Approaches to Increase Mechanistic Understanding and Aid in the Selection of Precipitation Inhibitors for Supersaturating Formulations- A PEARRL Review

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Abstract

Objectives Supersaturating formulations hold great promise for delivery of poorly soluble active pharmaceutical ingredients (APIs). To profit from supersaturating formulations, precipitation is hindered with precipitation inhibitors (PIs), maintaining drug concentrations for as long as possible. This review provides a brief overview of supersaturation and precipitation, focusing on precipitation inhibition. Trial-and-error PI selection will be examined alongside established PI screening techniques. Primarily, however, this review will focus on recent advances that utilise advanced analytical techniques to increase mechanistic understanding of PI action and systematic PI selection.

 Key Findings. Advances in mechanistic understanding have been made possible by the use of analytical tools such as spectroscopy, microscopy and mathematical and molecular modelling, which have been reviewed herein. Using these techniques, PI selection can instead be guided by molecular rationale. However, more work is required to see wide-spread application of such an approach for PI selection.

Conclusions PIs are becoming increasingly important in enabling formulations. Trial-and-error approaches have seen success thus far. However, it is essential to learn more about the mode of action of PIs if the most optimal formulations are to be realised. Robust analytical tools, and the knowledge of where and how they can be applied, will be essential in this endeavour.

1. Introduction

Among the various routes of administration 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. [1] Various dosage forms are available for oral delivery including solid formulations such as capsules and tablets, as well as liquid formulations such as solutions, suspensions and syrups. For the active pharmaceutical ingredient (API) to exert its pharmacological effect, it must be released from the dosage form and absorbed from the gastrointestinal (GI) tract into the systemic circulation, where it can be transported to its physiological target. Thus, the bioavailability of a drug relies, among other parameters, on its capability to dissolve in the GI milieu and pass through the intestinal membrane. [2] It is from these two parameters (solubility and permeability) that the Biopharmaceutics Classification System arose, a system which groups drugs into four classes, based on solubility and permeability. [3]

Due to recent scientific advances such as high throughput screening in combination with combinatorial chemistry; X-ray diffraction of target proteins and computational chemistry, we now have an increased understanding of how small molecules bind to targets. Therefore, it has become 'easier' to identify 'hits' that have therapeutic potential. In parallel, there has been a large increase in the number of "drugable" targets which have been discovered and validated using a broad spectrum of novel methods, such as proteomics, genomics and even gene editing. [2, 4-7] On the other hand, it is recognized that use of such discovery tools often results in the identification of a higher proportion of lipophilic, high molecular weight and poorly soluble molecules, which do not adhere to Lipinski's rules of 5. [5] Albeit positive for the industry, these shifts have also increased the number of drug candidates with poor physicochemical profiles (low solubility, high log P, high molecular weight, poor solubility) appearing in research and development pipelines. As a result, there is a higher risk of attrition during pharmaceutical research and development due to insufficient oral bioavailability, which represents a loss in therapeutic and economic potential. It has been reported that approximately 40% of all commercial drugs are classified as poorly soluble. [8] Extending this trend to those compounds still in the development pipeline, it has been reported that anywhere between 80-

70 90% are 'not highly soluble'. [8] Therefore, the need for effective formulation regimes for these compounds,

71 to avoid low bioavailability due to poor aqueous solubility, has never been greater.

72 Faced with these challenges, pharmaceutical scientists have developed a toolkit of strategies, which use

73 physicochemical knowledge of solvation and dissolution to enhance solubilization and to overcome poor

74 oral bioavailability. [7] These approaches can involve modifying the chemical form of an API for example

75 with (i) salt formation, [9] (ii) co-crystals, [10] or (iii) prodrugs. [11] Or, alternatively, formulation approaches

76 such as: (iv) solvents, co-solvents and lipids; [12-15[(v) micelle systems; [16-18] (vi) particle size reduction; [19]

77 (vii) complexation; [20] and, (viii) solid amorphous dispersions, produced by techniques as hot melt

78 extrusions (HMEs), spray-dried dispersions (SDDs), co-precipitates or mesoporous silica can be used. [21-26]

79 In recent years, formulations that generate and stabilize a supersaturated state *in vivo* have come to the

80 forefront when considering the delivery of poorly soluble drugs. [8, 27-28] Supersaturation is a state in which

81 the concentration of a solute exceeds the thermodynamic (equilibrium) solubility of the molecule. Such a

82 state is highly attractive for compounds with low aqueous solubility as artificially high API concentrations

83 can be generated in the GI tract. This increases the absorptive flux, which can increase subsequent

84 absorption and bioavailability. [27,29] Such an approach must, however, be considered from an energetic

85 perspective as well. [30] The free energy of the supersaturated state is significantly higher than that of the

86 saturated solution, and there is a strong driving force for the system to return to its thermodynamically stable

87 state *via* crystallisation and precipitation processes. [8] Therefore, successful supersaturating formulations

88 should not only generate increased API concentrations in solution, but should also be able to stabilise the

89 supersaturated state. Often, this stabilising factor takes the form of a precipitation inhibitor, which prevents

90 the recrystallisation and precipitation process. Therefore, PIs are an integral part of supersaturating

91 formulations, and a robust understanding of the mechanisms behind precipitation inhibition is essential for

92 effective formulation design. [8] This review offers a brief overview of the physical chemistry underpinning

93 supersaturation and precipitation before examining recent work utilising cutting edge analytical techniques

94 and methods that have led to an increased understanding of precipitation inhibition mechanisms, and how

95   such understanding can be used in the selection of optimal PI systems for supersaturating formulations.

96   **Finally, to the best of our knowledge, this is the first review that uses such an approach to address**

97   **precipitation inhibition and precipitation inhibitor selection.**

2. Supersaturating Formulations

99   In order to understand the process of precipitation inhibition, it is important to have an overview of the

100   physical chemical underpinnings of drug supersaturation. Since a complete treatment of supersaturated

101   solutions is beyond the scope of the present review, the interested reader is referred to an excellent review

102   recently published by Taylor and Zhang. [8]

103

104   Supersaturation, from a physicochemical perspective, is generally defined as a system in which the free

105   energy of the solute in solution is higher than that of the crystal form or amorphous solid phase of the drug

106   at equilibrium (*figure 1*). [8, 31-32] Practically speaking, this is any system in which the concentration of drug

107   in solution exceeds the thermodynamic solubility of the pure API. Increasing the concentration in the GI

108   tract *via* supersaturation can increase the overall absorption of the drug. As a result, supersaturating

109   formulations are highly appealing for drugs with low thermodynamic solubility, which often exhibit poor

110   bioavailability. [3]

111

112   The gap between the free energy of the supersaturated state and the equilibrium state can lead to stability

113   issues. [8, 30] Therefore, the absorption advantages previously mentioned are not always realised, as this

114   instability can lead to precipitation of amorphous or crystalline material from the supersaturated solution.

115   [27, 33] The precipitated drug would have to re-dissolve in the GI tract in order become available for absorption

116   and, therefore, the potential absorption advantage is typically diminished. [34] Consequently, an effective

117   supersaturating formulation needs not only to generate high supersaturation, but also to maintain this for a

118   physiologically relevant time. With typical upper GI transit time, this would be 2-4 hours, the time during

119   which most drug absorption takes place after gastric emptying when the drug is taken in the fasted state, [35]

120 In order to successfully develop such a formulation, it is important to have a good understanding of the

121 physical basis of supersaturation, and how this knowledge can be exploited to maximal effect.

122

123 During the dissolution of supersaturating formulations, drug concentration increases until the crystalline

124 saturation solubility is exceeded. After this point, supersaturation is established, which exists in a metastable

125 state. [36] This metastability is the driving force of nucleation and thus precipitation of either amorphous or

126 crystalline solids. [37-38] The rates of nucleation and precipitation, in relation to the dissolution rate of the

127 formulation, are the key parameters when considering whether the supersaturated state will be maintained.

128

129 Successful supersaturating formulations may also exceed the so-called "amorphous solubility", a metastable

130 state in which amorphous drug exists in a pseudo-equilibrium with the dissolution medium, eventually

131 returning to the crystalline form. [39-31] "Amorphous solubility" has been calculated theoretically based on

132 the crystalline saturation solubility and by considering the amorphous material as a supercooled liquid. [43-

133 44] High drug concentrations of slowly crystallizing drugs are typically limited by a kinetically favoured

134 liquid-liquid phase separation (LLPS), *(Figure 2 and 3)* in which separation of drug molecules into drug-

135 rich "droplets" (100-500 nm) from the aqueous phase occurs *via* spinodal decomposition. [8, 45-49]

136

137 At, or approaching, the theoretical amorphous solubility, the LLPS state is at a lower energy than the

138 supersaturated solution, and so is thermodynamically favored. LLPS proceeds *via* quick spinodal

139 decomposition and so can only occur when fast crystallization does not occur. Drug molecules in the drug-

140 rich regions exist in the amorphous state, and usually tend to fall back to the more stable crystalline state

141 over time. [50-51] Thus, the process of LLPS extends the lifetime of the molecule in solution, such that high

142 concentrations can be achieved over longer periods of time, compared to the unstable supersaturated state.

143 **Typically, precipitation inhibition would aim to avoid any precipitation from the supersaturated**

144 **state. However, given that precipitation inhibition is concerned with the biopharmaceutical**

145 **performance of a drug, the effect of a PI on sustaining LLPS is relevant to the current body of work.**

146 **In this instance,** LLPS can be considered a "reservoir" of supersaturation such that the initially unstable

147 supersaturation results in a plateau of supersaturation at the LLPS due to the separation of API in droplets.

148 [8] This is a direct analogy to the "Spring and Parachute" model proposed by Guzman [52] *(Figure 4)* and also

149 termed the "Spring and Plateu" approach. [8] Generally, this spring is generated either by delivering the drug

150 in a pre-solubilized form (e.g. SEDDS or lipid-based formulations) or in a rapidly dissolving form (e.g.

151 amorphous, less stable polymorphs, particle size engineering, amorphous dispersion, solid solutions and

152 prodrugs). [27] In order to complete the model, PIs act as 'parachutes' by hindering nucleation, thus arresting

153 precipitation [34, 53] or, maintaining the lifetime of the droplet state after LLPS, both of which increase the

154 concentration and lifetime of the supersaturated drug in solution.

155

156 The success of supersaturating formulations is dependent on appropriate selection of "spring-parachute"

157 combinations. Consequently, mechanistic understanding of the role of the PI in supersaturating

158 formulations is imperative for the educated choice of successful PI-drug combinations.

159

160 ## 3.1. Precipitation Inhibition: Theory and Practice

161 Precipitation is a process whereby a solid phase separates from a liquid phase. This can yield either

162 amorphous or crystalline materials, or mixtures of these. Although precipitation in the amorphous form is

163 possible from supersaturated solutions, the vast majority of work has focussed on crystallisation theory.

164 Fortunately, classical crystallisation theory can be applied to the precipitation of both crystalline and

165 amorphous solids. [27, 28, 55] In the following section, a short overview of the current theories about

166 crystallisation is presented. For more details, the reader is referred to the book by Mullin on "Crystallisation"

167 [30] and to an excellent review by Warren. [55]

168

169 ### *3.1.2. Crystallisation and Precipitation*

170 Crystallisation is an energy-driven process whereby a molecule in solution at supersaturated concentrations

171 precipitates to a solid, crystalline material. [56] As supersaturation concentrations approach a critical level,

172 the system becomes labile and "precipitation is self-influenced instantaneously without any external

173 influence. [30] Before this critical point, the supersaturation is said to be metastable and precipitation may

174 not occur instantaneously and/or spontaneously, but can be easily induced *via* mechanical activation or the

175 addition of seed crystals. [30, 57] Crystallisation from a supersaturated solution is a function of the

176 concentration, temperature and pressure of the solution. Practically speaking, however, it is generally only

177 temperature and concentration that are considered (***Figure 5***). A higher concentration of solute increases

178 the lability of the system; whereas an increased temperature decreases lability. For concentration, the higher

179 the concentration of a solute in solution, the higher the degree of supersaturation, which increases excess

180 energy of the system. Conversely, as temperature increases, the saturation concentration usually also

181 increases, and the degree of supersaturation is decreased *(**Figure 5**)*.

182

183 Crystallisation occurs in two key stages: nucleation and crystal growth. Simply put, solute molecules must

184 come together (nucleate) until a critical size of a nucleus is reached, after which crystal growth can occur.

185 During crystallisation, both nucleation and crystal growth are occurring simultaneously at different rates,

186 depending on the stage of the crystallisation process and the conditions of the system (*vis á vis* solute

187 concentration and solvent temperature). [55} Nucleation can occur spontaneously or artificially, [30] for

188 example *via* agitation, mechanical shock, pH change or dilution. The exact process of nucleation is still

189 unclear, although several mechanisms have been proposed in the literature, none have been fully validated.

190 [58] Nevertheless, it seems that nucleation can occur in a number of ways, either with or without seed crystals

191 (**secondary and primary nucleation**, respectively) and in the presence or absence of a cluster surface, e.g.

192 foreign particles or container defects (**heterogeneous/2D and homogenous/3D crystallisation,**

193 respectively). [30] The nucleation rate (*J*) can be described by the classical nucleation theory (CNT)

194 (***Equation 1***). [59]

195

196 $$J = A \exp\left(-\frac{B}{\ln^2 S}\right)$$ ***(Equation 1)***

197   S is the supersaturation of the system. The pre-exponential factor A is a kinetic measure that described how

198   the molecules come together to form nuclei. Given the random nature of this process, with a wide-range of

199   accessible clusters, which can grow or decay in multiple direction, the pre-exponential factor is often

200   calculated using Monte Carlo simulations. The thermodynamic factor, B, describes the free energy barrier

201   for the formation of a nucleus, which is also referred to as "nucleation work". For practical purposes, A and

202   B are considered to be constant in systems with fixed temperatures. This equation underscored the

203   importance and significance that supersaturation, S, plays in the nucleation process. [59-60]

204

205   After stable nuclei have been formed, they increase in size and form visible crystals *via* crystal growth. [30]

206   The process of crystal growth has been extensively described in the literature. [30, 38, 61-62] Various models

207   have been put forward to provide a theoretical mechanism for the process of crystal growth including: i)

208   Surface Energy Theory, ii) Diffusion Theory and, iii) Adsorption Layer Theory. [30] All three models have

209   significant failings; however, the most widely accepted and quoted model is the Adsorption Layer Theory.

210   [30]

211   Adsorption Layer Theory is based on the thermodynamic assumption that there is a layer of adsorbed

212   molecules on the surface of a growing crystal, which is in an equilibrium state with the bulk solution

213   surrounding the layer. The crystal will grow if the adsorbed molecules on the surface find a position that is

214   thermodynamically favourable and exhibits high attraction forces between the adsorbed molecule and the

215   crystal, enabling extension of the lattice. [30] This occurs at imperfections and kinks on the surface of the

216   growing crystal face. [30, 55] Despite this process, the resultant crystal may not be the thermodynamically

217   most favourable. It is common for metastable polymorphs to form, [63] based on the quick access of surface

218   adsorbed solutes to energetically stable, but not *the most* energetically stable sites. This phenomenon,

219   whereby the first crystal formed is simply kinetically favoured and leads via different stages to a more stable

220   solid, was first suggested by Wilhelm Ostwald, and is now referred to as "Ostwald's Rule". [64] That being

221   said, if left for a sufficient time period, the thermodynamically most stable polymorph should form in most

222   cases. [62]

223   For supersaturating formulations, nucleation of the generated supersaturated solution would be highly

224   probable if the formulation is not stabilised by addition of PIs. [34] If the process of precipitation is arrested

225   for physiologically relevant time-scales, the supersaturated state can be maintained for long enough to allow

226   for increased absorption. [55] Polymers, surfactants, and cyclodextrins have been investigated as excipients

227   that can maintain the supersaturated state. [34] Another consideration, is the effect that endogenous molecules

228   (e.g. bile salts) in the body can have on precipitation inhibition. [66]

229

230   *3.1.3. Polymers as Precipitation Inhibitors*

231   The most common PIs employed in the pharmaceutical industry are polymers. Table 1 lists a large range of

232   polymers, including cellulose derivatives, polyvinylpyrrolidones and methacrylates, which have been

233   evaluated as precipitation inhibitors in oral drug products.

234

235   Polymers function by slowing down the process of nucleation and crystal growth through interaction with

236   the dissolved API molecules and interaction with and adsorption onto growing crystals. [34,55] Given the

237   kinetic nature of this effect, the thermodynamic equilibrium solubility is usually not affected by the polymer,

238   except in a number of limited cases, where polymers can have a co-solvent effect as well. [55, 67-69] Kinetic

239   inhibition of nucleation relies on molecular interactions between the polymer and the drug, i.e. hydrogen

240   bonds, polar, or dispersion forces. [27, 28, 55] **Each of these interactions may contribute to varying degrees,**

241   **especially when considering the impact of water. This is an area that has seen increasing interest in**

242   **the literature lately [66,69,70,71] but more work is required to resolve the exact contributions and nature**

243   **of the PI-API interaction**. More generally, such interactions can be influenced by temperature, molecular

244   weight, polarity and hydrogen bonding capabilities of both the drug and the polymer. One potential mode

245   of action is the adsorption of the polymer onto the growing crystal surface, blocking the access of the solute

246   to the surface. Furthermore, polymers can disrupt the growth rate on crystal surfaces by binding onto

247   imperfection sites, thus flattening the surface and removing the interaction point. This is highly dependent

248   on the balance of interactions between the solid and the polymer and the interaction between the liquid and

249   the polymer. [72] The adsorption of the polymer onto the surface of the particle also introduces steric

250   hindrance, which disrupts the diffusion of the molecules at the solid-liquid interface. [73-75] It has previously

251   been reported that the hydrophobicity of the polymer influences this balance. [72,76] Additionally, the pH of

252   the solvent affects ionisable polymers and ionisable APIs. [59,77] Another potential mode of action for

253   polymeric PIs is *via* alteration of the solid-liquid interface, which can cause a change in surface energy and

254   hinders the diffusion of new molecules to the crystal surface. Furthermore, the solubility and surface tension

255   in the bulk solution can undergo changes and therefore, may contribute to the precipitation inhibition. [73,78]

256   However, for this purpose, it is not currently understood to what extent the polymer needs to be in a colloidal

257   state versus solubilized as random coils. [55] Another important factor to consider is viscosity; as the viscosity

258   of the solution increases, the molecular mobility of the drug in solution decreases. This increases the energy

259   required for the diffusion of drug through the solution and can have a profound effect on both nucleation

260   and crystal growth, both of which depend on diffusion of drug to another solute molecule or to the growing

261   crystal, respectively.

262   For a given polymer, a high molecular weight is associated with an increase in viscosity as well as an

263   increase in the number of binding sites. For this reason, it is often unclear which factor is responsible for

264   any increased inhibition when considering a range of molecular weight polymers. For example, Chavan and

265   co-workers concluded that viscosity was an important polymer characteristic in the precipitation inhibition

266   of nifedipine from supersaturated solution by HPMC, with higher viscosity samples of HPMC delaying the

267   induction time crystallisation for the longest periods of time. [79] But viscosity is not the only determinant of

268   nucleation inhibition by polymers, indeed, some reports in the literature were unable to show a significant

269   impact of polymer viscosity. [70] On the other hand, one must bear in mind, that viscosity will also effect

270   permeation of the drug. In any case, it is likely that the mechanisms(s) of interaction and the biggest

271   contributor to the inhibitory effect will vary with the individual system.

272

273

274

3.1.3. Surfactants and Cyclodextrins as Precipitation Inhibitors

Similar to polymers, surfactants and cyclodextrins have also been reported to sustain drugs in solution kinetically through molecular interactions [80,81]. However, they also have the potential to inhibit precipitation by increasing the solubility of the API, thereby reducing supersaturation. For surfactants and cyclodextrins, this can occur *via* micellar solubilisation and complexation, respectively. [80-83] Thus, surfactants and cyclodextrins have often been called thermodynamic precipitation inhibitors. [20, 55, 80-89] This distinction relates to the mechanism of action of the inhibitory affect. For conventional polymer PIs, the drug is sustained in solution only temporarily, and precipitation will eventually occur. Therefore, polymers have a kinetic effect on drug precipitation. For surfactants and cyclodextrins, however, increasing the solubility (i.e. reduction of supersaturation) is a more sustained effect. The classification of cyclodextrins and surfactants as precipitation inhibitors is therefore somewhat tentative and the distinction of kinetic inhibition versus solubilization effects has typically not been addressed in the literature. Therefore, further mechanistic work surrounding the mode of action of surfactants and cyclodextrins on precipitation inhibition will be required to better understand the relevance of these compounds as PIs. For a list of all surfactants and cyclodextrins reported in the literature to act as PIs, please refer to *Table 1*.

3.1.5 Endogenous Precipitation Inhibitors

Endogeneous surfactants in the GI tract can also inhibit the precipitation of supersaturating formulations during dissolution *in vivo*. In theory, surfactants such as bile salts and lecithin have the potential to inhibit crystallisation *via* the mechanisms mentioned in the previous sections. For example, Chen and co-workers showed that sodium taurocholate was able to extend nucleation time significantly (up to 11-fold) for a group of 11 structurally diverse compounds. [90] This may partly explain why many *in vitro* dissolution tests overestimate the precipitation of supersaturated API. [91] Further work by Li and co-workers expanded the precipitation screen to 13 different bile salts. [66] It was observed that most of the 13 bile salts investigated inhibited precipitation of celecoxib, nevirapine and flibanserin, with varying degrees. Further, it was

301   concluded that van de Waal and hydrogen bond interactions between the inhibitor and the molecule in

302   solution were the key factors determining PI effects. [66] In this respect, there are clear similarities with

303   formulation-based PIs. Although more work is required, it is clear already that it is important to understand

304   and take into account the effect of endogenous molecules on supersaturating formulation, especially in the

305   design of *in vitro* dissolution tests.

306

3.1.6 Precipitation Inhibitor Screening Methods

308   Given the large number of PIs reported in the literature, *(Table 1)* various screening methods to select API-

309   PI combinations have been developed. Invariably, these screening methods involve the generation of

310   supersaturation in combination with a variety of analytical techniques that can determine the rate and extent

311   of precipitation of a drug over time in a large number of samples. A wide variety of methods to generate

312   supersaturation are reported in the literature, including use of amorphous solids, shifts in temperature or pH,

313   use of salts or solvent shifts. [55] Of these techniques, the most common is solvent shift, this involves

314   dissolving the API in high concentrations in a favourable solvent (e.g. DMSO), a small volume of which is

315   then added an aqueous phase to generate a supersaturated state. Analytical techniques such as UV

316   spectroscopy, HPLC or nephelometry can then be used to assess API concentration or concentration of

317   precipitate over time, which in turn gives information about the efficiency of the inhibitor being studied. [55]

318   For example, during a drug development regime, two Johnson and Johnson drugs, A and B, required

319   addition of PIs to a surfactant-based bioenabling formulation that generated supersaturation but did not itself

320   prevent precipitation. [128] In order to select an appropriate PI candidate, supersaturation was generated in

321   the presence of a range of potential PIs for both compounds, and then HPLC was employed to determine

322   residual drug concentration after 24 hours. This screening platform identified Pluronic F127 as the most

323   efficient PI. [94, 128] As a side-note, this type of experimental set-up is particularly attractive when pursuing

324   surfactant-based formulations, as the methodology can simultaneously screen surfactant systems as well as

325   PIs. In this respect, one can simultaneously assess the extent of the supersaturation generated by the

326   surfactant and how sustained the profile is in the presence of PIs. [94] The main drawback of the given

327   experimental design is that barely any information is obtained about the kinetics of drug precipitation in the

328   presence of polymer by using a single time point.

329   An alternative screening platform, which utilised off-line chromatography was reported by Petrusevska and

330   co-workers. [129] In this study, supersaturation was generated for fenofibrate and carbamazepine, in the

331   presence of PIs, using a solvent shift from DMSO into aqueous buffer. The plates were sealed and incubated,

332   with samples being taken at 30, 90, 180 and 360 minutes, filtered and analysed with UPLC for API content.

333   This method provides more information about which PI is the most efficient over physiologically relevant

334   time scales. In this study, it was found that surfactants such as Tween® and Cremophor® were most efficient

335   for fenofibrate, whereas for carbamazepine cellulose derivatives such as HPMC and HPMCAS were the

336   optimal systems. [129]

337   In spite of the limited time-resolution that such 'off-line' methods can provide, they can often be very

338   reliable when it comes to predicting performance of the final formulation. Yamashita and colleagues

339   performed a similar high-throughput screen for a range of surfactants, oils and polymers in combination

340   with Itraconazole. [130] The screen demonstrated that HPMCAS was the most efficient 'anti-precipitator'.

341   When itraconazole-HPMCAS spray-dried dispersions were manufactured and compared to the

342   commercially available Sopranox® HPMC-based dispersions, the HPMCAS-based formulations

343   significantly outperformed the commercial product in dissolution tests. [130]

344   Analytical techniques that offer *in situ* analysis are very appealing as they can provide a real-time picture

345   of supersaturation-precipitation behaviour. It was demonstrated by Warren and colleagues that utilising *in*

346   *situ* nephelometry, a technique that uses light-scattering to measure particle concentration, can provide an

347   indirect measure of API concentration in a high-throughput screen. [55] In this instance, the nephelometer

348   measures light scattering of the samples, which directly relates to the total concentration of particular matter

349   in suspension and API in solution. A large number of species were screened for precipitation inhibition

350 using a plate reader, after which the researchers were able to sort the PIs into three distinct groups based on

351 the nephelometry data (*figure 6*). [55]

352

353 Chauan and co-workers expanded upon this technique by utilising an *in situ* UV probe that provided time-

354 resolved information about the concentration of API in solution. [71] This method was used to assess the

355 interactions between indomethacin and a wide range of polymers. After a solvent-shift to generate

356 supersaturation, the turbidity and API concentration was measured using an *in situ* UV-probe. This time-

357 resolved data is highly useful and allows further calculation and processing. Chauan and colleagues

358 subsequently used this data to calculate precipitation induction time (time delay between supersaturation

359 and precipitation) as well as rate of precipitation, in which they showed that PVP, HPMC and Eudragit E100

360 increased induction time and decreased the rate. Subsequently, successful solid dispersions of indomethacin-

361 PVP, indomethacin-HPMC and indomethacin-Eudragit® E100 were developed. [71] A similar study, with

362 dipyridamole was also carried out. [92] The downside of using nephelometry as a screening tool is the

363 difficulty of screening any systems that are insoluble. For example, some supersaturating formulations, e.g.

364 mesoporous silica, contain insoluble excipients that would interfere with the light scattering and make

365 analysis difficult.

366 Recent advances in PI screening have seen the introduction of smaller-scale dissolution techniques, such as

367 the µDISS profiler™ apparatus, produced by Pion. The µDISS utilises *in situ* UV in combination with liquid

368 handling, and can be used to efficiently study supersaturation and precipitation in real-time. Palmelund and

369 co-workers were able to study six different poorly soluble drugs in combination with HPMC or PVP at

370 different degrees of supersaturation. [131] This method was successful in discriminating between innate

371 solubility enhancements of the polymers vs. precipitation inhibition. For the BCS IV drug, aprepitant, for

372 example, both polymers increased solubility by approximately 150%, with the solubility being the same in

373 both polymer systems. That being said, there were distinct differences in the curves observed in the real-

374 time data display, with HPMC showing a more pronounced effect on the dissolution profile than PVP.

375 Therefore, for this system, HPMC acted as a more effective PI than PVP. [131] The µDISS profiler™ is

376 particularly appealing as the experimental protocols can be easily standardised to reduced inter- and intra-

377 lab variability. [132] The µDISS profiler™ has also been applied to investigate the effect of prandial state and

378 PIs on the precipitation of supersaturated zafirlukast. [134] Further methods have also been employed in small

379 scale precipitation testing. [134]

4. Approaches for Precipitation Inhibitor Selection and Increased Mechanistic Understanding

382 Although screening, and especially small-scale screening, of PIs is attractive from a throughput perspective,

383 it adds little to the mechanistic understanding of precipitation inhibitor. Detailed mechanistic investigations

384 usually require a comparatively larger testing scale that employs advanced analytical methods. In recent

385 years, such studies have come to the forefront as the need for precipitation inhibitors has become greater.

386 This section offers an overview of these recent advances as well as a more general overview of the analytical

387 tools that are important, and will continue to be of importance, in the design and selection of PI systems for

388 supersaturating formulations. **Also important to the development of PI formulations is their *in vivo***

389 **performance. Although this has been covered extensively in the literature, it is beyond the scope of**

390 **this review. The interested reader is referred to a number of recent papers that study the effect of PI**

391 **selection on *in vivo* performance of supersaturating formulation.** [(24, 119, 136]

4.1. Experimental Approaches

393 **Recently, there have been a wide range of novel experimental approaches applied to precipitation**

394 **inhibitor selection. In addition, these approaches often offer a wealth of mechanistic detail. For a**

395 **summary of the experimental approaches described in this section, please see table 2.**

4.1.1 NMR Spectroscopy

397   Nuclear magnetic resonance (NMR) spectroscopy is a spectroscopic technique that exploits the

398   electromagnetic emission of a nuclei in a magnetic field to gain structural information about the sample. [137]

399   Ueda and colleagues utilised 1D NMR spectroscopy as a tool to assess the impact of HPMCAS substitution

400   patterns on the precipitation behaviour of carbamazepine. [138] In their study, it was observed that HPMCAS

401   successfully inhibited the precipitation of carbamazepine, depending on the ratio of succinyl and acetate

402   groups in the polymer. Specifically, it was observed that the highest degrees of carbamazepine

403   supersaturation were sustained in the presence of HPMCAS grades with low succinyl and high acetyl

404   substitutions. The increased acetyl substitution was concluded to be essential for precipitation inhibition, in

405   line with the idea that the more hydrophobic the polymer, the higher the affinity for the growing crystal

406   surface as discussed above. In order to expand upon this assumption, the group utilised ^{1}H NMR

407   spectroscopy to provide information about the molecular mobility of carbamazepine in solution, with a

408   range of HPMCAS variants. A good correlation was observed between precipitation inhibition and the

409   molecular mobility. A lower molecular mobility corresponded to a more successful precipitation inhibition,

410   due to increased interaction between the drug and the polymer. It was hypothesized that this interaction was

411   the insertion of HPMCAS into growing aggregates before nucleation, which prevents the formation of the

412   crystal lattice. [138]

413   In a recent study by Prasad and co-workers, 1D ^{1}H NMR spectroscopy was utilised to probe the interactions

414   behind the inhibitory effect of a range of polymers on indomethacin precipitation after the generation of

415   supersaturation. [93] It was hypothesized that interactions between the polymers and the carboxylic acid

416   functionality of indomethacin were essential for precipitation inhibition. Thus, the chemical shift of the

417   carboxylic acid functional group, at 3.70 ppm, was closely monitored for changes that could indicate that

418   the chemical environment surrounding the protons had been altered. Eudragit® E100 and PVP, when

419   combined with drug in solution, shifted the carboxylic acid peak to a lower value, due to shielding effects.

420   [93] The investigators utilised this shift to quantify the strength of drug-polymer interaction and subsequent

421   precipitation inhibition effect. Eudragit E100 resulted in a larger downward shift than PVP, and for both

422   polymers this shift was directly proportional to the concentration of the polymer. Additionally, it was

423   observed that when the formulation was changed from a binary system (drug/polymer), to a ternary system

424   (drug/polymer 1/polymer 2), this shift was even more pronounced. This provided evidence for a synergistic

425   contribution of both polymers to the precipitation inhibition. Dissolution performance of the drug in the

426   presence of polymers revealed a larger and more sustained supersaturation was generated with Eudragit

427   E100 than with PVP, while the ternary system gave the best results. [93] Therefore, the change in chemical

428   shift was shown to be a useful parameter when assessing the effect of polymers on indomethacin

429   precipitation.

430   Although use of 1D NMR spectroscopy to gain information about molecular mobility and chemical shift

431   variations can be a useful tool when assessing the impact of PIs, it is somewhat limited in its ability to detect

432   weaker intermolecular interactions, which are likely to play a highly important role in precipitation

433   inhibition. Typical 1D NMR spectroscopy gives information about atoms that are chemically bonded and

434   can only provide limited information about intermolecular or supramolecular effects. Whereas, there are a

435   range of 2D NMR spectroscopic techniques that can yield information about correlations of different atoms

436   through space such as Nuclear Overhauser effect spectroscopy (NOESY) and diffusion ordered

437   spectroscopy (DOSY). [139-140]

438   Prior to their work surrounding the importance of substituent ratios for HPMCAS precipitation inhibition,

439   which utilised 1D NMR spectroscopy for the calculation of molecular mobility, Ueda and colleagues first

440   established the mechanism of interaction between HPMCAS and carbamazepine in solution by utilising

441   NOESY. [150] During this experiment, it was observed that HPMCAS-HF (a particular grade of HPMCAS,

442   relating to the ratio of acetyl to succinyl substituents) had cross-peak interactions with the aromatic protons

443   and amide protons of carbamazepine, suggesting the possibility of both hydrogen bond interactions and

444   hydrophobic interactions. After further inspection of the intensities of the cross-peaks, it was concluded that

445   the more predominant effect was a hydrophobic interaction between the HPMCAS acetyl substituents and

446   the aromatic region of carbamazepine. [150] This interaction was concluded to be essential for successful

447   precipitation inhibition.

448   NOESY has also been used in combination with High Resolution Magic-Angle Spinning (HR-MAS) NMR

449   spectroscopy, to understand the interactions between the poorly soluble drug mefenamic acid with

450   Eudragit® EPO in supersaturated solutions. [141] HR-MAS NMR spectroscopy is an enhanced spectroscopic

451   technique that can offer improved resolution for the study of complex solutions and has been used in the

452   pharmaceutical industry to detect and quantify API in complex formulations such as gels and creams. [142]

453   Higashi and co-workers were able to significantly improve the NMR spectra for mefenamic acid-EPO

454   solution under MAS conditions. This allowed cross-peaks to be observed during the NOESY experiments.

455   These cross-peaks showed evidence of multiple points of interaction between the API and the polymer,

456   indicating two different interactions: first, a hydrophobic interaction between the aromatic part of the API

457   and the EPO backbone, and, second, a hydrophilic hydrogen-bond interaction between the aminoalkyl

458   groups of EPO and the carbonyl groups of mefenamic acid was described. Furthermore, it was observed

459   that the intensities of the two sets of cross-peaks were similar, leading the authors to conclude that both

460   interactions played an important role in precipitation inhibition. [141]

461   DOSY was an essential part of a study assessing the suitability of a novel spray dried dispersion matrix,

462   HPMCAS and dodecyl (C_{12}) poly(N-isopropylacrylamide) (PNIPAm), for the systems suitability to enhance

463   the delivery of a poorly soluble drug, phenytoin. [151] After dissolution of the solid dispersion, the C_{12}-

464   PNIPAm polymers formed micelles with the dodecyl groups, which successfully sustained the

465   supersaturated state of phenytoin generated by the spray-dried dispersion (SDD). Furthermore, it was

466   observed that the C_{12}-PNIPAm inhibited precipitation of the supersaturated phenytoin by inclusion of the

467   drug within the corona of the micelles, rather than the core. It was also concluded that the HPMCAS in the

468   formulation had little effect on sustaining the supersaturation compared to C_{12}-PNIPAm, which instead, was

469   responsible for the enhanced dissolution of the drug from the SDD. [151] These conclusions were reached

470   using both NOESY and DOSY data. The NOESY spectra of the novel formulation showed cross-peak

471 interactions between the phenyl groups of the phenytoin and the isopropyl functionality on the PNIPAm

472 polymer. Conversely, NOESY spectra revealed no cross-peaks for phenytoin combined with HPMCAS. On

473 application of DOSY as an orthogonal approach, no reduction in diffusion coefficient for phenytoin was

474 observed in HPMCAS or C_2-PNIPAm. Conversely, the diffusion coefficient decreased dramatically in the

475 presence of C_{12}-PNIPAm, which was concentration-dependent. This provided the researchers with strong

476 evidence that the C_{12}-PNIPAm was responsible for the remarkable sustained supersaturation that was

477 observed upon dissolution of this novel SDD, as well as the mechanism taking place within the corona of

478 the micelles, which was not present in the C_2 variant. [151]

Solid-State NMR Spectroscopy

480 Chauhan and colleagues utilised carbon cross-polarization magic angle spinning SS-NMR spectroscopy to

481 investigate the interactions between indomethacin and polymers in solid dispersions. [71] Three different

482 polymers: Eudragit® S100, PVP and HPMCAS were screened for indomethacin interactions using this

483 method. Chemical shift changes of the indomethacin signal were recorded in the presence of the polymers,

484 with a larger chemical shift change indicating a stronger interaction. For the aromatic region, a slight

485 chemical shift change was observed for all three polymers, with the biggest shift occurring in the PVP SDD,

486 this correlated well with the observed performance of the SDD during biorelevant dissolution, which

487 outperformed all other polymers due to enhanced precipitation inhibition. [71]

4.1.2 IR/ FTIR Spectroscopy

489 FTIR spectroscopy is highly attractive from a drug development setting as it can be employed to study

490 interactions between compounds, even in complex mixtures. [142] Nie and co-workers performed an

491 experiment to determine interactions between clofazimine and hypromellose phthalate (HPMCP), which

492 has been previously reported to have very high drug loading capacity in solid dispersions. [152-153] IR spectra

493 were analysed in order to identify changes in the vibrational modes of clofazimine and HPMCP in a solid

494 dispersion. [152] A new peak, at 3310 cm^{-1}, was observed in the IR spectrum of the solid dispersion, which

495   corresponded to the stretching mode of the ionised imine in the clofazimine. This was presented due to

496   protonation by the carboxylic acid groups in the phthalate substituent of the HPMCP. A sensitivity analysis

497   showed that the effect was no longer observed at ratios less than 1:0.5 w/w (API/polymer). Additionally,

498   the intensity of the peak increased with increasing HPMCP concentration. This acid-base interaction

499   between HPMCP and clofazimine was further supported by the appearance of peaks at 1540 cm^{-1} and 1395

500   cm^{-1}, which both correspond to the formation of a carboxylate group. [152] Knowledge about solid state

501   interactions can be directly correlated to solid state stability and loading capacity, i.e. drug to polymer ratio,

502   as well as to enhanced precipitation inhibition and supersaturation. Indeed, the combination of HPMCP and

503   clofazimine in a solid dispersion resulted in a 10-fold increase in apparent clofazimine solubility. [152]

504   Petrusevska and colleagues also employed FTIR spectroscopy to investigate the mechanism of interactions

505   between a successful API-PI formulation, sirolimus and HPMC. [154] Solid dispersions of HPMC and

506   sirolimus demonstrated significant variation from the neat samples as well as a physical blend. Specifically,

507   the sirolimus peaks at 1680-1640 cm^{-1} (C=C) and 1760-1670 cm^{-1} (C=O) in the solid dispersion were far

508   broader than those from the pure drug, which the authors concluded was a result of interaction between the

509   two species. [154] This interaction was suggested to be partly responsible for the 2-fold increase in

510   supersaturation and dissolution in the sirolimus-HPMC solid dispersion versus the commercially available

511   Rapamune® nanoparticles. In a subsequent human pharmacokinetic study, the novel formulation

512   significantly outperformed the commercial formulation. This effect was attributed to the enhanced

513   precipitation inhibition properties of HPMC in the novel formulation. [154]

514   Another application of FTIR spectroscopy is the characterization of precipitates. In a recent study by

515   Chavan, in which IR spectroscopy was used to verify that potential polymeric PIs did not affect the solid-

516   state phase behaviour (polymorphism) of the drug, nifedipine. [148] In this instance, the FTIR spectra of the

517   precipitates for all three polymers (HPMC, PVP and HPC) aligned well with crystalline nifedipine,

518   indicating that no polymorphic change was induced by the polymers.

519

4.1.3 UV-Vis Spectroscopy

UV-vis spectroscopy enables quantitative analysis of any molecule that absorbs light in the UV-vis range, which make it a very useful spectroscopic technique; arguably one of the most widely used in pharmaceutical sciences. [143] This section will focus on instances where UV-vis spectroscopy has been applied for more advanced studies, such as in the determination of interactions between drug and polymer or in-depth studies looking at precipitation kinetics during dissolution of supersaturating formulations.

Nie and colleagues used UV-vis spectroscopy as an orthogonal technique to support their mechanistic hypothesis for clofazimine-HPMCP interaction. [152] This was especially useful as clofazimine is red in both crystalline and amorphous forms, but a colour shift to purple occurred in the presence of HPMCP in solid dispersions. Qualitatively, this was also observed for mixtures of drug and carboxylic acid analogues (e.g. glacial acetic acid), but not for polymers without carboxylic acids, such as HPMC. It was concluded that the bathochromic shift was associated with a proton-transfer from the carboxylic acid functional group of HPMCP. [152] In combination with principal component analysis, the API:PI ratio **at which** no interaction was observed was calculated to be at ratios below 1:0.5. Using the same approach, it was concluded that the API:PI ratio at which full deprotonation of the imine occurs, **i.e. the strongest interaction**, was at 1:1.5. [152-153] Such information can be valuable in the design and development of PI-based formulations. A similar study was conducted by Misic and co-workers in the investigation of acid-base interactions between the poorly soluble drugs, loratadine and carvedilol, and oleic acid. [155]

Patel and co-workers also utilized UV-vis spectroscopy in combination with mathematical modelling. [156] This study involved the combination of online second-derivative UV spectroscopy and modelling using the diffusion reaction model in order to give real-time concentration values and mechanistic insight for indomethacin in supersaturated solutions. This methodology was able to provide a large amount of information about the precipitation behavior, including that at high degrees of supersaturation the precipitation was bulk diffusion limited, which fits in well with the diffusion-reaction model. [156]

544 UV-vis spectroscopy can also be employed to increase the understanding of phase behaviour of

545 supersaturated solutions, which can aid in the selection of PIs. Jackson and co-workers utilised two

546 techniques, UV-vis spectroscopy and fluorescence spectroscopy to determine the phase change behaviour

547 of danazol during LLPS into drug-rich amorphous or drug-rich crystalline phases in the presence and

548 absence of PIs. [49] During phase separation (e.g. LLPS) danazol scatters light, which increases UV-

549 extinction. Therefore, it is possible to use UV-vis spectroscopy to determine the concentration at which

550 LLPS occurs in supersaturated solution. [48] It was reported that LLPS onset occurred at 13μg/mL, a value

551 which could be decreased to varying degrees in the presence of polymers. Furthermore, it was determined

552 that apparent decrease in the LLPS induction time in the presence of polymers correlated well with the

553 ultimate performance of the polymer, with HPMCAS and HPMC showing both the biggest decrease on

554 LLPS induction time (both to 8 seconds) and the biggest increase in precipitation induction time (277

555 seconds and 163 seconds, respectively). [49] Therefore, *in-line* UV-spectroscopy can be a valuable tool in

556 the assessment of precipitation inhibition efficiency *via* the study of LLPS induction times from

557 supersaturated solutions. This is a particularly attractive perspective as LLPS induction time can be

558 measured relatively easily using this method in a high-throughput experimental set-up.

559 **4.1.4 Raman Spectroscopy**

560 Raut and co-workers utilised an *in situ* Raman probe, placed inside a dissolution set-up, in order to

561 investigate the precipitation inhibition effect of vitamin E TPGS on two model compounds, probucol and

562 indomethacin, in a self-emulsifying drug delivery systems. [83] In order to achieve this, the formulations

563 were added to a solution at pH 1.2, followed by a pH shift to pH 6.8. The Raman probe enabled the collection

564 of time-resolved Raman spectra for both the solid precipitate and the species in solution, which were

565 analysed for molecular interactions between the drug and excipients. For probucol, Raman peaks were

566 observed at 540 and 1164 cm^{-1}, corresponding to the hydroxyl groups in the molecules. However, in the

567 presence of vitamin E TPGS, this peak dropped significantly in intensity, with 1164 cm^{-1} disappearing

568 completely. This was attributed to the interaction of the probucol hydroxyl groups with carbonyl groups of

569 the PI. These interactions had a profound effect on precipitation, with no precipitation observed in the

570 presence of vitamin E TPGS, in spite of the system being supersaturated to 100-fold of the thermodynamic

571 solubility of probucol. [83] Similar observations were made for indomethacin. Interestingly, in the case of

572 indomethacin, it was observed that interactions were only evident whenever a certain "supersaturation

573 threshold" was obtained, [83] below which interactions were not observable and precipitation occurred. This

574 is an important factor to bear in mind: although a drug and polymer may theoretically interact strongly, the

575 formulation in question must generate a particular concentration before interactions will occur.

576 In addition to probing interactions in solution, Raman spectroscopy is a useful tool for investigating short-

577 range interactions in the solid state. This can be particularly beneficial in the development of solid dispersion

578 formulations, such as hot melt extrusion (HME) and spray-dried dispersions (SDD), where both drug-

579 polymer miscibility and the precipitation inhibition performance of the polymer is based on these

580 interactions. Chauhan and co-workers utilised this technique, among a wide range of spectroscopic tools, to

581 develop solid dispersions of dipyridamole. [92] The team found that the most successful formulations

582 consisted of drug-HPMC and drug-Eudragit E100®, which performed significantly better than all other

583 polymers screened. Utilising solid-state Raman spectroscopy, it was revealed that interactions were present

584 between the drug and HPMC and Eudragit E100®. [92]

585 Raman spectroscopy can also be applied to study the extent of drug precipitation because dissolved and

586 solid drug generally differ in their spectra. Both types of spectra can be used in a multivariate calibration.

587 Thus, in-line dispersive Raman spectroscopy has been used to monitor drug precipitation from

588 supersaturated dipyridamole solutions using a transfer test [157]; and this spectroscopic approach has also

589 been reported to study drug precipitation from lipid-based formulations in the course of digestion. [158]

590 ## 4.1.5 Fluorescence spectroscopy

591 Fluorescence spectroscopy can be used to determine interaction mechanisms, changes in hydrophobicity

592 and phase change behaviour. This is achieved by utilising fluorescence probes, such as pyrene. [49] Pyrene

593   is a good probe for hydrophobicity, since when it preferentially partitions into hydrophobic

594   microenvironments a change in its fluorescence absorption bands, I_1 and I_3, occurs. [49] Jackson and co-

595   workers exploited this phenomenon in order to assess the concentration at which LLPS occurred in the

596   presence of danazol, and to determine whether a crystalline or non-crystalline phase was formed. For LLPS,

597   it was determined that no significant change in the I_1/I_3 ratio in the pyrene fluorescence spectra occurred

598   below danazol concentrations of 13 µg/mL in the pure sample. Above this, the change in I_1/I_3 aligned well

599   with the formation of a non-crystalline LLPS, suggesting an onset of LLPS at 13 µg/mL. Additionally, it

600   was observed that incorporation of a polymeric PI decreased the LLPS onset concentration, with HPMCAS

601   and HPMC having the biggest impact, decreasing LLPS onset concentration to 8 and 9 µg/mL, respectively.

602   Observing the changes in I_1/I_3 can provide information about the induction time of precipitation as, generally

603   speaking, the I_1/I_3 ratio returns to normal after recrystallisation. This is due to the disappearance of the drug-

604   rich phase and the expulsion of pyrene from the crystal lattice and back into the aqueous phase. [159] In the

605   study, all three polymers showed a sustained I_1/I_3 ratio for at least 15 minutes. These data correlated with

606   the induction times, with HPMCAS showing the longest induction time of 4 hours. [49]

607   Creasey and co-workers utilised this technique to assess the interaction between Pluronic and Labrasol in a

608   formulation being developed for two Johnson and Johnson compounds. Pyrene solution was added to

609   solutions of Labrasol, Pluronic and mixtures of the two. Fluorescence spectra for pyrene was then collected

610   to record the effect of Pluronic on the micropolarity of the Labrasol formulations. [128] In this instance, the

611   I_1/I_3 ratio in the mixture of Labrasol and Pluronic was significantly lower than Labrasol alone, which

612   indicates a more pronounced hydrophobic microenvironments in the sample. It was hypothesized by the

613   researchers that this increased hydrophobicity was the key factor for inhibiting precipitation, allowing the

614   drug to be held more tightly within the microstructures formed by the surfactant. Finally, this mechanism

615   had a profound effect both compounds with a 500- and 200-fold increase in concentration compared to no

616   excipients added. [128]

617   **4.1.4 Differential Scanning Calorimetry**

618   Differential scanning calorimetry (DSC) is a thermal analysis technique that sees wide application in

619   pharmaceutical sciences, for example in solid state characterization. [146] Although not as common, DSC can

620   also be used to investigate interactions between two species. However, traditional DSC cannot achieve this

621   and instead, modulated DSC (MDSC) must be used. MDSC differs from traditional DSC in that it operates

622   using two simultaneous heating rates, in contrast to the single linear heating rate used in DSC. MDSC utilises

623   both a linear heating rate and a modulated heating rate that allows simultaneous measurement of the heat

624   capacity of the sample.

625   Chauhan and co-workers used MDSC to investigate the mechanism by which dipyridamole (DPD) interacts

626   with a range of PIs. [92] Previously, MDSC has been used to assess miscibility between a drug and polymer,

627   based on changes in melting events. Expanding on this idea, the melting temperatures of the DPD and DPD

628   precipitates in the presence of polymers were used to identify whether any drug-polymer interaction was

629   taking place. For Eudragit® E100, Eudragit® S100 and HPMC, additional melting endotherms were

630   observed in the MDSC curves. The authors of the study reasoned that this change in the thermal behaviour

631   occurred due to interaction of the drug with the polymers in solution, which corresponded with an increased

632   precipitation inhibition. Indeed, out of the 6 polymers studied, only those polymers where a change in

633   melting temperature of the precipitates was observed were successful PIs. [92] Moreover, the authors also

634   cautioned that, although melting point changes were present, this was not a definitive proof of interaction

635   as certain polymers, e.g. PEG, can dissolve a drug and therefore alter the melting temperature. Rather, DSC

636   is a useful tool to determine lack of interaction, as was the case with the unsuccessful PIs, Eudragit S100,

637   Eudragit L100 and PEG 8000. In order to state that an interaction is definitely present, MDSC should be

638   used with complimentary analytical techniques.

639   4.1.5 Synchrotron Radiation

640   Synchrotron radiation is the electromagnetic radiation emitted from charged particles that are accelerated in

641   a curved fashion, for example in a circular particle accelerator. [147] For diffraction studies, synchrotron

642   radiation enhances the flux of X-ray radiation, [149] which leads to diffraction patterns with higher

643   resolutions, obtained in a shorter period of time. The technique is a highly sophisticated analytical tool, and

644   practical usage is limited due to the fact that the equipment is not common and is highly expensive.

645   Therefore, synchrotron light sources exist at dedicated sites, of which there are only a few around the world,

646   (a list of dedicated sites can be found at: http://www.lightsources.org/light-source-facility-information). [148]

647   It was reported by Van Eerdenbrugh and colleagues that synchrotron radiation could be utilised to study the

648   precipitation and crystallisation behaviour of API in a supersaturated solution. [160] This was achieved using

649   wide-angle X-ray scattering (WAXS) with synchrotron X-ray beams. The research group concluded that the

650   method could detect crystalline particles at a minimum *suspension* concentration of 2.6 mg/mL for all

651   samples in their 52-sample study, including a large proportion of poorly soluble model drugs, [161] which

652   translates to a sensitivity of around 0.26% w/w drug in aqueous suspension. This sensitivity is significantly

653   higher than all other conventional methods for detecting crystallinity and is also in agreement with previous

654   studies that showed crystals with a size of < 1 nm could be detected. [160]

655   Further work by this group incorporated PIs into the same experimental design, to study mechanistically the

656   effect of PIs on the crystallisation time of a range of compounds. [162] In this study, the solvent shift method

657   was applied to generate supersaturated solutions in the presence of a particular amount of pre-dissolved

658   polymer. The onset time of crystallisation was examined using several measurements over 24 h, to determine

659   the delay in onset of crystallisation. Combining the results with polarised light microscopy demonstrated

660   the presence of LLPS, which was prolonged in the presence of some polymers. Similar to other reports in

661   the literature, [74] that the most hydrophobic and hydrophilic polymers were not effective PIs, supporting the

662   hypothesis that an effective PI must interact with *both* the aqueous medium and API. [163]

663   Due to the high costs and low availability of the synchrotron methods and equipment, it is unlikely that this

664   approach will ever find wide-spread adoption for screening of precipitation inhibitors, and certainly not in

665   routine drug development. Additionally, although it is significantly more sensitive than standard methods,

666   there is still a limit to how easily one can detect the early crystallisation events taking place during

667   precipitation.

668

669

670

4.1.6 Video Microscopy

For screening of PIs, microscopy is an attractive approach as the efficiency of inhibitors can be visualised, offering insight into early crystallization events that would not be typically observable *via* conventional methods such as detection by UV-spectroscopy. However, the use of manual microscopy is not highly prevalent in the literature, probably due to the time and labour requirements. In order to circumvent this, various studies have combined conventional microscopy with video analysis. [118]

Recent work by Christfort and co-workers utilised such a system to investigate supersaturated solutions of tadalafil in the presence and absence of a PI, HPMC. Samples were viewed under video light microscopes, and multi-particle analysis was carried out on the videos in order to assess total precipitation as a function of time. [118]

4.1.7 Atomic Force Microscopy

Atomic force microscopy (AFM) is a type of high-resolution microscopy that can image on the nanometre scale. Due to this high resolution, AFM has been applied to study the effect of polymer surface coverage on growing crystals, and how this relates to precipitation inhibition. According to the adsorption layer model, before a solute molecule can join a growing crystal lattice, it must adsorb and interact with the surface at a site with high attraction forces. This interaction can be hindered or disrupted with the use of polymers, which can bind the solute in solution or adsorb onto the growing crystal surface. For the latter mechanism, it has been hypothesised that understanding the conformation of polymers on the surface of the growing lattice is key to inhibition of crystal growth. [76, 163]

690   Expanding upon this idea and early work by Roiter and Minko, [166] Schram and colleagues studied the effect

691   of the pH on the adsorption of HPMCAS on felodipine crystals and the subsequent effect on precipitation

692   inhibition, using AFM and *in situ* UV-spectroscopy, respectively. [76] It was observed during the AFM

693   experiments, that at pH 3.0 the polymer was adsorbed as small aggregates and at pH 6.8 the whole surface

694   was covered with a monolayer. The space between two polymer aggregates at pH 3.0 ranged from 25-50

695   nm, compared to 0.9 nm at pH 6.8. Therefore, at a higher pH there was a greater polymer surface coverage

696   and greater inhibition of crystal growth. Indeed, the ratio of crystal growth (crystal growth rate in the absence

697   of an inhibitor / crystal growth rate in presence of inhibitor) was 1.28 and 2.29 at pH 3 and 6.8, respectively.

698   This was explained by the intra- and intermolecular hydrogen bonding of HPMCAS at the different pHs

699   investigated. [76] With a pKa of 5.5, HPMCAS is unionized at pH 3. As a result, intermolecular interactions

700   between the succinyl groups can occur, which allows the polymer to form coils and aggregates. Conversely,

701   at pH 6.8, the polymer is ionized, and self-repulsion results in the molecules forming a monolayer on the

702   surface with no interaction between the succinyl groups. These observations led Schram and colleagues to

703   conclude that the conformation of a polymer, and more importantly the total surface coverage of a polymer,

704   is crucial when considering the observed inhibitory effect *(Figure 7).* [76]

705   Additional work by Schram and colleagues was dedicated to establishing a correlation between polymer

706   surface coverage and precipitation inhibition. [163] For this, the group again employed atomic force

707   microscopy (AFM) to study the interaction between a range of polymers and felodipine crystals. The

708   measurements were done in suspension, with height and phase contrast images recorded simultaneously.

709   The phase contrast in the images is dependent on the surface characteristics of the sample, and therefore

710   difference between surface properties can be detected between different samples. [164] The images were then

711   analysed using "imageJ", a software that can detect changes in digital images based on pixel changes, [165]

712   which could be used to calculate total surface coverage of the polymer on the growing crystal. In the study,

713   it was reported that polyacrylic acid (PAA), a hydrophilic polymer, was not adsorbed to the crystal surface,

714   while PVP, another hydrophilic polymer, formed aggregates to decrease the surface area exposed to the

715   crystal surface to 8% ± 0.8 coverage. At the other end of the scale, it was observed that hydrophobic

716 polymers like polyvinyl acetate (PVAc) also minimized their exposed surface area to the liquid by forming

717 aggregates adsorbed on the surface of the crystal (14% ± 0.7 coverage). [163] By contrast, polymers with both

718 properties (hydrophobicity and hydrophilicity), like HPMC, resulted in the best surface coverage by

719 adsorbing to the surface of a crystal in a more mono-layer-like fashion (54% ± 0.8 coverage). [163] There was

720 a linear correlation between surface area coverage and polymer effectiveness (based on the aforementioned

721 crystal growth ratio) was established, showing that a good surface coverage can predict good precipitation

722 inhibition.

723 AFM is a useful tool for studying polymer surface coverage and conformation, essential parameters when

724 considering the effectiveness of a PI in a supersaturating formulation. In combination with knowledge of

725 the PI conformations throughout the physiological pH range, such parameters have the potential to be

726 utilised routinely as part of a selection protocol for PIs. More work is required to bridge the gap between

727 these proof of concept studies towards a more wide-spread application which could operate in industrial

728 settings. Particular emphasis should be placed on demonstrating such correlations with different API -PI

729 systems, and on the development and validation of the *in silico* models to predict polymer performance

730 based on polymer surface coverage, which could offer a very attractive avenue in the pre-screening phase

731 of PI selection. One issue may be the accessibility and complexity of AFM, which is not often commonly

732 used in early stage development.

4.2. Computational Approaches

4.2.1 Mathematical Modelling

735 Mathematical modelling of physical, physicochemical and physiological processes can be a useful tool in

736 drug development with a wide range of applications. [166-168] For precipitation inhibition, models that are

737 related to adsorption, crystallisation and dissolution have been very valuable in a number of key studies.

738 However, the use of mathematical models to aid in the understanding of precipitation behaviour, and the

739 effect of inhibitors, has not yet been adopted widely.

740 **_Adsorption Modelling_**

741 Early work in the literature utilised well-established adsorption isotherms, including the Langmuir [169] and

742 Freundlich models, [166] to predict the adsorption of additives on crystal surfaces and consequently to

743 estimate the crystal growth kinetics inhibition through mathematical modelling. This approach is often used

744 in combination with crystallisation models such as the Kubota-Mullin model, which models crystal

745 growth. [168, 170-171]

746 The Kubota-Mullin model applies a step-growth model, which assumes that a crystal grows monolayer by

747 monolayer. The growth inhibition depends on the PI and the distance between the adsorbed species on the

748 surface. In the model, crystal growth is linked to fractional surface coverage, θ, (which is the ratio of the

749 average distance between the potential adsorption sites, L, and the average distance between the adsorbed

750 species, l; and the free energy of the unit length *(Equation 2)*. [168, 170-171]

751 $$\theta = \frac{L}{l} \quad \textbf{\textit{(Equation 2)}}$$

752 Expanding on their work using AFM to probe the polymer surface coverage of a range of PIs on felodipine

753 crystals, Schram and colleagues adapted the model in order to predict polymer performance from the

754 experimentally obtained surface coverage values. [163] In this case, polymer effectiveness, R_p/R_0, is the ratio

755 of crystal growth in the presence (R_p) and absence of polymer (R_0) and depends on the fractional surface

756 coverage, θ; the relative supersaturation, σ; the edge free energy per unit length, γ; the size of a growth unit,

757 a; the temperature, T, the Boltzman constant, k, and the average distance between absorbed polymers, l

758 *(Equation 3)*

759 $$\frac{R_p}{R_0} = 1 - \frac{\gamma a}{kT\sigma(\theta l)}\theta \qquad \textbf{\textit{(Equation 3)}}$$

760

761 The average distance between polymer molecule, is specific for each system and depends on the amount of

762 polymer adsorbed to the surface. Thus, l was proportional to the experimental polymer surface coverage

763   determined by AFM. Consequently, a correlation was established, with which l could be determined from

764   the polymer surface coverage. This allows the calculation of the fractional surface coverage in the Kubota-

765   Mullin model (*Equation 2)* and subsequently the theoretical effectiveness of crystal growth inhibitors

766   *(Equation 3)*. In this study, the theoretical effectiveness calculations were in good agreement with the

767   experimental values. [163]

768   Alonzo and co-workers also adopted the Kubota-Mullin model to study the effect of HPMC on crystal

769   growth and nucleation of felodipine in a supersaturated solution. [53] Using the Langmuir adsorption model,

770   the research team explored the effect of HPMC on felodipine crystallisation. Based on the model, it was

771   predicted that the effect of HPMC on the crystallisation of felodipine crystals was highly dependent on the

772   extent of supersaturation, with greater supersaturation reducing the effect of the inhibitor. The theoretical

773   predictions were in agreement with the experimental crystal growth rate in the presence of HPMC. It was

774   also predicted that polymers successfully interrupting the nucleation process would be more advantageous

775   than those that affect the crystal growth rate, which was supported experimentally. [53] Further work utilising

776   the Kubota-Mullin model was carried out by Ilevbare and co-workers, [48] who were able to successfully

777   estimate the crystallisation rate of the poorly soluble drug, ritonavir, in the presence and absence of a

778   polymer.

779   One of the most extensive studies on the use of adsorption and crystallisation models was carried out by

780   Patel et al.They studied the precipitation behaviour of indomethacin in the presence of PIs. [105-106, 156] The

781   effect of molecular weight on the inhibition potential of PVP and of N-vinylpyrrolidone on indomethacin

782   crystallisation [106] was investigated using adsorption isotherms generated *via* the solution depletion method.

783   Using a wide-range of molecular weights, the two inhibitors were combined with indomethacin in an

784   aqueous suspension and allowed to equilibrate for a set amount of time. The samples were then filtered and

785   analysed using size exclusion chromatography to determine the concentration of the adsorbed polymer,

786   which provided a value of the extent of inhibitor surface adsorption. These values were then used to

787   construct adsorption isotherms based on the Langmuir model. It was shown that the adsorption potential for

788   PVP was greater than N-vinylpyrrolidone. Further, the isotherms demonstrated that the adsorption capacity

789   for PVP was directly proportional to PVP molecular weight. These model predictions were then validated

790   experimentally, with PVP significantly outperforming N-vinylpyrollidone in the inhibition of indomethacin

791   precipitation, with increasing molecular weight of PVP also correlating to a higher degree of sustained

792   supersaturation. [105]

793   Further work by Patel and Anderson, employed the previously developed second-derivative UV method in

794   combination with a first-order crystal growth model to investigate the growth rates of indomethacin in the

795   presence of various PIs. [156] HPMC and PVP significantly outperformed HPβCD, in agreement with the

796   inhibition models. For HPβCD, precipitation inhibition was modelled using diffusion layer theory, based on

797   the assumption that HPβCD complexes with indomethacin in the diffusion layer of crystal growth. This

798   model successfully predicted that, at high degrees of supersaturation, HPβCD inhibition could be related to

799   the reversible complexation between the two species at the diffusion layer. [156]

800   *Molecular Modelling*

801   Many types of molecular modelling can be used in simulation of crystallisation or precipitation, i.e. from

802   quantum mechanical approaches, over Monte Carlo methods to molecular dynamics simulations. [172]

803   Molecular simulations of crystallisation and precipitation are of high interest as they can offer a high

804   throughput that can be applied to a wide range of systems. Mandal and co-workers developed a framework

805   that enabled the simulation of crystallisation behaviour of a range of organic molecules in the presence of

806   inhibitors. [167] In this study, a coarse-grained (CG) model for crystal growth [173] based on force fields

807   obtained from simulators, was applied to a range of molecules. Coarse-graining is an approach that allows

808   the simulation of complex systems without using extensive computation time due to the use of simplified

809   atomistic representations. Such an approach is often used to model the interaction of proteins and small

810   molecules. [174] There are many software packages that can carry out the CG process, such as MARTINI,

811   [173] however, such software packages often oversimplify the molecules such that information important to

812   understand crystallisation behaviour may be lost. In order to improve upon these established CG processes,

813   Mandal and colleagues utilised a CG model based on the radial distribution functions of the molecules,

814   which were obtained from atomic simulations carried out in the crystalline states. [167] As a result, the CG

815   model developed was able to simulate crystal growth of the organic molecule, phenytoin, in the absence and

816   presence of the polymer HPMCAS. [167, 173] Furthermore, the simulation was able to correctly predict that

817   inhibition of phenytoin by HPMCAS is highly dependent on the substitution of the polymer, with an

818   increased acetate substitution slowing crystal growth most effectively. This simulation proved to be very

819   robust in its prediction as these observations were also demonstrated experimentally. [167]

820 4.2.2 Chi Parameter and Interaction Enthalpy

821   A rigorous thermodynamic treatment of PI selection would have to consider the drug and PI in water as a

822   ternary system, where both the solid-liquid equilibrium of solubility and liquid-liquid phase separation of

823   amorphous solubility are considered. [8,41] Such a pursuit is very attractive given the high degree of

824   information one can learn about the system. An example of such a theoretical model is the perturbed-chain

825   statistical associating fluid theory (PC-SAFT), which bears much promise but is also demanding, due to the

826   various parameters that are required in order to fully describe a system. [41] Such highly parametrized

827   methods still have to be adopted in the area of precipitation inhibition to enable an early *in silico* screening

828   of excipients. Moreover, a focus on equilibrium thermodynamics may not be the most descriptive way to

829   model drug precipitation due to the importance of kinetics. Non-equilibrium thermodynamics is an even

830   more challenging approach and so far, attempts have only been made to consider interactions parameters of

831   simpler thermodynamic models for empirical kinetic considerations.

832   In order to consider the kinetics of precipitation, the interaction parameter χ of the Flory-Huggins (FH)

833   theory [175-176] can be employed. [177-178] The Flory-Huggins solution equation *(Equation 4)* describes the

834   thermodynamic behavior of polymers in solution. The equation is an adaption of the standard Gibbs energy

835   equation, introducing extra terms to adjust the entropy portion to account for the dissimilarity of molecular

836   sizes. In the Flory-Huggins equation the enthalpic portion of the Gibbs equation is represented by the Chi

837   parameter, χ:

838

$$\Delta G = RT[n_1 \, ln\varphi_1 + n_2 \, ln\varphi_2 + n_1 \, \varphi_2 \, \chi_{1,2}] \qquad \textbf{\textit{Equation 4}}$$

840

841   In the Flory-Huggins equation, R is the ideal gas constant and T is the absolute temperature. n_1, n_2 and φ

842   are the number of moles and volume fraction, respectively, for component 1 and 2 of the system, while χ is

843   the interaction enthalpy upon association of component 1 and 2.

844

845   Expanding upon the Flory-Huggins equation, one can consider mixtures of drug and polymers, whereby the

846   enthalpic contribution becomes, χ_{DP}. In this approach, it is assumed that the interactions considered in χ_{DP}

847   are also relevant for the kinetics of sustaining drug supersaturation. This is based on the understanding that

848   some of the mechanisms by which a polymer can inhibit precipitation are dependent on energetic

849   interactions such as hydrophobic, polar or hydrogen bond interactions between the drug and PI. [55] The χ_{DP}

850   parameter, can be determined experimentally by combining the Flory-Huggins equation with experimental

851   DSC measurements. [179] This parameter has been applied to other areas of drug development, for example

852   in the assessment of drug-polymer miscibility in the screening of candidate formulations. [180] It is possible

853   to utilize *in silico* predictions of the χ_{DP} parameter, which reduces the number of experiments required, this

854   allowing more focus on the most promising formulations. This can be achieved in a number of ways, with

855   one key strategy combining the total solubility parameters of the drug, δ_D, and polymer, δ_P, in relation to the

856   molar volume of the drug, V_m, the temperature, T, and the ideal gas constant, R (***Equation 5***). [181]

857

858    $$\chi_{DP} = \frac{V_m(\delta_D - \delta_P)^2}{RT} \textbf{\textit{(Equation 5)}}$$

859

860   The extended Hansen solubility parameters, δ_D, and δ_P, can be predicted based on chemical structure alone,

861   using group contribution methods. [182] Indeed, this determination of χ_{DP} via partial solubility parameters has

862   been used to construct entire phase diagrams for solid dispersions based on the Flory-Huggins theory. [183]

863

864 In addition to group contribution methods, it has been shown that solubility parameters can be predicted

865 based on quantitative structure property relationships (QSPR). [184-186] With rising computational power there

866 is also the option to use molecular dynamics (MD) simulations to calculate solubility parameters and hence

867 to determine χ_{DP}. A first calculation option is to simulate internal energy change due to vaporization, ΔE_v.

868 [187] This MD approach utilizes the original definition of the solubility parameter as a cohesive energy

869 density (CED) *(Equation 6)*: [188]

870

871
$$\delta = (CED)^{1/2} = \left[\frac{\Delta E_v}{V_m}\right]^{1/2} \textit{(Equation 6)}$$

872

873 In this instance, total energy difference for isolated molecules and for the bulk system with periodic

874 boundary conditions provides an estimate of ΔE_v. [188] That being said, calculation of the cohesive energy

875 difference and conversion of the solubility parameter into χ_{DP} is not the only way to obtain the Flory-

876 Huggins interaction parameter by means of molecular simulations. Fan and co-workers developed a

877 molecular simulation method to derive phase diagrams of binary mixtures *(Equation 7, 8)*. [189]

878
$$\chi_{1,2} = \frac{z\Delta w_{1,2}}{RT} \textit{(Equation 7)}$$

879
$$\Delta w_{1,2} = w_{1,2} - \frac{1}{2}\left(w_{1,1} + w_{2,2}\right) \textit{(Equation 8)}$$

880 The interaction parameter between two molecules, ($\chi_{1,2}$), is obtained directly from the corresponding pair-

881 wise interactions, *w,* and hence the energies $w_{1,1}$, $w_{2,2}$, $w_{1,2}$ *(Equation 7-8).* This can be achieved by averaging

882 a large number of configurations using a Monte Carlo approach, taking into consideration the number of

883 neighboring molecules (i.e. coordination number, z) in combination with the ideal gas constant, *R,* and

884 temperature, *T*.

885

886   There are numerous routes to obtain the FH χ_{DP}. That being said, there are only a few examples of this

887   parameter being used to understand precipitation inhibition. Baghel and co-workers studied solid

888   dispersions of dipyridamole (DPY) and cinnarizine (CNZ) with polyvinyl pyrrolidone (PVP) and

889   polyacrylic acid (PAA). [178] It was found that the combinations capable of forming hydrogen bonds (DPY-

890   PVP; DPY-PAA and CNZ-PAA) in the solution state were more effective at keeping the drug in

891   supersaturation than those not able to hydrogen bond (CNZ-PVP). In this instance, CNZ-PVP had the

892   highest predicted χ_{DP} parameter, suggesting a less stable interaction, in line the observed precipitation

893   inhibition results. However, it was noted that, despite their significantly different supersaturation

894   performance, the difference between the χ_{DP} parameters of CNZ-PVP and CNZ-PAA was not great, and

895   that other aspects such as the hydrophilicity of the polymer should also be considered.

896   Similar findings were also reported by Chen and co-workers who compared solid dispersions of

897   griseofulvin, felodipine, and ketoconazole with PVP vinylacetate (PVP-VA) and HPMC-AS. [177] Although

898   felodipine interacted much more effectively with PVP-VA in the solid-state (χ_{DP}= -1.9) than with HPMC-

899   AS (χ_{DP}= -0.21), this behavior was not replicated in aqueous dispersions, where the HPMCAS solid-

900   dispersion generated higher and more sustained supersaturation profiles upon dissolution. This was likely

901   due to the hydrophilic interactions of PVP-VA with water upon exposure to an aqueous environment, which

902   may have reduced or negated the favorable interactions with felodipine.

903

904   4.3 Mechanistic Design of Precipitation Inhibitors

905   The development and use of modern analytical techniques has provided an increased understanding of PI

906   interactions. Utilizing knowledge of potential binding modes with the drug, it has become possible to select-

907   or even design- PIs on a drug-by-drug basis.

908   The first example of such an approach was published by Ting and co-workers, who synthesised co-polymers

909   inspired by HPMCAS using reversible addition-fragmentation chain transfer (RAFT) polymerization. [69]

910   These novel polymers were then used in SDDs and studied for interactions with probucol, danazol and

911 phenytoin, in order to determine which structural motifs were important for inhibition of precipitation. For

912 probucol, the most efficient polymers were those with only succinyl and acetyl functionality, improving

913 dissolution up to 180-fold. Conversely, for danazol and phenytoin, polymers that exhibited a higher

914 prevalence of hydroxyl groups were able to better sustain supersaturation. This was supported by FTIR

915 spectroscopy. In the FTIR-spectra, –OH stretching absorptions of danazol shifted and broadened in the

916 presence of polymers with high proportions of –OH functionality, indicating the presence of hydrogen bond

917 interactions. [69]

918 Mosquera-Giraldo and co-workers also utilised a mechanistic design approach to fine-tune inhibition of

919 telaprevir precipitation by cellulosic polymers. [190] In this approach, a large number of chemically diverse

920 cellulosic polymers were synthesized containing different combinations of key functional group substituents

921 such as alcohols, amides, esters, ethers, carboxylic acids and sulfides. The novel polymers were grouped

922 into two distinct chemical groups: the ω-carboxyalkanoates, which were cellulosic polymers with a wide

923 variety of hydrocarbon chain lengths capped with a terminal carboxylic acid, and a second group which

924 included the aforementioned chemically diverse functional groups. The solubility parameters for the novel

925 polymers was calculated, and they were then screened using an *in situ* UV-probe to determine the onset of

926 precipitation in supersaturated telaprevir solutions generates with the solvent-shift approach. It was found

927 that for the ω-carboxyalkanoates both the solubility parameter range and the carboxylic acid functionality

928 were important for precipitation inhibition. Similar conclusions were reached in the second group of

929 polymers, in which it was found that the only functional group that appeared to influence precipitation was

930 the terminal carboxylic acid, with all other functionality showing little to no effect on precipitation

931 inhibition. Furthermore, there was also a direct correlation with hydrocarbon chain length and ultimate PI

932 performance. Ultimately, it was concluded that both a terminal carboxylic group and long chain length were

933 essential for effective inhibition, with the carboxylic acid providing hydrophilicity for the drug to remain in

934 solution, whilst the hydrocarbon chain was essential for hydrophobic interactions with the growing crystal

935 surface. [190]

936   Expanding on the aforementioned studies, Ting and co-workers were the first to demonstrate a *de novo*

937   design of polymeric inhibitors based on molecular interactions with phenytoin. [192] A high-throughput

938   excipient design process that could yield a wide-range of chemically diverse polymeric fragments, referred

939   to as "synthons", was applied. These synthons were selected based on the known binding interactions of

940   phenytoin in the formation of the crystal lattice, namely the hydrogen bond interaction between carbonyl

941   oxygen and cyclin imines. The fragments selected were N-isopropylacrylamide (NIPAm), diethyl amide

942   (DEA) and isopropyl methacrylate (IPMA), which could insert themselves into the growing crystal lattice

943   *via* chemical interaction with the phenytoin molecule, thereby disrupting its internal hydrogen bond

944   interactions and thus arrest crystallization. Hydrophilic partner fragments, i.e. dimethyl amide (DMA),

945   amide (AM) and hydroxyethyl methacrylate (HEMA), which can interact with water, were also included in

946   the screen. The resultant polymers thus had the ability to bind to the growing crystal, inhibit crystallisation

947   and maintain the drug in solution. [194] Based on this chemical library of 6 synthons, the team employed a

948   controlled, high-throughput RAFT polymerization was used to synthesise a large library of distinct novel

949   polymers, 60 in total. These polymers were then screened for precipitation inhibition of phenytoin in a high-

950   throughput screen utilising the solvent-shift method in combination with UV spectroscopy. Poly(NIPAm70-

951   *co*-DMA30) was able to maintain phenytoin supersaturation at >1000 µg/mL for over 3 hours. This was a

952   significant improvement relative to the commercially available excipient, HPMCAS, which is able to sustain

953   concentrations at only 100 µg/mL for 3 hours. [192]

954   NOESY and DOSY spectroscopy was used to further assess the potential interaction of the drug phenytoin

955   with the precipitation inhibitor synthons. NOESY data showed that the best performing polymer,

956   poly(NIPAm70-*co*-DMA30), exhibited strong cross-peaks with the aromatic portion of phenytoin and the

957   isopropyl portion of the polymer, coupled with complementary hydrophilic interactions offered by the

958   DMA. Further, the DOSY data showed a significant decrease in reduced diffusion coefficients for phenytoin

959   in the presence of the polymer. Together, the two spectroscopic techniques were able to elucidate the

960   mechanistic interaction between poly(NIPAm70-*co*-DMA30) and phenytoin and explain the highly

961   improved precipitation inhibition. [192]

962 Although the *de novo* design of PI to maximise interaction with API is an exciting prospect, there are some

963 key hurdles for this technology to be widely applicable. For example, from a regulatory perspective such an

964 approach could be very costly and restrictive, since additional safety studies would be required to

965 demonstrate an absence of polymer-related toxicity. Adopting a similar approach that is based on selection

966 of polymers already approved by health authorities such as the **FDA** for use in pharmaceuticals might be

967 more efficient. Such an approach would retain some of the advantages of a bespoke PI selection process

968 whilst avoiding any additional regulatory burden.

5. Concluding Remarks

969

970 In recent years, focus has been placed on understanding the stabilisation of the supersaturated state and the

971 importance of PI selection. Although classical crystallisation theory has been well described and applied to

972 in vitro crystallisation and precipitation inhibition, the relative importance of a wide range of PI properties

973 including: molecular weight, viscosity and number of hydrogen bond donors/acceptors is not yet entirely

974 clear, with different studies reaching different conclusions. Ultimately, it is accepted that precipitation

975 inhibition is not a "one-size-fits-all" process, and that each API will have different dependencies, which can

976 make *a priori* PI selection difficult. Although a diverse set of high-throughput screening methods are

977 available which can be used to identify suitable precipitation inhibitors, such an approach does not provide

978 any mechanistic information about how precipitation inhibitors function. With this in mind, huge strides

979 have been made in recent years towards elucidating the precipitation inhibition process for a wide range of

980 drug-inhibitor systems, as covered in this review.

981 Spectroscopic techniques such as NMR, IR, Raman, UV-vis and fluorescence spectroscopy can be

982 employed to study interaction mechanisms between API and polymer as well as to gain understanding about

983 the phase behaviour of supersaturated API solutions. Further work in this area, particularly on developing

984 new techniques to improve sensitivity of detection would be valuable in order to allow application to a wider

985 range of systems. From a thermal perspective, mDSC has seen limited application in detecting subtle

986 changes in melting points of drug-polymer mixtures, which can be indicative of molecular interaction.

987   Additional thermal tools, such as isothermal calorimetry could be investigated to gain insights into additive

988   effects on nucleation and growth inhibition using various excipients. Microscopic techniques, such as

989   atomic force microscopy, have been pivotal in building a picture of how polymers interact with the growing

990   surface. This has been particularly important when understanding the effect of pH and polarity on polymer

991   surface coverage, which can have a dramatic effect on PI performance. Furthermore, video microscopy has

992   emerged recently as an interesting technique that, when coupled with image analysis, can visualise the

993   crystallisation and nucleation behaviour of supersaturated API. This can be achieved both in the presence

994   and absence of polymer and can allow the detection of early-stage precipitation events that may not be

995   detectable by conventional methods. Another approach which can achieve this high sensitivity is

996   synchrotron radiation, which has been used to study nucleation in detail. It is clear that the application of

997   this technology is not feasible on a larger scale, so more work should be carried out on alternative approaches

998   that can provide increased sensitivity for detection of crystallinity. Further progress has also been made on

999   understanding the precipitation/inhibition interplay from a theoretical perspective, using thermodynamic

1000   modelling and molecular simulations. In particular, the molecular simulation of atomistic detail in

1001   crystallisation and inhibition is a highly interesting area. Such simulations can simultaneously decrease the

1002   amount of experimental work required in the development of the formulations whilst increasing the amount

1003   of understanding yielded about the systems studied, which could help lowering the risk when working with

1004   the development of supersaturating formulations.

1005   The combination of increased understanding of precipitation inhibition processes with advanced analytics

1006   has the potential to completely reshape how PIs are selected in drug development in the future. The

1007   possibility of bespoke polymer design, or at least bespoke PI selection for each individual supersaturating

1008   formulation has also become possible. In this approach, it will be possible to decrease the number of

1009   experiments required and perhaps increase the absorption performance of the final formulation, which may

1010   in turn reduce the required dose. This would have a downstream impact on the overall efficiency and cost

1011   of the development of poorly soluble drug candidates. These savings could ultimately be passed on to the

1012   patient or reinvested in innovative drug discovery and development and could lead to earlier access to

1013 breakthrough therapies for patients. Therefore, the need for increased mechanistic understanding in the

1014 selection of PIs has never been greater.

Declarations

Conflict of interest

The authors declare that they have no conflicts of interest to disclose.

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Table 1. *Examples of PIs studied in the literature*

Inhibitor Name	Reference(s)
Eudragit® S100	*[92]*
Eudragit® E100	*[92-93]*
Poly(ethylene oxide)-*b*-poly(propylene)-*b*-poly(ethylene oxide) (*Polaxamer) (Pluronic®)*	*[94-95]*
Poly(ethylene gylcol) *(PEG)*	*[92, 96]*
Poly(ethylene imine) (*PEI)*	*[55, 97]*
Eudragit RL100	*[55, 98]*
Poly(ether)-*co*-poly(ol) (*PEPO)*	*[97]*
Poly(propylene glycol) (*PPG)*	*[55, 99-100]*
Poly(styrene) sulfonic acid *(PSS)*	*[101-102]*
Poly(vinyl pyrollidone) *(PVP)(Povidone) (Copovidone)*	*[103-109]*
Poly(vinyl acetate)-*co*-poly/vinyl pyrollidone) *(PVA-PVP)*	*[109-110]*
Hydroxyethyl cellulose *(HEC)*	*[94-95]*
Poly(methyl methacyrlate) *(PMMA)*	*[55, 100]*
Poly(lactic acid) *(PLA)*	*[92]*

Table 1. *Continued*

Inhibitor Name	Reference
Poly(vinyl acetate) phthalate (*PVAP*)	*[55[*
Hydroxypropyl methyl cellulose acetate succinate *(HPMCAS)*	*[23-24, 69, 111-116]*
Cellulose Acetate Phthalate *(CAP)*	*[116]*
Hydroxypropyl methyl cellulose *(HPMC)*	*[117-121]*
Poly(vinyl alcohol) (*PVOH*)	*[28, 55]*
Poly(acrylic acid) (*PAA*)	*[28, 55, 122]*
Poly(acetylene)	*[28, 55, 120]*
Methyl Cellulose	*[28, 55]*
Poly(lactid-*co*-glycolid) (*PLGA*)	*[55]*
Sodium Carboxymethyl cellulose *(SCMC)*	*[55]*
Chitosan	*[55]*
Poly(urethane) *(PUR)*	*[123]*
Mannitol	*[55]*

Table 1. Continued

Inhibitor Name	Reference

Inhibitor Name	Reference
Poly(glycolide) (*PGA)*	*[55]*
Locust Bean Gum	*[55, 123]*
Alginic Acid Gum	*[55]*
Hydroxy propyl-β-cyclodextrin *(HPβCD)*	*[20, 55, 119, 125-127]*
Sulfobutyl ether- β –cyclodextrin *(SBEβCD)* *(Captisol®)*	*[55]*
Sodium Dodecyl sulfate *(SDS)*	*[55]*
PEG-40 Hydrogenated Castor Oil *(Cremophor®)*	*[55]*
Poly(ethylene glycol) sorbitan monolaurate *(Tween ® 20)*	*[55]*
Sorbitol	*[55]*
Sodium Cholate	*[66]*
Sodium deoxycolate	*[66]*
Sodium chenodeoxycholate	*[66]*
Sodium lithocholate	*[66]*
Sodium ursodeoxycholate	*[66]*
Sodium hyodeoxycholate	*[66]*
Sodium taurocholate	*[66]*
	[66]
Sodium glyocholate	

Table 1. Continued

Inhibitor Name	Reference
Sodium glycodeoxycholate	*[66]*

Sodium glycochenodeoxycholate	*[66]*
Sodium glycourseodeoxycholate	*[66]*
Sodium taurodeoxy cholate	*[66]*
Sodium taurochenodeoxycholate	*[66]*

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Table 2. *Summary of experimental techniques recently applied for selection and increased understanding of precipitation inhibition*

Method	Type	Theory	Application	Limitations	Ref.
1D Nuclear Magnetic Resonance (NMR) Spectroscopy	Spectroscopic	• Individual nuclei have unique response to magnetic fields • Molecules have a distinct NMR spectrum based on their response • Changes to the environment of the nuclei can affect the response	• Structural Information • Interaction between two species • Mobility	• Molecule must be sufficiently soluble • Weaker interactions cannot be studied • No information about intermolecular bonding	136-138
Nuclear Overhauser Effect Spectroscopy (NOESY)	Spectroscopic	• Cross relaxation and magnetic transfer during spin interactions leads to NOE effects • For NOEs to occur, the two nuclei must be close in space	• Determination of intermolecular interactions	• Molecular must be sufficiently soluble • Atoms of interest must not have overlapping spectra • Resolution can be poor	139
Diffusion Ordered Spectroscopy (DOSY)	Spectroscopic	• Uses pulses to measure the speed of travelling complexes • Diffusion coefficients can be calculated	• DOESY diffusion coefficients can be correlated to size of interacting species • Can differentiate between API in solution and an API-PI complex • Orthogonal confirmation of NOE interactions	• Molecular must be sufficiently soluble • Atoms of interest must not have overlapping spectra • Resolution can be poor	140

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Table 2. *Continued*

Method	Type	Theory	Application	Limitations	Ref.
Solid State (SS) NMR Spectroscopy	Spectroscopic	• NMR Spectroscopy applied to solids • Magic angle spinning (MAS) is used to limit the effects of directionally dependent interactions, also known as anisotropy	• MAS conditions can be applied to liquids • This can be used to improve resolution of interactions	• Not common • Equipment can be hard to access	141
FTIR Spectroscopy	Spectroscopic	• Infrared light absorption produces a different vibrational response depending on chemical environment	• Structural elucidation • Determination of ntermolecular interactions	• Difficult to carry out in solution due to water's individual response to IR light • Weak molecular interactions cannot be resolved	142
UV-vis Spectroscopy	Spectroscopic	• Absorption of light in the UV-visible range produces a different vibrational response depending on chemical environment	• Quantifying concentration in combination with the Beer-lambert law • Determination of intermolecular interactions • Dissolution kinetics • Precipitation kinetics	• Weak molecular interactions cannot be resolved • Molecular must absorb in the UV/Vis range	143

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Table 2. *Continued*

Method	Type	Theory	Application	Limitations	Ref.
Raman Spectroscopy	**Spectroscopic**	• **Absorption of inelastic Raman light from lasers produces a different vibrational response depending on chemical environment**	• **Structural elucidation** • **Determination of intermolecular interactions** • **Analysis in solution**	• **Only a small proportion of light will be in the Raman range (*ca.* 10^{-8}%)** • **Weak interactions cannot be resolved**	**144-145**
Fluorescence Spectroscopy	**Spectroscopic**	• **Measured fluorescence after adsorption of light**	• **Determination of intermolecular interactions** • **Highlight changes in hydrophobicity and hydrophilicity** • **Demonstration of phase change behaviour**	• **Required a fluorescent probe**	**49**
Differential Scanning Calorimetry (DSC)	**Thermal**	• **Measures the heat input required to raise the temperature of a sample**	• **Determination of the glass transition temperature** • **Solid state characterization** • **Determination of intermolecular interaction**	• **Does not consider the effect of water** • **Cannot measure weak interactions**	**150**

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Table 2. *Continued*

Method	Type	Theory	Application	Limitations	Ref.
Synchrotron	Diffractometry	• Electromagnetic radiation emitted from charged particles accelerated in a curved path • Synchrotrons can be used as an x-ray source • Enhances the flux of x-rays, which improves the resolution	• Detection of early stage crystals	• Expensive • Equipment is not widely available	147-149
Video Microscopy	Microscopy	• Video microscopes can be combined with image analysis to record precipitation in progress	• Image analysis can calculate precipitation initiation time • Detection of early crystallization events	• Complicated set up • Not well-established	118
Atomic Force Microscopy (AFM)	Microscopy	• High-resolution microscopy • Cantilever, or tip, interacts with the surface of the sample • This interaction deflects an electron beam • The pattern of the electron bean can provide information about the sample down to the nanometer scale	• Imagine of crystal surfaces • Can be used to study polymer surface coverage	• Expensive • Equipment is not common place	49

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