EXECUTIVE SUMMARY OF THE REPORT

The project report entitled, “Charge Transfer Complexes of drugs with \( \pi \) - acceptors” is divided into two parts. Part-I deals with the spectrophotometric studies of molecular complexes of drugs with TCNE and Paraquat and Part - II deals with Ion-pair charge transfer complexes of drugs with acidic triphenylmethane dyes. Each part is further divided into 1) Introduction 2) Experimental and 3) Results & Discussion.

PART - I : CHARGE TRANSFER COMPLEXES OF DRUGS

1. Introduction

Various physical methods such as conductivity, kinetics, distribution coefficient, magnetic susceptibility and spectroscopy viz., X-ray diffraction, IR, UV-Vis, ESR, NMR and Mass have been extensively used for the study of molecular complexes. Often molecular complex exhibits an absorption band, known as charge transfer (CT) band, in UV-Vis spectrum in the region where neither donor nor acceptor have any absorption, hence UV – Vis spectroscopy is widely used.

The energy of charge transfer band \( (E_{CT} = hc/\lambda_{CT}) \), is related to Ionization Potential of donor \( (I_D) \) by the equation

\[
E_{CT} = a I_D + b
\]

where a and b are constants dependent on the acceptor and solvent used. For example the values of a and b for TCNE in CHCl\(_3\) are 0.82 eV and -4.28 respectively.

The study of molecular complexes by UV-Vis spectroscopy has the advantage of determining ionization potential of donors which are otherwise difficult for molecules of low volatility. The position of charge transfer band varies from donor to donor and provides a method for qualitative analysis of donor. The absorbance of charge transfer band is sensitive to dilution and provides a method for the determination of stability constants of molecular complexes. The absorbance of charge transfer band is also sensitive to
temperature and enables the determination of thermodynamic parameters in normal experimental range of temperatures.

2. Experimental

The commercial sample of Tetracyanoethylene obtained from Lancerter, USA was twice recrystallised from pure chlorobenzene and twice sublimed under vacuum and preserved in a vacuum desciclator. Paraquat dichloride was extracted from the commercial herbicide, gramoxone, by repeated recrystallization from water, ethanol and ethanol-acetone mixture. Colourless crystals of the PQ dichloride obtained were TLC pure. The IR and UV spectra of the sample tallied well with those reported in literature. The drugs used in study are procured from various bulk drug and pharmaceutical industries like Hetero drugs, Symed Pharma, Neo Spark, Syn-finechem and Sreenivas Pharma in and around Hyderabad. Most of the drugs procured are in the form of their acid salts. They have been neutralized by adding calculated amount of NaOH / NH₄OH as required followed by extraction with ether or CHCl₃, then recrystallization is carried out from suitable solvent till TLC pure. A few drugs were salts of Na⁺ and Mg²⁺ are recrystallised before use. Solvent used in the study is spectrograde Chloroform (CHCl₃) without further purification.

Stock solutions were prepared for all the acceptors and drugs. The spectra of individual components and charge transfer complexes have been recorded on Shimadzu 140 and Elico SL 210 UV-Visible double beam spectrophotometers as well as on Thermo Nicolet 100 using Quartz cells of 10mm path length. Stability constants and thermodynamic parameters of molecular complexes were determined by Rose- Drago method.

3. Results & Discussion

a) Molecular Complexes of TCNE : When colourless solution of TCNE is mixed with drugs, characteristic colors were observed. Each of the solution exhibited Charge Transfer band (s) in their electronic spectra. Astemizole exhibited two CT bands while Terazosin, Ketoconozole, Pantoprazole, Irbesartan and Ramipril exhibited only one CT band. The appearance of color and exhibition of CT bands are attributed to the formation of charge transfer complexes between the drugs and TCNE since these absorption bands are uncharacteristic of the individual components.

The position of CT band (λₘₚₐₓ) of the drugs with TCNE is in the order: Terazosin > Astemizole > Ketoconozole > Pantoprazole > Irbesartan > Ramipril. The trends have been explained in terms of structures of the drugs. The ionization potentials of the donors have been calculated using the equation

\[ hν_{CT} = 0.82I_d - 4.28 \]
where $\nu_{CT}$ is the frequency of the CT band, $I_d$ is ionization potential of donor and $h$ is Planck's constant.

The stoichiometry of the complexes, Extinction co-efficients ($\varepsilon$), Oscillatory Strength ($f$) and Transition Dipolemoments ($D$) have been determined from the appropriate methods and equations. Stability constants of the complexes were determined from variation of absorbance with the concentration of drug at a fixed concentration of acceptor while the thermodynamic parameters from the temperature dependence of stability constants.

**b) Molecular Complexes of Paraquat (PQ) :** The colourless aqueous solution of PQ when mixed with 2,4,6-Trimethylphenol, 4-Benzylxyphenol, 4-Cyanophenol and 2-Cyanophenol in alkaline medium produced characteristic colors. The PQ with methanolic solution of Salmeterol, Diclofenac Na, Omeprazole, Esomeprazole Mg and Pantoprazole also produced characteristic colour changes indicating the formation of CT complexes. The formation of CT complexes of the former set of drugs is between PQ and anions of drug intermediates whereas the formation of CT complexes of latter set of drugs is between the PQ and neutral drug molecules. All the complexes exhibit one CT band each in the region where neither of the components have any absorption. The color changes observed and appearance of CT bands in their electronic spectra are attributed to the excitation of electron from the HOMO of donor to the LUMO of PQ.

The positions of CT bands of drugs with PQ are in the order: 2,4,6-Trimethylphenol > 4-Benzylxyphenol > Salmeterol > 4-Cyanophenol > 2-Cyanophenol > Diclofenac Na > Omeprazole > Esomeprazole Mg > Pantoprazole. The trends have been explained in terms of structures of the intermediates (former set) and the drugs (latter set).

The ionization potentials of the donors have been calculated using the equations

\[
h\nu_{CT} = 0.976I_d - 4.50 \quad \text{(for aqueous solutions)}
\]
\[
h\nu_{CT} = 0.90I_d - 4.19 \quad \text{(for methanolic solutions)}
\]

where $\nu_{CT}$ is the frequency of the CT band, $I_d$ is ionization potential of donor and $h$ is Planck's constant.

The stoichiometry of the complexes, Extinction co-efficients ($\varepsilon$), Oscillatory Strength ($f$) and Transition Dipolemoments ($D$) have been determined from the appropriate methods and equations as mentioned in molecular complexes of TCNE. Stability constants of the complexes were determined from variation of absorbance with the concentration of drug at a fixed concentration of acceptor while the thermodynamic parameters from the temperature dependence of stability constants.
PART–II  ION-PAIR CT COMPLEXES OF DRUGS WITH ACIDIC TRIPHENYLMETHANE DYES

1. Introduction

The most common pharmaceutical analysis is the quantitative measurement of the active ingredient and related compounds in the pharmaceutical product. These determinations require the highest accuracy, precision, and reliability because of the intended use of the data: manufacturing control, stability evaluation. Determination of drugs and their metabolites in biological samples, generally plasma or urine, is important in elucidation of drug metabolism pathways as well as comparing bioavailability of different formulations. Pharmaceutical analysis is an important subject for the quality control of raw materials, drugs, food stuffs and pharmaceuticals. This is due to the fact that drug, pharmaceutical and food stuffs of maximum purity are essential for the safeguard of the health of human beings. The purity and stability of the drugs are checked with the help of various physical, chemical and instrumental technologies. These techniques have been developed / modified day by day with the aim to increase the selectivity, sensitivity and accuracy of the method. The new techniques have replaced the old ones in the various pharmacopoeias.

Methods used for analysis of drugs

Various physical, chemical and instrumental methods used for quantitative determination of drugs have been enumerated and discussed briefly with suitable examples and references. The methods include: Titrimetry, Potentiometry, Conductometry, Chromatography, Mass spectrometry, Colorimetry, Fluorimetry and Spectrophotometry. Extractive spectrophotometry is applied for the quantification of selected drugs.

Overview on the validation of a method for quantification

The parameters for method validation have been defined in different working groups of national and international committees and are described in the literature. An attempt at harmonization was made for pharmaceutical applications through the International Conference on Harmonization (ICH) where representatives from the industry and regulatory agencies from the United States, Europe and Japan defined parameters, requirements and, to some extent, methodology for analytical methods validation. The parameters, as defined by the ICH and by other organizations and authors, are summarized in and are described in brief in the following paragraphs.
Possible analytical parameters for method validation

**Specificity:** The term specificity generally refers to a method that produces a response for a single analyte only.

**Selectivity:** The USP monograph defines the selectivity of an analytical method as its ability to measure accurately an analyte in the presence of interference, such as synthetic precursors, excipients, enantiomers and known (or likely) degradation products that may be expected to be present in the sample matrix.

**Precision:** The precision of a method is the extent to which the individual test results of multiple injections of a series of standards agree. Precision can be subdivided into 3 categories: repeatability, intermediate precision and reproducibility.

**Accuracy:** The accuracy of an analytical method is the extent to which test results generated by the method and the true value agree. Accuracy can also be described as the closeness of agreement between the value that is adopted, either as a conventional, true or accepted reference value, and the value found. The true value for accuracy assessment can be obtained in several ways. One alternative is to compare the results of the method with results from an established reference method.

**Linearity:** The linearity of an analytical method is its ability to elicit test results that are directly proportional to the concentration of analytes in samples within a given range or proportional by means of well-defined mathematical transformations.

**Limit of detection:** The limit of detection is the point at which a measured value is larger than the uncertainty associated with it. It is the lowest concentration of analyte in a sample that can be detected but not necessarily quantified. It is calculated from the residual intercept of calibration graphs.

\[
\text{LOD} = 3.3s/S \quad \text{where } s \text{ is the residual intercept of the calibration graphs and } S \text{ is the slope of the calibration graph.}
\]

**Limit of quantification:** It is calculated from the residual intercept of calibration graphs.

\[
\text{LOQ} = 10s/S \quad \text{where } s \text{ is the residual intercept of the calibration graphs and } S \text{ is the slope of the calibration graph.}
\]

**Robustness:** Robustness tests examine the effect that operational parameters have on the analysis results. For the determination of a method’s robustness, a number of method parameters, for example, pH, temperature, reagent volume, detection wavelength are varied within a realistic range, and the quantitative...
influence of the variables is determined. If the influence of the parameter is within a previously specified tolerance, the parameter is said to be within the method’s robustness range.

2.0 experimental

i) Instruments

The spectra of ion-pair complexes have been recorded on SHIMADZU 140 double beam spectrophotometer, Thermo Nicolet 1000 and also on ELICO 159 UV-Visible single beam spectrophotometer using quartz cells of 10 mm path length. An Elico model Li-120 pH meter was used for pH measurement.

ii) Materials

HPLC grade chloroform, Analytical grade (AR) dyes viz., a)BTB b) BPB c)BCG d) BCP e)Tropaeolin OO, and AR grade HCl, Sodium acetate KMnO₄ sulphuric acid and sodium hydroxide supplied by Sd Fine Chemicals, Mumbai were used in the study. Iodine (BDH, Poole, UK) was twice sublimed and preserved in vacuum desiccator (mp 113.6 °C).

The drugs analysed were procured from Dr.Reddy’s laboratories, Hetero drugs private limited, Symed laboratories Ltd. as gift samples.

iii) Methods

Different aliquots of drug solutions were added to a fixed volume of the solution of analytical reagent. The interaction / reaction product showed absorption band uncharacteristic of either drug or the reagent. The absorbance of the band varied linearly with the concentration of the drug and formed basis for the determination of the drug. The parameters like concentration of analytical reagent, pH, polarity of solvent and other parameters which can influence the absorbance have been optimised. Each of the method has been validated as per the guidelines of ICH. The stoichiometry of the interaction have been determined by Job’s continuous variation method while that of reactions by limiting logarithm method.

3. Results & discussion

1. Quantification of Ambroxol hydrochloride

Three simple and sensitive extractive spectrophotometric methods have been described for the assay of Ambroxol hydrochloride either in pure form or in pharmaceutical formulations. The developed methods involve formation of coloured chloroform extractable ion-pair complexes of the drug with bromothymol blue (BTB), bromophenol blue (BPB) and
bromocresol purple (BCP) in acidic medium. The extracted complexes showed absorbance maxima at 414, 412 and 407 nm with use of the cited reagents, respectively. Beer’s law is obeyed in the concentration ranges 2.5-25, 4.0-30 and 4.0-40 µg/ml with BTB, BPB and BCP respectively.

2. Quantification of Rasagiline mesylate

Three simple and sensitive extractive spectrophotometric methods have been described for the assay of Rasagiline mesylate either in pure form or in pharmaceutical formulations. The developed methods involve formation of coloured chloroform extractable ion-pair complexes of the drug with bromothymol blue (BTB), bromophenol blue (BPB) and bromocresol green (BCG) in acidic medium. The extracted complexes showed absorbance maxima at 414 nm for all three methods. Beer’s law is obeyed in the concentration ranges 3.0-30, 3.0-0-30 and 2.0-25 µg/ml with BTB, BPB and BCG respectively.

3. Quantification of Sibutramine hydrochloride

Three simple and sensitive extractive spectrophotometric methods have been described for the assay of sibutramine hydrochloride either in pure form or in pharmaceutical formulations. The developed methods involve formation of coloured chloroform extractable ion-pair complexes of the drug with bromothymol blue (BTB), bromophenol blue (BPB) and bromocresol green (BCG) in acidic medium. The extracted complexes showed absorbance maxima at 415 nm for all three methods. Beer’s law is obeyed in the concentration ranges 2.0-25, 2.0-20 and 2.5-25 µg/mL with BTB, BPB and BCG, respectively.

4. Quantification of Ondansetron hydrochloride

Three simple and sensitive extractive spectrophotometric methods have been described for the assay of ondansetron hydrochloride either in pure form or in pharmaceutical formulations. The developed methods involve formation of coloured chloroform extractable ion-pair complexes of the drug with bromothymol blue (BTB), bromophenol blue (BPB) and bromocresol green (BCG) in acidic medium. The extracted complexes showed absorbance maxima at 419 nm for all three methods. Beer’s law is obeyed in the concentration ranges 2.5-25, 2.5-0-25 and 3.5-22.5 µg/ml with BTB, BPB and BCG respectively.

The effect of concentration of dyes and pH have been studied and optimized. The limits of detection and quantification have been determined for all the methods. All the methods have been validated as per the guidelines of ICH. The methods have been applied to the determination of drug in commercial tablets and results of analysis were validated statistically through recovery studies.
Executive Summary of the Report
Major Research Project in Chemistry
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