Crystallisation of Aspirin via Simulated Pulmonary Surfactant Monolayers and Lung-Specific Additives

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Abstract

Pain is a prevalent condition that can have a serious impact upon the socioeconomic function of a population. Numerous methods exist to administer analgesic medication (e.g. aspirin) to the body however inherent drawbacks limit patient acceptability. The inhaled route offers promise to facilitate the administration of medication to the body. Here, we consider the crystallisation behaviour of aspirin, our *model* therapeutic agent, when in contact with material of relevance to the lung. Thus, our approach aims to better understand the interaction between drug substances and the respiratory tract. Langmuir monolayers composed of a mixed surfactant system were supported on an aqueous subphase containing aspirin (7.5mg/ml). The surfactant film was compressed to either 5mN/m (i.e. inhalation end point) or 50mN/m (i.e. exhalation end point), whilst located within a humid environment for 16 hours. Standard cooling crystallisation procedures were employed to produce control samples. Antisolvent crystallisation in the presence or absence of lung-specific additives was conducted. All samples were analysed via scanning electron microscopy (SEM) and X-ray diffraction (XRD). Drug-surfactant interactions were confirmed via condensed Langmuir isotherms. SEM analysis revealed plate-like morphology. The crystallisation route dictated both the crystal habit and particle size distribution. Dominant reflections were the (100) and (200) aspects. The main modes of interaction were hydrogen bonding, hydrophobic associations and van der Waals forces. Here, we have demonstrated the potential of antisolvent crystallisation with lung-specific additives to achieve control over drug crystal morphology. The approach taken can be applied in respirable formulation engineering.

Key Words

Inhaled drug delivery, Langmuir monolayers, aspirin, antisolvent crystallisation, X-ray diffraction (XRD) and scanning electron microscopy (SEM).

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1. Introduction

Pain is associated with a number of well-documented disease states; including for example rheumatoid arthritis, malignant disease and conditions of idiopathic origin [1]. Consequently, pain is a prevailing complaint that presents worldwide and within the United Kingdom it is estimated that thousands of people experience the issue on a daily basis [2]. The presentation of pain can result in a significant reduction in quality of life and may affect socioeconomic function [3]. Pain management is routinely achieved through the administration of analgesic medication via the oral, transdermal or parenteral routes. Although the more traditional routes of analgesic drug delivery to the body hold merit (e.g. ease of use and dose flexibility) several drawbacks do exist (e.g. discomfort at the injection site and variable bioavailability). Accordingly, interest in alternative, more patient friendly methods to administer analgesic medication to the body has gained pace; one such example is that of the inhaled route [4].

The lung serves as an effective portal for drug delivery to the body due to the large surface area for absorption, the highly vascularised nature of the organ plus the thin, moist air-blood barrier [5]. The delivery of particulate material to the respiratory tract is an inefficient process, with typically only 20% of the emitted dose reaching the lung and contacting pulmonary surfactant [6]. This notable inefficiency may be ascribed to a number of factors, including for example material cohesion / adhesion, particle size distribution along with particle morphology [7]. Thus, in order to maximise the availability of drug substance for therapeutic benefit, a formulation should exhibit the best possible combination of physicochemical characteristics (e.g. particle size (optimal range between 1µm - 5µm), shape, crystal form, solubility, bioavailability and stability). Of significance to the work presented herein, it is the morphology of the respirable material (and hence exposed chemical functionalities) that will principally govern the interaction profile between an active pharmaceutical ingredient (API) and pulmonary surfactant [8].

On delivery to the (deep) lung, an aerosolised formulation will initially interact with pulmonary surfactant [9 & 10]. This material is a complex biological mixture that lines the inner surface of the lung (i.e. the alveolar space) and reduces the surface tension term to maintain airway patency [11]. During the process of tidal breathing, variation in pulmonary surfactant physical characteristics occurs [12], which can in turn influence the processes of drug particle dissolution and subsequent absorption [13]. The principal components of pulmonary surfactant are saturated dipalmitoylphosphatidylcholine (DPPC), unsaturated phosphatidylcholines, phosphatidylglycerols (i.e. 1-palmitoyl-2-oleyl-sn-glycero-3-phosphatidylglycerol (POPG)) along with the surfactant proteins (SP) A, B, C and D [14 & 15]. Absence of this endogenous material in new born infants results in the precipitation of respiratory distress syndrome (RDS). This condition may be effectively managed by the intratracheal administration of commercially available pulmonary surfactant replacement preparations (i.e. Survanta®). Often such products are supplemented with palmitic acid (PA) to achieve a representative lipid – protein profile. The chemical structures of DPPC, POPG and PA are presented in Figure 1.

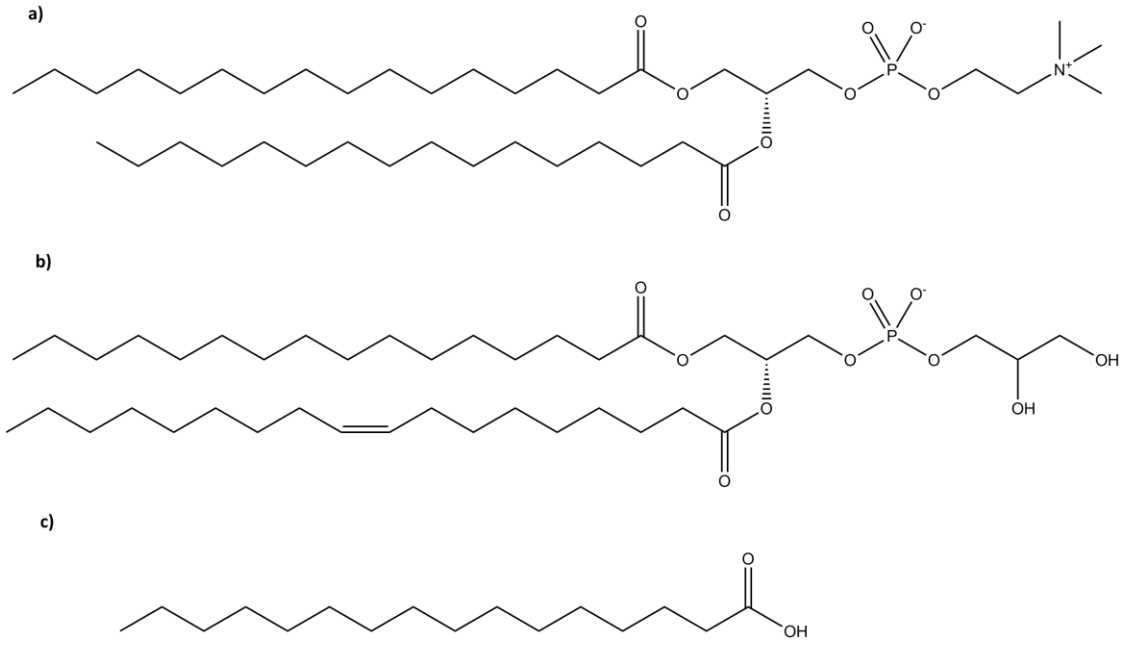


Figure 1. *The molecular structures of (a) DPPC, (b) POPG and (c) PA.*

Langmuir monolayers may be applied within the laboratory setting to investigate the structure-function activity of pulmonary surfactant [16, 17, & 18]. The experimental approach may also be exploited to stimulate epitaxial nucleation and facilitate controlled crystal growth [19]. Here, the presence of an ordered two-dimensional surface in proximity to a solubilised API can serve to reduce the activation energy necessary for crystal nucleation when compared to similar homogenous media. As such, the model may be applied to investigate the chemical complementarity between APIs / biologically relevant molecules at the alveolar air-liquid interface. Indeed, the potential of Langmuir monolayers to stimulate crystal formation was successfully demonstrated by Mu and co-workers during 2005, whereby the group employed DPPC monolayers to induce and control glycine crystal development [20]. Various operating parameters were noted to influence resultant crystal habits; including for instance the pH of the subphase and monolayer surface pressure. Flexibility in operating conditions can thus afford the formulator with scope to achieve control over crystalline properties (e.g. exposed surface chemistries).

Although Langmuir monolayers hold potential to inform understanding surrounding API nucleation and crystal growth within simulated pulmonary environments, several drawbacks (e.g. low crystal yield plus large particle size distribution) preclude use for industrial scale-up and patient end-use. Therefore, it is necessary to investigate alternative routes of crystallisation, such as antisolvent crystallisation with biologically relevant additives, to achieve controlled crystal growth and support chemical complementarity with appropriate yields. The strategy involves the addition of a second solvent (i.e. ethanol) to a supersaturated, drug-containing solution to reduce drug solubility and initiate crystallisation [21]. Typically, this approach is rapid and generates a large number of micron-sized drug particles that are suitable for delivery to the (deep) lung. This particular methodology was applied by Xie and co-workers in 2010, whereby a range of additives were used to achieve control over drug crystal size and form [22]. Here, the group crystallised salbutamol sulphate in the presence of compounds such as hydroxypropylmethylcellulose and demonstrated an influence on crystal development, morphology and size distribution. The authors linked the findings to the capability of the additives to interact favourably with specific chemical groupings on drug crystal surfaces and thus inhibit growth.

Aspirin has been selected as the *model* API for study. This agent is administered extensively via the oral route within the clinical setting to manage both acute pain and inflammation [23]. The molecule comprises of a benzene ring, carboxylic acid and acetylated phenol groupings. Here, the intention is to obtain an improved understanding as to how intrinsic chemical moieties within a drug molecule can influence the interaction with lung-specific material (i.e. DPPC, POPG and PA) and thus govern crystal presentation.

Aspirin exerts its effect by irreversibly inactivating both the cyclo-oxygenase (COX)-1 and (COX)-2 enzyme systems [24]. To date, the therapeutic compound has been subject to extensive investigation [25, 26, 27 & 28]. As such, aspirin is known to exist as two polymorphs (i.e. form I and form II) [25, 27 & 28]. Typically, form I dominates, however in 2005 Vishweshwar and co-workers solved the structure for aspirin form II, both being very similar in terms of internal molecular ordering [25, 26 & 29]. Aspirin form I exists with the monoclinic space grouping of P21/c [28] having unit cell parameters of a = 11.23(3), b = 6.54(10), c = 11.23(3) Å, β = 95.89°, V = 821.218 Å^3 [28 & 30].

This study aims to investigate the crystallisation behaviour of aspirin when exposed to simulated pulmonary surfactant monolayers and biologically relevant components of such. The work will provide an insight into the morphologies of resultant aspirin crystals and enable the determination of important functionalities at the dominant solid interfaces that may underpin the interaction with the alveolar air-liquid interface.

2. Materials and Methods

2.1 Materials

The starting material, aspirin with ≥99.0% purity (Lot: 080M0092V), was purchased from Sigma-Aldrich, UK. The surfactants DPPC (Avanti Polar Lipids, USA. Lot: 160PC-299), POPG (Avanti Polar Lipids, USA. Lot: 160-181PG-113) and PA (Sigma-Aldrich, UK. Lot: 087K1877) were of analytical grade and used as supplied. Chloroform ($CHCl_3$) (Sigma-Aldrich, UK) employed as the spreading solvent was of analytical grade (≥ 99.9%). Ethanol (HPLC grade, Sigma-Aldrich, UK) was used as the antisolvent in this work. Ultrapure water (Elga, UK), demonstrating a resistivity of 18.MΩcm, was used both during cleaning procedures and as the aqueous subphase.

2.2 Methods

2.2.1 Aspirin Batch Crystallisation

A saturated solution of aspirin with a concentration of 7.5mg/ml was produced in 20ml of ultrapure water heated to 40°C in a jacketed beaker connected to a circulating water bath (ThermoHaake DC10, USA) and subsequently stirred at 700rpm. The solution was rapidly cooled in an ice bath to 1.5°C for an hour and a half to facilitate crystallisation. The crystals were recovered via Buchner filtration and dried in an oven at 60°C overnight. The resultant crystals were later analysed via scanning electron microscopy (SEM) and X-ray diffraction (XRD).

2.2.2 Aspirin Antisolvent Crystallisation

A saturated solution of aspirin with a concentration of 100mg/ml was produced in 10ml of ethanol. Subsequently, a total of 30ml of ultrapure water (i.e. the antisolvent) was heated to 20°C in a jacketed beaker connected to a circulating water bath (ThermoHaake DC10, USA) and placed on a heating stage with a magnetic stirrer. The aspirin solution was added drop wise to the ultrapure water with vigorous stirring. The drug-containing solution was agitated for a period of 10 minutes. Resultant crystals were recovered via Buchner filtration and dried in an oven at 60°C overnight. The approach was repeated with DPPC (1% and 5%), POPG (1% and 5%) and PA (1% and 5%) in the aspirin solution.

The relatively low percentage strengths were chosen during this aspect of the study so as not to dominate the crystallisation environment (i.e. as apparent in the Langmuir trough) and also align with budgetary constraints.

2.2.3 Langmuir Monolayers

Surfactant monolayers were produced using a Langmuir trough (Model 102M, Nima Technology, UK). Surfactant free tissues (Kimtech Science, Kimberley-Clark Professional, 75512, UK) were soaked in chloroform and used to clean all the glassware and contacting surfaces. Test runs that monitor surface pressure during barrier compression were performed to ensure cleanliness. Trough cleanliness was confirmed at 0.4mN/m on complete barrier compression, in the absence of a surfactant monolayer.

A spreading solution composed of DPPC, POPG and PA in the ratio 69:20:11 was produced by dissolving the surfactant material in chloroform (1 mg/ml). In total, 10µl of this solution was delivered to the surface of the pure water subphase by drop-wise addition using a Hamilton microsyringe and left for 10 minutes to facilitate chloroform evaporation and surfactant spreading. The trough barriers were programmed to move to the centre of the trough at a rate of 30cm^2/min. Plots of surface pressure vs area per molecule (π-A) for the surfactant system at 25°C were collected using a Wilhelmy plate at the centre of the compartment.

2.2.4 Aspirin Recrystallisation beneath Langmuir Monolayers

Recrystallisation was performed using a drug containing aqueous subphase at concentrations of 7.5mg/ml aspirin at 25±1°C. The simulated pulmonary surfactant monolayer was compressed to surface pressures of 5mN/m or 50mN/m and left to stand for 16 hours to facilitate crystallisation. During collection, the inner section of the Langmuir trough was isolated then the solid material was collected and subsequently filtered via a Buchner filter.

2.2.5 Sample Characterisation

2.2.5.1 X-ray Diffraction (XRD)

To complete the XRD investigation, the aspirin crystals recovered from each system were mounted onto a quartz sample holder. Diffraction patterns were obtained using Cu radiation (λ = 1.54 Å) at a voltage of 30 kV and a current of 15 mA with an automatic, variable divergence slit (Rigaku Miniflex, Rigaku corporation, Japan). Samples were scanned from 3° to 50° 2θ. Subsequently, the data were compared with those presented in the literature to characterise the solid form [27].

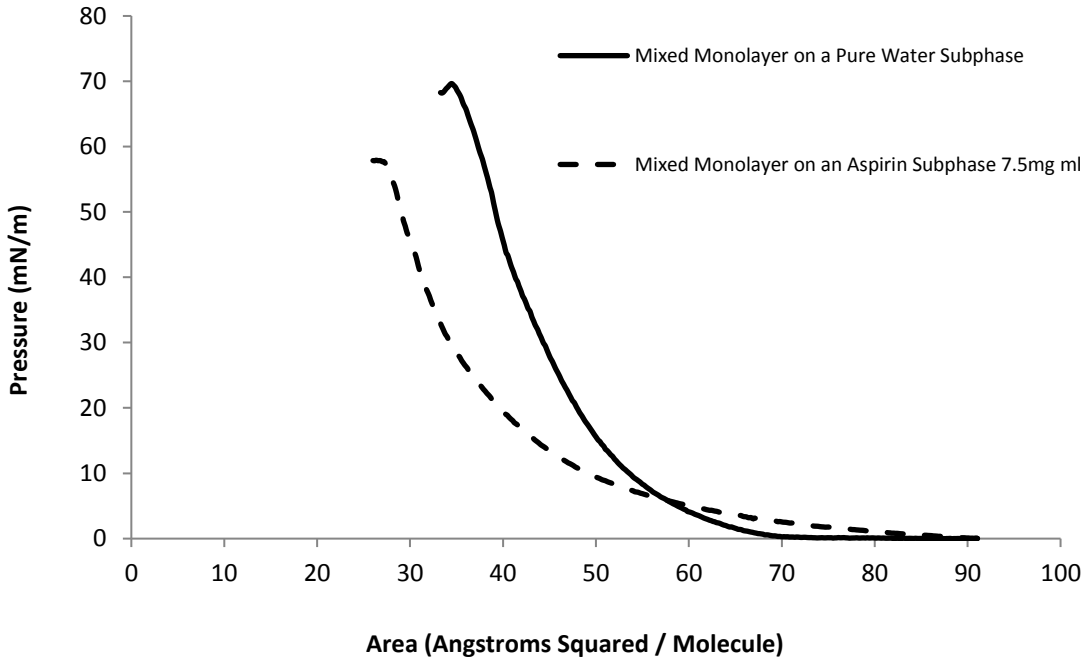
2.2.5.2 Scanning Electron Microscopy (SEM)

All samples were visualised via SEM analysis (Quanta 200 SEM, FEI, Holland). At the outset, the material was palladium coated using a K550X sputter coater (EMITECH, UK) and then scanned using an acceleration voltage of 10 kV at a working distance of approximately 10mm.

3. Results

3.1 Langmuir Monolayers

Langmuir pressure-area isotherms acquired for the mixed surfactant system when supported on either a pure water or an aspirin containing subphase are presented in Figure 2. Upon inspection, it is evident that the presence of the API within the supporting subphase did not adversely affect monolayer dynamics. Deviation between each trace does confirm aspirin – simulated pulmonary surfactant interaction.



228

229 **Figure 2.** *Langmuir π-A isotherms for the mixed surfactant systems at 25°C.*

230

231 Distinct phase changes over the course of compression are denoted by the variation in recorded gradients
232 within each trace. The limiting area per molecule for the mixed system in the absence of aspirin was
233 approximately 55Å^2. However, further to the inclusion of aspirin within the subphase, the limiting area per
234 molecule was estimated to be 42Å^2. The notable reduction in this term arises due to the interaction between
235 those aspirin molecules in solution and the surfactant molecules forming the monolayer structure [19]. The
236 result may be ascribed to the binding of drug molecules to the underside of the mixed surfactant monolayer,
237 in turn forming a condensed ensemble.

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3.2 Scanning Electron Microscopy

SEM images relating to aspirin crystals generated by various routes are presented in Figure 3. Crystalline particles formed by conventional cooling crystallisation (Figure 3a) exhibited equant morphology and were large in size (i.e. approximately 370μm x 270μm), thus precluding use for drug delivery to the lung. The samples produced via mixed surfactant monolayers at surface pressures of 5mN/m (Figure 3b) and 50mN/m (Figure 3c) displayed plate-like morphology, with bladed crystals being present in the sample generated at 50mN/m. In the case of both samples, the size of the resultant material would also not permit effective delivery to the (deep) lung; indicative sizes being 90μm x 140μm and 100μm x 390μm, respectively.

With respect to the material produced via antisolvent crystallisation, the particle size was significantly smaller. Antisolvent crystallisation in the absence of additives produced drug crystals of an irregular nature, which may be attributed to limited control over the crystallisation process (data not shown). The inclusion of lung-specific additives had a profound impact upon crystal size distribution and morphology. In terms of antisolvent crystallisation in the presence of DPPC 5% (Figure 3d), the crystalline material had a relatively smooth texture with the sample containing particulates demonstrating cohesive properties. Here, the typical size range was 35μm x 45μm. In terms of the material obtained via antisolvent crystallisation in the presence of POPG 5% (Figure 3e), plate-like morphology was apparent with some deviation in the size distribution; the representative particle size was estimated at 90μm x 55μm. Clearly, within this particular sample the particles were larger when compared to those obtained with DPPC as the lung-specific additive. Once again, the particles demonstrated cohesive properties with agglomerates clearly visible. The inclusion of PA at 5% (Figure 3f) within the antisolvent reaction vessel once more resulted in the generation of small drug-containing particulates similar to the DPPC sample; here, the typical size was 35μm x 45μm. Within this sample plate-like crystals with smooth surfaces were evident. Overall, the data indicate that antisolvent crystallisation, in combination with lung-specific additives, resulted in the presentation of drug-containing particulates that demonstrated plate-like morphology at geometric diameters within the 10's of micron size range. The route of crystallisation governed the crystal habit.

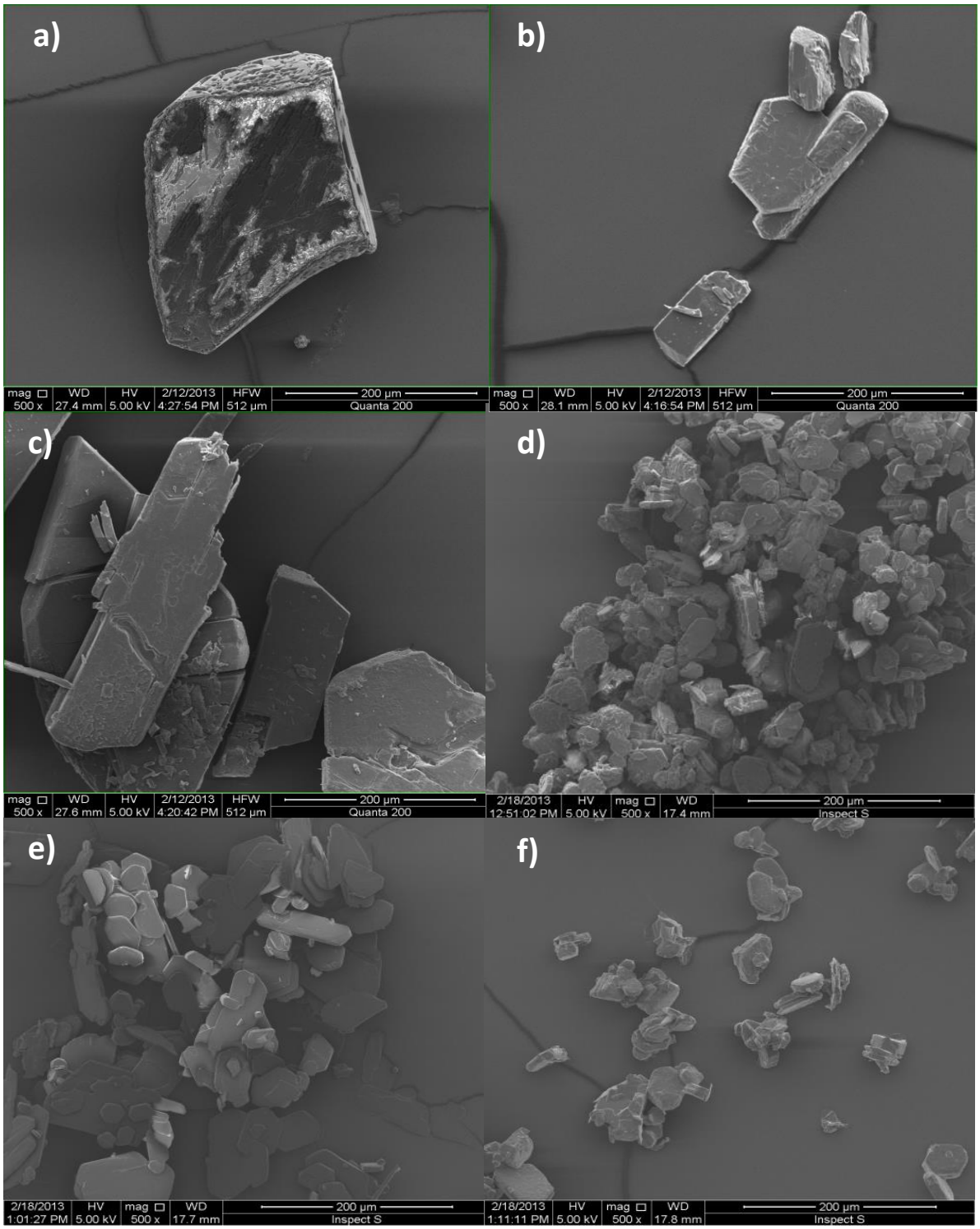

Figure 3. *SEM images of aspirin crystals produced under various study conditions: a) conventional cooling crystallisation, b) mixed monolayer at 5mN/m, c) mixed monolayer at 50mN/m, d) antisolvent crystallisation with DPPC 5%, e) antisolvent crystallisation with POPG 5% f) antisolvent crystallisation with PA 5%. Typical morpholgies included equant and plate-like. The crystallisation environment dictated gross particle morphology.*

3.3 Crystal Yield

Crystal yield data from the various systems under investigation are presented in Figure 4. Upon inspection of the data it is evident that the conventional cooling approach resulted in the smallest crystal yield. This result may be ascribed to the limited solubility of aspirin in ultrapure water under the predefined experimental conditions and confirms this route of crystal manufacture would be unsuitable at the industrial scale. The application of antisolvent crystallisation with lung-specific additives resulted in the generation of a greater crystal mass, which may be attributed to the increased capacity for drug solubilisation within each system. As the concentration of each specific additive was increased, the yield from each antisolvent system also increased.

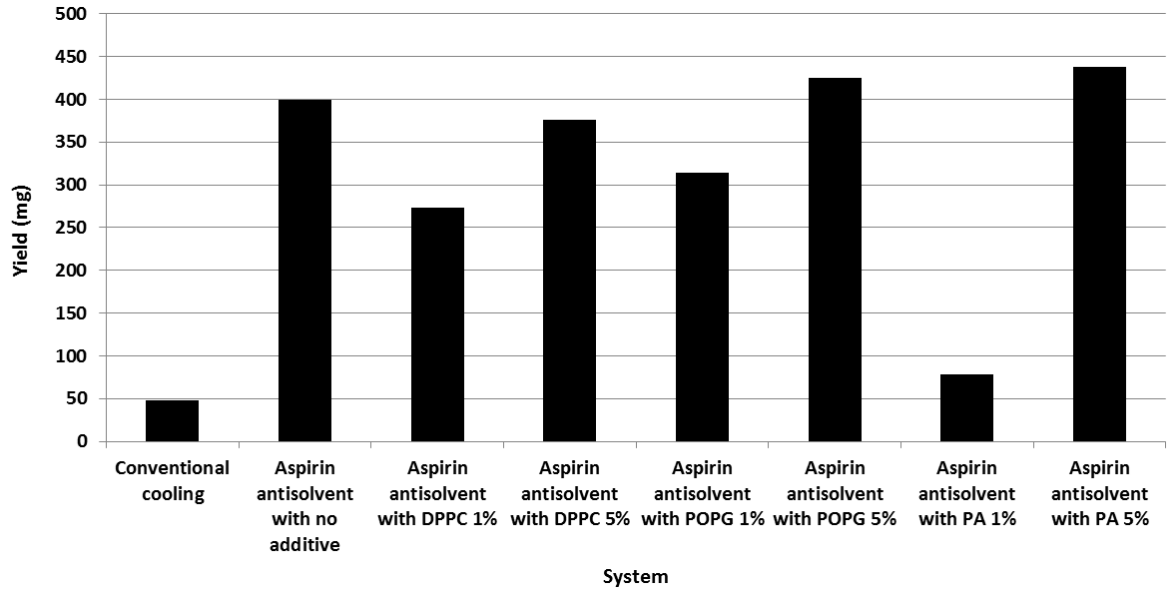

Figure 4. *Crystal yield data from the systems under investigation. The inclusion of lung-specific additives at higher percentages increased the crystal yield.*

Inhibition of crystal growth was most notable in the case of antisolvent crystallisation in the presence of PA at the 1% level, with 78.7mg of material generated. Conversely, antisolvent crystallisation in the presence of PA at 5% concentration resulted in the largest recoded yield. Of particular note is the mass obtained from the antisolvent crystallisation POPG 5% system. Here, a relatively large amount of drug-containing crystalline material (i.e. 424.7mg) was recovered. Interestingly, the XRD diffraction pattern for this particular sample reflected that obtained from the crystalline material obtained from the mixed surfactant monolayers at 5mN/m and 50mN/m.

3.4 X-ray Diffraction Analysis

The crystalline material recovered from each system under investigation was subject to XRD examination; representative traces are illustrated in Figure 5. Analysis was conducted on 'as synthesised' samples, such that dominance within the morphology could be determined; this approach has been applied in previous crystal elucidation studies [31 & 32]. All intensity values were converted to relative values (e.g. percentages) to highlight subtle changes in aspirin morphology under variable experimental conditions.

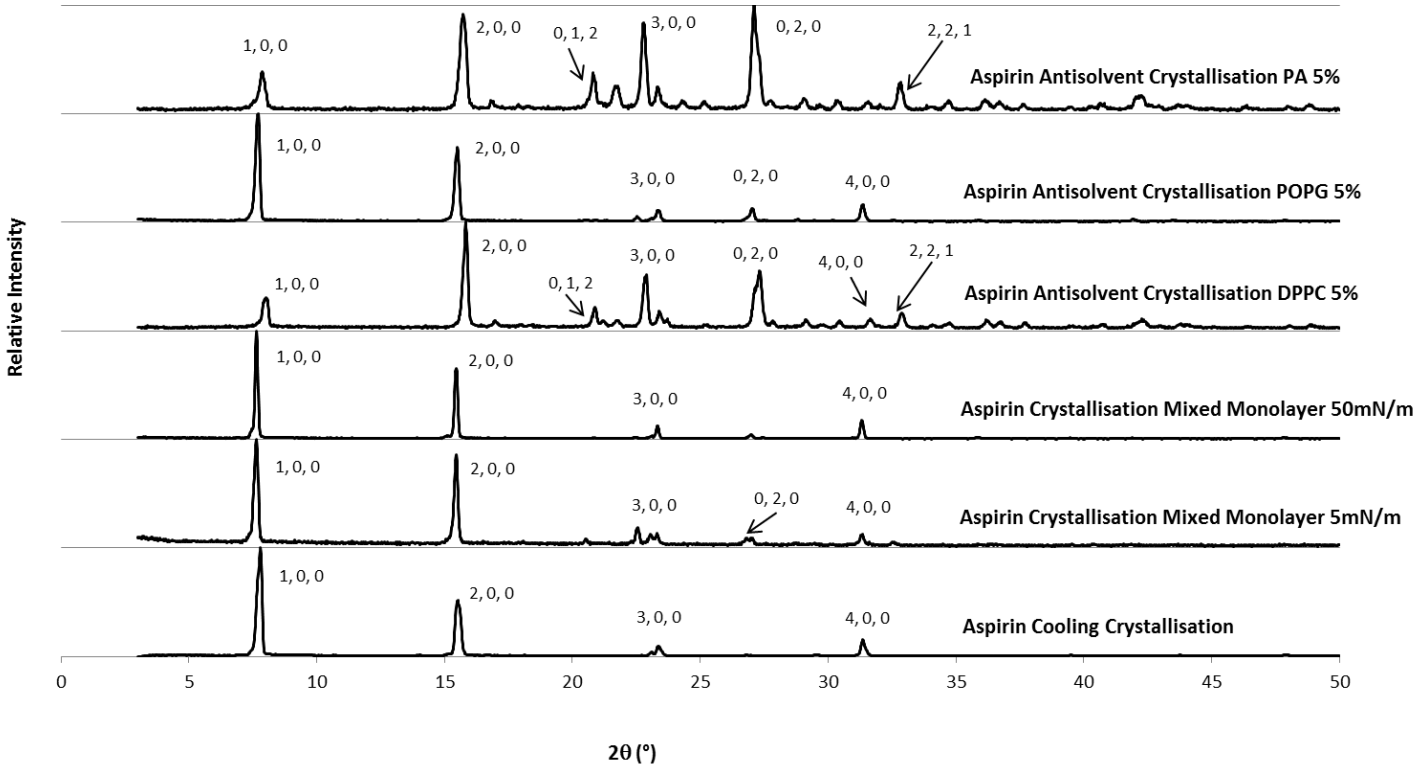

Figure 5. *XRD analysis of aspirin crystals recovered from each system under investigation. The route of crystallisation holds a significant bearing on the material acquired from each system. With the Langmuir trough crystallisation environment in mind, similarity is most evident in the case of the antisolvent system with POPG at the 5% level. Thus, the data indicate that the latter route of particle manufacture would be most suitable for material scale up to ensure internal lung surface complimentarity.*

354 The diffraction data highlight that there is a clear difference between the aspirin crystals produced under pre-
355 determined conditions. The preferred orientation noted reflects the area of the face on which the crystals are
356 lying and the morphology of the material. With respect to the conventional cooling sample, the (200) face
357 demonstrates a reflection of 52%, with the (300) and (400) facets showing reflections of 10% and 15%,
358 respectively; the XRD pattern matches those previously determined for single aspirin crystals [28]. The XRD
359 profile of the as synthesised sample by antisolvent crystallisation with PA 5% is comparable to the XRD pattern
360 for ground sample [32], this reflecting the size and morphology of the crystal.

361 The XRD patterns for the other systems show a clear difference in preferred orientation, which to a certain
362 extent reflects the difference in the morphology [31]. As anticipated, the dominant reflection presenting
363 within the sample produced via the conventional cooling method was the (100) facet. This particular reflection
364 is expected to govern the crystal morphology of aspirin form I because the material was grown in water and is
365 assigned a space grouping of P21/c [27 & 28]. The (100) reflection within the sample was applied to confirm
366 the remaining faces associated with the material. Here, additional readings at 2θ values of 15.81°, 23.83° and
367 31.9° suggest the presence of the (200), (300) and (400) reflections, respectively [27].

368 The XRD data acquired from the mixed monolayer system at 5mN/m demonstrates dominance in the (100)
369 reflection. This result may be ascribed to the conditions under which the crystals were allowed to grow (i.e. an
370 aqueous environment). An interesting point to note with this sample is the increase in percentage intensity
371 associated with the (200) face when compared to the cooling crystallisation sample. Here, the reflection
372 demonstrates 85% which represents a 23% increase. With regard to the crystalline aspirin sample acquired
373 from the mixed surfactant system at 50mN/m, it is apparent that close similarity exists with that obtained
374 from the lower surface pressure. That is to say, the (100) facet dominates the situation with the (200)
375 reflection being a close second. In this case, the (200) reflection presents with a 65% intensity, this figure
376 being 13% greater than the conventional cooling sample. The data suggest that the mixed surfactant
377 monolayers, at both low and high surface pressures, influence the reflection of the (200) face and as such this
378 face, with associated external chemistries, may indeed be important during the interaction with endogenous
379 pulmonary surfactant.

The XRD analysis for aspirin crystals recovered from the antisolvent crystallisation system with DPPC 5% as a lung-specific additive indicate that a number of reflections present (i.e. a random array of peaks are evident). Here, the (100) facet presents at 30%, the (200) face at 100%, (300) plane at 51% and (400) plane at 11% relative intensity. The application of DPPC at 5% within the antisolvent reaction vessel does not permit effective control over particle morphology to reflect the simulated pulmonary surfactant systems. On inspection of the XRD data for the crystalline material produced with POPG 5% as the lung-specific additive, it is clear that similarity exists between the diffraction data acquired for the simulated pulmonary surfactant systems at high and low surface pressure. We suggest, therefore, that this integral component of endogenous pulmonary surfactant would be suitable to guide the presentation of aspirin crystals complementary to the internal surface of the (deep) lung. Conversely, the inclusion of PA at the 5% level within the reaction vessel did not favour the production of crystalline particles demonstrating similar diffraction patterns to the mixed monolayer systems. In this case, the (100) reflection intensity diminished, whilst the (200), (300) and (020) reflections dominated within the morphology. Overall, the XRD data confirm that lung-specific additives can influence the synthesis of aspirin during the process of antisolvent crystallisation. Thus, in order to rationally engineer drug-containing particulates to support interaction with internal lung surfaces, the manufacturing process and additive must be carefully defined.

3 Discussion

During this work stable, simulated pulmonary surfactant monolayers were generated using DPPC, POPG and PA. We have previously detailed how the protrusion of related chemical functionalities into the supporting subphase may attract solubilised APIs and correspondingly reduce the activation energy required for crystallisation [8 & 18]. In order to better understand the crystallisation mechanism(s) associated with aspirin when in contact with simulated pulmonary surfactant monolayers or components thereof, consideration must be given to the underlying chemistries that govern the initial interactions between each species.

DPPC is a zwitterionic molecule that contains anionic hydrogen bond acceptors and a cationic ammonium group. The latter, which is at the termini and therefore exposed most prominently to the aqueous layer, has the potential to form strong interactions with aspirin molecules dissolved in solution. In particular, the carboxylate group arising from the dissociation of aspirin in water will associate with the ammonium group via charge-charge interaction. The polarised CH bonds of the methyl groups in the ammonium group may also interact through specific binding interactions with the carboxylate or other hydrogen bond acceptors in the aspirin. This functionality will therefore interact most favourably with the (100) face which presents the carboxylate group. By contrast, the POPG retains the phosphate oxygens but has the hydrogen bonding features of the glycerol group in place of the charged features of the ammonium group in DPPC. These groups are much better placed to interact with the acetyl group of aspirin with which they can form polar and hydrophobic interactions. This amphiphilic behaviour is one of the defining characteristics of glycerol that underpins its utility in stabilising many compounds during their crystallisation. The proposed mechanism for interaction between the species considered herein is presented in Figure 6. The PA molecule will primarily present carboxylate on the lower surface of the monolayer and is therefore unlikely to interact constructively with aspirin.

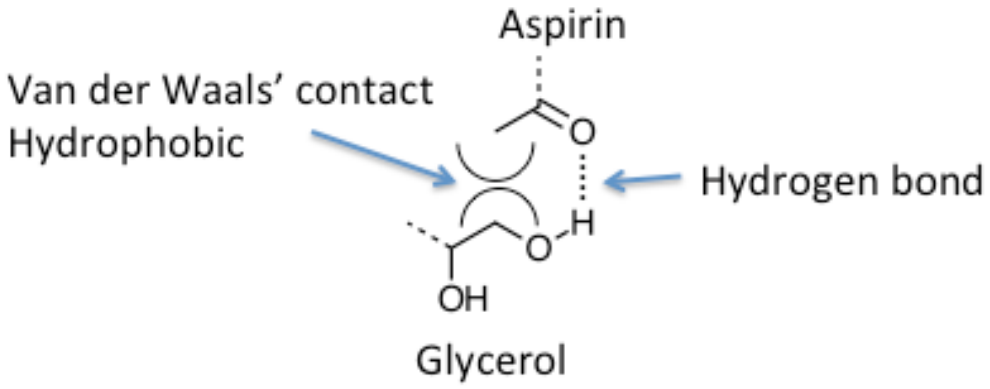

Figure 6. *The proposed mechanism of interaction between key aspirin and POPG functionalities within the simulated pulmonary surfactant system.*

With respect to aspirin crystals formed beneath the simulated pulmonary surfactant monolayers at 5mNm^{-1} and 50mNm^{-1}, it is clear that the (100) and (200) planes dominate the scenario. A similar trend was also noted in the case of those aspirin crystals recovered from the rapid cooling crystallisation vessel, although in this particular case the (200) plane was less prominent. The data confirm that both crystal faces, and associated chemistries, are important during material synthesis. The principal crystal faces identified via XRD analysis were visualised using the Mercury v3.0 software package. Here, the (100) face predominately involves the carboxylic acid group. As illustrated in Figure 7 panel a, this group is positioned across this face in a way that will maximise interactions between the carboxylic acid and solvent water. The related (200) face, illustrated in Figure 7 panel b, is dominated by the acetyl group. Specifically the carbonyl oxygen and the hydrophobic methyl group are presented. This pair of groups is likely to form attractive interactions with the glycerol head groups of the surfactant molecules, as shown in Figure 1. Glycerol is known to interact amphiphilically in this way [33].

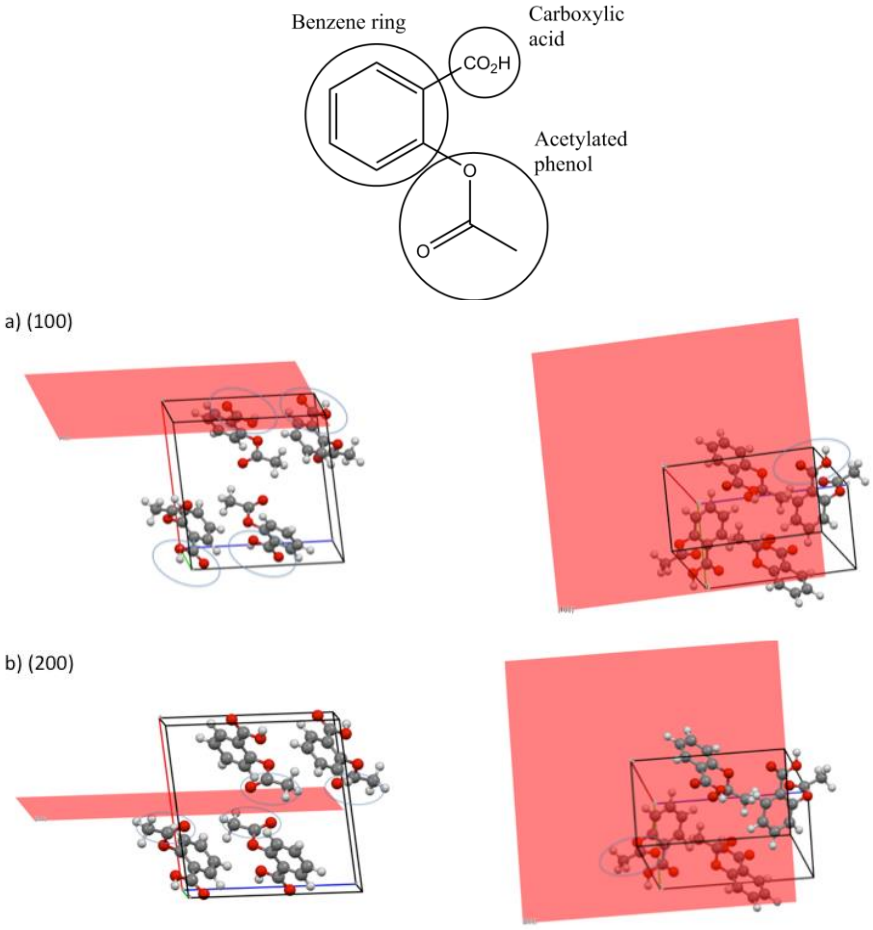

Figure 7. *The chemical structure of aspirin with key functionalities along with their presentation at the dominant (100) and (200) crystal planes.*

When considering drug particle delivery to the (deep) lung, the physicochemical changes associated with pulmonary surfactant during tidal breathing are of importance. This is so because on initial contact with internal surfaces in the (deep) lung, respirable drug-containing particles are wetted by pulmonary surfactant and the related hypophase then subsequently displaced towards the alveolar epithelium [11 & 13]. The extent of particle immersion is dependent on the surface pressure of the surfactant monolayer, with greater immersion apparent at a lower surface pressure [13]. During inhalation, the surface area of the alveoli increases, which leads to a related decrease in surfactant surface pressure; the net effect being lack of uniformity of the constituent molecules and the availability of polar head groups for interaction. Conversely, during exhalation the alveolar surface area decreases and a related increase in the surfactant surface pressure is noted. This effect results in a more ordered scenario whereby the hydrocarbon chains of the surfactant molecules are fully extended towards the alveolar lumen and the polar heads groups associate strongly with the supporting aqueous subphase. At this point, both the polar head groups and pulmonary hypophase are theoretically 'hidden' from descending drug particles.

With this in mind, during the breath holding stage of the accepted inhaler technique, rationally engineered drug-containing particulates may descend upon a pulmonary surfactant monolayer that is in the gaseous phase and interact with the polar head groups effectively. Here, we suggest that the extent of particle wetting and related immersion would be greater; hence, the time lag to reach the systemic circulation would be reduced leading to more effective systemic presence and related disease treatment (i.e. pain management). With commercially available respirable formulations in mind, there is no guarantee that such preferred external chemistries between a drug particle and pulmonary surfactant will come into contact, in effect the association between each species is essentially uncontrolled.

In general, drug particle manufacture within the pharmaceutical industry is often conducted via crude, uncontrolled crystallisation processes that typically utilise organic solvents. Further to crystallisation, a drug-containing suspension is typically filtered, dried and subjected to comminution procedures. Such stages can be costly, time consuming, inefficient and can have significant effects on powder stability, flow properties, energetics and may go so far as to destabilise the crystal structure [34]. As such, alternative methods for respirable particle manufacture have been investigated, for example spray drying [35]. Although this particular method is a one-step manufacturing process, the high temperatures that are often used prove restrictive for thermally liable compounds and often lead to amorphous particle generation [36]. As a result of such inherent drawbacks, attention is now focussed on alternative crystallisation techniques that involve single step particle production leading to material with narrow size distribution, optimal aerodynamic parameters and desirable surface properties, a prime example of this is antisolvent crystallisation.

In 2012 Park and Yeo successfully applied antisolvent crystallisation to synthesise carbamazepine-containing particulates [21]. Here, the authors considered a wide range of experimental parameters during particle generation such as solution concentration, crystallisation temperature, solution addition rate and the application of ultrasound. The data indicated that as the concentration of the carbamazepine solution was increased the resultant particle size decreased, which also held true when the temperature of the system was increased. Importantly, the group noted that smaller drug-containing particles were generated with rapid introduction of the antisolvent (10ml/min) compared to larger particles with slower introduction (1.4ml/min); the average particle size range was reported to be 62.1µm – 112.3µm, respectively. Moreover, the rate of antisolvent addition did not influence the crystal habit; hence, exposed chemical moieties remained constant. Thus, the authors demonstrate that antisolvent crystallisation can provide a route to manufacture drug-containing particulates within a narrow particle size range at the 10's of micron scale.

4 Conclusion

This study has provided opportunity to better understand the crystallisation behaviour of the commonly prescribed analgesic agent aspirin when in contact with material located at the alveolar air-liquid interface. Key crystal planes for consideration during the interaction with simulated pulmonary surfactant, and components thereof, include the (100) and (200) facets. Here, we have demonstrated that the crystallisation environment (i.e. heterogeneous nucleating surface vs antisolvent crystallisation) and the presence of additives are important in guiding the morphology of drug-containing particulate material. The understanding gained may be applied in the rational engineering of drug-containing respirable particulates for local or systemic disease management.

Whilst this exploratory study (i.e. the combination of unrelated crystallisation techniques to better understand drug chemical complementarity with the lung) was conducted under ambient conditions, potential exists to execute such work under physiologically relevant parameters via application of the lung biosimulator [37]. This new development within the field of Langmuir monolayer technology also provides the user with scope to investigate the impact of environmental toxins (e.g. cigarette / e-cigarette / cannabis smoke) on lung function [38]. Moreover, the approach has application in the dissolution profiling of orally inhaled products (OIPs) along with the implementation of *in vitro – in vivo* correlation studies.

5 Acknowledgements

MJD would like to thank LJMU for funding this research project. Special thanks go to Mr Paul Burgess and Mr Geoffrey Henshaw for technical support throughout.

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