ADSORPTION PROFILE OF ANTICARCINOGEN BOUND DNA

DISSERTATION SUBMITTED TO THE
JAWAHARLAL NEHRU UNIVERSITY
IN PARTIAL FULFILMENT OF
THE REQUIREMENTS FOR
THE AWARD OF THE DEGREE OF
MASTER OF PHILOSOPHY
1980

SUBHENDU GHOSH

67p. + fig.

SCHOOL OF ENVIRONMENTAL SCIENCES,
JAWAHARLAL NEHRU UNIVERSITY,
NEW DELHI

July 1980.

PREFACE

The research work embodied in this dissertation has been carried out in the School of Environmental Sciences, Jawaharlal Nehru University, New Delhi. The work is original and has not been submitted so far, in part or full for any other degree of diploma of any University.

Subhendu Ghosh

SUBHENDU GHOSH

Anjali Mookerjee

ANJALI MOOKERJEE
(Supervisor)

J.M. DAVE
(Dean)

School of Environmental Sciences,
Jawaharlal Nehru University,
NEW DELHI - 110 067.

March, 1980.

CONTENTS

ACKNOWLEDGEMENTS

I am indebted to Dr. Anjali Mookerjee for her inspiring guidance throughout this work. I am also thankful to her for her valuable suggestions and critical comments in preparing this manuscript.

I appreciate the interest taken by Prof. J.M. Dave, Dean, School of Environmental Sciences in this work.

I express my sincere thanks to Dr. D.K. Chattoraj Jadavpur University and Dr. Miss Rita Mukhopadhyay for their suggestions and inspirations.

I am also thankful to Mr. Sailen Nandi, Mr. A.C. Alexander, Mr. Nagendra Roy and Mr. Gajanan Hegde, for their cooperation in this work.

I am grateful to all my colleagues who gave me constant inspiration and assistance throughout this work.

The financial assistance provided by Jawaharlal Nehru University, New Delhi as fellowship is also acknowledged.

SUBHENDU GHOSH

INTRODUCTION

Intensive work has been done on the binding of simpler molecules to cellular macromolecules and their biological effects. Small organic molecules, denoted as ligands, are used as 'probe' to study the finer changes and distortion in the structure of the macromolecules (Peacocke, 1973). These small ligands include dyes and some antibiotics which usually exert some sort of pharmacological effect (Bloomfield et al., 1974) and hence may be considered under a common heading of 'drug'.

Diseases like cancer are believed to be accompanied by changes in genetic code, and this can be induced by chemicals as well as radiations in the environment and also by bacterial, fungal, viral interactions. Looking from a molecular point of view, the information contained by DNA is either altered or suppressed during the above processes so that there is error in the genetic message transferred which affects the entire biological process. Also there are chemical compounds which can resist the abnormal growth of living cells or the distortion of information contained by DNA, known as antibacterial, antiviral agents. The biological effectiveness of all these agents depends on the extent to which they are associated with genetic material. Therefore, study of binding of these agents with DNA is important.

Mode of Binding

In the drug DNA complex formation two types of binding are found :

i) a weak binding mode that takes place when number of drug molecules bound per nucleotide (r) is equal or greater than 0.2 ($r \gtrsim 0.2$), and

ii) a strong primary mode of binding that predominates till $r \lesssim 0.2$ (Peacocke and Skerrett, 1956).

The weak binding is an external attachment of the drug molecule to the DNA rodlike chain, where drug in its cationic state is electrostatically held by the negatively charged phosphates (Peacocke and Skerrett, 1956; Bradley and Wolf, 1959; Stone and Bradley, 1961). This process is called stacking and may be 'partiall or total' depending on the ratio of moles of DNA is to moles of drug present. However, it has been inferred that the complex is stabilised by Van der Waal's forces between the ligand molecules. It should be noted that the binding can occur as an interaction with either ligand molecule which only contribute to this mode of binding or cationic part of the drug molecule (which has already formed a strong

bond with DNA) projecting out as a branch on the DNA surface. The activation energy is only a few (2 to 3) Kcals/mole of acridine.

The strong binding is, from chemical point of view monomeric in nature and has essentially resulted from the interaction of electron clould of purine and pyrimidine bases of the nuclotides and the consequent charge-transfer. Obviously in such an interaction a specific geometry of the molecular system is demanded. Hydrophobic interactions between the base pairs and the dye rings stabilise the complex (Lochmann and Micheler, 1973). The free energy change is about 6 to 9 Kcal/mole of the drug.

Kinetic measurements (Li and Crothers, 1969) show that strong binding occurs in two steps: first the cation is rapidly and electrostatically attracted towards the external phosphate groups. This process requires little activation energy and is almost totally diffusion controlled. Next, the base pairs near the cation is geometrically strained, without breaking the hydrogen bonds to create a channel, and the drug molecules move in from a state of external binding. This gives rise to a sandwiched

complex where the drug molecule is held rigidly between the base-pairs with cationic ring and located centrally above and below the hydrogen bonds of the bases. This enables maximum interaction of the π-electrons. The activation energy is required mainly to dissociate the externally bound drug and to overcome the stacking forces between the neighbouring base-pairs.

For the double-stranded DNA, this mode of binding corresponds to an intercalation of the ligand molecule made possible by a contour extention (Freifelder, 1971; Dasgupta and Dasgupta, 1973) and local unwinding of the helix (Lerman, 1961). The distance between the bases is approximately doubled from 3.4 Å to 6.8 Å and the ligand molecule, in Van der Waal's contact with the adjacent base-pairs, is maximally protected from contact with the surrounding medium (Waring, 1975). The planes of the drug-rings are held more perpendicular than parallel to the helix axis, but deviates to within $\pm 30°$ of the DNA bases (Lerman, 1963; Nagata et al., 1966) forcing the bases to tilt slightly to accommodate the ligand. The tendency of the drug-molecules to bind externally

is enhanced by (a) denaturation of the DNA, and (b) decrease in ionic strength.

Daunomycin As a Ligand

Antibiotics have been found to inhibit tumour growth. Since 1870's (Lissaner) study of antibiotic took a definite turn with the discovery of Actinomycin D (Wakemann and Woodruff, 1940) the first of a series of prototype drugs, followed by identification of anthracyclines chromomycins, antinomycins etc. (review by Gottlieb and Shaw, 1967).

These antibiotics come into action via interaction with cellular macromolecules, e.g. especially DNA (Ranon et al., 1960; Kersten and Kersten, 1965; Newton, 1970; Horwitz, 1971; Hollstein, 1973). Investigation shows that compounds derivable from the three ring anthracene like system either planner and aromatic and heterocyclic, or partially saturated non-polar form, form stable complexes with DNA and inhibition of DNA dependent RNA synthesis. Again, the anthracycline group bears the strongest resemblance to the acridine dyes. Cinerubin, Nogalamycin and Daunomycin belong to this group of antibiotic anticarcinogens.

In the present studies, the antibiotic Daunomycin has been used as a ligand for complexation with DNA.

Radiation Effect on Complex Formation

In medical practice chemotherapy and radiation therapy are usually combined in treating cancer. Mukhopadhyay and Mookerjee (1976) have shown the effect of gamma-irradiation on the binding pattern of DNA with Acridine Orange, Proflavine and Daunomycin at various stages, before and after complex formation.

Adsorption of Biopolymers

The surface activity of biopolymers like nucleic acids, proteins and enzymes has been a subject of much investigation (Bull, 1956; 1957; McLeren, 1954, 1958; Zittle, 1953; Chattoraj, Chowrashi, Chakrabarty, 1967, 1968; Upadhyay and Chattoraj, 1970, 1974, 1972) as an increasing number of biological phenomena involve adsorption of surface active molecules and ions at interfaces. Direct adsorption experiments with DNA onto alumina-water interface have also been carried out by different workers (Upadhyay and Chattoraj, 1968; Chari and Mookerjee, 1975; Upadhyay and Mookerjee, 1977).

Systematic study on the adsorption of macromolecules from solution on to solid surfaces began about two decades ago but most of the work involved use of non-biological polymers. The helical secondary and tertiary structures of the biopolymers are rigid rod-like surface with polyelectrolyte and are usually more inflexible than the non-biological ones. Like in DNA, firstly the inherent polyfunctionality of the polymer suggests that if one segment of the molecule adsorbs on a solid surface, the probability of adsorption of neighbouring segment will be greatly enhanced; secondly, the unusual configurational behaviour of a macromolecule like DNA as evidenced by the properties of polymer solutes, suggests that the intramolecular configuration of the adsorbed molecules will be an important aspect of the adsorption process.

Frommer and Miller observed that denatured DNA adsorbs much faster than native DNA probably because of the higher diffusion coefficient of the denatured DNA. Chari and Mookerjee (1975) found that the quantity of gamma-irradiated DNA adsorbed on alumina was much greater than native and heat denatured DNA. Correspondingly, the adsorption of gamma-irradiated DNA was faster than the other forms of DNA studied.

Miller studied the interaction of DNA with a charged mercury surface by measuring the effect of DNA on the different capacity of the electrical double layer between the polarised mercury surface and 0.1 N sodium chloride solution containing varying concentrations of DNA. The lowering of the differential capacity by adsorbed DNA gave an idea of the adhesion of the different molecular residues viz. sugar, purines, pyrimidines to the surface. The lowering of the differential capacity varied with surface concentration at partially covered surface concentration at partially covered surfaces and reached a constant value when the surface was fully covered. Miller also suggested that DNA preserves its double helix at a negatively charged surface while unfolding occurred at a positively charged surface. He also has suggested a mechanism for the unfolding of the double helix at a positively charged surface, which is presumed to be a very fast process. According to Miller, one can speculate that unfolding of the DNA, double helix in biological system takes place at positive surfaces.

Miller followed up this work with adsorption studies on DNA on surfaces of copolymers of 4-vinyl pyridine and styrene. He studied the electrophoretic mobility of the particles coated with either native or heated denatured DNA adsorbed from aqueous solution of different salt concentration. He observed that the electrophoretic mobility of the coated particles depended only on the salt concentration and the state of DNA molecules in solution and not on the composition of the surface of the copolymer particles before DNA adsorption. The shape of DNA molecules interacting with monolayers of copolymers with different surface characterisation was determined by electron microscopy (Gordon, 1970). The influence of surface charge on adsorbent on the extent of adsorption and structural changes of adsorbed DNA was studied. The amount of DNA adsorbed was found to be depend both on copolymer concentration and surface treatment but not on the concentration of DNA remaining in solution. The interaction between DNA and electropositive or weakly electronegative copolymer surfaces were strong enough to break the structure of adsorbed DNA.

Chattoraj and Upadhyay (1968) have studied the adsorption of DNA and RNA at alumina-H_2O interface. They have used native, heat denatured and alkali and acid denatured DNA as adsorbates. The nature and shape of the adsorption versus concentration of DNA curve for native DNA indicated Langmuir type adsorption. Frommer and Miller (1966) standardized a method for measuring the adsorption of tritium labelled compounds to study DNA adsorption on a polypeptide monolayer. Gordon studied the adsorption of DNA on mice after replacing the cation by Al^{+3} through ion-exchange. The adsorption process monitored by electron microscopic observation showed that the amount of DNA adsorbed increased with ionic strength of solution. Of several multivalent cation tried only Al^{+3} was effective in causing adsorption. Chattoraj and Chaurashi studied the electrophoretic mobility of calfthymus DNA adsorbed on charged particles. Other studies on adsorption of nucleohistone by Upadhyay and Chattoraj (1972), Fasman (1970) and co-workers; Upadhyay and Mookerjee (1976) and Akinrimisi et al. (1965) are also of much significance.

The present work has been undertaken with a view to elucidate the adsorption profile of complexed DNA before and after gamma-irradiation at solid liquid interface. The drug used for binding is Daunomycin and the solid surface is that of alumina.

MATERIALS AND METHODS

1. **DNA:**

Highly polymerised calf thymus DNA Type I obtained from Sigma Chemicals, U.S.A., was used for all experiments.

2. **Daunomycin Hydrochloride:**

Daunomycin was obtained in the form of a hydrochloride from 'Calbiochem' under the trade name of Daunorubicine. Since this was available in the analytical grade, it did not require further purification.

3. **Sodium Chloride:**

Analar quality NaCl from B.D.H. was used.

4. **Alumina:**

Alumina supplied by B.D.H. had already been standardized for chromatographic adsorption analysis.

5. **Glassware:**

Corning glasswares were used throughout the experiments.

Source of Radiation:

Gamma chamber 4000 A (Co_{60} Isotope) supplied by the Isotopes division of Bhabha Atomic Research Centre (BARC) Bombay was used as the source of gamma

radiation. Frick Ferrous Sulphate dosimetry was used for the determination of dose. The dose was 127 rads/sec.

The graph of time of irradiation vs. O.D. is given herewith (Fig. I)

From graph

Slope of the plot of time of irradiation vs. O.D.

$$\Delta \text{O.D.} = \frac{.275}{1} \text{ rads./minute.}$$

Hence,

$$\text{Dose} = 2.774 \times 10^4 \times \Delta \text{ O.D.}$$

$$= 2.774 \times 10^4 \times .275$$

$$= 7628.5 \text{ rads/minute}$$

$$= 127 \text{ rads/sec.}$$

Spectrophotometer:

The optical densities in the U.V. range at 260 and 320 nm were measured in Karl Zeiss PMQ II 95358 and ECIL Spectrophotometers. The absorption spectra were taken in Shimadzu.

Preparation of Different DNA Samples

1) Native DNA: A stock solution of DNA at a concentration of 20 mgm/100 ml in 0.002M NaCl was prepared. This stock solution was then diluted to

concentrations ranging from 1 mgm to 7 mgm/100 ml and the diluted solutions used for adsorption studies.

2. Gamma-Irradiated DNA: The stock solution of native DNA was diluted to required concentration. 20 ml of the solution was taken in each case in test tubes of diameter 1.8 cms. and irradiated for 4 minutes. A total dose of 30.45 Krads was used.

Procedure for preparing DNA-Daunomycin Complex

The drug 'Daunomycin' was bound to DNA by mixing the two solutions in a test tube and gently shaking or rotating the test-tube between the two palms for 10 minutes followed by an interval of 30 minutes for the process to attain a steady state The macromolecule to ligand ratio, henceforth written P/D, was determined as follows:

If P_{st} μM = Stock conc. of DNA of total volume V_p ml.

D_{st} μM = Stock conc. of Daunomycin solution of total volume V_D ml.

P μM = concentration of DNA in the complex.

D μM = concentration of 'drug' in the complex

then, for a required ratio of (P/D), the volume of DNA to be taken from stock = $V_P \times \frac{P}{P_{st}}$

Volume of 'drug' to be taken = $V_D \times \frac{D}{D_{st}}$

If the required final volume is V ml, then,

volume of buffer solvent to be taken = $\left\{ V - V_P \times \frac{P}{P_{st}} - V_D \times \frac{D}{D_{st}} \right\}$

If $V_P = V_D = V$, then

volume of buffer solvent to be taken = $V\left\{ 1 - \frac{P}{P_{st}} - \frac{D}{D_{st}} \right\}$

Particle Size

The average size of alumina was measured using a micrometer and microscope. The diameter of Alumina particles ranged from 4 to 6 microns.

Procedure for Adsorption of Free and Complexed DNA At Alumina-H_2O Interface

I. Unirradiated Samples: The solutions of different concentrations were adjusted to pH 7.0 and required ionic strength by addition of requisite amount of HCl and NaCl respectively. 10 ml of the solution in each case were added to 1 gm. of Alumina in 50 ml stoppered conical flasks and shaken intermittently for 4 hours at 20°C. and the solution left undisturbed for another 20 hours. The supernatant-free or complexed DNA-solution was then decanted off. Optical densities of the free or complexed DNA

solutions were measured at 260 nm. The lower values of the optical densities were indicative of the fact that some macromolecules have been adsorbed by the alumina surface. The present decrease in optical density was evaluated in each case. This value corresponds to the percentage of macromolecules getting adsorbed.

Optical density measurements were recorded in spectrophotometer using 4 cm. quartz cuvettes with 1 cm. path length. Absorption was also measured at 320 nm to see whether any of the adsorbent particles interfered.

A graph giving the % fall in O.D. versus initial $\overset{DNA}{\wedge}$ concentration of free or complexed DNA was plotted (Fig IV).

II. Adsorption of Gamma-Irradiated free or Complexed DNA on Alumina: Adsorption was carried out as in the case of native samples after adjustment of pH to 7.0 and ionic strength with Gamma irradiated samples. Optical density measurements of the samples were recorded before and after irradiation, and also after adsorption. Graphs of per cent fall in O.D. vs. initial $\overset{DNA}{\wedge}$ concentration of DNA were plotted (Fig. Va)

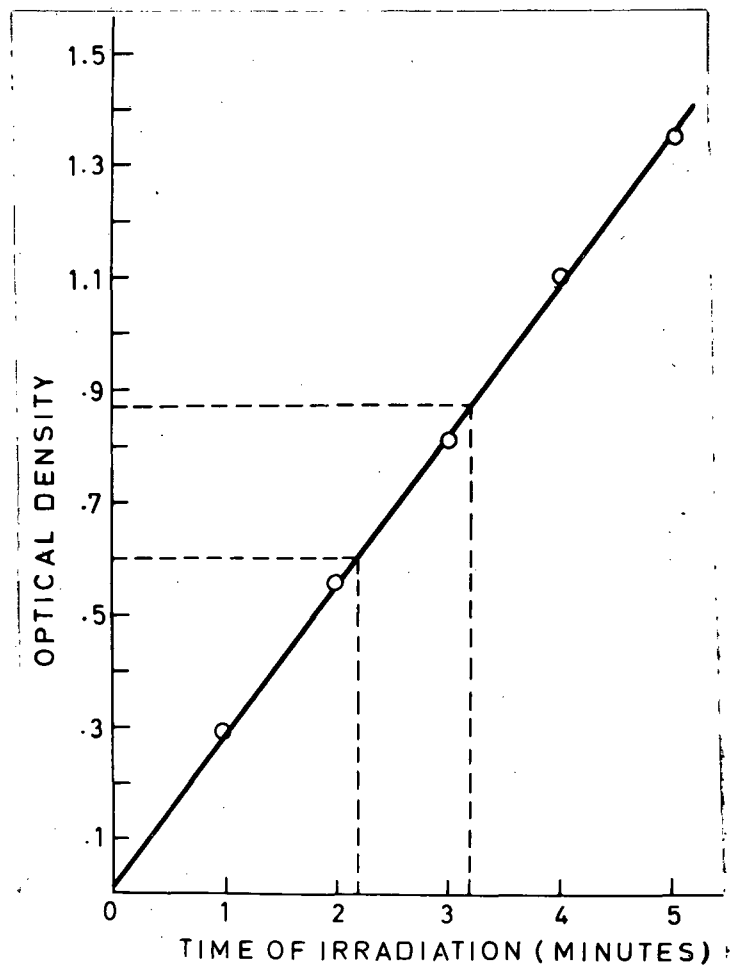

FIG. 1 FRICKE-FERROUS SULPHATE DOSIMETRY

RESULTS

Adsorption of DNA at Alumina - H_2O Interface

I) Adsorption of Native DNA on Alumina

Native DNA was adsorbed on Alumina as described earlier. Table I(a) gives the amounts of DNA adsorbed and the equilibrium concentration of DNA at ionic strength 0.05M and pH 7.

Table I(a)

Initial concentration of DNA µg/ml. Weighed/ (Calculated) (a)	Initial O.D. (Before adsorption)	Final O.D. (After adsorption) at equilibrium	Equilibrium conc. of DNA (µgm/ml) (b)	Change in DNA Conc. (µg/ml) (a-b)	DNA adsorbed (µg/gm of Alumina)
20 (17.5)	0.34	0.08	4.00	13.5	135
40 (33.5)	0.67	0.36	18.00	15.5	155
50 (42.5)	0.85	0.52	26.00	16.5	165
60 (54.5)	1.09	0.70	35.00	19.5	195
80 (68.0)	1.36	0.96	48.00	20.0	200
100 (86.0)	1.72	1.32	66.00	20.0	200

The concentrations of DNA calculated by using the relation $E_1^{1\%}$ cm, 1 ml = 200 are given in Table I(a) in brackets. Fig. II shows the variations in the amount of DNA absorbed (µg/mg of Alumina) with equilibrium concentration

of DNA. The graph shows an initial steep rise in adsorption with concentration. With further rise in concentration, the amount of DNA adsorbed (µg/gm of Alumina) tends to reach a constant value, i.e., a saturation point. At an equilibrium concentration of 4 µg/ml the weight of DNA adsorbed is 135 µg/gm Alumina, whereas the amount of DNA adsorbed at an equilibrium concentration of 18 µg/ml is 155 µgm, the corresponding figure at an equilibrium concentration of 35 µg/ml is only 195 µgm/gm of Alumina.

In order to study what fraction of the initial amount of macromolecule (DNA) is adsorbed on solid-surface one prefers to tabulate initial concentration of DNA with the values of percent change in optical densities at 260 nm. We define

$$\Delta \text{ O.D. } \% = \frac{\text{Initial O.D. - Final O.D.}}{\text{Initial O.D.}} \times 100$$

(Table I(b))

In Fig. III, initial DNA concentrations vs. Δ O.D.% have been plotted. The graph shows approximately an exponential fall with increased concentration.

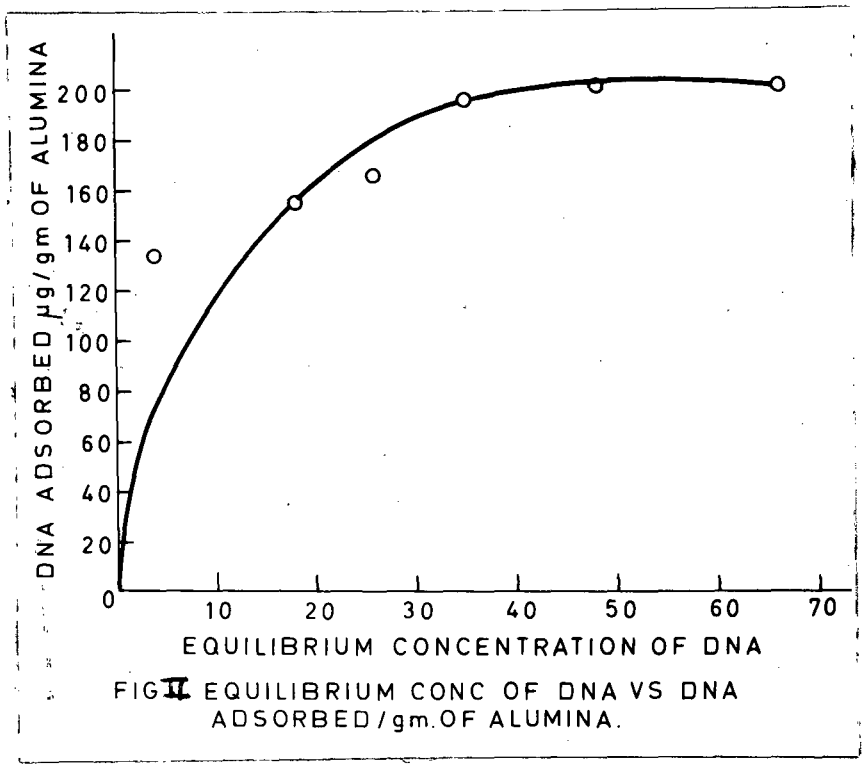

FIG II. EQUILIBRIUM CONC OF DNA VS DNA ADSORBED/gm.OF ALUMINA.

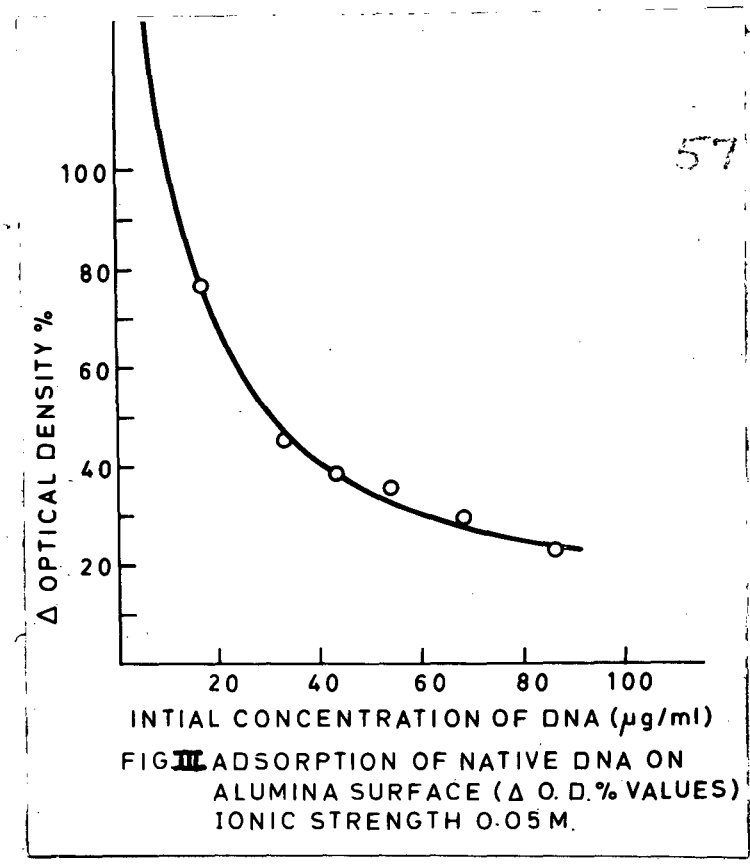

FIG III. ADSORPTION OF NATIVE DNA ON ALUMINA SURFACE (Δ O.D.% VALUES) IONIC STRENGTH 0.05 M.

Table I(b)

Initial Concentration of DNA (µg/ml weighed)	Calculated µg/ml	Δ O.D.%
20	17.5	76.471
40	33.5	46.269
50	42.5	38.824
60	54.5	35.780
80	68.0	29.412
100	86.0	23.256

II) Adsorption of Free and Complexed DNA on Alumina-H_2O Interface

a) At Ionic Strength 0.05M: Daunomycin-DNA complexes were prepared as described earlier. Both free and complexed DNA were adsorbed on alumina.

Table II shows the percentage changes in optical densities at 260 nm (equivalent to % of molecules adsorbed) of free DNA and Daunomycin bound DNA at different concentrations of DNA at ionic strength 0.05M and pH equal to 7. Micromolar ratio of DNA to drug was kept approximately at eight (P/D ≃ 8) in all the samples. P_i stands for DNA at apparent concentration i µg/ml and C_i stands for complex with the same concentration of

DNA keeping P/D=8. Calculated concentration in µg/ml of DNA (from O.D.) is written in brackets. 'P' ranges from 10 to 70 µg/ml.

Table II

Samples (DNA and Complexed DNA)	O.D. before adsorption (a)	O.D. after adsorption (b)	Δ O.D.% $\left(\frac{a-b}{a} \times 100\right)$	Difference in Δ O.D.% of P_i and Ci_1 sample
P_{10} (11.00)	0.33	0.048	78.180	
C_{10}	0.24	0.055	77.083	1.097
P_{20} (19.25)	0.385	0.141	63.377	
C_{20} (	0.465	0.173	62.796	0.581
P_{30} (30.00)	0.60	0.265	55.833	
C_{30}	0.69	0.305	55.791	0.042
P_{40} (37.8)	0.75	0.41	45.333	
C_{40}	0.885	0.480	45.763	- 0.430
P_{50} (52.5)	1.05	0.65	38.095	
C_{50}	1.21	0.765	36.77	1.325
P_{60} (65.0)	1.30	0.88	32.308	
C_{60}	1.57	1.07	31.847	0.461

Table II indicates no significant difference in the Δ O.D.% values of complex with the corresponding Δ O.D.% value of free DNA at various concentrations.

b) At Ionic Strength 0.002M: Tables III (a), III (b) and III (c) show the percentage changes in optical densities at 260 nm (equivalent to % of molecules adsorbed) of free DNA and Daunomycin bound DNA at different concentrations of DNA at ionic strength 0.002M and pH = 7, P/D = 8. P_i and C_i stand as in Table II. Concentration of DNA ranges from 10 µg/ml to 70 µg/ml as weighed.

Table III(a)

Sample (DNA and Complexed DNA)	Initial O.D. (before adsorption)	Final O.D. (after adsorption)	Δ O.D.%	Difference in Δ O.D.% of P_i and C_i samples
P_{10} (14.50)	0.290	0.105	63.79	
				4.33
C_{10}	0.37	0.150	59.46	
P_{20} (22.75)	0.455	0.20	56.04	
				4.32
C_{20}	0.58	0.28	51.72	
P_{30} (30.75)	0.615	0.35	43.09	
				2.96
C_{30}	0.76	0.455	40.13	
P_{40} (42.75)	0.855	0.490	42.69	
				3.11
C_{40}	0.96	0.58	39.58	
P_{50} (46.75)	0.935	0.65	30.48	
				5.26
C_{50}	1.15	0.68	25.22	
P_{60} (55.00)	1.100	0.80	27.27	
				3.48
C_{60}	1.345	1.025	23.79	
P_{70} (63.25)	1.265	0.96	24.11	
				4.24
C_{70}	1.56	1.25	19.87	

Table III (b)

Samples (DNA and Complexed DNA)	Initial O.D. (before adsorption)	Final O.D. (after adsorption)	Δ O.D.%	Difference in Δ O.D.% of P_i and C_i samples
P_{10} (14.75)	0.295	0.095	67.80	
				3.30
C_{10}	0.380	0.135	64.47	
P_{20} (22.50)	0.450	0.20	55.56	
				5.56
C_{20}	0.58	0.29	50.00	
P_{30} (31.00)	0.620	0.320	48.39	
				6.56
C_{30}	0.765	0.445	41.83	
P_{40} (42.25)	0.485	0.49	42.01	
				4.12
C_{40}	0.950	0.59	37.89	
P_{50} (46.50)	0.93	0.65	30.11	
				4.46
C_{50}	1.15	0.855	25.65	
P_{60} (54.00)	1.08	0.27	28.70	
				4.46
C_{60}	1.32	1.00	24.24	
P_{70} (62.50)	1.250	0.95	24.00	
				4.58
C_{70}	1.545	1.245	19.42	

Table III(c)

Samples (DNA and complexed DNA	Initial O.D. (before adsorption)	Final O.D. (after adsorption)	Δ O.D.%	Difference in Δ O.D.% of P_i and C_i samples
P_{10} (14.25)	0.285	0.105	63.16	
				2.89
C_{10}	0.365	0.145	60.27	
P_{20} (23.00)	0.460	0.210	54.35	
				3.07
C_{20}	0.585	0.285	51.28	
P_{30} (31.00)	0.620	0.335	45.97	
				5.18
C_{30}	0.760	0.450	40.79	
P_{40} (42.75)	0.855	0.485	43.27	
				4.41
C_{40}	0.965	0.59	38.86	
P_{50} (47.25)	0.945	0.65	31.22	
				7.08
C_{50}	1.16	0.88	24.14	
P_{60} (55.75)	1.115	0.815	26.91	
				5.59
C_{60}	1.360	1.070	21.32	
P_{70} (64.00)	1.28	0.95	25.78	
				2.92
C_{70}	1.575	1.215	22.86	

Table III(d) shows the average values of Δ O.D.% (average values shown in table III(a), III(b), III(c)) for different samples. Average initial DNA values are written in brackets.

Table III(d)

Concentration of macromolecule (DNA) in ug/ml. Weighed/ (calculated)	Average values of Δ O.D.%
P_{10} (14.50)	64.92
C_{10}	61.40
P_{20} (22.75)	55.32
C_{20}	51.00
P_{30} (30.92)	45.82
C_{30}	40.92
P_{40} (42.58)	42.66
C_{40}	38.78
P_{50} (46.83)	30.60
C_{50}	25.00
P_{60} (54.92)	27.63
C_{60}	23.12
P_{70} (63.25)	24.63
C_{70}	20.72

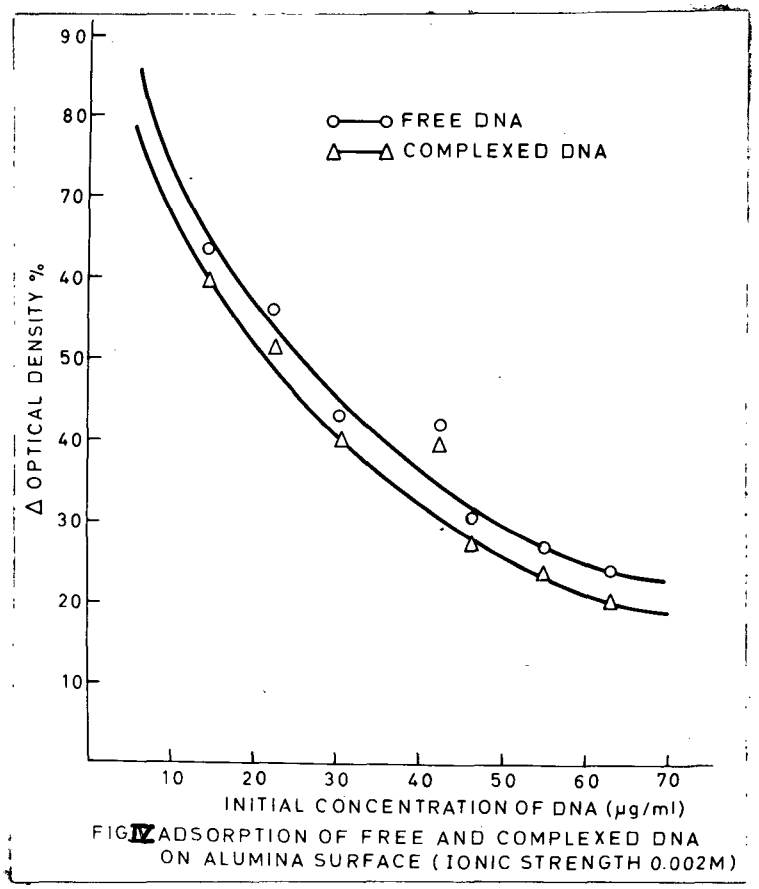

FIG. IV ADSORPTION OF FREE AND COMPLEXED DNA ON ALUMINA SURFACE (IONIC STRENGTH 0.002M)

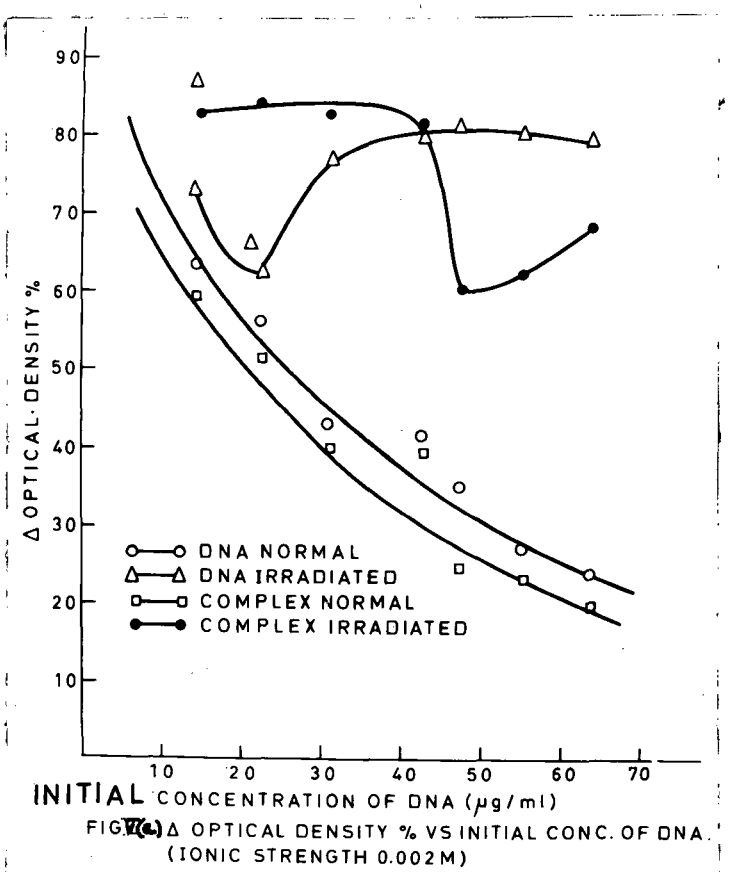

FIG. V(a) Δ OPTICAL DENSITY % VS INITIAL CONC. OF DNA. (IONIC STRENGTH 0.002M)

The above tables indicate decrease in Δ O.D.% value due to complexation. The graphs (Fig. IV) of initial concentration of DNA vs. Δ O.D.% have been plotted both for free and complexed DNA.

III. Adsorption of Gamma-Irradiated DNA on Alumina

a) At Ionic Strength 0.002M: DNA solutions (20 to 70 µg/ml) and the corresponding complexes with Daunomycin were gamma-irradiated as given in Materials and Methods and adsorbed on alumina surface at ionic strength 0.002M. The exact concentrations of DNA were calculated. TableIV(a) shows different values of irradiated samples (DNA and complexed DNA) at various concentrations of DNA and fixed P/D value of 8 for complexes.

Table IV(a)

Samples (DNA and complexed DNA)	O.D. before Irradiation (a)	O.D. after Irradiation (b)	O.D. after adsorption (c)	Δ O.D.% = $\frac{b-c}{b} \times 100$
P_{10} (14.75)	0.295	0.305	0.085	73.81
C_{10}	0.380	0.395	0.070	82.28
P_{20} (22.75)	0.455	0.360	0.135	62.50
C_{20}	0.58	0.56	0.09	83.93
P_{30} (30.75)	0.615	0.605	0.14	76.86
C_{30}	0.76	0.78	0.135	82.69
P_{40} (42.75)	0.855	0.780	0.16	79.49
C_{40}	0.96	0.980	0.18	81.63
P_{50} (46.75)	0.935	1.085	0.205	81.11
C_{50}	1.15	1.23	0.495	60.00
P_{60} (55.00)	1.100	1.145	0.225	80.35
C_{60}	1.345	1.385	0.525	62.09
P_{70} (63.25)	1.265	1.335	0.28	79.03
C_{70}	1.56	1.60	0.51	68.13

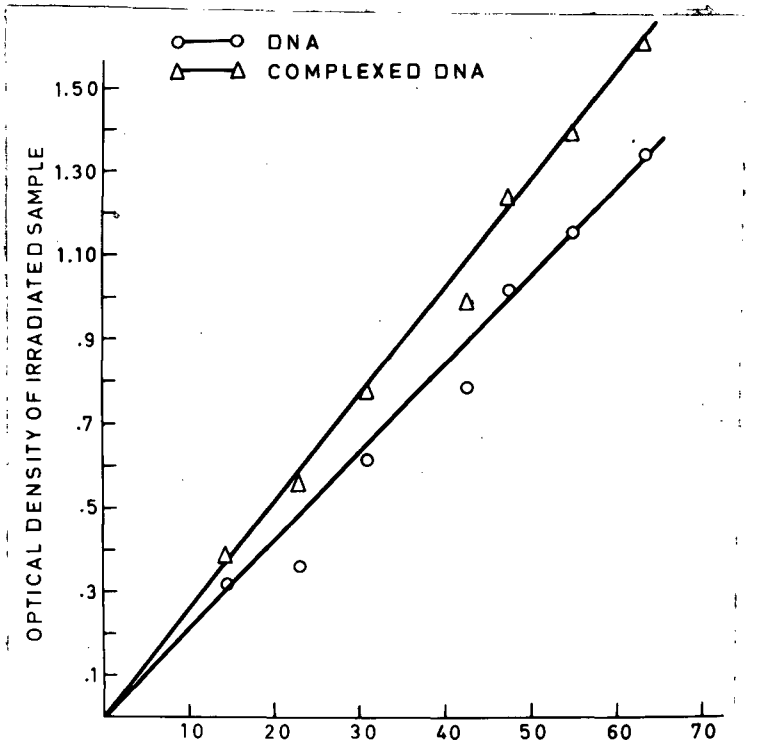

FIG. 6b OPTICAL DENSITY OF IRRADIATED SAMPLE VS CONC. (IONIC STRENGTH 0.002M)

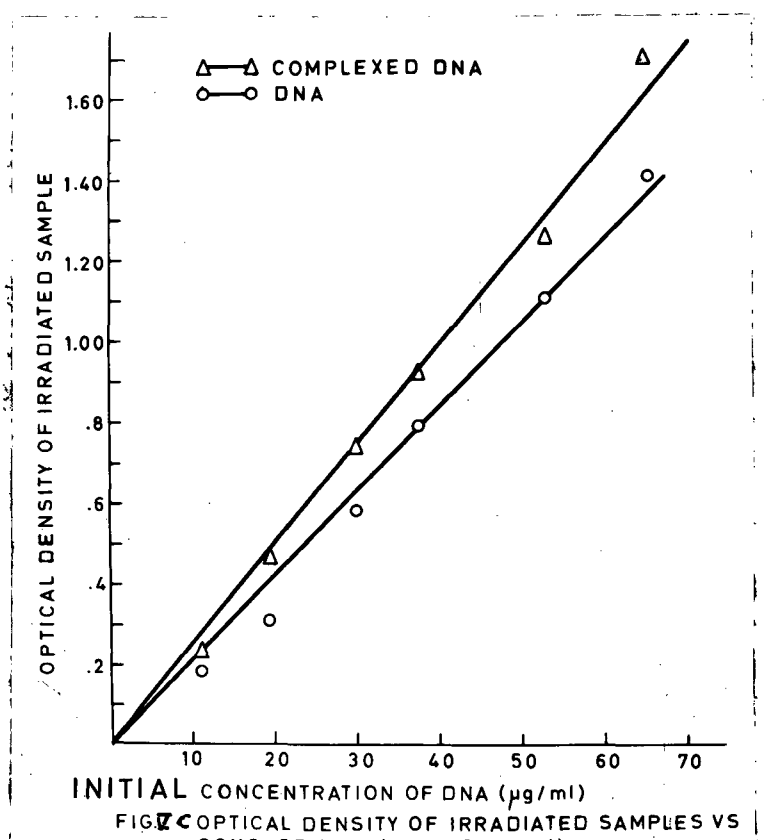

FIG. 6c OPTICAL DENSITY OF IRRADIATED SAMPLES VS CONC. OF DNA. (IN 0.05M Nacl)

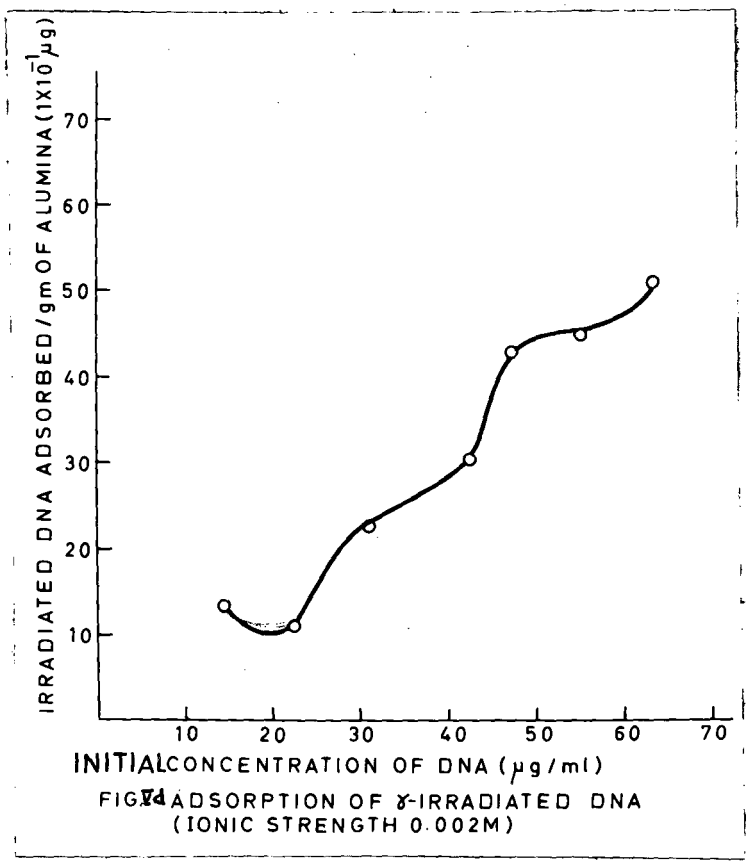

FIG. 21 ADSORPTION OF γ-IRRADIATED DNA (IONIC STRENGTH 0.002M)

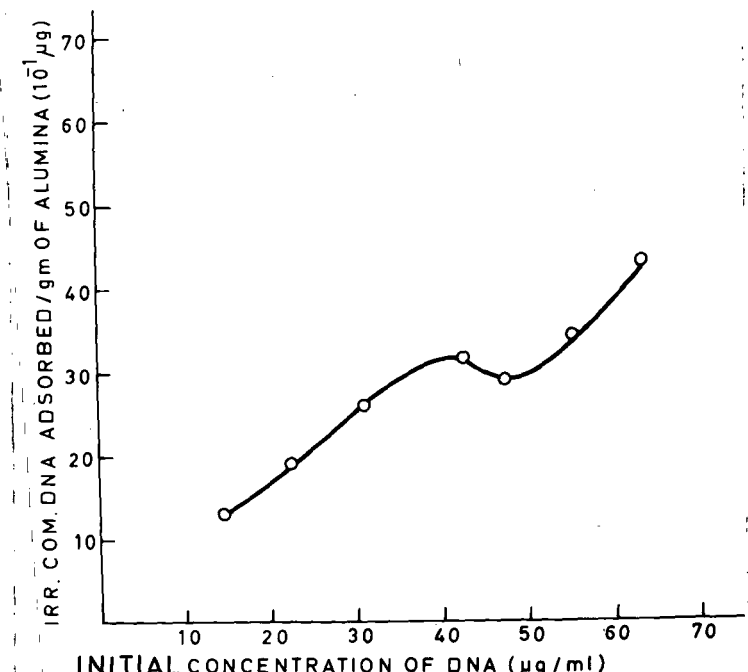

FIG. 22 ADSORPTION OF γ-IRRADIATED COMPLEXED DNA (IONIC STRENGTH 0.002M)

The above results show an increased value of Δ O.D.% than the corresponding unirradiated sample for each concentration of DNA.

The graphs of initial concentration of DNA vs. Δ O.D.% have been plotted in Fig. V for both the unirradiated free and complexed DNA samples and irradiated free and complexed DNA samples. (in the same scale) The standard plots of O.D of irradiated DNA & irradiated complexed DNA vs. initial conc. of DNA are given in Table V & the amounts of irradiated DNA & complexed DNA adsorbed vs. initial concentration of DNA have been plotted in Fig. E(d) & E(e) respectively.

b) At Ionic Strength 0.05M: Table IV(b) shows different values of Δ O.D.% for gamma-irradiated free and complex DNA samples at different concentrations of DNA (10 to 70 µg/ml) at ionic strength 0.05M due to adsorption on Alumina surface.

Table IV(b)

Samples (DNA and complexed DNA)	O.D. before Irradiation (a)	O.D. after Irradiation (b)	O.D. after adsorption (c)	Δ O.D.% = $\frac{b-c}{b} \times 100$
P_{10} (11.00)	0.22	0.18	0.095	47.22
C_{10}	0.24	0.245	0.08	67.35
P_{20} (19.25)	0.385	0.315	0.10	68.25
C_{20}	0.465	0.47	0.08	82.98
P_{30} (30.00)	0.60	0.58	0.142	75.52
C_{30}	0.69	0.74	0.085	88.51
P_{40} (37.50)	0.75	0.79	0.148	81.27
C_{40}	0.885	0.925	0.13	85.94
P_{50} (52.50)	1.05	1.10	0.205	81.36
C_{50}	1.21	1.25	0.190	84.80
P_{60} (65.00)	1.30	1.40	0.22	84.28
C_{60}	1.57	1.70	0.435	74.41

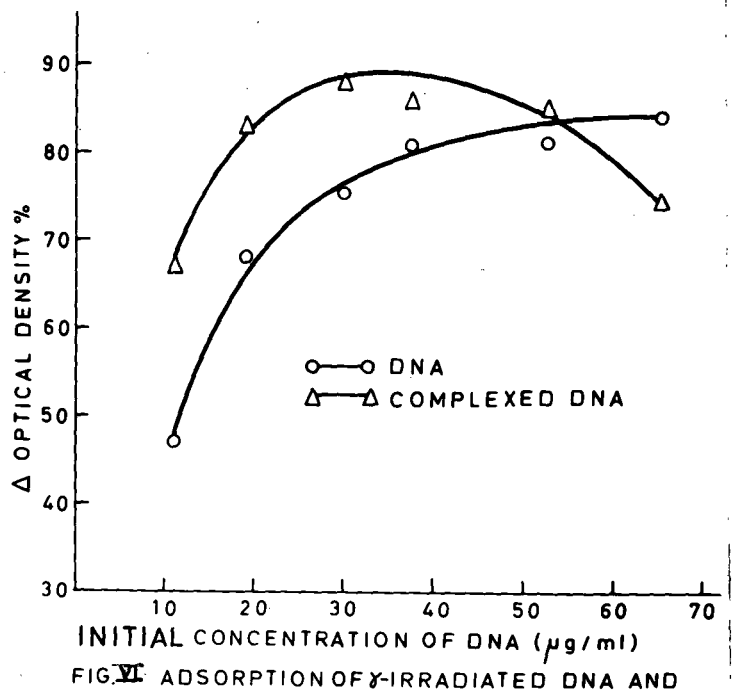

FIG. VI ADSORPTION OF γ-IRRADIATED DNA AND COMPLEXED DNA ON ALUMINA SURFACE (IONIC STRENGTH 0.05M.)

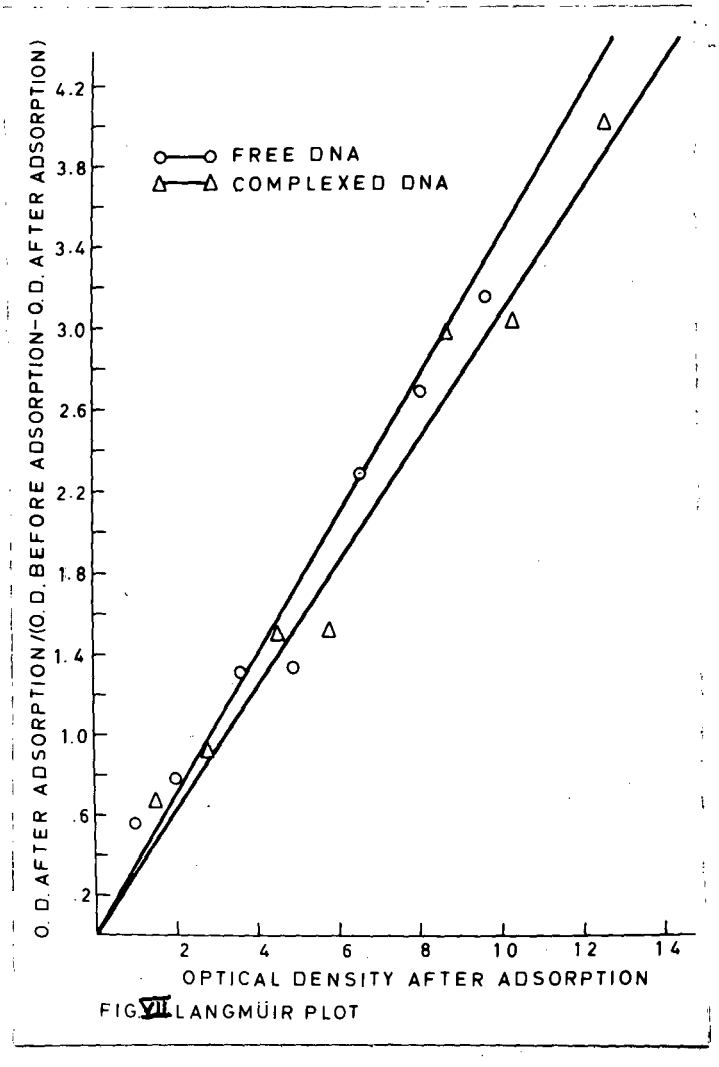

FIG. VII LANGMÜIR PLOT

The above table indicates that there is appreciable change in Δ O.D.% in each γ-irradiated sample although there was insignificant change in Δ O.D.% in case of unirradiated samples at 0.05M ionic strength. The graphs of initial concentration of DNA vs. Δ O.D.% at 0.05M ionic strength, for both irradiated free and complexed DNA, have been shown in Fig. VI.

The graphs have the following features:

i) The values of free DNA rises with increase in DNA concentration, whereas that of the complexed DNA initially rises and then falls with increase in concentration.

ii) The two curves intersect each other at a point corresponding to DNA concentration 52.5 µg/ml.

Langmuir Plot:

Langmuir plot is the plot of equilibrium concentration of DNA vs. $\left(\frac{\text{equilibrium concentration of DNA}}{\text{DNA adsorbed}}\right)$

i.e., C vs. $\frac{C}{X}$

where, C = equilibrium concentration of DNA

X = DNA adsorbed.

But, equilibrium concentration C is proportional to O.D. after adsorption and DNA adsorbed is proportional

to Δ O.D., i.e., (O.D. before adsorption - O.D. after adsorption)

Hence,

$$\frac{C}{X} = \frac{\text{O.D. after adsorption}}{(\text{O.D. before adsorption} - \text{O.D. after adsorption})}$$

Hence, the plot of O.D. after adsorption vs.

$$\left(\frac{\text{O.D. after adsorption}}{\text{O.D. before adsorption} - \text{O.D. after adsorption}}\right)$$

should follow the same nature as C vs. $\frac{C}{X}$ plot.

In order to avoid calculation of exact values of C and X in case of complexed DNA, we plot O.D. after adsorption vs.

$$\frac{\text{O.D. after adsorption}}{(\text{O.D. before adsorption} - \text{O.D. after adsorption})}$$

(Fig. VII) which is equivalent to Langmuir plot.

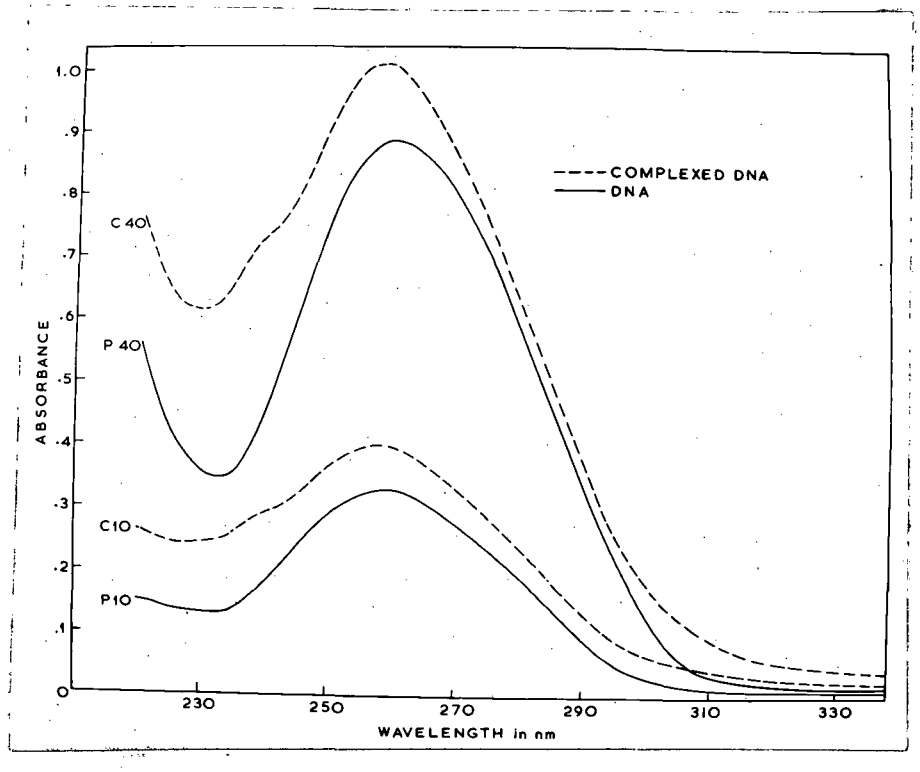

FIG. VIII UV ABSORPTION SPECTRA OF FREE DNA AND COMPLEXED DNA.

DISCUSSION

Adsorption phenomenon at solid-liquid interfaces are controlled in most cases by the electrical double layer. Hence, the charge on the solid surface is important as far as the behaviour of ions that adsorb as counter ions are concerned. In our work, the adsorption of DNA on a positively charged surface like that of Alumina has been studied. The point of zero charge of Alumina is at pH 9.0. Hence, below this pH value Alumina particles will be positively charged. The importance of the point of zero charge is that the sign of the surface charge has a major effect on the adsorption of all other ions. For oxides, hydrogen and hydroxyl ions have been considered to be potential determining (Wood, 1946). A hydroxylated surface is usually formed when oxide minerals come in contact with water vapour. Hence, it is assumed that a hydroxylated surface is formed when the solid oxide (e.g. Alumina) is equilibrated with an aqueous solution. Adsorption or dissociation of hydrogen ions from the hydroxylated surface can account for the surface charge on the oxide by the following mechanism (Healy and Fuerstenan, 1965)

$$MOH\ (surface) \rightleftharpoons MO^{-}\ (surface) + H^{+}(aq)$$

$$MOH\ (surface) + H^{+}(aq) \rightleftharpoons MOH^{+}\ (Surface)$$

where, M represents the metal. Parks and deBruyn (1962) have postulated a different mechanism for the charging of oxide surfaces involving partial dissolution of the oxide, formation of hydroxyl complexes in solution and subsequent adsorption of these complexes. Thus, Alumina particles can be expected to get a positive charge in contact with water.

The physical state of the dissolved DNA is very important as in adsorption studies, the equilibrium process:

Polymer in Solution $\rightleftharpoons$ Polymer adsorbed at surface

is being considered. The interaction of the DNA molecule with the solvent (in this case, water) determines the state of DNA in solution and this is also reflected in some way in the adsorbed state. The unique physical properties of DNA molecules in solution or at an interface are a result of their flexibility. Thus, DNA, being a long, chain-like molecule containing several hundreds of bonds in the backbone can assume a number of different configurations. Thus, the DNA molecule will have a very large configurational

entropy/mole. This has been confirmed by intrinsic viscosity and sedimentation coefficient measurements of the DNA solution which indicate that the DNA structure in solution is intermediate between the rod and the random coil. For a DNA molecule of high molecular weight there exist 200 to 450 statistical segments of length ranging from 1400 to 2700 Å (Gordon, 1970). Further refined analysis of hydrodynamic data and electron micrographs support the worm-like nature of DNA molecule in solution. However, adsorption at solid surface or the influence of other external forces can cause drastic conformational alteration in the DNA structure. The majority of forces responsible for biopolymer conformation is due to hydrophobic interactions (Upadhyay and Chattoraj, 1972). This is due to the presence of H_2O all round the DNA molecule. Any change in the aqueous environment during adsorption at a solid surface may cause a change in the conformation, of the molecule. Solvent water enmeshed in the macromolecular DNA coil is considered to be virtually trapped and carried along during rotational or translational motion of the DNA molecule. Hence, the voluminosity or degree of swelling of the DNA molecule will be determined by the degree of

interaction with the solvent. Since H_2O is being carried along with the DNA molecule, it may contribute to the binding of the DNA to the solid surface. It is suggested that the portion of the adsorbed DNA molecule, in close contact with solid surface, may have a rigid and compact structure to which the bound solvent H_2O molecule also contributes. The rigidity of the structure at interface inhibits relative motion of solid and liquid in this region. The residual part of the DNA molecule, which, in view of its distance from the adsorbent, is not bound to the solid surface is largely surrounded by H_2O molecules which results in swelling of these regions and the conformation is more extended. The charge and structure of the adsorbent molecules will play a significant role in determining the structural balance between the rigid part and the free or expanded part of the DNA molecule. Electrostatic interactions between DNA and the adsorbent particles are also important in determining the extent of adsorption.

It has already been mentioned in Introduction that drug molecules can bind with DNA in two ways, weak binding or stacking and strong binding or intercalation. On the other hand, it has been indicated

that the adsorption of DNA on Alumina surface is mainly due to electrostatic interaction between the negatively charged phosphate groups on the outer region of the macromolecular chain and the positively charged Alumina surface. Hence, the adsorption on Alumina surface is a function of the number of negatively charged sites on the DNA template.

Keeping in view the two types of binding modes, one can logically speculate that when the DNA is complexed with a ligand, specially when the drug molecules are stacked to DNA, then the number of negatively charged phosphate sites remaining vacant for adsorption is reduced. And hence the adsorption on Alumina surface should be comparatively less in the case of the complexed DNA than that of free DNA. As the adsorption profile of complexed DNA is not known, this work was undertaken on the above mentioned assumptions.

Adsorption process is dependent on the ionic environment. Initially the experiments were carried out in a solvent of 0.05M sodium chloride (Chattoraj and Upadhyay, 1968; Chari and Mookerjee, 1975). But,

in case of unirradiated Daunomycin-DNA complex no significant difference between the adsorption of pure DNA at P/D ratio equal to 8 was found. This is justified as follows.

Peacocke and Skerrett (1956) have shown in case of Proflavin-DNA complex that with increase in ionic strength number of dye-molecules bound per phosphate molecule (r) decreases. It has also been pointed out that in general, low values of r mainly correspond to intercalations and stacking takes place at higher r-values ($r \gtrsim 0.02$). But due to lack of any other data it has been assumed that it is only the stacking that is affecting the adsorption process. Therefore, it may be concluded that at an ionic strength 0.05M there has not been sufficient stacking to show significant difference in adsorption patterns between the free-DNA and the complexed DNA.

Next, a lower ionic strength 0.002M was chosen in order to get significant difference in stacking between free and complexed DNA. The UV adsorption spectra of Daunomycin-DNA complex and free-DNA with the same concentration as in complex were observed. (Fig. VIII) Comparison of these two spectra shows that although the optical density differs there is little change

in their contour patterns excepting a hump near 240 nm and a blue shift of the DNA peak at 260 nm by 2 nm due to complexation. This leads to the conclusion that due to stacking the molecular structure of DNA (the p π and d π orbitals) remains more or less unchanged. Intercalation leads to the changes in molecular structure (Peacocke and Skerrett, 1956) changing the UV absorption spectra whereas stacking does not. Therefore, it is wise to consider that in the above case stacking is dominant and intercalation is reduced considerably. Under such circumstances, when both the free and complexed DNA give absorption maxima near 260 nm, the optical density measurements at 260 nm can be taken to be proportional to the concentration or number of molecules per ml.

In case of DNA adsorption isotherms and adsorption models, Chattoraj and Upadhyay have shown that for native calf thymus DNA the Langmuir plot (C/X vs. C) gives a straight line, and later this was shown to hold for heat, U.V. and γ-irradiated DNA by Chari and Mookerjee (1975)(Fig. VII). Although for gamma-irradiated DNA there are two distinct linear regions which indicated the formation of two types of monolayers. This clearly shows that the adsorption of a

polyelectrolyte like DNA takes place through the same process as in Langmuir model. In fact, this model with modification in case of a polymer fits the adsorption of polynucleotides on solid surface.

Before adsorption of the polymer the small molecules and electrolytes occupy the adsorption sites on the solid surface and cover it by molecular layer formation. By means of continuous shaking with the polymer solution, the previous equilibrium is disturbed while the adsorption sites are more and more exposed to the polyelectrolytes. This enables the polyelectrolytes to get adsorbed till equilibrium is attained.

Let, on the average ν moles of solvent be displaced by 1 mole of phosphate,

if, A = polymer in solution of mole-fraction N_2

B_s= solvent adsorbed of mole-fraction N_1^s

A_s= polymer adsorbed of mole-fraction N_2^s

B = solvent in solution of mole-fraction N_1

then, $$A + \nu.B_s = A_s + y.B \quad \ldots\ldots (1)$$

The equilibrium constant for the process at the experimental temperature is given by

$$K = \frac{N_2^s \, a_1^{\nu}}{(N_1^s)^{\nu} a_2} \quad \ldots\ldots (2)$$

where a_1 and a_2 are the solvent and solute activities in solution. For dilute solutions $a_1 = N_1$; $a_2 = N_2$.

Let us apply modified Langmuir model for adsorption of polymer on solid surfaces. (Adamson)

We consider DNA as the chain of phosphate units which are negatively charged and adsorbed via electrostatic interaction on the positively charged Alumina surface. When undergoing adsorption a part of the chain remains in the solution unadsorbed. This means only a fraction of the total phosphate is bound to the solid surface. However, this takes place by replacement of solvent molecules already adsorbed on the solid surface.

Then, we have from modified Langmuir model

$$\frac{\theta/\nu}{(1-\theta^{\nu})} = \frac{K.C_2}{C_1^{\nu}} \qquad \ldots\ldots (3)$$

where, θ = fraction of surface covered by the macromolecule

C_1 = concentration of solvent in mole-fraction

C_2 = concentration of solute (macromolecule) in mole fraction.

Now,

$$\theta = \frac{\text{no. of moles of solute molecules adsorbed } (n_2^s)}{\text{no. of moles of solvent molecules adsorbed } (n_s)}$$

Now, for our system $n_s \gg n_2^s$ and $\nu \leq 1$, hence we can write, $1 - \theta^{\nu} \simeq 1$

Hence, $\frac{\theta}{\nu} = \frac{K.C_2}{C_1^{\nu}}$

or, $\frac{n_2^s}{n_s} = \frac{K \cdot \nu \cdot C_2}{C_1^{\nu}}$ . . . . . (4)

Now, C_1 = mole-fraction of solvent.

The solvent in the present set of experiments is 0.002M NaCl in water. The function of NaCl is mainly to stabilise DNA. The role of solvent in adsorption is mainly played by water molecules and one can neglect the role of sodium and chloride ions in adsorption. However, the experimental solutions are dilute so that mole-fraction of water remains constant for all practical purposes.

Therefore, $C_1 \simeq 1$

Again, C_2 = mole-fraction of macromolecule in solution at equilibrium

$$= \frac{\text{moles of macromolecule in soln.}}{\text{moles of macromolecule + moles of water in soln.}} \text{ (at equilibrium)}$$

For dilute solutions,

moles of macromolecules $\ll$ moles of water present.
(of the order of 10^{-4}M)

Hence, $C_2 = \frac{\text{moles of macromolecule in soln.}}{\text{moles of water in soln.}}$ (at equilibrium)

Therefore, from eq.(4),

$$\frac{n_2^s}{n_s} = K.\ \nu.\ \frac{\text{moles of macromolecule in soln.}}{\text{moles of water in soln.}}$$

or, $$\frac{n_2^s}{\text{moles of macromolecule in soln.}}$$

$$= \frac{K.\ n_s}{\text{moles of water in soln.}} \times \nu$$

Writing $$\frac{n_2^s}{\text{moles of macromolecule in soln.}} = f$$

we have $$f = \frac{K.\ n_s}{\text{moles of water in soln.}} \times \nu \quad . . (5)$$

The term f is equal to the ratio of moles of macromolecule adsorbed on solid surface to moles of macromolecule present in solution at equilibrium. But, the total volume of solution in contact with 1 gm solid was 10 ml. hence mole of macromolecule adsorbed is proportional to change in concentration.

And, moles of macromolecule in solution at equilibrium corresponds to the equilibrium concentration of the macromolecule in solution

$$C = \frac{1}{\epsilon . l} \cdot O.D.$$

where, ϵ is a constant at a particular wavelength.

and l is chosen to be a constant.

Then, macromolecule taken out/ml

= Initial concentration - Final concentration

$$= \left(\frac{1}{\epsilon} . l.\right) O.D.\ \text{initial} - \left(\frac{1}{\epsilon} . l.\right) O.D.\ \text{final.}$$

Therefore, fraction of macromolecule taken out to solid surface/ml $= \frac{k(O.D._{Initial} - O.D._{Final})}{K.O.D._{Initial}}$ where $k = \frac{1}{\epsilon . l}$

i.e., $\frac{O.D._{Initial} - O.D._{Final}}{O.D._{Initial}} \times 100$ or $\Delta O.D.\%$

By using this method of calculation, evaluation of 'k' may be avoided which is not known for Daunomycin-DNA complex.

Again, $f = \frac{\text{Initial O.D.} - \text{Final O.D.}}{\text{Final O.D.}}$

and this is determined from experimental UV adsorption data.

We rewrite from (5) for free-DNA:

$$f = \frac{K.\ n_s}{\text{moles of water in soln.}} \cdot \nu \quad \ldots (6)$$

for complexed DNA:

$$f' = \frac{K'.\ n_s}{\text{moles of water in soln.}} \cdot \nu' \quad \ldots (7)$$

deviding (7) by (6),

$$\frac{f'}{f} = \frac{K'}{K} \cdot \frac{\nu'}{\nu}$$

considering K and K' to be of the same order,

$$\frac{K'}{K} \simeq 1$$

so that,

$$\frac{f'}{f} = \frac{\nu'}{\nu} \quad \text{or,} \quad \frac{f - f'}{f} = \frac{\nu - \nu'}{\nu}$$

We consider the term $\frac{\nu-\nu'}{\nu}$ to be the measure of the fraction of phosphates stacked by Daunomycin.

The following tables (V(a), V(b), V(c)) give the calculated values of $\frac{f-f'}{f} \times 100$ for different samples (free and complexed DNA).

Table V(a)

Sample	O.D. before adsorption	O.D. after adsorption	f & f' values	$\frac{f-f'}{f} \times 100$
P_{10} (14.75)	0.295	0.095	2.1053	
C_{10}	0.380	0.135	1.8148	13.7985
P_{20} (22.50)	0.450	0.20	1.25	
C_{20}	0.58	0.29	1.00	20.00
P_{30} (31.00)	0.620	0.320	0.9375	
C_{30}	0.765	0.446	0.7191	23.296
P_{40} (42.25)	0.845	0.49	0.7245	
C_{40}	0.900	0.59	0.6102	15.7764
P_{50} (46.50)	0.93	0.65	0.4308	
C_{50}	1.15	0.855	0.3450	19.9164
P_{60} (54.00)	1.08	0.77	0.4026	
C_{60}	1.32	1.00	0.3200	20.5166
P_{70} (62.50)	1.250	0.95	0.3158	
C_{70}	1.545	1.245	0.2410	23.6858

Table V(b)

Sample	O.D. before adsorption	O.D. after adsorption	f & f' values	$\frac{f-f'}{f} \times 100$
P_{10} (14.25)	0.285	0.105	1.7142	
C_{10}	0.365	0.145	1.5172	11.4922
P_{20} (23.00)	0.460	0.210	1.1905	
C_{20}	0.585	0.285	1.0526	11.5834
P_{30} (31.00)	0.620	0.335	0.8507	
C_{30}	0.760	0.450	0.6889	19.0796
P_{40} (42.75)	0.855	0.485	0.7629	
C_{40}	0.965	0.59	0.6356	16.6863
P_{50} (47.25)	0.945	0.65	0.4538	
C_{50}	1.16	0.88	0.3182	29.881
P_{60} (55.75)	1.115	0.815	0.3681	
C_{60}	1.360	1.070	0.2710	26.3787
P_{70} (64.00)	1.28	0.95	0.3475	
C_{70}	1.575	1.215	0.2963	14.7338

Table V(c)

Sample	O.D. before adsorption	O.D. after adsorption	f & f' values	$\frac{f-f'}{f} \times 100$
P_{10} (14.50)	0.290	0.105	1.7613	16.7357
C_{10}	0.37	0.150	1.4667	
P_{20} (22.75)	0.455	0.20	1.275	15.9686
C_{20}	0.58	0.28	1.0714	
P_{30} (30.75)	0.615	0.35	0.7571	11.4648
C_{30}	0.76	0.455	0.6703	
P_{40} (42.75)	0.855	0.490	0.7449	12.0419
C_{40}	0.96	0.58	0.6552	
P_{50} (46.75	0.935	0.65	0.4385	23.1015
C_{50}	1.15	0.86	0.3372	
P_{60} (55.00)	1.160	0.80	0.375	16.7467
C_{60}	1.345	1.025	0.3122	
P_{70} (63.25)	1.265	0.96	0.3177	21.9389
C_{70}	1.56	1.25	0.2480	

The average values from table V(a), V(b), V(c) are shown in table VI

Table VI

Concentration of macromolecule (DNA) in ug/ml as weighed/ (calculated)	Average values of $\frac{f - f'}{f} \times 100$
10 (14.50)	14.0088
20 (22.75)	15.8203
30 (30.92)	17.9268
40 (42.58)	14.8344
50 (46.83)	24.2996
60 (54.92)	21.2140
70 (63.25)	20.1125

The values of % of phosphates stacked by Daunomycin as equated to the $\frac{f-f'}{f}$ values, lead to the following inferences:

1) At the specific ionic strength of 0.002M and P/D = 8, percentage of phosphates (conc. 10 to 70 µg/ml) ranges from14 to 25%.

2) While calculating the % of number of binding sites stacked $\frac{\nu-\nu'}{\nu}$ is evaluated. This is done on the basis of that even if the binding of the drug to DNA chain is not a random process, DNA chain can be considered to consist of regions of binding sites

and these regions are randomly distributed over the DNA chain. This allows us to conclude that the number of segments of the macromolecular chain getting adsorbed on the solid surface will be at random process. The same also holds in case of complexed DNA with bound phosphate sites due to drug interaction.

3) The values of stacked phosphate sites per 100 phosphates (Table VI) seems to be distributed around certain mean value (18.3166). This indicates that in the range of concentrations of DNA (10 µgm/ml to 70 µgm/ml) in 0.002M NaCl solution, where P/D ratio is maintained to be equal to a constant (P/D=8), the fraction of phosphates stacked by Daunomycin is roughly a constant, given by the mean value. This is quite likely in the sense that throughout all the concentrations, the drug molecules are exposed to DNA molecules in a constant proportion (P/D ≃ 8).

Table VII

Concentration of macromolecule (DNA) in ugm/ml.as weighed/ (calculated)	Average values of $\frac{f-f'}{f} \times 100$	Mean values of $\frac{f-f'}{f} \times 100$
10 (14.50)	14.0088	
20 (22.75)	15.8203	
30 (30.92)	17.9268	18.3166
40 (42.58)	14.8344	
50 (46.83)	24.2996	±3.4935
60 (54.92)	21.2140	
70 (63.25)	20.1125	

The second phase of the experiment was to study the adsorption patterns of gamma-radiated native and complexed DNA. The significant increase in the value of Δ O.D.% due to irradiation at each concentration of DNA indicates the increase in number of sites to be adsorbed. This may be due to strand separation and strand sission, which increase the number of openings for interaction. These are usually expected when DNA is exposed to gamma-radiation (Chari and Mookerjee, 1975). However, unlike the case of native DNA, Δ O.D.% vs. concentration of DNA curve for irradiated DNA shows a rise at the beginning and then a somewhat flat portion with increase in concentration. This means with increase in concentration the number of openings and hence the number of available adsorption sites increases so that more and more fraction of molecules undergo adsorption. Again, comparison of the radiation denatured curves for free DNA and complexed DNA show that at a lower concentration fraction of complexed molecules adsorbed is higher but it falls with increase in concentration and finally becomes lower than that of free irradiated DNA. This leads to the conclusion that with increase in concentration

drug binding protects the macromolecule from denaturation, which is expected also, for the bound drugs resist the radiation damage to DNA.

However, beyond a certain concentration, the curve shows further rise indicating greater fraction of molecules adsorbed. The plausible reason may be that of detachment of bound drug molecules from phosphate chain hence exposing the macromolecules more to the positive charges of the solid surface for adsorption.

The calculation for irradiated native and complex DNA are being done.

SUMMARY

The adsorption profile of free and Daunomycin bound DNA at Alumina-H_2O interface have been studied. Proper experimental conditions were maintained to reduce intercalation considerably and to make electrostatic binding predominant in the DNA-Daunomycin complex. Adsorption of drug bound DNA was less than that of free DNA. Similar studies have also been done after irradiating the samples by Gamma-radiation. The quantities of gamma-irradiated free and complexed DNA were much greater than the corresponding unirradiated free and complexed DNA. Model for adsorption of polymers was used to calculate average value of percent of phosphates stacked by Daunomycin.

REFERENCES

Adamson, A.W. (1967) Physical Chemistry of Surfaces, 2nd Ed. Interscience Publishers, U.S.A.

Akinrimisi, E.O., Bonner, J. and P.O.PTs'o (1965) Binding of basic proteins to DNA. J. Mol.Biol. 14, 129.

Arcos, J.C. and Argus, M.F. (1974) Structure activity relationships. In: Chemical Induction of Cancer; Vol. II-A, Academic Press, N.York and London.

Armstrong, R.N., Kuruscev, T. and Strauss, U.P. (1970) The interaction between acridine dyes and DNA. J. Am. Chem. Soc., 92 (10), p. 3174.

Barnett, L.B. and Bull, H.B. (1959) J. Am.Chem.Soc., 81, 5128.

Berenblum, I. (1974) Two stage mechanism on carcinogenesis in biochemical terms. In: The Physiopathology of Cancer, (ed.) F. Homburger, Vol. I, Biology and Biochemistry (Vol. Ed: P. Shubik), S. Karger Publishers, Basel, Munchen, Paris, London, N.York, Sydney.

Bergmann, E.D. and Pullmann, B. (1969) Ed. Physicochemical mechanism of carcinogenesis, Israel Academy of Science and Humanities, Jerusalem (Distrib: AP, N.York).

Bradley, D.F. and Wolf, M.K. (1959) Aggregation of Dyes bound to Polyanions. Proc. Natl. Acad. Sci., 45, p. 944.

Bull, H.B. (1947) Adv. Prot. Chem., 3, p.95.

Bull, H.B., (1956) Adsorption of bovine serum albumen on glass. Biochim. Biophys. Acta., 19, 464.

Bull, H.B. (1957) Adsorbed monolayer of egg albumen. Arch. Bio Chem. Biophys. 68, 102.

Bull, H.B. (1958) J. Am. Chem. Soc., 80, 1901.

Chan, E.W. and Ball, J.K. (1971a): Interaction of DNA with three Dimethyl Derivatives of Benz(c)Acridines. Biochim. Biophys. Acta., 238, p.31.

Chan, E.W. and Ball, J.K. (1971b) Polycyclic Aromatic Hydrocarbons - Covalent Binding to DNA and effects on template function. Biochim. Biophys. Acta., 238, 46.

Chargaff, E. and Davidson, J.N. (1955) The Nucleicm acids VI, Academic Press.

Chattoraj, D.K. and Bull, H.B. (1959) Electrophoresis of adsorbed protein, J. Am. Chem. Soc., 81, 5128.

Chattoraj, D.K. and Chaurashi (1967) Electrophoresis of adsorbed DNA, Bio-Polymers 5(2), 173.

Chattoraj, D.K. and Upadhyay, S.N. (1968) Adsorption of nucleic acids at the Alumina-H_2O interface. Bio-Chem. Biophys. Acta., 161, 561.

Cole, L.J. and Nowell, C. (1965) Radiation Carcinogenesis: The sequence of events. Science, 150, 1782.

Crothers, D.M. (1968) Calculations of binding isotherms for heterogenous polymers. Biopolymers, 6, 575.

Das, G.C., Dasgupta, S. and Dasgupta, N.N. (1974) Interaction of Nogalamycin with DNA. Biochim.Biophys. Acta., 353, 274.

Dasgupta, S. and Dasgupta, N.N. (1973) Electron Microscopic and Spectrophotometric studies of DNA - Proflavine complexes. Biochim. Biophys. Acta., 294, 38.

Dasgupta, S. and Misra, D.N. (1973) Conformation of Proflavine bound DNA molecules. Z. Naturforsch, 29(c), 128.

DiMacro, A., ~~xxxxxx~~ Zunino, F., Silvestrini, R., et al., (1971) Interaction of some Daunomycin Derivatives with DNA and their biological activity. Biochem. Pharmacol., 20, 1323.

Fontana, B.J. (1971) In: The Chemistry of Biosurfaces IV. Ed. Hair M.L., MarcelDekker Inc., New York.

Freifelder, D. (1971) Journ. Molec. Biol., 60, 401.

Fromer, M.A. and Miller, I.R. (1968) Adsorption of DNA at air-water interface. J. Phys. Chem., 72, 2862.

*Chari, R.V.J. and Mookerjee, A. (1975) Adsorption Profile of Native and Irradiated DNA at Solid-liquid Interface, Indian Jr. of Biochem. Biophys., 12, 219.

Frommer, M.A. and Miller, T.R. (1966) Method for measuring the adsorbed tritium labelled compound and its application in the investigation of surface activity of DNA. J. Colloid & Interface Sci., 21, 245.

Fuerstenau, D.W. (1971) In: The Chemistry of biosurfaces V.I. Ed. Hair M.L., Marcel Dekker Inc., New York.

Fuller, W. and Waring, M.T. (1964) A Molecular model for the interaction of ethidium bromide with DNA. Ber. Der. Bunsenges. 68, 805.

Gordon (1970) Electron microscopic observation of DNA adsorption on Alumina and mica surface. J. Coll. Interface Sci. 34(1), 131.

Goswami, D.N., Das, J. and Dasgupta, N.N. (1973) Eielectric Behaviour of DNA-Proflavine complex. Biopolymers, 12, 1047.

Gottlieb, D. and Shaw, P.D. (1967) Ed. Antibiotics - Mechanism of action, vol. I, Springer-Verlag, Berlin, Hydelberg, New York.

Healy, T.W. and Fuerstenan, D.W.,(1965) J. Coll. Sci., 20, 376d.

Hollstein, U. (1974) Actinomycin, chemistry and mechanism of action. Chemical Reviews, 74(6), 625.

Hummel, J.P. and Anderson, B.S. (1965) Arch. Biochem. Biophys., 112, 443.

Kersten, W. and Kersten, H? (1965) Die BindungVon Daunomycin, cinerubin und chromomycin A. An Nucleinsauren. Biochem. Z., 341 194.

Lerman, L.S. (1961) Structural considerations in the interaction of DNA and Acridines. Jour. Molec. Biol., 3, 18.

Lochmann, E.R. and Micheller, A. (1973) Binding of organic dyes to nucleic acids and the photodynamic effect. In: Physico chemical properties of nucleic acids, Vol. I, Ed. J. Duchesne, London, New York, Academic Press.

* Li, H.J., & Crothers, D.M., (1969): Relaxation studies of Proflavine-DNA complex: Kinetics of the intercalation reaction. Jour. Molec. Biol. 39, p. 461

Lofroth, G. and Angenstein L. (1967) Effect of adsorption on the radiation sensitivity of Trypsin Arch. Biochem. Biophys. 118, 73.

Miller, J.A. and Miller, E.C. (1976) The Metabolic activation of chemical carcinogenesis to reactive electrophiles. In: Biology of Radiation Carcinogenesis. Eds. J.M. Yuhas, R.W. Tennant and J.D. Regan, Raven Press, New York.

Miller, I.R. (1961) Structure of DNA and RNA at a H_2O/Hg interface. J. Mol. Biol., 3, 229.

Mookerjee, A. and Upadhyay, S.N. (1976) Conformational changes of native and reconstituted nucleoprotamin by heat and gamma-irradiation. The Nucleus, 19(3), 172.

Mukhopadhyay, R. and Mookerjee, A. (1976) Effect of gamma-irradiation on Dye-DNA binding. Int. J. Radiat. Biol., 30(6), 543.

Muller, W. and Crothers, D.M. (1968) Studies on the binding of actinomycin and related compounds to DNA. Jour. Molec. Biol., 35, 251.

Muller, W., Bunemann, H. and Dattagupta, N. (1975) Interaction of heteroatomic compounds with nucleic acids-2 - Influence of substituents on the base and sequence specificity of intercalating ligands. Eur. Jour. Biochem., 54, 219.

Nagata, C., Kodama, M., Tagashira, Y. and Imamura, A. (1966) Interaction of polynuclear aromatic hydrocarbons, 4-nitroquinoline 1-oxides, and various dyes with DNA.. Biopolymers, 4, 409.

Parks D.A. and deBruym, P.L. (1962) J. Phys. Chem., 66, 967.

Peacocke, A.R. (1973) The interaction of Acridines with nucleic acids. In: Acridines, Ed. Acheson, Interscience publishers, A Div. of John Wiley and Sons, New York.

Peacocke, A.R. and Skerrett, J.N.H. (1956) Interaction of Aminoacridines with nucleic acids. Trans. Farad. Soc., 52, 261.

Pitchard, N.J., Blake, A. and Peacocke, A.R. (1966) Modified Intercalation model for the interaction of Aminoacridines with DNA. Nature (Lond.), 212, 1360.

Schwarz, G. (1970) Cooperative binding to linear Biopolymers I. Fundamental static and dynamic propeties. Eur. Jour. Biochem., 12, 442.

Schwarz, G. and Balthabar, W. (1970) Cooperative binding to linear biopolymers: 3. Thermodynamic and kinetic analysis of the Acridine Orange - poly (L-glutamic acid) system. Eur. Jour. Biochem. 12, 467.

Schwarz, G., Klose, S. and Balthasar, W. (1970) Cooperative binding to linear biopolymers 2. Thermodynam analysis of the Proflavine - Poly (L-glutamic) system. Eur. Jour. Biochem., 12, 454.

Shapira, R. (1959) BioChem. Biophys. Res. Comm., 1 236.

Stone, A.L. and Bradley, D.F. (1961) Aggregation of Acridine Orange bound to Polyanions: The Stacking tendency of Deoxyribonucleic acids. Jour. Am. Chem., Soc., 83, 3627.

Waring, M. (1975) Stacking Interactions. Chemistry and Industry, 1, 105.

Watson, J.D. and Crick, F.H.C. (1953) The Structure of DNA. Cold Spr. Harb. Symp. Quant. Biol., 18, 123.

Wood, L.A. (1946) J. Am. Chem. Soc., 68, 437.

Weissbluth, M. (1971) Hypochromism, Quart. Rev. Biophys., 4(1), 1.

Zunino, F. (1971) Studies on the mode of interaction of Daunomycin with DNA. Febs Letters, 18(2), 249.

Zunino, F., Gambetta, R., DiMarco, A. and Zaccara, A. (1972) Interaction of Daunomycin and its derivatives with DNA. Biochim. Et. Biophys. Acta., 227, 489.

Zunino, F., Gambetta, R., DiMarco, A., et al., (1976) Effects of stereochemical configuration on the interaction of some Daunomycin derivatives with DNA. Biochem. Biophys. Res. Comm., 69(3), p.744.

*Upadhyay, S.N. and Mookerjee, A. (1977) Adsorption Characteristics and Some Physico-chemical Properties of Nucleoprotamine. Ind. Jr. of Biochem. Biophys., 14, 121.