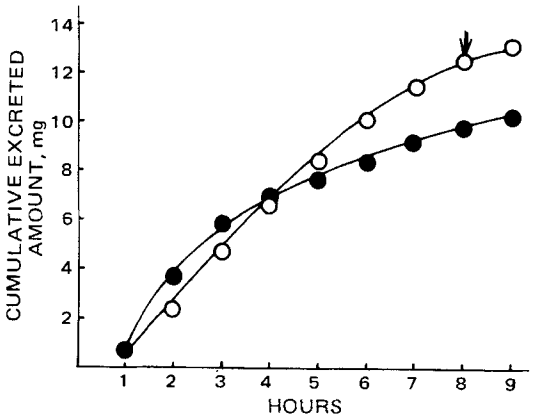

Figure 7—*Comparison of effect of suppository formulation of cumulative amount of nitrofurantoin excreted in Subject SK. Key: ○, lecithin and dried whey product; and ●, theobroma oil.*

from Formula T was greater than that from Formula B (Fig. 6); however, in this subject, both bases provided an adequate bacteriostatic concentration in the urine.

The amount of nitrofurantoin released from Formula D (Fig. 3) was so small that further investigation was not attempted.

Formula K provided a slightly greater amount of nitrofurantoin in the urine than the theobroma oil base suppository (Fig. 7). The dissolution profile of the theobroma oil base suppository suggested poor bioavailability, which was confirmed by *in vivo* data. In water at body temperature, the nitrofurantoin–theobroma oil suppository melted and formed a tacky mass from which the nitrofurantoin was slowly dissolved. With the limited fluid in the rectum, dissolution of the nitrofurantoin from the tacky mass was a very slow process. By mixing a lecithin and dried whey product with theobroma oil, it was hoped that the surface activity of the lecithin and the water solubility of the whey might enhance nitrofurantoin release. In measuring the dissolution profile, it was noticed that the mass was not tacky and that the release was slightly faster. Similarly, in the *in vivo* test, the amount of nitrofurantoin eliminated in the urine was slightly greater than that from the theobroma oil base.

SUMMARY

The usual adult dose of nitrofurantoin is 50–100 mg four times a day. If doses of nitrofurantoin several times the usual oral dose are administered with the same frequency rectally, adequate concentration of nitrofurantoin in the urine is attained. In this preliminary investigation, 400 mg of nitrofurantoin administered in a polyethylene glycol–polysorbate 80 suppository base and in a polyethylene glycol–silica suppository base provided an adequate concentration in the urine. Persons who exhibit nausea and emesis after oral administration of nitrofurantoin possibly could receive nitrofurantoin therapy by the rectal route.

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Electronic Absorption Spectra and Protolytic Equilibria of Doxorubicin: Direct Spectrophotometric Determination of Microconstants

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Abstract □ The ground- and excited-state dissociation constants and the electronic absorption and fluorescence spectra of doxorubicin were investigated by spectrophotometry. A general method for the direct calculation of individual microscopic dissociation constants was derived using the spectrophotometric data obtained. It was concluded that the protonated amino sugar group is slightly more acidic than the phenolic group. The spectrophotometric data were analyzed, and the macro- and microconstants for the various equilibria are reported.

Keyphrases □ Doxorubicin—electronic absorption and fluorescence spectrophotometric determination of dissociation constants and protolytic equilibria □ Spectrophotometry, electronic absorption and fluorescence—determination, doxorubicin dissociation constants and protolytic equilibria □ Dissociation constants—doxorubicin, electronic absorption and fluorescence spectrophotometric determination □ Protolytic equilibria—doxorubicin, electronic absorption and fluorescence spectrophotometric determination □ Antineoplastic agents—doxorubicin, electronic absorption and fluorescence spectrophotometric determination of dissociation constants and protolytic equilibria

Molecules containing a quinone moiety have been widely employed in trace analysis, dye manufacturing, and metal–ligand binding. In pharmaceutical applications, molecules such as the naphthoquinones (1) and the anthraquinones have shown promise as antifungal and antibacterial agents. Of more recent importance is the use of

Table I—Electronic Absorption (λ_{max}) and Fluorescence (λ_f) Maxima of the Prototropic Species of Doxorubicin in Water

Doxorubicin	λ_{max}, nm	log ϵ	λ_f, nm
Doubly charged cation	580	4.35	596
Monocation	495	4.00	552
Singly charged anion	550	3.81	588
Doubly charged anion	612	3.53	—

anthracycline antibiotics to treat carcinomas (2–4). The anthracyclines contain the anthraquinone nucleus linearly fused to a saturated six-membered ring.

Several fluorometric procedures for the analysis of doxorubicin have been reported (5–7), but little information is available about its acid–base properties and electronic absorption spectra. To obtain information of analytical, biological, and photochemical interest, it is desirable to know accurately in what prototropic form doxorubicin occurs in various media. Such studies are a necessary prerequisite to the spectrometric interpretation of the interactions of doxorubicin with biologically significant macromolecules such as proteins and nucleic acids.

EXPERIMENTAL

Reagents—Doxorubicin[1] was used without further purification.

Analytical reagent grade sulfuric acid[2] was diluted with distilled, deionized water for the solutions used to study the Hammett acidity region (8). Solutions in the pH range were citrate and phosphate buffers and sodium hydroxide solutions in distilled, deionized water.

Each sulfuric acid or buffer solution, in a 10-ml volumetric flask, was injected with 100 μl of a 4.4×10^{-4} *M* stock solution of doxorubicin in a mixed solvent of 10% (v/v) ethanol and 90% distilled, deionized water immediately prior to the taking of spectra to minimize decomposition errors. The tendency of doxorubicin to self-aggregate at higher concentrations dictated the low concentrations ($\sim 4 \times 10^{-6}$ *M*) employed.

Apparatus—Absorption spectra were taken on a spectrophotometer[3]. Fluorescence spectra were taken on a fluorescence spectrophotometer[4] whose monochromators were calibrated against the emission spectrum from a low-pressure mercury lamp and whose output was uncorrected for wavelength variable instrumental response. The pH measurements were made on a pH meter[5] employing a silver–silver chloride–glass combination electrode[6].

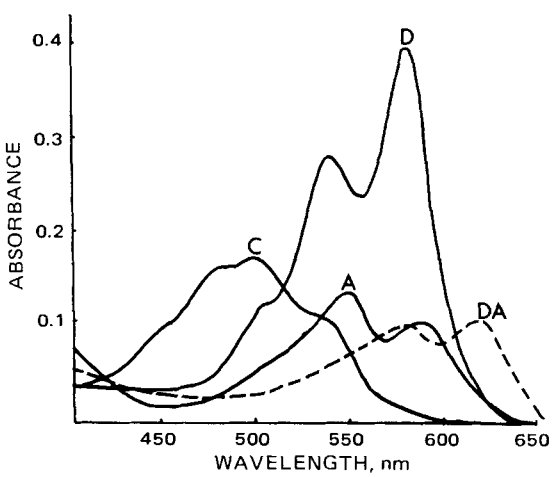


Figure 1—*Electronic absorption spectra of 1.6 × 10⁻⁵ M doxorubicin as the monocation (C), doubly charged cation (D), singly charged anion (A), and doubly charged anion (DA).*

[1] Adriamycin, courtesy of Adria Laboratories, Wilmington, Del.
[2] Mallinckrodt Chemical Works, St. Louis, Mo.
[3] Aminco DW-2.
[4] Aminco–Bowman SPF-1.
[5] Markson Electromark.
[6] Markson 888.

Table II—Negative Logarithms of Dissociation Constants of Doxorubicin

Constant	Value
pK_1	-5.9 ± 0.05
pK_2	8.15 ± 0.07
pK_3	10.16 ± 0.09
pK_{CN}	8.22
pK_{CZ}	9.01
pK_{ZA^-}	9.36
pK_{NA}	10.10
$\frac{[N]}{[Z]}$	6.27 ± 0.02
pK_4	13.2 ± 0.2

Calculations of macro- and microconstants were made employing three analytical wavelengths in the 465–495-nm region, where the molar absorptivity of the phenolate form is minimal compared to the undissociated phenolic form. Within experimental error, the results reported are independent of the wavelength selected.

RESULTS AND DISCUSSION

The electronic absorption and fluorescence spectra of doxorubicin and its various prototropic forms are shown in Figs. 1 and 2, respectively. The maxima along with the corresponding molar absorptivities (log ϵ) of the various species are presented in Table I.

To establish the optimal pH region for the analysis of aqueous solutions of doxorubicin, the ground-state dissociation constants (pKa) for the various prototropic species must be known. The pKa values (Table II) were determined for the dissociation in concentrated sulfuric acid of a proton from a protonated carbonyl group and for the dissociations in alkaline media of a protonated alkyl amino group and two phenolic groups, as shown in Scheme I. The pK_{CN} corresponds to the equilibrium between the singly charged cation and the neutral species; pK_{CZ} corresponds to the equilibrium between the singly charged cation and the zwitterionic species; pK_{AZ} corresponds to the equilibrium between the zwitterion and the singly charged anion, and pK_{NA} corresponds to the equilibrium between the neutral species and the singly charged anion.

In concentrated sulfuric acid, one of the three carbonyl groups of doxorubicin is protonated, the molecule existing as a dication (D). Dissociation of the protonated carbonyl group results in a blue shift of the long wavelength absorption maximum, from which the pKa of −5.9 may be calculated. The only species significantly present in the region between Hammett acidity −4.0 and pH 7.0 is the singly charged species with the positive charge at the amino sugar group. However, at a pH greater than 7.0, this monocation (C) can lose a proton either from a phenolic group to form a zwitterion (Z) or from the amino sugar group to form the neutral species (N). Either may then lose a proton to form the singly charged anion (A^-).

Doxorubicin, like numerous other drugs, contains several ionizable functions, including some of similar acidity. Therefore, overlapping

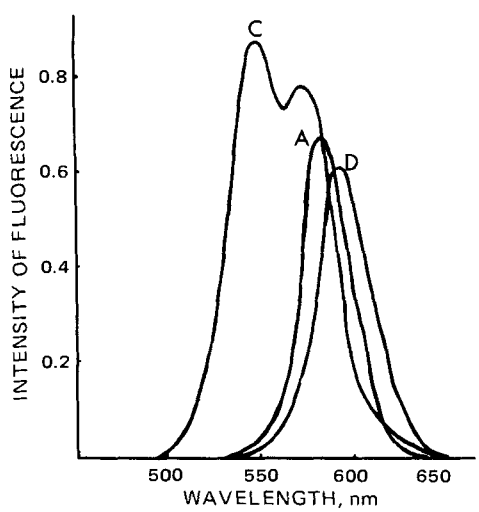


Figure 2—*Fluorescence spectra of doxorubicin as the monocation (C), doubly charged cation (D), and singly charged anion (A).*

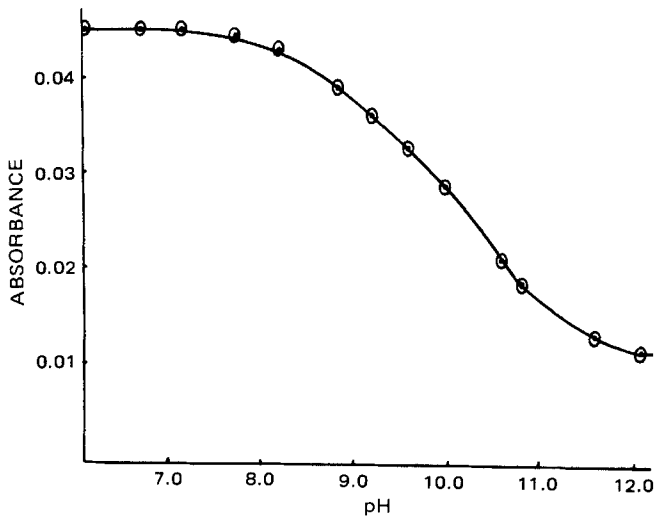


Figure 3—*Plot of absorbance at 495 nm* versus *pH for* 4.4×10^{-6} M *doxorubicin in the pH 7–12 range.*

protolytic equilibria are possible. In doxorubicin, the amino sugar group and a phenolic group both ionized in the pH region from 8 to 13. To calculate accurately the corresponding dissociation constants, the effects of the dissociations on the electronic absorption spectra must be followed carefully. Because the amino group of the amino sugar moiety is several atoms removed from the aromatic chromophore of doxorubicin, electrometric or inductive influences, due to the free or protonated amino group, upon the electronic spectrum of doxorubicin are negligible. Thus, only the dissociation of the phenolic group affects the spectrometric properties of the molecule in alkaline solutions. However, the competing protolytic equilibria involving the amino sugar group affect the rate of appearance of the various spectrometric species of doxorubicin during the pH titration.

While following the spectrometric changes resulting from variations in pH of solutions of doxorubicin in the pH 7–12 region, no distinct isosbestic point was observed. The lack of an isosbestic point is indicative of three or more absorbing species present in the solutions. The overlapping successive protolytic equilibria also result in a wider titration interval than would normally be expected if no competition was observed. Figure 3 shows a plot of absorbance *versus* pH for the titration of doxorubicin in the pH 6.0–12.5 range.

Since dissociation of the zwitterion to form the singly charged anion is spectrometrically undetectable, the molar absorptivities (ϵ_λ) of the monocation and neutral species are equal and those of the zwitterion and anion are equal at the analytical wavelength. The total absorbance (A_T) at the analytical wavelength may be written as the sum of the absorbances of the various species present:

$$A_T = A_C + A_Z + A_N + A_{A^-} = \epsilon_C[C]1 + \epsilon_Z[Z]1 + \epsilon_N[N]1 + \epsilon_{A^-}[A^-]1 \quad \text{(Eq. 1)}$$

The individual stages of ionization have microscopic dissociation constants, denoted as K_{CN}, K_{CZ}, K_{ZA^-}, and K_{NA^-} in Scheme I. The macroscopic constants, K_2 and K_3, have the following relation to the microscopic constants:

$$K_2 = K_{CN} + K_{CZ} \quad \text{(Eq. 2)}$$

$$K_3^{-1} = K_{ZA}^{-1} + K_{NA}^{-1} \quad \text{(Eq. 3)}$$

and since the free energy change in going from C to A^- is the same regardless of whether path C → N → A^- or C → Z → A^- is taken:

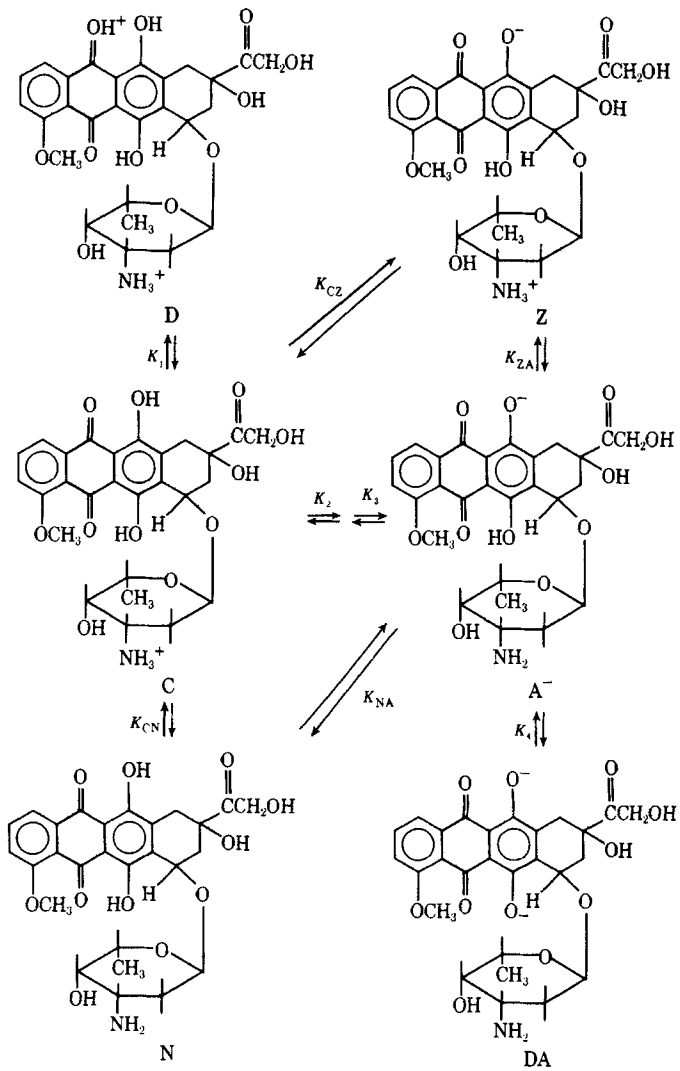
$$K_2K_3 = K_{CN}K_{NA} = K_{CZ}K_{ZA} \quad \text{(Eq. 4)}$$

By letting the sum of the concentrations of the neutral species and zwitterion equal [N′], Eqs. 2–4 may be written as:

$$K_2 = \frac{[H^+][N']}{[C]} \quad \text{(Eq. 5)}$$

$$K_3 = \frac{[A^-][H^+]}{[N']} \quad \text{(Eq. 6)}$$

$$K_2K_3 = \frac{[H^+]^2[A^-]}{[C]} \quad \text{(Eq. 7)}$$



Scheme I

At equilibrium, the mass balance equation states that the sum of the concentrations of all species must equal the total analytical concentration of doxorubicin (C_T):

$$C_T = [C] + [N'] + [A^-] \quad \text{(Eq. 8)}$$

By combining the mass balance equations and the equilibrium expressions, the concentrations of the cation, [C], zwitterion, [Z], neutral, [N], and anion, [A^-], may be written as:

$$[C] = \frac{C_T[H^+]^2}{[H^+]^2 + K_2[H^+] + K_2K_3} \quad \text{(Eq. 9)}$$

$$[N'] = \frac{C_TK_2[H^+]}{[H^+]^2 + K_2[H^+] + K_2K_3} \quad \text{(Eq. 10)}$$

$$[A^-] = \frac{C_TK_2K_3}{[H^+]^2 + K_2[H^+] + K_2K_3} \quad \text{(Eq. 11)}$$

Equation 1 can be rewritten as:

$$A_T = \epsilon_C[C]1 + \epsilon_{N'}[N']1 + \epsilon_{A^-}[A^-]1 \quad \text{(Eq. 12)}$$

Since [N′] is the sum of the concentrations of the neutral and zwitterionic species, $\epsilon_{N'}$ is the expedient molar absorptivity of N′. This ϵ value is essentially a weighted sum of the neutral and zwitterionic molar absorptivities with respect to the fraction of each uncharged species present.

By substituting Eqs. 9–11 into Eq. 12, the following equation results:

$$(A_T - \epsilon_CC_T)[H^+]^2 + (A_T - \epsilon_{N'}C_T)K_2[H^+] + (A_T - \epsilon_{A^-}C_T)K_2K_3 = 0 \quad \text{(Eq. 13)}$$

Equation 13 may now be solved at three different points in the titration

for the values of $\epsilon_{N'}$, K_2, and K_3 (9). With these values (Table II), the microconstants may then be determined. The K_{NZ}, the ratio of the concentration of the neutral species to that of the zwitterionic species, is a constant independent of pH. Since:

$$\epsilon_C[N] + \epsilon_A[Z] = \epsilon_{N'}[N'] \quad \text{(Eq. 14)}$$

and at 495 nm $\epsilon_{N'}$ is equal to 9.10×10^3 cm^{-1} M^{-1}, the following equation can be used to evaluate K_{NZ}:

$$K_{NZ} = \frac{[N]}{[Z]} = \frac{\epsilon_{N'} - \epsilon_A}{\epsilon_C - \epsilon_{N'}} \quad \text{(Eq. 15)}$$

Since [N] = 6.27 [Z], Eq. 2 may be solved for K_{CZ} and K_{CN} as follows:

$$K_2 = \frac{6.27[H^+][Z]}{[C]} + \frac{[H^+][Z]}{[C]} = 7.27\,K_{CZ} \quad \text{(Eq. 16)}$$

Similar substitution into Eq. 3 allows both K_{NA} and K_{ZA} to be calculated.

A similar method for the spectrophotometric evaluation of microconstants involving overlapping equilibria, in which the dissociation of one group affects the absorption spectrum and the other does not, was developed by Edsall *et al.* (10) in studies of the dissociations of tyrosine. This method was applied to the dissociation equilibria of alkanolamines (11). However, the latter approach is somewhat more complicated algebraically than the present one. Moreover, the macroconstants in Edsall's method are calculated from the microconstants. In the present approach, the preliminary evaluation of macroconstants from spectrophotometric data can be checked independently by potentiometry if solubility permits.

The fluorescence spectrum of doxorubicin in the pH region follows the same changes observed in the pH dependence of the absorption spectrum, indicating that protolytic reaction in the excited state is too slow to compete with fluorescence. However, in the Hammett acidity region the fluorescence spectrum of doxorubicin red shifts with increasing acidity in acid solutions more dilute than those in which the corresponding absorption spectral changes occur. This shift is indicative of protonation in the lowest excited singlet state, with the inflection point in the fluorometric titration representing the excited-state dissociation constant (pK*) of −3.1. Doxorubicin becomes more basic in the excited state due to the transfer of charge to a carbonyl group upon excitation.

The method allows the direct calculation of microconstants when overlapping dissociation equilibria are present in a system under spectrometric study. This method is general for any molecule with overlapping equilibria in which dissociation of only one functional group affects the electronic spectral properties of the molecule. The values of the microconstants show that the protonated amino sugar group is slightly more acidic than the phenolic group.

In developing or using any method of analysis for doxorubicin, careful choice of the pH and the analytical wavelength is necessary. The prevalence of only one absorbing species having intense, long wavelength absorption in the pH 4–7 region and one emitting species having intense fluorescence in the pH 1–7 region suggests that the spectrometric analysis of doxorubicin in aqueous solutions be carried out in dilute acidic solutions.

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Determination of Energy Change Associated with Dissolution of a Solid

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Abstract □ The dissolution of a solid immersed in a solvent was considered as a consecutive process, consisting of a primary surface interaction leading to the production of a new surface at the solid–liquid interface, solvation of the solid at the interface, and transfer of the solvated solid into the bulk of the solution. The energy changes involved in each step were studied for the dissolution of *m*-tolylacetamide in hexane and heptane. An energy diagram was constructed according to the proposed dissolution mechanism. The heats of dissolution determined from the energy diagram agreed well with those obtained experimentally.

Keyphrases □ Solids—dissolution in a solvent, energy changes determined □ Dissolution—solid in a solvent, energy changes determined □ Energy changes—determined for dissolution of a solid in a solvent

Several recent investigations concerned the energy changes involved in the dissolution process. Wadke and Reier (1) presented a rate equation, based on previously published results (2–4), involving both the heat of solution of the solid and the activation energy for diffusion. Szinai and coworkers (5, 6), after reviewing several articles on dissolution, proposed a rate equation incorporating an energy term comprised of the energy required to remove a solute molecule from the solid surface, the energy of solvation, and the activation energy for diffusion. A numerical study of the three energy terms was not reported.

The purpose of this study was to quantify the energy associated with initial solvent–solid interaction, the solvation of the solute, and the mass transfer steps of the dissolution process.