Degradative Behavior of Cadralazine in Aqueous Solution

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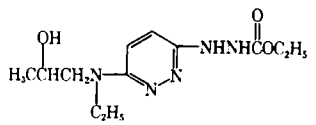
Abstract □ The degradation of cadralazine in aqueous solution was investigated in relation to pH, concentration, and temperature, under aerobic and anaerobic conditions in light and dark. The appearance of three main degradation products was also studied. Drug disappearance was dependent on temperature, pH, concentration, and oxygen and followed three different mechanistic pathways: thermal, hydrolytic, and oxidative. The individual rate constants were determined by nonlinear regression fitting.

Keyphrases □ Cadralazine—degradation kinetics, antihypertensive agent, identification of oxidative, hydrolytic, and thermal degradation products □ Antihypertensive agent—cadralazine, degradation kinetics, identification of oxidative, hydrolytic, and thermal degradation products □ Degradation kinetics—cadralazine, antihypertensive agent

Cadralazine, ethyl 6-[ethyl-(2-hydroxypropyl)amino]-3-pyridazinecarbazate (I) (1), is a new potent peripheral vasodilator (2, 3) which has been shown to be active in hypertensive patients (4–11). Like the chemically related antihypertensive drugs hydralazine (12) and pildnalazine (13), cadralazine is stable in the solid state, whereas it is susceptible to decomposition in water.

A study was therefore initiated to identify the degradation products of I; it was performed in stress conditions that are different than the physiological ones, in order to accelerate the decomposition processes. Further investigations were conducted to establish the kinetics of cadralazine degradation. The effects of pH, temperature, drug concentration, and other factors on cadralazine decomposition and on the appearance rate of the main degradation products were also investigated.



I

EXPERIMENTAL SECTION[1]

Cadralazine (I), the working standard (14) [MS: *m/z* 283 (37), 238 (100), 210 (50), 192 (53) and 164 (60)] was used as received. 6-[Ethyl-(2-hydroxypropyl)amino]-1,2,4-triazolo[4,3-*b*]pyridazin-3(2*H*)-one (II) was synthesized according to the method of Parravicini *et al.* (1) [MS: *m/z* 237 (12), 192 (38), and 164 (100)]. *N*-Ethyl-*N*-(2-hydroxypropyl)-6-oxo-3(1*H*)-pyridazinylamine (III), synthesized by the hydrolysis of 6-chloro-*N*-ethyl-*N*-(2-hydroxypropyl)-3-pyridazinylamine (15) by a literature method (13), was obtained as a white powder, mp 106–107°C; MS[2]: *m/z* 197 (15% $M^{\ddagger}$), 152 [100, $(M - CH_3CHOH)^+$], 139 (20), and 124 (40); ^{1}H-NMR[3] (60 Hz, Me_2SO-d_6): δ 7.25 and 6.78 (ABq, 2, *J* = 10 Hz, H-5 and H-4), 4.25 (br s, 1, OH), 4.00 (m, 1, CH), 3.46 (q, 2, *J* = 6.5 Hz, NCH_2CH_3), 3.22 (d, 2, *J* = 6.5 Hz, NCH_2CH), 1.15 (d, 3, *J* = 5.3 Hz, CH_3CH), and 1.10 ppm (t, 3, *J* = 6.5 Hz, CH_2CH_3).

Anal.—Calc. for $C_9H_{15}N_3O_2$: C, 54.81; H, 7.66; N, 21.30. Found: C, 54.56; H, 7.82; N, 21.51.

N-Ethyl-*N*-(2-hydroxypropyl)-3-pyridazinylamine (IV), synthesized by hydrogenolysis of 6-chloro-*N*-ethyl-*N*-(2-hydroxypropyl)-3-pyridazinylamine (15) by a literature method (13), was obtained as a pale-yellow oil; MS[2]: *m/z* 181 (22% $M^{\ddagger}$), 136 [100, $(M - CH_3CHOH)^+$], 123 (30), and 108 (90); ^{1}H-NMR[3] (60 Hz, $CDCl_3$): δ 8.44 (dd, 1, *J* = 4.6, 1.0 Hz, H-6), 7.18 (dd, 1, *J* = 4.6, 9.5 Hz, H-5), 6.90 (dd, 1, *J* = 9.5, 1.0 Hz, H-4), 5.00 (br s, 1, OH) 4.10 (m, 1, CH), 3.56 (d, 2, *J* = 6.2 Hz, NCH_2CH), 3.53 (q, 2, *J* = 7.0 Hz, NCH_2CH_3), 1.22 (d, 3, *J* = 7.0 Hz, CH_3CH), and 1.18 ppm (t, 3, *J* = 7.0 Hz, CH_2CH_3).

Anal.—Calc. for $C_9H_{15}N_3O$: C, 59.66; H, 8.34; N, 23.19. Found: C, 60.00; H, 8.04; N, 23.34.

Ethyl 2-[6-[ethyl-(2-hydroxypropyl)amino]-3-pyridazinyl]azocarboxylate (V) was synthesized according to Gaviraghi *et al.* (16) [MS: *m/z* 283 (28), 238 (48), 237 (32), 181 (36), and 136 (100)]. The following internal standards were used: 1-[3,4,5-trimethoxythiobenzoyl]-4-methylpiperazine hydrochloride (VI) synthesized according to Farina *et al.* (17) and (L)-α-(3,4,5-trimethoxythiobenzamido)caprolactam (VII) synthesized according to Farina *et al.* (18). All the compounds synthesized gave the expected NMR and mass spectral data.

Identification of the Degradation Products—Aliquots (25 mL) of a 0.1% (w/v) cadralazine (I) solution in phosphate buffer (pH 7.4) were kept overnight at 80°C in a water bath under constant tungsten light, then extracted three times with 40 mL of chloroform. The organic layer was evaporated to dryness, and the residue was dissolved in 5 mL of methanol; 200 μL of the solution was spotted and eluted on silica gel plates with the following eluants: chloroform–methanol–water (95:10:1), chloroform–methanol–water (70:27:5), 1-butanol–acetic acid–water (80:20:20), benzene–methanol (80:20), tetrahydrofuran–methanol (96:4); on aluminum oxide plates with methyl ethyl ketone–methanol–chloroform (40:5:55); on silanized silica gel plates with benzene–ethyl acetate–acetonitrile–methanol (35:45:15:5). The spots were detected under UV light, scraped, examined by MS[2] and compared with synthetic samples.

Decomposition Rate of Cadralazine—The degradations were performed on 0.1% solution of cadralazine buffered at pH 3.0, 5.0, 7.4, and 10.0, and stored at 30 ± 0.1°C, 50 ± 0.1°C, and 80 ± 0.1°C in air and light; analogous solutions buffered at pH 0.2 and 1 were followed at 50 ± 0.1°C in the same conditions. Degradations at pH 7.4 and 10 were also studied at 80°C in a nitrogen atmosphere (both in light and in the dark) and in a dark and aerobic atmosphere. Solutions of 0.093, 0.93, 5.15, 10.3, and 18.3 mg/mL of cadralazine in acetate buffer (pH 5.0) were tested at 80 ± 0.1°C in a light and aerobic atmosphere. All samples were taken at various intervals. The internal standard VI was added, and the samples were suitably diluted and assayed by TLC–photodensitometry[4] at 254 nm as previously described (19).

Appearance Rate of II, III, and IV—The appearance rates of II, III, and IV were tested on 0.1% solutions of cadralazine buffered at pH 7.4 and 10.0, submitted to decomposition at 80 ± 0.1°C in an aerobic or a nitrogen atmosphere, in light or dark. Quantitation was performed by adding VII (an internal standard for II and III) and VIII (an internal standard for IV) to the samples. The undiluted solutions were assayed by TLC–photodensitometry as previously described (19).

RESULTS AND DISCUSSION

Characterization of the Degradation Products—TLC of the degraded cadralazine mixture in several eluant systems indicated the presence of four major and three minor compounds. The degradation products were isolated (TLC) and characterized by comparison of their R_f values and MS data with those of authentic samples of I–V (1, 16). The four major compounds were identical with cadralazine (I) (1), triazolone II (1), pyridazinone III, pyridazine IV; one of the minor compounds was the azocarboxylate V (16, 20). Two

[1] All reagents and solvents were analytical grade. Hydrochloric acid–potassium chloride buffer (Normex, pH 0.2 and 1.0, respectively), sodium citrate buffer (pH 3.0), potassium phthalate buffer (pH 5.0), sodium acetate buffer (pH 5.0), potassium phosphate buffer (pH 7.4), and sodium borate buffer (pH 10.0) were purchased from Carlo Erba, Milan, Italy. Sodium chloride was added to all buffers to a constant ionic strength (μ = 0.115). TLC plates silica gel 60 F 254, HPTLC silica gel 60 F 254, silanized silica gel 60 F 254, and aluminum oxide type E F 254 were obtained from Merck, Darmstadt, West Germany.

[2] Varian Mat 112 spectrometer (70 eV, 1.5 mA).

[3] Perkin-Elmer R12B.

[4] Camag TLC scanner; Camag, Switzerland, equipped with a Perkin-Elmer 56 recorder.

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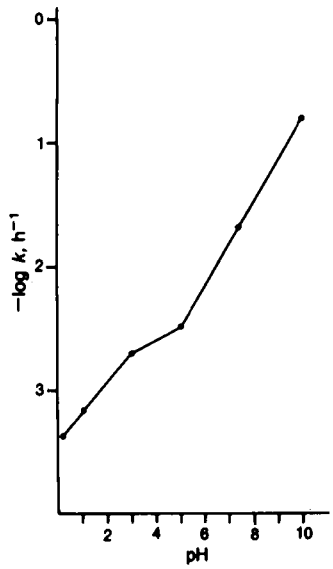


Figure 1—*The pH-rate profile of cadralazine (0.1%, w/v) in aqueous solutions at 50°C.*

of the minor products were not identified. Compounds II, III, IV, and V were treated under the same conditions used for cadralazine degradation, and no decomposition was found except for V, which rapidly converted to III and IV.

Order of Reaction—Semilogarithmic plots of residual concentrations of cadralazine *versus* time exhibited good linearity ($r^2 > 0.98$) for experiments at all pH levels studied. The disappearance of cadralazine from aqueous solutions followed pseudo-first-order kinetics under the experimental conditions. The degradation rate constants of cadralazine were computed by the least-squares linear regression method. The calculated pseudo-first-order rate constants at fixed concentration of cadralazine (0.1%, w/v), buffer ionic strength ($\mu = 0.115$) and temperature (50 ± 0.1°C) were: 4.17×10^{-4} h^{-1} at pH 0.2; 6.72×10^{-4} h^{-1} at pH 1; 2.00×10^{-3} h^{-1} at pH 3; 3.17×10^{-3} h^{-1} at pH 5; 2.14×10^{-2} h^{-1} at pH 7.4; 0.1570 h^{-1} pH 10.

pH Dependency—The pH-rate profile for the degradation of cadralazine was constructed from the logarithm of the pseudo-first-order rate constants and the pH values at 50 ± 0.1°C. The buffering capacities of all the solutions were sufficient to keep constant pH values (±0.05) throughout the experiment. The pH-rate profile (Fig. 1) showed a sigmoid curve superimposed on one branch of a V-graph and revealed a profound degradation rate increase in the neutral and basic pH region, where cadralazine was in the neutral form. In the acidic pH region, the degradation rate appeared low, whereas between pH 3 and 5 it was less sensitive to pH changes. At pH 7.4 and 50°C, the apparent first-order rate constant was 2.14×10^{-2} h^{-1}, which corresponds to a half life of ~32.4 h.

Concentration Effect—The effect of cadralazine concentration on its degradation constant was investigated at 80°C in pH 5 acetate buffer. As shown in Fig. 2, the highest concentrations stabilized the solutions of cadralazine.

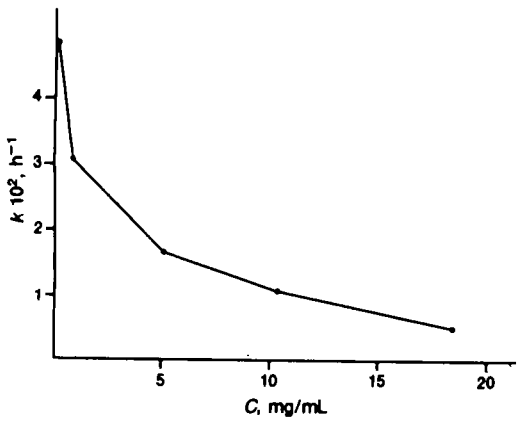


Figure 2—*Influence of cadralazine concentration on the rate degradation constants at 80°C and pH 5.0.*

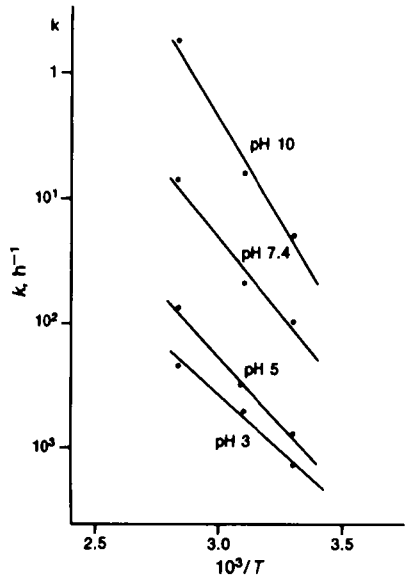


Figure 3—*Arrhenius plot depicting the temperature dependency of cadralazine degradation.*

Table I—Arrhenius Parameters for Degradation of Cadralazine

pH	$E^{\neq}$, kcal/mol	log A	r^2
3	7.72	2.46194	0.976
5	10.12	4.38242	0.997
7.4	11.00	5.89449	0.963
10	15.36	9.74349	0.983

Influence of Temperature, Light, and Air on Cadralazine—The Arrhenius plots of the pseudo-first-order rate constant (k) at varying temperatures (30, 50, and 80 ± 0.1°C) at different pH levels (10, 7.4, 5, and 3) are shown in Fig. 3. The apparent Arrhenius parameters for the disappearance of cadralazine, calculated from the regression equations, are reported in Table I. The degradation of cadralazine was greatly influenced by the presence of air and, to a lesser extent, by light, as shown in Fig. 4.

Light, pH and Air Effects on Formation of II, III and IV—The appearance rate of triazolone II at pH 7.4 and 80°C (Fig. 5A) was independent of the presence of air or light, whereas its formation rate was moderately pH dependent. Moreover, it has been reported that II is obtained from I by thermal cyclization (1). The weak influence of pH and the independence of the formation of II from light and air suggests that the mechanism of formation of II may be essentially thermal in degradative conditions.

In contrast, the appearance rate of pyridazinone III (Fig. 5B) was independent of the presence of air, greatly dependent on pH, and moderately influenced by the presence of light. This excludes an oxidative mechanism in its formation and suggests a hydrolytic pathway.

Finally, pyridazine IV was not detected in the absence of air, whereas its formation was independent of light, and moderately related to the increase in pH (Fig. 5C). This behavior is essentially in agreement with an oxidative reaction.

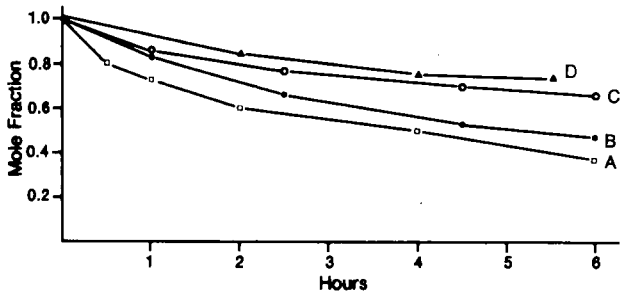


Figure 4—*Experimental time-concentration profile of cadralazine at 80°C and pH 7.4 in light and air (A), dark and air (B), light and nitrogen (C), and dark and nitrogen (D).*

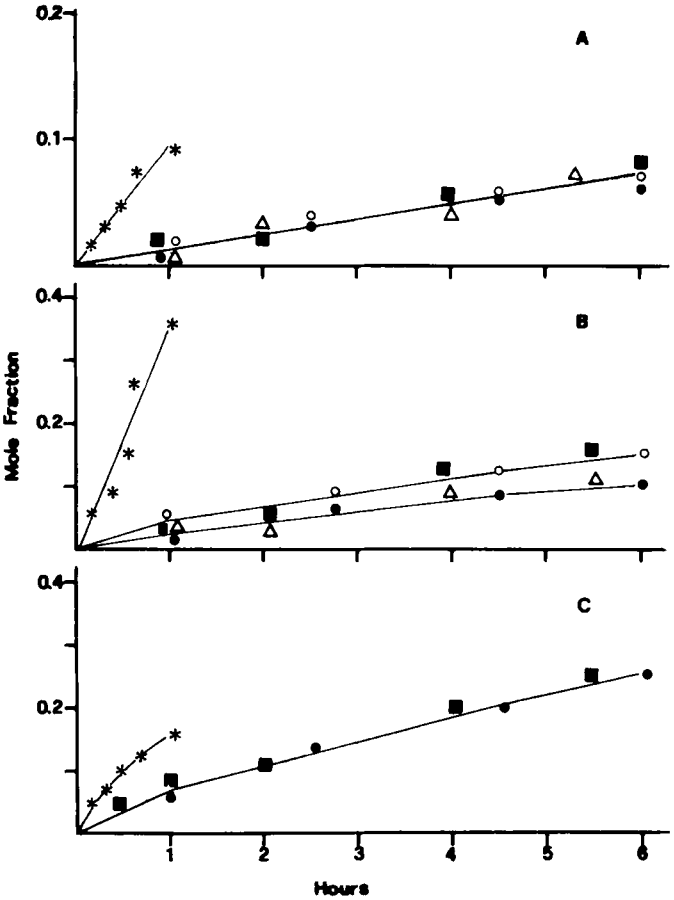
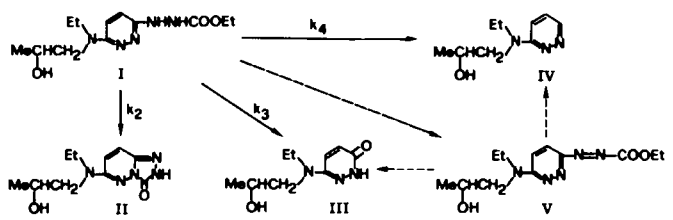


Figure 5—*Experimental time-concentration profile of II (A), III (B), and IV (C) at 80°C in pH 10, light and air (*), pH 7.4, light and air (■), light and nitrogen (○), dark and air (●), and dark and nitrogen (▲). No traces of IV were found at pH 7.4 in light and nitrogen, or in dark and nitrogen.*

Possible Degradation Pathway—The degradation of cadralazine yields triazolone II, pyridazinone III, pyridazine IV, and minor quantities of azocarboxylate V. Cadralazine decomposition at pH 7.4 and 80°C can be hypothesized as a combination of three parallel reactions (Scheme I). On the basis of this hypothesis, the disappearance of cadralazine can be described as a pseudo-first-order process expressed by:

$$-\frac{d[I]}{dt} = (k_2 + k_3 + k_4)[I] = k_1[I] \qquad \text{(Eq. 1)}$$



Scheme I—Proposed degradation pattern of cadralazine in aqueous solution.

Table II—Rate Constants for Cadralazine Degradation [a]

Constant	k, h^{-1} [b]	r^2
k_1	0.1346 ± 0.0190	0.989
k_2	0.0179 ± 0.0047	0.994
k_3	0.0452 ± 0.0049	0.990
k_4	0.0744 ± 0.0050	0.997
$k_2 + k_3 + k_4$	0.1375	

[a] Determined at pH 7.4, 80°C. [b] Confidence limits of ±95%.

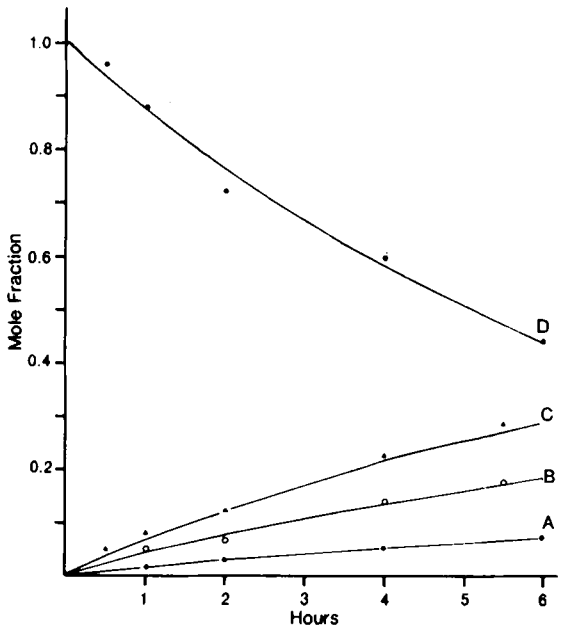


Figure 6—*Time-concentration profile of cadralazine (A) and its degradation products II (B), III (C), and IV (D) in an aqueous solution (0.1%, w/v) of cadralazine at pH 7.4 incubated at 80°C. The experimental data are superimposed on calculated curves.*

Rate expressions describing the appearance of II, III, and IV take similar forms (pseudo-first-order reactions):

$$\frac{d[II]}{dt} = k_2[I] \qquad \text{(Eq. 2)}$$

$$\frac{d[III]}{dt} = k_3[I] \qquad \text{(Eq. 3)}$$

$$\frac{d[IV]}{dt} = k_4[I] \qquad \text{(Eq. 4)}$$

These differential equations can be solved and the amount of each component in the mixture can be expressed as the mole fraction of the initial concentration of cadralazine as a function of time:

$$\frac{[I]}{[I]_0} = e^{-k_1 t} \qquad \text{(Eq. 5)}$$

$$\frac{[II]}{[I]_0} = \frac{k_2}{k_1}(1 - e^{-k_1 t}) \qquad \text{(Eq. 6)}$$

$$\frac{[III]}{[I]_0} = \frac{k_3}{k_1}(1 - e^{-k_1 t}) \qquad \text{(Eq. 7)}$$

$$\frac{[IV]}{[I]_0} = \frac{k_4}{k_1}(1 - e^{-k_1 t}) \qquad \text{(Eq. 8)}$$

Initial conditions were defined so that at $t = 0$ the amount of cadralazine was equal to $[I]_0$, and no degradation products were present, *i.e.*, [II], [III], and [IV] were equal to zero.

Equations 5–8 were used to calculate simultaneously k_1, k_2, k_3, and k_4 from the experimental data derived from the degradation of I carried out at 80°C and pH 7.4, in air and light, using a nonlinear regression program (NOLIN). The values obtained for k_i and their 95% confidence limits are reported in Table II. The quality of the fit between experimental and theoretical concentrations is shown in Fig. 6. The accuracy of the calculated k_i was demonstrated by comparing the pseudo-first-order rate constant k_1, determined experimentally, with the sum of the individually calculated rate constants $k_2 + k_3 + k_4$.

The minor compound, V, was not considered in the kinetic study, because its yield was small, near the range of detectability. However, this product could be a possible intermediate in the formation of III and IV.

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Binding Parameters of Interactions of Monomer–Polymer Systems Based on Quenching of Their Completely Overlapped Fluorescence: A Theory

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Received March 3, 1983, from the *Department of Pharmaceutical Chemistry, College of Pharmacy, University of Baghdad, Baghdad, Republic of Iraq.* Accepted for publication September 12, 1983.

Abstract □ A theory is presented which describes the interactions between completely overlapping fluorescing monomer–polymer systems and affords a method to calculate binding parameters. The theory is based on the assumptions that the complex formed from the fluorescing binding site and the monomer molecule does not fluoresce and that the fluorescence intensity is linearly related to concentration. The relationships derived from this theory have provided a sensitive and easy method for calculating the number of binding sites on a polymer molecule from only three emission-intensity values. The theory has been tested on systems containing trimethoprim–serum albumins and found to yield results which are consistent with a curve-fitting approach to the experimental data. This reflects the validity of the theoretical model presented and the various assumptions and approximations made.

Keyphrases □ Monomer–polymer systems—binding parameters, overlapped fluorescence quenching □ Binding parameters—overlapped fluorescence quenching, monomer–polymer system □ Fluorescence quenching—binding parameters, monomer–polymer systems

Fluorescence quenching has been applied to the study of complex formation (1); for example, the interactions of amino acids containing aromatic nuclei and their amines, and oligopeptides containing aromatic amino acids with nucleic acids, were studied by this method (2, 3). Recently, Koumriqian (4) and Borazan and Koumriqian (5) have applied the technique to study interactions between catecholamines and polyadenylic acid. In these studies, the emissions of the polynucleotides were negligible compared with those of the monomers. It was, therefore, easy to derive equations for calculating the binding parameters using the fraction remaining from the fluorescence intensity of the monomers or the observed quantum yield.

However, due to the inherent properties of many systems, both the monomers and polymers in the binary mixtures exhibit almost complete emission overlap over a wide range of excitation and emission wavelengths. Examples for such systems can be found in the fluorescence quenching studies of the interactions of steroids–polynucleotides (6), antineoplastics–polynucleotides (7), and antibacterials–serum albumins (8). Thus, these types of systems impose a difficult problem when analyzing the experimental data to calculate the binding constants. These authors (6–8) have applied an empirical approach involving a curve-fitting technique to analyze the fluorescence data.

The present study is concerned with a theoretical treatment which utilizes the fluorescence technique in situations where complete fluorescence overlap is taking place. This allowed us to determine the number of fluorescing binding sites on a polymer molecule available to the monomer molecules and to calculate the equilibrium constant for the process.

THEORETICAL SECTION

To calculate the binding constants for an interaction between a monomer, M, and a binding site on a polymer, Pb, the following simple mathematical model was assumed:

$$M + Pb = MPb \qquad \text{(Eq. 1)}$$

where MPb, is the complex formed. When using the fluorescence technique to calculate the binding parameters, the solution of the problem is straight-

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