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# *In vitro* evaluation of the dissolution behaviour of itraconazole in bio-relevant media

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ABSTRACT

Drugs in the gastrointestinal tract are exposed to a medium of partially digested food, comprising mixtures of fat, protein and carbohydrate. The dissolution behaviour of itraconazole was evaluated in bio-relevant media which were developed to take this into account. Media containing milk with different fat contents, protein (albumin, casein, gluten and gelatin), carbohydrates (glucose, lactose and starch) and amino acids (lysine, glycine, alanine and aspartic acid) to mimic a digested meal and bile components (sodium taurocholate and lecithin) to represent a key endogenous digestive material were investigated. The effect of medium composition on the intrinsic dissolution rate of itraconazole was evaluated as this drug has extremely poor solubility and its bioavailability is affected by food. Dissolution tests were carried out in simple compendial media based on dilute solutions of hydrochloric acid or neutral solutions of phosphate buffer and in more complex media containing the dietary components. The data obtained showed that most of the dietary components enhanced the solubility compared to simulated gastric fluid (SGF) but to differing extents. The greatest increase in dissolution was observed with the addition of milk and albumin although an increase was also seen with other proteins, amino acids and simulated gastrointestinal fluids.



## 1. Introduction

The pharmacopoeial dissolution test is a simple test using a medium composed of aqueous buffers which does not actually reflect what happens *in vivo* to a drug delivered in the solid-state. Thus, in order to develop a more predictive *in vitro–in vivo* correlation (IVIVC) model to use in drug development studies, better bio-relevant media are needed to simulate the gastrointestinal fluid more accurately. Several attempts have been made to address this issue by evaluating more realistic media, such as using milk as an integrated type of balanced nutrition (Macheras et al., 1986, 1987, 1989; Galia et al., 1998; Nicolaides et al., 1999), homogenised standard breakfasts (Klein et al., 2004), nutritional fluids such as the diluted intralipid emulsions (Ashby et al., 1989) or by more specifically exploring the effect of single proteins such as albumin and casein (Macheras and Reppas, 1987). Another aspect previously considered was creating conditions more akin to the *in vivo* conditions of fed and fast states by adding bile salts and fatty acids to the medium. The formulae of those two media were published in the International Pharmaceutical Federation (FIP) guidelines (Aiache et al., 1997) and subsequent studies have further investigated and developed these physiologically based media (Galia et al., 1998; Nicolaides et al., 1999; Dressman and Reppas, 2000; Persson et al., 2005).

Most of these studies investigating the effect of bio-relevant media on drug behaviour were performed on drug formulations or drug particles where factors such as excipient interactions or drug particle size and shape might interfere with the dissolution of the drug. Few examples exist in literature of the use of intrinsic dissolution rate (IDR), which allows the dissolution of drug substance from a compact disk with constant surface area to be investigated (USP, 2007). IDR can be performed either using rotating or stationary disk apparatus and by comparing the two methodologies, it was found that the stationary method offers some advantages over the rotating method: the rotating disk might form bubbles that cover the surface of the disk media leading to a decrease in the active dissolution surface causing low dissolution. Furthermore, with the rotating disk, less temperature control at the beginning of the experiment was observed due to heat loss when lowering the shafts which reduced the temperature by approximately 2 °C (Viegas et al., 2001). Previous studies in bio-relevant media used the rotating disk apparatus (Naylor et al., 1993; Pedersen et al., 2000; Persson et al., 2005; Aburub et al., 2008) whereas no publications appear to report the utilization of the stationary disk methodology

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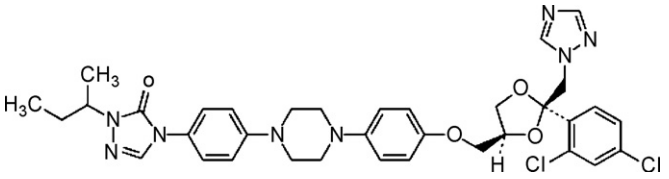

**Fig. 1.** Chemical structure of itraconazole.

to investigate the dissolution rate of drugs in bio-relevant media. Therefore, in the present study IDR (stationary disk apparatus) was employed to reduce variables influencing dissolution.

Itraconazole, an orally active synthetic anti-fungal drug, is weakly basic and very lipophilic ($pK_a = 3.7$) with a log partition coefficient in octanol/water of greater than 5 at pH 6 (Fig. 1). It is classified as class II in the biopharmaceutics classification system (BCS) due to its low aqueous solubility and good permeability (Amidon et al., 1995). It was chosen as a model drug for this study due to its low solubility and because its oral bioavailability is poor and food-correlated. The bioavailability of itraconazole was also observed to increase significantly after a standard breakfast meal (Van Peer et al., 1989; Barone et al., 1993; Zimmermann et al., 1994).

The purpose of the present study was to characterize the dissolution performance of itraconazole in different dissolution media, elucidate the effect of food on its behaviour and investigate the impact of gastrointestinal surfactants, representing fed and fasted conditions, on the dissolution of itraconazole.

## 2. Materials and methods

### 2.1. Materials

Itraconazole (BP 2000) was obtained from Medichem (China). Casein, gluten, gelatin, glycine, aspartic acid and Triton X-100 were purchased from Sigma (UK); albumin, alanine and lysine from Fluka (UK); glucose, starch and sodium dodecyl sulfate (SDS) from BDH (UK), lactose from Foremost (USA) and sodium taurocholate hydrate 97% from Alfa Aesar (UK). Egg–lecithin (Lipoid E P C, 96%) was kindly donated by Lipoid GmbH (Germany). Three types of fresh pasteurised cow milk, whole milk, semi-skimmed milk and skimmed milk (Express Diaries, UK) with fat contents of 3.6%, 1.7% and 0.1% respectively were used.

### 2.2. Composition of dissolution media

Compendial media without enzymes were prepared according to the United States Pharmacopeia (2007): Simulated gastric fluid (SGF) contained 2 g sodium chloride, 0.2 M hydrochloric acid (HCl) (to adjust pH to 1.2) and water to 1000 ml. SGF pH 3 contained 2 g sodium chloride, 0.1 M HCl (to adjust pH to 3) and water to 1000 ml. Simulated intestinal fluid (SIF) was prepared from 6.8 g monobasic potassium phosphate, 0.2 M sodium hydroxide (NaOH) (to adjust pH to 6.8) and water to 1000 ml.

Simulated gastric fluid media containing milk were prepared using an equal-parts mixture of milk and SGF pH 1.2. The final pH was taken to 3 with either 0.1 M HCl or 0.1 M (NaOH). Dietary media were composed of a single component dissolved or dispersed in SGF then the final pH was adjusted to 3 with a solution of HCl (1 or 0.1 M). Four different proteins were chosen on the basis of their availability in common foodstuffs: albumin from hen egg white (0.5%, 1%, 2%, 3% and 4%, w/v), casein from bovine milk (2%, w/v and saturated solution), gluten from wheat (2%, w/v and saturated solution) and gelatin from bovine skin (0.5%, 1%, and 2%, w/v). Saturated solutions were prepared, stirred overnight and then filtered through Whatman filter paper type: 1, the pH of the solutions was adjusted to pH 3 with 0.1 M HCl and sodium chloride was added ($2\,g\,l^{-1}$) to each solution. The concentration of casein in the filtered saturated solution was determined using the Bradford protein assay (Bradford, 1976).

Three different carbohydrates were chosen to represent examples of monosaccharides (glucose), disaccharides (lactose) and polysaccharides (starch) (1%, w/v). The amino acids: lysine, glycine and alanine (each at 1%, w/v) and a saturated solution of aspartic acid were chosen as examples of basic (LYS), neutral (GLY, ALA) and acidic (ASP) amino acids.

Two bile salt-containing media were prepared according to Marques (2004). Fasted state simulated intestinal fluid (FaSSIF) contained 3 mM sodium taurocholate and 0.75 mM lecithin in a pH 6.5 phosphate buffer. Fed state simulated intestinal fluid (FeSSIF) contained 15 mM sodium taurocholate and 3.75 mM lecithin in a pH 5.0 acetate buffer. The compositions of the media were identical to the composition prescribed by Vertzoni et al. (2004). Blank FaSSIF and FeSSIF without lecithin and taurocholate were used as controls, the composition of the former media: sodium dihydrogen phosphate 28.66 mM, sodium chloride 106 mM and approximately 13.8 mM sodium hydroxide to take the final pH to 6.5 and blank FeSSIF contained acetic acid 144 mM, sodium chloride 173 mM and approximately 101 mM of sodium hydroxide to make the final pH 5.

Fasted state simulated gastric fluid (FaSSGF) consisted of SGF pH 1.2 containing 80 μM sodium taurocholate and 20 μM lecithin. The formulation was modified from that of Vertzoni et al. (2005).

Finally, two simulated gastric fluids were prepared by adding synthetic surfactants to SGF pH 1.2; sodium dodecyl sulfate (SGF + 0.25%, w/v SDS) (Dressman et al., 1998) and Triton X-100 (0.1%, w/v in SGF) (Galia et al., 1999).

### 2.3. Determination of saturation solubility

Saturation solubility was determined using the following method (Sunesen et al., 2005). An excess of drug (approximately 30 mg) was added to closed-cap vials containing 10 ml of the test media and the vials were shaken for 24 h at 37 °C. Due to the complex media used for these studies and also the hydrophobicity of the drug which caused it to stick to the glassware and filters, centrifugation was performed prior to filtration to provide cleaner samples for analysis and prevent fine particles of undissolved material re-dissolving in the filtrate (Macheras et al., 1986). The suspension was centrifuged (4000 rpm for 10 min) and filtered through 0.2 μm PVDF syringe filters (Whatman) discarding the first 1 ml of each filtrate. Filter validation studies were performed on all filters. Hydrochloric acid caused precipitation of the albumin and milk media, yielding samples that were dispersions rather than solutions. For the albumin, 1 μm glass filters (Gelman Sciences) were used and 5 μm Acrodisc Versapor filters (Gelman Sciences, medium: acrylic polymer) were used for the milk samples. The filtrates were diluted with the mobile phase (where appropriate) and then analysed by HPLC to determine the concentration of drug in solution.

### 2.4. Intrinsic dissolution discs and dissolution conditions

Intrinsic dissolution rate testing of itraconazole was performed using the stationary disk apparatus. Approximately 150 mg of itraconazole was compressed for 30 s in a 9.5-mm diameter die against a steel plate under a pressure of 1000 p.s.i. using a punch and die system to yield a disk of known surface area (70.9 $mm^2$). The disks were blown with compressed air to remove any loose particles. The

die, containing the disk, was then positioned disk-up at the bottom of a dissolution vessel containing 500 ml of the dissolution medium. The dissolution apparatus used was USP apparatus II (Pharmatest PTW S3C, Pharmatest GmbH, Germany) set at 37 ± 0.5 °C and a rotation speed of 100 rpm.

The surfaces of a number of disks were analysed using scanning electron microscopy (SEM) (Model JSM-840, Jeol Technics Ltd., Japan) to confirm the surface was intact and so ensure dissolution would take place evenly across the entire surface.

Studies were carried out to determine whether compression of the drug into IDR disks led to any change in polymorphic form, since different polymorphs may exhibit different solubility behaviours. Differential scanning calorimetry, infra-red spectroscopy and powder X-ray diffractometry were used and no evidence that compressing the itraconazole powder affected the polymorphic form of the drug was observed.

### 2.5. Extraction of itraconazole from IDR sample solutions

Samples (3 ml) were withdrawn from the dissolution vessels either through 20 μm Cannula filters if using particulate media or filtered using Versapor filters if non-particulate was used. Aliquots (1 ml) were adjusted to pH 8.5 with 1 M phosphate buffer before being shaken for 10 min. They were then extracted with heptane–isoamylalcohol (98.5:1.5, v/v) and centrifuged at 4000 rpm for 10 min. Portions (2 ml) of the upper organic layer were dried under a stream of nitrogen at 60 °C before the residue was reconstituted with the HPLC eluent by sonication for 2 min.

### 2.6. HPLC assay

The drug content of the IDR sample solutions was determined by HPLC using a Waters Alliance 2695 chromatograph. 50 μl of sample solution was injected onto a Phenomenex LUNA® C18 (2) column (5 μm, 250 mm × 4.60 mm) held at ambient temperature and eluted with a mixture of acetonitrile and 0.02 M potassium dihydrogen phosphate (60:40, v/v) adjusted to pH 3.0 with 5 M HCl at a flow rate of 1.2 ml $min^{-1}$. Quantitation was based on peak area measurement by fluorescence ($\lambda_{Exc}$ = 252 nm, $\lambda_{Emm}$ = 360 nm). The retention time of itraconazole was 8.5 min.

### 2.7. Data analysis

Differences in solubility and dissolution data for itraconazole in the various media compared to blank solutions of SGF, FaSSIF and FeSSIF were statistically investigated using a *t*-test. A one-way analysis of variance (ANOVA) was used to compare the data from the three types of milk. Differences were considered significant at the 0.05 levels.

## 3. Results and discussion

The presence of striations on the surface of a disk can significantly affect the dissolution process (Levy, 1963) as such features would tend to dissolve or erode first during the test, leading to a change in the surface area. Initially, scanning electron micrographs of the freshly prepared disks exhibited a clearly striated surface with regular, raised ridges. These striations arose from a roughness of the surface of the plate against which the drug powder had been compressed and were due to the fabrication procedure of the base-plate itself. To tackle this problem, plates were polished and micrographs indicated that this reduced the striations but the ridges formed during production of the drug disk were reduced further by covering the steel plate, which the drug is compressed against, with aluminium foil to produce smoother surfaces. Thereafter, aluminium foil was routinely used to cover the steel plates for disk-production.

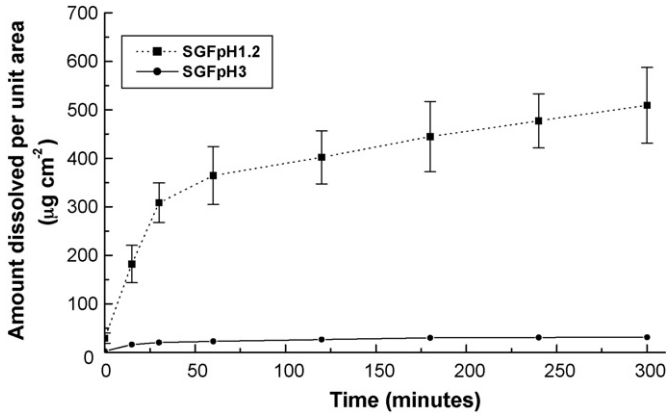


**Fig. 2.** The intrinsic dissolution profile of itraconazole in simulated gastric fluid (SGF) at pH 1.2 (■) and 3 (●). Each data point represents the mean ± S.D. of 6 measurements.

Due to the low solubility of itraconazole, it was not possible to achieve sink conditions in the dissolution experiments. Thus, all experiments were performed under non-sink conditions. The linear portion of each curve was quite brief as saturation solubility was quickly reached so for comparative purposes, data for amounts dissolved per unit area after 1 h were utilized. Although it is desirable to maintain sink conditions throughout the dissolution process (CDER/FDA, 1997), these conditions may not prevail in the GI tract. Attaining these conditions *in vivo* depends upon the permeability, volume and composition of luminal fluids (Dressman and Reppas, 2000). Itraconazole has a solubility of less than 1 μg $ml^{-1}$ so the volume of the GI fluids is not large enough to dissolve a typical dose of 100 mg, unless the mucosa permeability is high enough to compensate for this poor solubility. *In vivo,* the basic drug is expected to be dissolved mostly in the stomach, which acts as reservoir, while the main site for absorption is intestinal mucosa (Dressman et al., 1998). Since this report is investigating food-induced effects on the dissolution in SGF, it is more probable that sink conditions are not maintained in gastric fluids and so better IVIVC would be achieved under non-sink conditions for this drug.

### 3.1. Dissolution and saturation solubility in conventional media

To investigate the effect of pH on the dissolution of itraconazole, dissolution was performed in compendial media SGF pH 1.2, SIF pH 6.8 and modified SGF pH 3. The dissolution of the drug (Fig. 2) was strongly affected by the pH, with significantly greater dissolution observed at pH 1.2 than at pH 3. The dissolution at pH 6.8 was less than the limit of quantitation and cannot be reported. The saturation solubility data for these media and for water support this observation, with a marked decrease in solubility above pH 1.2 (Table 1).

**Table 1**
Solubility of itraconazole in simulated gastric fluid (SGF) at pH 1.2 and 3, simulated intestinal fluid (SIF) pH 6.8 and deionised water.

| Medium | Solubility (μg $ml^{-1}$) |
|---|---|
| SGF pH 1.2 | 3.9 ± 0.7 |
| SGF pH 3 | 0.035 ± 0.003 |
| Deionised water | ~0.002 |
| SIF pH 6.8 | ~0.003 |

Each data point represents the mean ± S.D. of 3 measurements.

Itraconazole is a weak base with four ionisable nitrogen atoms. Two of the p$K_a$ values are 4 and 1.5–2 whereas the other ionisable nitrogens are not protonated between pH 2 and 10 (Peeters et al., 2002). This explains the drop-in solubility above pH 3 and why changes in pH profoundly influenced both solubility and dissolution. Table 1 demonstrates that there is approximately a 100-fold decrease in solubility from pH 1.2 to 3 to extremely poor solubility at pH 6.8. *In vivo*, itraconazole would be ionised in the low pH of the stomach which underpins the importance of this elevated gastric acidity in solubilising the drug. The influence of gastric pH on the bioavailability of itraconazole has been previously investigated and it was revealed that when the drug was co-administrated to subjects with an antacid suspension or subjects were pre-treated with H2 blockers (ranitidine), the bioavailability decreased (Lange et al., 1997; Lohitnavy et al., 2005). Despite the fact that a meal would increase the pH of the stomach due to the buffering effects of food components, this increase in pH was not reflected in a decline in bioavailability, which might be attributed to the effect of food on the solubilisation of the drug.

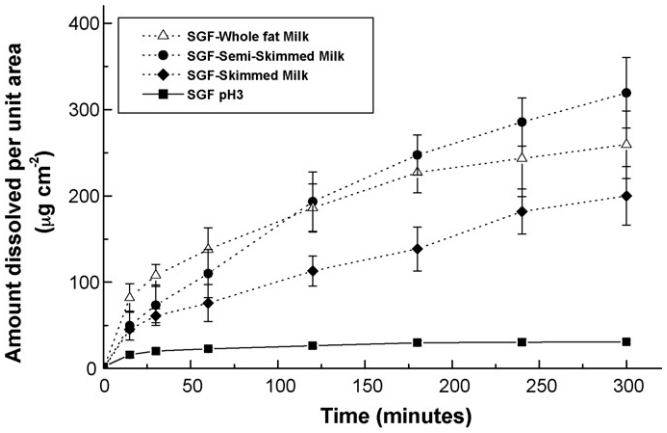


**Fig. 3.** The intrinsic dissolution profile of itraconazole in simulated gastric fluid (SGF) (■) and SGF containing 50% milk (whole fat (△), semi-skimmed (●), or skimmed (♦)). The pH of each media was 3. Each data point represents the mean ± S.D. of at least 4 measurements.

### 3.2. Dissolution and saturation solubility in milk

As milk contains the three basic nutritious components, proteins, carbohydrates and fat, it was considered to be a physiologically relevant dissolution medium to investigate.

The saturation solubilities for itraconazole in 1:1 mixtures of SGF and whole milk, semi-skimmed milk and skimmed milk were 0.74, 0.82, 0.90 μg ml$^{-1}$ respectively and statistical analysis of the results indicates there was no significant difference in solubility between the different types of milk. Fig. 3 shows that after 1 h the amount of itraconazole dissolved per unit area in SGF media containing whole milk, semi-skimmed milk and skimmed milk media, was 138, 109, 75 μg cm$^{-2}$ respectively, which is 6-, 4.8-, 3.3-fold respectively greater than in SGF alone. However, after 3 h the amount dissolved per unit area into SGF media containing semi-skimmed milk (247 μg) exceeded that in SGF media containing whole milk (227 μg). ANOVA analysis of the IDR data after 1 and 3 h exhibited a significant difference among the three media. However, the fat content of the milk appeared to have no systematic effect on the dissolution rate.

These results provide clear evidence of an increase in both dissolution rate and solubility in milk-containing media. Since itraconazole is lipophilic, the increased fat content of milk would be expected to aid solubility however the different fat content of the three types of milk did not show the expected systematic effect on drug behaviour. It is possible that some disruption of the milk emulsion could lead to separation of the fat, which would then float to the surface, making it unavailable to the itraconazole disk at the bottom of the vessel. Emulsions are known to be broken by extremes of pH, as was seen upon the addition of SGF to milk. Steps were taken to minimize this disruption, by adding acid to the milk rather than vice versa, but even this process still caused some visible change to the appearance of the milk. This destabilizing effect of acid on milk media could also explain why the results obtained were variable.

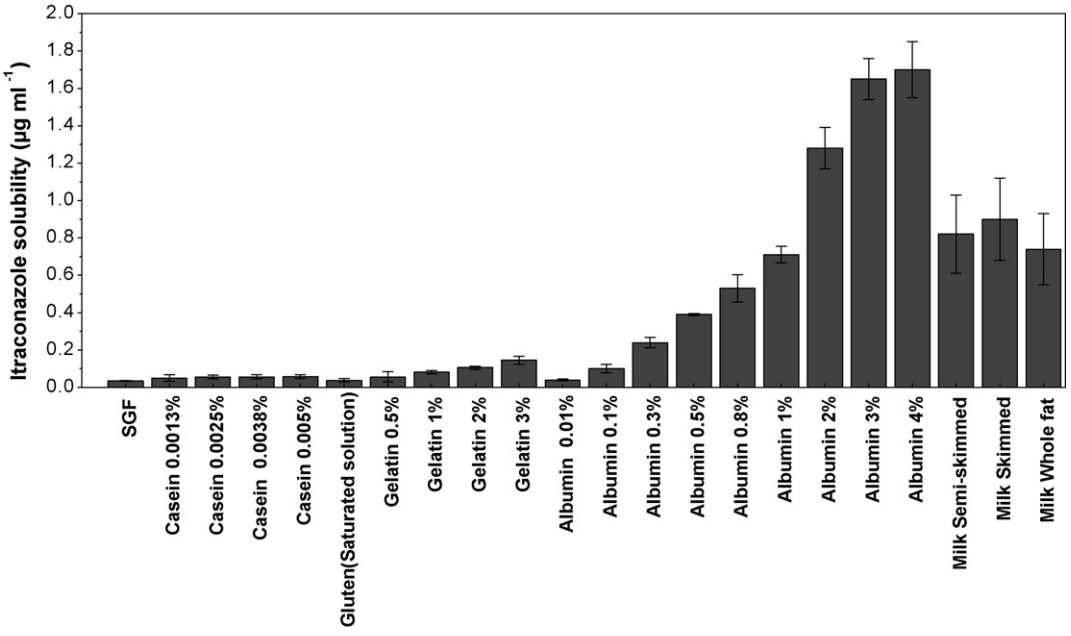


**Fig. 4.** Solubility of itraconazole in simulated gastric fluid (SGF) pH 3, SGF containing 50% milk (semi-skimmed, skimmed, whole fat) and (SGF) containing different concentrations of albumin, casein, gelatin and gluten. pH of each medium was 3. Each data point represents the mean ± S.D. of 3 measurements.

### 3.3. Dissolution and saturation solubility in the presence of proteins

Inclusion of albumin in SGF medium increased the solubility and dissolution of itraconazole with a quantitative relationship between concentration and rate of dissolution. The solubility of the drug was linear up to the concentrations of 2% (w/v) of albumin with coefficient of variance of 0.99; above 3% (w/v) a plateau level was reached (Fig. 4). The dissolution of the drug increased with the increase in albumin concentration (Fig. 5). For example, for the 0.5%, 1%, 2%, 3%, 4% (w/v) of albumin the amounts dissolved per unit area after 1 h were 3.5-, 5.1-, 6.7-, 7.1- and 7.8-folds (respectively) greater than that recorded with SGF.

Albumin was chosen for this investigation as it is a protein commonly available in food. The observed enhancement in itraconazole dissolution and solubility makes egg albumin a potential drug carrier for itraconazole as a safe alternative for synthetic polymers (Imai et al., 1989). The mechanism affecting drug behaviour in albumin-containing media is believed to be attributed to protein–drug binding. Egg albumin could interact with itraconazole through hydrophobic and electrostatic interaction, as in the case with human serum albumin where itraconazole exhibited significant protein binding *in vivo* (99.8%) (Heykants et al., 1989).

Suspensions of casein and of gluten (2%, w/v) did not appear to greatly enhance the dissolution of the drug as the profiles observed were not markedly improved over that using SGF alone (Fig. 6). This could be attributed to undissolved protein forming an occlusive layer on the surface of the disks, so limiting the effect of the protein. This hypothesis was tested by increasing the casein concentration to 4% (w/v). At this concentration, it was found that virtually no drug dissolved. To avoid this 'blinding' of the disk, saturated solutions (rather than suspensions) of casein and of gluten were prepared and the dissolution data for these solutions (Fig. 6) exhibited a marked increase in dissolution in casein media, confirming the probable effect of undissolved protein. Although the gluten solution appeared to increase the dissolution rate, this increase was not statistically significant compared to the SGF because the data was widely distributed.

Subsequently, three dilutions were prepared from a saturated casein solution (0.005%, w/v), to quantitatively study the relationship between casein concentrations and the solubility of itraconazole (Fig. 4). A statistical evaluation of these data (ANOVA) showed that adding casein slightly increases the solubility but that there was no statistically significant difference between the solubilities seen with the four different casein solutions, so there was no quantitative relationship between casein concentration and solubility over the range tested. Casein is believed to exert an effect through forming micelles where the drug can dissolve into the hydrophobic core (Macheras and Reppas, 1986). Although it is uncertain whether micelles were formed under the present experimental conditions, the use of casein media with its low surface tension demonstrates the ability of casein to solubilise itraconazole.

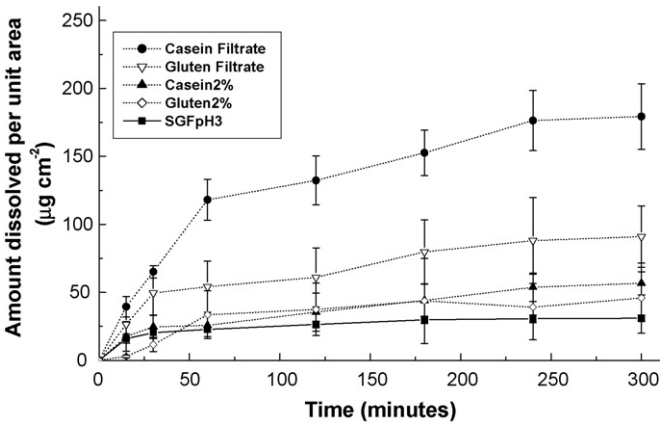


**Fig. 6.** The intrinsic dissolution profile of itraconazole in simulated gastric fluid (SGF) pH 3 (■) and SGF containing casein 2% (▲), casein as a filtered solution (●), gluten 2% (◊) and gluten as a filtered solution (▽), the pH of the media was 3. Each data point represents the mean ± S.D. of 3 measurements.

Dissolution profiles of itraconazole in three gelatin solutions of different concentrations depicted a correlation between the concentration of gelatin in the media and both the rate of dissolution and the saturation solubility of the drug (Figs. 4 and 7). A *t*-test showed the amount dissolved in 1 h per unit area in 0.5% (w/v) gelatin media did not increase significantly from that in the SGF but with media containing higher concentrations of gelatin a marked increase was recorded.

Gelatin increases the solubility of drugs and this effect was more pronounced with drug of high lipophilicity and low aqueous solubility (Kallinteri and Antimisiaris, 2001). The effect of gelatin was attributed to the mild surface activity of gelatin (Acarturk et al., 1992). This may explain the observed effect of gelatin on itraconazole which has extremely poor solubility and high lipophilicity.

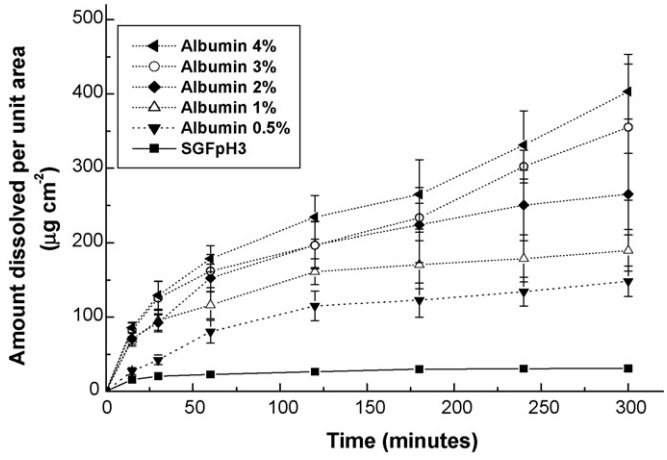


**Fig. 5.** The intrinsic dissolution profile of itraconazole in simulated gastric fluid (SGF) (■) and SGF containing egg albumin in a concentrations of 0.5% (▼), 1% (△), 2% (◆), 3% (○), 4% (◄) of egg albumin, pH of the media was 3. Each data point represents the mean ± S.D. of 5 measurements.

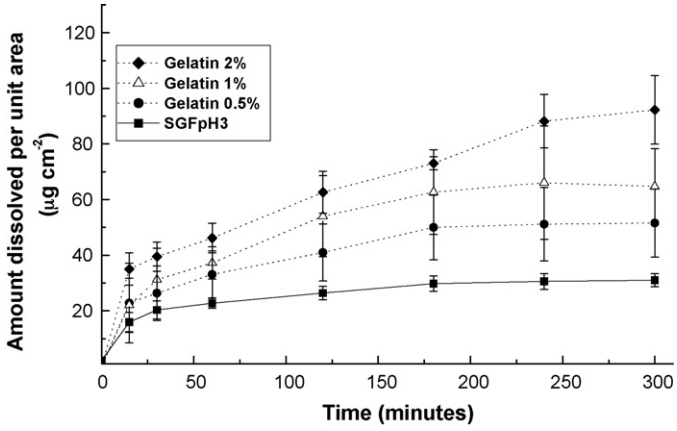


**Fig. 7.** The intrinsic dissolution profile of itraconazole in simulated gastric fluid (SGF) pH 3 (■) and SGF containing gelatin in a concentrations of 0.5% (●), 1% (△), 2% (◆) of gelatin, the pH of the media was 3. Each data point represents the mean ± S.D. of 3 measurements.

**Table 2**
Solubility and intrinsic dissolution of itraconazole in simulated gastric fluid (SGF) containing carbohydrates, the pH of the media is 3.

| Medium | Amount dissolved per unit area in 1 h (μg ml$^{-1}$) | Solubility (μg ml$^{-1}$) |
|---|---|---|
| SGF pH 3 | 22.7 ± 1.8 | 0.035 ± 0.003 |
| Glucose 1% in SGF | 31.4 ± 3.8 | 0.039 ± 0.002 |
| Lactose 1% in SGF | 35.2 ± 8.0 | 0.044 ± 0.006 |
| Starch 1% in SGF | 36.3 ± 11 | 0.042 ± 0.004 |

Each data point represents the mean ± S.D. of 3 measurements.

### 3.4. Dissolution and saturation solubility in the presence of carbohydrate or amino acids

The solubility and dissolution values of itraconazole in glucose, lactose and starch media exhibited a slight increase compared to the SGF. However, statistical studies for the amount dissolved in 1 h per unit area showed this increase was insignificant, (Table 2).

Media containing amino acids also showed a slight increase in both dissolution and saturation solubility (Table 3). After 1 h the amounts dissolved were slightly higher than the blank although were not statistically different. However, by 3 h the amounts dissolved in glycine and alanine media were significantly higher than that dissolved in SGF. This is in agreement with the solubility values which were also higher in glycine and alanine-containing media.

The mechanism by which carbohydrates and amino acids increased the dissolution of the drug remains to be explained. The slight effect noted with carbohydrates could be due to simple hydrogen bonding between the itraconazole nitrogens and sugar hydroxyl groups. With amino acids, the effect could be due to hydrogen bonding between the itraconazole nitrogens and the carboxylic acid group of the amino acid.

### 3.5. Dissolution and saturation solubility in simulated gastric and intestinal fluids

The solubility and dissolution characteristics of itraconazole were determined in FaSSGF, a medium containing sodium taurocholate and lecithin, and also in a medium containing 0.25% (w/v) of SDS (anionic surfactant) or 0.1% (w/v) of Triton X-100 (non-ionic surfactant). The amount of added surfactant was sufficient to make the solutions equivalent, in terms of surface tension, to the surface tension of gastric fluid. The dissolution data (Fig. 8) showed that the initial burst in FaSSGF was far greater than in SGF and thus the dissolution was also greater. Approximately 1271 μg dissolved in FaSSGF at 1 h compared to 324 μg in SGF. However, the dissolution profile in the presence of SDS did not demonstrate an initial burst but a steadier dissolution was observed with 778 μg dissolved at 1 h. The dissolution in media containing Triton X-100 (0.1%) exhibited only a slight increase in dissolution compared to the compendial SGF. These distinct differences between FaSSGF and the artificial surfactant-SGF could call into doubt the relevance of using an artificial medium component. Table 4 shows that SDS medium has the highest solubility value for itraconazole which might explain the steady rise in the dissolution in SDS medium (Fig. 8), as the amount dissolved within 5 h did not reach saturation solubility.

**Table 3**
Solubility and IDR in simulated gastric fluid (SGF) containing amino acids, the pH of the media was 3.

| Medium | Amount dissolved per unit area in 1 h (μg ml$^{-1}$) | Solubility (μg ml$^{-1}$) |
|---|---|---|
| Glycine 1% in SGF | 35.7 ± 1.5 | 0.060 ± 0.008 |
| Alanine 1% in SGF | 37.1 ± 4.1 | 0.073 ± 0.014 |
| Lysine 1% in SGF | 32 ± 6.8 | 0.056 ± 0.012 |
| Aspartic acid (filtered) in SGF | 23.5 ± 3.4 | 0.040 ± 0.004 |

Each data point represents the mean ± S.D. of 3 measurements.

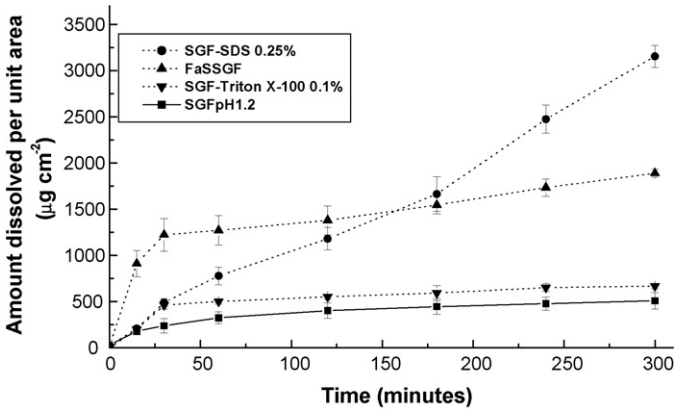


**Fig. 8.** The intrinsic dissolution profile of itraconazole in simulated gastric medium (SGF) pH 1.2 (■) and SGF containing sodium dodecyl sulfate (SDS) 0.25% (●), Triton X-100 0.1% (▼) and fasted simulated gastric fluid (FaSSGF) (▲), the pH of the media was 1.2. Each data point represents the mean ± S.D. of at least 3 measurements.

The data for medium composed of SDS in SGF appears high when compared to endogenous surfactants such as bile salts. This is in agreement with a recent study that showed the concentration of SDS (0.25%, w/v), suggested by Dressman et al. (1998) to mimic gastric fluid is too high as it exceeds the CMC concentration, which leads to 'artificial SDS-micellar solubilisation' (Aburub et al., 2008). Although Triton X-100 (0.1%) solution has the same surface tension as SDS medium, it did not show a marked effect on solubility and dissolution behaviour compared to SGF and proved to be a poor solubilising agent. The slight increase in dissolution is believed to be due to the wetting ability of Triton X-100 (Galia et al., 1999).

Fig. 9 illustrates the dissolution profiles of itraconazole in FaSSIF and FeSSIF. The presence of lecithin and sodium taurocholate produced an enhancement in dissolution rate. FeSSIF with its lower pH and more bile salts and lecithin induced a greater increase in dissolution and solubility of the basic drug than FaSSIF. The amount of itraconazole dissolved in 1 h in FeSSIF was approximately 3 times higher than in FaSSIF. In FeSSIF, the amount dissolved in 1 h increased 16-fold from the blank FeSSIF and the solubility values increased by a factor of 18 (Table 4). The data comparison could not be performed for FaSSIF with blank due to the very low results obtained in blank FaSSIF media which were less than the limit of quantitation. The bile components exert their effect on solubility by forming different colloidal phases that could solubilise lipophilic drugs. *In vivo*, food stimulates the release of bile salts and phospholipids, this highlights the importance of the fed state in solubilising this lipophilic compound and to consequently increase its bioavailability.

**Table 4**
Solubility of itraconazole in simulated media. The intestinal simulated fluids are fed simulated intestinal fluid (FeSSIF) pH 5, fast simulated fluid (FaSSIF) pH 6.5, blank (FeSSIF) and blank (FaSSIF).

| Medium | Solubility (μg ml$^{-1}$) |
|---|---|
| FaSSGF | 6.8 ± 0.13 |
| SDS 0.25% in SGF | 8.27 ± 1.9 |
| Triton X-100 0.1% in SGF | 4.84 ± 0.8 |
| Blank FaSSIF | ~0.003 |
| FaSSIF | 0.070 ± 0.005 |
| Blank FeSSIF | 0.011 ± 0.004 |
| FeSSIF | 0.20 ± 0.02 |

The simulated gastric fluids (SGF) are SGF containing sodium dodecyl sulfate (SDS) 0.25% (w/v), Triton X-100 0.1% (w/v) and modified fasted simulated gastric fluid (FaSSGF); the pH of the gastric media was 1.2. Each data point represents the mean ± S.D. of at least 3 measurements.

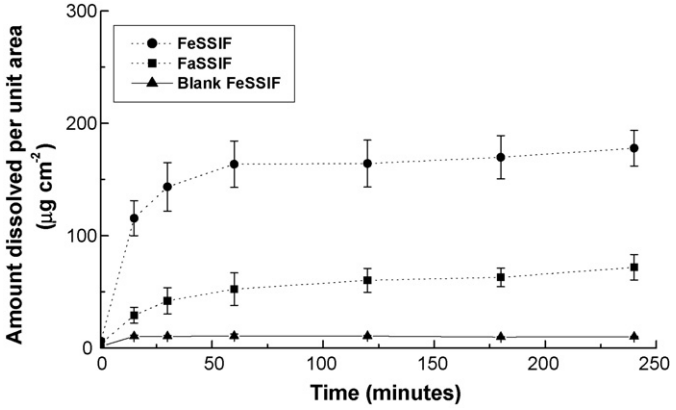


**Fig. 9.** The intrinsic dissolution profile of itraconazole in fed simulated intestinal fluid (FeSSIF) pH 5 (●), fast simulated intestinal fluid (FaSSIF) pH 6.5 (■) and blank fed simulated intestinal fluid pH 6.5 (▲). Each data point represents the mean ± S.D. of at least 3 measurements.

## 4. Conclusion

Zimmermann et al. (1994) observed that a high-fat meal provided the greatest bioavailability of itraconazole and hence suggested that itraconazole absorption was promoted at the post-prandial stage due to longer gastric retention and the high fat content of the co-ingested meal. The data presented here supports the potential effect of co-ingested food on drug dissolution. Moreover, the meals given to Zimmerman's subjects were composed of white bread, butter, eggs, bacon, sausages and decaffeinated coffee, which is a protein-rich diet. Therefore, in addition to the potential solubilisation of the lipophilic drug and bile secretion, the proteins in the diet might also promote the dissolution of the drug and consequently its absorption.

In conclusion, the increased dissolution of itraconazole in the presence of the investigated dietary media might account for the reported increases in its bioavailability when co-administered with meal. Future work may explain the mechanism behind this observed change in the presence of food.

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