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Spectrophotometric pK_a Determination of Ionizable Pharmaceuticals: Resolution of Molecules with Weak pH-Dependent Spectral Shift

Deren Dohoda[1], Konstantin Tsinman[1], Oksana Tsinman[1], Dave Kwajewski[1], Kin Y. Tam[2] [1]Pion Inc., 10 Cook Street, Billerica, MA01821, USA, [2]Faculty of Health Sciences, University Macau, Taipa, Macau, China

Pion

UNIVERSIDADE DE MACAU
UNIVERSITY OF MACAU

PURPOSE

Introduction

- The extent of ionization of a drug molecule at different pH values can be characterized by its pK_a (acid dissociation constants).
- pK_a is an important parameter to rationalize the distribution behaviors of the molecule at different in vivo environments.
- UV titration for pK_a determination has become one of the popular methods but the success of this method requires the molecule exhibiting strong pH-dependent spectral shift that related to the ionization process. Depending on the proximity between the ionizable group and the chromophore, the spectral shift may not be strong enough to warrant a successful determination.
- In this work we investigate a UV titration method for pK_a determination, with a particular emphasis on molecules with weak pH-dependent spectral shift.

Experimental

- UV spectra during a titration experiment with compound concentration of about 1μM were collected by using a newly developed automated potentiometric - optical system (PULSE, Pion Inc., see Figure 1).
- All experiments were carried out in 0.15M KCl solution at 25 ± 0.5 °C.

Data analysis

- The spectral data in the form of a data matrix was subjected to principal component analysis (PCA) to determine the number of independent light absorbing species,[1] which aids the establishment of an ionization model.
- Alternative least square (ALS) method[2] that we previously used to calculate the tautomer ratios of zwitterionic compounds was adopted for the determination of the unknown pK_a values from the spectra data.[3]

Figure 1. Pion Pulse™ automated potentiometric - optical system

RESULTS

Samples were selected based on the optical properties and the distance between the ionizable group and the chromophore to exemplify the method developed in this study. Figures 2 shows some representative results from the PCA-ALS analysis.

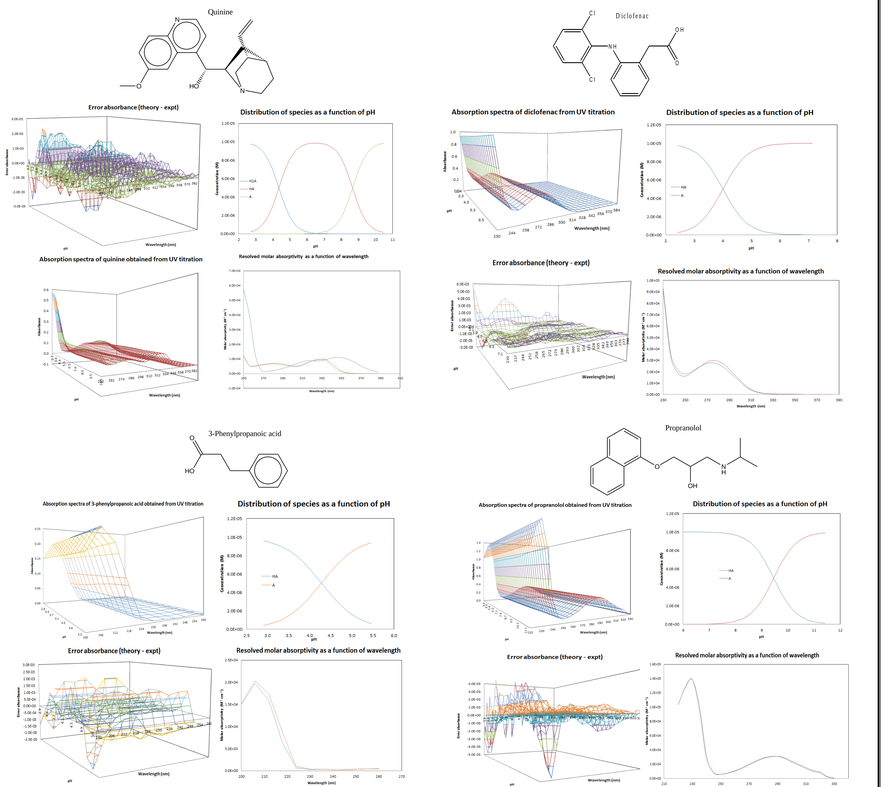

Figure 2. Representative results obtained from the PCA-ALS analysis developed in this study.

DISSCUSSION

Table 1 pK_a of the selected compounds determined using the PCA-ALS analysis.

Compound	Chromophore-ionization center distance	pK_a(s) determined by PULSE/PCA-ALS	pK_a(s) Literature	Refs.
Quinine	None, 3 σ bonds	4.37, 8.57	4.37, 8.60	4
Propranolol	5 σ bonds	9.46	9.53	5
Diclofenac	3 σ bonds	4.02	3.99	5
Aniline	1 σ bond	4.68	4.61	6
Benzylamine	2 σ bonds	9.33	9.34	7
Phenylethylamine	3 σ bonds	9.84	9.83	7
3-Phenyl-1-propylamine	4 σ bonds	10.40	10.01	7
Benzoic acid	2 σ bonds	3.98	3.98	8
Phenylacetic acid	3 σ bonds	4.09	4.29	9
3-Phenylpropanoic acid	4 σ bonds	4.30	4.37	10

- Results that show in Table 1 are in good agreement with literature pK_a values.
- The PULSE system is capable of generating spectra data of very low noise level (~5×10^{-4} a.u.), which enables an unambiguous resolution of overlapping spectra.
- We have shown that our optical system could determine the pK_a values where the distance between the chromophore and the ionization is less than 5 σ bonds.

CONCLUSIONS

We have developed a potentiometric - optical system, which is capable of collecting very clean spectral data during a UV titration experiment of an ionizable drug molecule. Data analysis procedure based on the PCA-ALS method has been implemented and has shown to be very sensitive in determining the pK_as of ionizable drug molecules even the ionizing species show very similar UV spectra. The results obtained from this novel system are found to be in good agreement with literature.

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