

Measuring pK_a values of thyroxine and glipizide by a fast pH-UV titration technique

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Objectives

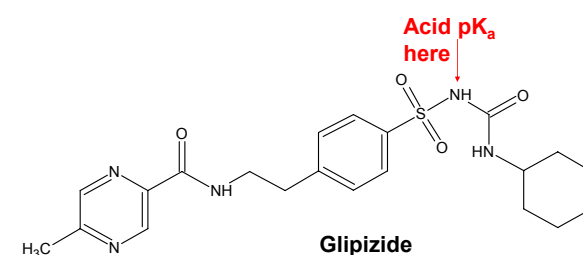
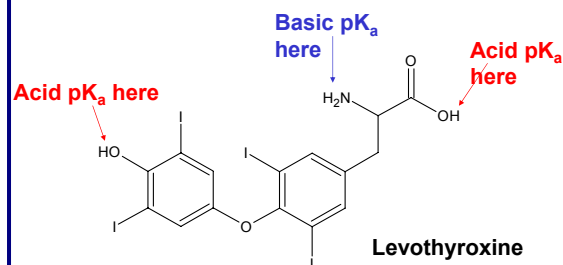
We measured the pK_a, logP (octanol-water) and intrinsic solubilities of 24 physicochemically diverse drug molecules. We anticipate that this data set can serve as a benchmark set for validation of new experimental techniques or *in silico* models. It can also be used as a diverse starting data set for the development of new computational models [1].

Because of the structural diversity, properties of some of the molecules were difficult to measure, for reasons like poor solubility, low logP or unusual kinetics. Two of the most difficult measurements turned out to be pK_a values of levothyroxine and glipizide. We sought a way to measure the pK_a values of these molecules, whose **low solubility has led to errors in reported pK_a values**.

Drug pK_a values are useful for a number of reasons: they allow chemists to specify the pH range where the molecule will be unionized, and thus more likely to be absorbed by passive diffusion; they influence pH-dependent properties like logD or lipophilicity and solubility, thus molecules are most lipophilic and least soluble in their unionized forms; they are involved in drug-receptor binding; they influence formulation and dosage forms.

Methods

Stock solutions (10mM) of levo-thyroxine and glipizide were prepared in methanol basified with KOH. For each pK_a assay, an aliquot of 50μL was pipetted into a vial containing 10mL of an aqueous linear buffer solution containing a mixture of weak acids and bases that did not absorb UV, whose pH had been basified to pH 12 with KOH. Solutions were titrated with 0.5M HCl, and multiwavelength UV spectra were collected after each pH adjustment. The solutions were monitored for light scattering at 600nm. Approximately 40 pH points with spectra were collected during titrations lasting less than three minutes. The pK_a values were calculated from the changes in UV absorbance of the samples as a function of pH. This assay is called **Fast D-PAS**.



Measuring pK_as of levothyroxine

Levothyroxine is a synthetic version of the principal thyroid hormone, thyroxine. Although it has three pK_a values, the unionized form of **levothyroxine is insoluble in water and in many cosolvents including methanol and DMSO** at pH-metric concentrations. It also precipitates in standard pH-UV assays, which take about 45 minutes to perform. The compound is only soluble at high pH. By using the **Fast D-PAS** method, it is possible to obtain the highest two pK_a values in aqueous conditions before the molecule has time to precipitate. The lowest pK_a (corresponding to the carboxylic acid) was inaccessible by all techniques because the compound precipitates below the second pK_a and also because this carboxylic acid pK_a is UV inactive. A value of 2.2 has been suggested [2].

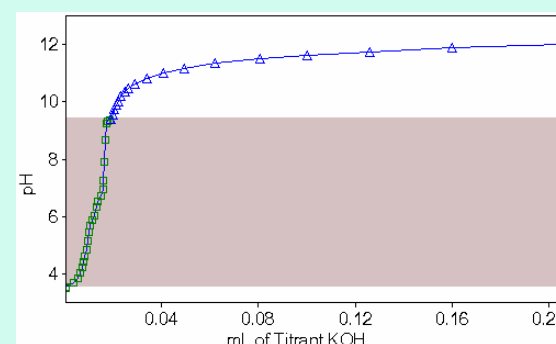
Measuring pK_a of glipizide

Glipizide is used to treat type 2 diabetes; it lowers blood sugar by stimulating the pancreas to secrete insulin and helping the body use insulin efficiently. Glipizide is water-insoluble when unionized, with very slow dissolution rate even at high pH where the compound is ionized. Given the low solubility of glipizide (1.4 μg/mL) it might be expected to precipitate even at pH-UV concentrations, but **glipizide stays in supersaturated solution** for long enough for pK_a values to be measured by the **Fast D-PAS** method. In this method, a 50 μL aliquot of alkaline methanolic stock solution of glipizide is titrated within two minutes over a pH range 12 to 2 in an aqueous solution of a linear buffer mixture. This fast method allowed for sufficient UV data in aqueous solution to be collected before the sample could precipitate. The change in UV absorbance versus pH was adequate for good results. Glipizide precipitated in the slower, standard pH-UV assays.

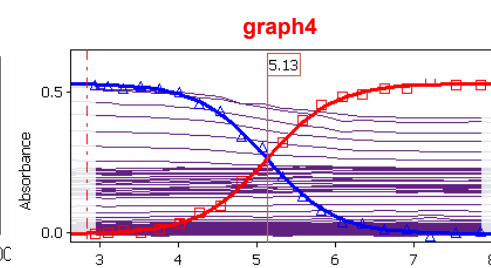
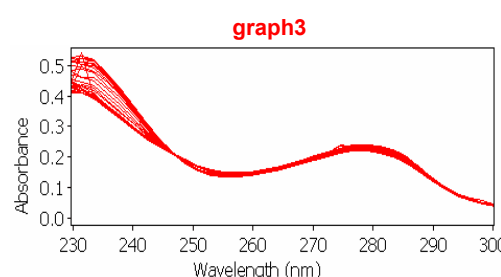
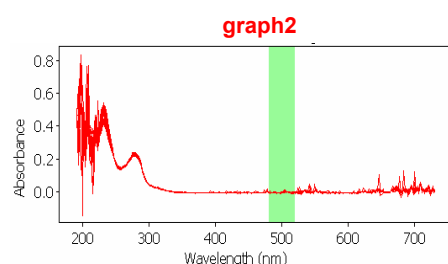
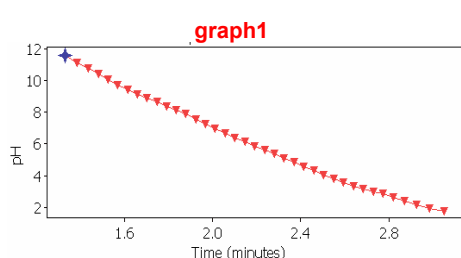
Other methods for measuring pK_a values. A molecule's pK_a represents the pH at which it is half-ionized, so pK_a can be measured by any technique that can assess the molecule's changing ionization state as a function of pH.

In the **pH-metric method**, a solution of the molecule is acid-base titrated, and its pK_a is determined as the value that will allow a computed titration curve to fit the experimental curve. High sample concentrations (at least 0.0005M) are required, and levothyroxine and glipizide are **too insoluble even in solvent-water mixtures for the standard pH-metric method**.

In the **pH-UV method**, a molecule is again acid-base titrated, and its pK_a is determined from multiwavelength spectra collected during the titration. This method requires lower concentrations than the pH-metric method, but nevertheless the sample must stay in solution throughout the titration, either in water or in solvent-water mixture. Both levothyroxine and glipizide were **too insoluble for the standard pH-UV method**.



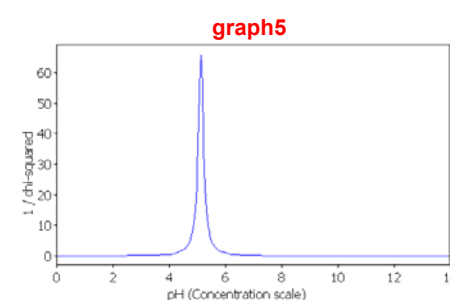
Failed attempt at pH-metric measurement of glipizide pK_a in 40% methanol. Sample concentration was 0.0007M. Precipitate was present throughout the shaded part of the titration curve.



Obtaining pK_as from experimental data

Glipizide is titrated in aqueous linear buffer solution starting from high pH where it is soluble (**graph1**). UV spectra were collected at 38 pH points in less than 2 minutes. Each red line in **graph2** represents absorbance measured at 256 wavelengths at a single pH. Absorbance data for wavelengths between 230 and 360nm is selected for further analysis (**graph3**). Absorbance changes with pH because glipizide converts from an ionized species to an unionized species with different UV absorption. The pK_a occurs in the region where the absorbance lines "cross over".

The Fast D-PAS calculation uses Principal Component Analysis and matrix algebra to separate signal from noise and to resolve the data into Absorption of each species vs. wavelength and Distribution of Species vs. pH (**graph4**). The pK_a (5.13) occurs where 50% of each species is present. The high quality of the TFA (Target Factor Analysis) fit is demonstrated by the single sharp, symmetrical peak in **graph5**.



Results

Values were determined for **levothyroxine pK_a = 6.83 and 8.84**, and for **glipizide pK_a = 5.13**. Results were reproducible, and values reported are the mean obtained from five experiments. No light scattering occurred at 600 nm, suggesting that the samples did not precipitate during the three-minute assays. Experiments using Cheqsol [3] confirmed that **thyroxine and glipizide form supersaturated solutions**, and the neutral form could thus remain in solution for a short time before precipitating. Assays were repeated using conventional pH-UV and pH-metric titrations in unbuffered solutions that lasted about 40 minutes, during which precipitation occurred both in aqueous solution and in mixtures of water with solvents. **Conventional results were not reproducible, and differed significantly from those obtained by Fast D-PAS**. Measured pK_a values for levothyroxine of 10.1 and 6.7 have been reported [4], as well as a value of 6.2 [5]. The 10.1 value is too high, and was probably deduced from titrations during which precipitation occurred. A pK_a of 5.9 for glipizide is widely cited. This value is too high, and was probably deduced from titrations during which it precipitated.

Conclusion

Fast D-PAS is an effective method for measuring aqueous pK_a values of poorly soluble samples.

References

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