Practical Kinetics I: Quantitation of Barbiturate Stability by TLC

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Abstract □ The purpose of this work was the application of established techniques to the quantitation of stability investigations of liquid formulations as well as in solution kinetic studies. The process consists of streaking aliquots of reaction solution on TLC plates, development, elution, and analysis of the substances in the eluate. Several barbiturates were investigated regarding their decomposition at elevated temperatures. Reasonable correlation is noted between obtained rate constants and literature data. This technique represents a utilitarian approach to stability screening of compounds in solution, aqueous or otherwise, where chromatographic separation and analytical methodology for the pure compound are available.

Keyphrases □ Barbiturates—stability, quantitation by TLC □ Stability—barbiturates, quantitation by TLC □ TLC—monitoring and quantitating barbiturate stability

This report outlines a rather general method for studying the kinetics of a wide variety of chemical compounds. It obviates the necessity for time-consuming development of analytical methodology where complications arise such as product interference with the analysis of intact substances. Although the basic method is well known, this report shows its general applicability to the formulating pharmacist and routine stability studies.

The two prerequisites for implementation of the method are: *(a)* an available assay for a solution of the starting material, and *(b)* a TLC separation of reaction product or products from the parent compound. These kinetic studies, utilizing quantitative TLC, have been carried out on diverse chemical classes with success in all of the cases considered. No particular expertise appears necessary beyond the normal requirements of technical work.

This method is well suited for evaluation of the stability parameters of solution and parenteral formulations, thus making it of particular value to the developmental pharmacist. It offers the advantage of being relatively inexpensive and within the price range of most laboratories, since the only equipment required is a plate streaker and syringe for spotting. Techniques such as spectrophotodensitometry and high-pressure liquid chromatography might serve the same purpose but both entail a much larger financial expenditure.

The technique is not a panacea and suffers from some inherent limitations. Compounds such as erythromycin, which defy chemical analysis in solution, are not readily examined by this method, nor are substances that are not easily separated by TLC from degradation products such as tetracycline. The method is somewhat tedious in that care and precautions must be taken to prevent loss in scraping, eluting, *etc.*

Several barbiturates were scrutinized in this work since this group of medicinal compounds is of considerable importance. Additionally, this class has sufficient published data available for comparisons regarding their degradation rate constants at various temperatures and pH values (1–4).

EXPERIMENTAL[1]

TLC—The general procedure utilizing thiamylal is given, but other barbiturates were separated by the same system. Solutions were made of the barbiturates in various buffers and sodium hydroxide solutions. A solution of 2 mg/ml thiamylal sodium was heated at 80.4 ± 0.1° in a constant-temperature oil bath. Samples were withdrawn periodically from the reaction solution, and 0.25 ml was streaked[2] on silica gel fluorescent plates[3] with occasional air drying to confine the bandwidth. Full plates were streaked[4] with a sample on each half of the normally scored plate. The development solution was isopropyl alcohol–chloroform–ammonium hydroxide (9:9:2), with a running distance of 15 cm (5).

The barbiturate, R_f 0.8, was visualized by short wavelength UV light (255 nm). Products could be observed on treatment with iodine vapor, followed by heating to remove the vapor. The band of silica gel containing the barbiturate was marked under the UV light, scraped by microspatula, and collected. The silica gel containing the barbiturate was washed with portions of 0.1 *N* NH_4OH to 50 ml in a volumetric flask, followed by filtration through a sintered-glass filter. In lieu of filtration, the silica gel was centrifuged with 50 ml of 0.1 *N* NH_4OH.

Spectrophotometric Analysis—The clear supernate from the centrifuged silica gel, or the solution following filtration, was read in the UV against a blank prepared from a corresponding blank plate treated in the same manner as the chromatograph containing the barbiturate. Solvent blanks could also be used with little discrepancy in results. Samples were read[5] in standard 1-cm cells with scans from 350 to 220 nm.

pH Measurements—The pH values of the sodium hydroxide solutions used were calculated from activity coefficient data available in the literature (6); other pH values were determined on a pH meter[6] standardized at the specified temperatures, utilizing phthalate and borate buffer solutions (7).

RESULTS AND DISCUSSION

A readily applicable stability screening method is of considerable value to the solution or parenteral formulator. Utilization of the presented technique, which represents a synthesis of well-established procedures, may save the time required for development of analytical methodology in situations where interactions or interferences exist or where the molecules do not lend themselves to routine techniques. Previously, the tendency with these substances has been to follow the rate of decomposition visually on TLC plates as a function of time at several pH values and temperatures. This qualitative type of analysis, while giving an estimation, does not allow arrival at legitimate quantitative data.

[1] The barbiturates were supplied by Ganes Chemical Co., Carlstadt, N.J., with the exception of thiamylal sodium, which was obtained from Parke, Davis and Co., Detroit, Mich. The substances were used "as is" with no further purification. All other materials employed were of analytical reagent grade.

[2] Streaked by means of a 0.5-ml syringe, Hamilton Co., Whittier, Calif.

[3] Silica Gel 60 F-254, 0.25-mm plates, 20 × 20 cm, EM Labs. Inc., Elmsford, N.Y.

[4] Plate holder for quantitative TLC made by Applied Science Laboratories Inc., State College, Pa.

[5] Cary 14 or 11 recording spectrophotometer.

[6] Metrohm.

Table I—Observed or Apparent First-Order Rate Constants (k in seconds^{-1} × 10^6) for Decomposition of Some Barbiturates (2 mg/ml) in Aqueous Solution at 80.4°

Barbiturate	Buffer	pH[a]	λ_{max}[b], nm	Rate Constant from TLC Plate Elutions[c]	Rate Constants from the Literature[d]
Thiamylal	Phosphate[e]	7.5	255	11.9	11.2
Thiamylal	Borate[e]	10.1	305	54.8	47.2
Thiamylal	0.1 *N* NaOH	11.5	305	276	257
Phenobarbital	0.033 *N* NaOH	10.99	240	152	171
Phenobarbital	0.1 *N* NaOH	11.5	240	302	363
Phenobarbital	0.1 *N* NaOH	11.5	240	311[f]	363
Butalbital	Phosphate	8.5	240	3.9	4.1
Butalbital	0.05 *N* NaOH	11.2	240	49	52
Butalbital	0.1 *N* NaOH	11.5	240	91	87
Amobarbital	Borate	8.0	240	6.5	6.7
Amobarbital	0.1 *N* NaOH	11.5	240	83.7	90.5

[a] Description of methods of pH measurement are given under *Experimental*. [b] Measured in 0.1 *N* NH_4OH. [c] Plates were run after spotting with 0.25 ml of solution and were eluted with 50 ml of 0.1 *N* NH_4OH. See *Experimental*. Runs were carried out in duplicate at least by two different operators on different days. Results are averages of the kinetic runs done in each case. [d] References 1 and 3. Temperature = 80°. [e] See Refs. 1 and 3 for preparation of buffers. [f] Run was carried out on 0.50 g phenobarbital/liter or one-fourth of normally used concentration.

Analytical problems may be circumvented with subsequent timesaving by elution of compounds from properly spotted thin-layer plates followed by classical analysis. This procedure has been employed successfully in this laboratory, although little specific documentation is found in the literature. However, it has been applied only to compounds of molecular weight up to 520. Undoubtedly, difficulties may be expected with higher molecular weight, more complex organic molecules.

Since first-order processes are approximated due to control of reaction parameters, absolute values regarding concentration terms are not necessary. As long as the same volumes of solution are spotted each time, apparent first-order kinetics will hold. The syringe used fulfilled this condition by allowing streaking of the plate with equivalent volumes of each sample.

Table I lists some barbiturates and the conditions under which they were studied. The preponderance of the work was carried out at ~80° due to the reaction velocities and general convenience of this temperature with these compounds. The correlation may be noted between the rate constants found experimentally and those previously derived by classical solution kinetics.

A statistical comparison of the two sets of data in Table I is negated because the temperature reported was 80° (2, 3) while these investigations were carried out at 80.4°.

Table II gives a typical set of runs with thiamylal carried out by three different operators. These runs were done on different solutions on different days. The data gave a standard error of 7 with a 95% confidence interval of 262–290 sec^{-1} × 10^6 for the rate constant k.

Figure 1 illustrates a typical absorbance loss of thiamylal in 0.1 *N* NaOH as a function of time. Experiments were generally carried out for two or more half-lives.

Figure 2 gives some first-order plots for thiamylal at pH 10.1 and in 0.1 *N* NaOH at 80.4° at both 255 and 305 nm. The same slope is observed for both wavelengths under each condition.

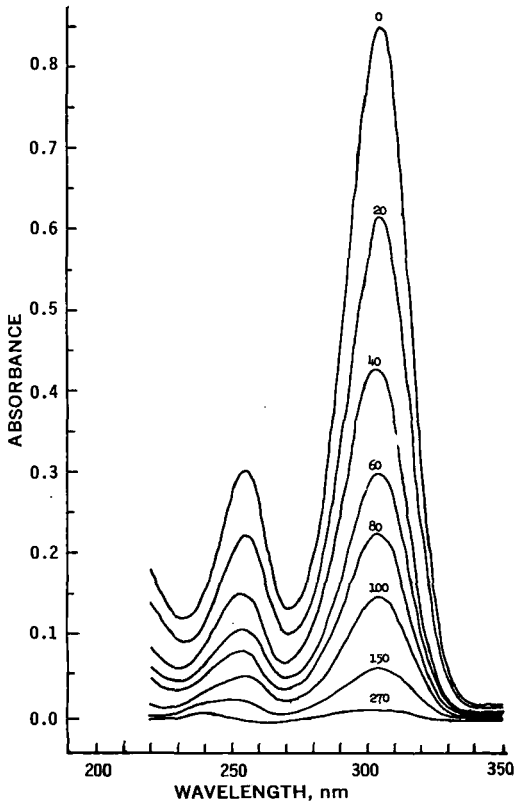

Figure 1—*Spectral change for hydrolysis of thiamylal sodium (2 mg/ml) in 0.1 N NaOH at 80.4° with time in minutes (listed on graph). Intact thiamylal was eluted from plates and read in 0.1 N NH_4OH.*

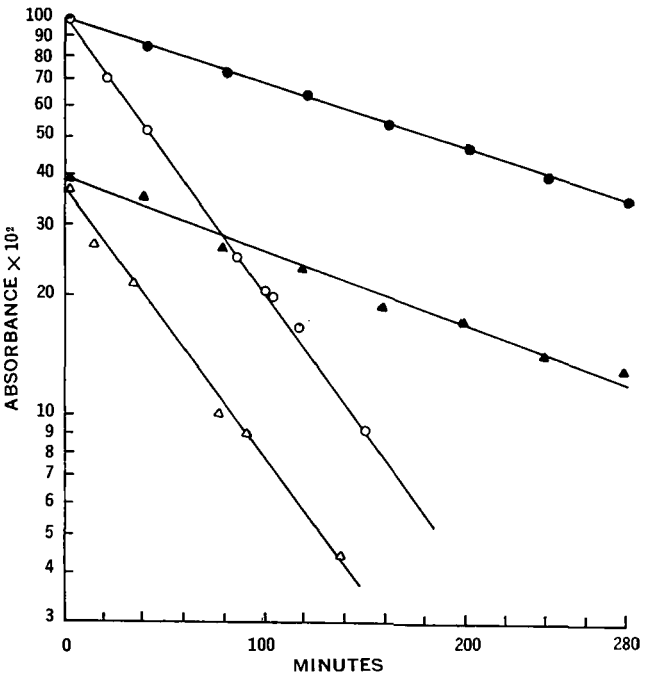

Figure 2—*Apparent first-order plots for hydrolysis of thiamylal sodium (2 mg/ml) in 0.1 N NaOH and at pH 10.1 and 80.4°. Rate constants were determined from absorbance values obtained at 255 and 305 nm. Thiamylal was extracted from silica gel thin-layer plates and read in 0.1 N NH_4OH. Key: ●, pH 10.1, 305 nm; ▲, pH 10.1, 255 nm; ○, 0.1 N NaOH, 305 nm; and △, 0.1 M NaOH, 255 nm.*

Table II—Rate Constants for Hydrolysis of Thiamylal[a] in 0.1 *N* NaOH at 80.4°

Run	k, $sec^{-1} \times 10^{6}$ [b]
1	291
2	288
3	284
4	263
5	256
Average	276[c,d]

[a] Thiamylal was read in 0.1 *N* NH_4OH at 305 nm (255 nm gives similar values). Initial concentration was 2 g/liter. [b] Results were determined by three operators at different times. [c] $SD = \sqrt{(X_i - \bar{X})^2/(n-1)} = 16$, $SE = SD/\sqrt{n} = 7$. [d] The 95% confidence interval = 262–290 $sec^{-1} \times 10^6$.

Barbiturates function as excellent models because they break down into several compounds—malonuric acids, acetic acids, urea, acylureides, diamides, *etc.* (2–4, 8–11). These products are easily separated from the parent molecule by the thin-layer system used (5). With some barbiturates, only the parent compound may be seen on fluorescent plates under short wavelength UV light. Other compounds may be observed using iodine vapor, followed by removal of the color on heating the TLC plates (2). Urea must be visualized in another manner (2). The anionic form of the pyrimidine ring system is easily extractable from the TLC plates into 0.1 *N* NH_4OH, which serves as a suitable solvent for many anionic substances. Methanol, methanolic hydrochloric acid, and 0.1 *N* HCl serve as eluants for amines and neutral molecules adsorbed onto silica gel.

Figure 3 shows absorbance diminution as a function of time for a 2-mg/ml solution of phenobarbital in 0.033 *N* NaOH at 80.4°. The spectra represented are typical, and no attempts were made to select the best curves for representation.

Several plots of log absorbance *versus* time are given in Fig. 4. This figure is a good illustration of the results obtained from the thin-layer plates for the various barbiturates. The four barbiturates examined exemplify most types currently used in therapy with regard to the nature of substituents at positions 2 and 5. There is no reason to expect that all barbiturates normally utilized medicinally cannot be analyzed in this manner.

Problems can arise in these studies when the substrate will not dissolve in amounts of 1–2 mg/ml, as occurs with barbiturates as the pH approaches pKa (pH ≈ pKa). In such cases, cosolvents may be used. Pseudo- or apparent first-order kinetics hold well in these concentrations (2 mg/ml, about 8×10^{-3} *M*) in the cases investigated. Phenobarbital was run in 10^{-4} *M* solution (Table I) with success, although working at this concentration was tedious as well as somewhat inconvenient.

When syrups containing relatively large quantities of sugars and/or polyhydric alcohols are analyzed, it may be best to extract the active compound from the aqueous solution into a suitable solvent for spotting (*i.e.*, chloroform, ether, *etc.*) due to difficulties encountered in spotting and poor running characteristics of chromatograms of syrups spotted directly without prior extraction.

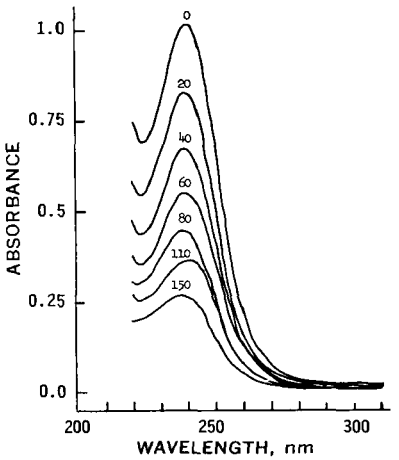

Figure 3—*Absorbance diminution of phenobarbital sodium (2 mg/ml) in 0.033* N *NaOH at 80° with time in minutes (listed on graph). Intact phenobarbital was extracted from silica gel thin-layer plates and read in 0.1* N *NH_4OH. See* Experimental.

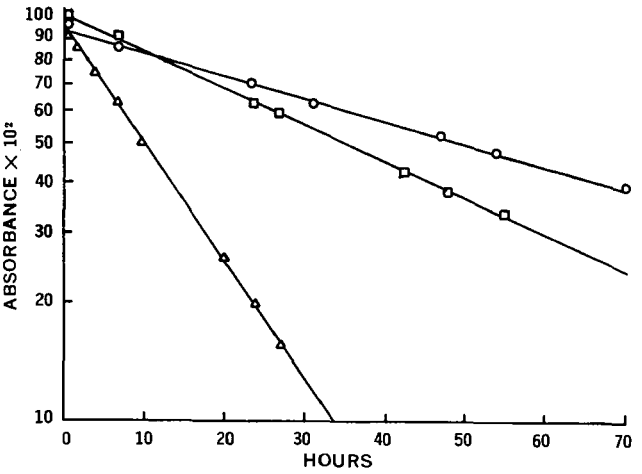

Figure 4—*Apparent first-order plots for hydrolysis of barbiturate (2 mg/ml) as a function of time. Reaction was followed by loss of absorbance at specified wavelengths (Table I). Samples were eluted from silica gel thin-layer plates as discussed in* Experimental. *Temperature was 80.4°. Key:* ○, *butalbital sodium, pH 8.5;* □, *amobarbital sodium, pH 8.5; and* △, *thiamylal sodium, pH 10.1.*

The quality of TLC plates used is critical. The plates specified under *Experimental* work satisfactorily. Problems can arise in the 230–260-nm range, because many plates tend to contain impurities that absorb in this spectral region upon elution. At higher wavelengths, most commercial plates are acceptable. When absorbance occurs, it may be circumvented by predeveloping the plates prior to spotting. Caution must be taken when streaking the plate to prevent scoring which interferes with proper development. The barbiturates generally ran to R_f 0.7–0.8; but when lower amounts were streaked (0.134 mg relative to the normally used 0.5 mg), the barbiturate phenobarbital ran considerably higher than normal, R_f 0.9+.

REFERENCES

(1) H. V. Maulding and M. A. Zoglio, *J. Pharm. Sci.*, **60,** 40(1971).

(2) H. V. Maulding, J. Nazareno, J. Polesuk, and A. Michaelis, *ibid.*, **61,** 1389(1972).

(3) E. R. Garrett, J. Bojarski, and G. J. Yakatan, *ibid.*, **60,** 1145(1971).

(4) F. Tishler, J. E. Sinsheimer, and J. E. Goyan, *ibid.*, **51,** 214(1962).

(5) J. P. Comer and I. Comer, *ibid.*, **56,** 413(1967).

(6) H. S. Harned and B. B. Owen, "The Physical Chemistry of Electrolytic Solutions," 3rd ed., Reinhold, New York, N.Y., 1958.

(7) A. Albert and E. P. Sergeant, "Ionization Constants of Acids and Bases," Wiley, New York, N.Y., 1962, pp. 17–68.

(8) F. Fretwurst, *Arzneim.-Forsch.*, **8,** 44(1958).

(9) H. Aspelund, *Acta Acad. Aboensis Math. Phys.*, **20** (3) (1955); through *Chem. Abstr.*, **50,** 11351e(1956).

(10) H. Aspelund and S. Stolt, *ibid.*, **20** (4) (1955); through *Chem. Abstr.*, **50,** 11352b(1956).

(11) H. Aspelund and B. Eklund, *ibid.*, **21** (3) (1957).

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