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REVIEW ARTICLE

Application of Analytical Techniques in Preformulation Study: A Review

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ABSTRACT

Analytical techniques are fundamental to Preformulation Studies. Without them, no evaluation of the quality of materials, product precursors, or final product can be made. No biological or pharmacologic responses in the preclinical or clinical stages can be measured. Selection of analytical techniques should be based on the elements of the study; specificity, accuracy, precision, sensitivity, and speed of a test must be justified for the method selected. To generate Preformulation data analytical techniques like Spectroscopic, Chromatographic, Thermal methods and some specific detection methods like Capillary electrophoresis are used. The present article is framed with the objective to provide an in-depth insight the application of Analytical Techniques in Preformulation Study.

Key word: Preformulation Study, Thermal Methods, Capillary electrophoresis, Precursors

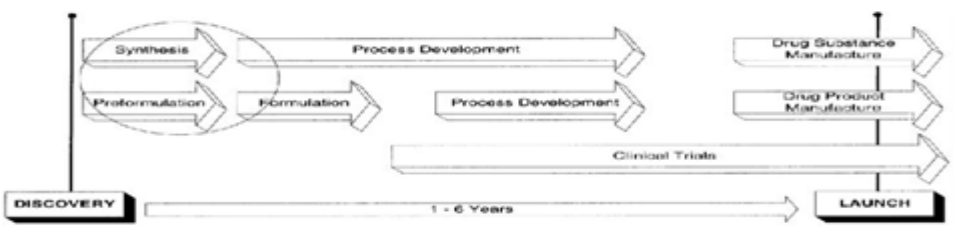
INTRODUCTION

Preformulation studies have been developed for supporting the dosage form design of a new drug and its quality control. Preformulation studies gained momentum in the 1950s and imposed scientific principles and rationale on formulation development to minimize trial-and-error efforts. Such a study is built on knowledge of physical pharmacy, the study of physical and chemical principles of pharmaceutical science and biopharmaceutics, the study of the influence of formulation on the therapeutic availability of a drug product. Form selection is commonly considered among the primary goals of a preformulation study. However, the investigative techniques discussed herein also have application in early drug substance and drug product development activities [1] shown by the circled area in (**Fig 1**).

A typical development track activity for preformulation monitoring may be divided into several phases,

1. Selection of a Drug Substance for Dosage Form Development
   a. Structure Modifications
   b. Purity
   c. Chirality
   d. Salt Forms Selection
   e. Prodrugs
   f. Metabolites
2. Intellectual Property Protection and Patent Filing.
3. Selection of Analytical Technique and Development.
4. Preparation and Submission of IND.
5. Clinical Trial Studies.
6. Development and Manufacturing of Dosage Forms.
7. Establishment of a QA/QC System.
8. Preparation of a New Drug Application.
9. Abbreviated New Drug Application.

Fig. 1 The drug development process [2].



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PREFORMULATION STUDIES

Preformulation is the study of the chemical and physical properties of the drug components prior to the compounding process of the formulation. The purpose of the study is to understand the nature and characteristics of each component and to optimize conditions of the dosage form manufacture. Before formulation development, preformulation data must be generated to aid the development process and the physicochemical properties must be defined.

A. Stages of Preformulation Studies [3]:

Timely preformulation data availability is critical because it is an essential prerequisite of development. Physical properties, such as melting point, ultraviolet spectrum, and thin-layer chromatography (TLC) from preformulation are essential for the preliminary specification. The preformulation is performed in several stages with different development cycles, which are discussed in the following.

1: Physicochemical Properties and Analytical Testing for Drugs

The data consist of physicochemical properties of the chemical substance and analytical properties useful in the development of analytical methods, the evaluation of material quality, and testing for the acceptance of the formulation developed. The portion of this report consisting of analytical data may be known as an "analytical profile".

2: Data Supporting the Development of Dosage Forms

Before formulation development stability, incompatibility, and solid-state characteristics of a drug must be studied to support product development and improvement. The selection of the appropriate methods for dosage form evaluation may also be considered as part of the preformulation studies. The evaluation of the dosage form is based on testing: pharmaceutical testing (friability, hardness, disintegration, and dissolution, etc.), bioburden testing (microbiology, etc.), and bioavailability studies.

3: Support for Quality Control and Finished Product Manufacturing

Analytical methods of the interim developed product, and issues regarding difficulty of QA/QC may be included in Part 3 of the preformulation report. It must be published before the marketed product is finalized in "Biobatch," a scale-up production of 10% of a manufacturing lot.

B. Preformulation drug characterization [4]

Before beginning the formal preformulation programs the preformulation scientist must consider the following factors:-

- The amount of drug available.
- The physicochemical properties of the drug already known.
- Therapeutic category and anticipated dose of compound.
- The nature of information, a formulation should have or would like to have.

Characterization of drug molecules is very important step at the preformulation phase of product development. Following studies are conducted as basic preformulation studies; special studies are conducted depending on the type of dosage form and the type of drug molecules.

1) Solubility determination
2) pKa determination
3) Partition co-efficient
4) Crystal properties and polymorphism
5) Practical size, shape and surface area.
6) Chemical stability profile.

Table 1: Preformulation drug characterization in a structured program

Test	Method/ function Characterization
Fundamental	
1) UV spectroscopy	Simple assay
2) Solubility	Phase solubility/ purity
a) Aqueous	Intrinsic & pH effect
b) pKa	solubility control , salt formation
c) Salt	Solubility, hygroscopicity & stability
d)Solvents	Vehicles & Extraction
e) $k_{o/w}$	Lipophillicity, structure activity
f) Dissolution	Biopharmacy
3) Melting point	DSC-polymorphism hydrate & solvent
4) Assay development	UV, HPLC, TLC
5) Stability	
In Solution	Thermal, hydrolysis, pH
In solid state	Oxidation, proteolysis metal ion
Derived	
6) Microscopy	Particle size and morphology

7) Bulk density	Tablet and capsule formation
8) Flow properties	Tablet and capsule formation
9) Compression properties	Acid / excipient choice
10) Excipient compatibility	Preliminary screen by DSC, Conformation by TLC

ANALYTICAL TECHNIQUES

For Preformulation Studies Analytical techniques divided into three types of Methods

A. Spectroscopic and specific detection Methods.
B. Separation Methods.
C. Thermal Analytical Methods.

A. Spectroscopic and specific detection Methods

The need for identification and structure elucidation for newly discovered compounds drives the progress of specific detection techniques with NMR and X-ray diffraction and MS. The detection of foreign metal contaminants is essential with inductively coupled plasma spectroscopy (ICP), atomic absorption (AA), and X-ray fluorescence. The analytical techniques commonly used in the preformulation study are discussed in the following.

1. UV Spectroscopy

UV absorption is an essential tool for qualitative and quantitative determination of a single component drug or isolated extract. In a preformulation study, solubility, dissolution rate, and some stability studies (when degradation products have a different absorption maximum from the parent compound) are performed with the UV technique. UV is extensively used for HPLC detection. Most of drugs have aromatic rings and/or double bonds as part of their structure and absorb light in UV range, UV spectroscopy being a fairly accurate and simple method is a performed estimation technique at early preformulation stages. The absorption Co-efficient of the drug can be determined by the formula:-

$E = AF / X$

Where, A = Asborbance
F= dilution factor
X = weight of drug (mg)

It is now possible to determine concentration of drug in any solution by measuring absorbance.

$C = AF / E$ mg/ ml

2. Visible Photometry and Colorimetry

Visible spectrometry is identical to UV spectrometry, with the exception of the wavelengths, which are 400–750 μm in visible spectrometry. A color product may be formed with a specific agent as a result of chemical reaction. Quantitative determination of the colored compound is based on this principle for drug assay. Another method of forming a color compound (subsequently separated by extraction) is the dye–salt method. In an ion-pair reaction forming a color complex in reaction to the drug with a dye of opposite polarity such as bromthymol blue, the complex is extracted into the organic layer and determined colorimetrically.

3. IR Spectroscopy

IR spectroscopy is used extensively in pharmaceutical analysis for fingerprint identification of a drug molecule and the proof of its structure. Infrared absorption spectroscopy, especially when measured by means of the Fourier transform method (FTIR), is a powerful technique for the physical characterization of pharmaceutical solids. In preformulation, IR may be applied to the study of polymorphism of solid crystals. Polymorphs pose different IR characteristics, and they may be used as a tool for fingerprint identification. In addition, solid-state vibrational spectra can be very useful in studies of the solvation phenomena associated with a solvatomorphic system [5].

Acquisition of solid-state FTIR spectra suitable for use in the characterization of different crystal forms can be performed using Nujol mull, diffuse reflectance, or (most preferably) attenuated total reflectance (ATR) techniques. Any use of pelleting techniques is to be strictly avoided, since too many complications and spurious effects can arise with compaction of the KBr pellet, and these can limit the utility of the spectroscopic method. The main drawback to the mull technique is that regions in the IR spectrum overlapping with carbon–hydrogen vibrational modes will be obliterated owing to absorbance from the oil.

4. Raman Spectroscopy

Another technique of vibrational spectroscopy that is ideally suited for the characterization of polymorphism or solvatomorphism in solids is Raman spectroscopy. In this methodology, the sample is irradiated with monochromatic laser radiation, and the inelastic scattering of the source energy is used to obtain a vibrational spectrum of the analyte. Since most compounds of pharmaceutical interest are of low symmetry, the Raman spectrum will contain spectra features at the same energies as those obtained using the FTIR method [6]. In general, symmetric vibrations and nonpolar groups yield the most intense Raman scattering bands, while antisymmetric vibrations and polar groups yield the most intense infrared

absorption bands. These differences can, at times, be quite profound and can therefore be successfully exploited in the characterization of solid materials.

Raman spectroscopy is a nondestructive tool and requires little or no sample preparation. A sample may be analyzed in solid or powder form or in an aqueous solution and placed in glass containers such as an NMR tube, GC vial, test tube, light-path cell, or glass bottle. Aside from structure elucidation and functional group analysis, FT-Raman may be used for quantitative determination of polymorphs in a preformulation study [7].

5. NIR Spectroscopy

The absorption bands found in the near-infrared (NIR) region of the spectrum (typically considered to cover 1000–2500 nm) are all due to overtones and combinations of fundamental molecular vibrational modes. The energies of the overtone bands are more affected by environmental details than are the energies of their fundamentals, so slight perturbations in the bonding can yield drastic frequency and amplitude changes in the NIR. The advantage of this technique is the rapidity of analytical determinations without sample preparation and the use of solvent. The application of NIR in the pharmaceutical industry can be qualitative or quantitative. Materials such as active drug substances, organic liquids and solvents, excipients, and packaging materials can be tested rapidly for identity in the receiving area [8]. The use of NIR for quantitative determination includes moisture determination for the drying process, assay of dosage form, and content uniformity, as well as dissolution rate monitoring. Since NIR spectra consist of overtone transitions of fundamental vibrational modes, they are not terribly useful for identity purposes without the use of multicomponent analysis and access to spectral libraries of known materials [9].

6. X-Ray Diffraction

The X-ray diffractometry technique obtains information on substance structure at the atomic level. This technique allows measurement of both crystalline and noncrystalline materials. The analysis is nondestructive in nature and handles samples in the form of powders, solids, and liquids. Powder diffraction is used for fingerprint purposes. Polymorphism may be identified by diffraction patterns with d-spacing that has broader and overlapping peaks. Quantitative ratios of two polymorphs and their percentage of crystallinity may also be determined. Besides the identification methods, other applications of X-ray powder diffraction methodology include the evaluation of polymorphism and solvatomorphism, the study of phase transitions, and evaluation of degrees of crystallinity. A very useful complement to ordinary PXRD is variable temperature XRD. In this method, the sample is contained on a stage that can be heated to any desired temperature. The method is extremely useful for the study of thermally induced phenomena and can be a vital complement to thermal methods of analysis [10,11].

XRPD has become exceedingly important to pharmaceutics because it represents the primary method whereby one can obtain fundamental structural information on the structure of a crystalline substance. The technique is ideally suited for the study of large numbers of polycrystalline samples and has found widespread use in the evaluation of crystal structures, comparison of polymorphism and solvate structures, evaluation of degrees of crystallinity, and the study of phase transitions [12]. When the phase identity, or degree of crystallinity, of a drug substance is important to its performance in a drug product, PXRD can serve as a vital stability-indicating method. For example, amorphous clarithromycin was prepared by grinding and spray-drying processes, and PXRD was used to follow changes in crystallinity upon exposure to elevated temperature and relative humidity [13].

7. NMR Spectroscopy

After X-ray crystallography, solid-state nuclear magnetic resonance spectroscopy can be considered as being the most powerful molecular level characterization technique for a pharmaceutical solid, since this spectroscopic method yields information regarding the individual chemical environments of each atom in the compound under study. NMR involves the absorption of electromagnetic radiation in the radiofrequency of a longer wavelength spectrum. The nuclei shift from the preferred orientation with lowest energy to a less preferred, high-energy orientation at a particular frequency. Thus a plot of frequency versus intensity of radiation results in the NMR spectrum of a material. The major application of broadline NMR is in the measurement of the internuclear distances and other crystal parameters important in the study of polymorphism as well as hydrates and solvates [14]. In addition to qualitative investigation of polymorphs and solvates, the quantitative measurement of polymorphs is also possible. In NMR analysis with liquids, the sample is commonly dissolved in deuterated solvents (such

as chloroform-d, benzene-d, or D2O) and fills a sample tube. The liquid technique is widely used for structure elucidation to provide detailed information on the presence or absence of certain magnetic nuclei in different functional groups, along with structural and geometric relationships among the magnetic nuclei but powder samples are suitable for generating a spectrum to illustrate the crystal structure by the solid NMR technique [15].

8. Metal Analysis

The methods of metal analysis of pharmaceuticals include X-ray fluorescence spectroscopy, AA spectroscopy, and ICP. High-sensitivity methods and techniques for metal analysis are essential for quality control. The classical method of detecting metal contamination is the heavy metal testing described in the USP [16].

a) X-Ray Fluorescence

When a beam of high-intensity X-rays strikes a sample, the elements in the sample are excited and emit their own characteristic X-rays. Powder samples, solutions, or liquids can be placed in a sample cup wrapped with Mylar film that is transparent to X-rays. This method is nondestructive and can be an automatic operation.

b) Atomic Absorption

In AA, the sample in solution is atomized in a flame, producing atomic vapor with elements from the solution. A monochromatic light source with a hollow cathode tube containing the element of interest emits light at the same wavelength as the element of interest passing through the atomic vapor sample in the flame. The amount of radiation absorbed is proportional to the concentration of the elements in the solution.

c) ICP-AES/ICP-MS

In ICP-AES the sample introduced in the form of aerosol by the nebulizer is instantaneously decomposed in the plasma (plasma temperature 6,000–10,000 K) to form analyte atoms that are simultaneously ionized. The ions produced are extracted from the plasma into the atomic emission spectrometer For ICP with a mass spectrometer (ICP-MS), ions are transferred to a high vacuum in an MS. and the analyte ions are then focused by a series of ion lenses into a mass analyzer. The analyzer separates the ions based on their mass/charge ratio. Finally, the ions are measured with an electron multiplier and collected by a counter for each mass number. In the mass spectrum, each elemental isotope appears at a different mass, with peak intensity directly proportional to the initial concentration in the sample solution isotope.

B. Separation Sciences

The range of analytical methodology suitable for the evaluation of chemical compatibility between a drug substance and proposed excipients is extremely large, and methods can range from the relatively simple to the extremely complex. The most frequently used methods for obtaining chemical composition information in the preformulation stage of development are based on various types of separation science, such as thin-layer chromatography (TLC) or high-pressure liquid chromatography (HPLC), with the occasional use of gas chromatography (GC). The latter two methods are often coupled with mass spectrometry (MS) when the identity of degradant species is required. Separation techniques such as counter current extraction (CCE), and capillary electrophoresis (CE) are extensively employed in preformulation studies [17].

1. Thin-Layer Chromatography

TLC is a separation technique characterized by high sensitivity and multiple detection, but its use has gone somewhat out of vogue owing to the development of newer instrumental methods. Nevertheless, TLC still can play an important role in preformulation characterization studies and has undergone a steady evolution in technology and capability over the years [18]. The general detection technique is to spray a sample with a detecting agent, which reacts chemically with the ingredient to be detected, so that a visible spot develops. Detection by visual observation under short- or long-wave UV light is employed. TLC can be used as a separation method to obtain impurities from dosage forms in a state suitable for further analysis [19]. The disadvantages of TLC include reproducibility, detection inconsistency, person-to-person variations, documentation, and electronic data reduction [20]. The modern practice of TLC is now distinguished as high-performance TLC (HPTLC) to eliminate these disadvantages of TLC.

2. High-Pressure Liquid Chromatography

HPLC methodology is unique in that the analytical separation step is coupled with on-line analysis instrumentation that senses all analytes as they elute out of the chromatographic system. The UV detector coupling with HPLC equipment is the most important analytical instrument for preformulation, QC/QA, and in-process control in pharmaceutical analysis [21]. HPLC is a basic and reliable analytical tool for preformulation study because of the high-resolution capacity, accuracy, and reproducibility of the equipment. Its primary function includes search for and detection of

impurities in drug substances, as well as stability evaluation of dosage forms in terms of detection and quantitation of degradation products [22]. The utility of HPLC analysis in a program of preformulation testing was demonstrated for a number of compounds, including fosinopril sodium, ceronapril, pravastatin sodium, sorivudine, and ifetroban sodium. A reversed-phase method for the determination of nicotine in immediate- and extended-release formulations has been reported that also was used in the analysis of drug–excipient compatibility samples [23].

3. LC/MS

The first HPLC methods are usually developed during the preformulation stage of development; the combination of this technology with MS probably represents the ideal combination of technologies for the detection and identification of drug–excipient interaction products. In usual practice, one must vaporize the analytes, convert these into charged species, allow the ions to undergo fragmentation, and finally separate and detect the ion fragments on the basis of their mass-to-charge (m/e) ratio. The m/e value of the molecular ion confirms the formula weight of the compound, while the structures of the various fragments are consistent with the structure of the compound [24]. The key element in developing an HPLC-MS method is that all components must be volatile and capable of carrying the analytes into the vapor phase.

4. Capillary Electrophoresis

Capillary electrophoresis (CE) has been widely used in physicochemical profiling and pharmaceutical analysis. Capillary electrophoresis (CE) is a simple, versatile, automated, and powerful separation technique and widely applied in physicochemical profiling for pharmaceuticals such as acid dissociation constant (p*K*a), octanol-water partition coefficient (logPow). The p*K*a determination of acids and bases by CE is based on measuring the electrophoretic mobility of charged species associated with the acid-base equilibria as a function of pH. A number of direct and indirect methods have been applied for log *P*ow measurement. Conventional shake-flask method was historically considered to be the standard assay for direct measurements of log *P*ow[25].

Micellar electrokinetic chromatography (MEKC) is an analytical technique with combined features of conventional chromatography and capillary electrophoresis, which enables the separation of neutral and charged analytes. An anionic surfactant, sodium dodecyl sulfate, is commonly used as a micellar agent. In addition, cyclodextrin, a chiral selector, is added to the system, which contains three phases: aqueous, micelle, and cyclodextrin. Detection is accomplished with UV light, a diode array, laser-induced fluorescence, or a mass spectrometer.

C. Thermal Analytical Methods

Thermal analysis and calorimetric methods have demonstrated a wide array of applications in the preformulation, and formulation development (Table 2). Thermal analysis and calorimetric techniques permit rapid characterization with small drug substance requirements. These techniques are critical in physical–chemical screening of early discovery leads, during salt form screening, and in the characterization of polymorphs to determine the thermodynamic relationships between the various crystal forms.

ROLE OF THERMAL ANALYTICAL METHODS IN PREFORMULATION STUDY [26]

1) They are unique methods in the field of polymer analysis & of high value for a solid state analysis.
2) They finds wide application in
   A) Detection of impurity
   B) Determination of moisture content in any drug substance or any excipient
   C) Study of polymorphism
   D) Characterization of hydrates & solvates
   E) Degree of Crystallinity
   F) Study of phase diagram
   G) Drug excipient compatibility study
   H) Study of complexation

1. Differential Scanning Calorimetry

Differential scanning calorimetry(DSC) is a widely used technique within the pharmaceutical industry because the range of phase transitions it can measure usually allows near complete physical characterization of a new active principal early during preformulation.

DSC technology is constantly evolving and improving and three recent derivatives have become popular[27]. These are:

- Temperature-modulated DSC
- High-sensitivity DSC
- Fast-scan DSC

Temperature-modulated DSC (TM-DSC) is particularly useful pharmaceutically for isolating and quantifying glass transitions while high-sensitivity DSC, (HS-DSC) was developed for

studying dilute solutions of macromolecules (usually biologicals). The main benefits of fast-scan DSC (FS-DSC) are simply to increase the size of the measured signal and to reduce the experimental time-frame. DSC techniques provide information regarding the melting point (temperature)/range, heat of fusion and crystallization, purity, polymorphism, pseudopolymorphism, glass transition, drug and excipient interaction/compatibility, thermal stability, etc. which is essential for preformulation studies of pharmaceuticals and the subsequent development of a stable and effective dosage form. The performance of DSC is dependent on a number of experimental factors. Some of the important factors to be considered are the sample size, the heating rate, the atmosphere, and crucible type.

2. Hot Stage Microscopy

Changes in thermal properties are observed through a microscope during the heating of a sample placed on a hot stage with a temperature-programming device. Melting point can be observed and the temperature at the time of the occurrence can be noted.

3. Thermal Gravimetric Analysis (TGA)

TGA may be used to determine moisture content related to weight loss in isothermal or nonisothermal stability studies. In the preformulation study, TGA is the appropriate technique for differentiation of polymorph from hydrate or identification of monohydrate from among other hydrates which may not be possible by DSC alone.

Table 2: Thermal Application for Preformulation Analysis

Thermal Methods	Measurement	Application
Differntial Scanning Calorimetry (DSC)	• Heat Flow/Heat Capacity • Energy of Transition as a function of temperature.	• Crystallanity • Polymorphism/Pseudopolumorphism • Glass Transition • Thermal Decomposition • Melting point • Drug-excipient compatibility
Thermogravimetric Analysis	• Weight changes as a function of temperature and/or Time.	• Characterization of solvates/hydrates • Loss on Drying • Decomposition • Sublimation
Modulated DSC	• Heat flow/heat Capacity as function of a sinusoidal temperature fluctuation.	• Glass Transition • Separation of reversible/nonreversible heat flow to deconvulate overlapping transition. • Measurement of relaxation enthalpy Stability
Thermomicroscopy (Hot Stage Microscopy)	• Photomicrography of a drug substance as a function of temperature.	• Melting point • Decomposition • Polymorphism • Crystallization • Desolvation
Isothermal Microcalorimetry	• Heat flows as a function of time/temperature with a high degree of sensitivity.	• Stability • Polymorphism • Characterization of Amorphous content
Solution Calorimetry	• Heat flows as a function of time/temperature	• Polymorphism • Amorphous content
Micro Thermal Analysis	• Surface Topography. • Heat flows as a function of temperature	• Melting point • Glass Transition • Amorphous Character in specific region of material surface
Thermo Mechanical Analysis	• Expansion Coefficient (Softening)	• Glass Transition
Dynamic mechanical Analysis	• Mechanical Strength/energy loss as a function of temperature.	• Glass Transition • Rheological properties

CONCLUSION

Preformulation Study is very important step for Drug Development Process. To Characterize the Physico-Chemical Properties of drug Substances different Analytical Techniques play very crucial role. Analytical Techniques specifically IR Spectroscopy, X-Ray Powder diffractrometry, HPLC, Capillary Electrophoresis (CE) and from Thermal Methods Differential Scanning Calorimetry (DSC) are very essential for Analytical Profiling of New drug Substance.

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