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Interaction study of 5- Fluorouracil with Bovine serum albumin by UV-Visible spectrophotometry

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Abstract

Introduction: 5-Fluorouracil (5-FU) is primary chemotherapeutic drug used for most colorectal, pancreatic and aggressive forms of breast cancers. Serum albumin is major carrier protein and its binding with drugs is important to examine the change in pharmacokinetic properties which directly influence the bio availability, sustained drug release and toxicity of drugs.

Aim and objectives: The aim of the project is to perform a study on interaction of 5 - fluorouracil with bovine serum albumin employing the UV-Visible spectrophotometric method.

Methods: Interaction of 5-FU with BSA has been studied systematically using UV spectroscopy by fixing the carbonate buffer of pH 7.4 as solvent and at a suitable wavelength 230 nm and 275 nm. To gain an insight into the interaction of 5-FU with BSA, the spectra of the 5-FU and BSA were examined in the absence and presence of various concentrations of the 5-FU and BSA respectively at zero minutes and repeated in 30 minutes, 60 minutes and 90 minutes time interval. The changes in peak intensity, absorbance and shift in peaks were observed. The detectable differences were recorded. This method can be used successfully for the interaction study of 5-FU with BSA.

Results: Interaction study of 5-Fluorouracil with bovine serum albumin revealed that appreciable changes in absorbance, peak area and shifts in peak were observed which may be due to binding of drug with protein BSA.

Keywords: 5-Fluorouracil; Bovine serum albumin; Chemotherapeutic; UV visiblespectroscopy; Interaction study

1. Introduction

1.1. Spectrophotometric methods

UV-Visible spectroscopy is a well-established analytical technique used in the development of active pharmaceutical ingredients, quantification of impurities in pharmaceutical development. It uses light over the ultraviolet range (185 - 400 nm) and visible range (400 - 700 nm) of the electromagnetic radiation spectrum.

Spectroscopy is defined as the interaction of light with matter. When light is absorbed by matter, there will be an increase in the energy content of molecules or atoms. When radiation is absorbed, there will be an excitation of electrons from the ground state to a higher energy state. The incident light of the spectrophotometer is in the range of visible and UV spectra of electromagnetic radiation.

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The absorbance of a compound depends on the number of excited electrons from the ground state, which is dependent on the concentration or number of molecules in the sample. The absorbance of radiation by a compound produces a distinct spectrum, which is helpful in serving as a marker or identifier of the compound. The easier the electrons get excited, the longer the wavelength of the light, the compound can absorb [1-6].

1.2. Cancer

Cancers are a group of diseases characterized by uncontrolled growth and spread of abnormal cells. Metastasis is the term for the spread of cancer cells, if not controlled it may become fatal. In addition to some internal factors (inherited mutations, hormones, immune conditions, and random mutations), cancer is brought on by numerous external factors (tobacco, chemicals, radiation, and infectious organisms). Numerous factors, such as dietary elements, specific infections, lack of physical activity, obesity, and environmental pollutants are known to raise the risk of cancer. The combination of these factors may initiate or promote carcinogenesis in humans, making cancer the leading cause of death [7-10].

1.3. Drug -Protein Interactions

Drug-protein interaction affects the pharmacological activities therefore research on drug-protein binding is important in pharmacology and pharmacokinetics, mainly for distribution and elimination, as well as its side effects. The unbound drug alone is supposed to exhibit the pharmacological activity and/or the side effect by diffusing from the blood to the extravascular active sites.

The free drug fraction also affects some crucial pharmacokinetic characteristics, including the rate of hepatic metabolism, renal excretion, bio membrane permeation, and steady- state distribution volume.

The advancement of these studies is aided by the creation of an easy-to-use method for calculating the concentration of free drug. Equilibrium dialysis and ultrafiltration are two popular techniques used to assess the binding of drugs to proteins.

Protein binding can influence the drug's biological half-life. The bound portion may act as a reservoir or depot from which the drug is slowly released in the unbound form. Since the unbound form is being metabolized and/or excreted from the body, the bound fraction will be released in order to maintain equilibrium.

Binding of drugs to proteins is generally reversible and irreversible. Reversible generally involves weak chemical bonds such as Hydrogen bonds, hydrophobic bonds, Ionic bonds, Vander waals forces. Irreversible drug binding results from covalent binding and is frequently the cause of the drug's carcinogenicity or tissue toxicity [11-13].

The protein contents of body fluids are considered to be a vital index for the clinical diagnosis of any drug. Bioavailability of the drug under testing is important for its direct regulation, interaction and involvement in immunity generation and metabolism. Blood proteins are the main factors for the transportation and homing of drugs to the target molecules.

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2. Material and methods

2.1. Standard drugs

5- Flurouracil and Bovine serum albumin was obtained as gift sample from Celon Laboratories, Telangana.

2.2. Chemicals and solvents used

- Distilled water

- Sodium bicarbonate
- Sodium carbonate
- Sodium hydroxide
- Hydrochloric acid

2.3. Instruments used

- Analytical balance – Wensar
- UV-Visible Spectrophotometer – Shimadzu -1900
- pH-meter – Eutech
- Sonicator

2.4. Analytical methodologies

- Samples were analysed by UV-Visible spectrophotometer using UV-1800 spectrophotometer (Shimadzu) with a 1 cm path length cuvette and 1 nm slit width quartz cells.
- Assessment of solubility of drug
- Selection of wavelength
- Preparation of standard solutions of 5- FU and BSA
- Study of spectral characteristics of 5- FU and BSA
- Calibration curve of 5- FU and BSA in carbonate buffer
- Statistical evaluation of calibration plot
- Interaction study of standard drug 5-FU with BSA
- Measurement of absorbance, peak area and shift in peaks at 0, 30, 60 and 90 minutes

2.5. Preparation of reagents

2.5.1. Preparation of carbonate buffer

Dissolve 8.4 gm of Sodium bicarbonate and 10.6 gm of sodium carbonate in sufficient water to produce 500 ml. 0.1 N Hcl and 0.1 N NaOH solutions were used for adjusting the pH of carbonate buffer to 7.4. Freshly prepared buffer solutions were used for the entire studies.

2.5.2. Preparation of standard drug (5-FU) solution

Weighed accurately 50 mg of the 5-FU RS, and transferred it into a 100 ml standard flask, dissolved completely and made up to 100ml in the freshly prepared carbonate buffer solution of 7.4 pH to get a concentration of 1000 µg/ml.

The solution is sonicated for 10 min and filtered using whatmann filter paper. An aliquot solution was then diluted with carbonate buffer to get final concentration of 10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml, 50 µg/ml and 60 µg/ml.

2.5.3. Preparation of standard BSA solution

Weighed accurately 100 mg of the BSA, and transferred it into a 100ml standard flask, dissolved completely and made up to 100 ml in the freshly prepared carbonate buffer solution of 7.4 pH to get a concentration of 1000 µg/ml.

The solution is sonicated for 10 min and filtered using whatmann filter paper. An aliquot solution was then diluted with carbonate buffer to get a final concentration of 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml, 100 µg/ml and 120 µg/ml.

2.5.4. Study of spectral characteristics of 5-FU and BSA in carbonate buffer of pH 7.4

Shimadzu UV-Vis spectrophotometer was used for scanning 5-FU RS and BSA in carbonate buffer of pH 7.4 after enabling blank correction in the above region. An absorption band ranging from 200- 400nm was observed with maximum absorption at 230 nm and 275 nm for 5-FU RS and BSA respectively.

2.6. Calibration plot of 5-FU in carbonate buffer

Accurately pipetted out 0.5 ml, 1 ml, 1.5 ml, 2 ml, 2.5 ml and 3 ml of standard solution into 6 labelled standard flasks of 25 ml and volume was made up to the mark with carbonate buffer. The absorbance of each solution was measured at 275 nm and 230 nm with carbonate buffer as blank and this data reveals that Beer-Lambert's law is obeyed from 10-60 µg/ml.

2.7. Statistical evaluation of calibration plot 5-FU

The calibration curve was plotted with absorbance on the Y-axis and concentration on the X-axis. A linear plot was obtained within this concentration range.

2.8. Calibration plot of BSA in carbonate buffer

Accurately pipetted out 0.5ml, 1ml, 1.5ml, 2ml, 2.5ml and 3ml of standard solution into 6 labelled standard flasks of 25 ml and volume was made up to the mark with carbonate buffer. The absorbance of each solution was measured at 275 nm and 230 nm with carbonate buffer as blank and this data reveals that Beer-Lambert's law is obeyed from 20-120 µg/ml.

2.9. Statistical evaluation of calibration plot of BSA

The calibration curve was plotted with absorbance on the Y-axis and concentration on the X-axis. A linear plot was obtained within this concentration range.

2.10. Stability profile

Stability of absorbance is of major importance in spectrophotometric methods. The period over which absorbance value of 5-FU and BSA at 230 and 275 nm in carbonate buffer of pH 7.4 remained stable was investigated by observing the absorbance of a single concentration of drug and BSA solution (within the linearity range of 5-FU and BSA) for a period of one hour.

2.11. Interaction study of standard drug 5-FU with BSA

Changes in spectral characters of the spectra of 5-FU and BSA due to interaction was examined by scanning the mixtures of solutions of constant concentrations of drug 5-FU (40 µg/ml) and variable concentration BSA (60 µg/ml, 80 µg/ml, 100 µg/ml) and vice versa, constant concentration of BSA (80 µg/ml) and variable concentration 5-FU (30 µg/ml, 40 µg/ml, 50 µg/ml). Changes in peak intensity, peak areas and shift in wavelength are studied.

2.12. Intraday study of Interaction of standard drug 5-FU with BSA

The mixtures of solutions of constant concentrations of drug 5-FU (40 µg/ml) and variable concentration BSA (60 µg/ml, 80 µg/ml, 100 µg/ml) and vice versa, constant concentration of BSA (80 µg/ml) and variable concentration 5-FU (30 µg/ml, 40 µg/ml, 50 µg/ml) were prepared, and changes in peak intensity, peak areas and shift in wavelength were studied at zero minutes and repeated in 30 minutes, 60 minutes and 90 minutes time interval [17-22].

3. Results and discussion

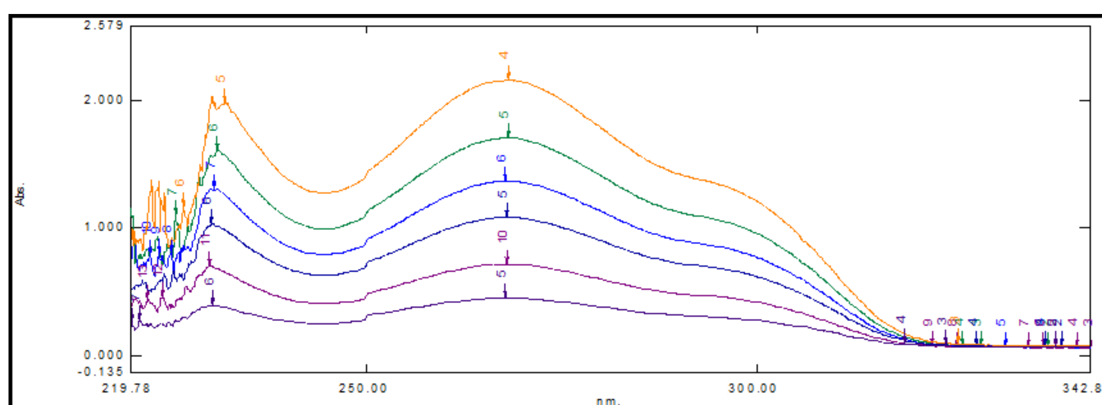

Figure 1 UV- Visible overlay spectra of 5- FU in carbonate buffer of pH 7.4

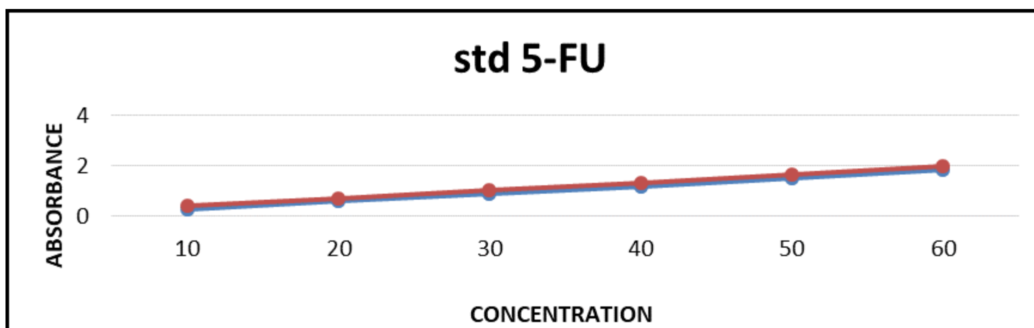

Figure 2 Calibration plot of 5- FU in carbonate buffer of pH 7.4

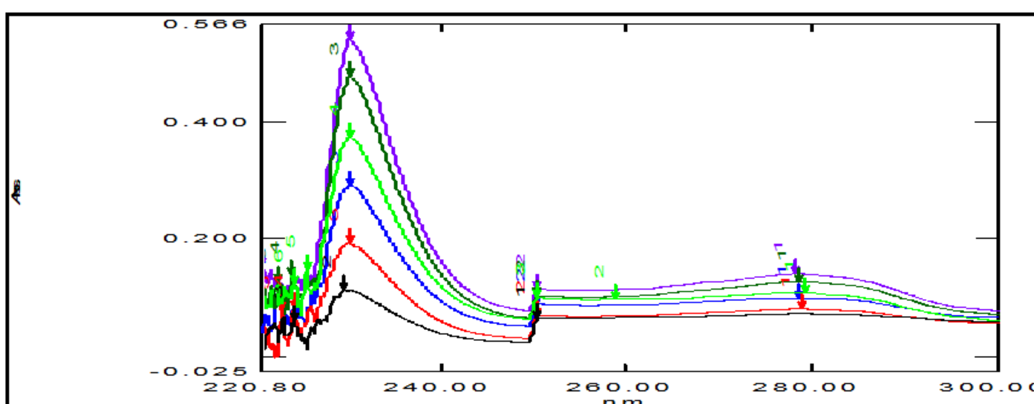

Figure 3 UV- Visible overlay spectra of BSA in carbonate buffer of pH 7.4

Table 1 Interaction study of standard drug 5-FU with BSA

Concentration of 5-FU and BSA in µg	Peak area		Absorbance		Lamda max	
	230 nm	275 nm	230 nm	275 nm	230 nm	275 nm
5-FU 30	2.676	7.687	1.079	1.072	230.0	270.0
5-FU 40	3.092	9.333	1.307	1.296	230.0	270.0
5-FU 50	3.508	11.339	1.658	1.491	230.0	270.0
BSA 60	2.021	0.146	0.303	0.062	230.0	278.0
BSA 80	2.311	0.220	0.400	0.092	230.0	278.0
BSA 100	2.712	0.233	0.450	0.121	230.0	278.0
5-FU 40 + BSA 60	2.039	4.644	0.781	0.653	229.4	269.4
5-FU 40 + BSA 80	1.953	4.605	0.819	0.650	229.3	269.5
5-FU 40 + BSA 100	2.026	4.567	0.876	0.660	229.2	269.0
BSA 80 + 5-FU 30	1.964	3.810	0.808	0.647	230.2	268.3
BSA 80 + 5-FU 40	1.953	4.605	0.819	0.650	229.3	268.5
BSA 80 + 5-FU 50	1.943	5.462	0.830	0.656	229.6	268.7

Table 2 Intraday interaction study of standard drug 5-FU with BSA

Concentration of 5-FU and BSA in µg	Peak area		Absorbance		Lamda max	
	230 nm	275 nm	230 nm	275 nm	230 nm	275 nm
5-FU 40+BSA 60 (0 min)	2.039	3.931	0.781	0.653	229.4	268.4
5-FU 40+BSA 60 (30min)	1.470	4.587	0.768	0.648	229.4	268.3
5-FU 40+BSA 60 (1 hr)	0.913	5.244	0.589	0.712	231.7	268.2
5-FU 40+BSA 80 (0 min)	1.953	4.605	0.819	0.650	229.3	268.4
5-FU 40+BSA 80 (30min)	1.914	4.641	0.857	0.657	229.4	268.5
5-FU 40+BSA 80 (1hr)	0.580	5.175	0.618	0.704	231.5	268.6
5-FU 40+BSA 100 (0min)	2.526	4.67	0.876	0.660	229.4	268.5
5-FU 40 + BSA 100 (30min)	1.612	4.603	0.879	0.654	229.3	268.5
5-FU 40+BSA 100 (1hr)	1.203	5.118	0.639	0.698	231.7	268.9
BSA80+5-FU 30 (0min)	1.964	3.810	0.808	0.647	230.2	268.3
BSA80+5-FU 30 (30min)	2.142	3.804	0.575	0.714	230.4	268.2
BSA80+5-FU 30 (1hr)	1.944	3.855	0.721	0.592	230.3	268.3
BSA80+5-FU 40 (0min)	2.374	4.723	0.951	0.770	230.3	268.7
BSA80+5-FU 40 (30min)	3.416	4.750	0.907	0.759	230.2	268.2
BSA80+5-FU 40 (1hr)	2.374	4.829	0.948	0.185	230.1	268.2
BSA80+5-FU 50 (0min)	2.082	6.062	1.133	0.934	229.6	268.3
BSA80+5-FU 50 (30min)	2.300	6.102	1.085	0.930	230.3	268.3

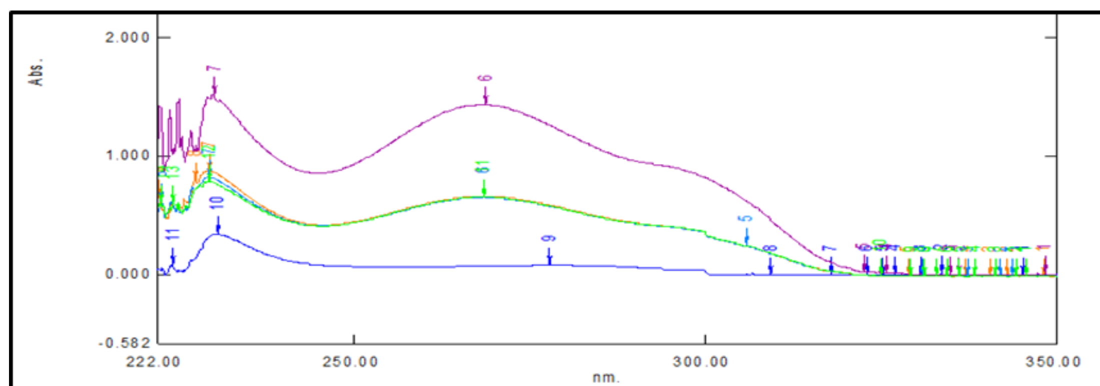

Figure 4 Interaction with of std 5-FU of constant conc. and variable conc. BSA

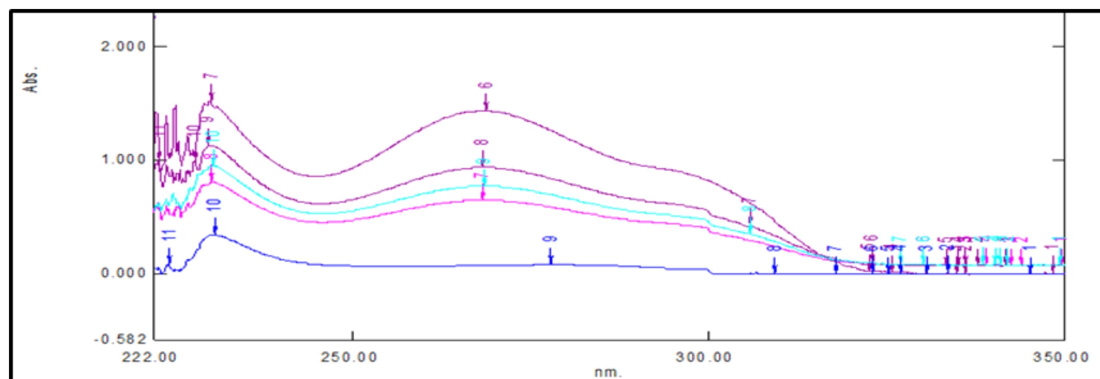

Figure 5 Interaction with std 5-FU of variable conc. and constant conc. of BSA

4. Conclusion

- 5-FU is a potential compound against various cancers so it is important to understand the pharmacokinetic and pharmacodynamic properties essential for its evolution as a more preferable anti-cancer agent. Ultraviolet absorption spectroscopy is a widely employed technique to understand protein-drug interactions and to analyse the corresponding structural changes.
- Interaction of 5-FU with BSA has been studied systematically using UV spectroscopy by fixing the carbonate buffer of pH 7.4 as solvent.
- Calibration curve of 5-FU with BSA were plotted in the obtained λmax and the curves found to be linear in the selected concentration range 10-60 µg/ml and 20-120 µg/ml for 5-FU and BSA respectively.
- To gain an insight into the interaction of 5-FU with BSA, the spectra of the 5-FU and BSA were examined in the absence and presence of various concentrations of the 5-FU and BSA respectively at zero minutes and repeated in 30 minutes, 60 minutes and 90 minutes time interval.
- The absorbance, peak area and shifts in peaks were observed in time intervals.
- The detectable distinction in absorption, shifts in absorption spectra and increase in peak intensity are indicative of a complex formation between 5-FU and BSA.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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