

Preparation of a stable amiodarone hydrochloride formulation using various nanotechnology methods and pharmaceutical studies

Research Article

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Abstract: Amiodarone hydrochloride (AD), a Class II antiarrhythmic agent, is widely prescribed for ventricular and supraventricular arrhythmias. However, its limited aqueous solubility constrains its clinical application. In this study, two chemically stable AD nanosuspensions were prepared via the evaporative precipitation into aqueous solution (EPAS) method and the wet-milling technique. The nanosuspensions were characterised by transmission electron microscopy, photon correlation spectroscopy, differential scanning calorimetry (DSC), and X-ray powder diffraction (XRPD). DSC and XRPD analyses confirmed the maintenance of the crystalline state. *In vitro* dissolution assays demonstrated that both nanosuspensions exhibited significantly improved dissolution rates compared with their coarse suspension counterparts. Pharmacokinetic studies in rats showed that the oral bioavailability of AD in nanosuspensions (75.91 and 64.72%) was two to three times higher than that of coarse suspensions (33.31%).

Keywords: Amiodarone hydrochloride • Evaporative precipitation into aqueous solution • Pharmacokinetic studies • Nanosuspensions • Wet-milling

1. Introduction

Amiodarone hydrochloride (AD), 2-butyl-3-benzofuranyl-4-(2-(diethylamino)ethoxy)-3,5-diiodophenylketone hydrate (Figure 1), is a multi-ion channel blocker that inhibits sodium and calcium inward currents as well as potassium outward currents [1]. Given its efficacy in preventing and treating atrial fibrillation, ventricular tachycardia, and ventricular fibrillation, AD is widely used in the management of life-threatening ventricular tachyarrhythmias [2]. Owing to its high lipid solubility, AD distributes extensively in tissues and has been detected in brain tissue following intravenous administration [3]. However, its poor aqueous solubility significantly hinders further development.

More than half of commercial pharmaceuticals and many promising candidates belong to Class II of the

Biopharmaceutics Classification System (BCS), a category in which low aqueous solubility constitutes a major constraint on clinical utility [4]. AD, characterised by high permeability but low solubility, is thus a BCS Class II compound. This unfavourable solubility profile commonly leads to poor oral bioavailability, underscoring the need to develop water-soluble formulations to enhance therapeutic efficacy.

Over the past decade, research on AD nanosystems has advanced significantly. This includes developing AD nanoparticles using various matrix materials to enhance solubility, reduce toxicity, optimise pharmacokinetics, and enable targeted delivery [5–7]. Subsequent engineering of AD liposomes and nanomicelles have improved both pharmacokinetic parameters and tissue distribution *in vivo*, thereby enhancing targeting precision and reducing off-target toxicity [3,8,9]. Moreover, solid lipid nanoparticles (SLNs) loaded with AD have been demonstrated to facilitate sustained and controlled drug release [3,10]. Beyond cardiovascular indications, pharmacodynamic

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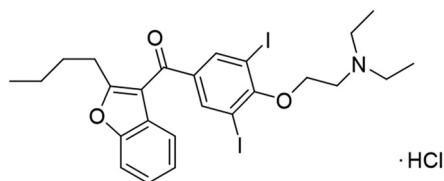


Figure 1. Chemical structure of amiodarone hydrochloride.

investigations have also explored the antibacterial and antitumour potential of AD [11,12]. Nevertheless, each of these strategies exhibits inherent limitations. Micronisation only increases the dissolution rate without improving intrinsic solubility; cosolvent approaches carry risks of residual organic solvent toxicity and particle precipitation; emulsion dispersion requires high drug lipophilicity; and SLNs suffer from low drug loading, physical instability, and poor storage stability. As a result, many drug candidates are discontinued due to inadequate bioavailability. The advent of nanosuspension technology offers a promising strategy for the redevelopment of such otherwise discarded agents [13–16].

Nanosuspensions have become an established technological platform for the delivery of poorly water-soluble drugs. These carrier-free colloidal systems comprise pure drug particles stabilised in suspension, with mean particle sizes in the nanometre range [17]. Extensive research has facilitated the commercialisation of numerous nanosuspension-based formulations. Particle size reduction confers a substantial increase in specific surface area, which in turn markedly enhances the dissolution rate. As described by the Noyes – Whitney and Ostwald – Freundlich equations, reducing particle size increases both the available surface area and the saturated solubility [18]. This enables nanocrystals to establish a steeper concentration gradient between the gastrointestinal lumen and bloodstream, thereby improving dissolution and bioavailability. Consequently, nanosuspension formulation represents a promising strategy for enhancing the stability of chemically labile, yet inherently water-insoluble drugs [13].

Techniques for the preparation of drug nanosuspensions are broadly classified into two principal categories: “bottom-up” and “top-down.” Bottom-up technologies rely on the precipitation of solute molecules from solution via non-solvents in the presence of stabilisers, encompassing evaporative precipitation into aqueous solution (EPAS), spray-freezing into liquid, supercritical fluid (SCF) technology, and liquid solvent exchange processes [18–20]. In contrast, top – down methods entail the mechanical comminution of coarser drug particles via techniques including ball milling, pearl milling, high-pressure homogenisation, and

microfluidisation. Such approaches obviate the need for harsh solvents but typically require high energy input and demonstrate low power efficiency [21].

The EPAS technique is predicated on the nucleation and subsequent growth of drug crystals to generate crystalline or semi-crystalline nanoparticles [22]. By facilitating the controlled evaporation of organic solvents – ethanol in this instance – it modulates supersaturation, thereby yielding particles of greater uniformity and sphericity. Conversely, conventional nanoprecipitation frequently produces irregular particles with a broad size distribution (polydispersity index (PDI) > 0.3), attributable to rapid, unregulated nucleation. Spray-freezing into liquid and SCF methodologies typically necessitate costly apparatus and entail intricate operational procedures, which contribute to a substantial elevation in production costs. Typically, a suitable solvent – such as methanol, ethanol, acetone, tetrahydrofuran, or *N*-methyl-2-pyrrolidone – is chosen to solubilise the active pharmaceutical ingredient. Stabilisers, including Pluronic F-127, lecithin, polyvinylpyrrolidone (PVP), Tween 80, and hydroxypropyl methylcellulose, are solubilised in the antisolvent (e.g., water) to mitigate excessive crystal growth and particle aggregation; to achieve optimal performance, these stabilisers are frequently employed in combination [23]. Drug nanocrystals are formed upon addition of the drug solution to the antisolvent. Within the EPAS technique, parameters such as the mixing regime, solvent selection, and stabiliser choice exert a pivotal influence on governing the particle size and stability of the resultant nanoparticles. Notably, the mixing stage is critical for the rapid generation of a homogeneous supersaturated solution, which promotes the formation of uniformly sized, small nanocrystals [22].

Ball milling represents an extensively employed technique for the fabrication of nanosuspensions. This wet-milling method utilises a ball mill fitted with glass or yttrium-stabilised zirconium oxide pearls as milling media, enabling size reduction of drug particles within an aqueous medium to yield nanosuspensions [24]. Intense high-energy shear forces are generated via vigorous high-speed agitation of the drug, aqueous stabiliser solution, and milling media; these forces supply the energy required for particle fracture and subsequent formation of nanoscale particulates [25]. The wet-milling offers substantial advantages over high-pressure homogenisation and microfluidisation, underpinning its widespread utility: it is operationally straightforward, eliminates the need for organic solvents, is readily scalable and modifiable, exhibits excellent process reproducibility, and is cost-effective [18,26,27]. Briefly, a coarse suspension comprising the drug, adjuvants, and milling beads is prepared in a vial and then pumped into a milling chamber mounted on a

stir plate regulated at a predefined speed for a set duration. Both operational parameters and dispersing medium composition are optimised to achieve nanosuspensions with high recovery rates, while particle size distribution is monitored routinely throughout processing to track milling progression.

This study aimed to prepare stable AD nanosuspensions using two contrasting strategies: EPAS (bottom-up) and wet-milling (top-down) methods. The resulting formulations were screened, optimised, and directly compared, with comprehensive physicochemical characterisation performed, including particle morphology, particle size distribution, differential scanning calorimetry (DSC), and X-ray powder diffraction (XRPD). Furthermore, *in vitro* dissolution rates were determined. *In vitro* dissolution profiles and *in vivo* pharmacokinetic investigations in rats were subsequently conducted to evaluate the bioavailability-enhancing performance of the nanosuspensions.

2. Experimental section

2.1 Materials

AD, sourced from Novartis Pharma, was employed as the model drug. It exhibits a melting point of 158–163°C and a molecular weight of 681.77, with its chemical structure depicted in Figure 1. Excipients including Poloxamer 188 and Pluronic F-127 (PF-127) were kindly provided by BASF (Ludwigshafen, Germany). PVP K30 and sodium dodecyl sulphate (SDS) were sourced from Sigma-Aldrich (Saint Louis, MO). Polysorbate-80 (Tween 80) and polysorbate-20 (Tween 20) were supplied by KeLong Chemical (Chengdu, China). Deionised water was used as the dispersion medium, while yttrium-stabilised zirconium oxide beads (0.8 mm diameter) were employed as the milling medium and purchased from Agitator Bead Milling (PMQW, Nanjing Chishun, China). All other chemicals and solvents were of analytical reagent grade, and deionised-distilled water was used throughout the study.

2.2 Preparation of Two AD nanosuspensions

2.2.1 EPAS process (Bottom-Up Method)

AD nanosuspensions were prepared via the antisolvent precipitation technique. Briefly, 100 mg of AD was completely dissolved in 8 mL of ethanol at 60°C to form the organic

phase. This phase was added dropwise into 10 mL of an aqueous solution containing PF-127 (0.5%, w/v) and SDS (0.1%, w/v) at 0°C under magnetic stirring at 800 rpm. Subsequently, the resultant suspension was stirred at 300 rpm for 1 h at room temperature. Finally, the suspension was concentrated *in vacuo* at room temperature to reduce ethanol content, yielding the EPAS nanosuspension [22].

2.2.2 The wet-milling process (top – down method)

For the wet-milling process, AD, PVP, SDS, grinding beads, and deionised water were weighed according to Table 1. The grinding beads used were yttria-doped zirconium beads with a diameter of 0.8 mm. The drug and other ingredients were mixed in a 50 mL scintillation vial. The resulting suspension was then pumped into the milling chamber, followed by the addition of the grinding beads. Milling was performed using an Agitator Bead Mill (PMQW, Nanjing Chishun, China) under ambient conditions, with effective particle size reduction attained after a specified duration. The milled suspension was collected by passing it through a screen that retained the grinding beads, with a recovery rate of >90% anticipated. To achieve nearly 100% recovery, two to three rinsing steps were performed using the formulation vehicle, prior to particle size analysis. Particle size reduction was optimised with respect to milling time, milling speed, and the ratios of steric and electrostatic stabilisers to drug (Table 2).

2.3 Characterisation of nanosuspension

2.3.1 Particle size measurement

The mean particle size and PDI of the nanosuspensions were determined by dynamic light scattering (DLS; Malvern Instruments, Malvern, UK) at room temperature, with all analyses conducted in triplicate.

2.3.2 Morphology observation by transmission electron microscopy (TEM)

The morphology of AD nanosuspensions was characterised by TEM (H-600, Hitachi, Japan). A droplet of the nanosuspension was placed on a 200-mesh copper grid, air dried at room temperature, and subsequently loaded into the TEM for analysis.

2.4 Lyophilisation

Nanosuspensions were freeze-dried for subsequent physicochemical characterisation. Approximately 2 mL of

Table 1. Composition of AD nanosuspensions by the EPAS method

Formulation	AD (mg)	PF-127 (mg)	PEG-6000 (mg)	PVPK30 (mg)	SDS (mg)	T-80 (mg)	Ethanol (mL)
1	100	0	0	50	20	0	10
2	100	0	0	50	0	0	10
3	100	0	0	0	50	0	10
4	100	0	0	0	20	0	8
5	100	0	0	50	10	0	10
6	100	50	0	0	0	0	8
7	100	50	0	0	10	0	10
8	100	50	0	0	20	0	8
9	100	80	0	0	0	0	10
10	100	80	0	0	10	0	10
11	100	0	0	50	0	10	8
12	100	0	0	0	0	80	10
13	100	0	50	0	10	0	10
14	100	0	50	0	0	0	10
15	100	50	0	0	10	0	10
16	100	50	0	0	15	0	10
17	100	50	0	0	10	0	8
18	100	50	0	20	20	0	10
19	100	50	0	0	10	0	8
20	100	50	0	0	10	0	8
21	100	0	0	0	0	0	10

aqueous samples were frozen at -20°C in 5 mL glass vials for 48 h, followed by freeze-drying using a freeze-dryer (FDU-1100, EYELA, Japan) with no secondary drying step implemented. Powders from the EPAS and wet-milling processes were designated NS-A and NS-B, respectively.

2.5 DSC analysis

Thermal properties were evaluated by DSC (DSC Q200 V24.2 Build 107; TA Instruments, New Castle, DE, USA). Approximately 5 mg of each sample (AD coarse powder, physical mixture (PM), and freeze-dried nanosuspension powders) was analysed in an open aluminium pan. The scanning temperature ranged from 25 to 170°C at a rate of $10^{\circ}\text{C}/\text{min}$ under a nitrogen atmosphere.

2.6 XRPD measurements

XRPD was employed to investigate the crystallinity of the samples. Experiments were performed using an X-ray powder

diffractometer (D/Max-2500PC, Rigaku, Japan) with Cu K α radiation (graphite monochromator) at 200 mA and 40 kV. The scanning rate was $4^{\circ}/\text{min}$ over the range $3^{\circ} \leq 2\theta \leq 60^{\circ}$.

2.7 Analytical method

AD concentration was determined by high-performance liquid chromatography (HPLC; UltiMate 3000 system, Dionex, USA) equipped with a variable-wavelength UV detector and an autosampler. Detection was performed at 241 nm. A 5 μm Thermo C₁₈ column (200 mm $\times$ 4.6 mm, Waters Technologies, Ireland) was utilised at 35°C , with a mobile phase consisting of methanol/water containing 2% triethylamine (80:20, v/v) at a flow rate of 1.0 mL/min.

2.8 *In vitro* dissolution studies of AD

Dissolution studies were conducted using a dissolution tester (ZRS-12G, Tianjin TDTF Technology Co., Ltd, China) following the paddle method (Chinese Pharmacopoeia

Table 2. Effect of the ratio of steric stabilizers to drug, the ratio of electrostatic stabilizers to drug, milling time, and milling speed on mean particle size $d(90)$ and PDI

Run	Ration of steric stabilizers to drug (mg)	Ration of electrostatic stabilizers to drug (mg)	Milling time (h)	Milling speed (Hz)	MPS $d(90)$ nm	PDI
1	300	150	8	30	279.1	0.317
2	300	80	8	30	215.3	0.221
3	300	50	8	30	221.9	0.298
4	300	80	6	30	228.0	0.271
5	300	80	12	30	205.6	0.214
6	350	80	8	30	246.4	0.230
7	400	80	8	30	288.7	0.252
8	300	80	8	35	210.1	0.197
9	300	80	8	40	195.7	0.248
10	300	80	6	35	204.5	0.217
11	350	80	6	35	224.9	0.247
12	350	80	8	35	212.4	0.235
13	400	80	8	35	237.1	0.217
14	400	50	8	35	255.3	0.240
15	400	150	8	35	287.0	0.371
16	350	150	8	35	321.9	0.298
17	300	150	8	35	390.7	0.383
18	300	50	8	35	226.7	0.224
19	350	50	8	35	217.0	0.222
20	350	50	8	40	195.4	0.256
21	350	50	12	40	189.6	0.247
22	350	80	12	40	225.3	0.295
23	350	80	8	40	243.4	0.276
24	350	150	8	40	289.5	0.354
25	300	150	8	40	274.5	0.342

mg, milligram; h, hours; nm, nanometer; MPS $d(90)$, mean particle size $d(90)$.

2020). Samples equivalent to 25 mg of AD were dispersed 900 mL of 0.5% SDS aqueous solution (dissolution medium) at $37.0 \pm 0.5^\circ\text{C}$ and 100 rpm. At predetermined intervals, 5 mL of the medium was withdrawn, filtered (0.45 μm cutoff), and replaced with 5 mL of blank medium. The AD content was analysed via HPLC as described in Section 2.7. All measurements were performed in triplicate.

2.9 Stability study

Freeze-dried powder samples were stored at 25°C and 75% relative humidity for 3 months. At predetermined intervals, approximately 10 mg of the powder was

reconstituted in deionised water for analysis. The mean particle and PDI were determined as detailed in Section 2.3.1, with all analyses performed in triplicate.

2.10 *In vivo* pharmacokinetics studies

Animal studies were conducted in accordance with the *Guidelines for the Care and Use of Laboratory Animals*, with protocols approved by the Institutional Animal Care and Use Committee of Sichuan University. Healthy male Wistar rats (220 ± 20 g) were randomly divided into four groups ($n = 6$ per group). Following overnight fasting, the groups received the following treatments: intravenous AD solution (20 mg/kg), oral nanosuspensions (wet-milling or

EPAS formulation), or oral coarse suspension (50 mg/kg). Blood samples (0.3–0.4 mL) were collected at 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12, 24, 36, 48, and 72 h postadministration into heparinised tubes and centrifuged at 6000 rpm for 10 min, and the resulting plasma was processed via acetonitrile protein precipitation. AD concentration was determined via HPLC, as described in Section 2.7. Pharmacokinetic parameters were analysed using DAS software.

2.11 *In vitro* cytotoxicity assay

Cellular safety was evaluated via the MTT assay on CT26 and HEK293 cell lines. Cells were seeded at a density of 2500 cells/well in 96-well plates and incubated overnight. Treatment groups included normal saline, AD-free NS-A and NS-B, PM, and AD nanosuspensions at various concentrations. After 72 h of incubation at 37°C, 20 µL of MTT solution (5 mg/mL in Phosphate-Buffered Saline) was added to each well. Following 4 h of incubation in the dark, the medium was discarded and 150 µL of Dimethyl Sulfoxide was added. Absorbance was measured at 490 nm using a microplate spectrophotometer (Molecular Devices). Cell viability was calculated using the following formula:

$$\text{Cell viability (\%)} = (A_x - A_0)/(A_c - A_0) \times 100\% \quad (1)$$

where A_x is the absorbance of the wells treated with drugs, A_0 was the absorbance of the wells containing medium only, and A_c is the absorbance of the normally cultured cells.

3. Results and discussion

3.1 Formulation analysis for the EPAS process

To identify an optimised formulation with long-term stability, a series of electrostatic and steric stabilisers with varying concentrations were systematically designed and evaluated. Specifically, PVP K30 and PF-127 were utilised to investigate steric stabilisation, whereas SDS and Tween 80 were employed for electrostatic stabilisation. The selection of stabilisers emerged as a critical determinant of nanoparticle stability. Given that excessive stabilisation may elicit adverse effects – such as anaphylactic reactions or irritability – this parameter must be carefully controlled during formulation optimisation.

Among the Pluronic® family, PF-127 (also known as Poloxamer 407) is the most extensively investigated member due to its excellent aqueous solubility and relatively long polypropylene oxide (PPO) block, which contributes to hydrophobic interactions and micellar aggregation. At sufficiently high concentrations (18–20% w/w) and temperatures above the critical gelation temperature, poloxamer micelles self-assemble into an ordered arrangement, triggering a sol-to-gel transition to form thermosensitive gels [28]. Moreover, Pluronics are Food and Drug Administration (FDA)-approved biocompatible polymers suitable for oral, injectable, topical, inhalation, and ophthalmic formulations.

Tween 80 is a synthetic non-ionic surfactant utilised as a solubiliser, stabiliser, or emulsifier across food, cosmetic, and pharmaceutical applications. As a component of commercial AD injections, it has a well-documented safety profile [29]. Its high hydrophilic – lipophilic balance and low critical micelle concentration confer sufficient surface activity, enabling effective stabilisation of aqueous formulations at low concentrations (0.001–0.01% w/v).

SDS is a commonly used anionic surfactant employed in pharmaceutical and food formulations. As an excipient, it serves to solubilise hydrophobic flavour compounds and various classes of preservatives. The SDS concentration is dependent on the specific product type and manufacturer and generally ranges from 0.01 to 50% (w/w). PVP K30 is a neutral polymeric excipient that is FDA approved used in oral, parenteral, and topical formulations. It is negligibly metabolised in the human body and predominantly excreted via renal excretion with no tissue accumulation. According to an industry survey, its concentration in commercial products typically ranges from 0.0005 to 12% (w/w). The excipients selected for the nanoformulation – PF-127, Tween 80, SDS, and PVP K30 are widely used in pharmaceutical products and possess well-established safety profiles that comply with relevant regulatory requirements [29–32].

The final formulation was selected based on critical quality attributes, including mean particle size, PDI, dissolution rate, and saturated solubility. To establish an optimised formulation with long-term stability, a range of stabilisers was systematically screened. Single-factor experiments demonstrated that Tween 80 was unsuitable for use as a surfactant. Following this screening, 21 formulations were prepared and assessed using the EPAS method, as summarised in Table 1. Among these candidates, formulation 7 displayed the most favourable stability profile, with the smallest mean particle size (approximately 225.7 nm) and a PDI of 0.223. On the basis

of these results, formulation 7 was selected as the optimal nanosuspension formulation.

3.2 Formulation analysis for the wet-milling process

Following single-factor optimisation, PVP K30 and SDS were identified as the most effective steric and electrostatic stabilisers, respectively. Their combination consistently produced particles within the submicron range, yielding the smallest mean sizes. As detailed in Table 2, the mean particle size decreased at lower stabiliser concentrations, which may be attributed to enhanced particle collisions resulting from stronger impaction forces during milling. Conversely, higher concentrations of the steric stabiliser increased system viscosity, thereby impeding the formation of nanosuspensions.

Mean particle size also decreased with increasing milling time and speed (Table 2). Longer milling durations yielded smaller particles, with the majority of size reduction occurring within the initial 8 h; no significant reduction was observed beyond this threshold, highlighting milling time as a key parameter for particle size control. The screening study further demonstrated the influence of milling speed on PDI. A PDI value of 0.1–0.25 indicates a narrow particle size distribution, whereas values above 0.5 correspond to a broad distribution. Increased milling speed led to higher PDI values, likely due to enhanced stabiliser adsorption that reduces particle mobility. Excessive adsorption of charged species onto particle surfaces can thereby increase particle size and broaden the size distribution. The minimum particle size (213.0 nm) was achieved at the maximum combination of milling time and speed, attributable to the higher energy and shear forces generated. Relative to alternative preparation methods, the proposed approach offers operational simplicity, ease of scale-up, and considerable cost-effectiveness.

Formulation optimisation identified the optimal ratios of steric stabiliser to drug (*A*) and electrostatic stabiliser to drug (*B*), along with milling time (*C*) and speed (*D*). For a 20 g batch, the target PDI and mean particle size were achieved under the following conditions: *A* = 300 mg, *B* = 80 mg, *C* = 6 h, and *D* = 35 Hz.

3.3 Morphology observation by TEM

TEM analysis revealed that unprocessed AD particles were irregular in shape and exceeded 10 µm in size. In contrast,

particles produced via the EPAS method were predominantly spherical with smooth surfaces and diameters below 500 nm, exhibiting a relatively uniform and encapsulated morphology. Wet-milling particles (Figure 2), however, displayed irregular shapes and heterogeneous size distributions, which tend to promote aggregation and result in larger effective particle sizes. Consequently, nanosuspensions prepared by EPAS may offer enhanced physical stability.

3.4 DSC analysis

The crystalline structure of the formulations was investigated by comparing the DSC thermograms of unprocessed AD, the PM, and the dried nanosuspension powders (NS-A and NS-B). As shown in Figure 3, the melting points of AD in NS-A, NS-B, and the PM were 157.25, 158.71, and 157.65°C, respectively. The thermogram of the PM exhibited two overlapping peaks, attributed to the superposition of the individual endotherms of AD and PVP K30. Compared with unprocessed AD, the nanosuspension powders displayed slight shifts in melting point and reduced peak areas, likely resulting from particle size reduction and the presence of formulation excipients. A lower melting point is generally associated with reduced lattice energy, which may enhance the dissolution rate [31].

3.5 XRPD analysis

XRPD analysis (Figure 4) further confirmed the crystallinity of the formulations. Characteristic diffraction peaks of AD at 2θ values of 11.66°, 18.56°, 21.86°, and 24.32° were observed in the PM, NS-A, and NS-B, indicating that the crystalline form of AD remained unchanged. Therefore, the enhanced dissolution rate can be attributed to the reduction in particle size and the presence of excipients, rather than to any alteration in crystallinity.

3.6 *In vitro* dissolution test

The dissolution profiles of NS-A and NS-B powders are presented in Figure 5. Nanocrystal formation markedly accelerated drug dissolution: at 120 min, the cumulative release from the four formulations reached approximately 24.62, 36.7, 82.28, and 87.56%, respectively. While surfactants in the PM improved solubility relative to pure AD, their contribution to the wet-milling and EPAS-derived powders was relatively minor. This observation was

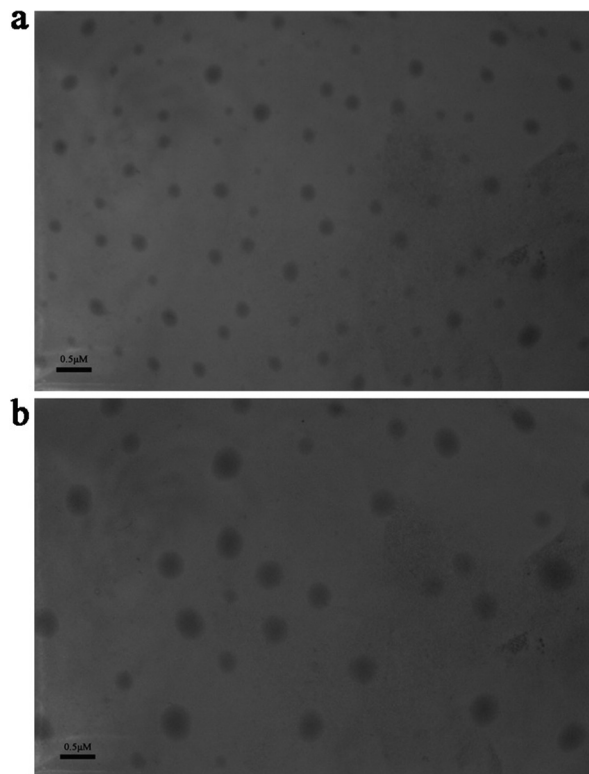


Figure 2. TEM images of the wet-milling nanosuspension (a) and the EPAS nanosuspension (b).

supported by the markedly faster dissolution of the nanocrystal formulations, which attained 67.1 and 72.59% release within 10 min, in sharp contrast to the 12.6 and 23.43% release achieved by unmodified AD and the PM at the 1 h time point, respectively.

In accordance with the Noyes – Whitney equation, this marked enhancement – particularly for nanosized

particles – is attributable to a substantial increase in the effective surface area available for dissolution. As nanocrystalline powders, NS-A and NS-B exhibited comparable rapid dissolution rates, with no statistically significant difference observed between them. DSC and XRPD analyses confirmed the absence of crystalline polymorphic transition, indicating that the improved dissolution

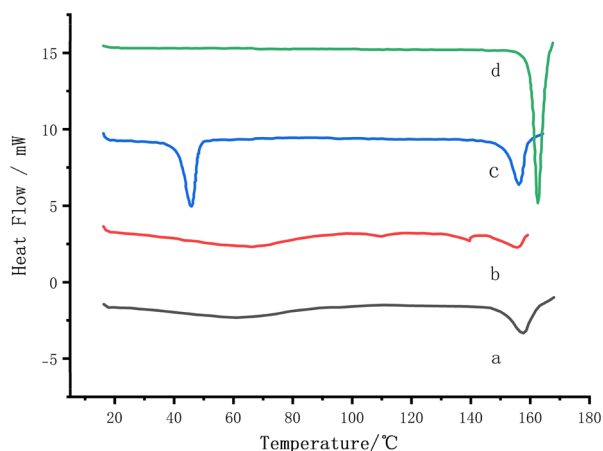


Figure 3. DSC spectra of (a) physical mixture (PM) powder, (b) the NS-A powders, (c) NS-B powders, and (d) AD.

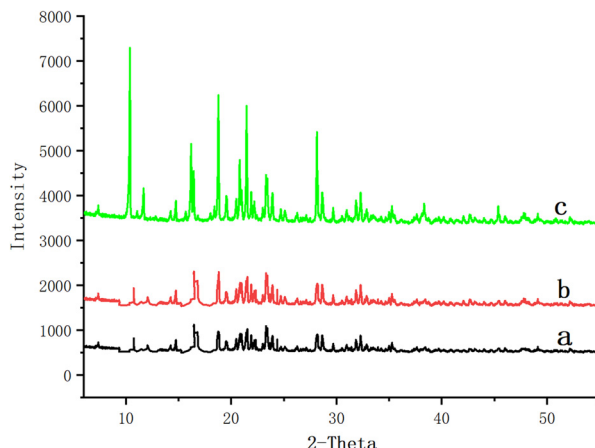


Figure 4. XRPD spectra of (a) NS-B, (b) NS-A, and (c) AD powders.

performance stemmed from the reduction in particle size to the nanometre range.

3.7 The stability study

NS-A and NS-B powders were stored for 3 months at 25°C and 75% relative humidity (RH). Particle size analysis indicated that humidity had a measurable effect on the samples (Tables 3 and 4). Following reconstitution, both the mean particle size and PDI of NS-A and NS-B showed slight increases. This is attributed primarily to moisture adsorption by the lyophilised powder under high humidity conditions, leading to non-uniform thickening of the surface hydration layer and particle aggregation via interparticle bridging. Additionally, humidity may promote slow oxidation or hydrolysis of the stabilisers (e.g., hydrolysis of SDS,

chain scission of PVP K30), thereby impairing their dispersing and stabilising capacity. Preliminary results indicate that the 3-month stability assessment has been completed, while data for the 5-month time point remain pending. Long-term stability studies, including a 6-month trial and evaluation at 25°C/<40% RH, have been scheduled to generate the data to support subsequent commercial development.

3.8 Pharmacokinetic study

Plasma concentration – time profiles following oral administration of coarse AD suspension, wet-milling nanosuspension, and EPAS nanosuspension are shown in Figure 6, alongside those obtained after intravenous administration; the corresponding pharmacokinetic

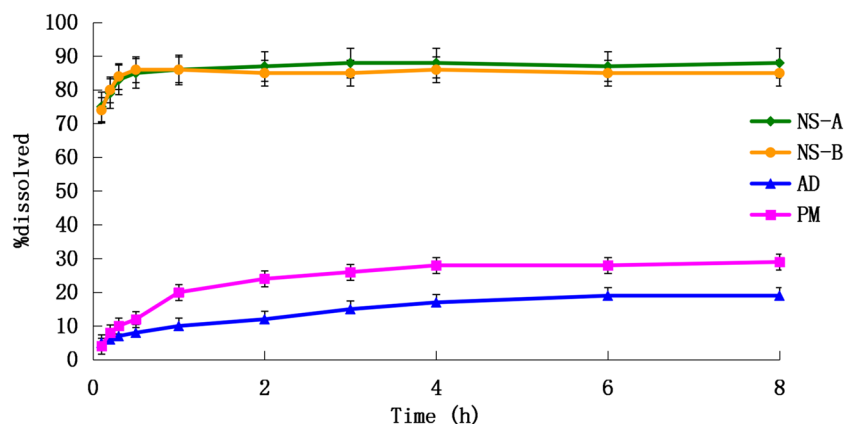


Figure 5. Dissolution profiles for raw AD (AD), physical mixture of AD (PM), the EPAS dried powder (NS-A) and wet-milling dried powder (NS-B) at 37°C and 100 rpm ($n = 3$).

Table 3. The stability of the EPAS dried powder (NS-A)

Time month	Physical appearance	Colour change	MPS <i>d</i> (90) nm	PDI
0 month	Homogenous	No change	226 ± 0.32	0.223 ± 0.005
1 month	Homogenous	No change	233 ± 0.86	0.237 ± 0.008
2 month	Homogenous	No change	252 ± 1.12	0.248 ± 0.014
3 month	Homogenous	No change	259 ± 1.35	0.265 ± 0.017

Stability studies at 0, 1, 2 and 3 months' time period at 25°C and RH 75%.

Table 4. The stability of the wet-milling dried powder (NS-B)

Time month	Physical appearance	Colour change	MPS <i>d</i> (90) nm	PDI
0 month	Homogenous	No change	205 ± 0.57	0.217 ± 0.004
1 month	Homogenous	No change	223 ± 1.07	0.235 ± 0.008
2 month	Homogenous	No change	235 ± 1.31	0.257 ± 0.016
3 month	Homogenous	No change	255 ± 1.62	0.268 ± 0.018

Stability studies at 0, 1, 2 and 3 months' time period at 25°C and RH 75%.

parameters are summarised in Table 5. Compared with the coarse suspension, both nanosuspensions exhibited markedly enhanced pharmacokinetic profiles, as reflected by higher mean $C_{\max}$ (54–68% increase, $p < 0.01$ vs. control), greater $AUC_{0-\infty}$ (94.3–125.4% increase, $*p < 0.05$ vs. control), and a significantly shorter $T_{\max}$ (reduced by 4 h, $*p < 0.05$ vs. control). Furthermore, the absolute oral bioavailability of AD from the nanosuspensions – 75.91% for NS-A and 64.72% for NS-B – was approximately two- to three-fold higher than that of the coarse suspension (33.31%), confirming markedly improved systemic absorption. Dissolution studies confirmed that AD nanosuspensions exhibited the highest release rates among all groups,

which likely underpins the observed improvement in systemic exposure. Consequently, the nanosuspensions achieved significantly higher $AUC_{0-\infty}$ and $C_{\max}$, alongside a reduced $T_{\max}$. Dissolution studies confirmed that AD nanosuspensions exhibited the highest release rates among all groups, which likely underpins the observed improvement in systemic exposure. Consequently, the nanosuspensions achieved significantly higher $AUC_{0-\infty}$ and $C_{\max}$, alongside a reduced $T_{\max}$.

Nanosuspensions enhance the solubility and bioavailability of poorly soluble drugs through streamlined manufacturing and formulation optimisation, thereby mitigating adverse effects associated with excessive

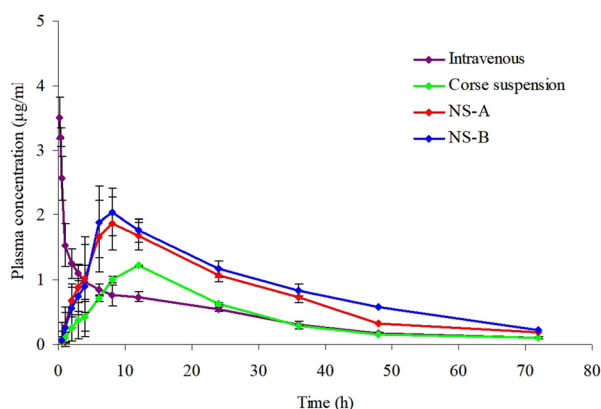


Figure 6. Plasma concentration-time profiles of AD after intravenous AD coarse suspension (20 mg/kg), oral nanosuspension and AD coarse suspension administrations of 50 mg/kg in the rats. Two different oral formulations were tested: nanosuspension and coarse suspension. Data are expressed as mean ± SD ($n = 6$).

Table 5. Pharmacokinetic parameters in three groups: coarse suspension of oral AD (50 mg/kg), nanosuspension of oral AD (50 mg/kg), and intravenous AD (20 mg/kg)

Parameter	Intravenous	Coarse suspensions	NS-A	NS-B
Dose (mg/kg)	20	50	50	50
C_{max} (µg/mL)	3.503 ± 0.329	1.217 ± 0.225	1.873 ± 0.31**	2.049 ± 0.29**
T_{max} (h)	—	12 ± 0	8 ± 0.765*	8 ± 1.155*
$AUC_{0-\infty}$ (µg h/mL)	37.446 ± 7.076	31.19 ± 4.594	60.59 ± 3.375*	70.304 ± 4.563*
$T_{1/2}$ (h)	23.20 ± 9.867	11.98 ± 0.11	18.98 ± 2.15*	20.013 ± 3.25*
F_r	—	33.31%	64.72%	75.91%

Data are expressed as mean ± SD ($n = 6$) (* $p < 0.05$, ** $p < 0.01$, vs. control).

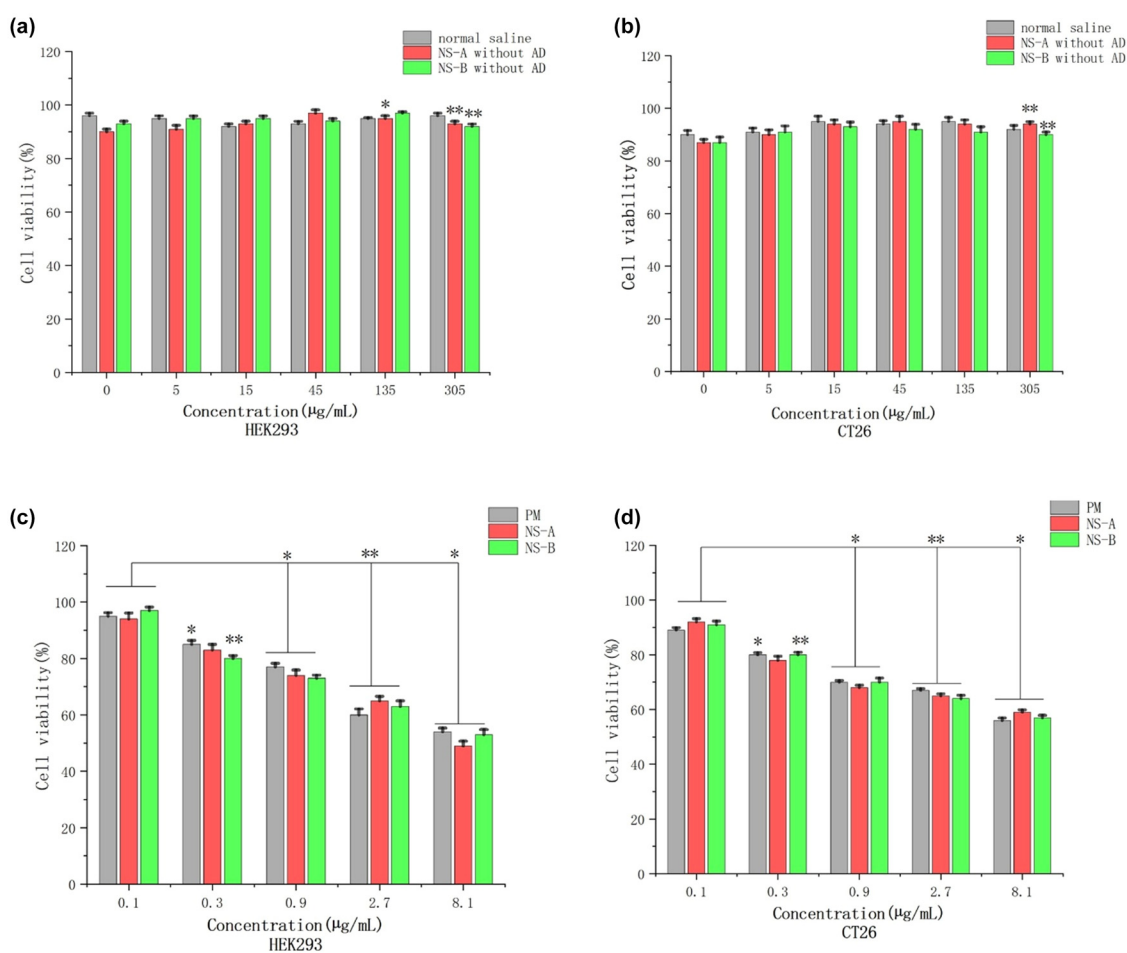


Figure 7. Cytotoxicity of normal saline, NS-A, and NS-B without AD, at different concentrations to HEK293 (a) and CT26 (b) cells was measured by MTT assay. Cells were treated with normal saline, NS-A, and NS-B without AD for 3 days. Each column represented the mean ± SD for three independent experiments (* $p < 0.05$, ** $p < 0.01$ vs. control). Cytotoxicity of PM, NS-A, and NS-B at different concentrations to HEK293 (c) and CT26 (d) cells was measured by MTT assay. Cells were treated with PM, NS-A, or NS-B for 3 days. Each column represented the mean ± SD for three independent experiments (* $p < 0.05$, ** $p < 0.01$ vs. control).

solubiliser use. In recent years, considerable research attention has been directed toward modulating the *in vivo* pharmacokinetic profiles of drugs. Nanosuspensions are thus poised to offer innovative strategies for pharmaceutical development.

3.9 Cytotoxicity assay

The *in vitro* cytotoxicity of the formulations was assessed in HEK293 and CT26 cell lines using MTT assays. As illustrated in Figure 7, neither the drug-free nanosuspension carriers (NS-A and NS-B) nor the negative control exhibited significant cytotoxicity in either cell line following 72 h of incubation, confirming the favourable safety profile of the EPAS and wet-milling vehicles. Furthermore, the cytotoxicity of the AD-loaded nanosuspensions was comparable to that of the PM across all tested concentrations in both cell lines, suggesting that the observed toxicity was attributable primarily to the high drug concentration rather than the nanocarrier itself.

4. Conclusions

The optimal formulation was established using the EPAS method, wherein excipient content and process parameters were tailored to achieve uniform particle size while minimising the use of hazardous solvents. Subsequently, wet-milling was performed using simplified apparatus to optimise the production conditions. A formulation optimisation system was employed to screen the ratio of steric to electrostatic stabilisers relative to the drug, together with grinding time and rotational speed, rendering the overall procedure straightforward to operate and cost-effective. The results further underscored the advantages of both strategies: operational simplicity, low organic solvent consumption, excellent scalability, exceptional process reproducibility, and marked cost-effectiveness.

DSC and XRPD analyses confirmed that the crystalline state of the nanosuspensions was preserved following preparation by both methods, suggesting that particle size reduction did not alter the lattice energy and that retention of the original crystalline form is critical to stability. The observed increase in dissolution rate was attributed to this size reduction, consistent with the greater surface area-to-volume ratio of smaller particles. Both EPAS and wet-milling proved to be effective techniques to prepare stable AD nanosuspensions. Pharmacokinetic studies confirmed that the resulting nanosuspensions were readily

absorbed. Collectively, these findings demonstrate that nanosuspensions prepared by EPAS and wet-milling offer superior solubility and bioavailability relative to previously reported AD nanocarriers (e.g., nanocrystals, SLNs, nanomicelles), alongside simplified manufacturing processes and enhanced safety profiles.

This study primarily focused on the preparation of nanosuspensions and preliminary pharmacokinetic investigations. While further research is required for clinical translation, particularly to develop final dosage forms (e.g., oral tablets, suspensions) with optimised flowability, reconstitution stability, and taste-masking properties, the present findings support the promise of these formulations for subsequent development into such oral dosage forms.

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Author contributions

Xinyu You: conceptualization, methodology, investigation, writing – original draft. Xianghua Gao: investigation, data curation; Bingbing Wu: investigation, formal analysis; Rongqiang Li: resources, supervision; Luoting Yu: conceptualization, supervision; Qijie Xu: writing – review & editing.

Conflict of interest statement

The authors declare no conflict of interest.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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