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Investigation of the physical stability of amorphous drug and drug / polymer melts using variable temperature solid state NMR

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Investigations into molecular mobility have become the focus of considerable amounts of work on stabilising the amorphous state of drugs or drug/polymer mixtures [1]. The glass transition (Tg) is generally regarded as the most important indicator of a major change in the molecular mobility of an amorphous system, describing the transition from the glassy to the rubbery state upon heating the amorphous sample. Differential scanning calorimetry (DSC) is routinely used to determine the Tg and thus to estimate amorphous stability. However, there are examples in the pharmaceutical literature of compounds for which the Tg is an inaccurate predictor of amorphous stability [2]. A few studies have investigated the application of solid-state NMR to the study of molecular mobility in amorphous compounds [3–6]. During an NMR experiment, nuclei are excited to a higher energy state. The excited nucleus loses energy via interactions between the spins of adjacent nuclei (spin-spin) and with the surroundings (spin-lattice). These relaxation processes can be used to probe molecular mobility [7]. This study compares the prediction of amorphous physical stability by DSC to proton relaxation measurements using variable temperature solid state NMR, for drug melts and drug/PVP co-melts.

All drug and drug/polymer melts were amorphous, when investigated by XRPD, showing only the typical halo for amorphous substances. Nifedipine drug melts recrystallised following light grinding with a mortar and pestle (appearance of Bragg reflections in the diffractograms of the pure drug), but remained amorphous for up to 24 h when stored intact and dry at ambient temperature. For the melted and quench-cooled pure drugs and drug/PVP mixtures the following Tg values (onset) were obtained by DSC: indomethacin 42.2 ± 0.1 °C, nifedipine 44.5 ± 0.5 °C, indomethacin/PVP 67.1 ± 1.3 °C, nifedipine/PVP 71.6 ± 0.7 °C ($n = 3$). Based on these Tg values, one would predict the stability of the two amorphous drugs and two drug/polymer formulations to be fairly similar (and the stability of the mixtures to be higher than that of the pure drugs), as Tg values for both drugs (alone and with PVP) are within the same temperature range.

In solid state NMR experiments, a direct comparison of the absolute relaxation values between compounds is not possible. However, comparison of the spin-lattice relaxation time in the rotating frame ($T_1\varrho$) allows the temperature dependence of different compounds to be probed. $T_1\varrho$ values for amorphous nifedipine show a large change in relaxation behaviour with an onset well below the Tg (Fig. 1, upper traces). The change in amorphous indomethacin relaxation values on the other hand corresponds to the Tg of the drug (Fig. 1, lower traces). The $T_1\varrho$ behaviour of drug/PVP solid solutions was similar to that of the drug melts, with respect to nifedipine/PVP co-melts, showing a change in $T_1\varrho$ well below the measured Tg (Fig. 2, upper trace), and indomethacin/PVP co-melts exhibiting a change in the relaxation values corresponding to the Tg of the amorphous drug/polymer mixture (Fig. 2, lower trace). $T_1\varrho$ values relate to slower motions (70 kHz) and may reflect an overall 'freeing up' of molecular mobility as the sample softens upon temperature increase. Based on the temperature dependent relaxation behaviour, one would predict the stability of the two amorphous drugs and the two drug/polymer formulations to be different, with the indomethacin samples showing a higher stability towards recrystallisation, both alone and in co-melts with PVP.

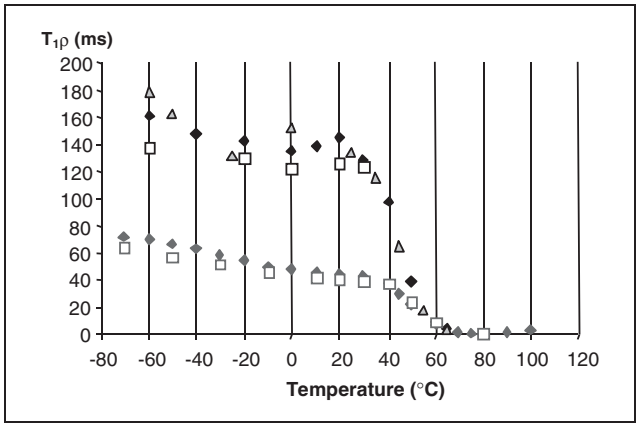

Fig. 1: $T_1\varrho$ values for nifedipine ($n = 3$, upper traces) and indomethacin ($n = 2$, lower traces)

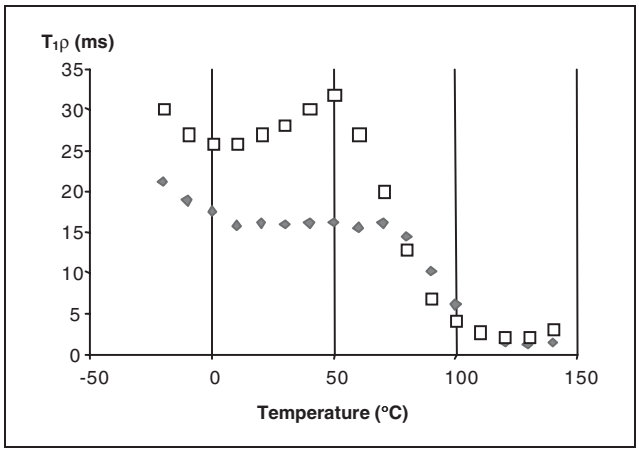

Fig. 2: $T_1\varrho$ values for nifedipine / PVP (1 : 1) co-melts (upper trace) and indomethacin / PVP (1 : 1) co-melts (lower trace)

Table: Physical stability of samples investigated by XRPD

Sample	5 weeks storage at		
	−20[a]	30 / < 10	30 / 75
Indomethacin melt	A	C	C
Indomethacin / PVP melt	–	A	A
Nifedipine melt	C	C	C
Nifedipine / PVP melt	–	A	C

a −20 °C, 30 °C / < 10% RH and 30 °C / 75% RH
A = amorphous as determined by XRPD, C = crystalline peaks of the drug detectable by XRPD

The results from the DSC and NMR studies were compared to the outcome of a storage study of drug melts and drug/PVP co-melts for 5 weeks at 30 °C/< 10% RH and 30 °C/75% RH and, in the case of drug melts, −20 °C. The stability data (Table) indicates that amorphous indomethacin and indomethacin/PVP formulations are more stable than the respective nifedipine formulations.

The results of this study show that proton relaxation measurements, using variable temperature solid state NMR, are a valuable additional tool to thermal analytical methods for predicting the amorphous physical stability. For amorphous drug melts and drug/PVP co-melts $T_1\varrho$ measurements provide an insight into amorphous stability that is different to that of DSC Tg measurements. NMR relaxation measurements lead to an improved estimate of amorphous physical stability compared to DSC Tg determination.

In proton NMR all the proton relaxation rates are averaged. Future work will use ^{13}C NMR to measure the relaxation rate of different protons of the molecule to discover potential 'hotspots', which may be linked to the decreased physical stability of some amorphous compounds.

Experimental

1. Materials

Indomethacin, nifedipine and polyvinylpyrrolidone k30 (PVP) were purchased from Sigma Aldrich (Dorset, UK).

2. Methods

2.1. X-ray Powder Diffraction (XRPD)

To confirm that quench cooling resulted in an amorphous product, samples were melted in an X-ray sample holder and then quench cooled with liquid nitrogen. Following cooling the samples were analysed with a Philips X'Pert MPD (count time 1 s, step size 0.04 °2θ, Ni-filtered Cu-kα radiation, 30 kV, 40 mA, sample size: approx. 300 mg).

2.2. Differential Scanning Calorimetry (DSC)

Amorphous samples were prepared by melting either pure drug or drug/PVP (1 : 1 w/w) mixtures in crimped aluminium pans using a TA Instruments 2920 DSC at a heating rate of 10 K min^{-1} (nitrogen purge: 20 ml min^{-1}, sample size: approx. 5 mg, temperature and enthalpy calibration with an indium standard) and then quench cooling the melt using a stainless steel cylinder filled with liquid nitrogen placed over the DSC cell. To determine the Tg (onset) value the samples were then reheated in the DSC at 10 K min^{-1}.

2.3. Solid State NMR

Amorphous samples were prepared by melting approximately 500 mg of drug or drug/PVP in an oven in 1 cm long glass tubes. Molten samples were then plunged into liquid nitrogen. All cooled melts were transparent yellow solids. $T_1\varrho$ proton relaxation measurements were performed over a wide temperature range for all samples. Following preparation, the samples were placed in the NMR probe and cooled to −70 °C. Subsequent measurements were made by heating to various temperatures at 5 K min^{-1} and then measuring relaxation times isothermally. A Varian Unity plus NMR operating at 300 MHz was used for the proton measurements. For $T_1\varrho$ measurements a radio frequency field equivalent to 70 kHz was used.

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A new picoline alkaloid from the pseudobulb of *Desmotrichum fimbriatum* Blume

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Desmotrichum fimbriatum Blume (Orchidaceae), is an epiphytic orchid commonly known as Jivanti, distributed up to 2,700 m. It is an important Ayurvedic drug used as astringent, aphrodisiac, expectorant, stimulant, cardiotonic and to relieve asthma, bronchitis and throat infections [1, 2]. The earliest observation was the presence of traces an alkaloid, α- and β-jivantic acids [3] and 3-methyl octacosane, 9-methyl deca (15′-hydroxy-15-methyl-n-nonadeca-1-oate) and octacont-3-en-1,5-olide [4] from the stem and root of this plant. The present paper describes the isolation and characterization of a new alkaloid.

Compound **1**, named desmotrichinine was obtained as a yellow crystalline solid from chloroform fractions. It responded positively to Dragendorff's reagent and 5% ferric chloride solution. The HREIMS of **1** showed the molecular ions peak at m/z 233.0142 corresponding to the molecular formula established as $C_{12}H_{11}O_4N$. The positive ion FAB MS showed $[M]^+$ at m/z 233 and further significant fragments ion peak at m/z 138 [M-C_5H_5NO, C_4-C_6 fission]$^+$, 125 [M-C_6H_6NO, C_6-O fission]$^+$ and 108 [M-$C_6H_5O_3$]$^+$. Its IR spectrum showed characteristic absorptions for hydroxyl group (3300 cm^{-1}) and aromatic rings (1670, 1523 cm^{-1}). The UV spectrum also exhibited the absorption maximum at 335 nm and bathochromic shifts were observed with sodium acetate (418 nm) and boric acid (+ 18 nm) indicating the presence of an m-hydroxylated benzenoid nucleus [5].

The ^{1}H NMR spectrum of **1** displayed the presence of a two proton broad signal at δ 9.62 assigned to H-1 and H-5. A three proton-meta-coupled doublet at δ 7.21 (J = 3.6 Hz) was ascribed to H-2′, H-4′, H-6′. A one-proton double doublet at δ 6.58 with coupling interactions of 3.6 and 3.6 Hz was associated with H-3. A two-proton broad signal at δ 4.63 was due to oxygenated C-6 methylene protons. The ^{13}C NMR data showed signals at δ 177.7 (C-1, C-5), 111.9 (C-3) and 121.9 (C-4) supporting thereby that the compound **1** possessed a pyridine nucleus and carbon signals for oxygenated methylene carbons at δ 64.6 and aromatic carbons between δ 177.7- 111.6.

On the basis of the forgoing account the structure of **1** has been established as 2-hydroxy-6-pyrogalloloxy-β-picoline.