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The use of constant volume ultrafiltration for dissolution studies

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Summary

In the course of an investigation into the dissolution characteristics of phenytoin it was required to follow the effect of added protein on the dissolution profile. The Amicon Diaflo ultrafiltration cell and membranes have been used for ultrafiltration of protein solutions. We felt that this system could be readily adapted for dissolution studies in the presence of protein, and indeed found that it provided a very useful method of investigating dissolution in general. We report here methods of analysis of the results of such experiments, using phenytoin and oxyphenbutazone as examples.

Introduction

A great number of reports dealing with the diffusion of a solute from a solid surface via the diffusion layer to the bulk solution indicate that active agents play an important role in the dissolution process. Macromolecules have been extensively used in this respect, being employed to enhance the dissolution characteristics of sparingly soluble drugs. Typical examples of such use are the dissolution studies using polyvinylpyrolidone (Florence et al., 1973; Najib et al., 1978), methylcellulose, hydroxypropylcellulose, guar gum and xanthan gum (Sarisuta and Parrot, 1982) and polyethylene glycol 4000 (Ravis and Chen, 1981).

The evaluation of the dissolution characteristics of drugs in media containing the macromolecule or from appropriate formulations of drug–macromolecule as co-precipitates (Sekikawa et al., 1978), ground mixtures (Yamamoto et al., 1976) or cyclodextrin complexes (Uekama et al., 1983) is routinely made by use of conventional dissolution apparatus. However, neither those apparati recommended in the official compendia nor those which have been reported in the literature are capable of distinguishing the free drug from that bound to the macromolecule. When the drug–macromolecule complex is soluble in the dissolution medium the estimated concentration in a conventionally filtered sample is that of the total drug. A further complication in the latter case can be the interference of the macromolecule in the normal estimation of drug concentrations.

The difficulties encountered with the normal dissolution techniques prompted us to utilize constant volume ultrafiltration for dissolution studies where the specific objective was the measurement

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of free drug concentrations (Rosen and Macheras, 1984). This system is capable of giving free drug dissolution profiles directly, and we report here details of the technique employed and of the methods used to analyse the data.

Materials and Methods

An Amicon Diaflo ultrafiltration apparatus (Model 202) was used for the diafiltration. This consists of a polycarbonate cylinder capped top and bottom with teflon ends. The bottom end holds a scintered glass disc which supports the ultrafiltration membrane (Amicon PM 10, cut off 10,000 rrm). A capillary tube leads from the bottom, allowing effluent to escape from the cell. This travels via capillary tubing to an optical flow cell within a UV spectrophotometer. The top cap holds the spindle of a bar magnet stirrer which is held just above the ultrafiltration membrane. There are two leads into the top cap; the first the inflow tube, the second a screw-in pressure valve. A stainless reservoir is connected to the diaflo cell and to a nitrogen cylinder by a selector valve. This allows pressure to be applied directly to the diaflo cell alone, in which case the volume of the contents within the diaflo cell drops; to the reservoir alone, in which case the volume of the contents of the diaflo cell increases; or equally to both, in which case the volume of the contents of the diaflo cell remains constant. The reservoir is placed in a water bath at constant temperature, and water from the bath circulated through a copper cylinder jacketing the diaflo cell.

With a known volume (110–160 ml) of solvent in the diaflo cell and the reservoir charged with the same solvent, the apparatus is left in the batch to obtain thermal equilibrium. When macromolecules are present in the diaflo cell this time may also be used to dialyze the macromolecular solution until zero or constant absorption is shown on the uv monitor. At the start of an experimental run the pressure relief valve at the top of the cell is unscrewed and a known sample of the compound under investigation introduced into the cell. The valve is then rapidly screwed back into position and the system pressurized. The cell contents are

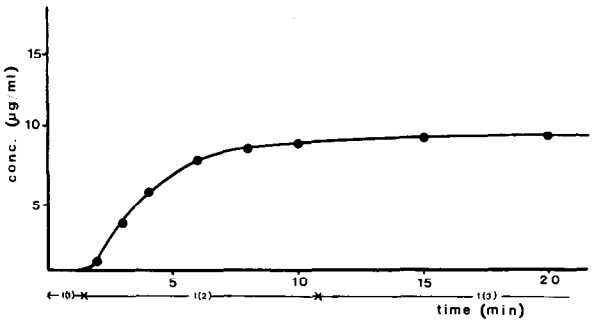

Fig. 1. Dissolution of phenytoin at pH 1 as a function of time. The periods outlined show; a lag time (t_1), a time to establish steady-state (t_2), and the steady-state time (t_3).

stirred at a constant rate throughout. Effluent from the cell passes through the recording spectrophotometer and thence to a flowmeter or some other means of measuring the flow rate.

The general shape of the experimental curves obtained using this apparatus is shown in Fig. 1. A short lag-time t_1 is caused by the time to pressurize the system and fill the void volume from the ultrafiltration membrane to the outflow of the optical cell. It also reflects any binding to the membrane which might occur. This time, of the order of 1 or 2 min, is followed by a further period t_2 during which an equilibrium concentration is being reached in the cell. The values of t_2 depends on a number of factors; the volume of solvent in the cell, the flow rate and the rate of dissolution of the sample. By varying the conditions of the experiment it can be made to be of the order of several minutes. A further period, t_3, represents a steady-state situation (see below). The length of this period again depends on the experimental factors of volume, flow rate and solubility. Not shown on Fig. 1 is the slow decline in the steady-state value, caused by changes in surface area, which is finally followed by a rapid exponential drop when all the solid has dissolved and only dilution is occurring.

Theoretical analysis

Assuming a constant surface area in contact with the solvent, the rate of increase of concentra-

tion is given by the Noyes-Whitney equation

$$\frac{dc}{dt} = k(c_s - c) \quad (1)$$

The rate of outflow of material from the cell and the dilution of the cell contents by an equal volume of solvent is given by

$$\frac{dc}{dt} = c \cdot u/V \quad (2)$$

where V is the volume of solvent in the diaflo cell and u is the flow rate through the cell. With both Eqns. 1 and 2 operating, the overall concentration change in the cell is given by

$$\frac{dc}{dt} = k(c_s - c) - c \cdot u/V \quad (3)$$

This is a linear differential equation which can be treated in a number of ways

(a) At steady-state the rate of change of concentration is zero. Eqn. 3 after rearrangement then gives

$$c_m = \frac{kc_s}{k + \frac{u}{V}} \quad (4)$$

where c_m is the concentration reached at steady-state. If u/V is very much smaller than k, then $c_m = c_s$. In all other cases Eqn. 4 is best expressed in the reciprocal form

$$\frac{1}{c_m} = \frac{1}{c_s} + \frac{1}{kc_s} \times \frac{u}{V} \quad (5)$$

A plot of the reciprocal of the steady-state concentration, c_m, reached during runs involving different values of the fraction u/V against these values then gives a straight line with intercept $1/c_s$ and gradient $1/kc_s$. This straight line, of course, only occurs as such if k is constant between runs. Since k is a pseudo-first-order constant which in truth depends on the effective surface area available for contact with solvent, it is important that similar amounts of compound of the same particle size are used in all determinations in which u/V is varied.

(b) Integration of Eqn. 3, rearranging and taking logarithms gives

$$\ln \frac{c_m}{c_m - c} = (k + u/V)t \quad (6)$$

A plot of the left hand side of eqn. 6 against t gives a line of slope $k + u/V$. For several such straight lines the value of the slope may be plotted against the value of the corresponding fraction u/V to give a straight line of intercept k. Alternatively, k may be calculated from the expression

$$k + u/V = 0.693/t_{1/2}$$

where $t_{1/2}$ is the time taken for the concentration to reach half the steady-state level. However, such a calculation is subject to errors caused by the lag time t_1.

The reciprocal plot of Eqn. 5 is preferred to the log plot used in Eqn. 6 providing k and c_s are small. Large values of these constants mean that only large changes in u/V cause a measurable difference in c_m. However, such changes are precluded by the need to keep a fairly constant ratio of surface area of powder to volume of solvent, which limits V, and the need to take effluent concentration as equal to the bulk concentration, which limits u to low values.

(c) The steady-state equation includes an assumption that k is truly constant. In fact, k includes a component of surface area. As material dissolves, the surface area decreases, so that there is a continual decrease in the dissolution rate 'constant'. Provided that the initial amount of solid taken is greatly in excess of the amount required to saturate the volume of solvent in the diaflo cell, the decrease in surface area is too small to be seen initially, and steady-state levels are attained. However, if the run is continued over a long time eventually the steady-state level begins to decline. This decline remains slow, and is to all intents and purposes linear. Finally, when all the solid matter is in solution, the concentration in the cell drops exponentially. If the solubility is such that the change in surface area needed to reach a steady-state is greater than 10% of the original, then rate constants should be obtained from the

initial portion of the concentration/time curve only.

(d) In an investigation of the effect of some other solute on the rate of dissolution of the material the dissolution rate constant is changed to some new value k′. All the equations related to the system, however, remain the same, so that the analysis carried out is not changed. If the other solute is a macromolecule it is placed at suitable concentration directly in the diaflo cell. If it is a solute capable of passing through the ultrafilter then it must be incorporated in the reservoir solvent.

Results and Discussion

We have applied Eqns. 5 and 6 in dissolution studies with a number of drugs and give here a few representative results to illustrate their use.

The type of dissolution profile shown in Fig. 1 can in practice be produced several times in one run. This is illustrated in Fig. 2, where the rise to a given plateau value is reached within a few minutes. Reduction of pressure, and hence flow rate, causes an increase in concentration to a new steady-state value. Such reductions can be carried out consecutively several times during one complete run, giving sufficient data for a reciprocal plot (Fig. 3) which can be analyzed to give both

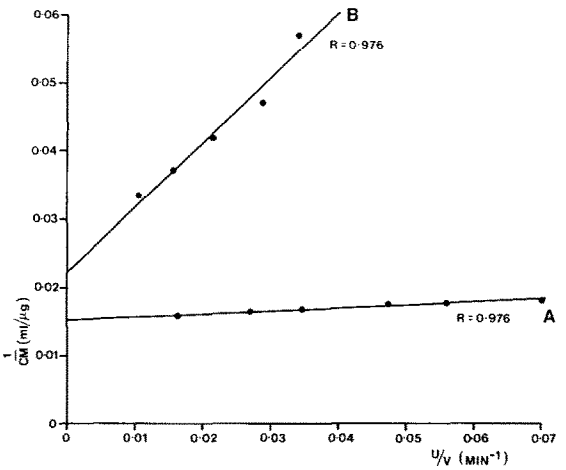

Fig. 3. Dissolution of oxyphenbutazone at pH 4. Data obtain from Fig. 2 were plotted according to equation 5. Lines A and B correspond to A and B from Fig. 2. Graphical values for solubility and apparent rate constants in absence and presence of protein were 65.232 μg/ml, 0.3265 min^{-1} and 43.72 μg/ml and 0.0244 min^{-1}, respectively.

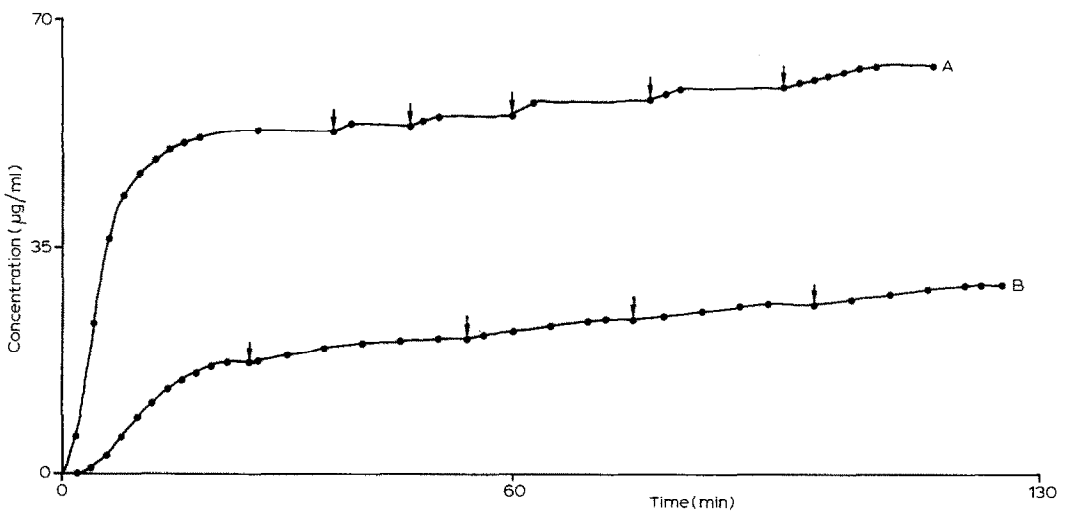

Fig. 2. Dissolution of oxyphenbutazone as a function of time. The arrows show the instant of consecutive pressure decreases. *Curve A*: 300 mg oxyphenbutazone in 0.02 M phosphate citrate buffer pH 4. The flow rates (with associated steady-state values shown in brackets) are, from left to right, 9.1 (54.44); 7.23 (55.34); 6.12 (56.54); 4.48 (58.35); 3.49 (60.15) and 2.11 (63.16) ml · min^{-1} (μg · ml^{-1}). *Curve B*: as above in presence of 50 mg % BSA. From left to right 4.6 (1.15), 3.9 (21.25), 2.9 (24), 2.1 (26.75) and 1.4 (29.75) ml · min^{-1} (μg · ml^{-1}).

the first-order rate constant for dissolution and the saturating solubility. We find experimentally that it is easier to work with sequential pressure decreases than with pressure increases. Figs. 2 and 3 show also that the same system can be used in the presence of protein. In Fig. 2 curve B experimental points are shown for oxyphenbutazone dissolution in the presence of 50 mg % BSA and values from these plots are replotted in Fig. 3. As noted previously, obtaining appreciably different steady-state values is only practicable when k and u/v are of the same order.

If $k \gg u/V$ then changing the ratio of flow rate-to-volume makes little difference to the steady-state concentration, which in this case is close to the saturating solubility. This is illustrated by Fig. 4, where despite different flow ratios the plot of $\ln C_m/(C_m - C)$ versus t results in a set of near parallel straight lines. The different intercepts of these lines are caused by differing lag times.

While the actual shapes of the dissolution profiles of different drugs may be altered in the presence of macromolecular species, the analysis of the results remains the same. Thus the technique of constant volume ultrafiltration can be readily applied to a comparison of dissolution behaviour in the presence and absence of polymer or indeed to other types of dissolution study (Jones and Parr 1983). From the physiological point of view the dissolution profiles of free drug obtained in this way are more meaningful than the corresponding values for total drug concentration obtained when ultrafiltration is omitted.

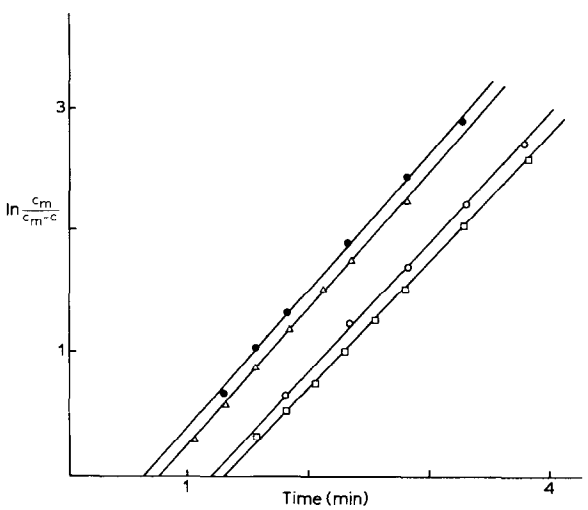

Fig. 4. Dissolution of phenytoin at pH 7.4. Data are plotted according to Eqn. 6. The ratios of flow rate-to-volume (u/v) for the various runs are: 2.58, 3.2, 2.32, 1.8×10^{-2} min^{-1}.

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