pH Indicator Titration: A Novel Fast pKa Determination Method

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ABSTRACT: This study describes a fast spectrophotometic titration method for apparent ionization constant (pKa) determination. In this method, a Universal pH indicator is utilized instead of the conventional pH electrode. An autoburette is set to add HCl at a constant rate to a vigorously stirred 1 cm UV cuvette which contains sample and indicator solution. A spectrophotometer continuously records the spectra. Acquired spectral data are processed by calculating the pH from the indicator spectra in the visible region and extracting sample spectra from the UV region. Five compounds possessing pKa values in the range 2–10 were investigated. These results differed from measurements by conventional spectrophotometric titration by ± 0.05 to ± 0.10 log unit. © 2007 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 96:2777–2783, 2007

Keywords: fast determination; ionization constants pKa values; protonation constants; spectrophotometic titration

INTRODUCTION

The ionization constant (pKa) is a fundamental physicochemical property of many compounds and has an important influence on the overall biological properties of pharmaceutically active molecules.

Recent advances in combinatorial technology have encouraged the development of pKa determination methods to be rapid, high throughput, and operational on the microscale. Although techniques, such as liquid chromatography,[1] CE,[2] and 96 well gradient pH methods[3] can accomplish assays within 10 min; the preparation for most of these methods can be time consuming. The microscale titrimetric method[4] for the determination of ionization constants decreases the amount of sample required but the duration of experiment is as long as that of a conventional titration. Historically, potentiometric titration is the most common technique employed for the determination of pKa values, the method typically uses pH electrodes. This requires the calibration of the pH electrode every day. Calibration procedures normally take 20 min and the E_0 and slope of the electrode remain valid for less than 1 day. Spectrophotometic titration has the advantage that it is not affected by CO_2 interference, less sample is required and it can provide

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multi-wavelength absorbance information for factor analysis.[5] However, it is only possible to apply the method to compounds with chromophores placed close to the titratable groups. Although spectrophotometic titration has gained popularity in recent years, the method also requires precise pH measurement.

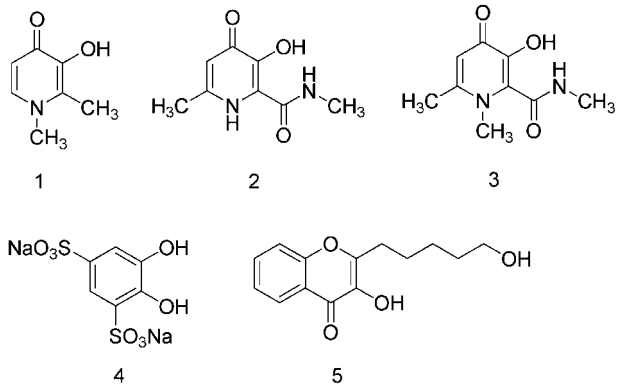
All methods involving the use of pH electrodes have the same limitation, namely relatively extensive experimental time. During titration, the average pH meter response time can be as long as 0.5 min for each recorded point even with the best pH electrodes. Therefore, a typical titration process takes approximately 1 h to cover the pH range 2–12, with the 0.1 pH unit intervals necessary to provide the high quality data suitable for analysis. As the uniformity of the solution can be achieved quickly by vigorous stirring, and typical proton dissociation processes are less than 10^{-8} s,[6] much time during pH titration experiments is wasted, waiting for the electrode to provide a stable response. Clearly, an alternative rapid method for the accurate measurement of pH is required.

Using acid–base indicators to measure pH has been widely adopted for many years.[7] Indicators are typically compounds with chromophores that can be detected in the visible range. The indicator spectra can be used to calculate the pH value of a solution from the pKa values, concentration, and molar extinction coefficients of the indicator species. In contrast to pH electrodes, indicators respond rapidly and do not require frequent calibration. In this study, we develop a spectrophotometic titration method using pH indicators to reduce the time of pH determination, and therefore to improve the efficiency of pKa determination.

MATERIALS AND METHODS

Universal pH indicator (Lot number 33460 Aldrich, Sigma-Aldrich, St. Louis, MO) was utilized in this study. Analytical grade volumetric HCl (0.2092 M) from Aldrich and HPLC grade water (Fisher Scientific, Leicestershire, UK) were used in the preparation of all solutions. Other reagents were purchased from Aldrich without further purification.

Samples **1** and **4** were purchased from Aldrich (172553) (379409) with purity >97.9% and 98%, respectively. The other samples (**2**, **3**, and **5**) were synthesized and characterized as previously reported with a purity >99%.[8,9]



INDICATOR TITRATION STRATEGY

Initially, the conventional spectrophotometric titration method was used to measure the pKa values of the Universal pH indicator solution and to calculate the effective molar extinction coefficient spectra of each species. Utilizing these parameters, it is possible to reconstruct the spectra absorbance data at any specific pH value (Eq. (2)). When a spectrum of a mixture of the sample and the indicator are analyzed together, the visible spectrum results from the indicator only while the UV spectrum results from the combination of both the sample and the indicator at the same pH value. Clearly, only samples lacking a visible absorbance spectrum can be used in this method.

In the present study, the visible region of the spectral data (normally from 450–700 nm) was used for pH determination. To realize this, the solve function of EXCEL was employed in a visual basic macro program to optimize the pH value and initial concentration of the indicator with the aim of minimizing the sum of the squares of observed and calculated spectra (Eq. (3)). Simulation spectra can be generated according to the pH value, concentration, and previously acquired effective molar extinction coefficient of each species of the indicator. This simulated spectra includes both the UV and visible region, the UV region (250–350 nm) is then subtracted from the experimental spectra of the sample plus indicator mixture, resulting in the sample-only spectra at a specified calculated pH value. These data can then be