml volumetric flask then filled to the mark with deionized distilled water (DDW).

The same way, all the following reagents those used for the preparation of the buffer solutions were of analytical grade and their standard solutions have been prepared as listed in (Table 2).

RESULTS AND DISCUSSION

The pKa of a medicinal pharmaceutical active material is of great importance since it affects its solubility in vivo and henceforth it's curing effectiveness. Poor solubility of a drug in physiological fluids results in a poor activity of it since it will not be capable of reaching the tissues or penetrating the cells walls. Thus, the capability of determining this important parameter (pKa) in a simple, fast, afford-

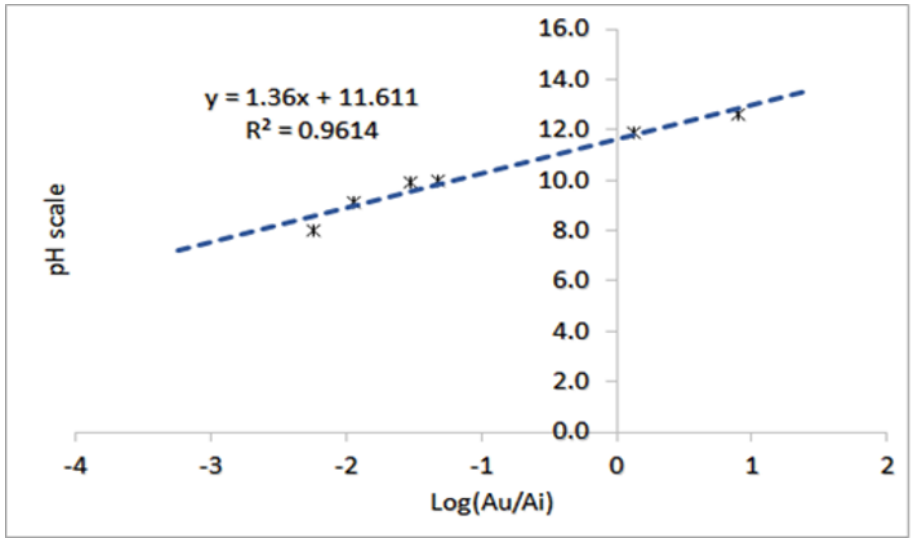

Figure 5: A UV plot at 250 nm representing the fraction of the second derivative of the absorbance spectrum of metformin at differentacidic pH mediums (8.0, 9.1, 9.9, 10.0, 11.9, 12.6) as a function of the pH ofmetformin solutions

able, and accurate approach is vital in the industry and development of pharmaceuticals.

UV/Vis spectrophotometry has been used to determine the value of pKa(s) of Metformin. The study suggests a methodology depends on the ratio of ionized to the neutral status of the analyte, which can be achieved by changing the pH of the medium. Upon that, the optimum wavelength will be the one that has the largest difference between the absorbance of the two analyte species.

The determination of metformin dissociation constants has been fulfilled using two spectrometric strategies; ST1 and ST2. The first strategy ST1, depends on a linear regression of the data acquired from graphing the second derivatives of the pH values against the log function of (A_u / A_i) , where A_u is the absorbance of the unionized analyte, while A_i is the absorbance of the ionized analyte at the λ_{max} . The Equation (1) concludes that the pKa value will approximately be equal to the value of the pH when the fraction of $(\log\ A_u/Ai)$ approaches zero.

$$pH = pK_a + \log\ A_u/A_i \qquad (1)$$

However, the second spectrometric strategy ST2 involves the application of Equation (2), which implicates $A, A_u\ and\ A_i$ species those representing intermediate, unionized, and ionized format of metformin molecule.

$$pK_a = pH + \log \frac{A_u - A}{A - A_i} \qquad (2)$$

Standard aqueous solutions of 0.0035 μg/mL metformin hydrochloride were measured at different buffer acidities (pH= 1.8, 2.1, 3.0, 4.0, 5.0, 6.0, 6.8, 8.0, 9.1, 9.9, 10.0, 11.9, 12.6) over the UV range of 190 – 350 nm. The pre-selected pH range was within the conventionally commonly used safe scaling. However, pH values out of the buffer range or at the extreme edges were reached and accomplished by the addition of a standard solution of HCl or NaOH (0.5 mol/L).

For specifying the pH at which the metformin molecule becomes un-ionized, the acidity adjustment reagents of HCl and NaOH were added to a (0.0035 μg/ml) metformin hydrochloride solution at the maximum absorption wavelength of the solution in DDW at 232.5 nm. The micro addition volumes of HCl and NaOH were plotted separately against the pH of the metformin hydrochloride solution. The resultant graph exhibited a pH value of 6.3, representing the acidity at which the metformin molecule become un-ionized (Figure 2).

Solution acidity is commonly altered due to the fluctuation of the temperature also and then need to be readjusted. Likewise, solutions with ultra-extreme pH values, i.e., come closer to the pH values of 0 or 14, usually avoided because of the errors mainly coming from the unpredictable changes in the activity coefficients of the solution and the potential impedance in the response of sensitive membrane of the glass electrode of the pH meter. Yet, mathematical models like the “Smaller ion Shell model of Debye-Hückel” (DH-SiS) (Fraenkel, 2018) and the model of Bromley (Cui *et al.*, 2019) can be implemented to estimate the pH value of concentrated solutions and may be verified by the comparison with experimental results.

Furthermore, in concentrated solutions, the ionic

interactions may become substantial and hence lead to considerable effects on the activity and the equilibrium coefficients. Such effects are challenging and very hard to estimate. Thus, the errors that may arise from using high concentrations of the pH controlling solutions of HCl and NaOH has been avoided to minimize the ionic interactions as possible.

Estimating the Dissociation Coefficients of metformin (pKa)

Measurement of UV absorption spectra of standard metformin solutions at various pH values (1.8-12.6) have exhibited a maximum absorption single peak at the wavelength of 232.5±0.5 nm and average molar absorptivity (ε) of $1.37 \times 10^4 L/mol$. The resultant spectra were interpolated mathematically and their second derivatives were overlaid drawn as in (Figure 3).

Two isobestic points were observed within the pH range of (1.8 – 12.6), which point out to the existence of two dissociation constants for metformin. The first isobestic point (iso-1) was at 225.5 nm, while the second (iso-2) was at 244 nm.

Nevertheless, all the derivatized curves were intersected into (iso-2) isobestic point, while the second derivatives for the pH mediums of 10, 11, and 12 were not passing through (iso-1). This important observation points out that the second pKa, which related to iso.2, is the dominant dissociation constant compared to the one related to iso.1 of the metformin.

The acidic pH values of standard metformin solutions were plotted against the logarithm function of the fraction of $(\log\ A_u/Ai)$ at the wavelength of 235.1 nm to determine metformin pKa1. The application of ST1 has come up with a chart of excellent linearity of $R^2 = 0.9916$ representing the $[pH = -521.05(\pm 0.04) \times \log\ A_u/A_i + 2.7202(\pm 0.05)]$ for n = 4, as a function of the mathematical fraction of $(\log\ A_u/A_i)$ as displayed in (Figure 4). The linear regression intersects with the (Y-axis) at $2.7202\ (\pm\ 0.05)$ when $(\log\ A_u/A_i) = 0$ which is the value of pKa1 of metformin. While pKa1= $2.7211(\pm\ 0.04)$ when the ST2 applied, which is very close to the value of pKa1 come out from ST1.

In the same way, basic pH values of metformin standard solutions were plotted against the logarithm function of the fraction of $(\log\ A_u/A_i)$ at the wavelength 250 nm to determine metformin pKa2. The application of ST1 has come up with a chart of excellent linearity of $R^2 = 0.9614$ representing the [$pH = 1.36(\pm 0.03) \times \log\ A_u/A_i + 11.611(\pm 0.11)$], for n = 4, as a function of the mathematical fraction of $(\log\ A_u/A_i)$as displayed in (Figure 5). The linear regression intersects with the (Y-axis) at $11.611(\pm 0.11)$ when $(\log\ A_u/A_i)$ which is the value of pKa2 of metformin. While pKa2 was equal to $11.621(\pm 0.05)$ when the ST2 applied, which is also close enough to the value of pKa2 come out from ST1.

The method was validated by assessing the reproducibility of the results using different approaches. A comparison for the estimated dissociation constants of (pKa1, pKa2) values of metformin, those resulting from the application of ST1 and ST2, came with an excellent match. The calculated values were $pKa1 = 2.7202(\pm\ 0.05), 2.7211(\pm\ 0.04)$ and $pKa2 = 11.611(\pm\ 0.11), 11.621(\pm\ 0.05)$ by applying ST1 and ST2 respectively. Furthermore, the whole procedure was repeated with a new set of freshly prepared analyte standard solutions and buffers. All the outcome data were verified and have shown excellent agreement.

CONCLUSIONS

The proposed method was validated as a fast, accurate, reproducible, and affordable spectrometric approach using the second derivative for the determination of the values of the dissociation constants of the metformin medicinal compound. Two different scientific procedures were applied and came out with an excellent agreement of the results. The approach can be applied for the estimation of the dissociation constants of other pharmaceutical molecules. However, there were other analytical approaches for the estimation of the dissociation constants of pharmaceutical analytes, but this analytical method has the privilege of simplicity along with the accuracy and affordability.

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