DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 15(3), 373-386 (1989)

APPLICATION OF A SECOND-ORDER DERIVATIVE SPECTROSCOPIC TECHNIQUE: DETERMINATION OF THE IONIZATION CONSTANTS OF PHENYTOIN AND PHENOBARBITAL

Leonard S. Rosenberg* and Jenny L. Jackson,
Preformulation Group, Product Development Section,
Du Pont Critical Care, Waukegan, Ill 60085

INTRODUCTION

Absorption spectroscopy can be used to determine the pK of a compound when a change in the spectral pattern can be related to a change in the protonation state of an ionizable group. The calculation of a pK becomes difficult (standard error increases) when these spectral changes are indistinct or are minimally resolved. In these cases, the ability of higher-order derivative spectroscopy to enhance the short-range fine structure of a spectra usually offers adequate spectral resolution for quantitative pK determinations.

Higher-order derivative spectroscopy consists of calculating the desired derivative of a spectrum (absorbance, fluorescence, etc.) with respect to wavelength or frequency. Most current instrumentation is capable of performing the mathematical and curve smoothing functions required for the determination of higher-order

derivatives. The choice of which higher-order derivative is most suitable for a particular experiment is based on the nature, complexity, and signal-to-noise ratio of the spectra and the accuracy and amount of information needed. Several excellent reviews on derivative spectroscopy have been published (1-4).

For the current work, second-order derivatives were found to provide an excellent enhancement of the spectra with an acceptable signal-to-noise ratio. In second-order derivative spectroscopy, spectral minima usually correlate with the zero-order absorbance maxima. The shifts in the second-order spectra were easier to work with as compared to the spectra produced by first- or third-order derivatives. Fourth-order spectra were also easy to work with, but were found to have an unacceptable signal-to-noise ratio.

EXPERIMENTAL

Absorption spectra were taken in a 1 cm quartz cell using a HP8450A double-beam diode array spectrometer. Each spectrum was the average of 30 individual spectral measurements, thus minimizing the signal-to-noise ratio in the derivative spectra. Second-order derivative spectra were processed using the HP8450A.

Spectroscopic pK titrations were performed using an in-the-cell (INC) titration method. In this method the drug solution of

choice is placed in a 1 cm quartz UV cell, and the entire pK titration is then completed in this cell. The pH of the drug solution, in the UV cell, is increased or decreased slowly using concentrated base (5N NaOH) or acid (2N HCl). The pH is monitored (ION 85 Radiometer) using a semi-micro Ross pH electrode which is immersed in the drug solution. At each desired pH a UV spectrum is taken. The volume of added titrant is negligible and does not affect the pK calculations. The pH is measured before and after each UV measurement to correct for possible pH drift. This method worked well in determining the pK of phenobarbital.

The minimal solubility of phenytoin in water ($pH \leq 6$ approximately 18 ug/ml or $7.1*10^{-5}$ M (5-8)), required a slight modification of the INC method. In these experiments, a saturated aqueous phenytoin solution ($pH<6.0$, no buffer) was diluted 1:3 with pH=6.0 deionized water. The titration was then completed with this single solution.

CALCULATIONS

The dissociation constant, K_a, for a weak acid in aqueous solution can be derived using the Henderson-Hasselbalch equation;

$$pK_a = pH - \log \frac{[B]}{[A]} \qquad (1)$$

where A and B are the acid and conjugate base forms of the drug in

question. The total concentration, C_t, of drug in solution is;

$$C_t = [A] + [B] \quad (2)$$

The total absorbance, A_t, for this solution is;

$$A_t = A_A + A_B \quad (3)$$

where A_A and A_B are the absorbances of the acid and conjugate base, respectively, at a specific wavelength. Equation 3 can be expanded to;

$$A_t = \varepsilon_A[A]\ell + \varepsilon_B[B]\ell \quad (4)$$

where ε_A,and ε_B are the molar absorptivities of the various species in solution, and ℓ is the pathlength of the UV cell. Equation 2 and 4 are combined with equation 1 and rearranged to give;

$$pK_a = pH - \log\left(\frac{\varepsilon_A C_t \ell - A_t}{A_t - \varepsilon_B C_t \ell}\right) \quad (5)$$

where $\varepsilon_A C_t \ell$ and $\varepsilon_B C_t \ell$ are the absolute absorbance of the neutral and anionic form of phenytoin, respectively. In those cases where the value of $\varepsilon_B C_t \ell$ cannot be determined experimentally, then equation 5 can be rearranged (in a non-logarithmic form) to give;

$$A_t = \frac{(\varepsilon_A C_t \ell - A_t)[H^+]}{K_a} + \varepsilon_B C_t \ell \qquad (6)$$

A plot of A_t vs. $(\varepsilon_A C_t \ell - A_t)[H^+]$ for all points in the titration curve will give a slope equal to the reciprocal of the ionization constant and an intercept equal to the absorbance of the conjugate base form.

RESULTS AND DISCUSSION

PHENOBARBITAL

Figure 1 and Figure 2 show the pH dependence of the zero- and second-order absorption spectra of phenobarbital, respectively. In the zero-order spectra, increasing pH causes a hyperchromic and bathochromic shift. Note that in the wavelength range of 200 to 285 nm, an isosbestic point is not found. However, the second-order spectra shows numerous spectral features in this wavelength range, including three isosbestic points (207, 224, and 250 nm). Interestingly, the maximum spectral separation in the second-order derivative is found between 207 nm and 224 nm, which in the zero-order spectral is a region of minimal separation.

The pK of phenobarbital was determined, using equation 5, at 238 nm in the zero-order spectra, and at 213 nm in the second-order spectra. Ionization constants were calculated at other wavelengths

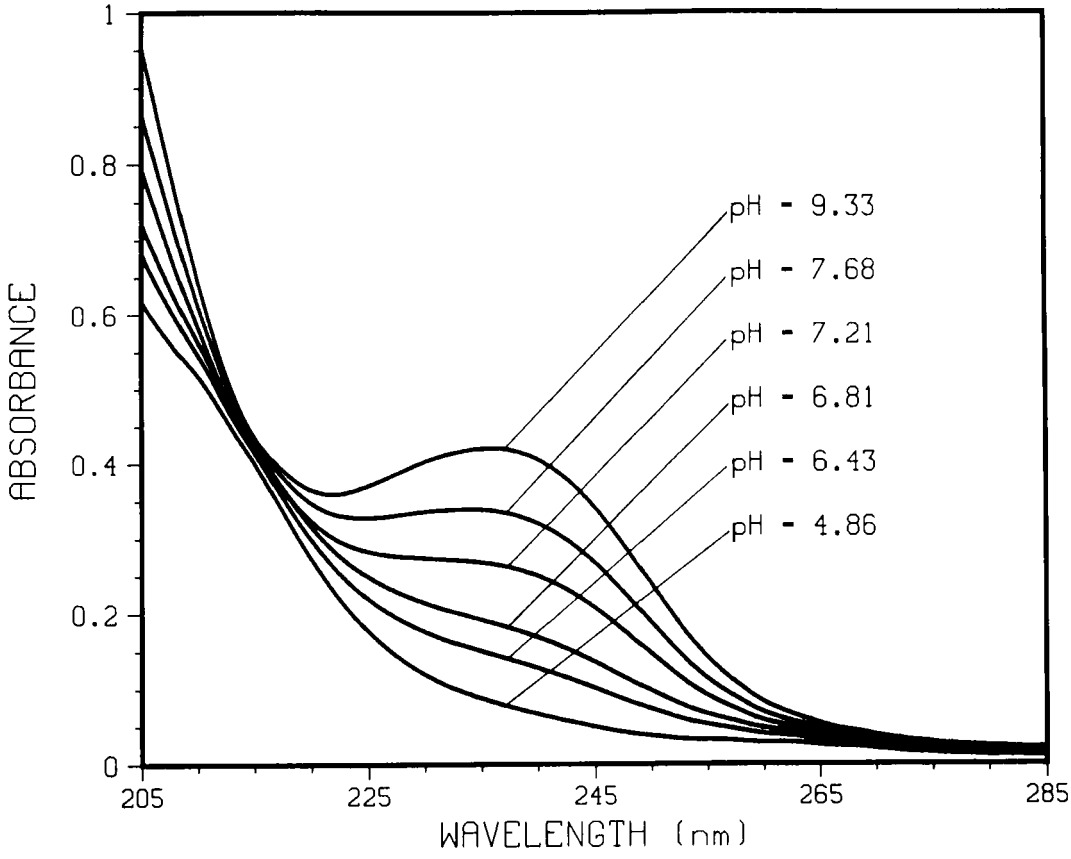

FIGURE 1
Phenobarbital: Zero-Order Derivative

with similar results. The pKs determined by the zero- and second-order derivatives (Table 1) were 7.18 and 7.20 respectively. These pKs agree well with each other and with the previously reported value of 7.40 (9). Although, the higher-order derivative did not provide an enhancement in the pK resolution, the appearance of isosbestic points and increased spectral structure may increase

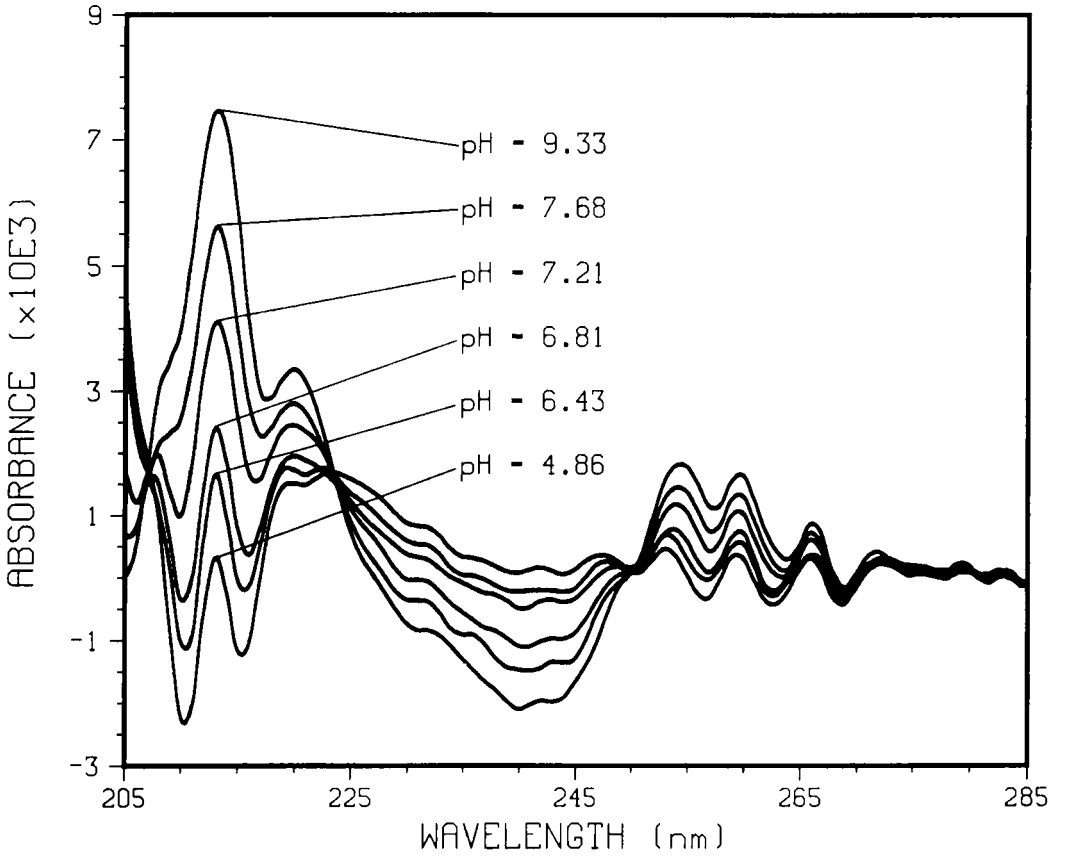

FIGURE 2
Phenobarbital: Second-Order Derivative

Table 1
pK of Phenobarbital

Derivative Order	Wavelength (nm)	n+	pK	Equation #
Zero	238	8	7.18 ± 0.04	5
Second	213	8	7.20 ± 0.04	5

+Number of points averaged to determine the pK.

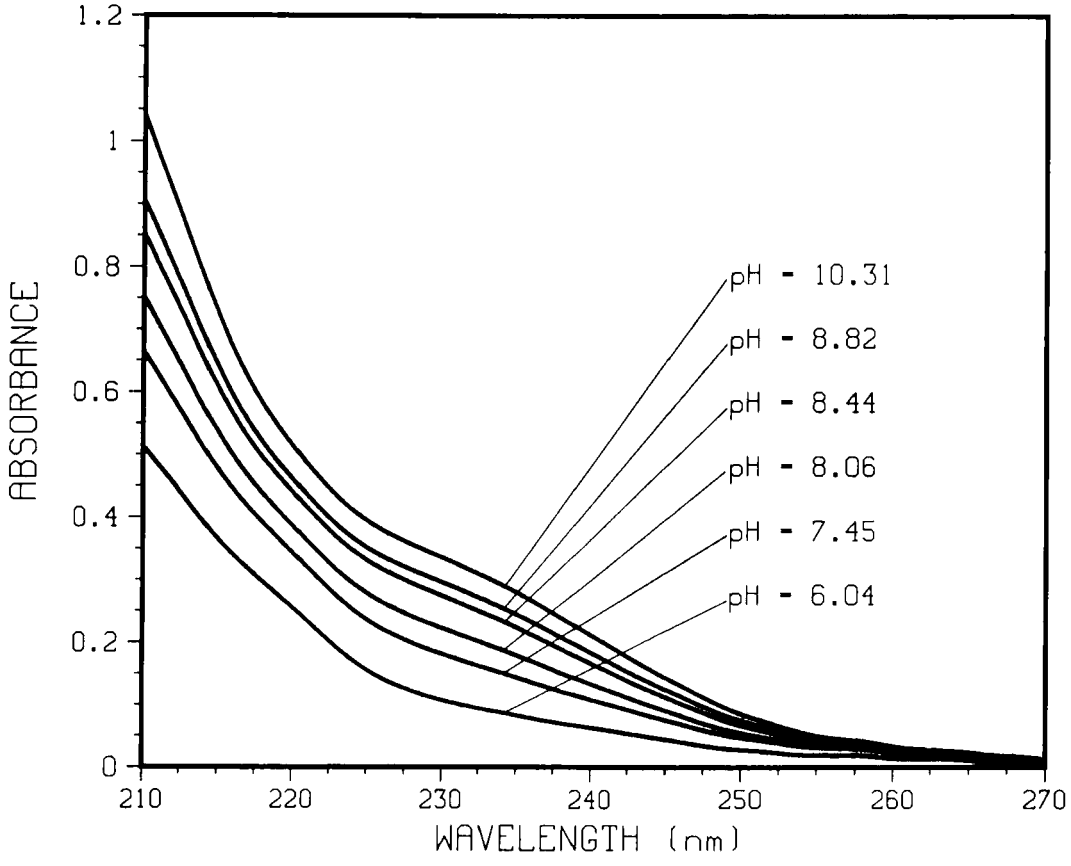

FIGURE 3
Phenytoin: Zero-Order Derivative

confidence in the information derived from the UV spectra. It also should be noted that there was no loss of accuracy in determining the pK by the use of second-order spectra.

PHENYTOIN

Figure 3 and Figure 4 show the pH dependence of the zero- and second-order absorption spectra of phenytoin, respectively. A

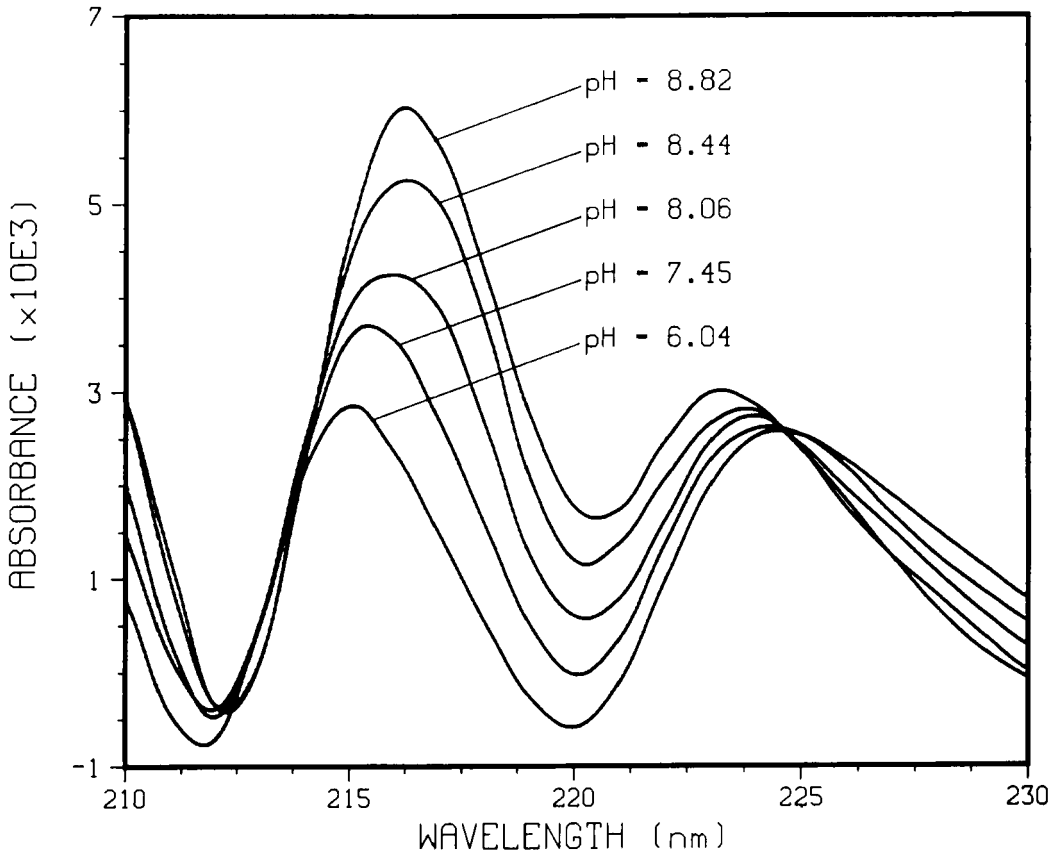

FIGURE 4
Phenytoin: Second-Order Derivative

hyperchromic and bathochromic shift is noted in the zero-order spectra as the pH is increased. The second-order derivative spectra shows a uniform bathochromic shift, with the 215 nm maxima and 223 nm minima both showing a hyperchromic shift. Also, in the wavelength range of 212 to 230 nm, a large change in the absorbance is noted, and two isosbestic points (215 and 225 nm) are found.

Table 2
pK of Phenytoin

Derivative Order	Wavelength (nm)	n+	pK	Equation #
Zero	230 & 235	30	8.14 ± 0.12	5
Second	217 & 220	30	8.24 ± 0.10	5
Zero	230 & 235	28	8.08 ± 0.14	6
Second	217 & 220	28	8.12 ± 0.05	6

*pK values from both reported wavelengths and three titrations were averaged. Individual pK values had similar values and standard deviations.
+Number of points averaged from three experiments and two wavelengths. Some points were lost using equation 6 because their absorbances were greater than the calculated absorbance maximum.

These pH-dependent absorbance changes were originally thought to relate exclusively to the dissociation of the N_3 nitrogen of phenytoin. However, the absorption spectra (both zero- and second-order) did not exhibit a maxima in the pH=10 to 14 range. The pK of phenytoin (Table 2) was therefore calculated, for several wavelengths in the 210 nm to 240 nm range, using equation 5 and an absorbance value for the anionic form of phenytoin determined at pH=10.5 ± 0.2. The pK of phenytoin determined in this manner, gave an unacceptably large standard deviation, despite the averaging of three experiments and two wavelengths.

Since it was not possible to experimentally determine a absorbance value for the conjugate base form of phenytoin (A_B), it was necessary to use equation 6 to determine a pK. The zero-order spectra provided little information on the nature of the pH-dependent changes occuring in the upper pH range. This made it

difficult to ascertain, even approximately, where the variation in the spectra became unacceptably large. In the second-order derivative spectra, two isosbestic points are visible in the pK titration. These isosbestic points were taken to indicate that there is a relationship between the pH of the solution and the ionization nature of phenytoin. As long as the isosbestic points were held 'tightly', the spectral shifts related to pH were ascribed directly to the pK of phenytoin. From pH 6, (or less), to the pH range of 8.6 to 8.8 these isosbestic points were consistent and 'tight'. At pHs above 8.8 the isosbestic points began to drift and were eventually completely lost. The loss of continuity in the isosbestic points seems to indicate that other physical factors are affecting the UV of phenytoin in this pH range. The possibility of a secondary ionization occurring was checked using a modified form of the Robinson-Biggs equation (10). Results from these experiments did not indicate a second pK in this pH region.

The ionization constant of phenytoin, determined using equation 6, was 8.08 $\pm$ 0.14 in the zero-order spectra, and 8.12 $\pm$ 0.05 in the second-order spectra. The pK value determined by the zero-order spectra had an unacceptably large standard deviation, however the pK determined by the second-order derivative gave an acceptable result. This pK value is in good agreement with a value of 8.06 reported by Schwartz et. al (8) using a solubility technique.

Table 3
Absorbance Ratio for Phenytoin

Derivative Order	Wavelength (nm)	Ratio A_B/A_A
Zero	230	1.64 ± 0.04
	235	1.80 ± 0.06
Second	217	2.38 ± 0.05
	220	3.27 ± 0.08

The relative absorbance value for the anionic form of phenytoin ($\varepsilon_B C_t \ell$) can also be determined from equation 6. In these experiments an accurate concentration of phenytoin was not determined, and therefore ε_B cannot be calculated. The relative absorbance increase, at specific wavelengths, can be derived as a ratio of A_B versus A_A (Table 3). This ratio can be used to indirectly calculate ε_B for phenytoin. It also shows that the second-order derivative spectra have a larger spectral separation, at the wavelengths chosen, than the zero-order spectra.

The inability to accurately determine an absorbance value for the conjugate base form of phenytoin (A_B) was unexpected. However, the literature shows that there are questions about the high pH behavior of phenytoin. Work by Salem et. al. (11) has shown that the solubility of phenytoin continues to increase in the pH range of 9.87 to 10.98. Since the pK of phenytoin is approximately 8.1, it would be expected that the solubility of phenytoin should maximize around pH=10 (12,13). This assumes that the solubility of phenytoin, in this pH range, is due strictly to

its ionization. It has also been reported that the maximum aqueous dissolution of phenytoin does not occur until a pH $\geq$ 11.7 (14,15). In the solubility experiments of Schwartz et. al., (8), the highest pH used to determine the pK of phenytoin was pH=8.3. Further work is ongoing to determine the exact value of ε_B and the nature of the changes occurring in higher pH solutions.

CONCLUSIONS

It is possible to use higher-order derivative spectroscopy to determine the ionization constant of compounds. This requires that the ionization of the drug in question causes a change in the absorption spectra. In those cases where the compound is sparingly soluble, and/or the zero-order spectra does not show enough spectral separation to allow an accurate pK determination, second-order derivative spectroscopy can be employed. It should be noted that second-order derivative spectroscopy does not provide new spectral information, but only enhances features in the absorbance spectrum which otherwise might go unnoticed.

REFERENCES

1) J.E. Cahill, Amer. Lab., 11(11), 79-85 (1979).

2) J.E. Cahill and F.G. Padera, Amer. Lab., 4, 101-112 (1980).

3) A.F. Fell, Trends in Analytical Chem., 2(3), 63-66, (1983).

4) T.C. O'Haver and G.L. Green, Amer. Lab., 3, 15-21, (1975).

5) A.J. Glazko, in "Antiepileptic Drugs", Ed. D.M. Woodbury, J.K. Penry, and R.P. Schmidt, Raven Press, New York, p. 103, (1972).

6) Unpublished Results, L.S. Rosenberg and C.K. Hostetler.

7) S.P. Agarwal and M.I. Blake, J. Pharm. Sci., 57(8), 1434, (1968).

8) P.A. Schwartz, C.T. Rhodes, and J.W. Cooper, J. Pharm. Sci., 66(7), 994, (1977).

9) M.E. Krahl, J. Phys. Chem., 44, 449-463 (1940).

10) R.A. Robinson and A.I. Biggs, Aust. J. Chem. 10, 128, (1957).

11) R.B. Salem, R.L. Yost, G. Torosian, F.T. Davis, and B.J. Wilder, Drug Intelligence and Clinical Pharmacy, 14, 605-608, (1980).

12) D.W. Newton and R.B. Kluza, Am. J. Hospital Pharmacy, 37, 1647-1651, (1980).

13) A. Albert and E.P. Serjeant, "The Determination of Ionization Constants", 3rd Ed., Chapman and Hall, New York, NY, 1984, p. 105.

14) J.K. Dale and R.E. Booth, in "Dispensing of Medication", J.E. Hoover, eds., Mack, Easton, PA, 1976, p. 940.

15) M. Windholz, "The Merck Index", 9th Ed., Merck & Co., Rahway, NJ, 1976, p. 952.