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Dissolution rate studies of pharmaceutical multisized powders – a practical approach using the Coulter method

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Abstract

The aim of this work is the study of the influence of particle size and related properties on the dissolution rate of a sparingly soluble drug – indomethacin. Different size fractions were fully characterized concerning particle size distribution, specific surface area, density, degree of crystallinity and solubility. A particle size counter – the Coulter Multisizer II — was used, not only to characterize the primary particle size distribution of the different fractions, but also to monitor the size and number of the suspended particles during the dissolution process. Although this information was applied to evaluate dissolution profiles (dissolution drug concentration vs. time) it can be further used to thoroughly study the dissolution phenomenon. The accuracy of this instrument to assess dissolution profiles was confirmed by comparing its results with those obtained by HPLC. As expected a strong influence of the fraction size on the dissolution rate was found. A correlation was established between the mean dissolution time (MDT) and the mean particle size of the various indomethacin fractions.

Keywords: Particle size distribution; Mean particle size; Coulter counter method; Indomethacin; Dissolution profile; Mean dissolution time

1. Introduction

The low bioavailability of sparingly soluble drugs is often limited by their dissolution rates in the gastrointestinal fluids (Abdou, 1989). Dissolution rate is normally described by the well known modified Noyes-Whitney equation (Brunner, 1904):

$$-\frac{dW_t}{dt} = \frac{DS}{h}(C_s - C_t) \quad (1)$$

where W_t represents the suspended solids weight at time t, S the interfacial surface area, D the drug diffusion coefficient, h the boundary layer thickness and (C_s-C_t) the driving force in

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terms of concentration, C_s corresponding to the saturation value. If a sufficiently dilute medium is used ($C_t \ll C_s$), which means working in the so-called sink conditions, the concentration gradient can be considered approximately constant and equal to C_s. On the other hand, under carefully controlled experimental conditions, namely temperature and agitation, D, C_s and h can be assumed constant, being the surface area available for dissolution, S, the only variable with time on the right-hand side of Eq. (1). Concerning this parameter and in order to calculate the drug dissolution rate, two strategies can be adopted: to keep the interfacial area constant, by using compressed flat disks, or to use the drug in the form of a multisized powder. The disk method (Nicklasson and Brodin, 1982) originates a simpler mathematical treatment, since all parameters can be considered constant, and is often used to calculate surface specific (or so called intrinsic) dissolution rates. Nevertheless, the latter procedure represents a more realistic approach of the in vivo situation, requiring, however, a previous knowledge of the change of surface area during dissolution and consequently a more complicated calculation procedure.

The methodology adopted in this work was that of using a powdered sparingly soluble drug, being the dissolution profile (dissolved drug % vs. time) calculated making use of a particle size analysis technique – the Coulter counter method. This technique has been used by Nyström et al. (1985) to determine surface specific dissolution rates and solubilities of sparingly soluble drugs. Its main advantage over the usual dissolution techniques (e.g. paddle or flow through methods) is that it is capable, not only of monitoring the quantity of solids remaining in suspension as a function of time, but also of following the variation of particle number and size. This information is of utmost importance to study the dissolution phenomenon, especially in polydisperse systems. It can be used either as an input in simulation studies or, otherwise, to validate already existing theoretical models.

The present paper represents the first stage of a fundamental study about the dissolution process of multisized drug powders, using indomethacin as a model drug. It describes the experimental approach needed to fully characterize this material in order to apply the Coulter method to calculate the dissolution rate. A systematic study of the influence of the primary particle size on this parameter was also performed by using several size fractions.

As an attempt to elucidate the mechanisms involved in the dissolution process all the information provided by this technique will be used in a forthcoming paper to test the applicability of the classical diffusional models (Hixson and Crowell, 1931; Higuchi et al., 1963; Niebergall et al., 1963) usually employed to calculate dissolution rate constants.

1.1. Determination of dissolution profiles using the Coulter counter method

The measuring principle of the Coulter counter technique has been extensively described in numerous papers and text books (Allen, 1981; Lines, 1992). This technique is normally used to measure particle size distributions (number or volume vs. particle volume, or equivalent volume diameter) but, in this case, it will also be used to measure solids concentration in terms of weight of suspended particles per unit of suspension volume.

Knowing the initial particle concentration (W_0) and the corresponding value at time t (W_t), it is possible to calculate the amount of dissolved solids (W_0-W_t) as a function of time and therefore to estimate the dissolution profile.

The weight of solids remaining in suspension at time t, is given by:

$$W_t = \rho V_t = \rho \sum n_i v_i = \rho \frac{\pi}{6} \sum n_i d_{vi}^3 \qquad (2)$$

where: ρ = density of the material; V_t = total volume of remaining suspended solids at time t; n_i = number of particles in class i; v_i = arithmetic mean volume of class i; d_{vi} = equivalent mean diameter of class i [$= (\Pi/6v_i)^{1/3}$].

The interfacial surface area can also be calculated as

$$S_t = \alpha_{s.v} \sum n_i d_{vi}^2 \qquad (3)$$

being S_t the interfacial surface area at time t and $\alpha_{s,v}$ the surface volume shape coefficient (Nyström et al., 1985). The calculation of this coefficient requires the knowledge of the surface specific area of the material (S_w) which is the surface area per unit weight, and is given by:

$$\alpha_{s,v} = S_w \frac{\rho\pi}{6} \frac{\sum n_i d_{vi}^3}{\sum n_i d_{vi}^2} \quad (4)$$

Several methods have been used to evaluate surface specific areas of powders, the results being strongly dependent on the method employed (Lowell and Shields, 1984).

As mentioned before, the great advantage of the Coulter counter method is that it supplies information about the quantity of suspended solids, their number, size distribution and mean diameter, simultaneously, as a function of time. These unique features make this technique an extremely valuable tool to follow the behaviour of the particles during the dissolution process.

2. Materials and methods

Indomethacin was the drug selected for this study mainly because it is sparingly soluble and because it has associated bioavailability problems.

The drug (Sigma lot 60H 448) was supplied with a broad size distribution, and will be referred to as bulk indomethacin. As one of the purposes of this work is the study of the effect of the initial particle size on the dissolution rate, the bulk indomethacin had to be fractionated. The fractions obtained by microsieving (precision test sieves with circular openings, Retsch type USG) and an additional micronized sample (Esteves Lot 3-901S), used as supplied, were characterized regarding particle size distribution, density, solubility and specific surface area. As explained before, this information was needed to establish the dissolution profiles applying the Coulter counter method. These results were, nevertheless, validated by HPLC as described later.

The chemicals and solvents used were of reagent grade. All determinations were performed at least 4 times to establish the reproducibility of the results.

As the indomethacin presents different polymorphs (Borka, 1974), differential scanning calorimetry was used to check the purity of the starting materials (bulk and micronized samples) and of the sieved fractions to ensure that the wet sieving process did not alter the drug degree of crystallinity.

2.1. Primary characterization of particle size

In spite of the fact that the Coulter technique is the one selected in this study, another technique – laser diffraction spectrometry – was used to determine the particle size distribution and mean diameter of the sieved and micronized fractions.

Due to the solubility (although sparing) of the drug, a pre-saturated free particle solution was used as suspending medium for both techniques. This solution was prepared by adding an excess of the drug to free distilled water containing 0.9% of NaCl and 0.01% of Tween 80. The NaCl was added to guarantee the solution electrical conductivity required by the Coulter method, and the polysorbate to facilitate the powder dispersion. This suspension was agitated for 48 h at room temperature and thereafter left undisturbed for 12 h and filtered through a 0.45 μm filter (Millipore-HV).

Stock suspensions were prepared for each size fraction by adding the powdered material to the saturated solution (35 mg/50 ml) followed by sonication for 3 min in an ultrasonic bath.

2.1.1. Coulter counter method

The model used in this study was the Coulter Multisizer II which is an accurate particle counter, fully automatic, capable of analyzing broad size ranges with high resolution (up to 256 size classes) in short time intervals. It was coupled to a personal computer which enabled the data to be recorded and statistically treated by an appropriate software (AccuComp from Coulter Electronics).

Two aperture tubes were used, 50 and 100 μm, suitable for the micronized and sieved fractions, respectively. A preliminary calibration was per-

formed using 3.1 and 14.0 μm latices recommended, respectively, for the smaller and larger tube (British Standards Methods for Determination of Particle Size of Powders, 1983).

The indomethacin saturated solution was used as the electrolyte. The samples to be analyzed were pipetted, under magnetic agitation, from the previously prepared stock suspensions, the final suspension being sonicated for 3 more min to eliminate eventual agglomerates. The agitation speed was adjusted to ensure a uniform concentration in the beaker but preventing the entrapment of air bubbles. The solids concentration corresponded to a coincidence correction level of approximately 5%.

2.1.2. Laser diffraction spectrometry

As mentioned before, and for the sake of comparison, all the fractions were also analyzed by a laser granulometer – the Malvern 2600c. The laser diffraction technique is widely used for particle size analysis since it is fast, versatile (not strictly applied to suspensions but also to dry powders or aerosols), users friendly and needs no calibration (Barth, 1984). However, it does not provide reliable values for particle concentration and the results are somehow particle shape dependent (Ferreira et al., 1993). The liquid dispersion unit (Malvern PS14A) was employed, which included a small mechanically agitated tank ($\approx$ 800 ml), provided with ultrasonic facilities, and a pump which enabled the recirculation of the suspension through a glass window cell located in the laser beam. A lens of 100 mm focal length was used for all fractions and the data was treated using the model independent method.

The sample preparation procedure was similar to that of the Coulter, the final particle concentration corresponding to nearly 20% of the obscuration level.

It should be pointed out that the working solids concentration required by both the Malvern and the Coulter Multisizer are different, being qualitatively indicated by the instrument software.

2.2. Differential scanning calorimetry

As referred to earlier, information about the degree of crystallinity of the samples is needed to make sure that the fractions under analysis are identical. For that, a heat flux calorimeter Shimadzu DSC 50 was used. After being dried under vacuum at 30°C, the samples were introduced in aluminum seals (Shimadzu Ref. 201-53090) and scanned from 25 to 190°C at a heating rate of 10°C/min.

2.3. Density

The true density was determined by dividing an accurate weight of sample by its volume measured with an helium pycnometer (Micromeritics Accupyc 1330). However, due to the considerable amount of material needed for these measurements, only the bulk indomethacin, the micronized sample and the 25–35 μm fraction were analyzed.

2.4. Gas adsorption surface area

The specific surface area of the test material was measured by a static gas adsorption technique (Micromeritics ASAP 2000), using krypton as adsorbate, applying the BET equation to the data (Lowell and Shields, 1984).

As in the true density measurements, due to the lack of enough material, only the 25–35 μm and the micronized fractions were analyzed by this technique.

2.5. Solubility determinations

Solubility studies are quite important in the present work. Indeed, if on the one hand the dissolution tests must be carried out at low solids concentration (i.e. under sink conditions), on the other hand the number of particles counted by the Multisizer (in short time intervals) must be statistically meaningful. As the indomethacin is a pH dependent drug, it was possible to adjust the pH of the dissolution medium so that both conditions were fulfilled.

Experiments were performed at different pH phosphate buffer aqueous solutions enriched with 0.01% Tween 80 and 0.9% NaCl. An excess of material was added to these solutions, which were shaken for 48 h at ambient temperature followed by 12 h rest. These suspensions were afterwards centrifuged at 6000 rpm, and the supernatant assayed by HPLC (Hewlett Packard 1050) at 254 nm. The analyses were made with a reversed phase Lichrosorb RP-18 column (200 × 4.6 mm) with a particle size of 10 μm. The eluent was prepared by degassing a mixture of 50% acetonitrile and 50% phosphate buffer 0.01 M of $NaHPO_4$ and Na_2HPO_4. The column temperature was ambient, the sample injection size being 20 μl and the flow rate of 1.0 ml/min.

Calculations of peak areas were made using a Hewlett-Packard 3369 A integrator. Results are mean of three determinations.

Since HPLC was to be used in this work as a reference method, a criterious validation of the technique was carried out. Linearity, accuracy, precision (repeatability and reproducibility) and recovery were the evaluated parameters (data not shown).

2.6. Dissolution studies

The dissolution tests were performed under sink conditions ($C_t \ll C_s$) at ambient temperature (23 ± 1°C). A known volume of the indomethacin saturated stock suspensions, prepared for each fraction as described earlier, was added to the coulter beaker containing the dissolution medium (phosphate buffer, pH 6.2 with 0.9% NaCl and 0.01% Tween 80). The total volume was 200 ml corresponding to a final concentration of 7 μg/ml ($\approx$ 10% C_s). The concentration-time profiles were obtained with the Coulter Multisizer II and later validated by HPLC.

2.6.1. Dissolution measurements with the Multisizer

Immediately after the addition of the stock suspension to the particle free electrolyte, sample volumes of 0.1 and 0.5 ml, respectively for the micronized (50 μm tube) and the other fractions (100 μm tube), were analyzed. The same sample volumes were analyzed at pre-determined time intervals until 80% of the initial solids amount (W_0) was dissolved. The value of suspended solids was automatically calculated by the Multisizer (according to Eq. (2)) for each measurement. Knowing the material density, the remaining weight of undissolved particles (W_t) and, consequently, the dissolved solids amount (W_0-W_t) can be calculated and the dissolution profiles evaluated.

A similar procedure can be followed to calculate the remaining particle surface area (Eq. (3)), as a function of time, if the particle shape factor (Eq. (4)) is known.

All the experiments were performed 5 times for each size fraction. The agitation speed was kept constant (position 4, corresponding to $\approx$ 900 rpm).

2.6.2. HPLC validation

As mentioned previously, it was decided to monitor the dissolved indomethacin concentration by HPLC, for one of the experiments performed with the Multisizer. Samples were withdrawn in between the Coulter sampling measurements, filtered at once through a 0.22 μm membrane to remove suspended particles, and analyzed by HPLC, as described earlier. The concentration-time profile could then be evaluated.

3. Results and discussion

3.1. Primary characterization of the drug

As stated earlier, all fractions were characterized regarding particle size, surface area, polymorphism and density. These results are summarized in Table 1 and suggest the following conclusions:

(1) As can be seen by comparison of the mass median diameters, the sieved fractions present distinct average sizes. They were also relatively narrow, as shown by the cumulative undersize distributions displayed in Fig. 1. There is a good agreement between the results obtained with the electrical sensing zone method and laser diffractometry. The mean size d_{sv} (defined as $\Sigma n_i d_{vi}^3 / \Sigma n_i d_{vi}^2$) was also

Table 1
Primary characteristics of the indomethacin fractions

Sample	$d_{50}^{a,b}$ (μm)	$d_{50}^{a,c}$ (μm)	d_{sv} (μm)	Specific surface area (m^2/g)	Fusion specific enthalpy (J/g)	Melting point (°C)	Density (g/cm^3)
Bulk					−107.32	159.30	1.374
Micronized	5.10	4.7	4.81	3.1	−108.58	158.81	1.352
5–15 μm	13.38	15.2	13.26	0.42[d]	−107.24	158.80	–
15–25 μm	17.98	20.3	19.20	0.33[d]	−106.45	159.26	–
25–35 μm	29.79	33.46	30.91	0.19	−104.76	159.44	1.374

[a]Mass median diameter.
[b]Electrical sensing zone method.
[c]Laser diffraction technique.
[d]Calculated from eq. 3 ($\alpha_{s,v}$ = 3.7).

calculated as it is considered the most adequate for these kind of studies (Allen, 1981);
(2) Concerning the specific surface areas (S_w) and as mentioned before, only the micronized fraction and the 25–35 μm sieved fraction were analyzed. The shape factor was calculated for the latter, according to Eq. (4), and assumed constant for the other sieved fractions (Nyström et al., 1985). This presumption enabled the calculation of S_w for the remaining fractions, also by using Eq. (4) ($\alpha_{s,v}$ = 3.7). Due to the low values obtained for S_w, krypton had to be used as adsorbate gas. This option increases the accuracy of the BET values but prevents the calculation of the sample porosity and pore size distribution. Preliminary results using nitrogen revealed, however, that the samples exhibit very low porosity. This indicates that the total surface area measured by BET should be similar to the external surface area, which is probably the surface area that takes part in the dissolution process (Bisrat and Nyström, 1988);
(3) The values obtained by DSC clearly indicate that the polymorph I (Borka, 1974) is the dominant form either in the bulk or in the micronized indomethacin. Moreover, it can also be concluded that the wet sieving process (followed by drying) did not introduce any change in the drug degree of crystallinity;
(4) The density of the samples was found to be independent of the size fraction.

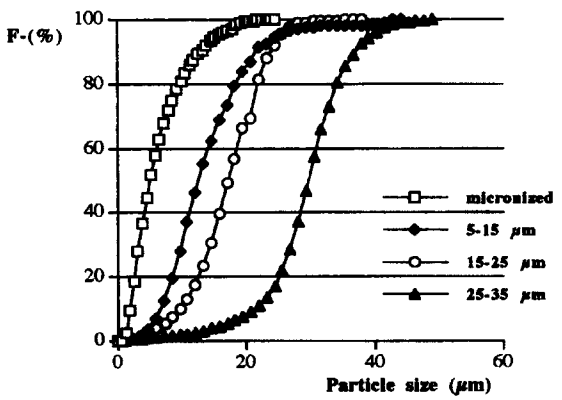

Fig. 1. Cumulative undersize distributions obtained with the Coulter Multisizer II for the various indomethacin fractions.

3.2. Solubility

Table 2 resumes the influence of pH on the equilibrium solubility of indomethacin.

A strong dependence on the solution pH was found with a significant increase when the pH was changed from 6.2 to 7.2. This was, somehow, expected since the drug behaves in solution as a weak acid (pKa = 4.5) (O'Brien et al., 1984). On the contrary, no significant changes were detected with particle size.

The pH value selected in this work was 6.2 since it represents a compromise between the low particle concentrations verified for the non-buffered solution and the fast dissolution rates observed at the highest pH.

Table 2
Solubility of indomethacin (μg/ml) at different pH solutions measured at room temperature (23 ± 1°C) for the various fractions (mean ± S.D.)

Dissolution medium	Bulk indomethacin	Micronized indomethacin	5–15 μm fraction	15–25 μm fraction	25–35 μm fraction
Aqueous solution without buffer	7.0 ± 0.6	8.0 ± 0.5	7.5 ± 0.6	6.8 ± 0.7	7.0 ± 0.6
Buffer solution pH 6.2	68 ± 5.0	84 ± 7.0	87 ± 6.6	72 ± 9.9	68 ± 5.0
Buffer solution pH 7.2	547.0 ± 43.0	635 ± 22.0	645 ± 37.0	535 ± 20.0	547 ± 43.0

3.3. Dissolution profiles

As mentioned previously the Coulter Multisizer is capable of measuring the number and the volume of the particles in a given sampled volume of suspension, as a function of time. A typical output is shown in Fig. 2 in terms of volume distribution versus equivalent volume diameter at various times of the dissolution process. This figure clearly indicates that this technique is sensitive enough to detect changes in the volume of suspended solids during dissolution.

However, to check the accuracy of the method, the dissolved indomethacin concentration was measured by HPLC as described earlier. The results so obtained are compared in Fig. 3 with those of the Coulter for the same experiment. In the case of the Coulter, the dissolved concentration at any instant was calculated from the difference between the initial and the remaining solids weight. The close agreement found be-

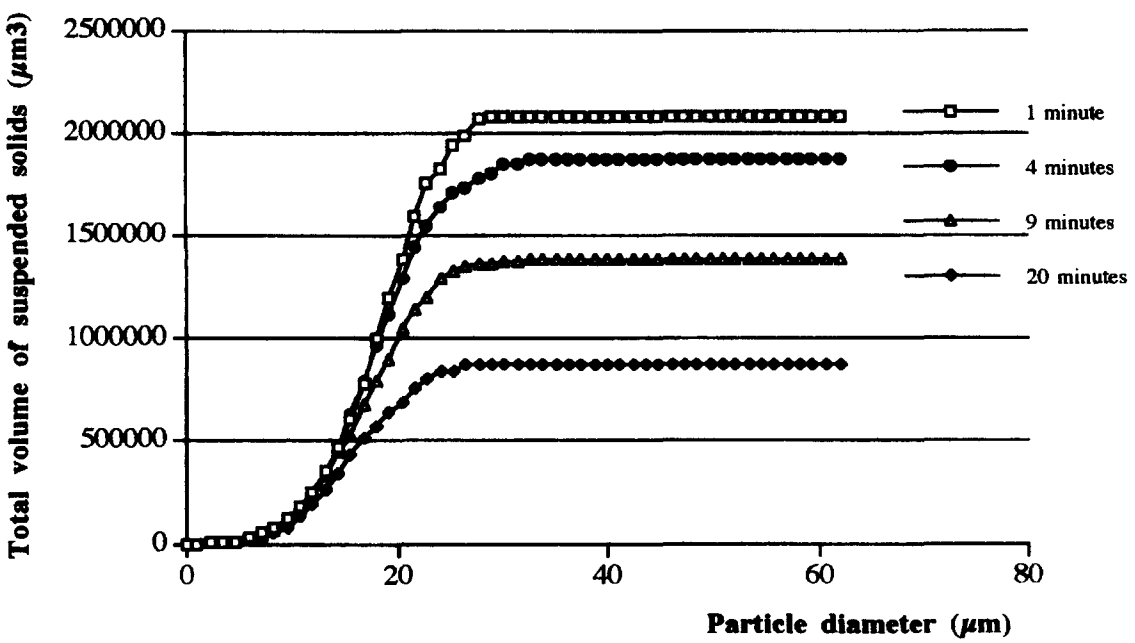

Fig. 2. Cumulative undersize distribution of the 15–25 μm indomethacin fraction at various dissolution times for a 0.5 ml sampling volume.

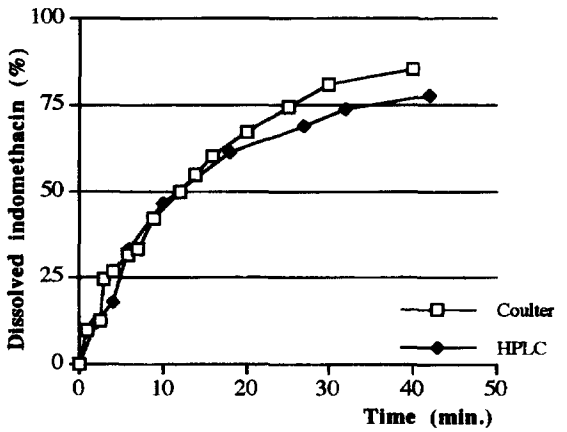

Fig. 3. Comparison between the dissolution profiles obtained with the Coulter and by HPLC for the 15–25 μm indomethacin fraction.

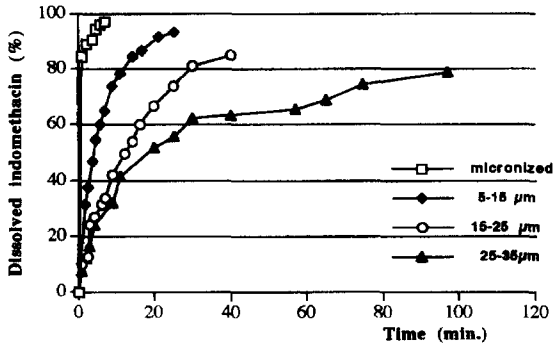

Fig. 4. Dissolution profiles of different fractions of indomethacin.

tween both methods validates the Coulter technique as appropriate to follow the dissolution process.

The concentration versus time profiles obtained for the various size fractions are displayed in Fig. 4. Each curve is the mean of 5 independent dissolution tests, as stated before, being the S.D. values lower than 5%. This figure demonstrates, as expected, the strong influence of the primary particle size on the dissolution profiles.

In order to quantitatively describe these profiles the concept of the mean dissolution time (MDT) was used, defined as (Brockmeier, 1986):

$$MDT = \frac{\sum_i \bar{t}_i \Delta M_i}{\sum_i \Delta M_i} \quad (5)$$

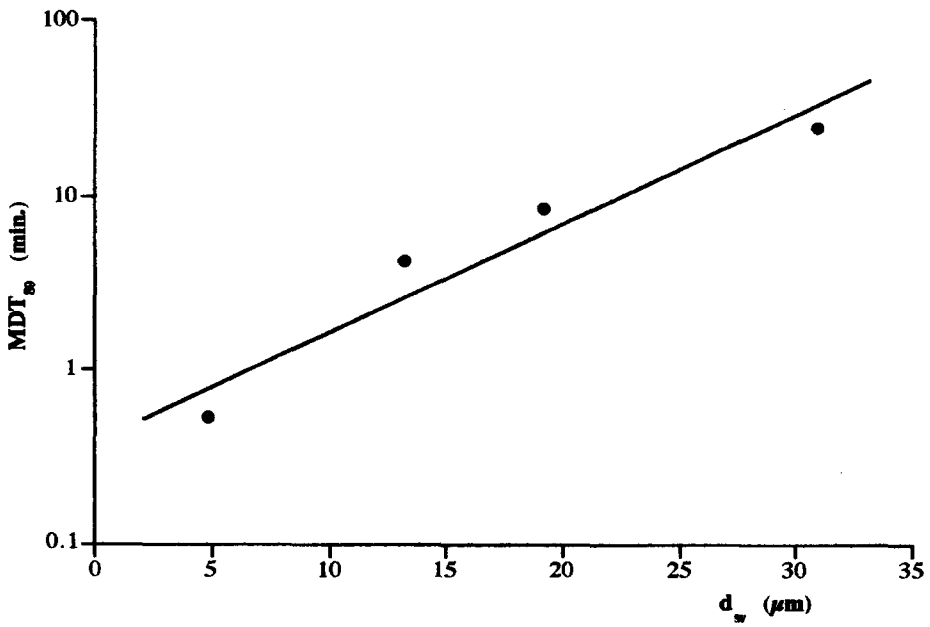

Fig. 5. Relationship between the mean dissolution time (MDT) and the mean particle size (d_{sv}) for each indomethacin fraction.

where $\bar{t}_i$ is the midpoint of the time interval corresponding to the released fraction ΔM_i.

Fig. 5 shows that a linear relationship can be established between the logarithm of MDT_{80} (corresponding to 80% of the total dissolution) and d_{sv} for the different fractions. However, the limited number of fractions analysed is not enough to establish an accurate correlation.

4. Conclusions

The first conclusion that emerges from these results is that the dissolution profile is strongly affected by the primary size of the particles. As expected, the dissolution rate increases with a reduction in particle size. The mean dissolution time was well correlated with the mean particle size of each fraction, however a broader range of size fractions should be tested to establish a more precise correlation.

The knowledge of this relationship is of utmost importance as it can be ultimately used for drug release control. Indeed, if there is a correlation between MDT and d_{sv}, it should be possible to manipulate the drug particle size in order to obtain a required dissolution rate.

However, the real goal of this paper was to show that the Coulter technique is a powerful tool for the study of dissolution of sparingly soluble drug powders. Besides enabling the evaluation of the drug dissolution profile, it is the only method capable of providing information about the undissolved solids weight, size distribution, number and surface area during the dissolution process. This information will be further applied to test the applicability of the commonly used dissolution models (Hixson and Crowell, 1931; Higuchi et al., 1963; Niebergall et al., 1963) which are generally accepted for monodisperse spheres, but questionable for multisized powders.

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