Chapter I

KINETIC STUDIES

Nature is, by its very nature, concerned with change. While Chemistry is, by its very virtue, concerned with understanding this change in nature. Chemical reactions convert substances with well-defined properties into other materials with different properties. Much of our study of chemical reactions is concerned with the formation of new substances from a given set of reactants. However, it is equally important to understand how rapidly chemical reactions occur. The rates of reactions span an enormous range, from those that complete within fractions of seconds, such as certain explosions, to those that take thousands or even millions of years, such as the formation of diamonds or other minerals in Earth’s crust.

The area of chemistry, that is concerned with the understanding speeds, or rates, of reactions is called chemical kinetics. It is to be contrasted with thermodynamics, which deals with the direction in which a process occurs but in itself tells nothing about its rate. Chemical kinetics relates to many aspects of cosmology, geology, biology, engineering and even psychology and thus has far-reaching implications. The principles of chemical kinetics apply to purely physical processes as well as to chemical reactions. Chemical kinetics is a subject of broad importance. It relates, for example, to how quickly a medicine is able to work, to whether the formation and depletion of ozone in the upper atmosphere are in balance, and to industrial problems such as the development of catalysts to synthesize new materials. Our goal in the thesis is not only to understand how to determine the rates at which reactions occur but also to consider the factors that control these rates. For example, what factors determine how rapidly drugs degrade? How does one design a probable reaction mechanism based on kinetic data? What controls the rate of oxidation? Although we won’t address these specific questions directly, we will see that the rates of all chemical reactions are subject to the same basic principles. In principle, molecules can be activated not only by molecular collisions and by light quanta but also by bombardment with electrons or ions. One of the objectives of the thesis is to find out the relation between the number of units involved and a number of molecules reacting.
History of chemical kinetics

Some landmarks in the history of chemical kinetics are associated with making mechanisms more realistic: the steady-state approximation of Bodenstein and Lind, the Lindemann mechanism involving activated molecules for unimolecular dissociation, and the Michaelis–Menten multistep mechanism for enzyme kinetics are three examples. The interaction of bodies is simpler when there is a similitude between them, this is the base idea of Chemical Affinities and come from ancient and medieval alchemy and naturalism doctrine. At the end of the 17th century, this intuitive principle becomes a theory, although qualitative, that justify and classify interactions between different substances. In the same period also the observation of time become important for the determination of the nature of chemical reactions. Time of recurrence was clearly contemplated for the preparation of substances with long reactions but it was seen as an ordinary technical factor. The Opera of Alchemy, for example in the transmutations of metals, was considered as a means for the acceleration of the millenary gestation of precious metals in the bowels of Mother Earth.

Kinetics and mechanistic study of redox reactions

The experimental part of the subject deals with ways of measuring precisely the rates of reactions at various varying conditions of the experiments. The interpretation of results leads to an understanding of the mechanism of the reaction. The combination of the results of a large number of experiments gives rise to general theories of chemical reactivity. The important steps in any kinetic investigations are; (1) collection of kinetic data, (2) establishment of relationships between the rate and reaction mixture composition, (3) study of structural effects and (4) interpretation of the collected data to arrive at reaction mechanism.

The award of Nobel prize for the year 1992 to Prof. R. A. Marcus on the “Electron Transfer Reactions” and 1999 Nobel prize to Prof. Ahmed Zewail for the discovery of “Femto chemistry” and 2001 Nobel prize to Profs. William Knowles, K. Bary Sharpless and Royji Noyori for their work on “Chirally Catalysed Hydrogenation Reactions” emphasize the importance of the field of reaction kinetics. Electron transfer reactions play a central role in physical, chemical and biological processes. Because of the ubiquity of electron transfer processes, the study of electron
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transfer reactions, perhaps more so than that of any other area of chemistry is characterized by a strong interplay of theory and experiment\(^1\), nonetheless the importance of electron transfer in transition metal redox chemistry has been recognised\(^2\) and more recently it has become increasingly obvious that many reactions in organic chemistry once thought to be concerted in nature also occur via sequential one-electron steps\(^3\). The study of reaction kinetics gives a wealth of information about the nature and course of reactions. The order of a reaction can be used to interpret the reaction on a molecular level. For example, the chemists by considering the order of a reaction with respect to different reactive species predict the sequence in which bonds break and atoms rearrange during the reaction and hence propose reaction mechanism.

The work of Henry Taube\(^4\) in redox systems unequivocally demonstrated the transport of electron from reductant to oxidant. This discovery certainly added many important features in the syntheses of coordination complexes and organometallics. It is such a subject, which has manifestations in almost all walks of life. As a result, oxidation-reduction reaction needs at least two reactants, one capable of gaining electrons (oxidant) and the other capable of losing electrons (reductant), i.e., a reducing agent (reductant) by losing electrons, gets oxidised and an oxidising agent (oxidant), by gaining the electrons, gets reduced.

**Oxidation - reduction in inorganic reactions**

Two general classes of transition states emerge for redox reactions involving metal complexes, the so called **outer-sphere and inner-sphere types**\(^5\). In the first of these, the inner coordination shells of both the metal ions are intact in the transition state. In the second case, the two metal ions are connected through a bridging ligand common to both the coordination shells. From Franck-Condon principle, it follows that, before electron transfer between two ions is possible, the energy of the electron must be the same in the two sites. There must also be sufficient orbital overlap between the two sites to provide for a reasonable probability of a transfer.

Considerations in an outer-sphere mechanism, (1) reactants must get close together for tunneling to occur (2) bond lengthening and shortening must occur and (3) Franck-Condon principle must be obeyed (4) electronic transitions (and electron transfer) occur on a far shorter time scale than molecular vibrations (nuclear motion)
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this means that electron transfer will only occur when the complexes are distorted to the appropriate geometry for the products - i.e., this imposes an electronic barrier on the rate of electron transfer.

The coordination shells of the complexes or metal ion remains intact, during this kind of electron transfer takes place. Such type of electron transfer is called as tunneling effect. Outer sphere electron transfer is generally enthalpically less favorable than inner sphere electron transfer because the interaction through space between the redox centers in outersphere electron transfer is weaker than the interaction through the chemical bridge present in the inner sphere mechanism. By the same token, outer sphere electron transfer is usually entropically more favorable than inner sphere electron transfer as the two sites involved do not have to go through the ordering processes associated with the formation of a bridge.

Such a mechanism is established when rapid electron transfer occurs between two substitution-inert complexes.

\[
[\text{Fe(CN)}_6]^{4-} + [\text{Ir Cl}_6]^{2-} \rightarrow [\text{Fe(CN)}_6]^{3-} + [\text{Ir Cl}_6]^{3-}
\]

\[
[\text{Fe(CN)}_6]^{4-} + [\text{Mo(CN)}_8]^{3-} \rightarrow [\text{Fe(CN)}_6]^{3-} + [\text{Mo(CN)}_8]^{4+}
\]

The inner-sphere mechanism should obey three distinct steps: (1) substitution to form a bridge between oxidant and reductant (2) actual electron transfer and (3) separation of the products (often with transfer of the bridge ligand). An inner-sphere mechanism is one in which the reactant and oxidant share a ligand transitorily in their inner or primary co-ordination spheres forming a bridged intermediate activated complex. The discoverer of the inner-sphere mechanism was Henry Taube, who was awarded the Nobel Prize in Chemistry in 1983 for his pioneering studies. A particularly historic finding is summarized in the abstract of the seminal publication.

Taube’s classical 1953 experiment (Nobel Prize 1983):

\[
[\text{Co(NH}_3)_5\text{Cl}]^{2+} + \text{Cr(H}_2\text{O)}_6]^{2+} + 5 \text{H}_2\text{O} \rightarrow [\text{Co(H}_2\text{O)}_6]^{2+} + [\text{Cr(H}_2\text{O)}_5\text{Cl}]^{2+} + 5 \text{NH}_3
\]

The electron being transferred across a bridging group. An example is given below

\[
[\text{CoCl(NH}_3)_5]^{2+} + [\text{Cr(H}_2\text{O)}_6]^{2+} \rightarrow [\text{Co(NH}_3)_5\text{(H}_2\text{O)})]^{2+} + [\text{CrCl(H}_2\text{O)}_5]^{2+}
\]
Oxidation - reduction in organic reactions

The oxidation - reduction concepts, however, are not so clearly applicable in organic chemistry, for when carbon compounds are oxidized their component atoms are very seldom deprived of their surrounding complete electron shells. Covalent bond fission is an essential feature of organic reactions and it can be affected by two different pathways, viz, “Homolytic reactions” in which electron pairs are symmetrically disrupted and “Heterolytic reactions” in which electron pairs are transferred from one molecule to another as an undivided entity. Electron removal by these two pathways has clearly distinguishable characteristics.

Homolytic fission is chemical bond dissociation of a neutral molecule generating two free radicals. That is, two electrons that are involved in the bond are distributed one by one to the two species. In homolytic reaction electrons are removed singly from organic molecules forming free radicals leading to chain reactions, dimerisations or disproportionations. All heterolytic organic chemistry reactions can be described by a sequence of fundamental mechanistic subtypes. The elementary mechanistic subtypes taught in introductory organic chemistry are $S_N1$, $S_N2$, E1, E2, addition and addition-elimination. Using arrow pushing, each of these mechanistic subtypes can be described. On the contrary in heterolytic reactions oxidants attack exposed electron pairs or loosely held $\pi$-electrons yield stable molecular or ionic products in one or at most two consecutive stages and very seldom lead to chain reactions.

Probable ways of electron transfer reactions

The oxidation - reduction reaction may involve one or more electron transfer. Depending upon the number of electrons transferred between oxidant and reductant, the reaction may proceed in one or more steps. Transition metals such as iron and cobalt and several others usually exhibit stable oxidation states differing by one electron and react with each other through one equivalent steps. However, the stable oxidation states in post-transition elements such as arsenic, antimony etc., differ by two electrons. Thus, on the basis of their pattern of reactivity, the reactions of these elements are classified into two main categories, which is Complementary and Non-complementary reactions.
Complementary reactions

Complementary reactions are those in which oxidant and reductant both undergo two equivalent changes or one equivalent change. These reactions generally obey a bimolecular rate equation and the electron transfer can take place in a single step or in two-step of one electron each. In Tl(I) - Tl(III) exchange reactions, electrons are transferred in a single step as the formation of the intermediate, Tl(II), is not detected. On the other hand, the oxidations of As(III) and Sb(III) by Tl(III) are other reactions where no evidence for the formation of Tl(II) has been obtained. The evidence obtained for the formation of As(IV) in pulse-radiolytic studies indirectly supports one-equivalent steps in As(III) oxidations. However, such an evidence in the reaction of As(III) - Tl(III) has not been observed. It is assumed that As(IV) is formed in the reaction (i) then it is very much likely that the two intermediates Tl(II) and As(IV) react with each other before they can diffuse out of the solvent cage in which they are found.

(i) One equivalent – One equivalent reactions

\[
\text{As(III) + Tl(III) } \quad \longrightarrow \quad \text{Tl(II) + As(IV)}
\]

\[
\text{As(IV) + Tl(III) } \quad \longrightarrow \quad \text{Tl(II) + As(V)}
\]

\[
\text{Ce(III) + Co(III) } \quad \longrightarrow \quad \text{Ce(IV) + Co(II)}
\]

(ii) Two-equivalent – Two-equivalent reactions

\[
\text{U(IV) + Tl(III) } \quad \longrightarrow \quad \text{U(VI) + Tl(I)}
\]

\[
\text{Sn(II) + Hg(II) } \quad \longrightarrow \quad \text{Sn(IV) + Hg(0)}
\]

Non-complementary reactions

Non-complementary reactions are those in which oxidant and reductant undergo unequal equivalent changes such as one-equivalent oxidant interacts with two equivalent reductant and two-equivalent oxidant interacts with one-equivalent reductant. There are a number of possibilities of electron transfer in non-complementary reactions and these are related to the nature of both oxidant and reductant. The most commonly observed kinetic Scheme is

\[
\text{Cr(VI) + Fe(II) } \quad \longrightarrow \quad \text{Cr(V) + Fe(III),}
\]
chromium(V) reacts with a ferrous ion in a rate determining step by one of the following Schemes.

(a) \[ \text{Cr(V)} + \text{Fe(II)} \rightarrow \text{Cr(IV)} + \text{Fe(III)} \quad \text{slow} \]
\[ \text{Cr(IV)} + \text{Fe(II)} \rightarrow \text{Cr(III)} + \text{Fe(III)} \quad \text{rapid} \]

(b) \[ \text{Cr(V)} + \text{Fe(II)} \rightarrow \text{Cr(III)} + \text{Fe(IV)} \quad \text{slow} \]
\[ \text{Fe(IV)} + \text{Fe(II)} \rightarrow 2\text{Fe(III)} \quad \text{rapid} \]

**Multi equivalent reactions**

Oxidizing agents such as Cr(VI) and Mn(VII) undergo net changes of 3 and 5 units in oxidation number respectively during their reactions in acidic solution. For the most part, these reactions occur by one or two electron steps, with the necessary intervention of unstable intermediate oxidation states of Cr or Mn. The reactions of Cr(VI) with transition metal complexes generally proceed by sequential one-electron step\(^{21}\), but with post-transition metal ions and with non-metallic compounds, two electron steps appear to be preferred.

**Electron transfer reactions are found to be governed by two classical principles**

(a) **Michaelis principle of compulsory univalent oxidation steps**\(^{22}\)

(b) **Shaffer’s principle of equivalent change**\(^{23}\)

**Unstable oxidation states**

The formation of unstable oxidation states during the course of non-complementary reactions has been now anticipated in a number of such reactions with sufficient proofs. For example, the reductions of Tl(III) by Fe(II)\(^{24}\), V(III) or V(IV)\(^{25,26}\) and Cr(VI) by Tl(I)\(^{27}\) can only be explained through the formation of unstable Tl(II) species. Similar unstable oxidation states have been observed in other studies\(^{26,28}\). The interconversions\(^{20}\) between Cr(III) and Cr(VI) always appear to involve the unstable states, Cr(IV) and Cr(V).
Active species

If a particular substance (oxidant, reductant or catalyst) is capable of existence in several forms in aqueous solution, all the species existing may not be active. Those species, which are involved in a slow step, will influence the reaction. The reaction conditions will determine the nature of the active species.

Application of chemical kinetics

Multiple chemical reactions occur in nature. Some of these reactions are necessary to synthesize new compounds which hold important responsibility in the field of food, textile, cosmetics, pharmaceuticals etc. while others result in spoilage, undesirable, harmful substances. Thus it is important to understand the mechanism of these reactions. Chemical kinetics plays an important role in determining the rate of reaction and establishes mathematical relationship and mechanisms describing such phenomena. Chemical kinetics is widely applied in the physical and chemical sciences to study reaction mechanisms and their engineering at the molecular level. Such mechanistic information is particularly valuable in the context of the development of strategies to combat diseases, pollution etc. Indeed, information about the microscopic processes underlying changes in macroscopic variables is crucial for understanding the mechanism of action of a given drug as well as for identifying strategies to change both the thermodynamics and the kinetics of disease associated processes. This general strategy has found widespread applications in many fields including enzymology, where chemical kinetics has become a standard tool for testing inhibition mechanisms, including identifying competitive, uncompetitive, and noncompetitive inhibition mechanisms. This approach has already resulted in significant progress in the understanding of the mechanisms of action of enzymes and has led, for example, to the development of anticancer drugs targeting kinase activity. However, the power of this approach in the area of protein aggregation disorders largely remains unexploited.
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DRUG-PROTEIN INTERACTION STUDIES

Many proteins have different functions in different cell types and in cells responding to different extracellular signals. The effects of the cellular environment on protein functions are often mediated by interactions with different partners under different conditions. Protein interactions also integrate signals from different signaling pathways and developmental programs and coordinate regulatory mechanisms in the cell. Studies of protein interactions in living cells can provide insights into these functions since interactions with different partners may occur in different cells, at different times and in different subcellular locations. The visualization of interactions in individual cells also enables analysis of differences among different cells in the population. Studies in intact cells also avoid the possibility of changes in protein interactions as a result of cell lysis and mixing of the contents of different cellular compartments. Consequently, the direct visualization of protein complexes in living cells provides a valuable complement to other methods for the study of protein interactions. Since proteins are polymers consisting of dozens to thousands of amino acids, folding must overcome an enormous amount of conformational entropy, and the fact that proteins self-assemble to an essentially unique fold are a triumph of natural selection. Furthermore, understanding how proteins fold has emerged as a central part of understanding the molecular mechanism of many diseases, such as Alzheimer’s disease or Huntington’s disease, where it is believed that incorrect folding of proteins (misfolding) is a critical part of the disease pathology. The biophysical and biomedical aspects of protein dynamics have created many challenges. First, even small changes, such as a mutation of a single amino acid, can lead to changes in protein dynamics. Moreover, studying protein dynamics experimentally is fraught with many difficulties, given the stochastic and heterogeneous nature of an ensemble of folding proteins. Beyond studying protein folding, protein dynamics within the native state is often critical for function, yet it too shares the challenges of sensitivity to details, long time scales, and complex dynamics, making protein folding a useful model system for protein dynamics more generally. As self-assembly is at the heart of many biological processes as well as the inspiration for much of modern nanotechnology, understanding how proteins fold can also have an impact on many other fields, and the methods used can serve as a paradigm for tackling complex problems in kinetics.
Fluorescence studies

Fluorescence is the result of a three-stage process that occurs in certain molecules called fluorophores or fluorescent dyes. A fluorescent probe is a fluorophore designed to localize within a specific region of a biological specimen or to respond to a specific stimulus. The process responsible for the fluorescence of fluorescent probes and other fluorophores is illustrated by the simple electronic-state diagram (Jablonski diagram) shown in below figure.

Stage 1: Excitation. A photon of energy $h\nu_{EX}$ is supplied by an external source such as an incandescent lamp or a laser and absorbed by the fluorophore, creating an excited electronic singlet state ($S_1'$). This process distinguishes fluorescence from chemiluminescence, in which the excited state is populated by a chemical reaction.

Stage 2: Excited-State Lifetime. The excited state exists for a finite time (typically 1–10 nanoseconds). During this time, the fluorophore undergoes conformational changes and is also subject to a multitude of possible interactions with its molecular environment. These processes have two important consequences. First, the energy of $S_1'$ is partially dissipated, yielding a relaxed singlet excited state ($S_1$) from which fluorescence emission originates. Second, not all the molecules initially excited by absorption (Stage 1) return to the ground state ($S_0$) by fluorescence emission. Other processes such as collisional quenching, Fluorescence Resonance Energy Transfer (FRET) and intersystem crossing may also depopulate $S_1$. The fluorescence quantum yield, which is the ratio of the number of fluorescence photons emitted (Stage 3) to
the number of photons absorbed (Stage 1), is a measure of the relative extent to which these processes occur.

**Stage 3**: Fluorescence Emission. A photon of energy $h\nu_{EM}$ is emitted, returning the fluorophore to its ground state $S_0$. Due to energy dissipation during the excited-state lifetime, the energy of this photon is lower, and therefore of longer wavelength, than the excitation photon $h\nu_{EX}$. The difference in energy or wavelength represented by $(h\nu_{EX} - h\nu_{EM})$ is called the Stokes shift. The Stokes shift is fundamental to the sensitivity of fluorescence techniques because it allows emission photons to be detected against a low background, isolated from excitation photons. In contrast, absorption spectrophotometry requires measurement of transmitted light relative to high incident light levels at the same wavelength.

**Interaction study**

Serum albumin, the most abundant protein in the circulatory system, has been one of the most extensively studied of all proteins\(^{29}\). It is synthesized in the liver, exported as a non-glycosylated protein and is present in the blood at about 40 mg/mL (~0.6 mmol/dm\(^3\))\(^{30}\). The most important physiological role of albumins is to bind numerous ligands viz., fatty acids, amino acids, steroids and metal ions in the blood stream to their target organs\(^{31}\). The remarkable binding properties of albumin accounts for the central role it can play in both the efficacy and rate of delivery of drugs\(^{32}\). Many drugs, including anti-coagulants, tranquilizers, and general anesthetics are transported in the blood while bound to albumin (often more than 90% of the drug is bound). This has stimulated much research on the nature of the drug binding sites and investigations of whether fatty acids, natural metabolites, and drugs compete with one another for binding to the protein. These studies may provide information about the structural features that determine the therapeutic effectiveness of drugs and become an important research field in life sciences, chemistry and clinical medicine\(^{33,34}\).

Bovine serum albumin (BSA) is one of the most widely studied proteins and is the most abundant protein in plasma. The primary structure of BSA is well known for a long time and its tertiary structure was determined a few years ago by X-ray crystallography\(^ {35,36}\). BSA has two tryptophan residues embedded in two different domains: Trp134, located in the proximity of the protein surface but buried in the hydrophobic pocket of domain I and Trp 214 located in an internal part of domain
II\textsuperscript{37}. It consists of a single chain 582 amino acid globular glycoprotein cross-linked with 17 cystine residues (8 disulfide bonds and 1 free thiol). In BSA, the disulphide bonds are located in the following positions: (1) 77 - 86 (2) 99 - 115 (3) 114 - 125 (4) 147 - 192 (5) 191 - 200 (6) 223 - 269 (7) 268 - 276 (8) 288 - 302 (9) 301 - 312 (10) 339 - 384 (11) 383 - 392 (12) 415 - 461 (13) 460 - 471 (14) 484 - 500 (15) 499 - 510 (16) 537 - 582 and (17) 581 - 590. BSA is divided into three linearly arranged, structurally distinct and evolutionarily related domains (I - III); each domain is composed of two subdomains (A and B)\textsuperscript{38,39}. Like other serum albumins, BSA has a wide range of physiological functions involving the binding, transport and delivery of fatty acids, porphyrins, bilirubins, tryptophan, tyrosine and steroids\textsuperscript{40}. It is home to specific binding sites for metals, pharmaceuticals, and dyes. It is known that many drugs bind to serum albumin. It is noteworthy that the presence of more than one tryptophan residues in BSA makes the system more complex than human serum albumin (HSA) that contains only one tryptophan residue unit. BSA structure is similar\textsuperscript{37}to HSA in 75%.

Drug-protein interactions are important since most of the administered drugs are extensively and reversibly bound to serum albumin and the drug is transported mainly as a complex with protein\textsuperscript{41}. The free drug (unbound drug) in the plasma can transfer freely to the target site producing pharmacological effect while the bound drug hardly passes through the blood capillary walls to reach the action site due to its larger molecular weight. Hence, the nature and magnitude of drug-protein interaction significantly influence the biological activity of the drug\textsuperscript{42,43}. The binding parameters are useful in studying the pharmacological response of drugs and design of dosage forms\textsuperscript{39,44}. Serum albumin being the major binding protein for the drugs and other physiological substances are considered as a model for studying drug-protein interaction in \textit{invitro}\textsuperscript{42}.

The spectroscopic techniques (UV-Vis absorption, fluorescence, and circular dichroism) are of great help in the study of interactions between drugs and plasma proteins in general and serum albumin in particular because of their high sensitivity, rapidity and ease of implementation. They have advantages over conventional approaches employed for studying the drug-protein binding such as affinity and size exclusion chromatography, equilibrium dialysis, ultrafiltration, and ultracentrifugation, which suffer from lack of sensitivity or long analysis time or both

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and use of protein concentrations far in excess of dissociation constant for drug-protein complex\textsuperscript{45,46} and for drug-protein interaction studies. Moreover, the fluorescent probe, 8-anilino-1-naphthalein-sulphonic acid (ANS), which could be used as a valuable tool to understand the nature of interaction involved\textsuperscript{47} was not employed by many researchers. In view of this and to overcome the limitations of classical techniques, we have employed spectrofluorimetric, spectrophotometric and FT-IR methods to investigate the mode of interaction of bioactive compounds with BSA. This is the first attempt wherein three spectroscopic techniques are employed to understand the mechanism of interaction of selected bioactive compounds with BSA. Hence, the investigator has made some attempts in this direction and succeeded in developing the interaction study of some bioactive drug with proteins.
Chapter-I

SUMMARY OF THESIS

Dynamics is an important entity to understand science and mechanism of life in general. In present investigation dynamics is important tool for us to understand kinetic studies and drug-protein interaction. If for no other reason than that drug-protein interaction is a reaction in which motion as a function of time is the essential element. In the present investigation, oxidation of some biomedically potent substances in alkaline as well as in acid media using different oxidants having unusual oxidation states have been studied. With ever growing research on the drug specificity to fight against new illness, the newer drugs arrive into the market, giving birth to newer fields of research to combat challenges arising out of these drugs. In this thesis an attempt has been made to identify research problems associated to the title through literature survey in the field. Degradation of drugs through oxidation and understanding the chemical kinetics of these redox reactions, products obtained thereafter, and proposing a probable mechanism was undertaken using various spectroscopic techniques.

This thesis comprises of two parts containing seven chapters including the general introduction. Chapter two to seven has been divided into two parts as; Part A: Kinetic Studies, Part B: Drug-Protein Interaction Studies. The details of such studies are given bellow.

I General Introduction

It introduces about the literature survey on theoretical concepts of kinetic studies, various principles, and applications of kinetics. This chapter sheds some light on the importance of fluorescence studies, serum albumins, drug-protein binding, and various techniques used in the study.
Chapter-I

PART - A
KINETIC STUDIES

II Oxidation of clindamycin phosphate by chromium(VI) in aqueous sulfuric acid medium - A kinetic and mechanistic study

Kinetics and mechanism of oxidation of clindamycin phosphate by potassium dichromate in aqueous sulfuric acid medium is studied spectrophotometrically at 25° C at a constant ionic strength of 3.60 mol dm$^{-3}$. The stoichiometry of the reaction is determined and it was found that one mole of clindamycin phosphate consumes two moles of chromium(VI) (1:2). The oxidation products are characterized and confirmed by spectral studies such as IR, GC-MS and LC-MS. The reaction is first order each in chromium(VI) and clindamycin phosphate concentrations. An increase in the sulfuric acid concentration causes an increase of the reaction rate. The order with respect to acid concentration is found to be 1.65. From the results of kinetic studies, reaction stoichiometry and product analysis a suitable free radical mechanism is proposed. Based on investigation of the reaction at different temperatures, computation of the activation parameters with respect to the slow step of the proposed mechanism is evaluated.

III Oxidation of amoxicillin by hexacyanoferrate(III) in aqueous alkaline medium- A kinetic and mechanistic approach

The kinetics and mechanism of oxidation of amoxicillin by hexacyanoferrate(III) in aqueous alkaline medium at constant ionic strength of 0.10 mol dm$^{-3}$ is studied spectrophotometrically at 25°C. The reaction exhibits 2:1 ([Fe(CN)$_6$]$^{3-}$: amoxicillin) stoichiometry. The reaction products have been identified with the help of TLC and characterized by FT-IR, GCMS and LCMS. The reaction is first order in hexacyanoferrate(III) concentration but fractional order in both amoxicillin and alkali concentrations. The effects of ionic strength and dielectric constant have been investigated. In a composite equilibrium step, amoxicillin binds to hexacyanoferrate(III) to form a complex that subsequently decomposes to the products. Based on investigation of the reaction at different temperatures, computation of the activation parameters with respect to the slow step of the proposed mechanism is evaluated.
IV Oxidative degradation of antitussive drug, dextromethorphan in aqueous acidic medium by permanganate - kinetic and mechanistic approach

Over the past few years, pharmaceuticals are considered as an emerging environmental problem due to their continuous input and persistence to the aquatic ecosystem even at low concentrations. In this investigation, the spectrophotometry is used to monitor the kinetics of permanganate oxidation of dextromethorphan (DXM) in acidic medium. The reaction exhibits 1:1, ([MnO₄⁻] : dextromethorphan), stoichiometry. The reaction products have been characterized. The MnO₄⁻ DXM reaction progress has been monitored at λₘₐₓ = 525 nm over the temperature range 15-35°C. The redox reaction follows first order in Mn(VII) concentration but the fractional order in both dextromethorphan and acid concentrations. Variations of the ionic strength and the solvent dielectric constant have been studied. Based on results thermodynamic parameters are evaluated. A mechanism consistent with the observed kinetic and activation data has been proposed and the rate-law has been derived.

PART - B
DRUG - PROTEIN INTERACTION STUDIES

V Fluorescent bovine serum albumin interacting with the antitussive quencher dextromethorphan: a spectroscopic insight

The interaction of dextromethorphan hydrobromide (DXM) with bovine serum albumin (BSA) is studied by using fluorescence spectra, UV–vis absorption, synchronous fluorescence spectra (SFS), 3D fluorescence spectra, fourier transform infrared(FTIR) spectroscopy and circular dichroism under simulated physiological conditions. DXM effectively quenched the intrinsic fluorescence of BSA. Values of the binding constant, Kₐ, are 7.159 ×10³, 9.398 ×10³ and 16.101× 10³ L/mol; the number of binding sites, n, and the corresponding thermodynamic parameters ΔG°, ΔH° and ΔS° between DXM and BSA were calculated at different temperatures. The interaction between DXM and BSA occurs through dynamic quenching and the effect of DXM on the conformation of BSA was analyzed using SFS. The average binding distance, r, between the donor (BSA) and acceptor (DXM) was determined based on Förster’s theory. The results of fluorescence spectra, UV–vis absorption spectra and
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SFS show that the secondary structure of the protein has been changed in the presence of DXM.

VI Interplay of luminescent bovine serum albumin with antihyperlipidemic quencher fenofibrate, a spectroscopic exploration

The interplay between fenofibrate (FNF) and bovine serum albumin (BSA) is studied by using fluorescence spectra, UV–visible absorption, synchronous fluorescence spectra (SFS), 3D fluorescence spectra, ATR-FTIR and circular dichroism under simulated physiological conditions. Fenofibrate effectively quenched intrinsic fluorescence of BSA. The binding constants $K_A$ values are $1.713 \times 10^3$, $2.028 \times 10^3$, $1.334 \times 10^3$ L mol$^{-1}$, the number of binding sites 'n' and corresponding thermodynamic parameters $\Delta G^\circ$, $\Delta H^\circ$ and $\Delta S^\circ$ between FNF and BSA were calculated at three different temperatures. The interaction between FNF and BSA occurs through dynamic quenching and the effect of FNF on the conformation of BSA was analyzed using synchronous florescence spectra. The average binding distance 'r' between the donor (BSA) and acceptor (FNF) was determined based on Förster's theory. The results of fluorescence spectra, UV–vis absorption spectra and SFS shows that the secondary structure of the protein has been changed in the presence of FNF while fluorescence quenching study of BSA with the addition of FNF indicates that there is a significant interaction between BSA and FNF. Furthermore, the data suggested that the association between FNF and BSA indicate that hydrophobic forces played a crucial role in the binding reaction and contributed to the stability of the complex.

VII Multi spectroscopic approach to unravel interaction of memantine with model transport protein

Bovine serum albumin (BSA) is a model protein which has been used to investigate the interactions between proteins and other substances. Prior work has shown that serum albumin plays a significant role in the body as a transport protein moving a broad range of substances through the bloodstream. The fluorescence of BSA is primarily dependent upon tryptophan. The binding of memantine to the protein is localized near a tryptophan residue, resulting in the deduction of fluorescence signal. The shape, size, and polarity of sites I and II in BSA are unique which account for the different binding specificities, and allowed us to determine the
preferred binding site of memantine through competitive site probe studies. Values of the binding constant, $K_A$, number of binding sites, $n$, and the corresponding thermodynamic parameters were calculated at different temperatures.
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