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ORIGINAL RESEARCH

Development of Amorphous Solid Dispersion to Improve the Dissolution and Antiproliferative Activity of Brazilin

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Introduction: Brazilin (BRZ), a major bioactive constituent of *Caesalpinia sappan*, exhibits promising anticancer activity but suffers from extremely poor aqueous solubility, limiting its oral bioavailability and therapeutic efficacy. Drug solubility is a critical determinant of absorption and therapeutic efficacy, so strategies to enhance the solubility of BRZ are essential. Amorphous solid dispersions (ASDs) have been widely employed to enhance the solubility and dissolution behavior of poorly soluble compounds. This study aimed to develop and characterize ASDs of BRZ using polyvinylpyrrolidone (PVP) as a polymer carrier, to investigate molecular interactions through in silico docking, and to evaluate their physicochemical properties, dissolution behavior, physical stability, and antiproliferative activity.

Methods: ASDs of BRZ with PVP were prepared using the solvent evaporation method with drug-to-polymer weight ratios of 1:1, 1:3, and 1:5. The formulations were characterized using powder X-ray diffraction (PXRD), differential scanning calorimetry (DSC), and Fourier-transform infrared (FT-IR) spectroscopy. Solubility, in vitro dissolution, and antiproliferative activity against DU145 prostate cancer cells were evaluated. Physical stability was evaluated over 30 days under dry (25 °C/0% RH) and humid (25 °C/90% RH) storage conditions using PXRD to detect any possible recrystallization.

Results: PXRD and DSC analyses confirmed the complete amorphization of BRZ within the ASD matrix, as evidenced by the disappearance of crystalline diffraction peaks and characteristic melting endotherms. FT-IR spectroscopy, supported by in silico studies, revealed hydrogen bond formation between the hydroxyl group of BRZ and the carbonyl group of PVP. The BRZ:PVP (1:5) formulation achieved a 5.7-fold increase in solubility compared to crystalline BRZ. Dissolution testing showed a characteristic spring-and-parachute profile, with ASDs maintaining supersaturation for a prolonged period. Moreover, enhanced antiproliferative activity against DU145 cells was observed for BRZ ASDs, with the lowest IC_{50} value of 14.02 µg/mL at a 1:5 ratio, compared to 20.12 µg/mL for crystalline BRZ. Stability testing showed that all ASDs maintained their amorphous form under dry storage conditions for 30 days, whereas samples stored at 90% relative humidity exhibited recrystallization.

Conclusion: BRZ ASDs using PVP effectively improved solubility, dissolution profile, and antiproliferative activity. The formulations demonstrated good stability under dry storage conditions but were sensitive to high humidity. These findings suggest that ASDs represent a promising strategy for enhancing the therapeutic potential of poorly soluble natural anticancer compounds.

Keywords: brazilin, amorphous solid dispersion, polyvinylpyrrolidone, dissolution, antiproliferative effects

Introduction

Drug solubility is an important factor in drug absorption and medication effectivity. Improving the solubility and maintaining the supersaturation of poorly water-soluble drugs are crucial for enhancing their oral bioavailability.[1] Drug substances with low water solubility are becoming increasingly common in pharmaceutical development; however, their limited solubility often results in poor bioavailability when administered orally.[2–5] Several formulation strategies have been widely explored to enhance the solubility of poorly water-soluble compounds. These include salt formation,

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co-solvent systems, inclusion complexation (such as cyclodextrin complexes), lipid-based formulations, and nanotechnology-based approaches, including nanoparticles and solid lipid carriers. Each method offers distinct advantages; however, their applicability depends on the physicochemical characteristics of the drug molecule.[6]

To enhance the dissolution and oral bioavailability of poorly water-soluble drugs, amorphization is one of the most effective pharmaceutical approaches available.[7] Amorphous drug formulations, characterized by their high Gibbs free energy, offer significant potential for enhancing drug delivery. This is attributed to their capability to form supersaturated aqueous solutions of drugs with low water solubility. In such a supersaturated state, drug absorption across biological membranes increases due to the elevated thermodynamic activity that surpasses the drug's inherent solubility.[8] Amorphization is considered a highly effective approach to enhance the dissolution rate and bioavailability of drugs with low water solubility. Unlike their crystalline forms, amorphous drugs possess a disordered molecular arrangement and elevated free energy, which contribute to improved solubility in water.[9] However, amorphous drugs are thermodynamically unstable and prone to recrystallization during storage or upon contact with water, making them challenging to use as standalone components in solid formulations.

To address this, excipients are often incorporated to prevent recrystallization. One effective strategy is the use of amorphous solid dispersions (ASDs), where the drug is embedded within a polymer matrix to maintain its amorphous state.[10] Intermolecular interactions between the drug and polymer in ASDs contribute to enhanced solubility and dissolution rates while also improving the physical stability of the drug.[11] These strong interactions effectively prevent the recrystallization of amorphous drugs, highlighting the importance of selecting an appropriate polymer carrier. A suitable polymer not only facilitates the transformation of a crystalline drug into its amorphous form but also stabilizes the dispersion by reducing molecular mobility and increasing the glass transition temperature (T_g).[12] In silico molecular docking can provide mechanistic insight into hydrogen bond formation and non-covalent interactions between BRZ and PVP, supporting the rational design of ASDs and complementing experimental evaluations. Such integration of computational and experimental approaches remains limited for BRZ, highlighting the novelty of the present study.

This study aims to explore the interaction mechanisms between brazilin (BRZ) and a polymer at the molecular level, and how these interactions influence key pharmaceutical properties such as dissolution behavior and physical stability. BRZ, a promising anticancer agent, was chosen as a model for poorly water-soluble drugs due to its low solubility and limited oral bioavailability. Polyvinylpyrrolidone (PVP) was selected as the polymer carrier, given its widespread use in ASDs formulations and its proven efficacy in preventing drug recrystallization both during storage and upon dispersion in aqueous environments.[13,14] In vitro cytotoxicity, dissolution, homogeneity, and apparent solubility were all improved by the successful preparation of amorphous solid dispersions based on PVP.[15] PVP has been reported to be particularly effective in inhibiting drug crystallization and forming a stable amorphous matrix, thereby enhancing the dissolution rate.[16]

BRZ is the main compound in *Caesalpinia sappan* that has potential as an anticancer agent, but its solubility is very low, resulting in low bioavailability. BRZ is soluble in organic solvents, has a boiling point above 150°C, and is used at relatively low concentrations, making it suitable for ASDs preparation using the solvent evaporation technique.[17] Amorphous solid dispersions not only improve the solubility and oral bioavailability of poorly water-soluble anticancer drugs, but also enhance their pharmacokinetic profiles, leading to improved therapeutic efficacy and safety.[9]

In this research, BRZ ASDs were developed utilizing PVP through the solvent evaporation technique. The resulting formulation was characterized using analytical instruments including X-ray Diffraction (XRD), Differential Scanning Calorimetry (DSC), and Fourier Transform Infrared Spectroscopy (FTIR). Additional evaluations will include solubility testing, entrapment efficiency, dissolution studies, physical stability assessments, and antiproliferative activity testing against DU145 prostate cancer cells. To our knowledge, studies on BRZ ASDs remain limited, particularly those integrating physicochemical characterization with biological performance evaluation. The novelty of this work lies in the development of a PVP-based BRZ ASD using solvent evaporation and the simultaneous assessment of drug-polymer interactions, dissolution behavior, physical stability, and antiproliferative activity. This integrated evaluation provides a comprehensive understanding of both formulation performance and molecular mechanisms, highlighting the potential of PVP-based ASDs for enhancing the oral bioavailability and therapeutic efficacy of poorly water-soluble anticancer compounds.

Materials and Methods

Materials

BRZ and PVP K30 were purchased from Chengdu Biopurify Phytochemicals (Sichuan, China) and JH Nanhang Life Sciences Co., Ltd. (Zhejiang, China), respectively. Methanol, acetonitrile (HPLC grade), and potassium dihydrogen phosphate were obtained from Merck (Darmstadt, Germany). Potassium bromide (KBr) was used for FTIR analysis. Dimethyl sulfoxide (DMSO), MTT reagent, and other cell culture reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA). Phosphate buffer solutions were prepared using analytical-grade reagents. All chemicals and solvents were of analytical grade and used without further purification. The chemical structures of BRZ and PVP are shown in Figure 1.

In silico Study

The 3D structures of BRZ and PVP monomer were retrieved from the PubChem database. Molecular docking simulations were performed to investigate the interaction between BRZ and PVP using AutoDock 4.2.[18,19] Before docking, ligand and receptor preparations were carried out using AutoDockTools version 1.5.6, including the addition of polar hydrogens, Gasteiger charges, and the conversion of structures into the required PDBQT format. The docking grid box was set to 40 × 40×40 points with a grid spacing of 0.375 Å, centered at coordinates x = 0.123, y = 0.069, and z = 0.172. The genetic algorithm (GA) parameters were configured with 100 independent runs, a maximum of 2,500,000 energy evaluations, and 27,000 generations per run. Docking results were analyzed based on binding energy and interaction types.

Preparation of ASDs

ASDs were prepared using the solvent evaporation method. BRZ and PVP were dissolved in methanol at drug-to-polymer weight ratios of 1:1, 1:3, and 1:5. The resulting solutions were evaporated using a Buchi Rotavapor-R (Buchi Corp., New Castle, DE, USA) attached to a water bath (BM200, Yamato Scientific America, Santa Clara, CA, USA) maintained at 30 °C. The residues were dried at 30 °C for 72 hours in an oven to obtain dry ASD powders.

Powder X-Ray Diffraction

PXRD patterns were collected using a Kristalloflex diffractometer (Bruker D8 Advance, Siemens, Berlin, Germany) with the following settings: 40 kV, 40 mA, Cu target with Ni filter, scanning rate of 1°/min, and a 2θ range of 10–40°.

Differential Scanning Calorimetry (DSC)

DSC measurements were performed using a Shimadzu DSC-60 Plus (Kyoto, Japan). Samples (3–5 mg) were sealed in aluminum pans and scanned from 0 to 280 °C at a heating rate of 10 °C/min under a nitrogen purge of 20 mL/min.

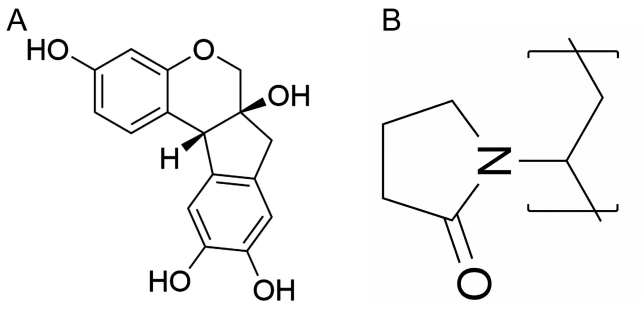

Figure 1 Chemical structure of (**A**) BRZ and (**B**) PVP K30 (ChemDraw).

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Fourier-Transform Infrared Spectroscopy (FTIR)

FTIR spectra were obtained using a Shimadzu Prestige-21 spectrometer (Kyoto, Japan). Each sample (1–2 mg) was mixed with 200–250 mg of KBr, ground, and compressed into a pellet under 60 psi. Spectra were recorded in the 4000–400 cm^{-1} range.

Solubility Studies

The crystalline and amorphous solubility of BRZ was determined in 50 mM phosphate buffer (pH 7.4) at 37 °C. Excess amounts of each sample were shaken for 48 hours, filtered through 0.45 μm membranes, and analyzed by HPLC after dilution with the mobile phase. Phosphate buffer at pH 7.4 was selected to simulate physiological conditions relevant to systemic circulation and to enable comparison with dissolution and antiproliferative studies conducted under near-physiological environments.

HPLC Analysis

HPLC analysis was performed using an Agilent system equipped with an Eclipse Plus C18 column (4.6 × 150 mm, 5 μm) maintained at 30 °C. The mobile phase consisted of methanol:water (95:5, v/v) at a constant flow rate, with detection at 254 nm. The injection volume was 20 μL. Calibration curves were constructed in the concentration range of 4–128 μg/mL, showing good linearity ($R^2 > 0.999$). The limit of detection (LOD) and limit of quantification (LOQ) were determined based on signal-to-noise ratios of 3:1 and 10:1, respectively. The analytical method was adapted from previously reported procedures for BRZ with minor modifications.

Entrapment Efficiency

The entrapment efficiency study was conducted to evaluate the extent of drug incorporation within the polymeric matrix and to assess the influence of drug-to-polymer ratio on formulation efficiency and content uniformity.

Entrapment efficiency was determined by dissolving each ASDs sample in methanol to achieve a BRZ concentration of 100 μg/mL. After stirring for 30 minutes, the solutions were filtered (0.45 μm), diluted with acetonitrile, and analyzed by HPLC.

$$\text{Entrapment efficiency}(\%) = (\text{Measured concentration} / \text{Theoretical concentration}) \times 100.$$

In vitro Dissolution

Dissolution studies were performed using USP Apparatus II (paddle method). ASD powders equivalent to 40 μg/mL of BRZ were directly dispersed into 500 mL of phosphate buffer (pH 7.4) at 37 °C. The medium was stirred at 150 rpm, a commonly used condition for poorly soluble compounds to ensure uniform dispersion without excessive hydrodynamic effects. Samples (5 mL) were withdrawn at predetermined intervals (1–150 min), filtered through 0.45 μm membranes, diluted with acetonitrile, and analyzed by HPLC.

Storage Stability

Physical stability of ASDs was tested under two conditions for 30 days: (a) 25 °C/0% RH in a desiccator with silica gel, and (b) 25 °C/90% RH using a desiccator with saturated potassium nitrate. PXRD was used to evaluate changes in crystallinity post-storage.

Cancer Cell Inhibition Assay

The cytotoxicity of BRZ and its ASDs was assessed using the MTT assay on DU145 (prostate cancer) and Vero (normal) cell lines. Cells (3×10^4/well) were seeded in 24-well plates and incubated for 24 h at 37 °C in a 5% CO_2 atmosphere. Media were replaced with fresh media containing BRZ or ASDs. After 48 h, MTT (0.5 mg/mL) was added and incubated for 4 h. Formazan crystals were dissolved in 1000 μL DMSO and absorbance was read at 595 nm using an ELISA reader. Cell viability was calculated as a percentage of control absorbance. Untreated cells served as the negative control, while

vehicle-treated cells were used as solvent controls. The effect of PVP alone was also evaluated at equivalent concentrations and showed no significant cytotoxicity within the tested range.

Results

In silico Study

Molecular docking between BRZ and the PVP polymer was performed using AutoDock 4.2, with 100 docking runs conducted to identify the optimal binding configuration based on interaction affinity. All predicted conformations clustered into a single group (cluster rank = 1), indicating a consistent binding orientation. The minimum binding free energy was −2.71 kcal/mol, with an average value of −2.63 kcal/mol. Visualization in both 3D and 2D formats (Figure 2) revealed a hydrogen bond between the hydroxyl (-OH) group of BRZ's aromatic ring and the carbonyl (C=O) group of a PVP monomer unit. The hydrogen bond distance was measured at 2.1 Å, which falls within the typical range for stable hydrogen bonds (1.5–3.2 Å).[20]

In addition, several hydrophobic interactions, including pi–alkyl and pi–sigma types, were identified between the aromatic rings of BRZ and the alkyl chains of PVP, with interaction distances ranging from 2.6 Å to 4.6 Å.[21] Such interactions contribute significantly to complex stabilization, particularly in polymer-based drug delivery systems, by enhancing the affinity between active molecules and the polymer matrix.[22]

The critical role of non-covalent forces, such as hydrogen bonding and hydrophobic interactions, in stabilizing molecular complexes has been extensively reported. Hydrogen bonds are key in dictating molecular orientation and

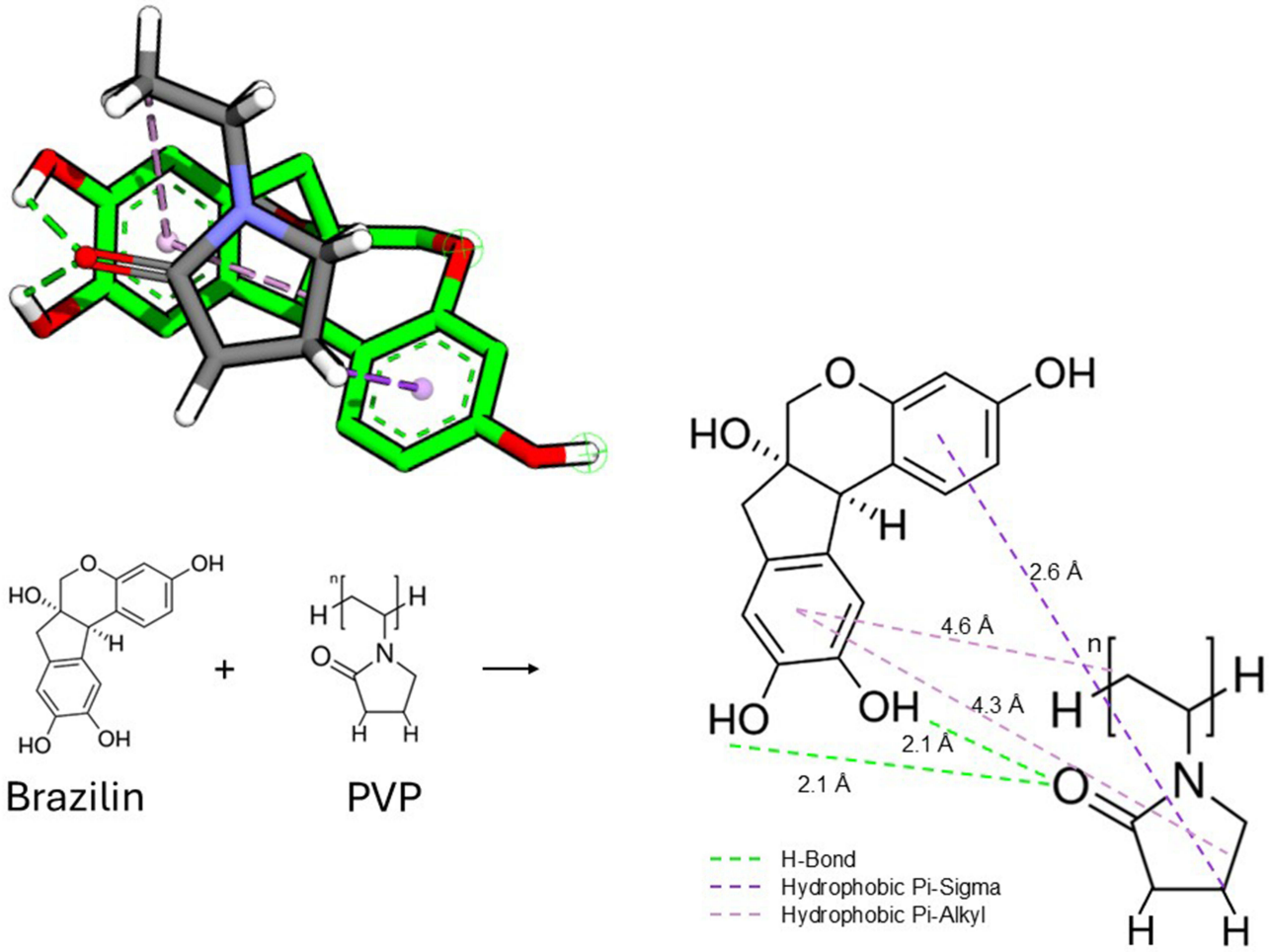

Figure 2 3D and 2D visualization of BRZ and PVP interactions after molecular docking simulation.

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complex formation, whereas hydrophobic interactions promote molecular alignment through water exclusion, thereby strengthening the overall stability of the complex.[17]

In comparison with the previous findings on the interaction between BRZ and PVP, hydrogen bonding also occurred at the carbonyl group, with a bond length of approximately 1.54 Å and a binding energy of −1.5 kcal/mol.[8] In contrast, the BRZ–PVP complex exhibited a binding energy of −2.71 kcal/mol, indicating a slightly stronger interaction, though still weak relative to strong drug–target bindings. These results suggest that PVP interacts with BRZ through both hydrogen bonding and hydrophobic forces, supporting its potential as an excipient in pharmaceutical formulations, such as nanoparticles or drug delivery matrices. Furthermore, these interactions imply that BRZ can be dispersed or encapsulated within a PVP matrix without undergoing covalent modification. These in silico findings provide mechanistic insight into the molecular interactions between BRZ and PVP, supporting the enhanced solubility, stability, and antiproliferative activity observed experimentally, and highlighting the novelty of integrating computational predictions with experimental evaluations in BRZ ASD development.

Powder X-Ray Diffraction

PXRD analysis revealed distinct diffraction peaks for crystalline BRZ, whereas PVP exhibited a halo pattern, indicative of its amorphous nature (Figure 3). Similar diffraction peaks were present in BRZ obtained via solvent evaporation, suggesting that the compound remained crystalline, likely due to its pronounced tendency to recrystallize.

In contrast, the physical mixture of BRZ with PVP exhibited distinct diffraction peaks in the PXRD profiles. Conversely, BRZ formulated as an ASDs BRZ with PVP via solvent evaporation displayed a halo pattern without detectable diffraction peaks across all weight ratios, confirming that amorphous ASDs BRZ can be successfully obtained through this method.

Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry (DSC) analysis was used to confirm the transformation of BRZ from a crystalline to an amorphous form. The crystalline BRZ exhibited an endothermic melting peak at approximately 250 °C, corresponding to its melting point (Figure 4). This peak was absent in all BRZ–PVP amorphous solid dispersions (ASDs) across the tested weight ratios after heating, indicating successful amorphization in the presence of PVP. Furthermore, the DSC data suggest that even at a 1:1 ratio, the polymer content was sufficient to stabilize the amorphous BRZ. Combined PXRD and DSC results confirm that the solvent evaporation method effectively produced BRZ–PVP ASDs. As shown in Figure 4,

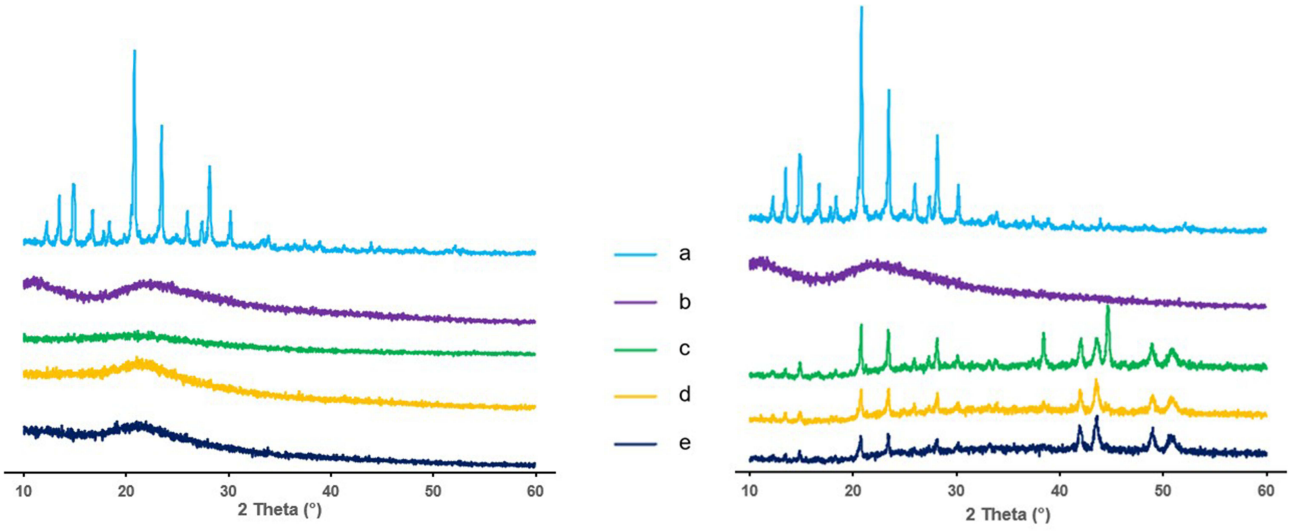

Figure 3 X-ray diffraction pattern of BRZ:PVP amorf solid dispersion (left) and physical mixture (right). (a) BRZ, (b) PVP, (c) BRZ:PVP (1:1), (d) BRZ:PVP (1:3), (e) BRZ:PVP (1:5).

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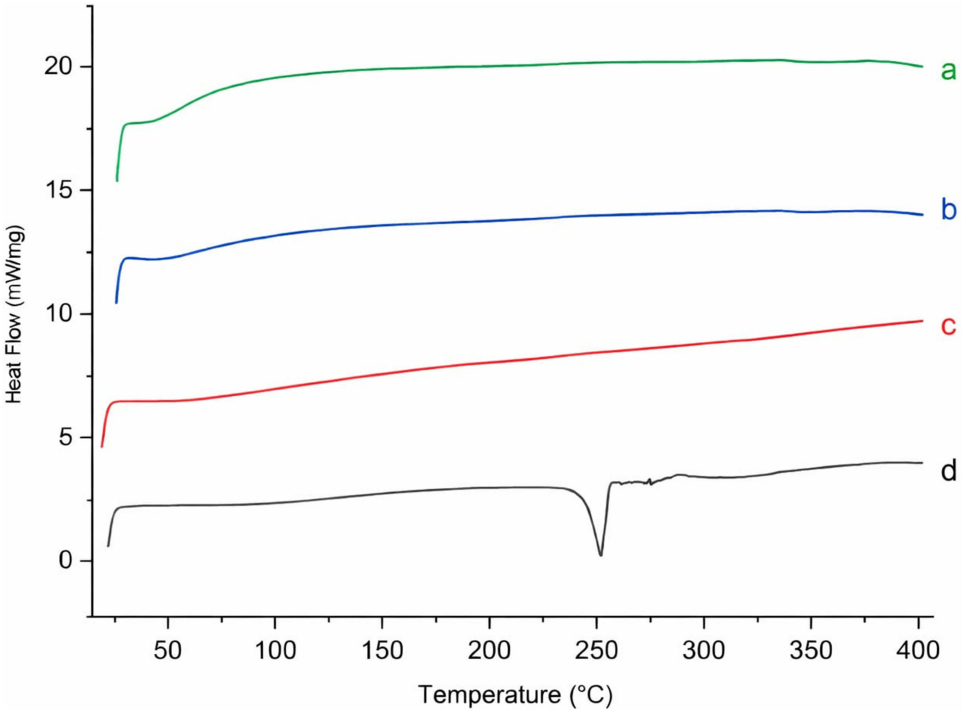

Figure 4 DSC curves of BRZ and BRZ-PVP ASDs with various weight ratios. (a) BRZ:PVP (1:5), (b) BRZ:PVP (1:3), (c) BRZ:PVP (1:1), (d) BRZ.

the characteristic melting peak of crystalline BRZ at around 250 °C disappears in the BRZ–PVP ASD systems at different weight ratios (1:5, 1:3, and 1:1), indicating successful amorphization.

Fourier-Transform Infrared Spectroscopy (FTIR)

BRZ possesses several key functional groups that influence its chemical behavior (Figure 5). The hydroxyl (-OH) groups attached to the aromatic ring contribute to polarity and hydrogen-bonding capacity, while methoxy (-OCH_3) substituents

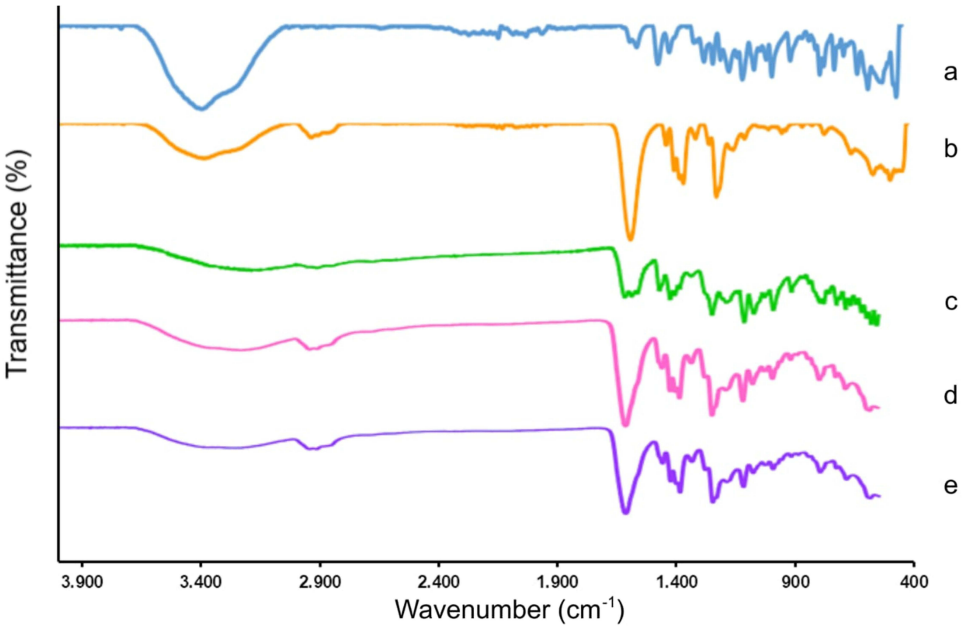

Figure 5 FTIR spectra of BRZ, PVP, and BRZ-PVP with various weight ratios. (a) BRZ, (b) PVP, (c) BRZ-PVP (1:1), (d) BRZ-PVP (1:3), (e) BRZ-PVP (1:5).

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present in certain derivatives interact with the aromatic system, enhancing solubility in polar solvents.[20] Its conjugated aromatic ring system further increases molecular stability and imparts distinctive optical properties. Interaction with PVP was evidenced by a shift in wave number, consistent with hydrogen bond formation between BRZ's hydroxyl groups and the carbonyl groups of PVP. This interaction suggests that PVP facilitates improved solubility of BRZ by forming a more stable and soluble complex.

Solubility Studies

The solubility test was conducted to evaluate the relative hydrophobicity between the polymer and the model compound. The equilibrium solubility of BRZ was measured in 50 mM phosphate buffer (pH 7.4) at 37 °C. The crystalline form of BRZ, where the molecules predominantly exist in the un-ionized state, exhibited an equilibrium solubility of 9.88 ± 0.2211 μg/mL, indicating extremely poor aqueous solubility. As shown in Table 1, the presence of PVP markedly enhanced solubility. In other words, the solubility of amorphous BRZ increased proportionally with higher polymer concentrations in the ASDs. This observation is consistent with previous findings reporting that PVP improves drug solubility through a solubilization effect.[23]

At 48 hours, the 1:5 BRZ-to-PVP ratio achieved a 5.7-fold increase in solubility compared to the pure compound, suggesting a sustained improvement over extended periods. The enhancement can be attributed to drug solubilization, reducing the solute's thermodynamic activity, allowing amorphous drug solubility to be increased by adding solubilizing agents such as polymers.[24,25] The solubility improvement of BRZ in the presence of PVP may involve multiple mechanisms, including complex formation with PVP, transformation from a crystalline to an amorphous state, reduction in particle size, and self-assembly into nanomicelles. Previous reports also indicate that the BRZ–PVP complex facilitates the encapsulation of hydrophobic molecules within its core, thereby enhancing aqueous solubility.[26,27] Nevertheless, amorphization is likely the primary factor for solubility enhancement, as earlier studies showed that PVP alone exerts only a minor effect on thermal equilibrium solubility, particularly at concentrations above 2000 μg/mL.[8]

The solubility of crystalline and amorphous BRZ in phosphate buffer is presented in Table 1.

Entrapment Efficiency (EE)

This study was conducted to assess the influence of the ASD (drug-to-polymer amorphous dispersion) ratio on the entrapment efficiency of BRZ using PVP as the polymer. The findings revealed a decreasing trend in AUC, concentration, and entrapment efficiency as the polymer content in the formulation increased.

The entrapment efficiency of BRZ–PVP was determined for all prepared formulations by dispersing the samples in methanol. The results demonstrate that BRZ was effectively incorporated into PVP resulting in a high concentration within the ASDs system.

The entrapment efficiency of BRZ amorphous solid dispersions is shown in Table 2

The results indicated that the lowest entrapment efficiency was obtained at an ASDs BRZ ratio of 1:1 (70.99 ± 0.42%), with a progressive increase at ratios of 1:3 (87.50 ± 0.62%) and 1:5 (89.86 ± 0.38%). This increase can be attributed to variations in the interaction between BRZ and the PVP polymer. At a 1:5 ratio, the polymer content appears sufficient to establish an optimal entrapment network through hydrogen bonding and van der Waals interactions, thereby enhancing trapping efficiency.

Table 1 Crystalline ($S_{c,B}$) and Amorphous ($S_{a,B}$) Solubility of BRZ (B) in 50 mM Phosphate Buffer at pH7.4

Sample	Sc B	Sa B	Sa B/Sc B
ASD BRZ:PVP (1:1)	9.88 ± 0.22	44.51 ± 0.47	4.5
ASD BRZ:PVP (1:3)	9.88 ± 0.22	51.66 ± 0.61	5.2
ASD BRZ:PVP (1:5)	9.88 ± 0.22	56.13 ± 0.47	5.7

Table 2 Entrapment Efficiency (EE) of BRZ Amorphous Solid Dispersion

Sample	x1	x2	x3	Mean EE (%)	SD	RSD
ASD BRZ:PVP (1:1)	71.11	71.34	70.53	70.99	0.42	2%
ASD BRZ:PVP (1:3)	86.91	87.46	88.14	87.50	0.62	2%
ASD BRZ:PVP (1:5)	89.62	89.66	90.30	89.86	0.38	2%

In vitro Dissolution

The dissolution behavior of BRZ in ASD was evaluated in 50 mM phosphate buffer (pH 7.4) at 37 °C under non-sink conditions (Figure 6). The crystalline form of BRZ dissolved slowly, reaching only about 40 µg/mL. In comparison, BRZ–PVP ASD showed enhanced dissolution relative to the crystalline material, presenting a spring–parachute profile typical of amorphous formulations. This indicates that BRZ–PVP ASD disperses well in the medium; however, the concentration gradually decreased over time due to recrystallization. The higher concentration observed for the ASDs sample can be explained by the greater amorphous solubility compared to the crystalline state. Under humid conditions, water uptake lowered the glass transition temperature (Tg) of BRZ i ASDs, thereby facilitating recrystallization.

Furthermore, the sticky mass detected in ASDs BRZ under humidified environments is likely associated with the samples being in a supercooled liquid state. The generally low dissolution of both crystalline and amorphous forms may also be influenced by their poor wettability, although this assumption still requires verification through additional experiments.

Statistical analysis revealed significant differences in dissolution performance between crystalline BRZ and all ASD formulations ($p < 0.05$). Among the tested ratios, the 1:5 BRZ:PVP formulation exhibited the highest dissolution rate, demonstrating superior performance compared to the other groups.

Storage Stability

The formulation of BRZ as an amorphous solid dispersion with PVP exhibited halo patterns even after 30 days of storage at 25 °C under both 0% and 90% relative humidity (RH) conditions, as shown in Figure 7. This stability may be

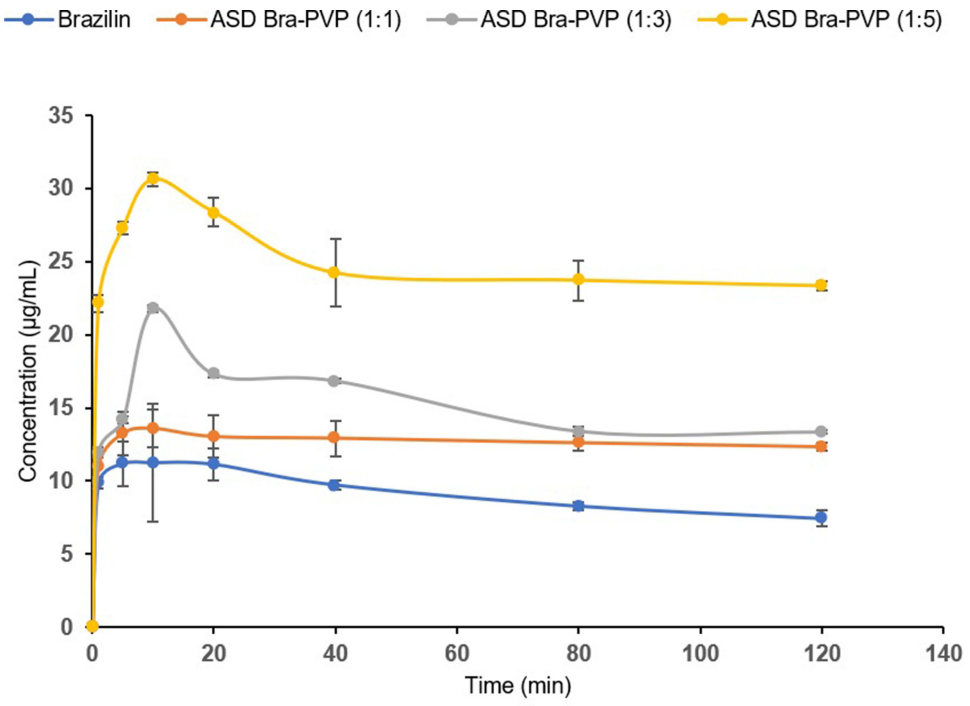

Figure 6 Dissolution profiles of each sample in 50 mM phosphate buffer (pH 7.4) at 37 °C (n = 3, mean SD).

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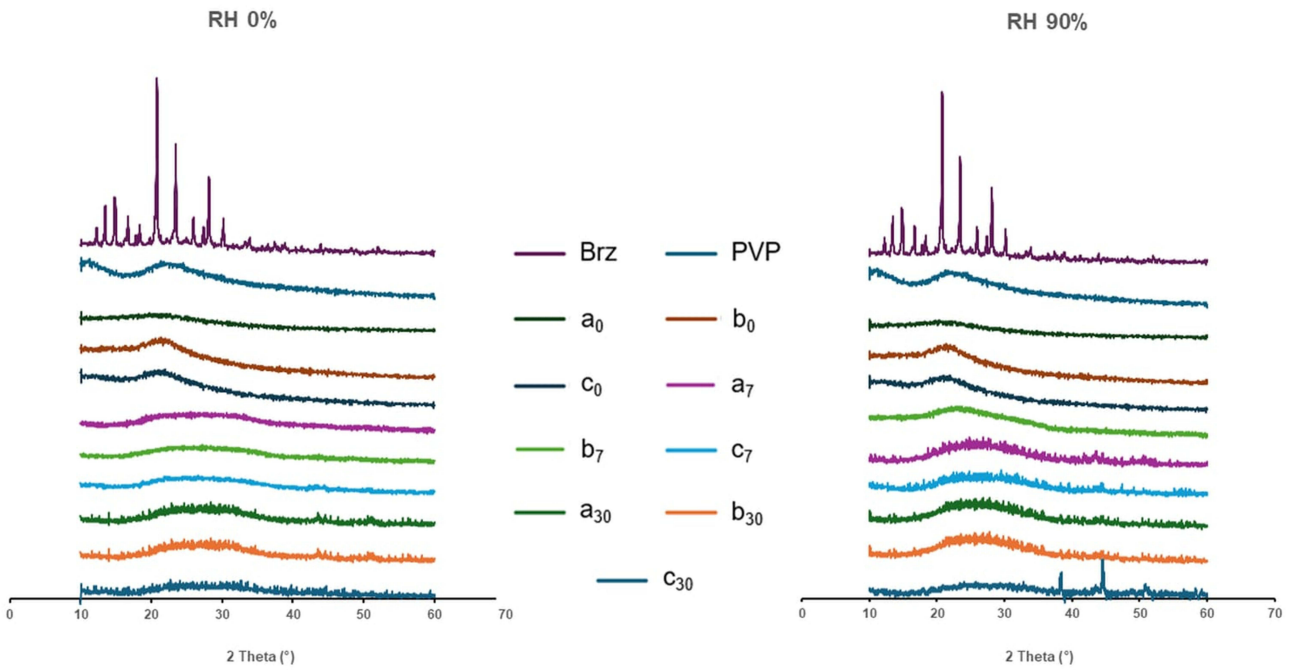

Figure 7 PXRD patterns of BRZ, PVP, and Brz-PVP with various weight ratios after storage at 25°C 0% RH (left) and 90% RH (right). Brz = brazilin, PVP = polyvinylpyrrolidone, a_0 = ASD Brz:PVP (1:5) 0 day, b_0 = ASD Brz:PVP (1:3) 0 day, c_0 = ASD Brz:PVP (1:1) 0 day, a_7 = ASD Brz:PVP (1:5) 7 days, b_7 = ASD Brz:PVP (1:3) 7 days, c_7 = ASD Brz:PVP (1:1) 7 days, a_{30} = ASD Brz:PVP (1:5) 30 days, b_{30} = ASD Brz:PVP (1:3) 30 days, c_{30} = ASD Brz:PVP (1:1) 30 days.

attributed to the self-assembly of the system into nanomicelles, which could hinder water penetration and interaction with BRZ within the PVP matrix.

The appearance of characteristic diffraction peaks after storage under high humidity conditions indicates partial moisture-induced recrystallization. Water absorption can plasticize the amorphous system, reduce the glass transition temperature, and increase molecular mobility, thereby facilitating crystal nucleation and growth. This phenomenon is commonly observed in amorphous pharmaceutical systems exposed to elevated humidity. The PXRD patterns of BRZ–PVP ASDs remained largely amorphous after storage at 25 °C under both 0% RH and 90% RH conditions, indicating the physical stability of the ASD systems.

Cancer Cell Inhibition Assay

The amorphous solid dispersion formulation of BRZ with PVP demonstrated a marked enhancement in antiproliferative activity against DU 145 prostate cancer cells compared to crystalline BRZ.[28] Antiproliferative assay results revealed a significant improvement in the activity of the solid dispersion relative to the pure crystalline form, showing a positive correlation between increased solubility and enhanced biological performance. The IC_{50} values were 16.63 µg/mL for the ASDs BRZ:PVP (1:1) formulation, 15.33 µg/mL for the ASDs BRZ:PVP (1:3) formulation, and 14.02 µg/mL for the ASD BRZ:PVP (1:5) formulation. All ASD formulations exhibited higher antiproliferative activity than pure BRZ crystals, which had an IC_{50} of 20.12 µg/mL. Among these, ASDs BRZ:PVP (1:5) showed the greatest solubility enhancement and the strongest biological effect.[29] Improving the bioavailability of poorly water-soluble anticancer drugs could also enhance their pharmacokinetics, efficacy, and safety.[17] This study highlights the potential of BRZ in ASDs formulations, particularly at a 1:5 ratio, as a promising candidate for a more effective and selective anticancer agent. Statistical analysis demonstrated that ASD formulations significantly enhanced antiproliferative activity compared to crystalline BRZ ($p < 0.05$), with the 1:5 formulation showing the most pronounced effect. As shown in Figure 8, BRZ–PVP amorphous solid dispersions exhibited enhanced anticancer activity against DU-145 cells compared with pure BRZ.

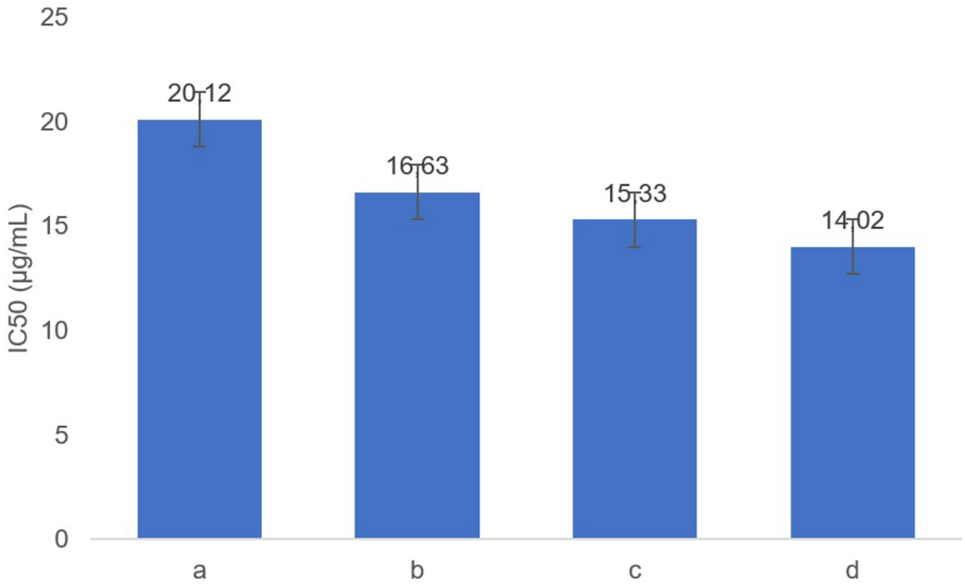

Figure 8 Inhibition concentration (IC 50) of BRZ amorphous solid dispersion against DU 145 cancer cells. (a) brazilin (Brz), (b) Brz:PVP (1:1), (c) Brz:PVP (1:3), (d) Brz:PVP (1:5).

Discussion

Enhancing drug solubility is a crucial strategy for improving therapeutic efficacy. The amorphous form of a compound provides higher free energy than its crystalline counterpart, resulting in superior solubility.[30] BRZ, a natural compound with promising antiproliferative activity, particularly against prostate cancer, suffers from low solubility that limits its oral bioavailability and clinical effectiveness. Transforming BRZ into an amorphous solid dispersion can significantly increase its dissolution and bioavailability.

Amorphous drugs face thermodynamic instability and a high tendency to recrystallize. Solid dispersions using hydrophilic polymers, such as polyvinylpyrrolidone (PVP) K30, are widely employed to stabilize amorphous drugs by preventing nucleation and crystal growth. In this study, solvent evaporation was used to produce BRZ-PVP solid dispersions at weight ratios of 1:1, 1:3, and 1:5. PVP was selected because of its strong hydrogen-bonding capacity, hydrophilicity, and chemical stability.

Physicochemical characterization confirmed successful amorphization. PXRD patterns showed broad halos without any diffraction peaks, indicating the absence of crystalline domains. DSC thermograms revealed the disappearance of sharp melting endotherms, confirming the transformation into the amorphous state. FTIR spectra demonstrated significant shifts in hydroxyl and carbonyl bands, confirming hydrogen-bond formation between BRZ and PVP. The molecular docking study predicted the formation of a hydrogen bond between the -OH group and the C=O PVP at a distance of 2.1 Å, which confirms the FTIR result by the shift in the number of wavelengths in the same functional group. Both methods consistently identify hydrogen bonds as the main interaction mechanisms responsible for increasing the solubility and stability of complexes in amorphous solid dispersion systems. The mechanism of increased solubility of BRZ formulated with PVP involves the transformation from a crystalline form to an amorphous one, which increases free energy and accelerates the solubility of the compound in a liquid solvent. This amorphization process is evidenced by PXRD analysis, which shows the disappearance of crystalline peaks in BRZ after formulation, indicating that the crystalline structure has transformed into an amorphous state. This is further supported by DSC testing results, which show no significant endothermic peaks in the BRZ-PVP formulation, confirming that BRZ is in an amorphous state. The amorphous structure, which has higher free energy compared to the crystalline form, allows this compound to dissolve more easily in liquid media. The addition of PVP to the formulation not only plays a role in transforming the crystalline form into amorphous, but also stabilizes the compound, preventing its return to the crystalline form and enhancing its

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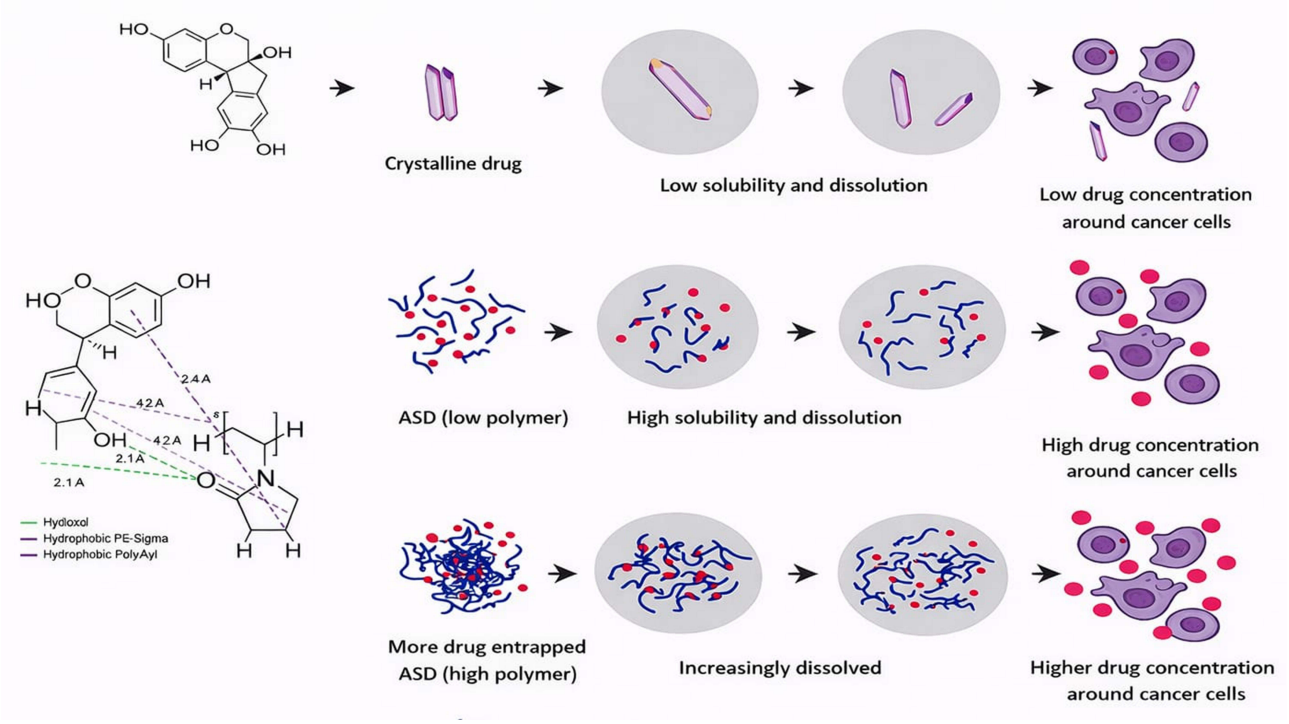

Figure 9 Schematic Illustration Comparing Crystalline and Amorphous Solid Dispersions for Enhanced Therapeutic Effect against Cancer Cells.

solubility. This is in line with previous studies that show that polymers like PVP can bind active molecules through hydrogen bonds, improving their solubility and stability in pharmaceutical formulations.[23]

Dissolution studies revealed a typical "spring and parachute" effect, which is characteristic of successful amorphous solid dispersions. The initial rapid dissolution (spring) generated supersaturation, while the presence of PVP maintained this state (parachute) by inhibiting nucleation and crystal growth. The 1:5 ratio formulation exhibited the most sustained improvement in solubility, maintaining up to a 5.7-fold increase after 48 hours.

Although all formulations exhibited an amorphous state, polymer content plays a crucial role in maintaining supersaturation during dissolution. Lower polymer ratios (1:1 and 1:3) may provide insufficient inhibition of nucleation and crystal growth, leading to faster recrystallization and reduced apparent solubility. Similar observations have been reported in previous amorphous solid dispersion systems, where higher polymer fractions improved dissolution stability and sustained supersaturation.

Antiproliferative assays against DU145 prostate cancer cells demonstrated significantly enhanced activity for the amorphous solid dispersions compared to crystalline BRZ. This improvement reflects the combined effects of increased solubility, prolonged supersaturation, and potentially preferential uptake of drug–polymer complexes by cancer cells. Figure 9 illustrates the proposed mechanism by which BRZ–PVP amorphous solid dispersions improve dissolution and subsequently enhance the anticancer activity compared with crystalline BRZ.

Conclusion

This study successfully produced BRZ in the form of a stable amorphous solid dispersion with PVP polymer using the solvent evaporation method. FTIR analysis and molecular docking confirmed the formation of hydrogen bonds between BRZ and PVP, which is key to stabilizing the system. The resulting solid dispersion increased BRZ solubility by 5.7-fold compared to BRZ crystals through the "spring and parachute" mechanism, which maintains supersaturation over an extended period. The increased solubility also enhances the antiproliferative activity against prostate cancer cells, with an IC_{50} value of 14.02 µg/mL. This indicates that the solid dispersion formulation improves not only the physicochemical properties, but also the biological activity of BRZ. Stability testing over 30 days under various temperature and humidity

conditions demonstrated that the solid dispersion remains stable without recrystallization. This makes the solvent evaporation method worthy of further development as a strategy to enhance BRZ's potential as an anticancer agent. In conclusion, amorphous solid dispersions of BRZ with PVP effectively improve solubility, sustain supersaturation, and enhance antiproliferative activity. This formulation strategy offers a promising approach for developing more effective anticancer agents from poorly soluble natural compounds.

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Rocha B, de Morais LA, Viana MC, Carneiro G. Promising strategies for improving oral bioavailability of poor water-soluble drugs. *Expert Opin Drug Discov*. 2023;18(6):615–627. doi:10.1080/17460441.2023.2211801
2. Dahan A, Miller JM, Amidon GL. Prediction of solubility and permeability class membership: provisional BCS classification of the world's top oral drugs. *AAPS J*. 2009;11(4):740–746. doi:10.1208/s12248-009-9144-x
3. Ku MS, Dulin W. A biopharmaceutical classification-based right-first-time formulation approach to reduce human pharmacokinetic variability and project cycle time from first-in-human to clinical proof-of-concept. *Pharm Dev Technol*. 2012;17(3):285–302. doi:10.3109/10837450.2010.535826
4. Okada H, Ueda K, Yasuda Y, et al. Correlation between drug dissolution and resistance to water-induced phase separation in solid dispersion formulations revealed by solid-state NMR spectroscopy. *Int J Pharm*. 2020;577:119086. doi:10.1016/j.ijpharm.2020.119086
5. Takagi T, Ramachandran C, Bermejo M, Yamashita S, Yu LX, Amidon GL. A provisional biopharmaceutical classification of the top 200 oral drug products in the United States, Great Britain, Spain, and Japan. *Mol Pharm*. 2006;3(6):631–643. doi:10.1021/mp0600182
6. Deshmukh M, Ingole PG, Sharma MR, Gite TN. Enhancing the solubility of poorly soluble drugs: strategies and advances in pharmaceutical formulations. *Asian J Pharm Res*. 2025;309–315. doi:10.52711/2231-5691.2025.00048
7. Shi Q, Li F, Yeh S, et al. Recent advances in enhancement of dissolution and supersaturation of poorly water-soluble drugs in amorphous pharmaceutical solids: a review. *AAPS PharmSciTech*. 2022;23(1):16. doi:10.1208/s12249-021-02137-0
8. Budiman A, Citraloka ZG, Muchtaridi M, Sriwidodo S, Aulifa DL, Rusdin A. Inhibition of crystal nucleation and growth in aqueous drug solutions: impact of different polymers on the supersaturation profiles of amorphous drugs—the case of alpha-mangostin. *Pharmaceutics*. 2022;14(11):2386. doi:10.3390/pharmaceutics14112386
9. Budiman A, Nurani NV, Laelasari E, Muchtaridi M, Sriwidodo S, Aulifa DL. Effect of drug–polymer interaction in amorphous solid dispersion on the physical stability and dissolution of drugs: the case of alpha-mangostin. *Polymers*. 2023;15(14):3034. doi:10.3390/polym15143034
10. Brough C, Williams RO. Amorphous solid dispersions and nanocrystal technologies for poorly water-soluble drug delivery. *Int J Pharm*. 2013;453 (1):157–166. doi:10.1016/j.ijpharm.2013.05.061
11. Ueda K, Okada H, Zhao Z, Higashi K, Moribe K. Application of solid-state 13C relaxation time to prediction of the recrystallization inhibition strength of polymers on amorphous felodipine at low polymer loading. *Int J Pharm*. 2020;581:119300. doi:10.1016/j.ijpharm.2020.119300
12. Pandi P, Bulusu R, Kommineni N, Khan W, Singh M. Amorphous solid dispersions: an update for preparation, characterization, mechanism on bioavailability, stability, regulatory considerations and marketed products. *Int J Pharm*. 2020;586:119560. doi:10.1016/j.ijpharm.2020.119560
13. Qin Y, Xiao C, Li X, Huang J, Si L, Sun M. Enteric polymer–based amorphous solid dispersions enhance oral absorption of the weakly basic drug nintedanib via stabilization of supersaturation. *Pharmaceutics*. 2022;14(9):1830. doi:10.3390/pharmaceutics14091830
14. Fan N, Ma P, Wang X, et al. Storage stability and solubilization ability of HPMC in curcumin amorphous solid dispersions formulated by Eudragit E100. *Carbohydr Polym*. 2018;199:492–498. doi:10.1016/j.carbpol.2018.07.036
15. Liu W, Zhai Y, Heng X, et al. Oral bioavailability of curcumin: problems and advancements. *J Drug Target*. 2016;24(8):694–702. doi:10.3109/ 1061186X.2016.1157883
16. Shi Q, Chen H-M, Wang Y, Wang R, Xu J, Zhang C. Amorphous solid dispersions: role of the polymer and its importance in physical stability and in vitro performance. *Pharmaceutics*. 2022;14(8):1747. doi:10.3390/pharmaceutics14081747
17. Budiman A, Handini AL, Muslimah MN, et al. Amorphous solid dispersion as drug delivery vehicles in cancer. *Polymers*. 2023;15(16):3380. doi:10.3390/polym15163380
18. Morris GM, Huey R, Lindstrom W, et al. AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility. *J Comput Chem*. 2009;30(16):2785–2791. doi:10.1002/jcc.21256
19. Bhujbal SV, Mitra B, Jain U, et al. Pharmaceutical amorphous solid dispersion: a review of manufacturing strategies. *Acta Pharm Sin B*. 2021;11 (8):2505–2536. doi:10.1016/j.apsb.2021.05.014
20. Cools L, Derveaux E, Reniers F, Dehaen W, Adriaensens P, Van den Mooter G. Exploring the influence of hydrogen bond donor groups on the microstructure and intermolecular interactions of amorphous solid dispersions containing diflunisal structural analogues. *Int J Pharm*. 2024;661:124438. doi:10.1016/j.ijpharm.2024.124438
21. Tabandeh S, Lemus CE, Leon L. Deciphering the role of π-interactions in polyelectrolyte complexes using rationally designed peptides. *Polymers*. 2021;13(13):2074. doi:10.3390/polym13132074

22. Tang TH, Deretey E, Jensen SK, et al. Hydrogen bonds: relation between lengths and electron densities at bond critical points. *Eur Phys J D*. 2006;37(2):217–222. doi:10.1140/epjd/e2005-00317-0
23. Xiang TX, Anderson BD. Molecular dynamics simulation of amorphous indomethacin-poly(vinylpyrrolidone) glasses: solubility and hydrogen bonding interactions. *Pharm Drug Deliv Pharm Technol*. 2013;102(3):876–891.
24. Saal W, Ross A, Wyttenbach N, Alsenz J, Kuentz M. A systematic study of molecular interactions of anionic drugs with a dimethylaminoethyl methacrylate copolymer regarding solubility enhancement. *Mol Pharm*. 2017;14(4):1243–1250. doi:10.1021/acs.molpharmaceut.6b01116
25. Indulkar AS, Mo H, Gao Y, Raina SA, Zhang GGZ, Taylor LS. Impact of micellar surfactant on supersaturation and insight into solubilization mechanisms in supersaturated solutions of atazanavir. *Pharm Res*. 2017;34(6):1276–1295. doi:10.1007/s11095-017-2144-0
26. Lu J, Ormes JD, Lowinger M, et al. Impact of endogenous bile salts on the thermodynamics of supersaturated active pharmaceutical ingredient solutions. *Cryst Growth Des*. 2017;17(3):1264–1275. doi:10.1021/acs.cgd.6b01664
27. Abdul-Fattah AM, Bhargava HN. Preparation and in vitro evaluation of solid dispersions of halofantrine. *Int J Pharm*. 2002;235(1–2):17–33. doi:10.1016/S0378-5173(01)00941-3
28. Sethia S, Squillante E. Solid dispersion of carbamazepine in PVP K30 by conventional solvent evaporation and supercritical methods. *Int J Pharm*. 2004;272(1–2):1–10. doi:10.1016/j.ijpharm.2003.11.025
29. Liu Y, Wang T, Liu J, Wang N, Zhang L. Solid dispersion of curcumin-phospholipid complex with enhanced oral bioavailability and anti-inflammatory activity. *Int J Pharm*. 2019;564:96–105.
30. Hancock BC, Parks M. What is the true solubility advantage for amorphous pharmaceuticals? *Pharm Res*. 2000;17(4):397–404. doi:10.1023/A:1007516718048