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A preformulation study on the in vitro dissolution characteristics of the organophosphorus poisoning antidote HI-6

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Summary

The in vitro dissolution of the new organophosphorus antidote HI-6 has been investigated by using the rotating disc technique. By applying centrically rotating discs, the diffusion controlled dissolution rates at different laminar flow rates, pH and temperatures have been obtained. From the well-known Levich relationship, a diffusion coefficient was obtained. The enthalpy of activation of the dissolution rate indicates that the ability of HI-6 to dissolve is very high. This is also supported by the value of the initial mass transfer from solid to aqueous phase, i.e. the intrinsic rate of dissolution, which was found to be 7.4 $mg \cdot cm^{-2} \cdot s^{-1}$. This rate constant was obtained by using a modified rotating disc method. The intrinsic dissolution rate of HI-6 was, however, much higher than expected from solubility data. The crystal form of HI-6 seems to change when equilibrated with water as indicated by scanning electron microscopy and thermal analysis, which thus might explain the observed deviation from the general solubility – dissolution rate relationship.

Introduction

Many different experiments are needed before a routine formulation work can start on a new chemical entity, intended for medical use. Among several things, physicochemical characterisation plays an important part for making proper decisions regarding dosage from design. One important physicochemical aspect to consider is the in vitro dissolution of the chemical entity under various experimental conditions. Not only does it answer biopharmaceutical questions such as pH dependency and dissolution rate-limited absorption, but also several pharmaceutical issues.

When studying in vitro dissolution, it is of utmost importance that the experimental conditions are well defined and reproducible. One of the most pertinent techniques which indeed fullfills these requirements is the rotating disc method. The hydrodynamics of the rotating disc system have been thoroughly described in several papers (Grijseels et al., 1981; Levich, 1962; Millsaps and Pohlhausen, 1952; Riddiford, 1966). The rotating disc method is most widely used in electrochemistry but the method has also been used successfully in many pharmaceutical and biopharmaceutical investigations (Carless and Jordan, 1974; Friedman and Asherov, 1981; Graffner et al., 1985; Kaplan, 1972; Levy and Tanski, 1964;

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Nicklasson and Nyqvist, 1983; Nicklasson and Brodin, 1984; Nicklasson and Magnusson, 1985; Nyqvist and Nicklasson, 1985; Prakongpan et al., 1976; Wood et al., 1965). The application of the centrically rotating disc technique, like the one described by Levich (1962) and Wood (1965) has become well accepted for pharmaceutical applications. However, recently a modified rotating disc technique was described using the concept of excentrically rotating discs, in order to obtain the initial maximum mass transfer from solid to liquid phase without any influence of a diffusion process (Nicklasson et al., 1982b; Nicklasson et al., 1983; Nicklasson et al., 1985). The theoretical considerations regarding this modified rotating disc technique has been discussed in a paper, applying a molecular approach (Nicklasson et al., 1985).

The oxime HI-6 has been shown to be an efficient organophosphorus antidote. Experiments on rats showed that HI-6 caused functional recovery of neuromuscular transmission in vivo and in vitro when given 60 min after the acetylcholinesterase inhibitor soman (Wolthuis and Kepner, 1978). The pharmacokinetics of HI-6 has been studied in beagle dogs (Simons and Briggs, 1983). The results showed a 100% bioavailability from the i.m. route of administration compared to i.v. administration.

One way to increase the stability of a parenteral dosage form is to use a sterile powder of the drug which then is reconstituted immediately before administration. In such cases, dissolution properties are of fundamental interest in order to design an efficient parental dosage form system. It is the aim of this paper to demonstrate the in vitro dissolution properties of HI-6 under various experimental conditions such as pH, temperature and hydrodynamics using both the centrically and the modified excentrically rotating disc techniques. Correlations between dissolution kinetics and aqueous solubility will also be discussed.

Materials and Methods

Chemicals

HI-6 (1-(2-hydroxyiminomethyl- pyridinium)-2-(4-carboxyamidopyridinium)-dimethyl ether dichloride) was used as obtained. HCl, NaCl, H_3PO_4, NaH_2PO_4, Na_2HPO_4, CH_3COONa, NaOH and boric acid of analytical grade were used to prepare the buffer solutions. The pH-values of the buffer solutions were measured using an Orion 601A pH-meter equipped with a combination pH electrode.

Thermoanalysis and thermodynamics

Crystals of HI-6 equilibrated with water for 20 h were collected and allowed to dry overnight at room temperature. Samples of 5 mg were placed in a 910 Differential Scanning Calorimeter (Du Pont Instruments). The samples were run at 10 °C/min. Thermograms were also recorded for HI-6 crystals which had not been in contact with water.

The dissolution rates of HI-6 in water from centrically mounted discs were determined at 500 rpm as a function of temperature (18–34.4 °C). The activation energy (E_a) for the dissolution process was calculated by linear regression analysis from Arrhenius plots. The enthalpy of activation for the dissolution process at 25 °C (ΔH^*) was calculated according to Eqn. 1:

$$E_a = \Delta H^* + R \cdot T \tag{1}$$

where $R = 8.314$ J · mol^{-1} · K^{-1} and T = temperature, K.

By knowing the ΔH^* for the dissolution process, the free energy of activation (ΔG^*) can be obtained by applying the following equation (Brodin et al. 1976; Nyqvist and Nicklasson, 1985):

$$\Delta G^* = \Delta H^* - T\Delta S^* \tag{2}$$

where ΔS^* is the entropy of activation ($J \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$). The value of ΔS^* can be calculated according to Eqn. 3 (Brodin et al., 1976; Nyqvist and Nicklasson, 1985):

$$J = \lambda \cdot e\frac{K \cdot T}{h} \cdot e^{\Delta S^*/R} \cdot e^{-E_{a/RT}} \tag{3}$$

where

J = dissolution rate, mol · cm^{-2} · s^{-1}

λ = distance between equilibrium positions for the solutes, Å

K = Boltzmann constant, $1.38 \cdot 10^{-23}$ J · K^{-1}
h = Planck constant, $6.63 \cdot 10^{-34}$ Js.

From the intercept of an Arrhenius plot, the value of $\lambda \cdot e^{\Delta S^*/R}$ can be obtained as can be seen from Eqn. 3 and from this expression, ΔS^* is obtained. The data reported here has been calculated by arbitrarily assuming $\lambda = 10$ Å, which is the approximate length of the solute molecules as estimate from bond lengths (Brodin et al., 1976).

Dissolution rates

Discs of HI-6 having a diameter of 11.3 mm and a thickness of about 1–1.5 mm were compressed in a Diaf excenter press using flat-faced tablet punches. The discs were attached to round steel plates using a water-resistant tape with a circular hole of 0.50 cm^2. The experimental procedure has been described in (Nicklasson et al., 1985; Nicklasson and Magnusson, 1985). The steel plates with the discs were attached to a motor (IKA RW20 DZM) and while rotating it was lowered into 200 ml of solution thermostated at 25 °C. At the excentric mounting (Nicklasson and Magnusson, 1985), the distance between the centre of the steel plate and the centre of the compressed disc varied from 1.2 to 2.4 cm. At each run, the dissolution medium was analysed by continuous recirculation through a spectrophotometer (Beckman DU-7) using a 10 mm flow cuvette and a peristaltic pump. Samples were assayed at wavelength of maximum absorption e.g. water and pH 1.2–6.4 = 303 nm, pH 6.9–7.1 = 305 nm and pH 7.6–9.0 = 355 nm. The observed dissolution rate of HI-6, G (mg · cm^{-2} · s^{-1}), at a given rotation speed, pH and temperature was calculated by linear regression analysis of the amount dissolved per cm^2 as a function of time. The maximum rate of dissolution of HI-6 i.e., the intrinsic rate of dissolution at 25 °C in water was determined by means of excentrically rotating discs using Eqn. 4 (Nicklasson et al., 1983)

$$\frac{1}{G} = \frac{1}{k_1} + \frac{k'}{R\sqrt{\omega}} \qquad (4)$$

where
k_1 = intrinsic rate of dissolution, mg · cm^{-2} · s^{-1}
k' = proportionality constant
R = distance form the centre of the steel plate to the centre of the disc surface, cm
ω = angular velocity, s^{-1}.

This expression verifies that, for laminar flow G^{-1} is a linear function of $(R/\omega)^{-1}$ which for $(R\sqrt{\omega})^{-1} = 0$ extrapolates to k_1^{-1} i.e. the reciprocal value of the intrinsic rate of dissolution.

The dissolution rates at 25 °C of HI-6 were also determined from centrically mounted discs as a function of pH (pH 1.2–9.0 at 500 rpm) and as a function of angular velocity (120–1000 rpm in water). For the latter experimental conditions the well acknowledged Levich relationship can be applied (Levich, 1962), i.e.

$$G = 0.61 \cdot D^{2/3} \cdot \nu^{-1/6} \cdot C_s \cdot \omega^{1/2} \qquad (5)$$

where
D = diffusion coefficient, cm^2 · s^{-1}
ν = kinematic viscosity, cm^2 · s^{-1}
C_s = aqueous solubility, mg · cm^{-3}.

By plotting G versus $\sqrt{\omega}$, a straight line is obtained from which it is possible to calculate the diffusion coefficient. A kinematic viscosity of $8.931 \cdot 10^{-3}$ cm^2 · s^{-1} was used (water 25 °C).

Solubility

HI-6 in excess of solution saturation quantity was added to water. The resulting suspension was equilibrated at 25 °C for 24 h using a magnetic stirrer. Samples of 5 ml were filtered through 0.1 μm polycarbonate filters (Nuclopore, U.S.A.) and diluted to suitable concentrations for spectrophotometry.

Scanning electron microscopy

Crystals of HI-6 were covered with approximately 300 Å of gold using a JEOL Fine Coat ion sputter (JFC-1100). Pictures of the prepared crystals were taken in a JEOL JSM-25 II scanning electron microscope (SEM).

Results and Discussion

Fig. 1 shows the Levich relationship for HI-6 in water at 25 °C. As can be seen from Eqn. 5, a plot

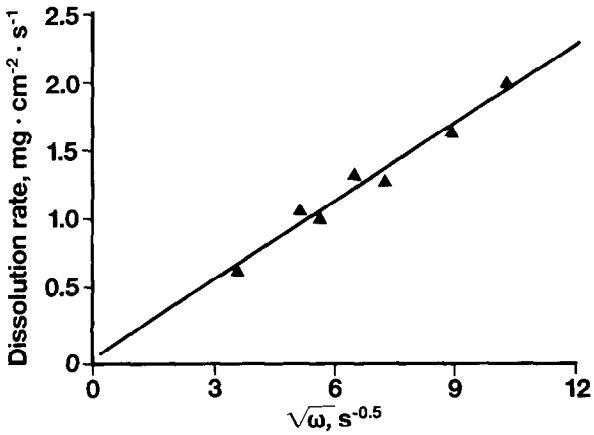

Fig. 1. Dissolution rates of HI-6 as a function of the square root of the angular velocity. Centrically rotating discs, water, 25 °C.

of the dissolution rate (G) vs. the square root of the angular velocity for centrically rotating discs will give a straight line. The intercept should be very close to origo. This requirement is obtained for HI-6. The intercept calculated by means of linear regression analysis was found to be 0.0099 $\pm$ 0.10 ($\pm$S.E.M., $n = 7$). The data shown in Fig. 1 demonstrate the diffusion-controlled dissolution rate of HI-6 at various laminar flow rates past the disc surface. At a moderate rotating speed, i.e. 500 rpm (corresponding to a $\sqrt{\omega}$ value of 7.2 $s^{1/2}$) the diffusion-controlled dissolution rate of HI-6 is 1.28 $mg \cdot cm^{-2} \cdot s^{-1}$ and at 1000 rpm ($\sqrt{\omega} = 10.2$) the dissolution rate is 2.00 $mg \cdot cm^{-2} \cdot s^{-1}$, which indicates that HI-6 is a rapidly dissolving compound in water. This interpretation is supported by data recently obtained at identical experimental conditions from other freely soluble compounds (Nicklasson and Magnusson, 1985). For the most rapidly dissolving compound reported in that paper, i.e. buciclovir hydrochoride, a value of 1.5 $mg \cdot cm^{-2} \cdot s^{-1}$ was obtained at 500 rpm. However, the temperature was 37 °C which normally means that the corresponding value at 25 °C should be 2–3 times lower. From the slope in Fig. 1 it is possible to calculate the diffusion coefficient of the solute (D) since the kinematic viscosity of water and the aqueous solubility of HI-6 are constants at 25 °C (cf. Eqn. 5). The slope calculated by means of linear regression analysis was found to be 0.19 $\pm$ 0.015 ($\pm$S.E.M., $n = 7$, $r = 0.986$). The diffusion coefficient thus obtained was calculated to be $7.7 \cdot 10^{-6}$ $cm^2 \cdot s^{-1}$. This value of D is in good agreement with other data reported for compounds having similar molecular weights, i.e. around 400–600 (Nicklasson et al., 1982a; Nicklasson et al., 1982c; Nicklasson and Magnusson, 1985).

The diffusion controlled dissolution rate from centrically mounted discs, was also determined as a function of pH. The pH–dissolution rate relationship for HI-6 at 500 rpm is shown in Fig. 2. Similar relationships for other compounds have been demonstrated in papers by Nicklasson et al. (1981) and Nicklasson and Magnusson (1985). Since the rotating disc method is relatively simple and rapid, valuable information can easily be obtained within a wide pH-range. From the relationship in Fig. 2, it is quite evident that HI-6 behaves both as an acid and as a base. Two pK_a-values can be estimated graphically, i.e. 7.1 and 7.8. A dissolution rate of about 1.28 $mg \cdot cm^{-2} \cdot s^{-1}$ was obtained at pH between 1.2 and 6.9 which is the same rate as obtained in water at the same experimental conditions. At pH near 7 a lower dissolution rate is obtained. However, still a very high release rate of HI-6 was found between pH 7.1 and 7.9 (0.97 $mg \cdot cm^{-2} \cdot s^{-1}$). Above pH 7.9, the dissolution rate of HI-6 increases and at pH 9 the value is nearly the same as the one found at pH less than 6.9.

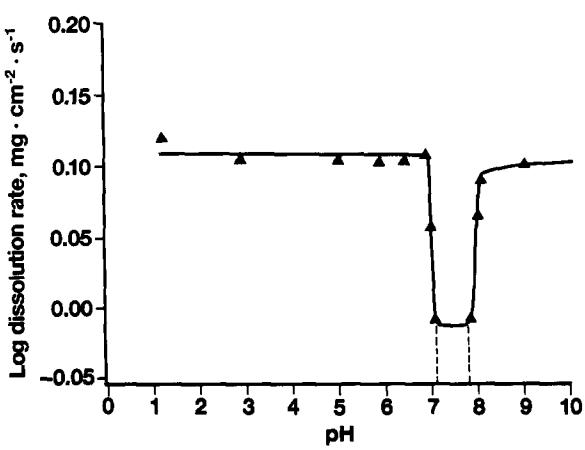

Fig. 2. Log dissolution rates vs pH for HI-6 using centrically rotating discs (500 rpm, 25 °C).

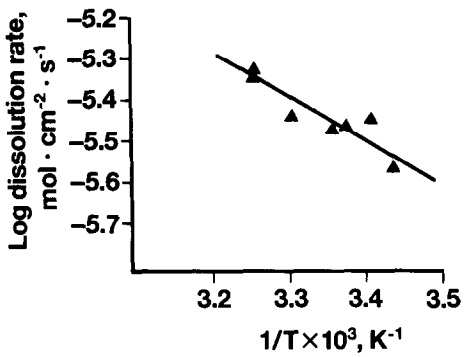

Fig. 3. A plot of log dissolution rates of HI-6 using centrically rotating discs (500 rpm, water) as a function of the reciprocal value of the absolute temperature.

Fig. 3 shows an Arrhenius plot of the dissolution rate of HI-6 from centrically rotating discs in water (500 rpm). Previous papers have reported similar relationships for other substances, from which it is possible to calculate the activation energy of the dissolution process (Tsuji et al., 1979; Nicklasson et al., 1982a; Nicklasson and Magnusson, 1985). The activation energy for HI-6 was calculated to be 20.3 kJ · mol^{-1} and the enthalpy of dissolution, ΔH^*, was calculated to be 17.9 kJ · mol^{-1} at 25 °C, cf. Eqn. 1. This ΔH^* value indicates that the energy difference between solid and liquid phase is relatively small and thus supports the conclusion that HI-6 is a compound with a high ability to dissolve in water. Data on enthalpies of activation of the dissolution process have been reported for various epimer forms of bacampicillin hydrochloride (Nyqvist and Nicklasson, 1985) and for alaproclate hydrochloride, acetylsalicylic acid and sulfamethizole (Nicklasson et al., 1982a). For the freely soluble compound bacampicillin hydrochloride, a ΔH^*-value of about 17 kJ · mol^{-1} was obtained corresponding to a dissolution rate of about 0.8 mg · cm^{-2} · s^{-1} at 22 °C at a rotating speed of 400 rpm (centrically rotating discs). At 25 °C and 500 rpm a value of about 1 mg · cm^{-2} · s^{-2} can be estimated. The data reported here for HI-6, i.e. 17.9 kJ · mol^{-1} and 1.28 mg · cm^{-2} · s^{-1}, thus seems to be relatively close to the values found for bacampicillin hydrochloride. For less soluble compounds like e.g. sulfamethizol, ΔH^* values of about 40–50 kJ · mol^{-1} have been reported (Nicklasson et al., 1982a) which indicates a higher energy difference between solid and liquid phase and therefore a lower ability to dissolve. The free energy of activation for the dissolution of HI-6, ΔG^*, at 25 °C was calculated to be 64.1 kJ · mol^{-1}, cf. Eqns. 2 and 3. When comparing the ΔG^* value for HI-6 with the corresponding ones obtained for various crystal forms of bacampicillin hydrochloride (Nyqvist and Nicklasson, 1985) it can be concluded that there is a relatively high energy content in the HI-6 crystals.

The initial mass transfer from solid to aqueous phase at 25 °C was calculated according to Eqn. 4. The extrapolation procedure and linear regression analysis are shown in Fig. 4. An intrinsic rate of dissolution (k_1) of 7.4 mg · cm^{-2} · s^{-1} was calculated for HI-6. This value once again confirms the extremely rapid dissolution rate of HI-6 in water. For freely soluble compounds such as remoxipride hydrochloride monohydrate or buciclovir hydrochloride, corresponding values (at 37 °C) of about 4 and 4.5 mg · cm^{-2} · s^{-1} have been reported respectively (Nicklasson et al., 1983; Nicklasson and Magnusson, 1985). These values are normally about 2 – 2.5 times lower at 25 °C. The aqueous solubility of HI-6 in water at 25 °C was determined to be 366 mg · ml^{-1}. This, however, is not in agreement with what is expected from the very high intrinsic rate of dissolution. A linear relationship between solubility and intrinsic rate of dissolution (k_1) has been reported for various compounds in the water-ethanol binary system at

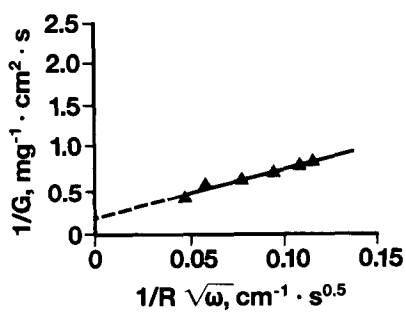

Fig. 4. Plot of the reciprocal of the dissolution rate vs the reciprocal of the distance from the centre of rotation and the square root of the angular velocity for HI-6 in water at 25 °C according to Eqn. 4.

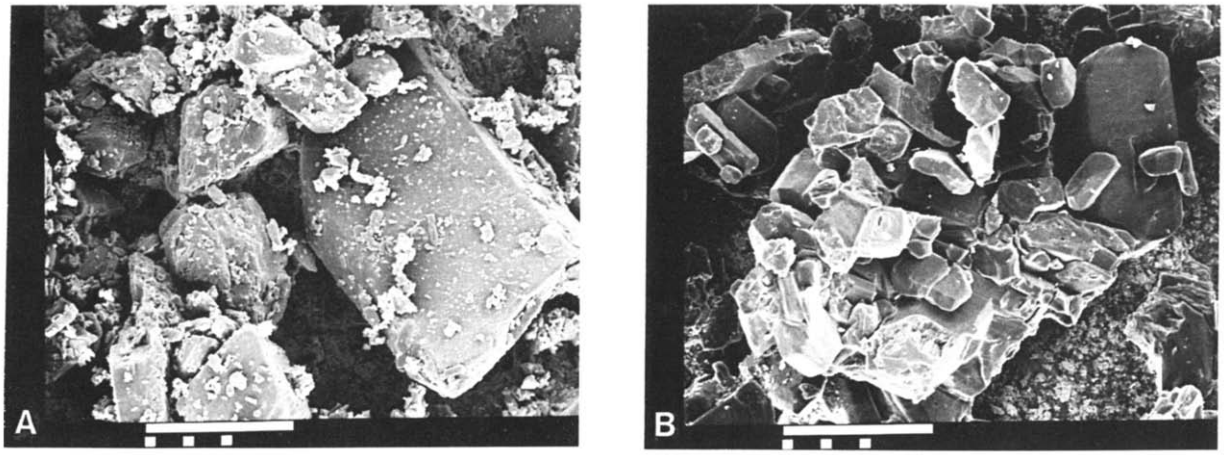

Fig. 5. SEM pictures of HI-6 crystals (A) before equilibrium with water (B) after equilibrium with water for 24 h and then dried overnight. bar 100 μm.

22° C (Nicklasson and Brodin, 1984). By applying that regression equation i.e. $\log C_s = 0.99 \cdot \log k_1 + 2.27$, $n = 16$, $r = 0.990$, inserting the k_1-value for HI-6, a calculated solubility of 1351 mg · ml^{-1} is obtained. It is assumed that the intrinsic rate of dissolution k_1 reflects the highest possible mass transfer for the system, and at such conditions there are no transformations of the compound as it goes from solid to liquid phase. At an equilibrium experiment, however, changes may occur as reported by Nicklasson and Brodin (1984) For dextropropoxyphene napsylate dissolved in ethanol. For dextropropoxyphene napsylate, a significant decrease in ethanol solubility occurred with time and the final equilibrium concentration did not correspond to the calculated value obtained from the general solubility–intrinsic rate of dissolution relationship. A similar phenomenon thus seems to happen for HI-6 in water. In Fig. 5, SEM-pictures are shown for HI-6 crystals before the solubility experiment and after equilibrium in water for 24 h, respectively. As can be seen, there is an obvious difference in the crystal appearance after contact with water which might indicate some kind of change of the crystal structure. Several other papers have reported changes in drug solubility due to the formation of various pseudo-polymorphs such as prednisolone (Wurster and Taylor, 1965), theophylline (Fokkens et al., 1983) and phenobarbital (Eriksson, 1961). The assumption that HI-6 undergoes some kind of change in crystal form in contact with water at equilibrium conditions is further supported by the DSC-thermograms shown in Fig. 6. After equilibrium with water for 24 h followed by drying at room temperature, an obvious shifting of the endothermic peak is obtained. As mentioned previously, the solubility of HI-6 was found to be 366 gm · ml^{-1} at 25° C. Assuming the same activation energy during the solubility experiment as the one found for the dissolution rate measurements (20.3

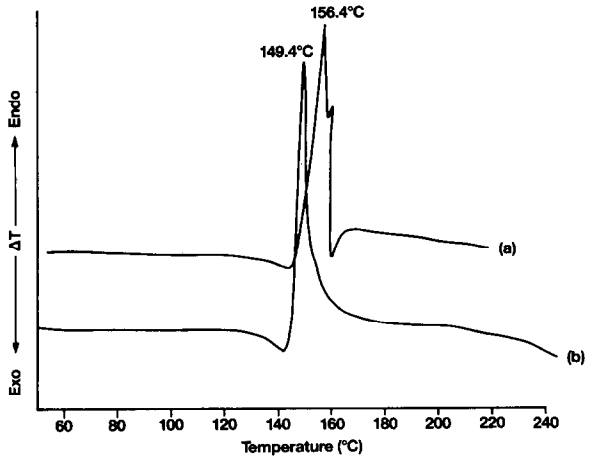

Fig. 6. DSC-thermograms on HI-6 crystals (a) before equilibrium with water (b) after equilibrium with water for 24 h and then dried overnight.

$kJ \cdot mol^{-1}$), a calculated solubility of about 500 $mg \cdot ml^{-1}$ is obtained at 37 °C. The diffusion controlled dissolution rate of HI-6 (500 rpm) is calculated to be about 2 $mg \cdot cm^{-2} \cdot s^{-1}$ in water at 37 °C (cf. Fig. 3). By applying the general solubility–dissolution rate relationship at 37 °C reported by Nicklasson and Magnusson (1985) a predicted HI-6 solubility of 475 $mg \cdot ml^{-1}$ is obtained using the regression line $\log C_s = 0.892 \cdot \log G_{500} + 2.4$. Thus, there seems to be a good agreement between predicted and observed solubility when using data for a diffusion controlled dissolution process. Since there was no agreement between the initial mass transfer for solid to aqueous phase (k_1) and the aqueous solubility from these hypothetical calculations give support to the assumption that for a diffusion controlled dissolution condition, the transformation of HI-6 seems to occur very rapidly within the diffusion layer adjacent to the crystal surface.

It can be concluded that generally, care must be taken when interpreting results of solubility analysis since many compounds show remarkable sensitivity to the solvents used. However, the application of the rotating disc technique and the concept of initial mass transfer from solid to liquid phase in combination with thermal analysis and thermodynamics can serve as a helpful instrument for the investigation and in some cases also the explanation of these kind of phenomena.

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