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Interaction study of Glutamic Acid with double-stranded calf thymus DNA by using Cyclic Voltammetric, Ultraviolet-visible and Fluorescence spectroscopy

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Abstract

Interaction of glutamic acid (Glu) was studied with double-stranded (ds) calf thymus DNA (CT-DNA) by cyclic voltammetry (CV) then obtained results were further correlated by ultraviolet-visible (UV-Vis) and fluorescence spectroscopies. Calculated binding constant from all techniques was very close to each other and it is around 3.54×10^3 M L^{-1}. Further binding sites were also calculated and it is near to 1, which indicates appropriate binding of Glu with CT-DNA. The result shows Glu binds in groove modes of DNA. Further binding free energy (ΔG) of the complex was also calculated and it is -4.76 kCal M^{-1}. In our work a correlative intractability pattern for Glu with DNA has been identified.

Keywords: Glutamic Acid; calf thymus DNA; Intercalating binding; Binding Constant

1. Introduction

Near to 500 types of amino acids (AA) exist in which 20, α-amino acids, are used as building blocks of protein [1]. Recent studies have witnessed that AA are not only used for cell signaling purpose for molecule but also used as a regulator for gene expression [2]. Glutamic Acids usually exist as glutamate because the conditions in human's body favors to loss of a hydrogen atom from glutamic acid [3, 4]. Glu is a non-essential AA [4]. Most oftenly L-Glu is applied for flavor enhancer in fruits, seafood, meats, poultry, soups and snacks [5, 6, 7, 8]. Glu also useful as stability enhancer of several proteins [9, 10] and it protects intestinal wall to gastric attack [11],

gut function, active brain nuclei related to appetite, memory, thermoregulation [12]. It is also used in nutrition diet in patient of poor nutrition [13]. DNA is building block of cell and it holds all genetic information that is obligatory for cellular functions [14, 15]. Thus interaction inbetween DNA and other molecules have a unique consequences as it is associated to the replication, mutation and transcription [16]. More often three types of binding modes are reported for interaction of AA to DNA, these are: electrostatic interaction, intercalation of aromatic heterocyclic groups between the base pairs and Van der Waals interaction [17,18,19]. Interaction between DNA and Cu and Ru complexes of Glu as ligand has been studied and disclosed that the formed complexes can be lead analogues for the development of an effective anticancer drug [20].

In the present study, the interaction of Glu and DNA was studied by CV and obtained results were correlated with UV-Vis and fluorescence spectroscopies. The binding constant, binding sites and binding free energy of Glu-DNA complex were evaluated.

2. Experimental

2.1. Chemicals and reagents

Calf thymus DNA (CT-DNA) and L-Glutamic acid (Glu) was purchased from Sigma, India and used without further purification. An appropriate amount of the DNA and deionized water was used to prepare stock solution of DNA further it was stored overnight at 4 $^{\circ}$C temprature. The concentrations of DNA solutions were determined by using UV-Vis spectrophotometry with the average extinction coefficient value of 6600 M^{-1} cm^{-1} for a single nucleotide at 260 nm [21]. L-Glutamic acid of 0.04 ML^{-1} solution was prepared by using deionize water and stored at 4 ^{0}C. Tris-buffer solution (0.04 ML^{-1}) of pH 7.0 were prepared by using Systronics µpH 361 digital analyzer. All other reagents and chemicals were used of analytical grade and prepared by using deionized water. All the experiments were carried out at room temperature.

2.2. Cyclic Voltammetric study

The cyclic voltammograms were recorded in Metrohm Autolab B.V. PGSTAT128N computer controlled with software NOVA version 1.10.1.9 assembled with conventional three electrode system having bare and modified GCE as working electrode, Ag/AgCl/KCl as reference electrode and a platinum wire as counter electrode. The voltammetric measurement of Glu was carried out with 10 mL of 0.02 M tris-buffer solution (pH 7.0), KCl as supporting electrolyte using clean and dried voltammetric cell. The accumulation potential of -0.2 V vs. Ag/AgCl was applied to a modified electrode for 60 s by keeping modulation amplitude constant. Following the preconcentration period, the voltammograms were recorded in potential range of -0.5 V to -1.5 V with Scan rate: 50 mVs-1, Modulation amplitude: 25mV, Modulation time: 0.01 s, Step potential: -5mV.

2.3. Fabrication of modified GCE

Prior to the modification of bare glassy carbon electrode (b-GCE), the surface of b-GCE was mechanically polished by alumina powder (Al_2O_3 0.05 µm) and cleaned by sonication in 1:1 HCl and HNO_3 (0.1 M) using Frontline sonicater. The multi-walled carbon nanotubes (MWCNT) were dispersed in 10 ml ethanol and sonicated for 20 min to form a homogeneous suspension. Then 7 µL of this suspension was casted over the polished GCE surface and the solvent was allowed to evaporate at room temperature. The fabricated MWCNT-GCE was electrochemically activated by 10times cyclic voltammetric sweeps in the potential range of -1.0V to +2.0V in 0.1 M nitric acid solution at the scan rate of 50 mVs^{-1}.

The surface features of b-GCE and MWCNT-GCE were characterized by cyclic voltammetry (CV). Cyclic voltammograms were recorded for 1.0 mM $K_3[Fe(CN)_6]$ probe in 0.1 M KCl solution at bare GCE, MWCNT-GCE. The surface area of the electrodes were calculated using Randles-Sevick equation [22, 23, 24] by performing cyclic potential sweeps at different scan rates:

$$I_{pa} = 0.4463 \left(\frac{F^3}{RT}\right)^{1/2} n^{3/2} A_0 D_0^{1/2} C v^{1/2} \quad (1)$$

Where, I_{pa} is Anodic peak current, n is the number of electrons transferred, A_0 is surface area of the electrode (cm^2), D_0 is diffusion coefficient, C is concentration of $Fe(CN)_6^{3-/4-}$ and υ is the scan rate, R is molar gas constant (8.314 $JK^{-1}mol^{-1}$) and F is Faraday's constant (96480 C mol^{-1}). For 1.0 mM $K_3[Fe(CN)_6]$ in 0.1 M KCl at Temperature, T = 298 K, n = 1 and $D_0 = 7.6 \times 10^{-6}$ cm^2s^{-1}. The surface area is calculated from the slope of the plot of I_{pa} versus $v^{1/2}$, and was calculated to be 0.0174 cm^2, 0.186 cm^2, for bare GCE and MWCNT-GCE respectively. This indicates the modification of electrode surface which causes easier and faster electron transfer.

2.4. UV-Visible Spectrophotometry

UV-Vis spectroscopy is one of the most utilized technique to detect the binding strength and to predict the mode of binding exists in a complex [25]. If a small molecule interacts with CT-DNA, which alters the absorbance and the position of the signal. UV-Vis spectra for Glu were recorded by Systronics Double Beam UV-Vis spectrophotometer, with the constant concentration of Glu (1×10^{-4} M) and the addition of variable concentration of CT-DNA (from 1×10^{-5} M to 1×10^{-4} M).

2.5. Fluorescence emission spectroscopy

The fluorescence spectroscopic techniques also play an important role to study the interaction of molecules. The fluorescence emission measurements were carried out on RF-5301PC Spectrofluorophotometer. The emission spectra in the fixed concentration of Glu (30 µM L^{-1}) in tris-buffer solution (0.04 ML^{-1}) and by adding aliquots of variable concentration of

CT-DNA was recorded in the range of 270 to 400 nm at an excitation wavelength of 266 nm. The quenched fluorescence intensity was represented as F_0/F, where F and F_0 were fluorescence intensities of the system with and without DNA, respectively.

3. Result and discussion

3.1. Voltammetric studies of Glu-DNA interaction

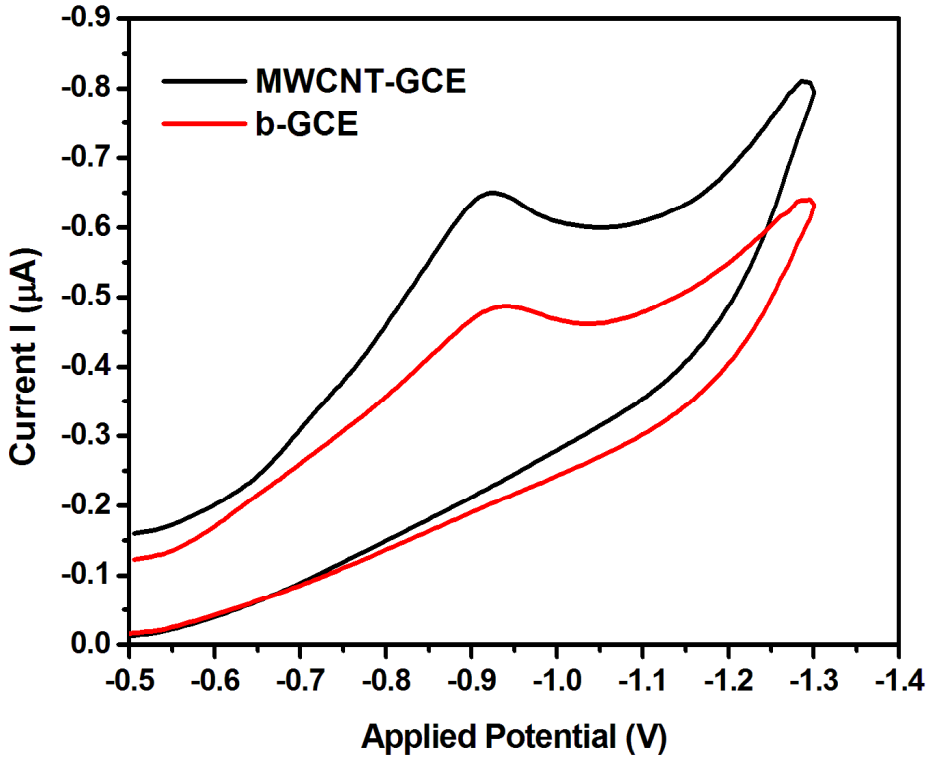

Fig. 1: Cyclic Voltammogram of 1.0×10^{-4} mol L^{-1} Glu at bare GCE and MWCNT-GCE in scan rate 50 mVs^{-1}.

The cyclic voltammograms of Glu in tris-buffer solution (pH 7.0) were recorded at MWCNT-GCE in the absence and presence of DNA. The cyclic voltammogram of Glu shows single well defined, irreversible, reduction peak at cathodic sweep in the potential range from -0.5 to -1.4 V **(Fig. 1).** The irreversible cathodic peak at -0.638 V is related to the reduction of the NH2 group. **Fig. 1** shows the comparison of cyclic voltammogram of Glu on MWCNT-GCE and b-GCE. MWCNT-GCE shows better peak response in terms of peak intensity due to high electron transfer rate and better electroactive surface area. To evaluate the interaction of Glu with DNA, cyclic voltammograms were recorded. The variation in peak potential and peak current with the addition of DNA in Glu was exploited for the determination of binding parameters.

With the addition of DNA in Glu and tris-buffer(pH 7.0) solution, the reduction peak current decreases without shift in potential which is clearly indicate the mode of interaction is groove between Glu and DNA [26, 27]. The decay in peak current (Ip) of the drug by increasing the amounts of DNA can be used for the determination of binding constant.

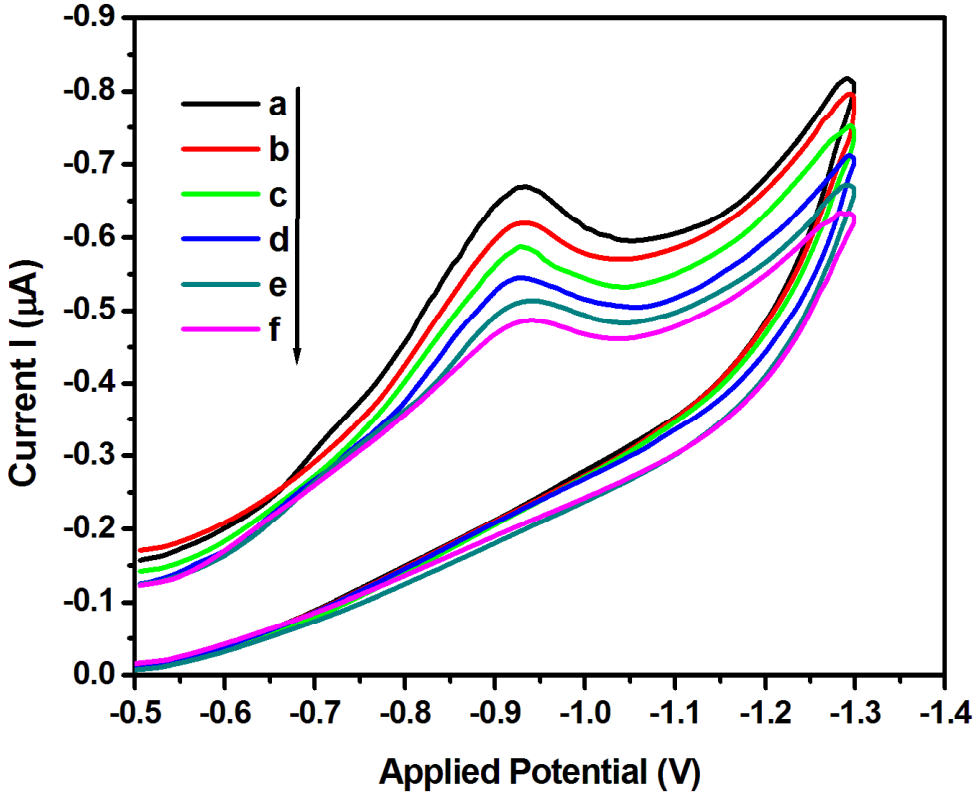

Fig. 2: Cyclic voltammogram of Glu in tris-buffer (pH 7.0) in absence on MWCNT-GCE (a) at the scan rate 50 mVs^{-1} and (b-f) with increasing concentration of DNA (0.1×10^{-5} mol L^{-1} , 0.2×10^{-5} mol L^{-1}, 0.3×10^{-5} mol L^{-1}, 0.4×10^{-5} mol L^{-1}, 0.5×10^{-5} mol L^{-1})

The binding behavior of Glu with DNA was measured by recording the cyclic voltammograms of Glu with the standard addition of DNA. **Fig. 2** shows a typical cyclic voltammograms of Glu-DNA at MWCNT-GCE. The binding constant is quantified by the following Bard's equation [28]:

$$log\frac{1}{C[DNA]} = logK + log\frac{I}{(I_0 - I)} \quad (2)$$

where, K is the binding constant, I_0 and I are the peak currents of the drug in the absence and presence of DNA, respectively. The plot of *log (1/[DNA])* vs. *log (I/(I₀ -I))* becomes linear with the intercept *(log* K*)*. The binding constant, K is obtained from the intercept *(log K)* of the plot (**Fig. 3**). The binding constant (K) value 3.95×10^3 M^{-1} was obtained.

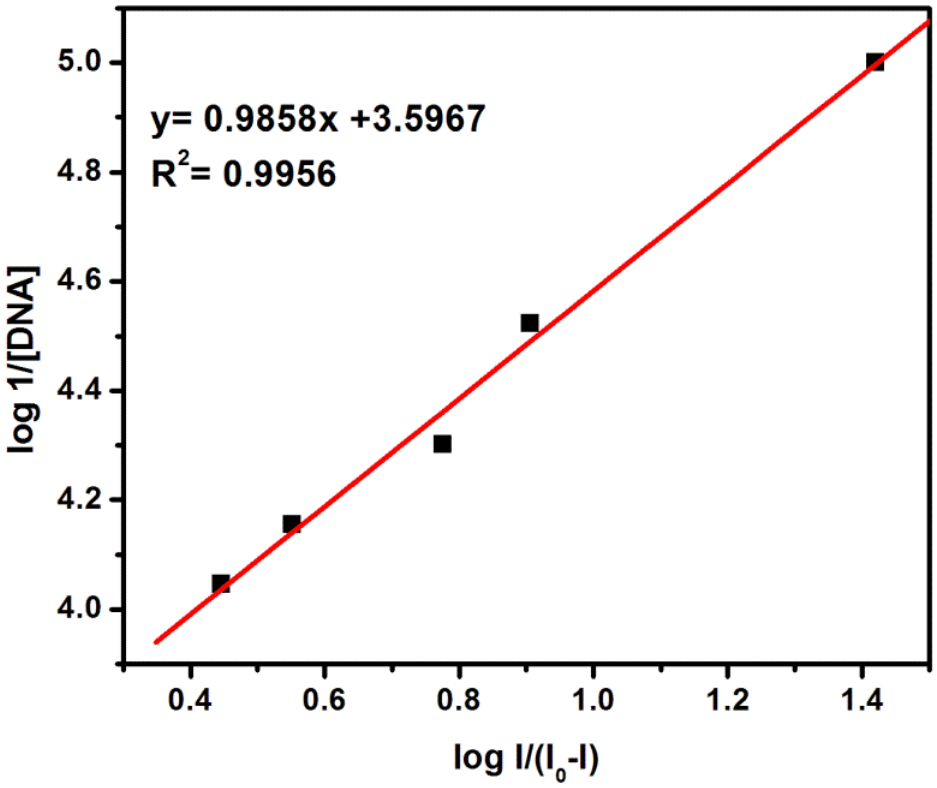

Fig. 3: Linear plot of log $I/(I_0-I)$ Vs. log 1/[DNA].

3.2. UV-Vis spectroscopy

The interaction of Glu with CT-DNA was characterized by monitoring a titration using UV-Vis absorption in the tris-buffer solution (pH 7.0). The absorption spectra were recorded for the fixed concentration of Glu and the interaction was studied with varying concentration of CT-DNA. Glu shows two absorption peaks at 219 nm and 266 nm. The absorption peak at 266 nm was decreases with the addition of DNA as shown in **Fig. 4**.

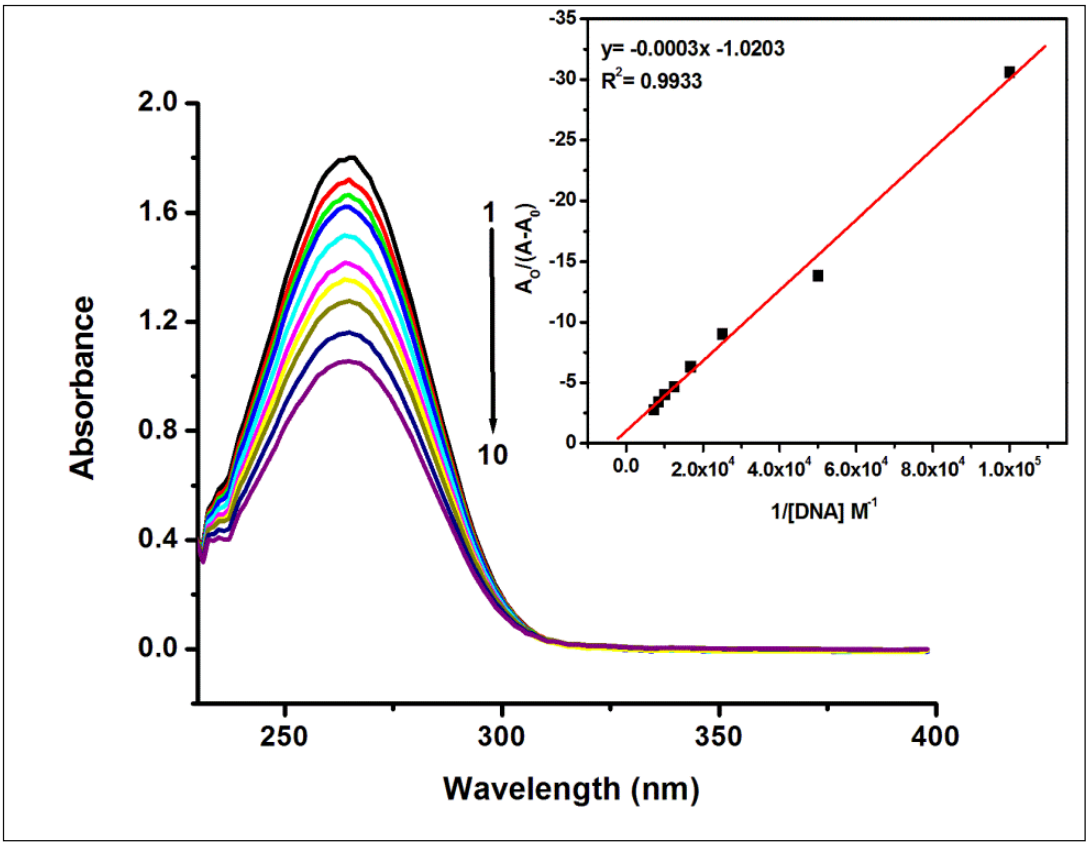

Fig.4: The UV spectra of Glu and Glu-DNA complex in tris-buffer solution (pH 7.0). From 1 to 10: (1) GLU (1.0×10^{-4} mol L^{-1})in absence of DNA and (2-10) with increasing concentration of DNA: (0.1×10^{-5} mol L^{-1}, 0.2×10^{-5} mol L^{-1}, 0.3×10^{-5} mol L^{-1}, 0.4×10^{-5} mol L^{-1}, 0.5×10^{-5} mol L^{-1}, 0.6×10^{-5} mol L^{-1}, 0.7×10^{-5} mol L^{-1}, 0.8×10^{-5} mol L^{-1}, 0.9×10^{-5} mol L^{-1}); **Inset**: The linear plot of $A_0/(A-A_0)$ *vs* 1/[DNA].

The λ_{max} was obtained constant and absorption intensity was changed due to changes in conformation and structure of Glu and DNA via their interaction. The hypochromic incorporated with bathochromic shift indicates the existence of intercalative binding modes **[**26**]**. But the absorption results of interaction between Glu-DNA shows hypochromic shift with minor change in bathochromic shift probably indicates the existence of groove binding mode of interaction **[**29**]**. The intrinsic binding constant, K_b, was determined from equation [28]:

$$\frac{A_0}{A-A_0} = \frac{\varepsilon_G}{\varepsilon_{H-G}-\varepsilon_G} + \frac{\varepsilon_G}{\varepsilon_{H-G}-\varepsilon_G} \times \frac{1}{K_b[DNA]} \quad (3)$$

Where, A_0 and A are the absorbance of Glu and its complex with DNA, respectively. ε_G and $\varepsilon_{H\text{-}G}$ are the absorption coefficients of Glu and Glu-DNA complex, respectively and K_b is the binding constant. The value of K_b was calculated from the intercept of linear plot (A_0/A-A_0) vs. 1/[DNA]

(Fig. 4, inset). The value of the binding constant (K_b) for Glu-DNA complex was obtained to be 3.39×10^3 M^{-1}.

3.3. Fluorescence spectroscopy

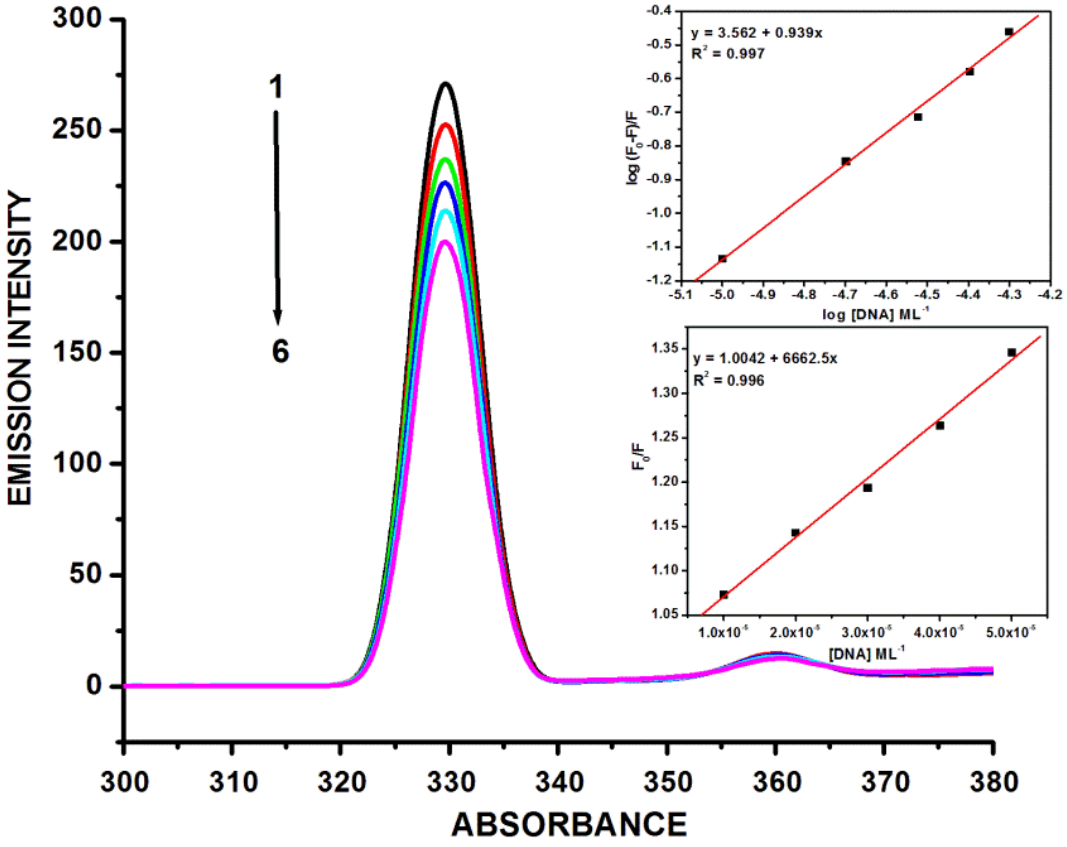

Fig. 5: The Fluorescence spectra of Glu-DNA in tris-buffer (pH 7.0) from 1 to 5 : (1) Glu (1.0×10^{-4} mol L^{-1}) in absence of DNA and (2-5) with increasing concentration of DNA: (0.1×10^{-5} mol L^{-1}, 0.2×10^{-5} mol L^{-1}, 0.3×10^{-5} mol L^{-1}, 0.4×10^{-5} mol L^{-1}, 0.5×10^{-5} mol L^{-1}); **Inset**: Linear plot of F_0/F vs. concentration of DNA and the linear plot of log $[(F_0 - F)/F]$ *vs* log C_{DNA}.

Fluorescence emission spectroscopy also gives the information for binding mode and sites of interactions between Glu and CT-DNA. Quenching of fluorescence provided valuable information about the interaction of quencher and fluorophore. The Glu solution in tris-buffer (pH 7.0) gives intrinsic fluorescence emission spectra when excited at 266 nm with emission maxima at wavelength 331 nm (**Fig. 5**). The intensity of fluorescence spectra of 1.0×10^{-4} mol L^{-1} Glu was decreases successively with addition of CT-DNA. The decline in fluorescence intensity indicates the quenching of Glu during binding with DNA. Since the spectra shows that quenching of Glu with DNA shifts the intensity but shifts in the wavelength (λ) was not

observed. This result suggests that Glu interacts with DNA through groove binding. The Stern–Volmer equation (Eq. 4) was used to study quenching process and to calculate quenching constant [27]:

$$\frac{F_0}{F} = 1 + (K_D + K_S)[Q] + K_S K_D [Q]^2 \quad (4)$$

where, K_D and K_S are the dynamic and static quenching constants, respectively. F_0 and F are the fluorescence intensities in the absence and presence of quencher respectively. [Q] is the molar concentration of quencher. In the case of combined static and dynamic quenching, plot between F_0 / F vs. [DNA] should be non-linear. But **Fig. 5 inset** clearly shows a linear plot with R^2 = 0.997. Thus the quenching mechanism was static or dynamic in nature. The nature of quenching, static or dynamic can be determined by using linear classical Stern-Volmer equation [28].

$$\frac{F_0}{F} = 1 + kq\, \tau_0 [Q] = 1 + K_{SV}[Q] \quad (5)$$

where, k_q and K_{SV} are the bimolecular quenching constant and Stern-Volmer quenching constant, respectively. The value of K_{SV} and kq calculated from the linear Stern-Volmer plot between F_0/F versus concentration of CT-DNA as shown in **Fig 5, inset**. The value of K_{SV} was calculated from the slope of the curve, 6.662×10^3 M^{-1} L s^{-1}. The value of K_{SV} was in the range of typical groove binders [30]. After putting the value of K_{SV} and fluorescence lifetime (τ_0) of biological macromolecules as 10^{-8} s in equation 5, it gives the value of k_q, 6.662×10^{11} M^{-1} L s^{-1}. It is larger than the limiting diffusion rate constant of bimolecule (2.0×10^{10} M^{-1} L s^{-1}) indicates the static quenching occurred in Glu quenching by DNA. The binding constant 'K_b' and the binding sites 'n', calculated by using the following equation [31]:

$$log\, \frac{F_0 - F}{F} = \log K_b + n log[DNA] \quad (6)$$

The intercept of the plot of *log* $(F_0 - F)/F$ vs. *log [DNA]* gives the value of K_b and the n were evaluated from the slope value (**Fig. 5, inset**). The calculated binding sites were 0.939 and binding constant was 3.655×10^3 M^{-1}. These values are close to the calculated values of binding parameters from UV-Vis spectroscopy. The binding Gibb's free energy (ΔG_b*) for Glu-DNA was calculated from the following relation and it is obtained as -4.86 kCal M^{-1} [32]:

$$\Delta G_b^* = -RT\, ln\, K_b \quad (7)$$

where, R represents the gas constant and T for absolute room temperature.

Table 1: The calculated value of binding constants obtained from voltametric and spectroscopic techniques.

Techniques	Cyclic Voltammetry	UV-Vis Spectroscopy	Fluorescence Spectroscopy
Binding Constant (K_b)	3.95×10^3 M^{-1}	3.39×10^3 M^{-1}	3.65×10^3 M^{-1}

4. Conclusion

Interaction of Glu with CT-DNA was studied by performing cyclic voltammetric and the results obtained from the detailed experiment have been further correlated with UV-Vis and fluorescence spectroscopy to identify and explore its interaction pattern. Obtained results from different experimental techniques are mentioned in table 1. All results prove that the groove mode of interaction exists between Glu and DNA complex. The combination of the cyclic Voltametric and spectroscopic methods shows potential importance in understanding the mechanism and mode of action of this important class of amino acid with DNA which can contribute to the further exploitation of the interaction for understanding the basics to use the pattern in different field of DNA study, besides these, can be used for the generation of drug molecule having prior mechanism of action in DNA, in different aspect of disease related to DNA molecule interaction or DNA mutation.

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REFERENCES

[1] K. Tao, A. Levin, L. Adler-Abramovichab E. Gazit, "Fmoc-modified amino acids and short peptides: simple bio-inspired building blocks for the fabrication of functional materials", *Chem. Soc. Rev.* **45**, 3935-3953 (2016).

[2] S.K. Paul, M.M. Goldar, M. Yakura, Y. Oowatari, M. Kawamukai, "Glutamyl tRNA Synthetases and Glutamic Acid Induce Sexual Differentiation of Schizosaccharomyces pombe"., *Biosci. Biotechnol. Biochem.* **73**, 1339-1347 (2009).

[3] V.A. Najjar, "The Biological Effect of Glutamic Acid and Its Derivatives", *Mole. Cellul. biochem.* **38**, 167-170 (1981).

[4] M. Watford, "Glutamine and glutamate: Nonessential or essential amino acids?", *Animal Nutrition.* **1**, 119–122 (2015).

[5] S. Yamaguchi, K. Ninomiya, "Umami and food palatability", *J. of Nutr*. **4**, 246-251 (2000).

[6] S. Fuke, T. Shimizu, "Sensory and preference aspects of umami", *Trends in F.ood Sci. Tech.* **4,** 246-251 (1993).

[7] T. Populin, S. Moret, S. Truant, L.S. Conte, "A survey on the presence of free glutamic acid in foodstuffs, with and without added monosodium glutamate", *Food Chem.* **104** 1712-1717 (2007).

[8] F. Bellisle, "Glutamate and the umami taste. Sensory, metabolic, nutritional and behavioural considerations. A review of the literature published in the last 10 years". *Neurosci. Biobehav. Rev.* **23,** 423-438 (1999).

[9] T. Arakawa, K. Tsumoto, Y. Kita, B. Chang, D. Ejima, "Biotechnology applications of amino acids in protein purification and formulations", *Rev. Art. Amino Acids.* **33,** 587-605 (2007).

[10] A.K. Mandal, S. Samaddar, R. Banerjee, S. Lahiri, A. Bhattacharyya, S. Roy, "Glutamate Counteracts the Denaturing Effect of Urea through Its Effect on the Denatured State", *J. Bio. Chem.* **278**, 36077-36084(2003).

[11] Y. Akiba, C. Watanabe, M. Mizumori, J. D. Kaunitz, "Luminal L-glutamate enhances duodenal mucosal defense mechanisms via multiple glutamate receptors in rats", *A. J. Physio.: Gastro. and Liver Physio.* **297**, G781-G791 (2009).

[12] T. Tsurugizawa, A. Uematsu, E. Nakamura, Hasumura, M. Hirota, "Mechanisms of neural response to gastrointestinal nutritive stimuli", *Gastroenterology.* **37**, 262-273 (2009).

[13] K. Wachirasiri, S. Wanlapa, D. Uttapap, V. Rungsardthong, "Use of amino acids as a phosphate alternative and their effects on quality of frozen white shrimps (Penaeus vanamei)", *LWT - Food Science and Technology.* **69**, 303-311(2016).

[14] S. Yamamoto, M. Tomoe, K. Toyama, M. Kawai, H. Uneyama, "Can dietary supplementation of monosodium glutamate improve the health of the elderly?", *A. J. Clin. Nutr.* **90**, S844-S849 (2009).

[15] J. Rhodes, A.C. Titherley, J. A. Norman, R. Wood, D.W. Lord, "A survey of the monosodium glutamate content of foods and an estimation of the dietary intake of monosodium glutamate", *Food Addit. Contam.* **8**, 663-672 (1991).

[16] Y. Zhousheng, "Electrochemical behaviour of gallic acid interaction with DNA and detection of damage to DNA", *J. Ele.Ana. Chem.* **624**, 91-96 (2008).

[17] T. Bivera, "Use of UV-Vis Spectrometry to Gain Information on the Mode of Binding of Small Molecules to DNAs and RNAs", *Appl. Spectro. Rev.* **47**, 272-325 (2012).

[18] A. Mehdinia, S.H. Kazemi, S.Z. Bathaie, A. Alizadeh, M. Shamsipur, M. Fazlollah, "Electrochemical DNA nano-biosensor for the study of spermidine–DNA interaction", *J. Phar. Bio.* **49,** 587-593 (2009).

[19] J. Szejtli, "Past, present and future of cyclodextrin research", *Pure Appl. Chem.* **76**, 1825-1845 (2004).

[20] A. Imran, A.W. Waseem, S. Kishwar, W. Diana, "Syntheses, DNA Binding and Anticancer Profiles of L-Glutamic Acid Ligand and its Copper(II) and Ruthenium(III) Complexes", *Med. Chem.* **9**, 11-21 (2013).

[21] M.A. Husain, Z. Yaseen, S.U. Rehman, T. Sarwar, M. Tabish, "Naproxen intercalates with DNA and causes photocleavage through ROS generation", *FEBS J.* **280**, 6569-6580 (2013).

[22] E. Jin, X. Bian, X. Lu, C. Wang, "Fabrication of multiwalled carbon nanotubes/polypyrrole/Prussian blue ternary composite nanofibers and their application for enzymeless hydrogen peroxide detection", *J. of Materials Science*. **47**, 4326–4331 (2012).

[23] I. Khan, U. J. Pandit, S. Wankar, R. Das, S. N. Limaye; "Fabrication of electrochemical nanosensor based on polyaniline film-coated AgNP-MWCNT-modified GCE and its application for trace analysis of fenitrothion", *Ionics.* **23**, 1293–1308 (2017).

[24] Ahmed M. Abdul-Lettif, "Determination of diffusion coefficients in Au/Ni thin filmsby Auger electron spectroscopy", *Phys. stat. sol. (a)* **201,** 2063–2066 (2004).

[25] T. Sarwar, M.A. Husain, S.U. Rehman, H.M. Ishqi, M. Tabish, "Multi-spectroscopic and molecular modelling studies on the interaction of esculetin with calf thymus DNA", *Mol. Bio. Syst.* **11,** 522-531 (2015).

[26] M.L. Yola, N. Ozaltin, "Electrochemical studies on the interaction of an antibacterial drug nitrofuranation with DNA", *J. Eletanal. Chem*. **653,** 56-60 (2011).

[27] X. Hua , Y. F. Zhang, J. H. Chen, "Studies of the interaction between Aloe-emodin and DNA and preparation of DNA biosensor for detection of PML-RARα fusion gene in acute promyelocytic leukemia", *Talanta.* **74**, 944-950 (2008).

[28] J.A. War, S.K. Srivastava, S.D. Srivastava, "Synthesis and DNA-binding study of imidazole linked thiazolidinone derivatives", *Luminescence.* **32,** 104–113 (2017).

[29] C.V. Kumar, E.H. Punzalan, W.B. Tan, "Adenine-thymine base pair recognition by an anthryl probe from the DNA minor groove", *Tetrahedron.* **56**, 7027–40 (2000).

[30] L. Fotouhi, R. Tabatabaee, "A study of the interaction tyrosin and DNA using voltammetry and spectroscopy methods", *Spectrochim, Acta Part A: Mol, Biomol. Spectro.* **121** 152-156 (2014).

[31] G.A. Holdgate, W.H.J. Ward, "Measurements of binding thermodynamics in drug discovery", *Drug Dis. Today*. **10**, 1543-1550 (2005)