PREFACE

The rapid growth in instrumental techniques has enabled spectroscopy to be developed into an indispensable tool for scientific research and industrial quality control in a wide field of applications. The atomic and molecular structure and the nature of chemical bonding existing in matter have been brought to light with the different types of spectroscopic techniques. Vibrational spectroscopy serves as a valuable tool for the elucidation of molecular structure, thus enabling characterization and identification of molecules. Raman and Infrared spectroscopies are complementary techniques which together provide a more complete impression of the biochemical information within a sample. Both these techniques are successfully employed in the study of various biomolecules. The UV-Visible spectroscopy throws light on the group characters of the molecule. The choice of a particular spectroscopic method for a specific problem depends on the kind of information sought. With the involvement of the scientific community in the development and use of new instrumental techniques, spectroscopy is gaining prominence as one of the most powerful, versatile and fascinating tool for the investigation of matter.

The present thesis is a spectroscopic approach for the analysis of some materials of pharmaceutical and biological interest. The thesis is organized as follows.
Chapter one gives an overview of the role of spectroscopy in the fields of pharmaceutical and biological sciences. This chapter details about the various fields of pharmaceutical and biological sciences where spectroscopy is extensively used for research and quality control.

Chapter two enumerates the theory and instrumentation of FTIR, FTRaman, UV-Visible spectroscopy and HPLC techniques, which have been employed in the current work. The FTIR spectral measurements of the pharmaceutical samples studied in the current work has been measured using ABB Bomem Series spectrophotometer and the blood plasma samples were measured using Perkin-Elmer spectrophotometer. Raman spectrophotometer Nexus 670 and Bruker IFS 66V spectrometer equipped with FRA 106 FT Raman module were used for recording the Raman spectrum of the samples. The qualitative analysis of the chosen pharmaceutical compounds was done using Shimadzu-1601 double beam spectrophotometer. Reverse phase high performance liquid chromatographic (RP-HPLC) methods for the assay of the various chosen pharmaceutical formulations in this study have been carried out with Shimadzu HPLC VP series system. A brief note on instrumentation and various sampling techniques followed is presented in this chapter.

Chapter three gives an account of the qualitative and quantitative analysis of a few antianginal drugs Metoprolol Tartrate and Trimetazidine Hydrochloride and antipsychotic drugs Sertraline Hydrochloride and
Olanzapine, carried out using FTIR, FTRaman and UV-Visible spectroscopic techniques. The vibrational band assignment was made based on the position, shape and relative intensity of the recorded spectra and in correlation with the vibrational bands of structurally related molecules. By employing FTIR and UV-Visible spectral techniques, the change in the quality of the drugs under various storage conditions has been studied. The estimation of the active substance of a drug is an important quantity assessment technique. The assay of the tablets of these drugs, Meto Er-50mg, Taz-20mg, Serta-25mg and Olanz-10mg were performed using the UV-Visible spectroscopy and was compared with the labeled amount.

Chapter four is a study on the quality of drugs in their pharmaceutical formulation by employing HPLC and UV-Visible spectroscopic techniques. The three cardiac drugs namely Atenolol, Verapamil and Losartan were chosen for the study. The amount of drug present in tablet formulations must comply with the labeled amount. This ensures that there is no loss of activity when the prescribed dosage is taken by the patients. For this the evaluation of the amount of drug present in the tablet formulations becomes essential. In the present investigation RP-HPLC method was employed for estimation of cardiac drugs in tablet dosage form before and 10-12 months after its expiry. The results obtained have been substantiated by employing UV-Visible spectroscopic method. Recovery studies have been carried out to ensure the accuracy of the
procedure adopted in each case. The high recovery percentage highlights the accuracy of the method followed.

**Chapter five** discusses the semi-empirical and Density Functional computations of the molecules methionine, homocysteine and cysteine in comparison with the experimentally recorded vibrational frequencies observed in FTIR and FTRaman spectra. The optimized geometry, wave number and other properties were studied using semi-empirical and density functional computations. The Modified Neglect of Differential Overlap (MNDO) and Austin Model I (AM1) were adopted in semi-empirical analysis. The ab initio and density functional study based on Hartree-Fock and Becke3-Lee-Yang-Parr(B3LYP) level using 6-31G(d,p) basis set was performed. A complete vibrational assignment aided by the theoretical harmonic wave number analysis was proposed. The calculated harmonic vibrational frequencies were compared with experimental FTIR and FTRaman spectra. Based on the comparison between calculated and experimental results and the comparison with related molecules, assignments of fundamental vibrational modes were made. The X-ray geometry and experimental frequencies were compared with the results of theoretical calculations.

**Chapter six** is an attempt to use FTIR spectroscopy for the analysis of blood plasma to detect elevated levels of homocysteine. Those with cardiovascular disease, renal failure, smokers and alcoholics are prone to have
elevated levels of plasma homocysteine. Hence their blood samples were subjected to clinical and spectral analysis. The analysis led to the identification of specific modes of vibration pertaining to homocysteine in blood plasma. The internal ratio parameter was calculated. The absorbance values at these specific modes of vibration varied significantly from that of healthy volunteers. It provided an excellent classification between healthy individuals and those with elevated homocysteine levels, which correlated completely with the clinical data. This indicates that spectroscopic methods can be successfully applied for studying pathological changes in blood and characterization of blood as healthy and diseased.

**Chapter seven** is entirely devoted to the study of efficacy of multivitamins on patients with elevated homocysteine levels. For the present study atherosclerotic, hyperlipidemic, hypertensive and epileptic patients were enrolled. Five from each of the four categories with hyperhomocysteinemia were chosen. Before the initiation of vitamin supplements along with their regular medication, the FTIR spectra of the blood plasma was recorded and the homocysteine levels were clinically tested. They were orally administered a daily dosage of folic acid(5 mg), vitamin B12(250mcg) and vitamin B6(25mg) supplements for a period of two months. Efficacy of these vitamin supplements on plasma homocysteine was analyzed both clinically and spectroscopically. The FTIR spectra were recorded at the end of the first and the second month
and also the homocysteine levels were clinically tested. The absorption values of the specific modes of vibration pertaining to homocysteine of both pre and post-treatment spectra were noted and the percentage of efficacy of the multivitamins was calculated. The spectral and clinical investigation showed that the addition of these vitamins can markedly reduce the homocysteine levels in blood plasma. This study forms a promising basis for employing spectroscopy in the follow-up of patients undergoing treatment for various ailments.

**Chapter eight** summarises the results and conclusions arrived at, on the spectroscopic investigation undertaken on the chosen samples of pharmaceutical and biological importance.

Summing up, the present work furthermore confirms the reliability and utility of spectroscopic techniques in the analysis of pharmaceutical and biological samples.
A part of the material of the thesis has been published in reputed journals and presented in National/International Conferences.

**Papers published**


**Papers Communicated**

8. Efficacy of vitamin supplementation on plasma homocysteine levels among hyperlipidemic patients – A Spectral and Clinical Analysis, *Indian Journal of Clinical Cardiology*. (Communicated)
9. Plasma homocysteine levels and efficacy of vitamin supplementation among patients with atherosclerosis – A Spectral and Clinical Follow up, Asian Journal of Clinical Cardiology. (Communicated)

Papers presented

1. FTIR spectral analysis of plasma homocysteine levels among smokers, International Conference on Photonics, Nanotechnology and Computer Applications, PRIST University, Thanjavur on 27th and 28th February 2009.


4. Characterisation of plasma homocysteine and study of efficacy of vitamin supplements by FTIR spectroscopy, International Conference on recent frontiers in applied spectroscopy, Annamalai University, Annamalainagar, Chidambaram, from 22nd – 24th September 2010.