

# SPECTROSCOPIC STUDY FOR DETERMINING THE INTERACTION BETWEEN BACITRACIN AND FISH SPERM DNA

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## ABSTRACT

In this paper, the interaction of bacitracin, an antispasmodic drug with fish sperm DNA (fsDNA) was studied. The interaction between bacitracin and fsDNA was investigated by fluorescence and UV-vis absorbance methods. The binding constants between bacitracin and fsDNA were calculated based on fluorescence quenching data at different temperatures. The negative  $\Delta G^\circ$  implied that the binding process was spontaneous and negative  $\Delta H^\circ$  and negative  $\Delta S^\circ$  suggested that hydrogen bonding force most likely played a major role in the binding of bacitracin and fsDNA. Moreover, the results obtained from viscometric method corroborate the experimental results obtained from spectroscopic investigations.

**Keywords:** *Bacitracin, Binding Process, Fish Sperm DNA, Spectroscopic*

## I INTRODUCTION

Bacitracin was first discovered in 1943 and named after a culture of bacillus and the last name of a seven-year-old American girl, Margaret Tracey, from whose wounds the Bacillus was isolated [2,3]. It is a potent peptide antibiotic of narrow spectrum directed primarily against Grampositive cocci and bacilli, including Staphylococcus, Streptococcus, and Clostridium difficile as well as some Archaeobacteria such as Methanobacterium, Mathanococcus, and Halococcus [1–7] and the oyster-infecting Perkinsus marinus [8]. This antibiotic has also been shown to exhibit an interesting metal-dependent, particularly  $\text{Cu}^{2+}$  ion, inhibition toward the growth of the mold Neurospora crassa [9,10]. It is one ingredient in several commercially available topical “triple antibiotic” ointments (along with neomycin and polymyxin B) such as Polysporin and Neosporin that are used to prevent infections in minor cuts and burns [11]. Although this antibiotic has been generally considered safe for topical use, it has recently been found in a few cases to generate delayed hypersensitivity, topical irritation, acute IgE-mediated allergic reactions, and even lifethreatening anaphylaxis [12–16]. Bacitracin is not administered systemically as it is nephrotoxic (toxic to

kidney cells), and is used only as a last resort. It is considered safe when taken orally as the gastrointestinal tract does not absorb significant amount of the drug [4, 11] (however, the absorption was found significant in rainbow trout [17]). Bacitracin has thus been used for the treatment of gastrointestinal infections (such as antibiotic-associated colitis and diarrhea caused by *C. difficile* [18–20] and was found to be as effective as vancomycin [21]), vancomycin-resistant *Enterococcus faecium* [22] (however, a recent study shows its inefficiency [23]), and intestinal infections by *Entamoeba histolytica* [24]. It has been widely utilized as an animal feed additive to improve animal body weight and to prevent diseases in farm animals [25–27]. Consequently, bacitracin is important in both the pharmaceutical and livestock industries and is produced in large quantities throughout the world

The literature survey reveals that so far, the studies on the interactions between Bacitracin and DNA/BSA have not been reported. In the present study, we investigated the interactions of Bacitracin with fsDNA by UV-visible, fluorescence spectroscopic methods.

## II EXPERIMENTAL

**Materials and apparatus:** All chemicals were of analytical or pharmaceutical grade and quartz processed high-purity water was used throughout. Pure Bacitracin was obtained from Global Calcium Ltd., Hosur, India. The stock solution ( $1 \times 10^{-3} \text{ mol L}^{-1}$ ) of Bacitracin was prepared in doubly distilled water. A solution of fsDNA ( $5 \times 10^{-4} \text{ mol L}^{-1}$ ) was prepared (Sigma-Aldrich, India) and stored at  $4^{\circ}\text{C}$ . Buffer solution was prepared by following the standard methods.

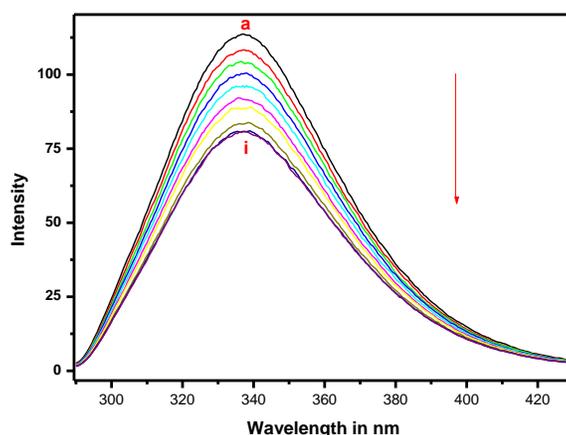
**Absorption Measurements:** The UV–vis spectra were recorded on a double beam Ellico UV-visible spectrophotometer (INDIA) in matched quartz cell of 1- cm path length by adding the increments of fsDNA stock solution into a fixed concentration of Bacitracin. The solutions were allowed to incubate for 10 min before the absorption spectra were recorded.

**Fluorescence Measurements:** All fluorescence measurements were performed on a HITACHI F-4500 spectrofluorimeter equipped with a 150W Xenon lamp and a quartz cuvette of 1 cm path length. 2.0 mL pH 7.4 of Tris–HCl buffer solution, certain volume of drug and varying volume of fsDNA solution were transferred to a 10 mL volumetric flask, and diluted to the final volume with doubly distilled water. The fluorescence emission spectra were measured at 287, 297 and 307 K in the wavelength range of 350–750 nm with an excitation wavelength at 256 nm.

## III RESULTS AND DISCUSSION

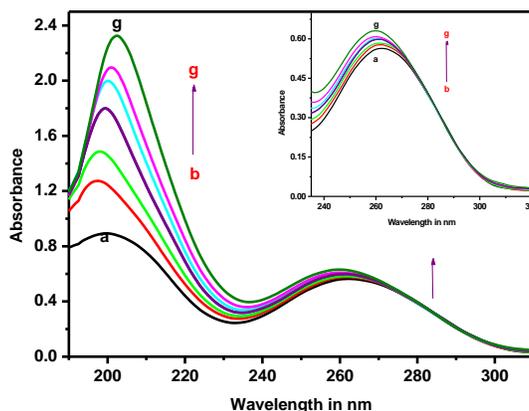
**Spectral characteristics of Bacitracin binding to fsDNA:** It is known that the intrinsic fluorescence of DNA is of little practical use, whereas the titled molecule displays luminescent property ( $\lambda_{\text{ex}}/\lambda_{\text{em}} = 256/340 \text{ nm}$ ). Hence, the fluorescence emission spectra of Bacitracin in the absence and presence of fsDNA were studied. As shown in Fig. 1, with the increasing amounts of fsDNA, the fluorescence intensity of Bacitracin increases without apparent shift of  $\lambda_{\text{em}}$ , implying that the microenvironment around the chromophore of Bacitracin is changed. The fluorescence

intensity increases due to increase in the molecular planarity of the complex and decreases the collision frequency of solvent molecules with Bacitracin, which indicated the binding of Bacitracin to fsDNA indeed existed.



**Fig. 1 Fluorescence spectra of bacitracin in the presence of fsDNA.  $C_{fsDNA} = 0, 5.0, 10.0, 15.0, 20.0, 25.0, 30.0$  and  $35.0 \mu\text{ML}^{-1}$  for curves a – i;  $C_{Bacitracin} = 1.5 \times 10^{-4} \text{ M}$  in pH-7.4**

The formation of Bacitracin-fsDNA complex was further confirmed by UV absorption spectra (Fig. 2). The UV absorption spectra of Bacitracin showed an intense absorption band at 256 nm. It was apparent that as the concentration of fsDNA increased, the absorption peak at 256 nm increases.



**Fig. 2. UV-visible spectra  $2.8 \times 10^{-6} \text{ M}$  fsDNA in absence of a) bacitracin and presence of 20, 40, 60, 80, 100, 120  $\mu\text{M}$  (b to g) of bacitracin in physiological condition.**

### The quenching mechanism of fluorescence of Bacitracin by fsDNA

The mechanisms of fluorescence quenching are usually classified as dynamic quenching and static quenching, which can be distinguished by examination of the temperature on the Stern-Volmer equation:

$$F/F_0 = 1 + K_q \tau_0 [\text{DNA}] = 1 + K_{SV} [\text{DNA}]$$

where,  $F_0$  and  $F$  are the fluorescence intensities in the absence and presence of fsDNA, respectively,  $K_q$  is the quenching rate constant of biomolecular,  $\tau_0$  is the average lifetime of the fluorophore and  $K_{SV}$  is the Stern-Volmer quenching constant which can measure the fluorescence quenching efficiency. It was assumed that the interaction of Bacitracin with fsDNA proceeded via a dynamic quenching. The  $K_{SV}$  value was obtained from the slope of the  $F_0/F$  versus  $[DNA]$  linear plot and the values of  $K_{SV}$  and  $K_q$  at the three temperatures (287, 297 and 307 K) are listed in Table 1. As can be seen, the values of  $K_{SV}$  decreased with the increasing temperature, indicating that the fluorescence quenching of Bacitracin by fsDNA was static.

### Binding constant and number of binding sites:

For a static quenching interaction, it is often assumed that the binding capability of DNA at each binding site is equal. The binding constant  $K$  and the number of binding site  $n$  can be calculated by using the double logarithm regression equation,

$$\log (F_0 - F)/F = \log K + n \log [DNA]$$

The values of  $K$  and  $n$  were obtained from the intercept and slope of the plot of  $\log [(F_0-F)/F]$  versus  $\log [DNA]$ . The corresponding results at different temperatures are summarized in Table 2. The value of  $n$  approximately equal to 1 indicated that there was just a single binding site between fsDNA and Bacitracin. The increasing trend of  $K$  with temperature indicated that the capacity of Bacitracin binding to DNA was enhanced with the temperature rising.

### Thermodynamic parameters and nature of binding forces:

To further characterize the interaction forces, the thermodynamic parameters which are the main evidence to determine the binding mode were analyzed. If the enthalpy changes ( $\Delta H$ ) does not vary significantly within the range of temperature, the thermodynamic parameters  $\Delta H$  and  $\Delta S$  can be estimated using van't Hoff plots:

$$\ln K = -\Delta H/RT + \Delta S/RT$$

The free energy change ( $\Delta G$ ) was estimated from the Gibbs equation

$$\Delta G = \Delta H - T\Delta S = -RT \ln K$$

The values of  $\Delta H$  and  $\Delta S$  were obtained from the slope ( $-\Delta H/R$ ,  $R$  is the gas constant) and intercept ( $\Delta S/R$ ). The values of  $\Delta H$ ,  $\Delta S$  and  $\Delta G$  at different temperature are given in Table 2. The negative values of  $\Delta G$  revealed that the interaction processes was spontaneous, and the positive  $\Delta H$  and  $\Delta S$  values associated indicated that the hydrophobic interaction played a major role. Melting Curve studies also done which is shown in Fig. 3

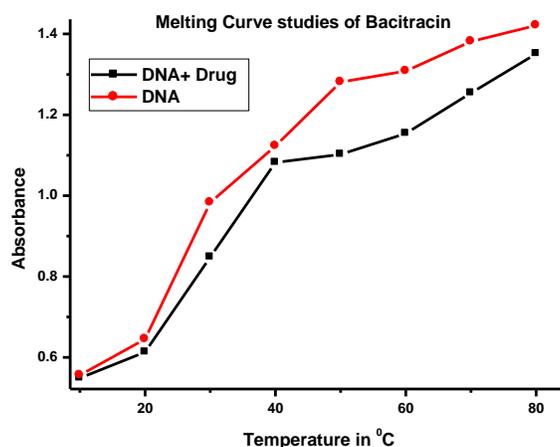


Fig. 3 Melting Curve studies

Table: Thermodynamic parameter ( $K_b$ ,  $\Delta G^0$ ,  $\Delta H^0$ ,  $\Delta S^0$ ) and number of binding constant of fsDNA in the interaction with Bacitracin using spectrofluorimetric measurements.

Temperature	$K_b$ $10^4 M^{-1}$	$\Delta H^0$ $kJM^{-1}$	$\Delta S^0$ $JM^{-1}K^{-1}$	$\Delta G^0$ $kJM^{-1}$	Linear regression	$R^2$
297	1.851	-16.825	138.343	-24.263	$Y=7.833+54.61824x$	0.99818
303	1.96607		137.70	-24.898	$Y= 6.678+ 51.0079$	0.99909
309	2.0		136.787	-25.442	$Y=4.974+ 0.5859x$	0.9985

#### IV. CONCLUSION

The interaction between Bacitracin and fsDNA was studied by spectroscopic analysis. The spectral changes in UV and fluorescence spectroscopy showed that Bacitracin could interact with fsDNA through static quenching mechanism. The binding constants and the number of binding sites of Bacitracin binding to fsDNA were measured at different temperatures and the thermodynamic parameters were calculated as well. It was found that hydrophobic force played a significant role in the binding. The intercalative binding was much more reasonable by taking account of ionic strength effects, tests for the ability of Bacitracin binding with fsDNA. These spectroscopic methods were expected to provide important insight into the interactions of the physiologically important DNA with Bacitracin congeners.

#### V. ACKNOWLEDGMENT

We gratefully acknowledge the financial support of University Grants Commission, New Delhi, India (F. No. 42-308/2013 (SR) Dated, 28/03/2013). Thanks are also due to Global Calcium Ltd., Hosur, India for supplying gift samples of Bacitracin.

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