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Dissolution testing of a poorly soluble compound using the flow-through cell dissolution apparatus

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Abstract

Dissolution of Pfizer Compound PD198306, a poorly soluble compound, was studied in 25 mM pH 9 sodium phosphate solution with 0.5% SLS using the flow-through cell dissolution apparatus. Unmicronized and micronized drug powders were tested. Several methods of loading the drug powder into the flow-through dissolution cells and their impact on dissolution were investigated. The influence of flow rate of the dissolution medium on the rate and extent of dissolution were studied. PD198306 has poor wettability even in the presence of 0.5% SLS. It was found that loading the drug powder into the dissolution cell in the form of a suspension provided the best dissolution profile in terms of the rate and extent of dissolution. The flow rate of 4 ml/min resulted in good particle size discrimination.

Keywords: Dissolution testing; Flow-through dissolution method; High throughput screening

1. Introduction

Compounds with less than optimal physicochemical properties are increasingly encountered in pharmaceutical research. This is primarily due to the use of high throughput screening (HTS) techniques in the discovery process that tend to bias toward compounds of higher molecular weight and lipophilicity (Lipinski et al., 1997). A number of these compounds moving from HTS into development display low bioavailability due to poor aqueous solubility and dissolution rate. In many instances, these compounds must be further processed to increase the dissolution rate and eventual bioavailability. This is often accomplished using particle size reduction techniques (micronization or nanosizing) or more aggressive enabling technologies including lipophilic drug delivery using liquid filled capsules (Gershanik and Benita, 2000), solid state modifications such as conversion of the crystalline drug substance to an amorphous material (Crisp et al., 1984; Ichikawa et al., 1997), or solubilization in surfactant systems (Serajuddin, 1997). Therefore, information on the dissolution parameters of these compounds

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is important and needs to be determined early in the discovery phase using very small quantities of material. It is critical that the dissolution method employed be capable of demonstrating the extent of improvement in dissolution that may be achieved by further processing of the drug substance. The flow-through (USP Type IV) dissolution apparatus (U.S. Pharmacopeia XXIV, 2000) appears to be suitable for this application.

The flow-through dissolution apparatus has successfully been used to study dissolution of conventional and controlled release tablets and hard and soft capsules, (Moller, 1986; Neisingh et al., 1986; Moller and Wirbitzki, 1990; Qureshi et al., 1994; Zhang et al., 1994). The reproducibility and ruggedness of this dissolution technique has been established in collaborative experiments at several independent laboratories using USP calibrator tablets (Nicklasson et al., 1987; Wennergren et al., 1989). The results from these studies demonstrate that the flow-through apparatus is a useful tool to study oral solid dosage form dissolution.

There have been few reports in the literature, however, regarding the testing of powders using the flow through dissolution apparatus (Moller, 1983; Nicklasson et al., 1991; Moller and Wirbitzki, 1990). Moreover, no systematic evaluation of powder loading into the flow cell to achieve maximum dissolution and reproducibility has been conducted. Powders with very low solubility and wettability present unique problems that necessitate optimized methods of sample loading into the flow-through dissolution cell in order to achieve acceptable results. The flow-through dissolution apparatus is specially designed to have a small holdup volume compared with other USP dissolution apparatus, that helps to minimize spreading of drug particles to undefined sites of the apparatus. This feature is useful in the testing of drug powders, especially those with poor solubility and wettability, as spreading results in erratic and highly variable dissolution profiles (Langenbucher et al., 1989). For a given ratio of drug substance to dissolution medium, dissolution profiles obtained from the flow-through dissolution apparatus are, within reasonable limits, unaffected by the actual quantities of the drug substance or the medium. This flexibility is an added advantage in working with small quantities of powder, especially during the discovery phase of development.

This work was carried out to evaluate how different patterns of sample loading into the flow through dissolution cell affect the rate and extent of dissolution. Studies were done using Pfizer compound PD198306 (*N*-Cyclopropylmethoxy-3,4,5-trifluoro-2-(4-iodo-2-methyl-phenylamino) $\times$ -benzamide). PD198306 is a selective and potent inhibitor of MEK (Mitogen activated protein kinase (MAPK)-ERK-Kinase), for treating both the symptoms (pain, swelling) and connective tissue destruction associated with rheumatoid arthritis and osteoarthritis (OA). It is a poorly soluble compound with poor wetting properties. The structure of the compound is shown in Fig. 1. The compound is weakly acidic with a pK_a of 8.31. The molecular weight of the compound is 476.24 g/mol. The compound is crystalline with a melting point of approximately 140 °C. The solubility of the compound in 50 mM phosphate buffer pH 6.5 is 0.18 μg/ml.

2. Materials and methods

2.1. Materials

PD198306 was received as unmicronized and micronized material. Micronization was performed by Micron Technologies, Inc., (Exton, PA). The particle size of the unmicronized and mironized powders was determined using a Malvern Mastersizer. Particle size analysis showed that for the unmicronized powder, 90% of the particles were under 49.8 μm. For the micronized powder, 90% of the particles were under 7.93 μm. Sodium phosphate tribasic ($Na_3PO_4 \cdot 12H_2O$), or-

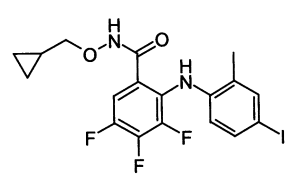

Fig. 1. Structure of PD198306.

thophosphoric acid (both Mallinckrodt) and sodium lauryl sulfate (SLS) USP/NF, were used to prepare 25mM pH 9 sodium phosphate solution with 0.5% SLS. This solution was used as the dissolution medium. Hydroxypropylmethylcellulose (HPMC) and Polysorbate 80 were obtained from Pfizer Central Raw Materials Group. Deionized water was obtained from Ricca Chemical Co. A suspension medium containing 0.3% w/v HPMC and 0.2% w/v Polysorbate 80 in water was used, as described later, to make a suspension of the drug for certain dissolution experiments.

2.2. Methods

2.2.1. UV spectrophotometry

A standard curve of absorbance versus concentration was constructed using solutions of PD198306 in the dissolution medium, ranging in concentration from 5 to 30 μg/ml. Absorbance versus concentration plot was linear over this concentration range and was used to determine percent drug dissolved in the dissolution experiments.

2.2.2. Flow-through dissolution

A schematic of the flow-through dissolution apparatus (Erweka Instruments Inc., Milford CT) is illustrated in Fig. 2. The apparatus is fitted with six 12 mm (internal diameter) cells. A brief description of the cell design helps better understand the various sample loading patterns used in the experiments. The flow-through cell that was used in the experiments may be described has having 3 parts: the lower *cone*, the middle *cylindrical portion*, and the *filter head* on top. Dissolution medium enters the cone through a capillary bore on the bottom and flows up the cell. The cone is separated from the cylindrical portion by a # 40 mesh screen and a glass microfiber filter. The filter head on top also holds a glass microfiber filter. In all cases, the lower cone holds a glass bead 6mm in diameter, which serves to equalize the jet of fluid entering the cell.

Powder was loaded into the flow-through cell in four different patterns according to the following experimental design to investigate the effect of sample loading on the dissolution profiles of unmicronized drug substance (please refer to Fig. 3). In all experiments the cylindrical portion of the flow cell contained 2 g of 1 mm round glass beads. The powder was positioned in four different ways relative to the glass beads contained in the dissolution cells as described below, but was not compacted to any extent by the presence of the beads or the method of loading.

Pattern-A, drug substance homogeneously mixed with the 1 mm round glass beads. Mixing was carried out very gently with the help of a spatula.

Pattern-B, drug substance layered midway across the bed of 1 mm round glass beads to sandwich the powder in the bed of glass beads.

Pattern-C, drug substance layered on the bottom of the cylindrical portion below the bed of 1 mm round glass beads.

Pattern-D, same as Pattern-C, but with the lower cone also filled with 1 mm round glass beads.

The amount of drug used per cell in most experiments was 10 mg with 400 ml of dissolution medium. (In some experiments, however, 20 mg of drug was used with 800 ml of dissolution medium). Thus at 100% dissolution, the concentration of drug in the dissolution medium would be approximately 40% of the saturation solubility value. (Equilibrium solubility of the compound in the dissolution medium was measured and found to be 62 μg/ml).

All experiments were carried out in a closed loop setup. The flow rate of the dissolution medium through the cells was either 8 or 4 ml/min as described under the Section 3. Samples were withdrawn automatically from the flow-through cell every 15 min for 3 h. Absorbance was measured at 280 nm, against a reference cell containing only the dissolution medium. The absorbance values were used to calculate the percentage of drug dissolved at each time point.

3. Results and discussion

The dissolution profiles obtained from the experiments are shown in Figs. 4–8. Error bars on the graphs represent the standard deviation (S.D.)

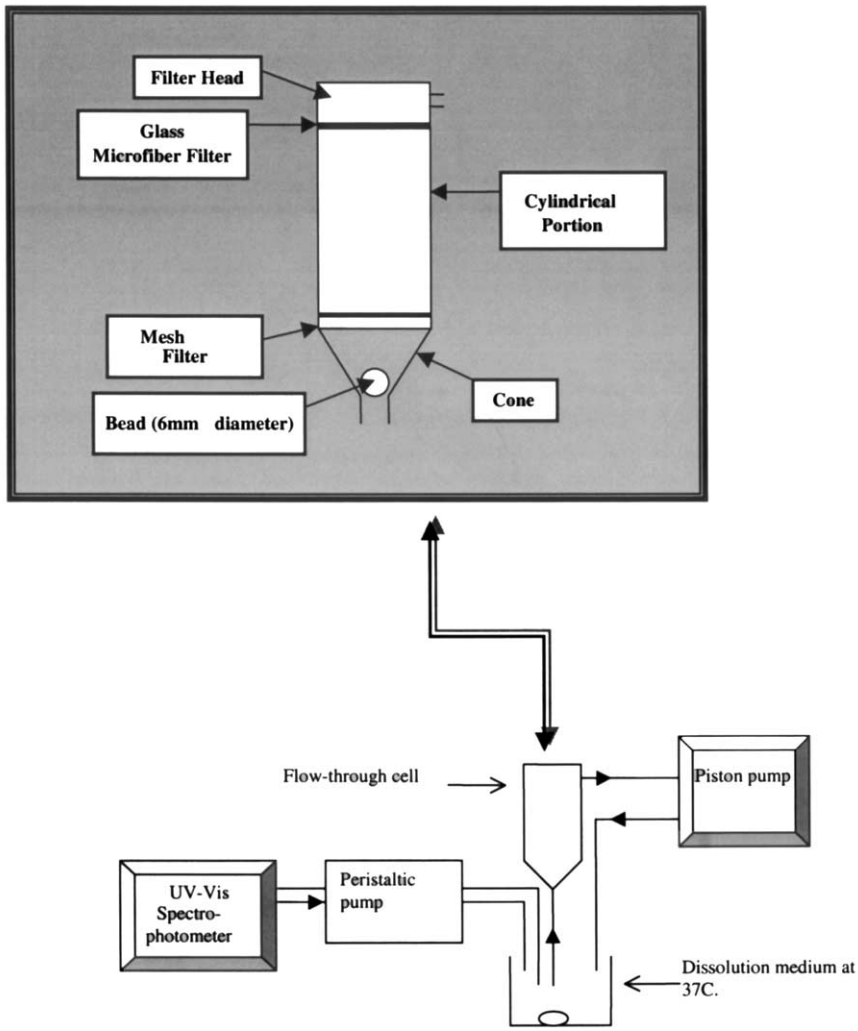

Fig. 2. Schematic diagram of the flow-through dissolution apparatus.

of the mean. The dissolution profiles obtained using a flow rate of 8 ml/min and sample loading Patterns A–D described above are illustrated graphically in Fig. 4. The graph shows that at the end of 3 h, the rate and extent of dissolution of unmicronized PD198306 was greatest ($77.4 \pm 0.9\%$) when drug substance was loaded according to Pattern-A (Drug substance homogeneously mixed with 1 mm round glass beads). The rate and extent of dissolution at the end of 3 h were reduced when the drug was either 'sandwiched' between the 1 mm round glass beads (Pattern-B, $64.9 \pm 7.6\%$ dissolved), or layered on the bottom of the cylindrical portion below the bed of 1 mm round glass beads (Pattern-C, $67.3 \pm 2.4\%$ dissolved) and was the lowest ($53.8 \pm 8.1\%$ dissolved) when the 1 mm round glass beads were packed in the lower cone *and* over the layer of drug (Pattern-D). The reproducibility of results was best when the drug was loaded according to Pattern-A, showing lower S.D. as compared with the alternative sample loading methods (Patterns B–D).

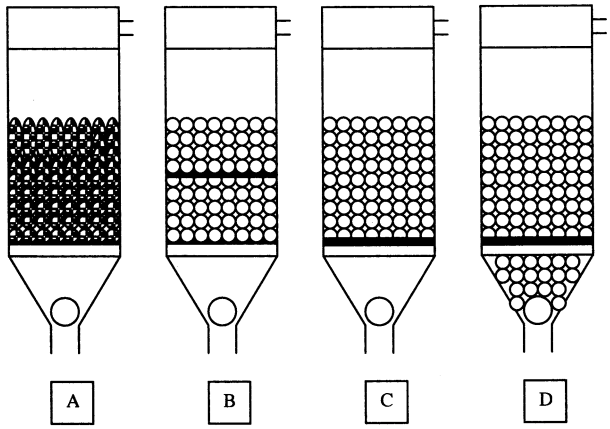

Fig. 3. Schematic diagrams showing the position of drug in the flow-through cell.

Homogeneously mixing the drug with glass beads according to Pattern-A was experimentally inferred to be the best method of drug powder loading into the dissolution cell in order to achieve maximum dissolution with minimum variability of results based on unmicronized drug data. Micronized drug was, therefore, tested using Pattern-A, with the flow rate of the dissolution medium, as before, at 8 ml/min.

The dissolution profile obtained using micronized drug compared with the dissolution of unmicronized drug using the flow cell loading scheme according to Pattern-A is shown in Fig. 5. The profiles in Fig. 5 demonstrate that the rate and extent of dissolution of micronized drug is reduced compared with that of unmicronized drug using the cell loading technique previously shown to be successful (Pattern-A). The dissolution of unmicronized drug achieved values of 77% dissolved in 3 h versus approximately 40–50% dissolved for micronized material. Per the Noyes–Whitney relationship, dissolution rate is directly proportional to particle surface area and should, therefore, increase with reduced particle size. However, these results illustrate a slower dissolution with micronized drug substance. The anomalously reduced dissolution of micronized compared with unmicronized drug particles was attributed to the observation that micronized material, owing to very poor wettablity, had been carried to the top of the flow through cell and deposited in the filter. The deposition of the drug

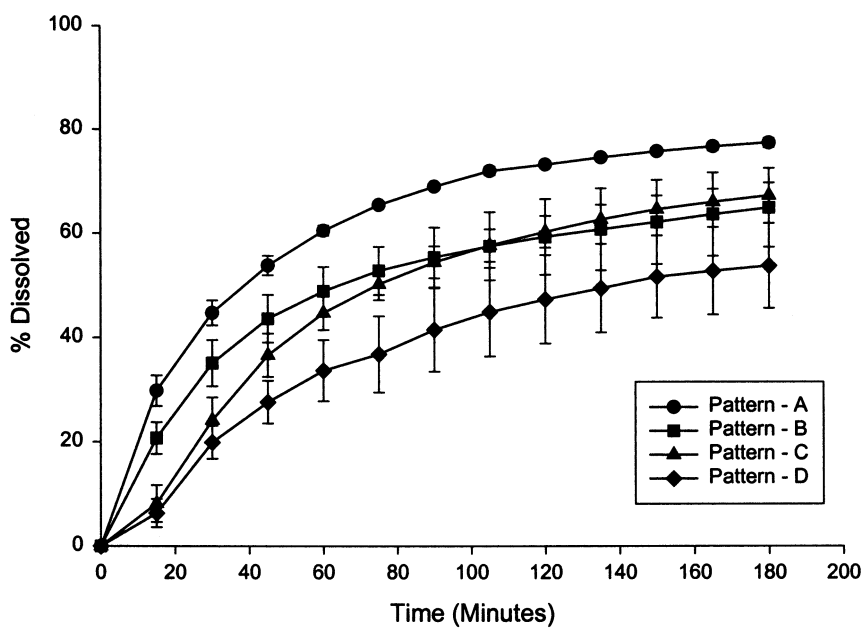

Fig. 4. Dissolution profiles obtained from unmicronized powder loaded into the flow-through dissolution cell according to patterns A–D, ($n = 3$).

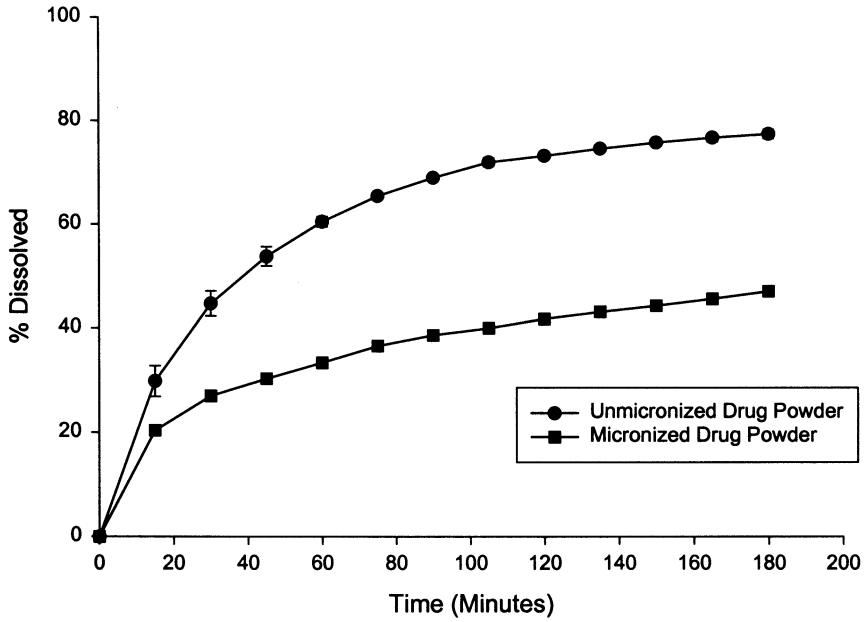

Fig. 5. Dissolution profiles of micronized ($n = 2$) and unmicronized ($n = 3$) drug powders using sample loading Pattern-A.

substance onto the filter resulted in incomplete dissolution of the particles. It was concluded from the above study, that when both micronized and unmicronized drug were taken into consideration, homogeneously mixing the drug powder with glass beads according to Pattern-A was not an appropriate method of loading sample into the flow-through dissolution cell. This unexpected result led to the exploration of alternative methods of sample loading in order to achieve acceptable rate/extent of dissolution, low variability of results, and particle size discrimination.

The next series of experiments examined the effect of loading the unmicronized and micronized powder as a suspension in an aqueous medium containing 0.3% HPMC and 0.2% Tween 80 to achieve a concentration of 10 mg of drug in 1 ml of suspension. PD198306 has no appreciable solubility in the medium and forms a uniform suspension in it. The rationale behind this approach was that a suspension containing pre-wetted particles in a dispersion would minimize the problems caused by poor wetting and, therefore, achieve suitable dissolution properties and particle size discrimination. The suspension was loaded on the bottom of the cylindrical portion of the cell below the bed of glass beads before the start of the experiment. Keeping the drug particles at the bottom of the cell was also expected to further control the migration of the drug particles to the top and subsequent deposition in the filter. Glass beads were not packed into the bottom lower cone of the cell in this experimental design because the previous data showed no beneficial effect. The flow rate of the dissolution medium was maintained at 8 ml/min. The results of the suspension loading technique are displayed in Fig. 6 showing that $71 \pm 4.8\%$ of the unmicronized drug and $57.7 \pm 2.9\%$ of the micronized drug had dissolved in 3 h.

In comparing Figs. 5 and 6 the following observations are noted. The dissolution of micronized drug increased from 47.1% with dry powder homogeneously mixed with beads to $57.7 \pm 2.9\%$ with micronized drug in suspension. Unmicronized drug, however, displayed a reduction in dissolution under similar conditions, going from $77.4 \pm 0.9\%$ (drug powder homogeneously mixed with beads) to $71 \pm 4.8\%$ (suspension). In Fig. 6, the difference between the dissolution profiles of

micronized and unmicronized drug powder was smaller compared with that in Fig. 7.

The following may be inferred from the results described above. Mixing the drug powder with beads facilitates dissolution by keeping the particles well dispersed within the cell. Wetting of the drug particles is also important, lack of which leads to the upward migration of particles. The suspension method of drug loading was an effort to pre-wet the drug particles. While the benefit of dispersing the drug in the beads is seen with the unmicronized drug, it is not evident with the micronized drug because the problem of upward migration of the particles was much worse with the latter. The suspension method of loading the drug into the dissolution cell helped with the dissolution of the micronized drug by controlling the upward migration of micronized particles and it appeared, therefore, to be better suited for testing the dissolution of the micronized powder. When the unmicronized drug was loaded into the cell as a suspension at the bottom of the cell, dissolution was less than optimum although the drug was pre-wet because the drug was not well dispersed in the beads.

It is apparent from the data that the suspension loading method did achieve a smaller difference in terms of extent of dissolution of micronized and unmicronized drug powder compared with when the samples are homogeneously mixed with the glass beads as a dry powder. This is potentially due to the control of migration of particles to the top of the cell. This method of sample loading, however, still did not show particle size discrimination predicted by the Noyes–Whitney equation that would dictate micronized particles display a greater rate of dissolution compared with unmicronized particles. Potentially, particle size discrimination could be achieved by reducing flow rate of solution through the dissolution cell thereby further reducing the migration of micronized particles to the top of the cell.

Fig. 7 shows a comparison of the dissolution profiles of micronized drug obtained at 8 and 4 ml/min using the suspension method of drug loading. With the flow rate at 4 ml/min, $70.5 \pm 7.5\%$ of the drug had dissolved at the end of 3 h whereas only $57.7 \pm 2.9\%$ had dissolved when the flow rate was 8 ml/min. This is contradictory to previously reported results (Zhang et al., 1994)

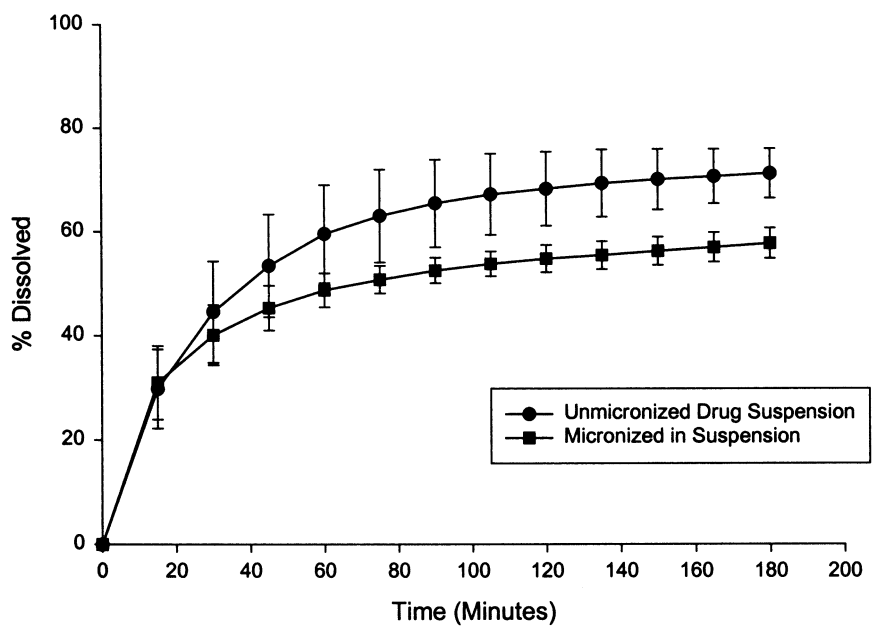

Fig. 6. Dissolution profiles of micronized and unmicronized drug in suspension ($n = 3$).

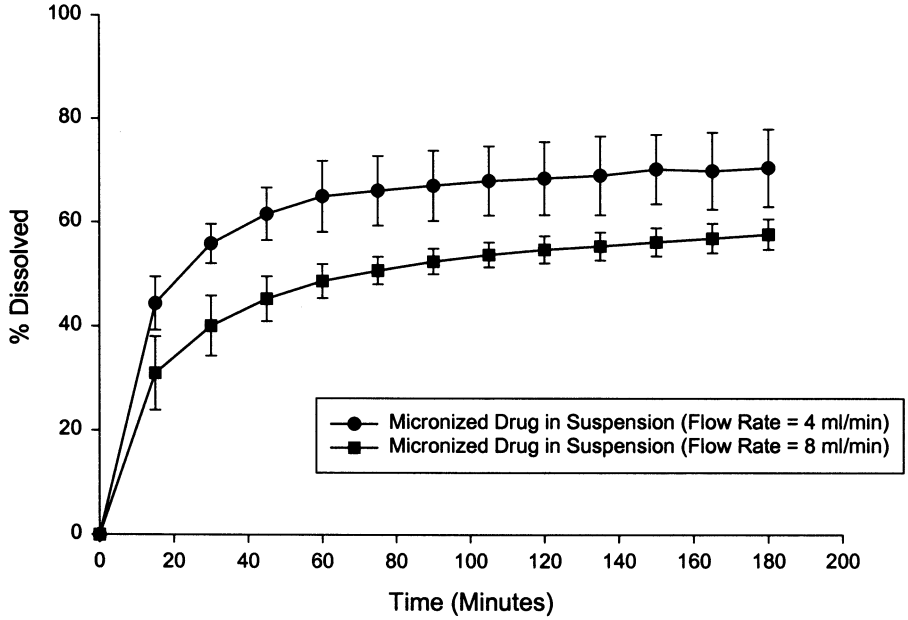

Fig. 7. Dissolution profiles comparing micronized drug in suspension using flow rates of 4 and 8 ml/min ($n = 3$).

with solid dosage forms of poorly soluble compounds, where increasing flow rates consistently caused an enhancement in dissolution. We attribute our results to the fact that the poor wettability of the drug in the dissolution medium caused the drug particles to float and to be swept up the cell that increases with increasing flow rate of the dissolution media. Although the suspension method of loading the drug into the cell was better than loading the dry powder, the migration of drug particles to the top of the cell was only completely controlled when the flow rate of the dissolution medium was reduced to 4 ml/min.

Fig. 8 shows the dissolution profiles of unmicronized and micronized drug loaded as suspension and with the flow of the dissolution medium at 4 ml/min. The dissolution profiles clearly demonstrate particle size discrimination showing that the micronized material clearly displays an increased rate of dissolution compared with unmicronized drug. The extent of dissolution in both cases was about the same (60–70%).

4. Conclusion

In order to successfully employ the flow-through dissolution cell for testing of drug powders, it is critical that the drug substance be maintained in the body of the cell during testing. As seen from the example of PD198306, this is not easy to achieve with poorly wettable drug substances. In the absence of a better cell design, the method of sample preparation and loading into the cell need to be carefully chosen in order to obtain reliable results. The intent of this work was to determine how well this method of dissolution testing and the pattern of powder loading can discriminate between unmicronized and micronized drug powders. Achieving 100% dissolution, although desirable, was not a set goal. The data presented demonstrates that for a drug substance of poor wettablity, preparing the sample as a suspension before it is loaded into the flow through dissolution cell is a useful approach that can be applied to determine the influence of particle size on dissolution. Improper methods of sample loading may result in confusing or erroneous data if not analyzed carefully.

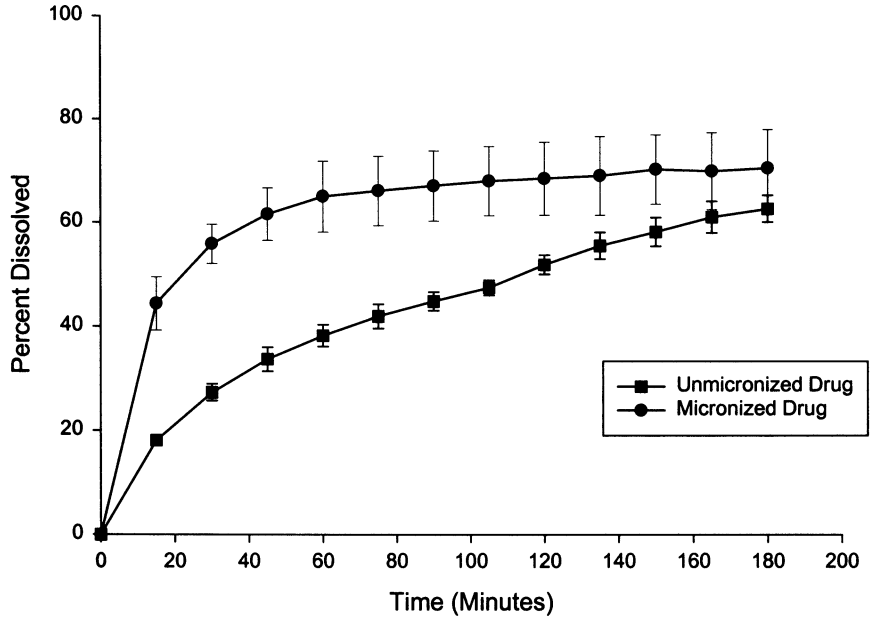

Fig. 8. Dissolution profiles of micronized and unmicronized drug, using suspension loading method and 4 ml/min flow rate.

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