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The impact of substrate properties on the formation of spherulitic films: a case study of salbutamol sulfate

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Abstract

Spherulitic assemblies have applications as carriers for drug delivery and as targeting vectors. Presently, the driving force behind the formation of spherulites comprising small drug molecules is not fully understood. Herein, the impact of different substrate types on spherulitic crystallization of salbutamol sulfate (SS) was investigated. Freshly cleaved mica, silicon (111) wafer (SiW), uncoated borosilicate glass (UG), silane coated glass (CG) and stainless steel (MS) were used as substrates. It was demonstrated that the spherulite growth can be controlled via the substrate selection. Spherulite formation was inhibited on hydrophilic substrates, such as mica, possibly due to stronger intermolecular interactions between SS and the substrate than SS-SS interactions. Contact angle measurements established that mica possessed the lowest contact angle ($15.1 \pm 0.5^\circ$) and the values of this parameter increased in the order of UC < SiW < CG < MS. Substrate roughness also played a key role in controlling spherulite formation. SiW, UC and CG had isotropic surfaces with low average roughness, facilitating spherulite formation. Overall, this work demonstrates that it is possible to successfully produce SS spherulites using a single step process at room temperature. Furthermore, the formation of SS spherulites can be tuned by the hydrophobicity of the substrate, an approach that could be applied to assembling spherulites of other small organic molecules.

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Spherulitic assemblies have applications as carriers for drug delivery and as targeting vectors. Presently, the driving force behind the formation of spherulites comprising small drug molecules is not fully understood. Herein, the impact of different substrate types on spherulitic crystallization of salbutamol sulfate (SS) was investigated. Freshly cleaved mica, silicon (111) wafer (SiW), uncoated borosilicate glass (UG), silane coated glass (CG) and stainless steel (MS) were used as substrates. It was demonstrated that the spherulite growth can be controlled via the substrate selection. Spherulite formation was inhibited on hydrophilic substrates, such as mica, possibly due to stronger intermolecular interactions between SS and the substrate than SS-SS interactions. Contact angle measurements established that mica possessed the lowest contact angle ($15.1\pm0.5^\circ$) and the values of this parameter increased in the order of UC<SiW<CG<MS. Substrate roughness also played a key role in controlling spherulite formation. SiW, UC and CG had isotropic surfaces with low average roughness, facilitating spherulite formation. Overall, this work demonstrates that it is possible to successfully produce SS spherulites using a single step process at room temperature. Furthermore, the formation of SS spherulites can be tuned by the hydrophobicity of the substrate, an approach that could be applied to assembling spherulites of other small organic molecules.

Keywords: spherulite, self-assembly, salbutamol sulfate, roughness, wettability, molecular interactions, nucleation.

1. Introduction

Spherulitic self-assembly is one of the intriguing properties of both inorganic and organic materials. The size of these polycrystalline, often spherical in shape, suprastructures can range from a few nanometers to a few millimeters or centimeters; thus larger spherulites can be easily viewed by the naked eye. The organized spherulitic assembly has constituted an ongoing and growing research domain since the publication of Lehmann's work on radially arranged fibrillary aggregates in 1888¹. The spherulitic assembly in organic materials is mainly observed in macromolecules, particularly polymers².

The growth of spherulites is regarded as extraordinary and anomalous, especially from the point of view of crystallization mechanisms³. One of the concepts explaining the formation of spherulites is that of growth front nucleation provided by Granasy⁴ and co-workers, who suggested that newly nucleating crystals growing at the surface have a different lattice orientation to that of the parent crystal. The formation of spherulites is linked to fast crystal growth and caused by the presence of static inhomogeneities, such as impurities and defects, critically high viscosities of systems⁵, or "non-crystallographic branching" observed in systems with lower viscosities containing no impurities⁶.

Spherulites have been shown to have several medical applications such as drug release carriers for low and high molecular weight agents with good entrapment efficiency of up to 60%⁷, targeted vectors that facilitate the internalization across specific cells when they are functionalized⁸ and also as probes to study the physiological significance of amyloid spherulites formed in Alzheimer's disease⁹. Spherulitic growth has also been observed in a number of solutions containing proteins, such as lysozyme, insulin and, most recently, interferon¹⁰. Interferon spherulites showed no protein aggregates and a significantly prolonged pharmacokinetic profile, when compared with the soluble protein formulation¹⁰.

While different morphologies of spherulitic arrangements have been reported, the driving force leading to their formation is not yet fully understood¹¹. Furthermore, Hendler et al. reported different film morphologies formed on different surfaces using diphenylalanine, which was claimed to be the shortest spherulite-forming organic biomolecule¹¹. Nevertheless, no detailed investigation of the effect of substrate nature on the spherulitic assembly was carried out.

In this work, we investigate the impact of different types of substrates, varying in surface composition, hydrophilic-hydrophobic properties and morphologies, on spherulitic crystallization of salbutamol sulfate, used as a model organic molecule. Salbutamol sulfate (SS) is an anti-asthmatic agent¹² which has been reported to adopt different crystal habits¹³ but no spherulitic morphologies. Thus this work highlights the importance of surface properties of the substrates in relation to supporting the spherulitic growth of SS, and attempts to elucidate the type and mechanism of the SS spherulite formation were made.

2. Materials and methods

2.1 Materials

Salbutamol sulfate (SS) was purchased from Camida Ltd. (Clonmel, Ireland). The substrates used were as follows: i) freshly cleaved 12 mm atomic-force microscopy (AFM) mica disc (Agar Scientific, Essex, UK), ii) silicon (111) wafer (SiW) (Ted Pella Inc., USA), iii) uncoated borosilicate glass (UG, Scientific Laboratory Supplies (SLS) coverslips No. 2, 13 mm in diameter purchased from Gerhard Menzel GMBH), iv) silane coated glass (CG) which was obtained after immersion of the SLS coverslips in Sigmacote solution, a siliconizing reagent for glass¹⁴ (Sigma-Aldrich, Ireland) for 5 seconds and v) atomic-force microscopy (AFM) stainless steel (MS) supports, 15 mm in diameter (alloy 430, product code 16218, Ted

Pella, Inc., USA). Deionized water was produced by a Millipore Elix Advantage (USA) water purification system.

2.2 Methods

2.2.1 Spherulite formation by evaporation crystallization

SS was dissolved in deionized water to form aqueous solutions at different concentrations ranging from 2.5 to 30% w/v. A drop (50 μ l) of the aqueous drug solution was drop cast on a range of substrates including: i) freshly cleaved mica, ii) SiW, iii) UG, iv) CG and v) MS. The drop of SS solution on the substrate was then incubated at room temperature (25 ± 2 °C) until complete evaporation had occurred.

2.2.2 Imaging

The growth and morphology of the salbutamol sulfate (SS) spherulites were imaged using a 9MP 2-200 x digital microscope (Conrad Electronics, Germany) and images were processed by Image J v1.46 image analysis software¹⁵. An Olympus BX35 (Tokyo, Japan) upright polarizing microscope and Lynksys 32 software were used to visualize the grain boundary of the spherulites deposited on uncoated borosilicate glass (UG) and silane coated glass (CG). The surface morphology of the films, previously coated with gold/palladium, was evaluated using scanning electron microscopy (SEM)¹⁶.

2.2.3 Solid state characterization

Powder X-ray diffraction (PXRD) patterns were measured in a Rigaku MiniFlex II, desktop X-ray diffractometer (Japan). PXRD scans, over a 2θ range of $5-40^\circ$, of the spherulites were carried out on the films formed by the polycrystals still attached to the substrates on which they were deposited¹⁷. Near infrared (NIR) spectra were collected with interleaved scans in the $10,000-4,000\text{ cm}^{-1}$ range with a resolution of 8 cm^{-1} , using 32 co-added scans using a

Buchi NIR (Flawil, Switzerland) with an NIR reflectance attachment¹⁸. Substrate spectra subtraction, normalization and baseline correction were applied.

2.2.4 Wettability studies and contact angle measurements

Contact angles for deionized water against each of the substrates were measured using the sessile drop method and imaging using a camera of 9MP 2-200 x digital. Images were processed by Image J v1.46 image analysis software¹⁵ and the contact angle was calculated based on the Young-Laplace equation¹⁹. The slope of the tangent to the drop at the liquid–solid–vapor interface line was calculated. All measurements were performed in triplicate under ambient conditions of 25±2 °C.

2.2.5 Surface roughness of substrates

The surface roughness of the substrates were measured by white light interferometry using a MicroXAM 3D (KLA-Tencor, USA) non-contact surface profilometer²⁰.

2.2.6 Molecular modelling

Bravais, Friedel, Donnay and Harker (BFDH) morphology and interaction maps were generated using Mercury[®] 3.5.1 software utilizing the refcode SALBUT01 and SALBUT02, available in the Cambridge Structural Database. The surface interaction maps were built using the Superstar approach²¹. Molecules on the surface of interest were split into IsoStar central groups and appropriate 3D scatterplots were then superimposed on the molecules of interest followed by removal of any clashing data points. Normalized density maps were then generated and overlapping maps from the same probe were combined by multiplication followed by the generation of the final interaction map^{22,23}. Herein, carbonyl, uncharged NH and aromatic CH were employed to probe the interaction maps indicative of H-bond acceptor, H-bond donors and hydrophobic interactions, respectively.

3. Results and discussion

3.1. Spherulite formation

We first observed the formation of SS spherulites under non-equilibrium conditions after water evaporation from a 10% w/v aqueous solution using a rotary evaporator at 80 °C (Figure SI.1). Observing this behavior, it was of a great interest to probe the ability of this substance to self-assemble at room temperature on surfaces with different physical and chemical natures.

For the drop casting experiments, the minimum concentration of SS in the solution required to form spherulites varied between the different types of surfaces. Figure 1 presents the various spherulitic morphologies obtained. A 2.5% w/v SS solution was sufficient for the spherulite formation on CG. MS and SiW supports required a higher concentration of SS (5% w/v) for the spherulites to form, while at least 7.5% w/v of SS in the solution was necessary for the spherulitic assembly to occur on UG. The spherulites did not form on mica at the concentrations investigated, and an increase in SS concentration up to 30% w/v led to unordered crystalline depositions (Figure 1h).

Figure 1. Images of SS spherulites formed from a 10% w/v solution: a) microscopy image on a silicon (111) substrate (SiW); b) microscopy image on an uncoated borosilicate glass (UG); c) microscopy image on a silane coated glass (CG); d) microscopy image on an AFM stainless steel (MS); e) scanning electron (SE) micrograph on a silicon (111) substrate. External layer and inner core of the spherulite can be observed; f) polarized optical image on UG; g) polarized optical image on CG; h) SE micrograph of mica support with a dried droplet of 30% w/v SS solution; j) SE micrograph on UG; k) SE micrograph on CG and l) SE micrograph on MS.

The kinetics of spherulite growth from 5 and 10% w/v SS solutions indicated that the spherulites formed faster on SiW, CG and MS compared to UG (Figures SI.2 and SI.3). At a low drug concentration (5% w/v), opened spherulites developed, characterized by structures with large widely spaced fibers diverging outwards from the central nucleus²⁴. At a higher drug concentration (10% w/v), open spherulitic centers were observed at earlier stages and continued to grow, forming closed spherulites following the complete evaporation of liquid after 4 h. The closed spherulites consist of acicular microfibers radially aggregated around the center (Figure SI.2). Figure 1 illustrates the assembly of SS on CG, SiW, UG and MS using a 10% w/v solution and the unordered assembly of SS on mica (from a 30% w/v SS solution)

indicating that the surface itself affects the assembly process. The spherulites presented in Figure 1 are composed of circular structures with well-defined grain boundaries that can be observed with the naked eye as their size ranged from 500 μm to several millimeters. A nucleation center surrounded by a fan-like assembly was observed in all spherulites grown on the surfaces investigated, except mica. Using a 10% w/v SS solution, closed spherulites were observed (Figure 1a-d). Birefringence was observed using a polarized light microscope (PLM) confirming the crystalline nature of the SS assemblies (Figure 1f-g). In addition, PL micrographs demonstrate the grain boundaries between the adjacent spherulites (Figures 1f-g). A Maltese cross was observed in Figure 1g forming with the center of the spherulite and the radiating fibrous structure. SE micrographs showed both the morphology of the nucleation center and the edge of the spherulitic assemblies. The spherulites were characterized by a 3D layered growth resulting in the formation of an apex at the center (vertical growth), which was similar for all the substrates on which the spherulites formed, while the periphery, which resulted from the horizontal growth, revealed the interaction of the lower layer with the substrate (Figure 1e and i-l). The shape of crystals at the growth front of the spherulites was dependent on the nature of the surface. The spherulites grown on SiW exhibited a characteristic, well defined, feather-like pattern (Figure 1e and i), whereas spherulites formed on MS displayed a less ordered edge growth (Figure 1l). The lower layer of the spherulites on UG covered a larger surface compared to the coverage of CG (Figure 1j and k). External dendritic layer of the spherulite was observed on UG substrates (Figure 1j) whereas spherulites characterized by external layer with flat edges and internal fish scale layer were shown on CG substrates (Figure 1k). Also, the spherulites grown on CG exhibited shorter, but smoother, growth front compared to those formed on UG.

3.2 Properties of the substrate

In order to correlate the nucleation and spherulite growth with the surface properties, the hydrophilicity and surface roughness of the substrates were examined. Wettability studies using contact angle (θ) measured against deionized water provided an indication of the hydrophilicity of the surfaces, where the smaller the contact angle the higher the hydrophilicity of the surface (Figure SI.4). Mica possessed the lowest contact angle of $15.1 \pm 0.5^\circ$ and the values of this parameter increased in the order of $UG < SiW < CG < MS$ (θ values in Figure 2). The formation of spherulites was hindered when the substrate was more hydrophilic, possibly due to the stronger intermolecular interactions between SS and the substrate than interactions between the molecules of the drug (SS-SS). This may explain why spherulites were not formed on mica. Ion-exchange reactions usually take place on the surface of freshly cleaved mica²⁵. Mica is negatively charged and ionic interactions with the tertiary amine of SS may occur during the deposition stage promoting interactions between SS molecules and the substrate, preventing spherulite formation²⁵. The three major components of UG are silicon, boron and sodium oxides, resulting in the relatively low contact angle of this substrate with deionized water and thus the inability of the spherulites to form on such a surface using low SS concentrations²⁶. In contrast, the chlorinated organosiloxane used to prepare CG interacts covalently with the silanol groups of glass to produce a water repellent film, thus inhibiting interactions with a SS solution^{14,27}. Similarly, the SiW (111) surface is terminated by SiH groups, rendering it hydrophobic. It should be highlighted that the SiW (100) plane is slightly more hydrophobic in nature than its (111) counterpart due to the presence of SiH₂²⁸. MS is non-hardenable chromium stainless steel composed of iron, 16-18% chromium and up to 1% manganese and silicon each imparting its hydrophobic character²⁹.

Apart from the hydrophile-hydrophobe properties, the surface roughness also played a key role in the assembly of spherulites. SiW, UC and CG exhibited isotropic surfaces with a low

average roughness compared to MS. MS was the only substrate investigated that exhibited anisotropic topology³⁰ (showing a striated pattern) with a greater average deviation of the peaks and valleys in the measured surface profile, as observed in Figures 2 and SI.4. During the film deposition stage, SS solution could penetrate along the valleys increasing the number of contact points and friction between SS and the substrate leading to a higher number of nucleation centers (static homogeneities) (Figure SI.3). This can explain the irregular edges of the spherulites observed in the SE micrographs (Figure 11) and the superior adhesion (qualitatively determined as the force required to detach the spherulite from the substrate surface) observed between the spherulite and the substrate. However, at this point it is also important to bear in mind the substrate hydrophilicity as it could also affect the solvent-substrate interaction. If the MS substrate was very hydrophilic, the penetration of the SS solution along the valleys would be much higher which probably would hinder the formation of the spherulites. This could explain why the spherulites were formed onto MS substrate despite its higher surface roughness.

Figure 2. 3D surface mapping profiles of different substrates along with roughness and contact angle measurements: a) SiW, b) UG, c) CG and d) MS. S_a =average roughness, applies to 3D area, S_{min} =value of the lowest point in the area measured, S_{max} =value of the highest point in the area measured and θ =contact angle using deionized water.

3.3 Intermolecular interactions

Mercury[®] 3.5.1 software was used to calculate the Bravais, Friedel, Donnay and Harker (BFDH) SS crystal morphology and a full interaction map of the SS molecule to get an insight into the possible interactions during the spherulite formation process. The results are shown in Figure 3 along with the powder X-ray diffraction (PXRD) patterns of the spherulites.

Figure 3. Molecular interactions involved in the SS spherulites growth: (a) predicted BFDH crystal morphology of SS showing the hydrophobic nature of the (002) and (200) facets bearing functional groups with H-bond interaction capability; (b) full interaction map showing the functional groups involved in the H-bond and hydrophobic interactions of the SS molecule with its surroundings, (c) PXRD pattern of powder SS starting material and SS spherulites on different substrates formed using solutions with 10 and 20% w/v SS concentration and (d) NIR spectra of spherulites deposited on CG (2.5 to 20% w/v SS) and UG, MS and SiW (20% w/v SS).

The positions of the Bragg peaks of the spherulites were in agreement with those of the simulated powder patterns (using SALBUT01 and SALBUT02, available in the Cambridge Structural Database). The SS molecule is capable of interacting through hydrogen bond formation, both donor and acceptor, and hydrophobic interactions such as π - π stacking. The BFDH simulation, with the H-bonds included, indicates that the (002) facet has the hydrophobic groups exposed and the (200) facet has the hydrophilic groups available, with the potential to form hydrogen bonding necessary for spherulitic growth. In addition, the (400), (600) and (800) planes are parallel to the (200) facet (seen in the UG PXRD patterns, Figure 3c), thus they are expected to exhibit a higher surface area when interacting with a hydrophilic substrate. However, the intensity of the PXRD peaks varied depending on the concentration and type of the substrate, indicating a different preferred growth orientation. Bragg reflections (hkl) with zero second and third Miller indices (k and l) of SS grown on UG exhibited higher intensities, which could be related to the longer and wider edges of the lower layer of spherulites observed by SEM.

NIR analysis was performed to probe the molecular interactions in the spherulites and spectra were acquired for the spherulites deposited on each substrate (Figure 3d). Similar bands were observed for all the spherulites, but with apparent differences in intensity. The increase in SS concentration was associated with a concomitant increase in absorbance of the bands in the 6,600–7,200 cm^{-1} range and at around 4,750 cm^{-1} , assigned to the first overtone of the OH stretching vibration and OH stretching/bending combination, respectively. This increase in intensity indicates that the H-bond formation is responsible for the spherulitic assembly. Moreover, the intensity of the abovementioned peaks was lower for the spherulites grown from a 20% w/v SS solution on UG compared to those formed on other substrates due to the more hydrophilic nature of UG. This further supports the earlier hypothesis of the impact of hydrophilicity of the substrate on the spherulitic formation: the higher the hydrophilicity,

such as for mica, the less chance for spherulites to assemble. This is because SS will preferentially form H-bonds with the substrate, inhibiting the spherulitic assembly. In addition to the H-bonding interaction, an aromatic C-H first overtone stretching at around $6,000\text{ cm}^{-1}$ was observed in spherulites grown on all substrates, which could be correlated with the π - π stacking within the assembly.

3.4 Mechanism of spherulitic formation

Apart from geological materials (like oxides, alloys and metallic glasses), spherulites can be found in organic materials, mainly macromolecules such as polymers and proteins, which are relatively large structures. To the best of our knowledge, SS is the smallest organic drug molecule that has been reported to form spherulitic assemblies. The formation of spherulites commonly occurs under non-equilibrium conditions. The growth of spherulitic assemblies has been frequently reported to be caused by rapid crystallization from solutions containing high solute concentration or crystallization by solvent evaporation at high temperatures, whereas slow crystallization of the same solutions was reported to lead to the growth of dendrites in many cases³¹. However, we have shown that spherulites can be formed at milder non-equilibrium conditions (in a single step at room temperature and organic solvent-free process) compared to what has been previously claimed to be a mild conditions (temperature of $60\text{ }^{\circ}\text{C}$) for the formation of spherulites^{11,32}.

SS spherulites grew radially from the nucleation site branching intermittently to maintain a space filling growth (Figure SI.2), characteristic of category 1 spherulites⁵. We propose that homogeneous primary nucleation of single crystallites is combined with heterogeneous nucleation due to roughness of the surface of the substrate, which can explain the reason for the observed higher number of nuclei on the MS substrate, exhibiting an anisotropic surface³³. At the edges of the growth front, however, secondary misoriented nuclei can grow due to the milder non-equilibrium conditions utilized in the manufacture of the spherulites.

Their growth can be enhanced because the free perimeter of the growing spherulite faces a continuous supply of SS from solution³⁴.

Based on our findings, the SS spherulitic formation mechanism is driven by the slow evaporation process that leads to local rises in SS concentration in the aqueous medium triggering the drug deposition under non-equilibrium conditions (Figure SI.6). The deposition stage depends on the interactions between the drug molecules and the substrate involving both H-bond and π - π interactions. During the evaporation stage the fate of individual crystals within an aggregate is determined by the crystal orientation with respect to the substrate surface and the growth rates. It has been reported that at a faster growing rate, crystals will be easily oriented to the aggregate interface and the spherulite assembly has a better chance of formation³¹. However, it is crucial to inhibit the interactions between the assembling molecules and the substrate in order to facilitate the molecule grouping from individual crystals to the spherulitic arrangement, especially at lower growing rates under mild non-equilibrium conditions. 2D spherulitic assemblies of small organic molecules could potentially be applied as a transdermal technology³⁵⁻³⁶, where SS can permeate across the skin through passive diffusion³⁷. One of the advantages of using spherulites is that they possess an enhanced mechanical strength compared to their non-spherulitic deposits³⁸ which indicates that the growth of SS spherulites on the surface of different substrates employed in the fabrication of mucoadhesive transdermal or buccal patches could be used to develop drug delivery systems for the control of pulmonary diseases.

4. Conclusions

The current work shows that the growth of SS spherulites can be controlled by selecting a suitable surface. Substrates which were morphologically isotropic and hydrophobic in nature were superior in supporting the growth of spherulitic assemblies consisting of the model polar drug. It may be assumed that the opposite will apply and hydrophilic substrates will be

more suitable to growing spherulites of hydrophobic materials. SS spherulites described in this work were successfully produced using a simple and mild process. This process of spherulite preparation could be applied to other organic molecules, which may be useful when designing core technological applications, such as biosensors and biocoating materials and developing drug delivery systems which could facilitate the transport across biological membranes, not only of the drug that form the spherulite itself but also of other molecules that could be encapsulated within the cavities of the spherulites.

The authors declare no competing financial interest.

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The impact of substrate properties on the formation of spherulitic films: a case study of salbutamol sulfate

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Synopsis

This work describes the formation of salbutamol sulfate (SS) spherulites. The spherulitic growth was controlled via the substrate selection and was inhibited on hydrophilic substrates. Substrate roughness also played a key role in controlling the spherulite formation with isotropic surfaces facilitating the spherulite assembly. It was possible to produce SS spherulites using a single step process at room temperature.

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