INTRODUCTION

The ionization constant, pKₐ, helps medicinal chemists understand a drug’s bioavailability as it dissolves and permeates through lipophilic barriers of the intestinal tract into the bloodstream. Flavonoids, which are naturally occurring antioxidants with beneficial activity, are under represented in some modeling software.

The determination of pKₐ by potentiometric titration is a well-established technique.¹ Methanol or DMSO cosolvents can improve the solubility of sparingly soluble drugs during the titration. For optimum accuracy, it is most desirable to use low concentrations of cosolvent to minimize the extrapolation distance to the aqueous intercept at 0% cosolvent.

MATERIALS AND METHODS

Potentiometric Titrations

In the potentiometric method, titration data are converted to Bjerrum plots which express bound protons versus pH. The Bjerrum plot is an especially useful tool, since the pKₐ occurs at the pH corresponding to the half-integral amounts of bound protons.

The Gemini Profiler™ includes two stepper-motor driven syringes capable of delivering as little as 20 nL acid and base titrants. A third stepper-motor syringe delivers ionic strength adjusted 0.15 M KCl in water. (Figure 1).

Since a number of the flavonoids were poorly soluble in aqueous solution, the methanol cosolvent procedure was used, where the apparent pKₐ values at various ratios of cosolvent to water were extrapolated to zero-cosolvent to estimate the aqueous value. For each compound, three replicate titrations were performed at 25.0 ± 0.5°C in 0.5 M KCl medium. The titrated solutions were bathed with argon, to minimize the ingress of ambient carbon dioxide. The double-junction pH electrode (pION) was calibrated in situ (Avdeef-Bucherer 4-parameter “ABC” procedure) under precisely the same conditions as used for the pKₐ determination. (The traditional “blank” titrations are no longer necessary.) As typical procedures, the flavonoids were titrated from pH 1.8 to 12.2 with 0.5 M KOH.

RESULTS

While many of the flavonoids studied had three ionizable protons, the –OH at the C₂ position (β in Fig. 2 below) was of greatest interest. This proton is available for hydrogen bonding with the adjacent carbonyl, thus forming a more stable, six-membered structure with a relatively higher pKₐ than might be otherwise predicted.

The structures and predicted values from a few vendors is shown for the flavonoids considered is presented in Fig. 2 below.

![Fig. 2. The structures for the five flavonoids examined with their expected values are shown for three vendors of predictive software.](image)

![Fig. 1. Gemini Profiler.](image)

Therefore, the work then focused on biochanin A, hesperetin and naringenin. Three titrations with different amounts of methanol were conducted using the same sample of flavonoid. As shown in figure 3 below, the pKₐ values obtained for each compound followed a well-behaved, regular pattern throughout, with no evidence of decomposition as previously mentioned. The extrapolated pKₐ values for biochanin A were 6.17, 9.9; for hesperetin 6.21, 9.01, 11.4; for naringenin 6.26, 9.41, 12.0.

Tentative structural assignments for the measured pKₐs are based on the following stepwise approach. Given the large body of data on phenols, it’s weakly acidic pKₐ is consistently observed between 9-10, thus suggesting that the substituents in hesperetin and naringenin are measured at 9.01 (γ and δ), respectively. Assignment of the α and β hydroxyls is guided by resorcinol, a 1,3-dihydroxy phenol, in which one phenolic group exhibits a pKₐ at 9.3 and the other at 11.1. Given this reasoning, the pKₐ for the α substituent in flavonoids of Figure 3 is assigned between 9.0 and 11.4, thus suggesting that the substituent in biochanin A, hesperetin and naringenin are assigned at 9.9, 11.4 and 12.0, respectively. The predicted values for the β substituent in Figure 2 ranges between 6.5-9.7 for Vendor A, 10-10.7 for Vendor P and 9.7-11.3 for Vendor M. It would appear that internal hydrogen bonding of the hydrogen on this group into a six membered ring may be incorporated into the algorithm for the various software vendors.

CONCLUSION

As discussed, flavonoids exhibit several challenges to accurate pKₐ measurement, including low solubility and potential oxidative degradation. Cosolvents ensured that the compounds were completely in solution during the titration. While the assignments made herein are tentative, they suggest that the β hydroxyl group may undergo internal hydrogen bonding that may be incorporated into some commercially available software programs, thus providing better agreement between measured and predicted values. Future work will focus on investigating some of the underlying chemistry further.

REFERENCES