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Research Article

Interactions Evaluation of 5-Fluorouracil Drug with Amino Acids Least Abundance in Protein

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Abstract

In the beginning, the 5-FU drug (0.1 mM) and amino acids (tryptophan and cysteine; 1.0 mM) were prepared separately in potassium phosphate buffer at a physiological conditions (pH=7.4 and T=37°C). The acidity function and temperature of prepared solutions were checking continuously, and the spectral measurements were achieved for these solutions individually. The experimental spectra of reactants (5-FU and amino acids) at pH=7.4 and T=37°C were showed identical values for the standards (λ_{max} = 266; 280, 190 nm for 5-FU, tryptophan and cysteine respectively). Then, the spectral lines of molecular complexes were followed as first order interactions for tryptophan (at 277 nm; k_f =30.48×10^{-3} min^{-1}; $t_{1/2}$= 17.67 min) and cysteine (at 264 nm; k_f =29.43×10^{-3} min^{-1}; $t_{1/2}$= 22.28 min). The mathematical calculations showed that, the interaction of cysteine with 5-FU was proceed in forward direction simply by regularity mode with a high stability (Keq=17.67). While, the molecular complex of tryptophan was behaved as a complicated reversible interaction by manner less stability than in cysteine (with; Keq=3.49). The non essential peaks of amino acids interactions (at 287 nm in tryptophan and 320 nm in cysteine) were explained as aggregation for transient molecular complexes, or it may be represent different interactions for the more one kind of reactants. The molecular complexes interactions (5-FU drug with tryptophan and cysteine) were occurred spontaneously, with free energies favourable for a physical forces (Van Der Waals forces) or hydrogen bonding ($\Delta G^{\circ} < 10$ kJ/mol) by a high chemical affinity.

1. Introduction

5-fluorouracil (5-FU) drug can be represented the one of chemotherapy compounds which used the most widely for treatment of the different cancers yet (especially, those associated with the aerodigestive tract, breast, and colorectal system) [1]. It phase specific drug interferes with nucleic acids synthesis (RNA and DNA) to prevent the replication of cells in human body. 5-FU drug as a pyrimidine analog has a scientific name 5-fluoro-1H-pyrimidine-2,4-dione (according to IUPAC system; Figure 1) [2]. The chemotherapy compound (5-FU) have been important data half time in plasma was small 5−20 min, the metabolized dose in liver and extra hepatic tissues about 60−90% and the unchanged ratio which excreted in urine about 10−20%. So, the smaller amount of 5-FU drug would be remained in human body to represent its anticancer activity. Therefore, the bioavailability of this drug (5-FU) was estimated by 18%, while its binding with protein in human body about 8−12% [1–3]. Here, the spotlighted of importance for achieving the different studies to understanding binding 5-FU drug with proteins (abundant biological macromolecules).

The protein represents polymers of amino acids, it can be broken down (hydrolyzed) to residue of amino acids by a variety of methods, and earliest studies of proteins focused on free amino acids derived from them [1, 4, 5]. The proteins (as a biological macromolecules) can

be described as a distinguished player in many cellular functions including gene expression, catalyzing metabolic reactions, DNA repair and replication [6]. As well as, the proteins can be operated as a complexes with different biomolecules such as proteins, nucleic acids, carbohydrates, or lipids. These are complexes can be transient (as a molecular complexes), stable or dynamic, and the association between them would be controlled by the cellular conditions [7].

Generally, twenty different amino acids were classified into five main classes based on properties of their R groups; and it can be founded commonly in proteins with different ratios. The two amino acids (under study) tryptophan and cysteine have been existing with a least abundance in proteins in human body; it can be estimated by 1.4% and 1.9% respectively. The percentage and important biological functions of these amino acids were initial step in selection for evaluation its interaction with anticancer drug (5–FU) [4, 5].

Tryptophan (an aromatic amino acid) has unique physicochemical properties, it was encountered frequently in membrane proteins (especially at the level of water-bilayer interface). Although its hydrophobic character, it can be appointed in many types of interactions such as $\pi-$cation, $\pi-\pi$ interaction or hydrogen bonds. This was supported by the presence of nitrogen in indole ring as shown in Figure 1 which may facilitate the solubility of proteins, and it has a dipole moment molecule similar to tyrosine (but not phenylalanine) [8]. The distribution of amino acids in proteins was showed that tryptophan has preferential location in transmembrane proteins in both α-helix and β-sheet structures (which is called the aromatic belt) [8, 9].

Cysteine (a polar uncharged amino acid) can be classified the semi essential proteinogenic amino acid with the thiol side chain. It has ability on the formation of disulfide bonds (as a disulfide derivative cystine by oxidation process) [10]. After its convert into cystine, it can be served in important structural role in many proteins, and often participates in the enzymatic reactions as a nucleophile [11]. Although, cysteine was classified as a nonessential amino acid; but it may be responsible for reduced blood pressure and stroke risk in high protein diets partially [12]. As well as, proteins containing cysteine have a high affinity for heavy metals, and it has redox-active properties which lead to contribution with complexes of respiratory chain [13, 14].

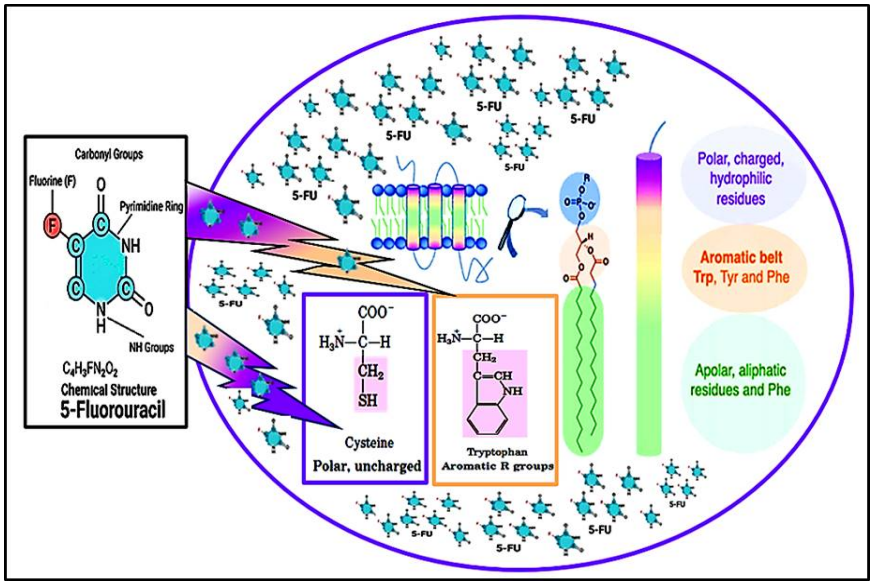

Figure 1: shown the position of amino acids in transmembrane protein and structures of 5-FUdrug, tryptophan and cysteine at a physiological conditions (pH=7.4 and T=37°C) [2, 4, 5, 8, 9]

2. Methods

2.1. Materials

All chemicals were obtained from commercial sources, with a highest available purity. 5-FU was purchased from Sigma Aldrich, USA, with purity of 99.99%. KH_2PO_4 and K_2HPO_4 of phosphate buffer solution (has pH=5 $\sim$ 8) were obtained from Sdfine chemical limited-Mumbai, India. Tryptophan (Trp:$C_{11}H_{12}N_2O_2$; Aromatic Amino Acid), Cysteine (Cys:$C_3H_7NO_2S$; Polar uncharged Amino Acid) were supplied by GCC-England with the purity of 99.99%.

2.2. Instruments and method

The all electronic absorption spectra of ultraviolet-visible spectroscopy (UV-Vis) were recorded by Shimadzu (Japan) 1800PC-computrace spectrophotometer, version 2.42,by using 1cm matched quartz cells in aqueous solution of potassium phosphate buffer as a solvent. Measurements of pH were adjusted by using WTW (Germany) Inolap720 pH meter with electrode Sentix41. The distilled water produced by GFL (Germany) apparatus was purified via SG ionic exchangers. The prepared deionized water was measured in WTW (Germany) Inolap720 Conductivity meter. The temperature of all prepared solutions was regulated within 37°C, by using Julabo (Germany) circulator water bath. All weights were taken by Mettler Toledo (Switzerland) SAB204S, sensitive electronic balance. Some apparatus were used in different preparation processes such as heater with magnetic stirrer (IKA; India) and Oven with controlled temperature (Heidolph; Germany).

All glassware was thoroughly cleaned with aqua region, and rinsed with distilled water as well as deionized water prior its use. The deionized water (has conductivity=0.5 $\sim$ 0.7µS/cm) was used to prepare all the solutions.

2.3. Preparation of drug and amino acids solutions

In the beginning, solution of potassium phosphate buffer was prepared by mixing suitable volume of potassium dihydrogen phosphate (0.0666M) with dipotassium hydrogen phosphate (0.20M). Continuously, the prepared solution of phosphate buffer was adjusted by using a calibrated pH meter. Then, the solution of 5-FU drug (M.wt.=130.08 gm/mol) was prepared with concentration of 0.1 mM in potassium phosphate buffer solution which prepared previously by weight of 0.0013 gm in 100 mL calibrated flask. Then, the amino acids (Trp; 204.23 and Cys; 121.15 gm/mol as a standard materials with concentration 1.0 mM) were prepared in same potassium phosphate buffer solution which it used to prepared the 5-FU drug (at pH=7.4 and T=37°C). The prepared method of 5-FU drug was used with a different types of amino acids with simple continuous shaking through the preparation process. As well as, the acidity function and temperature of the prepared solutions were be measured continually (for guarantee, the achieved of work at a physiological conditions; pH=7.4 and T=37°C).

3. Results and Discussion

Analysis of 5-FU drug (0.1 mM) in phosphate buffer solution must be performed at physiological conditions (pH=7.4 and T=37°C), for ensuring absorption peak corresponded to a standard value Figure 2. The drug of 5-FU has distinguished spectral data involved a maximum wave length (λ=266 nm) with absorption (A=0.673) [2, 15]. Individually, the checking of amino acids (1.0 mM) which prepared previously by potassium phosphate buffer (at pH=7.4 and T=37°C) was achieved, without any signal or absorption peak interference with 5-FU drug. Therefore, the experimental applied studies of 5-FU drug with amino acids can be occurred spectrally; for assessment the type of relationship between them as follow :

3.1. Interaction of 5-FU drug with Tryptophan (as an Aromatic amino acid)

The spectral electronic report of tryptophan (1.0 mM) was involved distinguished absorption peak at 280 nm (with absorbance; A=1.87) in the solution of phosphate buffer (pH=7.4 and T=37°C). Knowingly, tryptophan was classified as a essential amino acid with aromatic group; which it is believed main cause for absorption peak in the ultraviolet region [16]. The formation of molecular complex between 5-FU drug (0.1 mM) and tryptophan (1.0 mM) in potassium phosphate buffer solution (pH=7.4 and T=37°C) was occurred by direct mixing without any complicated. As shown in Figure 2, The new absorption peak of molecular complex (with a main peak at λ=277 nm) has maximum wave length differ from starting substances (5-FU drug and tryptophan) with a small distinguished peak (as a inside shoulder at 287 nm) prior main peak.

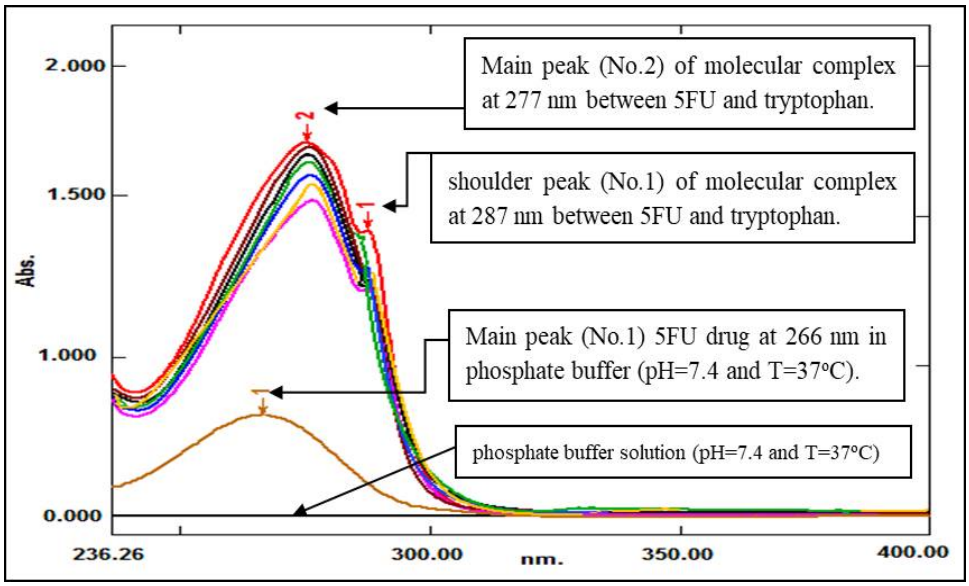

Figure 2: The interaction 5-FU drug (0.1 mM) and tryptophan (1.0 mM) with time in potassium phosphate buffer solution at a physiological conditions (pH=7.4 and T=37°C)

For attention, the inside shoulder peak of molecular complex at 287 nm had changed absorbance value through different measurements with time (so, it can not depend on it in this study). This is irregular peak may be formed as result of the aggregation for transient molecular complex (which it is reformatted continuously) between 5-FU drug and tryptophan in the aqueous solution (it is needed more studies by another techniques in order to its understanding correctly). In oppositely, the regular absorbance of main peak at 277 nm was encouraged following the interaction between 5-FU drug and tryptophan at physiological conditions in potassium phosphate buffer solution.

3.2. Interaction of 5-FU drug with Cysteine (as a polar amino acid)

The interaction study between 5-FU drug (0.1 mM) and cysteine (1.0 mM) was achieved at a physiological conditions (pH=7.4 and T=37°C). This means, the same followed conditions and procedures in tryptophan experimental would be repeated in cysteine case.

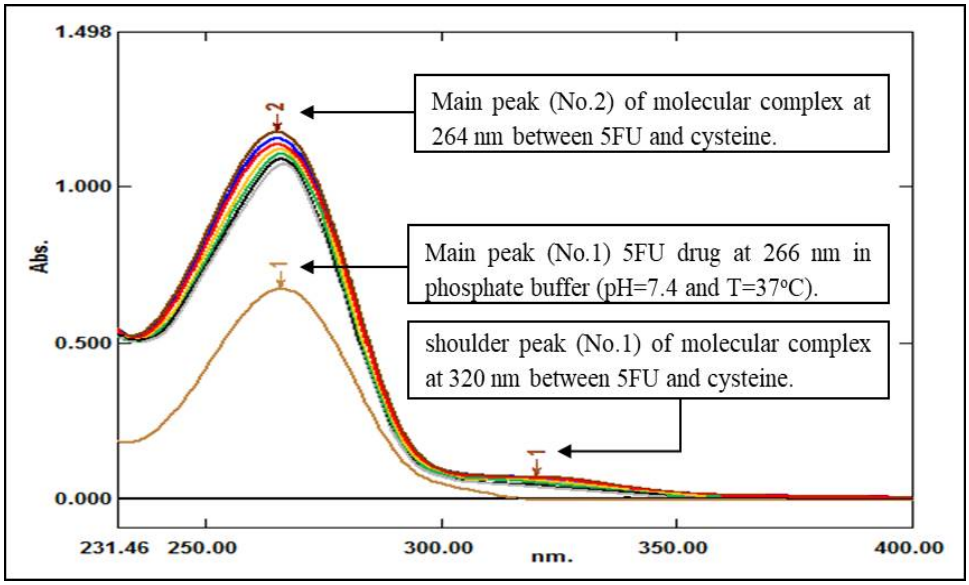

Figure 3: The interaction 5-FU drug (0.1 mM) and cysteine (1.0 mM) with the time in potassium phosphate buffer solution at a physiological conditions (pH=7.4 and T=37°C)

The small peak of molecular complex at 320 nm peak No.1 in Figure 3 was not increased by regularity in absorbance value with time (although the repeat of spectral measurements for more one time). The small wave may be attributed into the transient molecular complex that reformatted continuously between interaction 5-FU drug and cysteine in the aqueous solution. Generally, the thiol group (–SH) of cysteine amino acid is absorbed in ultraviolet region at 190 nm, which it can be interfered with resulted wave from dissolved oxygen in aqueous solutions. As well as, disulfide group (S–S) of the cystine (as a dipeptide that derivative from bind two molecules of cysteine) has absorbed peak at 250 nm [17]. Accordingly, the peak of transient molecular complex (at 320 nm) can not be returned into either cysteine or cystine individually, and it may be represent different interactions for the more one kind of reactants. However, the main peak (No.2; at 264 nm) on the molecular complex was distinguished by regular sequenced absorbance, it is encouraged on follow up of the interaction between 5-FU drug and cysteine at a physiological conditions.

By comparison between Figures 2 and 3, the interaction 5-FU drug with cysteine was more regular than tryptophan in wave lines with time. Especially, the spectral wave of molecular complex for tryptophan at equilibrium (1440 min) was irregular simply (at top of wave) although repeating the measurement three times. This confuse in molecular complex of tryptophan may be back into the contribution negative charge that located on 5-FU drug (as a result presence of fluoro atom) with resonance of aromatic system in indole ring Figure 1.

3.3. kinetic interaction of 5-FU drug as a reversible equilibrium from first order

The apparent values of absorption for 5-FU drug interaction with amino acids were repeated more than three times, in order to ensure the equilibrium state occurred between interacted molecules. Therefore, the results obtained could be applied in the equation of reversible equilibrium from first order reaction of 5-FU drug interaction with amino acids Eq.1; Table 1. It can binding between kinetic and thermodynamic behavior to determine the equilibrium constant Eq. 2 for interaction 5-FU drug with amino acids as a physicochemical constant [18, 19] as shown in Table 2.

$$ln(A_e – A_x) = ln(A_e) – (k_1 + k_{-1})t \quad (1)$$

$$Keq = \frac{A_e}{A_a - A_e} \quad (2)$$

where A_e: absorbance of complex at equilibrium (1440 min), A_x: absorbance of produced complex with time, k_1: forward rate constant, k_{-1}: backward rate constant, t :time of measurement (min), $(A_a - A_e)$ = (a - X_e): the absorbance which equivalent to remaining quantity of molecular complex at equilibrium state, and Keq: equilibrium constant.

Mathematically, the absorbance value of molecular complexes at origin time (A_o) can be extracted by intercept of straight line resulted from the direct plot of (A_x) versus time (as shown in Table 1; A_x at origin time (A_o) = 1.2511; 1.1255 of tryptophan and cysteine respectively). Then, the value $(A_a - A_e)$ at equilibrium state between period times (the origin time into 1440 min) of molecular complex can be determined by equivalent value for it $(A_e - A_o)$ to extract equilibrium constant. Therefore, the obtained experimental results can represented according to reversible equilibrium first order equation as a function to absorption within a limited times as following :

Table 1: Time effect on absorbance of 5-FU (0.1 mM) with tryptophan and cysteine (1.0 mM) at the same conditions (pH = 7.4 and T = 37° C), as a reversible equilibrium of first order interaction

Molecular complex of 5-FU drug with amino acids molecules as a reversible equilibrium						
Tryptophan (at 277 nm)				Cysteine (at 264 nm)		
t / min	A_x	$A_e - A_x$	ln $(A_e - A_x)$	A_x	$A_e - A_x$	ln $(A_e - A_x)$
0	1.2511	0.5009	– 0.69134	1.1255	0.0675	– 2.69562
10	1.301	0.451	– 0.79628	1.134	0.059	– 2.83021
20	1.414	0.338	– 1.08470	1.144	0.049	– 3.01593
30	1.503	0.249	– 1.39030	1.155	0.038	– 3.27016
40	1.585	0.167	– 1.78976	1.165	0.028	– 3.57555
50	1.654	0.098	– 2.32278	1.175	0.018	– 4.01738
60	1.686	0.066	– 2.71810	1.18	0.013	– 4.34280
Equilibrium	1.752	——	——	1.193	——	——

The plotting of ln (Ae – Ax) within limited time period (10 – 60 min) would produce straight line with slope represents kinetic behavior equal to [– $(k_1 + k_{-1})$], as shown in Figure 4.

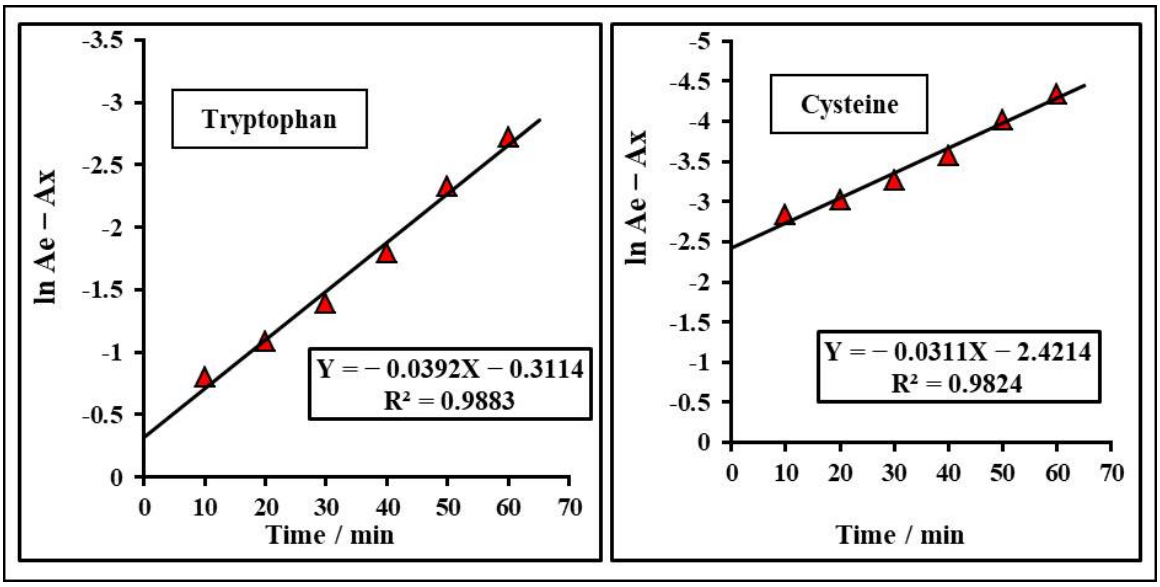

Figure 4: Diagrams show the rates of reversible equilibrium interaction of 5-FU drug (0.1 mM) with amino acids (1.0 mM) time in potassium phosphate buffer solution (at pH=7.4 and T=37°C)

Subsequently, the rate constants k_1 and k_{-1} respectively can be calculated according to equation of reversible first order interaction as shown in Table 2 by combining between the thermodynamic and kinetic behavior represented by Eq. 1 and Eq. 2.

Table 2: The parameters of thermodynamic and kinetic of interaction 5-FU drug (0.1mM) with amino acids (1.0 mM) at pH =7.4 and T = 37°C; as a reversible equilibrium first order interaction

5-FU drug with Amino Acids	Y = a X – b	R^2	$k_1 \times 10^{-3}$ (min^{-1})	$k_{-1} \times 10^{-3}$ (min^{-1})	Keq
Tryptophan	Y = – 0.0393X – 0.3114	0.9883	30.48444	8.71555	3.49770
Cysteine	Y = –0.0311X – 2.4214	0.9824	29.43458	1.66541	17.67407

The physicochemical properties (k_1, k_{-1} and Keq) were gave important indicators of interaction process between 5-FU drug and amino acids Figures 2 and 3. The backward rate constant (k_{-1}) of interaction 5-FU drug with amino acid cysteine was less than its similarities of tryptophan. This is mean that the interaction of 5-FU drug with cysteine is proceed in forward direction only, but with tryptophan can be consider as a reversible interaction. This matter was ensured the belief that 5-FU drug has a net negative charge can be contributed eminently in effect on the interaction with molecules. As well as, the same mentioned reason explains the decrease of equilibrium constant for interaction with tryptophan (Keq= 3.49) comparison with cysteine (Keq= 17.67).

The half time of 5-FU interaction with amino acids could be determined : $(t_{1/2})_{total} = 0.693/k_1 + k_{-1}$ where; A_x=0.5A_e in Eq.1, since the physicochemical processes from the first order. Then, the forward half time $(t_{1/2})_f = 0.693/ k_1$ and the backward half time $(t_{1/2})_b = 0.693/ k_{-1}$ could be extracted respectively, by the same method and assumption Table 3 [18–20].

Table 3: Half time of 5-FU drug (0.1 mM) with amino acids (1.0 mM) in aqueous solution of phosphate buffer at pH=7.4 and T=37°C, as a reversible equilibrium from first order interaction

5-FU drug with Amino Acids	Equilibrium interaction $(t_{1/2})_{total}$(min)	Forward interaction $(t_{1/2})_f$ (min)	Backward interaction $(t_{1/2})_b$(min)
Tryptophan	17.678	22.732	79.512
Cysteine	22.282	23.543	416.113

The half life of interaction with amino acids did not excess half time of presence 5-FU drug in plasma (5 – 20 min) [1, 2] (as shown in Table 3). The half time of interaction in forward direction $[(t_{1/2})_f]$ was equal approximately for both amino acids. But the backward half time was ensured that interaction of cysteine in forward direction and revisable with tryptophan. Additionally, the interaction of 5-FU drug with cysteine more stability as explained in Table 2; Keq=17.67) and extra regularity as shown in Figure 3 from tryptophan according to Figure 2.

3.4. Gibbs free energy and chemical affinity of 5-FU interaction

The physicochemical property of free energy is considered as a thermodynamic parameter for known the spontaneously of chemical reaction, it has zero value at equilibrium state (ΔG=0). So, the standard Gibbs free energy (ΔG°) consider is more useful in biophysicochemical processes, which can be calculated depending on equilibrium constant value.

$$\Delta G^{\circ} = -RT\,lnKeq \tag{3}$$

where; R : gas constant (8.314 J/K.mol), and T : absolute temperature (310°K).
Then, chemical affinity (A_h; or Helmholtz constant) can be extracted depend on free energy but with different sign, this means A_h; +ve of spontaneous processes (ΔG°; –ve) and vice versa as shown in Table 4 [2, 19].

$$A_h = -(\Delta G^{\circ})_{T,P,n} \tag{4}$$

Table 4: Gibbs free energy and chemical affinity of 5-FU (0.1 mM) with amino acids (1.0 mM) as reversible equilibrium from first order interaction in phosphate buffer at pH=7.4 and T=37°C

5-FU with Micelle	Keq	ln Keq	ΔG ° (J/mol)	A_h (J/mol)
Tryptophan	3.49770	1.25210	– 3227.10491	3227.10491
Cysteine	17.67407	2.87209	– 7402.37518	7402.37518

The high values of Gibbs free energy were indicated on occurrence of interaction between 5-FU drug with amino acids (under study; tryptophan and cysteine) spontaneously. The only difference, the molecular complexes obtained from interactions between 5-FU molecules with cysteine were more regular and formed easier by comparison with its similarities in tryptophan.

Generally, tryptophan (as essential amino acid; human body can not synthesis it) is necessary for human nutrition and physiological functions, it plays a essential role in gut brain functions, bone health, immune regulation, mitochondrial functions, and energy metabolism. Tryptophan as daily requirement is very important for children and adult, and decrease it effect on health (minimum daily requirement; 250 mg for men and 150 mg for women) [21, 22].

While, cysteine (as a non or semi essential amino acid) can be synthesized by the human body at a physiological conditions [23]. The thiol group (–SH) of cysteine has a various biological functions (such as; SARS-CoV-2 [24]), and it may be partially responsible for reduced blood pressure and stroke risk [13]. Cysteine can be oxidized easily to form cystine with disulfide bonds (S–S), it has role in folding and stability of some proteins (it form 10 –14% in human hair and skin) [23, 25].

4. Conclusions

Experimentally, the obtained results shown occurrence active interaction between 5-FU drug and amino acids in phosphate buffer at pH=7.4 and T=37°C. The spectral measurements of cysteine interactions were more regularity than it similarities in tryptophan. The shoulder peaks of amino acids interactions are explicated as aggregation for transient molecular complexes, or it may be represent different interactions for the more one kind of reactants. The resulted molecular complex of cysteine was proceed in forward direction, but interactions of tryptophan as a reversible form first order. The molecular complex of cysteine exhibits as a simple interaction with high stability, while tryptophan was more complication and less stability for complete the interaction with 5-FU. The mathematical calculations of amino acids were ensured that interactions with 5-FU occurred spontaneously by van der Waals forces or by hydrogen bonding (with a high value of affinity).

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Data Availability: Data availability is not applicable to this article because no datasets were generated or analyzed.

Disclaimer (Artificial Intelligence): The author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.), and text-to-image generators have been used during writing or editing of manuscripts.

References

[1] H. Verma, G. Narendra, B. Raju, P. K. Singh, and O. Silakari. Dihydropyrimidine Dehydrogenase-Mediated Resistance to 5-Fluorouracil : Mechanistic Investigation and Solution. *ACS Pharmacology & Translational Science*, 5(11):1017–1033, 2022. URL https://pubs.acs.org/doi/abs/10.1021/acsptsci.2c00117.

[2] R. A. Ibrahim, F. S. Suhail, and H. K. Al-Hakeim. Stability of Anticancer Drug 5-Fluorouracil in Aqueous Solution: An Assessment of Kinetic Behavior. *Nano Biomedicine and Engineering*, 10(3):224–234, 2018. URL https://doi.org/10.5101/nbe.v10i3.p224-234.

[3] H. L. Kindler and R. L. Schilsky. Eniluracil : An Irreversible Inhibitor of dihydropyrimidine dehydrogenase. *Expert Opinion on Investigational Drugs*, 9(7):1635–1649, 2000. URL https://doi.org/10.1517/13543784.9.7.1635.

[4] D. L. Nelson and M. Michael. *Lehninger Principles of Biochemistry*. John Wiley and Sons, New York, 4th edition, 2010. URL http://www.whfreeman.com/lehninger4e.

[5] K. Murray, K. Granner, A. Mayes, and W. Rodwell. *Harper's Illustrated Biochemistry*. McGraw-Hill Companies, London, 26th edition, 2003. URL https://dpbck.ac.in/wp-content/uploads/2023/06/6.pdf.

[6] Z. Ahdash1, A. M. Lau1, C. Martens1, and A. Politis. Analyzing Protein Architectures and Protein-Ligand Complexes by Integrative Structural Mass Spectrometry. *Journal of Visualized Experiments : JoVE*, 140:57966, 2018. URL https://pmc.ncbi.nlm.nih.gov/articles/PMC6235531/.

[7] I. Wittig and P. F. Malacarne. Complexome Profiling: Assembly and Remodeling of Protein Complexes (Review). *International Journal of Molecular Sciences*, 22(15):7809, 2021. URL https://www.mdpi.com/1422-0067/22/15/7809.

[8] S. Khemaissa, S. Sagan, and A. Walrant. Tryptophan: an Amino-Acid Endowed with Unique Properties and its Many Roles in Membrane Proteins. *Crystals*, 11(9):1032, 2021. URL https://www.mdpi.com/2073-4352/11/9/1032.

[9] M. B. Ulmschneider and M. S. Sansom. Amino acid distributions in integral membrane protein structures. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, 1512(1):1–14, 2001. URL https://doi.org/10.1016/S0005-2736(01)00299-1.

[10] R. E. Semenza, M. M. Harraz, E. Abramson, P. A. Efrat, V. Chirag, M. M. Gadalla, D. M. Kornberg, H. S. Snyder, and R. Roychaudhuri. D-cysteine is an endogenous regulator of neural progenitor cell dynamics in the mammalian brain. *Proceedings of the National Academy of Sciences*, 118(39):e2110610118, 2021. URL https://doi.org/10.1073/pnas.2110610118.

[11] J. Lee, M. Hitzenberger, M. Rieger, R. N. Kern, R. Nathan, M. Zacharias, and I. Wonpil. CHARMM-GUI supports the Amber force fields. *The Journal of Chemical Physics*, 153(3), 2020. URL https://doi.org/10.1063/5.0012280.

[12] C. S. Larsson, N. Håkansson, and A. Wolk. Dietary cysteine and other amino acids and stroke incidence in women. *Stroke*, 46(4), 2015. URL https://www.ahajournals.org/doi/abs/10.1161/STROKEAHA.114.008022.

[13] E. C. Paulsen and S. K. Carroll. Cysteine-mediated redox signaling: chemistry, biology, and tools for discovery. *Chemical Reviews*, 113(7):4633–4679, 2013. URL https://pubs.acs.org/doi/full/10.1021/cr300163e.

[14] B. Moosmann, P. Hajieva, and C. Behl. The Antioxidant Function of Protein Methionine Explains About the Evolution of a Non-Standard Genetic Code in Mitochondria : 402. *Free Radical Biology and Medicine*, 41:S149–S150, 2006. URL https://doi.org/10.1016.

[15] S. Cheralayikkal, K. Manoj, and K. S. Hussan. Formulation and evaluation of a smart drug delivery system of 5-fluorouracil for pH-sensitive chemotherapy. *Heliyon*, 8(7), 2022. URL https://doi.org/10.1016/j.heliyon.2022.e09926.

[16] S. Barik. The uniqueness of tryptophan in biology: properties, metabolism, interactions and localization in proteins. (Review). *International Journal of Molecular Sciences*, 21(22):8776, 2020. URL https://doi.org/10.3390/ijms21228776.

[17] S. Prasad, I. Mandal, S. Singh, A. Paul, B. Mandal, R. Venkatramani, and R. Swaminathan. Near UV-Visible electronic absorption originating from charged amino acids in a monomeric protein. *Chemical science*, 8(8):5416–5433, 2017. URL https://doi.org/10.1039/C7SC00880E.

[18] P. Atkins, J. de Paula, and J. Keeler. *Atkins' Physical Chemistry*. Oxford University Press, Edinburgh, UK, 12th edition, 2023.

[19] P. M. Monk. *Physical Chemistry : understanding our chemical world*. John Wiley and Sons Ltd, England, p. 385–460, 2004.

[20] U. Saha, R. De, and B. Das. Interactions between loaded drugs and surfactant molecules in micellar drug delivery systems. *A critical review, Journal of Molecular Liquids*, 382:121906, 2023. URL https://doi.org/10.1016/j.molliq.2023.121906.

[21] S. Klaessens, V. Stroobant, E. De Plaen, and B. J. V. Eynde. Systemic tryptophan homeostasis (Review). *Frontiers in Molecular Biosciences*, 9(897929), 2022. URL https://doi.org/10.3389/fmolb.2022.897929.

[22] B. Poeggeler, S. K. Singh, K. Sambamurti, and M. A. Pappolla. Tryptophan in Nutrition and Health 3.0. *International Journal of Molecular Sciences*, 26(17):8443, 2025. URL https://doi.org/10.3390/ijms26178443.

[23] G. Bulaj, T. Kortemme, and D. P. Goldenberg. Ionization-reactivity relationships for cysteine thiols in polypeptides. *Biochemistry*, 37 (25):8965–8972, 1998. URL https://doi.org/10.1021/bi973101r.

[24] B. Nanda, A. Maity, R. P. Nandi, S. Dolui, R. Misra, U. Pal, and N. C. Mait. Conformational Heterogeneity and Redox Switching of the Cysteine Residues in SARS-CoV-2 Main Protease : A Raman Molecular Fingerprint. *The Journal of Physical Chemistry B*, 130(6): 1755–1773, 2026. URL https://doi.org/10.1021/acs.jpcb.5c06195.

[25] C. S. Sevier and C. A. Kaiser. Formation and transfer of disulphide bonds in living cells. *Nature reviews Molecular cell biology*, 3(11): 836–847, 2002. URL https://www.nature.com/articles/nrm954.