Brief/Technical Note

Instrumentation of Flow-Through USP IV Dissolution Apparatus to Assess Poorly Soluble Basic Drug Products: a Technical Note

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Abstract. Supersaturation and precipitation are common limitations encountered especially with poorly soluble basic drugs. The aims of this work were to explore the pattern of dissolution and precipitation of poorly soluble basic drugs using a United States Pharmacopoeia (USP) IV dissolution apparatus and to compare it to the widely used USP II dissolution apparatus. In order to investigate the influence of gastric emptying time on bioavailability, tables of two model drugs (dipyridamole 100 mg and cinnarizine 15 mg) were investigated and pH change from 1.2 to 6.8 were achieved after 10, 20 or 30 min using USP II or USP IV dissolution apparatuses. Using USP II, dipyridamole and cinnarizine concentrations dropped instantly as a result of drug precipitation with drug crystals evident in the dissolution vessel. At pH change times of 10, 20 and 30 min, the total amount of dissolved drug was dependent on pH change time. Using USP IV, at a flow rate of 8 ml/min, it was possible to have comparable release to agitation at 50 rpm using USP II suggesting that comparable hydrodynamic forces are possible. No drop in drug percentage occurs as the dissolved fraction was readily emptied from the flow cell, preventing drug accumulation in the dissolution medium. However, a negligible percentage of drug release took place following pH change. In conclusion, the use of the flow-through cell dissolution provided laminar flow, use of realistic fluid volumes and avoided precipitation of dissolved drug fraction in the gastric phase as it is discharged before pH change.

KEY WORDS: USP 4; USP 2; Flow-through; Antihistamine; Anticoagulant; Gastric emptying.

INTRODUCTION

pH-dependent solubility is a major consideration for poorly soluble basic drugs, whose solubility is significantly reduced upon gastric emptying (1). This leads to supersaturation, which may be followed by precipitation of the basic drug in intestinal fluids where it is likely to be absorbed. This can result in erratic bioavailability and variable onset of action following oral administration (2). For instance, an elevated gastric pH has led to a slower and compromised oral bioavailability of dipyridamole with less than half C_{max} and T_{max} observed (3). In another report, the oral bioavailability of ketoconazole, a poorly soluble basic drug, was significantly compromised in achlorhydric patients (4).

Several dissolution models have been proposed to simulate changes in dissolution that occur following gastric emptying. One study has reported the use of a 96-well microtiter plate to study drug precipitation in different simulated GI fluids (5). Also, a United States Pharmacopoeia (USP) dissolution test with single medium for each test has been utilised (6). Despite the merits of these methods, they do not describe the changes in pH that occur following gastric emptying. The USP has also described a pH change method utilising the USP II apparatus that has been

applied to dosage forms (2,7). Mathias *et al.* suggested a two-stage micro-dissolution method using a low-volume compartment by dissolving the drug in simulated gastric fluid (SGF) for 20 min followed by the addition of fasted state simulated intestinal fluid (FASSIF) (8). A general concern about these methods is the possible overestimation of drug precipitation, since these systems do not simulate the absorption step (9,10).

One approach towards overcoming this is to use multi-compartments where gastric and basic chambers are connected with tubing and the speed of gastric emptying is simulated by changes in flow rate (11–13). Although this approach is increasingly receiving attention, its tailored nature makes it difficult to validate and reproduce.

The USP IV is a well-established standardised flow-through dissolution system that has also been extensively used to assess the drug release pattern from extended-release formulations (14–16). It has also been increasingly used to study release pattern from microsphere and film-based systems (17–19). It allows laminar flow, use of different sequences of dissolution media and continuous replenishing of drug saturated media with a fresh drug-free medium, hence limiting the risk of supersaturation. Despite these advantages of using the USP IV apparatus, little information is available on its use for mimicking the *in vivo* dissolution of poorly soluble basic drugs.

The aims of this work were to explore the pattern of dissolution and precipitation for poorly soluble basic drugs using the USP IV dissolution apparatus and to compare it to the widely used USP II dissolution apparatus.

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MATERIALS AND METHODS

Materials

A brand of dipyridamole tablets 100 mg (Persantine®, Boehringer Ingelheim, UK) and 15 mg cinnarizine tablets (Stugeron®, Janssen-Cilag Ltd., UK) were purchased from local pharmacies. Dipyridamole and cinnarizine standards were purchased from Sigma-Aldrich, UK. All other chemicals were commercially available and of analytical grade.

pH Change Dissolution Test Using the USP II Apparatus

Immediate-release tablets are likely to disintegrate within minutes in the stomach (20) and the disintegrated and dissolved parts will empty in the fasted state in a mono-exponential pattern (21) with a gastric emptying time for calorie-free liquids of less than 30 min (22,23). In order to simulate different gastric emptying times, three different pH change systems were utilised with an emptying time of 10, 20 or 30 min. The USP II paddle apparatus (Erweka® DT700LH dissolution system, Heusenstamm, Germany) was employed to assess the tablet dissolution profiles. One tablet was placed in a vessel containing 750 ml of 0.1 M HCl as a dissolution medium at $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$. After 10, 20 or 30 min, 250 ml of 0.215 M tri-sodium phosphate (maintained at $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$) was added to the dissolution vessel, and the pH of the solution was adjusted to 6.8 ± 0.05 when necessary using 3 N HCl or 3 N NaOH solutions. Following this, the dissolution experiment was continued for another 50, 40 or 30 min, respectively. The total time per run was 60 min, during which samples were collected every 3 min for the first 30 min and then every 5 min. The speed of the paddle was set at 50 rpm and each dissolution test was replicated three times. These were filtered and then measured by inline UV spectrophotometer (Automated Lab SP700, UK) at λ_{max} of 282 and 254 nm for dipyridamole and cinnarizine, respectively. Data was processed using Icalis software (Icalis Data Systems Ltd, Berkshire, UK). A standard calibration curve was prepared for each drug in acidic and buffer media and the limit of detection for both drug molecules was 2 mg/l. The percentage of released drug was calculated based on the amount of detected drug at each time point in comparison to the labelled dose.

pH Change Dissolution Test Using the USP IV Apparatus

In order to obtain comparable results following pH change between the USP II and USP IV, the same dissolution medium used in the gastric phase of the USP II has been utilised. Phosphate buffer (pH 6.8) was prepared as a 3:1 v/v mixture of hydrochloric acid (0.1 M) and phosphate buffer (0.215 M), adjusted to pH 6.8.

A USP IV Apparatus (Sotax CE7 Smart, Switzerland), connected to a SOTAX fraction collector, was equipped with 12-mm cell size containing one 5-mm sphere and 1.86 g of glass beads (1 mm) to provide laminar flow. Each tablet was placed on the beads within the cell with 25 mm (FG/B) (Whatman, USA). The apparatus was used in an open-loop system, where new dissolution medium was continuously introduced into the system. The temperature of the water bath was set at $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ and the flow rate of media was set at 8 ml/min. After 10, 20 or 30 min, the medium was switched

from HCl to phosphate buffer to mimic different gastric emptying time with a total run time of 60 min.

The samples obtained from the fraction collector were processed using a Jenway Spectrophotometer (Bibby Scientific Ltd, UK) for dipyridamole. In the case of poorly soluble cinnarizine and in order to ensure that all drug had been dissolved, the collected samples were diluted at 1:1 volume ratio with acetonitrile and analysed by HPLC. The concentration of dipyridamole and cinnarizine was assessed at 0, 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 35, 40, 45, 50, 55 and 60 min. The percentage of released drug was calculated based on the amount of detected drug at each time point in comparison to the labelled dose.

HPLC Analysis

Dipyridamole

The amount of released dipyridamole was analysed on a HPLC system (Agilent Technologies 1200 series, Germany).

Fig. 1. *In vitro* release of dipyridamole from tablets using pH change in the USP II dissolution systems with change of pH **a** after 10 min, **b** after 20 min and **c** after 30 min

Fig. 2. *In vitro* release of cinnarizine from tablets using pH change in the USP II dissolution systems with change of pH **a** after 10 min, **b** after 20 min and **c** after 30 min

Each sample was dissolved in a medium containing surfactant before the injection of 10 μ l into the HPLC system. For the chromatographic conditions, the mobile phase comprised phosphate buffer pH 6.8 and acetonitrile (50:50) at a flow rate of 1.5 ml/min. The XTerra column (Waters Inc., Ireland) was of 4.6 μ m ID $\times$ 150 mm length with 185 μ m particle size. The

Table I. Percentage of Maximum Drug Release from Poorly Water-Soluble Basic Drug Tablets in USP II and IV pH Change Dissolution Tests

pH change time (min)	Dipyridamole 100-mg tablet		Cinnarizine 15-mg tablet	
	USP II	USP IV	USP II	USP IV
10	73.4	98.3	37.9	54.5
20	81.8	100	75.4	82.7
30	83.9	100	87.7	87.9

USP United States Pharmacopoeia

column temperature was maintained at 40°C. Detection was performed using UV-visible spectrophotometry and the wavelength of detection was set at 285 nm. A run time of 6 min was utilised and each sample was run in triplicates and the limit of detection was 0.5 mg/l.

Cinnarizine

Samples were analysed using HPLC (Agilent Technologies 1200 series, Germany). A reversed-phase column (C18 Synergi Max-RP80, Phenomenex, USA) was used with dimensions 250 mm $\times$ 4.60 mm, particle size 4 μ m. The mobile phase, which consisted of acetonitrile (90% v/v) and 10 mM ammonium acetate buffer (10% v/v) adjusted to pH 7 by ammonium hydroxide with distilled water (1:4), was eluted at a flow rate of 1.5 ml/min. The injection volume was 20 μ l, and a UV detector employed an excitation wavelength of 254 nm. The temperature in the column was maintained at 30°C and the run time for each sample was 8 min. Under these conditions, the retention time for cinnarizine was about 5.5 min and the limit of detection was 0.1 mg/l. The assay was validated in

Fig. 3. *In vitro* release of dipyridamole from tablets using pH change in the USP IV dissolution systems (flow rate 8 ml/min) with change of pH **a** after 10 min, **b** after 20 min and **c** after 30 min

terms of accuracy and precision using standard methodologies.

RESULTS AND DISCUSSION

The dissolution profile of dipyrindamole immediate-release tablets on simulating a gastric emptying time of 10, 20 and 30 min in the USP II apparatus is illustrated in Fig. 1. For dipyrindamole, more than 70% of the drug was released within 10 min for the various pH change times. However, the proportion of dissolved cinnarizine was mainly affected by the timing of gastric emptying (Fig. 2, Table I). This indicates the critical importance of gastric emptying times of these formulations.

Upon pH change, both cinnarizine and dipyrindamole showed an instant drug-to-drug concentration in the USP II dissolution apparatus. Such a drop is directly related to the precipitation drug crystals within the dissolution vessel. As the pH of the media in the intestinal phase exceeds the pKa of both basic molecules (pKa 6.4 for dipyrindamole (21) and pKa

1.94 for cinnarizine (24)), their solubility drops significantly (from 29,000 mg/l (25) to 6 mg/l at pH 6.8 (26) for dipyrindamole and from 2100 mg/l at pH 1.2 (25) to <2 mg/l at pH 6.8 (2) for cinnarizine). This results in drug supersaturation in dissolution media followed by drug precipitation. It could also be related to the tablet composition, where solubility enhancer and/or precipitation inhibitor is often included in the additives of tablet formulation (11). However, such precipitation is unlikely to be realistic as basic drugs dissolve in gastric fluids and quickly emptied where they are mainly absorbed in the upper small intestine.

In order to assess the influence of in flow-through cell on drug release profile, a dissolution method using the dissolution media and pH change time were carried out using the USP IV. When flow rates of 2 and 4 ml/min were utilised, it was visually noticed that very slow disintegration took place and limited or no drug release was detected following 1 h. This could be due to the weak disintegrating effect of flow-through cell in comparison to paddle-based dissolution tests (27). In flow-through systems, these agitation forces are mainly generated from the pulses of the peristaltic pump and they are likely to be minimal when a

Fig. 4. *In vitro* release of cinnarizine using pH change in the USP IV dissolution systems (flow rate 8 ml/min) with change of pH **a** after 10 min, **b** after 20 min and **c** after 30 min

Fig. 5. Dipyrindamole concentration-time plot for the USP IV flow-through cell (flow rate 8 ml/min) with change of pH **a** after 10 min, **b** after 20 min and **c** after 30 min

Fig. 6. Cinnarizine concentration-time plot for the USP IV flow-through cell (flow rate 8 ml/min) with change of pH **a** after 10 min, **b** after 20 min and **c** after 30 min

flow rate of 2 or 4 ml/min was applied. This is in contrast with USP disintegration tests for example where more major shear force is generated (28). However, at a flow rate of 8 ml/min, it was possible to have a comparable release to 50 rpm of paddle-based USP II dissolution (Figs. 3 and 4), suggesting that comparable hydrodynamic forces are possible to obtain at this flow rate (29). The majority of drug dissolution (>90%) was achieved using a biologically realistic volume of gastric medium (240 ml after 30 min).

The design of the flow-through dissolution cell allows laminar and more homogeneous flow to be readily achieved due to the pressure drop imparted when beads are used (18). No drop in drug percentage occurs as the dissolved fraction was readily emptied from the flow cell, preventing drug accumulation in the dissolution medium. The flow-through appeared to have significantly higher percentage of dissolved drug compared to paddle system using the realistic volume of gastric medium (240 ml after 30 min) composition (Table I).

Figures 5 and 6 illustrate the concentration of drugs within the flow-through cell in the USP IV apparatus. Unlike the USP II

where the drug stays in the media after pH change, all dissolved drug is transferred from the flow-through cell in the USP IV; this will allow the assessment of dissolving drug fraction (if any) in the intestinal phase. However, only a limited amount of drug dissolved in the intestinal phase of the test. It is clear that most of the dissolution in the intestinal phase took place within the first few minutes after pH change. In the first few minutes after pH change, the flow-through cells are likely to contain a mixture of acidic and neutral buffer before it becomes completely dominated with intestinal medium (pH 6.8). It is possible that some drug dissolved in this medium before the increase in pH to the intestinal values.

CONCLUSION

Unlike paddle-based dissolution methods, the flow-through cell dissolution provided laminar flow, used realistic fluid volumes and avoided precipitation of dissolved drug fraction in the gastric phase as it is discharged before pH change. Using both dissolution systems, the percentages of dissolved drug at the end of the dissolution test was significantly influenced by the pH change time. Incomplete drug dissolution due to shorter residence time in the stomach may compromise oral bioavailability.

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