Differential Pulse Polarography of Some Degradation Products of Tetracycline

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Received October 6, 1981, from the *Arnold and Marie Schwartz College of Pharmacy and Health Sciences, Long Island University, Brooklyn, NY 11201.* Accepted for publication January 19, 1982.

Abstract □ In an attempt to develop a more rapid, convenient, and precise method for the direct detection and analysis of the degradation products of tetracycline, a study of those products utilizing differential pulse polarography was initiated. The investigation was concentrated on the subject of the kinetics of the epimerization of anhydrotetracycline to 4-epianhydrotetracycline in acetate buffer. The reaction was followed at 25 and 50°. Duplicate experiments were run at each temperature. The apparent rate constants obtained were $4.17 \pm 0.13 \times 10^{-1}$/hr (25°) and $6.97 \pm 1.00 \times 10^{-2}$/hr (50°).

Keyphrases □ Tetracycline—differential pulse polarography, degradation products, kinetics, epimerization □ Kinetics—differential pulse polarography, degradation products of tetracycline, epimerization □ Epimerization—differential pulse polarography of degradation products of tetracycline, kinetics □ Degradation—differential pulse polarography, products of tetracycline, epimerization, kinetics

A unique method is reported for the determination of degradation products of tetracycline. It is well known that tetracycline undergoes spontaneous degradation to an epimer, 4-epitetracycline and dehydration to anhydrotetracycline. In addition, both of the latter compounds undergo further transformation to produce 4-epianhydrotetracycline (1–3). There has been much concern over the kinetics and mechanisms (4–6) of these reactions and methods for determining the various products (7–15). The application of differential pulse polarography to the detection of tetracycline and a number of its derivatives has been described previously (16). This report describes the use of differential pulse polarography for detecting anhydrotetracycline in the presence of epianhydrotetracycline and for following the conversion of the former compound to the latter.

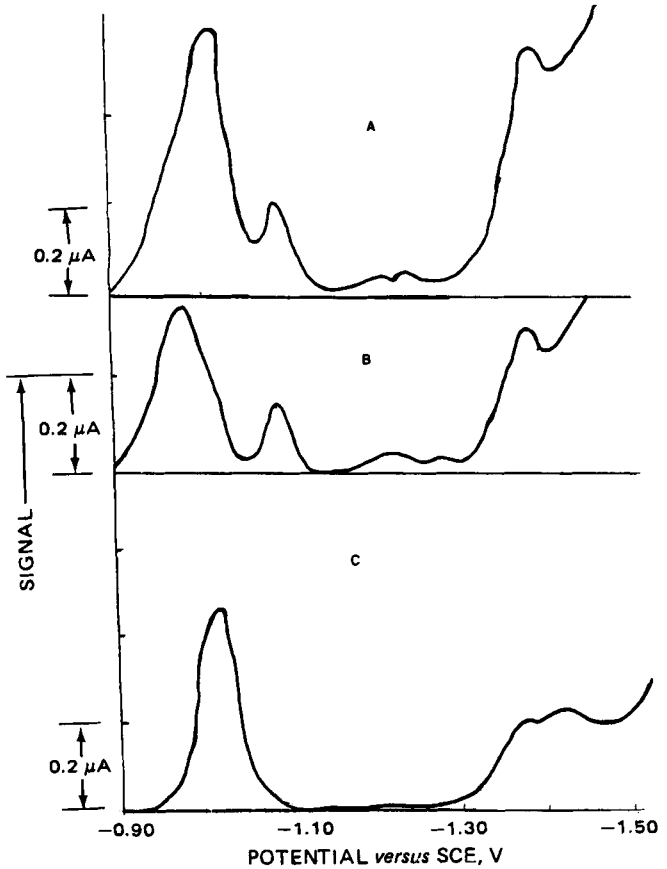

Figure 1—*(A) Differential pulse polarogram of a mixture of anhydrotetracycline and epianhydrotetracycline; (B) differential pulse polarogram of anhydrotetracycline; (C) differential pulse polarogram of epianhydrotetracycline.*

EXPERIMENTAL

Apparatus—The apparatus and technique used in this work have been described previously (17).

Materials—Anhydrotetracycline and 4-epianhydrotetracycline reference grade[1], were used as received. Acetate buffer (pH 4.31) was freshly prepared from reagent grade acetic acid and sodium acetate[2].

Procedure—Known amounts of anhydrotetracycline hydrochloride and/or 4-epianhydrotetracycline hydrochloride were added to a 250-ml volumetric flask. The materials were dissolved and brought to volume with acetate buffer solution. A fixed volume (25 ml) of each solution was withdrawn, deaerated, and assayed polarographically. Polarograms were recorded between −0.9 and −1.5 V.

In the rate studies, four different concentrations of anhydrotetracycline hydrochloride ($5.0–8.0 \times 10^{-5}$ *M*) in acetate buffer solution (pH 4.31) were followed as a function of time at the desired temperature. Polarograms of each sample were run immediately upon preparing the solution. Each solution was transferred into two separate 56.8-ml prescription bottles. The bottles were sealed with plastic covers and immediately placed in a thermostated shaker bath.

At appropriate time intervals, each sample bottle was withdrawn and immersed in ice to reduce the possibility of further reaction. The samples were analyzed by differential pulse polarography as described. Duplicate kinetic experiments were run at 25 and 50°.

RESULTS AND DISCUSSION

Figure 1A is a typical polarogram of a mixture of anhydrotetracycline and epianhydrotetracycline showing separate peaks attributable to the two compounds. The polarograms demonstrate clearly diverse curves at a peak potential of −1.09 V (Figs. 1B and 1C). A linear relationship between current and concentration exists at the peak potential at which these currents were measured. This is the major reduction potential in the polarographic spectrum for the compound.

At appropriate time intervals, solutions of anhydrotetracycline kept at various temperatures and concentrations were analyzed polarographically by the measurement of peak currents of the polarograms at the peak potential of −1.09 V.

The epimerization was observed to follow first-order kinetics as reported previously (18). The rate constants obtained from semilogarithmic plots are 0.0417 ± 0.0013 at 25° and 0.0697 ± 0.0100 at 50°. The values obtained in this study are somewhat lower than those reported (18) for the same reaction in phosphate buffer at pH 1.5. It is suspected that acid catalysis plays a role in the conversion.

Estimation of the activation energy for this reaction based on the two

[1] Lot Ph Eur 1, European Pharmacopoeia.
[2] Scientific Products, Inc. Edison, N.J.

0022-3549/82/1100-1284$01.00/0

temperatures studied leads to a low apparent value. It may be speculated that the reaction involves a low-energy bond rotation. However, it is not the intent of this report to provide a detailed mechanistic evaluation of the process. Such studies are to be conducted.

The technique of differential pulse polarography has been applied to the detection of anhydrotetracycline in the presence of epianhydrotetracycline and used to study the rate of conversion of anhydrotetracycline to its epimer. The authors feel this is a unique application of this technique. It is also felt that this work proves that this method, which is somewhat simpler to utilize than most commonly employed analytical procedures, might be useful in studying the reactions of other tetracycline derivatives.

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Extended Hansen Approach: Calculating Partial Solubility Parameters of Solid Solutes

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Received October 13, 1981, from the **Drug Dynamics Institute, College of Pharmacy, University of Texas, Austin, TX 78712*, and the ‡*Energy Center, University of California at San Diego, La Jolla, CA 92093.* Accepted for publication January 21, 1982.

Abstract □ A multiple linear regression method, known as the extended Hansen solubility approach, was used to estimate the partial solubility parameters, δ_d, δ_p, and δ_h for crystalline solutes. The method is useful, since organic compounds may decompose near their melting points, and it is not possible to determine solubility parameters for these solid compounds by the methods used for liquid solvents. The method gives good partial and total solubility parameters for naphthalene; with related compounds, less satisfactory results were obtained. At least three conditions, pertaining to the regression equation and the solvent systems, must be met in order to obtain reasonable solute solubility parameters. In addition to providing partial solubility parameters, the regression equations afford a calculation of solute solubility in both polar and nonpolar solvents.

Keyphrases □ Solubility, partial—extended Hansen approach, parameters of solid solutes, naphthalene, decomposition □ Naphthalene—extended Hansen approach, partial solubility parameters of solid solutes, decomposition □ Decomposition—extended Hansen approach, partial solubility parameters of solid solutes, naphthalene

A multiple regression method using Hansen partial solubility parameters, δ_d, δ_p, and δ_h, was reported (1) for calculating the solubility of naphthalene in pure polar and nonpolar solvents.

THEORETICAL

The method, called the extended Hansen solubility approach, uses a regression equation of three terms involving solvent and solute solubility parameters:

$$-\log X_2 = -\log X_2{}^i + A[C_1(\delta_{1d} - \delta_{2d})^2 + C_2(\delta_{1p} - \delta_{2p})^2 + C_3(\delta_{1h} - \delta_{2h})^2] + C_0 \quad \text{(Eq. 1)}$$

where X_2 and $X_2{}^i$ are the mole fraction solubility and mole fraction ideal solubility, and A is a term from regular solution theory:

$$A = \frac{V_2\phi_1{}^2}{2.303RT} \quad \text{(Eq. 2)}$$

where V_2 is the molar volume of the solute in the supercooled liquid state, ϕ_1 is the volume fraction of solvent, R is the gas constant, and T is the absolute temperature.

The partial solubility parameters for dispersion, δ_d, dipolar interaction forces, δ_p, and hydrogen bonding and other Lewis acid–base interactions, δ_h, are found in Eq. 1 for solvent (subscript 1) and solute (subscript 2). The coefficients C_0, C_1, C_2, and C_3 are provided in the computer output resulting from the least-squares analysis.

The equation obtained for naphthalene in 24 solvents by the extended Hansen solubility approach was (1):

$$\log \alpha_2 = \log \frac{X_2{}^i}{X_2} = 1.0488A(\delta_{1d} - \delta_{2d})^2 - 0.3148A(\delta_{1p} - \delta_{2p})^2 + 0.2252A(\delta_{1h} - \delta_{2h})^2 + 0.0451 \quad \text{(Eq. 3)}$$

This equation provided solubilities of naphthalene in polar and nonpolar solvents at 40° with <30% error (except for *tert*-butanol, 53% error); for ~50% of the cases results were obtained within <5% error. The method allowed the calculation of the solubility of naphthalene in solvents not included in the series under investigation. The extended Hansen solubility approach was tested against the UNIFAC method (2) and the extended Hildebrand solubility approach (3), two alternate methods undergoing recent development.

RESULTS AND DISCUSSION

The partial solubility parameters of Hansen and Beerbower (4) are available for a large number of liquids, but the values for only a few solids (represented as supercooled liquids) are found in the literature. A table was prepared of group contributions for calculating partial solubility