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Chapter

Preformulation Studies: A Versatile Tool in Formulation Design

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Abstract

The physicochemical properties of pharmacological molecules have a tremendous effect on safety and efficacy. Poor physicochemical properties can often make it hard to set up a reliable structure-activity relationship (SAR) with no prominent efficacy in preclinical and clinical models. This can lead to more variability in capability and higher drug development costs in the entire development process, and in the worst case, even to stop the clinical trials in the later period. Understanding the basic physicochemical properties makes it possible to separate and untangle investigational observations hence poor molecular properties can be changed or fixed during the design phase. This makes it more likely that the molecule will make it through the long and difficult development process. The decline in innovator pharmacotherapeutics number registrations decline each year and the industry is under even more pressure than in the past to speed up the drug development process. This reduces the length of time required for development and introduces innovative pharmaceutical products. To do this, it is imperative to proceed with an organised approach and act appropriately the first time. The current chapter aims to focus on the important physicochemical properties of the selected molecule, along with how those properties are evaluated and implicated in both discovery enablement and final dosage form development.

Keywords: conventional drug delivery, novel drug delivery, new chemical entities, generics, biopharmaceutical

1. Introduction

Preformulation studies are synonymously known as "Learning before doing". The Preformulation concept emerged between 1950 and 1960 and since then the pharmaceutical product development emphasis has transformed. Therefore, preformulation studies are required for the early step of data collecting relating to the physicochemical properties of the therapeutic molecule, assessing possible salts and excipient appropriateness. Hence, preformulation is the interface between new chemical entities (NCE) and the formulation development phase therefore it is the sole study to provide a complete pathway of drug formulation development [1]. These studies are most and much important before formulation development and they give us an idea about the stages of drug formulation development related

to the physicochemical properties of new chemical entities (NCEs) or any drug substances [2–4]. During drug development stages, the physical and chemical properties are categorised and standardised to establish the optimum mark for formulation development and ensure that these properties are helpful for formulation development [5, 6]. Key preformulation factors are thermal particle size, shape, dissociation and partition coefficient, drug/API stability, absorption behaviour, and solid-state characters like polymorphism. Furthermore, the structural, degradable, biophysical, and physicochemical characteristics of the macromolecules are also evaluated at the preformulation stage [3, 7].

Characterisation of the drug at the initial stage is the most important step for an early stage of drug product development to have the understanding and behaviour of drug molecules at the entry level in the cycle of drug development (**Figure 1**). Hence, the preformulation studies are the optimum available tool for API and drug product development. It comprises a set of physicochemical parameters and biopharmaceutical principles to design and develop appropriate drug delivery systems [8, 9]. Therefore, the experimental confirmation of the interaction of drug molecule and excipients are well studied at the preformulation level hence it gives an idea about their interaction and adduct formulation results in intelligent selection of drug molecules and excipients. The primary drug degradation profile is also studied to guide the formulation stability and storage conditions for final dosage forms leads the quick development of desired dosage forms [10].

Post-drug discovery with strong basic knowledge of the physicochemical behaviour of candidate molecules, solid state, and relevant powder characteristics of drug molecules are required to develop the most appropriate intended form of formulation to be administered to humans. Each drug molecule proceeds through various physical and chemical checkpoints before being developed into a final dosage form. Furthermore, these properties are helpful to get information about the combination of drug molecules and ingredients. Preformulation studies were performed for NCEs or any extracted compounds to get information about toxicity, pharmacokinetics, drug distribution, degradation process, adverse conditions, and finally bioavailability [11].

Deep understanding and thorough information of physicochemical and related biopharmaceutical properties of the drug direct scientists to design optimum and appropriate formulation delivery methods to get strong proof of concept (POC) and

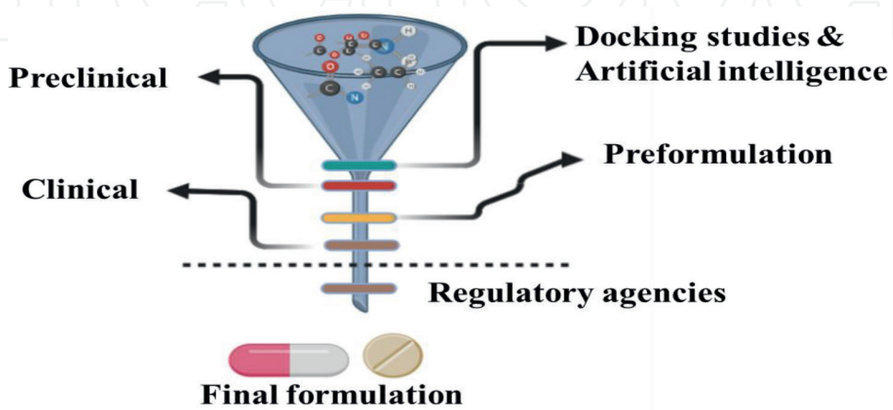

Figure 1.
Stages of preformulation studies.

pre-clinical studies to have a clear understanding of active pharmaceutical ingredients (API) characters and relevant additives that might affect the final formulation and drug performance [5].

Preformulation studies performed post-scientific literature survey of some type of drug molecules to recognise the following parameters [2, 12–14]:

- Chemistry of API, the synthetic pathway of API, drug product manufacturing processes.
- Degradation process and metabolites.
- Development of analytical methods.
- Distribution kinetics and bioavailability of same kind molecules.
- Toxicity profile of candidate drug.
- Preformulation influences, drug candidate selection, formulation adjuvants.
- Optimum storage containers and conditions selection.

Preformulation studies provide a path for formulation development and drug product development in respect of drug form, adjuvants, composition, physical structure, and chemistry of drug molecules, facilitating pharmacokinetic and biopharmaceutical properties evaluation, adjustments, and their implementation to get an appropriate end product, product process development support for Process Analytical Technology to get qualified products, harvest essential and suitable scientific facts for analytical method development and validation [15]. Preformulation research is performed to generate data related to biopharmaceutical, physicochemical, and physic-mechanical properties of molecules, and adjuvants (**Figure 2**) [16, 17]. These analytical investigations inspire formulation strategies and manufacturing methods for drug substances and drug products [18].

Various regulatory guidelines like the ICH and US-FDA support and recommend basic concepts for preformulation development. Preformulation studies are advised by NDA, IND, and ANDA and released by the ICH and the US FDA regulatory agencies. ICH guidelines state that all technical requirements for drug approval applications should be submitted in a standardised e-CTD format. This format, known as QOS-QbR, is even more comprehensive technically and is used by the US-FDA. Additionally, the Quality by Design method, which improves the effectiveness of the FDA review process, is the foundation of the QbR format.

There are various scientific and regulatory descriptions available for acquiring preformulation data that are helpful for the predetermination of the final dosage form. These are 1. Setting up drug product specifications for clinical supply preparations and toxicological testing. 2. Creating initial specifications for clinical supplies and their formulation. 3. Provide scientific evidence to support the creation of dosage forms and assessments of the effectiveness, quality, stability, and bioavailability of products. 4. Review of the stability of early dosage formulations produced. 5. Meeting the CMC section's requirements for the IND and any subsequent NDAs or ANDAs submissions [19, 20].

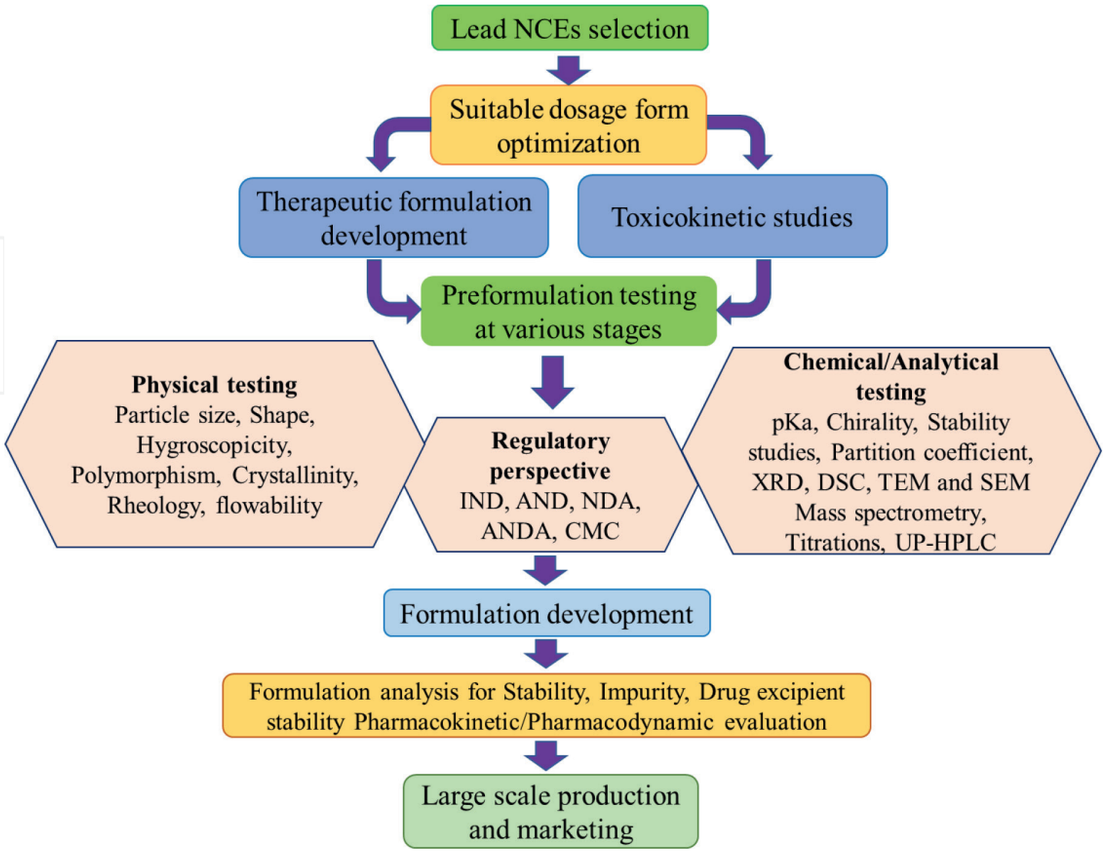

Figure 2.
Preformulation study schematic representation.

2. Overview of preformulation studies

2.1 Preformulation studies: an essential concept in formulation development

Preformulation is the vital concept for final formulation dosage form development intended to target disease as a result preformulation studies are carried out to generate usable data and uncover crucial information that innovators and industry professionals may utilise to produce dosage forms that are stable effective and safe for end users patients it is the main step toward the creation of final dosage form it encompasses comprehensive knowledge of physicochemical aspects of the drug and mixing with appropriate adjuvants to the final design of effective, safe and stable drug delivery system [21]. But it is noteworthy to have data evidence about the basic properties of the drug molecule, its stability information, pharmacokinetic data of lead drug molecules or the same kind of molecule available in the market and bioavailability profile, and feasible route of drug administration before preformulation studies [22]. Additionally, it includes the molecular optimization of the API to change its dissolution and solubility behaviour, for example, salt production techniques have been commonly used to boost solubility, its rate of dissolution (diclofenac sodium salt) and prodrug approach (Levodopa and Enalapril). Also, it determines the connection between physicochemical variables and the kinetic profiles of a novel drug moiety and investigation several aspects of an API's bulk, solubility, stability and compatibility of drug and excipients [23, 24].

Biotherapeutics (Vaccine, proteins and peptides preformulation studies) development into a drug candidate has many difficulties associated with a drug's clinical effectiveness in patients depending on the unique physicochemical and biological features of a given biotherapeutic molecule, including stability, viscosity, manufacture ability, bioavailability, and immunogenicity. To overcome these challenges before being developed a final dosage form is covered under preformulation studies for monoclonal antibodies, peptides, and proteins. The methods used to analyse the candidate macromolecules' primary, secondary, and tertiary structures, along with tests to determine the types and concentrations of contaminants, are the first steps in the process. Then, for the best possible potential to develop the intended biological products, the functions of the various compounds are evaluated using various screening procedures, along with research on their solubility and stability [25]. As a result, it will eventually open the door for the creation of dosage forms that are acceptable to patients and have high levels of stability, safety, efficacy, and are low-cost affordable dosage forms for the patients.

2.2 Pharmaceutical drug product life cycle

Nowadays the century of the internet and artificial intelligence have changed things very fast. For pharmaceutical industry considerations, drug discovery and data mining tools have made the industrial process quick to reach. This is a kind of opportunity used by the scientists working in various research and development teams of the pharmaceutical industry are working at foremost for generating the new drug concept and brand development for the industry [18]. Using modernised data science tools, the entire product life cycle is being transformed by this technology like discovery biology and medicine research and development, production, and validation. Industrial operations must show that the process is compliant with the specified, validated acceptable limits for both isolated process parameter control strategies and comprehensive production control strategies once the process has been validated. ICH recommendations specify in ICH-Q12 that technological and legal aspects of pharmaceutical product lifecycle management should be taken into account [26].

The key funding from product life cycle management:

- Provide comprehensive and detailed knowledge of the product to the company's product pipeline.
- Determine the product specifications continuously within the purview of the regulatory framework and know about product specifications.
- Determine batch variability by trend analysis resulting from differences in the raw materials and ageing of the equipment, and establish the proper preventive maintenance and corrective action and preventive action.
- Manage post-commercialization data related to regulatory approvals, change management, data publication from other sources and sales.

2.3 Preformulation: objectives

The preformulation tool can be viewed as a decisive tool for making decisions during the drug discovery and development phases. Thorough knowledge

of physicochemical characteristics and how they affect biological performance enables the choice of possible lead molecules and related drug delivery challenges detection [3, 27].

- To produce the helpful data required for the development of desired, convenient, stable, efficacious, and products produced at a large level.
- Establish a fine understanding of the physicochemical properties of new drug substances before being developed into a final dosage form.
- Physical characteristics establish the selection of the appropriate form of a drug substance.
- Selection of accurate excipients and additives which are compatible with drug substances.

2.4 Preformulation: goals

- The establishment of the physicochemical properties leads to a drug candidate.
- Establish pharmacokinetics and biodistribution profile of selected drug molecule.
- Establishment of drug molecule common excipients compatibility.
- Significant roadblocks to the development of successful pharmaceutical products

2.5 Preformulation study challenges and mitigation

There are various hurdles associated with successful drug product development. That's why a question that comes around scientists working in pharmaceutical product development is Why do 90% of drug development fail at the clinical stage, and what can be done to change that? [11]. The baseline difficulties related to drug candidate evaluation at the research and development level like market potency of a drug candidate, poor project plan, pre-submission challenges, drug development timelines, and cost of drug product development and thereafter the regulatory filing need to be investigated carefully [28]. Drug development starting from discovery is a long and costly process with high chances of failure. Generally, molecules failed to make a place in the market because they do not perform well in clinical phases II and III (40–50%), show unmanageable toxicity (30%), poor drug-like properties and strategic planning (10–15%), and fewer business opportunities (10%). Some of the drug discovery and developmental challenges are listed in **Table 1** [35].

3. Physical characteristics

3.1 Organoleptic properties

Organoleptic properties of new chemical entity preformulation start with a detailed description of drug substances' organoleptic properties including colour, odour, flavour, texture, and taste and these properties should be recorded at the

Discovery team	Developmental challenges	Strategies to overcome drug discovery challenges	Reference
Drug discovery	Poor drug-like properties	Use of "Lipinski rule of 5" for chemical structure design of candidate drug. Robust selection criteria with certain cut-off values for solubility, stability, permeability, protein binding, and in vitro permeability of more than 2–3 × 10^6 cm/s are favoured for oral drug absorption, and in vivo pharmacokinetic parameters.	[22]
Discovery unit	Poor drug potency and specificity	Apply SAR to get drugs with low IC50 (nmol/L) values for lower toxicity and improved effectiveness. STR-mediated structural modifications are useful for clinical efficacy, and safety dose selection in clinical studies.	[29]
Clinical	Lack of clinical efficacy	The top leading candidate for clinical trials is identified by the ratio of their IC50 value and kinase profile selectivity which is 10-fold higher for disease vs. other kinases.	[11]
Safety (clinical toxicity)	Off-target toxicity	Toxicogenomics as a tool for early toxicity determination	[30, 31]
Safety (clinical toxicity)	On-target toxicity	Inhibition of disease-related targets and required titration of dose, toxicogenomics used for early assessment of potential toxicity.	[32, 33]
Business development	Poor strategic planning	Industries have a multidisciplinary business development team which make a detailed roadmap and milestone for new compound development from laboratory to market authorization. Nowadays, Artificial Intelligence with the help of large data analytics enables pharma industries to quickly choose emerging disease areas, and new disease diagnoses cost-effectively.	[34]

Table 1.
List of drug product development challenges and strategies to overcome the challenges.

preformulation stage and also described using expressive terminology. These properties vary as vendor changes and hence they are evaluated with reference standards to confirm the API purity. Further, once these organoleptic properties are established, they may analyse for batch-to-batch consistency mentioned in **Table 2** [14, 20].

3.2 Bulk properties and characterisation: physical, analytical, and physicochemical

3.2.1 Solid-state properties

They include crystallisation, salt formation, polymorphisms, and solvates that profoundly impact solubility, stability, permeability, and finally bioavailability. These are the most crucial parameters of drugs that are necessary for the effective development of drug candidates for patients [36]. For example, powders are masses of solid particles encased in the air (or another fluid), these two systems are significantly bearing on the bulk properties of the powders. The fluid content and other variable parameters are associated with the powder formulations that may affect flow

Organoleptic properties	Characters	Challenges	Mitigations/acceptance
Colour	Off-white, cream yellow, tan, shiny, yellowish white	In the case of low-dose coloured API, maintaining colour homogeneity in the finished uncoated tablets is particularly challenging	Uncoated tablets need to be coated for colour uniformity
Odour	Pungent, sulphurous, fruity, aromatic, odourless	API with bad odour	Use a flavouring agent or capsules/coated tablets to mask the odour
Taste	Acidic, bitter, bland, intense, sweet, tasteless	API with unpalatable taste should not consider for mouth-dissolving or chewable tablets	Sweeteners or flavours are added to get taste-masking properties by covering the entire surface (capsule or coated tablet)

Table 2.
List of various organoleptic properties.

performance which is impacted by the physical features of particles like shape, size, size inconsistency, angularity, and rigidity. Some outside factors including humidity, aeration, vibration, and conveying environment intensify the problem [37].

3.2.2 Flow properties

The flow characteristics of powders are essential to successful tabletting operations. For effective mixing and tolerable weight consistency for the compressed tablets, an optimum flow of granules/powders is required. Suppose, at the preformulation stage a drug is categorised as "poor flowable" therefore the proper selection of excipients can resolve this problem. Precompression and granulation methods are utilised for powder drugs to improve their flow behaviour. A preformulation test of the granule mass for the measurement of flow properties is performed with the help of the angle of repose, flow through an orifice, Hausner ratio, bulk and tapped density, inter-particle porosity, Carr's index and ideal flowability. Generally, a uniform shape or large crystal displays a narrower angle of repose and a low Carr's index resulting from changes in particle size and shape [38].

The angle of repose can be understood as the maximum angle formed between the free-standing surface of the powder heap and the horizontal plane of the powder at the base. It may use to evaluate the interparticle force of powder particles and bulk characterisation of solids.

The range of angle of repose can vary from 0° to 90°, the angle of repose value below 25° represents excellent flow properties however, on the other if the angle of repose value ranges between 25° and 45°, the flow is considered poor, the values of angle of repose indicated in **Table 3**. The formula for the calculation of the angle of repose is as follows [39].

$$\mathrm{AOR} : \tan\theta^{-1}\left(2\,h / d\right) \tag{1}$$

h is heap height, and d is the horizontal base diameter.

Preformulation test name	Formula	Observations	Properties	Reference
The angle of repose (flow behaviour of granule)	AOR = $\text{Tan}\theta^{-1}(2\ h/d)$	<20	Excellent flow	[39]
		20–30	Free-flowing	
		30–34	Passable flow	
		>40	Poor flow	
Bulk density (individual particle arrangement)	Pb = M/Vo	> 0.5 g/ml (high value)	Limitation to flow	[39]
Tapped density (degree of powder packing, cohesiveness)	Pt = M/Vt	High density	Better flow	[40]
Inter-particle porosity (Ie)	Ie = {(Pb – Pt)/(Pb × Pt)}			[41]
Carr Index (CI%, strength, stability, and compressibility)	CI = ((Pb – Pt)/Pt)	<0.10	Excellent flow	[42]
		0.26–0.31	Poor flow	
Hausner ratio (HR, inter-particulate friction, and compressibility)	HR = Pt/Pb	1.00–1.11	Free-flowing	[43]
		1.35–1.45	Poor flow	

Vo: volume, M: known weight of the granule's mixture, Vt: tapped volume in measuring cylinder, Pb: bulk density, Pt: tapped density, AOR: angle of repose

Table 3.
Preformulation tests for measurement of granule flow properties.

3.2.3 Particle size distribution

The particle size of dosage form affects the physicochemical properties and biopharmaceutical behaviour of drug substances. Drug solubility is frequently inversely proportional to the particle size; for example, a smaller particle size dosage form has a large surface area, similarly surface area to volume ratio as well. A stronger contact between the surface area and the solvent increases the solubility. Particle size reduction methods like milling and grinding frequently subject the drug product to high levels of physical stress, which could lead to degradation [43]. Moreover, micronization is the conventional technique used to make particles smaller and increase the surface area of drugs thereby enhancing solubility and dissolution.

However, micronization milling techniques (rotor-stator colloid mill, and jet mill) are not appropriate for drugs because they do not change the saturation solubility.

Griseofulvin, progesterone, phenacetin, and fenofibrate are examples where smaller particle with large surface area enhances drug bioavailable concentration [44]. Spray drying, active compound milling, and grinding are common methods of particle size reduction. These techniques frequently subject the therapeutic product to severe physical stress, which could lead to degradation.

3.2.4 Compressibility

Compressibility is the ability of drug powders to decrease in volume under pressure and compress into tablet dosage forms with specific tensile strength. It is

calculated by the Hausner ratio and Carr's index (formula mentioned in **Table 3**) to determine the flow behaviour of powder-based drugs to calculate the density at the preformulation stages [45].

3.2.5 Crystallinity and polymorphism

Polymorphism and crystalline behaviour of solid-state drugs are important for formulators because most of the drugs exist in the solid states and are suitable for the intended use. In a solid state, drugs may exist in salt form, cocrystals, hydrates, polymorphs, amorphous form, solid solutions, and eutectics. Liquid states drugs like valproic acid and general anaesthetics as gaseous phases. In a crystal, lattice atoms are arranged in a unique pattern and they are highly ordered arrangement based on this arrangement they are categorised as crystalline or amorphous. Both forms show different physical and chemical properties, therefore, they have different solubility and stability performances which influence the delivery system and activity of the drug [38].

When the crystalline and amorphous drug substances are analysed and confirmed that they show different X-ray patterns, vapour pressure, melting point, crystal shape, density, hardness, dissolution, solubility, and bioavailable concentration at the site of action. Furthermore, other properties also fluctuate from their standard values for example limited free energy, high melting point and decrease in solubility behaviour can be considered for stable polymorph. A metastable polymorph has a lower melting point, increased solubility, and is more bioavailable than a stable polymorph. Crystals and polymorphs are analysed by advanced techniques such as the X-ray diffraction method, FTIR, NMR, Optical crystallography, hot stage microscopy, SEM/TEM, and differential scanning calorimetry [46, 47]. The polymorphism and crystal habit behaviour affect solubility, for example, anhydrous Carbamazepine has three different polymorphic forms and these forms are different in their physicochemical properties hence, they show different solubility behaviour at different conditions of temperature and melting points. However, the hydrous form of Carbamazepine as such will not have any polymorphic forms. The melting point may also significantly impact the selection of polymorphic forms like most stable form is polymorph III [44].

3.2.6 Hygroscopicity and deliquescence

Hygroscopicity can be demonstrated as the potency of a drug or salt to gain moisture or water vapour. Compounds can be interacted with moisture by retaining it in bulk or absorbing it on the surface, capillary condensation, and chemical reactions. Atmospheric conditions and the surface area of drug substances are responsible for the amount of moisture absorption. Moisture level variation highly affects the stability, compressibility, and flow properties that's why these attributes should be studied cautiously.

The moisture uptake measurement is done by Karl Fischer titration, Thermogravimetric analysis (TGA), and gas chromatography techniques. As per European Pharmacopoeia, four separate grades of hygroscopicity are defined when a drug substance is stored at 80% relative humidity and a temperature of 25°C for 24 h. The four classes of hygroscopicity are described here as per the above-mentioned storage conditions [48, 49].

Class I: Non-hygroscopic drug substances, when no moisture is detected below 90% relative humidity condition.

Class II: Slightly hygroscopic drug substances are those when their weight increases between 0.2% but less than 2% w/w in presence of moisture.

Class III: Hygroscopic drug substances produces weight increases between 0.2% and less than 15% w/w.

Class IV: Very hygroscopic when the weight increases more than 15% w/w.

Moisture is a significant element that may have an impact on the stability of potential medications and their formulations. Hydrolysis is frequently seen by the adsorption of water molecules onto a potential medication (or excipient). Water molecules have hydrogen bonds and high polarity, this property of water made it absorb the surface of drug substances and affects crystal habit properties, compaction, flow properties, dissolution rate, lubricity, and drug permeability across biological membranes. Therefore, to get rid of the hygroscopicity problems it is necessary to select an appropriate adjuvant, stabile drug compounds, and optimum storage conditions during the preformulation phase. The substances absorb moisture to the highest quantity and liquefy themselves. Deliquescent substance absorbs moisture to a greater extent and liquefies themselves hence, they dissolve solids and leave a thin water film on their surface. This process is linked with relative humidity conditions, this condition measured by vapour diffusion and heat transport rates [18].

3.2.7 Pseudo-polymorphism

Pseudopolymorphism refers to the phenomenon in which the drug molecules get incorporated into the crystal lattice of solids. The solids can exist in different crystal forms known as pseudo polymorphs and the process is pseudopolymorphisms. These forms contain a secondary heterostructure within the crystal lattice, with the same chemical makeup (examples are water, solvent, co-former, etc.). These forms are also known by other names like hydrates, solvates, and cocrystals, and FDA considered them polymorphs [50].

3.3 Preformulation solubility parameters

A significant barrier to product development is solubility. Poor drug solubility (shown in **Table 4**) is a common cause of medication discovery and development failure.

Insufficient solubility can hinder the molecule's capacity to be developed since it can make it difficult to design assays and negatively affect how the compound behaves in vivo. Therefore, inadequate solubility may prove to be a barrier to therapeutic development. In general, drug solubility is influenced by several factors such as lattice energy, molecular arrangement, bond strength, weak bonding forces, lipophilicity, ionisation potential, pH, cosolvency, additives, dielectric constant, solubilisation by surfactant, hydrotrophy, complexation, temperature, pressure, and molecular volume. If these factors have been studied well during preformulation studies, they can be useful for final formulation development with fewer chances of drug failure [51].

In summary, the variables that might alter a drug's solubility profile include the temperature, pH conditions, diluents, additives, solvent system, and the physical condition of drug molecules. The common ion releases in the medium in presence of

BCS Class	Solubility	Permeability	Formulation strategies	Examples
I	High	High	Solid oral conventional dosage forms	Metoprolol, Paracetamol
II	Low	High	Increase solubility by nano-crystallisation, surfactant and co-solvents	Glibenclamide, Bicalutamide
III	High	Low	Permeability enhancers	Cimetidine, Acyclovir
IV	Low	Low	Both class II&III applies here	Chlorothiazide, Furosemide

Table 4.
BCS classification system for solubility and permeability.

solubilising agents responsible for drug molecules crystallisation [52, 53]. Solubility, particularly water solubility, is a crucial physical-chemical property of a medicinal ingredient. For a medication to be therapeutically effective in the physiological pH range of 1–8, considerable water solubility is required.

If a drug substance's solubility is less than ideal, consideration must be paid to increasing it. 10 mg/ml is considered as poor solubility which may result in irregular or partial drug absorption from 1 to 7 pH range at 37°C. But for novel molecules, an understanding of two fundamental qualities is necessary [14].

3.3.1 pH measurement during the preformulation stage

The negative logarithm of hydrogen ion concentration of drug substances is known as pH. The formula for calculation of pH is pH = − Log [H+] and based on the pH scale it is categorised as an acidic drug with 1–7 pH, ±7 is a neutral drug, and 7–14 pH is alkaline/basic drugs.

Most of the drugs are available in salt form or they are either weak bases or acids. Therefore, it is very important to have a complete understanding of molecule ionisation behaviour at specific pH values. Hence, at the preformulation stage, the effect of drug ionisation, ionic strength, pH, and temperature are simultaneously studied to understand dosage form stability, solubility, bioavailability, and efficacy of drug molecules [54].

Importance of pH in preformulation

1. Injectable formulation should be in the range of pH 3–9 to prevent tissue damage and pain at the injection site.

2. Oral syrups cannot be formulated too acidic for palatability reasons.

3. More alkali formulations may attack the glass container.

4. If the drug is susceptible to degradation in acidic pH, then its delayed-release formulation is to be prepared.

5. The pH of the formulation must not sensitise the site of application. For example pH for buccal application should be in the range of 6.6 to 6.8. 6. GIT shows a variety of pH ranges like pH 1.2 for the stomach, pH 6.6 buccal, and pH 7–8 for

the small intestine throughout its length from the oral cavity to the colon. As a key point note, the drug formulation should be stable at the pH intended for the target site of absorption [55, 56].

3.3.2 Dissociation constant (pKa) assessment

pKa is the dissociation constant of a drug and they are available as weak bases or acids in the solution, hence drugs can exist in the ionised or un-ionised form at particular pH. The aqueous solubility of drugs depends on the ionisation and fraction of ionised to the unionised form of the drug. The un-ionised form of drugs is lipophilic, thus permeating through the bilayer membrane however, the ionised substances are lipid insoluble hence permeation is slow. Three parameters that are crucial for absorption are the ionisation constant, which is the un-ionised form of drug at the gastrointestinal tract site available for absorption to show its efficacy [57].

The degree of ionisation depends on the Henderson-Hasselbalch equation and can be determined by UV spectroscopy, potentiometric titration, and titrimetric methods from intrinsic solubility data. A parameter that also considers a compound's ionisation state is the ionisation constant (pKa). Understanding pKa is crucial for predicting the absorption route of weakly acidic or basic medications [3, 58].

The equations for basic and acidic compounds are mentioned below:

$$\text{For acidic drugs}: \text{pH} = \log \text{pKa} + \text{ratio of un} - \text{ionised to ionised drug} \quad (2)$$

$$\text{For basic drugs}: \text{pH} = \log \text{pKa} + \text{ratio of ionised to un} - \text{ionised drug} \quad (3)$$

$$\%\text{Ionisation} = 10(\text{pH-pKa})/1+10(\text{pH-pKa}), \text{where } Ka \text{ is the dissociation constant} \quad (4)$$

Weakly acidic drugs with around 4 pKa value are absorbed fast from the stomach because drug molecules available here in an un-ionised state at this pKa value in the stomach. Similarly, basic drugs with an 8 pKa value are available in an unionised form and easily absorbed from the intestine [58]. Various methods used for the determination of the partition coefficient are a shake-flask method, chromatographic determination, microelectrometric titration, counter current and filter probe. pKa determination at the preformulation stage is important because of the following reasons [59].

- Based on the pKa value, particular pH can be selected to obtain optimum solubility and suitable salt form to get improved bioavailability, and stability.
- pKa is helpful for a selection of buffer, temperature, ionic strength, and co-solvent.
- The extent of drug dissociation can be determined.

3.3.3 Partition coefficient (Log P) assessment

The ratio of unionised drugs dispersed in the aqueous and organic phases is known as the partition coefficient. This is helpful to predict drugs' ability to cross the bilayer. Lipinski's Rule of 5 has been used to predict solubility and permeability.

$$\text{The log P can be determined with the formula } \log P = (\text{oil/water}) \text{ at equilibrium.} \quad (5)$$

A compound's lipophilicity is represented by its Log P value, 0 log P value indicates that the drug substance is similarly soluble in both n-octanol and water. While 2 Log P values indicate the hydrophilic nature of the drug and 5 indicates the lipophilic nature. A suitable absorption profile ranges between log P values of 1 and 3, while a Log P value of less than 1 and more than 6 indicates poor permeability. The software tools are nowadays very helpful for Log P value determination for example Molecular Modelling Pro™ 6.27 software [60].

3.3.4 Distribution coefficient (Log D)

Log D provides an approximation of the lipophilicity of drug molecules in blood plasma at pH 7.4. It can be determined by correlating the drug retention time compared with a similar compound with a known log P value. Log D values consider the possibility of drug molecules in an ionic state [3, 14].

3.3.5 Thermal effect (enthalpy of solution)

The effect of heating on drug solubility can be measured in the form of heat of solution. The heat released or absorbed during the dissolution of a mole of solute in a large volume of solvent is referred to as the heat of the solution. The ideal temperature range should typically include 5, 25, 37, and 50°C. For the endothermic process, the heat of the solution is considered positive and negative for exothermic. Positive heat of solution with an increase in temperature leads to an increase in the drug solubility hence at the preformulation stage, with the use of the heat of solution formula, the optimum drug solubility can be determined. The heat of solution between 4 and 8 kcal/mol indicates un-ionised forms of weak bases and acidic drugs dissolved in water [36].

3.3.6 Common ion effect (Ksp)

Pre-formulation evaluation performed for solubility determination must not avoid the common ion effect since the common ions are responsible for salt solubility reduction.

Le Châtelier's principle states that when an equilibrium is out of balance, the reaction will change to put it back in balance. An equilibrium between a weak acid or base and a common ion will shift in favour of the reactants. The common ions suppress the ionisation of a weak acid in the presence of a weak base or acid by producing more comparable product ions [61]. Hence, adding common ions to the solution may shift the reaction toward the reactant to dismiss excess product stress in the form of precipitation leading to a decrease in the solubility. For example, the solubility of weakly basic drugs in acidic (HCl) solution is diminished when they are administered as HCl salts due to Cl common ions. Hydrochloride salt's intrinsic dissolution rate evaluation between water, and water containing 1.2% w/v NaCl, and 0.9% w/v NaCl in 0.05 M HCl medium suggest a common ion interaction pathway. Following this, if the drug's solubility has not decreased, the drug can suitable to administer as a chloride salt; otherwise, it should be discarded. Hence, to get optimum solubility common ions effect must be avoided [14].

3.3.7 Dissolution

The dissolution rate is defined as the quantity of drug substances dissolve per unit of time with specific circumstances of temperature and solvent conditions for liquid or solid interface. The process is termed dissolution. The dissolution rate can be determined with the help of the Noyes-Whitney Equation. The dissolution rate is the rate-limiting step at the site of absorption for drugs in solution. At the preformulation stage, scientists understand, how excipients, surface area, and particle size affect the dissolution behaviour of drug substances and ascertain whether the rate-limiting behaviour is dissolution mediated. The drug dissolution is followed by reaching into the systemic circulation and is dependent on the type of dosage forms like solid oral (tablets, capsules, and suspensions) and intramuscular (pellets or suspensions); the rate-limiting factor governs which type of drug administration route is optimum for a selected dosage form [3, 14].

3.4 Permeability assessment at the preformulation stage

After dissolution, when a drug is present in the physiological fluids, such as in gastric juices, the small intestine or in plasma, it must have to penetrate cells and tissues to reach the intended site for action. Drug penetration is mediated by various transport mechanisms both passive and active. The drug must diffuse through aqueous pores in tissues or partition with the lipid components of cells to cause passive diffusion, however, active diffusion requires energy. The early drug development process; involves the in vitro models to forecast drug permeation because they offered a simple, repeatable way of monitoring drug absorption rate and mechanism with a favourable cost-benefit ratio. Some of the drug permeability assessment models are listed here.

In-vitro permeability assessment models:

- Caco-2 cells assay for oral permeability assessment
- Parallel Artificial Membrane Permeability Assay
- Madin-Darby canine kidney cells assay
- Franz diffusion cells model

Artificial intelligence-based models (In silico):

- Corneal PAMPA-based in-silico models
- Hierarchical support vector regression (HSVR) scheme

The human epithelial colorectal carcinoma cells-based model is known as the Caco-2 monolayer assay for permeability assessment. This is a diversified in-vitro permeability assay that covers P-glycoprotein efflux transporters, a variety of enzymes like esterases and peptidases, and tight cellular junctions that resemble the small intestine. These in-silico methods are applied to assess drug permeability with the ability to filter massive amounts of sample data while producing precise and accurate results quickly and accurately. Caco-2 cell permeability approach can be used for in-vitro pharmacokinetic research for oral dosage forms. This cell-based assay is suitable for P-glycoprotein efflux and intestinal enzyme metabolism studies [62].

3.5 Stability analysis as per ICH guidelines

According to ICH (International Conference on Harmonisation) Q1A (R2) regulatory guidelines, the purpose of these guidelines is to test drug substances under different stress conditions such as long-term stability testing, accelerated stability testing for a minimum three different time points, and sometimes the intermediate testing also done for some special cases. These stability testing depend upon the climatic zones where the testing is to be done and follow the criteria of that particular zone. The testing includes the effect of pH temperature, humidity, and photolysis under stress conditions. Stability studies during the preformulation stage are most important to check chemical stability and product degradation for the solid-state and liquid-state formulations [2]. Furthermore, some drugs which are prone to degradation produce toxic substances hence it is important to determine the conditions under which this drug degradation happens. This degradation pattern of the drug substance can suggest a way to mitigate or stabilise or to determine the optimum storage, climatic and shelf-life improvement conditions. The physical observation at product development to check, caking, liquefaction, discolouration, odour, and gel formation. After physical observation, degradation can be identified by mass spectroscopy, HPLC or DSC, NMR, FTIR or other relevant sophisticated analytical techniques.

3.5.1 Photostability

The photostability standard conditions are well mentioned in ICH-Q1B guidelines. The photostability of drug substances and drug products must be understood to specify handling, packaging, labelling, adverse effects analysis, and to consider innovative formulation strategies. The optimum exposure for simulation during drug manufacture is casually 1.2 million lux as per the European Federation of Pharmaceutical Industries Expert Working Group. Further, during drug/API manufacture, the cumulative exposure of the compounds can be accepted as 100 Klux of visible light without UV exposure, however, the ICH guideline is not applicable here and this data may be helpful for internal audits.

3.5.2 Solid-state stability

When it comes to solid-state stability, environmental elements including temperature, light, and moisture, as well as the packing materials that come into contact with the dosage form, are the first to have an impact on drug stability. Excipients may affect different chemical interactions with the drug/formulation if selected improperly. The excipients' bound or free moisture, pH, and microclimate can start the deterioration of the dosage forms. Therefore, excipients with low moisture content and low hygroscopicity are preferred for medications that are resistant to hydrolysis-induced deterioration. Various physical properties of the drug molecules like particle shape, size, and surface area, morphology, presence of impurities can play a significant responsibility in drug degradation, either alone or in the presence of excipients [63]. For heat-sensitive drug substances, the processing conditions should maintain at low-temperature conditions to escape from drug degradation. It is possible to use a variety of unique formulation techniques, such as multi-layer particles in capsules or tablets, tablets with moisture-proof coatings, compression coating, tablets inside tablets, or tablets inside capsules. Stressed settings, such as high-temperature studies,

high humidity, exposure to high wetness, and high-intensity light environments, can be used to assess the solid-state stability of an active substance. It provides an early warning of stability issues that could affect product development [61].

As a case study, published by Carney et al. the degradation pattern of Ciclosidomine in solution due to temperature, buffer constituents, and ionic strength are described. The first-order hydrolysis rate appears in absence of light, again at pH 3.0, 5.0, and 6.0 with the absence of light and in presence of air or nitrogen, reported drug degradation. Hence, optimum light and air conditions should be maintained during small-scale and manufacture stages [14].

3.5.3 Solution state stability studies

Compared to solid-state reactions, liquid-state reactions are simpler to spot. The methodologies for detecting unidentified liquid incompatibilities are the same as for solid dosage forms. For suspensions and solutions formulations of bulk drugs, the investigations include listed conditions and they must be evaluated as per FDA stability guidelines, these conditions are higher nitrogen and oxygen environment, alkaline/acidic pH conditions, and the existence of chelating agents and stabilisers [64]. The results of stability studies can help guide stabilisation strategies, giving feedback to the chemistry team on how to modify labile groups to improve stability, assisting researchers in determining the compound's developability, and providing instructions on how to handle and store compounds [2].

3.5.4 Drug-excipients compatibility

The medicine is in close contact with one or more excipients in the tablet dosage form; they could have an impact on the drug's stability. Therefore, understanding how drugs and excipients interact helps the formulator choose the best excipients. It's possible that this knowledge already exists for well-known drugs. The preformulation scientist must produce the necessary data for new medications or excipients. Binders, disintegrants, lubricants, and fillers are typically found in tablets [65]. A new drug's compatibility screening must take into account two or more excipients from each class. The preformulation scientist has a great deal of control over the medication-to-excipient ratio employed in these studies. The following conclusions are made from the drug-excipient compatibility studies [9].

- DECS data submission is essential for new formulation as per FDA regulatory agencies.
- Provide ideas for long-term drug stability.
- Mitigation of difficulties before formulating the final dosage form.
- Determine any chemical and physical interactions with the drug and excipient and how they can affect the stability and bioavailability of the formulation.

Various sophisticated analytical techniques are used to detect drug-excipient compatibility are differential FT-IR spectroscopy, scanning calorimetry, fluorescence spectroscopy, differential thermal analysis, osmometry, diffuse reflectance spectroscopy, high-pressure liquid chromatography, radiolabelling [66].

4. Chemical properties optimization

4.1 Hydrolysis

Hydrolysis reactions are very commonly observed chemical reactions responsible for drug degradation due to the high dipolar water molecules availability which binds to the drug molecules and leads to hydrolysis. It involves nucleophilic reaction the of the labile group [67]. The PH can be adjusted to stop hydrolysis. Since the majority of drugs are weak acids or bases in compositions. Formulate the drug solution so that it has a pH that will ensure its stability, add a water-soluble solvent to the formulation, use the optimal buffer concentration to prevent ionisation, or add a surfactant to stabilise against enzyme catalysis. Drug solubility decreased by creating less soluble salts or esters of the drug which are susceptible to ester hydrolysis. The carbonyl functional groups of esters, lactones, amides, lactams, carbamates and imides are susceptible to hydrolysis. By adjusting the pH conditions of liquid dosage form its shelf life can be lengthened however, stability and solubility should not be compromised.

The hydrolysis problem can be prevented by different approaches, such as drug hydrolysis should be checked to stop drug degradation and this can be achieved with the addition of bulky alkyl groups near the functional group by chemical modifications to hinder the action of a nucleophile or enzyme and decreases drug hydrolysis. Similarly, the labile ester functional group is replaced with urethane or amide can increase chemical and metabolic stability [68].

4.2 Oxidation

A high-oxygen environment can be used to examine a drug's oxidation sensitivity. Rapid evaluation is typically feasible in an atmosphere with 40% oxygen. Samples are kept in desiccators with three-way stop cocks, which are then alternately evacuated and submerged in the desired environment. To ensure the desired environment is created, the method is repeated three or four times. The outcomes could be used to decide if the formulation needs an antioxidant or whether the final drug product has to be packaged in an inert environment [67].

4.3 Reduction

Reduction is a more prevalent mechanism for drug metabolism. Nicotinamide adenine dinucleotide phosphate is necessary for hepatic microsomes to perform a variety of reductive chemical reactions. Cytochrome P450 catalyses the reduction of azo and nitro compounds. Alcohol dehydrogenase converts chloral hydrate to its active metabolite, trichloroethanol. The active metabolite hydrocortisone is produced when prednisolone and cortisone are reduced. Azo dyes are used as colouring additives in drug products or food items and this can be degraded by liver and intestinal flora to create amines [48].

4.4 Chirality

The chirality determination at the preformulation stage is considered during the drug development strategy. The undesirable enantiomer should be removed from the therapeutic formulation since, in most cases, one of the enantiomers lacks the

necessary pharmacological characteristics. When a separation method is available, the inactive form should be eliminated from considerations of cost. Optical activity evaluation for drug molecules is an essential analysis performed during the early discovery process for NCEs. This would suggest the active enantiotropic form of drug molecules. The drug must be in the enantiopure form as it is a legal requirement for IND filing. Therefore, choosing the appropriate enantiomeric form for the market product must be made before IND filling or a patent. As a case study, the Sirolimus [20] branded as Rapamune in liquid solution was marketed by Pfizer and approved in 1999 by the FDA for immunosuppression. Sirolimus contains Phosal 50-PG active ingredient and polysorbate 80 as an inactive ingredient which is nonaqueous. Further, in solid-state Sirolimus is a chiral compound, however, in aqueous solutions, this drug is found in A, B, and C isomers hence the nonaqueous vehicle is selected for drug product development [69].

5. Preformulation studies for biopharmaceuticals development: proteins, peptides, and vaccines

The concept of recombinant DNA technology and clustered regularly interspaced short palindromic repeats (CRISPR) associated protein 9 is flourishing for biopharmaceutical product development. These technologies when coupled with artificial intelligence gel large genome data for the proper designing of peptide, protein, and vaccine products [70]. Proteins are classified into secondary, tertiary, and quaternary structures based on their kinds, which in turn affects each structure's molecular size. With the help of the prodrug approach, the vulnerable peptide backbones undergone proteolytic cleavage can be mitigated. Therefore, peptides can be chemically altered to create more stable and high plasma-concentration prodrugs. The prodrug can be produced by chemical modification and substitution reactions like dehydroamino acid substitution, D-amino acid substitution, thio-methylene modification, carboxyl reduction, and PEG-amino acid joining [71].

Preformulation studies are facilitated the right path of selecting the appropriate adjuvants and other formulation conditions required during manufacture. For example, the stability profile of a live attenuated Ty21a bacterial typhoid vaccine performed by spectroscopic techniques provides time-dependent real-time high throughput information at a broad range of temperatures (10–85°C) and pH (4–8). The above information is useful during preformulation studies of other similar type of peptide drugs. An empirical phase diagram, which was created using data from circular dichroism and fluorescence techniques suggests Ty21a cells exist in various physical states and the most stable state occurs at pH 6–7 below 30°C. Among other potential stabilisers, 10% sucrose and 0.15 M glutamic acid show the strongest protective properties, increasing Ty21a cells' transition temperature by roughly 10°C each. Foam-dried formulations have also been the subject of preliminary research as a potential alternative strategy for further stabilising Ty21a cells. Additionally, in-process stability can be improved by 10% sucrose and trehalose solutions [20, 72].

6. Role of artificial intelligence in preformulation studies

A large, multidisciplinary discipline known as artificial intelligence gives machines the ability to think, learn, and reason. Artificial intelligence has two

subsets: machine learning and deep learning. Scientists frequently integrate computer-added drug design tools with artificial intelligence, powered decision-making at crucial stages of drug discovery programs [1, 73]. Deep learning-based artificial neural networks and machine learning-based expert systems are currently very well-liked for predicting interactions between drugs and their targets as well as physicochemical properties, quality, stability, toxicity, safety, and biological activity of formulations. Medical diagnostics, epidemic breakouts, and individualised treatments are all examples of how AI is used in the healthcare industry [34]. The healthcare industry pursues exceptional advancements with the help of AI tools for example Adaptive neuro-fuzzy inference system (ANFIS) performance is satisfactory for excipients selection hence the AI-based algorithms made drug research simple and shorten the drug discovery and development timelines. In-silico models found their way as successful tools for determining drugs' aqueous solubilities. These factors include molecular size, molecular shape, and hydrogen bonding capacities [74].

7. Preformulation studies: a regulatory perspective

A preformulation report for a novel molecule includes information on stability, excipient compatibility tests, solid-state characteristics, physicochemical characters, biopharmaceutical features, thermal behaviour, mechanical properties, and analytical profiling. In the area of the IND devoted to CMC/pharmaceutical development, these drug substance features have to be highlighted. For example, the medicine must be in the enantiopure form as it is a legal requirement for IND filing. Therefore, choosing the appropriate drug enantiomeric form should be indicated with its activity before filing an IND or a patent [75].

The characteristics of a specific drug material must now be thoroughly explored early in the initial development process, and the findings from these investigations must be provided in the CMC part of an IND. As part of robust regulatory guidelines, the drug excipient additives, manufacturing procedures, and storage conditions, utilised to make the drug substance and drug product are all included in the CMC section. This data is analysed to make sure that the business can effectively manufacture and supply the drug consistently. Nowadays, regulatory agencies have made a common format of documents for submission as indicated by ICH guidelines to harmonise CMC requirements for global marketing. A common technical document that will harmonise CMC regulatory requirements for global development and marketing. The creation of CMC sections by European Union and American standards yields two formally distinct NDAs. The CMC sections of the EU Marketing Authorization Applications and the US-FDA largely share the same components [33].

8. Preformulation studies for nano-based therapeutics

Nano-based therapeutics are suitable formulation strategies for the delivery of active drug ingredients because of their harmonious morphological design and features. The novel drug delivery systems are [76]. The objectives of the preformulation study for nano-based formulations are to design and demonstrate kinetic profile, acceptability with the other substances, physicochemical parameters, and

polymorphism of the new drug entity to design an elegant dosage form [77]. At the preformulation stage, the shape, size, amorphous or crystal structure, and size variability are some of the important features which are evaluated for nano-based delivery systems hence the synthesis of nano-based therapeutics systems is truly based on the physiochemical characteristics [78]. The diluents and solvents also play a significant role in inhomogeneity, size and shape in the nanoprecipitation methods. The profiling of active moieties is crucial in terms of their solubility studies, melting point, thermal properties, and pKa behaviour. In the preformulation phase, physiochemical properties and the compatibility of the drug with excipients of the formulation are regulating the nano-system behavioural selected for the final formulation design. Preformulation studies are conducted to create an appropriate dosage form by establishing the pharmacokinetics and pharmacodynamic profile parameters [79, 80]. Moreover, the dissolution profile, polymorphic forms, the pharmacokinetics of the drug, and its bioavailability, details on the drug's deterioration process, undesirable drug-related conditions, and pharmacodynamic effects are also an important part of preformulation studies which are provided by physicochemical properties of the drug. The nano-based formulations can be used for topical, transdermal, injectable and oral deliveries for compound development, screening, therapeutic, imaging and diagnostic purposes therefore they are novel systems for targeting cancer, inflammatory diseases and autoimmune disorders [81, 82]. For example, these considerations are helpful for justification for the preparation of nanoparticles, lipid-based nanoparticles, polymeric nanoparticles, concerning polymer, adjuvant selection, crucial formulation profiling, preparatory techniques, process-related variables optimization for favourable formulation parameters, nanoparticle characterisation, stability profiling and entrapment efficiency enhancement [28].

9. Conclusion

Preformulation studies are all about selecting a good drug candidate with the help of various supporting parameters. The utmost requirements are starting from the optimum drug candidate selection, API form of drug, compatible excipients, drug manufacturing process, stability studies as per regulatory guidelines, container and closure selection, solubility parameters, pharmacokinetics and biopharmaceuticals behaviour, and safety strategies all come up in physical and chemical properties optimization as per current industrial regulatory perspectives. Preformulation studies provide a strong and optimum scientific background of a drug molecule concerning "proof of concept" implementation in drug development, public safety concerns, new technologies of dosage forms, improvement of quality standards, and regulatory perspectives. All the preformulation parameters are should be studied for biological drug candidates and NCEs as well in every dimensional approach to have fewer chances of molecule failure. This review chapter summarises the path of drug candidate development facilitated by preformulation studies.

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Conflict of interest

The authors declare no conflict of interest among themselves.

Abbreviations

ANDA	abbreviated new drug application
API	active pharmaceutical ingredient
CADD	computer aided drug design
CMC	chemistry, manufacturing, and controls
eCTD	electronic common technical documents
FT-IR	Fourier transform infrared spectroscopy
HCl	hydrochloric acid
HSVR	hierarchical support vector regression
IC50	inhibitory concentration
ICH	international conference on harmonisation
IND	investigational new drug
Ksp	solubility product constant
Log D	distribution constant
Log P	partition coefficient
NaCl	sodium chloride
NDA	new drug application
PAT	Process Analytical Technology
pH	potential of hydrogen
pKa	dissociation constant
QbR	question-based review
QoS-QbR	quality overall summary-question-based review
RP-UHPLC	reverse-phase ultra-high performance liquid chromatography
SAR	structure-activity relationship
TGA	thermogravimetric analysis
US-FDA	United states food and drug administration

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