Summary of the works done

Forensic science is a multidisciplinary subject and it encompasses the different branches of science like chemistry, biology, physics, medicine etc.

However, most of the materials of forensic interest are organic molecules. Those are explosives, drugs (scheduled or unscheduled), pesticides, DNA, RNA etc.

In forensic science, much emphasis is given to develop suitable analytical techniques to identify and estimate the forensic compounds of interest. The limitations are: very small amount of samples are available and samples are usually not available to repeat the experiments.

Therefore, most forensic laboratories use sophisticated instruments of high precision and reproducibility for the detection and quantification of compounds of forensic interest. Naturally, the development of new methodologies and techniques based on physico-chemical properties and interactions of compounds of Forensic interest and depending on the requirements of the laboratory are greatly needed.

The thesis entitled, “Physico-Chemical Properties and Interactions of Compounds of Forensic Interest (Explosives, Drugs and Biomolecules)” is divided into two parts Part-I and Part-II.

Part-I consists of six chapters (I-VI). Part-I deals with the development of innovative analytical techniques for the identification and estimation of compounds of interest in forensic exhibits based on physico-chemical principles.

Part-II consists of five chapters (VII-XI). Part-II is devoted to the study of the charge-transfer interactions of compounds of forensic interest (explosives, drugs and biomolecules).

TLC, HPTLC, UV-Visible spectrophotometry, FTIR, GC-MS, LC/MS/MS have been extensively used for the identification and estimation of alcohols, explosives and drugs (seized by law enforcing authorities) as such.
The thesis ends with some concluding remarks. The contents (or summary of works done) of the different chapters of Part-I (Chapters I-VI) and Part-II (Chapters VII-XI) are described as what follows.

**Part-I**

**Chapter-I:** A brief description on the basic principles of forensic science and functions of a forensic scientist is given. A short review on the different aspects of forensic science and some relevant aspects pertained in the present thesis has been made. The need for new innovative experiments based on physico-chemical interactions and instrumental techniques has been the subject matter of the Chapter-I.

**Chapter-II:** Identification of petroleum hydrocarbons from crude and distilled oils by GC-MS is well known. The distilled and crude oils contain specific group of hydrocarbons are known as biomarkers bearing the information of their geographical source or origin and the geographical past. These are diamondoids (adamantanes, diadamantanes, terpanes, steranes, sesquiterpanes, hopanes, norhopanes etc.). The present article describes the identification of hydrocarbons using their IR spectra in the molecular fingerprinting region where every molecule shows its unique and characteristic individual vibrational frequencies. GC-FTIR spectrum of each of the individual components present in distilled oils was identified by proper standard library matching with the spectrum of the individual component. The GC-MS profiles of the biomarkers of different light and middle distilled fine products of samples from three leading oil companies, namely Indian Oil Corporation (IOC), Indo-Burma Petroleum (IBP) and Bharat Petroleum Corporation (BPC) differ from each other, indicating the variation of the sources of the crudes used by the companies. But nothing specific can be said about the origin of crudes from this limited data.

**Chapter-III:** Numerous methods are being used to identify and quantify methanol and ethanol in alcoholic beverages, including country liquors. Some of the known methods are density and refractive index measurements, and using Schiff’s reagent or chromatropic acid. Other advanced techniques involve head space gas chromatography...
(GC), GC–flame ionization detection, high-performance liquid chromatography, enzymatic reactions, and biosensors. However, identification and quantification of methanol and ethanol in beverages can be accurately done using GC-Fourier transform infrared spectroscopy (FTIR) and horizontal attenuated total reflectance (HATR)-FTIR. Identification of alcohols is possible from library matching of the IR spectra obtained from GC-FTIR. In water, methanol and ethanol show a very strong peak for C–O, stretching at 1015.3 and 1044.2 cm$^{-1}$, respectively. The strong absorption of vibrational stretching frequency of C–O present in alcohols was used for quantification purposes. The absorptions of C–O group frequency of alcohols in water mixtures were measured using HATR-FTIR with a zinc–selenide crystal. Samples were placed directly on the HATR crystal, with alcohol concentrations ranging from 0.2 to 50.0% (v/v). The plot of absorptions against concentrations of methanol and ethanol obeyed Beer’s law ($r^2 = 0.9998$ and 0.9987, respectively), from which alcohol in the mixtures was quantified. Propan-2-ol and $n$-butanol showed no interference. The method was validated from absorption measurements of known mixtures of standard ethanol in water. This is a simple, specific, rapid, accurate, and nondestructive method of identification and quantification of methanol and ethanol in mixtures. It can be used to ascertain methanol contamination in alcoholic beverages that can lead to death or methanol poisoning by alcohol consumption.

Chapter-IV: Numerous methods like distillation followed by Iodometric titrations, GC-FID, GC-MS, GC-Headspace, Breath analyzer and biosensors including Alcohol dehydrogenase (enzymatic) have been utilized to determine blood alcohol concentration (BAC). In the present study HATR- FTIR had been used to determine BAC in whole blood. The asymmetric stretching frequency of C-C-O group of ethanol in water (1045 cm$^{-1}$) had been used to calculate BAC using Beer’s Law. A seven point calibration curve of ethanol was drawn at the concentration range 24 -790 mg dl$^{-1}$. The curve showed good linearity over the concentration range used ($r^2 = 0.9999$, SD = 0.0023). The method is accurate, reproducible, rapid, simple and non-destructive in nature.
Chapter-V: Different methods of detecting and estimating traces of phenolphthalein in forensic exhibits and commercial formulations were examined. Most of the methods suffered from limited sensitivity and require elaborate sample preparations. Liquid-chromatography-tandem-mass-spectrometry has been found to be most sensitive, accurate and capable of detecting and estimating phenolphthalein in traces in colorless forensic exhibits and commercial formulations. The method requires no elaborate sample preparations and is free from interferences from other compounds. The detection limit of phenolphthalein is 1.6 ppb and the calibration curve shows good linearity ($r^2 = 0.9974$).

Chapter-VI: In this study the FTIR spectra of 4-mononitrotoluene (p-MNT), 3,5-dinitrotoluene (m-DNT) and 2,4,6-trinitrotoluene (α - TNT) were measured at gaseous state (473K), solid (298K), solid (77K) and the experimental vibrational frequencies were compared with those computed using DFT. The basis set was 6-311 with B3LYP correlation functions. Differences between gas phase\textsuperscript{2} spectra and computed spectra of the compounds were found to be low. The vibrational frequencies were shifted towards higher energy in gas phase and towards lower energy in solid (77K) with respect to solid (298K) spectrum.

Chapter-VII: Chapter VII contains a brief review of charge-transfer interactions. The use of CT complexes in the detection and estimation of explosives and drugs has been emphasized. Understanding of CT interactions of drugs with different acceptors may be useful to understand drug-receptor interactions. However, the important draw back is that the works are usually carried out in non-biological, organic solvents and the information derived are to some extent limited.

Chapter-VIII: 1,3-dinitrobenzene formed colored 1:1 complexes with chromogenic agents like isopropylamine, ethylenediamine, tetaethylpentamine and bis(3-aminopropyl)amine in DMSO having absorption maxima at 563 nm, 584 nm, 580.5 nm and 555 nm respectively. The complexes were stable for more than 24hrs. The accurate association constants $K_{DA}$ and other thermodynamic parameters were determined with D and A usually in stoichiometric ratios. But in case of m-DNB and bis(3-aminopropyl)amine, the association constants $K_{DA}$ and the thermodynamic parameters...
were also determined using Benesi-Hildebrand equation to show the variations of $K_{DA}$ under different conditions. $\Delta G^\circ$ values were found to be negative in all cases resulting from exothermic enthalpy changes and favourable entropy changes. The energies of transition for the CT complexes $h\nu_{CT}$ found experimentally were considerably different from the energies of transition (from HOMO of donor to LUMO of acceptor) calculated using AM1 but the differences were considerably reduced using DFT calculations. The vertical electron affinity of m-DNB was calculated using the method suggested by Mulliken.

Chapter-IX: Lamotrigine, a well-known and extensively used antiseizure drug was found to form beautifully colored charge-transfer complexes with o-Chloranil, Chloranilic acid and dichlorodicyanobenzoquinone in acetonitrile. The absorption maxima of the complexes were 543nm and 576nm; 521nm; 408nm,459nm and 587nm respectively (where Lamotrigine had no absorption but the acceptors had absorptions in these regions). The compositions of the complexes were determined to be 1:1 from the Job's method of continuous variations. FTIR measurements of the complexes and the corresponding acceptors were compared. The complexes showed considerable shifts in absorption peaks, change in intensities of the peaks and formation of new band probably due to H-bonding.

The thermodynamic association constants of the complexes and other thermodynamic properties were determined spectrophotometrically taking D and A in stoichiometric ratios. The complex formations were found to be spontaneous and associated with negative changes of $\Delta G^\circ$, $\Delta H^\circ$ and $\Delta S^\circ$ values.

The energies $h\nu_{CT}$ of the charge-transfer complexes and vertical ionization potential $I_D^V$ of Lamotrigine were compared with the theoretical values of $h\nu_{CT}$ obtained from HOMO and LUMO of the donors and acceptors calculated using Density Function Theory utilizing different basis sets. The agreement between the results can be regarded to be reasonable. Oscillator strengths and dipole strengths of the complexes were determined theoretically and experimentally and the limitations of the calculations were outlined.
A physico-chemical approach has been put forward to explain the causes for the onset of seizures and suggest a probable molecular mechanism underlying the effects of Lamotrigine to counter seizures based on the physico-chemical properties of drugs.

**Chapter-X:** Lamotrigine (LTG) is an important anticonvulsant drug used in the treatment of epilepsy and all kinds of seizures (consisting of different types of seizures including severe seizures like Lennex-Gastaut syndrome [a disorder of childhood characteristic by multiple seizure types] mental retardation and refractoriness to anti-seizure medication), bipolar disorder or mood stabilizer.

Due to its importance, the assay of LTG in commercial tablets attracted much attention. The chloranillic acid in acetonitrile was found to be most suitable for the assay of LTG in different pharmaceutical dosage forms in a simple way, which is described in this article. The CT complex between p-chloranilic acid and lamotrigine at 521 nm was used to determine accurately the concentration of lamotrigine in tablets.

**Chapter-XI:** Methyldopa is a much used antihypertensive drug and is the subject matter of study mostly on the determination and estimation of methyldopa in pharmaceutical properties. These considerations led us to study the charge-transfer interactions between methyldopa, a centrally acting antihypertensive agent of limited use due to side reactions with the known acceptors like o-chloranil(o-CIN), chloranilic acid(CIA) and dichlorodicyanobenzoquinone (DDQ). Methyldopa (MDP) formed beautifully colored complexes(having absorption maxima at 581 nm and 368 nm; 519 nm; 583.5 nm, 547 nm and 346 nm respectively) with these acceptors and their physico-chemical properties were studied using UV-Visible spectrophotometry and FTIR measurements. The composition, the accurate association constants and thermodynamics of the complexes were determined spectrophotometrically. Attempts were made to interpret the thermodynamics of complexes in terms of $I_D^V$, $E_A^V$ and $h_{CT}$. Solid CT complexes between MDP + o-CIN, MDP + CIA and MDP + DDQ were prepared and FTIR spectra of the complexes were studied. The energies $h_{CT}$ of the charge-transfer complexes and vertical ionization potential $I_D^V$ of Methyldopa were compared with the theoretical
values of $h\nu_{CT}$ obtained from HOMO and LUMO of the donors and acceptors calculated using Density Function Theory utilizing different basis sets. The agreement between the results can be regarded to be reasonable. Oscillator strengths and dipole strengths of the complexes were determined theoretically and experimentally and the limitations of the calculations were outlined.

Each chapter of the thesis is complete with references and other relevant data.