Incorporation of HPMCAS during loading of glibenclamide onto mesoporous silica improves dissolution and inhibits precipitation

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Graphical abstract

API/PI Co-loaded Silica Formulations: The Optimal Method for PI Incorporation

Solid State and Stomach
✓ Formation of drug-polymer interactions
✓ Loaded silica confined to polymer plate
✓ No release in stomach

Intestines
✓ Breakdown of polymer plate
✓ Release of supersaturated API
✓ Improved precipitation inhibition
Abstract
Mesoporous silica has emerged as an enabling formulation for poorly soluble active pharmaceutical ingredients (APIs). Unlike other formulations, mesoporous silica typically does not inhibit precipitation of supersaturated API therefore, a suitable precipitation inhibitor (PI) should be added to increase absorption from the gastrointestinal (GI) tract. However, there is limited research about optimal processes for combining PIs with silica formulations. Typically, the PI is added by simply blending the API-loaded silica mechanically with the selected PI. This has the drawback of an additional blending step and may also not be optimal with regard to release of drug and PI. By contrast, loading PI simultaneously with the API onto mesoporous silica, i.e. co-incorporation, is attractive from both a performance and practical perspective. The aim of this study was to demonstrate the utility of a co-incorporation approach for combining PIs with silica formulations, and to develop a mechanistic rationale for improvement of the performance of silica formulations using the co-incorporation approach. The results indicate that co-incorporating HPMCAS with glibenclamide onto silica significantly improved the extent and duration of drug supersaturation in single-medium and transfer dissolution experiments. Extensive spectroscopic characterization of the formulation revealed that the improved performance was related to the formation of drug-polymer interactions already in the solid state; the immobilization of API-loaded silica on HPMCAS plates, which prevents premature release and precipitation of API; and drug-polymer proximity on disintegration of the formulation, allowing for rapid onset of precipitation inhibition. The data suggests that co-incorporating the PI with the API is appealing for silica formulations from both a practical and formulation performance perspective.
1. Introduction

Among the various administration routes for drugs, oral administration is the most commonly employed. It is cost-effective and convenient for the patient, leading to a very high patient compliance (Krishnaiah, 2010). APIs must be absorbed to become orally bioavailable, a process which relies in turn on sufficient solubility and permeability of the API (Zheng, 2012). In recent years there has been an exponential increase in drugs exhibiting poor solubility: it is reported that approximately 60% of all drugs on the market are poorly soluble (Taylor and Zhang 2016). It has been suggested that anywhere between 80 and 90% of compounds in development also demonstrate low solubility (Loftsson, 2010). These estimates highlight the need for effective formulation approaches to avoid low bioavailability associated with poor aqueous solubility.

To overcome these challenges, formulators have developed a series of promising formulation strategies (Ditzinger, 2018). These approaches include: (i) solvents, co-solvents and lipids; (ii) micelle systems; (iii) particle size reduction; (iv) complexation; and (v) amorphous technologies (Zheng, 2012). One of the most common approaches for improving bioavailability is via the generation of supersaturated solutions in the GI-tract, which can drive improved absorption (Zheng, 2012). However, these systems are metastable due to the energetic propensity of the compound to precipitate (Price, 2018). Therefore, precipitation inhibitors (PIs) are often used to sustain the supersaturated state by inhibiting or slowing down precipitation of drug (Warren, 2010). Successful PI systems can sustain drug supersaturation over physiologically relevant time-scales by interfering with the crystallization process (Price, 2018). Precipitation inhibitors can kinetically prevent re-crystallization via a number of mechanisms, including: viscosity, co-solvency and drug-polymer interactions, with the latter widely being reported to being especially important (Warren, 2010; Price, 2018). Recent advances in precipitation inhibition design and selection include de novo precipitation inhibitor design (Ting, 2017) and in silico calculation of drug-polymer mixing enthalpies for precipitation inhibitor selection (Price, 2019).

One under-utilized formulation technology to generate drug supersaturation is mesoporous silica. Mesoporous silica is a silicon dioxide excipient that has a highly porous network, consisting of mesopores between 2 and 50 nm in diameter (Barbe, 2009). These materials have very high specific surface areas and are used in catalysis, environmental clean-up, chromatography, and drug delivery (McCarthy, 2016). Poorly soluble APIs can become molecularly adsorbed on the surface of the silica and sterically confined such that recrystallization cannot occur (Knapik, 2016). Indeed, this is one of the most widely reported
advantages of mesoporous silica, in its enhanced stabilization capabilities due to nanoconfinement in the porous network (McCarthy, 2016). Mesoporous silica-based ‘spring and parachute’ formulations have been widely demonstrated in the literature, from both an in vitro and in vivo perspective (Ditzinger, 2018; McCarthy, 2016). Van Speybroeck and colleagues originally described how such precipitation inhibitors, including HPMC and HPMCAS, can enhance the oral absorption of itraconazole released from mesoporous silica in rats (Van Speybroeck, 2010). This was also demonstrated in pigs, with O’Shea and colleagues using the precipitation inhibitor HPMCAS to improve the oral absorption of fenofibrate released from mesoporous silica (O’Shea, 2016). Recent work on precipitation inhibitors for mesoporous silica has also taken place, Price and co-workers developed an in silico screening approach which calculates drug-polymer mixing enthalpy for the optimized selection of precipitation inhibitors for mesoporous silica formulations (Price, 2019). In spite of these recent advances in mesoporous silica and precipitation inhibition, the method of combining precipitation inhibitors with mesoporous silica remains relatively inefficient. Typically, PIs are mechanically blended with the API-loaded silica formulations after the drug is loaded (usually with a mortar and pestle). However, it has recently been shown that incorporating the PI into the API loading process itself can dramatically improve both in vitro and in vivo performance of a celecoxib loaded silica formulation (Laine, 2016). In light of this proof of concept, there is a need for further mechanistic research. This study aims to demonstrate the utility of a co-incorporation approach for combining PIs with silica formulations, and to develop a mechanistic rationale to explain the improvement in performance of silica formulations using the co-incorporation approach.

2. Experimental

2.1 Materials

Crystalline glibenclamide (GB), reagent grade acetone, HPLC grade acetonitrile and HPLC grade methanol were all purchased from MilliporeSigma (St Louis, MO, USA). AQOAT (HPMCAS-MF) was purchased from ShinEtsu (Japan). Parteck® SLC was a gift sample from Merck KGaA (Germany). FaSSGF/FaSSIF/FeSSIF powder to make biorelevant dissolution medium, Fasted Simulated Intestinal Fluid (FaSSIF), was obtained from Biorelevant.com (UK).

2.2 Methods

2.2.1 Determination of thermodynamic solubility

FaSSIF was prepared by weighing 45 mg of FaSSGF/FaSSIF/FeSSIF powder into 45 mL of phosphate buffer (pH 6.5) (Galia, et al. 1998). SGF (pH 1.2) was prepared according to USP monographs. Glibenclamide (2-
3mg) was accurately weighed into a Uniprep® syringeless filter (5mL; 0.45µm). 2 mL of either FaSSIF (pH 6.5) or SGF (pH 1.2) was added and the samples were agitated at 450 rpm for 24 hours at 37 °C. The pH was checked at 7 hours and adjusted with 0.1 N NaOH or 0.1 N HCl, if a deviation greater than +/- 0.05 pH units was observed. The final pH was also recorded after 24 hours.

Samples were filtered with PTFE 0.45 µm Whatman filters after 24 hours. Filtrates were diluted with acetonitrile and water (1:4) to avoid precipitation from the saturated solution. Samples were analyzed with ultra-high performance liquid chromatography (UPLC) (Thermo Dionex Ultimate 3000, Thermo Fisher, MA, USA) to determine the API concentration. API concentration was determined based on a standard calibration curve of nine standard concentrations (50, 30, 10, 5, 3, 1, 0.5, 0.3, 0.1 µg/mL). Three quality control samples of known concentrations (30, 3, 0.3 µg/mL) were prepared and used to check the robustness of the calibration curve. The determination was carried out in duplicate.

2.2.2 UPLC method

UPLC analysis was performed using a Thermo Dionex Ultimate 3000 (Thermo Fisher, MA, USA) equipped with a diode array detector at 240 nm (Thermo Fisher, MA, USA). Chromatographic separation was achieved on an Acquity UPLC BEH column C8 (2.1 x 50 mm, 1.7 µm, Waters, MA, USA). The mobile phases A and B consisted of water: formic acid 99:1 (v:v) and acetonitrile : formic acid 99:1 (v: v), respectively. Gradient and flow rate is shown in Table 2. System management, data acquisition and processing were performed with the Chromeleon™ software package, version 7.2 (Thermo Fisher, MA, USA)

<table>
<thead>
<tr>
<th>Time (mins)</th>
<th>Flow rate (mL/min)</th>
<th>% (v:v) Mobile phase A</th>
<th>% (v:v) Mobile phase B</th>
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<tbody>
<tr>
<td>0</td>
<td>0.83</td>
<td>90</td>
<td>10</td>
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<tr>
<td>0.83</td>
<td>0.83</td>
<td>10</td>
<td>90</td>
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<tr>
<td>1.2</td>
<td>1.5</td>
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<tr>
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<td>1.5</td>
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<td>10</td>
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<tr>
<td>2.01</td>
<td>0.83</td>
<td>90</td>
<td>10</td>
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2.2.3 Parteck SLC® standard loading procedure and standard PI incorporation

Glibenclamide loaded silica was prepared using the solvent impregnation rotary evaporator method (Laine, et al. 2016) as follows: A solution (10 mg/mL) of API in acetone was added to Parteck SLC (1:2 w/w API/Parteck SLC®) under magnetic stirring for 30 minutes. The suspension was then transferred to a rotary
The solvent was removed under reduced pressure at 40° C. After complete removal of the solvent, the powder was left to dry in the rotary evaporator under reduced pressure for a further 2 hours. The formulation was then physically combined with HPMCAS (API: Parteck SLC®: HPMCAS 1: 2: 3 w/w) using a pestle and mortar.

2.2.4 Parteck SLC® API/PI co-incorporation procedure

Glibenclamide/HPMCAS co-incorporated Silica samples were prepared using the solvent impregnation rotary evaporator method. A solution of API (10 mg/mL) and HPMCAS (30 mg/mL) in acetone was added to Parteck SLC (1 : 2 : 3 API: Parteck SLC®: HPMCAS) under magnetic stirring, which was continued for 30 minutes. The suspension was transferred to a rotary evaporator, and the solvent was removed under reduced pressure at 40° C. After complete removal of the solvent, the powder was left to dry in the rotary evaporator under reduced pressure for a further 2 hours.

2.2.5 Preparation of an API-HPMCAS sample as control

A control sample consisting of only of API and HPMCAS was also prepared. A solution with the same concentrations of API (10mg/mL) and HPMCAS (30 mg/mL) as described above for the API – silica – PI system in acetone was prepared under magnetic stirring for 15 minutes. The solution was then transferred to a rotary evaporator, and the solvent was removed under reduced pressure at 40° C. After complete removal of the solvent, the powder was left to dry in the rotary evaporator under reduced pressure for a further 2 hours. Residual solvent concentration was recorded with 2D ¹H NMR to ensure residual solvent was below the ICH limit of 0.5% (data not shown).

2.2.6 Determination of glibenclamide loading onto mesoporous silica

To determine the % (w/w) of API in the mesoporous silica, the loaded samples were dispersed and stirred in DMSO as this solvent is known to dissolve glibenclamide readily. Samples were taken after 1 hour, centrifuged, filtered and diluted before being quantified by UPLC, according to the method described in 2.2.2. The API content was calculated relative to the mass of loaded samples dispersed within the DMSO. The study was performed in triplicate.

2.2.7 Powder X-Ray Diffraction (PXRD)

Samples were prepared between X-ray amorphous films and measured in transmission mode using Cu-Kα1-radiation and a Stoe StadiP 611 KL diffractometer equipped with Dectris Mythen1K PSD. The measurements were evaluated with the software WinXPow 3.03 by Stoe, Crystallographica Search/Match Version 3.1.0.2, the ICDD PDF-4+ 2014 Database and Igor Pro Version 6.34 by Wavemetrics Inc.
2.2.8 FaSSIF mini-dissolution experiment

Around 5 mg of API (or the equivalent of API-loaded silica) was weighed accurately into a glass vial. 5 mL of FaSSIF was added. The vials were agitated at 37 °C and 450 rpm in a shaker for 2 hours. Samples were taken at 2, 15, 60 and 120 minutes, filtered (0.45 PTFE Whatman filters), diluted, and analyzed by UPLC. Solid residues at the end of the experiment were collected via centrifugation and analyzed for crystallinity with powder X-ray diffraction (PXRD). This was carried out on the following samples: API, API + polymer, API loaded silica and API loaded silica + PI. The mini-dissolution trials were conducted in duplicate for all samples.

2.2.9 Biorelevant transfer experiments

The experimental set-up for the transfer experiments is demonstrated in **Figure 1**.

![Figure 1. Experimental diagram showing mini-transfer set-up](image)

**Figure 1.** Experimental diagram showing mini-transfer set-up

Around 150 mg of API or equivalent was accurately weighed in a 100 mL stoppered flask - the exact sample masses varied dependent on the formulation (see Table 1).

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Weighed mass (mg)</th>
<th>API mass (mg)</th>
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*Table 1. Transfer dissolution sample preparation*
25mL of simulated gastric fluid (pH 1.2) prepared according to USP monographs was added to the flask and agitated at 450 rpm and 37 °C. 50mL of FaSSIF were added to a separate flask, which was also agitated at 450 rpm and 37 °C. After 30 minutes, the API suspension in SGF pH 1.2 was transferred at a zero-order rate of 0.85 mL/min using a peristaltic pump, until the complete gastric contents were transferred (~30 minutes) into the FaSSIF compartment. Samples were withdrawn from the intestinal compartment at regular time points using a 1 mL syringe to a sampling tube fitted with a pre-filter of 10 µm and filtered again using a 0.45 µm PTFE Whatman syringe filter and diluted. Samples were then analyzed by UPLC for API content. The post-dissolution residues were then collected and analyzed for crystallinity with XRPD.

2.2.10 Single medium SGF dissolution assay (in tandem to transfer assay)

Around 150 mg of API or equivalent was accurately weighed into a 100 mL stoppered flask. The exact sample masses varied dependent on the formulation (see Table 1). 25mL of SGF (pH 1.2, gastric compartment) was added to the flask and the contents agitated at 450 rpm and 37 °C. Samples were withdrawn at regular time points using a 1 mL syringe to a sampling tube fitted with a pre-filter of 10 µm and filtered again using a 0.45 µm PTFE Whatman syringe filter and suitably diluted. Samples were then analyzed with UPLC for API content. The post-dissolution residues were directly collected and analyzed for crystallinity with PXRD.

2.2.11 Scanning Electron Microscopy (SEM) and Energy-Dispersive X-ray Spectroscopy (EDX)

Samples were prepared on copper tape and imaged using a Hitachi TM3000 Tabletop Microscope, W cathode, low vacuum, accelerating voltage 5 kV and 15 kV, 4-Quadrant BSE detector, magnification 15x – 30,000x. For the energy dispersive X-ray spectroscopy (EDX) data, a standard-less quantitative analysis was performed by using the ZAF correction, considering the correction for light elements standardless element coefficient factors (SEC).

2.2.12 Solid-state Nuclear Magnetic Resonance (NMR) spectroscopy
Solid-state NMR experiments were performed under magic-angle-sample (MAS) spinning using a Bruker 4 mm MAS HXY probe in double resonance mode in combination with a Bruker Avance 600 MHz wide bore NMR spectrometer (Bruker). The sample spinning frequency was 10 kHz, and the readout on the probe thermocouple was set to 290 K. 

$^{13}$C-CP experiments were performed using a contact time of 1 ms and 100 kHz high power proton decoupling following the SPINAL64 scheme was applied during acquisition. The recycle delay was 3 s. The spectra were indirectly referenced to DSS via the CH2 signal of Adamantane at 40.49 ppm. Solid-state NMR measurements were repeated on multiple batches to ensure reliability of the interpretation.

3. Results

3.1 Solid-state form of glibenclamide in formulations

The glibenclamide powder used in this work is crystalline in the solid-state as shown by XRPD (Figure 2a). Successful loading of glibenclamide onto mesoporous silica was demonstrated by the absence of distinct Bragg peaks in XRPD patterns, which indicated a shift from the crystalline to the amorphous state (Figure 2b). The co-incorporation process did not interfere with the solid-state conversion of glibenclamide: the co-incorporated sample exhibited the same shift from crystalline to amorphous post-loading (Figure 2c). However, the control sample, which consisted of HPMCAS/GB prepared by solvent evaporation, showed partial crystallinity, which aligned with the XRPD pattern for the unmodified crystalline glibenclamide (Figure 2d).
Figure 2. XRPD pattern for crystalline glibenclamide (GB) (a), glibenclamide loaded silica (b), GB and HPMCAS co-incorporated silica (c) and GB and HPMCAS prepared by rotary evaporation (d)

3.2 Loading content of glibenclamide in mesoporous silica formulations

The % loading of glibenclamide determined by UPLC is shown in Table 3. The final glibenclamide content in the final mesoporous silica formulations was around 15%, irrespective of whether the drug was first loaded onto the silica and then combined with HPMCAS, or the HPMCAS was incorporated during drug loading. Drug loading levels are modest, which could be a limitation for drugs that are administered at high doses. However, they are in line with usual supersaturating drug formulations that require precipitation inhibitors (Price, 2019; Ditzinger, 2018).

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Theoretical loading (%)</th>
<th>Actual loading (UPLC) (%)</th>
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</table>
Glibenclamide loaded silica (without HPMCAS) & 30 & 30.1 ± 0.1 \\
Glibenclamide and HPMCAS co-incorporated silica & 15 & 15.9 ± 0.2 \\
Glibenclamide loaded silica + HPMCAS blend & - & 15.1 \\

### 3.3 Scanning Electron Microscopy (SEM) and Electron Dispersive X-ray Spectroscopy (EDX)

SEM images for glibenclamide loaded silica, glibenclamide loaded silica + HPMCAS Blend and SEM and EDX images for glibenclamide and HPMCAS co-incorporated silica are shown in Figure 3. The unloaded silica is shown in Figure 3a. In glibenclamide loaded silica, the characteristic silica particles are also present (Figure 3b). This is also the case for glibenclamide loaded silica + HPMCAS physical mixture (Figure 3c), where the particles are simply ‘diluted’ by the addition of the polymer, which is depicted as the dark texture in between the silica particles. However, large platelet particles were observed when HPMCAS was incorporated during the loading step onto the silica (Figure 3d). The EDX images show that the platelet particles are carbon based and therefore likely composed of HPMCAS. The silica particles appear to be embedded in the HPMCAS plate, as when the image was zoomed to the same resolution as Figures 3, the images looked similar to characteristic silica particles. Chlorine, used as a marker for glibenclamide, was observed within the silica particles on the HPMCAS plate, with no API observable outside of the platelets. These observations suggest that the formulation is a solid dispersion of API loaded silica in HPMCAS (Figure 3 bottom).
Figure 3. Top: SEM (left) of unloaded mesoporous silica (a), glibenclamide loaded silica (b), glibenclamide loaded silica + HPMCAS physical mixture (c) and HPMCAS incorporated during loading of glibenclamide onto silica (d).

Bottom: SEM EDX of HPMCAS incorporated during loading of glibenclamide onto silica. Carbon (a), silicon (b) and chlorine (c) atoms are highlighted. Chlorine is a marker for glibenclamide.

Based on this potential combination of silica loading and classical solid dispersion, it was important to assess what role the silica plays in the formulations. As can be seen in Figure 4, the particles produced in the solvent evaporation of glibenclamide and HPMCAS (control sample) are similar to the particles produced when the HPMCAS is incorporated during the drug loading step onto the silica. However, EDX
analysis shows a key difference between the formulations, in that the drug marker, chlorine, is no longer confined within the polymer (plates), but is freely distributed in the control sample.

**Figure 4.** SEM (left) and EDX (right) images of glibenclamide and HPMCAS prepared by solvent evaporation shows the same particle size and morphology as the co-incorporated samples. However, in this sample the drug (indicated by green) is no longer confined within the polymer plate and is freely distributed throughout the sample.

### 3.4 FaSSIF mini-dissolution

In FaSSIF mini-dissolution experiments, the concentration of the pure drug approached the thermodynamic solubility value of 8.1 µg/mL. *(Appendix 1) (Figure 5a).* From the glibenclamide loaded silica formulations, a significant improvement in dissolution was observed in the FaSSIF mini-dissolution experiments, reaching a 25-fold supersaturation *(Figure 5).* However, due to the metastable nature of the supersaturation, these extremely high concentrations were short-lived and the concentration reverted to the thermodynamic solubility within 60 minutes *(Figure 5).*
**Figure 5.** Mini-dissolution profiles of glibenclamide (●), and glibenclamide loaded silica (★) in FaSSIF, pH 6.5 at 37°C (n=2). Mean Glibenclamide thermodynamic solubility in FaSSIF is represented by the dashed horizontal line. In the insert on the right, the dissolution of crystalline glibenclamide has been magnified for better comparison.

Physically blending the glibenclamide loaded silica with HPMCAS prolonged the duration of supersaturation to at least 2 hours, although the degree of supersaturation was lower (about 3-fold) (**Figure 6**). Co-incorporating the HPMCAS with the glibenclamide onto the silica further improved the dissolution and precipitation performance, with higher supersaturation (about 6-fold) achieved over the time course of the experiment (**Figure 6**). Finally, the control sample, which used the same process as the co-incorporated in the absence of silica, showed almost no improvement in the FaSSIF mini-dissolution relative to the crystalline API. This result is in agreement with the partial crystallinity observed in the XRPD (**Figure 6**).

**Figure 6.** Mini-dissolution profiles of glibenclamide (●), glibenclamide loaded silica + HPMCAS blend (★) and glibenclamide and HPMCAS Co-incorporated silica (▲) and control: glibenclamide/HPMCAS prepared by solvent evaporation (□) in FaSSIF, pH 6.5 at 37°C (n=2).

Post-dissolution residues were collected for each of the samples and analyzed by XRPD. Crystalline glibenclamide precipitated in all samples except the co-incorporated formulation, in which the solid residue at the end of the experiment was amorphous (**Appendix 2**).

3.5 Transfer model experiments
During transfer model dissolution experiments with pure glibenclamide, no concentrations were detected in the SGF portion of the assay (Figure 7). This is in line with the thermodynamic solubility results, which indicated that the solubility of glibenclamide was under the limit of detection of the UPLC method (Appendix 1). After transfer into the FaSSIF portion of the experiment, the concentration profile closely overlapped with the mini-dissolution profile, suggesting that the dissolution of crystalline glibenclamide was largely unaffected by pre-wetting in SGF (Figure 7).

Comparison of results from transfer model and mini-dissolution experiments of glibenclamide loaded silica in the absence of any precipitation inhibitors suggests that single-medium dissolution may lead to different expectations of formulation performance. In the transfer model experiments with glibenclamide loaded silica (Figure 7); the performance of the loaded silica formulation was even poorer than the unmodified, crystalline parent.

Figure 7. Biorelevant transfer dissolution of glibenclamide loaded silica (●), crystalline glibenclamide (▲), glibenclamide Loaded Silica + HPMCAS Blend (♦) and glibenclamide and HPMCAS co-incorporated silica (▲) (n=2). Transfer from SGF to FaSSIF occurred at 30 minutes. N.B. no API was detectable during the SGF dissolution for glibenclamide loaded silica and crystalline glibenclamide. FaSSIF thermodynamic solubility is shown by the dotted line.

Crystallinity was observed in the post-SGF dissolution residues for glibenclamide loaded silica (Appendix 3). This suggests that, although no release was detectable in SGF, the drug did indeed release but then rapidly precipitated to the crystalline form.
Combination of glibenclamide loaded silica with HPMCAS significantly improved the transfer dissolution performance, with the formulation generating supersaturation in the intestinal phase of the assay (Figure 7). It was also possible to detect glibenclamide in the SGF portion of the assay, suggesting that supersaturation occurred in this medium. Similarly to the glibenclamide loaded silica, crystallinity was observed in the post-SGF residue for the sample containing a physical mixture of HPMCAS with the drug loaded silica (Appendix 3). This finding was in agreement with the XRPD patterns obtained post-FaSSIF mini-dissolution (Appendix 2).

The transfer dissolution of the sample where HPMCAS was incorporated during the drug loading step is shown in Figure 7. Unlike the sample where HPMCAS was added post-loading, no release of glibenclamide was observed in the SGF of the portion of the assay. This is likely explained by the immobilization of the drug loaded silica onto the HPMCAS platelets, which do not disintegrate in the gastric environment. As observed in the mini-dissolution experiments, (i) the supersaturation of glibenclamide during the FaSSIF portion of the experiment was significantly greater and more sustained from the co-incorporated formulation compared to the blend. In this case (unlike the pure drug), similar concentrations were achieved in the mini-dissolution and transfer model experiments.

Visually, the transfer dissolution of the sample in which HPMCAS was incorporated during the drug loading step was also quite different from the glibenclamide loaded silica and physical mixture of glibenclamide loaded silica with HPMCAS samples. For glibenclamide loaded silica with and without post-loading addition of HPMCAS, the powder was immediately dispersed in the dissolution vessel, creating a suspension. Conversely, no such dispersion was observed within the sample in which HPMCAS was incorporated in the drug loading step and the dispersion remained clear (Figure 8).

\[ \text{Figure 8. Images of glibenclamide loaded silica (a) and glibenclamide loaded silica + HPMCAS (b) dispersed in SGF; and glibenclamide and HPMCAS co-incorporated silica dispersed in SGF (c) and FaSSIF (d)}\]

Unlike the other silica formulations, the post-SGF dissolution residue for the co-incorporated formulation remained amorphous (Appendix 3).
The control sample was not investigated during the transfer dissolution as it was fully crystalline and behaved identically to pure crystalline glibenclamide during dissolution in FaSSIF. Furthermore, given that the thermodynamic solubility of crystalline glibenclamide is < LOD it was not anticipated that any useful observations could be made from the control sample during the SGF portion of the transfer dissolution.

3.2.6. Post-dissolution SEM

To examine the physical behavior of the formulation with HPMCAS incorporated during the drug loading step, the post-SGF and post-FaSSIF residues were characterized with SEM. Post-SGF dissolution, the large platelets (Figure 3) were unchanged (Figure 9). Increasing the magnification, one can still observe the loaded silica particles immobilized within the polymer platelets. Conversely, in post-FaSSIF dissolution, the only observable particles are of silica, suggesting the polymer platelets had dissolved, allowing the drug to be released from the silica (Figure 9).

![SEM Images](image)

**Figure 9.** SEM images of glibenclamide and HPMCAS co-incorporated silica after dissolution in SGF (top) and FaSSIF (bottom)

3.2.7 Solid-state NMR spectroscopy

SS-NMR spectroscopy was carried out on all samples (Figure 10). The full spectra are provided in Appendix 4. The $^{13}$C peaks for the API were identical in all samples except the co-incorporated formulation. In the co-incorporated formulation, a low field shift of 0.2 – 0.3 ppm for all API peaks was observed. For example, the characteristic API peak at 53 ppm was observable in all samples except the co-incorporated formulation, in which the peak shifted to 53.5 ppm. This is indicative of an interaction taking place between...
the drug and the polymer in the solid-state, which can take place once the drug is immobilized in the silica and subsequently in the HPMCS plate. By contrast, no peak shift was observed in the control sample, GB/HPMCAS, which was prepared by rotary evaporation. The results suggest that solid-state drug-polymer interactions and hence dissolution performance can be altered by changing the method used to manufacture the formulation.

Figure 10. A section of the $^{13}$C NMR spectra for all samples showing characteristic peaks for API $^{13}$C atoms at 43.5, 53 and 58 ppm. Analysis was carried out on multiple batches (n=2) of co-incorporated formulation and in all cases a 0.2 - 0.3 ppm peak-shift was observed for the co-incorporated formulation versus all other samples, with the co-incorporated formulation showing API $^{13}$C peaks at 44, 53.5 and 58.5 ppm. Given that the spectra were unchanged for different batches and repeats, only one dataset is show. Full spectra are available in Appendix 4.

4. Discussion

Mesoporous silica is an emerging oral delivery technique to formulate low soluble drugs. Upon impregnation of the silica with a concentrated API solution, drug can be molecularly adsorbed onto the surface of the silica. Due to the size of the pores, which have an approximate mean diameter of 4 nm, the molecularly adsorbed API is locally and sterically confined, preventing recrystallization (Ditzinger, Price,
More understanding is required to fully resolve the relative importance of the various considerations in the design and development of mesoporous silica formulations. Particularly critical is incorporation of precipitation inhibitors in the final formulation, since without such additives, the supersaturated state of the API is barely stabilized.

To date there has been no systematic study of how best to incorporate precipitation inhibitors in mesoporous silica formulations. Current practice for preparation on a small scale involves combining PIs in a physical mixture with the API loaded silica, either by mortar and pestle or overhead stirring. Due to the lack of a standard protocol, there is uncertainty about the reliability of this approach and how well the PI is blended with the loaded silica. In addition to the practical limitations of incorporating the PI post-loading, it represents a further step in product manufacture. By contrast, incorporation of the PI during the loading step removes these limitations while maintaining improvement in dissolution of the API. Laine and co-workers demonstrated that incorporation of HPMCAS during loading of celecoxib onto mesoporous silica substantially improved both the in vitro and in vivo performance of this poorly soluble API (Laine, 2016). In the current study, we have not only demonstrated a marked improvement in dissolution of the BCS II compound, glibenclamide, by the co-incorporation approach, but have additionally proposed a mechanistic hypothesis of how this enhanced performance is achieved.

**Understanding the effect of adsorption onto mesoporous silica on release in a transfer experiment**

In the current study, a successful conversion of glibenclamide to the amorphous form after loading onto mesoporous silica was confirmed with XRPD. This conversion led to 25-fold supersaturation during FaSSIF mini-dissolution (*Figure 2b* and *Figure 5*). Given the instability of the supersaturated state, the system rapidly precipitated and returned to its thermodynamic solubility, in line with previous studies with mesoporous silica (McCarthy, 2016; Laine, 2016; Price, 2019). Although precipitation was observed in the single-medium FaSSIF dissolution test, the full effect of precipitation on the overall performance was only realized by considering transfer dissolution data. In these experiments, no dissolution of crystalline glibenclamide (i.e. pure API) was observed in SGF, because its thermodynamic solubility is below the limit of detection at this pH. By contrast, in the transfer dissolution of the supersaturating silica formulation, API was detected in the SGF phase, suggesting that supersaturation occurred (*Figure 7*). This supersaturation of API in the SGF portion of the assay allowed precipitation to commence, along with the generation of seed crystals. This resulted in significantly poorer dissolution performance of the API-silica formulation in the FaSSIF portion of the experiment, relative to the single-medium approach (*Figure 7*). Therefore, one should consider the effect of transfer from the stomach to the intestine when assessing
the dissolution performance of supersaturating formulations, especially mesoporous silica-based formulations.

Application of HPMCAS as a precipitation inhibitor: blending vs. co-incorporation

For the current study, HPMCAS was selected as model precipitation inhibitor. HPMCAS is a well-established PI and has a track record in the literature of successfully sustaining supersaturated solutions for a range of APIS (Warren, 2010; Price, 2018; Laine, 2016; Udea, 2015).

From a practical perspective, the co-incorporation of precipitation inhibitor in the same formulation step is appealing, however, one potential concern for the co-incorporation approach is the accessibility of the pores for the API so that adsorption and nanoconfinement can still occur (Laine, 2016). Encouragingly, co-incorporating HPMCAS with glibenclamide onto mesoporous silica successfully converted the solid-state form of the API from the crystalline to the amorphous phase. This is in line with previous experience with celecoxib (Laine, 2016). Previous literature, which describes the incorporation of a polymer into the loading process as a “co-load” might infer the adsorption of the polymer inside the porous network. However, the molecular weight of the HPMCAS polymer used is approximately 18,000 Da. This is 36-times larger than the API, glibenclamide, which has a molecular weight of 484 Da. Given the very small size of the pore, 6 nm in diameter, it is highly unlikely that the polymer is actually co-loaded inside the pore.

Further, the particles in samples where HMPCAS has been incorporated into the formulation appear to be larger and different in shape than API-loaded silica samples without HMPCAS (Figure 3) data confirmed that these plate-like particles were composed of carbon and, therefore, it was concluded that the plate-like particles were comprised of HPMCAS.

The next important consideration, on the location of the API within the formulation, was addressed with EDX spectroscopy. EDX is a useful tool to envisage the distribution of a drug within a formulation. In the samples where HMPCAS was incorporated during the drug loading step, it was observed that drug was adsorbed onto the mesoporous silica particles and partly within the HPMCAS plate. Crucially, there was no API observed outside of these newly present HPMCAS plates. Therefore, it was concluded that co-incorporating the PI resulted in a solid dispersion of glibenclamide as the loaded silica. This appears to be the first example of such a solid dispersion in the literature. Given the novelty of this system, further work should be carried out to investigate the solid-state stability of the amorphous API in the system, which is an essential consideration for amorphous formulations (Ditzinger, 2018). Specifically, future work is
planned to assess the amorphous stability of the API in the formulation, in line with the ICH Q1 conditions for accelerated stability.

Neither the formulation in which HPMCAS was incorporated during the loading step nor the sample where it was added post-loading was able to capture the extremely high 25-fold supersaturation generated by simply loading the drug onto the silica. However, it has often been observed that the efficiency of precipitation inhibition is not able to capture the full supersaturation potential generated by the enabling formulation alone (Price, 2018; Price 2019). In spite of this, it was observed that when HMPCAS was incorporated during rather than after the drug loading step, the dissolution profile was much higher. Addition of HPMCAS post-loading improved the performance of glibenclamide loaded silica during both single-medium and transfer dissolution experiments, but there was some evidence of re-crystallization, suggesting that in a simple physical mixture HPMCAS is not able to completely inhibit precipitation. Indeed, incorporating the HPMCAS during the drug loading step demonstrated a 3-fold enhancement in dissolution performance compared to the simple physical mixture (Figure 6 and Figure 7). Such an improved precipitation inhibition effect could be related to the formation of drug polymer interactions already in the solid-state, which appears to be crucial for maximum precipitation inhibition (Price, 2018). This was supported by solid-state NMR data, in which a peak-shift was observed for co-incorporated formulations but not for other samples (Figure 10). Although the peak-shift was small (0.2 – 0.3 ppm), it was consistently observed for different batches. Alternative methods for obtaining information about drug-polymer interaction, for example 2D NOESY NMR, were unsuccessful because sufficiently concentrated solutions of drug-polymer could not be achieved. Another potential mechanism for enhanced precipitation inhibition in the formulation in which HPMCAS was incorporated during the drug loading step is the generation of an increased viscosity in the microenvironment surrounding the dissolving plates in FaSSIF. Such an increased viscosity would decrease the diffusion time out of the formulation and allow drug and polymer to remain in close proximity, both of which have been shown to be crucial factors in nucleation time in the presence of precipitation inhibitors (Price, 2018; Warren, 2010). However, further work would be required to fully confirm this hypothesis.

During the transfer experiment, it was observed that the HPMCAS plates do not disperse in SGF (Figure 8). This is a significant benefit, given that the HPMCAS plates did not break down, the API-loaded silica remained immobilized and API could not be released from the silica. Therefore, the formation of seed crystals in SGF was prevented. Ultimately, this has a significant effect on the dissolution performance and provides an additional mechanism by which formulations with HPMCAS incorporated during the drug
loading step can improve dissolution performance. In addition, it is interesting to observe that a change in manufacturing process - without a change in the qualitative and quantitative composition of the formulation - can introduce new properties to the product. By incorporating the HPMCAS during the loading step rather than post-loading, premature release of the drug from the formulation was circumvented without the need to add extra excipients, coating processes or special capsules, which are typically otherwise required (Qiu and Lee, 2017). This property should be especially advantageous in the delivery of poorly soluble basic compounds, whose premature release and supersaturation in the stomach (due to ionization in acidic conditions) with subsequent precipitation in the intestine could be avoided. Although Van Speybroeck and colleagues described an improved oral absorption of itraconazole loaded silica in rats, they found that silica formulations with post-loading incorporation of HPMCAS were unable to prevent the release of API in the stomach and therefore absorption was reduced (Van Speybroeck, 2010). The potential for incorporation of HPMCAS during the drug loading step on the dissolution performance of poorly soluble weak base drugs should be further explored.

Co-incorporated formulations: just a solid dispersion?

Given the improvement of the formulation performance when HPMCAS was incorporated during the loading step rather than post-loading, it was important to rule out that a simple solid dispersion was formed directly, and that the silica in the formulation plays an important role in the dissolution enhancement. EDX indicates that the drug is localized in the silica particles and on the HPMCAS plate when the polymer is incorporated during the loading step (Figure 3, bottom panel), but is distributed freely throughout the entire sample when no silica is present (Figure 5). The results suggest that drug is confined within the mesoporous silica particles, which are in turn were immobilized in the polymer platelets when HPMCAS is incorporated in the drug loading step. Without the nanoconfinement effects of the silica (Ditzinger, 2018), the drug can re-crystallize, as observed in the XRPD (Figure 7). Ultimately, this resulted in the control sample showing no improvement in FaSSIF dissolution versus crystalline API (Figure 6). Furthermore, if a portion of the sample was able to remain amorphous in the polymer platelets, the absence of drug-polymer interaction (as shown in the solid-state NMR spectra) would reduce the precipitation inhibition effect of the polymer (Figure 10).

5. Conclusions

A novel co-incorporated formulation of glibenclamide and the precipitation inhibitor, HPMCAS, onto mesoporous silica is described. By co-incorporating the precipitation inhibitor, the formulation significantly outperformed the commonly applied simple physical blend, regarding improved
supersaturation and dissolution in both single-medium FaSSIF and transfer dissolution assays. Furthermore, the co-incorporation approach allows the removal of a time-consuming and inefficient blending step. To provide a physical mechanistic basis is for the improved performance the co-incorporated formulation, a range of spectroscopic tools were utilized. It was concluded that the improved dissolution performance is a synergistic effect related to two key factors: formation of drug-polymer interactions in the solid state, and lack of release and premature precipitation under gastric conditions due to the immobilization of API-loaded silica particles within the enteric HPMCAS plates. Crucially, both of these properties are absent in a simple HPMCAS blend. Ultimately, the co-incorporation of precipitation inhibitors with the API on mesoporous silica formulations has the potential to improve both the process and formulation efficiency in the development of poorly soluble drugs.

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Appendix 1: thermodynamic solubility values for glibenclamide

**Appendix 1, Table 1. glibenclamide thermodynamic solubility values**

<table>
<thead>
<tr>
<th>Medium</th>
<th>Solubility (µg/mL)</th>
</tr>
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<tbody>
<tr>
<td>FaSSIF</td>
<td>8.1 ± 0.1</td>
</tr>
<tr>
<td>SGF</td>
<td>&lt;LOD*</td>
</tr>
</tbody>
</table>
Appendix 2: Post-FaSSIF dissolution XRPD

Appendix 2. XRPD patterns for post-FaSSIF dissolution residues for (a) glibenclamide loaded silica, (b) glibenclamide loaded silica + HPMCAS blend, (c) glibenclamide and HPMCAS co-incorporated silica and (d) glibenclamide and HPMCAS prepared by rotary evaporation (control)
Appendix 3: Post-SGF transfer dissolution XRPD patterns

Appendix 3. XRPD pattern for Glibenclamide loaded silica (a), glibenclamide loaded silica + HPMCAS blend (b) and GB/HPMCAS co-incorporated silica (c) residues post-SGF transfer dissolution
Appendix 4. SS-NMR spectra

Appendix 4. Full solid-state NMR spectra for all samples showing peak shift in co-incorporated samples. The section highlighted corresponds to the section included in the main body of text.
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