Comparison of Bioavailabilities and Dissolution Characteristics of Commercial Tablet Formulations of Sulfamethizole

G. L. MATTOK▲ and I. J. McGILVERAY

Abstract □ Bioavailabilities of samples from one lot of two commercial tablet formulations and two lots of another commercial tablet formulation of sulfamethizole were found not to be significantly different. Blood levels and urinary excretion of drug correlated satisfactorily; thus, bioavailabilities were calculated from areas under the blood level curves and total urinary excretion of drug. The reference dosage form was a suspension of the drug in water. Absorption rate coefficients for the tablet formulations and the suspension were close to the mean (0.5 $hr.^{-1}$) value. Drug was absorbed rapidly from the suspension, while there was a delay (0.8 hr.) in the onset of absorption from the tablets. Dissolution times were measured using four methods, but no correlation of predictive value was obtained between dissolution and absorption parameters. The stationary basket method discriminated best between formulations and is the most useful of the methods for quality control purposes. The delay in absorption of drug from the tablets was not reflected in their *in vitro* dissolution profiles.

Keyphrases □ Sulfamethizole tablets—comparison of bioavailability parameters with dissolution rates □ Bioavailability of sulfamethizole tablets—compared to dissolution rates □ Dissolution rates of sulfamethizole tablets—compared to bioavailability parameters

Many sulfa drugs, such as sulfadiazine (1), sulfamethoxazole (2), sulfisoxazole (3, 4), and sulfamethazine (5), in tablet formulations appear to be susceptible to bioavailability and absorption rate variations. The chemical similarity of sulfamethizole to the other sulfa drugs and its relatively low solubility in water suggest that sulfamethizole in tablet formulations may also be associated with variable absorption. The objective of this study was to compare drug bioavailability parameters of sulfamethizole formulations with dissolution characteristics.

EXPERIMENTAL

Materials—Samples (B, C, D, and E) from commercial production lots of three different products were obtained directly from the manufacturer. Samples D and E, while having different lot numbers, were made by the same manufacturer. Samples B, D, and E had label claims of 500 mg. sulfamethizole/tablet, while C had a label claim of 250 mg./tablet. The pure drug substance was obtained[1] for preparation of the control suspension (A, 500 mg. in 200 ml. water).

***In Vivo* Studies**—Volunteers who had been screened by a physician were asked to refrain from taking any drug or alcohol for 48 hr. prior to drug administration. A standard breakfast of coffee (1 cup), orange juice (100 ml.), cornflakes (individual pack) with milk, and one slice of toast with butter was eaten 1.5 hr. before each dose of sulfamethizole. The various formulations were administered in 500-mg. doses with water (200 ml.) to five volunteers at weekly intervals over 5 weeks according to a 5 × 5 Latin square design. Volunteers also drank 200 ml. of water 1.5 hr. before drug ingestion and then 100 ml. every hour for the next 5 hr. Blood (0.8 ml.) was collected at 0.5, 0.75, 1, 1.5, 2.5, 4, and 6 hr. and urine was collected at 1, 2, 3, 5, 7, 10, 14, and 24 hr. after ingestion of the drug. Blood was obtained from finger micropunctures and processed immediately. Urine samples were stored without preservative in a refrigerator, since the drug had been shown to be stable under these conditions. Unchanged sulfamethizole in blood and urine and total sulfamethizole in urine were estimated in duplicate by the Bratton–Marshall procedure (6).

Treatment of Data—The results were analyzed for variance using an ANOVA computer program. The output gave the variance ratios, *F*, for treatment, person, and time; the standard deviation (*SD*); the standard error (*SE*); and the coefficient of variation percent (*CV*). Application of Tukey's multiple-range test (7) gave a *W* value or least allowable difference, which showed differences significant at the 95% confidence level. The label claim was assumed to represent the total amount of drug potentially available for absorption. Areas under the blood level curves were calculated by the trapezoidal rule. Absorption rates and lag times were calculated graphically from blood level curves by the Wagner–Nelson method (8), which assumes a single-compartment model. There was no evidence of a distribution phase, and the model seemed to fit the data.

Dissolution Rates—The resin kettle (9) and stationary basket (10) methods were used with the modifications and sampling procedures described previously (11). The USP–NF method I, *i.e.*, rotating-basket method (12, 13), was used under the compendial conditions, *i.e.*, dilute hydrochloric acid, 1 in 12.5, and 100 r.p.m. for sulfisoxazole (12). Simulated gastric (0.1 *N* HCl) and intestinal

Table I—Mean Areas under Blood Level Curve (*AUC*) and Bioavailabilities (*BA*) Obtained with Various Commercial Sulfamethizole Tablets

Lot	*AUC*	*BA(B)*[a]	*BA(U)*[b]
A	58.23	100.0	100
B	56.31	96.7	92
C	63.98	110.0	107
D	56.18	96.5	100
E	56.68	97.3	109
SE	3.75	—	—
CV	14.53	—	—
W	16.91	—	—
F (treatment)	1.12	—	—
F (person)	2.32	—	—

[a] % *BA(B)* = (*AUC* for dosage form/*AUC* for control) × 100. [b] *BA(U)* is bioavailability calculated from total urinary excretion of drug.

Table II—Mean Blood Levels of Free Sulfamethizole (mcg./ml.) after Administration of Each Formulation to Five Subjects

Lot	Minutes 30	45	60	90	150	240	360
A	12.70	15.86	17.84	16.86	11.74	6.82	1.78
B	2.68	7.50	14.74	17.50	14.50	6.84	2.34
C	2.14	3.56	8.12	18.74	16.84	9.86	3.44
D	1.00	2.36	4.76	15.74	16.02	8.00	2.30
E	2.68	5.58	8.72	11.96	13.04	10.64	3.56
SE	1.13	1.80	1.93	2.72	1.73	1.11	0.44
CV	59.73	57.88	39.80	37.60	26.86	29.56	36.30
W[a]	15.11	8.14	8.70	12.26	7.82	5.03	1.97
F (treatment)	7.80	8.76	7.60	0.91	1.46	2.46	3.19
F (person)	1.18	1.84	4.23	2.79	0.24	1.01	2.99

[a] Tukey's test of significance (7).

[1] Lot No. 2588-NF, Ayerst, McKenna and Harrison Ltd., Montreal, Canada.

Table III—Mean Absorption and Elimination Characteristics of Sulfamethizole from Different Formulations

Lot	Lag time, hr.	$t_{1/2}$ (Absorption), hr.	$t_{1/2}$ (Elimination), hr.	Peak Blood Level[a], mcg./ml.	Time to Peak, hr.[b]
A	0	0.56	1.32	18.8	1.1
B	0.86	0.38	1.26	21.7	1.5
C	0.71	0.59	1.34	21.5	2.4
D	0.84	0.53	1.24	18.0	2.1
E	0.91	0.66	1.37	17.8	2.6[c]

[a] $W = 8.15$. [b] $W = 1.46$. [c] Significantly different than control (A).

(0.05 *M* phosphate buffer, pH 7.4) fluids were also used. Assays were based on absorbance measurements at 267 nm. In this paper, t_x indicates the time for $x\%$ of the labeled amount of drug in a tablet to dissolve; $\bar{t}_x$ is the mean of six successive t_x determinations.

Disintegration Times—The official method (14) was used.

RESULTS AND DISCUSSION

Blood Data—No drug was detected in the blood 8 hr. after ingestion of sulfamethizole; bioavailabilities of each formulation were calculated, therefore, from the mean values of the areas under the blood level curves from 0 to 8 hr. No significant differences ($p < 0.05$) between the bioavailabilities of the control suspension (A) and the four commercial formulations were detected (Table I). However, mean concentrations of the drug in blood observed at several times (Table II) showed that there were differences in the extent of absorption in the 1st hr. after ingestion. Relatively high concentrations of drug from the control (A) were detected at the time of the first sample (30 min.), and it was not until 90 min. after ingestion that the mean drug concentrations from the four tablet formulations and the suspension were comparable. This did not appear to be due to differences in rates of absorption but rather to a lag between ingestion of the tablets and the attainment of the maximum rate of absorption (Table II). Similar delays were reported previously for sulfamethizole (15) and sulfisoxazole (4, 16) tablet formulations.

Absorption rate (8) coefficients for all of the formulations tested were close to the mean (0.5 $hr.^{-1}$), while the control showed no lag time and the four tablet formulations showed a mean lag time of 0.8 hr. These values reflect the peak blood level data and times to achieve these levels (Table III). The similarity of the absorption rate coefficients for the control suspension and the tablet formulations suggests that this value (0.5 $hr.^{-1}$) may be the maximum that can be achieved with this drug. There were no significant differences in the maximum concentrations of drug in blood, but Formulation E required a significantly longer time (2.6 hr.) to reach the maximum level than did the control (A, 1.1 hr.). The mean elimination (β) half-life from blood (1.3 ± 0.12 *SE* hr.) was shorter than previously reported values, *i.e.*, 1.6 (17), 1.8 (15), and 2.1 hr. (18). Mean values for four of the five volunteers were in close agreement, but one volunteer showed a longer half-life of elimination (1.8 hr.) than the overall mean (1.3 hr.).

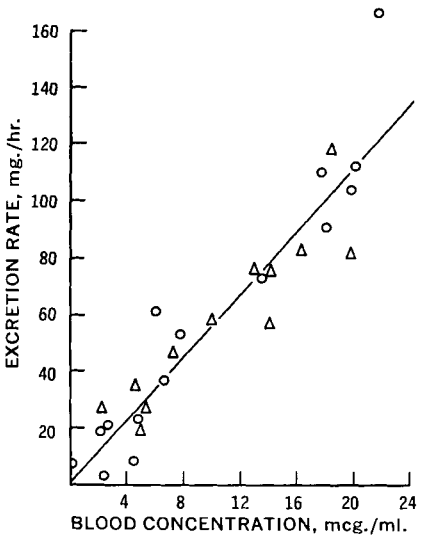

Figure 1—*Linear relationship between concentration of sulfamethizole in blood and urinary excretion rate for two subjects* (○ *and* △).

Urine Data—Urinary excretion rates correlated well with the corresponding blood concentrations (Fig. 1). The average renal clearance rate of the drug was 91 ml./min., which is within the range (67–188 ml./min.) found by Frisk (19). These results indicate that urinary excretion data are useful for estimating the bioavailabilities of the different formulations of sulfamethizole. There were no significant differences in the total amounts of drug excreted after ingestion of the control suspension or the four tablet formulations (Table IV). Urinary recoveries of total sulfamethizole ranged from 74 to 100%, with 12–18% present as the acetylated metabolite. Robinson *et al.* (18) reported 61–99% total excretion of sulfamethizole, with 6–23% present as the metabolite. Nelson and O'Reilly (20) found

Table IV—Mean Cumulative Urinary Excretion of Unchanged (*U*) and "Total" (*T*) Sulfamethizole for Five Different Formulations

Lot	Assay	Milligrams Excreted at Time (hr.) after Ingestion: 1.0	2.0	3.0	5.0	7.0	10.0	14.0	24.0
A	*U*	63.4	186.6	261.2	333.0	361.0	374.4	381.9	382.9
	T	70.1	205.8	291.3	377.5	423.6	443.9	456.3	458.3
B	*U*	15.2	120.2	217.1	310.0	347.9	364.9	370.8	371.1
	T	18.8	135.0	239.1	342.1	389.1	412.1	421.1	423.0
C	*U*	7.4	106.6	222.5	335.6	379.8	401.3	410.2	413.2
	T	8.4	121.3	255.6	391.6	444.9	472.5	485.1	491.5
D	*U*	3.5	83.8	183.7	288.0	337.1	363.6	371.5	371.7
	T	4.2	89.8	231.7	352.8	415.5	447.3	454.8	459.3
E	*U*	29.5	111.3	210.1	333.3	393.7	414.9	425.9	434.2
	T	31.9	116.4	225.9	372.5	446.8	474.6	488.4	497.8
SE	*U*	8.6	16.4	18.7	13.0	14.6	15.3	16.7	18.4
	T	9.1	15.6	23.9	17.4	16.5	17.0	18.3	19.8
CV	*U*	80.7	30.1	19.1	9.1	9.0	8.9	9.5	10.4
	T	76.6	26.0	21.5	10.6	8.7	8.5	8.9	9.5
W[a]	*U*	38.8	74.0	84.1	58.9	65.8	69.1	75.3	82.8
	T	41.2	70.2	107.9	78.5	74.2	76.7	82.6	89.4
F (treatment)	*U*	8.0	5.6	2.2	2.5	2.5	2.3	2.2	2.3
	T	8.4	7.8	1.2	1.3	2.1	2.2	2.2	2.3
F (person)	*U*	1.9	3.6	2.9	5.5	5.0	4.6	3.7	2.9
	T	1.9	4.3	1.7	1.7	2.0	1.2	0.9	0.6

[a] Tukey's test of significance (7).

Table V—Dissolution Times and Coefficients of Variation (*CV*) of Sulfamethizole from Four Different Commercial Formulations

Formulation	Method[a]	t_{50}	*CV*
B	SB	86	54
	RK	4.2	6
	RK(intestinal)[b]	5,5	—
	USP	5.8	10
C	SB	4.9	46
	RK	3.5	30
	RK(intestinal)[b]	6,4	—
	USP	3.0	0
D	SB	43	26
	RK	26	15
	RK(intestinal)[b]	13,13	—
	USP	6.1	28
E	SB	31	33
	RK	7.4	25
	RK(intestinal)[b]	8,6	—
	USP	5.4	15

[a] RK, resin kettle; SB, stationary basket; USP, USP XVIII (rotating basket). The dissolution fluid was diluted hydrochloric acid solution. [b] t_{50} values in intestinal buffer.

80–100% of the dose present in urine, with up to 23% present as the acetylated metabolite.

Differences in apparent absorption rate are also evident from the mean urinary excretion profiles (Table IV). The control suspension (A) yielded significantly greater amounts of drug than did B, C, and D in the 1-hr. urine sample and D and E in the 2-hr. sample. The mean half-life of elimination (β) of the drug from urine was 1.5 ± 0.05 *SE* hr., which is in agreement with 1.5 hr. reported by Nelson and O'Reilly (20). This value is probably more precise than that (1.3 hr.) calculated from the blood data, since more data points were obtained in the urine study. All formulations and subjects showed mean half-lives of elimination of unchanged drug that are close to the overall mean value.

Dissolution Data—There were pronounced differences in dissolution rates of the four tablet formulations when the stationary basket method (10) (gastric buffer) was used. In the other methods, dissolution times were relatively fast and comparable for all tablets tested (Table V). Dissolution times were not affected markedly when intestinal buffer was used instead of the gastric fluid in the resin kettle method (Table V).

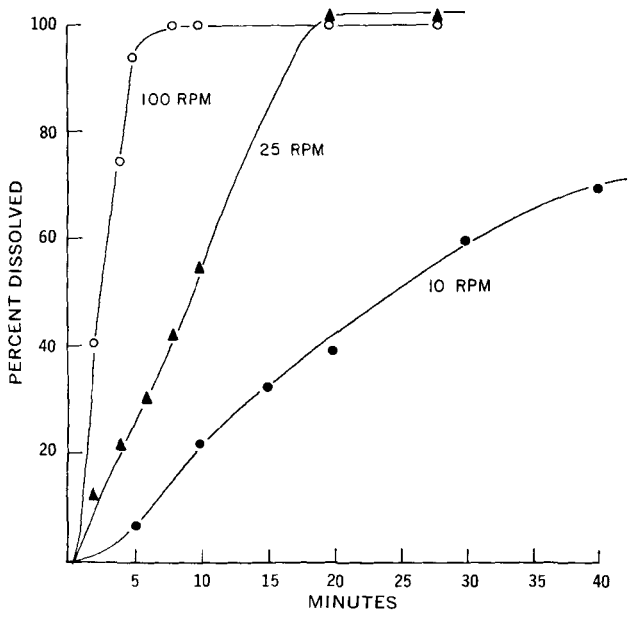

Figure 2—*Single-tablet dissolution profiles for a sulfamethizole formulation (C) obtained in dilute hydrochloric acid (1:12.5) with the USP XVIII method and three stirring rates.*

The *in vivo* studies showed that the control suspension and tablet formulations had comparable bioavailabilities and rates of absorption but that the tablets were distinguished from the suspension by a delay before the onset of absorption of drug from the tablets. Aguiar *et al.* (21) pointed out that often the aggregates formed during the preparation of the dosage forms are difficult to disperse and the deaggregation or dispersion rate may then be the rate-limiting step in the absorption sequence. The delay observed in the absorption of sulfamethizole may be attributable to the time required for the tablet to disintegrate and disperse the drug so that it is in a similar state to the suspension after administration. Thus, any dissolution test that correlates with the *in vivo* biopharmaceutical parameters should ideally show comparable dissolution times for the four tablet formulations and a lag time before dissolution. The latter would correspond to the disintegration and dispersion of the tablet. None of the dissolution tests used met these requirements. For the most part, dissolution was so rapid that even if a relatively slow disintegration or dispersion step existed, it would be masked. However, a disintegration step was not evident when the stirring rate in the USP XVIII apparatus was reduced from 100 to 25 and then to 10 r.p.m.; the t_{50} for Formulation C increased from 3 to 9 to 24 min. (Fig. 2). When the stationary basket method (10) was applied, two of the tablet formulations (D and B) had t_{50} values of approximately 40 and 90 min., respectively (Table V). Even under these conditions of slow dissolution, there was no suggestion of delayed dissolution of the drug.

Disintegration times did not help in the appreciation of the *in vivo* observations. Disintegration times for B, C, and E were 2, 4, and 3 min., respectively. The corresponding value for D was 22 min., which is longer than its dissolution time (t_{50}) with the USP and resin kettle methods. This distinctive disintegration time for D was not reflected by differences from the other formulations in its dissolution profiles with any of the methods used, nor in the *in vivo* delay in, or extent of, absorption. Since the various dissolution tests do not detect established *in vitro* differences in disintegration time, it is not surprising that the same tests do not show *in vivo* differences. Immediate and rapid absorption of the drug is a desirable feature of sulfamethizole dosage form design. Therefore, an appropriate dissolution test should disclose the delay in dissolution as well as the rate of dissolution. When such a test, with a good *in vivo/in vitro* correlation, is devised, it would be possible to establish an appropriate dissolution time limit. In view of the reported delayed absorption of sulfisoxazole from tablet formulations (4, 15), similar considerations may well apply to this drug.

Both the stationary basket (10) and resin kettle (9) methods discriminated among and within the different lots more efficiently than the USP XVIII method (Table V). Thus, the stationary basket and resin kettle methods detected differences that were not apparent from the *in vivo* data. If formulation effects on dissolution rate or quality control are the main considerations, then the stationary basket appears to be the method of choice. The USP XVIII method gave similar dissolution times (3–6 min.) for the four formulations. This method provided a good rank-order correlation and the best linear correlation [*BA(B) versus* t_{50}]. However, the latter had a correlation coefficient (r = 0.36; $100r^2$ = 13.0) indicating that the correlation is not of predictive value (*cf.*, *Reference 22*).

REFERENCES

(1) G. R. Van Petten, G. C. Becking, R. J. Withey, and H. F. Lettau, *J. Clin. Pharmacol.*, **11**, 27(1971).

(2) G. H. Schneller, *Amer. J. Hosp. Pharm.*, **27**, 485(1970).

(3) C. M. Martin, M. Rubin, W. E. O'Malley, V. F. Garagusi, and C. E. McCauley, *Pharmacologist*, **10**, 167(1968).

(4) G. R. Van Petten, G. C. Becking, R. J. Withey, and H. F. Lettau, *J. Clin. Pharmacol.*, **11**, 35(1971).

(5) M. J. Taraszka and R. A. Delor, *J. Pharm. Sci.*, **58**, 207 (1969).

(6) A. C. Bratton and E. K. Marshall, Jr., *J. Biol. Chem.*, **128**, 537(1939).

(7) G. W. Snedecor, "Statistical Methods," Iowa State College Press, Ames, Iowa, 1956, p. 251.

(8) J. G. Wagner and E. Nelson, *J. Pharm. Sci.*, **53**, 1392(1964).

(9) R. A. Castello, G. Jellinek, J. M. Konieczny, K. C. Kwan, and O. R. Toberman, *ibid.*, **57**, 485(1968).

(10) D. Cook, H. S. Chang, and C. A. Mainville, *Can. J. Pharm. Sci.*, **1**, 69(1966).
(11) G. L. Mattok, I. J. McGilveray, and C. A. Mainville, *J. Pharm. Sci.*, **60**, 561(1971).
(12) "The United States Pharmacopeia," 18th rev., Mack Publishing Co., Easton, Pa., 1970.
(13) "The National Formulary," 13th ed., Mack Publishing Co., Easton, Pa., 1970.
(14) *Ibid.*, p. 878.
(15) M. Hanano, *Chem. Pharm. Bull.*, **15**, 994(1967).
(16) A. P. Goosens and M. C. B. Van Oudtshoorn, *S. African Med. J.*, **43**, 1370(1969).
(17) F. H. Dost, *Arzneim.-Forsch.*, **3**, 182(1953).
(18) M. J. Robinson, A. Bondi, and J. V. Swintosky, *J. Amer. Pharm. Ass., Sci. Ed.*, **47**, 874(1958).
(19) A. R. Frisk, *Acta Med. Scand.* (Suppl. CXLII), 1943.
(20) E. Nelson and I. O'Reilly, *J. Pharm. Sci.*, **50**, 417(1961).
(21) A. J. Aguiar, J. E. Zelmer, and A. W. Kinkel, *ibid.*, **56**, 1243(1967).
(22) J. G. Wagner, *Drug Intel. Clin. Pharm.*, **4**, 160(1970).

ACKNOWLEDGMENTS AND ADDRESSES

Received October 21, 1971, from the *Research Laboratories, Food and Drug Directorate, Ottawa, Ontario, K1A 0L2, Canada.*
Accepted for publication January 26, 1972.
The authors thank Miss A. Latour and Mr. C. A. Mainville for technical assistance.
▲ To whom inquiries should be directed.

Phototautomerism of Warfarin Cation in Lowest Excited Singlet State *via* an Intramolecular Hydrogen Bridge

G. J. YAKATAN*, R. J. JUNEAU, and S. G. SCHULMAN▲

Abstract □ The pH and Hammett acidity dependence of the fluorescence spectra of 4-hydroxycoumarin and warfarin were studied. Warfarin demonstrates an anomalous long wavelength emission in moderately concentrated sulfuric acid solutions. The emission properties of methylated derivatives of 4-hydroxycoumarin and warfarin were employed to show that the anomalous fluorescence of warfarin is due to a protonated zwitterionic excited species, formed by intramolecular proton transfer in the excited state of the warfarin cation from the hydroxyl group to the acetonyl oxygen atom. The long wavelength of the warfarin fluorescence in sulfuric acid may be useful in the selective fluorometric determination of warfarin in the presence of other 4-hydroxycoumarin derivatives.

Keyphrases □ Warfarin—pH and Hammett acidity dependence of fluorescent spectra, phototautomerism □ 4-Hydroxycoumarin—pH and Hammett acidity dependence of fluorescent spectra □ Phototautomerism—warfarin cation in the lowest excited singlet state □ Spectrophotofluorometry—analysis, warfarin and 4-hydroxycoumarin

Coumarin and several of its hydroxylated derivatives have found wide applications in analytical biochemistry and pharmacy. 7-Hydroxycoumarin (umbelliferone) has been employed as a fluorescent indicator in fluorometric titrations and as a fluorogenic substrate in the study of the kinetics of hydrolytic reactions catalyzed by phosphatase and sulfatase (1–4). Several derivatives of 4-hydroxycoumarin, notably warfarin and dicumarol (bishydroxycoumarin), are used extensively as anticoagulants in the prevention and therapy of thromboembolic disease.

Studies of the pH dependence of the fluorescences of 4-methylumbelliferone (hymecromone) and some related model compounds (5) have recently been employed to show that the red shifting of the blue fluorescence of the umbelliferone anion in the region near pH 2 is due to the formation of a zwitterion in the lowest excited singlet state, a species that is not measurably present in the ground electronic state of umbelliferone.

Solvent and acidity dependence studies of the fluorescences of drugs and related molecules are important from the standpoints of both the analytical chemistry and the photochemistry of pharmaceutically important compounds. From the analytical point of view, solvent and acidity studies are useful for the determination of the optimal emission wavelengths at which to carry out fluorometric analyses. Because the acidity dependence of fluorescence wavelengths frequently depends upon the thermodynamic parameters (*e.g.*, dissociation constants) of the excited molecules, rather than (or as well as) the ground state thermodynamic parameters, it is important to determine the excited state dissociation constants (pKa*) as well as the ground state dissociation constants (pKa) of drug molecules (having dissociable protons or basic groups capable of accepting protons). Due to the differences in electronic distributions between ground and electronically excited molecules, the pKa* values are generally quite different from the pKa values; thus, the pH regions in which the fluorescence spectra change are generally different from the pH regions in which the absorption spectra change as one prototropic form is converted to another. The knowledge of pKa* values of molecules is critical to the selection of the optimal solution acidity in which to carry out fluorometric determinations.

From the photochemical point of view, the shelflives of drugs and the phototoxic actions of some drugs are dependent upon the action of light on these compounds. While the photochemical reactions leading to decomposition and phototoxic action of drugs are probably primarily the results of the reactivities of the excited