Dissolution rate measurements of sparingly soluble compounds with the Coulter Counter model TAII§

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A Coulter Counter, Model TAII was used to determine both solubility and dissolution rate from suspensions of griseofulvin and hydrocortisone acetate, where the initial particle size and shape had been characterized. The method assesses the weights and surface areas of particles remaining undissolved as a function of time. A comparison with a conventional method, assessing dissolved quantities of materials, showed that solubility data of the same order of magnitude can be obtained. The TAII data fitted a previously established equation that linearly relates log solubility and log of the intrinsic dissolution rate and indicated that the dissolution process was not diffusion controlled. The TAII method described is a rapid and convenient means of estimating the solubility and dissolution rate where no adequate technique for analysing the dissolved fraction exists.

The intrinsic dissolution rate constant for any drug can be obtained by measuring the dissolution rate at constant surface area or when the decrease in surface area can be measured as a function of time. The most common of the methods available for the determination of dissolution rates is the rotating disc method of Wood et al (1965) which purports to maintain a constant surface area. The release from a rotating disc is referred to as a diffusion controlled process, thus any change in hydrodynamics caused by varying the rotation speed will influence the thickness of the diffusion layer and hence the dissolution rate. By using an extrapolation procedure, Nicklasson et al (1982) obtained 'true' intrinsic dissolution rates corresponding to infinite rotation speeds. Some inherent disadvantages are that the preparation of the disc by compression produces changes in solid structure that may influence the dissolution behaviour, and that the assumption of a constant surface area is only valid for a short time at the beginning of the determination.

Measurement of the dissolution rate on suspended materials does not normally include an assessment or measurement of inherent changes in surface area. The Hixon-Crowell cube root law has been stated by Carstensen (1980) to be applicable to monodisperse materials, where it is assumed that the particles dissolve in an isotropic manner and the surface area therefore decreases in a predictable way. Higuchi & Heistand (1963) claimed that the cube root law was applicable to polydisperse systems provided that the particle size distribution of the material was log-normal.

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Edmundson & Lees (1965) used the Coulter Counter to determine the loss in count of particles of hydrocortisone acetate of given diameters as a function of time and calculated from this the dissolution rate of the polydispersed material. The total particulate count was constant and the changes in sizes of the particles changed with time, so the method could be considered to give an intrinsic dissolution rate. The limitation was instrumental as only one size fraction could be counted in any one time interval, therefore the overall changes in particle size distribution could not be monitored.

We describe a means of overcoming the problem by using the Coulter Counter Model TAII for accurate monitoring of the changes in size distribution of two polydispersed materials at frequent and short time intervals so that changes in weight and surface area are accurately determined.

The objective was to assess the applicability of the TAII for determining both intrinsic dissolution rates and solubilities of sparingly soluble materials.

MATERIALS AND METHODS

Materials
Micronized griseofulvin (Glaxo, UK) and hydrocortisone acetate (Sigma Chemical Co, USA) were used. The hydrocortisone acetate was size reduced in a pin disc mill and fractionated with an air classifier to give a fine particle material with a size distribution as shown in Fig. 1, along with that of griseofulvin.

Coulter Counter method
A Coulter Counter Model TAII fitted with a 30 μm aperture tube and calibrated with 8·06 and 2·02 μm latex spheres was used.
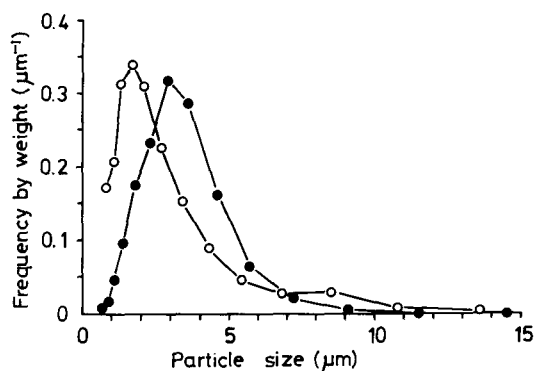
Fig. 1. Particle size distributions obtained by the Coulter Counter TAII of hydrocortisone acetate (O) and griseofulvin (●).

Sample preparation

Stock suspensions containing 15 and 30 mg/100 ml were prepared for each of the materials by suspending them in distilled, particle-free water containing 0·9% sodium chloride and 0·01% polysorbate 80 for which at this concentration it was verified that any interaction between drug molecule and a micellar structure could be neglected. An ultrasonic bath was used to disperse the materials, assurance of complete dispersion being checked by photometric measurements of specific surface area where no increase of the latter occurred with time.

This dispersion medium was also used as the electrolyte for the TAII. For each determination a known volume of the suspension was added to the electrolyte solution, previously equilibrated to ambient temperature, with the total volume always constant at 300 ml.

The volumes of stock suspensions added represented final concentrations of 0·5, 5 and 10 μg ml⁻¹ for both griseofulvin and hydrocortisone acetate.

Size distribution determination

After addition of the requisite volume of stock suspension to the electrolyte, 0·05 ml samples were analysed each minute for 20 min for hydrocortisone acetate and up to 30 min for griseofulvin. Two rotational speeds, 200 and 800 rev min⁻¹ were used in preliminary studies on the dissolution of griseofulvin by the TAII method to confirm that different stirring rates did not affect the dissolution. All subsequent experiments were carried out using a rotational speed of 800 rev min⁻¹. The numbers of particles in 14 size classes were recorded and used to calculate the total weight and surface area of the remaining particles per millilitre of suspension on a

Hewlett Packard 9825T computer. All results presented are mean values of five determinations.

Calculation of remaining weight and surface area

The total weight (w_t) of the remaining particles per millilitre of suspension at any time (t) was calculated from the equation:

$$w_t = \rho_s \cdot (\pi/6) \cdot \Sigma(n_r \cdot \bar{d}_{vr}^3) \tag{1}$$

where ρ_s is the true density of the material, $\pi/6$ is the volume shape factor for a sphere (Heywood 1954), n_r is the number of particles in class r per ml and $\bar{d}_{vr}$ is the arithmetic mean volume diameter in class r, i.e. the diameter of a sphere having the same volume as the measured particles. It was assumed that the material density did not vary with particle size or with time.

The total surface area (S_t) of remaining particles per millilitre of suspension at time t was calculated (Allen 1981):

$$S_t = \alpha_{s,dv} \cdot \Sigma(n_r \cdot \bar{d}_{vr}^2) \tag{2}$$

where $\alpha_{s,dv}$ is a volume diameter (v) based surface shape factor.

$$\alpha_{s,dv} = \frac{s}{v} \cdot \frac{\pi}{6} \cdot \frac{\Sigma(n_r \cdot \bar{d}_{vr}^3)}{\Sigma(n_r \cdot \bar{d}_{vr}^2)} \tag{3}$$

where s/v is the volume specific surface area determined by permeametry, using a Blaine apparatus as described by Kaye (1967). Due to the fine particulate materials used, the calculations for surface area were corrected for 'slip flow' according to the equations of Carman & Malherbe (1950). The use of equation (2) is based upon the assumption that the shape of the particles is not substantially changed during dissolution i.e. that the particles are isometric (Carstensen 1980).

Calculation of solubility

The weights of materials remaining at specified time intervals were determined by the Coulter Counter technique described earlier. For each of the materials the addition of 10 μg ml⁻¹ is in excess of the total amount that could dissolve (Table 1) and

Table 1. Primary characteristics of test materials.

Material	Density[a] ρ_s (g cm⁻³)	Specific surface area S/V (cm² cm⁻³)	Surface shape[b] factor $\alpha_{s,dv}$	Solubility[c] C_s (g g⁻¹)
Griseofulvin	1·44	31 000	5·06	8·7 10⁻⁶
Hydrocortisone acetate	1·29	100 000	9·63	7·7 10⁻⁶

[a] Measured with an Air Comparison Pycnometer.
[b] Calculated from eqn 3.
[c] Obtained from HPLC data.

therefore the weight remaining at equilibrium conditions was used to calculate the solubilities (C_s) of both materials.

Calculation of intrinsic dissolution rate

The intrinsic dissolution rate, G_t ($\mu g\ min^{-1}\ cm^{-2}$) for a specific time interval, Δt (min) was calculated as:

$$G_t = \Delta w_t/(\bar{S}_t \cdot \Delta t) \tag{4}$$

where Δw_t is the amount dissolved in μg during the time interval and $\bar{S}_t$ is the mean surface area (cm^2).

Reference methods

HPLC determination of solubility. The solubilities of griseofulvin and hydrocortisone acetate were determined by the following method. An excess of material was added to a 0·9% sodium chloride solution containing 0·01% w/w polysorbate 80 and shaken at ambient temperature for 48 h. The suspensions were then centrifuged at 6000 rev min^{-1} and the supernatant assayed by HPLC using a variable-wavelength uv detector which was operated at 295 nm for griseofulvin and 254 nm for hydrocortisone acetate. The HPLC analyses were made with a reversed phase Lichrosorb RP-18 column (250 × 4·5 mm i.d., particle size 5 μm). The eluant for griseofulvin was prepared by degassing a mixture of 45% acetonitrile and 55% of 0·1 M acetic acid. Chromatographic analysis using the above mobile phase was at a flow-rate of 1·0 ml min^{-1}. The column temperature was ambient and the sample injection size was 20 μl. The mobile phase for hydrocortisone acetate was 65% of methanol and 35% of 0·01 M potassium dihydrogen phosphate. Under these conditions the retention times of griseofulvin and hydrocortisone acetate were about 9·0 and 7·4 min respectively. Calculations of peak areas were made using a Hewlett-Packard 3390A integrator. Results are mean values of three determinations.

HPLC determination of dissolution. To validate the TAII method for determining the weights of undissolved material, the HPLC method was used to assay samples from the TAII experiments. This experiment was carried out for griseofulvin only by using two separate additions in the range 3–5 μg ml^{-1}.

The HPLC method gave data on material dissolved which could be equated with the TAII data. Samples were taken at 1, 5, 10, 15, 20 and 25 min simultaneously from the Coulter beaker and analysed by both methods. The HPLC samples were withdrawn from the Coulter beaker through a filter (0·22 μm membrane) and analysed in triplicate.

Theoretical calculation of intrinsic dissolution rate. Hamlin et al (1965) have demonstrated a linear relation between solubilities and intrinsic rate of dissolution utilizing a log/log scale. This has been confirmed by Nicklasson & Brodin (1984) but with a smaller value of the intercept on the solubility axis caused by the absence of any substantial diffusion layers. This means that higher dissolution rates were achieved. By using this relation intrinsic dissolution rates (G) corresponding to sink conditions were calculated from the determined solubility values (C_s) as follows:

$$\log C_s = \log G + 1·94 \tag{5}$$

where C_s = solubility in mg ml^{-1}; G = 'true' intrinsic dissolution rate in mg s^{-1} cm^{-2}.

RESULTS AND DISCUSSION

For the TAII technique described earlier to be valid, it is necessary to ensure that the materials to be characterized are within the measurement range of the aperture tube being used. From Fig. 1 it can be seen that both materials are log normally distributed and only small weight fractions of the materials are below 0·6 μm, which is the approximate lower limit of the 30 μm aperture tube.

Solubility

The solubilities of griseofulvin and hydrocortisone acetate derived from the Coulter Counter TAII data were 6·9 μg ml^{-1} and 5·9 μg ml^{-1} respectively. Solubilities from the HPLC method were calculated as griseofulvin 8·7 μg ml^{-1} and hydrocortisone acetate 7·7 μg ml^{-1}. Although the differences between the methods are significant, the numerical values are of the same order, and closer results may be obtained by extending the dissolution times on the TAII determinations to ensure that a saturated solution is obtained. However, the TAII method described is a rapid and convenient means of estimating the solubilities of materials where no adequate technique for analysing the dissolved fraction exists. The method requires only that the material can be suspended in a particle-free solution which is sufficiently electrically conductive for Coulter analysis. Fig. 2 shows a comparison of the amounts of griseofulvin dissolved against time as determined by the TAII and HPLC methods. Both methods gave the same results with respect to weight dissolved against time. This shows that the TAII method could be used for an accurate estimation of the weight remaining in a suspension in which rapid dissolution is taking place. In the estimation of the solubility, the
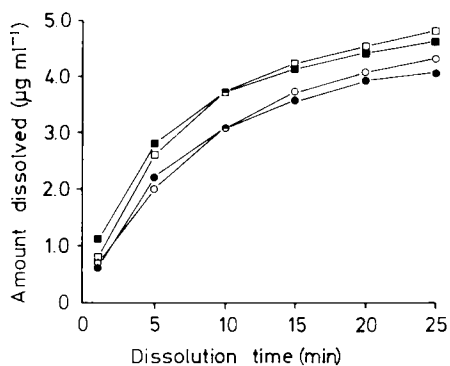
FIG. 2. Comparison between Coulter Counter method (closed symbols) and HPLC method (open symbols) for monitoring the dissolution of two griseofulvin samples.

apparent errors found were probably not due to incorrect weight estimations but because saturation had not been achieved.

Intrinsic dissolution rate

Figs 3 and 4 show intrinsic dissolution rates of griseofulvin and hydrocortisone acetate for the three concentrations used calculated from equation 4. At the lowest concentration of 0·5 µg ml^{-1}, most of the materials had dissolved after approximately 6 min and subsequent values of the dissolution rate became erratic (dotted lines) due to the very small weights and surface areas of the particles remaining in the suspensions.
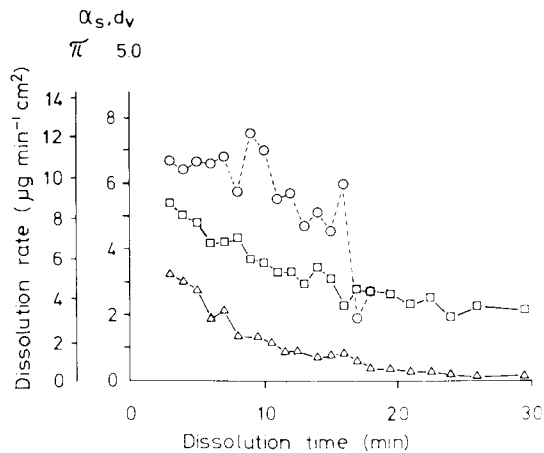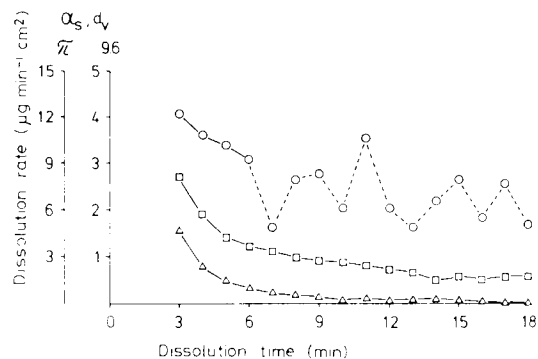
At the lowest concentrations, where sink condi-

FIG. 4. Intrinsic dissolution rates of hydrocortisone acetate obtained by Coulter Counter method. Symbols as in Fig. 3.

tions exist, only a slight decrease in dissolution rate would be expected. This is found for griseofulvin, but an apparently greater change in dissolution rate occurs for hydrocortisone acetate. Reference to SEM photographs (Fig. 5B) shows a high surface roughness for hydrocortisone acetate, which is reflected in a high specific surface area and surface shape factor (Table 1). Although it is assumed that

FIG. 3. Intrinsic dissolution rates of griseofulvin obtained by Coulter Counter method: ○, addition of suspension corresponding to 0·5, □, 5 and △, 10 µg ml^{-1}. The two ordinate axes represent the use of assumed (π) and calculated (5·06) surface shape factor.
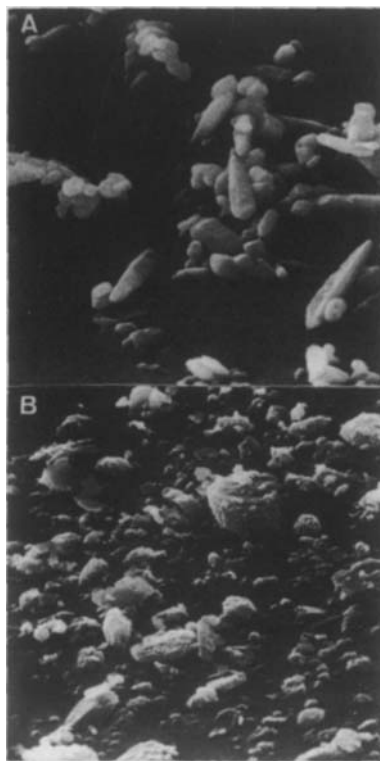

FIG. 5. Photomicrographs obtained by scanning electron microscopy (SMU-3, JEOL, Japan). (a) Griseofulvin; (b) Hydrocortisone acetate.

dissolution of particles takes place isometrically, the possibility of a rapid decrease in surface roughness giving an over estimate of the surface shape factor cannot be excluded. Such an over estimation produces an intrinsic dissolution rate smaller than the real value. For griseofulvin with its smooth surface (Fig. 5A) the shape factor apparently remains constant with time.

For the intermediate and highest concentrations, 5 and $10 \mu g \, ml^{-1}$, representing non-sink conditions, the intrinsic dissolution rate decreases with time as saturation is approached. At $10 \mu g \, ml^{-1}$ the dissolution rate curves approach zero for both materials.

Intrinsic dissolution rates were calculated using two different surface shape factors ($\alpha_{s,dv}$). One surface shape factor was assumed to be π, corresponding to spherical particles, while the other was calculated from equation 3 and represents the actual shape of the particles. The influence of these shape factors on the dissolution rates is demonstrated by the use of two separate axes on the ordinate in Figs 3 and 4.

The initial values of intrinsic dissolution rates corresponding to sink conditions ($0.5 \mu g \, ml^{-1}$) were compared with the data calculated from equation 5. These values are shown in Table 2. Nicklasson et al (1982) obtained dissolution data from which the intrinsic dissolution rate at infinite rotating speed

was calculated. Their results showed that dissolution under these conditions was not a diffusion controlled process. The experimental data obtained from the TAII method shows good agreement with the calculated values from equation 5. This implies that in the TAII method for characterizing sparingly soluble materials, dissolution is also not significantly diffusion-controlled. This hypothesis is supported by the results of the preliminary studies using two widely different stirring speeds, where no significant differences in dissolution rates were obtained. The use of a calculated shape factor instead of an assumed spherical shape factor gives better agreement with the calculated values, from equation 5.

Acknowledgements

Table 2. Experimental and theoretical intrinsic dissolution rates.

| Material | Intrinsic dissolution rate, G ($\mu g \, min^{-1} cm^{-2}$) | | |
	Theoretical data from eqn 5	Experimental data from eqn 4 assumed $\alpha_{s,dv}$	calculated $\alpha_{s,dv}$
Griseofulvin	5·9	11	6·5
Hydrocortisone acetate	5·3	13	4·2

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