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Formulation of cinnarizine for stabilization of its physiologically generated supersaturation

Running title: Formulation of cinnarizine

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Abstract

Physiologically generated supersaturation and subsequent crystallization of a weakly basic drug in the small intestine leads to compromised bioavailability. In this study, the pH-induced crystallization of cinnarizine (CNZ) in the presence of different polymers was investigated. Inhibitory effect of Eudragit L100 (Eu) on crystallization of CNZ at varying supersaturation ratios was examined. The effect of Eu on the dissolution behaviour of CNZ from CNZ/Eu physical mixtures (PMs) and solid dispersions (SDs) was assessed. Results showed that both Eu and HPMC have a considerable maintenance effect on supersaturation of CNZ but Eu was more effective than HPMC. When Eudragit was used the phenomenon of liquid-liquid phase separation (formation of colloidal phase) was observed at supersaturation ratio of 20 times above PMs showed a higher area under the dissolution curve (AUDC) the solubility of the drug. compared to plain CNZ. In contrast, SDs showed a lower AUDC than plain CNZ. For SDs, the AUDC was limited by the slow release of the drug from Eu in acidic pH which in turn hindered the creation of CNZ supersaturation following the transition of acidic to neutral pH. From these findings, it can be concluded that the ability of the formulation to generate supersaturation state and also maintaining the supersaturation are vital for improving the dissolution of CNZ.

Introduction

Weakly basic drugs have gained much interest due to their physiologically generated supersaturation and subsequent crystallization in the small intestine. As the weakly basic drugs might crystallize in the small intestine, therefore, the oral bioavailability of these types of drugs is a widespread challenge for scientists. It is well known that pH of the environment affects the solubility of weakly basic drugs in a manner that depends on its pK_a , according to the Henderson-Hasselbalch relationship. Generally, at acidic pH of the stomach, the ionized form of the weakly basic drugs displays higher solubility compared to the free base. Therefore, partial or complete drug solubilization can be achieved in the stomach. Following a pH change from the stomach to the small intestine (pH is closer to neutral) the solubility of the weakly basic drug can drop as a result of the generation of the free base in the small intestine. This indicates that in the small intestine, the weakly basic compounds may undergo supersaturation due to lower solubility of the free base (1-4). It is worth noting that the higher thermodynamic activity of a drug in the supersaturated state enhances its permeation across the biological membrane. It is well demonstrated that if the time to maintain the drug supersaturation is prolonged for an adequate time under physiological conditions, then this can lead to an improvement of drug transition across the intestinal epithelia and consequently increased drug absorption (5-10). Although, the prolongation of the supersaturated state can be beneficial, due to excess free energy of the supersaturated solutions (this leads to the precipitation of API), erratic absorption and low bioavailability for weakly basic drugs may be attained (5). In fact, crystallization of weakly basic drugs in the small intestine is behind poor oral bioavailability with large intra- and inter-individual variations. The main challenge is the inhibition of crystallization of weakly basic drugs following the passage from the stomach into the small intestine. In order to get

benefit from the high thermodynamic activity of a drug in supersaturated state and consequently 64
gaining high drug concentration, the presence of an inhibitor to prevent crystallization is needed 65
which preferably would not limit diffusion of the drug in the solution (having micelles or 66
cyclodextrin in the formulation)(11-13). 67

Polymer-based drug crystallization inhibitors have been used to inhibit or retard the crystallization 68
of drugs. The use of polymers is a progressively more widespread way for inhibiting crystallization 69
from the supersaturated state used to improve the dissolution performance of poorly water-soluble 70
drugs. It has been proven that a variety of polymers are effective to delay crystallization of drugs 71
from the supersaturated state for a sufficient time so that the drug absorption in the small intestine 72
can take place [5,14–19]. 73

Cinnarizine (CNZ), as a model compound, was examined in the present study. CNZ is a weakly 74
basic drug with pK_{a1} 1.94 and pK_{a2} 7.47 which is soluble 2 $\mu\text{g/ml}$ at pH 6.5 but dissolves more 75
(2110 $\mu\text{g/ml}$) at low pH (20,21). The poor solubility at higher pH indicates that it is highly likely 76
the proposed drug may partially crystallize in the small intestine following gastric emptying. 77
Previous works on the bioavailability of CNZ have revealed that the absorption of CNZ is erratic 78
and absolute bioavailability is very low (22). In this work, the inhibitory effect of various polymers, 79
including HPMC K4M, Eudragit L100 (Eu), NaCMC, HPC, PEG, and PVP, on pH-induced 80
crystallization of CNZ was quantified. The choice of polymers was based on their existence as a 81
crystallization inhibitor published in the literature (15,23). On the basis of the above introduction, 82
the main purpose of this work was to explore the best polymer with a capability to maintain 83
supersaturation of CNZ for a long period of time when the formulation is transmitted from an 84
acidic (the stomach) to neutral pH (the small intestine). 85

Materials and methods 86

Materials	87
Cinnarizine (Osvah Pharmaceutical Co, Iran), hydroxypropyl methylcellulose (HPMC) K4M	88
(Sigma, Germany), Eudragit L100 (Eu) (Evonik GmbH, Germany), hydroxypropyl cellulose	89
(HPC) with MW of 370000 (Sigma, Germany) was used. Polyvinylpyrrolidone K30 (PVP K30),	90
sodium carboxymethyl cellulose (NaCMC), polyethylene glycol 400(PEG 400), monobasic	91
potassium phosphate USP-standard (KH_2PO_4), dibasic potassium phosphate (K_2HPO_4),	92
trisodium phosphate (Na_3PO_4), ethanol analytical grade, dichloromethane analytical grade were	93
obtained from Merck, Germany.	94
Supersaturation experiment	95
A stock solution of CNZ was prepared by dissolving 10 mg of CNZ in 10 ml HCl solution (0.1N).	96
1 ml of this solution was then added to 50 ml 100 mM potassium phosphate buffer (pH 6.8; ionic	97
strength, 0.2M) containing 20 mg of polymer to induce an initial drug solution concentration of 20	98
$\mu\text{g}/\text{ml}$ corresponding to a supersaturation ratio of 10. The total time of supersaturation test was 2	99
h, and the buffer solution was kept in a water bath shaker at $37 \pm 0.5^\circ\text{C}$ at 100 rpm.	100
After adding HCl solution to the buffer solution, samples were taken at predetermined time points	101
(10, 20, 30, 60 and 120 min) and filtrated through a $0.45\mu\text{m}$ nylon membrane syringe filter. The	102
obtained filtrate was directly diluted in a 1:1 ratio with the buffer solution to avoid CNZ	103
crystallization and analyzed using UV-Vis spectrophotometer (UV-160A, Shimadzu, Kyoto,	104
Japan) at 253 nm. The concentration of CNZ ($n = 3$) was plotted as a function of time. No	105
interference between the polymers and CNZ assay was detected at 253 nm.	106
In order to evaluate the influence of supersaturation ratio on the crystallization of CNZ in the	107
presence of Eu (0.4 mg/ml), the supersaturation experiments at different supersaturation ratios of	108

5 and 20 were also performed. The same supersaturation tests were carried out in the buffer solution in the absence of polymer.

Solubility

The equilibrium solubility of CNZ was determined in phosphate buffer (pH 6.8) at 37°C using an excess of CNZ in the presence of Eu and HPMC (concentration of 0.4 mg/ml). The solubility was achieved after 48 h by using UV-Vis spectrophotometer at 253 nm following filtration through a 0.45µm nylon membrane syringe filter.

Dynamic light scattering (DLS)

DLS measurements were performed using laser- scattering spectrophotometer (Nano ZS, Malvern, UK) yields the mean particle size and the polydispersity index (PI) as a measure of the width of the distribution. Light scattering was monitored at 90° and measurements were obtained at 25 °C. Sample solutions were filtered through a 0.45µm nylon membrane syringe before measurement. All measurements were done in triplicate.

Preparation of solid formulations

Physical mixtures (PM) of CNZ and polymers at different weight ratios were prepared by weighing out the accurate amount of CNZ and polymers and triturating for at least 10 min in the mortar and pestle. Preparation of CNZ-Eu solid dispersions (SD) (CNZ: Eu 20:50, 20:100, and 20:200 w/w) was carried out by a rotary evaporation method. CNZ and Eu were dissolved in 1:1 ethanol /dichloromethane (concentration: 2% w/v) under stirring. Rotary evaporation was performed at 80 °C and under reduced pressure (0.09 MPa). Temperature and pressure were maintained by a water bath and vacuum pump respectively. After that, samples were dried in a vacuum oven overnight at 40 °C to

remove any remaining solvent. The solid dispersions were subsequently ground with a mortar and pestle and then sieved to separate a particle size fraction of 150-250 μm .

In vitro dissolution studies

Dissolution experiment was conducted using the USP II paddle method. Firstly, samples were subjected to the acid phase examination in 750 ml of 0.1 N HCl for 2 hours. After that, 250 ml of 0.2 M Na_3PO_4 was added to create the neutral pH and the dissolution was carried out for another 3 h. Samples equivalent to 20 mg of CNZ were calculated, weighted and added to each vessel. This amount of drug represented a theoretical 20 $\mu\text{g}/\text{ml}$ CNZ concentration for the neutral stage of dissolution testing which corresponds to a 10-fold higher than it is needed to create supersaturation (solubility of the drug at neutral pH, 6.8, is supposed to be 2 $\mu\text{g}/\text{ml}$) (20,21). The dissolution media was stirred at 100 rpm and held at 37 ± 0.5 $^{\circ}\text{C}$ throughout the experiment. Samples were taken at predetermined time points by withdrawing 5 ml from each vessel; then the aliquots were filtered using a nylon membrane syringe filter (0.45 μm , Whatman, Florham Park, NJ). The filtered samples were immediately diluted to avoid drug precipitation. CNZ concentrations were assessed as mentioned above. All measurements were carried out in triplicate.

Viscosity Measurements

A Brookfield RVT viscometer was used to measure the viscosity (in cps) of the solutions containing various concentration of Eu (0.05, 0.1 and 0.2 mg/ml). A spindle (no. 2) was rotated at a shear rate of 150 rad/s and 37 $^{\circ}\text{C}$.

Differential Scanning Calorimetry (DSC)

DSC analyses of plain CNZ, Eu, PM and SD formulations were performed using an automatic thermal analyzer system (DSC-60, Shimadzu, Tokyo, Japan). Samples were weighed (around 5

mg) in aluminium crimped pans and heated with a heating rate of 10 °C/min from 25 to 300 °C. 152

The instrument was calibrated using Indium as standard. 153

Fourier Transform Infrared (FT-IR) Spectroscopy 154

Fourier-transform Infrared (FTIR) Spectroscopy was carried out using a Spectrometer (M-B-100, 155

Bomem, Canada) (32 scans at 4 cm⁻¹ resolution). The plain CNZ and SD with Eu were mixed with 156

KBr, compressed into a disc, and analysed directly over a wavenumber range of 400–4000 cm⁻¹. 157

Statistical evaluation of data 158

The data were reported as the mean ± standard deviation (SD) of at least 3 determinations. 159

Statistical analysis was performed using the analysis of variance (ANOVA) followed by the Mann- 160

Whitney U test with statistical significance evaluated at P < 0.05. 161

Results and discussion 162

Supersaturation experiment 163

Polymers with different physicochemical properties were examined to identify excipients that 165

retard the crystallization of CNZ from neutral solution. In the supersaturation experiment, the 166

polymers were pre-dissolved at a concentration of 0.4 mg/ml in neutral solution, and a 167

concentrated solution of CNZ in acidic (i.e., the “spring”) was added to the neutral solution to 168

provide an initial degree of supersaturation where the concentration of drug 10 times higher than 169

the determined solubility, and then the solution concentration was monitored as a function of time 170

(Figure 1). As shown in this Fig, after 10 min, the concentration of CNZ in the solution without 171

any polymer decreased rapidly and reached a concentration close to the equilibrium solubility of 172

the crystalline CNZ (2 µg/ ml). This fast decrease in CNZ concentration is an indication of an 173

immediate crystallization of CNZ from the supersaturated solution. Similar findings have been 174

reported in other studies wherein celecoxib and felodipine quickly crystallized in the lack of polymers (24,25).

According to Fig.1, the CNZ concentration decreased more slowly in the presence of Eu and HPMC compared to the solution without any polymer.

The CNZ concentration measured after 20 min for the solution containing 0.4 mg/ml Eu was 9.1, whereas this value was 6.6 μ g/ml when HPMC with a concentration of 0.4 mg/ml was used. In order to take into account the possible solubilizing effect of Eu and HPMC, the equilibrium solubility of CNZ was determined in a neutral solution containing 0.4 mg/ml of the polymers. According to the results, no significant difference was found between the equilibrium solubility of CNZ in Eu and HPMC solutions (1.98 $\pm$ 0.08 and 2.13 $\pm$ 0.06 μ g/ml, respectively) and non-polymer solution (2.07 $\pm$ 0.07 μ g/ml) ($p>0.05$). This implies that Eu and HPMC have no solubilization effect, and reveals that supersaturated state of the drug in the presence of Eu and HPMC are attributed to the maintenance effect of the polymers on CNZ supersaturation. After 120 min, CNZ reached a concentration of 5.5 μ g/ml in Eu solution and 2.7 μ g/ml in HPMC solution. The CNZ concentrations in the polymer solution after 120 min were still higher than in the solution without any polymer. The results demonstrated that both Eu and HPMC (i.e., the “parachutes”) efficiently suppressed the crystallization of CNZ, but Eu was more effective compared to HPMC ($p<0.05$). The area under the concentration-time (AUC) profile of the supersaturation curve was evaluated to give more quantitative data for supersaturation results. The AUC provides an estimation of both degree and duration of supersaturation then evaluating the extent of supersaturation in the solutions. AUC values for different polymers are presented in Table 1. The AUC values also clearly confirmed the superiority of Eu with respect to stabilizing CNZ supersaturation and

inhibiting the crystallization of the drug. The Eu solution showed about 1.8-fold and 6.2-fold greater mean AUC values than HPMC solution and the solution with no polymer, respectively. The results of supersaturation experiments revealed that Eu and HPMC acted as effective anti-crystallizer to prevent the crystallization of CNZ when compared to other polymers. It has been demonstrated that stabilization of drug supersaturation by the polymers is mainly due to the intermolecular interaction between drug and polymer (26-28). When there is an attractive intermolecular interaction between the drug and the polymer in the solution, this interaction can slow down the nucleation process hence crystal growth of the drug. The above phenomenon inhibits the crystallization and maintains the supersaturated state (29).

According to previously published findings, the ability of a polymer to interact with a drug is dependent on several factors, such as functional groups of polymer and location of these groups and hydrophobicity, rigidity and molecular weight of polymer molecules. According to previous research, higher polymer's hydrophobicity leads to an improved inhibition efficiency of polymer on drug crystallization because of its stronger interaction with drug molecules. Several other research also confirmed that the hydrophilic polymers such as PEG had no crystallization inhibition efficiency which was attributed to the fact that hydrophilic polymers interact more favorably with water molecules (30,31). Apart from hydrophobicity, the rigidity of polymers may influence their inhibition efficiency on drug crystallization. It has been found that polymers with relatively rigid structures inhibit drug crystallization more effectively compared to polymers with flexible structures (32). Additionally, the improved inhibition capability of polymers with high molecular weight on drug crystallization can be related to high existing functional groups on the polymer chains for improved interaction of polymers with drug molecules (30).

Effect of supersaturation ratio on crystallization of CNZ

The influence of the supersaturation ratio on the crystallization of CNZ in the presence of 0.4 mg/ml Eu is shown in Fig. 2. As shown in this Figure the maximum concentration of the drug increased as the supersaturation ratio was increased ($p < 0.05$).

Also, it is clear that the initial crystallization rate was proportional to the supersaturation ratio. This can be explained by the fact that the driving force for crystallization rises with increasing supersaturation ratio (33). Therefore, the higher initial concentration will not consistently result in better performance. A pale solution was generated at a supersaturation ratio of 20 (CNZ concentration of 40 $\mu\text{g/ml}$), while a transparent solution was formed at supersaturation ratios of 5 and 10. To verify the appearance of colloidal species at different supersaturation ratios, a few millilitres of the sample was withdrawn after 1 h of supersaturation experiment and filtered through a 0.45 μm PTFE filter to eliminate crystallized particles. Dynamic light scattering analysis of the obtained supernatant revealed no colloidal species were present at supersaturation ratios of 5 and 10 after supersaturation experiment, but it was not the case when the supersaturation ratio was 20 and colloidal particles were seen in this sample. The determined diameter of the colloidal species at supersaturation ratio of 20 was approximately 170 nm, with a polydispersity index (PDI) smaller than 0.2 (Figure 3). It is interesting to note that at supersaturation ratio of 20, colloidal particles are formed immediately following pH transition. The generation of these colloidal particles during supersaturation experiment could be explained by liquid-liquid phase separation (LLPS) phenomenon (34). It is well known that, generally, a colloidal solute-rich phase is evolved above a definite level of supersaturation (35).

Such LLPS can occur when the highly supersaturated solution is quickly formed and crystallization is efficiently inhibited (36). The LLPS threshold concentration has been reported to occur at a

similar concentration to the amorphous solubility of the drug. According to the reported finding of LLPS determination tests, the practically measured LLPS onset concentration is in good agreement with the calculated solubility of the amorphous form of a drug (34,37). The solubility of the amorphous form of the drug can be predicted using thermodynamic data of the crystalline and amorphous forms of the drug (38). The theoretically predicted solubility of the amorphous form of CNZ is around 12 times than that of the crystalline CNZ (around 2µg/ml). As it was expected, LLPS was observed at concentration of 40 µg/ml of CNZ (supersaturation ratio of 20) which was supersaturated regarding the predicted amorphous solubility of CNZ in the presence of 0.4 mg/ml Eu which was added to inhibit crystallization of CNZ.

In the absence of Eu, no LLPS was observed for the solution of CNZ at a supersaturated ratio of 20 which indicates that the presence of Eu can efficiently inhibit the crystallization of CNZ, and provide the potential to the occurrence of LLPS. According to this result, it is reasonable to conclude that LLPS cannot occur in the solution that undergoes fast crystallization. Similar results have been reported for the supersaturated solution of nifedipine (39,40).

It is well known that the formation of drug –rich phase is beneficial for absorption enhancement of drug. It has been supposed that drug-rich phase can operate as a drug reservoir, becoming easily accessible in the solution, replenishing drug molecules that are diffused through membranes, leading to a highest possible flux as long as the drug-rich phase is present (41,42).

In vitro dissolution of PMs

The addition of CNZ “springs” to a neutral solution containing excipients is a valuable method for initial screening to find an excipient that would stabilize supersaturation of the drug. However, this method cannot promise that the efficient excipient dissolves quickly enough under gastrointestinal conditions to function appropriately when used in the solid formulation. It is

possible that the drug in solid formulation dissolves and subsequently crystallizes before the
 polymers dissolve in an adequate amount to inhibit the crystallization of the drug. To prove this,
 the dissolution of solid formulations consisting of CNZ with effective polymers was carried out.
 Taking into account the results of supersaturation experiments, dissolution tests were firstly carried
 out for the plain drug and its PMs with Eu and HPMC (drug/polymer 20:100). To evaluate the
 dissolution performance of CNZ from solid formulations, the tests were performed by a pH switch
 dissolution method, starting with an acidic pH of 1.2 for two hours followed by a pH adjusting to
 6.8 for additional three hours. The pH switch in this dissolution model was preferred based on
 previous studies with the intention to simulate biorelevant pH conditions (43,44). Taking into
 account the high solubility of CNZ in pH 1.2 (2110 mg/L), it is supposed that the applied amount
 (20 mg) of CNZ would be wholly soluble in acidic medium and produce supersaturated solution
 with starting neutral phase as a consequence of dramatic decrease in CNZ solubility at pH 6.8 (2
 $\mu\text{g/ml}$) (21). Thus, this pH switch in the dissolution test clearly implies the effect of different
 formulations on stabilizing supersaturation of CNZ in neutral solution.

The *in vitro* dissolution results of the samples are depicted in Figure 4. Complete dissolution of
 the plain drug was achieved within 10 min at acidic pH. These findings were consistent with
 previous observation of the rapid dissolution behaviour of CNZ in acidic condition (45). Drug
 release pattern of CNZ from Eu and HPMC PMs were almost identical to that of the plain drug in
 acidic medium. Therefore, at the beginning of the neutral phase, the extent of supersaturation was
 similar for plain CNZ and its PMs with Eu and HPMC ($p>0.05$). Following the acid –to– neutral
 pH change, in the absence of any polymer, significant crystallization of CNZ was seen as the mean
 value of 3.5 $\mu\text{g/ml}$ CNZ was determined 20 min after the pH transition and over 80% of the drug
 crystallized during this time. However, the Eu-based and HPMC –based PMs showed the

inhibition effect against CNZ crystallization (the concentration of CNZ in Eu and HPMC based PM was 11.5 and 6.5 µg/ml in solution 20 min after pH change respectively). These formulations kept on to provide obvious stabilization of supersaturated CNZ up to 3 h after the pH transition. As mentioned before, CNZ solubility was not significantly changed in the presence of these polymers. Therefore, the positive effect of the polymers on dissolution performance of CNZ was not likely attributable to an increase in the equilibrium solubility of CNZ. To give a more quantitative evaluation of the dissolution profile, the area under the dissolution curve (AUDC) for the neutral phase was considered because the main difference between dissolution performance of CNZ and PMs was in this phase. Moreover, AUDC neutral is the key metric by which to assess the different formulations as it is likely to correlate to *in vivo* absorption. AUDC neutral for different samples are shown in Table 2.

The Eu-based PMs showed AUDC neutral values of 1741 µg.min/ml, while the plain drug and its PM with HPMC produced significantly lower AUDC neutral value of 525 and 932 µg.min/ml, respectively ($p < 0.05$). The AUDC neutral values further showed the superiority of the Eu physical mixture with regard to the promotion of CNZ supersaturation in a neutral phase. HPMC provided supersaturation to a lesser extent than observed with Eu, which could either be a consequence of the lower dissolution rate of HPMC (as it tends to swell before it dissolves) compared to Eu or alternatively because Eu is a better crystallization inhibitor than HPMC.

These findings were consistent with the results obtained from supersaturation tests examining the inhibitory effects of the polymers against CNZ crystallization in solutions containing pre-dissolved Eu and HPMC. Dissolution results of PMs consistent with the results of supersaturation experiments demonstrated the superiority of Eu in respect to inhibiting crystallization of CNZ. Therefore, in this study, only Eu was considered for further study. The influence of Eu amount in

PMs on the dissolution profiles of CNZ was evaluated using various CNZ:Eu ratios (20:50, 20:100 and 20:200). The dissolution results signified that Eu-based PMs at all concentrations stabilized supersaturated levels of CNZ maintaining drug concentration significantly higher than the equilibrium solubility of CNZ ($2\mu\text{g/ml}$) for the whole period of neutral phase (3h) ($p < 0.05$ in all cases) (Fig.5). However, as can be seen from this Figure a direct correlation between the Eu content and the maximum CNZ concentration was not observed. The maximum CNZ concentration for PM (drug/polymer 20:200) and PM (drug/polymer 20:100) was relatively similar (around $11.5\mu\text{g/ml}$ after 20 min) ($p>0.05$), while PM (drug/polymer 20:50) differed with a lesser concentration of CNZ (almost $8.9\mu\text{g/ml}$ after 20 min) ($p<0.05$). Although, PM of drug/polymer with ratios of 20:100 and 20:200 showed >2.5 fold improvement in AUC_{0-3h} neutral for the plain drug, AUC_{0-3h} statistical analysis demonstrated no significant difference among these PMs ($p>0.05$). PM of drug/polymer 20:100 was chosen as the most promising PM for achieving the greatest CNZ concentration, with the highest drug loading and lowest polymer proportion. As mentioned previously, the interaction of the polymer with drug is the main factor which affects the crystallization of a drug from supersaturated solution. However, according to some studies, the viscosity should also be regarded as a potential factor contributing to the effect of the polymers on stabilizing supersaturation (46). This possibility was addressed in relation to the maintenance effect of the polymers on drug supersaturation by evaluation of the solution viscosity containing various concentrations of Eu (0.05, 0.1 and 0.2 mg/ml). Eu concentrations of 0.05, 0.1 and 0.2 mg/ml represent the concentration of Eu that would be obtained in solution subsequent the dissolution of Eu- based PMs at drug/ polymer ratios of 20:50, 20:100 and 20:200, respectively. At the Eu concentrations

of 0.05, 0.1 and 0.2 mg/ml, the viscosity of the solutions was determined and it is appeared to be below 1cP (at a reference shear rate of 150 rad/s, 37°C). According to the results of AUDC and viscosity, increasing the Eu amount in PMs resulted in higher AUDC values up to 20:100 (CNZ: Eu) ratio, whereas the solution viscosity was still very low for the solution containing 0.1 mg/ml of Eu. So, it can be concluded that viscosity is an insignificant factor in the Eu mediated stabilizing supersaturation. This appears to reveal that intermolecular interaction between CNZ and Eu only governs the stabilizing of supersaturation in a neutral phase.

According to previously published findings, the ability of a polymer to interact with a drug is mainly dependent on its functional sites (32). As Eu has the numerous acidic groups on the polymer backbone, thus, it is reasonable to assume that Eu with an abundance of hydrogen bond donor sites provides the suitable condition for interaction with CNZ molecules having hydrogen bond acceptor sites (C-N groups). Similarly, Hydrogen bonding has been accounted as a most important mechanism to stabilize supersaturation of other drugs in presence of the polymer (17).

However, it is worth pointing out that the mechanism of drug supersaturation maintenance by the polymer is still not comprehensively understood. In another study, the researchers compared the effect of three polymers namely hydroxypropylmethyl cellulose acetate succinate (HPMCAS), PVP and HPMC in inhibiting crystallization of felodipine and they showed that HPMC AS was more effective than PVP and HPMC in maintaining supersaturation of the drug and the reasons for that remained unknown (25).

In vitro dissolution of SDs

In order to investigate the influence of the process of making Eu with CNZ on their capability in maintaining supersaturation of the drug, the dissolution properties of Eu-based SDs were also evaluated. As shown in Fig.6, the dissolution profiles for Eu-based SDs were very different from

that of their PM counterparts. The dissolution profiles for CNZ revealed that the Eu- based SD 358
generated lower drug concentration in neutral phase than was obtained for a Eu- based PM at the 359
same drug/polymer ratio ($p < 0.05$). This may be explained as follows. According to previous 360
research, the induction and maintenance of a supersaturation state are essential to enhance the 361
dissolution properties of weakly basic drugs (47). 362

Dissolution results revealed that in SDs, the supersaturation was not generated in the neutral phase 363
and dissolution enhancement was not achieved in spite of the presence of Eu with efficient 364
supersaturation stabilizing effect. 365

In these formulations, in acidic phase very low drug release was obtained (less than 10% of the 366
drug released within 120 min at 20:100 drug /polymer ratio) which may be related to insolubility 367
of Eu in acidic phase (as Eu is soluble at $pH > 6.0$) and to the high molecular weight of CNZ (369.5 368
Da) (20). In fact, the Eu-based SD inhibited the release of CNZ under gastric pH while at neutral 369
pH it dissolved quickly and released the drug which crystallized out in the medium. In the case of 370
PMs, complete dissolution of CNZ in acidic phase resulting in substantial CNZ supersaturation 371
from these formulations following the pH change to 6.8. This suggests that Eu-based SD 372
formulation of CNZ would not be effective in promoting the dissolution of the drug, as this type 373
of formulation eliminates the acid-phase dissolution of CNZ and subsequent supersaturation upon 374
the acid to neutral pH change. On the other hand, it is obvious from the DSC thermograms that 375
Eu-based SDs do not show any signs of crystallinity (Fig.7). This demonstrated that CNZ was 376
dispersed in the SDs in an amorphous state. SD formulations containing amorphous drug has a 377
high energy and such a higher energy can increase the apparent solubility compared to 378
formulations containing the crystalline drug. 379

In previous studies, it has been demonstrated that amorphous SDs containing pH-dependent polymers may produce concentrations higher than equilibrium solubility of the drug as a supersaturation burst at pH in which the polymers dissolved (16,48-51). Therefore, it is reasonable to expect that Eu-based amorphous SD of CNZ could trigger the high initial concentration to form a supersaturated state at pH 6.8, considering the fact that Eu L100 is soluble at this pH (25,52). However, contrary to our expectation, there was no spring dissolution model of CNZ from Eu-based amorphous SDs in neutral phase (pH 6.8).

In the work published by Dereymaker *et al.* (53) they demonstrated that CNZ SDs have similar dissolution properties when various polymers were used and the amorphous CNZ crystallizes so rapidly during the dissolution in acetate buffer at pH 4.5. They concluded that formulation of CNZ as a solid dispersion does not appear to be suitable due to fast crystallization of CNZ. In another study performed by Baghel *et al* wherein crystallization tendency of dipyridamole and CNZ were determined, CNZ was also defined as a fast crystallizing drug (54).

These findings could be explained as follows. Upon dissolution, there are two different pathways for the crystallization of amorphous drugs. One is crystallization through the solid-to-solid pathway. The other is crystallization from supersaturated solution generated upon the dissolution of amorphous drug (50). It was assumed that if solid to solid transformation is the dominant crystallization mechanism of amorphous CNZ in SDs upon dissolution, then the dissolution profile of SDs observed should be similar to that of the crystalline CNZ. This similarity between the dissolution profiles of SDs and that of the crystalline CNZ in neutral phase can be clearly seen in Fig. 6 as expected. Therefore, the dissolution profiles of CNZ from Eu- based SDs with lack of supersaturated state upon dissolution in neutral pH may be attributed to this fact that crystallization of amorphous CNZ happens immediately in the solid amorphous state. It seems

that amorphous CNZ already crystallized by solid- to solid way upon exposure to aqueous
dissolution media so that only the dissolution behaviour of the crystallized CNZ was observed.
The rapid crystallization of amorphous CNZ upon dissolution may be attributed to sorption of
water with plasticization effect into the amorphous CNZ which led to higher molecular mobility
and hence accelerated crystallization. Indeed, CNZ as a fast crystallizing drug which possibly
suffers from solid to solid transformation upon dissolution compromised the benefits of
amorphous solubility of CNZ. Thus, this drug does not appear to be appropriate to be formulated
as Eu- based SD.

Similar results were reported for some other drugs including nifedipine, glipizide, sulfadoxine and
sucrose. It has been reported that for these drugs, crystallization initiates by solid- to- solid
transformation on the surface of their amorphous forms (55-58).

DSC and FT-IR analysis

The DSC thermograms of the samples are shown in Fig.7. In the case of Eu-based SD, contrary to
plain CNZ and its physical mixture with Eu, no melting peak of the drug was seen suggesting that
CNZ was dispersed within Eu to generate dispersion at a molecular level.

To investigate a possible interaction between CNZ and Eu, the FT-IR spectra of Eu, treated CNZ
and Eu-based solid dispersion were measured (Fig. 8). The pure CNZ has bands at 3066 cm^{-1}
(aromatic CH stretch), 3021 cm^{-1} (alkene CH stretch), 2956 cm^{-1} (aliphatic CH stretch), 1141 cm^{-1}
(C-N stretch), 1001 cm^{-1} (=C-H alkene) and 963 cm^{-1} (=C-H aromatic) (59). Eu has important
functional peaks at 1700 and 1730 cm^{-1} for the C=O bonds in the case of the carboxylic acid group
and the esterified carboxylic group, respectively (60). It was noticed that C-N peak of CNZ at
 1141 cm^{-1} for CNZ-Eu formulation was changed which could be an indication of an interaction

between Eu and CNZ during the processing. This change could be as a result of the interaction
between carboxylic acid group (donor) of Eu with C-N group (acceptor) of CNZ via hydrogen
bonding. FT-IR results confirmed the expected interaction between Eu and CNZ which was
mentioned previously. According to DSC and FT-IR results, it can be postulated that the CNZ-Eu
interactions are stronger than CNZ-CNZ interactions which appear to be valuable for stabilizing
CNZ molecules in solution.

Conclusion

The current study assessed CNZ supersaturation and excipient-mediated crystallization inhibition
in neutral pH. Following pH change from acidic to neutral, the effect on maintaining
supersaturation of CNZ was assessed using different polymers including HPMC, Eu, NaCMC,
HPC, PEG and PVP. It was demonstrated that maximum maintaining CNZ supersaturation was
achieved by Eu. The stabilizing effect of Eu on CNZ supersaturation in the neutral phase may be
related to drug-polymer interaction which was confirmed by FT-IR. The dissolution results
demonstrated that the physical mixture of Eu with CNZ markedly improved dissolution behaviour
of plain CNZ, however, Eu was not efficient in Eu-based solid dispersions, because of its hindering
effect on the generation of CNZ supersaturation, revealing the importance of formulation type over
the effectiveness of Eu in maintaining the supersaturation of CNZ. According to the results of this
study, a physical mixture of CNZ with Eu is expected to provide a higher chance for CNZ
absorption in the small intestine and it may be valuable to carry out further *in vivo* test on this
formulation.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and
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References	452
1- Stahl HP, Nakano M. Pharmaceutical aspects of the drug salt form. In Stahl PH, Wermuth CG, editors. Handbook of pharmaceutical salts: Properties, selection, and use, 1st ed., Zurich, Switzerland: VHCA, Verlag Helvetica Chimica Acta; Weinheim, Federal Republic of Germany: Wiley-VCH; 2002. p 83–116.	453 454 455 456
2- Zhou R, Moench P, Heran C, Lu X, Mathias N, Faria TN, Wall DA, Hussain MA, Smith RL, Sun D. pH-dependent dissolution in vitro and absorption in vivo of weakly basic drugs: development of a canine model. Pharm Res. 2005;22:188-92. doi: 10.1007/s11095-004-1185-3.	457 458 459
3-Hsieh YL, Ilevbare GA, Van Eerdenbrugh B, Box KJ, Sanchez-Felix MV, Taylor LS. pH-Induced crystallization behavior of weakly basic compounds: determination of extent and duration of supersaturation using potentiometric titration and correlation to solid state properties. Pharm Res. 2012;29:2738-53. doi:10.1007/s11095-012-0759-8.	460 461 462 463
4- Kostewicz ES, Wunderlich M, Brauns U, Becker R, Bock T, Dressman JB. Predicting the crystallization of poorly soluble weak bases upon entry in the small intestine. J Pharm Pharmacol. 2004;56:43-51. doi:10.1211/0022357022511.	464 465 466
5-Brouwers J, Brewster ME, Augustijns P. Supersaturating drug delivery systems: the answer to solubility-limited oral bioavailability?. J Pharm Sci. 2009;98:2549-72. doi:10.1002/jps.21650.	467 468

6-Stillhart C, Kuentz M. Trends in the Assessment of Drug Supersaturation and Crystallization	469
In Vitro Using Lipid-Based Delivery Systems. J Pharm Sci. 2016;105:2468-76.	470
doi:10.1016/j.xphs.2016.01.010.	471
7- Brouwers J, Tack J, Augustijns P. In vitro behavior of a phosphate ester prodrug of	472
amprenavir in human intestinal fluids and in the Caco-2 system: illustration of intraluminal	473
supersaturation. Int J Pharm. 2007;336:302-309. doi:10.1016/j.ijpharm.2006.12.011.	474
8-Mellaerts R, Mols R, Kayaert P, Annaert P, Van Humbeeck J, Van den Mooter G, Martens JA,	475
Augustijns P. Ordered mesoporous silica induces pH-independent supersaturation of the basic	476
low solubility compound itraconazole resulting in enhanced transepithelial transport. Int J	477
Pharm. 2008;357:169-79. doi:10.1016/j.ijpharm.2008.01.049.	478
9- Miller JM, Beig A, Carr RA, Spence JK, Dahan A. A win-win solution in oral delivery of	479
lipophilic drugs: supersaturation via amorphous solid dispersions increases apparent solubility	480
without sacrifice of intestinal membrane permeability. Mol Pharm. 2012;9:2009-16.	481
doi:10.1021/mp300104s.	482
10-Takano R, Takata N, Saito R, Furumoto K, Higo S, Hayashi Y, Machida M, Aso Y,	483
Yamashita S. Quantitative analysis of the effect of supersaturation on in vivo drug absorption.	484
Mol Pharm. 2010;7:1431-40. doi:10.1021/mp100109a.	485
11-Dahan A, Miller JM. The solubility-permeability interplay and its implications in	486
formulation design and development for poorly soluble drugs. AAPS J. 2012; 14: 244–251.	487
doi:10.1208/s12248-012-9337-6.	488

12-Dahan A, Miller JM, Hoffman A, Amidon GE, Amidon GL. The solubility–permeability interplay in using cyclodextrins as pharmaceutical solubilizers: Mechanistic modeling and application to progesterone. J Pharm Sci. 2010;99:2739–2749. doi:10.1002/jps.22033.	489 490 491
13-Miller JM, Beig A, Krieg BJ, Carr RA, Borchardt TB, Amidon GE, Amidon GL, Dahan A. The solubility–permeability interplay: Mechanistic modeling and predictive application of the Impact of micellar solubilization on intestinal permeation. Mol Pharmaceutics. 2011; 8: 1848–1856. doi: 10.1021/mp200181v.	492 493 494 495
14-Vandecruys R, Peeters J, Verreck G, Brewster ME. Use of a screening method to determine excipients which optimize the extent and stability of supersaturated drug solutions and application of this system to solid formulation design. Int J Pharm. 2007; 342:168-175. doi:10.1016/j.ijpharm.2007.05.006.	496 497 498 499
15-Brewster ME, Vandecruys R, Verreck G, Peeters J. Supersaturating drug delivery systems: effect of hydrophilic cyclodextrins and other excipients on the formation and stabilization of supersaturated drug solutions. Pharmazie. 2008;63:217-220. doi: 10.1691/ph.2008.7326 .	500 501 502
16-Curatolo W, Nightingale JA, Herbig SM. Utility of hydroxypropylmethylcellulose acetate succinate (HPMCAS) for initiation and maintenance of drug supersaturation in the GI milieu. Pharm Res. 2009;26:1419-31. doi:10.1007/s11095-009-9852-z.	503 504 505
17- Raghavan SL, Trividic A, Davis AF, Hadgraft J. Crystallization of hydrocortisone acetate: influence of polymers. Int J Pharm. 2001;212:213-21. doi: 10.1016/S0378-5173(00)00610-4.	506 507

18- Miller DA, DiNunzio JC, Yang W, McGinity JW, Williams RO 3rd. Enhanced in vivo	508
absorption of itraconazole via stabilization of supersaturation following acidic-to-neutral pH	509
transition. Drug Dev Ind Pharm. 2008;34:890-902. doi:10.1080/03639040801929273.	510
19- Gao P, Akrami A, Alvarez F, Hu J, Li L, Ma C, Surapaneni S. Characterization and	511
optimization of AMG 517 supersaturatable self-emulsifying drug delivery system (S-SEDDS)	512
for improved oral absorption. J Pharm Sci. 2009;98:516-28. doi:10.1002/jps.21451.	513
20- Alhnan MA, Cosi D, Murdan S, Basit AW. Inhibiting the gastric burst release of drugs from	514
enteric microparticles: the influence of drug molecular mass and solubility. J Pharm Sci.	515
2010;99:4576-83. doi :10.1002/jps.22174.	516
21- Gu CH, Rao D, Gandhi RB, Hilden J, Raghavan K. Using a novel multicompartment	517
dissolution system to predict the effect of gastric pH on the oral absorption of weak bases with	518
poor intrinsic solubility. J Pharm Sci. 2005;94:199-208.: doi 10.1002/jps.20242.	519
22-Järvinen T, Järvinen K, Schwarting N, Stella VJ. beta-cyclodextrin derivatives, SBE4-beta-	520
CD and HP-beta-CD, increase the oral bioavailability of cinnarizine in beagle dogs. J Pharm Sci.	521
1995;84:295-9.: doi:10.1002/jps.2600840306.	522
23- Warren DB, Benameur H, Porter CJ, Pouton CW. Using polymeric crystallization inhibitors	523
to improve the absorption of poorly water-soluble drugs: A mechanistic basis for utility. J Drug	524
Target. 2010;18:704-31. doi: 10.3109/1061186X.2010.525652.	525
24-Abu-Diak OA, Jones DS, Andrews GP. An investigation into the dissolution properties of	526
celecoxib melt extrudates: understanding the role of polymer type and concentration in	527

stabilizing supersaturated drug concentrations. Mol Pharm. 2011;8:1362-71.	528
doi:10.1021/mp200157b.	529
25-Konno H, Handa T, Alonzo DE, Taylor LS. Effect of polymer type on the dissolution profile of amorphous solid dispersions containing felodipine. Eur J Pharm Biopharm. 2008;70:493-499.	530 531
doi:10.1016/j.ejpb.2008.05.023.	532
26- Savolainen M, Kogermann K, Heinz A, Aaltonen J, Peltonen L, Strachan C, Yliruusi J. Better understanding of dissolution behaviour of amorphous drugs by in situ solid-state analysis using Raman spectroscopy. Eur J Pharm Biopharm. 2009;71:71-79.	533 534 535
doi:10.1016/j.ejpb.2008.06.001.	536
27-Alonzo DE, Zhang GG, Zhou D, Gao Y, Taylor LS. Understanding the behavior of amorphous pharmaceutical systems during dissolution. Pharm Res. 2010;27:608-618.	537 538
doi:10.1007/s11095-009-0021-1.	539
28- Chauhan H, Hui-Gu C, Atef E. Correlating the behavior of polymers in solution as crystallization inhibitor to its amorphous stabilization ability in solid dispersions. J Pharm Sci. 2013;102:1924-1935. doi:10.1002/jps.23539.	540 541 542
29- Yokoi Y, Yonemochi E, Terada K. Effects of sugar ester and hydroxypropyl methylcellulose on the physicochemical stability of amorphous cefditoren pivoxil in aqueous suspension. Int J Pharm. 2005;290:91-9. doi:10.1016/j.ijpharm.2004.11.020.	543 544 545
30- Zimmermann A, Millqvist-Fureby A, Elema MR, Hansen T, Müllertz A, Hovgaard L. Adsorption of pharmaceutical excipients onto microcrystals of siramesine hydrochloride: effects	546 547

on physicochemical properties. Eur J Pharm Biopharm. 2009; 71:109–116.	548
doi:10.1016/j.ejpb.2008.06.014.	549
31- Douroumis D, Fahr A. Stable carbamazepine colloidal systems using the cosolvent technique. Eur J Pharm Sci. 2007;30:367–374. doi:10.1016/j.ejps.2006.12.003.	550 551
32- Ilevbare GA, Liu H, Edgar KJ, Taylor LS. Understanding polymer properties important for crystal growth inhibition-impact of chemically diverse polymers on solution crystal growth of ritonavir. Cryst Growth Des. 2012;12: 3133–3143. doi: 10.1021/cg300325p.	552 553 554
33- Augustijns P, Brewster ME. Supersaturating drug delivery systems: fast is not necessarily good enough. J Pharm Sci. 2012;101:7-9. doi:10.1002/jps.22750.	555 556
34- Ilevbare GA, Taylor LS. Liquid–Liquid Phase Separation in Highly Supersaturated Aqueous Solutions of Poorly Water-Soluble Drugs: Implications for Solubility Enhancing Formulations. Cryst Growth Des. 2013;13:1497-1509. doi: 10.1021/cg301679h.	557 558 559
35-Brick MC, Palmer HJ, Whitesides TH. Formation of colloidal dispersions of organic materials in aqueous media by solvent shifting. Langmuir. 2003;19:6367–6380. doi: 10.1021/la034173o.	560 561
36- Tung H-H, Paul EL, Midler M, McCauley JA. Critical issues in crystallization practice. In Crystallization of organic compounds: An industrial perspective, 1st ed., Hoboken, New Jersey: John Wiley & Sons, Inc; 2009.p 107	562 563 564
37- Almeida e Sousa L, Reutzel-Edens SM, Stephenson GA, Taylor LS. Assessment of the amorphous "solubility" of a group of diverse drugs using new experimental and theoretical approaches. Mol Pharm. 2015;12:484-95. doi: 10.1021/mp500571m.	565 566 567

38-Baghel S, Cathcart H, O'Reilly NJ. Understanding the generation and maintenance of	568
supersaturation during the dissolution of amorphous solid dispersions using modulated DSC and	569
¹ H NMR. Int J Pharm. 2018 ;536:414-425. doi:10.1016/j.ijpharm.2017.11.056.	570
39- Raina SA, Van Eerdenbrugh B, Alonzo DE, Mo H, Zhang GGZ, Gao Y, Taylor LS. Trends	571
in the crystallization and crystallization behavior of supersaturated aqueous solutions of poorly	572
water-soluble drugs assessed using synchrotron radiation. J Pharm Sci. 2015;104:1981-92.	573
doi:10.1002/jps.24423.	574
40- Raina SA, Alonzo DE, Zhang GG, Gao Y, Taylor LS. Using Environment-Sensitive	575
Fluorescent Probes to Characterize Liquid-Liquid Phase Separation in Supersaturated Solutions	576
of Poorly Water Soluble Compounds. Pharm Res. 2015;32:3660-73. doi:10.1007/s11095-015-	577
1725-z.	578
41-Indulkar AS, Gao Y, Raina SA, Zhang GG, Taylor LS. Exploiting the phenomenon of liquid–	579
liquid phase separation for enhanced and sustained membrane transport of a poorly water-soluble	580
drug. Mol Pharm. 2016;13:2059-69. doi:10.1021/acs.molpharmaceut.6b00202.	581
42- Raina SA, Zhang GGZ, Alonzo DE, Wu J, Zhu D, Catron ND, Gao Y, Taylor LS.	582
Enhancements and limits in drug membrane transport using supersaturated solutions of poorly	583
water soluble drugs. J Pharm Sci. 2014;103:2736-48. doi:10.1002/jps.23826.	584
43-Heigoldt U, Sommer F, Daniels R, Wagner KG. Predicting in vivo absorption behavior of	585
oral modified release dosage forms containing pH-dependent poorly soluble drugs using a novel	586
pH-adjusted biphasic in vitro dissolution test. Eur J Pharm Biopharm. 2010;76:105-111.	587
doi:10.1016/j.ejpb.2010.05.006.	588

44-Kalantzi L, Goumas K, Kalioras V, Abrahamsson B, Dressman JB, Reppas C.	589
Characterization of the human upper gastrointestinal contents under conditions simulating	590
bioavailability/bioequivalence studies. <i>Pharm Res.</i> 2006;23:165-76. doi:10.1007/s11095-005-	591
8476-1.	592
45-Alhnan MA, Murdan S, Basit AW. Encapsulation of poorly soluble basic drugs into enteric	593
microparticles: a novel approach to enhance their oral bioavailability. <i>Int J Pharm.</i> 2011;416:55-	594
60. doi:10.1016/j.ijpharm.2011.05.079.	595
46- Usul F, Maeda K, Kusai A, Nishimura K, Yamamoto K. Inhibitory effect of	596
water soluble polymers on crystallization of RS-8359. <i>Int J Pharm.</i> 1997;154:59–66.	597
doi: 10.1016/S0378-5173(97)00129-4.	598
47-Guzmán HR, Tawa M, Zhang Z, Ratanabanangkoon P, Shaw P, Gardner CR, Chen H,	599
Moreau JP, Almarsson O, Remenar JF. Combined use of crystalline salt forms and crystallization	600
inhibitors to improve oral absorption of celecoxib from solid oral formulations. <i>J Pharm Sci.</i>	601
2007;96:2686-702. doi:10.1002/jps.20906.	602
48- Albers J, Alles R, Matthée K, Knop K, Nahrup JS, Kleinebudde P. Mechanism of drug	603
release from polymethacrylate-based extrudates and milled strands prepared by hot-melt	604
extrusion. <i>Eur J Pharm Biopharm.</i> 2009;71:387-94. doi:10.1016/j.ejpb.2008.10.002.	605
49-Overhoff KA, Moreno A, Miller DA, Johnston KP, Williams RO 3rd. Solid dispersions of	606
itraconazole and enteric polymers made by ultra-rapid freezing. <i>Int J Pharm.</i> 2007;336:122-32.	607
doi:10.1016/j.ijpharm.2006.11.043.	608

- 50- Zecevic DE, Meier R, Daniels R, Wagner KG. Site specific solubility improvement using solid dispersions of HPMC-AS/HPC SSL--mixtures. *Eur J Pharm Biopharm.* 2014;87:264-70. doi:10.1016/j.ejpb.2014.03.018.
- 51- Kojima T, Higashi K, Suzuki T, Tomono K, Moribe K, Yamamoto K. Stabilization of a supersaturated solution of mefenamic acid from a solid dispersion with EUDRAGIT(®) EPO. *Pharm Res.* 2012;29:2777-91. doi:10.1007/s11095-011-0655-7.
- 52- Loftsson T, Fridriksdottir F, Gudmundsdottir T K. The effect of water-soluble polymers on aqueous solubility of drugs. *Int J Pharm.* 1996;127:293–296. doi: 10.1016/0378-5173(95)04207-5.
- 53- Dereymaker A, Cinghia G, Van den Mooter G. Eudragit® RL as a stabilizer for supersaturation and a substrate for nanocrystal formation. *Eur J Pharm Biopharm.* 2017;114:250-262. doi:10.1016/j.ejpb.2017.02.002.
- 54-Baghel S, Cathcart H, Redington W, O'Reilly NJ. An investigation into the crystallization tendency/kinetics of amorphous active pharmaceutical ingredients: A case study with dipyridamole and cinnarizine. *Eur J Pharm Biopharm.* 2016;104:59-71. doi:10.1016/j.ejpb.2016.04.017.
- 55- Edueng K, Mahlin D, Larsson P, Bergström CAS. Mechanism-based selection of stabilization strategy for amorphous formulations: Insights into crystallization pathways. *J Control Release.* 2017;256:193-202. doi:10.1016/j.jconrel.2017.04.015.

56-Mehta M, Kothari K, Ragoonanan V, Suryanarayanan R. Effect of Water on Molecular Mobility and Physical Stability of Amorphous Pharmaceuticals. <i>Mol Pharm</i> . 2016;13:1339-46. doi:10.1021/acs.molpharmaceut.5b00950.	628 629 630
57- Aucamp M, Milne M, Liebenberg W. Amorphous Sulfadoxine: A Physical Stability and Crystallization Kinetics Study. <i>AAPS PharmSciTech</i> . 2016;17:1100-9. doi:10.1208/s12249-015-0436-4.	631 632 633
58- Yu X, Kappes SM, Bello-Perez LA, Schmidt SJ. Investigating the moisture sorption behavior of amorphous sucrose using a dynamic humidity generating instrument. <i>J Food Sci</i> . 2008;73:E25-35. doi:10.1111/j.1750-3841.2007.00596.x.	634 635 636
59- Haress NG. Cinnarizine: Comprehensive Profile. <i>Profiles Drug Subst Excip Relat Methodol</i> . 2015;40:1-41. doi :10.1016/bs.podrm.2015.01.001.	637 638
60- Cilurzo F, Minghetti P, Selmin F, Casiraghi A, Montanari L. Polymethacrylate salts as new low-swellable mucoadhesive materials. <i>J Control Release</i> . 2003;88:43-53. doi: 10.1016/S0168-3659(02)00459-5.	639 640 641 642
Table 1	643 644
The area under the concentration–time (AUC) profile of the supersaturation curve in presence and absence of polymers	645 646 647

Polymers	AUC ($\mu\text{g}\cdot\text{min}/\text{ml}$)
Without polymer	141.07 $\pm$ 13.2
Eu L100	873.11 $\pm$ 24.3
HPMC K4M	471.39 $\pm$ 6.9
HPC(Mw=370000)	269.59 $\pm$ 24.1
PVP	246.19 $\pm$ 28.6
PEG	116.34 $\pm$ 7.0
NaCMC	124.76 $\pm$ 10.4

Table 2

The area under the dissolution curve (AUDC) for the neutral phase for different formulations

samples	AUDC ($\mu\text{g}\cdot\text{min}/\text{ml}$)
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671	Plain CNZ	525.84±25.3
	PM(CNZ/HPMC) 20:100	932.42±26.8
	PM(CNZ/Eu) 20:50	1458.25±17.0
	PM(CNZ/Eu) 20:100	1741.90±11.5
674	PM(CNZ/Eu) 20:200	1723.45±23.6
675	SD(CNZ/Eu) 20:50	485.87±24.9
	SD(CNZ/Eu) 20:100	376.64±15.3
676	SD(CNZ/Eu) 20:200	240.51±24.9

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Fig.1. Inhibitory effects of polymers on precipitation of a supersaturated solution of CNZ (20µg/ml) at pH 6.8.

Fig. 2. Inhibitory effect of Eu (0.4 mg/ml) on precipitation of CNZ at different supersaturation ratios

Fig. 3. DLS particle size measurement of CNZ colloidal particles at super saturation ratio of 20 in presence of Eu

Fig. 4. In vitro release of plain CNZ and PMs with Eu and HPMC

Fig. 5. In vitro release of Eu-based PMs at different CNZ-Eu ratios

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Fig. 6. In vitro release of Eu-based SDs at different CNZ-Eu ratios

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Fig. 7. DSC scans of the samples: plain CNZ and PM and SD prepared with Eu

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Fig. 8. FT-IR of spectrum of samples (a) Eu, (b) treated CNZ, and (c) Eu-based SD

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