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Stability, characterization, and manufacturing optimization of ferrous Ascorbate: A Comprehensive Study by West Bengal Chemical Industries Ltd., Kolkata, India

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Abstract
Ferrous ascorbate, an essential pharmaceutical compound used in the treatment of iron deficiency anemia, exhibits unique physicochemical and solid-state properties that influence its manufacturability and clinical efficacy. This study investigates the stability and characterization of ferrous ascorbate produced by West Bengal Chemical Industries Ltd. Kolkata, India (WBCIL), focusing on preformulation studies, force degradation assessments, and analytical techniques including UV, IR spectroscopy, and HPLC. Key challenges such as hygroscopicity, flow properties, and tablet integrity were addressed through rigorous testing protocols, including flow parameter studies and swelling index evaluations. Comprehensive force degradation studies under various conditions revealed the compound's susceptibility to thermolysis and photolysis while highlighting its stability in acidic, basic, and oxidative environments. Flow properties, UV, and IR characterization studies confirmed alignment with established literature values, ensuring the reproducibility and quality of the compound. These findings underscore the significance of optimizing manufacturing processes and packaging solutions to enhance the stability and efficacy of ferrous ascorbate formulations.

Keywords: Ferrous ascorbate, iron deficiency anemia, stability, preformulation studies, degradation assessments, uv spectroscopy, flow properties

Introduction

Background

Drugs are typically not administered as pure chemical entities; rather, they are formulated into various pharmaceutical dosage forms such as compressed tablets, sustained-release products, solutions, and injections [1]. The physicochemical properties of a drug play a crucial role in determining its bioavailability and final dosage form. The solid-state form of a compound impacts several solid-state properties including particle size, density, flowability, wettability, surface area, solubility, and hygroscopicity [1]. These properties significantly influence the manufacturability of the drug product and its clinical performance. Key parameters such as wettability, surface area, and solubility can affect the dissolution rate of the drug product without altering its equilibrium solubility. Consequently, preformulation studies are essential to develop a stable and efficacious pharmaceutical product [1, 2].

Ferrous ascorbate, chemically designated as L-(+)-Ascorbic acid iron (II) salt, is synthesized using ferrous sulphate as the precursor. It is prescribed for the treatment of iron deficiency anemia. Ferrous ascorbate is a hygroscopic, fine dark violet powder that is odorless and tasteless [3]. Iron is optimally absorbed in its ferrous state, although most dietary iron exists in the ferric form. Absorption of iron in the stomach is minimal; however, gastric secretions (HCl) facilitate the dissolution of iron, enabling it to form soluble complexes with ascorbic acid. Vitamin C and other substances aid in reducing iron to its ferrous form, which is essential for patients with partial gastrectomy, who often experience iron deficiency anemia [4]. Iron absorption predominantly occurs in the upper part of the small intestine, specifically the duodenum and upper jejunum.

Within the mucosal cells, an intracellular iron carrier aids in the transport of some iron to the mitochondria. The remaining iron is distributed between apoferritin within the mucosal cells and transferrin, the iron-transporting polypeptide in plasma [5]. Apoferritin, which is also found in various other tissues, combines with iron to form ferritin. Ascorbic acid plays a vital role in the mobilization of plasma iron to storage sites within tissues and is also believed to enhance iron utilization through its reducing action. Additionally, ascorbic acid may have a direct impact on erythropoiesis, further supporting its role in the treatment of iron deficiency anemia [6].

Problems associated with Ferrous Ascorbate packaging

The packaging of Ferrous Ascorbate presents unique challenges due to its hygroscopic nature, which causes it to readily absorb moisture from the surrounding environment. This moisture uptake can lead to cracking, swelling, and compromised tablet integrity during storage and transportation. To mitigate these issues, it is crucial to maintain strict environmental controls, such as low humidity and stable temperature conditions, during the manufacturing and packaging processes [7]. Additionally, reducing the size of the granules could improve tablet compactness and minimize the likelihood of swelling. Poor tablet hardness further exacerbates the problem, indicating a need to enhance adhesion by optimizing the formulation with appropriate binders or excipients. Implementing these measures, coupled with the use of moisture-resistant packaging materials like desiccants and specialized blister packs, can significantly improve the stability and quality of Ferrous Ascorbate products [8].

Superiority of Ferrous Ascorbate Manufactured by WBCIL: The manufacturing process of ferrous ascorbate by West Bengal Chemical Industries Ltd., Kolkata, India (WBCIL), is distinguished by its comprehensive approach to ensuring product stability and efficacy through various rigorous testing methodologies. The process includes stress (force) degradation studies to evaluate the stability of ferrous ascorbate under various physical and chemical stress conditions. These studies are critical for the following reasons:

Degradation Potential: Identifying the potential degradation pathways of the active pharmaceutical ingredient (API), such as hydrolysis, oxidation, and photolysis.

Degradation Mechanism: Discovering the degradation mechanisms to ensure the formulation remains effective and safe throughout its shelf life.

Molecular Structure of Degradation Products: Elucidating the molecular structure of degradation products, which helps solve problems related to the API's stability.

Susceptibility to Degradation: Identifying the conditions under which the API or drug product may be more susceptible to degradation, ensuring the quality of the final product.

Stable Formulations: Aiming to create more stable formulations that meet stringent pharmaceutical standards.

Analytical Methods: Developing analytical methods to quantify both the API and its degradation products without interference.

In addition to the degradation studies, other critical testing protocols are followed, such as

Flow Parameter Assessment: Studying the flow characteristics of ferrous ascorbate powder to ensure optimal manufacturability and efficient production.

UV and IR Spectroscopy Studies: UV spectroscopy is used to quantify the active ingredient and monitor its stability, while IR spectroscopy helps confirm the molecular structure and detect impurities or changes in chemical composition.

Bulging or Swelling Index Study: Evaluating the physical stability of ferrous ascorbate tablets by assessing their tendency to absorb moisture and swell, which could affect mechanical integrity and dissolution rate.

Advantages of ferrous ascorbate

Ferrous ascorbate is preferred over ferric compounds in many therapeutic applications due to its superior bioavailability and tolerability. Unlike ferric salts, which require reduction to the ferrous state in the gastrointestinal tract before absorption, ferrous ascorbate is readily absorbed as it directly provides iron in the ferrous (Fe^{2+}) state, the biologically active form utilized by the body [12]. The ascorbate component not only stabilizes the ferrous iron, preventing its oxidation to the less absorbable ferric (Fe^{3+}) state, but also enhances iron absorption by reducing ferric iron in the intestinal lumen. Additionally, ferrous ascorbate has been associated with fewer gastrointestinal side effects, such as nausea and constipation, commonly observed with ferric compounds [13]. Its non-ionic nature minimizes interactions with dietary inhibitors of iron absorption, such as phytates and tannins, ensuring a more efficient delivery of iron. These properties make ferrous ascorbate a preferred choice for managing iron deficiency anemia, particularly in populations where enhancing absorption and reducing adverse effects are critical for patient compliance and therapeutic success [14].

Materials and Methods

Materials: Ferrous ascorbate samples were obtained from West Bengal Chemical Industries Ltd., Kolkata, India. Analytical-grade reagents, including 0.1 N hydrochloric acid (HCl), 0.1 N sodium hydroxide (NaOH), hydrogen peroxide (30% v/v), and deionized water, were sourced locally. All solutions were prepared using deionized water, and pH adjustments were made using HCl or NaOH, as required.

Forced Degradation Studies

Forced degradation studies were conducted to evaluate the stability of ferrous ascorbate under different stress conditions. The stressors included acidic, basic, neutral, thermal, oxidative, and photolytic conditions as outlined below:

1. Acidic Conditions: Samples were exposed to 0.1 N HCl at 80°C for 4 hours.
2. Basic Conditions: Samples were treated with 0.1 N NaOH at 80°C for 4 hours.

3. Neutral Conditions: Samples were incubated in deionized water at 80°C for 4 hours.
4. Thermal Conditions: Samples were heated in a dry environment at 120°C for 4 hours.
5. Oxidative Conditions: Samples were treated with hydrogen peroxide (30% v/v) at room temperature for 4 hours.
6. Photolytic Conditions: Samples were exposed to ultraviolet (UV) radiation under controlled light intensity at room temperature for 4 hours.

Each condition was optimized to ensure degradation levels between 10-30%, as recommended by International Council for Harmonization (ICH) guidelines. The importance of understanding the efficacy and safety of drugs through their toxicological and pharmacological profiles, emphasizing the potential risks posed by impurities. These impurities are defined by the International Council for Harmonization and Technical Requirements for Pharmaceuticals for Humans Use (ICH) as any component not identified as the active pharmaceutical ingredient (API) or excipient. Impurities are classified based on their origin-such as inorganic (e.g., heavy metals or salts), organic (e.g., starting materials, by-products), and solvents used in synthesis.

Analytical Methods

Degraded samples were analyzed using the following methods:

- Titration: Iron content was quantified through titrimetric analysis, ensuring minimal interference from degradation products.
- UV-Vis Spectroscopy: The absorbance of ascorbic acid was measured at 280 nm using a UV-Vis spectrophotometer.
- High-Performance Liquid Chromatography (HPLC): Separation and quantification were conducted on an Agilent Zorbax SB-C8 column with a phosphate buffer and methanol mobile phase (98:2 v/v), and detection at 264 nm.

Swelling Index Study

The moisture absorption characteristics of ferrous ascorbate (FAS) powders were investigated over a 30-day period. This study aimed to determine the swelling behavior of the powders in response to moisture absorption. The samples were stored under ambient conditions (temperature and humidity) and weighed at regular intervals to observe any changes. The pre-weighed tablets were submerged in 500 mL of the medium (deionized water, DIW, or simulated gastric solution, 0.1N HCl) and kept at 37.0 ± 0.5°C for 8 hours. At specific time intervals (0, 0.5, 2, 4, 6, and 8 hours), the swollen tablets were taken out of the solution, gently wiped with a paper towel to eliminate any surface droplets, and then reweighed. The swelling index (Sw) of each sample was calculated at each time point using the following formula:

$$Sw = \frac{W_t - W_0}{W_0}$$

Where:

W0 is the initial dry weight of the sample,

Wt is the weight of the sample at time t.

The process was repeated in triplicate to ensure the accuracy and reliability of the data. The resulting swelling indices were analyzed to assess how the powder interacts with moisture over time, which can affect its flow properties and compressibility, key factors in pharmaceutical manufacturing processes. The study also provides insights into the stability of ferrous ascorbate under varying environmental conditions, particularly in terms of its handling and storage.

Flow Parameter Studies

The flowability of the ferrous ascorbate powders was evaluated using a set of critical parameters: bulk density, tap density, Hausner's ratio, percentage compressibility, and angle of repose. These parameters were measured for two different batches of ferrous ascorbate (FAS13782407B and FAS13782312A), following standard pharmaceutical procedures:

- Bulk density measures the mass of powder per unit volume under normal conditions.
- Tap density is the density of the powder after being tapped into a fixed volume.
- Hausner's ratio is the ratio of tap density to bulk density. A value close to 1 indicates excellent flowability, while values greater than 1 suggest poor flowability.
- Percentage compressibility provides an estimate of the ease with which the powder compacts under pressure.
- Angle of repose is the maximum angle at which a powder can be piled without slumping, giving insight into its flow characteristics.

The results were compared with literature values to assess the manufacturability of the ferrous ascorbate powders and to determine their suitability for use in pharmaceutical processing, particularly in tableting and capsule filling. Although the qualitative description of powder flow using the angle of repose may vary slightly, most pharmaceutical literature aligns with Carr's classification, as presented in the Table. Some formulations with an angle of repose between 40 and 50 have been successfully manufactured. However, when the angle of repose exceeds 50, the flow is generally considered unacceptable for manufacturing purposes.

Table 1: Flow Properties

Flow Property	Angle of Repose (degrees)
Excellent	25-30
Good	31-35
Fair-aid not needed	36-40
Passable-may hang up	41-45
Poor-must agitate, vibrate	46-55
Very poor	56-65
Very, very poor	>66

Table 2: Scale of Flowability

Compressibility Index (%)	Flow Character	Hausner Ratio
≤10	Excellent	1.00-1.11
11-15	Good	1.12-1.18
16-20	Fair	1.19-1.25
21-25	Passable	1.26-1.34
26-31	Poor	1.35-1.45
32-37	Very poor	1.46-1.59
>38	Very, very poor	>1.60

UV and IR Spectroscopy Characterization

Ultraviolet (UV) and infrared (IR) spectroscopy were employed to characterize the ferrous ascorbate powders and

confirm their chemical identity and purity. UV spectra were recorded for samples dissolved in distilled water and 0.1 N HCl, with the observed peak wavelengths compared to reference spectra for validation. The absorption peaks were analyzed to confirm the presence of specific functional groups, and any deviations from the expected spectra were considered for further investigation.

Fourier-transform infrared (FTIR) spectroscopy was also performed to identify functional groups present in the ferrous ascorbate molecules. The analysis confirmed the presence of key functional groups such as hydroxyl (OH), carbonyl (C=O), and ether (C-O-C) bonds. These findings not only validate the identity of the samples but also ensure their chemical purity, which is crucial for the consistency and safety of the final pharmaceutical product.

Statistical Analysis

To ensure the reliability and reproducibility of the results, all experiments were performed in triplicate. The data were presented as mean values ± standard deviation to express variability within the samples. Statistical significance was determined using a one-way analysis of variance (ANOVA) test. A p-value of less than 0.05 ($p<0.05$) was considered statistically significant, indicating that the observed differences were unlikely to have occurred by chance. This rigorous statistical approach ensures the validity of the experimental outcomes and their applicability to real-world pharmaceutical manufacturing processes.

Results and Discussion

Degradation Studies

While impurities are often associated with degradation products, they represent a broader category, which also includes compounds that may arise due to chemical instability. The degradation of APIs (Active Pharmaceutical Ingredients) occurs through chemical reactions like oxidation, reduction, hydrolysis, and thermolysis, often accelerated by external factors such as light, heat, or humidity. The United States Pharmacopoeia refers to these degradation products and impurities as "Related Compounds." This highlights the complex and varied sources of impurities and their significant impact on drug quality and safety.

Ferrous ascorbate demonstrated high stability under acidic and oxidative conditions, with degradation percentages of 0.54% and 0.08%, respectively, as determined by titration. In contrast, photolysis and thermolysis resulted in significant degradation, with percentages of 33.82% and 29.09%, respectively. HPLC results indicated severe degradation under thermolysis (99.88%) and photolysis (99.91%).

Table 3: Stability study under different degradation conditions

Condition	Reagent	Reaction Condition	Initial Assay (%)	Final Assay (%)	% Degradation
Acid	0.1 (N) HCl	80°C, 4 hrs	76.54 (W/W)	0.54 (W/W)	99.29
Base	0.1 (N) NaOH	80°C, 4 hrs	76.54 (W/W)	0.45 (W/W)	99.41
Neutral	H2O	80°C, 4 hrs	76.54 (W/W)	0.81 (W/W)	98.94
Thermolysis	120°C, 4 hrs	80°C, 4 hrs	76.54 (W/W)	54.27 (W/W)	29.09
Oxidation	H2O2 (30% V/V)	RT, 4 hrs	76.54 (W/W)	0.08 (W/W)	99.89
Photolysis	UV	RT, 4 hrs	76.54 (W/W)	50.65 (W/W)	33.82
Acid	0.1 (N) HCl	80°C, 4 hrs	76.54 (W/W)	0.98 (W/W)	98.71
Base	0.1 (N) NaOH	80°C, 4 hrs	76.54 (W/W)	2.36 (W/W)	96.91
Neutral	H2O	80°C, 4 hrs	76.54 (W/W)	2.33 (W/W)	96.95
Thermolysis	120°C, 4 hrs	80°C, 4 hrs	76.54 (W/W)	64.04 (W/W)	16.33
Oxidation	H2O2 (30% V/V)	RT, 4 hrs	76.54 (W/W)	0.17 (W/W)	99.77
Photolysis	UV	RT, 4 hrs	76.54 (W/W)	59.17 (W/W)	22.69
Acid	0.1 (N) HCl	80°C, 4 hrs	94.27	45.53	51.70
Base	0.1 (N) NaOH	80°C, 4 hrs	94.27	22.81	75.80
Neutral	H2O	80°C, 4 hrs	94.27	49.55	47.34
Thermolysis	Dry Heat	120°C, 4 hrs	94.27	0.11	99.88
Oxidation	H2O2 (3% V/V)	RT, 4 hrs	94.27	8.79	90.67
Photolysis	UV	RT, 4 hrs	94.27	0.08	99.91

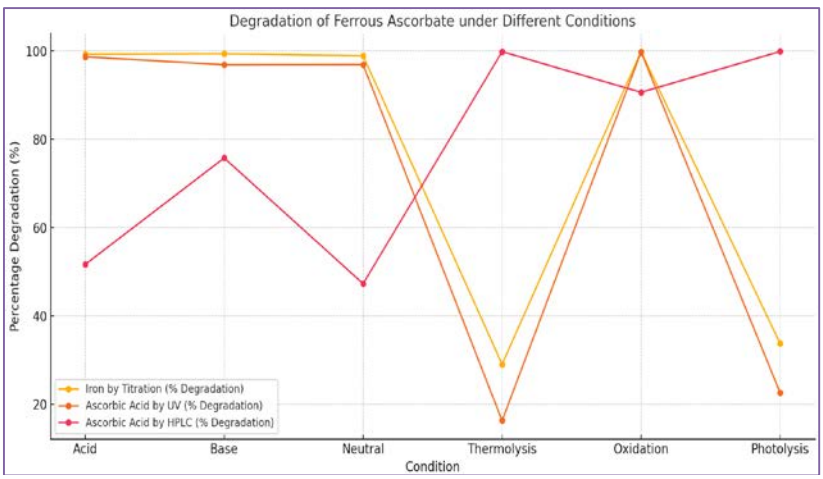


Fig 1: Degradation Studies of two batches of Ferrous Ascorbate

Flow Parameters

Powder flow properties are crucial for ensuring the consistency and efficiency of pharmaceutical manufacturing processes, such as mixing, granulation, tableting, and filling. These properties are assessed using various standardized methods, each with its own principles and applications. The angle of repose measures the resistance to movement between particles by determining the angle formed by a conical pile of powder. This can be assessed in variations like the drained or dynamic angle of repose, though challenges like material segregation and aeration can affect results. The compressibility index and Hausner ratio provide indirect measures of bulk density and flowability, with lower ratios indicating better flow. The flow rate through an orifice and shear cell testing also offer insights into powder behavior. However, no single method can fully characterize powder flow properties due to their complexity, necessitating the use of multiple techniques to gain a comprehensive understanding. Among these, the angle of repose remains a key metric in predicting potential manufacturing challenges, despite its limitations. Batch FAS13782407B exhibited a bulk density of 0.11 g/mL, tap density of 0.14 g/mL, Hausner's ratio of 1.27, and compressibility of 21.43%, while batch FAS13782312A demonstrated higher bulk and tap densities but lower compressibility (16.0%). Both batches met acceptable flow criteria but showed deviations from literature values. Hausner's ratio and compressibility index were measured by utilisng the formula mentioned below:

$$\text{Hausner's Ratio} = \frac{\text{Tapped Density}}{\text{Bulk Density}}$$

$$\text{Compressibility Index} = \frac{\text{Tapped Density} - \text{Bulk Density}}{\text{Tapped Density}} \times 100$$

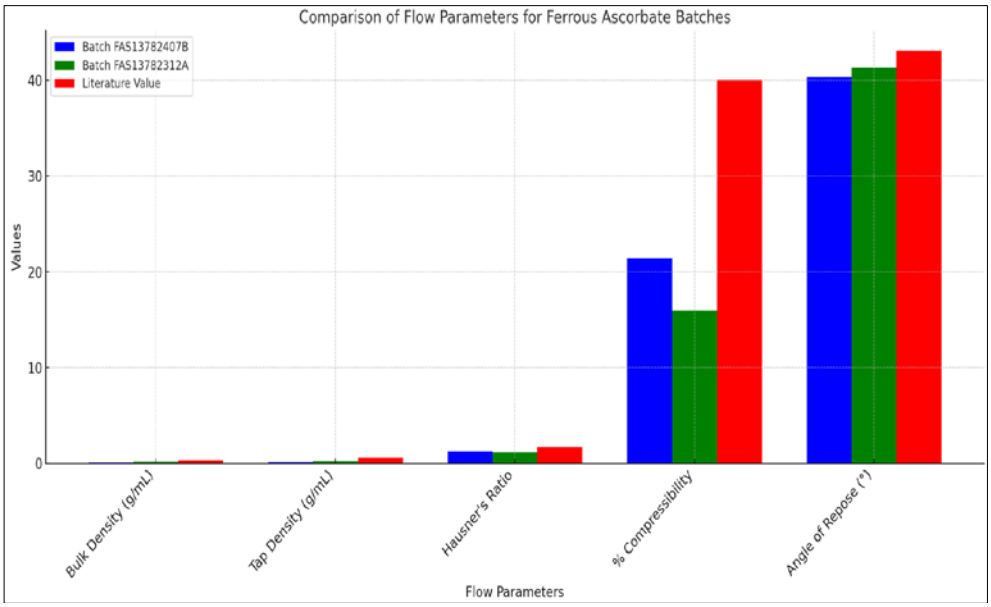


Fig 2: Comparison Studies for Flow Parameters of Different Ferrous Ascorbate Batches

UV and IR Characterization

The UV spectrum of ferrous ascorbate in distilled water showed a peak at 265 nm with absorbance values closely matching literature (257 nm). Similar alignment was observed in 0.1 N HCl (243 nm). FTIR spectra confirmed the presence of functional groups (OH, C=O, and C-O-C) consistent with reference compounds.

Table 4: Absorbance variations in different mediums and wavelengths.

Batch	Medium	Wavelength (nm)	Absorbance	Literature Wavelength (nm)
FAS13782407B	Distilled Water	265	0.2678	257
FAS13782312A	Distilled Water	265	0.2518	257
FAS13782407B	0.1 N HCl	243	0.3827	243
FAS13782312A	0.1 N HCl	243	0.2568	243

Swelling Index

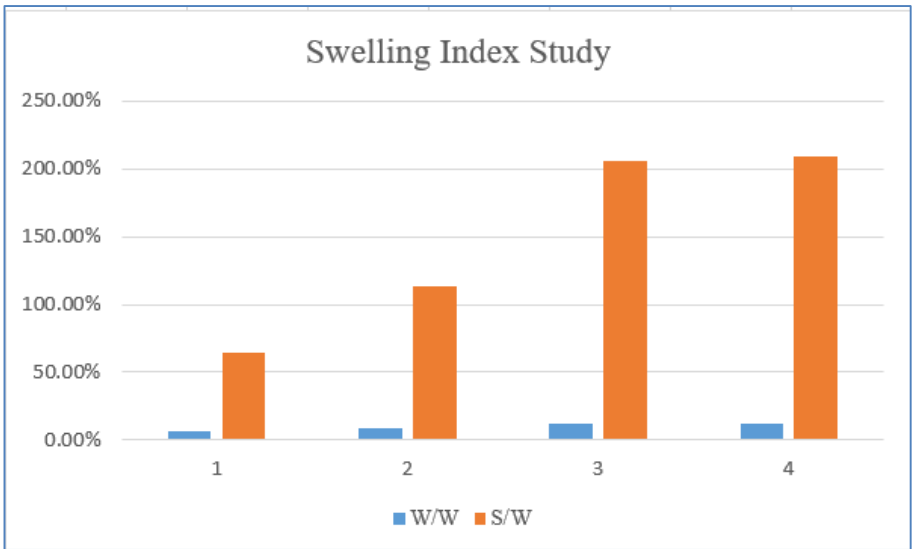
The swelling index increased significantly over the first 21 days, from an initial LOD of 4.08% to 12.51%, with a maximum swelling index of 2.06 observed at 21 days. Beyond this period, moisture absorption stabilized, with negligible changes in LOD. Swelling study of Ferrous Ascorbate was conducted by measuring LOD (105 DEG C 04 HRS) at different time interval, keeping the powder in open Petridis. [14] The swelling index (SW) was calculated according to the following formula:

Swelling Index (SW) = WT-W0/W0,

Where WT = weight of the powder after certain time (initial LOD)

W0= initial weight (final LOD)

Initial LOD = 4.08% W/W



- LOD after 07 days = 6.73% (W/W): SW (07 D) =0.649
- LOD after 14 days = 8.71% (W/W): SW (14 D) = 1.13
- LOD after 21 days = 12.51% (W/W): SW (21 D) = 2.06
- LOD after 30 days= 12.61% (W/W): SW (30 D) = 2.09

Conclusion

The study highlights the critical role of physicochemical characterization and rigorous stability assessments in developing ferrous ascorbate as a pharmaceutical dosage form. WBCIL's comprehensive approach, including degradation studies, flow parameter evaluations, and spectroscopic analyses, ensures the compound's quality and performance. Challenges such as hygroscopicity and poor flowability necessitate stringent environmental controls, optimized formulations, and advanced packaging solutions to maintain tablet integrity and therapeutic efficacy. The results demonstrate that ferrous ascorbate exhibits significant stability under acidic and oxidative conditions but is highly sensitive to thermolysis and photolysis, emphasizing the need for protective storage and handling practices. By addressing these factors, WBCIL sets a benchmark for producing high-quality, stable, and effective ferrous ascorbate products, contributing to improved outcomes in the treatment of iron deficiency anemia.

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