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Dissolution test for felodipine tablets using chemical oxidation *in situ* to maintain 'sink conditions'*

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Abstract: A test model is described for the determination of the dissolution rate of the vasodilator, felodipine, a derivative of dihydropyridine that is practically insoluble in water. 'Sink conditions' are maintained by means of an oxidizing agent, ceric sulphate, which reacts rapidly with dissolved drug molecules in the dissolution fluid. A pyridine derivative is formed quantitatively in the oxidation reaction. The amount of dissolved felodipine is calculated from the concentration of the pyridine derivative, as determined by reversed-phase liquid chromatography. Dissolution rates depend on the concentration of the oxidizing agent so that high concentrations accelerate dissolution. The dissolution test suggested for 25-mg felodipine tablets is performed in 500 ml fluid that contains 5 mM ceric sulphate in 0.12 M sulphuric acid. The test is performed on single tablets with USP paddle equipment. Dissolution rates for nine different tablet compositions are correlated to such bioavailability parameters as maximum plasma concentration and total area under the plasma concentration–time curve. Interferences and limitations of the method are discussed.

Keywords: *Ceric sulphate; dissolution rate; felodipine;* in vivo–in vitro *correlation; oxidation; reversed-phase liquid chromatography.*

Introduction

Pharmaceutical analysis during the development of a new solid dosage form usually includes a test for the dissolution rate of the drug. Standardized methodology for dissolution testing has been described in some pharmacopoeias [1, 2] and in a joint report issued by the Fédération Internationale Pharmaceutique [3]. It is recommended that the dissolution rate be determined under so-called 'sink conditions'; this term has been interpreted to mean that the concentration of the drug in the dissolution fluid must not exceed 10–20% of the saturation concentration [3]. The use of dissolution tests has been

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questioned in the case of lipophilic drugs of extremely low water-solubility. But it is easy to understand that effective means to predict the bioavailability are of special interest in the formulation of solid dosage forms of such drugs. Sufficient solubility of a lipophilic substance can be attained by the use of a dissolution fluid that contains a certain proportion of an organic solvent or a surfactant. Opinions differ as to whether or not these methods should be recommended for dissolution tests, although potentially both methods appear to have general application.

Felodipine is a derivative of dihydropyridine that has vasodilator properties. It is practically insoluble in water, the solubility at 37°C being about 1 mg/l. The difficulties associated with the dissolution testing of lipophilic drugs became evident during the process of formulating a tablet that contained 25 mg felodipine. A method based on pure water as a dissolution medium would have required more than 100 l. of fluid to ensure 'sink conditions'. The handling of such large volumes is by no means practical.

Instead, initial dissolution tests were performed in 30% ethanol in which the solubility of felodipine is about 4 g/l, this being more than enough to maintain 'sink conditions'. However, bioavailability studies gave results which could not be correlated with the dissolution *in vitro*. An alternative dissolution method was therefore desired, which would generate data that could be correlated with the various bioavailability parameters.

It was assumed that the new test model ought to be based on a dissolution liquid containing water as the only solvent. One approach to the problem was the removal of dissolved felodipine through a chemical reaction taking place in the dissolution fluid itself. The property utilized for this purpose was the oxidizability of felodipine to its more soluble pyridine derivative. The present report describes the development of this new method based on the principle of oxidative conversion of the drug in the dissolution liquid. The performance of this method is presented together with a discussion of its applicability to various dosage forms.

Experimental

Reagents

The sample of felodipine, 5-ethyl-3-methyl-4-(2,3-dichlorophenyl)-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylate, was a reference substance of 99.6% purity. The pyridine derivative, 5-ethyl-3-methyl-4-(2,3-dichlorophenyl)-2,6-dimethyl-3,5-pyridine-dicarboxylate, was used in the form of its hydrochloride salt with a purity of 98.0%. All chemicals used were of analytical grade. Solutions of ceric sulphate were made from a 0.1 M standard solution (Merck) in 0.5 M sulphuric acid, which was diluted with 0.1 M sulphuric acid to give the desired cerium concentrations. Ethanol 95% (v/v) was used. Nine different tablet compositions (A–I) of felodipine 25 mg were tested in addition to a number of other preparations of the drug.

Apparatus and methods

Dissolution tests were performed on single tablets using equipment in accordance with the requirements of USP XX apparatus 2 (paddle method) [2]. Experimental conditions were as follows, if not otherwise stated: volume, 500 ml; temperature, 37.0°C; and rotational speed, 100 rpm. The amount of dissolved felodipine was expressed as a percentage of declared content. The initial tests were carried out in a liquid which contained 30% v/v ethanol in water. First, the basket method was used at 150 rpm (for tablets B–E) and then the paddle method was used at 100 rpm (for tablets A and F–I). A

few experiments were performed in 1000 ml of 10% v/v ethanol using the paddle method at 100 rpm. Only a small piece of tablet (¼s) was used in these measurements because of the low solubility of felodipine in this medium.

Sample treatment for the determination of felodipine and the pyridine derivative included a filtration step. Filtration of aqueous solutions of felodipine was associated with adsorption losses; least adsorption occurred when using a thin membrane filter with capillary holes (Nucleopore polycarbonate). Solutions of the pyridine derivative did not give rise to any adsorption problems.

Felodipine and the pyridine derivative were determined by liquid chromatography on a reversed-phase solid support with a mean particle diameter of 5 μm (Spheri-5 RP8), on a 30 × 4.6 mm i.d. column. The mobile phase comprised methanol–0.02 M perchloric acid (60:40 v/v).

The liquid chromatograph consisted of a single-piston pump (Altex Model 110 A), a sample injection valve (Rheodyne Model 7010) and a variable wavelength detector (LDC SpectroMonitor III). The flow rate was 1.0 ml/min and the injection volume was usually 20 μl. The detector was operated either at 275 nm, at which the pyridine derivative has maximum absorbance, or at 254 nm. The retention time was about 3 min for the pyridine derivative and about 4 min for felodipine.

The concentration of Ce^{4+} in the dissolution fluid was determined by titration of 40-ml samples with 0.01 M sodium thiosulphate using starch as indicator. Solubilities were determined by dispersion of an excess of solid substance with the solvent in an ultrasonic bath, followed by agitation for at least 12 h to achieve equilibrium. The solutions were then filtered and the concentration of dissolved substance was determined by liquid chromatography.

The regression equation for concentrations of the pyridine derivative from 7–140 μg/ml ($n = 6$) was: $y = 2.222x - 0.121$; standard error in gradient, $S_m = 4.0 \times 10^{-3}$; correlation coefficient, $r = 1.0000$; ordinate y measured in cm at 0.01 a.u.f.s. and 275 nm. The relative standard deviation (RSD) at 70 μg/ml was 0.5% ($n = 6$). The detection limit (SNR = 3) for felodipine at 254 nm was 1 ng on-column, and for the pyridine derivative at 275 nm it was 3 ng.

The bioavailability of the tablets was investigated in three different studies. The first involved four different tablet formulations and five or six healthy volunteers; the second concerned a further three tablet formulations and six healthy volunteers; and the third study involved two additional formulations and 16 healthy volunteers. The concentration of felodipine in the plasma samples was determined by capillary gas chromatography using electron capture detection [4].

Control method

The procedure for determining the dissolution rate for quality control purposes was as follows. Six tablets were tested individually using a USP paddle apparatus operated at 100 rpm. The dissolution medium, which consisted of 500 ml 5.0 mM ceric sulphate in 0.12 M sulphuric acid, was maintained at 37°C. About 8 ml of the solution was sampled by a glass syringe 30 min after the addition of the tablet to the liquid. The sample was filtered immediately through a 0.4-μm Nucleopore filter. The first 4 ml of the filtrate was discarded and 20 μl of the subsequently collected filtrate was injected on to the liquid chromatograph. The peak height of the pyridine derivative was measured and the amount of dissolved felodipine was calculated by reference to external standards run consecutively.

Results and Discussion

Oxidation of felodipine

Felodipine, which is a dihydropyridine derivative, can be oxidized to the corresponding pyridine derivative (Scheme 1). The solubility of the pyridine derivative in dilute acids, which was assumed to be approximately equal to that of its hydrochloride, was high enough to allow dissolution tests on 25-mg felodipine tablets in volumes less than 1 l, provided that it was possible to oxidize the dissolved felodipine to the pyridine derivative continuously during the experiment (Table 1). A suitable oxidizing agent for that purpose had to fulfil a number of requirements among which were stoichiometric yield, high reaction rate and stability of the pyridine derivative in the dissolution medium. Of the various oxidizing agents tested initially, only ceric sulphate gave quantitative formation of the pyridine derivative (Table 2). The use of Ce^{4+} as the

Scheme 1

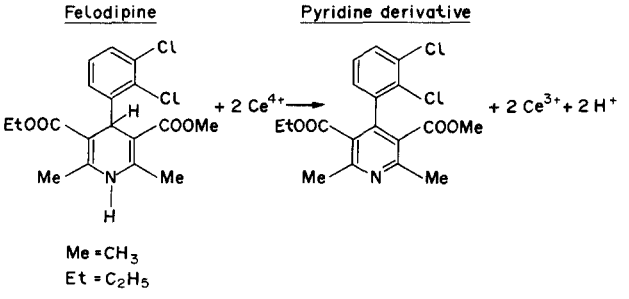

Table 1
Solubility of felodipine and the corresponding pyridine derivative in various solvents

Solvent	Temperature (°C)	Solubility (mg/l)	
		Felodipine	Pyridine derivative
Water	37	1.2	—
30% v/v ethanol	37	4000	—
0.1 M HCl	22	0.5	59
Phosphate buffer, pH 6.5	22	0.5	16
5 mM $Ce(SO_4)_2$ in 0.12 M H_2SO_4	37	—	300

Table 2
Oxidation of 0.31 μM felodipine with various oxidizing agents at room temperature

Oxidizing agent (1 mM)	Medium (0.1 M)	Reaction time (min)	Final concentration (μM)	
			Felodipine	Pyridine derivative
$Ce(SO_4)_2$	H_2SO_4	1	*	0.32
$Ce(SO_4)_2$	H_2SO_4	73	*	0.31
$KMnO_4$	HCl	1	*	0.11
$KMnO_4$	HCl	30	*	0.12
$K_2Cr_2O_7$	HCl	120	0.21	0.12
H_2O_2	HCl	50	0.21	0.11

* Concentration was less than the detection limit (~0.01 μM).

oxidizing agent implied that the dissolution fluid had to be acidic; this condition favoured the solubility of the pyridine derivative.

The oxidation rate at room temperature was investigated by pumping either water, or a solution of 5 mM ceric sulphate in 0.12 M sulphuric acid, at 1.5 ml/min through a 4-mm i.d. glass tube filled with crystals of felodipine. The length of the felodipine column was 60 mm. An injection valve with a 100-μl loop was connected directly to the outlet of the glass tube, so that the solution from the felodipine column could be injected on to the liquid chromatographic column with as little time delay as possible. The sum of the void volume of the felodipine column, the dead volume in the connecting capillaries and the injection volume was about 300 μl. This volume corresponded to a time interval of about 10 s between passage through the felodipine column and injection on to the chromatograph.

When water was pumped through the glass tube, the solution at the outlet contained 0.7 mg/l felodipine, a concentration that is somewhat higher than the estimated saturation concentration at room temperature. No pyridine derivative could be detected in that solution. When the cerium reagent was passed through the felodipine column, the resultant solution contained 300 mg/l of the pyridine derivative, but no felodipine could be detected. These results indicated that the oxidation reaction was fast. Further evaluation of the reaction rate was beyond the scope of this work.

The quantitative yield of the pyridine derivative and the stability of the product were determined in the following way. About 25 mg felodipine dissolved in 2.00 ml acetonitrile was added at a rate of 0.03 ml/min to 500 ml 5 mM ceric sulphate in 0.12 M sulphuric acid, to avoid precipitation of the added drug. The amount of pyridine derivative was measured 1 and 12 h after complete addition (Table 3). The presence of tablet constituents in the reaction medium did not affect the quantitative formation of the pyridine derivative or its stability.

Experimental conditions for the dissolution test

Theoretically, the oxidation of all felodipine from a 25-mg tablet would consume 0.13 mmol Ce^{4+}. This would correspond to 0.26 mM as the minimum concentration of oxidant in 500 ml dissolution medium. When the concentration of ceric sulphate was

Table 3
Oxidation of 25 mg felodipine in 500 ml dissolution fluid containing 5 mM ceric sulphate in 0.12 M sulphuric acid at 37°C, with and without the addition of tablet constituents

Starting material	Added amount of felodipine (μmol)	μmol pyridine derivative formed	
		1 h	12 h
Felodipine	64.9	65.5	65.5
Felodipine + constituents of tablet E*	59.8	62.1	—
Felodipine + constituents of tablet G*	59.6	59.8	—
Felodipine + constituents of tablet I*	64.9	64.0	64.5

* Main constituents of formulations E, G and I: lactose, microcrystalline cellulose and polyvinylpyrrolidone.

varied from 0.50 to 50 mM, it became evident that the initial concentration of oxidizing agent had a strong influence on the rate of release of felodipine (Table 4). High concentrations of oxidant accelerated dissolution.

This effect was interpreted according to a simplified model which divides the dissolution process into three consecutive steps: mass transfer across the solid–liquid interface, diffusive transport and convective transport. The reaction between dissolved felodipine molecules and the oxidant was assumed to take place in the bulk of the solution and in the outer part of the diffusion layer adjacent to the phase boundary. High concentrations of oxidizing agent would correspond to a higher oxidation rate and would result in a larger concentration gradient of felodipine in the diffusion layer. In turn, this would cause a higher diffusive flux of dissolved felodipine molecules from the solid–liquid interface into the bulk of the solution.

Table 4
Dependence of dissolution rate on initial concentration of ceric sulphate in dilute sulphuric acid for 25-mg felodipine tablets (formulation G)

Ce^{4+} (mM)	H_2SO_4 (M)	Dissolved felodipine as % stated content						
		5	10	15	30	60	120	240 min
0.5	0.10	—	—	31	38	47	61	72
1.0	0.10	—	—	34	46	60	72	84
2.0	0.11	20	33	42	55	72	84	—
5.0	0.12	23	35	44	60	76	—	—
10.0	0.15	34	54	65	87	99	—	—
50.0	0.35	74	83	84	—	—	—	—

At low concentrations of ceric salt, felodipine was released at about the same rate as observed in a preliminary experiment in 45 l. of water (Fig. 1). In this experiment half a tablet was placed in a USP basket at 100 rpm and a large paddle, operated at 90 rpm, served as stirrer. The dissolution rates of felodipine in highly concentrated ceric sulphate solutions were similar to those of the drug in mixtures of ethanol and water (Fig. 2).

The use of 5 mM ceric sulphate in the proposed control method was a compromise between two criteria. A low initial concentration of ceric sulphate would ensure a time-scale of the experiment long enough to allow safe discrimination between slowly and rapidly dissolving tablets. On the other hand, the effect of a small decrease in cerium

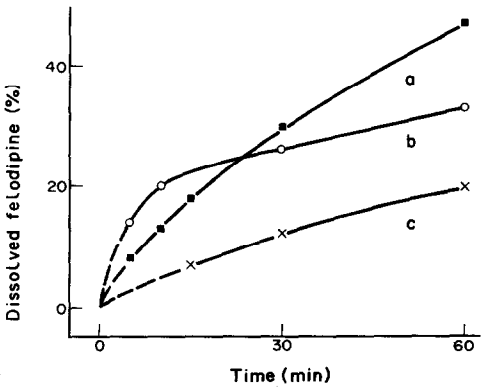

Figure 1
Dissolution rate of 25-mg felodipine tablets (formulation E) in: a, 5 mM ceric sulphate in 0.12 M sulphuric acid (■); b, water (○); and c, 0.5 mM ceric sulphate in 0.10 M sulphuric acid (×).

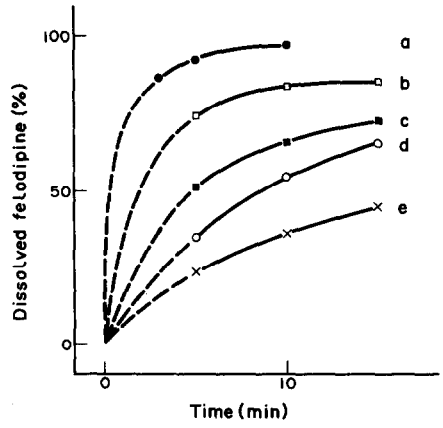

Figure 2
Dissolution rate of 25-mg felodipine tablets (formulation G) in: a, 30% ethanol (●); b, 50 mM ceric sulphate in 0.35 M sulphuric acid (□); c, 10% ethanol (■); d, 10 mM ceric sulphate in 0.15 M, sulphuric acid (○); and e, 5 mM ceric sulphate in 0.12 M sulphuric acid (×).

concentration during the experiment should be minimized by a high initial concentration of oxidant.

The concentration of sulphuric acid was 0.12 M in the selected dissolution medium containing 5 mM ceric sulphate. Small variations (±0.01 M) in the sulphuric acid concentration around 0.12 M did not cause any significant differences in dissolution rate. The influence of stirring was investigated on felodipine tablets H in 5 mM ceric sulphate. The proportion of felodipine dissolved was 51% at a rotational speed of 50 rpm, 63% at 100 rpm and 69% at 200 rpm. A medium speed of 100 rpm was chosen for the procedure used to determine dissolution rate.

Performance of dissolution method

The precision of the entire dissolution test was estimated from the individual results of 10 tablets of the same production batch I. The analyses were distributed over 3 days. The proportion of dissolved felodipine was in the range 44–50% after 10 min, 78–83% after 30 min and 93–100% after 60 min. The RSD of the average amount of dissolved felodipine was 4% after 10 min, 2% after 30 min and 3% after 60 min.

Dissolution rates measured by the oxidation method were examined for correlation with the following bioavailability parameters: maximum plasma concentration (Fig. 3a) and total area under the plasma concentration–time curve (Fig. 4a). The correlation coefficients were 0.786 and 0.811 for each plot respectively. The corresponding data for the method using 30% ethanol are illustrated in Figs 3b and 4b. These data points were scattered and indicated no significant correlation. Plots of the time for maximum plasma concentration against per cent dissolved felodipine showed no clear correlation between results *in vivo* and *in vitro*. However, results obtained by the cerium method did indicate that a high dissolution rate corresponded to a short time to attain a maximum concentration *in vivo*.

Interferences

The possibility of a reaction between the oxidizing agent and the inactive ingredients of the tablet has to be considered whenever the proposed dissolution method is applied, since this would lead to a corresponding decrease in the concentration of the oxidizing

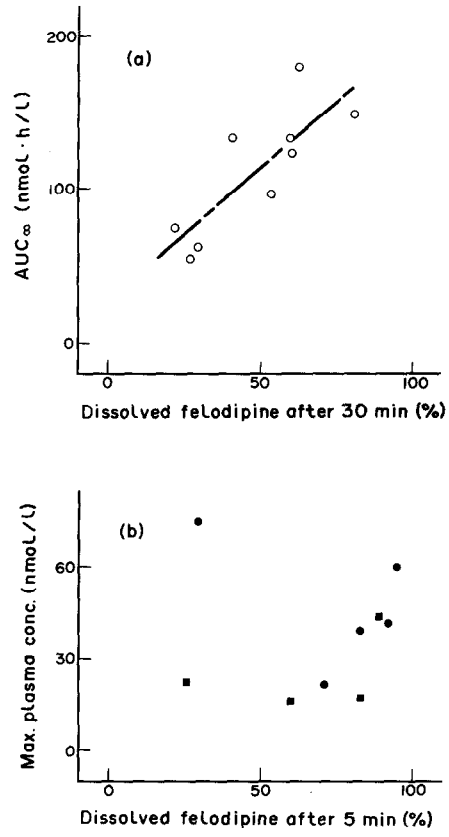

Figure 3
Plot of maximum plasma concentration against per cent dissolved felodipine: a, after 30 min in 5 mM ceric sulphate (○); and b, after 5 min in 30% ethanol using the basket method (■) or the paddle method (●).

agent available, which in turn would result in a decreased dissolution rate (Table 4). This effect is of special importance when dissolution rates of different tablet compositions are to be compared.

Of the components other than felodipine in the tablets A–I, only lactose gave rise to an appreciable consumption of ceric ions. Typically, the total consumption of ceric ions by the lactose and felodipine in a tablet formulation (G) was equivalent to a decrease in concentration of the oxidizing agent from 5.0 to 4.3 mM in 60 min. From the plot of dissolved felodipine after 30 min against initial ceric salt concentration for a study on tablet G (Fig. 5), it was estimated that the final concentration of oxidant should be 3 mM or higher for an initial ceric salt concentration of 5 mM. This would ensure that the hypothetical decrease in dissolution rate for acceptable final concentrations of oxidant would then be less than one standard deviation of the method, so that dissolution data for other tablet formulations can be regarded as strictly comparable.

Conclusions

A new method has been developed for the determination of the dissolution rate of the poorly soluble drug felodipine in tablet formulations. 'Sink conditions' are maintained by means of a chemical reagent, which reacts rapidly with dissolved drug molecules in the dissolution fluid. Measurements are performed with standardized USP equipment using

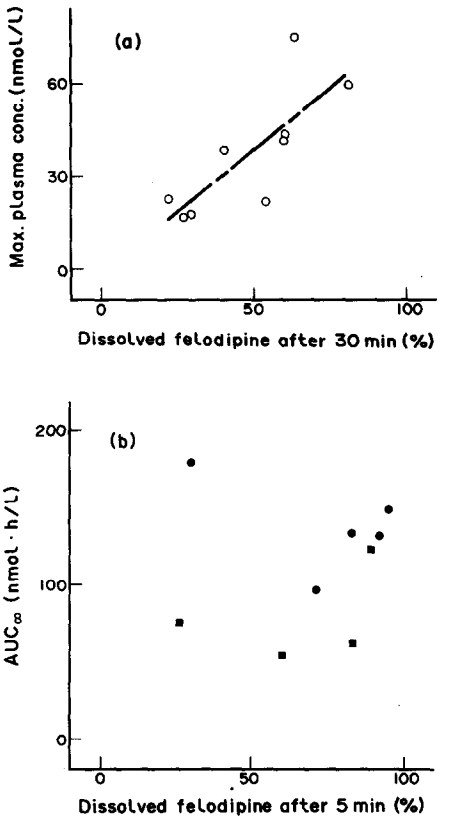

Figure 4
Plot of total area under plasma concentration–time curve (AUC_∞) against per cent dissolved felodipine: a, after 30 min in 5 mM ceric sulphate (○); and b, after 5 min in 30% ethanol using the basket method (■) or the paddle method (●).

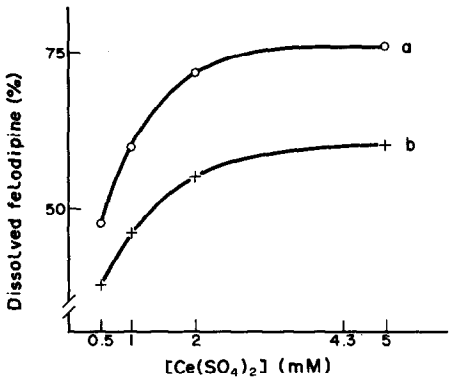

Figure 5
Influence of initial concentration of Ce^{4+} on the amount of dissolved felodipine from 25-mg felodipine tablets G after; a, 60 min (○); and b, 30 min (+); data taken from Table 4.

normal volumes of dissolution medium. A satisfactory correlation has been established betwen the dissolution rate *in vitro* and certain bioavailability parameters. Application of the method to structural analogues such as nifedipine should in principle be possible.

The limitations of the method are mainly determined by the properties of the oxidizing agent, ceric sulphate in this case. The useful pH range of the method is limited to acid media and the method must be applied with caution to formulations which contain

excipients readily oxidized by ceric ions. These limitations have been especially troublesome in work with so-called 'controlled release' preparations. Work is currently in progress to overcome these difficulties in a number of ways. One alternative method involves conversion of dissolved felodipine molecules by the use of electrochemical oxidation, which can be applied over a wide pH range.

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