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Amorphism and thermal decomposition of Salicylsalicylic acid - a cautionary tale.

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Abstract

Salicylsalicylic acid ('Salsalate') is a non-steroidal anti-inflammatory drug (NSAID) with anti-rheumatic properties, whose amorphous form offers the potential for enhanced dissolution rates and improved bioavailability compared with its crystalline counterpart. It has been reported to form a stable glassy phase on heating and rapid quenching. A number of the existing studies of the solid-state structure of salsalate and of its thermal decomposition contain information that is difficult to reconcile. In this manuscript we review much of the existing literature in light of our own recent studies using solution-state NMR, Mass Spectrometry and solid-state IR, and conclude that much of the literature data relating to melting and the glassy state is questionable due to failure to take into account the effects of thermal decomposition.

Introduction

In the last fifteen years or so interest in the amorphous state of APIs and, ultimately, new medicines has risen dramatically. Poor water-solubility of drugs, when produced in the crystalline state, has long been a potential issue, and production of an amorphous phase/drug offering enhanced dissolution behaviour and ultimately significantly enhanced bioavailability is seen as a viable approach to solving solubility issues. [1] A review considering all aspects of the risks and advantages of developing an amorphous API/drug was produced by Craig *et al*. [2] The use of different preparative techniques for generating amorphous materials is also a factor that requires careful understanding and consideration. [3] For example, and in the experience of one of us, cefuroxime axetil developed by Glaxo in the 1980s was produced by a high-temperature spray drying process to produce material that was both chemically and physically stable. Material produced by say, milling, whilst chemically stable lacks the physical stability of spray dried material as the elimination of seed crystals is difficult to ensure.

The Cambridge Structural Database [4] contains two reports of structures for salsalate [5, 6] that are essentially in agreement. They both report a structure that is disordered between two conformers in a 72:28 ratio (Figure 1). There is also some evidence for a polymorphic form but, to date, this has not been isolated free of the usually observed form, which corresponds to the commercially available material.

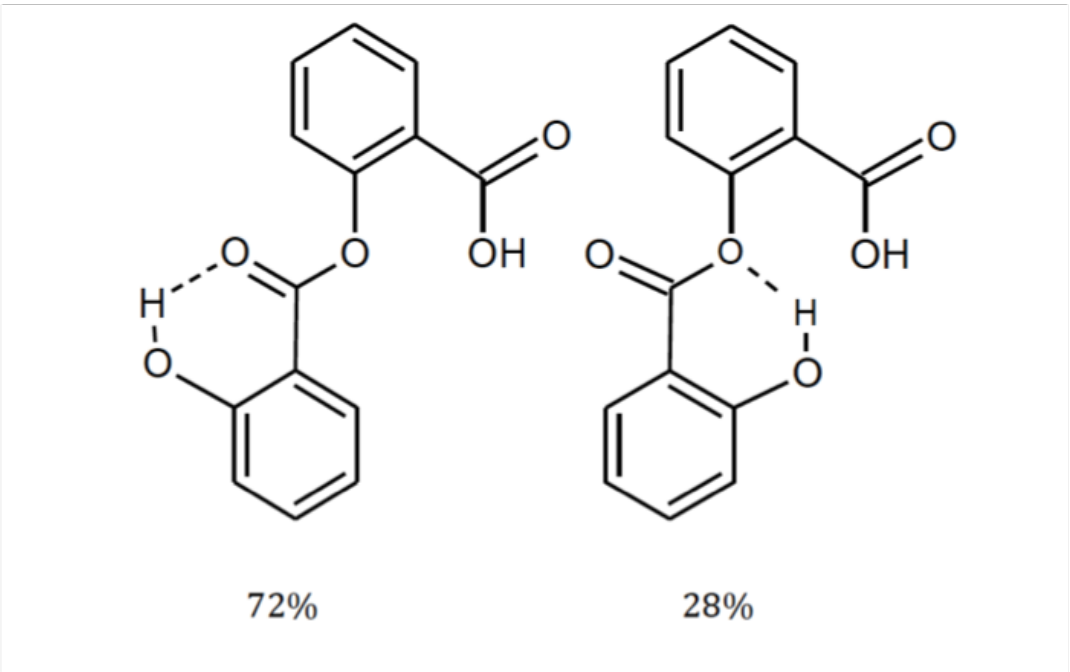

Figure 1 Conformers of Salicylsalicylic acid found in the disordered crystal structure

In addition to the crystalline form there is an often-reported amorphous (glassy) form, which can supposedly be reliably produced by heating a crystalline sample to its melting point and then quenching it by rapid cooling. Its properties are that it is a clear glass that can be moulded and that stress fractures at room temperature. [5, 7] There has also been a report of evidence for a nematic phase close to the melting point, [7] and it has recently been the subject of computational studies looking at the amorphous form to evaluate the possible utility of Crystal Structure Prediction (CSP) in relation to such materials. [8]

In view of the relative ease with which the glassy amorphous phase can be produced and its reluctance to subsequently recrystallize without dissolution and re-precipitation, it has been considered by a number of workers as a model compound for a stable amorphous phase. Recently, Graeser *et al*. [9] carried out a correlation of thermodynamic and kinetic

parameters with amorphous stability, and salsalate was shown to be a strong glass former, and the least 'fragile' (ie least prone to spontaneous crystallisation) of 12 model compounds chosen for the study.

Results and discussion

The starting point for our study was a preliminary investigation in the solid state using ^{13}C CP/MAS spectroscopy to look at what was supposed to be a sample of amorphous salsalate produced by heating a sample of the commercial material to its melting point (or slightly above) and then rapidly cooling it to room temperature. The ^{13}C CP/MAS spectrum (not shown) showed many more signals than could be accounted for by the molecular structure, which agreed with the results reported by Greener *et al*. This was initially attributed by Greener *et al*. [5] to the presence of a wide variety of conformers and/or hydrogen bonding patterns in the amorphous material, although this claim was later retracted in a corrigendum [Angew. Chem. Int. Ed., **39**, 3736, (2000) - N.B. not available in electronic format].

We investigated this 'amorphous' sample further by running solution-state ^{1}H and ^{13}C NMR in $CDCl_3$, both of which showed many more peaks than could be accounted for on the basis of the molecular structure of salsalate, suggesting that the sample contained a number of closely related, but distinct, molecular species, rather than a range of conformers. By comparison of the chemical shifts with the spectra of genuine samples it was shown that some of the peaks observed corresponded to the starting material (salsalate), and that another group of peaks corresponded salycilic acid. Further NMR studies showed that thermal decomposition of salsalate and formation of oligomers began at temperatures below the melting point, both in the solid-state and in solution in high-boiling point solvents such as dimethyl sulfoxide (DMSO) and tetrachloroethane (TCE).

The signals in the ^{1}H spectra of the mixture showed coupling patterns in groups of 4, each group showing the expected coupling patterns for an ortho-disubstituted phenylene ring with non-equivalent substituents. Using Constant Time Total Correlation Spectroscopy (CT-TOCSY) experiments it was possible to identify numerous sets of such groups of 4 signals and group them together by reference to the sizes of the integrals on the 1-dimensional ^{1}H spectrum (Figure 2).

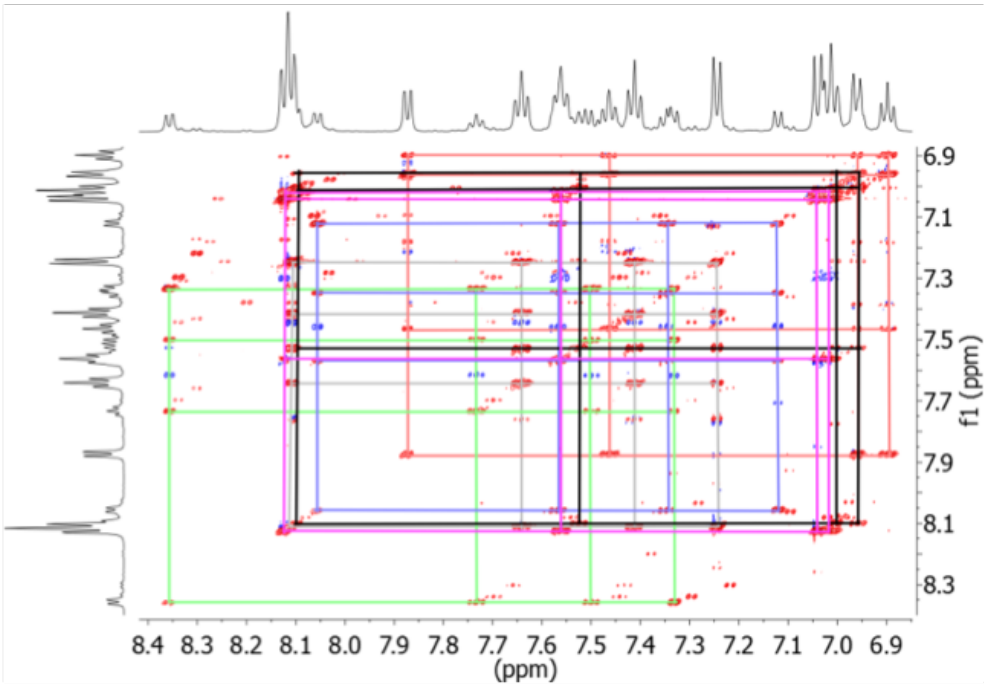

Figure 2 ^{1}H- ^{1}H CT-TOCSY spectrum of a heated sample of salsalate, with assignment of the 4x4 grids corresponding to the individual ring systems of salicylic acid (red), salsalate (grey and pink), and the linear trimer of salicyclic acid (blue, green and black)

Using this method, it was possible to tentatively assign signals due to the linear trimer and linear tetramer of salicyclic acid, with a significant number of lower intensity peaks unassigned, which were presumed to be due to longer oligomers. These tentative

assignments were all shown to be consistent with a range of other two-dimensional experiments, such as CT-COSY, HSQC, HMBC, and NOESY. Linear tetramers of salicyclic acid have been previously reported [10] with subsequent conversion to cyclic species at higher temperatures (> 300 °C).

To investigate further, previously heated samples were analysed by LC-MS. It proved possible to resolve a significant number of species corresponding to oligomers of salicylic acid up to the order of n=12. A typical chromatogram is shown in Figure 3 for the deprotonated molecules. Here the extracted ion chromatograms for $[M_n - H]^-$ where n = 1 to 12 clearly demonstrate the increase in retention time as the larger oligomers elute. In many of these chromatograms, the multiple occurrence of some *m/z* values at more than one retention time is a reflection in the labile nature of these ions. In-source dissociation occurring during or shortly after the electrospray process causes sequential fragmentation with the loss of discrete monomer units (n=1).

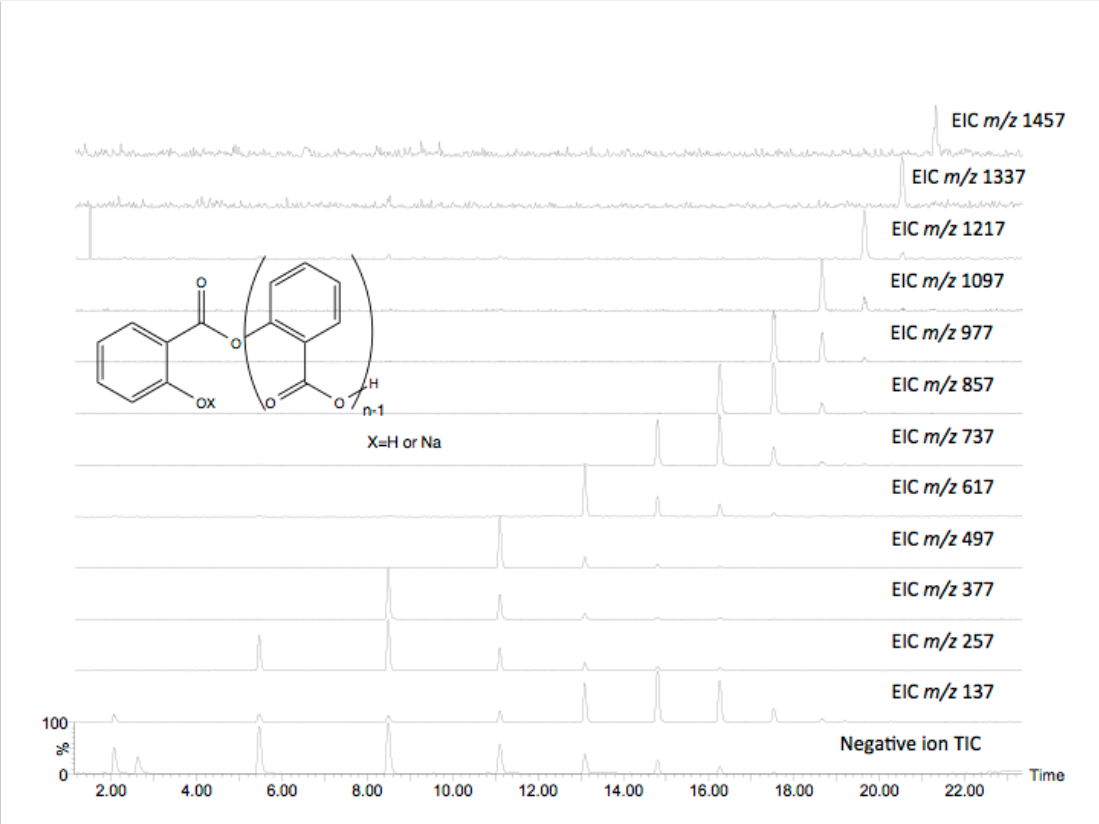

Figure 3 Total negative ion chromatogram (TIC) and extracted ion chromatograms (EIC) for the $[M_n\text{-}H]^-$ where n=1-12 from bottom to top

For each oligomer eluting, the mass spectral evidence weighs heavily toward open-ended or 'linear' structures. The negative ion mass spectrum shown for the tetramer (Figure 4a) clearly shows $[M_4 - H]^-$ at *m/z* 497 (x=H) and the sodium equivalent (x=Na) at *m/z* 519 which can only be accounted for by open-ended or 'linear' ions which then undergo dissociation by loss of sequential monomer units (n=1), similar to observations made by Shulga *et al*. [10] The open-ended or linear nature of the molecules is further supported by the positive ions $[M_4 + NH_4]^+$, $[M_4 + Na]^+$, $[M_4 + K]^+$ observed at the same retention time. (Figure 4b).

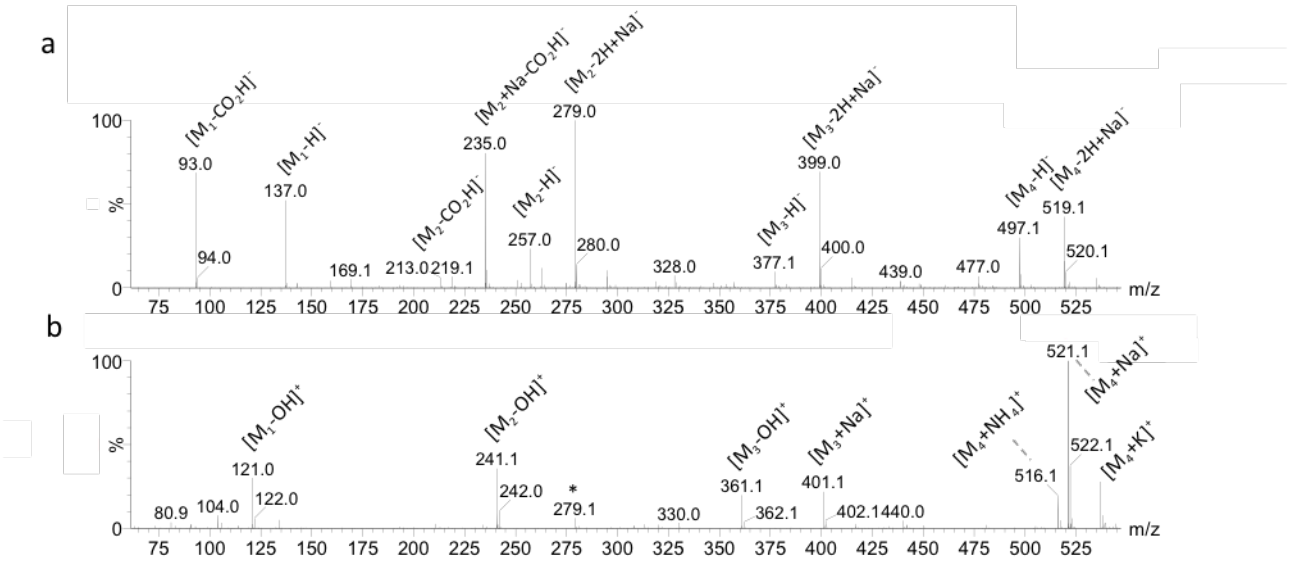

Figure 4 a) negative ion mass spectrum and b) positive ion mass spectrum for the tetramer. *indicates known solvent impurities.

A low intensity peak at *m/z* 481 in the positive ion mass spectrum is assigned as $[M_4 - OH]^+$ resulting from the loss of water from the protonated species. Although too low in intensity to easily be seen in Figure 4b, the isolation and collision-induced dissociation of *m/z* 481 shown in Figure 5b demonstrates sequential neutral mass losses of 120 Da corresponding to $C_7H_4O_2$ from $[M_4 - OH]^+$ to give $[M_3 - OH]^+$, $[M_2 - OH]^+$ and $[M_1 - OH]^+$, the relative intensities reflecting the relative stabilities of each ion, and confirming assignments made from the full scan data (Figure 4b). The exact match in retention time to the linear deprotonated tetramer indicates only one molecule is eluting at this time that, when ionised, follows a fragmentation mechanism that would require the cleavage of only one bond, not the two that would be required for a cyclic species.

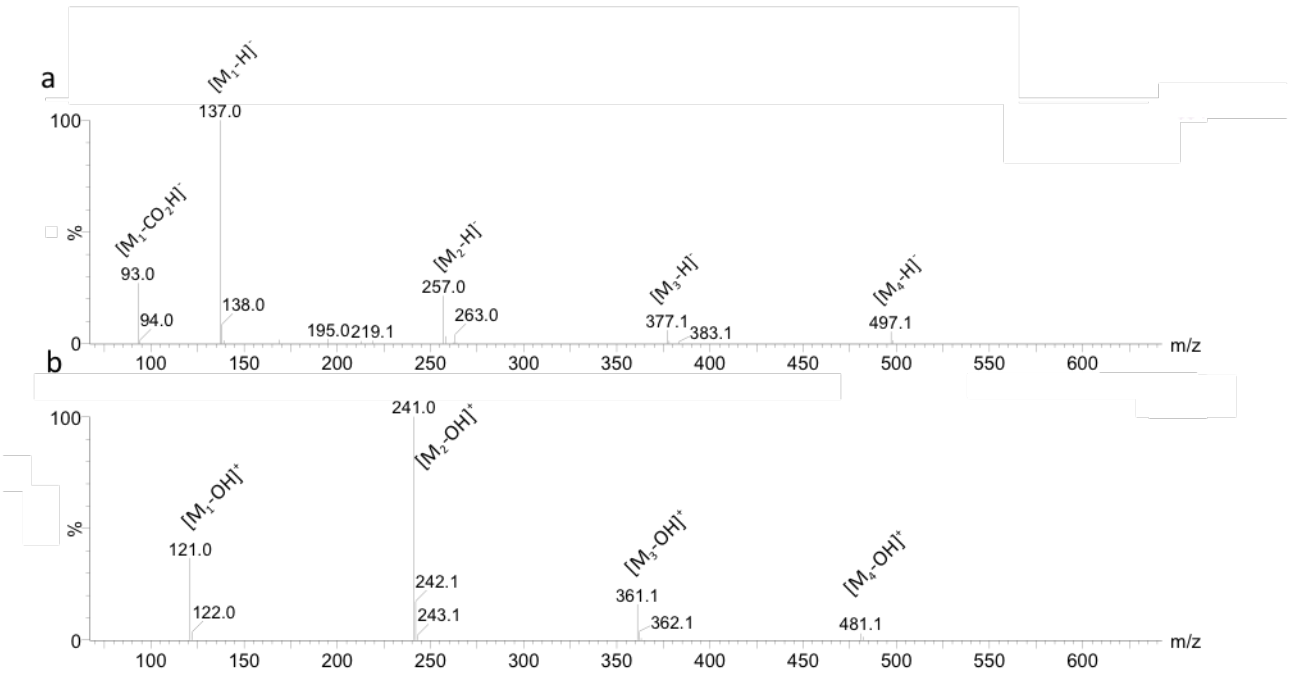

Figure 5 Tandem mass spectra for a) $[M_4 - H]^-$, b) $[M_4 - OH]^+$.

There is no evidence of a series of molecules corresponding to a cyclic structure; for the tetramer the deprotonated molecule would appear at *m/z* 479 if there were 'reasonable' protons to remove, and the positive ion adducts would appear at *m/z* 498, 503 and 519 respectively for the ammonium, sodium cation and potassium cation. The cyclic nature of such a molecule and its aromaticity may be reasonably expected to facilitate alkali cation adduction. [11] Cyclic species have been prepared previously [12] and observed as thermal decomposition products of both salicylic acid and acetylsalicylic acid (aspirin) [13, 14] but only at much higher temperatures (> 300°C). We therefore decided to investigate more closely the peaks that appeared as possible cyclic species using ion mobility measurements. [15] Our results showed that the species identified as 'cyclic' by Shulga & Dunn have essentially the same collisional cross section (CCS) as the corresponding linear oligomer (sodium adduct), which provides the final evidence to suggest that the species is not cyclic, but is produced by loss of water in the mass spectrometer.

Subsequently, based on the favourable LC-MS results, preparative HPLC was undertaken to try to obtain pure samples of some of the components of the mixture. It was only possible to obtain reasonably pure samples of the first four components in quantities suitable for further NMR investigation, but these were readily confirmed as salicylic acid, salsalate, the linear trimer and linear tetramer of salicylic acid respectively. Further supporting evidence was obtained from NMR diffusion measurements using DOSY [16] experiments on samples corresponding to the first three peaks eluted from the preparative HPLC. Values for the diffusion coefficients of 4.91×10^{-10}, 3.05×10^{-10}, and 2.69×10^{-10} m^2 s^{-1} were obtained. The first two were shown to correspond to the diffusion coefficients of known samples of salicylic acid and salsalate respectively. The monotonic decrease observed is the behaviour expected for the increasing hydrodynamic radius of oligomers where n = 1 to 3. [17] Importantly, the diffusion measurements on the third eluting peak also confirmed that all of the peaks observed in the NMR spectrum of that sample came from the same molecule.

These results suggest that thermal decomposition of salsalate leads to the formation of linear oligomeric species of the form shown in Figure 3 with n in the range 1 to 12, but with the bulk of the material in the range n = 1 to 4. Many additional minor signals are also visible in the aromatic region of the 1H NMR spectrum, but it was not possible to make detailed assignments because of the extensive peak overlap. However, by adjusting the pH of the sample and the temperature of the NMR experiment [18] it was possible to slow the rate of proton exchange for the phenolic -OH groups such that relatively sharp lines could be observed for the -OH protons and this confirmed the presence of at least eight distinct species. Obviously, mass spectrometry with its far higher sensitivity is better at detecting minor components of the mixture.

Further investigations were carried out to determine whether the reaction(s) involved were specific to the solid state. Samples were heated to specific temperatures for specified periods of time in the solid state and the resulting products were subsequently dissolved in high-boiling NMR solvents such as tetrachloroethane-d_6 (TCE) and DMSO-d_6. NMR spectra from these samples were compared with those from samples of salsalate dissolved in the same solvents and then put through the same heating process (but this time already in solution). While differences in the populations of products were observed, no signals due to new species were observed, nor was there conspicuous absence of any species. It was therefore concluded that the same thermal decomposition processes occurred both in the solid and/or melt, and in solution. Further experiments were conducted to investigate both the time course of the decomposition reaction(s) and their temperature dependence. Typical results are shown in Figures 6 and 7.

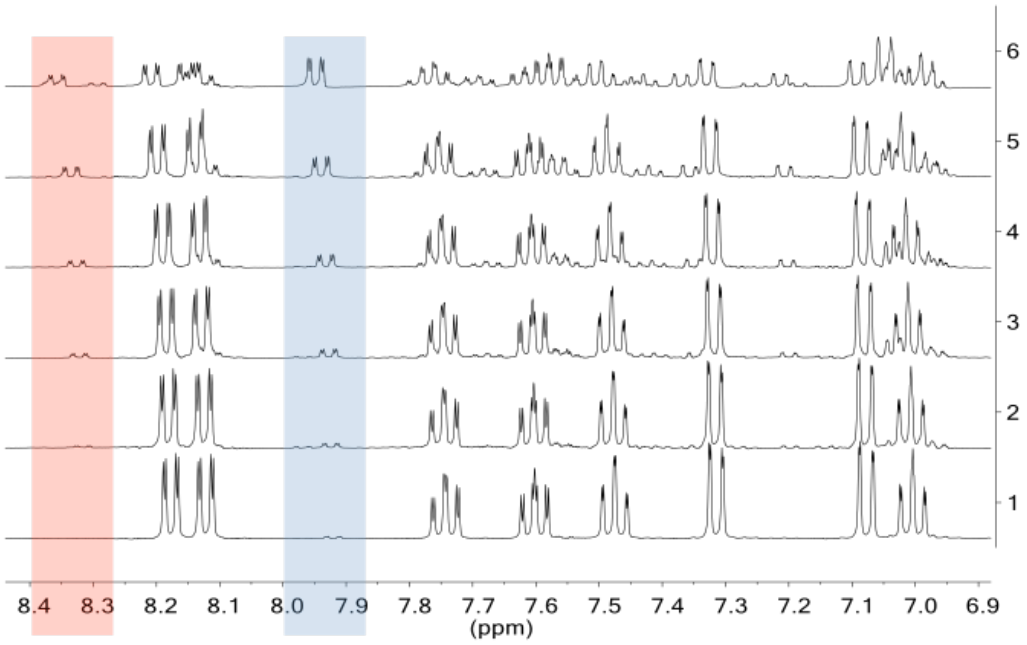

Figure 6 ^{1}H NMR spectra taken after heating salsalate in TCE-d $_{2}$ solution. Spectra 1-6 correspond to heating the solution for 0, 2, 5, 10, 20 and 30 mins respectively at 130 °C. The blue region corresponds to peaks from salycilic acid (n=1), the red region corresponds to peaks from the linear trimer decomposition product (n=3).

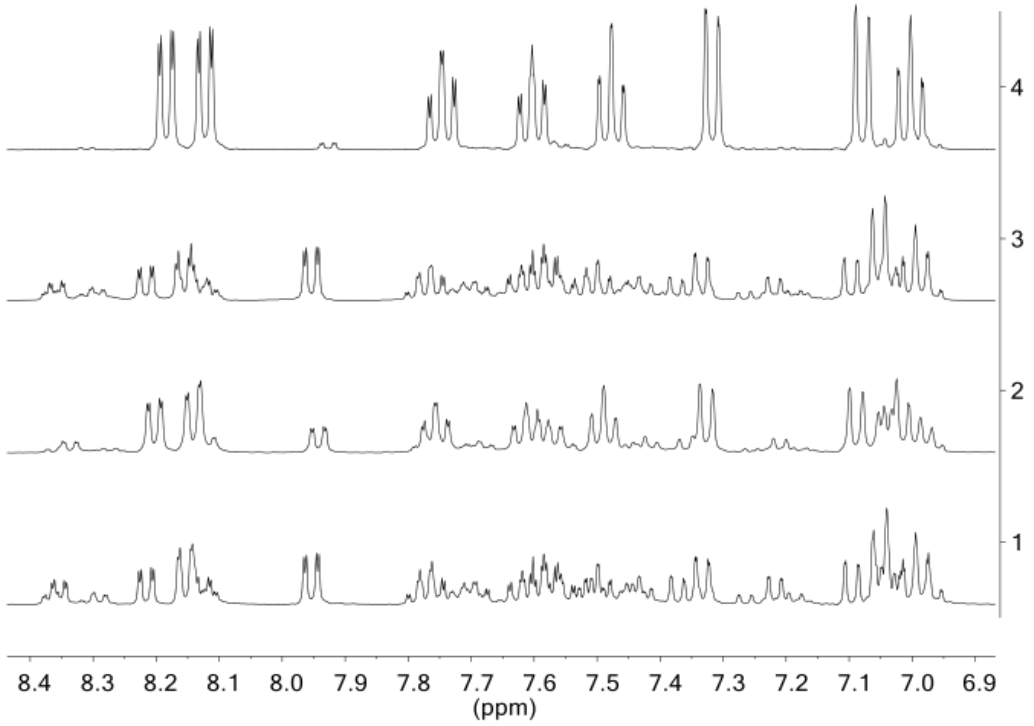

Figure 7 ^{1}H NMR spectra taken after heating a solid sample at 110 °C for 30 mins (4), 110 °C for 20 hours (3) and at 130 °C (2) and 150 °C (1) for 30 mins.

Literature values for the melting point of salsalate vary over quite a wide range. The manufacturer of the sample used in this work (Alfa Aesar) quotes a melting point of between 132 and 138°C , but other sources quote values as high as 148°C (Merck index, 11th Edition). However, even taking a conservative value of 135°C as the melting point of the sample, it is clear that thermal decomposition begins at temperatures significantly below the melting point. Attempts to determine the rate of thermal decomposition at a particular temperature showed limited reproducibility, and the source of variation was eventually traced to levels of incipient water in the samples. This makes sense since the decomposition from salsalate to salicylic acid requires uptake of water, while the formation of higher oligomers (n>3) requires elimination of water. The amount of water initially present can therefore be expected to influence both the rate of initial reaction and the

distribution of products observed. Both of these were confirmed experimentally, but it was not possible to determine water levels with sufficient accuracy to allow quantitative determinations of the various rate constants.

Infra-red spectra of samples of the commercial material and an amorphous sample (Figure 7) produced by quenching from the melt show notable differences, both in the fingerprint region and, significantly, in the -OH stretching region, where the -OH stretching band at 3648 cm^{-1} observed in the spectrum of the commercial (crystalline) material and believed to correspond to the intra-molecularly hydrogen bonded -OH is absent in the spectrum of the amorphous material. This confirms that the hydrogen bonding in the amorphous material is predominantly inter-molecular, but it is not possible to interpret the infra-red spectra in sufficient detail to comment on the purity of the sample.

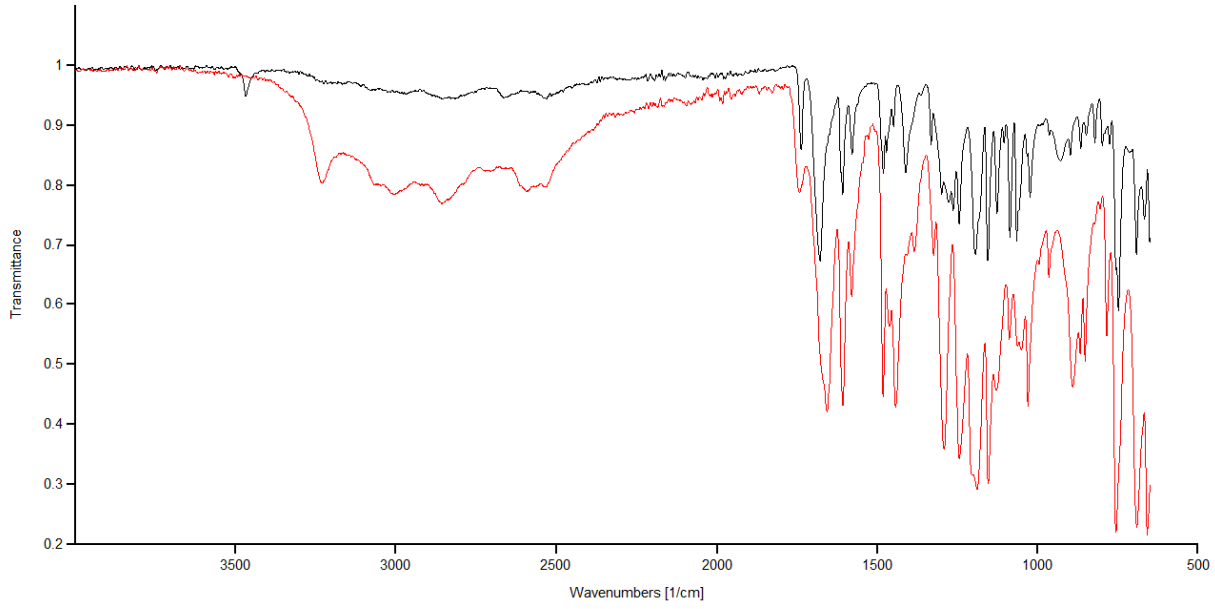

Figure 8 IR spectra - Black trace: commercial material; Red trace: quench cooled melt.

In our hands, it proved to be impossible to produce a sample of the amorphous form that was free of contamination by salicylic acid and oligomers of salicylic acid. Heating bulk salsalate to the melting point and then immediately quenching in liquid nitrogen still

resulted in material that contained a few weight% of material other than salsalate. It is highly probable that their presence in a mixture of structures closely related to salsalate is a major factor contributing to the stability of the amorphous form by inhibiting crystallisation, since the similarities in structure (isostructural "monomer" unit) would allow strong interaction with the existing hydrogen bonding patterns, while the differences in structure (chain length) would tend to preclude the establishment of long-range order. It is significant that the crystalline form can only be regenerated from the amorphous form by recrystallisation from solvent, a classic method of purification. The role of impurities in potentially stabilising solid-state forms (amorphous or crystalline) is an area that has not received widespread attention so far, but some work has been reported in relation to polymorphism. [19]

Experimental

Salicylsalicylic acid, 98%, was purchased from Alfa Aesar and 1,1,2,2-tetrachloroethane-d_2 (TCE-d_2) (D, 99.6%) was purchased from the Cambridge Isotope Laboratories, Inc. Samples were initially prepared to a concentration of 10-40 mg/mL and heating was carried out in NMR tubes using a custom-made heating block.

The LC MS/MS instrument used throughout this study was the Synapt G2-S HDMS (Waters Corp.) equipped with an Acquity UPLC. The LC separation was achieved with a reverse phase gradient such that 5% MeCN:95% (0.1% formic acid in water) to 95% (0.1% formic acid in water):5% MeCN over 9 min at a flow rate of 0.4 mL min^{-1} through a BEH C_{18} column: 2.1 x100 mm (Waters Corp.). The mass spectrometer was operated in resolution mode and both positive and negative ion mass spectra were taken across the range of 50-2000 Da using the following electrospray ion source conditions: capillary voltage 2.0 kV, source temperature 150 °C, sampling cone 70.0 V, source offset 30.0 V, desolvation

temperature 350 °C, cone gas flow 60 L Hr^{-1}, desolvation gas flow 600 L Hr^{-1}, nebuliser gas flow 6.0 Bar and a scan time of 1.0 s. For MSMS the ion of interest was isolated in the quadrupole and fragmented in the trap region of the triwave using a collision energy ramped from 10.0 to 30.0 eV. Ion mobility separation was performed in the triwave with nitrogen gas and a wave velocity of 800 m s^{-1} and wave height of 40 V.

1D ^{1}H NMR spectra were recorded using a 400 MHz Bruker Avance spectrometer with an acquisition time of 4.1 s, recycle delay of 1.0 s, and flip angle of 45°; ^{13}C NMR spectra were recorded on a 600 MHz Varian VNMRS spectrometer at 150 MHz with a relaxation delay of 3.0 s, acquisition time of 1.0 s, and flip angle of 45°.

2D NMR spectra were recorded using a 600 MHz Varian VNMRS spectrometer. Spectra were recorded at 25.0 °C unless stated otherwise with ^{1}H spectra acquired at 600 MHz and ^{13}C spectra at 150 MHz. The individual parameters used for the 2D experiments were: CT-TOCSY; [20] relaxation delay of 1.0 s, acquisition time of 1.14 s, mixing time 80 ms, using 2 repetitions over 2 x 256 increments. DOSY; the DBPPSTE convection compensated pulse sequence [21, 22] was used to acquire datasets with 16 gradient amplitudes ranging from 2 to 53 G cm^{-1} in equal steps of gradient squared, using 8 transients, 8192 complex data points, a total diffusion-encoding gradient duration of 1.5 ms, and a diffusion time of 210 ms.

NMR assignments for the linear trimer product are:

δ_H (600 MHz, TCE-d_2) 8.33 (1 H, dd, *J* 7.8, 1.7, H(12)), 8.09 (2 H, m, H(5,19)), 7.73 (1 H, td, *J* 7.8, 1.7, H(10)), 7.64 (1 H, td, *J* 7.8, 1.8, H(17)), 7.53 (1 H, td, *J* 7.7, 1.8, H(3)), 7.45 (1 H, t, *J* 7.7, H(11)), 7.39 (1 H, t, *J* 7.6, H(18)), 7.33 (1 H, d, *J* 8.1, H(9)), 7.17 (1 H, d, *J* 8.2, H(16)), 7.01 (1 H, d, *J* 8.5, H(2)), 6.95 (1 H, t, *J* 7.6, H(4)).

δ_C (150 MHz, TCE-d $_2$) 168.68 (C(7)), 167.24 (C(21)), 162.78 (C(14)), 161.69 (C(1)), 150.56 (C(15)), 150.22 (C(8)), 136.80 (C(3)), 134.97 (C(17)), 134.86 (C(10)), 132.67 (C(12)), 132.61 (C(19)), 130.78 (C(5)), 126.92 (C(11)), 126.66 (C(18)), 124.02 (C(9, 16)), 122.58 (C(13)), 122.32 (C(20)), 119.92 (C(4)), 117.84 (C(2)), 111.85 (C(6)).

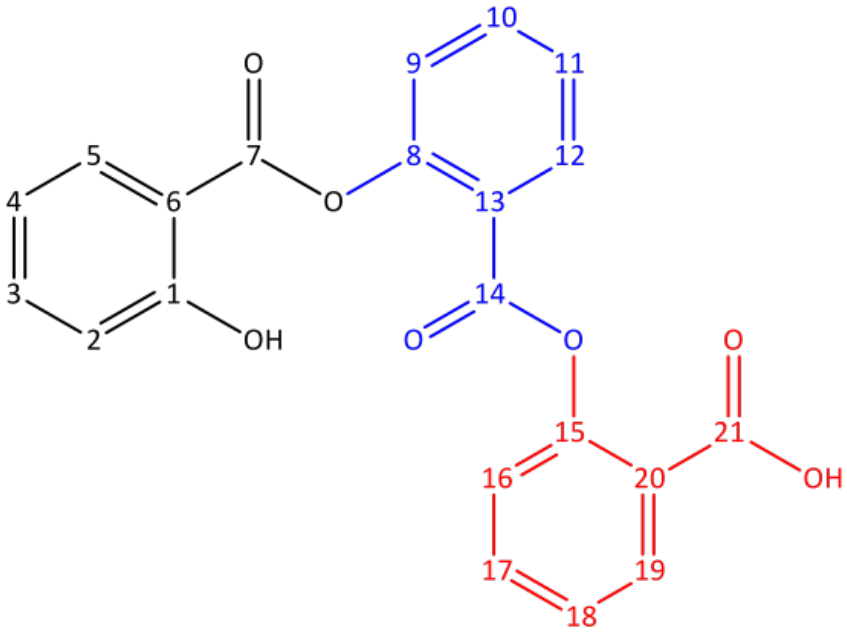

Figure 9 Molecular structure of the linear trimer decomposition product, showing the numbering system used for the NMR assignment of the trimer (black, red and blue), SSA (black and blue) and SA (black).

Conclusions

When an amorphous material has been produced by any means, and certainly if elevated temperatures are involved, the chemical purity should be monitored even if an apparently

clear glass has been formed; indeed the apparently stable glass may be 'stabilised' by the presence of impurity(s). We have shown that salsalate undergoes facile thermal decompostion to yield a mixture of salsalate, salicylic acid, and linear oligomers of salicyclic acid. The onset of thermal degradation occurs at temperatures well below the melting point of the pure material and takes place both in the solid state and in solution. The rate of thermal degradation and the distribution of products depend, *inter alia*, on the amount of incipient water present in the sample. We could find no evidence for the production of cyclic species at the temperatures used (< 150 °C). The fact that we were unable to produce samples of amorphous salsalate uncontaminated by degradation products following procedures reported in the literature suggests that published reports relating to the characteristics of the amorphous form of salsalate should be evaluated critically since they probably relate to ill-characterised material. Similarly, the report of a nematic phase produced by heating the crystalline material to just below its melting point may need to be re-evaluated.

Acknowledgements

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