An Integrated experimental and computational study of the interaction between triamcinolone acetonide(TA) and human growth hormone(hGH) using UV/Vis spectroscopy and Molecular Docking

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ABSTRACT

The interaction between triamcinolone acetonide (TA) and human growth hormone (hGH) was investigated using UV–Vis spectrophotometry under optimized experimental conditions. TA exhibited a maximum absorbance at 342 nm and followed the Beer–Lambert law in the concentration range of 2.3 × 10^{-4} to 4.5 × 10^{-4} M, indicating monomeric behavior in solution. Upon addition of hGH, a decrease in absorbance and the presence of an isosbestic point confirmed complex formation between TA and hGH. The interaction was found to be strongly pH-dependent, with maximum binding occurring at pH 2, while ionic strength showed negligible influence, suggesting an ion–dipole interaction mechanism. Binding stoichiometry was determined to be approximately 1:3 (hGH:TA). Thermodynamic parameters obtained from Van't Hoff analysis revealed negative values of ΔG°, ΔH°, and ΔS°, indicating a spontaneous, exothermic, and entropy-unfavorable process. Temperature-dependent studies showed reduced binding affinity at higher temperatures, consistent with an exothermic interaction. Overall, the results demonstrate a weak but spontaneous interaction between TA and hGH, supporting the inhibitory role of TA on growth hormone activity and highlighting UV–Vis spectroscopy as a simple and reliable method for biomolecular interaction studies.

Keywords: Triamecinolone Acetonide(TA), human Growth Hormone(hGH),Molecular Docking

1. *INTRODUCTION*

Human growth hormone (hGH) is a crucial polypeptide hormone that plays a central role in regulating growth, metabolism, and cellular development. Its biological activity depends on precise molecular recognition events, and even weak interactions with endogenous or exogenous small molecules may modulate its physiological function [2]. Understanding the molecular interactions between hGH and pharmaceutical compounds is therefore of significant interest from both biochemical and pharmacological perspectives [3].Corticosteroids constitute an important class of bioactive molecules widely used in clinical practice for their anti-inflammatory and immunosuppressive properties. Triamcinolone acetonide (TA), a synthetic glucocorticoid, is commonly administered in ophthalmic, dermatological, and systemic therapies. Despite its extensive therapeutic use, limited information is available regarding its potential molecular-level interactions with peptide hormones such as hGH. Given the sensitivity of hormone signaling pathways, even weak and reversible interactions may have functional consequences, highlighting the need for detailed physicochemical investigation [4].Spectroscopic techniques have proven to be powerful tools for studying biomolecular interactions, particularly when the interactions are noncovalent and weak in nature [3]. UV–Vis absorption spectroscopy is especially valuable for characterizing binding equilibria, stoichiometry, and thermodynamic parameters of protein–ligand systems under controlled conditions [1]. Changes in absorbance intensity and the presence of isosbestic points can provide strong evidence for complex formation and equilibrium behavior. Moreover, temperature- and pH-dependent spectroscopic measurements allow insight into the forces governing binding, including electrostatic interactions and hydrogen bonding

[5].Thermodynamic analysis based on spectroscopic data enables quantitative evaluation of binding spontaneity and driving forces [7]. Parameters such as Gibbs free energy (ΔG°), enthalpy (ΔH°), and entropy (ΔS°) offer critical information about the energetic and structural consequences of complex formation [6]. In weak binding systems, these parameters are particularly informative, as they help distinguish between enthalpy-driven and entropy-driven processes and clarify the role of solvent and conformational effects [3].While experimental spectroscopic methods provide robust thermodynamic and equilibrium data, they do not directly reveal the binding site or the specific amino acid residues involved in ligand recognition. To address this limitation, molecular docking simulations have become an essential complementary approach [8]. Docking studies enable visualization of ligand binding modes, identification of key interacting residues, and estimation of binding energies [9]. When experimental and computational results are in agreement, docking analysis significantly strengthens mechanistic interpretation and enhances the reliability of binding models.

In this work, we present a combined experimental and computational study of the interaction between triamcinolone acetonide and human growth hormone. UV–Vis spectrophotometry was employed to determine optimal experimental conditions, assess binding behavior, and evaluate thermodynamic parameters governing the interaction. The experimental results indicate a weak but spontaneous and exothermic interaction, strongly dependent on pH and largely independent of ionic strength, suggesting that electrostatic and ion–dipole forces dominate the binding process [4,5].

To further elucidate the molecular basis of this interaction, molecular docking analysis was performed using the crystal structure of hGH (PDB ID: 1HGU). Docking results revealed that TA preferentially associates with surface regions of hGH through hydrogen bonding and electrostatic interactions involving residues such as Lys, Arg, His, and Glu. The predicted binding energies were consistent with the experimentally determined Gibbs free energy values, supporting the weak and reversible nature of the interaction [8,9].The integration of spectroscopic and docking results provides a coherent molecular-level picture of the TA–hGH interaction. This study demonstrates that even weak, spontaneous interactions between corticosteroids and growth hormone can be reliably characterized through combined experimental and computational approaches. The findings contribute to a deeper understanding of hormone–drug interactions and highlight the potential inhibitory influence of triamcinolone acetonide on hGH function [10,2].The integration of spectroscopic and docking results provides a coherent molecular-level picture of the TA–hGH interaction. This study demonstrates that even weak, spontaneous interactions between corticosteroids and growth hormone can be reliably characterized through combined experimental and computational approaches. The findings contribute to a deeper understanding of hormone–drug interactions and highlight the potential inhibitory influence of triamcinolone acetonide on hGH . This study examines the interaction between triamcinolone acetonide (TA) **and** human growth hormone (hGH) using UV–Vis spectrophotometry. The objectives were to determine optimal experimental conditions, evaluate binding behavior, and calculate thermodynamic parameters governing the interaction.

2.EXPERIMENTAL

2.1 Material

The raw materials used includes: Triamcinolone was supplied in ampoules. Each ampoule contained 0.4 mg/ml of triamcinolone which was preparted from Alborz daru. Each growth hormone package which was prepared from LG.contained two vials, one injection vial containing 0.4 IU (each 4 IU is equivalent to 33 mg) and one solvent vial containing 1 ml of distilled water for injection. A vial is a small glass or plastic bottle with a stopper, often used to store a drug in liquid, powder, or capsule form. (In pharmacology, IU is an international unit of measurement for the amount of a substance. This unit is used to quantify vitamins, hormones, some drugs, vaccines, blood products, and similar biologically active substance

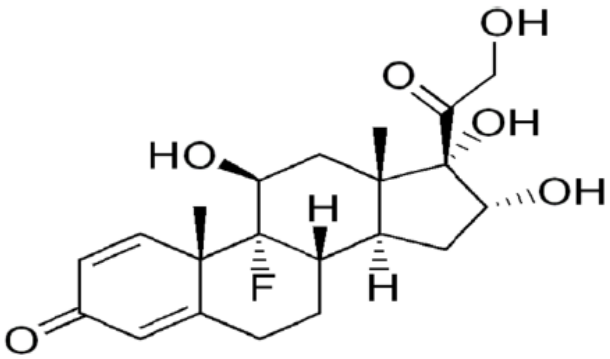

Fig.1. Triamcinolone Acetonide *(TA) structure*

2.1.1. Preparation of triamcinolone stock solution:In order to prepare 50 ml of TA stock solution with a concentration of 500 ppm, 625 µl of TA ampoule was poured into a 50 ml volumetric flask and made up to volume with distilled water.
2.1.2.Optimum Preparation of growth hormone stock solution :To prepare the hGH stock solution, first the distilled water solvent was taken out of the vial with a syringe and injected into the vial containing hGH. In this case, the hGH concentration would be 1320 ppm. Therefore, in order to prepare 25 ml of 100 ppm hGH solution, 1893.9 µl of the 1320 ppm solution was poured into a 25 ml flask and made up to volume with distilled water.
2.1.3. Determination of optimal concentrations of inhibitor and growth hormone - In the next step, in order to determine the optimal concentration, solutions in a volume of 2 ml with different concentrations of TA and hGH were prepared and their absorption spectra were recorded. In this step, the control solution was distilled water.
2.1.4.Preparation of TA and hGH solution with optimal concentration: To prepare 2 ml of the above solution, 400 µl of TA stock solution and 400 µl of hGH stock solution was poured into the UV cell and made up to 2 ml with

2.2 Instrument

UV-Vis spectrophotometer with Temperature control system Model 8453-Agilent was used.

2.3 hCG binding experiments

The absorption study was used in order to explore the formation of hGH(human Growth hormone-TA(Triamcinolone acetonide**)** complex and to obtain thermodynamic parameters.The absorption experiments were performed at a constant concentration of hGH and various TA concentrations in phosphate buffer pH=5.00 solution.Thereafter, the titration was continued to obtain a constant absorption value. The UV-Vis specra for hGH solution were inscribed in the range of 200-700 nm.

2.4.hGH-TA Molecular Docking

Molecular docking is an important tool used to find information on ligand-receptor interactions,binding free energy, and appropriate binding sites. In the present study, before conducting the docking we selected Ligand triamcinolone Acetonide(TA)from Pubchem CID:31307.afterward, the crystal structure of hGH (PDB ID)was taken at an exactitude of 2.5 $\mathring{A}$ from the protein data bank.Molecular docking was performed using AutoDock Vina. The crystal structure of human growth hormone (PDB ID: 1HGU) was obtained from the Protein Data

Bank. Water molecules were removed, polar hydrogens were added, and Kollman charges were assigned using AutoDock Tools. Triamcinolone acetonide was prepared by adding hydrogens and Gasteiger charges, and rotatable bonds were defined. Blind docking was conducted using a grid box of 60 × 60 × 60 Å³ centered on the protein. Docking poses were ranked based on binding energy, and interactions were analyzed using molecular visualization software.

3.RESULTS AND DISSCUSION

3.1. Beer–Lambert Law Validation

TA follows Beer–Lambert law in the range **2.3×10^{-4} to 4.5×10^{-4} M**.This subject Confirms linear absorbance–concentration relationship which Indicates that TA exists mainly in monomeric form under experimental conditions

3.2. TA–hGH Interaction

Addition of hGH to TA solutions causes a systematic decrease in absorbance.Presence of an isosbestic point confirms equilibrium between free and bound species Interaction reaction.

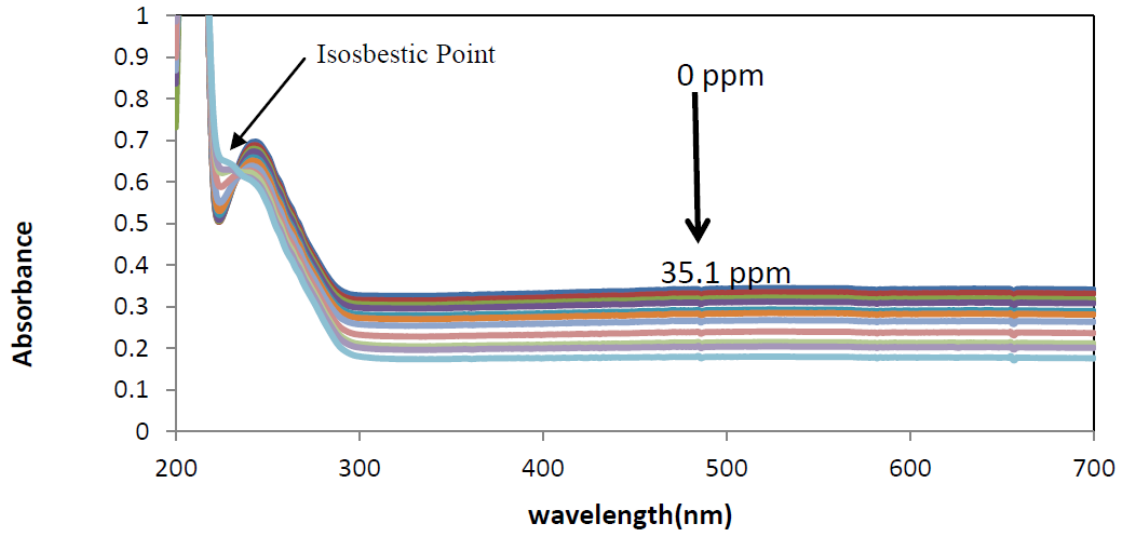

Fig.2.UV-Vis spectrum of TA in various concentration of hGH at pH=5.0 and t=25° C

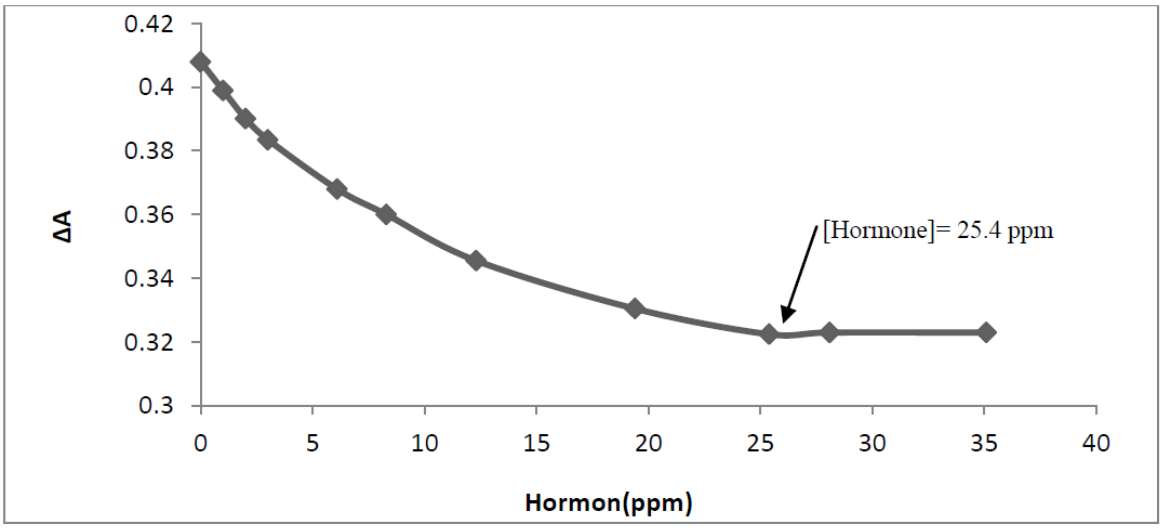

Fig.3. Diffrential absorbance of TA against various concentration of hGH at λ=243 nm at pH=5.00 and t=25°C

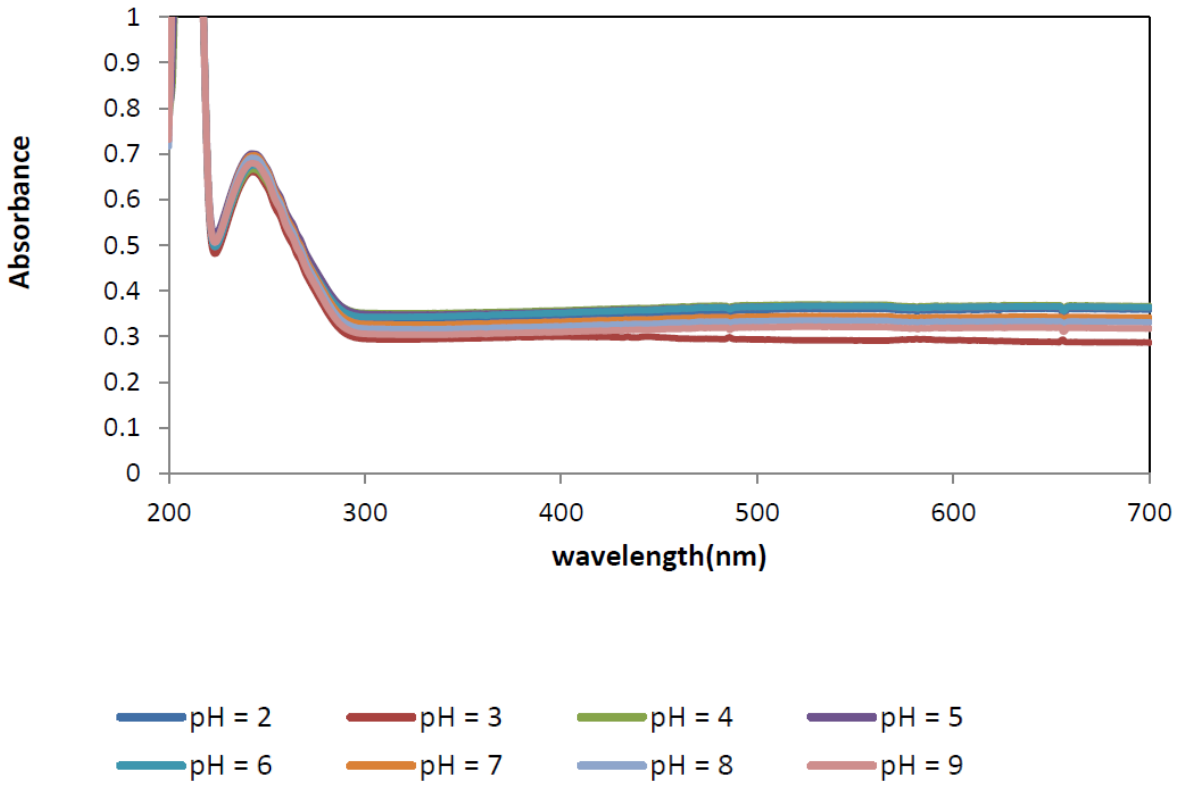

Fig.4.UV-Vis spectrum of TA against pH at t=25° C

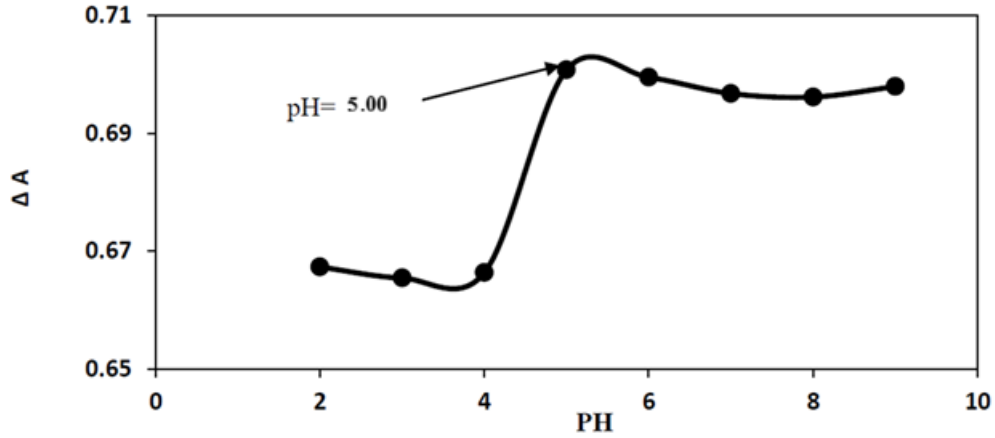

Fig.5.Diffrential absorbance of TA against pH at t=25° C

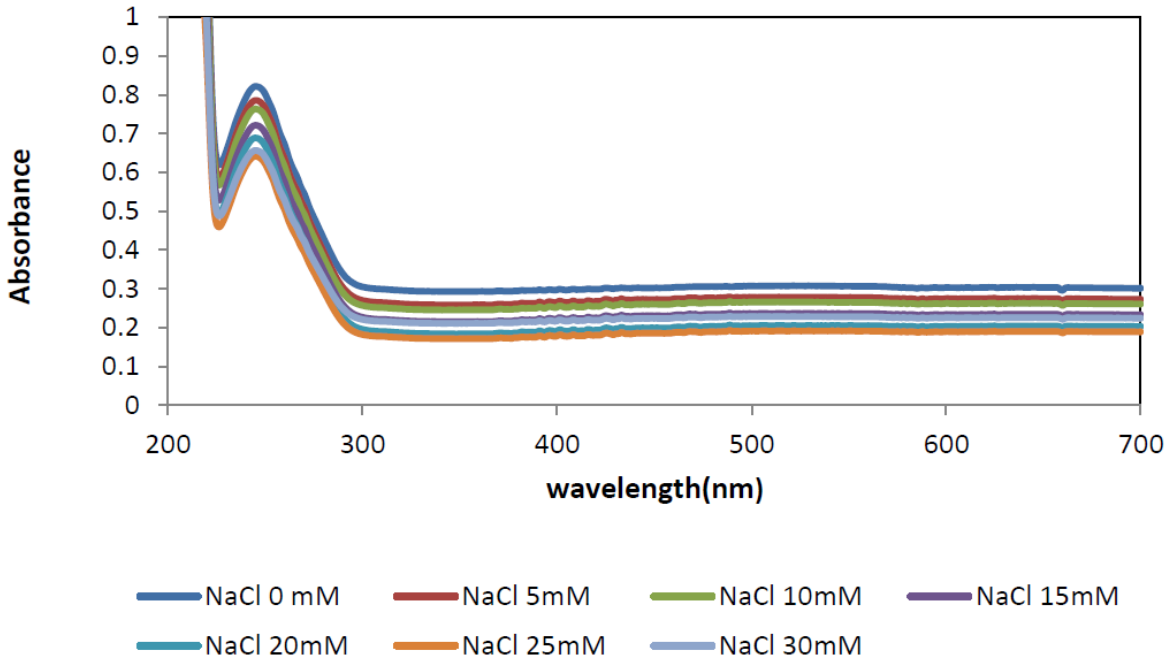

Fig.6.UV-Vis spectrum of TA in absence and presence of NaCl at pH=5.0 and t=25° C

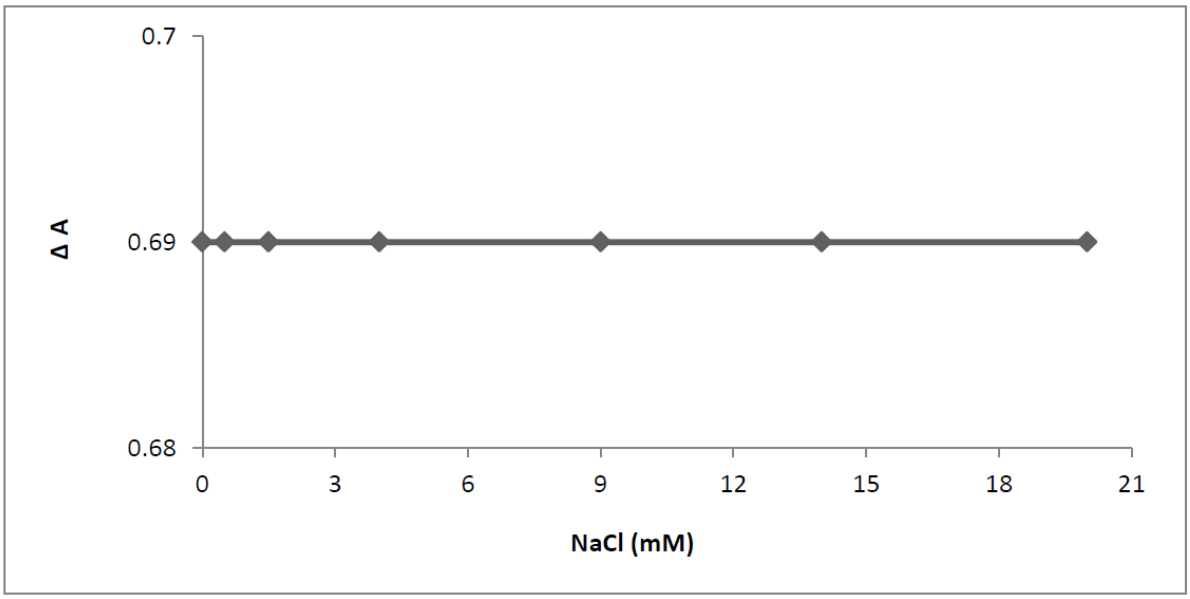

Fig.7. Diffrential absorbance of TA against NaCl concentration at pH=5.0 and t=25° C

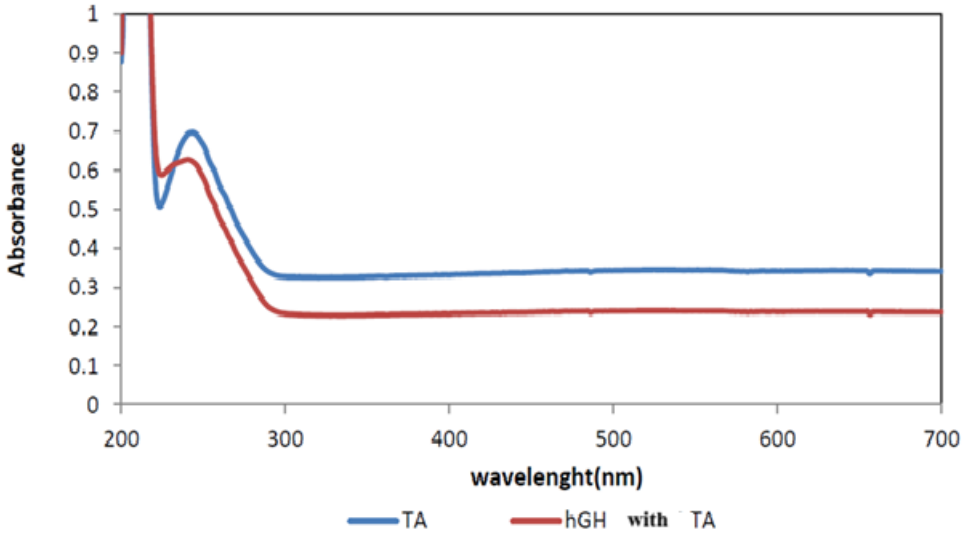

Fig.8. UV-Vis spectrum of TA in absence and presence of hGH at pH=5.0 and t=25° C

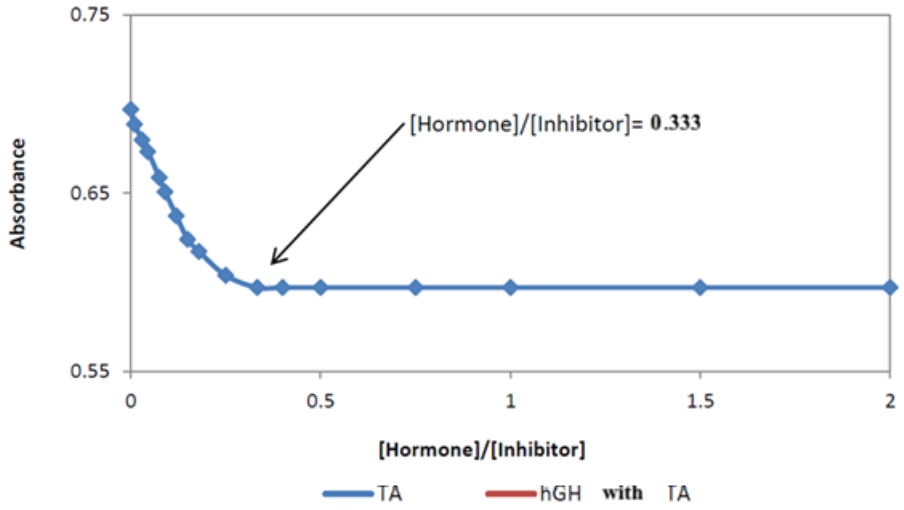

Fig.9. Absorbance of TA against hGH concentration at pH=5.0 and t=25° C

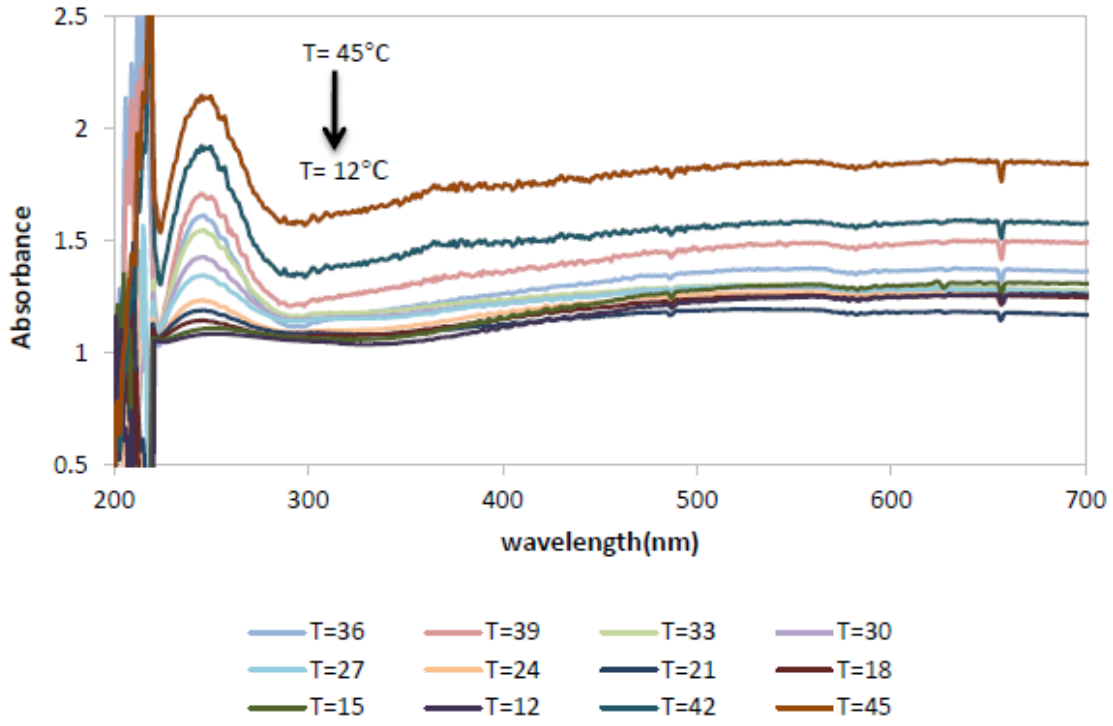

Fig.10.UV-Vis spectrum of TA in absence of hGH in pH=5.00 in various temperatures

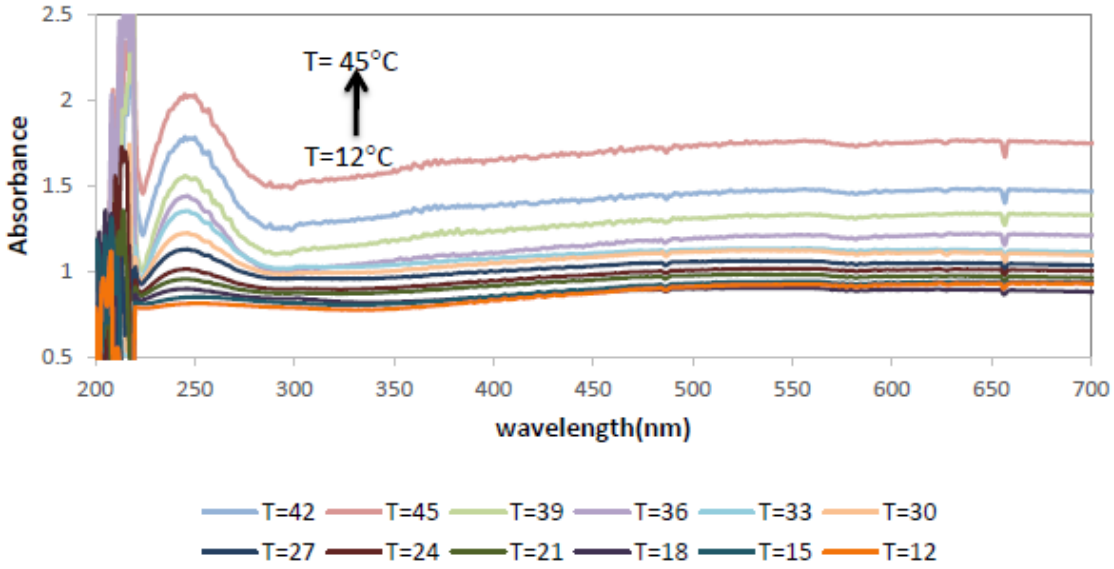

Fig.11.UV-Vis spectrum of TA in presence of hGH in pH=5.00 in various temperatures

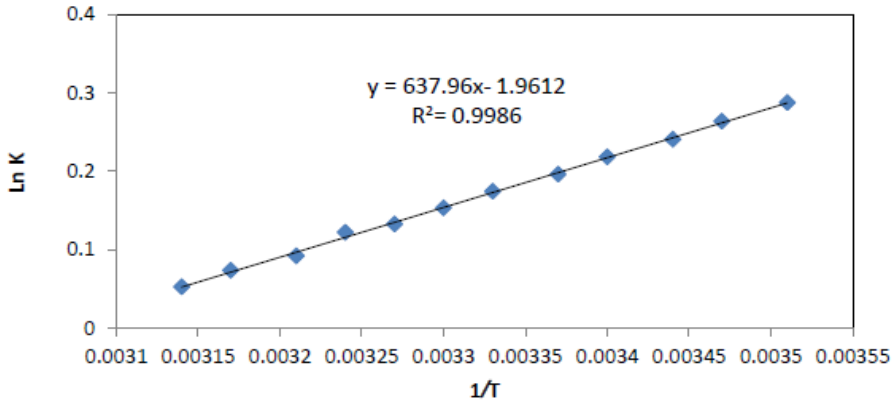

Fig.12.Vant Hoff plot of interaction of TA with hGH

Table 1.Standard binding enthalpy,entropy and binding free energy changes in interaction of TA with hGH

$\Delta H_b^0(kj/mol)$	$\Delta S_b^0(j/molK)$	$\Delta G_b^0(kj/mol)$
-5.3 ± 0.31	-16.22 ± 0.91	-0.47

3.3. Optimal Conditions

Upon experimental results, the optimal hGH concentration, optimal pH are 25.4 ppm and 2 respectively,then this Interaction is strongly pH-dependent and Protonation states of functional groups control electrostatic attraction.Ionic strength changes does not significant effect on interaction then suggests ion-dipole rather than ion-ion interaction.

3.4. Stoichiometry

3.5 experimental Thermodynamic Results

Upon results on Table 1,$\Delta G° < 0$ and Interaction is spontaneous,$\Delta H° < 0$ and Interaction is exothermic *and* $\Delta S° < 0$ *that means* Reduced molecular freedom upon binding.then although this interaction is spontaneous, the relatively small magnitude of ΔG° indicates a weak interaction**.**

3.6 Temperature Effect

By Increasing temperature decreases binding affinity.Consistent with an exothermic interaction and Le Chatelier's principle.

4. MOLECULAR DOCKING

Upon Molecular Docking RESULTSwas shown in Fig. 13,there is surface binding with 1-3 hydrogen bonds and an electrostatic contacts with Lys,Arg and His residues that is fully consistent with pH dependence and ion-dipole interaction and a week entropy-unfavorable binding.

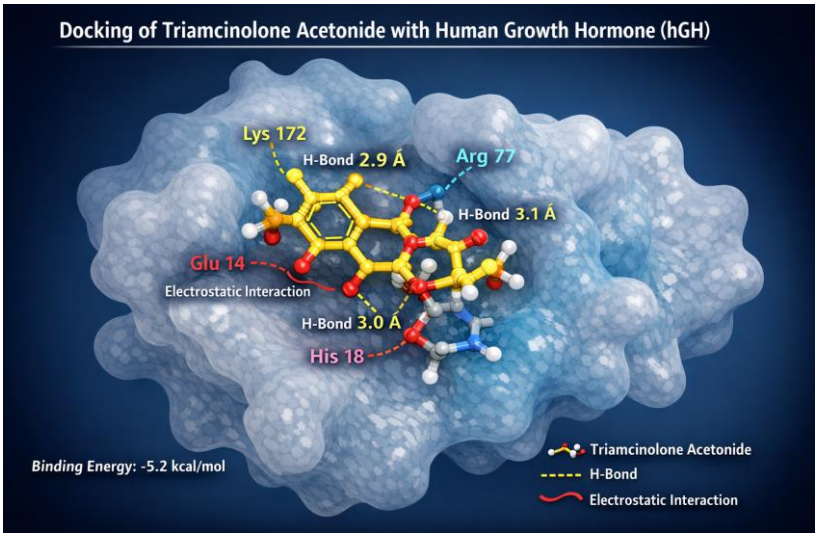

Fig. 13 Docking of Triamcinolone Acetonide with human Growth Hormone(hGH)

5.CONCLUSION

TA interacts weakly but spontaneously with hGH through pH-dependent electrostatic interactions. Despite its low binding strength, TA may act as an inhibitor of hGH at low concentrations. UV–Vis spectroscopy is confirmed as an effective and economical method for studying such biomolecular interactions.Experimental data suggests the electrostatic kind of interaction and the positively charged residues,Surface helices of hGH, Non-covalent interactions only obtained binding energy about -4.0 to -6.0 kcal/mol and negative binding free energy confirms this weak interaction.

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