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Spectrophotometric Determination of pK_a and Log P of Risperidone

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ABSTRACT

Objective: Determination of pKa and Log P of Risperidone by spectrophotometric technique.
Materials and Methods: From the perspective of dosage form design and pharmaceutical analysis, the dissociation constant and partition coefficient are the most important physicochemical properties of a drug which need to be determined. The pK_a determination of Risperidone was carried out using a simple UV–Visible spectrophotometric method and Log P was estimated using shake flask method followed by UV analysis.
Results and Conclusion: Experimental value of pK_a and log P for Risperidone, an anti-psychotic drug, has been reported for the first time. The pK_a value of Risperidone was found to be 8.62 and log P of Risperidone was estimated to be 3.50.

INTRODUCTION

Risperidone is an antipsychotic drug which belongs to the benzisoxazole derivatives class. IUPAC name of Risperidone is 4-(2-(4-(6-Flurobenzo[d]isoxazd-3yl]1-piperidyl] ethyl]-3-methyl-2,6 diazabicyclodeca-1,3-dien-5-one with molar formula $C_{23}H_{27}FN_4O_2$and molar mass of 410.485 g/mole. Its antipsychotic action is through selective antagonism of dopamine D2 and serotonin 5HT2 receptors (Hardman et al., 2001). As per biopharmaceutics classification system (BCS), Risperidone is classified as Class II drug whose metabolism takes place by alicyclic hydroxylation and oxidative N-dealkylation (Tripathi, 2003).

The oral bioavailability of Risperidone is reported to be 70% and the volume of distribution is 1-2 L/kg which suggest that it is readily distributed in body. In plasma, it is bound to α1-acid glycoprotein and albumin.

Ionization state of a drug molecule with respect to the pH of a solution is determined by its dissociation constant, pK_a and hydrophilicity is measured by partition coefficient, log P, hence pK_a and log P have fundamental roles in defining the pharmacokinetic profile of the drug. A drug molecule is more soluble in water but less membrane permeable in its ionized state than its counter form. From the viewpoint of pharmacokinetics, pK_a and log P becomes even more significant in case of poorly water soluble drug. Since ionization constant and partition coefficient also play critical roles in electrophoresis characteristics and chromatographic retention of a compound, pK_a and log P values are pivotal in the analytical method development for the separation and estimation of active pharmaceutical ingredients (APIs) by the liquid chromatography and capillary electrophoresis. Also, Log P is widely used by medicinal chemists in early drug development stages to check the efficiency of drug to reach its target, efficiency of binding at target and likely time for which it will remain in body. Hence, pK_a and Log P are the cardinal factors which have to be considered in the pharmaceutical analysis and dosage form design of the drug. While predicted pKa and Log P values of Risperidone are reported, exhaustive search of literature did not reveal strong evidence regarding experimental pKa and Log P values of Risperidone.

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A variety of methods such as reverse phase high performance liquid chromatography, potentiometry, conductimetry, UV–Visible spectrophotometry, capillary electrophoresis can be employed for carrying out the determination of the dissociation constant and log P of a compound. But when it comes to accuracy and producing reliable values, UV–Visible spectrophotometric method has a leading edge over other methods, although it requires a condition that compound should exhibit pH dependent UV spectrum. We have chosen this method for Risperidone as it meets the above criteria. Log P estimation is carried out by traditional shake flask method, followed by UV method analysis.

We carried out two experiments, one for pKa determination and other for log P estimation followed by their respective validation as per given guidelines. The derived pKa value from the experiment is validated for its ruggedness, considering primitiveness and applicability of pKa. We have followed a standard protocol for validation (Singh et al., 1999) for the pK_a determination of Nimesulide.

The entire experiment was repeated on two different spectrophotometers by two different individuals along with the inter-day validation with two different drug concentrations. Log P experiment was repeated thrice to get the accurate and precise results.

MATERIALS AND METHODS

Materials

Risperidone was obtained as gift sample. Hydrochloric acid and sodium hydroxide was procured from Fisher scientific (Mumbai, India). Potassium dihydrogen orthophosphate, methanol and ortho-phosphoric acid (85% pure) was received from Merck (Mumbai, India). Sodium chloride was received from SD finechem limited (Mumbai, India). Octanol was obtained from Sigma Aldrich. Milli-pore water was used throughout the study.

Instrumentation

The pH of the buffer solutions were determined using digital pH meter (Eutech pH meter). pH meter was calibrated using standard buffer solutions of pH 4.0, 7.0, 10.0 at room temperature. The spectra and absorbance readings were registered on two spectrophotometers, UV-Jasco and UV-1800 Shimadzu.

Method for pKa estimation

The fact that Risperidone has only one pKa, was established by analysing the absorbance diagram which is a basic graph between absorbance of the drug at one wavelength versus that of another wavelength at various pH. One value of pKa means a single equilibrium governing the system and hence a linear curve is observed without any change in the course of absorbance diagram (Blanco et al., 2005). We have plotted the absorbance of Risperidone at 238 nm against absorbance at 240 nm in various buffers of pH ranging from 2.0 to 10.5. Absorbance values are shown in Table 1 and the related graph is shown in Fig.1.

Table 1: Absorbance value of Risperidone at 238 and 240 nm in respective buffer solutions of pH 2 to 10.7.

pH	Absorbance at 238 nm	Absorbance at 240 nm
2	0.432	0.442
3.5	0.407	0.423
5	0.421	0.436
6.5	0.346	0.375
7.5	0.33	0.354
8.6	0.397	0.424
9.7	0.288	0.311
10.5	0.323	0.346
HCl	0.485	0.475
NaOH	0.390	0.384

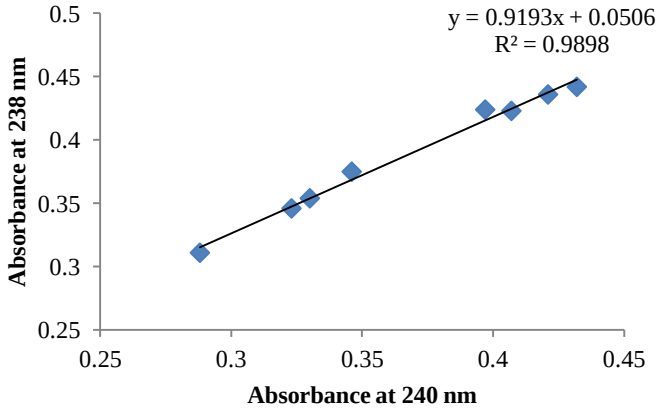

Fig. 1: Absorbance diagram of risperidone in buffer solutions of pH 2.0 to 10.5.

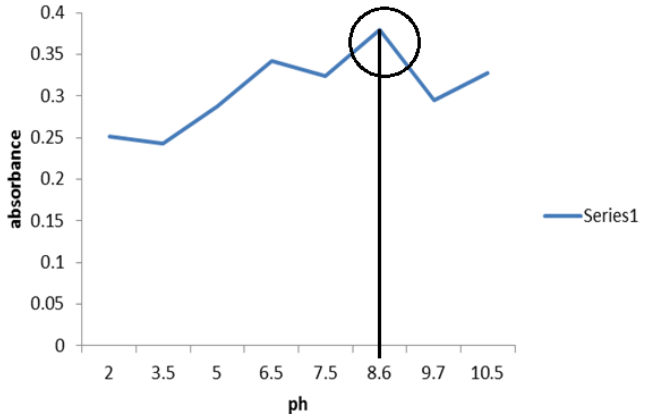

Fig. 2: Graph between pH and absorbance of Risperidone in buffer solutions of pH 2 to 10.5.

The methodology for determination and calculation of pKa was derived from the developed method (Albert and Serjeant, 1962). The application of this method is limited to the fact that we need to have a rough estimate of the pKa of our compound. Since there was no evidence available for confirming the experimental pKa of Risperidone, we have used the inflection method (Cox and Nelson, 2008) as a preliminary study for approximate

determination of pKa of Risperidone. In inflection method, we have basically plotted the absorbance of drug solution against their corresponding pH and noted the point of inflection as approximate pKa value of Risperidone. As shown in Fig. 2, a plot between Absorbance and pH for different buffers was drawn and it was clear from the graph that the biggest deflection was around pH 8.6.

After determining the approximate pKa value, we followed the protocol developed by Albert and Serjeant to deduce the exact value for pKa of Risperidone. A series of five buffer solutions were prepared around the pH 8.6. pH of these five buffers were kept at 8.2, 8.3, 8.4, 8.5 and 8.6. Appropriate amounts of 0.1 M ortho phosphoric acid (H_3PO_4) and 10 mM potassium dihydrogen orthophosphate (KH_2PO_4) were used to get desired pH for the buffer solution along with a relevant amount of sodium chloride (NaCl) for maintenance of ionic strength of all solutions at 0.02. Since Risperidone is poorly soluble in water, a primary stock solution of strength 100 µg/ml Risperidone was prepared in 0.1 N HCl.

From this stock solution, five 10 µg/ml working solutions were prepared using each of the five buffer solutions. These working solutions were then analysed under UV Spectrophotometer to record their respective UV spectra and absorbance at 275 nm. As depicted by Fig. 3 and 4, optical density of an ion and a molecule is considerably different. Along with the mentioned five buffers, we have also analysed absorbance of neutral and ionic molecule using 0.01 M Sodium Hydroxide (NaOH) and0.01 M hydrochloric acid (HCl) respectively. Table 2 shows the absorbance values for corresponding pH at 275 nm and pKa calculations.

Table 2 : Determination of pKa using absorbance values at respective pH.

pH	Absorbance (275 nm)	pK_a
8.2	0.285	8.29177
8.3	0.281	8.485637
8.4	0.27	8.877121
8.5	0.286	8.568716
8.6	0.277	8.883997

Result pk_a= 8.6214± 0.2570

d_m = 0.327 (in 0.01N NaOH); d_i = 0.251 (in 0.01 N HCl)

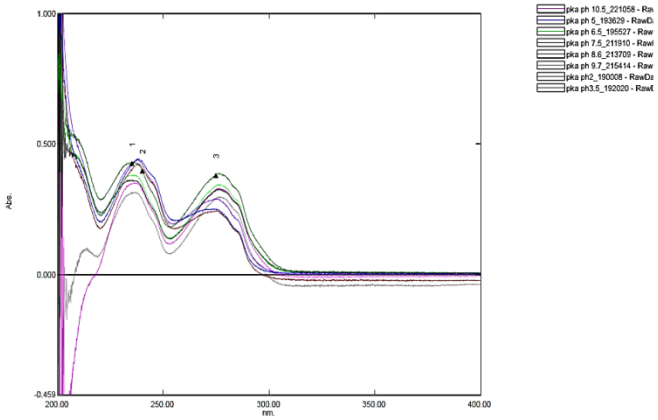

Fig. 3: UV spectrum of Risperidone in different pH from 2 to 10.5.

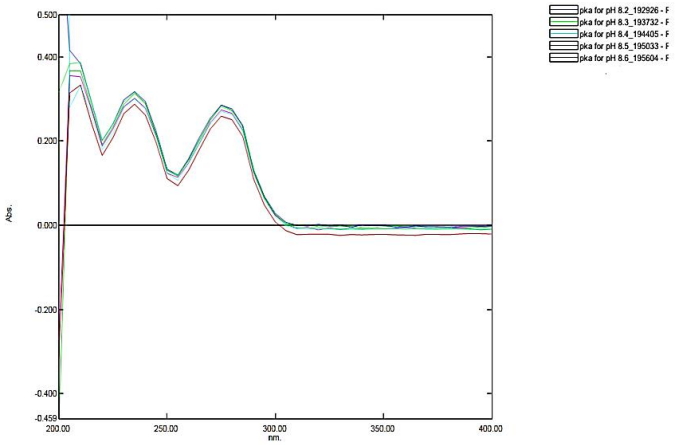

Fig 4 : UV spectrum of Risperidone in different pH from 8.2 to 8.6.

Method for Log P estimation

For estimating the Log P of Risperidone, we have used the traditional shake-flask method at 25.0 ± 0.1 ºC. We have taken 1-octanol as organic phase and phosphate buffer (pH = 6.8) as the aqueous phase. Equal amounts of both phases were mixed and kept for saturation for 24 hours. After saturation, a known amount of drug was loaded into the aqueous phase such that the concentration of our final dilutions lies in the range of developed analytical UV method. The drug loaded aqueous phase was again mixed with organic phase (volume was again the same) in a conical flask and flask was kept for shaking in thermostatic shaker at 100 rpm and 25 ºC. Since our drug is lipophilic in nature, some of the drug amount will get partitioned into organic phase and we analysed the aqueous phase for the drug amount left in UV to get log D and ultimately log P. Table 3 shows the absorbance data and calculated log D and log P.

Table 3 : Absorbance, Log D and Log P of Risperidone.

	Experiment 1	Experiment 2	Experiment 3
Absorbance	0.0356	0.0358	0.0355
Concentration in aqueous phase (µg/ml)	0.4859	0.4915	0.4831
Concentration in organic phase (µg/ml)	24.5141	24.5085	24.5169
log D	1.7029	1.6978	1.7055
log P	3.5097	3.5046	3.5123

Calculation

The following formula (Pandey et al., 2013) is used to determine the pKa of a weakly basic drug -

$$pKa = pH + log\frac{d_m - d}{d - d_i}$$

Where pH is the value recorded on pH meter, d_i is the absorbance of ionized molecule, d is the absorbance of the molecule in respective buffers tested and d_m is the absorbance of unionized molecule.

Log D of a drug is determined by the following formula (Pliska *et al*., 1996)–

$$log\ D = \log(\frac{Co}{Ci})$$

Where Co is the concentration of drug in organic phase and Ci is the concentration of drug in aqueous phase. Using the result of above calculation, we have find out log P with following relationship for weakly basic drugs –

$$\log D = \log P + \log\left(\frac{1}{1 + 10^{pKa-pH}}\right)$$

Validation of the pKa result was carried out by repeating the experiment using another working solution of 10 µg/ml concentration. To be insured from the manual errors, validation experiment was performed by a different team member on a different spectrophotometer. The validation results are shown in Table 4. Validation of log P results was done by repeating the experiment thrice.

RESULTS AND DISCUSSION

Approximate value of pK_a of Risperidone was inferred as 8.62 because inflection point in the plot of absorbance of drug solution versus its pH was found at pH value of 8.62.

Sufficient evidences were found for the existence of a single value of pKa of Risperidone as the absorbance diagram (Fig. 1) for the absorbance at 238 nm and 240 nm exhibits a typical linear nature which proves existence of a single equilibrium in ionisation state. It is also clear from Fig.2 that pH dependent UV-absorption is exhibited by Risperidone.

By using equation for determination of pK_a, we found out the value of pKa for each of the5 buffers of pH ranging from 8.2 to 8.6 and containing 10 µg/ml drug. These calculations are shown in Table 2.Then the average of those five pK_a values were taken to conclude the final pKa value of Risperidone to be 8.62. Entire experiment was repeated for the same buffer solutions containing 10 µg/ml of drug. The validation of ruggedness of the study was carried out as per the discussion in introduction part. The validation results are shown in Table 4.

Table 4: Validation of ruggedness of the pKa value.

Instrument 1			Instrument 2
Person 1 day 1 Drug strength= 10µg/ml 8.6214± 0.2570	Person 1 day 2 Drug strength = 10µg/ml 8.6213±0.2550	Person 1 day 3 Drug strength = 10µg/ml 8.6215±0.2510	Person 2 day 4 Drug strength = 10µg/ml 8.6218±0.2490

In partition coefficient study, we found out the absorbance for the aqueous phase to be 0.0356 after 24 hour shaking with organic phase which gave us the aqueous concentration as 0.4859 µg/ml from the developed calibration curve. It is clear that the remaining amount from the loaded concentration of 250 µg/ml has been permeated into organic phase. We used these concentrations to calculate the log P as 3.50 with the help of equation for log D and relationship between log D and log P.

CONCLUSION

We performed this experiment in order to gain insights about the molecular behaviour of Risperidone. In the present study, the pK_a and log P of Risperidone was calculated experimentally for the first time. pK_a and log P are the two important physicochemical parameter helpful in drug development. We conclude our study by inferring the pK_a of Risperidone to be 8.62 and log P value to be 3.50 as calculated from our experiments, all the values falls within a spread of ±0.25 which proves the preciseness of our results.

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Conflict of Interests: There are no conflicts of interest.

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