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An Investigation Into the Low Temperature Thermal Behaviour of Vitamin E Preparation USP Using Differential Scanning Calorimetry and Low Frequency Dielectric Analysis

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Abstract

The thermal and dielectric responses of Vitamin E Preparation USP have been examined to further understand the melting and solidification of this material. A TA Instruments 2920 Differential Scanning Calorimeter was used to examine the thermal response of the sample at a range of scanning speeds. Isothermal dielectric studies were performed using a Novocontrol Dielectric Spectrometer over a range of temperatures down to −70°C and a frequency range of 10^6–10^{-2} Hz.

The differential scanning calorimetry (DSC) studies showed an anomalous response whereby at slow heating rates (2°C min^{-1}) a small exotherm followed immediately by an endotherm was observed. This response was considerably diminished in magnitude at higher rates (5°C min^{-1}) and was not observed at the fastest heating rate of 10°C min^{-1}. No thermal events were seen on cooling the sample to −60°C. It was suggested that the material formed a glass on cooling, with a predicted transition temperature of approximately −100°C. Further studies using a liquid nitrogen cooling system indicated that the system did indeed exhibit a glass transition, albeit at a higher temperature than predicted (ca −63°C). Low frequency dielectric analysis showed a clear relaxation peak in the loss component, from which the relaxation time could be calculated using the Havriliak-Negami model. The relationship between the relaxation time and the temperature was studied and was found to follow the Vogel-Tammann-Fulcher (VTF) modification of the Arrhenius equation.

It is therefore concluded that Vitamin E Preparation USP is a glass-forming material that exhibits kinetically-hindered recrystallisation and melting behaviour. The study has also indicated that DSC and low frequency dielectric analysis may be powerful complementary tools in the study of the low temperature behaviour of pharmaceuticals.

A persistent problem facing the pharmaceutical industry is the formulation of drugs that are in the liquid state under normal operating conditions. One such material, Vitamin E, is widely used within the pharmaceutical and food industries as an antioxidant and as a nutritional supplement, where its biological activity is related to its antioxidant properties, specifically in terms of prevention of lipid peroxidation in biological membranes (Traber & Packer 1995; Theriault et al 1999). When given as a food supplement, vitamin E is usually formulated as a liquid-filled soft gelatin capsule. The study reported here is part of a wider investigation into the preparation of solid or semi-solid dosage forms containing therapeutic levels of vitamin E, hence it was of considerable interest to have a more detailed knowledge of the physicochemical properties of this material.

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The term vitamin E covers a range of naturally occurring tocopherols, of which α-tocopherol is the most biologically active (Theriault et al 1999) and also the most commonly used. The native form is (2 *R*,4′ *R*,8′ *R*)-α-tocopherol, commonly denoted as *d*-α-tocopherol, which consists of the heterocyclic chromanol core with a saturated 16-carbon branched side chain. Despite the extensive use of vitamin E in formulation science for its antioxidant properties, very little information has been published to date regarding its physicochemical

properties (Burton & Ingold 1986), particularly in terms of the melting and solidification behaviour. The Handbook of Pharmaceutical Excipients (2000) gives the melting point of *d*-α-tocopherol as 2·5–3·5°C and that of γ-tocopherol as −30°C. Given the minor chemical difference between α-tocopherol and γ-tocopherol (γ-tocopherol contains a hydrogen atom rather than a methyl group at position 5 on the heterocyclic ring), this difference in the melting points indicates a strong dependence of the solidification on minor structural alterations.

Knowledge of the melting and solidification properties of the various tocopherols is relevant not only for quality control of vitamin E, but also for formulation purposes. More specifically, an understanding of solid-state behaviour is important for the development of solid dosage forms containing vitamin E both as a nutritional supplement and as an antioxidant. Furthermore, the recent interest in the development of excipients for freeze dried formulations has generated a requirement to extend the current knowledge base regarding the cryogenic behaviour of pharmaceutical excipients (e.g. Levine & Slade 1988; Pikal et al 1991; Abblett et al 1992; Franks 1994; Craig et al 1999).

Given that, for reasons of expense and availability, the pharmaceutical and nutraceutical industries are more likely to utilise a semi-formulated, standardised material than pure α-tocopherol, we have used Vitamin E Preparation USP (*d*-α-tocopherol, 1000 IU g^{-1}) as the source of *d*-α-tocopherol in this investigation. Vitamin E Preparation USP is a combination of a single form of vitamin E with one or more inert substances, containing not less than 95% of the labelled amount of vitamin E (USP 24). The *d*-α-tocopherol was derived from soybean oil, which was also used as the diluent to give the final concentration.

Differential scanning calorimetry (DSC) and low frequency dielectric analysis have been used as complementary techniques for the characterisation of Vitamin E Preparation USP. DSC has been widely used within the pharmaceutical sciences for a considerable period of time (Ford & Timmins 1989), while dielectric analysis is attracting increasing attention as a means of characterising a range of pharmaceutical materials, including glasses, colloids, gels and liquid crystalline systems (Binns et al 1992; Barker et al 1994; Craig 1995; Duddu & Sokoloski 1995; He & Craig 1998). The method involves the application of an oscillating electrical signal and the measurement of the response in terms of the in-phase and out-of-phase components of the complex permittivity ε* where

$$\varepsilon^*(\omega) = \varepsilon'(\omega) - i\varepsilon''(\omega) \quad (1)$$

with $\varepsilon'(\omega)$ and $\varepsilon''(\omega)$ representing the real and imaginary permittivities at frequency ω, and i being the square root of −1. More details of the principles and applications of the technique may be found in a number of texts (McCrum et al 1967; Jonscher 1983; Craig 1995). Both DSC and dielectric analysis have been used in the study of cryogenic systems (e.g., Jain & Johari 1988; Ablett et al 1992). However, their combined use for low temperature work is relatively rare, particularly within the pharmaceutical sciences, hence, over and above the information yielded on the system under study, the merits of using these two techniques in conjunction as an approach to characterising the cryogenic behaviour of pharmaceutical systems will be evaluated.

Materials and Methods

Materials

Vitamin E Preparation (*d*-α-tocopherol, 1000 IU g^{-1}) USP (Lot SB0499-16) was received from Archer Daniels Midland, IL and used as received.

Differential scanning calorimetry (DSC) studies

DSC studies were performed using a TA Instruments model 2920 calorimeter. Helium was used as the carrier gas for all experiments, due to its greater thermal conductivity than nitrogen at low temperatures. Matched hermetic aluminium pans sourced from TA Instruments were used for all runs. Baseline calibration was performed using matched, empty pans. Temperature calibration runs were performed using *n*-octadecane (theoretical mp 28·24°C), cyclohexane (theoretical mp 6·54°C) and *n*-decane (theoretical mp −26·66°C). Full calibration was performed at each scanning speed used. Samples of 6–16 mg were used. The sample and reference pans were loaded into the DSC cell at ambient temperature and the chamber sealed. The sample chamber was then flash-cooled to −60°C, a process taking approximately 15 min, and then held at this temperature for 10 min. The sample temperature was then increased at a rate of 2, 5 or 10°C min^{-1} to 5°C. Three measurements were performed at each scanning rate using this protocol, each using a fresh sample. Additionally, cycling experiments were performed at 2 and 5°C min^{-1}, whereby the sample was treated as above, then held for 10 min at 5°C, cooled to −60°C, held there for 10 min and then reheated to 5°C. The cycling experiments were performed in duplicate. These studies were performed using a standard refri-

gerated cooling system that permits study of temperatures down to −60°C. Further lower temperature studies were performed using an equivalent instrument equipped with a liquid nitrogen cooling system.

Low frequency dielectric analysis

Low frequency dielectric analysis was performed using a BDC-N broad band dielectric converter (Novocontrol GmbH, Germany) and a SI 1255 Frequency Response Analyser (Solartron-Schlumberger, Germany) linked to a Quatro temperature control system (Novocontrol GmbH, Germany). A parallel-plate sample cell with stainless steel electrodes of area (A) 254·5 mm^2 and separation distance (d) 0·5 mm was used for all measurements. The applied voltage was 0·5 V r.m.s. Sample temperature was controlled to ±0·1°C. Following temperature equilibration, measurements were made at 20, 10, 0, −10, −20, −30, −40, −50, −60 and −70°C, as the temperature was decreased. The sample temperature was reduced at a rate of 2°C min^{-1} between measurement temperatures. A total of four measurements were made, each on a fresh sample. Temperature calibration of the cryostat was performed electronically.

The sample response was measured in terms of the capacitance $C(\omega)$ and dielectric loss $G(\omega)/\omega$, that are related to the real and imaginary permittivities via equations 2 and 3.

$$C(\omega) = [A\ \varepsilon_0\ \varepsilon'(\omega)]/d \quad (2)$$

$$G(\omega)/\omega = [A\ \varepsilon_0\ \varepsilon''(\omega)]/d \quad (3)$$

where ε_0 is the permittivity of free space.

Results and Discussion

DSC results

Figure 1 shows representative DSC responses for the samples of Vitamin E Preparation USP heated at 2, 5 and 10°C min^{-1}. A small exotherm followed immediately by an endotherm was observed for the samples heated at 2°C min^{-1}. A similar response was observed for the samples heated at 5°C min^{-1}, but this was of smaller magnitude and occurred at slightly higher temperatures. At a heating rate of 10°C min^{-1}, no transitions were observed over the temperature range studied.

On cooling the samples back to −60°C at either 2 or 5°C min^{-1}, no reverse transitions were observed. However, on reheating these samples at either heating rate, both the exotherm and the endotherm were observed. Values of the peak temperature for both transitions and the enthalpy of the endotherm were effectively identical to those on the first heating cycle. Figure 2 shows the cycling data for a Vitamin E Preparation USP sample cycled at 2°C min^{-1}. The peak temperatures for the exotherm and endotherm and the enthalpy of the endotherm are shown in Table 1. To reduce errors arising from a lack of a baseline before the endotherm, the onset was taken to be the peak of the exotherm for calculation of the enthalpy values.

These data were unexpected for several reasons. Firstly, melting is a first-order thermodynamic process and should not be dependent on heating rate, assuming appropriate calibration has taken place. Secondly, the magnitude of the endothermic peak (mean 1·21 J g^{-1} at 2°C min^{-1}) is much smaller than would be expected for the melting of a low-molecular-weight material. For example, paracetamol has a melting enthalpy of 182·8 J g^{-1} (Lloyd et al 1997). Thirdly, it is expected that sensitivity should increase when using faster scanning rates. This is because the temperature difference between the sample and reference, from

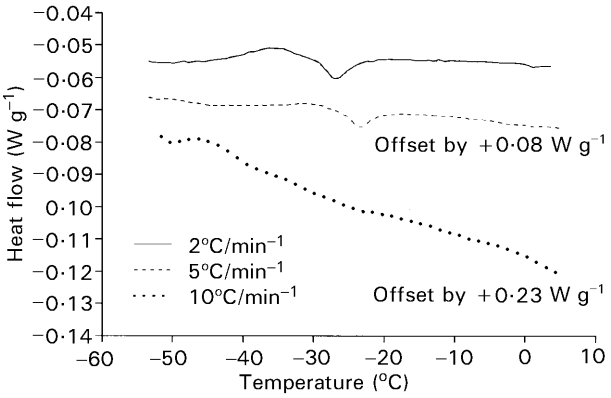

Figure 1. The DSC heating response of Vitamin E Preparation USP at different scanning rates.

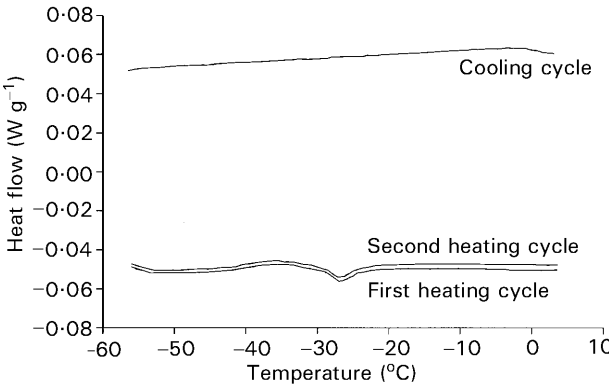

Figure 2. The DSC response of Vitamin E Preparation USP cycled at 2°C min^{-1}.

Table 1. Thermal data for Vitamin E Preparation USP.

Analysis	Heating rate	
	$2°C\,min^{-1}$	$5°C\,min^{-1}$
Peak of exotherm on first heating cycle (°C) (n = 5, mean ± s.d.)	−35·9 ± 0·5	−31·9 ± 0·6
Peak of exotherm on second heating cycle (°C) (n = 2, mean)	−35·8	−31·7
Peak of endotherm on first heating cycle (°C) (n = 5, mean ± s.d.)	−26·6 ± 0·3	−23·4 ± 0·2
Peak on endotherm on second heating cycle (°C) (n = 2, mean)	−26·7	−23·3
Enthalpy of endotherm on first heating cycle ($J\,g^{-1}$) (n = 5, mean ± s.d.)	1·21 ± 0·04	0·35 ± 0·02
Enthalpy of endotherm on second heating cycle ($J\,g^{-1}$) (n = 2, mean)	1·23	0·31

which the heat flux is calculated, is greater when going through a thermal event at rapid rates. Consequently the opposite trend to that observed here would intuitively be expected.

These observations suggested the following hypothesis, which is illustrated by the diagram shown in Figure 3. The absence of any discernible thermal event on cooling suggests that the system is not crystallising during this part of the cycle. Instead, the material is supercooling and remains in the non-equilibrium liquid state down to −60°C. It is, of course, possible that the material is recrystallising during the annealing period at this temperature. However, the appearance of an exotherm followed by an endotherm on reheating at low rates suggests that the material is undergoing recrystallisation followed immediately (or perhaps more probably simultaneously) by melting during the heating cycle, rather than in the equilibration period at −60°C. At more rapid heating rates, such as $10°C\,min^{-1}$, the kinetically controlled recrystallisation does not take place and the sample passes directly from the thermodynamically unstable supercooled state to the stable liquid state without forming a solid phase.

If the above hypothesis is correct, then a glass transition associated with the supercooled liquid would be expected to be seen. The value of the glass transition temperature (Tg) may be estimated before measurement by using the empirical observation noted by several authors (Gujrati & Goldstein 1980; Kerč & Srčič 1995) that the relationship between Tg and the melting point (Tm) may be approximated by

$$Tg/Tm \approx 0 \cdot 7 \qquad (4)$$

depending on the nature of the molecule in question. Kerč & Srčič (1995) reported values of approximately 0·6–0·8 for a wide range of drug systems. On this basis, the glass transition temperature is predicted to occur at approximately −100°C for the material under study here.

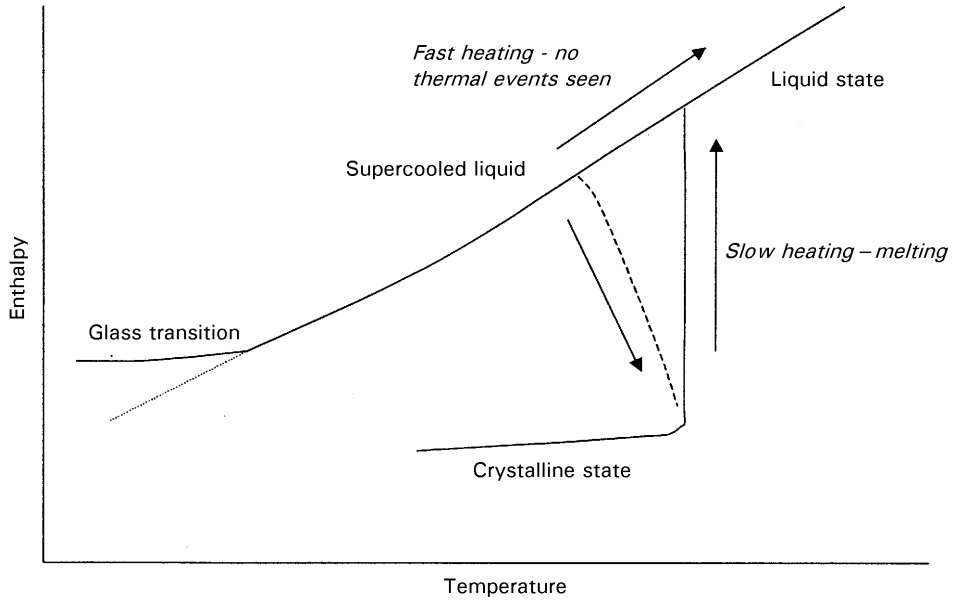

Figure 3. Schematic representation of the phase changes undergone by Vitamin E Preparation USP as a function of heating rate from a supercooled liquid.

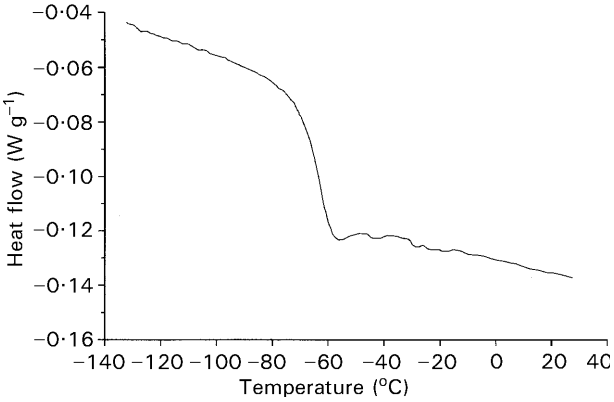

Figure 4. The low temperature DSC heating response of Vitamin E Preparation USP.

To assess the validity of this hypothesis, further studies were conducted on an equivalent calorimeter equipped with a liquid nitrogen cooling system, thereby allowing the sample to be studied at temperatures down to approximately −140°C. At a scanning speed of 5°C min^{-1}, a glass transition could be clearly seen at approximately −63°C. This transition was observed whether the sample had been flash-cooled or cooled at 5°C min^{-1}. On the cooling cycle, the reverse transition was also seen in the same position, therefore supporting the argument that the thermal event is indeed a glass transition. Figure 4 shows the response of Vitamin E Preparation USP to the 5°C min^{-1} heating cycle, clearly indicating the glass transition.

Dielectric studies

The dielectric spectrum of a representative sample of Vitamin E Preparation USP at −30°C is shown in Figure 5. The response shows the classic Debye behaviour, in that a maximum is seen in the loss peak accompanied by a step change in the capacitance (Debye 1945). As the temperature was

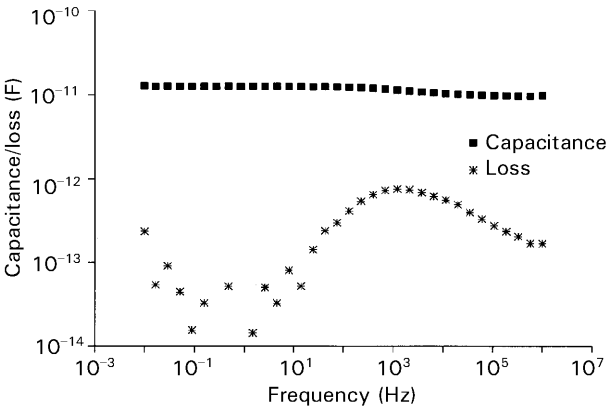

Figure 5. The dielectric spectrum of Vitamin E Preparation USP at −30°C.

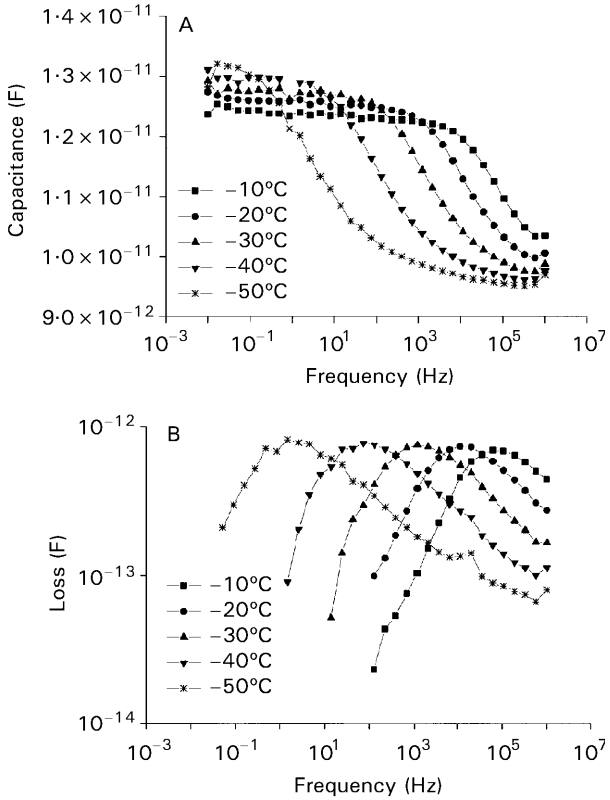

Figure 6. The capacitance (A) and dielectric loss (B) of Vitamin E Preparation USP as a function of temperature.

reduced, the position of the loss peak maximum shifted to progressively lower frequencies. Figures 6A and 6B show the capacitance and dielectric loss over the temperature range −10°C to −50°C. At temperatures above this range, the loss peak maximum appeared to be above the highest frequency studied here and at temperatures below this range, the loss peak maximum appeared to be below the lowest frequency studied here.

The frequency at which the loss peak occurs is inversely related to the relaxation time, hence it is possible to model the data so as to allow accurate evaluation of the loss peak frequency. This was achieved using the Havriliak-Negami equation (Havriliak & Negami 1966), given by equation 5.

$$\varepsilon(\omega) - \varepsilon(\infty) = \chi_0/[1 + (i\omega\tau)^{1-\alpha}]^{1-\beta} \qquad (5)$$

where $\varepsilon(\infty)$ is the permittivity at infinite frequency, χ_0 is the static susceptibility, τ is the relaxation time and α and β are constants. The relaxation time as a function of temperature is shown in Figure 7A. The calculation and use of relaxation times has attracted considerable interest within the pharmaceutical sciences, particularly in terms of the possibility of predicting chemical and physical stability (Fukuoka et al 1986; Hancock et al 1995; Shamblin et al 1999). The relationship between the relaxation time

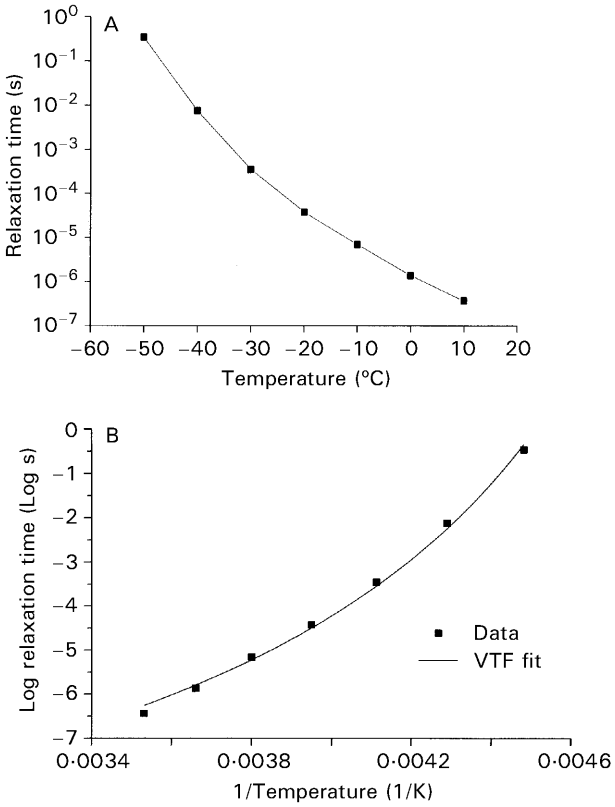

Figure 7. The relaxation times of Vitamin E Preparation USP as a function of temperature (A) and the VTF fit of the relaxation times of Vitamin E Preparation USP (B).

and temperature is highly dependent on the physical state of the material under study. More specifically, systems that are below the glass transition temperature usually exhibit Arrhenius behaviour, while systems immediately above Tg exhibit Vogel-Tammann-Fulcher (VTF) or WLF behaviour (Wong & Angell 1976). The VTF equation is given by

$$\log \tau = A + [B/(T - T_0)] \qquad (6)$$

where A, B and T_0 are constants derived from the experimental data. The VTF fit for the experimental data is shown in Figure 7B, with the fitted values of A, B and C being −12·60, 756·20 and 160·83, respectively. The satisfactory fit provides further evidence that the system is in the $>$Tg amorphous state over the temperature range under study.

Conclusions

The investigation has indicated that Vitamin E Preparation USP is a glass-forming system that undergoes kinetically hindered recrystallisation and melting on heating in the temperature region above Tg, with a glass transition identified at approximately −63°C. Dielectric investigations indicated that the relaxation behaviour of this material could be quantified, with a satisfactory fit to the VTF equation noted.

Overall, the study has indicated that this pharmaceutically important material displays more complex low temperature behaviour than was originally supposed. This has implications for understanding how this material may be incorporated into dosage forms, both as an excipient and as the primary active constituent. We believe, however, that there may be broader implications to these findings in that there is considerable interest in the use of materials for cryopreservation (e.g., Franks & Skaer 1976). More specifically, there has been particular emphasis on the relevance of the glass-forming properties of materials for the protection of biological tissues and delicate biomolecules such as proteins. To our knowledge, this is the first study that has suggested that vitamin E may also form a glassy state. Given the well known role of this material as a chemical protectant, the findings described here suggest that this material may merit further investigation as a cryoprotective agent.

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