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Evaluation of electrical conductivity-temperature curves using a mathematical model: temperature-dependent changes during thawing of frozen aqueous pharmaceuticals

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The information contained in the electrical conductivity curves of pharmaceuticals measured as a function of temperature can be represented by a small set of parameters. This is achieved by approximating the electrical conductivity curve as a number of consecutive steps, using a suitable empirical model. The three parameters describing each step are: transition temperature, slope factor and step height. The validity of the calculated transition temperatures was established by applying the model to electrical conductivity curves measured on aqueous solutions of KCl, NaCl and on a KCl–NaCl mixture. It appears that the transition temperatures calculated for these inorganic salts are in good agreement with the respective eutectic temperatures reported in the literature. Subsequently, the method was applied to the corticosteroids prednisolone sodium succinate and prednisolone disodium phosphate. The mathematical model yields a satisfactory fit for both experimental conductivity curves. The actual consequences of freeze-drying an aqueous solution of prednisolone sodium succinate below and above the respective transition temperatures calculated are discussed in relation to the experimental conductivity data.

Phase transitions can play a crucial role in the manufacture of lyophilized products. The narrow temperature intervals (e.g. within 3 °C), in which large structural changes can occur upon freezing (Mackenzie 1976), underline the significance of phase transitions. These structural changes can drastically affect the dissolution properties of the freeze-dried product. In some cases this results in an unacceptable reconstitution of the product, reflected by long dissolution times or by permanent opalescence of the solution formed upon addition of solvent to the product. Knowledge of phase transitions is to understand as well as design freeze-drying processes for pharmaceuticals. Prednisolone sodium succinate has been used as a representative compound in the study of the relation between conductivity curves and the actual freeze-drying behaviour. To calculate the parameters describing these conductivity curves, a mathematical model was used and a non-linear least square fitting method applied.

MATERIALS AND METHODS

Procedure

A freezing analyser (Edwards) was used to determine the freezing behaviour of prednisolone sodium succinate (PSS) and prednisolone disodium phosphate (PDP). A small sample (5·0 mL) of the dissolved product was placed in the analyser, the sample was then frozen and subsequently heated slowly under atmospheric pressure or under slightly reduced pressure to avoid condensation on the probe. During this process the temperature and electrical conductivity (AC current) were recorded simultaneously as a function of time. The calibration curve for the conductivity cell was obtained from the manufacturer (Edwards). During measurement no sublimation occurs. The analogue curves thus obtained were digitized using a plotter (Hewlett Packard 9872) and a desktop calculator. As the freezing analyser generates signals which are not linearly related to temperature as well as to conductivity, corrections were applied to the data read into the desktop calculator. Typically, 150–180 pairs of digitized points were sufficient to describe the analogue curves.

Powder diffraction spectra were recorded (Philips pw 1050 goniometer) with CuK_α radiation (λ = 0·154 nm). Moisture contents of freeze-dried PSS were determined by injecting Karl Fischer solvent into the vial through the rubber closure. After dissolving the powder, a portion of the solution was withdrawn for coulometric determination of the

† Correspondence.

residual moisture content (Mitsubishi CA-05/VA-05). Differential scanning calorimetry (DSC Mettler TA2100) and scanning electron microscopy (SEM S 180, Cambridge) were performed on PSS after freeze-drying (Lyovac GT20/LPC1, Leybold-Heraeus GmbH). The opalescence of reconstituted solutions of PSS was determined by turbidimetry (TRM-L, M.R. Drott K.G.) or by fluorimetry (Perkin Elmer 204).

Data reduction

To extract the information contained in the analogue curves, attempts were made to describe the conductivity curves by a small set of parameters.

The following empirical model was used to calculate the parameters describing the conductivity curves:

$$-\log EC = P0 + \sum_{i=1}^{N} \{Pi,1/(1 + \exp(Pi,2(Pi,3 - T)))\} \quad (1)$$

in which: EC = electrical conductivity measured in mS (milli Siemens); T = temperature (°C); N = number of steps; P0 = − log S at maximum temperature of experiment; Pi,1 = height of step i, = h_i; Pi,2 = slope factor of step i = S_i; Pi,3 = transition temperature (Tt) of step i = T_i.

The parameters (P) are calculated by a non-linear regression program (Draper & Smith 1966; Jordan-Engeln & Reutter 1974). A desktop calculator of 24 kbyte memory was used for relatively small datasets of about 100 points with at most two transition steps. For larger datasets or more transition steps the data were transferred to a computer.

The program checks for convergence by calculating the relative shift in the parameters after each iteration. If all absolute values of these relative shifts are less than 1×10^{-5}, the program stops. If this is not the case but the relative difference in successive sums of squared residuals is less than 1×10^{-7}, the program stops and offers the possibility to restart with different estimates. If neither of these conditions are met within 20 iterations, the program stops and returns the estimates corresponding to the smallest sum of squared residuals encountered in the 20 iterations.

The slope in each point of the curve is given by:

$$\sum_{i=1}^{N} [(Pi,1 \times Pi,2 \times (\exp(Pi,2 \times (Pi,3\text{-}T))))/((1 + \exp(Pi,2 \times (Pi,3 - T)))^2)] \quad (2)$$

This equation is valid regardless of the separation of the individual steps in the curve.

For curves with well-defined and well-separated steps this reduces to

$$\text{transition slope} = (Pi,1 \times Pi,2)/4 \quad (3)$$

Small positive or negative slope values for steps adjacent to step i yield a negligible contribution at $T = P_{i,3}$. Equation 3 can be used to obtain a starting value for $P_{i,2}$.

RESULTS AND DISCUSSION

Validation of the empirical model

To establish the validity of the empirical model of equation 1, the transition temperatures $P_{i,3}$ of the binary systems NaCl–H_2O and KCl–H_2O as well as of the ternary system NaCl–KCl–H_2O as model compounds were calculated by non-linear regression and compared with the values of their eutectic temperatures reported in the literature. In Fig. 1A, B the digitized points of the conductivity curves for NaCl–H_2O (120 mM) and KCl–H_2O (120 mM) are represented by (+++).

Both curves show one major transition, which is due to melting of crystallized salt. The curves for NaCl and KCl have been recorded up to 12 and 6 °C so as to include the melting of ice crystals at 0 °C, which is visible as a separate transition in both curves. Thus, both curves have been simulated using two steps in the model of equation 1 (solid line).

Simulation of the NaCl–H_2O curve yielded a transition temperature $P_{2,3}$ equal to −21·3 °C, which is in excellent agreement with the eutectic temperatures of −21·8 and −21·1 °C reported in the literature (Campbell & Smith 1951; Ito 1971). The transition temperature $P_{1,3}$ corresponds to the melting of ice crystals and will not be further discussed here.

Simulation of the KCl–H_2O curve yielded a transition temperature $P_{2,3}$ equal to −9·8 °C. The latter value also corresponds well with the reported values of the eutectic temperature of KCl–H_2O, i.e. −11·1 °C and −10·7 °C (Campbell & Smith 1951; Ito 1971). In Fig. 1C the digitized points of the conductivity curve for the NaCl–KCl–H_2O ternary system (30 mM NaCl; 120 mM KCl) are represented by (+++). The curve has been simulated using four steps in the model of equation 1 (solid line). Simulation of the curve yielded a lowest transition temperature $P_{4,3}$ equal to −22·6 °C. This value corresponds well with the reported eutectic temperature of −23·7 °C (Ito 1971).

In conclusion, the data analysis of equation 1 yields transition temperatures $P_{i,3}$ in close agreement

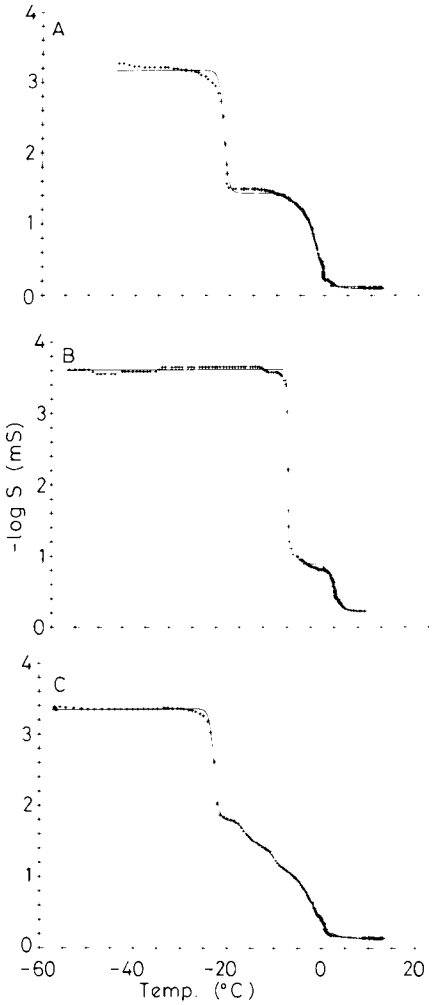

FIG. 1. Digitized form (+++) of the conductivity curves for A: NaCl–H_2O (120 mM), B: KCl–H_2O (120 mM) and C: NaCl–KCl–H_2O, together with the respective simulated curves (solid lines).

Table 1. Calculated transition temperature (°C) for the binary systems NaCl–H_2O and KCl–H_2O as well as for the ternary system NaCl–KCl–H_2O in comparison with their eutectic temperatures reported in the literature. Calculated transition slopes ($°C^{-1}$) are also given.

	Calculated transition temp. $P_{i,3}$ (°C)	Eutectic temp.* (°C)	Slope at $P_{i,3}$ ($°C^{-1}$)
NaCl–H_2O	−21·3	−21·8; −21·1	−0·82
KCl–H_2O	−9·8	−11·1; −10·7	−4·67
NaCl–KCl–H_2O	−22·6	−23·7	−0·78

* Reported in the literature (Campbell & Smith 1951; Ito 1971).

with the respective eutectic temperatures reported in the literature. In Table 1, the calculated and literature values of the transition temperatures are given,

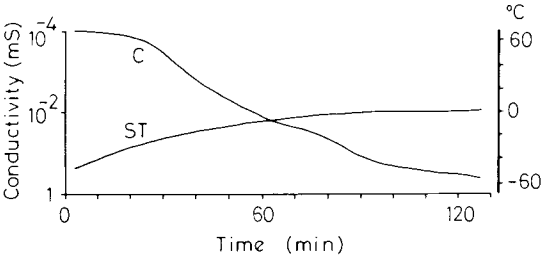

FIG. 2. Original conductivity curve recorded on prednisolone disodium phosphate solution (210 mM PDP, pH = 6·6) between −60 and 20 °C. C, conductivity; ST, sample temperature.

together with the calculated values of the transition slopes.

Prednisolone disodium phosphate

The original conductivity curve for prednisolone disodium phosphate (210 mM PDP, pH = 6·6), measured between −60 and 20 °C, is shown in Fig. 2. Fig. 3 gives the digitized points of this conductivity curve (+++), together with the corresponding simulated curve (solid line). Two steps in the model are used, because two transitions can be distinguished. The calculated transition temperatures and transition slopes are given in Table 2.

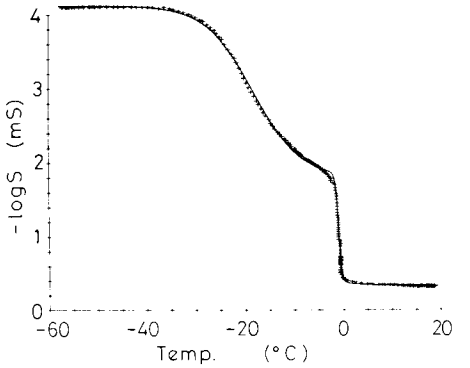

FIG. 3. Digitized form of Fig. 2 (+++) together with the simulated curve (solid line) for prednisolone disodium phosphate solution (210 mM PDP, pH = 6·6).

Table 2. Calculated transition temperatures (°C) and transition slopes ($°C^{-1}$) for prednisolone sodium succinate and prednisolone disodium phosphate.

Prednisolone sodium succinate		Prednisolone disodium phosphate	
Transition temp. $P_{i,3}$ (°C)	Transition slope at $P_{i,3}$ ($°C^{-1}$)	Transition temp. $P_{i,3}$ (°C)	Transition slope at $P_{i,3}$ ($°C^{-1}$)
−0·1	−0·50	−1·0	−1·15
−12·6	−0·06	−18·5	−0·12
−36·8	−0·06		

Prednisolone sodium succinate

The original conductivity curve for PSS (210 mM PSS, pH = 6·6), measured between −60 and 20 °C, is shown in Fig. 4. In Fig. 5, the digitized points of this curve are represented by (+++). In this curve three transitions can be distinguished. On this basis the curve has been calculated using three steps in the model as given by equation 1 (solid line). The three transition temperatures calculated are −0·1, −12·6 and −36·8 °C, respectively. These values are given in Table 2 together with the associated transition slopes (°C^{-1}). The transition at −0·1 °C is assigned to the melting of ice. This transition is associated with a high value for the transition slope (−0·50 °C^{-1}). As the average rate of heating was ~1 °C/15 min between −4 and +4 °C, equilibrium melting can be assumed. Nail & Gatlin (1985) have reported the electrokinetic and thermal analysis data on methylprednisolone sodium succinate and observed a transition at −13 °C for this drug, which is in good agreement with the present data on PSS.

The very shallow transition slope (−0·06 °C^{-1}) below 0 °C in Fig. 5 at $P_{2,3}$ and $P_{3,3}$ is in sharp contrast to the high slope values for NaCl (−0·82 °C^{-1}) and KCl (−4·67 °C^{-1}) in Fig. 1. The high slope values for NaCl and KCl are associated with their crystallization behaviour.

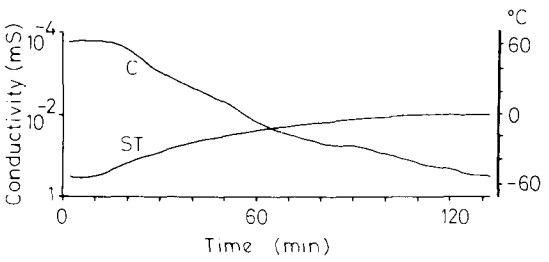

FIG. 4. Original conductivity curve recorded on prednisolone sodium succinate solution (210 mM PSS, pH = 6·6) between 60 and 20 °C. C, conductivity; ST, sample temperature.

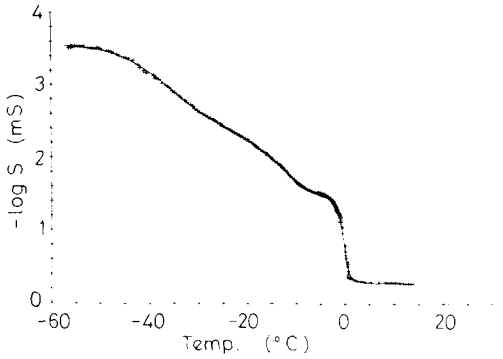

FIG. 5. Digitized form of Fig. 4 (+++) together with the simulated curve (solid line) for prednisolone sodium succinate solution (210 mM PSS, pH = 6·6).

Therefore, X-ray diffraction spectra were recorded on freeze-dried PSS as well as on prednisolone hemisuccinate (PHS), which is the starting material to prepare the compound for freeze-drying. The X-ray diffraction spectra are given in Fig. 6. From these it can be concluded that PHS is crystalline (upper spectrum) and that PSS after freeze-drying remains in an unorganized, amorphous state (lower spectrum). This is based on the observation that the spectrum of PHS shows a large number of discrete Bragg reflections, whereas the spectrum of PSS only yields a single, broad scattering peak. Crystallization of PSS may have been inhibited by the high concentration (210 mM) as well as by its micellar properties. Its critical micelle concentration (CMC) was estimated to be 50 mM on the basis of surface tension measurements (Brinks et al 1983). Consequently, freezing of PSS was performed above its CMC. For the similar drug methyl-PSS, a CMC of 20 mM has been reported (Anderson et al 1983). The combined data suggest that upon freezing the micelles of PSS become concentrated due to the continuing crystallization of ice and condense at a critical temperature, eventually leaving small amorphous particles after freeze-drying (Mazer et al 1980; Roe & Barry 1983). Evidence on the micellar compound nafcillin-sodium in the literature suggests that as a result of condensation a liquid crystalliza-

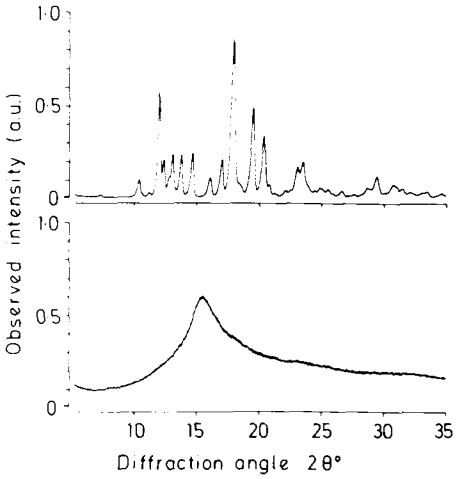

FIG. 6. X-ray diffraction spectra recorded on prednisolone hemisuccinate powder (upper spectrum) and prednisolone sodium succinate powder after freeze-drying (lower spectrum).

tion phase is obtained (Bogardus 1982). From Fig. 5 it is evident that minimum conductivity for PSS upon freezing is reached only after the solution is frozen to −50 °C, at which temperature the conductivity has a constant value.

To investigate the actual consequences of freeze-drying an aqueous solution of PSS below and above the respective calculated transition temperatures, PSS (210 mM) was frozen at different temperatures, and subsequently freeze-dried. The temperatures chosen were taken from the conductivity curve of Fig. 5: i.e. −11 °C (close to the 2nd transition temperature), −35 °C (close to the 3rd transition temperature) and −60 °C (far below the 3rd transition temperature). After freezing down to the indicated temperature, freeze-drying was performed with a standard program. Then the morphology (SEM, DSC) and residual moisture content of PSS were determined as well as the opalescence after reconstitution. SEM photographs reveal that the different freezing temperatures do not appear to affect the gross morphology after freeze-drying. A representative photograph is given in Fig. 7 (freezing at −60 °C).

The ice needle capillaries are visible as footprints in the PSS powder, yielding an estimated capillary ice needle radius of 50 μm. Similar capillary radii were found at the other freezing temperatures. Residual moisture contents (mg H_2O/vial) and opalescence after reconstitution (FTU) were of similar magnitude. The data are given in Table 3. The differences observed are small and it is hard to establish their significance.

DSC spectra were taken from 140 to 210 °C with a scan speed of 1 °C min^{-1} and 0·1 °C min^{-1}. With the lower scan speed, two DSC peaks become resolved with T_m values of 152·6–153·2 °C and 153·9–154·8 °C respectively (Fig. 8). It is well-known that PSS has an incongruent melting point. However, the presence of

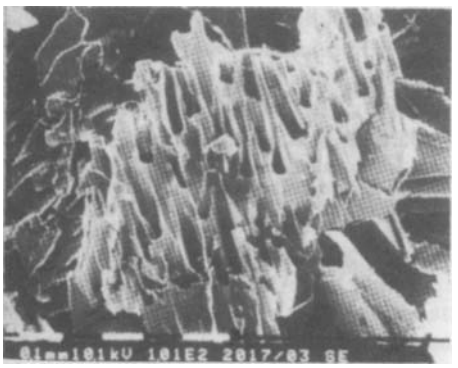

FIG. 7. SEM photograph of prednisolone sodium succinate powder after freeze-drying. The freezing temperature was −60 °C.

Table 3. Physicochemical properties of PSS, frozen to −11, −35, −60 °C and subsequently freeze-dried using a standard freeze-drying program.

Freezing temp. (°C)	Opalescence after reconstitution* (FTU)	Residual moisture content* (mg H_2O/vial)	Peak ratio calc. from DSC spectra†
−11	0·6 ± 0·1 (5)[a]	1·9 ± 0·2 (5)	1·4 ± 0·1
−35	0·6 ± 0·1 (5)[a]	1·4 ± 0·2 (5)	1·3 ± 0·1
−60	0·15 ± 0·05 (10)[b]	2·5 ± 0·2 (3)	1·1 ± 0·1

* The values given are the average of a number of determinations on one vial each; the number of determinations in each case is given between brackets. The opalescence is expressed in Formazine Turbidity Units (FTU) and measured with (a) the fluorimeter and (b) the turbidity meter.

† Height of DSC peak at T^1_m (low temperature melting peak) divided by height of DSC peak at T^2_m (high temperature melting peak).

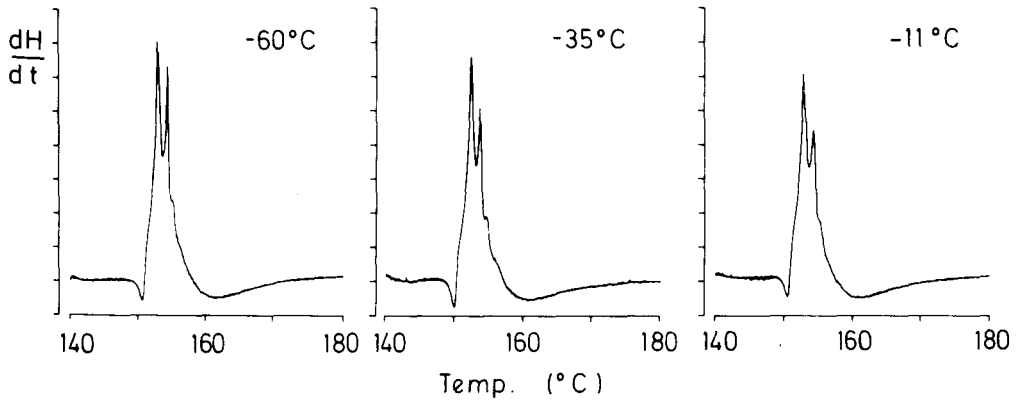

FIG. 8. DSC spectra recorded of a prednisolone sodium succinate powder after freeze-drying. The freezing temperatures were −60, −35 and −11 °C. The scan speed was 0·1 °C min^{-1} for the 3 spectra.

two peaks probably reflects the existence of two different polymorphic forms after freeze-drying. From Table 3 it is seen that there is a tendency for the calculated DSC peak ratio to decrease with decreasing freezing temperature. This may imply a shift in the relative contributions of both polymorphic forms.

Conclusions

An empirical model has been employed to describe the experimental conductivity curves of PSS and PDP. This it does adequately (van Gorp et al 1983). The validity of this mathematical model was proven by applying the model to aqueous solutions of KCl, NaCl and of a KCl–NaCl mixture. It was shown that the calculated transition temperatures for these three model systems are in excellent agreement with reported eutectic temperature values in the literature (Campbell & Smith 1951; Ito 1971). The digital plot presenting the conductivity vs temperature curve is a definite improvement over the conductivity curves recorded by the freezing analyser with respect to the ease of interpretation. In addition the distortion of these curves caused by recording the temperature and conductivity as a function of time is eliminated. The major advantage of the approach, however, is that complete conductivity-temperature curves are represented as a small set of relevant parameters, which are subsequently employed in actual freeze-drying.

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