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Effect of Oxygen and Water on the Stability of Imipenem and Cilastatin Sodium for Injection

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Abstract

The study aimed to investigate the factors affecting the stability of imipenem and cilastatin sodium for injection (IMI/CIL) to improve the quality and stability in IMI/CIL preparation. In this study, the effects of headspace oxygen (HO), water content, particle shape, and particle size on the stability of IMI/CIL were investigated. IMI/CIL was purged with air, premixed oxygen/nitrogen gas (5%/95%), or high-purity nitrogen (99.999%) at 20, 5, or 2% oxygen levels to prepare IMI/CIL with different HO levels. IMI/CIL was stored at 30, 45, and 75% relative humidity for 30 days to prepare IMI/CIL with different water contents. High-performance liquid chromatography method was used for analysis. The results showed that oxygen, water, particle shape, and particle size had significant effects on the stability of IMI/CIL, and free water content is a better predictor of the safety and stability of imipenem and cilastatin sodium than the total water content. The optimization scheme of the above parameters is proposed, which significantly improves the stability of IMI/CIL. This study led to a better understanding of the degradation mechanism of imipenem and cilastatin sodium, and could provide a reference for the selection and control of IMI/CIL process conditions. This study would contribute to the development of IMI/CIL with improved stability.

Keywords
- imipenem
- cilastatin sodium
- stability
- oxidation
- free water

Introduction

Imipenem and cilastatin sodium for injection (IMI/CIL), as the first carbapenem drug used in clinic, is used to treat complicated intra-abdominal infections and complicated urinary tract infections including pneumonia and other serious bacterial infections in adults. Compared with other types of antibacterial drugs, imipenem has higher sensitivity to pathogenic bacteria while bringing better safety. Therefore, it has important clinical value.[1] However, as a β-lactam drug, the chemical stability of IMI/CIL is very unstable, hence it is necessary to pay more attention to the factors affecting its stability.

Oxidation is a common pathway for drug degradation.[2] Active pharmaceutical ingredients (APIs) exposed to the air tend to deteriorate due to the influence of oxygen,[3] thus decreasing drug potency and greatly reducing their shelf life. In addition to oxygen, water is another important factor that influences the chemical stability of solid pharmaceuticals. Usually the degradation rate of a drug is dependent on the

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water content.[4] Although some studies have assessed the effects of oxygen and water on the degradation of compounds,[3,5] however, the influence of oxygen and water on the stability of IMI/CIL has not been evaluated.

In previous studies, it was generally believed that total water was the main factor affecting drug degradation, and few studies have studied the effect of free water on drug degradation. In this study, the effects of headspace oxygen (HO) level and water content on IMI/CIL were investigated. Moisture-mediated interactions between cilastatin and imipenem were also explored. This study demonstrated that free water content is a better predictor of the safety and stability of imipenem and cilastatin sodium than total water content. Moreover, the degradation mechanism of imipenem and cilastatin sodium exposed to oxygen and moisture has been proposed based on molecular structure information. The effects of shape and size of drug particles on the stability of imipenem and cilastatin sodium were also investigated. Our data suggested that the greater the surface area of imipenem and cilastatin sodium exposed in the air, the greater the effect of sodium cilastatin on imipenem. This work would contribute to the production of IMI/CIL with improved stability.

Materials and Methods

Materials

Imipenem, cilastatin sodium, and sodium bicarbonate were provided by manufacturer A. IMI and CIL from three batches (2021–1, 2021–2, 2021–3) were prepared in different processes. Acetonitrile was purchased from Honeywell International (New Jersey, United States) and phosphoric acid (H_3PO_4) was from Sinopharm Group Co. Ltd (Shanghai, China). Anhydrous sodium dihydrogen phosphate and anhydrous sodium dihydrogen phosphate were obtained from General Reagent. All laboratory water was prepared by using a Millipore milli-Q water purification system (Merk KGaA, Darmstadt, Germany). All other chemicals were of reagent grade and used without further purification.

Analysis of Imipenem and Cilastatin Sodium Impurities

A high-performance liquid chromatography (HPLC) method was used for analyzing the contents of imipenem, cilastatin sodium, and their related substances. Analyses were performed on a Thermo DIONEX Ultimate 3000 HPLC system (Thermo Fisher Scientific, Bremen, Germany) equipped with an LPG-3400SDN Ternary Pump, a TCC-3000RS column compartment, a WPS-3000TSL ANALYTICAL autosampler, and a diode array detector (all the above instruments were from Thermo Fisher Scientific). Chromatography was conducted on a C18 column (250 mm × 4.6 mm, 5 µm, Waters, United States). Eluent A was composed of potassium dihydrogen phosphate–acetonitrile (99.3:0.7, v/v) with pH = 7.3 adjusted by H_3PO_4, and eluent B was potassium dihydrogen phosphate–acetonitrile (50:50, v/v) with pH = 7.3. A gradient elution was performed using the method described in ►**Table 1**. The injection volume was 10 µL and the column temperature was maintained at 30°C. The flow rate was set to 1.0 mL/min. The UV detection was performed at 260 nm. Parameters such as retention time, resolution, and symmetry factor verified the suitability of the system. All the sample solutions must be prepared immediately before analysis.

Table 1 Gradient elution method

Time (min)	Mobile phase A%	Mobile phase B%
0	100	0
3	100	0
28	90	10
38	90	10
63	50	50
78	30	70
88	30	70
89	100	0
97	100	0

IMI/CIL Preparation

IMI/CIL was prepared according to a reported study, as well as the prescription including 500 mg of imipenem, 500 mg of cilastatin sodium, and 20 mg of sodium bicarbonate.[6]

Effect of HO Levels on Stability

Preparation of IMI/CIL with Different HO Levels

To prepare IMI/CIL with different HO levels, vials filled with imipenem and cilastatin from 2021–1 were purged with air, premixed oxygen/nitrogen gas (5%/95%), or high-purity nitrogen (99.999%) at 20, 5, or 2% oxygen levels, respectively, and then sealed and capped promptly. These samples were stored at 60°C and analyzed for the major degradation substances by HPLC on day 0, 10, and 30.

Preparation of IMI/CIL from Different Batches at the Same HO Level

IMI/CIL samples with the same HO level of 2% from 2021–1, 2021–2, and 2021–3 were prepared by purging with nitrogen. The samples were kept at 60°C and analyzed for the major degradation substances by HPLC on day 0, 5, 10, and 30.

Effect of Water Content on Stability

To explore the effect of water on the stability of imipenem and cilastatin sodium, imipenem, cilastatin sodium, and IMI/CIL were placed in different humidity environments. Water contents were measured by thermogravimetric analysis (TGA) and loss on drying (LOD) method. The major degradation substances of imipenem-cilastatin sodium were detected by HPLC, and the effect of water on the stability of imipenem-cilastatin sodium was studied. Imipenem, cilastatin sodium, and IMI/CIL were dried to investigate the effect of reducing their free water content on the stability of IMI/CIL.

Treatment of Imipenem, Cilastatin, and IMI/CIL

To evaluate the effect of moisture on the stability of imipenem and cilastatin sodium, imipenem, cilastatin sodium, and IMI/CIL were stored at 30, 45, and 75% relative humidity (RH) for 30 days. The samples were analyzed for the major degradation substances by HPLC on day 0, 5, 10, and 30.

Analysis of Water Content

TGA and LOD were used to assess the water content of imipenem, cilastatin sodium, and IMI/CIL. Karl Fischer titration was not suitable for the determination of the water content of IMI/CIL because IMI/CIL contained sodium bicarbonate which interfered with the measurement. These two methods were compared to identify and discuss the most suitable method for providing valuable information.

TGA thermograms of imipenem and cilastatin sodium were recorded using the TGA Q500 V20.13 Build 39 thermoanalyzer apparatus (Lairui Instrument, Shanghai, China). The weight loss of the samples was determined. The experiments were performed in alumina crucibles and the samples were heated under a dynamic nitrogen atmosphere at a heating rate of 10°C/min from 25 to 350°C with a sample mass of 5 to 10 mg.

For LOD assay, samples were dried in a vacuum oven (101–0ES, Keheng Co., Ltd., Shanghai, China). The atmospheric pressure of the vacuum oven was set to below 2.67 kPa (20 mm Hg). The temperature was set to 60°C and 100 g of samples were dried on a flat weighing bottle for 3 hours until the weight of the samples stabilized (the difference between the last two measurements was <0.02 g).

Drying Imipenem, Cilastatin Sodium, and IMI/CIL

The effect of decreasing the free water content in imipenem, cilastatin sodium, and IMI/CIL on the stability of imipenem was evaluated. The free water in imipenem, cilastatin sodium, and IMI/CIL was reduced by vacuum oven drying at 60°C. This experiment was divided into the following three groups:

- Group 1: the dried and undried imipenem were filled into two vials, respectively, and named L1 and L2.
- Group 2: the undried and dried cilastatin sodium were mixed with undried imipenem, respectively, and named L3 and L4.
- Group 3: the dried and undried IMI/CIL were filled into two vials, respectively, and named L5 and L6.

All the above vials filled with powder were purged with nitrogen to keep HO below 2%, and then sealed promptly. These samples were stored at 60°C and analyzed for the major degradation substances by HPLC on day 0, day 5, day 10, and day 30.

Characterization of IMI/CIL from Different Batches

Imipenem, cilastatin sodium, and IMI/CIL from 2021–1, 2021–2, and 2021–3 were characterized by X-ray powder diffraction (XRPD), polarized light microscopy (PLM), and laser diffraction particle size distribution (PSD), and the relationship between physical properties and stability of preparations were investigated.

XRPD Assay

The crystalline/amorphous nature of imipenem and cilastatin sodium in the solid state (powder form) was estimated by an X-ray diffractometer (D8 Advance, Bruker AXS, Karlsruhe, Germany). The patterns were collected in step scan mode at the rate of 0.02°/min. Cu Kα radiation (1.5418 Å) was used at 40 kV and 40 mA and the 2-theta scan range was set to 5°–60°. The diffraction data were analyzed with Origin 8.0 software (OriginLab, Massachusetts, United States).

PLM Assay

The microscopic morphology of imipenem and cilastatin sodium was examined using a XPN-990E polarized light microscope (Changfang Optical Instrument Co., LTD, Shanghai, China). The imipenem and cilastatin sodium samples were sprinkled onto a glass slide covered with a drop of dimethyl silicone oil to prevent exposure of the sample entities to the atmosphere, and then covered with another glass coverslip. Observed samples were determined to be in the crystalline form if they displayed uniform extinction, and amorphous materials if they did not display uniform extinction. Images of samples were collected using 100 × magnification, and TCapture software (Pooher Optoelectronics Technology Co., LTD. Shanghai, China) was used to obtain the measurements.

PSD Analysis

Imipenem, cilastatin sodium, and IMI/CIL were characterized by a TOPSIZER laser diffraction particle size analyzer (OMEC Instruments Co., Ltd, Zhuhai, China). PSDs were calculated on a volume basis following the Mie theory.[7] The particle refractive index and particle absorption index used were 1.52 and 0.1, respectively. All the measured particle diameters represent the area-equivalent or volume-equivalent spherical diameter based on the technique of size measurement.

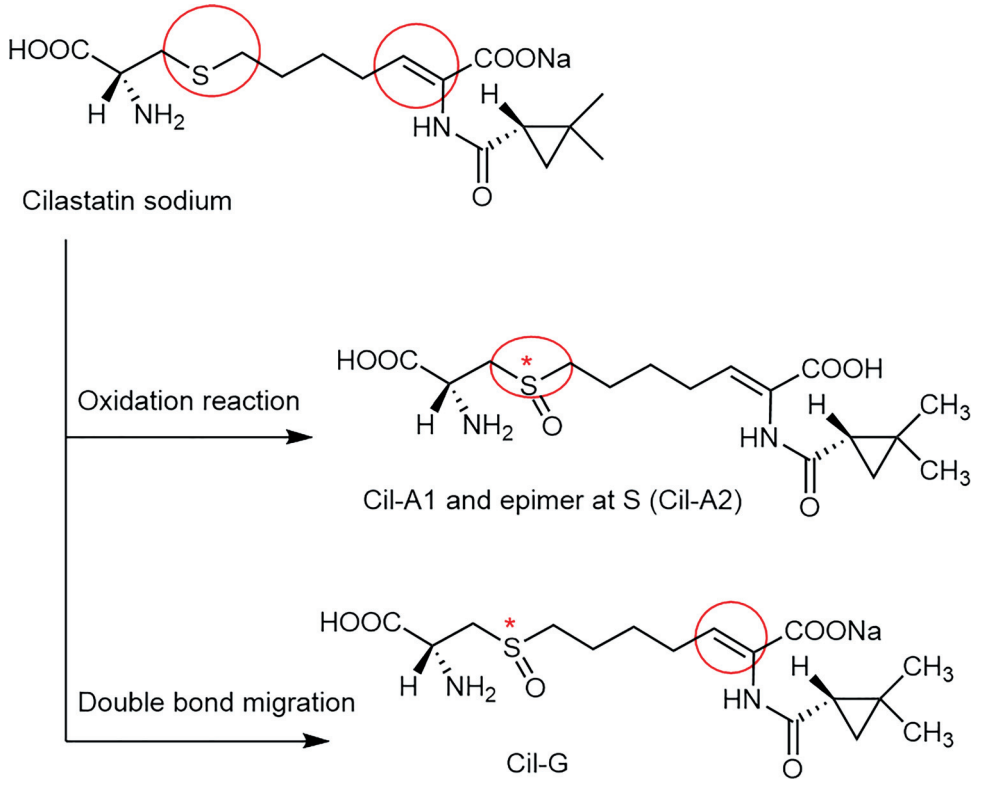
Statistical Analysis

Data were presented as mean of at least three trials, and value was recorded as mean ± standard error of the mean. To evaluate the statistical significance of the differences between groups, Student's *t*-test or one-way analysis of variance (ANOVA) was performed by using Excel (Microsoft Office 2016, United States). $p < 0.05$ level of significance was used for all analyses.

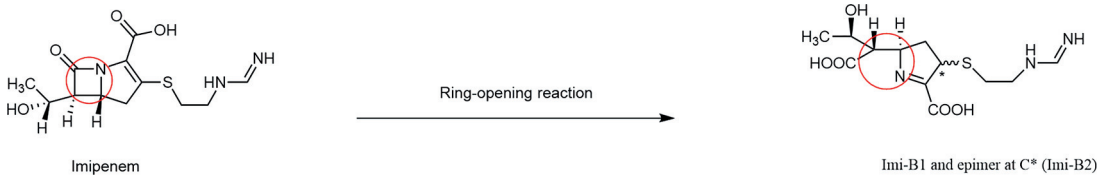
Results and Discussion

Degradation Products

Imipenem and cilastatin sodium could be degraded into many impurities in the stability studies. Three major degradation products of cilastatin sodium were identified as (*Z*)-7-[(*RS*)-[(2*R*)-2-amino-2-carboxyethyl]sulfinyl]-2-[[[(1*S*)-2,2-dimethylcyclopropyl]carbonyl]amino]hept-2-enoic acid (**Scheme 1**, Cil-A1), epimer (**Scheme 1**, Cil-A2) at S, and (*E*)-(2*RS*)-7-[[(2*R*)-2-amino-2-carboxyethyl]sulfanyl]-2-[[[(1*S*)-2,2-dimethylcyclopropyl]carbonyl]amino]hept-3-enoic acid (**Scheme 1**, Cil-G). Cil-A1 and Cil-A2 are oxidative



Scheme 1 Proposed degradation mechanism of cilastatin sodium.



Scheme 2 Proposed degradation mechanism of imipenem.

degradation products. The main impurities degraded by imipenem were imipenemoic acid (**Scheme 2**, Imi-B1) and epimer (**Scheme 2**, Imi-B2), which are hydrolytic degradation products. The above five impurities are the focus of this study.

HO Levels

Stability of IMI/CIL at Different HO Levels

The effect of oxygen on the stability of IMI/CIL is shown in ►**Fig. 1**. After storing at 60°C for 30 days, the total impurities of samples under 2, 5, and 20% of HO were $0.93 \pm 0.02\%$, $1.75 \pm 0.26\%$, and $2.71 \pm 0.19\%$, respectively. The degradation was significantly inhibited when HO was reduced ($p < 0.05$). This result indicated that HO has a significant effect on the degradation of IMI/CIL. Among the many degradation impurities of IMI/CIL, three impurities Cil-A1, Cil-A2, and Cil-G were identified to vary significantly with HO levels ($p < 0.05$). Impurities Cil-A1 and Cil-A2 are a pair of diastereoisomers, which are oxidative degradation products of cilastatin sodium. According to the molecular structures of the three impurities, the oxygen-mediated degradation mechanism of cilastatin sodium was speculated as shown in **Scheme 1**. Cilastatin sodium contains a sulfur atom, which makes it easy to oxidize. When cilastatin sodium is in an aerobic environment, the lone pair of electrons on the oxygen atom acts as a nucleophile to oxidize cilastatin sodium to produce Cil-A1 and Cil-A2. Cil-G is derived from the double bound migration of cilastatin sodium. Those results suggested that oxygen is involved in the degradation of cilastatin sodium. The stability of IMI/CIL can be greatly improved by controlling HO with nitrogen purging.

Stability of IMI/CIL from Different Batches at the Same HO Level

As shown in ►**Fig. 2**, the degradation rate of IMI/CIL from 2021–1 and 2021–2 was still higher than that from 2021–3 ($p < 0.05$) when the HO level of IMI/CIL was controlled at around 2% and stored at 60°C for 30 days. This result indicated that the stability of IMI/CIL is affected not only by HO levels, but also by other factors. It was found that two major impurities Imi-B1 and Imi-B2 increased faster in

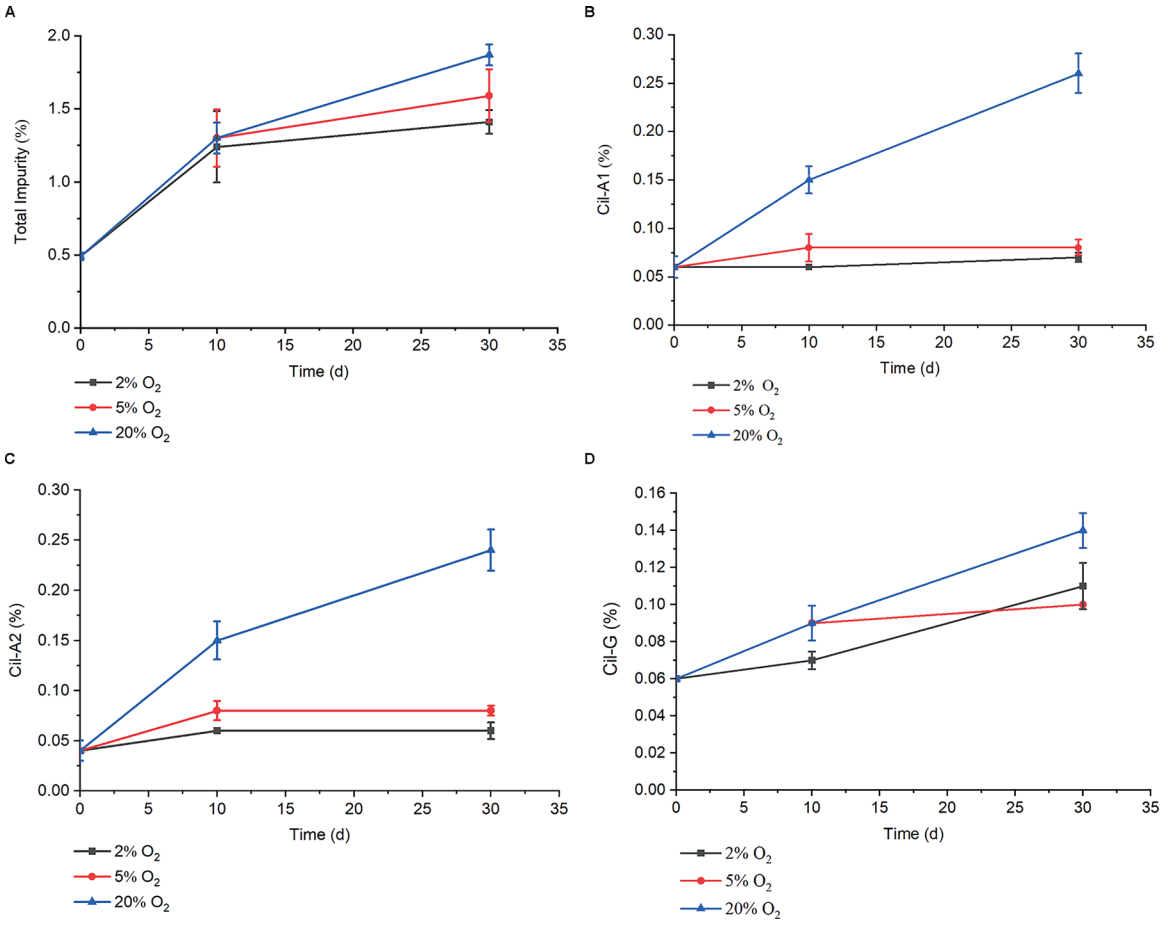


Fig. 1 Effect of HO levels on the stability of IMI/CIL from 2021–1 induced by stress condition at 60°C. **(A)** Increase in total impurity content (%). **(B)** Increase in Cil-A1 content (%). **(C)** Increase in Cil-A2 content (%). **(D)** Increase in Cil-G content (%). Cil-A1, cilastatin-impurity A1; Cil-A2, cilastatin-impurity A2; Cil-G, cilastatin-impurity G.

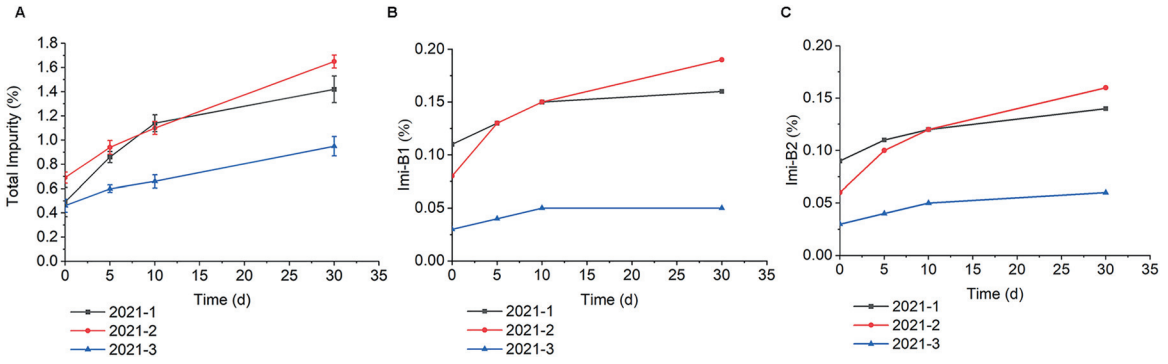


Fig. 2 Stability of IMI/CIL from different batches induced by stress condition at 60°C for 30 days. **(A)** Increase in total impurity content (%). **(B)** Increase in Imi-B1 content (%). **(C)** Increase in Imi-B2 content (%). Imi-B1, imipenem-impurity B1; Imi-B2, imipenem-impurity B2.

IMI/CIL from 2021–1 and 2021–2 than those from 2021–3 ($p < 0.05$), as shown in ►**Fig. 3**. Imi-B1 and Imi-B2 are a pair of diastereoisomers and hydrolytic degradation products of imipenem. Imipenem contains a β-lactam ring that is particularly sensitive to water. Many studies have shown that the degradation of β-lactam ring is related to water.[8,9] The β-lactam ring could be affected by OH^- and H^+ when it was exposed to water, resulting in the rupture of the amide bond on the quaternary ring.[10] The hydrolytic degradation mechanism of imipenem is related to the opening of the quaternary ring as shown in **Scheme 2**.

Effect of Water Content on Stability

Stability of Imipenem and Cilastatin at Different Relative Humidity

The stability of imipenem, cilastatin sodium, and IMI/CIL in different RH environments is shown in ►**Figs. 3** to **5**,

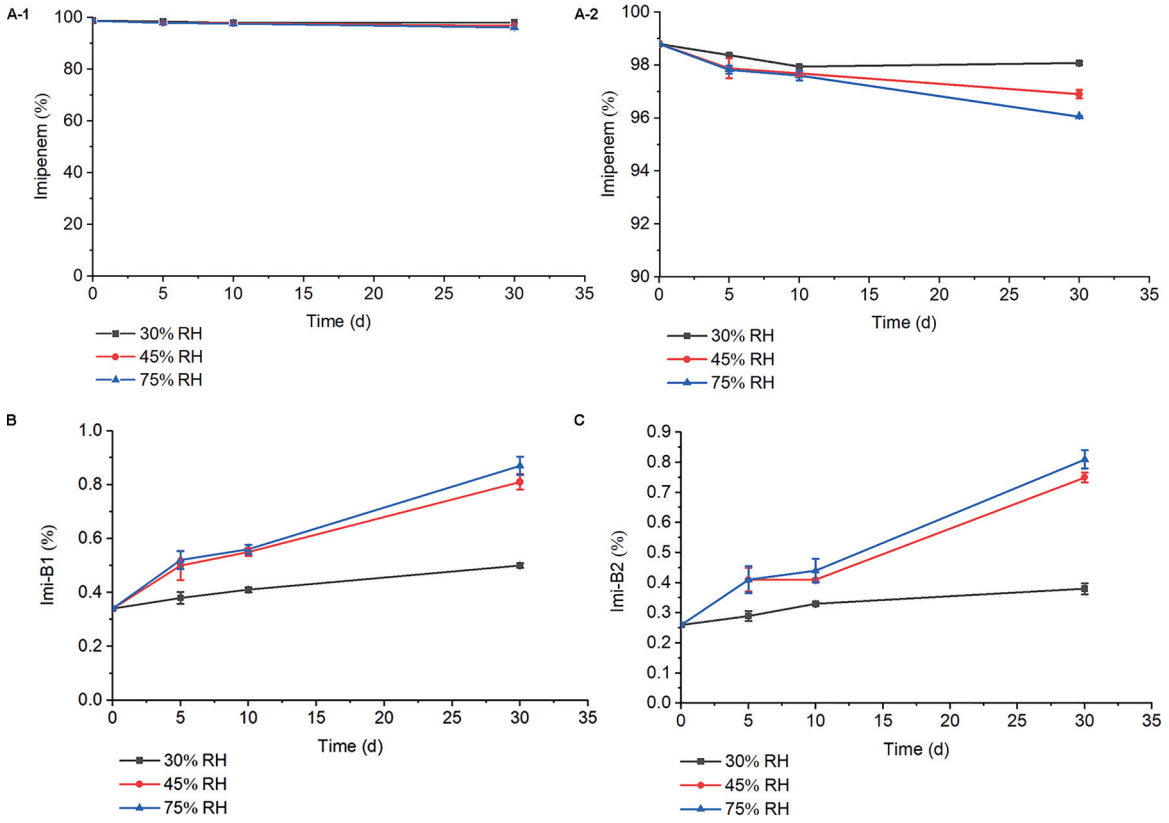


Fig. 3 Effect of RH on the stability of imipenem. **(A-1)** Decrease in imipenem content. **(A-2)** Partial enlarged view of (A-1). **(B)** Increase in Imi-B1 content (%). **(C)** Increase in Imi-B2 content (%). RH, relative humidity.

respectively. As shown in ►**Fig. 3**, after 30 days, the content of imipenem under RH 30, 45, and 75% were 98.07 ± 0.07%, 96.90 ± 0.2%, and 96.05 ± 0.03%, respectively. The higher the air humidity, the more obvious the decrease of imipenem content ($p < 0.05$). This result confirmed that the stability of imipenem was indeed related to water. Imi-B1 and Imi-B2 were the most significant growth impurities.

►**Fig. 4A** shows the stability of imipenem in IMI/CIL in different humidity environments after 30 days. Our data showed that the degradation of imipenem in IMI/CIL is more severe than that in imipenem alone ($p < 0.05$). After 30 days, the imipenem content in IMI/CIL was close to null at 45%RH and 75%RH, while the imipenem content in imipenem was still above 95%. These results indicated that cilastatin sodium had a significant effect on imipenem under high humidity ($p < 0.05$). Imi-B1 and Imi-B2 in IMI/CIL grew rapidly in the first 10 days, but decreased dramatically from day 10 to day 30 ($p < 0.05$), and were further degraded to other impurities (note: there was no such decrease in imipenem API at this time). Cilastatin sodium is an amorphous ingredient with hygroscopicity. The moisture absorbed by cilastatin may have allowed enough water to be in contact with crystalline imipenem under controlled RH conditions and induced its hydrolytic degradation.

Another phenomenon observed in this study, as shown in ►**Fig. 5**, is that imipenem could also affect the stability of cilastatin sodium. The degradation of cilastatin sodium in IMI/CIL was more severe than that of cilastatin sodium alone

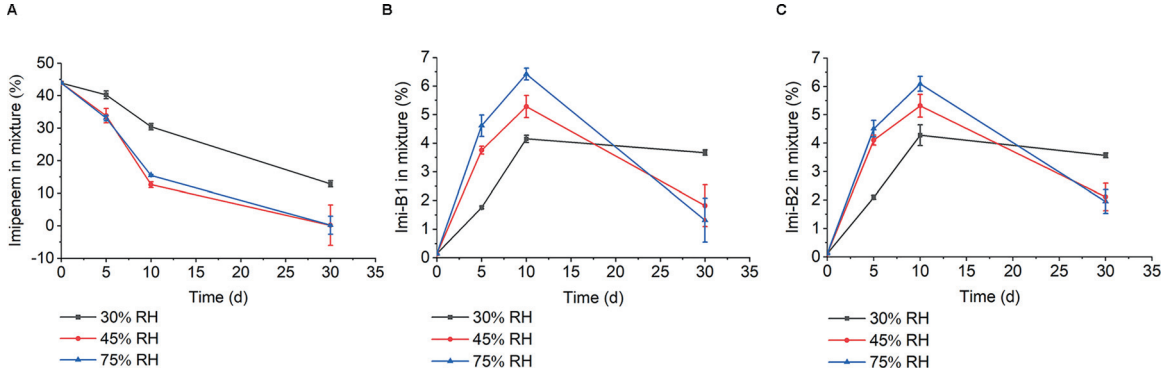


Fig. 4 Effect of RH on the stability of imipenem in 2021–1 preparation. **(A)** Decrease in imipenem content. **(B)** Increase in Imi-B1 content (%). **(C)** Increase in Imi-B2 content (%). RH, relative humidity; Imi-B1, imipenem-impurity B1; Imi-B2, imipenem-impurity B2.

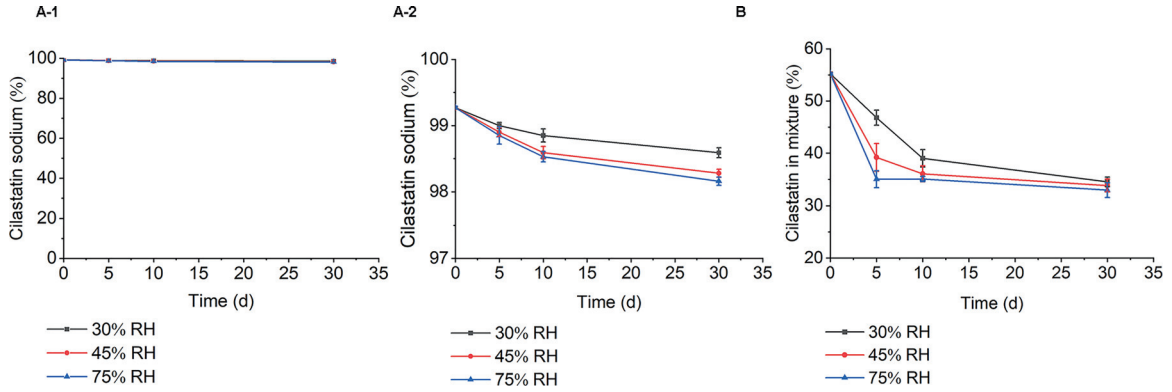


Fig. 5 Effect of RH on the content of cilastatin sodium in cilastatin sodium and IMI/CIL from 2021–1. **(A-1)** Decrease in cilastatin sodium content in cilastatin sodium. **(A-2)** Partial enlarged view of (A-1). **(B)** Decrease in cilastatin sodium content in IMI/CIL. RH, relative humidity.

under controlled RH conditions ($p < 0.05$), indicating that the presence of imipenem also accelerated the degradation of cilastatin sodium. Given above, water does have a significant effect on the stability of imipenem and cilastatin sodium. Imipenem and cilastatin sodium in IMI/CIL are more sensitive to moisture than individual imipenem or cilastatin sodium. Synergistic moisture sorption and degradation occurred under controlled RH conditions.

Water Content of IMI/CIL

The total water contents of IMI/CIL from 2021–1, 2021–2, and 2021–3 are shown in ►**Table 2** and ►**Fig. 6**. The total water contents of samples measured by TGA are as follow: 2021–2 > 2021–3 > 2021–1. However, as shown in ►**Fig. 3**, IMI/CIL from 2021–3 with the higher total water content had the best stability, rather than IMI/CIL from 2021–1 with the lowest total water content. The degradation rate of IMI/CIL is consistent with the free water content of IMI/CIL measured in a vacuum oven drying at 60°C as shown in ►**Table 3**: 2021–2 > 2021–1 > 2021–3 ($p < 0.05$). Total water content is a measurement of the total amount of water in samples, which includes free water, adsorbed water, and bound water.[11] The absorbed and bound water has reduced energy and different properties than pure water because it adsorbed on the drug surface or directly binds to the matrix through hydrogen or ionic binding.[11] Adsorbed water and bound water are not detrimental to the stability of the drug.[12,13] Simply knowing

Table 2 TGA results

Sample	Batches	TGA weight loss (%)
IMI/CIL	2021–1	4.41
	2021–2	4.84
	2021–3	4.65
Imipenem	2021–1	5.39
Cilastatin sodium	2021–1	1.25

Abbreviation: TGA, thermogravimetric analysis.

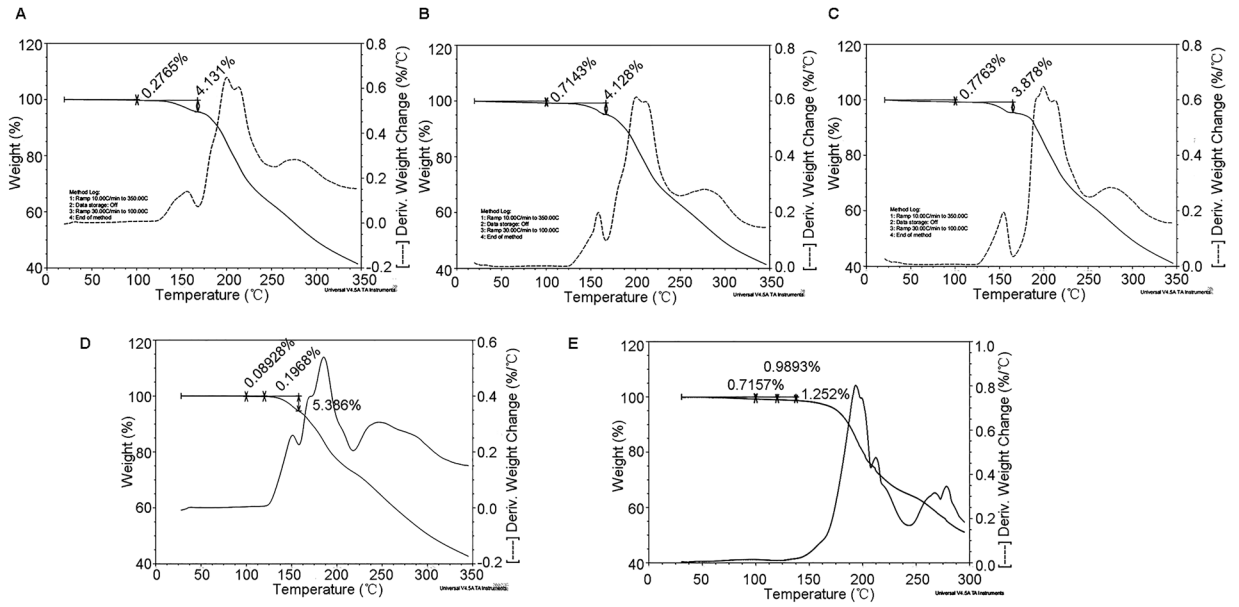


Fig. 6 TGA results of IMI/CIL imipenem and cilastatin sodium. **(A)** IMI/CIL from 2021–1. **(B)** IMI/CIL from 2021–2. **(C)** IMI/CIL from 2021–3. **(D)** Imipenem of from 2021–1. **(E)** Cilastatin sodium from 2021–1. TGA, thermogravimetric analysis.

Table 3 Results for vacuum drying at 60°C

Sample	Batches	Loss on drying (%)
IMI/CIL	2021–1	0.53 ± 0.05
	2021–2	0.77 ± 0.04
	2021–3	0.45 ± 0.05
Imipenem	2021–1	0.27 ± 0.03
Cilastatin sodium	2021–1	0.83 ± 0.07

Note: Each experiment was repeated three times.

the total water content may not be the most reliable method for understanding the effects of water on drug stability. Unlike adsorbed and bound water, free water has the same energy and properties as pure water, which is available for chemical reactions. The rate of drug reactivity increases with increasing content of free water. Consistent with this, this study further demonstrated that free water content is a better predictor of the safety and stability of imipenem and cilastatin sodium than total water content.

Drying Imipenem, Cilastatin Sodium, and IMI/CIL

The effects of reducing the free water content of imipenem, cilastatin sodium, and IMI/CIL on the stability of imipenem were investigated. As shown in ►**Fig. 7**, after storing at 60°C for 30 days, the total impurities in L1 and L2 were 3.09 ± 0.2% and 2.77 ± 0.05%, respectively. The stability of L2 (dried) is better than that of L1 (undried) ($p < 0.05$). Imi-B1 and Imi-B2 also decreased with the decrease of free water. This implies that the degradation of imipenem can be effectively controlled by reducing the free water content in imipenem. Although the free water content of undried imipenem was only 0.27 ± 0.03%, the presence of trace free water can still affect the growth of Imi-B1, Imi-B2, and total impurities ($p < 0.05$).

As shown in ►**Fig. 8**, the growth of Imi-B1 and Imi-B2 was significantly inhibited by drying cilastatin sodium ($p < 0.05$). This result confirmed that free water in cilastatin sodium could also affect the stability of imipenem, and the stability of imipenem could be improved by controlling the free water content of cilastatin sodium. As previously discovered by Yoshioka and Aso,[14] there is a phenomenon of water migration between different drug molecules. Cilastatin sodium exists in an amorphous state in the preparation, and it is easy to absorb moisture. Free water content of cilastatin sodium in IMI/CIL of 2021–1 was 0.83%, which was the main source of free water in IMI/CIL. Our data suggested that the stability of imipenem was affected by the intermolecular migration of water when imipenem was in contact with cilastatin sodium.

The effect of reducing the free water content of IMI/CIL on the stability of imipenem was investigated. As shown

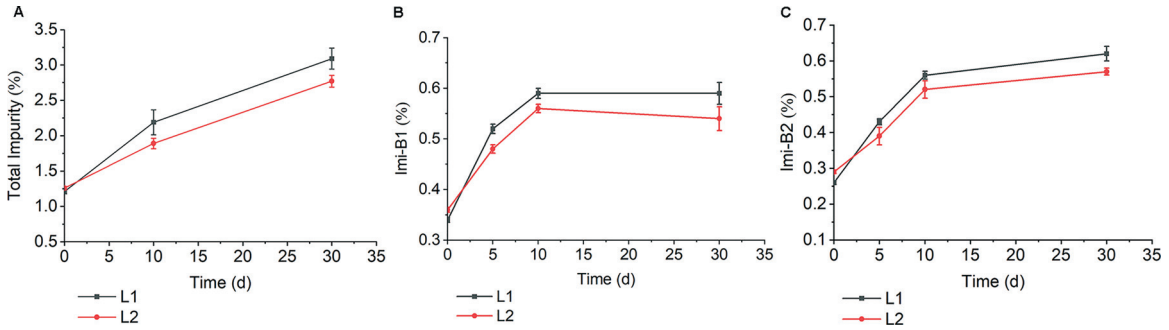


Fig. 7 Effect of reducing the free water content of imipenem on the stability of imipenem. **(A)** Increase in total impurity content (%); **(B)** Increase in Imi-B1 content (%); **(C)** Increase in Imi-B2 content (%). Imi-B1, imipenem-impurity B1; Imi-B2, imipenem-impurity B2.

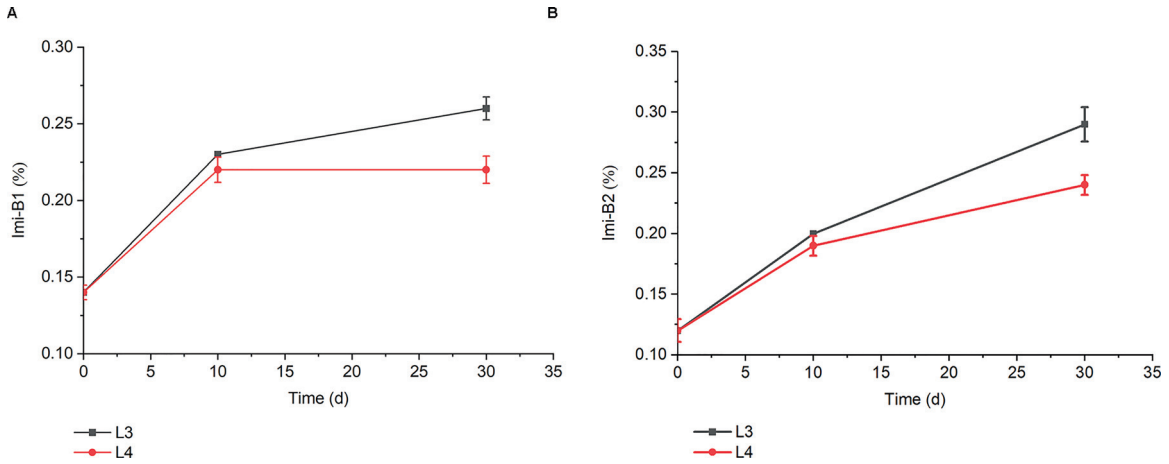


Fig. 8 Effect of reducing the free water content of cilastatin sodium on the stability of imipenem. **(A)** Increase in Imi-B1 content (%). **(B)** Increase in Imi-B2 content (%).

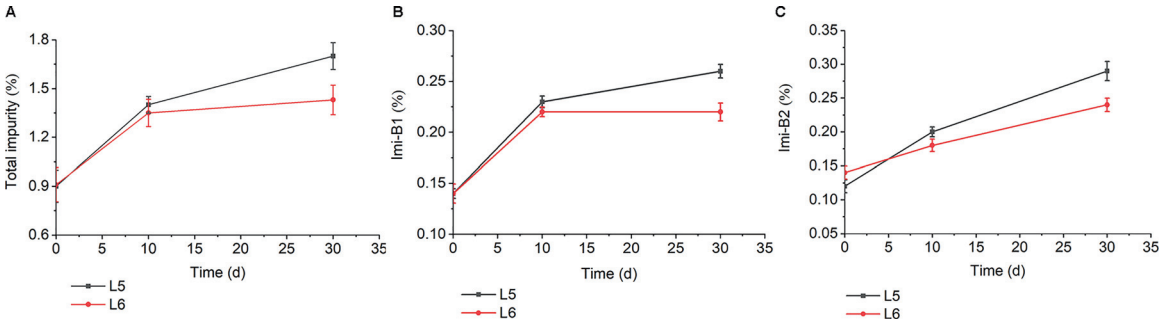


Fig. 9 Effect of reducing the free water content of preparation on the stability of imipenem. **(A)** Increase in total impurity content (%). **(B)** Increase in imi-B1 content (%). **(C)** Increase in imi-B2 content (%).

in ►**Fig. 9**, the stability of L6 was better than that of L5. The growth of total impurities, Imi-B1, and Imi-B2 was inhibited after drying IMI/CIL ($p < 0.05$). This result showed that the degradation of imipenem in IMI/CIL could be effectively prevented by reducing the free water content of IMI/CIL.

Characterization of IMI/CIL from Different Batches

Result from XRPD Analysis

The XRPD patterns of imipenem and cilastatin sodium are shown in ►**Fig. 10A** and **B.** XRPD analysis of imipenem revealed the presence of the sharp diffraction peak signals characteristic for the crystalline form, and the diffractogram of cilastatin sodium displayed a broad amorphous halo with the absence of well-defined and intense peaks, indicating that imipenem was crystalline in nature and cilastatin sodium was an amorphous substance. As shown in ►**Fig. 10C**, it is clear that the XRPD pattern of IMI/CIL is almost a superposition of the patterns contributed by imipenem and cilastatin sodium. The XRPD pattern of IMI/CIL from 2021–3 was consistent with that of 2021–1, indicating that the crystalline forms of the two IMI/CIL were consistent.

Result from PLM Analysis

As drugs' properties strongly depend on the chemical and physical constitution, the morphology of imipenem, cilastatin sodium, and IMI/CIL was explored with PLM. As shown in ►**Fig. 11**, a birefringence phenomenon was obviously observed in crystalline imipenem, whereas no visible birefringence phenomenon was observed in amorphous cilastatin sodium. The result was in a good agreement with the results obtained by XRPD. ►**Fig. 11E** revealed that imipenem produced by 2021–3 is granular in shape and is relatively uniform in size, neither elongated nor flat, and cilastatin sodium of 2021–3 is plate-shaped and relatively thick, not elongated. As shown in ►**Fig. 11(A–C)**, imipenem from 2021–1 formed in an elongated, fiber-like and needle-like shape, and cilastatin sodium was scale-like and angular and relatively thin. The form of imipenem and cilastatin sodium from 2021–2 is similar to that of 2021–1, but the particle size is much smaller. Hence, imipenem and cilastatin sodium from 2021–1 and B have larger specific surface areas.

For drugs with different specific surface areas, the free water content and the number of reactive groups exposed on the surface of drug particles are different. The larger the

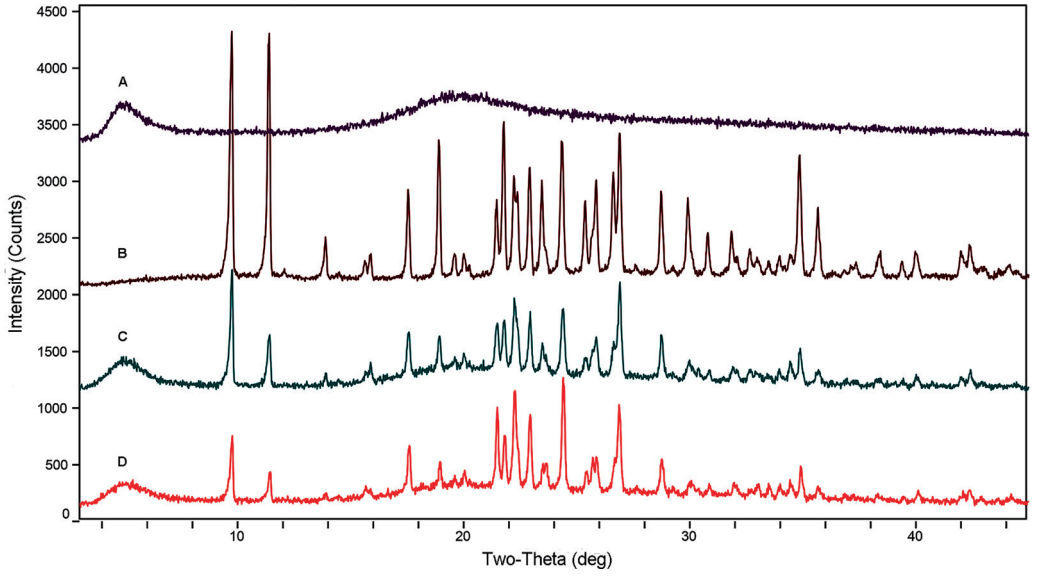


Fig. 10 XRPD diffractograms of cilastatin sodium, imipenem, and IMI/CIL. **(A)** Cilastatin sodium. **(B)** Imipenem. **(C)** IMI/CIL from 2021–1. **(D)** IMI/CIL from 2021–3. XRPD, X-ray powder diffraction.

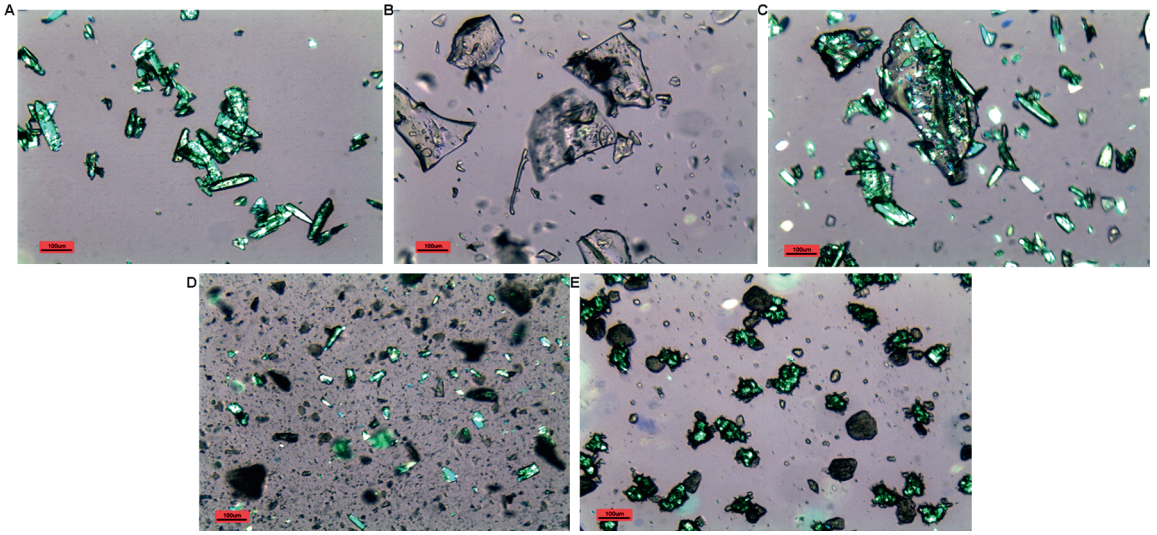


Fig. 11 Polarized microscopy images of imipenem, cilastatin sodium, and IMI/CIL. (A) Imipenem of from 2021–1. (B) Cilastatin sodium from 2021–1. (C) IMI/CIL from 2021–1. (D) IMI/CIL from 2021–2. (E) IMI/CIL from 2021–3.

specific surface area is, the higher free water content could be taken up and the more reactive groups are exposed on the surface. In addition, another phenomenon observed in this study is that large amounts of fine imipenem particles were adsorbed on the surface of cilastatin sodium from 2021–1 and 2021–2, which was probably caused by the electrostatic interaction. Consequently, the contact area between cilastatin sodium and imipenem from 2021–1 and 2021–2 was greatly increased, which could result in enhanced interaction between imipenem and cilastatin sodium. The adsorption kinetics is related by energy barriers associated with electrostatic interactions. The larger the specific surface area, the higher the free surface energy of cilastatin sodium; the smaller the imipenem particles, the more imipenem particles adsorbed on cilastatin sodium, thus, the greater the contact area between CIL and IMI. The water molecule mobility often plays an important role in the degradation rate.[15] Materials that contain more moisture were capable of transferring it to other ingredients.[16] In addition, in water adsorption, the water taken up is dependent on the available surface area.[16] The exposed surface of API particles is determined by the form of the drug.[17] Therefore, the larger the contact area between CIL and IMI, the more easily IMI absorbs free water migrating from CIL, and then CIL has a greater influence on IMI. This study confirmed that the micromeritic properties of drug particles, such as shape and size, are of essential importance for the stability of drugs.

Result from Laser Diffraction Particle Size Analysis

►**Fig. 12** illustrates the PSD curves of IMI/CIL from 2021–1, 2021–2, and 2021–3. It can be seen that the PSD of IMI/CIL from 2021–1 was relatively wide, and the PSD of IMI/CIL from 2021–3 was relatively narrow. Their largest volume fractions were both around 10 to 100 µm. However, due to the variability of particle shape, specific surface area may be different. The IMI/CIL of 2021–1 might have a larger specific surface area because imipenem of 2021–1 is elongated, fiber-like, and needle-like shape, and cilastatin sodium was scale-like, angular, and relatively thin. The particle size of IMI/CIL from 2021–2 was the smallest, mainly below 10 µm, and the specific surface area was the largest. Therefore, the specific surface areas of preparations from 2021–1, 2021–2, and 2021–3 were in the order of 2021–2 > 2021–1 > 2021–3, which was correlated well with the growth of Imi-B1 and Imi-B2 as shown in ►**Fig. 3**.

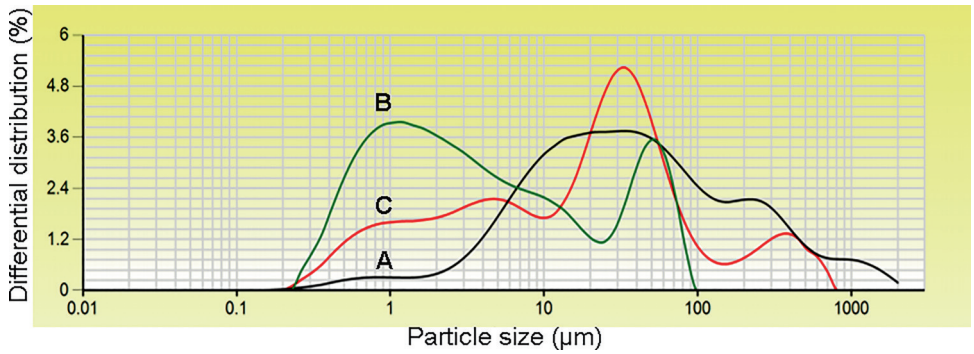


Fig. 12 Particle size distribution of IMI/CIL from different batches. (A) IMI/CIL of 2021–1. (B) IMI/CIL of 2021–2. (C) IMI/CIL of 2021–3.

Conclusion

In this study, the effects of HO levels, water content, particle shape, and particle size on the stability of IMI/CIL were investigated. Student's *t*-test and ANOVA were used to analyze the statistical significance of the differences between groups. It was shown that reducing HO level by nitrogen purging could significantly inhibit the degradation of cilastatin sodium. Oxygen caused some chemical reactions of cilastatin sodium, such as oxidation reaction and double bond migration. It was confirmed that moisture was detrimental to the stability of the imipenem. The higher the RH, the more serious the degradation of imipenem. Compared with 2021–3 with a higher total water content, the degradation of IMI/CIL was higher in 2021–1 with the lowest total water content. Moreover, free water content was responsible for the degradation of imipenem instead of the total water content. Free water in cilastatin sodium can also affect the stability of imipenem through water molecule migration. Controlling the free water content in imipenem or cilastatin sodium by vacuum oven drying can effectively improve the stability of imipenem. The shape and size of imipenem and cilastatin sodium particles also had a significant effect on the stability of IMI/CIL. The greater the surface area of imipenem and cilastatin sodium exposed in the air, the greater the effect of sodium cilastatin on imipenem. Based on the results obtained above, limiting HO level, controlling the content of free water, as well as the shape and size of imipenem and cilastatin sodium particles are highly recommended for the production of IMI/CIL in the future.

Conflicts of Interest

The authors declare no conflict of interest.

Acknowledgments

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