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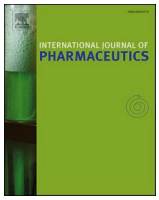

Non-salt based co-amorphous formulation produced by freeze-drying

Mohammed Suleiman Alsalhi [a,b], Paul G. Royall [a], Hisham Al-Obaidi [c], Alyaa Alsalhi [b], Agostino Cilibrizzi [a], Ka Lung Andrew Chan [a,*]

[a] Institute of Pharmaceutical Science, King's College London, SE1 9NH, UK
[b] College of pharmacy, King Saud University, Riyadh, Saudi Arabia
[c] School of Chemistry, Food and Pharmacy (SCFP), University of Reading, Whiteknights, Reading RG6 6AP, UK

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ABSTRACT

Amino acids-based co-amorphous system (CAM) has shown to be a promising approach to overcome the dissolution challenge of biopharmaceutics classification system class II drugs. To date, most CAM formulations are based on salt formation at a 1:1 M ratio and are prepared by mechanical activation. However, its use in medicinal products is still limited due to the lack of in-depth understanding of non-ionic based molecular interactions. There are also limited studies on the effect of drug-to-co-former ratio, the development of more scalable, less aggressive, manufacturing processes such as freeze drying and its dissolution benefits. This work aims to investigate the effect of the ratio of tryptophan (a model non-ionic amino acid) to indomethacin (a model drug) on a non-salt-based CAM prepared via freeze-drying with the *tert*-butyl alcohol-water cosolvent system. The CAM material was systemically characterized at various stages of the freeze-drying process using DSC, UV–Vis, FT-IR, NMR, TGA and XRPD. Dissolution performance and physical stability upon storage were also investigated. Freeze-drying using the cosolvent system has been successfully shown to produce CAMs. The molecular interactions involving H-bonding, H/π and π–π between compounds have been confirmed by FT-IR and NMR. The drug release rate for formulations with a 1.5:1 drug: amino acid molar ratio (or 1:0.42 wt ratio) or below is found to be significantly improved compared to the pure crystalline drug. Furthermore, formulation with a 2.3:1 drug:amino acid molar ratio (or 1:0.25 wt ratio) or below have shown to be physically stable for at least 9 months when stored at dry condition (5% relative humidity, 25 °C) compared to the pure amorphous indomethacin. We have demonstrated the potential of freeze-drying using *tert*-butyl alcohol-water cosolvent system to produce an optimal non-salt-based class II drug-amino acid CAM.

1. Introduction

Co-amorphous systems (CAMs) have recently emerged as a highly promising alternative approach for improving the dissolution properties of poorly water-soluble drugs (PWSDs). The term "co-amorphous" was highlighted to distinguish the molecularly mixed glass solutions of low molecular weight (LMW) components. This formulation combines two or more components that form a homogenous amorphous single-phase, either a drug-drug or drug-excipient CAM (1). Among the investigated LMW excipients, amino acids have received the most attention to date. Amino acids offer the advantages of being a natural compounds and generally regarded as safe (GRAS), with high drug loading capacity and miscibility (Chavan et al., 2016; Larsen, 1980). CAM can stabilize amorphous drugs via either specific ionic or non-ionic intermolecular interactions, or no interactions by physically separation of similar molecules (Liu et al., 2021).

To date, the preparation of CAM systems has mostly been done by mechanical activation (e.g., ball milling) as a direct kinetic disorder method (Liu et al., 2021). The technique is relatively aggressive, which may lead to the degradation of the original materials and, consequently, the generation of impurities. Furthermore, this technique is more suited to laboratory-scale production and is of limited interest for industrial-scale production. Therefore, recently, more focus has been placed on scalable thermodynamic production techniques, such as spray-drying and freeze-drying. These methods have higher throughput, lower mechanical stress (i.e., shear forces), and relatively low processing temperature (Singh and Van den Mooter, 2016). However, spray drying may not be applicable for systems with a low glass transition temperature (T_g), as undesirable sticky products could be produced if the T_g falls below the outlet temperature of the process, leading to low product

* Corresponding author.
E-mail addresses: mohalsalhi@ksu.edu.sa (M. Suleiman Alsalhi), ka_lung.chan@kcl.ac.uk (K.L.A. Chan).

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recovery. Additionally, the processing temperature in spray-drying may still be too high for thermolabile products.

Freeze-drying is widely exploited to achieve long-term stable protein formulations with a low risk of loss of biological activity (Rey and May 2020). This technique has been of high interest in the development of fast dissolving oral formulations due to the high porosity and high surface area of the freeze dried products (Dixit et al., 2011). The system is a gentle sublimation process without heating, making it suitable for thermolabile substances. Importantly, freeze-drying has not yet been widely studied for formulating PWSDs in CAM, particularly for non-salt based system (ElShaer et al., 2011; Wostry et al., 2020; Zhu et al., 2018), because there is a limited number of solvents that can freely dissolve PWSD whilst having high enough melting points. Fortunately, among the investigated organic solvents for PWSD, *tert*-butyl alcohol (TBA) has a relatively high freezing temperature, low toxicity and high vapor pressure, which may provide shorter drying time and improved freeze-dried product quality (Ni et al., 2001; Kunz et al., 2018).

Whilst ion-pairing was the major contribution to the salt CAM production for acidic drug and basic amino acids, we have recently demonstrated that some contributions of non-ionic interactions between drugs and amino acids were also involved (Alsalhi et al., 2022). Zhu et al. have also shown the potential of producing non-salt-based CAM drug-amino acids using aqueous freeze-drying, but the drug used in their demonstration was class I BCS rather than class II (Zhu et al., 2018). Furthermore, the use of non-ionic interactions (e.g., π-π) has been shown to be a promising method for the controlled release of oral drugs (Zhuang et al., 2019). The system has the advantages of not altering the structural or functional properties of drugs and being less affected by the pH compared to salt-based CAMs. Thus, it may overcome the disproportionation issue during storage and dissolution. Limited studies have also investigated the optimal ratio between drug and amino acid (Alsalhi et al., 2022; Liu et al., 2020; Jensen et al., 2016).

In this work, we will investigate TBA-water as the freeze-drying solvent for the first time to co-dissolve indomethacin and tryptophan (the model drug and amino acid) to produce a non-salt based CAM. Tryptophan as the co-former has shown to be a generally good amorphous stabiliser and the most superior solubilising agent for PWSDs compared to other amino acids (Alsalhi and Chan, 2022; Kasten et al., 2019). The chemical structures of these compounds are shown in Fig. 1. Additionally, we will investigate the effect of drug: amino acid ratio on the stability and dissolution properties of the CAMs.

2. Materials and methods

2.1. Materials

Indomethacin (IND, γ-polymorph, M_w = 357.79 g mol^{-1}, pKa $_{IND}$ = 4.45 (Brien et al., 1984), caffeine (CAF) and L-tryptophan (TRP), M_w = 204.22 g mol^{-1}, pI = 5.89 (Brandão-Lima et al., 2022) at 99% purity were obtained from Alfa Aesar (Heysham, England). Sodium chloride (≥99% purity) and phosphate-buffered saline (PBS) were purchased from Acros Organics (Geel, Belgium) and OXOID LTD (Hampshire, England), respectively. All powders were used as received. Ultrapure water was produced by a Pure Lab Ultra-System (London, United Kingdom), *tert*-butyl alcohol (TBA), ≥ 99.5% purity, was purchased from Alfa Aesar (Lancashire, United Kingdom), hydrochloric acid (37%) was purchased from Sigma Aldrich. pH meter was calibrated and used with standard buffer solutions: phosphate buffers; pH 7 and 4 ± 0.2 at 25 ± 1 °C (OXOID limited, England). Temperature probe (YC-747UD data logger thermometer) was performed to accurately determine the solution temperature (Resolution 0.1 °C).

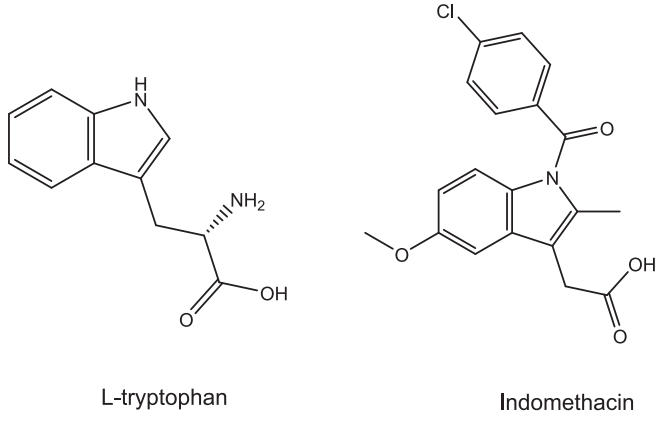

Fig. 1. Chemical structure of Indomethacin and L-tryptophan.

2.2. Method

2.2.1. Preparation of indomethacin TBA and tryptophan aqueous solution mixture

Mixtures of indomethacin-TBA solution and tryptophan aqueous solution at all ratios were investigated for miscibility. Stock solutions of indomethacin in TBA were prepared at 1%, 2%, 3% and 4% w/v. Stock solution of tryptophan in water was prepared at 1% w/v as described above. An appropriate volume of each stock solution was transferred into labelled 7 mL glass vials to produce 0, 10, 20, … 100% w/w of drug and amino acid mixtures. The samples were visually examined for phase separation such as changes in colour, turbidity, or precipitation.

2.2.2. Preparation of feed solution

Stock solutions of indomethacin in TBA and tryptophan in water were prepared at 1% w/v. Drug loading range from 30 to 90% w/w drug/ co-former was chosen for feed solutions.

2.2.3. Sub ambient differential scanning calorimetry (DSC) on freeze concentrate samples

2.2.3.1. Preparation of binary (TBA-water), tertiary (indomethacin-TBA-water) and quaternary (indomethacin-tryptophan-TBA-water) solutions. DSC thermograms were obtained using a Q20 DSC instrument (TA Instruments, New Castle, DE, USA) with a refrigerated cooling accessory (RCS). Solutions of 10 μL of either pure TBA, water or their binary mixtures (10–90% v/v TBA/water), tertiary (indomethacin-TBA-water, 1% w/v indomethacin in TBA-water cosolvent systems (50–90% v/v TBA)) and quaternary (indomethacin-tryptophan-TBA-water at ratios of 30–90% w/w indomethacin/tryptophan and v/v TBA/water) were placed in T-zero aluminium pans with T-zero lids, which are not creamed with pinholes (TA instrument, United Kingdom). The DSC was calibrated and validated using indium (average melting temperature 156.36 ± 0.03 °C and enthalpy 29.81 ± 0.05 J g^{-1}). The thermal cycles were designed by equilibrating the samples at 25 °C, followed by an isothermal temperature for 5 min, and then cooling from 25 °C to −40 °C, held for 10 min, then heated to 30 °C with a heating rate and nitrogen flowrate of 5 °C/min and 50 mL/min, respectively. 10% and 50% v/v TBA solutions were further investigated for their metastable transition by annealing the frozen solution at −9 °C for 1 h. All results were analysed using the TRIOS software.

2.2.4. Freeze-drying cycle

The freezing vials of feed solutions were immersed in liquid nitrogen until completely frozen. The frozen solutions were transferred directly from the liquid nitrogen to the freeze drier shelf (FDS), within the drying chamber of a bench top freeze dryer (Lyotrap freeze dryer; LTE Scientific Ltd). Both displayed temperature and pressure were monitored and recorded throughout the primary drying cycle (-52 ± 1 °C and at ≤ 0.2 ± 0.006 mbar in the absence of samples). The primary drying cycle was lasted for 24 h. Then, the samples were transferred into a vacuum

desiccator containing phosphorous pentoxide (P_2O_5) attached to nitrogen gas (0.3 L/min) at 23 ± 2 °C for 24 h in the secondary drying stage.

2.2.5. Visual appearance of freeze-dried cake

Freeze-dried indomethacin from TBA, tryptophan from water, and their mixtures (30–90% w/w drug) were visually inspected for cake appearance, including melt back, shrinkage and collapse (Patel et al., 2017). Vials were randomly selected for the inspection.

2.2.6. X-ray powder diffraction (XRPD)

XRPD spectra were collected using an X-ray diffractometer (a Philips FW 1700, Philips, Netherlands). The samples were irradiated with monochromatized Cu Kα radiation (1.542 Å) and analysed between 4° and 45° (2θ). The voltage and current used were 40 kV and 30 mA, respectively with chart speed at 10 mm/s.

2.2.7. Fourier transform Infrared (FT-IR)

Infrared spectra were collected using an FT-IR spectrometer (Spectrum One, Perkin Elmer, Seer Green, UK) with a diamond attenuated total reflectance (ATR) accessory (Golden Gate™, Specac Ltd, UK). Samples were collected with the Spectrum 10 software over a spectral range of 4000–400 cm^{-1} and an accumulation of 64 scans with 4 cm^{-1} resolutions. A background spectrum was collected with a clean ATR surface. Reference amorphous indomethacin was obtained by quench cooling (melt quench) from the melting temperature (161 ± 0.9 °C) to room temperature.

2.2.8. Nuclear magnetic resonance (NMR)

NMR spectra were collected using a Bruker Ascend 400 MHz NMR spectrometer. Sample solutions of indomethacin were prepared at their saturated solubility (excess amount of drug) in deuterium oxide (D_2O) at 25 ± 2 °C (approximately 20 μg/mL). 10 mg/mL of tryptophan in D_2O or combined with the saturated indomethacin were prepared. The samples were bath sonicated for 2 min and stirred for 24 h until equilibrium was established. The samples were then filtered using a membrane filter (syringe filter, 0.45 μm sterile). Samples were collected with 2048 accumulation scans. The D_2O signal at 4.79 ppm was used as a chemical shift reference and subtracted from all spectra.

2.2.9. Differential scanning calorimetry (DSC)

Powdered samples of 1 to 5 mg were weighed in T-zero aluminium pans with T-zero lids. The as received pure amino acids and physical mixtures of drug-amino acids 1:1 M ratio samples were measured under a flow of nitrogen purge gas at 50 mL/min from room temperature to 300 °C with a heating rate of 10 °C/min, except for the pure as received indomethacin and the freeze-dried complexes indomethacin-amino acids, where these samples were heated to 200 °C due to the instability of indomethacin at above the melting point (Shimada et al., 2018). Indomethacin (as received) was analysed in two heating cycles to produce an amorphous form.

2.2.10. Thermogravimetric analysis (TGA)

TGA thermograms were conducted using a TA instrument Q500 TGA. The system was calibrated using an empty platinum pan. Powder samples of 3 to 10 mg were weighed in a platinum pan. The thermal cycle was conducted from 25 °C to 120 °C with a heating rate of 10 °C/min, followed by an isothermal period of 2 min at 120 °C.

2.2.11. Uv–vis analysis

The UV–Vis spectra (400–200 nm) were measured using a double beam PerkinElmer Lambda 2 UV–Vis spectrophotometer. Model drugs were measured in phosphate buffer (PBS) pH 6.8 or/and the cosolvent system (TBA-water 50% v/v TBA). The same solvent without the drug/amino acid was used as the reference. Samples were analyzed at identical conditions: 1 nm slit width, 0.5 nm bandwidth and 1 cm path length.

2.2.12. Preparation of stock solution and calibration curve

A calibration curve was obtained by measuring solutions of concentration range from (5 μg/mL–50 μg/mL) for indomethacin prepared by diluting from a stock solution of 100 μg/mL in TBA-water 50% v/v cosolvent. Two stock solutions of caffeine and indomethacin were also prepared at 100 μg/mL and 30 μg/mL, respectively in phosphate buffer (PBS) pH 6.8. Serial dilution was performed to produce solutions of concentration range from (4 μg/mL–20 μg/mL) for caffeine and (0.5 μg/mL–14 μg/mL) for indomethacin. Samples were analysed through UV spectrometry at 320 nm (indomethacin) and 273 nm (caffeine).

2.2.13. Uv–vis spectrometry validation

The method was validated for accuracy, precision, the limit of detection (LOD), the limit of quantification (LOQ), linearity and range according to the international conference on harmonization (ICH) guidelines (International Council for Harmonization Guidline, 2014), Supplementary materials, Tables S1-S3. The linearity of calibration curves was determined over the concentration range of (5 μg/mL–50 μg/mL) for indomethacin in cosolvent system, (4 μg/mL–20 μg/mL) for caffeine in PBS and (0.5 μg/mL–14 μg/mL) for indomethacin in PBS. Precision was studied as repeatability. Repeatability was performed by running the same concentration of drug three times. The limit of detection and quantification (LOD and LOQ) were calculated based on standard deviation formula: LOD = (3.3 × STD / S) and LOQ = (10 × STD / S). STD = Standard deviation, S = slope of calibration curve.

2.2.14. Percentage drug loss

Indomethacin-tryptophan mixtures at ratios of 30–100% w/w indomethacin prepared by freeze-dried and physical mixing were examined using UV–Vis spectroscopy for their drug content. The samples were prepared at 100 μg/mL in 100 mL of 50% v/v TBA-water cosolvent system and analysed at 320 nm (n = 6). The percentage drug loss through the process was calculated by Equation 1:

$$DrugLoss(\%) = \left[1 - \left(\frac{measured\ drug\ content}{theoretical\ drug\ content}\right)\right] \times 100$$

2.2.15. Solubility study

The saturated solubility of indomethacin in phosphate buffer at pH of 6.8 and 37 ± 0.5 °C was conducted with the shake flask method, proposed by Higuchi and Connors (Higuchi, 1965). An excess drug was added to 10 mL of phosphate buffer solution. The samples were sonicated and then stirred for 24 h. The samples were filtrated using a membrane filter (syringe filter, 0.45 μm sterile). The filtered solutions were diluted within the range of the calibration curve and assayed - spectrophotometrically at 320 nm. The experiment was repeated independently for three times (n = 3).

2.2.16. Physical stability upon storage

The freeze-dried samples were stored in open vials in two conditions: 75% ± 5 relative humidity (RH) at 25 ± 2 °C and 5% ± 5 RH at 25 ± 2 °C in a closed vessel containing either a saturated sodium chloride salt solution or phosphorus pentoxide (P_2O_5) over 9 months, similar to the ICH stability storage guidelines (International Council for Harmonization, 2003). The humidity and temperature were recorded and monitored during the whole experiment. The samples were analysed immediately after freeze drying and intervalley at 1, 3, 6 and 9 months using thermal and spectroscopy techniques.

2.2.17. Dissolution studies

Pure crystalline indomethacin and freeze-dried formulations at ratios of 40–90% w/w indomethacin were examined for their dissolution rates under sink conditions. Formulation containing 10 mg of indomethacin was dispersed into 10 mM phosphate buffer dissolution media (pH 6.8 and 37 ± 0.5 °C) in 50 mL mini-size glass baker vessels of nonstandard dissolution apparatus (for validation, see supplementary materials,

Figure S1). The agitation rate was investigated at 200 rpm using different slide round stirrer bars (8 mm diameter – 25 mm length) in a multi-position magnetic stirrer with an integrated temperature control plate (RT 10 power, IKA, England LTD). The high-speed agitation was chosen to allow the media to interact with powder dosage form and minimize undesired effects such as coning (Hellberg et al., 2021). The pH and temperature were monitored using the pH meter and digital thermocouple indicator type K. Aliquots of 4 mL were withdrawn at 5, 10, 15, 30, 45, 60 and 120 min and replaced with fresh dissolution media. All samples were filtered using a membrane filter (syringe filter, 0.45 μm sterile). The concentration of the dissolved drug was determined spectrophotometrically at 320 nm (indomethacin) and 273 nm (caffeine). All dissolution experiments were conducted independently four times (n = 4).

2.2.18. Validation of UV–Vis for dissolution studies

The mini-size nonstandard dissolution apparatus was validated via assessing the dissolution profiles of pure crystalline model drug and at verifying pharmacopeia compliance using caffeine as a reference standard of fast-dissolving compound class I BCS, Supplementary materials, Figure S2. The dissolution profiles of pure crystalline drug obtained by both the mini size non-standard dissolution apparatus (50 mL) and the USP standard paddle dissolution apparatus (900 mL) are correctly declared as similar and caffeine standard drug as a complaint with respect to its specifications. The agitation rate for the mini nonstandard apparatus was tested at 200 rpm, while 100 rpm and 150 rpm for the paddle standard apparatus at identical conditions of drug concentration (0.2 mg/mL), dissolution media (PBS), temperature (37 ± 0.5 °C) and pH (6.8). Sample volumes of 4 mL were withdrawn at specific time points (2, 5, 10, 15, 30, 45 and 60 min). All samples were filtered using a membrane filter (syringe filter, 0.45 μm sterile). Quantification of the dissolved indomethacin or caffeine concentration was performed by UV spectrophotometrically at 320 nm and 273 nm, respectively. All dissolution experiments were conducted independently three times (n = 3). The highly soluble model drug is expected to release 85% within 15 min according to the guidance of dissolution testing of the immediate release solid oral dosage form (Shah et al., 1997).

2.2.19. Statistical evaluation of dissolution profile

The in-vitro dissolution profiles were tested for analysis of variance (ANOVA) and multiple comparisons t-tests. AVOVA test was applied to investigate whether there are significant differences among the percentage dissolved at each time level, multiple *t*-test comparisons between freeze-dried formulation against reference indomethacin were applied separately to each formulation for the comparison of percentage dissolved at the sequential times (Yuksel et al., 2000; Hara et al., 1997).

3. Results and discussion

3.1. Characterization of the liquid-state

The low solubility of indomethacin in water and amino acids in organic solvents necessitate the use of an alternative solvent system for freeze-drying. The TBA-water cosolvent system has been selected as it is extensively used for dissolving PWSDs (Wei et al., 2012). The mixability of the TBA-water systems was first examined and all combinations were found to be completely miscible at room temperature and atmospheric pressure, as expected (Woznyj and Lüdemann, 1985). Fig. 2 shows the miscibility of 1% w/v tryptophan in water as a function of various indomethacin concentrations in TBA. At the highest concentration of indomethacin (4% w/v), all mixtures were phase separated. However, a single-phase system was generally observed at lower concentrations of indomethacin and/or a lower ratio of tryptophan aqueous solution in the mixture. A drug loading between 30% and 90% w/w of 1% w/v drug and tryptophan concentrations was chosen for further investigation because it has the widest range of solutions that are mixable compared to the other drug concentrations.

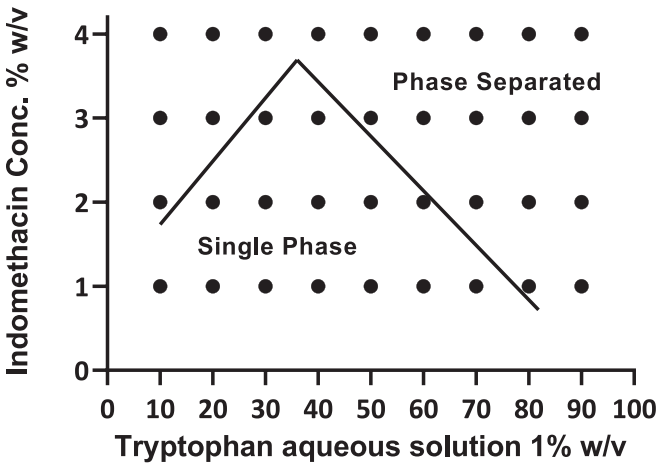

Fig. 2. Diagram showing region of phase separation of tryptophan aqueous solution 1% w/v as a function of various concentrations of indomethacin TBA solution, based on visual examinations of liquid mixtures.

3.2. Characterization of the freeze concentrate solutions

3.2.1. Tba-water

Frozen samples were analysed to determine their critical temperatures, including collapse temperature (T_c), eutectic melting temperature (T_{eu}) or primary glass transition temperature (T'_g), using DSC. These critical temperatures help define the maximum allowable product temperature during primary drying without the risk of losing their cake structure through melting back or collapsing. The water thermogram shows one broad melting endothermic transition at 0 °C corresponding to the melting of ice crystals. Glassy water was previously reported at ~ -133 °C (Angell, 2002). The TBA thermogram shows two melting endothermic transitions. The major event occurred at ~ 20 °C, attributed to the melting of TBA crystals, while the minor event at ~ -8 °C might be due to the eutectic melting temperature of TBA hydrate as a result of absorbed moisture (Kasraian and DeLuca, 1995). Contrastingly, upon heating the 10% v/v TBA solution, two endothermic events were observed at ~ -10 °C and 0 °C. The first event was due to the TBA dihydrate-ice eutectic, while the second event was related to ice melting. For the 50% anda70% v/v TBA solutions, three endothermic events were observed. The first two endotherms occurred at ~ -10 °C and −8 °C, which might be due to the TBA dihydrate-ice eutectic and TBA heptahydrate-ice eutectic, respectively (Sonje et al., 2020). The third event at ~ 0 °C is suggested to be caused by ice melting. For the 90% v/v TBA, however, the thermogram shows one broad endothermic event occurring at ~ -8 °C (Supplementary materials, Figure S3).

Several thermal events were observed associated with the frozen TBA-water binary solutions at different ratios, making it challenging to unambiguously attribute to the specific phases. The TBA-water binary system has been explored for its use in freeze-drying by several groups (Sonje et al., 2020; Ogienko et al., 2019; Bhatnagar et al., 2020; Thakral et al., 2021). However, there is still some ambiguity in the phase behaviours. Kasraian and Bhatnagar reported that the lower melting point (-10 °C) was due to the formation of metastable TBA phase (Bhatnagar et al., 2020; Kasraian and DeLuca, 1995). To understand this behaviour, annealing of frozen solutions at −9 °C, held for 1 h was performed (Pansare and Patel, 2016). The endotherm observed in both samples annealed at ~ -10 °C is not consistent with the endotherm arising from the metastable state but is more likely a stable state (Supplementary materials, Figure S4), which is consistent with previously reported findings (Wittaya-Areekul et al., 2002). However, the metastable transition could be overlapping with the melting of the stable transition. In

fact, several complex thermal events were observed over a short range of temperatures, and therefore some overlapping can be expected. This is considered an intrinsic limitation of DSC for characterizing frozen solution of multicomponent system.

3.2.2. Indomethacin in TBA-water

The heating curves of indomethacin in the solvent system show that the thermal events of the TBA-water systems were not affected by adding indomethacin. Interestingly, the indomethacin in pure TBA sample shows an exothermic event at −37 °C due to drug recrystallization. This event was completely disappeared after the second heating cycle. However, this was not observed in the indomethacin-TBA-water systems at all ratios examined (Supplementary materials, Figure S5).

3.2.3. Indomethacin-Tryptophan in TBA-water

The thermograms of frozen tryptophan show an endothermic melting peak of the eutectic mixture at −1 ± 0.7 °C (Supplementary materials, Figure S6) with no sign of a metastable event. This is consistent with previous reports that aqueous solution of most amino acids tend to crystallize and form eutectic mixtures (Horn et al., 2018; Lueckel et al., 1998; Steven et al., 2012). Interestingly, the thermograms of multiple solutes (i.e., indomethacin and tryptophan) in all cosolvent systems demonstrate that the thermal events of TBA-water systems were not affected by adding the solutes. Furthermore, no visible metastable event was detected, suggesting that this event could happen at a lower range of temperature than the current experiment or overlapped with the eutectic events (Supplementary materials, Figure S6).

Prior determination of the physical and thermal behaviours, such as phase transitions and critical temperatures of frozen multicomponent solutions is an integral goal for developing an efficient freeze-drying cycle. Previous studies on phase diagrams of eutectic melting have mainly focused on the binary TBA-water system, whilst there have been insufficient studies examining the effect of a multicomponent system (e.g., drug and amino acid) (Bhatnagar et al., 2020; Kasraian and DeLuca, 1995; Woznyj and Lüdemann, 1985). Fig. 3 shows the observed phase behaviour of indomethacin-tryptophan in the TBA-water cosolvent systems. Interestingly, the addition of these two substances (indomethacin and tryptophan) has shown no influence on the thermal behaviour of the TBA-water cosolvent system. Furthermore, the glass transition temperature (T'_g) of this system was not apparent within the range of temperature examined in the TBA-water mixture, while the highest eutectic melting temperature (T_{eu}) occurred at ~ -11 °C. This temperature can be used to estimate the crystallization phenomenon, which is assumed to be equal to the collapse temperature (T_c). Therefore, drying below this temperature would be desired to avoid melting back and destroying the properties of a freeze-dried solid, which may further impact on product quality, such as residual water content and stability. In general, the product temperature should be kept below the critical temperatures (i.e., T'_g if it is in amorphous state or below T_{eu} if it is in crystalline state) during primary drying to produce an acceptable and elegant cake appearance with no major defects (e.g., melt back, collapse and shrinkage) (Tang and Pikal, 2004; Khairnar et al., 2013). However, it has been reported that drying an amorphous formulation above its T'_g, while remaining below the collapse temperature (T_c) could be possible without impacting the physical stability, appearance and performance (Depaz et al., 2016).

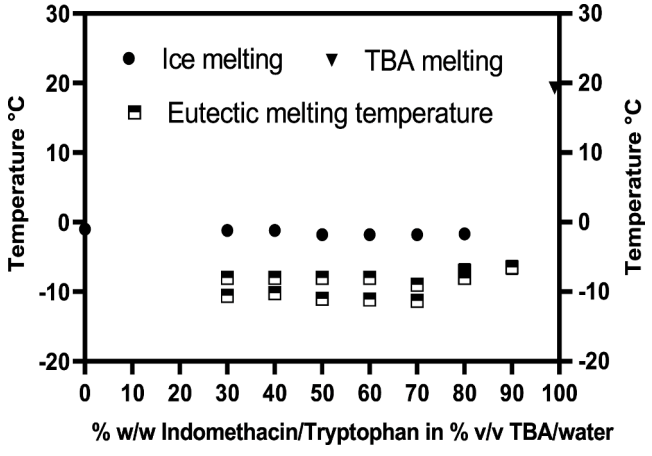

Fig. 3. The observed phase behaviour of the heating cycle of frozen TBA containing 1% w/v indomethacin, water containing 1% w/v tryptophan and their quaternary mixtures indomethacin (IND)-tryptophan (TRP) in TBA-water at ratios of 30–90% w/w indomethacin and v/v TBA as mean of n = 3 ± Std, highlighting TBA, ice and eutectic melting temperatures.

3.3. Characterization of the freeze-dried solid-state

3.3.1. Visual appearance of freeze-dried cake

The finished pharmaceutical products after freeze-drying were visually examined for their cake appearance. Changes in cake appearance could be indicative of change in the product quality, including residual solvent levels and stability. Fig. 4 shows the cake appearance of freeze-dried formulations. Inconsistent appearances were observed, which is expected due to their different compositions. Freeze-dried tryptophan from water (Fig. 4A) showed a volcano formation in the middle of the vial, suggesting it was due to a sudden contraction after the fast freezing method using liquid nitrogen (Esfandiary et al., 2016). The freeze-dried indomethacin from TBA (Fig. 4 I), however, exhibited poor cake quality with melt back and extensive collapse observed at the bottom of the vial (Patel et al., 2017). Melt back is a form of cake collapse caused by the change from solid to liquid state. Increasing residual solvent level can significantly affects the physical and chemical stability of the product, such as the rate of crystallization and degradation. Fig. 4 B (30% w/w indomethacin) demonstrated a blowing out of powder during primary drying, suggesting that the freeze-dried cake was not cohesive enough to stand and was ejected from the matrix as the solvent vapor escape. This might be due to phase separation between drug and amino acid. Freeze-dried formulations with 40–80% w/w indomethacin have shown successful production of elegant cakes without major defects (Fig. 4 C-G) (Patel et al., 2017). The slight cracks on the cake structure or splashing on the inside walls of vails were observed and increased with an increasing ratio of drug-TBA in the formulations. The crack issue, in general, is considered a relatively minor cosmetic defect with a neglectable impact on the stability of the final lyophilised product. However, extensive cracks sometimes can lead to a major cake collapse as seen with the 90% w/w indomethacin formulation (Fig. 4 H), which could impact product quality and stability.

3.3.2. XRPD

Fig. 5 shows the XRPD diffractograms of pure indomethacin, tryptophan, freeze-dried indomethacin from TBA, and tryptophan from water, and their freeze-dried formulations. The diffractogram of pure indomethacin shows sharp peaks identified at 10.2, 11.7, 16.7, 19.6, 20.5 and 21.9°, indicative of the γ-polymorph form of the drug, while peaks at 10.0, 14.8 and 20.0° are associated with L-tryptophan (Li et al., 2015; Van Duong et al., 2018). However, the diffractograms of freeze-dried indomethacin from TBA and tryptophan from water show clear differences compared to pure materials as received, suggesting that different polymorphs are formed when the pure drug and amino acids are freeze-dried from TBA and water. The peaks observed at 6.9, 8.5, 11.5, 13.9, 14.2, 17.6, and 18.0° indicate the formation of the α-form of the drug after freeze-drying (Van Duong et al., 2018). Both pure indomethacin and tryptophan have shown to crystallize after freeze-drying. However, the diffractograms of all freeze-dried drug-amino acid mixtures showed no crystalline peaks, indicating that co-amorphization was successfully produced after freeze-drying.

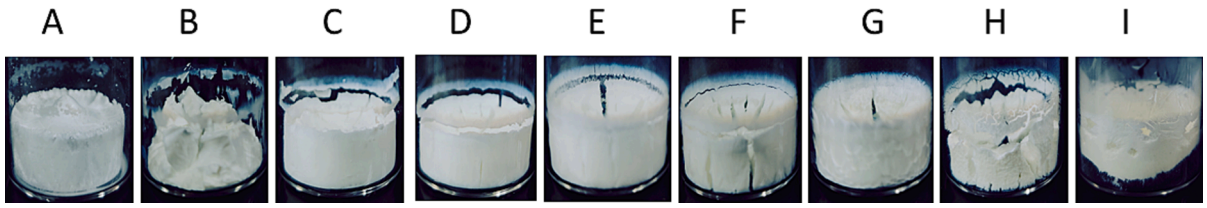

Fig. 4. Freeze dried products by using liquid nitrogen as freezing method; (A) showing tryptophan from water; (B-H) showing mixtures of indomethacin-tryptophan (30% to 90% w/w indomethacin) from TBA-water (30% to 90% v/v TBA). (I) showing indomethacin from TBA.

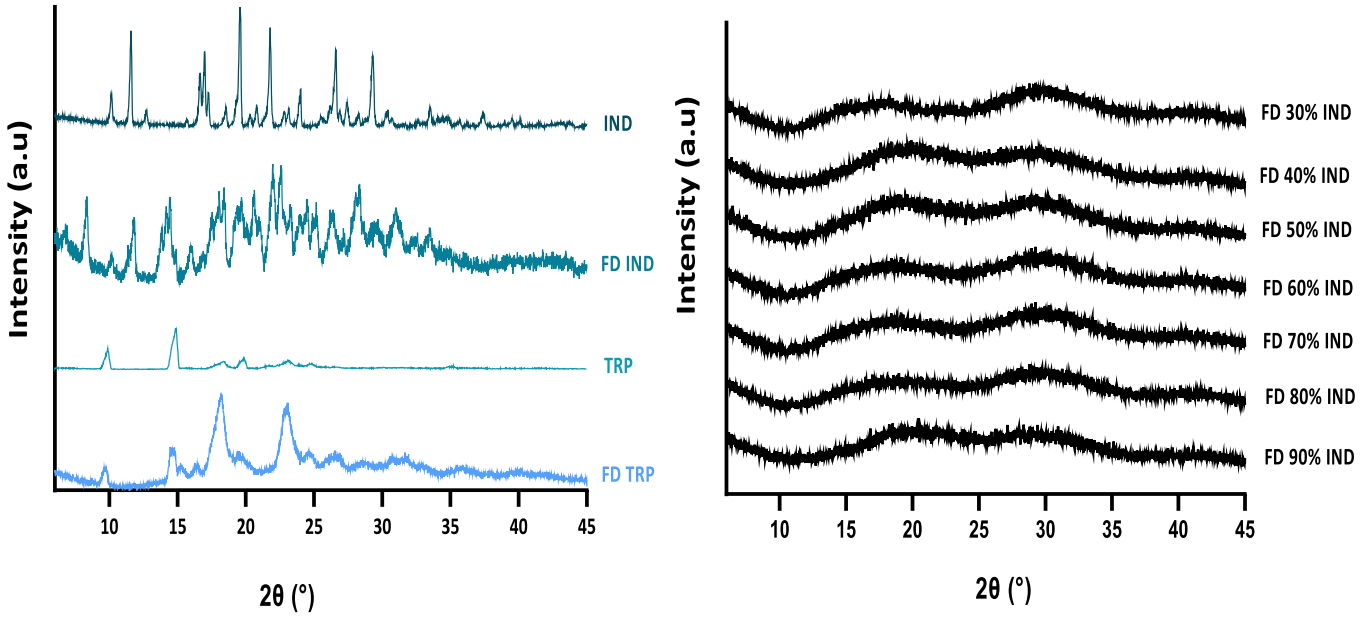

Fig. 5. XRPD diffractograms of pure γ-indomethacin (IND), tryptophan (TRP), freeze died indomethacin from TBA (FD IND), tryptophan from water (FD TRP), and their mixtures prepared by freeze drying at ratios of 30% to 90% w/w indomethacin in tryptophan (FD 30% to FD 90% w/w IND).

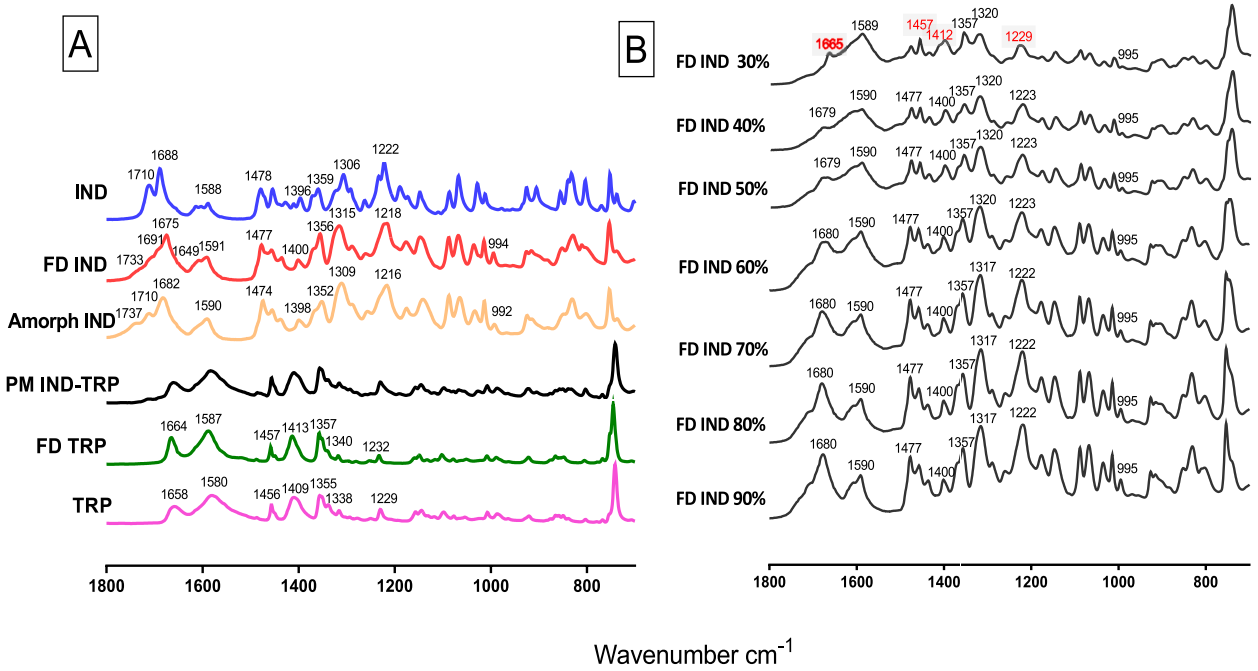

Fig. 6. FT-IR spectra of (A) pure γ-indomethacin (IND), L-tryptophan (TRP), freeze dried indomethacin from TBA (FD IND) and tryptophan from water (FD TRP), amorphous indomethacin (Amorph IND), physical mixture indomethacin-tryptophan 1:1 wt ratio (PM IND-TRP). (B) freeze-dried indomethacin-tryptophan at ratios 30% to 90% w/w indomethacin (FD 30% to FD 90% w/w IND).

3.3.3. FT-IR

Fig. 6 (A-B) shows the FTIR spectra of pure indomethacin, tryptophan, the freeze-dried drug from TBA, amino acid from water, and their mixtures (prepared by freeze-drying or physical mixing). The most significant changes can be observed in the region between 1800 cm^{-1} to 700 cm^{-1}. Pure indomethacin (Fig. 6A) shows two intense peaks at 1710 cm^{-1} and 1688 cm^{-1}, associated with the carbonyl stretching mode vibration (ν(C = O)) and peaks at 1588 cm^{-1}, 1478 cm^{-1}, 1396 cm^{-1}, 1359 cm^{-1}, 1306 cm^{-1} and 1222 cm^{-1} from the indole ring stretching, CH_3 deformation, indole ring deformations, acetic –COO coupled with the C-O and CCC stretching, CH_2 wagging and benzene-CH bending mode, respectively, (Badawi and Förner, 2014) confirming that the drug is in the γ-crystalline form (Van Duong et al., 2018). However, the freeze-dried indomethacin from TBA shows clear differences in peak positions at 1733 cm^{-1}, 1691 cm^{-1}, 1675 cm^{-1}, 1649 cm^{-1}, 1591 cm^{-1}, 1477 cm^{-1}, 1400 cm^{-1}, 1356 cm^{-1}, 1315 cm^{-1}, 1218 cm^{-1} and 994 cm^{-1}, indicating that the α-crystalline form of the drug is produced. The α-form is commonly reported to be the first metastable form after desolvation (Van Duong et al., 2018). In contrast, the amorphous indomethacin (Fig. 6A) shows broader bands with peaks at 1737 cm^{-1}, 1710 cm^{-1}, 1682 cm^{-1}, 1590 cm^{-1}, 1474 cm^{-1}, 1398 cm^{-1}, 1352 cm^{-1}, 1309 cm^{-1}, 1216 cm^{-1}, 992 cm^{-1}. The first two bands at 1737 cm^{-1} and 1710 cm^{-1} are due to the hydrogen and non-hydrogen bonded carbonyl group (Ewing et al., 2014). In general, the most significant change with all indomethacin forms can be seen in the ν(C = O) region at ~ 1700 cm^{-1}.

The spectrum of pure tryptophan (Fig. 6A) shows peaks at 1658 cm^{-1}, 1580 cm^{-1}, 1355 cm^{-1}, 1338 cm^{-1} and 1229 cm^{-1}. The first band corresponds to the ν(C = O) peaks, and the remaining peaks correspond to benzene ring stretching and bending mode vibration (Wolpert and Hellwig, 2006; Cao and Fischer, 1999). The intense band observed at 1409 cm-^{1} is assigned to $\nu_{sym}(COO)^-$. The spectrum of the freeze-dried tryptophan from aqueous solution, however, shows clear peaks at 1664 cm^{-1}, 1587 cm^{-1}, 1413 cm^{-1}, 1357 cm^{-1}, 1340 cm^{-1} and 1232 cm^{-1}, which are slightly shifted compared to pure tryptophan, suggesting a change in the crystallinity after freeze drying.

Fig. 6B shows the spectra of the freeze-dried indomethacin-tryptophan formulations (30–90% w/w indomethacin). At higher drug loadings, the freeze-dried formulations show spectra with a stronger contribution from the drug. The spectrum of the freeze-dried formulation (30% w/w drug) shows no differences in peak positions at 1665 cm^{-1}, 1457 cm^{-1}, 1412 cm^{-1} and 1229 cm^{-1} compared to freeze-dried tryptophan, suggesting that the structure of tryptophan has not been affected, indicating a non-homogeneous or phase separated system (Löbmann et al., 2013). However, peak shifts are observed for 40–90% w/w drug, suggesting molecular interactions were detected (Fig. 6B). Furthermore, the spectra of the freeze-dried indomethacin-tryptophan formulations show clear differences in the indomethacin peaks when compared to the pure amorphous indomethacin or freeze-dried indomethacin, indicating that specific interactions might be involved between indomethacin and tryptophan (Ewing et al., 2015).

The peaks at 1309 cm^{-1} and 1216 cm^{-1} in the amorphous indomethacin spectrum (Fig. 6A) can be assigned to the CH_2 wagging and benzene-CH bending mode vibrations, respectively. These peaks are shifted to 1315 cm^{-1} and 1218 cm^{-1} in the freeze-dried indomethacin and further to 1320 cm^{-1} and 1223 cm^{-1} in the freeze-dried indomethacin-tryptophan formulations (Fig. 6B). These small but reproducible shifts suggest the involvement of weak non-ionic interactions such as CH-π and π-π stacking between aromatic rings of indomethacin and tryptophan. Other apparent shifts could be found at 1682 cm^{-1} and 1352 cm^{-1} in the amorphous indomethacin spectrum. These peaks are assigned to the C = O stretching and acetate COO– coupled with the C-O and CCC stretching. They are shifted to 1675 cm^{-1} and 1356 cm^{-1} in the freeze-dried indomethacin and further to 1679 cm^{-1} and 1357 cm^{-1} in the freeze-dried indomethacin-tryptophan formulations. Furthermore, the plateau-like feature at ~ 1650 cm-^{1} region has been observed in the freeze-dried (40–70% w/w drug), but less with freeze-dried formulation (80–90% w/w drug), suggesting that complex H-bonds interactions are involved between the drug and tryptophan. These interactions are likely to consist of H-bonding, CH-π and π-π interactions because both hydrophobic and amide regions are significantly shifted in their respective bands.

3.3.4. NMR

^{1}H NMR spectroscopy was used to confirm the functional groups that are involved in the interactions between the amino acid and drug in water. Due to the poor aqueous solubility of the drug, a weak NMR signal was expected, but this issue was overcome by obtaining the saturated concentration and increasing the number of scans. The peak shift, which indicates changes in the environment of the neighbouring protons, was used to assess the molecular interactions (Nandy et al., 2007; Malta et al., 2009). All resonance bands were referenced to the deuterium oxide solvent signal at 4.79 ppm.

The ^{1}H NMR spectra of pure amino acid and model drug (Supplementary material, Figures S7 and S8) were in good agreement with previously reported data (Brien et al., 1984; Dubinnyi et al., 2020). Fig. 7 shows the ^{1}H NMR spectra of the model drug with and without tryptophan, highlighting the major changes. The bands at 6.77 ppm – 6.79 ppm were shielded and shifted to 6.72 ppm – 6.74 ppm in the indomethacin-tryptophan complex. These reproducible changes indicate interactions are present between the drug and the amino acid, potentially through H-π or π-π stacking, among the aromatic moieties. This result supports our finding from the FTIR and also is consistent with data previously reported (Alsalhi and Chan, 2022).

3.3.5. DSC

DSC was used to confirm that the amorphous formulation of indomethacin-tryptophan has successfully produced. The results of the phase transitions (glass or melting temperatures) of pure indomethacin, tryptophan, the freeze-dried drug, amino acid and 1:1 physical mixing (by weight) are shown in Supplementary material, Figures S9. Pure crystalline indomethacin shows a sharp endothermic melting peak at 160 ± 0.2 °C, confirming its γ-crystalline form (Surwase et al., 2013). The glass transition temperature (T_g) of the pure amorphous drug (42.1 ± 0.5 °C) was obtained via the quench cooling method. However, the freeze-dried indomethacin from TBA shows a melting peak followed by recrystallization peak, the two events occurred closely together, which might be due to the formation of indomethacin and TBA solvate (Joshi

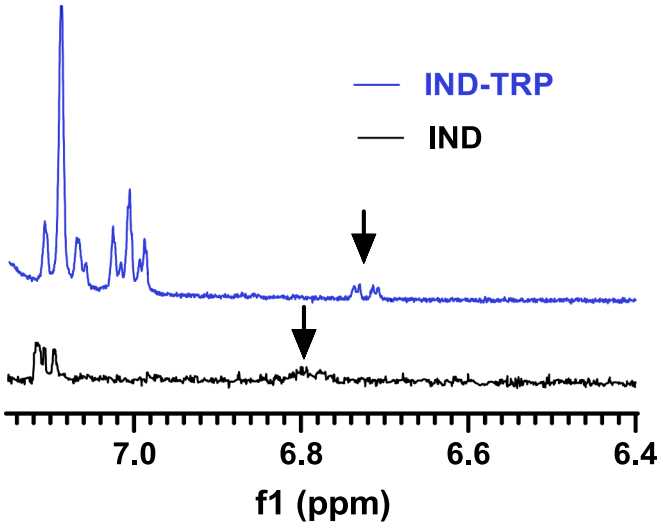

Fig. 7. ^{1}H NMR spectra of pure indomethacin and indomethacin-tryptophan complex in D_2O, highlighting the main changes in the aromatic regions.

et al., 1998). This was followed by transforming to α-crystalline form with an endothermic melting peak at 154 ± 0.5 °C, consistent with previously reported (Van Duong et al., 2018; Andrusenko et al., 2021). However, no glass transition was observed, suggesting that amorphous indomethacin has weak glass transition and was difficult to stabilise at close to room temperature (Kasten et al., 2019). The pure tryptophan shows an endothermic melting peak at 292 ± 0.3 °C (Rodante et al., 1992; Fulem, 2021), while the freeze-dried tryptophan from an aqueous solution shows a melting peak 284 ± 0.2 °C, indicating that tryptophan crystallised during primary drying into another polymorphic form. The physical mixtures, in contrast, show a broad melting peak of indomethacin at the same onset near 160 °C, and a shifted melting peak of tryptophan. This suggests that tryptophan was dissolved in the molten indomethacin.

The thermograms of freeze-dried formulations (30–90% w/w indomethacin) were shown in Supplementary material, Figure S10. The heating cycle was performed up to 180 °C due to instability issues of the drug above 200 °C (Shimada et al., 2018). All freeze-dried indomethacin-tryptophan formulations show no melting peak of the drug at 160 ± 0.2 °C, confirming that they are amorphous. The T_gs of formulations (30–90% w/w drug) were, unfortunately, not observed due to the weak glass transition except for 50% w/w drug. Dynamic mechanical analysis or modulated DSC could be used in future to determine the T_gs for these formulations. For the FD IND 30% sample, an exothermic event (recrystallisation peak) was detected but without an endothermic event (melting of IND), suggesting this might be due to the recrystallization of the amino acid. Whilst the T_g of an amorphous system may be advantageous for long term stability, it is not guaranteed (Craig et al., 1999; Moynihan et al., 1976).

3.3.6. Residual content

Fig. 8 shows the moisture content of the pure drug, amino acid and the freeze-dried samples measured by TGA (Matejtschuk et al., 2016). The pure drug and amino acid show no weight loss, which is expected due to their hydrophobic nature. However, the freeze-dried tryptophan from water and indomethacin from TBA shows average weight losses of 1% and 5% w/w, respectively (Mattern et al., 1999). The higher weight loss observed in freeze-dried indomethacin suggests melt back occurred during sublimation and primary drying process as shown in Fig. 4 I. The lower residual solvent of the freeze-dried tryptophan suggests that amino acid was crystallised from aqueous solution, which is consistent with the previous reports (Horn et al., 2018; Lueckel et al., 1998). The high solvent residual content may impact the physical stability of the amorphous system by increasing molecular mobility and thereby promoting recrystallization.

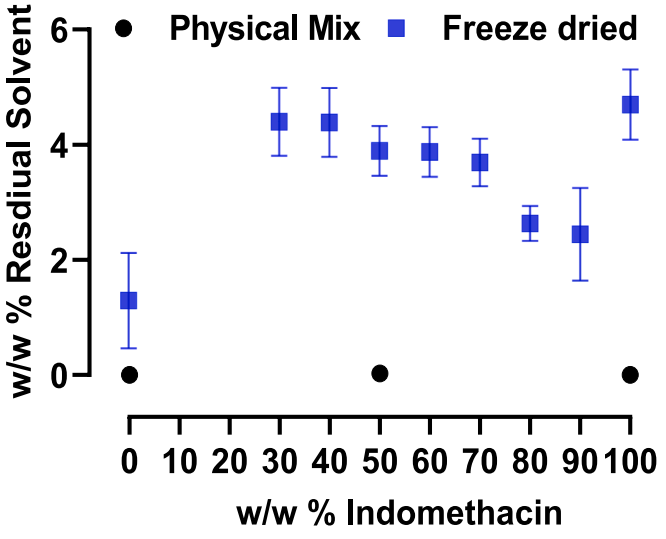

Fig. 8. Average w/w % of residual solvent (TBA-water) at different ratios of the FD and physical mix control of indomethacin in tryptophan samples as mean n = 3 and Std. Secondary drying was carried out for 24 h at a shelf temperature of 25 °C.

Nevertheless, the rapid freezing method (i.e., liquid nitrogen) used in this study has the potential to produce small ice crystals that creates the fine porosity structure in the freeze-dried materials with a high surface area. This structure provides many advantages such as fast dissolution and promoting the efficient removal of solvent during drying. Fig. 8 also shows the average mass loss (residual solvent level) of the freeze-dried formulations. As expected, more residual solvent was found in the amorphous solid materials compared to the crystalline solid (Wittaya-Areekul and Nail, 1998).

3.3.7. Drug content

The indomethacin contents in the drug-amino acid formulations (prepared by freeze-drying or physical mixing) at ratios of 30–100% w/w drug are determined via the UV–Vis method using Equation 1. Table 1 shows the drug content in the freeze-dried drug-amino acid formulations.

3.3.8. Saturated solubility

The aqueous solubility of indomethacin is mainly driven by the ionisation of its carboxylic acid group, which was found to be 32.1 ± 3 μg/mL (pH 6.3, 37 °C). The saturated solubility of indomethacin in phosphate buffer (pH 6.8) also was found to be 575.0 ± 3.4 μg/mL at 37 °C. This was performed to correctly choose the dissolution media, where the sink condition can be achieved to maintain the concentration gradient during the dissolution studies.

3.3.9. Dissolution studies

The dissolution method conducted in this study uses a reduced size nonstandard dissolution apparatus (50 mL) due to the small quantities of the freeze-dried materials produced in the laboratory. The method was validated via either comparing the dissolution profiles to the USP standard paddle dissolution apparatus or by compliance with the dissolution requirements for immediate-release drug forms (Rozet et al., 2012) and the accuracy, precision and reproducibility have been demonstrated to fit the ultimate goal of assuring consistent product quality in the defined set of specification criteria (Supplementary materials, Figure S2).

The in vitro dissolution profiles of pure indomethacin and freeze-dried formulations (40%–90% drug) are shown in Fig. 9. In general, formulations with high drug loading have slower release rates compared to low drug loading formulations due to poor wettability of the hydrophobic drugs. This can result in particles fusing together and agglomerate to form large crystals that hinder the dissolution process (Han et al., 2019).

The result shows significant difference in the percentage of the

Table 1
Average drug content in tryptophan at ratios of 30–100% w/w drug, physical mixtures (PM) as control and freeze dried (FD) formulation, % mean weight ± standard deviation (std) (n = 6).

Content	Physical mix		Freeze dried	
Drug in amino acid %w/w	Average Drug Loss (%w)	STD %	Average Drug Loss (% w)	STD %
30	0	± 1.1	12.8	± 2.4
40	0	± 0.5	4.1	± 2.3
50	0	± 0.5	4.4	± 2.0
60	0	± 1.2	4.2	± 1.5
70	0	± 1.3	4.0	± 2.2
80	0	± 0.4	3.6	± 2.7
90	0	± 0.9	1.0	± 2.1
100	0	± 0.3	0.8	± 1.0

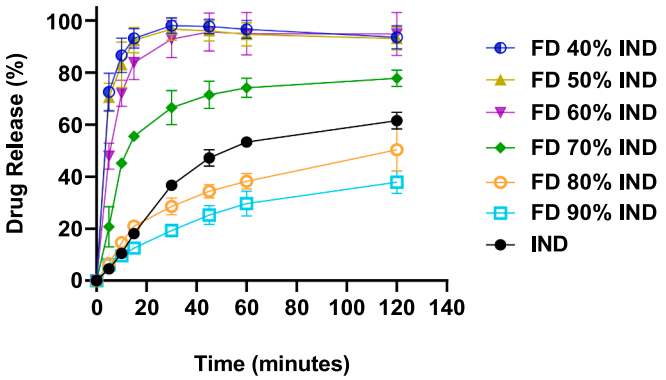

Fig. 9. Dissolution profiles of pure powder γ-polymorph indomethacin (IND) and freeze-dried indomethacin in tryptophan at ratios of 40–90% w/w IND in phosphate buffer dissolution media (pH 6.8, 37 ± 0.5 °C).

dissolved drug at each time point (15, 30, 60 and 120 min) for the freeze-dried formulations compared to pure indomethacin crystals ($p < 0.05$), as shown in Fig. 9. The percentage of the dissolved drug for the 40–50% w/w drug loading reached ~ 90% during the first 15 min, which is the fastest dissolving formulations compared to the others, while for the 60% w/w drug loading, ~80% was dissolved in the first 15 min and fully dissolved by 45 min. These formulations represent a fast-release oral dosage form for the neutral class II drug. In contrast, the 70% w/w drug loading formulation exhibited slower dissolution kinetics, with approximately 50% of the drug dissolved in the first 15 min, 70% dissolved at 45 min, and 80% dissolution by the end of the 120-minute dissolution test. For the highest drug loading formulations (80–90% w/w indomethacin), the percentages of the dissolved drug were not significantly different ($p > 0.05$) when compared to the reference crystalline indomethacin after 5 min and by 45 min. Only 34% (80% w/w drug loading) and 25% (90% w/w drug loading formulation) of the drug were dissolved, compared to 47% for the reference crystalline indomethacin. The slow dissolution profile of these formulations, despite of being amorphous at the beginning, is due to the fusing of particles together to form larger lumps and agglomerates. The increase in dissolution profiles of the low drug loading formulations could be explained by the formation of non-ionic interactions between drug and amino acids in the co-amorphous non-salt system. The time taken for 70% of the drug to be released for the freeze-dried formulation, for example, is approximately 24-fold faster for the 40% w/w drug loading than the pure crystalline drug.

3.3.10. Physical stability

The solid state stability of freeze-dried co-amorphous indomethacin-tryptophan powders at ratios of 50–90% w/w indomethacin were investigated at different conditions (moisture and temperature). The samples were stored at room temperature (25 °C) and relative humidity conditions (5% and 75%), followed by FT-IR and DSC analysis. The two conditions were selected to mimic conditions when the formulation is stored in a desiccated (5% RH, 25 °C) and when stored in an opened environment (75% RH, 25 °C). Physical instability is often associated with molecular mobility that increases with temperature or humidity, which can cause crystallization of amorphous drug. This often happens when the temperature is above the T_g of the amorphous substance or when stored in a high humidity environment. The freeze-dried pure indomethacin from TBA is highly unstable and rapidly recrystallised to the α-polymorph form after freeze drying, which is commonly observed in the desolvation process (Joshi et al., 1998). Fig. 10 (A and B) shows the DSC thermograms and the FT-IR spectra of the freeze-dried co-amorphous indomethacin-tryptophan at ratios of 50–90% w/w drug loading stored over 9 months and freeze-dried indomethacin from TBA stored for one week and month at dry condition (5% RH, 25 °C) for comparison purpose. The features of amorphous and crystalline IND could be distinguished from the FTIR spectra of the stored samples, which is consistent with previously reported (Van Duong et al., 2018; Löbmann et al., 2013). The co-amorphous mixtures (80% w/w drug loading and below) showed excellent physical satbiltiy and remained in a homogenous amorphous form over 9 months, while 90% drug loading shows drug recrystallisation to the α-crystalline form after 6 months. The DSC results in Fig. 10B have shown a recrystallisation peak in each of the amorphous samples (FD 50%-80% IND) but they were not followed by a melting peak at 160 °C. This suggests that the amino acid, which has a melting point above the scanned temperature range, in the co-amorphous system recrystallised during the DSC measurement whilst the drug remain amorphous. Under the more stressed condition (75% RH, 25 °C), however, the storage of freeze-dried co-amorphous indomethacin-tryptophan leads to transform into sticky materials and recrystallize at all ratios within less than a month to α-form of the drug (data not shown). This was due to the moisture absorbed by the hygroscopic amorphous material.

This work highlights the potential of freeze drying as a preparation method to produce the non-salt CAM system that has weak non-ionic interactions using the TBA-water cosolvent system. Freeze-drying indomethacin in TBA and tryptophan in water did not produce stable amorphous forms. However, the combination of TBA and water was found to be a successful cosolvent for producing CAM system of

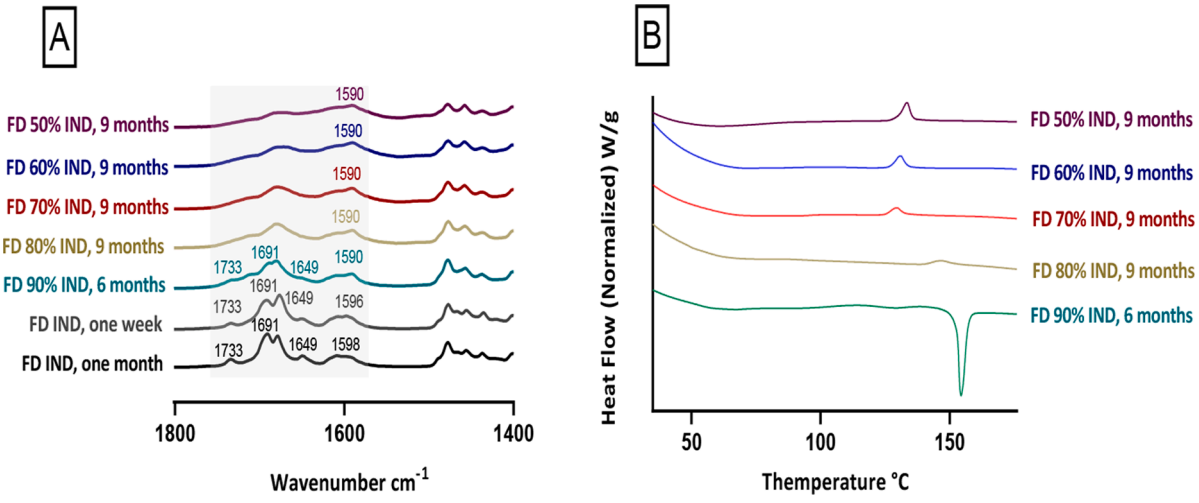

Fig. 10. FTIR spectra (A) and DSC thermograms (B) of freeze-dried formulations at ratios of 50–90% w/w indomethacin stored over 9 months at 5% RH and 25 °C and freeze-dried indomethacin from TBA after one week and month recyclization as α form indomethacin reference.

indomethacin with tryptophan via freeze-drying that is physically stable for up to 9 months at dry storage condition (5% RH, 25 °C).

Amino acids have shown to be able to effectively stabilise amorphous drug and improve dissolution properties of PWSDs via the complex formation between indomethacin and tryptophan. Non-ionic interactions between compounds and the absence of either crystalline or melting peaks of freeze-dried formulations were confirmed via FT-IR, NMR, DSC and XRPD. This is important as these interactions do not alter the structural or functional properties of the drug, and are less dependent on of the pH the solution, which helps to overcome issues such as disproportionation during storage and dissolution (Zhuang et al., 2019). The results obtained in our studies are consistent with the previously reported CAM system prepared via ball milling, but not in organic spray drying (Kasten et al., 2019; Lu et al., 2019). The non-toxic and low molecular weight co-former also has shown advantages such as improved drug loading (up to 90%) and miscibility compared to polymeric co-formers.

The drug release rate of formulations with 70% w/w drug loading (or 1.5:1 drug: amino acid molar ratio) and below was significantly improved when compared to the pure crystalline drug, while it has shown to prolong the drug release rate at 80% w/w drug loading (or 2.3:1 drug: amino acid molar ratio) and above. Tryptophan can co-form with class II BCS drugs to improve their dissolution profiles. An increase in the physical stability of this type of CAM may be due to the reducing molecular mobility via the weak non-ionic interactions.

4. Conclusion

Freeze-drying has successfully produced a non-salt-based CAM of a poorly water soluble drug (indomethacin) and a neutral amino acid (tryptophan) at drug ratios ranging from 30 to 90% w/w. The freeze-dried CAM formulations show an elegant cake appearance without major defects. Molecular interactions, involving H-bonding and H/π or π–π interactions between aromatic moieties, have been confirmed by FT-IR and NMR. The drug release rate and physical stability of freeze-dried formulations were significantly improved when compared to the pure crystalline drug. The optimal ratio for the highest drug loading, whilst maintaining a good dissolution profile and physically stability between indomethacin and tryptophan, is 70% w/w drug loading, which is higher than the 1:1 M ratio (1:0.56 wt ratio drug: amino acid) that is often assumed. Freeze drying appears to be a feasible technique to produce a stable non-salt CAM system class II drug (indomethacin) with tryptophan for the first time using TBA-water cosolvent with a significant improvement in the dissolution rate and physical stability upon long-term storage.

CRediT authorship contribution statement

Mohammed Suleiman Alsalhi: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Validation, Visualization, Writing – original draft. **Paul G. Royall:** Conceptualization, Methodology, Validation, Writing – review & editing. **Hisham Al-Obaidi:** Conceptualization, Methodology. **Alyaa Alsalhi:** Methodology, Validation. **Agostino Cilibrizzi:** Data curation, Methodology, Validation.**Ka Lung Andrew Chan:** Conceptualization, Formal analysis, Methodology, Project administration, Investigation, Resources, Software, Supervision, Validation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpharm.2023.123404.

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