ELSEVIER

International Journal of Pharmaceutics 132 (1996) 45–51

international journal of pharmaceutics

Solid state reactions studied by isothermal microcalorimetry; the solid state oxidation of ascorbic acid

Richard J. Willson, Anthony E. Beezer*, John C. Mitchell

The Chemical Laboratory, University of Kent, Canterbury, Kent CT2 7NH, UK

Received 17 July 1995; revised 15 September 1995; accepted 22 September 1995

Abstract

This paper reports a new method for the analysis of isothermal calorimetric data, for reactions in the solid state. As an illustration for this method of analysis, the solid state oxidation of L-ascorbic acid (vitamin C) has been studied. The investigation considers the role that water plays in the solid state oxidation of ascorbic acid and hence poses questions of the generality of water in solid state reactions. Isothermal heat conduction microcalorimetry (e.g., TAM, Thermometric, Sweden) has, for some time, been proposed as a general and rapid analytical technique that allows the calculation of the kinetic and thermodynamic parameters of chemical reactions. A new method for the determination of the kinetic and thermodynamic parameters from calorimetric data allows the quantitative study of reactions without preconceptions of the reaction mechanism and quantities of material reacting. This allows the quantitative study of reactions, especially reactions in the solid state, which, because of the inherently complex nature of reaction, are very difficult to analyse by any other means. From the analysis of the calorimetric data for the solid state oxidation of ascorbic acid at 298.15 K, we have determined that the reaction has a change in enthalpy of -195 ± 10 kJ mol^{-1} with an associated rate constant of 4.1×10^{-6} s^{-1}. The study was carried out as a function of the quantity of water, between dry (i.e. under ambient conditions) to 200 μl, added to 0.5 g ascorbic acid. The dry sample of ascorbic acid was used directly from the container, no special attention was paid to prevent atmospheric moisture entering the sample before loading the sample. Between these water quantities the kinetic and thermodynamic parameters remained the same, indicating that the reaction mechanism was unchanging throughout the range studied.

Keywords: Microcalorimetry; Solid state reaction; Ascorbic acid; Kinetics

1. Introduction

The isothermal microcalorimeter (for example, the TAM, Thermometric, Sweden) has previously been shown (Willson et al., 1995a) capable of detecting the reaction of a compound that has a first order rate constant of 1×10^{-11} s^{-1}. Such a reaction will have a half-life of 2200 years, and will have an annual degradation rate of 0.03% at 298 K and 1 bar pressure. Traditionally the degradation rates for pharmaceutical compounds have been determined using techniques such as HPLC (Constantinescu et al., 1993), NMR (Fleming et

* Corresponding author.

SSDI 0378-5173(95)04339-C

al., 1983), DSC (Buckton and Beezer, 1991), etc., following long term storage of the compound, often at elevated temperatures to accelerate the rate of reaction. All these techniques have problems associated with the experimental procedure for the analysis of degradation products and, because of the sensitivity of the detection methods, an extended length of time or elevated temperatures are required before analysis can be accurately performed. The isothermal microcalorimeter is a non-invasive and non-destructive analytical tool. Samples can be loaded into the calorimeter in any, or multiple, phases and the reaction that occurs can be studied under conditions which can be precisely controlled (temperature, pressure, humidity, gas partial pressure, with addition of scavengers, etc.). Importantly the sample's environment can be controlled and maintained under designated ambient storage conditions. The high sensitivity of the calorimeter coupled with direct and continuous observation of the reaction process means that the degradation data can be compiled within, say, 50 h of the start of the experiment (Willson et al., 1995a), 50 h being the time period required to differentiate between a first order rate constant of 1×10^{-11} and of 2×10^{-11} s^{-1}. The use of the isothermal microcalorimeter is therefore gaining an increasing reputation as an analytical tool for the prediction of *the long term stability* of drugs and compounds manufactured in the pharmaceutical industry.

There is a surprising lack of good kinetic data together with the associated thermodynamic parameters for chemical reactions published in the literature; this is especially true for long, slow reactions and for reactions that occur in the solid state. There have been several attempts to formally analyse chemical reactions (Byrn, 1982; Frank, 1985; Taylor and Shivji, 1987), and in particular solid state reactions (Byrn, 1982; Ng, 1975), however none of these methods seem totally satisfactory. The analytical procedure for solid state reactions is a much more complicated process because of the complexity of solid state reactions compared with that for reactions which occur in a solution or gas phase. In a previous paper (Willson et al., 1995a) a new method for the analysis of isothermal calorimetric data was proposed through which the kinetic and thermodynamic parameters for reactions can be determined directly from calorimetric data. The isothermal calorimeter produces two types of data, heat flow (power, Φ, in Watts) and heat output (q in Joules). The first term, heat flow, is a kinetic term and the second term, heat output, is a thermodynamic term. By using these two term in conjunction, the thermodynamic and kinetic parameters for the reaction under study can be determined; the rate constant, change in enthalpy and, for compounds in the solid state, m and n, the fitting constants for the reaction (see Section 2). It should be noted that for reactions in the solid state, m and n are not strictly the order of reaction, but are fitting parameters, relating to the mechanism by which the compound reacts. A previous study from this laboratory reported the use of an isothermal heat conduction microcalorimeter to study the oxidation of ascorbic acid in solution (Willson et al., 1995b). This study was carried out under controlled and varied conditions of pH, temperature, oxygen partial pressure, concentration of ascorbic acid, the addition of radical scavengers and the control of metal ions in solution. We demonstrated that the reaction process was dependent upon the concentration of oxygen in the system and that the degradation process involves metal ions (trace quantities inherent in the water supply) to initiate the reaction that proceeds via the formation of radicals together with the liberation of hydrogen peroxide. The results of this calorimetric study showed that by careful analysis of the calorimetric data the rate constant, change in enthalpy and order of reaction could be determined. These calculated parameters were found to be consistent with separately published data from independent research.

We now report a further study of the oxidation of ascorbic acid in which the quantity of water present in the ascorbic acid sample is controlled. The water content within the reaction ampoules, containing 0.5 g ascorbic acid, was varied from 200 to 20 μl. In the presence of 200 μl of water, the ascorbic acid reaction sample had a gel like appearance, and in the presence of 20 μl of water, the sample was free flowing and was indistinguish-

able from dry ascorbic acid. We therefore consider the calculated kinetic and thermodynamic parameters for the reaction to be essentially that for the reaction of ascorbic acid in the solid state.

To gain access to the true reaction parameters for the reaction studied in the calorimeter, it is important that the isothermal microcalorimeter is properly calibrated. It has been shown that the most useful method of calibration available is that of chemical calibration (Willson et al., 1995c). The characterisation of the solid state ascorbic acid reaction in terms of rate, enthalpy change and the fitting parameters m and n will allow this reaction to be considered as a chemical calibrant for medium term (a solid state reaction rate of about 8% per day) solid state reactions.

2. Experimental

Ascorbic acid (supplied by Sigma, 99.9% pure) was kept sealed in a desiccator. *Un-sieved* samples (0.5 g) were weighed out into glass ampoules (supplied by Thermometric, AB, Jarfalla, Sweden) and loaded in the isothermal calorimeter (TAM, Thermometric, AB, Jarfalla, Sweden). The operation of this instrument was as described in the manufacturer's manual (Thermometric AB, Jarfalla, Sweden). For additional operational detail refer to Suurkuusk and Wadso (1982). A 30 min thermal equilibrium period was allowed before data collection, using the dedicated Digitam software, began. The isothermal microcalorimeter (TAM) was housed in a constant temperature room (21 ± 0.1°C) and was operated at $25 \pm 1 \times 10^{-4}$°C. Calorimetric experimental runs lasted typically for 50 h before the experiment was terminated. The data collected using the Digitam software was then processed as described previously (Willson et al., 1995a) using the Origin graphics package. This was used to calculate the thermodynamic and kinetic parameters.

The kinetics of solid state degradation have previously been described (Willson et al., 1995a). The general equation proposed by Ng (1975), Eq. (1), can be manipulated so that it can be applied to calorimetric data.

$$\frac{d\alpha}{dt} = k(\alpha)^{1-m}(1-\alpha)^{1-n} \quad (1)$$

where m and n are the fitting constants for the reaction, indicating the mechanism for the degradation path, α is the fraction of the compound degraded as a function of time, $d\alpha/dt$ is the rate of reaction and k is a general form of the rate constant for the reaction and has the units s^{-1} for all solid state reaction schemes. Therefore, as $\alpha = x/A$, where x is the quantity of starting material (A) that has reacted over time, x/A can be substituted for α, Eq. (2)

$$\frac{dx}{dt} = Ak\left(\frac{x}{A}\right)^{m}\left(1-\frac{x}{A}\right)^{n} \quad (2)$$

From thermodynamics, $q = \Delta H\, x$, where q is the heat output for the reaction, ΔH is the change in enthalpy for the reaction and x is the amount of starting material reacted over time. So by substituting $q/\Delta H$ for x in the above equation (Eq. (2)), we obtain

$$\frac{dq}{dt} = \Phi = Ak\ \Delta H\left(\frac{q}{A\ \Delta H}\right)^{1-m}\left(1-\frac{q}{A\ \Delta H}\right)^{1-n} \quad (3)$$

From a plot of Φ vs q (the calorimetric data) we obtain a graph that is dependent on the values of k, A, ΔH, m and n. Using an iterative curve fitting procedure, such as the Origin graphics package, the constants k, ΔH, m and n can be determined.

This equation (Eq. (3)) can be simplified for convenience. Assuming the reaction goes to completion, Q (the total heat output for the reaction) $= A\ \Delta H$, therefore extrapolation of the power (Φ) heat graph to $\Phi = 0$, the ordinate value at $\Phi = 0$ has the value of Q. This value of Q can then be substituted for $A\ \Delta H$ in Eq. (3). The values for k, m and n can then be determined, Eq. (4)

$$\Phi = kQ\left(\frac{q}{Q}\right)^{1-m}\left(1-\frac{q}{Q}\right)^{1-n} \quad (4)$$

Equations for reactions in the solution phase were derived from solution phase kinetic equations

$$\frac{dx}{dt} = k(A-x)^{m}(B-x)^{n}$$

Table 1
The kinetic and thermodynamic parameters for the oxidation of 0.5 g L-ascorbic acid as a function of water concentration. The equation used for the calorimetric analysis is:

$$\frac{dq}{dt} = \Phi = Ak\,\Delta H\left(\frac{q}{A\,\Delta H}\right)^m\left(1-\frac{q}{A\,\Delta H}\right)^n$$

	Rate constant (s^{-1})	Change in enthalpy ($kJ\,mol^{-1}$)	Concentration of reactant [A] (mol)	m^a	n^a
Dry ascorbic acid	1.15×10^{-6}	−199	4.39×10^{-7}	−0.01	0.9
20 μl	4.10×10^{-6}	−199	2.25×10^{-6}	−1.41	0.59
30 μl	4.35×10^{-6}	−199	2.39×10^{-6}	−0.01	0.91
50 μl	5.21×10^{-6}	−197	3.93×10^{-6}	−0.01	0.98
100 μl	5.62×10^{-6}	−180	4.27×10^{-6}	−0.12	0.76
200 μl	4.22×10^{-6}	−188	8.045×10^{-6}	−0.09	0.34
500 μl					

[a] m and n are the fitting parameters for the solid state reaction, the values of which can be used to determine the mechanistic pathway of the reaction for the compound

and, as before, $q = \Delta H\,x$, therefore $q/\Delta H$ can be substituted for x

$$\frac{dq}{dt} = \Phi = k\,\Delta H\left(A-\frac{q}{\Delta H}\right)^m\left(B-\frac{q}{\Delta H}\right)^n \qquad (5)$$

Again from a plot of Φ vs q, the constants of the equation can be determined. It is not necessary to specify values of A, k, ΔH, m and n before applying this method. Solid state reactions where α and A are often unknown, therefore, present a severe test to this proposed method of analysis.

3. Results

Samples (0.5 g) of un-sieved ascorbic acid, with the addition of 0, 10, 20, 30, 50, 100 and 200 μl of water, were analysed using the isothermal microcalorimeter operated at 298.15 K. The calorimetric data were analysed as described in Section 2. From the experimental results we have determined that the rate constant and change in enthalpy for the oxidation process is *independent* of the quantity of water in the reaction sample over the range studied, see Table 1.

The power–time curves for the ascorbic acid reaction as a function of water (Fig. 1) show the *rate* of reaction increases with increasing amounts of water in the reaction sample. There is an initial heat flow recorded from ca. 1.5 h that increases as a function of water present in the reaction sample. This initial heat flow period is possibly due to the oxygen dissolved in the water phase reacting at the ascorbic acid crystal surface. After this period, the kinetics for the reaction appear to be dependent on the rate at which oxygen can diffuse from the head space, through the aqueous boundary at the crystal surface, to the un-reacted 'clean' layer of the ascorbic acid crystal.

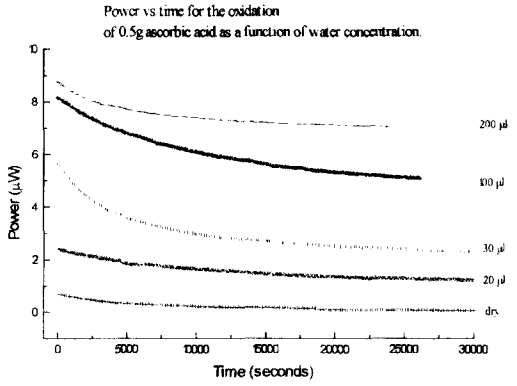

Fig. 1. A power time graph for the oxidation of ascorbic acid as a function of water quantity added to a 0.5 g ascorbic acid sample. The reaction *rate* for the oxidation of ascorbic acid in the solid state is seen to increase as a function of water quantity added. Although power vs time is not strictly the rate of reaction, the calorimetric signal (Φ) is proportional to the rate of reaction if the change in enthalpy is constant.

The *rate* of the oxidation process is dependent on the concentration of oxygen at the crystal surface that has the ability to react. Therefore, we should expect that decreasing the amount of water around the crystal structure, the rate of reaction will decrease, because of the reduction in available oxygen dissolved in the water at the crystal surface. This is seen from the initial values of power at $t = 0$, see Fig. 1 (although power is not strictly the rate of reaction, it is proportional to the rate because the change in enthalpy for the reaction is constant as long as the reaction mechanism is unchanging). Note that, although the rate of reaction is dependent on the quantity of water, and hence the available oxygen concentration, the *rate constant*, k, for the reaction remains, within experimental error, the same. The rate constant will only change if there is a change in the reaction mechanism, or temperature.

4. Discussion

From the experimental data we conclude that the oxidation of ascorbic acid in the solid state has an overall rate constant of 4.1×10^{-6} s^{-1} (see Table 1), with an associated change in enthalpy of -195 ± 10 kJ mol^{-1}. This is the first time, that we know of, that this solid state reaction has been characterised.

Compared to the solution phase oxidation of ascorbic acid (Willson et al., 1995b), the rate determining step for the solid state reaction is slower (seen by a longer reaction half-life; the $t_{1/2}$ for solution phase oxidation is about 1 h). We suggest that the reason for this is that the experiment in the solution phase was constructed such that there was no significant head space above the reaction sample, so all the available oxygen was contained within the water phase. In the solid state, these reaction conditions could not be reproduced because of the limited quantities of water present. Therefore, in the solid state the rate determining step is probably the rate at which oxygen can diffuse, from the surrounding space, into the water layer at the crystal surface and react with the ascorbic acid. Analysing the calorimetric data, as described in the Section 2, a value

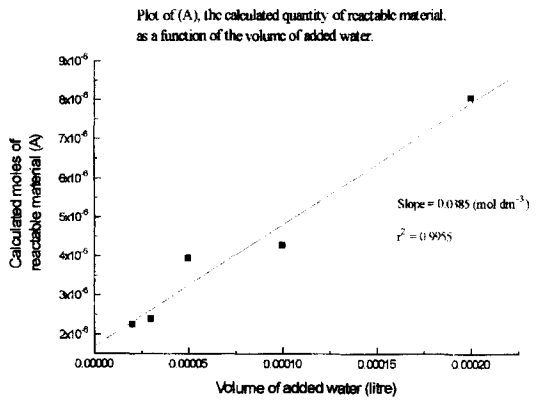

Fig. 2. A plot of A, the calculated total quantity of reactable material, vs the volume of added water, showing a linear relationship with a slope of 0.0385 mol dm^{-3}.

of A, the total concentration of material that has reacted when the reaction has gone to completion, can be determined. It was found that by plotting the calculated value of A vs the volume of water added for each reaction (see Fig. 2) there was a linear relationship with a slope of 0.0385 (mol dm^{-3}), with an r^2 value for the line of 0.9955. The significance of 0.0385 is so far unclear, although it is apparent that it is a concentration term associated with the rate determining step for the reaction. We have calculated that this value is not the water quantity (at 20 μl, there are 1.1×10^{-3} moles of water present, compared to the value of A, 2.2×10^{-6} moles). It is not the concentration of oxygen in the water phase around the crystal; 1×10^{-5} moles in 20 μl; even in a saturated solution of ascorbic acid, this value decreases by only 10%. It is also not the solubility of ascorbic acid in water; 2×10^{-5} moles in 20 μl. However, possibilities include the solubility of dehydroascorbic acid in water (the oxidation product of ascorbic acid). If the rate determining step is the removal of dehydroascorbic acid from the crystal reaction site, then the water solubility of dehydroascorbic acid will determine the total concentration of material that can react. Another possibility is the effective area of the ascorbic acid crystal that is exposed to oxidation. If one considers a cubic crystal that has reaction sites at regular depths within the lattice, the volume that is required to contain the concentration of 0.0385

mol dm^{-3} of ascorbic acid can be estimated. We have calculated using mathematical models that for 0.5 g of cubic crystals of uniform size, with the addition of 100 μl water, the depth required to be penetrated into the crystal surface to have sufficient area to obtain 0.0385 mol dm^{-3} ascorbic acid is 200 nm. This depth, when applied to a model of the ascorbic acid crystal (Davies et al., 1991), gives access to two layers of ascorbic acid molecules in the crystal lattice. The calculated average values of m and n, the fitting parameters, for this reaction were found to be $m = -0.01$ and $n = 0.8$. This is consistent with a contracting sphere type of reaction as described by Ng (1975) where $m = 0$ and $n = 0.77$.

Consideration must now be given to the *role* of water in solid state reactions. It is evident from the experimental data that the *rate* of reaction increases with increasing amounts of water in the reaction sample. The experiments were performed at differing water volumes up to 200 μl. However, the point at which the reaction ceases to be characterised as solid state, and becomes more characteristic of reactions in the solution phase, is unclear and subject to future experimentation. In the presence of up to 200 μl of water, the reaction kinetic and thermodynamic parameters were unchanging, suggesting that the reaction mechanism is the same as that for the 'dry' (i.e. surface properties determined by ambient RH) ascorbic acid sample. However, no special treatment was given to the dry sample to ensure that it remained dry during the loading of the sample into the calorimeter and so it probably acquired atmospheric moisture. The solid state analysis for water quantities above 200 μl gave increasingly variable results, and by 500 μl, the kinetics and thermodynamics calculated using solution phase equations (Willson et al., 1995a) gave results that were consistent with the kinetics and thermodynamics for 'pure' solution phase reactions as previously published (Willson et al., 1995b). The water boundary thus acts as a transport phase, allowing access of water to the ascorbic acid surface and the removal of oxidised products away from the surface.

The change in enthalpy for the reaction in the solid state is about 58 kJ mol^{-1} more exothermic than that found for the reaction in the solution phase. The reason for this is probably because the *overall* ΔH is a combination of the energies required to break the crystal lattice in the solid state to dissolve the ascorbic acid, to dissolve oxygen and the oxidised products at the interface, as well as the ΔH for the reaction. The ΔH for the dissolution of these species was not a component of the enthalpy measured during the solution phase study previously reported. Further experimentation is required, firstly, to determine if water is required for the solid state oxidation of ascorbic acid, i.e. what effect would completely removing all traces of water have on the reaction. Secondly, to determine how general the role of water is with respect to other types of solid state reactions.

A proposed chemical calibrant for the calorimeter is required to be fully characterised, have a medium to long term life span and to have a simple mechanism of degradation. The solid state oxidation of ascorbic acid, although it has now been characterised, is still not ideal as a calibrant because of its relatively short reaction time and the complex nature of its degradation. However, the fact that characterisation was accomplished gives confidence in the data analysis proposed and offers, therefore, the prospect that a better calibration model can be found in the future.

Acknowledgements

One of us (R.J.W.) is grateful to Pfizer Central Research, Pfizer Ltd, Sandwich, Kent, for financial support: in particular, to Dr T. Dolan and Dr P. Brewer for helpful discussions. We are all grateful to Mr R.P. Lipscombe for specialist technical advice on use of the isothermal calorimeter, and for keeping it running at its optimal performance level.

References

Buckton, G. and Beezer, A.E., The applications of microcalorimetry in the field of physical pharmacy. *Int. J. Pharm.*, 72 (1991) 181–191.

Byrn, S.R., *Solid State Chemistry of Drugs*, Academic Press, New York, 1982, pp. 3–26.

Constantinescu, A., Han, D. and Packer, L., Vitamin E recycling in human erythrocyte membranes. *J. Biol. Chem.*, 268 (1993) 10906–10915.

Davies, M.B., Austin, J. and Partridge, D.A., *Vitamin C, its Chemistry and Biochemistry*, Royal Society of Chemistry Paperbacks, 1991.

Fleming, J.E., Miyashita, K., Quay, S.C. and Bensch, K.G., Bicyclic ring formation is not necessary for the (auto)oxidation of ascorbic acid. *Biochem. Biophys. Res. Commun.*, 115 (1983) 531–535.

Frank M.J., Drug degradation studies. *Anal. Chem.*, 57 (1985) 69A–74A.

Ng, W.L., Thermal decomposition in the solid state. *Aust. J. Chem.*, 28 (1975) 1169–1178.

Suurkuusk, J. and Wadso, I., A multi channel microcalorimetry system. *Chem. Scripta*, 20 (1982) 155–163.

Taylor, R.B. and Shivji, A.S.H., A critical appraisal of drug stability testing methods. *Pharm. Res.*, 4 (1987) 177–180.

Willson, R.J., Beezer A.E., Mitchell, J.C. and Loh, W., Determination of thermodynamic and kinetic parameters from isothermal heat conduction microcalorimetry; applications to long term reaction studies. *J. Phys. Chem.*, 99 (1995a) 7108–7113.

Willson, R.J., Beezer A.E. and Mitchell, J.C., A kinetic study of the oxidation of L-ascorbic acid (vitamin C) in solution, using an isothermal microcalorimeter (TAM). *Thermochim. Acta*, 264 (1995b) 27–40.

Willson, R.J., Beezer A.E. and Mitchell, J.C., The imidazole catalysed hydrolysis of triacetin; a medium term chemical calibrant for calorimeters; the characterisation of the hydrolysis reaction and a long term stability study of the isothermal microcalorimeter (TAM), (1995c), in preparation.