

# Influence of preparation method and choice of phospholipid on co-amorphization, physical stability, and dissolution behavior of equimolar indomethacin-phospholipid systems – A case study

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## ABSTRACT

This case study aimed to evaluate the feasibility of forming equimolar co-amorphous drug-phospholipid systems by different preparation methods. Indomethacin (IND) was chosen as a model drug and combined with three phospholipids of natural origin (soybean phosphatidylcholine (SPC), hydrogenated phosphatidylcholine (HPC), and mono-acyl phosphatidylcholine (MAPC), at equimolar ratios. The systems were prepared by mechanochemical activation-based, melt-based, and solvent-based preparation methods, *i.e.*, ball milling (BM), quench cooling (QC), and solvent evaporation (SE), respectively. X-ray powder diffraction (XRPD), polarized light microscopy (PLM), and differential scanning calorimetry (DSC) were used to determine the solid state form of the prepared IND-phospholipid systems. The long-term physical stability of the co-amorphous systems and the amorphous drug alone were investigated under dry conditions at room temperature. The dissolution behavior of the formed co-amorphous drug-phospholipid systems was also studied. Our findings indicate that SE-prepared co-amorphous drug-phospholipid systems can be successfully prepared across all systems, whereas the QC method could only form co-amorphous drug-phospholipid systems for IND-SPC and IND-MAPC. In contrast, the BM method failed to produce co-amorphous drug-phospholipid systems, but the drug alone could be fully amorphized. The physical stability of SE-prepared co-amorphous drug-phospholipid systems was significantly prolonged under dry conditions (172–236 days) compared to pure amorphous IND (8–15 days) and all other systems (22–38 days). Furthermore, the co-amorphous drug-phospholipid systems formed by the SE method demonstrated improved dissolution behavior in comparison to crystalline IND, amorphous IND, and physical mixtures of IND and phospholipids. However, the phospholipids did not inhibit precipitation in the formed supersaturated IND solutions.

## 1. Introduction

Developing effective formulations for poorly water-soluble drugs is a significant challenge in pharmaceutical research and development. One promising strategy to enhance the solubility and dissolution rate of such drugs is to convert their crystalline state into an amorphous form, which offers higher apparent solubility and dissolution rates than its crystalline counterparts [1–3]. Formation of an amorphous form can be achieved through kinetic or thermodynamic pathways [3]. The kinetic pathway may be chosen for thermolabile crystalline solids, employing methods such as ball milling to induce defects in the crystal lattice and eventually transform it into an amorphous structure [3–5]. In contrast, for thermostable compounds, the thermodynamic pathway is usually preferred.

Techniques such as rotary solvent evaporation and quench cooling are used at the laboratory scale to dissolve the crystalline solid and rapidly remove the solvent or to rapidly cool the melt to obtain the amorphous form [3,4,6]. Despite the solubility advantages an amorphous form offers, it is thermodynamically unstable and tends to revert to the crystalline state over time, leading to a loss of the initial solubility advantage [7,8]. The physical stability of amorphous drugs is, *inter alia*, influenced by humidity, storage temperature, and preparation methods [1]. It is, therefore, usually necessary to stabilize the amorphous form of drugs since the physical stability of neat amorphous drugs is, in most cases, too low for industrial application in pharmaceutical dosage forms.

A common approach to stabilize the drug in its amorphous form is the formation of a solid dispersion using different types of excipients,

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such as polymeric carriers, mesoporous materials, or low molecular weight co-formers, to form so-called co-amorphous systems [1,2,9]. Among these excipients, phospholipids have emerged as promising co-formers for amorphous drugs [10]. Phospholipids possess polar head groups and lipophilic tail groups, enabling them to interact with drugs through various forces and thus to promote conversion of a crystalline drug into an amorphous form [10]. Moreover, phospholipids are amphiphilic polar lipids that form colloidal structures in the fluids of the gastrointestinal tract after oral administration. This may have advantages in the solubilization of the drug after administration and might aid in drug absorption. As such, phospholipids as co-formers for amorphous drugs can be considered as excipients to form amorphous solid dispersions (or more precisely, co-amorphous systems) when they are prepared, but as a lipid-based drug delivery system once in contact with the fluids of the gastrointestinal tract.

Several studies have shown that phospholipid-based drug delivery systems can enhance solubility, permeability, and oral bioavailability compared to pure crystalline drug [11,12]. These systems thus offer potential advantages over polymer-based amorphous solid dispersions, as they are not limited by the drug's solubility in the polymer matrix, which frequently is low and thus necessitates large quantities of polymer, which can introduce downstream hygroscopicity and stability issues [13].

To further explore the potential of phospholipids as co-formers for co-amorphous drug-phospholipid systems, this study focuses on investigating the technical feasibility of utilizing the zwitterionic phospholipids, soybean phosphatidylcholine (SPC), hydrogenated phosphatidylcholine (HPC), and mono-acyl phosphatidylcholine (MAPC), as well as the different preparation methods ball milling (BM), quench cooling (QC) and solvent evaporation (SE), using the poorly water-soluble model drug indomethacin (IND).

This study aims to address the knowledge gap in determining the optimal preparation method with respect to the physical stability of drug-phospholipid systems [12,14,15]. Thus, it is hypothesized that the kinetic (BM) and thermodynamic (QC, SE) preparation methods will be suitable for preparing co-amorphous drug-phospholipid systems. Secondly, it is hypothesized that the different phospholipids will influence the formation and the physical stability of the resulting co-amorphous drug-phospholipid systems. The solid-state analytical techniques differential scanning calorimetry (DSC), polarized light microscopy (PLM), and X-ray powder diffraction (XRPD) are employed to confirm the formation of co-amorphous drug-phospholipid systems.

## 2. Materials and methods

### 2.1. Materials

SPC ( $M_w = 782.0$  g/mol) (Lipoid® S 100, purity 98.9%), HPC ( $M_w = 790.0$  g/mol) (PHOSPHOLIPON® 90H, purity 95.8%), and MAPC ( $M_w = 508.0$  g/mol) (Lipoid® S LPC 80, purity 80%) were kindly donated by Lipoid GmbH (Ludwigshafen, Germany). IND ( $M_w = 357.8$  g/mol) was obtained from Fagron A/S (Copenhagen, Denmark). Ethanol (Ph. Eur. grade) was obtained from VWR (Herlev, Denmark). Fasted state simulated intestinal fluid (FaSSIF v1) powder was purchased from Bio-relevant Ltd. (London, United Kingdom).

### 2.2. Preparation of physical mixtures

Physical mixtures (PM) of the IND-phospholipid systems were prepared by mixing and grinding. The total mass of the PMs was 500 mg, and they were weighed and mixed at equimolar ratios with mortar and pestle for 60 s. The obtained semi-solid/waxy (SPC/MAPC) or powder (HPC) mixtures were collected and ground for another 60 s. Since the natural phospholipids were not pure (see section 2.1 Materials), their purity was considered in calculating the equimolar ratios. The obtained mixtures were stored in Eppendorf tubes at  $-20$  °C.

### 2.3. Quench-cooling as preparation method for Co-amorphous drug-phospholipid systems

The PMs were evenly spread on aluminum foils placed on a hotplate or inside an oven. The hotplate and the oven were heated above the drug's melting temperature ( $T_{m, onset}$ , 160 °C) to 170 °C. After the melting process was visually observed to be completed, the melt was stirred with a spatula to obtain a homogenous mix and to prevent phase separation. Subsequently, the mixtures were cooled at room temperature (21 °C) and then gently ground using a mortar and pestle. All preparations were performed in triplicates ( $n = 3$ ).

### 2.4. Mechano-chemical activation as preparation method for Co-amorphous drug-phospholipid systems

BM was used as a mechano-chemical activation method to prepare the IND-phospholipid mixtures. The ball mill (Mixer mill MM400, Retsch GmbH & Co., Haan, Germany) was placed in a cold room (6 °C, 80–87% RH). The PMs were BM in 5 mL (200 mg) jars with one stainless steel ball with a 5 mm diameter. The binary mixtures were milled for 120 min at a frequency of 30 Hz. BM was stopped every 10 min and not restarted before the jars reached a temperature below 10 °C, measured with an infrared thermometer. All preparations were performed in triplicates ( $n = 3$ ).

### 2.5. Rotary solvent evaporation as preparation method for Co-amorphous drug-phospholipid systems

The PMs were dissolved at room temperature in ethanol at a low batch volume of 30 mL. The solutions were prepared under magnetic stirring at room temperature and then sonicated for 30 min to obtain a clear solution (observed visually). The solutions were poured into round-bottom flasks, and the solvent was removed by SE at 40 °C and 179 mbar (to avoid foam formation). Once most of the solvent was removed, the pressure was set to 10 mbar for 30 min. Finally, the mixtures were left overnight in a vacuum chamber at 10 mbar to remove the remaining solvent residues. All samples were prepared in triplicates ( $n = 3$ ).

### 2.6. Differential scanning calorimetry

Thermal analysis was performed using a Discovery differential scanning calorimeter (TA Instruments Inc., New Castle, DE, USA). Samples (4–8 mg) were analyzed in Tzero Aluminum pans sealed with perforated lids. The furnace was purged with 50 mL/min nitrogen gas. The temperature and enthalpy of the DSC instrument were calibrated using indium as a standard. The  $T_m$  (onset) and glass transition temperatures ( $T_g$ , midpoint) were determined using the TRIOS software v5.1.1 (TA Instruments Inc., New Castle, DE, USA). The samples were heated from  $-40$  °C to 170 °C at a heating rate of 10 °C/min.

### 2.7. Polarized light microscopy

Polarized light microscopy (PLM) was conducted on a Leica DM LM microscope (Leica Microsystems GmbH, Wetzlar, Germany) equipped with an Evolution MP Camera (Media Cybernetics, Rockville, MD, USA) controlled by Image-Pro Insight software (Media Cybernetics, Rockville, MD, USA). The microscope was operated in both transmitted-light and transmitted cross-polarized-light modes produced by a halogen lamp, 12 V, 100 W. The samples were spread and squeezed evenly between glass coverslips to observe possible birefringent textures at a 10x objective magnification.

### 2.8. X-ray powder diffraction

The solid-state properties of the crystalline materials and co-

amorphous mixtures were analyzed by X-ray powder diffraction (XRPD) with an X'Pert PRO diffractometer (PANalytical, Almelo, Netherlands) using Cu K $\alpha$  radiation ( $\lambda = 1.5406 \text{ \AA}$ ) at 45 kV and 40 mA. Samples were placed on aluminum sample holders and measured over the angular range of 5–35° 2 $\theta$  at a scanning speed of 0.058° 2 $\theta$ /min and a step size of 0.026° 2 $\theta$ . The diffractograms were analyzed using X'Pert HighScore Plus (v2.2.4) software (PANalytical, Almelo, Netherlands).

### 2.9. Physical stability

Physical stability studies were performed on the mixtures, which formed co-amorphous drug-phospholipid systems and samples containing amorphous IND after preparation. The samples were stored in desiccators at close to 0 % relative humidity (RH) using phosphorus pentoxide (P<sub>2</sub>O<sub>5</sub>). The physical stability was investigated by DSC, XRPD, and PLM weekly. The occurrence of Bragg peaks and diffraction of polarized light originating from IND (in the co-amorphous drug-phospholipid systems) were deemed the time point of recrystallization. All preparations and measurements were performed in triplicates ( $n = 3$ ).

### 2.10. Determination of equilibrium solubility

The  $S_{eq}$  of IND in fasted state simulated intestinal fluid (FASSIF v1) was determined by adding an excess of IND to 1 mL of FASSIF v1. The suspensions were kept in a rotator (RCT basic, IKA) at 37 °C. To determine the drug  $S_{eq}$  in FASSIF v1, after 24 h a clear supernatant was obtained by centrifugation at 19,000 $\times$ g for 15 min at 25 °C. Appropriate dilution with acetonitrile + TFA 0.05% was performed. The IND content was quantified by HPLC as described in section 2.12.

### 2.11. In vitro dissolution

*In vitro* dissolution studies under non-sink conditions were conducted in a miniaturized USP2 apparatus. 100 mL of FaSSIF v1 (pH 6.5 and at 37 °C) as dissolution medium was used. Due to the poor wetting and dispersibility of the co-amorphous drug-phospholipid systems, all samples were initially subjected to a pre-dispersion step. Samples containing 10 mg of IND were pre-dispersed in 10 mL FaSSIF v1 and vortexed for 30 s. The dissolution study was initiated by transferring the mixtures into dissolution glass vials containing 90 mL FaSSIF v1. At predetermined time points (1, 5, 10, 15, 20, 30, 45, 60, 120, and 240 min), aliquots of 2 mL were withdrawn and filtered through 0.45  $\mu$ m membrane syringe filters. The first filtrate of 1 mL was discarded, and then 1 mL was collected and centrifuged (20 min at 13,000g, room temperature). The drug content was quantified by high-performance liquid chromatography (HPLC). After the dissolution test, the undissolved content in the dissolution vessels were collected, centrifuged, and the formed pellet was analyzed by XRPD.

### 2.12. High-performance liquid chromatography

Quantification of IND was conducted by HPLC coupled to ultraviolet (UV) detection using an Agilent 1260 Infinity chromatographic system from Agilent Technologies (Santa Clara, CA, USA) equipped with an Agilent 1290 Diode Array Detector. A Phenomenex® C18 (4.60 mm  $\times$  150 mm, 5  $\mu$ m) column (Torrance, CA, USA) was used. The analysis was performed using a gradient of mobile phase A (acetonitrile + TFA 0.05%) and mobile phase B (purified water + TFA 0.05%). An injection volume of 10  $\mu$ L, a 1.0 mL/min flow rate, and a column oven temperature of 25 °C were employed. A linear gradient from 90% to 20% of mobile phase A over 12 min was used, and IND was detected at a wavelength of 254 nm. The LOD and LOQ were estimated from the calibration curve,  $LOD = 3.3\sigma/S$ , and  $LOQ = 10\sigma/S$ , where  $\sigma$  is the standard deviation of the response, and  $S$  is the slope of the calibration curve. LOD and LOQ were found to be 1.25 and 3.79  $\mu$ g/mL, respectively.

## 3. Results and discussion

Developing co-amorphous drug-phospholipid systems to enhance drug solubility and absorption of poorly water-soluble drugs and thus improve their therapeutic efficacy is an area of increasing interest [16, 17]. This study aimed to investigate which preparation method, BM, QC, or SE is a suitable preparation technique for co-amorphous drug-phospholipid systems. To investigate this, an equimolar ratio of IND to phospholipid (1:1), using three phospholipids (SPC, HPC, and MAPC), was used.

### 3.1. Solid state characterization of pure components and Co-amorphous IND-phospholipid systems

#### 3.1.1. Model drug

Initially, the starting materials were investigated as received. Fig. 1a–c shows the obtained diffractograms, micrographs, and thermograms of pure crystalline IND and amorphous IND produced by all three methods, BM, QC, and SE. The diffractogram of crystalline IND (Fig. 1a) showed characteristic Bragg peaks of the polymorphic  $\gamma$  form [18]. In contrast, amorphous IND showed a diffuse halo, confirming successful amorphization. These findings are further supported by the PLM micrographs (Fig. 1b) showing characteristic crystals of the  $\gamma$  form under polarized light [18]. In contrast, amorphous IND formed a yellow transparent product that did not diffract light, confirming successful amorphization.

The thermal analysis of crystalline IND (Fig. 1c) showed an onset of melting at 160 °C belonging to the  $\gamma$  form. In contrast, its amorphous forms showed a  $T_g$  at 45 °C for the BM and QC samples, whereas the SE sample had a slightly lower  $T_g$  of 42 °C, confirming successful amorphization of pure IND by all preparation methods.

#### 3.1.2. Phospholipids

The zwitterionic phospholipids used in this study (SPC, HPC, and MAPC) are all of natural origin [19] and are thus a mixture of components with different lengths of hydrocarbon chains; such mixtures

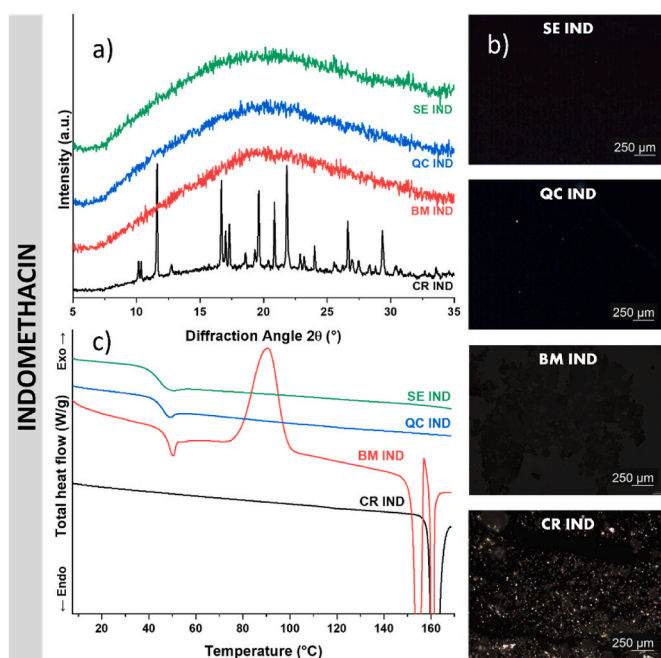


Fig. 1. X-ray powder diffraction (XRPD) diffractograms (a), Polarized light microscopy (PLM) micrographs (b), and differential scanning calorimetry (DSC) thermograms (c) of indomethacin (IND). Starting material (CR IND) and material after preparation by BM, QC, and SE.

produce broad and overlapping thermal events [20] (Fig. S1) resulting in complex thermograms. Therefore, the solid-state properties of the phospholipids are interpreted here based on their diffractograms and micrographs. The diffractograms of SPC (Fig. 2a) indicate a disordered crystal with a diffuse halo in the higher  $2\theta$  range but distinct peaks at  $5.8$  and  $7.7^\circ 2\theta$ . These peaks were barely visible for the QC and SE SPC. The micrographs (Fig. 2b) showed birefringence under polarized light for bulk, QC, and SE SPC, whereas the BM showed micronized crystals.

The diffractogram of HPC showed a diffuse halo with a distinct peak at  $5.8^\circ 2\theta$  and a broad peak at  $21.4^\circ 2\theta$  (Fig. 2c), but with reduced intensity after BM, QC, and SE. Under polarized light, birefringence can be seen in all samples (Fig. 2d). Similarly, for MAPC, the diffractograms (Fig. 2e) showed a diffuse halo with distinct peaks at  $5.8$  and  $7.7^\circ 2\theta$ . Whilst the peak positions of these two peaks remained similar to the starting crystalline form after BM, QC, and SE, indicating that the MAPC samples are in the same solid-state form, they appear with reduced intensity. The micrographs of all MAPC samples again showed birefringence under polarized light (Fig. 2f).

These solid-state characterizations of the phospholipids show that it is generally not feasible to convert and keep the bulk phospholipids in an amorphous form after BM, QC, and SE.

### 3.1.3. IND-SPC

The diffractogram of PM IND-SPC (Fig. 3a) showed an addition of the diffraction peaks of the crystalline IND and SPC. Upon BM, no peaks corresponding to the IND crystalline lattice can be seen, and the characteristic peaks for SPC at  $5.8$  and  $7.7^\circ 2\theta$  have vanished. However, a peak at  $5.0^\circ 2\theta$  was now seen. The PLM micrographs (Fig. 3b) confirmed residual crystallinity due to birefringence, which is likely due to the phospholipid. In contrast, the QC and SE systems formed transparent glasses showing no Bragg peaks or polarized light birefringence, indicating that co-amorphous drug-phospholipid systems were formed.

These results are supported by the DSC thermograms (Fig. 3c). The

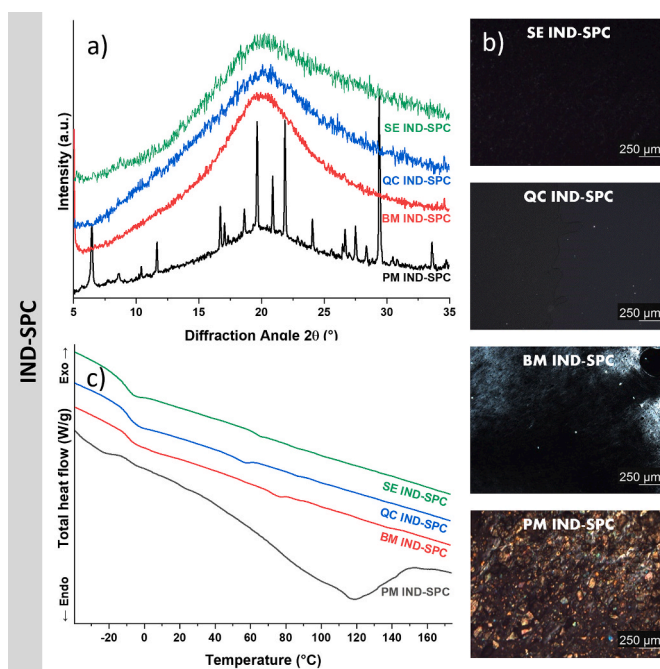


Fig. 3. XRPD diffractograms (a), PLM micrographs (b), and DSC thermograms (c) of IND-SPC at equimolar ratio. Physical mixture (PM) and samples after preparation by BM, QC and SE are shown.

PM of IND-SPC showed a broad endothermic peak from around  $40^\circ\text{C}$  to around  $150^\circ\text{C}$ , indicating that crystalline IND was dissolving into SPC during the phase transition of the phospholipid ( $30$ – $60^\circ\text{C}$ ) and was fully dissolved before reaching the  $T_{m, \text{onset}}$  of IND ( $160^\circ\text{C}$ ). In contrast, all

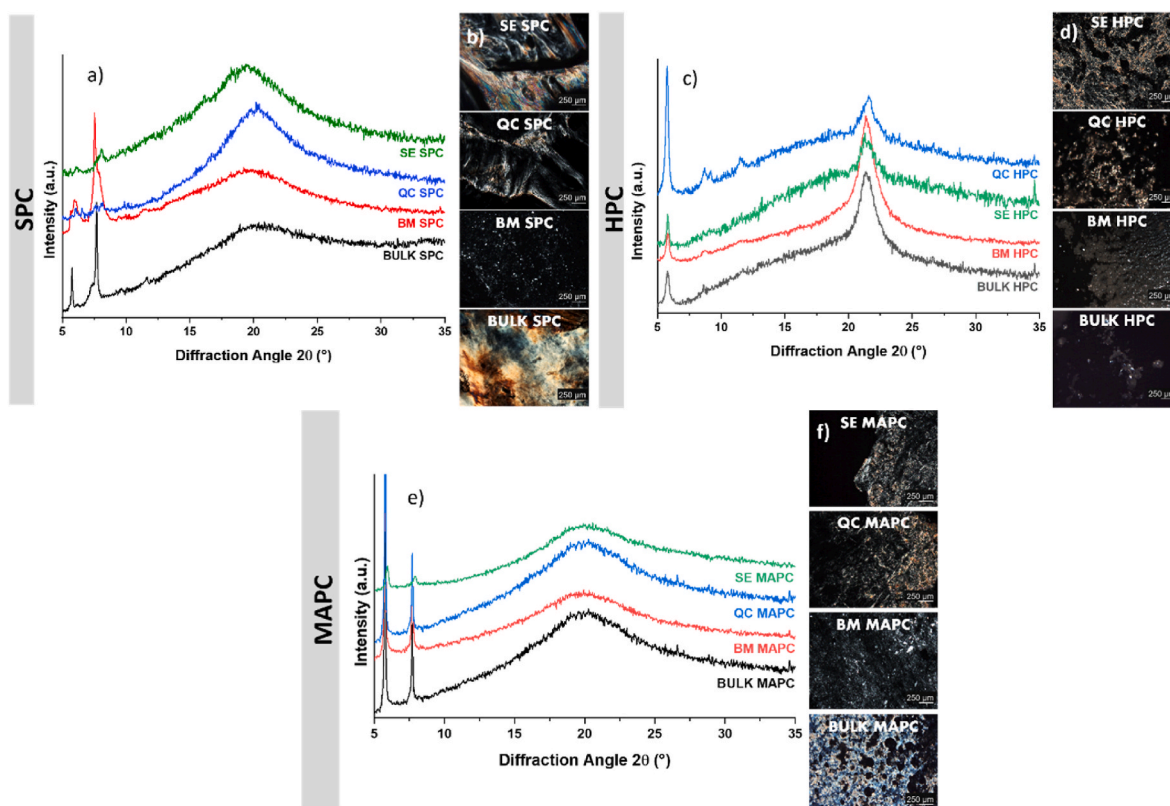


Fig. 2. XRPD diffractograms of SPC (a), HPC (c) and MAPC (e) and PLM micrographs of SPC (b), HPC (d) and MAPC (f). Starting material (Bulk) and material after preparation by BM, QC, and SE are shown.

systems that underwent amorphization (including the BM sample) exhibited a  $T_g$  at  $-8^\circ\text{C}$ , indicating that SPC acts as a plasticizer for amorphous IND. Overall, SPC can form co-amorphous drug-phospholipid systems with IND by SE and QC, whilst for the BM samples, residual phospholipid crystallinity remained.

### 3.1.4. IND-HPC

The diffractogram of PM IND-HPC (Fig. 4a) again showed an addition of the diffraction peaks of the crystalline starting materials, indicating the absence of interactions between the two components after mixing. The BM systems showed peaks corresponding to the characteristic HPC peaks at  $5.8^\circ 2\theta$  and  $21.4^\circ 2\theta$  (Fig. 2c), indicating residual crystallinity of the phospholipid, whilst no peaks belonging to IND could be found, indicating that the drug was fully amorphous. In contrast, the QC systems showed broad peaks at  $7, 8,$  and  $21^\circ 2\theta$ , indicating residual crystallinity of HPC with a possible partial polymorphic conversion. Similar to the BM samples, no residual crystallinity of the drug could be detected. Only the SE IND-HPC system formed a halo in the diffractograms, indicating the formation of a fully amorphous system. These results obtained by XRPD are confirmed by the PLM micrographs (Fig. 4b).

The DSC thermograms (Fig. 4c) of PM IND-HPC showed a broad endothermic peak from around  $35^\circ\text{C}$  to around  $115^\circ\text{C}$  that can be interpreted in a similar way as for the PM IND-SPC system (see above). In contrast to the respective IND-SPC systems, however, the BM and QC IND-HPC samples exhibited several endothermic events (around  $45$  to around  $90^\circ\text{C}$ ) corresponding to the melting of HPC, suggesting that the drug was amorphized, but the phospholipid remained crystalline. No clear  $T_g$  could be observed. Only the SE system exhibited a  $T_g$  at  $\sim 15^\circ\text{C}$ , indicating that HPC acts as a plasticizer.

The PLM micrographs, thermograms, and diffractograms were similar after subjecting IND-HPC to BM and QC, but with a higher residual crystallinity of the phospholipid after QC. Overall, it was only feasible to form co-amorphous drug-phospholipid systems by SE, whereas only IND turned amorphous after BM and QC.

### 3.1.5. IND-MAPC

The diffractogram of PM IND-MAPC (Fig. 5a) showed again an

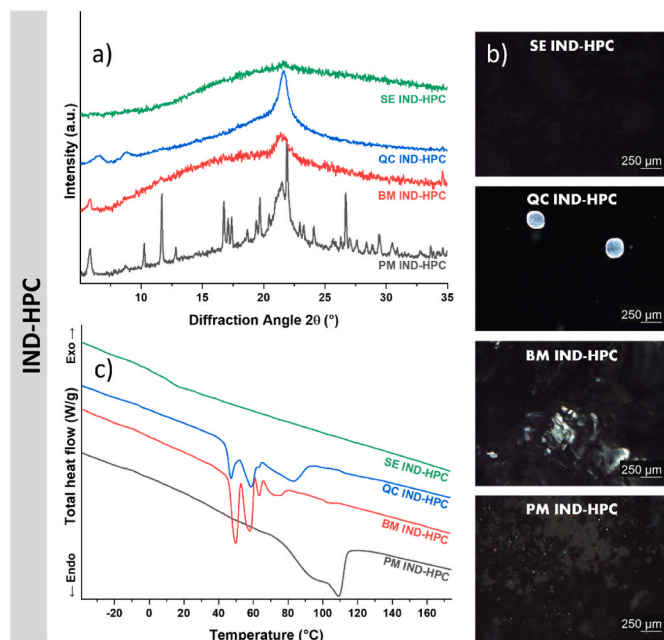


Fig. 4. XRPD diffractograms (a), PLM micrographs (b), and DSC thermograms (c) of IND-HPC at equimolar ratio. Physical mixture (PM) and samples after preparation by BM, QC, and SE are shown.

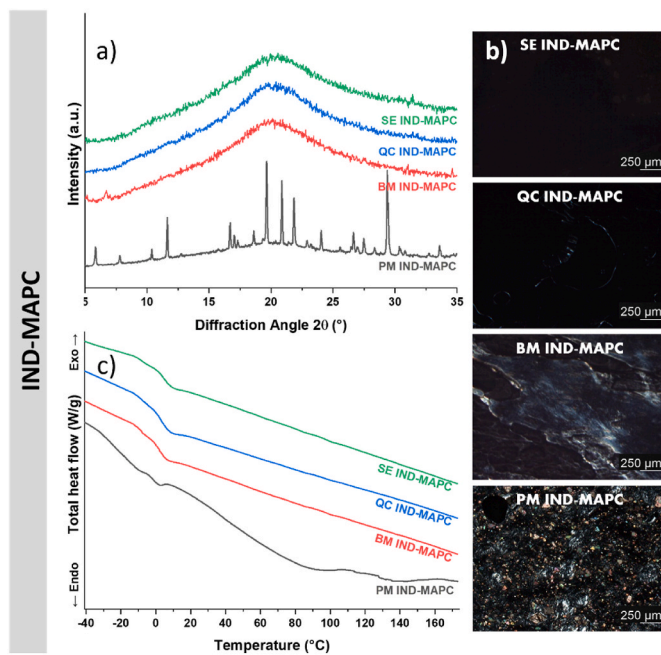


Fig. 5. XRPD diffractograms (a), PLM micrographs (b), and DSC thermograms (c) of IND-MAPC at equimolar ratio. Physical mixture (PM) and samples after preparation by BM, QC and SE are shown.

addition of the diffraction peaks of the crystalline starting materials. Upon BM, no peaks corresponding to the IND crystalline lattice can be seen, and the characteristic peaks for MAPC at  $5.8$  and  $7.7^\circ 2\theta$  have vanished. However, a peak at  $6.7^\circ 2\theta$  was now seen. The PLM micrographs (Fig. 5b) confirmed residual crystallinity due to birefringence still being visible, which is likely due to the phospholipid. In contrast, the QC and SE systems formed transparent glasses showing no Bragg peaks or polarized light birefringence, indicating that co-amorphous drug-phospholipid systems were formed.

These results are supported by the DSC thermograms (Fig. 5c). The PM of IND-SPC showed a broad endothermic peak from around  $40^\circ\text{C}$  to around  $150^\circ\text{C}$ , indicating that crystalline IND was dissolving into MAPC, during the phase transition of the phospholipid ( $25$ – $160^\circ\text{C}$ ) and was fully dissolved around the  $T_{m, \text{onset}}$  of IND ( $160^\circ\text{C}$ ). In contrast, all systems that underwent amorphization (including the BM sample) exhibited a  $T_g$  at  $\sim -5^\circ\text{C}$ , indicating that MAPC acts as a plasticizer for amorphous IND. Overall, MAPC can form co-amorphous drug-phospholipid systems with IND by SE and QC, whilst for the BM samples, residual phospholipid crystallinity remained.

Table 1 summarizes the findings for the three different phospholipids and the three preparation methods used.

IND alone could be made amorphous by all three preparation methods, whereas the phospholipids themselves did not become amorphous with any of the methods used. At an equimolar ratio of IND to phospholipid, it was, however, possible to form co-amorphous drug-phospholipid systems with all phospholipids using SE and with two out of the three phospholipids with QC. Only the drug (but not the phospholipids) converted to an amorphous form when using BM as a preparative technique. The findings suggest that the most promising preparation method forming co-amorphous drug-phospholipid systems is SE, followed by QC. The most promising phospholipids are SPC and MAPC over the fully hydrogenated HPC.

### 3.2. Influence of Co-amorphization and preparation method on the physical stability of the amorphous form of the drug

The obtained systems presented in Table 1 were stored at 0% RH at room temperature and evaluated by XRPD, PLM, and DSC over time. All

**Table 1**

Characterization of equimolar drug-phospholipid systems prepared by BM, QC, and SE. XRPD, PLM, and DSC were used to identify if samples were fully amorphous, i.e. formed co-amorphous drug-phospholipid systems (☑) or had visible birefringence from (residual) crystallinity of the phospholipid only (☐).

	BM	QC	SE
IND	☑	☑	☑
SPC	☐	☐	☐
HPC	☐	☐	☐
MAPC	☐	☐	☐
IND-SPC	☐	☑	☑
IND-HPC	☐	☐	☑
IND-MAPC	☐	☑	☑

systems contained IND in an amorphous form, with SE IND-HPC, QC and SE IND-SPC, and QC and SE IND-MAPC all confirmed to be co-amorphous drug-phospholipid systems. The systems were deemed recrystallized when IND had begun recrystallizing. The physical stability of equimolar co-amorphous drug-phospholipid systems in 0% RH at room temperature shows that the SE IND-phospholipid systems had a significantly higher physical stability than the pure amorphous drug and the systems prepared by other preparation methods (172–236 days), as shown in Fig. 6. Interestingly, the co-amorphous drug-phospholipid systems formed by QC (IND-SPC and IND-MAPC) did not show higher physical stability of the amorphous IND (28–39 days) than the systems in which only the IND was amorphous (22–38 days). However, all phospholipid systems showed a higher stability for IND than the pure amorphous IND, with 8–15 days (see Fig. 6).

This indicates that the preparation method, rather than the fact that a co-amorphous drug-phospholipid system or a system in which only the IND was amorphous is formed, has the dominant influence on the physical stability of the amorphous drug. Hence, from a viewpoint of physical stability, a solvent evaporation technique is much preferred over QC or BM. For the SE systems, SPC and MAPC may be preferred over the hydrogenated HPC.

### 3.3. Influence of phospholipids on the *in vitro* dissolution behavior of IND

The outcome of the physical stability study prompted us to choose SE as the preferred preparation technique for testing the co-amorphous

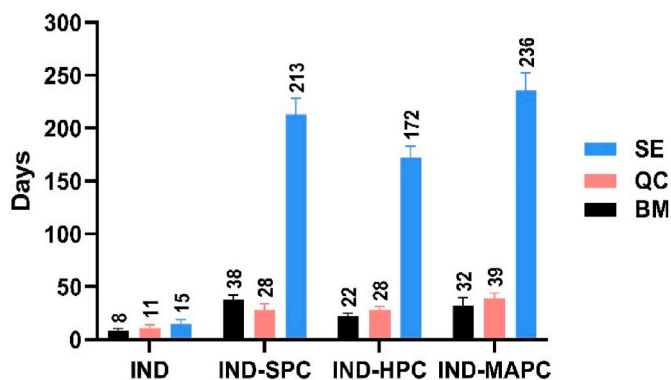


Fig. 6. Physical stability of IND and equimolar IND-phospholipid systems prepared by ■ BM, ■ QC, and ■ SE after storage in 0 % RH at RT. Physical stability refers to the time until the drug crystallizes. Data is represented as mean  $\pm$  SD ( $n = 3$ ).

drug-phospholipid systems by non-sink *in vitro* dissolution. The dissolution profiles of crystalline IND and the PMs show that the physical addition of the phospholipids had no (in the case of SPC (Fig. 7a) and HPC (Fig. 7b)) or only little (in the case of MAPC (Fig. 7c)) influence on the dissolution profile for the crystalline IND. The slightly positive influence of MAPC on crystalline IND dissolution from the PM may be explained by the fact that MAPC is more hydrophilic and micelle-forming than SPC and HPC, which may aid in the solubilization of the drug [14].

In contrast, amorphous IND showed a significantly higher initial drug release rate for the first 5 min. However, this supersaturation was followed by precipitation (after 5 min), resulting in IND reaching a concentration equal to the equilibrium solubility of IND. The dissolution profiles of the co-amorphous drug-phospholipid systems showed a more gradual increase in the dissolved IND concentration, reaching about the same level of supersaturation as the pure amorphous drug. However, gradual precipitation was also observed for the drug when dissolving from the co-amorphous drug-phospholipid systems until reaching concentrations equal to the equilibrium solubility of the drug. Hence, it can be concluded that the phospholipids from the co-amorphous drug-phospholipid systems did not provide a significant precipitation inhibition for the supersaturated IND.

## 4. Conclusion

This case study investigated the feasibility of preparing IND co-amorphous drug-phospholipid systems using different preparation techniques, assessing their physical stability, and drug dissolution. SE successfully prepared co-amorphous drug-phospholipid systems for all systems. QC could only form co-amorphous drug-phospholipid systems for IND-SPC and IND-MAPC, while BM, even for up to 120 min, could not form co-amorphous drug-phospholipid systems. Regardless of the preparation method, the physical stability under dry conditions of SE co-amorphous drug-phospholipid systems was significantly longer than for the pure amorphous drug and, indeed, for all other systems, indicating that the preparation method (SE) together with the formation of a co-amorphous drug-phospholipid systems is responsible for high stability.

The co-amorphous drug-phospholipid systems formed by SE showed improved dissolution behavior compared with the corresponding crystalline IND, amorphous IND, and IND-phospholipid PMs, but the phospholipids failed to impart precipitation inhibition to the supersaturated IND. In contrast to the preparation method, the choice of phospholipids had less influence on forming co-amorphous drug-phospholipid systems with respect to increased physical stability and improved dissolution behavior for the SE prepared IND-phospholipids.

Our original hypothesis that the kinetic (BM) and thermodynamic (QC, SE) preparation methods will be suitable for preparing co-amorphous drug-phospholipid systems, was thus not fulfilled and SE clearly was the best preparation method. In contrast, our second hypothesis that the different phospholipids will influence the formation and the physical stability of the resulting co-amorphous drug-phospholipid systems, was fulfilled with SPC and MAPC being better than hydrogenated HPC.

The current study has the limitation that only one model drug at 1 M ratio was used. Future studies should investigate more drug candidates for co-amorphous drug-phospholipid systems and different molar ratios to confirm if the found differences and similarities in preparation methods and phospholipid type can be generalized. The pathway of investigation used in this study could act as a guide in this future work.

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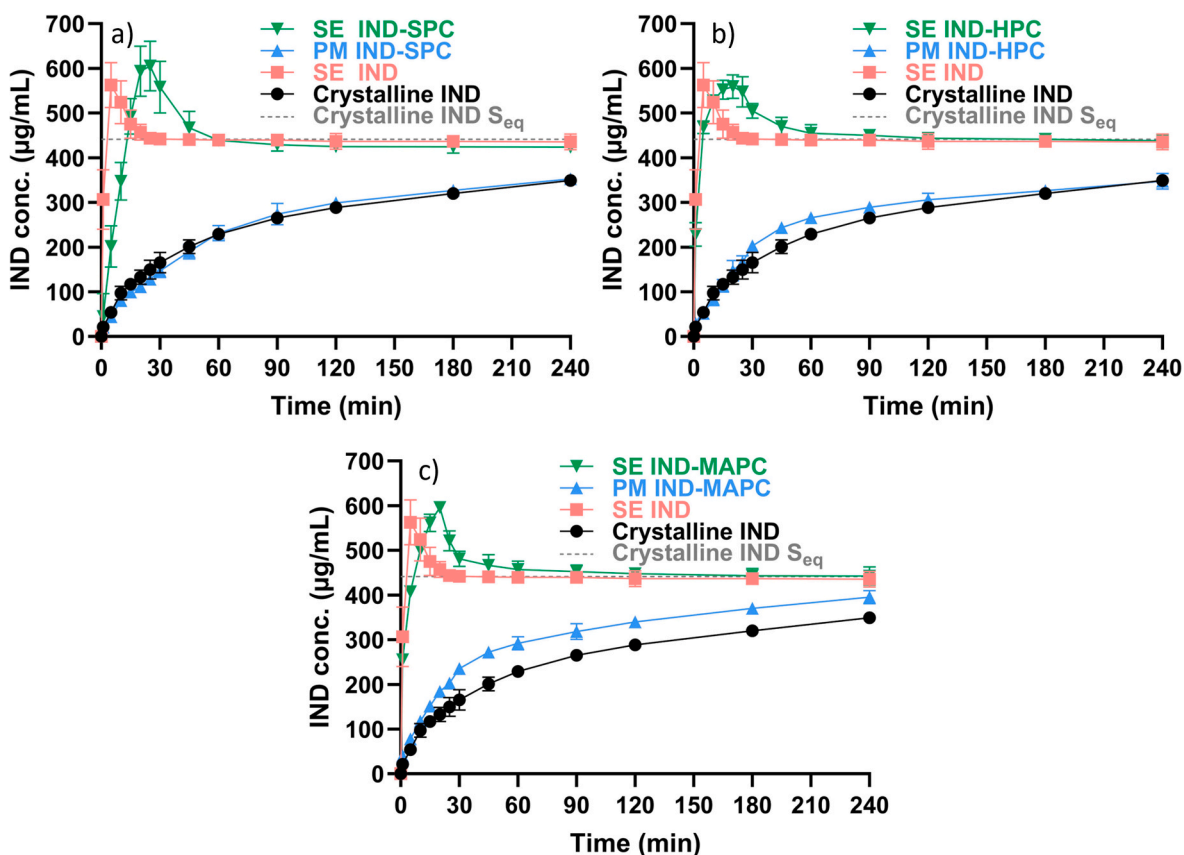


Fig. 7. In vitro non-sink dissolution profiles of crystalline IND, amorphous SE IND, and equimolar IND-phospholipid systems in FaSSiF v1 (pH 6.5) at non-sink conditions for 0–240 min. (a) IND-SPC systems, (b) IND-HPC systems and (c) IND-MAPC systems. (–●–) crystalline IND, (–■–) amorphous SE IND, (–▲–) PM IND-phospholipid, and (–▼–) SE IND-phospholipid. Data is represented as mean  $\pm$  SD ( $n = 3$ ).

#### CRedit authorship contribution statement

**Keyomars Khorami:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Anette Müllertz:** Writing – review & editing, Supervision, Resources, Project administration, Conceptualization. **Thomas Rades:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

#### Declaration of competing interest

The authors declare that they have no known financial interest or personal relationships that could 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.jddst.2024.105433>.

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