CONCLUSIONS

An enteric formulation of levodopa can prevent drug absorption in the stomach and so can reduce the side effects of the drug on the stomach. Application of III-50 as an enteric coating film material resulted in an enteric tablet with rapid disintegration characteristics after passing through the stomach. Addition of an effervescent component to the tablet reduced the lag time of *in vitro* dissolution in intestinal fluid, and the rate of levodopa dissolution was accelerated. The conventional round shape was suggested as a suitable tablet shape and also a smaller size was recommended to minimize the lag time for the transit of the tablet into the intestine.

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ACKNOWLEDGMENTS

The authors thank Dr. T. Higuchi for valuable discussions, Drs. H. Negoro, S. Akagi, and T. Miki for encouragement and useful discussion. The authors thank Mr. S. Takamura and Mrs. E. Takayama for their assistance.

Dissolution and Ionization of Warfarin

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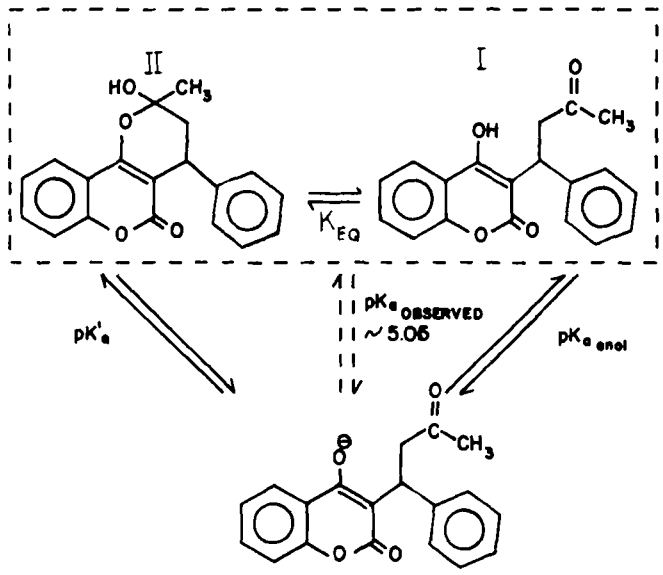
Received January 18, 1983, from the *Department of Pharmaceutical Chemistry, The University of Kansas, Lawrence, KS 66045.* Accepted for publication July 19, 1983. Present addresses: *American Cyanamid Co., Pearl River, NY 10965 and ‡Squibb Institute for Medical Research, New Brunswick, NJ 08903.

Abstract □ It has been shown in recent studies that warfarin exists in the solid state and in some nonaqueous solvents as a cyclic hemiketal. The present study was undertaken to investigate the ionization and ionization kinetics of warfarin, to confirm the probable existence of the cyclic hemiketal in aqueous solution, and to determine the possible consequences of the cyclic hemiketal to acyclic enol equilibrium and ionization kinetics on the dissolution rate of warfarin. The equilibrium aqueous solubility of un-ionized warfarin acid at 25°C and ionic strength 0.5 (with potassium chloride) was found to be 1.28×10^{-5} M, and its observed macroscopic pK_a was 5.03–5.06, depending on the method of determination. By comparing the aqueous pK_a of warfarin to phenprocoumin, a hydroxycoumarin that cannot exist in the cyclic hemiketal form, the hemiketal–acyclic enol ratio was estimated to be ~20:1. By stop-flow spectrophotometry, the ionization rate of warfarin (pH 3.5 jumped to pH 6.5) was found to have a $t_{1/2} < 1\text{–}2 \times 10^{-3}$ s. The dissolution rate of warfarin from a rotating disk (600 rpm), as a function of pH, was measured under nonbuffered but pH-stat conditions (μ = 0.5 with potassium chloride). The pH-dissolution rate profile for warfarin agreed with that calculated from an equation derived previously to describe the dissolution of instantaneous ionizing acids, *i.e.*, the profile was not perturbed from that expected from an acid of aqueous solubility 1.28×10^{-5} M (un-ionized form) and pK_a 5.06.

Keyphrases □ Dissolution—warfarin, ionization kinetics □ Warfarin—dissolution, ionization kinetics □ Ionization kinetics—warfarin, dissolution

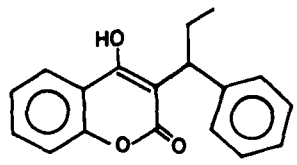
The structure of the anticoagulant warfarin is usually depicted in the open-chain form (I) whereas it is known to exist in the solid state in the cyclic hemiketal form (II) (1, 2). Spectrometric studies (3–6) have confirmed that II is also the predominant form of warfarin in solution in various nonaqueous solvents. In water, un-ionized warfarin (I and/or II) exists in equilibrium with the enolate (III) (Scheme I).

Since warfarin exists as the hemiketal in the solid state and its ionization appears to be complex, we decided to study the dissolution rate *versus* pH (unbuffered, pH maintained by pH-stat) profile of warfarin to observe whether it behaved as an instantaneously ionizing acid (7, 8). To achieve this, the dissolution rate from a compressed rotating disk of warfarin at pH 2 and in the pH range 7–9.5 was studied along with its solubility, ionization characteristics, and ionization rate. By



Scheme I

comparing the pK_a of warfarin with that of phenprocoumin (IV), a hydroxycoumarin which can exist only in an acyclic form, it was also possible to test for the existence of II as the predominant form of warfarin in aqueous solution.



IV

0022-3549/84/0700-0946$01.00/0

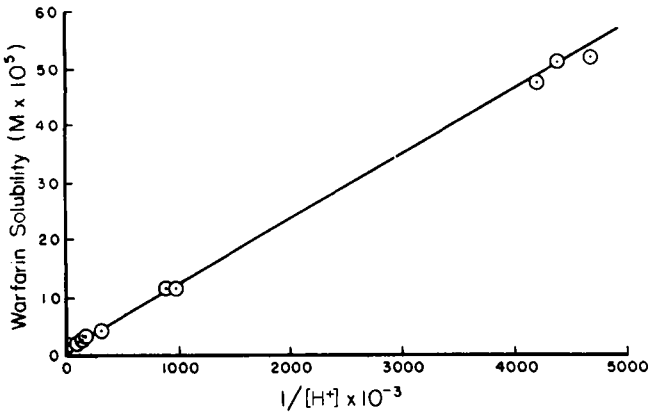


Figure 1—*Plot of the total solubility (S_T) of warfarin at 25°C* versus *$1/[H^+]$ for equilibrated 0.1 M acetate buffers, μ = 0.5 (potassium chloride): $S_T = 1.28 \times 10^{-5} + (112 \times 10^{-10}/[H^+])$ ($r = 0.999$; n = 29). $[HA]_0$ is obtained from the intercept, and K_a is obtained from slope/intercept.*

EXPERIMENTAL SECTION

Materials—Warfarin[1] and phenprocoumin[2] were used as supplied. All reactions, dissolution rates, and pK_a determinations were carried out in glass-distilled or doubly deionized carbon dioxide- and oxygen-free water. The buffer components, potassium chloride, and sodium hydroxide were of reagent or analytical reagent quality and were used without further purification.

pK_a and Solubility Determinations—*Method A*—The pK_a values of warfarin and phenprocoumin were determined spectrophotometrically[3] (9) at 282 (or 272) and 275 nm, respectively, at 25 ± 0.2°C and μ = 0.1 (with potassium chloride). The pK_a of warfarin was also determined spectrophotometrically at μ = 0.5 (with potassium chloride).

Method B—The solubility and pK_a of warfarin at 25 ± 0.5°C and μ = 0.5 (with potassium chloride) were determined by the solubility method (7, 10, 11). While determining solubility as a function of pH, care was taken to deoxygenate the solutions and to protect them from light. Analysis of the amount of dissolved warfarin was by spectrophotometry[3] of filtered samples, and the pH was adjusted to 11–12 with sodium hydroxide. A standard curve was prepared just before the measurements were taken.

Ionization Kinetics of Warfarin—The pH jump technique for studying ionization by stop-flow spectrophotometry[4] as previously described for the ionization of phenylbutazone (12), and phenindione (13, 14) was used to determine whether warfarin ionization might have a half-life measurable by this method ($t_{1/2} > 1\text{-}2 \times 10^{-3}$ s). Specifically, a 1.5×10^{-5} M (pH 3.5) solution of warfarin in 0.5 M KCl was jumped to pH 6.5 (0.1 M phosphate buffer; μ = 0.5 with potassium chloride), and transmission was recorded as a function of time (on the stop-flow spectrophotometer oscilloscope) at 272 nm[5] immediately after mixing (mixing time, $\sim 2 \times 10^{-3}$ s). Transmission changes as a function of time were observed down to 1×10^{-3} s/division on the oscilloscope.

Dissolution Study—Individual disks of 250 mg of warfarin were prepared by the method previously described for other acids (7, 8). Preslugging of the commercial warfarin and regrinding of the powder was necessary to produce disks which did not cap or crack on expulsion from the die. Dissolution rates were determined by previously described procedures (7, 8, 15). The continuous monitoring of dissolution was carried out at 285 nm for pH 2 (λ_{max} at pH 2) and at 292 nm (an isosbestic point) for all other pH values.

RESULTS AND DISCUSSION

The observed pK_a of warfarin and the macroscopic p$K_{a_{obs}}$, defined by Scheme I and Eq. 1:

$$K_{a_{obs}}{}^{warfarin} = \frac{[III][H^+]}{([I] + [II])} \qquad \text{(Eq. 1)}$$

and determined by method A at 25°C and μ = 0.1, was calculated to be 5.03 ± 0.01 (±*SD*). This was calculated from individual data points (in triplicate) for seven pH values in the range of 4.00–7.82, with the mean absorbance at pH 2.08, 2.88, and 3.02 representing the absorbancy of un-ionized warfarin and the absorbance at pH 11.77 representing the absorbancy of the enolate (9). A plotting technique described earlier (12–14) gave an identical result. The pK_a of phenprocoumin, under identical conditions, was determined to be 3.77 ± 0.01 (±*SD*). These determinations were carried out at μ = 0.1 for the purpose of comparison with earlier studies (12–14).

Inverting Eq. 1 and substituting the formulas for $K_{a_{enol}}{}^{warfarin}$ and K_a', as defined in Scheme I, gives Eq. 2 and 3, respectively:

$$\frac{1}{K_{a_{obs}}{}^{warfarin}} = \frac{[I]}{[III][H^+]} + \frac{[II]}{[III][H^+]} \qquad \text{(Eq. 2)}$$

$$= \frac{1}{K_{a_{enol}}{}^{warfarin}} + \frac{1}{K_a'} \qquad \text{(Eq. 3)}$$

The only difference in structure between warfarin enol (I) and phenprocoumin (IV) is the presence of a butanone group compared with an ethyl group at C-3, respectively. The presence of the carbonyl group in the butanone side chain of warfarin should have a negligible electron-withdrawing effect on the enol p$K_{a_{enol}}$ relative to the ethyl side chain in phenprocoumin. If it is also assumed that there is a negligible effect on p$K_{a_{enol}}$ by intramolecular hydrogen bonding and that warfarin and phenprocoumin do not exist significantly in a diketo form (12–14), then:

$$K_{a_{enol}}{}^{warfarin} \simeq K_a{}^{phenprocoumin} \qquad \text{(Eq. 4)}$$

By substituting $K_a{}^{phenprocoumin}$ for $K_{a_{enol}}{}^{warfarin}$ in Eq. 3, an estimate K_a' can be made. By using values of 9.33×10^{-6} for $K_{a_{obs}}{}^{warfarin}$ and 1.70×10^{-4} for $K_a{}^{phenprocoumin}$, Eq. 3 gives a value for K_a' of 9.87×10^{-6} or pK_a' of 5.00. Therefore, the fraction of un-ionized warfarin present in aqueous solution as II, which is defined by Eq. 5, is 0.95 or:

$$\frac{[II]}{[I] + [II]} = \frac{K_{a_{obs}}{}^{warfarin}}{K_a'} \qquad \text{(Eq. 5)}$$

The cyclic hemiketal–acyclic enol ratio is ~20:1 at 25°C and μ = 0.1. This conclusion is consistent with earlier spectrometric findings in other solvents (3–6).

Since the dissolution rate of warfarin was to be determined at μ = 0.5 for comparison with earlier studies (7, 8, 15), the pK_a of warfarin at this ionic strength was determined by both methods A and B. The observed pK_a of warfarin by method A was found to be 5.03 ± 0.02 (±*SD*).

The equilibrium solubility of a monoprotic weak acid in aqueous solution is given by:

$$S_T = [HA]_0 + [A^-] \qquad \text{(Eq. 6)}$$

where S_T is the total solubility of all the possible species, $[HA]_0$ is the saturated solubility of the un-ionized form(s) of the weak acid, *i.e.*, [I] + [II] in the case of warfarin, and $[A^-]$ is the amount of warfarin enolate, [III], present at equilibrium at the particular pH in question. Combining Eq. 6 with the K_a expression (Eq. 1) for warfarin yields:

$$S_T = [HA]_0 + \frac{K_{a_{obs}}{}^{warfarin}[HA]_0}{[H^+]} \qquad \text{(Eq. 7)}$$

A plot of S_T *versus* $1/[H^+]$ yields an intercept of $[HA]_0$ and a slope from which $K_{a_{obs}}{}^{warfarin}$ can be calculated. Figure 1 depicts this relationship for warfarin. From the intercept, the intrinsic solubility of un-ionized warfarin at 25 ± 0.5°C and μ = 0.5 was found to be 1.28×10^{-5} M, the $K_{a_{obs}}{}^{warfarin}$ was 8.75×10^{-6} ($pK_{a_{obs}}{}^{warfarin}$ = 5.06). The correlation coefficient for the plot was 0.999 (10 pH values, 2.20–6.67, and a total of 29 individual solubility determinations). The pK_a value of 5.06 correlates well with the value determined spectrophotometrically in this study and previous determinations (16, 17), and the aqueous solubility of the un-ionized warfarin acid of 1.28×10^{-5} M is similar to a recorded value in 0.1 M HCl of 1.43×10^{-5} (18).

Based on the findings that the predominant form of warfarin in the solid state and the probable predominant, un-ionized form of warfarin in solution is II, it was interesting to speculate that the conversion of II to III by whatever mechanism might not be a diffusion-controlled process. By using a stop-flow spectrophotometer and a pH jump technique, the transmission *versus* time oscilloscope tracing from mixing of an unbuffered pH 3.5 warfarin solution with a 0.1 M phosphate buffer solution of pH 6.5 (final pH on mixing, 6.5) gave a straight line (slope = 0). The absorbance calculated from the transmission tracing was consistent with a solution of 0.75×10^{-5} M warfarin of pH 6.5, *i.e.*, at all oscilloscope settings down to 1×10^{-3} s/division, the reaction (ionization) was found to be complete within the mixing time (2×10^{-3} s) of the stop-flow spectrophotometer, *i.e.*, $t_{1/2} < 1\text{-}2 \times 10^{-3}$ s. Under similar circumstances, the carbon acid phenylbutazone has a half-life of $\sim 12 \times 10^{-3}$ s (12).

[1] Sigma Chemical Co., St. Louis, Mo.
[2] Organon Pharmaceuticals, West Orange, N.J.
[3] Cary 219, Cary 118, or Zeiss PM 6 UV spectrophotometers.
[4] Durrum stopped-flow spectrophotometer, with a thermostated cell and syringes maintained at 25 ± 0.2°C.
[5] A wavelength at which there is a maximum transmission difference between un-ionized and ionized warfarin.

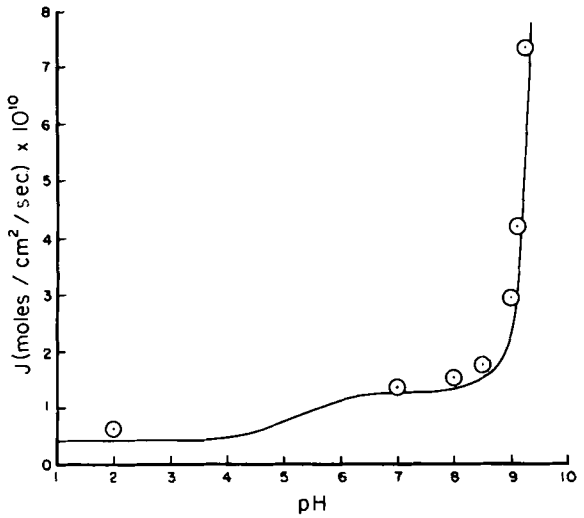


Figure 2—*Plot of the initial dissolution rate (flux) of warfarin from a rotating disk (600 rpm) as a function of pH, unbuffered but maintained by a pH-stat. Key: (⊙) experimental points (mean of 3–4 determinations at each pH); (—) predicted by Eq. 8.*

The dissolution rate of organic acids undergoing rapid ionization, dissolving from a rotating disk into nonbuffered aqueous solution under sink conditions in which the bulk solution pH has been maintained by a pH-stat, has been mathematically defined assuming a film model (7, 8) by:

$$J = \frac{D_{HA}}{h}[HA]_0 + \frac{D_H}{h}([H^+]_0 - [H^+]_h) + \frac{D_{OH}}{h}([\overline{O}H]_h - [\overline{O}H]_0) \quad \text{(Eq. 8)}$$

where J is the initial dissolution rate or flux with units of mol/cm²/s; D_{HA} is the diffusivity of the dissolving acid species in cm²/s; h is the film thickness of the diffusion resistance layer in cm; $[HA]_0$ is the molar saturated solubility of the dissolving acid under suppressed ionization conditions and is the amount of HA assumed to exist at the solid-liquid interface; D_H and D_{OH} are the diffusion coefficients (diffusivity) for hydrogen ion and hydroxide ion, respectively [2.8×10^{-5} cm²/s, (7)]; $[H^+]_0$ and $[H^+]_h$ are the hydrogen ion concentrations at the solid-liquid interface and in the bulk solution, respectively; and $[\overline{O}H]_h$ and $[\overline{O}H]_0$ are the hydroxide ion concentrations at the bulk solution and at the solid-liquid interface, respectively (calculated from the equation $[\overline{O}H] = K_w/[H^+]$). The aqueous diffusion layer thickness, h, can be calculated from (7, 8, 19):

$$h = 1.612 D_{HA}^{1/3} \nu^{1/6} \omega^{-1/2} \quad \text{(Eq. 9)}$$

where ν is the kinematic viscosity of the dissolution medium [9.77×10^{-3} stokes (7, 8)], and ω is the rotation rate of the disk in rad/s ($\omega^{1/2}$ at 600 rpm is 7.93). The value for $[H^+]_0$ can be calculated by solving Eq. 10 (7) for varying $[H^+]_h$ concentrations, as measured by the pH of the bulk medium:

$$-D_H[H^+]_0^2 + (D_H[H^+]_h - D_{OH}[\overline{O}H]_h)[H^+]_0 + K_w(D_{OH} + D_A K_1[HA]_0) = 0 \quad \text{(Eq. 10)}$$

where K_w is the dissociation constant for water at 25°C (10^{-14} M²); D_A is the diffusivity of the anionic form of HA and was assumed to be numerically equal to D_{HA} (7, 8); and K_1 is equal to K_a/K_w, where K_a is the dissociation constant of the acid. In the present case, K_a was assumed to be $K_{a_{obs}}^{warfarin}$ determined from the solubility study. Therefore, K_1 was numerically equal to 8.75×10^8.

An estimate of D_{HA} (and D_A), the diffusivity of the acid, is required to calculate J as a function of bulk solution pH (nonbuffered) with Eq. 8-10. In an earlier study, this was done by following J, the flux, as a function of $\omega^{1/2}$ under pH conditions in which ionization of the dissolving acid was suppressed and the diffusivity was calculated from the Levich equation (19). It was not possible to determine accurately the initial dissolution rate of warfarin at low pH because of its low solubility. For example, a saturated aqueous solution of warfarin at pH values < pK_a has an absorbance of only 0.16 at 285 nm, which is the absorbance maximum at pH values < pK_a. Therefore, the initial dissolution rate would require measuring very small absorbance readings[6]. The diffusivity of warfarin was, therefore, calculated from the earlier work by assuming the square root relationship to molecular weight using benzoic acid, 2-naphthoic acid, and indomethacin as standards (7). A mean value for D_{HA} for warfarin of 5.7×10^{-6} cm²/s at 25°C and $\mu = 0.5$ was therefore assumed in calculating J from Eq. 8.

Figure 2 is a plot of the initial dissolution rate of warfarin from a rotating disk (600 rpm) as a function of pH, unbuffered but maintained constant by a pH-stat (μ maintained at 0.5). The theoretical line tends to underestimate the dissolution rates by ~20%. The major differences appear to be between the estimated and calculated values for the dissolution rate at pH 2. However, as already discussed, it is very difficult to experimentally determine the initial dissolution rates at this or any acidic pH with certainty. Various parameters within Eq. 8 could be manipulated to give a better fit between the experimental and theoretical values. However, it was felt that this would serve no meaningful purpose at this stage, since the overall fit was reasonably good using parameters that were either determined experimentally or estimated from earlier studies.

If the dissolution of warfarin were to behave nonclassically due to slow ionization (15), the experimental dissolution rates would be overestimated by Eq. 8 since it is assumed in Eq. 8 that the dissolving acid dissociates at diffusion-controlled rates. If anything, Eq. 8 underestimated by ~20% the observed results in the present study. Therefore, it seems safe to conclude, based on the data in Fig. 2 and the ionization kinetics mentioned earlier, that warfarin behaves as if it were a fast-ionizing acid with a solubility in its unionized form of 1.28×10^{-5} M and a pK_a of 5.06.

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ACKNOWLEDGMENTS

This work was supprted by Grant GM 22357 from the National Institutes of Health. J.D.P. acknowledges the support of the American Foundation for Pharmaceutical Education.

[6] For example, true initial dissolution rates require measuring J under conditions at which <10% of the saturated solubility has been reached. Thus, accurate absorbance measurements of <0.02 are required. Under these restraints, problems such as fines on the pellet can lead to significant overestimation of the initial fluxes.