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Fluorometric studies of: I. daunomycin and myricetin interactions with l-tryptophyl-l-trytophan, l-tryptophyl-l-tyrosine, and adenosine triphosphate II. daunomycin-deoxyribonucleic acid interactions in the presence and absence of divalent metal ions

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II. DAUNOMYCIN-DEOXYRIBONUCLEIC ACID INTERACTIONS IN THE PRESENCE AND ABSENCE OF DIVALENT METAL IONS

A THESIS SUBMITTED TO THE FACULTY OF ATLANTA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

BY

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DEPARTMENT OF CHEMISTRY

ATLANTA, GEORGIA

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ABSTRACT

CHEMISTRY

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FLUOROMETRIC STUDIES OF: I. DAUNOMYCIN AND MYRICETIN INTERACTIONS WITH L-TRYPTOPHYL-L-TRYPTOPHAN, L-TRYPTOPHYL-L-TYROSINE, AND ADENOSINE TRIPHOSPHATE

II. DAUNOMYCIN-DEOXYRIBONUCLEIC ACID INTERACTIONS IN THE PRESENCE AND ABSENCE OF DIVALENT METAL IONS.

Advisor: Professor Yitbarek H.-Mariam

Thesis dated July 1983

Fluorescence quenching has been used to study the interactions of daunomycin and myricetin with various quenchers — adenosine triphosphate (ATP), L-tryptophyl-L-tryptophan, L-tryptophyl-L-tyrosine myosin, and deoxyribonucleic acid (DNA), at various pH levels. DNA, although producing a quenching effect on the fluorescence of daunomycin, enhanced the fluorescence of myricetin. Fluorescence quenching has also been used to study the interactions of DNA in the presence and absence of divalent metal ions, with daunomycin. The values of the Stern-Volmer quenching constant ($k_{SV}$), and static quenching constants ($V$) obtained, indicate that the quenching of daunomycin and myricetin fluorescence by the quenchers studied proceed via collisional, static, as well as, selective quenching mechanisms, and that they are good quenchers for daunomycin fluorescence. The
studies also revealed the pH-dependence of daunomycin fluorescence quenching. Physiologically high levels of Fe$^{++}$, Cu$^{++}$, and Mg$^{++}$, increased the apparent association constant ($K_{app}$) for daunomycin-DNA complex, and changed the number of available binding sites per drug molecule. The addition of Fe$^{++}$, Mg$^{++}$ and Cu$^{++}$ did not produce any change in the binding site model of daunomycin-DNA.
DEDICATION

My two years of study at Atlanta University, the period this research was done, was punctuated at several intervals by different forms of hard times; each new day arrived with greater cloudness, and my mind remained wandering in the desert of uncertainty awaiting the result of tomorrow's pregnancy. When things turned from bad to worse, my mind would pause and re-echo the words of my mother to me when I was a child: "When the darkness deepens, day break is nigh."

This thesis is dedicated to my wife, Carrie, and to the entire family of Jack Ochuwa Umesi for their contributions in several forms including but not limited to financial support, and words of encouragements throughout the period I have spent at Atlanta University.
My sincere thanks to Dr. Yitbarek H. Mariam, my advisor (academic program and medical option), Dr. Kofi B. Bota and Dr. Malcolm B. Polk, my advisors (Industrial Chemistry Program), the Atlanta University Chemistry Department and the Atlanta University Center's BISRIP Program grant number RR08006 from the Minority Biomedical Research Support Program of the National Institutes of Health for providing the funds, Mrs. Bettie Wright for her assistance in making most of the solutions, and Ms. Carol Johnson for her great assistance in typing this thesis. My special and unquantifiable appreciation goes to Mr. Joshua Ulonnam Ogbonna for his unalloyed effort and contributions, in the forms of time, cash and kind, towards the successful conclusion of this research.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>ii</td>
</tr>
<tr>
<td>DEDICATION</td>
<td>iv</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>v</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>vii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xi</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>EXPERIMENTAL</td>
<td>13</td>
</tr>
<tr>
<td>RESULTS AND DISCUSSION</td>
<td>19</td>
</tr>
<tr>
<td>CONCLUSION</td>
<td>47</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>48</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>--------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>1</td>
<td>Structure of daunomycin</td>
</tr>
<tr>
<td>2</td>
<td>Structure of myricetin</td>
</tr>
<tr>
<td>3</td>
<td>Modified Jablonski diagram showing transitions between excited states and the ground state. Radiative processes are shown by straight lines; radiationless processes by wavy lines</td>
</tr>
<tr>
<td>4</td>
<td>Stern-Volmer plot for the ATP quenching of daunomycin, at daunomycin concentration of $1.35 \times 10^{-7}$ M; the sample was excited at 466 nm; $F_0$ and $F$ are the fluorescence intensities (arbitrary units) in the absence and presence of a quencher respectively</td>
</tr>
<tr>
<td>5</td>
<td>Stern-Volmer plot for the ATP quenching of daunomycin, at daunomycin concentration of $1.35 \times 10^{-5}$ M; the sample was excited at 466 nm; $F_0$ and $F$ are the fluorescence intensities (arbitrary units) in the absence and presence of a quencher respectively</td>
</tr>
<tr>
<td>6</td>
<td>Stern-Volmer plot for the selective ATP quenching of daunomycin, at daunomycin concentration of $1.3 \times 10^{-5}$ M; the sample was excited at 466 nm; $F_0$ and $F$ are the fluorescence intensities (arbitrary units) in the absence and presence of quencher respectively</td>
</tr>
<tr>
<td>7</td>
<td>Stern-Volmer plot for the ATP quenching of daunomycin at daunomycin concentration of $1.35 \times 10^{-5}$ M; the sample was excited at 466 nm; pH 7.5; $F_0$ $F$ are the fluorescence intensities (arbitrary units) in the absence and presence of a quencher respectively</td>
</tr>
</tbody>
</table>
Stern-Volmer plot for the L-tryptophyl-L-tryptophan quenching of myricetin; the sample was excited at 375 nm; solvent used is ethanol; $F_0$ and $F$ are the fluorescence intensities (arbitrary units) in the absence and presence of a quencher.

Estimation of $F_\infty$ which is employed in the calculation of $k_d$ for the myosin enhancement of daunomycin fluorescence in Fig. 10. Fluorescence data (enhancement) collected at emission $\lambda_{\text{max}} = 590$ nm, excitation at 466 nm; solvent used is piperazine and NaCl; pH = 7.0; $F_0$ and $F$ are the fluorescence intensities (arbitrary units) in the absence and presence of a quencher respectively.

Estimation of $k_d$ for the myosin enhancement of daunomycin; $F_\infty$ (fluorescence at saturation) estimated from Fig. 9 was used; $F_0$ and $F$ are the fluorescence intensities in the absence and presence of a quencher respectively.

Estimation of $F_m$ which is employed in the calculation of $k_d$ for the deoxyribonucleic acid enhancement of myricetin fluorescence in Fig. 12. Fluorescence data (enhancement) collected at emission $\lambda_{\text{max}} = 580$ nm, excitation at 375 nm; solvent used is piperazine and NaCl; pH = 7.0; $F_0$ and $F$ are the fluorescence intensities (arbitrary units) in the absence and presence of a quencher respectively.

Estimation of $k_d$ for the deoxyribonucleic acid enhancement of myricetin $F$ estimated from Fig. 11 was employed; $F_0$ and $F$ are the fluorescence intensities in the absence and presence of a quencher respectively.
13 Quenching effects of deoxyribonucleic acid on daunomycin fluorescence (excitation at 466 nm) in the absence and presence of divalent metal ions ........................................... 34

14 Scatchard analysis of the quenching effect of deoxyribonucleic acid on the fluorescence of daunomycin. The parameters \( r_b \) (moles of ligand bound per nucleotide), and \( C \) (moles per liter of free ligand), were calculated by the method of Scatchard (1949) ........................................... 36

15 Scatchard analysis of the effect of Mg\(^{++}\) on the interactions between daunomycin and deoxyribonucleic acid. The parameters \( r_b \) (moles of ligand bound per nucleotide), and \( C \) (moles per liter of free ligands), were calculated by the method of Scatchard (1949) ........................................... 37

16 Scatchard analysis of the effect of Cu\(^{++}\) on the interactions between daunomycin and deoxyribonucleic acid. The parameters \( r_b \) (moles of ligand bound per nucleotide), and \( C \) (moles per liter of free ligands), were calculated by the method of Scatchard (1949) ........................................... 38

17 Scatchard analysis of the effect of Fe\(^{++}\) on the interactions between daunomycin and DNA. The parameters \( r_b \) (moles of ligand bound per nucleotide), and \( C \) (moles per liter of free ligands), were calculated by the method of Scatchard (1949) ........................................... 39

18 Stern-Volmer plots for the deoxyribonucleic acid quenching of daunomycin; the sample was excited at 466 nm. \( F_0 \) and \( F \) are the fluorescence intensities (arbitrary units) in the absence and presence of a quencher (DNA) respectively ........................................... 40
Figure

19 Stern-Volmer plots for the deoxyribonucleic acid quenching of daunomycin–Mg$^{++}$ complex; the sample was excited at 466 nm; $F_0$ and $F$ are the fluorescence intensities (arbitrary units) in the absence and presence of a quencher (DNA) respectively.

20 Stern-Volmer plots for the deoxyribonucleic acid quenching of daunomycin–Cu$^{++}$ complex; the sample was excited at 466 nm. $F_0$ and $F$ are the fluorescence intensities (arbitrary units) in the absence and presence of a quencher (DNA) respectively.

21 Stern-Volmer plots for the deoxyribonucleic acid quenching of daunomycin–Fe$^{++}$ complex; the sample was excited at 466 nm. $F_0$ and $F$ are the fluorescence intensities (arbitrary units) in the absence and presence of a quencher (DNA) respectively.
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Quenching and Enhancement Parameters for Daunomycin</td>
<td>25</td>
</tr>
<tr>
<td>2</td>
<td>Quenching and Enhancement Parameters of Myricetin</td>
<td>28</td>
</tr>
<tr>
<td>3</td>
<td>Scatchard Analysis of Deoxyribonucleic Acid</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>Fluorescence Quenching of Daunomycin in the Presence</td>
<td></td>
</tr>
<tr>
<td></td>
<td>and Absence of Divalent Metal Ions—Fe$^{++}$, Cu$^{++}$, and Mg$^{++}$</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Binding Parameters and Intrinsic Binding Constants</td>
<td>45</td>
</tr>
</tbody>
</table>
INTRODUCTION

Daunomycin\(^1\) (Fig. 1), is an anthracycline antibiotic isolated from streptomycetes peucetius, which possesses antitumor activity against certain animal and human neoplasms.\(^1-5\) Due to its clinical value in cancer chemotherapy, extensive studies have been devoted to elucidating the mechanism of action of the drug in the hope of improving its chemotherapeutic index and eliminating its cytotoxic effect. The accumulated evidence suggests that this drug binds to the cell genome\(^2,6,7\) and perturbs cellular DNA and ribonucleic acid (RNA) synthesis.\(^6,8-13\) It remains uncertain, however, whether the antimitotic activity of daunomycin arises exclusively from interactions with DNA and inhibition of nucleic acid synthesis. Indeed, studies with synchronized cell cultures have shown that the antimitotic effect of daunomycin may occur at different phases of the cell cycle\(^11,12\) and at daunomycin concentrations which do not affect DNA synthesis.\(^13\) N-acetyl-daunomycin, while possessing only a weak effect on nucleic acid synthesis, is capable of inhibiting cell mitosis.\(^14\) Furthermore, cross resistance of cells has been observed toward daunomycin and vinca alkaloids, a class of potent antimitotic drugs known to act through binding to microtubule protein with ensuing spindle poisoning. The last observation suggests the possible existence of a common site of interaction between these two different kinds of antimitotic drugs. It has in fact been speculated that the mechanism of the antimitotic effect of daunomycin could involve the perturbation of spindle fibre function.\(^15\)

The possibility of the existence of a common site of interaction\(^15\) between daunomycin and vinca alkaloids is of special interest. To pursue deeper into this expressed interest, myricetin (3,3',4',5,5',7-hexa hydroxyflavone), (Fig. 2), has been
Fig. 1. Structure of daunomycin.

Fig. 2. Structure of myricetin.
selected as a model for comparative studies. Daunomycin binds to nucleic acids by intercalation, as well as by electrostatic binding. Two major requirements for these modes of binding is that the drug molecule exhibits planarity and aromaticity, characteristic features shared by both daunomycin and myricetin. Also, it has been recently observed that myricetin inhibits glycolysis in tumor cells when the mitochondrial ATPase supplied Pi (inorganic phosphate) and adenosine diphosphate. This mitochondrial ATPase inhibition by myricetin is also exhibited by daunomycin, a possible means by which the energy necessary for RNA-based DNA synthesis is depleted. Although daunomycin and myricetin share similar characteristic features and effects, it remains uncertain whether the two antimitotic drugs would in fact affect similar biomolecules in the same manner, and, to the same degree.

Interaction of daunomycin with actin and myosin (muscle components) has been studied. Heavy meromyosin exhibited an emission $\lambda_{\text{max}}$ at 350 nm when excited at $\lambda_{\text{ex}}$ of 300 nm. The emission wavelength of 350 nm is due to the tryptophanyl and/or the tyrosine residues of the proteins. Even though it has been substantiated that daunomycin quenches the fluorescence of the tryptophan and tyrosine moieties of several biopolymers the exact nature of the interaction is not known. Model studies with the dipeptide of tryptophan or tryptophan-tyrosine may be helpful in this regard. Elucidating the interaction of myricetin with either of the two dipeptides, will also be appropriate.

The clinical use of daunomycin, as mentioned earlier, has been limited due to its cardiotoxicity. The intense efforts to date to ameliorate this cardiotoxicity involve, in addition to a search for less cardiotoxic derivatives, antagonists of the
cardiotoxicity;\textsuperscript{4} on the other hand, understanding the mechanism of action of the drug would help in design of strategies to alleviate this problem.

Physiological levels of certain divalent metal ions have shown interesting interactions with daunomycin, and daunomycin-deoxyribonucleic acid complexes.\textsuperscript{4,5} The exact trends of the effects of these divalent metal ions on daunomycin-deoxyribonucleic acid complexes, with a major interest on how the association constants of the complex vary—a factor which could possibly be related to the residency time of the drug in the tissue—and, a general interest on the variation of the number of binding sites available—a factor which could throw some insight into the drug-metal ion-DNA complex—remains to be determined. In order to assess these interactions of daunomycin with various cellular components (the major thrust of this thesis), studies have been undertaken using fluorescence methods.

**Fluorescence Phenomena.** The principles of fluorescence spectroscopy are by now well established, and after a rather lengthy gestation period, the technique is now routinely applied to a broad spectrum of problems, ranging from mechanistic photochemistry to chemical analysis in biomedical and environmental systems, to probes of structure, function, and complexations in biological macromolecules.

When a ray of polarized light passes through a solution medium of thickness, $\ell$, which contains an absorbing species at a concentration, $c$, the loss of intensity is proportional to the thickness, the concentration, and the intensity of the incident light. This process is summed up in the Beer–Lambert Law\textsuperscript{19} which is represented by:

$$I_f = I_i \exp (-\alpha c \ell) \quad (1)$$
where $I_f$ is the intensity after the medium, $I_i$ is the intensity before passing through
the medium, $\mu$ is the absorption coefficient which depends both on the molecule
under study and the frequency of the light, $c$ is the concentration of the solution,
and $l$ is the thickness of the sample. The loss of intensity is due to the loss of
energy to some of the chromophores (in the solution) which now go from their
ground states to their excited states. These excited molecules will eventually
return to the ground state through loss of the absorbed energy by one of several
ways, including a slow loss of energy by emission of light which forms the
fundamental basis for fluorescence spectroscopy.

**Type of Probe.** Since a probe of a biological system is considered to be a
molecule, small relative to that system, and providing information about the
limited volume in which it is found, fluorescent probes are therefore able to
reflect their environments by the emission they produce upon excitation.
Throughout this research, concentration is focused on the role played by intrinsic
probes, i.e., molecules which are already fluorescent. The $\lambda_{\text{max}}$ for such
molecules in the visible or ultraviolet region of the electromagnetic radiation gives
an absolute indication of the wavelength at which the excitation wavelength should
be set.

At a given excitation wavelength, temperature and pH, a given chromophore
will exhibit a spectrum which is directly proportional to the intensity of energy
absorbed. Upon interaction with molecules other than those present in the homo-
gegeneous solutions, a spectrum which is different from the natural spectrum is
observed. The new spectrum may depict lower intensity (quenching) or a higher
intensity (enhancement). A change in intensity is therefore a good indication of molecular interaction, and forms a basis for the investigations that follow in subsequent sections.

The Fate of an Excited Molecule, M. When a molecule, M, is excited by absorption of energy, the excited state, M*, differs from the ground state with respect to both energy and electronic wave function.\textsuperscript{19,20-22} During the lifetime, of M* several processes could occur—dissipation of the excitation energy, wholly or partly, by thermal collisional processes, e.g., radiationless return to the ground state, or decomposition of the molecule, or a chemical reaction (the excited state is more reactive), or formation of a complex (with ground state of same species, excimer, or another species, exciplex), or ionization of the molecule, or, during the transition from the excited singlet to the ground state, the energy may be emitted as light, a phenomenon known as fluorescence.\textsuperscript{19,21,22} Also possible is the occurrence of intersystem crossing—the molecule crossing over to one of the vibrational levels of a triplet state. This process competes with fluorescence, and when it occurs, the radiative transition from the lowest triplet state to the ground state is known as phosphorescence (Fig. 3).\textsuperscript{21}

Although the excited state is in general more reactive than the ground state, a reaction can be observed by fluorescence measurements only if the reaction time is comparable to or shorter than the mean lifetime of the unperturbed excited state. Quenching of fluorescence and complex formation are two such reactions that satisfy this kinetic requirement.\textsuperscript{22}
Fig. 3. Modified Jablonski diagram showing transitions between excited states and the ground state. Radiative processes are shown by straight lines; radiationless processes by wavy lines. ISC = intersystem crossing; IC = internal conversion; vc = vibrational cascade; hγ_f = fluorescence; hγ_p = phosphorescence. *From Ref. 21.
Fluorescence Quenching. Quenching is essentially a decrease in fluorescence intensity of a fluorophor via "physical contact" of the fluorophor with a quencher, Q. A number of important processes come under the general heading of "quenching." They have often been divided into viscosity-dependent (dynamic) and viscosity-independent (static) types.

All quenching processes can be considered as bimolecular processes in competition with the emission, internal conversion, and intersystem crossing phenomena from the first excited singlet state of the fluorophore. Five types can be distinguished: collision quenching (diffusion controlled), collision quenching (non-diffusion controlled), energy transfer quenching, concentration quenching (excimer formation), and radiative migration (self-absorption). Radiative migration is a property of the bulk solution, and since its effect is not a source of much information, normally it is to be avoided. Concentration quenching, on the other hand, is potentially a very useful indicator of local fluorophore concentrations. The effect is caused by association of an excited fluorophore with a ground-state fluorophore to yield an excited state dimer (excimer) with altered fluorescence properties.

\[
\begin{align*}
M + M^* & \xrightarrow{k_{dm}} (M \ldots M)^* 
\end{align*}
\]

The concentration-quenching process is conveniently described in terms of the concentration, \(c_{Q}\), of M needed for 50% decrease in the fluorescence yield of \(M^*\). In liquid solution, the process is diffusion controlled, hence,
where $\phi^0$ and $\phi$ are, respectively, the fluorescence quantum yield for $M^*$ in the absence of and presence of quenching by ground-state, $M$. Pyrene derivatives constitute the classical example of fluorescence quenching via excimer formation.20

Energy-transfer quenching can occur over much greater distance than "collisional" quenching. It involves a resonance between the transition dipoles. It depends on the relative orientation of the two molecules, the degree of overlap of the emission band of a sensitizer and the absorption band of the emitter and the molecules.23 The transfer is thus possible over distances of the order of $50 \AA$. Since the two reactants, $M$ or $M^*$ and $Q$ in solution would be required to form an encounter complex, they must approach to a distance within $50 \AA$, where any kind of interaction may become significant for quenching of fluorescence to take place.22

The encounter complex, $(M^* ... Q)$, arises via the two modes of collisional quenching mentioned above, diffusion controlled and non-diffusion controlled (static) as shown below:

\[
\frac{\phi^0}{\phi} = 1 + \tau k_{dm} [M], \quad c_\phi = \frac{1}{\tau k_{dm}}
\]  

\[\text{(3)}\]

where $\phi^0$ and $\phi$ are, respectively, the fluorescence quantum yield for $M^*$ in the absence of and presence of quenching by ground-state, $M$. Pyrene derivatives constitute the classical example of fluorescence quenching via excimer formation.20

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The encounter complex, $(M^* ... Q)$, arises via the two modes of collisional quenching mentioned above, diffusion controlled and non-diffusion controlled (static) as shown below:

\[
\text{Diffusional} \quad M^* + Q \xrightleftharpoons[k_d]{k_d} (M^* ... Q)
\]

\[
\text{Static} \quad M + Q \rightarrow (M ... Q)
\]

\[\text{(4)}\]
(M* ... Q) is formed with a diffusion-controlled rate constant, $k_d$, and the reaction completes the quenching of the fluorescence with a reaction constant, $k_2$

$$k_2 \quad (M* \ldots Q) \rightarrow M + Q + hv \quad (5)$$

The observed rate constant for this quenching, $k_2$, is equal to $\alpha k_d$, where $\alpha$ is the efficiency of the quenching process.\(^{22}\) If the efficiency is less than one, $k_2 = k_d k_2 / k_d$ (i.e., not every encounter between M or M* and Q leads to quenching).\(^{22}\) $k'$ is the formation constant for (M ... Q) and therefore viscosity independent. If $k_d$ approaches zero and (M ... Q) is non-fluorescent, then\(^{23}\)

$$F_0 / F = (1 + k_q \tau_0 [Q])(1 + k'[Q]) \quad (6)$$

where $F_0$ and $F$ are fluorescence intensities in the absence and presence of Q respectively, $k_q$ is apparent constant for the collisional quenching given by Eq. 4, and $\tau_0$ is fluorescence lifetime in the absence of a quencher, Q. Two cases of interest follow from Eq. 6. First, if ground-state complex formation is negligible, then $k' = 0$ and Eq. 6 reduces to

$$F_0 / F = 1 + k_{sv}[Q] = \tau_0 / \tau \quad (7)$$

where $k_{sv}$ is equal to $k_q \tau_0$, and $\tau$ equal fluorescence lifetime in the presence of a quencher. This is the classical Stern-Volmer\(^{24}\) equation for diffusion controlled
collisional quenching. At the other extreme, if \( k_d = 1/\tau_0 \), then the observed quenching is entirely due to ground-state complexation and

\[
F_0/F = 1 + k'[\mathcal{Q}]
\]  

(8)

where \( k_{sv} = k' \). It is seen that in both the limiting cases, the observed quenching obeys a Stern-Volmer relation.

A plot of \( F_0/F \) vs. \([\mathcal{Q}]\) would yield \( k_q \tau_0 \), from which \( k_q \) could be determined, (if \( \phi_0 \) is known) and Eq. 8 predicts a linear increase in the Stern-Volmer quenching constant, \( k_{sv} \), with increasing \([\mathcal{Q}]\). When both processes are operative simultaneously, plots of \( F_0/F \) against \([\mathcal{Q}]\) cease to be linear. Positive deviation (upward curvative) as well as negative deviations have been observed for such plots.\textsuperscript{22}

According to Weller\textsuperscript{22} such deviations arise because the reaction time is comparable to the time required to reach steady-state (for diffusion-controlled reactions) and only a certain fraction, \( W \), of the excited molecules obey the Stern-Volmer equation. \( W \) is related to the fluorescence quantum yield, \( \phi \), by

\[
\frac{\phi^0}{\phi} = \frac{W}{1 + k_d \tau_0 [\mathcal{Q}]}
\]  

(9)

where \( \phi^0 \) and \( \phi \) are the quantum yield in the absence and presence of a quencher, and the residual fraction \((1 - W)\) does not contribute to the fluorescence yield. \( W \) is also related to the molar volume of diffusion by
where \( V_D \) is the molar volume of diffusion.\(^{22}\)

To account for the excited states which are instantaneously deactivated by \( Q \) (i.e., the residual fraction, \( 1 - W \)), the Stern-Volmer equation is modified as:

\[
\frac{F_0}{F_1} = (1 + k_{sv}[Q])\exp(V[Q])
\]

or

\[
\frac{F_0}{F_{expV[Q]}} = 1 + k_{sv}[Q] = \frac{\tau^0}{\tau}
\]

where \( V \) is the static quenching constant and describes the quenching of \( M \) by \( Q \) which is randomly positioned in the vicinity of \( M \) at the time \( M \) is excited. The positive deviation from the classical Stern-Volmer plot is attributed to static quenching.\(^{22}\)

In this thesis, the interaction of daunomycin with adenosine triphosphate, L-tryptophyl-L-tryptophane, L-tryptophan-L-tyrosine, and the interactions of myricetin with L-tryptophyl-L-tryptophan, as well as the interactions of both molecules with deoxyribonucleic acid and myosin will be discussed. Also the results of the effects of some divalent metal ions—Fe\(^{++}\), Cu\(^{++}\), Mg\(^{++}\)—upon the interaction of daunomycin with deoxyribonucleic acid will be presented.
EXPERIMENTAL

**Materials.** Daunomycin (Dm), myricetin (3,3',4',5',7-hexahydroxyflavone) crystals, adenosin triphosphate (ATP), L-trytophyll-L-trytophan, L-trytophyll-L-tyrosine, and CuSO₄·5H₂O were obtained from Sigma Chemical Company, St. Louis, Missouri. Calf thymus DNA was a generous gift from Dr. David Wilson of Georgia State University, Atlanta, Georgia. Anhydrous piperazine was purchased from Aldrich Chemical Company. NaCl, and MgCl₂·6H₂O were obtained from J. T. Baker Chemical Company. All the commercially obtained chemicals were used without further purification.

ATP samples were made by dissolving in Tris buffer; L-trytophyll-L-trytophan, and L-trytophyll-L-tyrosine samples were made by dissolution in 50:50 (v/v), water-ethanol mixture, and stirred for three hours at room temperature. Daunomycin sample was prepared in piperazine buffer, and its concentration was determined spectroscopically employing a Varian Cary 210 from its known extinction coefficient of 11,500 M⁻¹ cm⁻¹ at 480 nm. Myricetin samples were prepared by dissolving in 50:50 (v/v) water-ethanol mixture, and the resulting mixture stirred for 30 minutes at room temperature. Solutions of CuSO₄, MgCl₂, and FeCl₂ were prepared by dissolving the solids in deionized water, and diluting to concentrations of interest. In making the buffer, 0.01 M piperazine and 0.1 M NaCl were diluted in deionized H₂O, and pH was adjusted with HCl.

**Fluorescence Intensity Measurements.** Fluorescence intensity measurements at ambient temperatures were made on a Perkin-Elmer MPF-44A fluorescence spectrophotometer, equipped with a magnetic stabilizer for Xenon arc light source,
differential corrected spectra unit, a model 2000 X-Y recorder, and temperature controls. Intensity of the lamp was found to be extremely stable over the time course of the quenching experiments. Band widths of 10 mm were routinely used. In every study pursued involving ATP, L-tryptophyl-L-tryptophan, L-tryptophyl-L-tyrosine, and DNA, the shape and position of the emission spectrum of (Dm) was not noticeably changed. The intensity of the emission maximum at 592 nm was therefore monitored in the quenching studies. Optical density measurements were made on a Varian Cary 210 spectrophotometer.

The quenching experiments were performed by using a couple of different routines, both giving similar results. In one, the fluorescence of different samples of daunomycin of the same concentration, and a graduated amount of quencher, was measured. However, as a matter of mere convenience and for purposes of monitoring the stability of the complexes formed, small aliquots (ranges: 0.5 - 20μl) of quencher solutions were added to series of sample solutions, using a Rainin 20 μl micropipette, in a 3 ml teflon fluoremetric cuvette, having an insignificant optical density at the excitation wavelengths of interest. After each addition, the solution was gently agitated by inversion, and air bubbles removed by bubbling nitrogen through the sample, before fluorescence intensity was measured. For the daunomycin fluorescence quenching, fluorescence measurements were made at excitation wavelengths of 300 nm and 466 nm. Fluorescence quenching measurements for the myricetin quenching were made at excitation wavelength of 375 nm. pH was varied by using different buffer solutions.

Scatchard Analyses. The quenching experiment of daunomycin vs. DNA was performed by progressive additions of small aliquots (ranges 0.5 to 2μl) of DNA
stock solutions. The quenching experiments of Dm + M^2+ vs DNA were performed by initial addition of M^2+ to Dm after scanning Dm alone, followed by subsequent and progressive additions of small aliquots of DNA as outlined above. Each cuvette contained 3.33 μM drug in 3 ml of solution, and DNA concentration varied such that [DNA] / [Dm] ratio varied from 0 to 8. The [DNA] / [Drug] ratio of 8 was taken as the end point in this titration, with the drug considered to be maximally bound, based on the observation that the fluorescence intensity reached a plateau at this point. The concentrations of the divalent metal ions—Fe^{++}, Cu^{++}, Mg^{++} was always 3.33 μM such that [Drug] / [M^{2+}] = 1, throughout the experiment.

The binding data were analyzed by the Scatchard^{24} method. The Scatchard variables r_b (moles of ligand bound per nucleotide) and c (the molar concentration of free ligand) were calculated from the fluorescence data according to the method of Peacocke and Skerrett.^{25} Binding parameters were determined from plots of r_b vs r_b/c, in which K_{app} (apparent association constant) is the negative slope and n_{app} (the apparent number of binding sites per nucleotide) is the x-intercept.

To obtain the Scatchard variables, r_b and r_b/C (C = free drug concentration) were necessary, and they were calculated as discussed below. When a spectrum of the drug (daunomycin) in the absence of a quencher, Q, is scanned, the fluorescence intensity of emission at λ_{max} is recorded as F_0. A spectrum of the drug in the presence of the quencher is then scanned, with the fluorescence intensity recorded this time as F. Subsequent scans at higher Q concentration, with, of course, more reduced intensities of fluorescence, F, are recorded. In this study, ten scans were made for each experiment. The concentration of bound drug, C_b, is given by:

\[ C_b = F_b X C_T, \]
where \( F_b \) and \( C_T \) are fraction of drug bound and total concentrations of drug respectively. The fraction of bound drug, \( F_b \), is given by:

\[
F_b = \frac{F_0 - F}{F_0 - F_\infty}
\]

where \( F_0 \) and \( F \) are fluorescence intensities in the absence and presence of a quencher respectively, and \( F_\infty \) is the fluorescence intensity when the drug is assumed to be maximally bound, and this is taken from the plateau region of the plot of intensity vs. quencher concentration (vide infra). The free drug concentration, \( C \), is therefore given by:

\[
C = C_T - C_b
\]

\( r_b \) is given by

\[
r_b = \frac{C_b}{[Q]}
\]

where \([Q]\) is the concentration of the quencher, DNA. A plot of \( r_b/C \) vs. \( r_b \) (Scatchard plot) is expected to give a linear curve, but concave, or even, convex curves have been observed. If a linear curve obtains, the negative slope of the curve gives \( K_{\text{app}} \) (apparent association constant), and the intercept on the x-axis gives \( n_{\text{app}} \) (apparent number of binding sites per nucleotide). If a deviation from linearity obtains, the slopes of the two tangents which intercept at the the point of
curvature give the respective $K_{\text{app}}$'s (two apparent associations constants $K_1$ and $K_2$ now exist). If $K_2$ is insignificant, a single $K_{\text{app}}$ is chosen. On the other hand, if $K_1 - K_2$ is a large value, the different $K_{\text{app}}$'s are determined.

For relative fluorescence studies, the fluorescence intensity, $F_0$, in the absence of a quencher, $Q$, was taken as a 100% fluorescence, 0% fluorescence was recorded when the drug was totally bound, in the plateau region of the spectra.

**Determinations of $k_{\text{SV}}$ and $V$**. All the analyses were made on a Tektronix terminal of the Prophet system. The Stern-Volmer and the modified Stern-Volmer equations were analyzed using an in-fitted fit function program. In this program, the classical Stern-Volmer equation:

$$\frac{F_0}{F} = 1 + k_{\text{SV}} [Q],$$

is replaced by the function:

$$(1 + B_1 X),$$

where $B_1$ now stands for $k_{\text{SV}}$, and $X$ represents $[Q]$, the quencher concentration. In the same manner, the modified Stern-Volmer equation:

$$\frac{F_0}{F} = (1 + k_{\text{SV}} [Q]) \exp(V [Q]),$$

now becomes

$$(1 + B_1 X) \exp(B_2 X),$$
where $B_2$ now stands for $V$, the static quenching constant. In accordance with the Stern-Volmer prediction, a plot of $F_0/F$ vs. $[Q]$ would give a linear curve from Eq. 8, the slope of which is $B_1$, or $k_{sv}$. The value of $B_2$ or $V$ is also given by the computer. Also, when the function is changed to $(1 + B_1 x) \exp(B_2 x)$, a linear plot is obtained from Eq. 11 and this represents the modified Stern-Volmer plot.

**Fluorescence Enhancement Studies.** Enhancement studies were performed following basically the same procedures used in the quenching experiments (progressive addition of aliquots of the quencher). The fluorescence intensity of the drug alone was recorded as $F_0$, and subsequent intensities (enhancement) were recorded as $F$ after each addition of $Q$, respectively. A plot of $1/\Delta F$ ($\Delta F = F_{(\text{drug-Q})} - F_{\text{drug}}$) vs. $1/[Q]$ would give a linear curve. By extrapolating the curve to $1/[Q] = 0$, i.e., at infinite concentration of $Q$, $F_\infty$ could be calculated. Using the $F_\infty$ thus obtained, a plot of $\log \frac{F_\infty - F}{F - F_0}$ vs. $-\log [Q]$ would also yield a linear curve, the extrapolation of which to $\log \frac{F_\infty - F}{F - F_0}$ equals zero would give $k_d$ (apparent dissociation constant).
RESULTS AND DISCUSSION

Quenching of Daunomycin Fluorescence by ATP. Figs. 4, 5, and 6 represent the results, plotted in Stern-Volmer fashion (Eq. 8) of ATP quenching studies of daunomycin. The amount of fluorescence observed in the presence of quencher (ATP) was not time dependent, indicating that permanent photochemistry is not involved, to any great extent, in the quenching process.

It is apparent, from Figs. 4, 5, and 6 that as you increase the concentration of daunomycin from $10^{-7}$ M (Fig. 4) to $10^{-5}$ M (Fig. 6), the plot of $F_o/F$ vs. $[Q]$ changes from a positive deviation (Fig. 4) to a negative deviation (Fig. 6), and in fact to almost a linear curve (Fig. 5) in conformity with (Eq. 8). By treating the data according to Eq. 11, that is, by plotting $F_o/F \exp(V[Q])$ vs. $[Q]$ for varying $V$ until linear plot is obtained, the static and collisional quenching modes can be dissected. From analysis of the plots (Figs. 4, 5, and 6) the following $k_{sv}$ and $V$ values were found - $k_{sv} = 0$, $V = 469.19$ M$^{-1}$ (Dm = $10^{-7}$ M), $k_{sv} = 89.27$ M$^{-1}$, $V = 4.217$ M$^{-1}$ (Dm = $10^{-5}$ M), and $k_{sv} = 182.7$ M$^{-1}$, $V = 0$ M$^{-1}$ (Dm = $10^{-6}$). At higher $k_{sv}$ values, collisional quenching is the major mode of quenching, and static quenching predominates at high $V$ values. The upward curvature (Fig. 4) is similar to those found in many other detailed quenching studies. This type of deviation from the expected linear plot can be explained by the fact that only a certain fraction of the excited states is actually quenched by the collisional mechanism as described by Stern and Volmer. In contrast to Fig. 4, the $F_o/F$ vs. $[Q]$ plot at higher Dm concentrations (Fig. 5) shows a negative deviation from linearity. Similar quenching patterns have also been observed.
Fig. 4. Stern-Volmer plot for the ATP quenching of daunomycin, at daunomycin concentration of $1.35 \times 10^{-7}$ M; the sample was excited at 466 nm; $F_0$ and $F$ are the fluorescence intensities (arbitrary units) in the absence and presence of a quencher respectively.
Fig. 5. Stern-Volmer plot for the ATP quenching of daunomycin, at daunomycin concentration of $1.35 \times 10^{-5}$ M; the sample was excited at 466 nm; $F_0$ and $F$ are the fluorescence intensities (arbitrary units) in the absence and presence of a quencher respectively.
Fig. 6. Stern-Volmer plot for the selective ATP quenching of daunomycin at daunomycin concentration of $1.3 \times 10^{-5}$ M; the sample was excited at 466 nm; $F_0$ and $F$ are the fluorescence intensities (arbitrary units) in the absence and presence of a quencher respectively.
reflects the selectivity in quenching, and indicates that selective quenching overwhelms any positive deviations caused by the static component.

**Quenching of Daunomycin Fluorescence by L-tryptophyl-L-tryptophan.** Presented in Fig. 7 is an example of the results of L-trp-L-trp quenching of daunomycin plotted in Stern-Volmer and modified Stern-Volmer fashions. All the curves show positive deviations—upward curve. This could mean that there is a very strong contribution to quenching by static mechanisms, a fact which is very much supported by the high V-values obtained (Table 1). The concentration dependence of $k_{sv}$ and V (Table 1) is probable as well as justifiable, since V, a parameter that is related to the probability of finding a quencher molecule close enough to a newly formed excited state molecule to quench it immediately, or statically, would naturally decrease with concentration. $k_{sv}$ and V were also found to be pH-dependent (Table 1). Although no regular pattern was observed for the dependence of the quenching of daunomycin fluorescence on pH range 7-7.5, the significant changes in the values of $k_{sv}$ could mean that either the donor-acceptor ability and the interaction of the molecules in the ground state is changed by pH variation, or, that the ground state daunomycin and quencher complexes are formed at pH ≥ 7.5.

**Quenching of Daunomycin Fluorescence by L-tryptophyl-L-tyrosine.** The Stern-Volmer plots of L-trp-L-tyr quenching of daunomycin fluorescence showed neither negative nor positive deviations from linearity (figures not shown). Linear Stern-Volmer plots (Eq. 8) are indicative of insignificant contributions of quenching of fluorescence by static mechanism. At pH ≥ 6.5, low values of $k_{sv}$ were observed (Table 1). The observed insignificant contributions to quenching of fluorescence by static mechanism and the low values of $k_{sv}$ at pH ≥ 6.5, indicate
Fig. 7 Stern-Volmer plot for the ATP quenching of daunomycin at daunomycin concentration of 1.35 x 10^{-5} M; the sample was excited at 466 nm; pH 7.5; $F_0$ and $F$ are the fluorescence intensities (arbitrary units) in the absence and presence of a quencher respectively.
Table 1. Quenching and Enhancement Parameters for Daunomycin.*

<table>
<thead>
<tr>
<th>Quencher</th>
<th>$k_{sv} \ (M^{-1})$</th>
<th>$V \ (M^{-1})$</th>
<th>$k_d \ (M^{-1})$</th>
<th>$\lambda_{ex} \ (\text{nm})$</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-trp-L-trpa</td>
<td>0</td>
<td>17.55</td>
<td>—</td>
<td>466</td>
<td>7.0</td>
</tr>
<tr>
<td>L-trp-L-tyr</td>
<td>15.76</td>
<td>5.45</td>
<td>—</td>
<td>466</td>
<td>7.5</td>
</tr>
<tr>
<td>ATP</td>
<td>163.23</td>
<td>0.65</td>
<td>—</td>
<td>466</td>
<td>6.5</td>
</tr>
<tr>
<td>ATP</td>
<td>178.09</td>
<td>0.47</td>
<td>—</td>
<td>466</td>
<td>6.5</td>
</tr>
<tr>
<td>Myosin</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>6309.57</td>
<td>7.0</td>
</tr>
</tbody>
</table>

*Studies at room temperature; fluorescence monitored at the emission $\lambda_{max} = 590 \ \text{nm}$.  

aL-tryptophyl-L-tryptophan; [daunomycin] = 1.35 x 10^{-6} M  
bL-tryptophyl-L-tyrosine; [daunomycin] = 1.30 x 10^{-5} M.  
cAdenosine triphosphate; [daunomycin] = 1.30 x 10^{-5} M.  
dAdenosine triphosphate; [daunomycin] = 1.35 x 10^{-6} M.
the dependence of L-trp-L-tyr quenching of daunomycin fluorescence on pH, and an overwhelming predominance of dynamic quenching mechanism.

**Quenching of Myricetin Fluorescence by L-tryptophyl-L-tryptophan.** Fig. 8 is representative of Stern-Volmer plots of L-trp-L-trp quenching of myricetin fluorescence. Significant differences in the values of $k_{sv}$ and $V$ were observed (Table 2) with variation in solvents. In ethanol, and at $\lambda_{ex} = 375$ nm, $k_{sv} = 1265.25$ M$^{-1}$, $V = 219$ M$^{-1}$. But in piperazine and NaCl buffer, and at the same excitation wavelength $= 375$ nm, $k_{sv} = 109.29$ M$^{-1}$, $V = 21.39$ M$^{-1}$ (Table 3). This dependence of fluorescence quenching of myricetin on solvent may probably be due to factors which include, but are not limited to, solvent polarity.

**Enhancement of Daunomycin Fluorescence by Myosin.** Fig. 9 shows the plot of $1/\Delta F (F_{Dm-myosin} - F_{Dm})$ against $1/[$myosin$]$. By extrapolating the curve to zero concentration of myosin, $F_\infty$ was obtained. The monophasic behavior of the curve is indicative of the presence of only one class of binding sites. With this value of $F_\infty$, a log—log plot was constructed (Fig. 10). $k_d$ (dissociation constants) value of $6.3 \times 10^3$ M$^{-1}$ was obtained. The progressive enhancement (a dequenching effect) produced by the progressive increase in the concentration of myosin is probably due to the increasing unavailability of some group or groups for quenching of one or more tryptophan residues in the complex. This probability is in line with energy transfer processes.

**Enhancement of Myricetin Fluorescence by DNA.** Figs. 11 and 12 are plots for the calculation of DNA enhancement parameters ($pk_d$ and $k_d$) for myricetin fluorescence. From the plot of $1/\Delta F (F_{myricetin-DNA} - F_{myricetin})$ vs. $1/[$DNA$]$,
Fig. 8. Stern-Volmer plot for the L-tryptophyl-L-tryptophan quenching of myricetin; the sample was excited at 375 nm; solvent used is ethanol; $F_0$ and $F$ are the fluorescence intensities (arbitrary units) in the absence and presence of a quencher (L-tryptophyl-L-tryptophan) respectively.
Table 2. Scatchard Analysis of Deoxyribonucleic Acid Fluorescence Quenching of Daunomycin in the Presence and Absence of Divalent Metal Ions — Fe^{++}, Cu^{++}, and Mg^{++}.*

<table>
<thead>
<tr>
<th>Ligand</th>
<th>$k_{\text{app}}^{(1)}$ x 10^{-4} M^{-1}</th>
<th>$k_{\text{app}}^{(2)}$ x 10^{-4} M^{-1}</th>
<th>$k \times 10^{-4}$ M^{-1}</th>
<th>$V \times 10^{-4}$ M^{-1}</th>
<th>$k_{sv}$ M^{-1}</th>
<th>$n_1$</th>
<th>$n_2$</th>
<th>$L_1$</th>
<th>$L_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dm</td>
<td>2.18</td>
<td>0.52</td>
<td>5.88</td>
<td>1.69</td>
<td>0.000</td>
<td>0.26</td>
<td>0.06</td>
<td>3.85</td>
<td>16.67</td>
</tr>
<tr>
<td>Dm + Mg^{++}</td>
<td>2.59</td>
<td>0.49</td>
<td>6.56</td>
<td>2.55</td>
<td>0.000</td>
<td>0.23</td>
<td>0.03</td>
<td>4.35</td>
<td>33.33</td>
</tr>
<tr>
<td>Dm + Cu^{++}</td>
<td>9.61</td>
<td>0.39</td>
<td>7.35</td>
<td>2.70</td>
<td>0.0039</td>
<td>0.19</td>
<td>0.06</td>
<td>5.12</td>
<td>16.67</td>
</tr>
<tr>
<td>Dm + Fe^{++}</td>
<td>10.17</td>
<td>0.54</td>
<td>7.45</td>
<td>2.70</td>
<td>0.0016</td>
<td>0.19</td>
<td>0.01</td>
<td>5.29</td>
<td>71.43</td>
</tr>
</tbody>
</table>

*Studies at room temperature; [Dm] = 1.33 x 10^{-6} M; fluorescence intensity monitored at emission $\lambda_{\text{max}} = 590$ nm, and the excitation $\lambda_{\text{ex}} = 466$ nm. Daunomycin is abbreviated as Dm; apparent association constant is abbreviated as $k_{\text{app}}$ ($k_{\text{app}}^{(1)}$ — for intercalation mode of binding, $k_{\text{app}}^{(2)}$ — for electrostatic binding mechanism); number of available ligands (drug and drug + divalent metal ions) is abbreviated as L. $k_{sv}$ is the Stern-Volmer constant; $V$ = static quenching constant; $k$ = intrinsic binding constant and is calculated as in Table 5; an isolated potential binding site is assumed.
Table 3. Quenching and Enhancement Parameters of Myricetin.$^a$

<table>
<thead>
<tr>
<th>Quencher</th>
<th>$k_{SV}$ (M$^{-1}$)</th>
<th>$k_d V$(M$^{-1}$) x 10$^{-2}$ (M$^{-1}$)</th>
<th>$V$ (M$^{-1}$)</th>
<th>$\lambda_{ex}$ (nm)</th>
<th>Solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-trp-L-trp$^b$</td>
<td>1265.28</td>
<td>—</td>
<td>219</td>
<td>375</td>
<td>ethanol</td>
</tr>
<tr>
<td>L-trp-L-trp$^b$</td>
<td>109.29</td>
<td>—</td>
<td>21.39</td>
<td>375</td>
<td>$H_2O^C$</td>
</tr>
<tr>
<td>DNA$^d$</td>
<td>—</td>
<td>6.31</td>
<td>—</td>
<td>375</td>
<td>$H_2O$</td>
</tr>
</tbody>
</table>

$^a$Studies at room temperature; [myricetin] = 4.9 x 10$^{-5}$ M; fluorescence monitored at the emission $\lambda_{max}$ = 542 nm.

$^b$L-tryptophyl-L-tryptophan.

$^c$H$_2$O, pH 7.0.

$^d$Deoxyribonucleic Acid. DNA enhanced myricetin fluorescence.
Fig. 9. Estimation of $F_\infty$ which is employed in the calculation of $k_d$ for the myosin enhancement of daunomycin fluorescence in Fig. 10. Fluorescence data (enhancement) collected at emission $\lambda_{\text{max}} = 590$ nm, excitation at 466 nm; solvent used is piperazine and NaCl; pH = 7.0; $F_0$ and $F$ are the fluorescence intensities (arbitrary units) in the absence and presence of a quencher respectively.
Fig. 10. Estimation of $k_d$ for the myosin enhancement of daunomycin; $F_\infty$ (fluorescence at saturation) estimated from Fig. 9 was used; $F_0$ and $F$ are the fluorescence intensities in the absence and presence of a quencher respectively.
Fig. 11. Estimation of $F_0$ which is employed in the calculation of $k_d$ for the deoxyribonucleic acid enhancement of myricetin fluorescence in Fig. 12. Fluorescence data (enhancement) collected at emission $\lambda_{max} = 580$ nm, excitation at 375 nm; solvent used is piperazine and NaCl; pH = 7.0; $F_0$ and $F$ are the fluorescence intensities (arbitrary units) in the absence and presence of a quencher respectively.
Fig. 12. Estimation of $k_d$ for the deoxyribonucleic acid enhancement of myricetin; $F_\infty$ estimated from Fig. 11 was employed; $F_0$ and $F$ are the fluorescence intensities in the absence and presence of a quencher respectively.
F_\infty (intensity at infinite concentration of DNA) was obtained. With the F_\infty obtained, a log—log plot was constructed (Fig. 12) from which k_d (apparent binding) was obtained. One class of binding sites was evident from the monophasic behavior of the curve.

**Quenching of Daunomycin Fluorescence by DNA in the Absence and Presence of Divalent Metal Ions.** Presented in Fig. 13 are the results of relative quenching effects of DNA on daunomycin fluorescence, in the absence and presence of the divalent metal ions Fe^{++}, Cu^{++}, Mg^{++}, at different DNA/drug ratios. Fluorescence intensity at saturation, F_\infty, due to DNA in the absence of the divalent metal ions is 10%. The presence of the divalent metal ions resulted in a reduction of fluorescence intensity, especially in the presence of Fe^{++} and Cu^{++}. The observed F_\infty's in the presence of Fe^{++}, Cu^{++} and Mg^{++} are 2.2%, 3.2%, and 8.6% respectively. The fluorescence intensities tend to level off at DNA/drug ratio of 1:2. The curvature of the Scatchard plots (Fig. 13-16) is indicative of a two site binding model. The presence of the divalent metal ions did not effect any significant change in the site model of the drug-DNA complex (Figs. 15-17). Figs. 18-21 show the Stern-Volmer and modified Stern-Volmer plots for the DNA quenching of daunomycin fluorescence in the absence and presence of divalent metal ions-Fe^{++}, Cu^{++}, Mg^{++}. Table 3 presents the results of the analysis of binding parameters. K_{app}'s were obtained by the method of Scatchard,
Fig. 13. Quenching effects of deoxyribonucleic acid on daunomycin fluorescence (excitation at 466 nm) in the absence and presence of divalent metal ions. ○ = DNA; ● = Mg^{++}; △ = Cu^{++}; ▲ = Fe^{++}; [M^{2+}]/[Dm] = 1 M; M^{2+} = Mg^{++}, Cu^{++}, or Fe^{++}. 
Fig. 14. Scatchard analysis of the quenching effect of deoxyribonucleic acid on the fluorescence of daunomycin. The parameters $r_b$ (moles of ligand bound per nucleotide), and $C$ (moles per liter of free ligand), were calculated by the method of Scatchard (1949).
Fig. 15. Scatchard analysis of the effect of Mg\(^{++}\) on the interactions between daunomycin and deoxyribonucleic acid. The parameters \( r_b \) (moles of ligand bound per nucleotide), and \( C \) (moles per liter of free ligands), were calculated by the method of Scatchard (1949).
Fig. 16. Scatchard analysis of the effect of Cu$^{++}$ on the interactions between daunomycin and deoxyribonucleic acid. The parameters $r_b$ (moles of ligand bound per nucleotide), and $C$ (moles per liter of free ligands), were calculated by the method of Scatchard (1949).
Fig. 17. Scatchard analysis of the effect of Fe$^{++}$ on the interactions between daunomycin and DNA. The parameters $r_b$ (moles of ligand bound per nucleotide), and $C$ (moles per liter of free ligands), were calculated by the method of Scatchard (1949).
Fig. 18. Stern-Volmer plots for the deoxyribonucleic acid quenching of daunomycin; the sample was excited at 466 nm. $F_0$ and $F$ are the fluorescence intensities (arbitrary units) in the absence and presence of a quencher (DNA) respectively; $Q$ = DNA; $\bullet$ = Stern-Volmer plot; $\circ$ = modified Stern-Volmer plot.
Fig. 19. Stern-Volmer plots for the deoxyribonucleic acid quenching of daunomycin-Mg$^{++}$ complex; the sample was excited at 666 nm; $F_0$ and $F$ are the fluorescence intensities (arbitrary units) in the absence and presence of a quencher (DNA) respectively; $Q =$ DNA; $\bullet =$ Stern-Volmer plot; $\circ =$ modified Stern-Volmer plot; $Mg^{++}/[Dm] = 1$; and *fluorescence intensity measurements were recorded at $\lambda_{em} = 590$ nm.
Fig. 20. Stern-Volmer plots for the deoxyribonucleic acid quenching of daunomycin-Cu$^{++}$ complex; the sample was excited at 466 nm. $F_0$ and $F$ are the fluorescence intensities (arbitrary units) in the absence and presence of a quencher (DNA) respectively; $Q$ = DNA; $\bullet$ = Stern-Volmer plot; $\bigcirc$ = modified Stern-Volmer plot.
Fig. 21. Stern-Volmer plots for the deoxyribonucleic acid quenching of daunomycin-Fe$^{2+}$ complex; the sample was excited at 466 nm. $F_0$ and $F$ are the fluorescence intensities (arbitrary units) in the absence and presence of a quencher (DNA) respectively; $Q =$ DNA; $\bullet =$ Stern-Volmer plot; $\bigcirc =$ modified Stern-Volmer plot.
$k_{sv}$ and $V$ were calculated from the Stern-Volmer plot, and $K$ was calculated as shown in Table 4. The apparent association constant ($K_{app}$), the static quenching constant ($V$), as well as the intrinsic binding constant ($k$) for daunomycin-DNA binding increased in the presence of the divalent metal ions - Fe$^{++}$, Cu$^{++}$, Mg$^{++}$ (Table 4). Fe$^{++}$, with $K_{app} = 1.02 \times 10^5$ M$^{-1}$, $V = 2.70 \times 10^4$ M$^{-1}$, and $k = 7.45 \times 10^4$ M$^{-1}$, showed the greatest increase in the constants. The corresponding constants in the absence of the divalent metal ions are $K_{app} = 2.18 \times 10^4$ M$^{-1}$, $V = 1.69 \times 10^4$ M$^{-1}$, and $k = 5.88 \times 10^4$ M$^{-1}$. $K_{app}$ of $2.18 \times 10^4$ M$^{-1}$ for DNA is small compared to that obtained by other methods. Hence the fluorescence method may not be very accurate. Although no regular sequence was observed in the change or variation of the number of available binding sites per drug molecules, $n_1$, the presence of the divalent metal ions decreased the number of available binding sites per drug molecule (fairly insignificant), thereby increasing the number of drug molecules per site, $L_1$, when the mode of binding is intercalative. When the mode of binding changed from intercalation to electrostatic binding, via the negative phosphate ions of the DNA and the cationic daunomycin molecule, there was an irregular, yet, a significant difference in the values of the number of available binding sites per drug molecule, $n_2$, and the number of drug molecules per site, $L_2$, in the presence of Fe$^{++}$ and Cu$^{++}$. This difference in the values of $n_2$ and $L_2$ for Fe$^{++}$ and Cu$^{++}$ may suggest that $n_2$ and $L_2$ could not be determined with any reasonable accuracy.

In both cases (in the absence and presence of divalent metal ions), the primary mode of quenching is static (Figs. 18-21). Quenching by the collisional mode is essentially insignificant as depicted by the values of the Stern-Volmer
Table 4. Binding Parameters and Intrinsic Binding Constants.*

<table>
<thead>
<tr>
<th>rb</th>
<th>rb/c</th>
<th>K</th>
</tr>
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<tbody>
<tr>
<td>0.4687</td>
<td>0.1549</td>
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<td>0.1946</td>
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</tr>
<tr>
<td>0.3274</td>
<td>0.24</td>
<td>83196.04</td>
</tr>
</tbody>
</table>

*The intrinsic binding constant, K, is calculated from the equation

\[ K = \frac{r_b}{c} \frac{(1 - 4r_b)^2}{(1 - 2r_b)} \]

where \( r_b \) is the moles of drug bound per nucleotide, and \( c \) is the concentration of free drug. K calculated above is for the effect of Mg++ on the daunomycin-DNA interactions.
constants, $k_{sv}'s$ (Table 4). This is reasonable because the ligand is expected to form a ground state complex. The observed increasing trend in the static quenching constant, $V$ (Table 4), is in agreement with the increasing trends of the apparent association constant ($K_{app}$), and the intrinsic binding constant, $k$. In this case, it is reasonable to take $V$ as an association constant, $k$. No particular trend was observed, however, in the variations of $K_{app}^2$, the apparent association constant when the mode of binding is predominantly by electrostatics, an observation which supports the fact that this mode of binding of daunomycin to DNA has not yet been as fully established as the competing intercalative mode. This is the first time that a comparative study of $K_{app}$, $K$, $k_{sv}$, and $V$, using fluorescence techniques has been undertaken.
CONCLUSION

From the values of the collisional quenching constants \( k_{sv} \) for the fluorescence quenching of daunomycin observed, it is justifiable, to conclude that ATP, L-tryptophyl-L-tryptophan, and L-tryptophyl-L-tyrosine are good quenchers. It is even more justifiable to establish that DNA and L-tryptophyl-L-tyrosine, are also good quenchers especially for the fluorescence quenching of myricetin. The increase in the apparent association constants for the daunomycin-DNA interaction produced by the addition of the divalent metal ions suggests that these metal ions interact with either daunomycin or DNA to enhance the formation of the daunomycin-DNA complex. The metal ions possibly exert their effect by the formation of a ternary metal-drug-DNA complex. Results from this research also confirms that drug-macromolecular interaction is pH-dependent.

Since drug effects, of daunomycin in particular, are governed by factors which include, but are not limited to, acceptor membrane permeability, pH of the medium, \( pK_a \), and effectiveness of binding, it may be of pharmacological interest to study the relationship between daunomycin residency time when daunomycin is administered alone, and when it is administered along with the quenchers studied, \( \text{Fe}^{++} \), \( \text{Cu}^{++} \), and \( \text{Mg}^{++} \). If possibly the daunomycin residency time is reduced by the addition of metal ions such as \( \text{Fe}^{++} \), \( \text{Cu}^{++} \), and \( \text{Mg}^{++} \), then we may in fact be making progress toward possible elimination of the cardiotoxicity associated with daunomycin.
REFERENCES


