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The DNA threat probing of some chromophores using UV/VIS spectroscopy

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Authors' Contribution: **Khan, S.** prepared DNA for processing, **F. Rahman** interpreted the data, **H. Rahman** performed UV-spectroscopy, **M. Rahman**, **A. B. Shah**, **A. Haq** and **M. Zahoor** performed UV-visible spectrum of bromophenol blue, bromocresol green and malachite green.

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ABSTRACT

Chromosphere such as triaryl methane family of dyes used in various industries can affect the body of living organism system upon the interaction with the most biochemical molecule is *the DNA. In this research,* the binding mode of triaryl methane family of chromophore dyes that is Bromothymol blue, Bromophenol blue, Bromocresol green and Malachite green with double strand DNA in phosphate buffer at pH 6.9 was investigated through UV/VIS spectroscopy. The study revealed that Bromothymol blue undergo noncovalent interaction with DNA, bromocresol green interact through groove binding and malachite green and bromophenol blue interact through intercalative and partially through electrostatic mode of interaction with DNA. The studies on the binding nature of chemicals/dyes, which are regarded as small molecules to DNA are important and fundamental issues in life sciences. These dyes were found to be more toxic and care should be taken during disposing them off. Proper disposal of these dyes is needed for safe environment and healthy life.

Keywords: Bromothymol blue; bromophenol blue; bromocresol green; malachite green; phosphate buffer.

INTRODUCTION: Dyes are used for many purposes. Besides dyeing they have wide range of medicinal values. Natural dyes and plants used as sources of them are becoming popular day by day among people. Due to their non-hazardous properties, less side effects and more medicinal values, they are used in daily food products and in pharmaceuticals (Chengaiah *et al.*, 2010). Based on greater demand and lesser supply of the natural dyes, now a day's synthetic dyes are in practice. Synthetic dyes are used in the textile, plastic, paper, pharmaceutical, rubber, cosmetics and food industries because of the fact that they are easy to use, accessible, cheap to synthesize, stable and has mixture of colors compared with natural dyes (Chagas and Durrant, 2001; Malik, 2003; Crini, 2006). Mostly synthetic dyes are organic in nature such as *Triarylmethane dyes* which is used as a colorant for foods and other substances. Besides food, it is also used in other brands, commonly in mouthwash and shampoos. These dyes have very low resistance to light and are used mainly in copying papers, in hectograph and printing inks, and in textile applications for which light fastness is not an important requirement. These commercial dyes have a serious drawback that most of them are not uniformly susceptible to microbial attack due to their stable chemical structures (Wong and Yu, 1999). Recently more than 10,000 dyes are available commercially, most of which are nonbiodegradable due to their complex molecular structure and synthetic origin. Very minute quantity of dyes in water (< 1 ppm for some dyes) is greatly visible and affects the quality of water bodies (Banat *et al.*, 1996). These dyes have a severe effect on health such as allergic and irritant contact dermatitis (most common), leukoderma, photosensitivity, purpuric eruptions, angioedema, urticarial, asthma and syncope (Sahoo, 2000; Pongpairoj *et al.*, 2016; Li and Li, 2021). The basic genetic material DNA play very important role in protein synthesis, cell proliferation and genetic information etc. it is quite good and often consider main molecular targeted chemical substance. Sometime unwanted molecules cause dramatic changes in the DNA structure lead to cancer, upon interaction (Kashanian *et al.*, 2012). The techniques of DNA-targeted ctenophores or chemotherapeutics have been wildly applicable for designing more effective product or drugs. The chemotherapeutics substantially increase the survival rate of patients(Cuya *et al.*, 2017). But sometime the effect may be adverse, for example double stranded ds-DNA with anticancer drugs, is responsible for their side effect at therapeutic dosage (Portugal, 2018). Various chromophores like bis(phenazinecarboxamide) possess a unique property in binding with DNA, in the same manner the selected chromophores Bromothymol blue (BTB), Bromophenol blue (BPB), Bromocresol green (BCG) and Malachite green (MG) were examined for their binding capability with DNA. This class of chromophores binds with DNA duplex, shows exceptional anti-turmeric activities, with transcription inhibition. It can further be employed for exploring mechanism of action (potential of pharmacopeia) and to seek new targets. In the binding interactions, linkers like diamine can also provide assistance. The two phenazine chromophores through bis-intercalation with DNA was assisted via linkers, showing tuned properties. Such type of novel interactions (interactions via linkers) with DNA exploring transcription inhibition mechanism, by limiting DNA groove interactions, also these novel interactions overcome drugs limitations (for estrogen treatment), which currently marked. Similarly in combination therapy various anticancer drugs of different action mechanisms are employed at decrease drug resistance and enhance efficacy at reduced dosage, which one of the benefit of this unique integration (Mokhtari *et al.*, 2017) For example a chromophore bis-9-methylphenazine-1-carboxamide is a potent anti-tumor agent, and capable of novel model of binding and interaction with DNA named as ds-DNA—bis-intercaltion with transcription inhibition (Dai *et al.*, 2004; Verborg *et al.*, 2007). It shows excellent cytotoxic potential against human cancer including leukemia and solid tumors of colon, lungs etc, within vitro EC50 in the range 0.04–0.4 Nm (Gamage *et al.*, 2001; Stewart *et al.*, 2001; Buric *et al.*, 2021). Various approaches are there in the literature which can be employed for the examination of DNA binding capabilities with dyes. The chemometrich approach for the interaction of agriculture pesticide metalcarb (MTMC) with calf thymus deoxyribonucleic acid (ctDNA) under buffer condition of pH 7.4 was evaluated with use of acridine orange. The probe was checked out by spectroscopic method which includes UV-visible, FT-IR, circular dichrorism molecular docking and fluorescence. It was found that MTMC molecules interact competitively with ctDNA to that of dye acridine orange, while molecular docking were used for clear visualization of binding sites (Li *et al.*, 2014). Keeping in view the extensive use of dyes in various industries it is voice of the day that their adverse or useful effects on life should be explored. Because DNA is the main target for drugs and other chemicals which are the most important biomacromolecules in life processes, it has a key role in the process of storing, copying and transmitting gene messages. Studies on the binding nature of chemicals/dyes, which are regarded as small molecules to DNA are important and fundamental issues in life sciences (Zhang *et al.*, 2008). Dyes and chemicals can significantly control the genetic information appearance and result in some diseases related to the cell proliferation and differentiation (Wang *et al.*, 2011). The detailed investigations on the interaction of DNA with small molecules are also helpful to design highly efficient antitumor drugs (Deligeorgiev *et al.*, 2007; Zhang and Liu, 2011) to understand the toxic mechanisms of harmful chemicals, such as pesticides and other pollutants etc. (Shen *et al.*, 2010; Shen *et al.*, 2011). It is pertinent to mention here that a little concentration has been paid to the biological influence of dyes, especially its binding properties with the biological macromolecules such as proteins and nucleic acids. The clarifications on the binding mechanism of these stains to biological macromolecules could help us to

recognize the biological values and/or toxicity of them. Some studies reveal that small molecules interact with DNA through non covalent influence by the effect of ionic strength variation (Deligeorgiev *et al.*, 2007). Some dyes provide permeant coloring to materials, keeping in mind this property the selected dyes were examined. The ability of interactions with another adsorbent surface will also be correlated in future. Similarly, the toxicity of dyes can also be finding out from degree of damages in the DNA molecules caused by these dyes. Also, to elaborate the interaction modes (i.e non-covalent interactions, groove-binding, electrostatic-interactions) of chromophores with DNA stand. Non-covalent interactions are also responsible for helping two different coformer stuck together (Khan *et al.*, 2023). The different chromophoric dyes that are used and available commercially like Bromothymol blue (BTB), Bromophenol blue (BPB), Bromocresol green (BCG), Malachite green (MG), di disodium hydrogen phosphate and sodium dihydrogen phosphate were purchased from Sigma Aldrich with CAS number 76-59-5, 115-39-9, 76-60-8 and 569-64-2 respectively. The DNA is used as its sodium salt which was purchased from across organics

OBJECTIVES: The objective of this research was, to elaborate the chemical nature of selected dyes and their interactions with DNA molecules.

MATERIALS AND METHODS: **The DNA binding studies:** The stock solution of sodium salt of DNA was prepared by dissolving its small amount in double distilled water and its concentration was determined by keeping molar absorption coefficient 6600L M^{-1} cm^{-1} at 260 nm this is the stock solution of DNA, the solution was buffered by phosphate buffer (0.1M KH_2PO_4 + 0.1M K_2HPO_4) at pH= 6.9 and this solution was stored below 4℃

The UV-Visible spectroscopy: UV-Visible absorption spectra were measured on a UV–Visible spectrophotometer model; Shimadzu 1800. The process involving interaction of triaryl methane class of dyes with DNA were carried out in double distilled buffer (0.1M KH_2PO_4 + 0.1M K_2HPO_4 at pH 6.9) using 20% aqueous ethanol (Parida *et al.*, 2018). UV-Visible absorption spectra of the known concentration of triaryl methane class of dyes were obtained without using DNA and then the same amount of selected dyes were studied with variable concentration of DNA. All the samples were allowed for five minutes to equilibrate previous to every spectroscopic measurement (Jing *et al.*, 2009).

RESULTS AND DISCUSSION: **The DNA binding studies: UV-visible spectrometric titration:** The binding and interaction of triaryl methane class of dyes with DNA were studied by UV-Visible absorption titration for getting information about the mode of binding, interaction and its strength. UV-Visible spectroscopy is an effective tool for quantification of binding strength of DNA with small molecules. The change in the absorption spectrum of chromophoric dye molecule upon addition of DNA molecule is used for determining their interaction. The chromophoric dye molecules interact with biologically important macromolecules like protein and DNA, and investigating their interactive mode is important both from toxic and beneficial point of view of these chromophoric molecules. The literature study shows that small molecules interact with DNA through non covalent mode of interactions like electrostatic, groove binding and intercalative causing structural and functional changes in DNA molecule (Rehman *et al.*, 2015) and the DNA binding constant was calculated according to the following Host Guest equation 1 (Gul *et al.*, 2014). The binding of chromophores with DNA were found as faction of pH, in other words we can say that different binding patterns and binding abilities were generated with change in pH. Here we selected pH 6.9 because this is very near to pH of human body and maximum binding for all chromophore were found at this value. From the binding constant values, we can get future idea that chromophores can be bind with DNA stand either with via non-covalent interactions, groove binding, electrostatic interactions or combinations of these. Here it was found that bromothymol blue interacts via noncovalent interactions with DNA stand, bromocresol green by groove binding and remaining two chromophores (bromophenol and malachite) via electrostatic interactions.

$$\frac{Ao}{A-Ao} = \frac{E_G}{E_{H-G}-E_G} + \frac{E_G}{E_{H-G}-E_G}\frac{1}{K[DNA]} \qquad \textit{Equation 1}$$

The UV-visible spectrum of bromothymol blue (BTB): The UV/visible spectrum of Bromothymol blue was recorded on Shimadzu UV-1800 model 240V spectrophotometer, showing maximum absorption at wavelength 431 nm having absorbance of 1.122 and a small peak at 616 nm having absorbance 0.251 and another small peak at279 nm with absorbance .656 The maximum intensity band at 431 nm and the small peak at 279 nm correspond to π – π* transition, while the less intense peak at 616 nm result from n – π* transition (figure 1a) (Al-Asfar *et al.*, 2018)

The UV-visible spectrum of bromophenol blue (BPB): The UV/visible spectrum of Bromophenol blue solution was taken on UV/VIS spectrophotometer, showing maximum absorption at wavelength 591 nm having absorbance 0.628 in the presence of phosphate buffer at pH 6.9, and other small peaks at 380 nm and 259 nm. The peak at 591 nm and 259 nm correspond to π – π* transition and the peak at 380 nm represent n – π* transition in Bromophenol blue molecule (figure 1b) (Khan *et al.*, 2016).

The UV-visible spectrum of bromocresol green (BCG): The UV/VIS spectrum of Bromocresol green solution showing maximum absorption at wavelength 616 nm having absorbance 1.713 in the presence of phosphate buffer at pH 6.9, and other less intense peak at 399 nmand 308 nm having absorbance 0.421and .587 respectively. The peak of high intensity at 616 nm and low intense peak at 308 nm are due to π – π* transition and the peak of lesser intensity at 399 nm is due to n – π* transition (figure 1c) (Murmu *et al.*, 2019).

The UV-visible spectrum of malachite green: The UV/VIS spectrum of Malachite green solution, show maximum absorption peak at wavelength 617 nm having absorbance 0.685 in the presence of phosphate buffer at pH 6.9, and other less intense peak at 425 nm and 316 nm having absorbance 0.184.and 0.215 respectively The peak at 617 nm and 316 nm correspond to π – π* transition and the peak at 425 nm is due to n – π* transition (figure 1d) (Lavand et al., 2019).

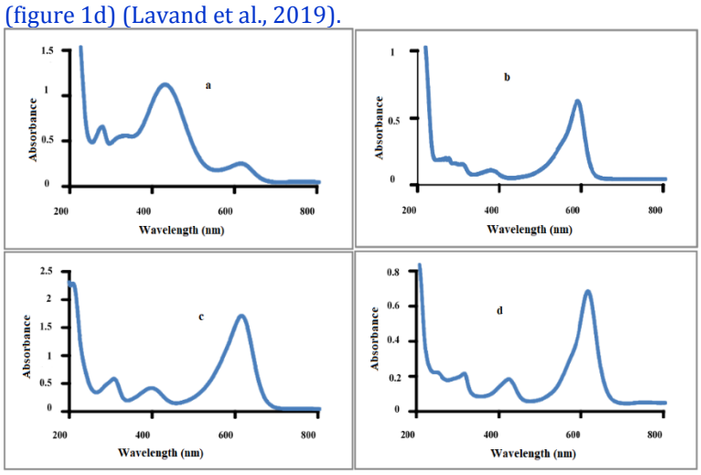

Figure 1: UV- visible spectrum of Bromothymol blue(a), Bromophenol blue(b), bromocresol green(c), malachite green(d).

Interaction of bromothymol blue with DNA: The Bromothymol blue solution was treated with different concentration of DNA solution. The DNA concentration of solution was increased from solution 7 toward solution 1 (8.63μM, 17.26μM , 25.89 μM, 34.52μM, 43.15μM, 51.78μM, 60.41μM) to Bromothymol blue solution. There occur a decrease in the intensity of peak (hypochromism) at 431 nm and occur an increase in intensity (hyperchromism) at 279 nm and also occur a slight blue shift (hypsochromic shift) towards shorter wavelength. The blue shift and hyperchromism at 279 nm is an indication of non- covalent mode of interaction with DNA shown in (figure 2a).

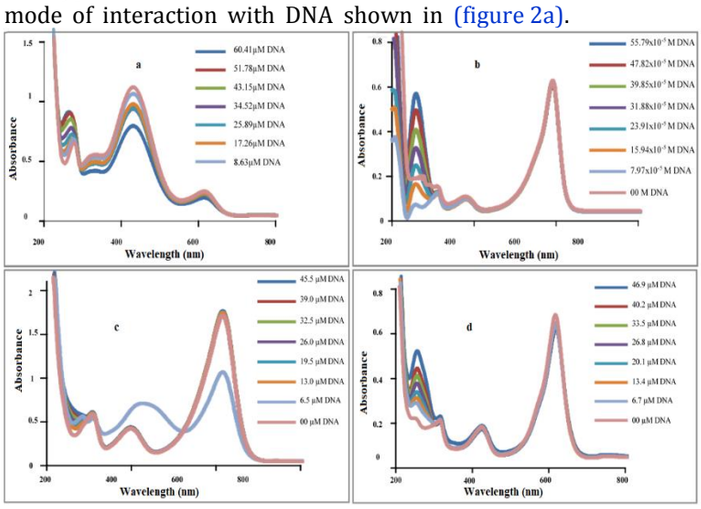

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Figure 2: Interactions of bromothymol blue (a), bromophenol blue (b), bromocresol green (c), and malachite green (d) with DNA.

The change in the absorption peak of Bromothymol blue solution, with increasing concentration of DNA solution can be exploited for determining the binding constant value of Bromothymol blue through the following mathematical equation. Where A_o and A mean the absorbance of free compound and compound-DNA complex respectively while ε_G and $\varepsilon_{H\text{-}G}$ is the molar extinction coefficients of free compound and compound-DNA complex respectively. The plot of A_o/ (A-A_o) were plotted against 1/ [DNA] which give slope ($\epsilon_G/\epsilon_{H\text{-}G}\text{-}\epsilon_G K$) and intercept ($\epsilon_G/\epsilon_{H\text{-}G}\text{-}\epsilon_G$) yielding the binding constant value 1.85×10^4 L mol^{-1} (figure 3a).

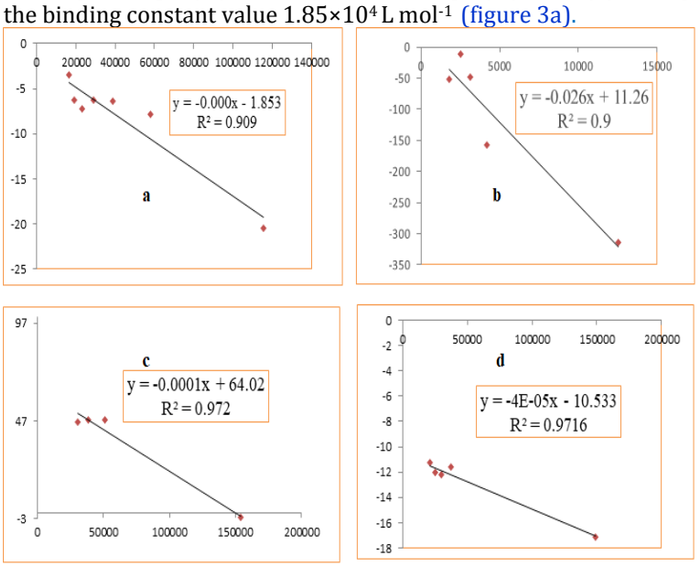

Figure 3: Binding constant value of Bromothymol blue Kb=1.85×10^5 L/mol (a), Bromophenol blue Kb=4.33×10^2 L/mol (b), Bromocresol green Kb=6.40×10^5 L/mol (c), and Malachite green blue Kb=2.6×10^6 L/mol (d), A plot of A_o/ (A-A_o) plotted against 1/ [DNA] which give slope ($\epsilon_G/\epsilon_{H\text{-}G}\text{-}\epsilon_G K$) and intercept ($\epsilon_G/\epsilon_{H\text{-}G}\text{-}\epsilon_G$) yielding the binding constant values of selected dyes.

Interaction of bromophenol blue with DNA: The Bromophenol blue solution was titrated with different concentration of DNA. The DNA concentration was increased from solution 7 toward solution 1 that is $7.9\text{x}10^{-5}$, $15.94\text{x}10^{-5}$, $23.91\text{x}10^{-5}$, $31.88\text{x}10^{-5}$, $39.85\text{x}10^{-5}$, $47.82\text{x}10^{-5}$, $55.79\text{x}10^{-5}$, respectively, with increasing concentration of DNA solution there occur a small increase in the wavelength (bathochromic shift) at 258 nm and also occur an increase in the intensity (hyperchromism) The small red shift (bathochromic shift) along with increase in absorption intensity is attributed to intercalative mode of interaction (figure 2b) (Tornaletti and Pedrini, 1988). The change in the absorption peak of Bromophenol blue solution, with increasing concentration of DNA solution can be exploited for determining the binding constant value of Bromophenol blue through the mathematical equation 1. The binding constant value of Bromophenol blue obtained through equation 1 were found to be $4.33\text{x}10^2$L mol^{-1} (figure 3b).

Interaction of bromocresol green with DNA: The Bromocresol green solution was titrated with different concentration of DNA that is 6.5 µM, 13.0 µM, 19.5 µM, 26.0 µM, 32.5 µM, 39.5 µM, 45.5 µM from solution 7 to solution 1 respectively. With increasing concentration of DNA to Bromocresol green there occurs an increase in the intensity (hyperchromism) at 308 nm while no change in wavelength take place. The above hyperchromism show groove binding in the major and minor groove through hydrogen bonding (figure 2c) (Chen *et al.*, 2005). The change in the absorption peak of Bromocresol green solution, with increasing concentration of DNA solution can be exploited for determining the binding constant value of Bromocresol green through mathematical equation 1. The binding constant value of Bromocresol green obtained through equation 1 were found to yield the binding constant value $6.40\text{ x }10^5$L mol^{-1} (figure 3c).

Interaction of malachite green with DNA: The Malachite green solution was titrated with increasing concentration of DNA that is 6.7µM, 13.4 µM, 20.1 µM, 26.8 µM, 33.5 µM, 40.2 µM and 46.9 µM from solution 7 towards solution 1. With increasing concentration of DNA solution there occur no change in wavelength at 316 nm while intensity increases(hyperchromism) slightly while there occur a small red shift (bathochromic shift) and a decrease in intensity(hypochromism) at 617 nm with increasing DNA concentration. These results suggest an intercalative mode of interaction and partially electrostatic mode of interaction due to cationic nature of malachite green with negative sugar phosphate backbone of DNA molecule (figure 2d) (Hu *et al.*, 2006). The change in the absorption peak of Malachite green solution, with increasing concentration of DNA solution can be exploited for determining the binding constant value of malachite green through the mathematical equation 1. The binding constant value of Malachite green obtained through equation 1 was found to yield the binding constant value $2.6\text{x}10^6$L/ mol (figure 3d).

CONCLUSION: The interaction between commercially available triarymethane dyes, Bromothymol blue, Bromophenol Blue, Bromocresol Green and Malachite Green with different concentrations of DNA in the presence of buffer ($K_2H_2PO_4$) the data obtained from spectrophotometric studies would be used for calculating the binding constant in order to determine carcinogenicity of the concerned dye. As evident from the data, the Bromothymol Blue and Malachite Green show less binding affinity towards DNA as compared to Bromophenol Blue and Bromocresol Green. The binding mode of triaryl methane family of chromophore dyes that is Bromothymol blue, Bromophenol blue, Bromocresol green and Malachite green with double strand DNA in phosphate buffer at pH 6.9 was investigated through UV/VIS spectroscopy. The study shows that Bromothymol blue undergo noncovalent interaction with DNA, bromocresol green interact through groove binding and malachite green and bromophenol blue interact through intercalative and partially through electrostatic mode of interaction with DNA. Seven different concentration of solution were prepared for each dye in order to fully examine of DNA binding. It was concluded that the value of binding constant for Bromothymol blue, Bromophenol blue, Bromocresol green and Malachite green is1.85×10^4 L mol^{-1} , 4.33 x 10^2 L mol^{-1}, 6.40 x 10^5 L mol^{-1} and $2.6\text{x}10^6$ L.mol^{-1} respectively. The pH of all solutions was kept close to physiological pH of the body, in order to know about adverse effects of dyes on health. The binding constants obtained for dyes discussed above show strong affinity towards carcinogenicity of the concerned dye. Among these dyes was found to be more toxic and care should be taken during disposing it off. Proper disposal of these dyes is needed for safe environment and healthy life.

CONFLICT OF INTEREST: Authors have no conflict of interest.

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