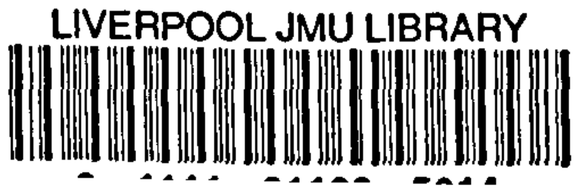
THE IMPACT OF BIORELEVANT MEDIA ON THE IN-VITRO DISSOLUTION OF AZOLE ANTI-FUNGAL DRUGS

Heba Ghazal

A thesis submitted in partial fulfilment of the requirements of Liverpool John Moores University for the degree of Doctor of Philosophy

School of Pharmacy and Biomolecular Sciences
2009



ABSTRACT

Dissolution of drugs is a prerequisite for oral absorption and bioavailability. Drugs in the gastrointestinal tract are exposed to a medium of partially digested food, comprising mixtures of fat, protein and carbohydrate in addition to bile components. Despite this, the compendial media are still simple hydrochloric acid solutions or phosphate buffers. In this investigation, biorelevant dissolution media were developed to examine the impact of fat, protein, carbohydrate, amino acids and bile components on the dissolution behaviours of two azole anti-fungal drugs (itraconazole and ketoconazole). The drugs are Biopharmaceutics Classification System Class II (poorly soluble with good permeability) and so they are more likely to exhibit dissolution rate-limited absorption. Furthermore, their bioavailability is affected by food ingestion.

The intrinsic dissolution rates of the drugs were evaluated using the stationary disk method in USP Apparatus II. Powders of the drugs were compressed to form disks with a constant surface area. No evidence of polymorphic change was observed due to drug compaction.

The dissolution and solubility of the drugs were assessed in simulated gastric fluid (SGF) containing milk with different fat contents, SGF containing albumin (hen egg white), gelatin (bovine skin), casein (bovine milk), gluten (wheat), carbohydrates (glucose, lactose and starch) and amino acids (glycine, alanine, leucin, lysine and aspartic acid) to mimic gastric fluid at fed state. Most of the dietary components enhanced the solubility compared to SGF but to differing extents. The greatest increase in dissolution of itraconazole was observed with the addition of milk or albumin. The greatest enhancement of ketoconazole dissolution was observed in media containing neutral amino acids or milk. The rate and extent of the increase in solubility and dissolution varied between the two drugs and this was attributed to differences in their physical and chemical structure, in particular their lipophilicity. The formation of complexes with food additives likely accounted for the solubilising effect and in milk-containing media the effect was attributed to the whole complex structure of milk.

The dissolution and solubility were assessed in simulated gastric fluid at fasted state (FaSSGF) using formulations based on natural (bile salts and phospholipids) and synthetic surfactants. The inclusion of surfactants increased the dissolution however

variable dissolution profiles in the media were obtained. This was presumably due to different solubilising mechanisms of the surfactant-containing media which rendered the formulations of synthetic surfactants as inappropriate alternative for FaSSGF which contained natural surfactants.

The dissolution was also assessed in the presence of key endogenous surfactants in conditions representing fasted and fed state simulated intestinal fluids (FaSSIF and FeSSIF). The dissolution in the fed simulated medium was higher than the fasting simulated medium due to the greater amount of bile components and the lower pH of the fed simulating medium. The dissolution profiles of the drugs in FaSSIF and FeSSIF were compared to the profiles acquired in simplified media containing synthetic surfactants, sodium dodecyl sulphate and hexadecyltrimethyl ammonium bromide. These simplified media did not provide identical dissolution to that in FaSSIF and FeSSIF, however, approximate simulation was achieved which may be of value for replacing FaSSIF and FeSSIF for the quality control tests of the dosage forms.

The physical properties of the media were assessed in terms of viscosity and surface tension. The viscosity of the media did not show important variations, apart from media containing milk, albumin, and gelatin. Surface tension was an important determinate of dissolution as media with high surface activity exhibited high solubilising capacity of the drugs in addition to enhancing the wettability of drugs.

Thus, the results confirm the importance of using biorelevant media in dissolution studies and highlight the potential effect of the ingested meals on drug dissolution and subsequent bioavailability.

ACKNOWLEDGEMENTS

The author would like to express her appreciation and gratitude to Dr Gillian A. Hutcheon and Dr A. Mark Dyas, who supervised this work and provided invaluable support during the period of the investigation.

Special thanks with gratitude and appreciation are due to Prof. James L. Ford for the guidance and advice through his constructive comments. His support made the successful conclusion of this investigation possible.

Special thanks are due to:

Ms. Nicola Dempester, Senior Research Officer at JMU, for her kind help and friendship. Her help with the thermal analysis is highly appreciated.

The staff in the School of Chemical Engineering of the University of Manchester for allowing access to the surface tensiometer device and special thanks are due to Dr Linda Seton for arranging this.

Dr. Francis Lilley of the School of Engineering, JMU, for his help in setting up the contact angle measurements.

Dr Elsie Gaskell for providing the vibration viscometer.

Lipoid GmbH for kindly donating Egg–lecithin (Lipoid E P C, 96%).

Janssen Pharmaceutica for providing the itraconazole drug substance which was used for the initial investigations of this research.

Finally I would like to record my appreciation to:

Damascus University for providing the financial support for this investigation.

My beloved husband, Dr Hussam Jouhara, for his continuous support, encouragement and never ending love.

Sam, my lovely son, who relieved my hard times with his lovely smile and innocent phrase "mummy go study".

My parents for their continuous love and perpetual encouragement, my sister for her peaceful prayers and my brothers for their support.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
ASP	Aspartic acid
ATR	Attenuated total reflectance
AUC	Area under curve
BA	Bioavailability
BP	British Pharmacopoeia
BCS	Biopharmaceutics Classification System
C	Concentration
cm	Centimetre
C_{max}	Maximum peak plasma concentration
CMC	Critical micelle concentration
C_s	Saturation solubility
C_t	Concentration at time t
CDs	Cyclodextrins
CTAB	Hexadecyltrimethyl ammonium bromide
D	Diffusion coefficient
DIF	Dog intestinal fluid
DSC	Differential scanning calorimetry
FaSSGF	Fasted state simulated gastric fluid
FaSSIF	Fasted state simulated intestinal fluid
FeSSIF	Fed state simulated intestinal fluid
FDA	Food and Drug Administration
FT-IR	Fourier transform infrared
g	Gram
GI	Gastro-intestinal
GIT	Gastro-intestinal tract
GLY	Glycine
h	Thickness of boundary layer
h	Hour
HCl	Hydrochloric acid
HIF	Human intestinal fluid

HPLC	High performance liquid chromatography
HPMC	Hydroxylpropylmethylcellulose
IDR	Intrinsic dissolution rate
IVIVC	*In vitro-in vivo* correlation
J	Mass flux or intrinsic dissolution rate
J g^{-1}	Joule per gram
Ka	Ionisation constant
L	Litre
LEU	Leucin
L.O.D	Limit of detection
L.O.Q	Limit of quantification
LYS	Lysine
M	Molar
mg	Milligram
min	Minute
mEq h^{-1}	Milli-equivalents per hour
ml	Millilitre
m.p.	Melting point
mm	Milli-metre
mM	Milli-Mole
MMC	Migrating motor complex
mN m^{-1}	milli-Newtons per metre
mPa.s	milli-Pascal second
N	Normality
NaCl	Sodium chloride
NaOH	Sodium hydroxide
NaTC	Sodium taurocholate
NMP	1-methyl 2-pyrrolidone
P	Distribution coefficient
pH	Negative log of the hydrogen ion concentration
PI	Isoelectric point
pKa	Negative log of the ionisation constant
PTFE	Poly-tetrafluoroethylene
PVDF	Poly-vinylidene fluoride
PVP	Polyvinylpyrrolidone
PXRD	Powder X-ray diffraction
r^2	Coefficient of determination

R.S.D	Relative standard deviation
Rev min^{-1}	Revolution per minute
s	Second
S	Surface area
SEM	Scanning electron microscopy
S.D.	Standard deviation
SDS	Sodium dodecyl sulphate
SIF	Simulated intestinal fluid
T	Temperature
t_{max}	Time to reach peak plasma concentration
™	Registered trade name
USP	United States Pharmacopoeia
UV	Ultraviolet
ν	Kinematic viscosity
V	Volume of the dissolution media
Ω	Angular velocity
w/v	Weight per volume
~	Approximately
%	Percent
ΔH	Enthalpy change
ΔG	Gibbs free energy
ΔS	Entropy change
$\gamma_{L/V}$	Liquid-vapour surface tension
$\gamma_{S/L}$	Solid-liquid interfacial tension
$\gamma_{S/V}$	Solid-vapour interfacial tension
γ	Surface tension
η	Viscosity
θ	Contact angle
λ_{Exc}	Excitation wavelength
λ_{Emm}	Emission wavelength
µl	Microlitre
µm	Micrometer
$\dot{\gamma}$	Shear rate
σ	Shear stress

Chapter 1: Introduction

1.1 Introduction to dissolution and solubility

Dissolution is a prerequisite for the oral absorption of solid dosage forms because for a drug to be absorbed it must dissolve first. Dissolution can be the rate-limiting step for the absorption of poorly soluble drugs administered orally, consequently limiting their bioavailability and systemic efficacy (Kramer et al., 2005).

The dissolution test has proved to be a useful tool for many purposes and at many stages of formulation development. It is used in quality control, batch to batch similarity and during drug development to help in choosing appropriate excipients and formulations. The test allows prediction of drug behaviour and determination of bio-equivalency *in vivo* which makes it a potential tool for *in vitro-in vivo* correlation (Figure 1.1).

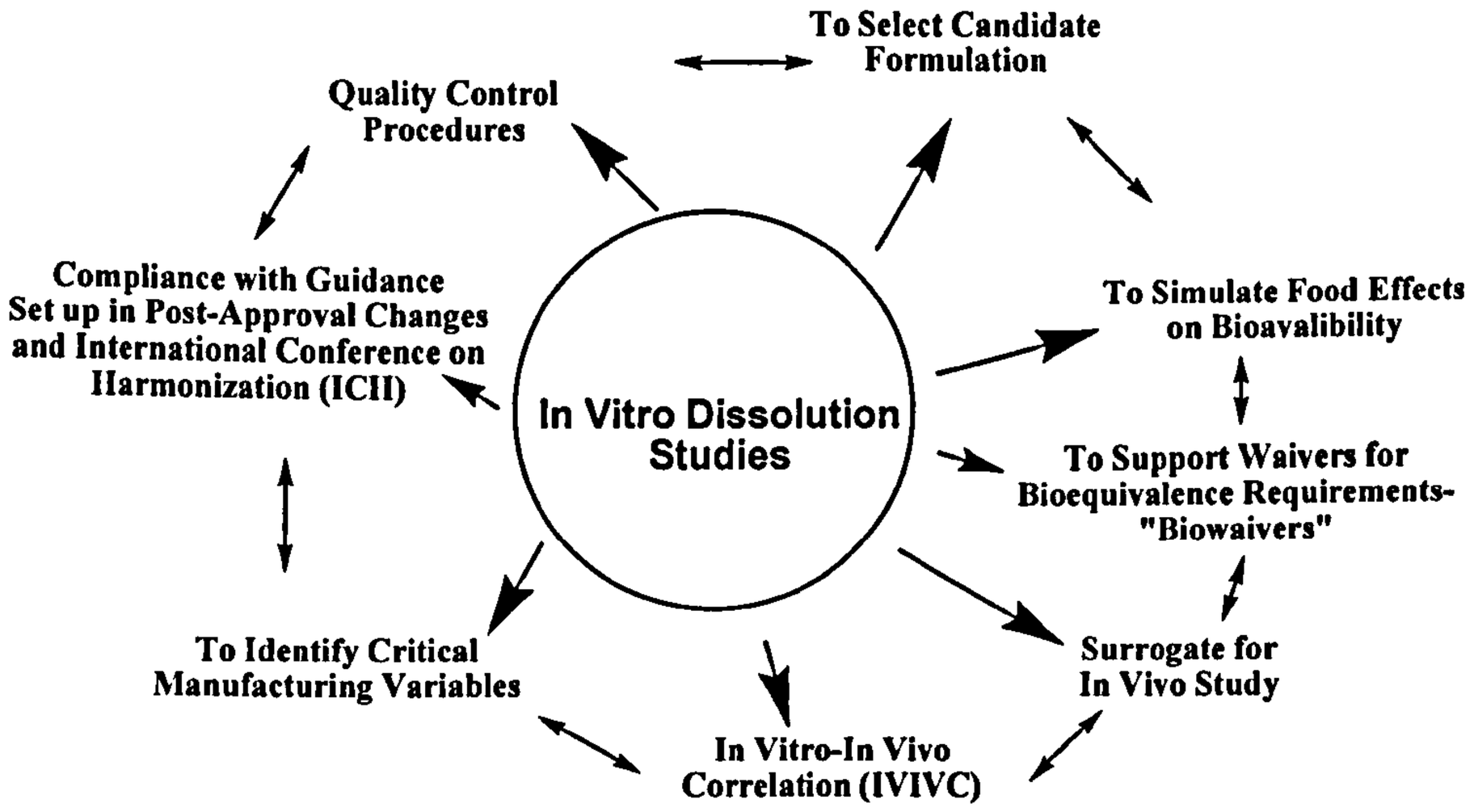


Figure 1.1 The applications of dissolution testing, from Jamzad and Fassihi (2006)

When a solid dosage form is immersed in dissolution media or in the fluids of the gastrointestinal (GI) tract, there are three possible steps for drug dissolution (Figure 1.2):

1) disintegration to granules or aggregates

2) deaggregation into fine particles

3) dissolution.

These three steps can also occur simultaneously or the dissolution process can start directly from the intact solid or granules without going through all stages (Martin, 1993c).

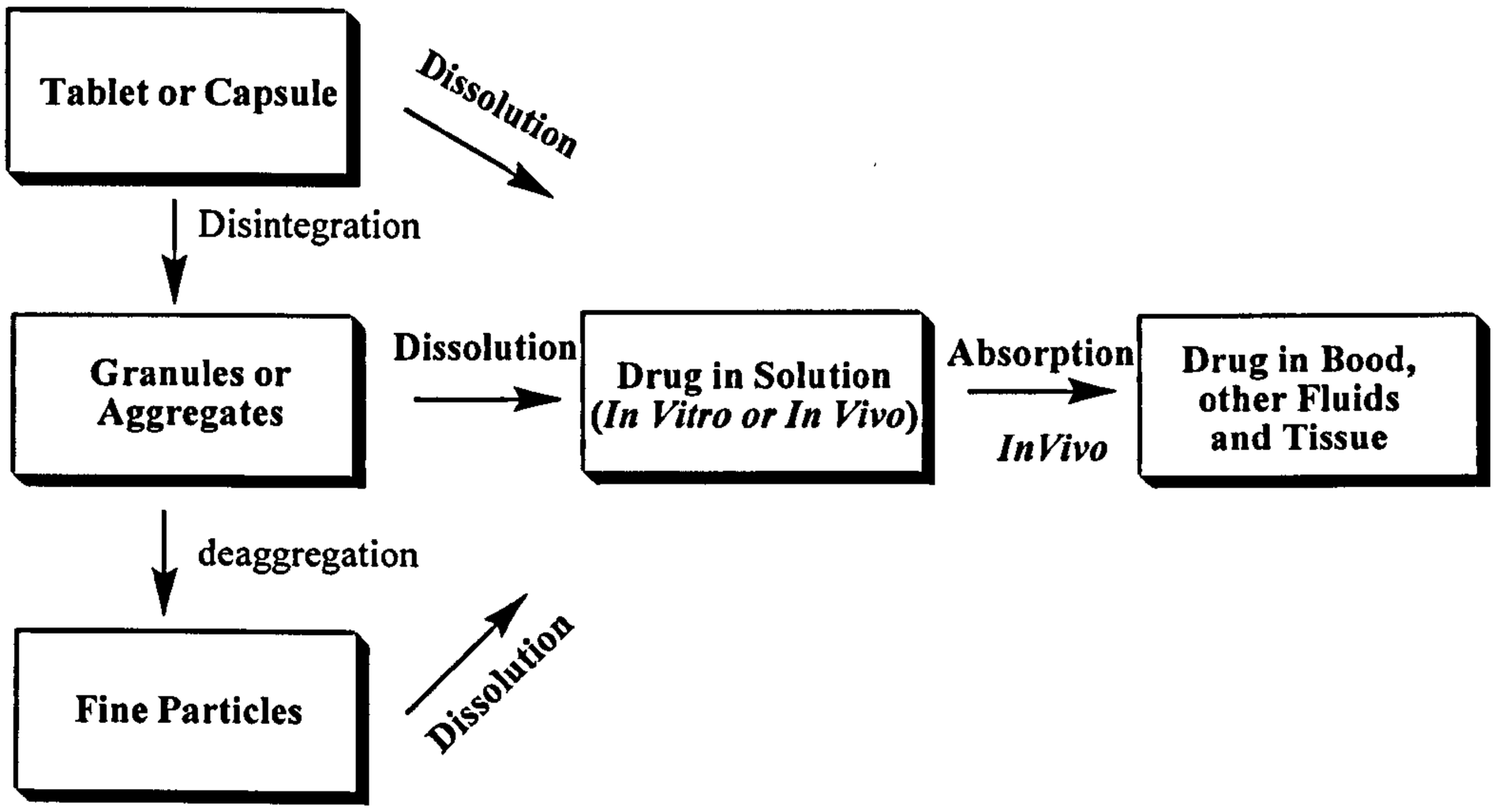


Figure 1.2 Steps for drug dissolution from a solid dosage, from Wagner (1971)

Dissolution can be explained as the process where solid particles leave the solid phase and become emerged in the solvent phase. It was defined by Viness and Reza (1999) as "Dissolution of the drug substance is a multi-step process involving heterogeneous reactions/interactions between the phases of the solute–solute and solvent–solvent phases and at the solute–solvent interface". Dissolution was expressed as a quantitative term called dissolution rate which was defined as "the amount of drug substances that goes into solution per unit time under standardized conditions of liquid/solid interface, temperature and solvent composition" (Abdou, 1989b).

A saturated solution is the state where a maximum amount of solute is dissolved in solvent and so equilibrium is maintained between the solution and the solid phases. Based on that, solubility was described by Martin (1993c) as the concentration of the dissolved substance in its saturated solution at a certain temperature.

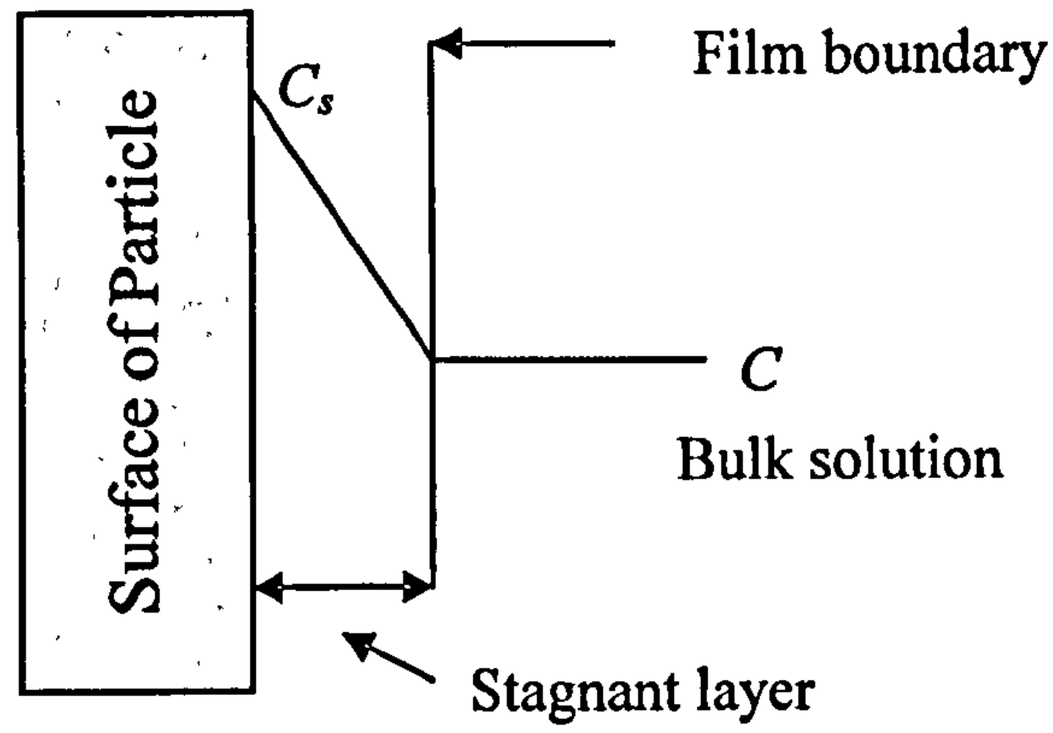
There is another term for solubility called the intrinsic solubility which was defined as the solubility of an un-dissociated species, i.e. the solubility of a compound in its free form as an acid or a base (Alsenz and Kansy, 2007).

To distinguish between solubility and dissolution, dissolution is a kinetic parameter quoted as the amount dissolved per unit time, so it is a dynamic process whereas solubility is an equilibrium parameter that expresses the maximum amount of the substance dissolved per volume unit.

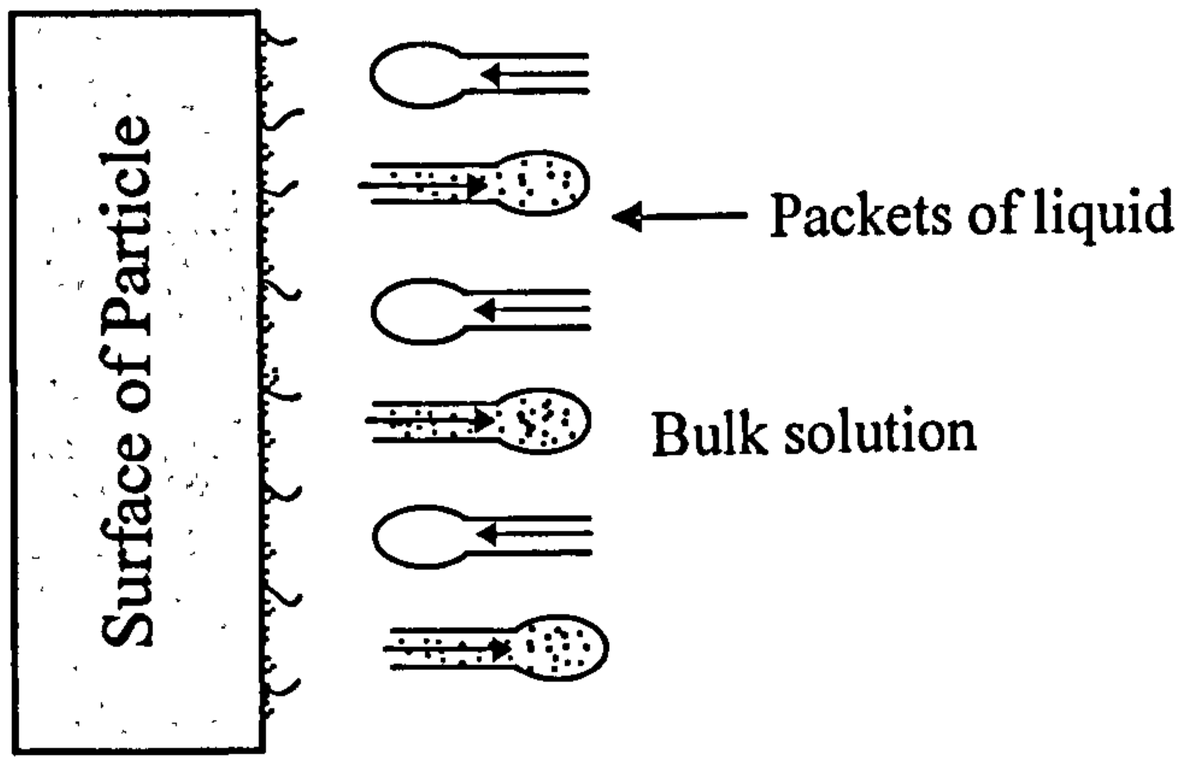
1.2 Theory of dissolution mechanisms

There are three prevailing models that describe the dissolution process (Figure 1.3). The dissolution concept, in terms of theories and historical development were reviewed thoroughly by Abdou (1989b) and Banakar (1992a).

a. Diffusion layer model



b. Danckwerts' model



c. Interfacial layer model

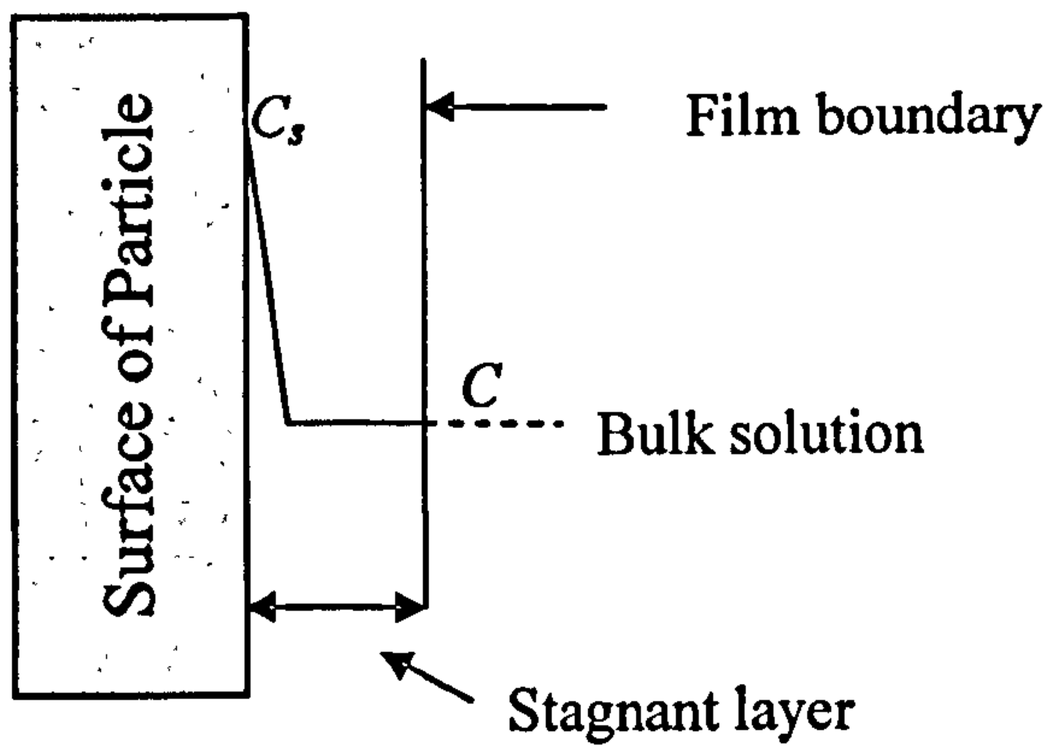


Figure 1.3 Schematic diagram illustrating different mechanisms of the dissolution process. *C* refers to the concentration in the bulk solution and C_s to the saturation solubility, reproduced from Higuchi, (1967)

1.2.1 Diffusion layer model (film theory)

This diffusion layer model is probably the most commonly used. It considers the process of dissolution to be composed of two consecutive steps; the first step, which occurs quickly, is the formation of a layer of solution around the solid which becomes a stagnant film adjacent to the solid surface. During the next step, the particles move from the surrounding layer to the bulk solution.

One of the first equations modelling dissolution was Fick's law for diffusion under constant conditions (Topp, 2000). Dissolution was expressed by the term "diffusion current" (*J*) which was defined as the amount of drug that passes vertically per unit of time per unit of area (Eq. (1.1)). D is the diffusion coefficient and $\partial c/\partial x$ is the concentration gradient in the x direction which is considered constant.

$$J = -D\frac{\partial c}{\partial x} \qquad \textbf{Eq. (1.1)}$$

Since the first equation of Fick's law assumed steady state, whereas the concentration of the drug will change with time, another equation (Eq. (1.2)) was derived and called Fick's second law, which express the dissolution rate $\partial c/\partial t$ as:

$$\frac{\partial c}{\partial t} = D\left(\frac{\partial^2 c}{\partial x^2}\right) \qquad \textbf{Eq. (1.2)}$$

Noyes and Whitney (1897) studied the kinetics of the dissolution of benzoic acid and lead chloride using rotating cylinders of the substances. By using slightly soluble compounds placed around cylinders, a constant surface area was maintained throughout the course of the experiment. It was noticed that the rate of the dissolution was proportional to the difference between the saturation solubility at the solid-liquid interface and the instant concentration in the bulk medium (Eq. (1.3)).

$$\frac{dc}{dt} = k(C_s - C_t) \qquad \textbf{Eq. (1.3)}$$

Where *dc/dt* is the change in concentration as a function of time, C_s is the saturation solubility, C_t is the concentration at time t and k is the dissolution rate constant. This equation is expressed schematically in Figure 1.4.

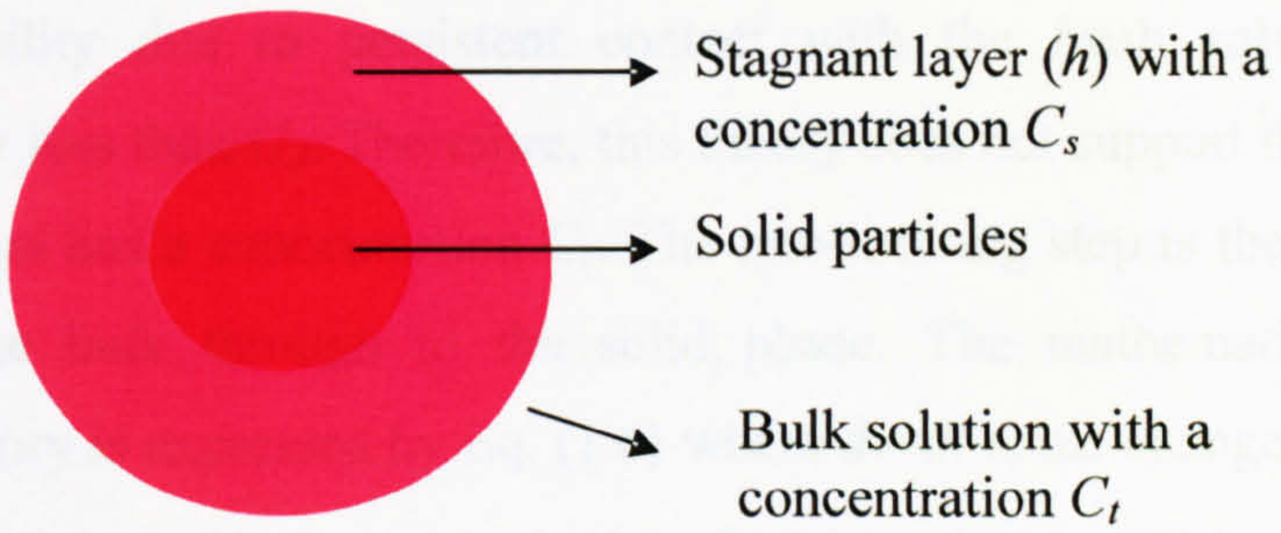


Figure 1.4 Schematic diagram for the dissolution process from a solid particle representing the Noyes-Whitney equation

Bruner and Tolloczko (1900) and Nernst (1904) incorporated a diffusion layer perception by proposing a thin film formed around the particles with thickness h and considered that the diffusion process took place from that layer to the bulk solvent. Based on Fick's second law, the Nernst–Brunner equation was developed (Eq. (1.4)). D is the diffusion coefficient of the substance, S is the surface area of the particles, h is the thickness of the diffusion layer and V the volume of the dissolution medium.

$$\frac{dc}{dt} = \frac{DS}{Vh}(C_s - C_t) \qquad \textbf{Eq. (1.4)}$$

Instead of expressing the dissolution rate as *dc/dt,* Hixson and Crowell (1931) expressed the dissolution rate in terms of a change in the weight of dissolved particles with time. Thus, Hixson and Crowell's cubic root model was proposed which relates time to the cube root of the weight. In a special case, when C_t was too small and considered constant and consequently (C_s - C_t) was constant, the equation took the simple form (Eq. (1.5)) assuming that the shape of the particles is not changing during the dissolution.

$$w_0^{1/3} - w^{1/3} = k_i t \qquad \textbf{Eq. (1.5)}$$

Where w_0 is the initial weight of solid particles, w is the weight of the undissolved particles at time t and k_i is the cube-root dissolution constant.

1.2.2 Penetration or surface renewal theory (Danckwerts' Model)

Danckwerts (1951) suggested a new model for dissolution based on the hypothesis that the surface is continuously being replaced and assumed that the agitated solvent is composed of packets that are continuously exposed to solid particles. These packets absorb the solute molecules and carry them into the solution; thus these solvent packets

are constantly renewed. This model proposes that the equilibrium occurs at the solid-solution interface. The concentration of the solute on the interface, C_A, is less than the saturation solubility due to persistent contact with the fresh solvent that has a concentration far less than C_A. Therefore, this theory does not support the existence of a stagnant layer that has a concentration C_s. The rate-limiting step is the diffusion of the solvent from the bulk through to the solid phase. The mathematical equation of Danckwerts' theory is expressed by Eq. (1.6) where *dw/dt* is the change of the weight of the dissolved particles with time, γ is the interfacial tension and C_t is the concentration of the solute in the bulk medium.

$$\frac{dw}{dt} = S(\gamma D)^{1/2}(C_s - C_t) \qquad \textbf{(Eq. 1.6)}$$

1.2.3 Interfacial barrier model (double barrier mechanism or limited solvation theory)

This interfacial barrier model is based on the assumption that the reaction occurs at the interfacial barrier between a solid surface and a liquid. This barrier has an intermediate concentration of the solute, which is less than the saturation concentration. Different faces of crystals may have different interfacial barriers and thus different dissolution profiles. Higuchi (1961) considered the rate limiting step as the diffusion of solute molecules from the solid interface, which demands high energy, rather than diffusion through the static film.

These three models were also employed in combination to explain the dissolution process. One of the new theories considered the dissolution as a heterogeneous mass transfer process composed of three steps " (i) removal of the solute from the solid phase, (ii) accommodating the solute in the liquid phase, and (iii) diffusive and/or convective transport of the solute away from the solid/liquid interface into the bulk phase" (Kramer et al., 2005).

1.3 The process of dissolution: energy changes

In order for the dissolution process to occur, the interaction between the solute-solvent should be stronger than the interaction between solute-solute and solvent-solvent (Brittain, 2007). The molecule must be able to move from an environment where it is surrounded by identical molecules to the solution where it is surrounded by non-identical molecules.

For the dissolution process to take place spontaneously the change in the free energy of the system at constant pressure should be negative. In this case, the attraction forces between solute-solvent should exceed the forces of attraction of solid-solid and solvent-solvent. Thereby, the solvation energy released during the dissolution process must be greater than the sum of the crystal lattice free energy and the free energy of cavity formation in the solvent (Brittain, 1999a). The general thermodynamic equation (Eq. (1.7)) applies where ΔG is Gibbs free energy, ΔH is the change in the enthalpy that occurs due to the thermodynamic change while absorbing or evolving energy, ΔS is the change in entropy which indicates the change in the disorder of the system and T is the thermodynamic temperature.

$$\Delta G = \Delta H - T\Delta S \qquad \textbf{Eq. (1.7)}$$

ΔS is always positive during a dissolution process and this can be explained by the particles leaving the solid mass as a process that increases the randomness of the system. Therefore, for ΔG to be negative, ΔH must less than $T\Delta S$ (Aulton, 2002).

The changes in ΔH during the dissolution process is the sum of two enthalpies: i) the heat absorbed to separate the molecules of solute against the intermolecular interactions which is expressed as crystal lattice enthalpy (ΔH_{cl}) and it is always positive, ii) the heat absorbed when the solute is immersed in solvent (ΔH_{solv}) which is normally negative (Eq. (1.8)).

$$\Delta H = \Delta H_{\text{cl}} + \Delta H_{\text{solv}} \qquad \textbf{Eq. (1.8)}$$

Dissolution overall can be either endothermic or exothermic. In most cases, $\Delta H_{cl} > \Delta H_{solv}$ so the dissolution process will be endothermic. If the affinity of the solute to the solvent is very high, ΔH becomes negative and the process will be exothermic (Aulton, 2002).

1.4 Compendial dissolution test

1.4.1 History

Studies carried out in the period of 1950-1970 highlighted the importance of the dissolution test in evaluating drug performance and bio-equivalency among generic products. The trigger for these studies was the perceived inconsistency in bioavailability of identical generic products such as digoxin tablets. Since digoxin is poorly soluble with a narrow therapeutic index and a narrow absorption window, any slight variation in

its bioavailability will have a serious impact. Shaw et al. (1973) suggested that differences in the bioavailability of digoxin could be attributed to different *in vitro* dissolution patterns. Accordingly, these findings demanded more extensive dissolution studies to standardize drug products to achieve bio-equivalency.

The disintegration process is the step prior to dissolution where the dosage form breaks up into granules or small particles. The disintegration test is an indicator for dosage form performance but it does not evaluate the drug release. It was introduced as an official quality control test by the USP Edition 14 in 1950, however, it was noticed that it was not sufficient to standardize products or give information about drug bioavailability (Campbell et al., 1957). Consequently, these concerns led to the introduction of dissolution requirements for tablet and capsule monographs in pharmacopoeias and the proliferation of dissolution apparatus designs. A detailed review of the history of pharmaceutical dissolution evolution is described by Kramer et al. (2005) and Dokoumetzidis and Macheras (2006).

One of the first methods utilized to study the dissolution of dosage forms was the Beaker method. It was initially developed by Levy and Hayes (1960) where a pellet of the drug was centred in a beaker containing the dissolution medium and a rotating motion was applied. The main problem with this system was that the pellets formed a mound at different positions on the base of beaker and this change in pellet position led to variable results. Thus, a round bottom flask was utilised and this method was called the flask-stirrer method. Thereafter rotating and basket methods were developed and described in most pharmacopoeias.

Of all dissolution apparatuses, Apparatus I (rotating basket) and II (paddle assembly) are the most widely utilized because they are simple, robust, and are sufficiently standardized.

It is important to note that the Food and Drug Administration (FDA) and the pharmacopoeias recommended the maintenance of sink conditions throughout the dissolution test period, which was defined when the concentration of the drug substance dissolved in the bulk medium (C) was less 1/3 of the saturated concentration (C_s) in that medium (USP, 2008). A stricter definition considered that sink conditions applied when the bulk concentration was <10% of the C_s (Aulton, 2002). This could be met when the dissolving medium was sufficiently diluted, i.e. by using a volume of dissolution medium that is at least three times greater than that required to form a saturated solution

of the drug or by using a method that allows constant removal of the solutes from the medium.

1.5 Intrinsic dissolution rate

The intrinsic dissolution rate (IDR) is the dissolution rate of a pure drug substance under constant conditions of surface area, temperature, agitation-stirring speed, pH and ionic strength of the dissolution medium. Therefore, the IDR is a useful tool to characterise a drug substance without the interaction of excipients and it also allows screening of new drug candidates to understand their behaviour in physiological fluids (Abdou, 1989b). In addition, it allows the study of the dissolution of excipients and their effect on the dissolution of the dosage form.

The IDR gives an indication of drug performance and potential bioavailability; if it is less than 0.1mg min^{-1} cm^{-2} then the dissolution rate is low and probably the rate-limiting step to absorption. If is greater than 0.1mg min^{-1} cm^{-2}, drug dissolution is not expected to be an impediment for absorption (Kaplan, 1974).

1.5.1 Methodology

The general procedure for determining IDR is to compress the pure drug powder to form a non-disintegrating disk under specific conditions of pressure and die dimensions without any additive to the drug powder. Thereafter, these disks are transferred to the dissolution apparatus where only one face of the compressed solid remains exposed to the dissolution medium. Dissolution then occurs under certain conditions of temperature and agitation rate. Samples are withdrawn and analyzed to determine drug concentration and calculate the IDR.

1.5.1.1 Rotating disk methods

The rotating disk method was initially described by Levy and Sahli (1962). In this method, a flat-faced tablet was mounted in a plexiglass holder which was rotated at 555rev min^{-1} in 200ml of dissolution medium placed in a 500-ml three necked flask. Drawbacks of this method included high rotating speeds and some drugs producing fragile disks that could easily break when handled. Thus, the Wood apparatus was developed (Wood et al., 1965). In this apparatus, the die containing the compressed pellet was attached to a rotor where one surface was facing the bottom of the beaker (Figure 1.5). The advantage of this system was the utilization of a compression assembly to form a compressed tablet that served as a tablet holder within the

dissolution apparatus. A further design of the rotating apparatus was developed by Underwood and Cadwallader (1978), where a disk of the drug was produced and held in a cup attached to a shaft that could rotate. Another type of apparatus that allows dissolution from a constant surface area was devised by attaching a cup containing drug pellets into USP basket apparatus where the rotation speed was set at 100rev min^{-1} (Hanson, 1991). Further variations of designs for the determination of IDR were reviewed by Abdou (1989b).

The USP (2008) considered a modification of the Wood apparatus for determining IDR. Drug powder was placed in the die cavity and compressed forming a smooth compact pellet with a predetermined surface area. The die was attached to the holder which was mounted on the shafts of a stirring device such as the modified USP Apparatus II. The USP (2008) recommended plotting a graph of the cumulative dissolved amount per unit area, until 10 % of drug is dissolved, versus time and so the slope of the regression line represents the IDR.

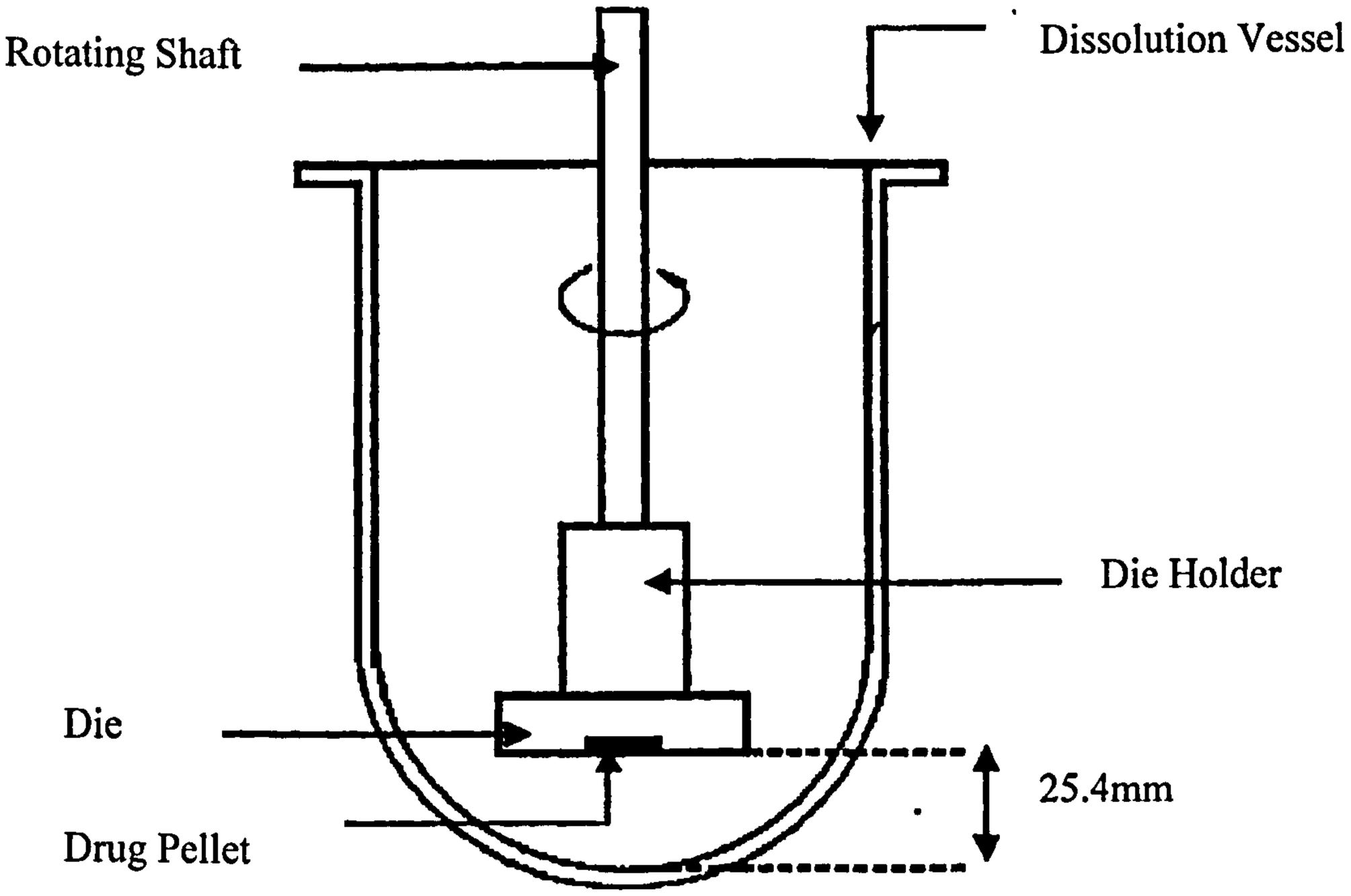


Figure 1.5 Schematic diagram for rotation disk apparatus, from Dyas and Shah (2007)

Recently, a miniaturized rotating disk apparatus was developed (Persson et al., 2008). This apparatus has the advantage of working on a small scale as it consumes only a small amount of drug substance, 5mg, compared to a minimum of 100mg required in the above methods and it only needs a small volume of dissolution medium (15ml). The

drug powder was compressed directly into a gold-plated magnetic bar holder and then a continuous flow system was pumped over the disk. However, a problem with this arrangement was the formation of a reproducible disk surface.

1.5.1.2 Stationary disk methods

Milosovich (1964) devised an apparatus that used stationary pellets in a stirred dissolution system. Samples were prepared by compressing 300mg of drug powder in a die using a punch and a hardened steel block. One surface of the disk was covered and the opposite one was left exposed to dissolution medium. This configuration was inserted into a stainless steel beaker where the disk surface was vertical on the beaker base. Stirring at 300rpm was produced using shafts driven by a motor. Khoury et al. (1988) used an apparatus which consisted of a holder unit for a tablet die with magnet-driven stirring. The drug powder was compressed in a die against the plate and then the die containing the pellet was introduced into the neck of a 200ml 3-necked flask and mounted via the die holder unit. The last two configurations allowed securing a fixed position and avoiding misalignment problems.

Viegas (2001) illustrated a stationary system provided by Distek. Inc. that easily fits into the USP apparatus II. The drug was compressed using a punch and die system and then the die was immersed in a dissolution vessel where only one face was subjected to the dissolution medium. The stirring was produced by rotating shafts with the distance between the bottom of the stirring paddle and the pellet face was specified as 2.54cm (Viegas, 2001).

The stationary method was recommended by the USP (2007) for cases where the use of rotating apparatus was not appropriate due to the formation of bubbles. However, the method was later adopted with the rotating disk in the USP (2008) (Figure 1.6). The drug substance was compressed using a steel punch, die, and a base plate. Then, the die was placed at the bottom of the flat bottomed-vessel of the dissolution tester II with one smooth surface facing upwards.

1.5.1.3 Stationary apparatus versus rotating disk apparatus

Viegas (2001) compared the two apparatus used for determining IDR, namely, the rotating disk (Wood) and the stationary disk (Distek Inc.), and noticed that different mechanisms controlled the dissolution operation; a forced shear-like process in the former and a solvent shear-like process in the latter.

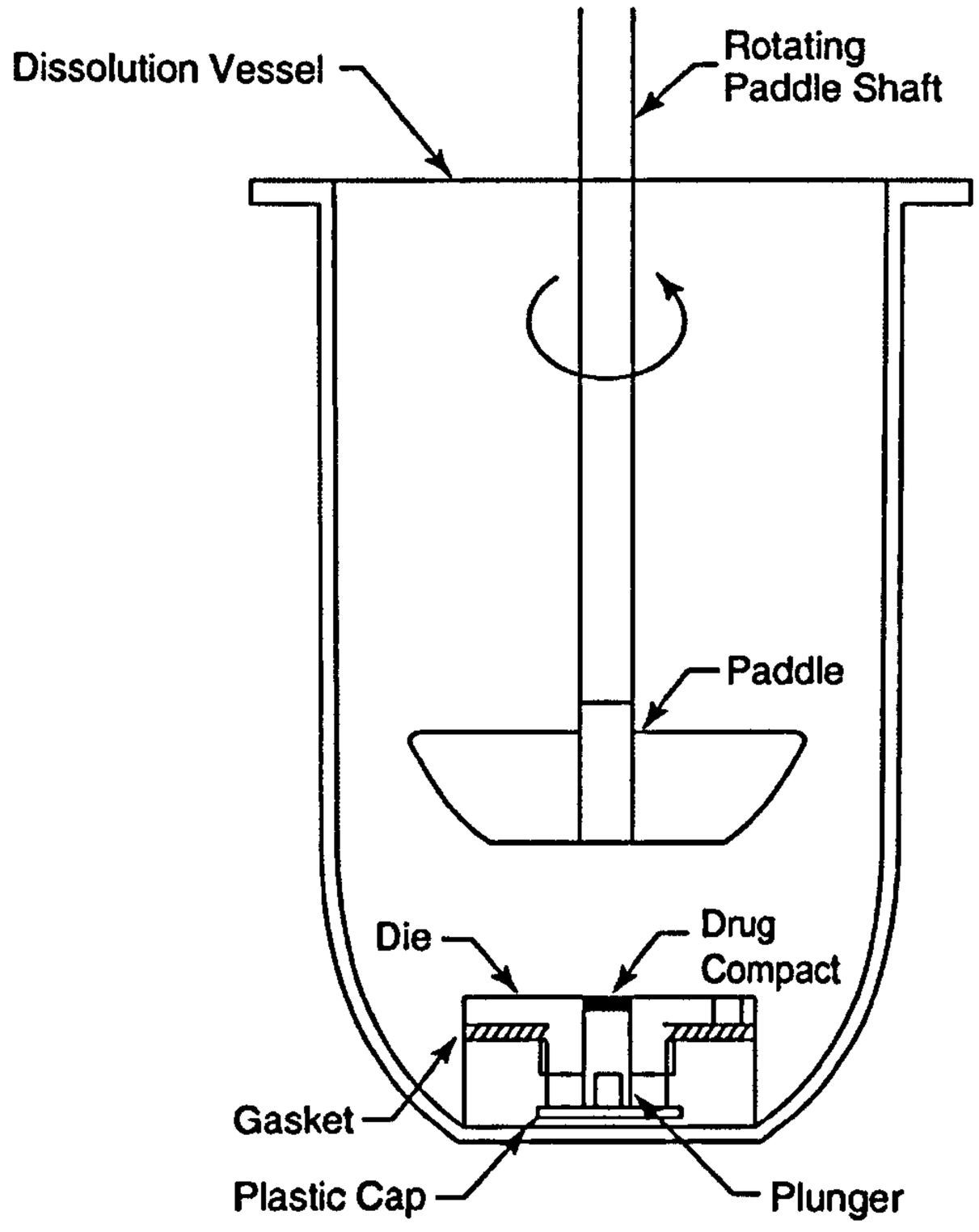


Figure 1.6 Schematic diagram for stationary disk apparatus, from the USP (2008)

The stationary disk method is a simple system that could fit into the USP Apparatus II, as it only requires the use of flat vessels, whereas a rotating disk needs special arrangements such as a shaft with a hollow die holder so that the disk can be attached to it and the surface of the compressed drug will face downward.

Two drawbacks were observed with the rotating apparatus; one due to the hydrodynamics which were more likely to cause bubbles that cover the surface of the disk, decrease the active dissolution surface and consequently lead to a low dissolution rate. The second drawback was due to less temperature control at the beginning of the experiment caused by the relatively big assembly. As by lowering the shafts heat loss through the shafts occurred which consequently decreased the temperature by approximately 2°C. No obvious changes in temperature were observed in the stationary system since the device was small and was totally immersed in the fluid in the vessel.

1.5.2 Calculations

Intrinsic dissolution rate per unit of surface area, *J*, (mass flux) was calculated by integrating the modified equation of Noyes and Whitney. Under sink conditions *C* is very small, so it can be neglected and since the surface area *S* is constant, *J* was

expressed by Farraj et al. (1989) by Eq. (1.9) where m is the mass of dissolved drug at time t.

$$J = \frac{D}{h} Cs = \frac{dm}{dt} \frac{1}{S} \qquad \textbf{Eq. (1.9)}$$

By integrating this equation, it was found that plotting of m/S against t yielded J (Eq. (1.10))

$$\frac{m}{S} = Jt \qquad \textbf{Eq. (1.10)}$$

Levich (1962) deduced Eq. (1.11) that can be applied for calculating J from rotating disk methodology, where v denotes the kinematic viscosity and Ω the angular velocity of the disk.

$$J = 0.62 D^{2/3} v^{-1/6} \Omega^{1/2} (C_s - C_t) \qquad \textbf{Eq. (1.11)}$$

The mass flux according to the Levich equation included both processes that usually occur during the dissolution process; convection and diffusion (Wall et al., 1985). Eq. (1.12) indicated that the dissolution rate from the rotating disk apparatus was proportional to the square root of the rotating velocity, where a is the proportionality constant.

$$\frac{dc}{dt} = a\Omega^{1/2} \qquad \textbf{Eq. (1.12)}$$

Colton and Smith (1972) computed the average of dissolution flux from the stationary disk in rotating fluid where a new term was introduced (ϕ): a dimensionless ratio of the fluid velocity at some axial distance below the paddle to the maximum paddle tip velocity. Based on their findings the following relationship for the dissolution flux from static disk was developed by Mauger et al. (2003) (Eq. (1.13)).

$$J = 0.77 D^{2/3} v^{-1/6} \Omega^{1/2} \phi^{1/2} (C_s - C_t) \qquad \textbf{Eq. (1.13)}$$

1.6 Factors affecting the dissolution of drug

There are various factors that affect the dissolution behaviour of drugs. These are classified as factors related to; the physicochemical properties of the drug, type of dissolution device, hydrodynamic conditions, experimental conditions such as temperature and agitation, factors related to the dosage form and its formulation such as

the excipients, coatings and the manufacturing procedures. Furthermore, *in vivo*, the dissolution process will also be affected by physiological factors.

This section will focus only on the physicochemical and physiological factors that influence drug dissolution in the upper GI tract. A detailed review is described by Banakar (1992b) and Horter and Dressman (2001).

1.6.1 Physicochemical factors

The physicochemical parameters affecting the dissolution rate of drugs are discussed herein through the modified form of Noyes-Whitney equation (Eq. (1.14)).

$$DR = \frac{dm}{dt} = \frac{DS}{h}(Cs - \frac{m}{V}) \qquad \textbf{Eq. (1.14)}$$

Where DR is the dissolution rate, m is the mass of the dissolved substance, C_s is the saturation solubility, V is the volume of the dissolution media, S is the area of the dissolving interface, D is the diffusion coefficient of the solute in solvent and h is the thickness of the diffusion layer.

1.6.1.1 Drug solubility

Saturation solubility is a fundamental factor in determining dissolution rate. Under sink conditions where $C_s >> C_t$, a linear relationship is expected from a plot of the dissolution rate versus solubility. Various physicochemical and physiological factors can affect the solubility:

1.6.1.1.1 Solid phase characteristics

Solids may exist in multiple physical forms such as crystals, amorphous forms, hydrates and solvates, and even the crystalline form can adopt different crystal structures, known as polymorphs. Polymorphism is defined as the ability of a substance to exist as two or more crystalline phases with different arrangements and/or conformations in the crystal lattice (Grant, 1999). These forms can behave differently when they are in the solid state which leads to different physicochemical properties such as melting point, chemical reactivity and density. A difference in the internal energy of the polymorph states can lead to different lattice energy and consequently different solubility. Critically, this can change the dissolution pattern and result in a different bioavailability.

In a polymorphic system, the polymorphs with the lowest energies are thermodynamically more stable while the polymorphs with the highest free energies are

the metastable forms. Because of their high energy, the solubility of the metatable forms is higher than the corresponding stable forms and they tend to dissolve faster (Gong et al., 2007).

Amorphous forms are more disordered and so their entropy and enthalpy are greater than the crystalline states, resulting in higher free energy and so greater solubility. Hancock and Parks (2000) predicted that the solubility of amorphous forms was higher than the most stable crystal form by a range of 16-fold to 1600-fold. Consequently, a solution of the metastable form had a higher concentration (supersaturated) compared to a saturated solution of the stable form. However, the less stable forms tend to revert to the stable form during the solubility experiment as the time required to reach equilibrium is relatively long which allows nucleation and so the formation of more stable forms. Therefore, in general, it is difficult to measure the solubility of the metastable form using the typical equilibrium methods. For instance, carbamazepine was found to exist in both anhydrate and dihydrate forms, with the former spontaneously converting to the latter upon contact with water. The dissolution rate of the anhydrous phase is normally higher than the hydrates but this statement is not necessarily applicable to solvates (Brittain, 1999a).

Ueda et al. (1984) demonstrated that chlorpropamide has many polymorphs of which four metastable forms exhibited higher dissolution rates. The dissolution study showed that the metastable form II of chlorpropamide instantly converted to the stable form under the dissolution conditions. Manipulation of the dissolution medium may allow inhibition of the transformation between the polymorphs. For example, hydroxylpropylmethylcellulose (HPMC) showed an ability to inhibit the conversion of the anhydride form of carbamazepin into the hydrated form (Tian et al., 2007).

1.6.1.1.2 pKa of the drug and pH of the medium

The pH of the dissolution medium plays a crucial role in the dissolution of ionisable drug substances because the solubility increases due to the contribution of the charged species. The charge state of the substance at a particular pH is expressed by its ionisation constant (Ka).

The solubility-pH profile can be predicted using simple Henderson–Hasselbalch equations. The relationship between the solubility of acidic compounds versus the pH is illustrated by Eq. (1.15) (Avdeef, 2007). At pH << pKa, a horizontal line is produced as

log S is approximately equal to the logarithm of the intrinsic solubility (log S_0). When the pH >> pKa, logarithm of solubility (log S) increases linearly with an increase in pH of the medium.

$$\log S = \log S_0 + \log\left(10^{-pKa+pH} + 1\right) \qquad \textbf{Eq. (1.15)}$$

For basic compounds, at pH << pKa the solubility increases with a decrease in pH (Eq. (1.16)). However, at pH >> pKa a horizontal line between log S and pH reflects the similarity between log S and log S_0.

$$\log S = \log S_0 + \log\left(10^{pKa-pH} + 1\right) \qquad \textbf{Eq. (1.16)}$$

Correlating the pH of the gastro-intestinal system with the pKa of a drug can explain why, when a basic drug is taken after food it is less soluble in the gastric medium which has a relatively high pH in the fed state. As an example, the bioavailability of the basic drug, ketoconazole, was reduced in hypochlorhydria (Blum et al., 1991). As a result, a basic drug delivered from the stomach to the duodenum might precipitate due to the change in pH. Kostewicz et al. (2004) developed an *in vitro* system composed of gastric-intestinal chambers where basic poorly soluble drugs were dissolved in simulated gastric fluid (SGF). The solution was then pumped into a chamber containing simulated intestinal fluid (SIF) representing fasted and fed conditions at pH 6.5 and 5, respectively. Precipitation was monitored in the acceptor. The study showed the possibility of drug precipitation and supersaturation upon entry to the small intestine at the fasted state.

The pH of the surrounding layer affects the dissolution of ionisable drugs. Different pH values were observed in the bulk medium from that recorded on the surface of the dissolving solid. Moreover, different dissolution profiles were obtained in buffered and unbuffered systems which indicated that the buffer capacity of the media can affect the dissolution (Ozturk et al., 1988).

Furthermore, different salts of the same compound can exhibit different dissolution. Li et al. (2005) found that different salts of haloperidol, the basic drug had different dissolution and solubility. Haloperidol mesylate had a higher dissolution rate between pH 2 and 5 than its hydrochloric salt or the free base.

1.6.1.1.3 Surfactants in dissolution media

Surfactants are amphiphilic molecules composed of a hydrophilic or polar moiety known as the head and a hydrophobic or nonpolar moiety, usually a lipid or a long-chain hydrocarbon, known as the tail (Malmsten, 2002). The surfactant head can be charged (cationic, anionic or dipolar) or non-ionic. In dilute solutions surfactant molecules are positioned at the interface and at a certain concentration the surfactant molecules organize, forming micelles. This point is called the critical micellar concentration (CMC) and at this point the physicochemical characteristics of the system change suddenly. Surfactants can enhance the dissolution of drugs by lowering the interfacial tension of the media and forming micelles and at concentrations below the CMC the surfactant can allow better penetration of the solving liquid to the dosage form.

The fluid in the GI system has a low surface tension due to the presence of bile salts and phospholipids, aiding the dissolution and subsequent absorption of drugs with limited aqueous solubility. Therefore, to achieve better prediction of drug behaviour, adding various surfactants to the dissolution media has been investigated extensively. One of the first dissolution studies that utilized surfactant was a comparative study of the dissolution of two drugs in gastric juice and hydrochloric acid media containing different amounts of polysorbate 80 (Tween 80) (Finholt and Solvang, 1968).

For example, the USP (2008) recommended the addition of sodium dodecyl sulphate (SDS) to study the dissolution of carbamazepine tablets (1%, w/v SDS in water) and danazol capsules (0.75%, w/v SDS in water). However, using synthetic surfactants could be problematic as they might interact with the drugs forming insoluble salts (Chen et al., 2003). SDS is the most frequently used surfactant due to its good solubilising capacity, low cost and ease of use. However, drawbacks were observed with its use as it can hydrolyze in solutions with a pH value lower than 4. Furthermore, it may interact with gelatin which makes it inapplicable for use with gelatin capsules (Zhao et al., 2004).

In addition to synthetic surfactants, the effect of natural surfactants was investigated by utilizing bile salts such as sodium taurocholic with or without phospholipids. These systems also influenced the dissolution by forming mixed micellar systems (Bakatselou et al., 1991; Mithani et al., 1996)

1.6.1.1.4 Complexation

The complexation of drugs with co-solvent materials leads to an increase in drug dissolution. The mechanism of forming complexes is due to intermolecular interactions such as dipolar and ionic bonds, London dispersion, π-bonding and hydrophobic interactions. For example, polyvinylpyrrolidone (PVP) was utilized as a complexing agent and it enhanced the solubility and dissolution of a variety of drugs. Different theories have been suggested to explain this phenomenon, for example, O'Driscoll and Corrigan (1982) attributed the enhancement in dissolution of chlorothiazide to a soluble complex formed with PVP. 1-methyl 2-pyrrolidone (NMP) was used as a solubilizing agent for itraconazole as it formed a complex with the aromatic ring and halogen groups of the drug. An aqueous solution of 8μg $ml^{-1}$of itraconazole was prepared from the poorly soluble drug (<0.1μg ml^{-1}) by adding NMP. Soluble vitamins and amino acids also increased the solubility of poorly soluble nucleosides and their derivatives such as adenine, guanosine and acyclovir through complex formation (Chen et al., 1994).

Nicotinamide and caffeine have been demonstrated to solubilize a variety of drugs by complexation through π donor–π accepter of the aromatic rings and moreover through hydrotropic effects. Lim and Go (2000) found that both agents increased the solubility of the aromatic anti-malarial agent halofantrine and caffeine did this to a greater extent. This was explained by the degree of aromaticity that affects the solubilizing capacity; caffeine has two aromatic rings while the nicotinamide has only one ring.

Cyclodextrins (CD), as cyclic oligosaccharides, can form non-covalent dynamic inclusion complexes by accommodating drug molecules in their core cavities, resulting in large increases in solubility. Chemical modification of the CD structure, such as ß-CD derivatives, resulted in an even greater solubilization capability (Challa et al., 2005). Alkylated cyclodextrins such as dimethyl-ß-CD were the most efficient in solubilizing itraconazole compared to other types of cyclodextrins (Brewster et al., 2007). Cyclodextrin's solubilisation properties have been exploited in drug formulations; for example 3-hydroxypropyl-CD was used in Sporanox™ (itraconazole) oral and parenteral solution (Challa et al., 2005).

Complexation with co-ingested food has a significant effect on drug dissolution *in vivo.* For instance, milk was found to increase the dissolution of drugs through drug binding to milk components (Macheras et al., 1990).

1.6.1.2 Surface area

1.6.1.2.1 Particle size

The dissolution rate is proportional to the surface area of the dissolving solid and since the surface area increases with decreasing particle size then the dissolution is proportional to 1/particle size. Consequently, the smaller the particle, the higher the surface area exposed to the dissolution medium (Aulton, 2002).

This was further evidenced by micronizing particles which led to an obvious increase in drug dissolution and bioavailability. For example, metronidazole dissolution increased as a result of reducing the size of drug particles (Lauwo, 1985).

Dispersibility of solids in the dissolving medium affects the active surface area because some solid powders of hydrophobic drugs tend to form aggregates during the dissolution process. For example, Finholt and Solvang (1968) revealed that although reducing the size of solid particles usually increases the dissolution rate, it did not in the case of phenacetin in 0.1N HCl. This was attributed to the poor wetting properties of the medium. By using diluted human gastric juice, which had lower surface tension, the rate of dissolution increased with a decrease in particle size. The porosity of solids also affects dissolution as more open porous particles will have a greater effective surface area exposed to the dissolving medium. Aulton (2002) stated that large pores permit fluid to penetrate and allow the diffusion of the solutes to the bulk solution.

By utilizing IDR methodology, which provides a constant surface area, the effect of variability resulting from the size of the particles, the ongoing reduction in particle size and porosity effects will be minimised.

1.6.1.2.2 Wettability

The wettability of drug particles by a liquid can be estimated by measuring the contact angle which is defined as the angle between a droplet of the liquid and the horizontal surface over which it spreads (Figure 1.7). When the contact angle is 0°, ideally, the drug is completely wetted with the liquid and if the angle is approaching 180° then the wetting is negligible.

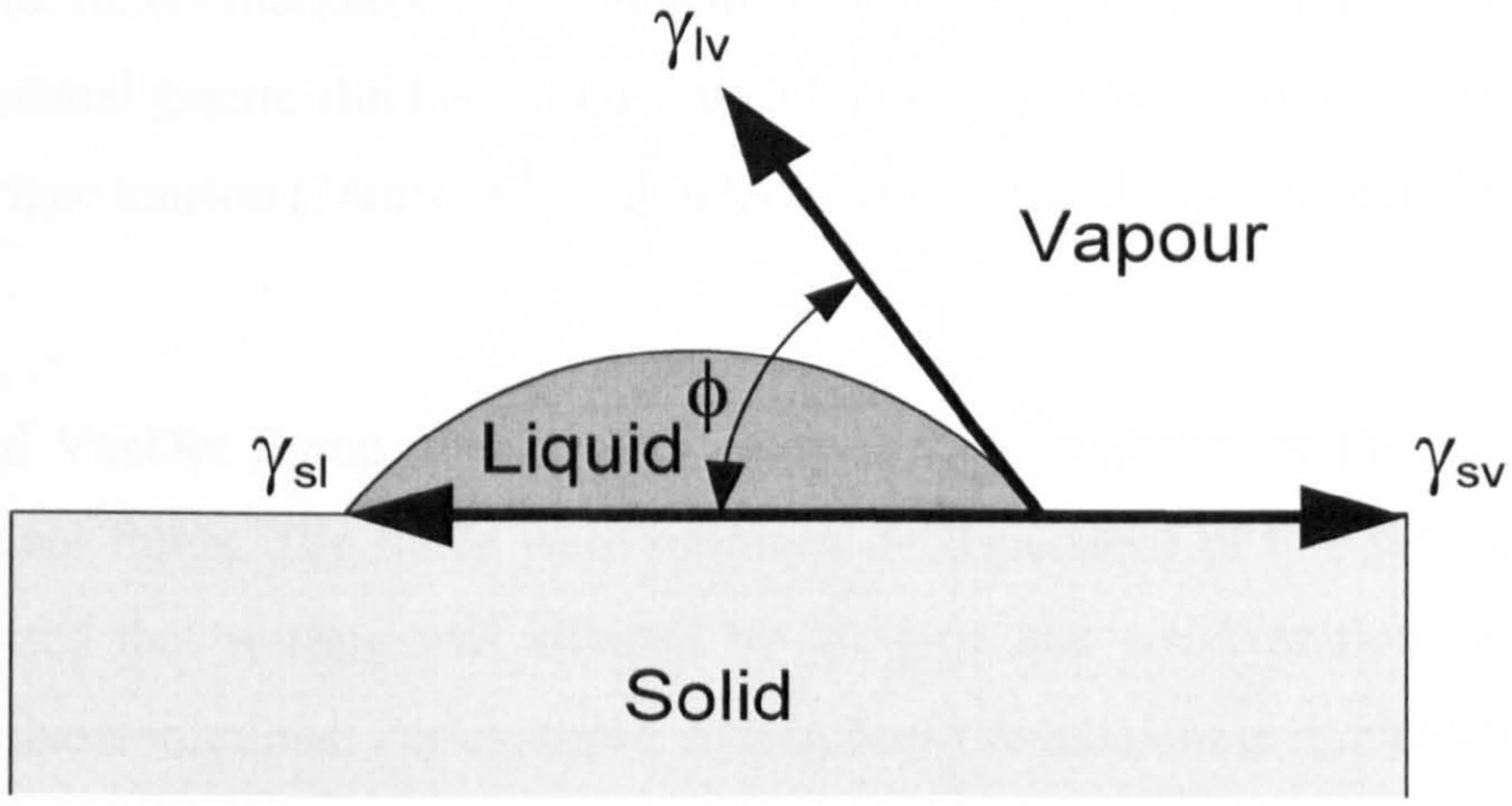


Figure 1.7 Contact angle between a drop of liquid and a solid surface

The contact angle, θ, is obtained from a balance of interfacial energy between three intersections of solid, liquid and gas and is expressed by Young's equation (Eq. (1.17)), where γ_{SL} , γ_{LV} and γ_{SV} are the solid–liquid, liquid–vapour and solid-vapour interfacial energies, respectively.

$$\cos\theta = \left(\frac{\gamma_{SV} - \gamma_{SL}}{\gamma_{LV}} \right) \qquad \textbf{Eq. (1.17)}$$

Thus, surfactants enhance the wettability by inducing changes in the surface energy to replace solid-vapour interfaces with solid-liquid interfaces.

Bile salts, which are believed to exist in the stomach due to reflux, play an important role in wetting poorly soluble drugs. Several formulations have been suggested to simulate the gastric fluid using synthetic surfactants instead of bile salts, such as Triton X-100 (Galia et al., 1999). This nonionic surfactant improved the dissolution of several dosage forms of albendazole and this effect was attributed to an enhanced wettability of the drug.

Luner and VanDer Kamp (2001b) assessed the effect of changes to the gastro-intestinal environment on the wettability of a model surface of poly (methyl methacrylate) (PMMA). This surface was chosen as representative of a polar low energy surface that might behave like a poorly soluble drug. The fluids were formulated to be physiologically-relevant to the gastric region in the fed and fasting conditions. It was found that adding lecithin (0.025mM) to taurodeoxycholate solution (0.1mM) decreased the surface tension from 58mN m^{-1} to 45mN m^{-1}. However, this decrease in surface tension was not reflected in the wettability, as the contact angle for taurodeoxycholate

solution (0.1mM) measured 58° and with lecithin added (0.025mM) it was 64°. Fed state simulated gastric fluid (FeSSGF), which is richer in bile and phospholipids, had a lower surface tension (26mN m^{-1}) and induced better wettability compared to the fasting fluids.

Luner and VanDer Kamp (2001a) also assessed the wettability of fluids that simulate the intestinal fluids. The fluids were solutions or dispersions of bile salts and lipids. It was reported that wetting was affected by the type and concentration of lipid in the micellar phase solutions. For example, adding 5mM monoolein to a micellar solution of 10mM of taurodeoxycholate in simulated intestinal fluid (SIF) resulted in a 21° decrease in wettability, whilst upon the addition of 5mM of lecithin to the same solution the contact angle increased by 7°. A significant difference in the wetting properties was observed also between fluids representing fed and fasting fluids.

1.6.1.3 Factors affecting diffusivity

The solubility of a drug depends on temperature; therefore it is very important to keep the temperature constant throughout the dissolution process. Temperature is usually maintained at 37 ± 0.5°C for oral dosage forms. The diffusion of a solute molecule also depends on the temperature of the medium. This relationship is expressed by the Stock-Einstein equation (Eq. (1.18)) where D is the diffusivity, T is the temperature, η is the viscosity, r is the radius of the drug molecule and k_b is the Boltzmann constant.

$$D = \frac{k_b T}{6\pi\eta r} \qquad \textbf{Eq.(1.18)}$$

It can be deduced from the Stock-Einstein equation for small particles that viscosity is inversely proportional to D and consequently to the dissolution rate. This phenomenon is more obvious when the dissolution of the drug is via a diffusion controlled process. The effect of viscosity on the dissolution rate is believed to be through increasing the thickness of the boundary layer (Aulton, 2002) and decreasing the penetration rate of the dissolving fluid into the particles which reduces the wetting (Parojcic et al., 2008).

The influence of viscosity on dissolution was investigated by Reppas et al. (1998) who studied the effect of elevating the viscosity of dissolution media on the behaviour of drugs. For example, when guar (2%) was added to normal saline the dissolution rate constant decreased by 2- to 7-fold. Parojcic et al. (2008) used HPMC K4M (0.5-1%) as a viscosity enhancing agent and studied the dissolution of paracetamol tablets, where it

was found that increasing the viscosity greatly delayed and prolonged the dissolution process.

1.6.1.4 Volume of dissolution media

The volume of dissolution medium is supposed to be sufficient to maintain sink conditions throughout the test. This requires the volume to be at least three times more than the saturation volume (USP, 2008). Keeping sink conditions for very poorly soluble drugs is difficult, requiring a large volume of liquid. Therefore, different approaches were developed, such as continuous flow-through cell apparatus or the addition of surfactants to the medium (Abdou, 1989a).

1.6.1.5 Boundary layer thickness

The dissolution rate is inversely proportional to the thickness of the boundary layer *h* around the dissolving particles, which depends on the hydrodynamic conditions (Levich, 1962). Factors affecting *h* are degree of agitation, including stirring speed, position and shape of stirrer, shape and dimension of dissolution vessels, the volume and viscosity of the dissolution medium (Aulton, 2002).

1.6.2 Physiological factors

The physiological conditions of the gastro-intestinal system can have a vital effect on the dissolution of drugs. Consequently, these conditions were considered by investigators for predicting the dissolution kinetics of drugs *in vivo* and developing physiologically-relevant dissolution media (Mullertz, 2007).

1.6.2.1 Surface tension of the luminal fluids

The surface tension of GI fluids is low and it varies within the different GI regions and according to fed state. The surface tension of fasting gastric aspirate was reported as 35-45mN m^{-1} (Finholt and Solvang, 1968; Efentakis and Dressman, 1998). Kalantzi et al. (2006a) measured the surface tension of the liquid in fasted stomach as 41.9-45mN m^{-1} and in the fed state, the values dropped to 30-31mN m^{-1}. The reason for this low surface tension is still a controversial issue. It has been attributed to the duodenal reflux of the bile salts to the gastric medium although some subjects showed a very low level of bile salt (0-1mM) in their gastric fluid (Efentakis and Dressman, 1998). Therefore, Kalantzi et al. (2006a) suggested that other gastric components such

as pepsin may affect the surface tension. A 0.1mg ml^{-1} pepsin solution in SGF has low surface tension (57mN m^{-1}) (Vertzoni et al., 2005).

In the small intestine, the surface tension of fasting duodenal fluid was reported as being 32.3mN m^{-1}, whereas after having a meal the value dropped to 28.1-28.8mN m^{-1} (Kalantzi et al., 2006a). This lower surface tension was attributed to the bile components. The endogenous surfactants that exist in the small intestinal fluids are bile acids, bile salts, phospholipids and cholesterol which are secreted by the bile, and fatty acids and monoglycerides produced from lipid digestion. The average bile salt concentration in intestinal fluids in fasted state was reported as being 4 to 6mM, while in the fed state the concentration is two to four times higher (Rautureau et al., 1981). The ratio between bile salts and phospholipid in the fasted conditions is between 2:1 and 10:1 and in the fed conditions it varies from 2:1 to 5:1 depending on the phospholipid level in the food (Persson et al., 2005; Persson et al., 2006).

Both bile salts and lecithin play an important role in digesting food and solubilizing poorly soluble drugs. Bile may increase the dissolution of poorly soluble drugs by enhancing the wettability of the drug and/or by increasing the solubility via micellar solubilisation (Charman et al., 1997). Wetting usually occurs when the concentration of the bile salts is below the CMC and at higher concentrations; the effect of the micelles will be predominant. For example, hydrocortisone dissolution was studied in sodium taurocholate (NaTC) solution or a mixture of 4:1 NaTC with lecithin (Naylor et al., 1993). The bile mixture formed a mixed micellar system which increased the solubility of hydrocortisone 2 times compared to the solubility in NaTC alone. Mixing NaTC with lecithin reduced the CMC from 4.7 to 0.25mM (25°C) which indicated that the mixed micelles had a more effective solubilisation capacity. Consequently, even in the fasted state micelles are expected to be formed and so both wetting and solubilisation may affect the dissolution of poorly soluble drugs (Pedersen et al., 2000a). Furthermore, during lipid digestion lipolytic products, such as fatty acids and monoglycerides are produced. These compounds interact with the bile salts and form mixed micelles that solubilise lipophilic drugs (Christensen et al., 2004).

1.6.2.2 pH profile of the gastrointestinal tract

The pH of the gastric fluid in fasting state is highly variable, pH values were measured over a range from 1 to 7 (Kalantzi et al., 2006a) exhibiting a median value of 1.7 (Dressman et al., 1990). Efentakis and Dressman (1998) found that these values are

usually in the range of pH 1 to 2. Hydrochloric acid output in a healthy human at fasted state is in the range of 0–11mEq h^{-1} whereas following ingestion of a meal this output is elevated to 10–63mEq h^{-1} (Barrett, 2006a). Despite this, the gastric pH rises to a value ranging from 3 to 7 due to the buffering capacity and diluting effect of food and this increase depends on the type of the meal. For example, thirty minutes after having a meal of Ensure plus™ the average pH of the aspirated gastric fluid was 6.4 (Kalantzi et al., 2006a). The time needed to restore the fasting pH of the stomach depended on the ingested meal. Thus, this shows the importance of gastric residence at fasted state for weak basic drugs as they are expected to be primarily dissolved in the stomach. For example, cimetidine, a H2-receptor blocker, reduced the amount of ketoconazole absorbed by about 60% by elevating the gastric pH (Van Der Meer et al., 1980). In general, the gastric medium contributes little to the dissolution of acidic drugs.

The intestinal pH is normally within the range of 4.9 to 8, but there is a pH gradient in the intestinal regions. This higher pH compared to the gastric fluid is due to neutralization of the received gastric fluid by carbonate ions that are secreted by the pancreas (Barrett, 2006a). The median duodenum pH at the fast state measured 6.2 and 120min following food ingestion the pH had dropped to 5.2 (Kalantzi et al., 2006a).

Buffer capacity of the dissolving medium mainly affects the pH of the boundary layer adjacent to the drug particle surface and consequently the C_s of the ionisable drug. The dominant buffer species in human body fluids, including the gastrointestinal fluids, are bicarbonates (Boni et al., 2007).

1.6.2.3 Viscosity of the luminal fluid

The viscosity of the luminal fluids can influence the dissolution by affecting the diffusivity of the dissolving drug. The viscosity of the luminal contents can be altered by administering food that contains soluble polymers or water soluble fibres such as guar gum, that usually work as regulators for bowel movement, or by giving a suspension of a dosage form that contains thickeners (Reppas et al., 1998).

1.6.2.4 Hydrodynamic patterns

The motility of the GIT affects the dissolution of drugs, mainly by affecting boundary layer thickness. Hence a brief introduction is provided herein outlining the motility in the proximal GIT. Between meals, the motility undergoes a cyclic motor pattern, known as the migrating motor complex (MMC). Phase I is characterized as quiescent (not

active), it lasts about 40min. Then, activity recommences by a gradual increase in contractions (phase II), which reaches a peak of intense contractions (phase III) that lasts only 10min. Phase III allows clearing of the remaining gastric contents to the intestine, and thereafter the initial conditions of phase I quiescence is returned. This whole cycle takes from 90 to 120min. After having a meal, motility events switch to the fed patterns of intermittent phasic contractions in the small intestine and the distal stomach accompanied by a tonic relaxation in the proximal stomach (Barrett, 2006b).

The absorption of a dosage form is affected by the motor pattern and meal ingestion. By analyzing the MMC stages, the first phase allows drug to dissolve since it is the longest, however if fluid is stagnant an increase in the boundary layer (h) occurs. In contrast, contractions at phase III reduce h but with a very short resident time which does not allow significant drug dissolution, and at phase II dissolution is expected to be intermediate (Horter and Dressman, 2001).

1.6.2.5 Gastric emptying time

Most drugs are not absorbed from the stomach, which serves as a reservoir, but from the intestine whose anatomical structure and mucosa means it serves as the main site for absorption. Consequently, delayed gastric emptying will delay the absorption of drugs that are absorbed mainly in the small intestine. However, for drugs which are poorly soluble in the gastric environment, longer residence in the stomach might increase bioavailability by dissolving more before passing to the absorption site in the intestinal region.

The time needed for gastric emptying is highly variable and can take from a few minutes up to 3h, depending on the dietary conditions including the type, volume of the meal and the conformation of the dosage form. Gastric emptying is usually faster at the fasting state than at the fed state. The size of the solid dosage particles affects their emptying time whereby undissolved particles with a diameter less than 0.4mm take 34-57min and particles bigger than 10mm can take up to 6 hours (Hunter et al., 1982). Emptying of drug solutions from the stomach exhibits a rapid pattern that is relatively unaffected by food digestion state (Davis et al., 1986).

1.6.2.6 Effect of Food

The effect of food on drug dissolution and absorption can be through physicochemical interactions or physiological changes. For instance, it was reported that a protein-

containing meal elevated the gastric pH due to the high buffering capacity of protein which led to a reduction in the absorption of the basic drug, indinavir (Carver et al., 1999).

Complexation and chelating interactions can occur with metal ions present in a meal such as the chelating of tetracycline with calcium in milk which led to limited absorption of the drug due to formation of insoluble complexes (Sweetman, 2009b).

Food affects the physiology by delaying gastric emptying, stimulating intestinal motility and elevating pancreatic and bile secretions, digestive enzymes and blood flow (Fleisher et al., 1999). These complex responses to food ingestion can increase or decrease absorption, which makes it difficult to predict the likely overall effect of food on systematic bioavailability (Charman et al., 1997). For example, itraconazole, which is a highly lipophilic drug, exhibited a marked increase in bioavailability when taken with food. This was attributed to an increase in drug solubility and solubilization and a longer gastric residence time. No significant effect was seen when fluconazole was co-administered with food which was postulated to be due to its hydrophilic character and weak lipophilicity (Van Peer et al., 1989).

1.6.2.7 Volume of fluid

The volume of the liquid in which the dosage form can dissolve depends on the fluid co-administrated with the dosage form and on the secretions output from the GI system. The volume of fluid in the gastric medium is 15-20ml at fasting conditions and depending on the intake volume, this can increase up to 1.5L (Horter and Dressman, 2001).

1.6.2.8 Permeability of gut wall

The permeability of the intestinal mucosa can be an indirect factor that affects drug dissolution, since the driving force for dissolution is the gradient of solubility and the concentration of drug in solution. Hence, drugs with high permeability are expected to have a constant low concentration in intestinal fluids (C_t) due to absorption which leads to more dissolution (Dressman and Reppas, 2000). The permeability is mainly governed by the chemical structure of a drug and in particular its lipophilicity expressed by the octanol-water partition coefficient (log P) (Martinez et al., 2002).

1.7 Biopharmaceutics Classification System (BCS)

1.7.1 BCS concept

In 1995, Amidon et al. (1995) introduced the Biopharmaceutics Classification System (BCS) framework, and five years later the FDA's centre for Drug Evolution and Research (CDER) adopted this system. The BCS takes into account three major factors that govern the rate and extent of drug absorption from immediate release solid oral dosage forms (IR): dissolution, solubility and intestinal permeability. Drugs were categorized by this system according to their solubility as related to dose, and permeability into four classes:

Class 1: High solubility—high permeability.
Class II: Low solubility—high permeability.
Class III: High solubility—low permeability.
Class IV: Low solubility—low permeability.

BCS guidelines (FDA, 2000) defined the terms of high solubility, permeability and rapid dissolving as follows:

"A drug substance is considered highly soluble when the highest dose strength is soluble in $\leq$ 250ml water over a pH range 1 to 7.5."

"A drug substance is considered highly permeable when the extent of absorption in humans is determined to be $\geq$ 90% of an administered dose, based on mass-balance or in comparison to an intravenous reference dose."

"A drug product is considered to be rapidly dissolving when $\geq$ 85% of the labelled amount of drug substance dissolves within 30 minutes using USP apparatus I or II in a volume of $\leq$ 900 ml buffer solutions."

Therefore, to find out whether a drug is highly soluble or not according to those definitions, the dose is divided by the solubility to provide the dose:solubility ratio (ml) and then compared to the FDA criterion for a highly soluble drug, which is 250ml. The solubility test is recommended to be performed by either the shake-flask method or acid or base titration methods.

Since the bioavailability (BA) and bioequivalence (BE) of drug products have an important role in drug development and post approval changes, the FDA included definitions for those two terms. BA is defined as "The rate and extent to which the

active ingredient or active moiety is absorbed from a drug product and becomes available at the site of action" (FDA, 2003).

Two pharmaceutical products were considered bioequivalent if they exhibited comparable rates and extents of absorption when given at the same molar dose under same conditions.

The aim of the BCS is to utilize the dissolution test as a prognostic tool for drug behaviour *in vivo* which may then be able to replace certain BE studies. So if two drug products exhibit the same dissolution profiles under all luminal conditions then it is likely that they are bioequivalent.

For high solubility–high permeability compounds (Class I) which are formulated in immediate release solid oral dosage forms and exhibit rapid dissolution *in vitro*, the BE clinical studies may be replaced by dissolution data under certain conditions (FDA, 2003). These conditions are; drug stability in the GIT, drug does not have a narrow margin of therapeutic index and the formulation does not contain any excipients that affect drug absorption. Furthermore, this biowaiver does not include drugs designed to be absorbed in the oral cavity. For some cases, considering the *in vitro* dissolution test as a replacement for *in vivo* studies would decrease the cost and time of clinical studies and reduce the unnecessary administration of drugs to healthy human volunteers.

Research studies were carried out after the inception of this system to classify drugs according to the BCS criteria. For example Lindenberg et al. (2004), classified 61 drugs from the essential medicines listed by the world health organization (WHO) based on data available from literature. Monographs of acetaminophen reported the drug as a Class III compound (Kalantzi et al., 2006c) and prednisolone as a Class I (Vogt et al., 2007).

Yu et al. (2004) suggested using the IDR instead of solubility to classify drugs since the IDR expresses the kinetics of the solubility rather than the equilibrium. Therefore, it was believed that IDR might correlate better with the *in vivo* dissolution. The IDR of a set of drugs classified in the BCS were determined and a good relationship between the IDR and BCS classification was found where a value of 0.1mg min^{-1} cm^{-2} was considered as a class boundary, taking into consideration the dose of the drug was not extremely high or low.

1.8 In vitro–in vivo correlation (IVIVC)

In vitro–in vivo correlation relates the *in vitro* dissolution of the drug with the *in vivo* pharmacokinetic data. Considering the BCS, an IVIVC is unlikely to occur with Class I drugs. High solubility means drug is dissolved in gastric media and waiting gastric emptying to be transferred to the intestine. Since the gastric site is not the main site for absorption then gastric emptying, which is not represented in the dissolution process, is the rate-limiting step for absorption rather than dissolution (Lennernas and Abrahamsson, 2005). Whereas with Class II drugs, IVIVC is likely since poor dissolution is the rate-limiting step for absorption. Class III correlation is also unlikely since the dissolution is high so dissolution is not the rate-limiting step, in fact it is the barrier properties of the GI which are controlling the absorption. With class IV drugs where both dissolution and permeability are limited, correlation is not expected (Gray et al., 2009).

There are two cases mentioned in the literature where IVIVC may not be achieved with Class II drugs (Lennernas and Abrahamsson, 2005). Firstly, when poor soluble drugs are manipulated in formulations that make them soluble, the drug would fall under the characteristics of Class I drugs instead. Thus, it was suggested to create an intermediate category, called intermediate Class II. Secondly, there are some cases where the saturation solubility and not the dissolution rate will be rate-limiting for drug absorption so the concentration of drug in the GI will be close to the saturation solubility. However, the *in vitro* standard dissolution test does not consider reaching the saturation solubility since it requires testing in non-sink conditions, which does not always prevail in the GI system. Drugs with extremely poor solubility could be examples for this case. Thus, more physiologically relevant media might improve IVIVC even though sink conditions are not maintained.

Dressman et al. (1998) confirmed the importance of the dissolution test as a predictive tool of absorption for all four BCS categories and accordingly recommended well-designed dissolution experiments where conditions simulate the GIT environment as closely as possible. For example, for Class II drugs dissolution is the limiting-factor for absorption for two reasons. Firstly, equilibrium considerations, where the fluid volumes are not enough to dissolve the dosage form. Secondly, kinetic considerations, because the time the dosage form spends before reaching its absorption window is not long enough for complete dissolution. Thus, this showed the importance of gaining

information regarding the dissolution in simulating conditions to establish meaningful IVIVC.

1.9 Biorelevant dissolution media

The dissolution media recommended by the pharmacopoeias are simple aqueous buffer systems, composed mainly of hydrochloric acid or phosphate buffer with or without enzymes (Table 1.1). The pharmacopoeias aim to achieve quality control by using simple media, which are easy to prepare and standardize and, moreover, with low costs. However, the dissolution test is supposed to evaluate the behaviour of dosage forms *in vivo* and consequently the dissolution media used should reflect the composition of the fluids encountered in the GI tract, especially when the test is used for developmental purposes.

Table 1.1 Examples of dissolution media recommended by the BP (2008)

pH	Dissolution media
pH 1.0	0.1M HCl
pH 1.2	0.05M NaCl, 0.085M HCl
pH 1.5	0.05M NaCl, 0.041M HCl
pH 5.5	0.2M Phosphate buffer or 0.05M acetate buffer
pH 6.8	0.2M Phosphate buffer
pH 7.5	0.33M Phosphate buffer

One of the early studies that formulated artificial gastric juice (Filleborn, 1948) to assess tablet disintegration took into consideration the conditions prevailing in the empty stomach and in fed conditions, such as pH, peristalsis, presence of pepsin and gastric mucin. Thereafter, efforts have been employed in this direction to develop more biorelevant dissolution media. This section gives examples of research studies developing media simulating GIT fluids and investigating their effect on drug dissolution.

1.9.1 Gastric simulated media

1.9.1.1 Simulated fasting gastric fluids

The composition of simulated gastric fluid (SGF) in the USP (2008) and BP (2008) is hydrochloric acid, sodium chloride and it may contain pepsin. However, the gastric medium has a lower surface tension (41.9 to 45.7mN m^{-1}) (Kalantzi et al., 2006a).

Therefore, to mimic the surface tension inside the stomach, surfactants were added to reduce the surface tension such as SDS (0.25%) (Dressman et al., 1998) and Triton X-100 (0.1%) in SGF (Galia et al., 1999). However, media with synthetic surfactants may lead to overestimation of the solubility of drugs (Pedersen et al., 2000b). Consequently, a formulation containing relevant physiological components was developed: NaTC (80μM), lecithin (20μM) and pepsin (0.1mg ml^{-1}) in SGF pH 1.6 (Vertzoni et al., 2005). Thereafter, it was suggested to perform the dissolution in two media; simple medium of HCl (pH 1.6) and the aforementioned physiologically-relevant medium and then combine the obtained data to achieve good simulation for drug performance in gastric fluid (Vertzoni et al., 2007).

1.9.1.2 Simulated fed gastric fluids

The composition of the gastric fluid at postprandial conditions is very variable according to the nature of the meal and the time after food ingestion. Initially, the composition of the stomach is similar to the composition of the ingested meal in terms of pH, surface tension and osmolality. However when the stomach starts the digestion process by the secretion of gastric juices followed by gastric emptying, the fasted state will be re-established (Kalantzi et al., 2006a; Mullertz, 2007). Therefore, to tackle the problem of variability of the gastric composition in fed conditions, some media were suggested to standardize simulated stomach fluids in fed conditions.

1.9.1.2.1 Milk

The use of milk as a biorelevant medium was suggested since it is a type of nutrient that contains the three basic components of fat, protein and carbohydrates in a ratio resembling the ratio in a typical diet (Dressman et al., 1998). Macheras et al. (1990) found that the solubility of a range of drugs increased in milk compared to in phosphate buffer, pH 6.5 and this increase was proportional to drug lipophilicity, fat content and temperature. The solubility of diazepam, a highly lipophilic drug, increased 7 times in low fat milk (0.75%) and 14 times in high fat milk (3.5%) compared to the buffer at 37°C. The solubility of hydrochlorothiazide and chlorothiazide also increased in milk compared to the buffer; however, the greatest increase was recorded in skimmed milk (fat content less than 0.1 %) (Macheras et al., 1989). It was demonstrated that drug entities had the ability to bind milk constituents and this binding increased when milk had a high fat content (Macheras et al., 1986). Furthermore, binding was independent of drug concentration which suggested that the drug binding was non-specific. Binding

was correlated to the lipophilicity of the drugs where a linear relationship was found between log P and log (bound drug / free drug). Although binding to milk components and solubilisation into casein micelles affected the solubility of drugs, this solubilising effect was attributed to the entire complex structure of milk (Macheras et al., 1989).

The dissolution of dosage forms in milk was investigated, for instance, the dissolution of mefenamic acid capsules increased in milk. This was explained by the effect of lipids in milk since this drug is highly lipophilic and moreover the effect of the milk pH (6.6) helped to dissolve the acidic drug (Galia et al., 1998).

The dissolution of BCS Class I drugs such as paracetamol decreased in milk whereas a significant increase was recorded with class II drugs such as danazol capsules (Galia et al., 1998). The same observations were reported by Nicolaides et al. (1999) where the dissolution of the investigated Class II dugs such as atovaquone tablets increased in milk compared to water or SIF without enzymes. However, Macheras et al. (1989) reported that low fat milk increased the dissolution of Class I drugs represented by theophylline in controlled release dosage forms.

It is worthy of note that milk may interfere with the disintegration process of dosage forms. The disintegration time of tested tablets was extended by more than five times in milk media compared to simulated gastric media at fasting state. This was attributed to the physical characteristics of milk such as high viscosity and low surface tension, which led to slow penetration of the fluid into tablets (Anwar et al., 2005).

Recently, Jantratid et al. (2008b) noticed that gastric post-prandial conditions could be represented by the stages of food digestion in the stomach. Subsequently, three compositions of simulated media were developed: early medium (pH 6.4) composed of milk with NaCl, middle medium (pH 5) composed of milk-acetate buffer (1:1) and late medium (pH 3) composed of milk-phosphate buffer (3:1). For practical interest, the solution that represented the middle stage was suggested as a global medium to reflect the composition of FeSSGF.

Although milk was considered a representative physiologically-relevant medium, in particular for the starting point of digestion (Dressman et al., 2007), drawbacks were observed with the use of milk as a standard medium where the variability in composition from region to region or even throughout the year led to difficulty in

standardisation (Klein et al., 2004). Furthermore it has a relatively high pH (6.7) which is unrepresentative of the fed gastric state.

1.9.1.2.2 Homogenised breakfast

Klein et al. (2004) discussed the possibility of using homogenised standard breakfasts as biorelevant media. The preparation of these mixtures was a difficult, time-consuming process and the composition of standard breakfasts varied among regions. Table 1.2 shows the composition of one of the standard breakfasts used in that investigation.

Table 1.2 Composition of a standard breakfast meal, from Klein et al. (2004)

Composition of standard breakfast meal
2 Slices of toasted white bread with butter
2 Eggs fried in butter
2 Slices of bacon
2 Ounces of hash browned potatoes
8 Ounces of whole milk
Carbohydrate 58g, 232kcal, 24% of calories
Protein 33g, 132kcal, 14% of calories
Fat 67g, 603 kcal, 62% of calories

1.9.1.2.3 Protein

Dissolution was investigated in media containing albumin or casein; these proteins were chosen to simulate the possible effects of food proteins on drug dissolution. Human serum albumin affected the dissolution of drugs through drug binding and casein exerted a solubilising effect through the formation of aggregates. For example, the dissolution of phenytoin powder increased in the presence of both proteins (Rosen and Macheras, 1984).

Drug solubility increased proportionally to albumin concentration. While in casein solutions, the increase in solubility was slow with low concentrations of casein until the concentration of the protein exceeded a certain point then a more significant increase in drug solubilisation was observed (Macheras and Reppas, 1986). Although both proteins affected drug solubility positively, the dissolution rate of dicoumarol was decreased. This delay in dissolution was attributed to protein increasing the interfacial barrier and formation of a drug-protein complex whose diffusivity was slower than the free drug (Macheras and Reppas, 1987).

Imai et al. (1989b) noticed that egg albumin increased the solubility of several drugs with a more pronounced effect on acidic drugs. For example, the solubility of flurbiprofen (acidic drug) in a 0.1% egg albumin solution at 20°C increased 3.2 times while the solubility of pindolol (basic drug) increased 1.2 times. The mechanism of this increase in solubility was attributed to albumin-drug interactions where two binding sites in albumin were found (Imai et al., 1991). The solubility of α-tocopherol increased 300-fold in the presence of 0.2% egg albumin at 37°C (Imai et al., 1997).

The effect of gelatin on drug solubility was investigated because firstly, gelatin as a natural polymer was considered a candidate drug carrier and secondly the bioavailability of digoxin increased when administrated in gelatin soft capsules compared to tablets. This raised the question of whether gelatin was increasing the solubility of the drug (Johnson et al., 1986). Gelatin increased the solubility of a range of drugs which was explained by the ability of gelatin to enhance the wettability of the drug. The maximum increase was recorded with lipophilic and poorly water-soluble drugs (Kallinteri and Antimisiaris, 2001). The solubility of the acidic drugs was greater when the drugs were not ionised, for example, the solubility of nitrofurantion in 0.5% gelatin-containing solution increased by 85% at pH 3.7 and increased only by 15% at pH 7. Acarturk et al. (1992) reported that gelatin and egg albumin did not induce a marked increase on the solubility of nifedipine. It was previously noticed that the solubility of basic drugs was enhanced in 0.0075% gelatin solution but acidic drugs were unaffected (Imai et al., 1989a).

1.9.1.2.4 Nutrional liquids

Intralipid™, Nutridrink™ and Nutrison™ emulsions are artificial nutritional substitutes with the same basic composition of purified soya oil, egg phospholipids and glycerol and a final pH of 7-8. Ashby et al. (1989) suggested utilizing diluted Intralipid™ emulsions to simulate fed gastric fluid since it could be a better standardized solution compared to milk or homogenized meals. It was noted that the release profiles of tetracycline tablets were altered in Intralipid™ compared to a solution of HCl (Buckton et al., 1989). Junginger et al. (1990) employed Nutridrink™ as a dissolution medium and noticed that a decline in the release of theophylline occurred. This was attributed to food particles being adsorbed onto the surface of the dosage form preventing the release of the drug from the carrier. Abrahamsson et al. (2004) examined the effect of a diluted nutritional drink (Nutrison™) on the disintegration process. Tablet disintegration was delayed in the test medium compared to a simple buffer, which was elucidated by a

precipitation of components, in particular proteins, that formed a film on the surface of tablets.

Another suggestion for simulating the fed stomach was to use the nutritional drink Ensure plus™. Klein et al. (2004) noticed that this drink resembled the composition of a typical western diet and matched the physiochemical parameters of homogenized breakfast when it was mixed with a viscosity enhancer (0.45% pectin). A mixture of Ensure™ with buffer or HCl was found to alter the dissolution of theophylline and tetracycline dosage forms (Ashby et al., 1989; Buckton et al., 1989)

1.9.1.2.5 Fat

To mimic a high fat meal, the contents of propranolol capsules were pre-treated with peanut oil prior to dissolution which resulted in a decrease in dissolution rate of the dosage forms (El-Arini et al., 1989). Fatty media containing 10% peanut oil were utilized to study the dissolution of theophylline controlled release formulations which induced variable effects on the dissolution (El-Arini et al., 1990).

1.9.2 Simulated intestinal fluids

Based on the composition and the physiochemical parameters of the intestinal fluids including pH, buffer capacity, surface tension and osmolality, simulated intestinal fluids were developed. Two formulae representing fast and fed states were published in the International Pharmaceutical Federation (FIP) guidelines (Aiache et al., 1997). Thereafter, simulated intestinal fluid in fasting (FaSSIF) and fed states (FeSSIF) were further developed (Galia et al., 1998). These media were employed in studying the dissolution of dosage forms of Class I drugs, such as acetaminophen and metoprolol which did not show a significant difference in their dissolution rate compared to that in water. The dissolution of Class II drugs increased in the biorelevant media. For instance, dissolution of danazole (Class II) capsules increased 30-fold in FaSSIF and 100-fold in FeSSIF, compared to water or SIF. Nicolaides el al. (1999) investigated the dissolution of weak acid and non-ionisable lipophilic compounds from BCS Class II. An increase in dissolution rate due to the presence of bile salts/lecithin was observed. Good agreements were revealed between the *in vitro* dissolution of the dosage forms and their bioavailability at pre- and postprandial conditions. Kostewicz et al. (2002) evaluated the dissolution of four poorly soluble basic drugs in these media and found good correlation between the dissolution and bioavailability in both fed and fast states.

The possibility of using FaSSIF and FeSSIF to forecast the *in vivo* behaviour of drugs was further demonstrated in several studies (Dressman and Reppas, 2000; Dinora et al., 2005). Wei and Lobenberg (2006) could predict the bioavailability parameters of glyburide (Class II) based on drug dissolution in FaSSIF combined with permeability measurements.

Some modifications to FaSSIF and FeSSIF were considered in order to develop media to achieve better IVIVC. Potassium cations were replaced by sodium ions to better simulate the physiology, this substitution did not affect the dissolution of the tested dosage forms (Vertzoni et al., 2004). Furthermore, the replacement of the expensive pure bile salts with cheaper mixtures of crude sodium taurocholate from ox bile and lecithin with partially hydrolysed Soybean phosphatidyl choline was suggested (Vertzoni et al., 2004). In some cases, such as ketoconazole, where the drug was highly lipophilic, using crude bile salts produced profiles more similar to the *in vivo* performance. Nevertheless it was difficult to standardize the crude bile salts due to batch to batch variation and the presence of other substances that may indirectly affect dissolution. The type of fatty acid affected only the dissolution of highly lipophilic compounds, which suggested the possibility of substituting the lecithin with partially hydrolysed phosphatidylcholine in the simulated fluids. The use of the crude products was also advised by Sunesen et al. (2005) but assaying the bile salt components was essential prior to their use in the dissolution media.

It is important to note that most of the developed simulated intestinal formulations used phosphate buffer due to its buffer stability, even though this buffer does not exist in natural fluids (Dressman and Reppas, 2000). Bicarbonate buffer is the predominant buffer in the upper GI in fasting conditions; however, its use is impractical because it has to be freshly prepared, has poor stability and requires a constant supply of CO_2 to the dissolution medium. Boni et al. (2007) reported that bicarbonate buffer caused a problem with reproducibility of drug release because bubble formation caused variations due to mechanical stress and a change in pH. Furthermore, when bicarbonate buffer was employed in FaSSIF and FeSSIF, problems of extreme foaming of the dissolution media made the use of this buffer impossible.

Vertzoni et al. (2004) demonstrated that the type of the buffer used in the biorelevant medium can affect the dissolution of basic drugs with pKa > 5 and extremely lipophilic compounds. Moreover, the buffer type may affect the stability of the compounds. It was

found that hydrolytic and oxidative reactions were decreased when replacing acetate by citrate buffer in FeSSIF.

Using aspirated human intestinal fluid (HIF) as a natural medium could produce the best IVIVC but it is not easily available (Dressman et al., 2007). Therefore, canine intestinal medium was studied as an alternative natural fluid. In general, the solubility and dissolution in canine fluids did not show good correlation with that in human fluids due to the higher bile salt and phospholipid contents in canine fluids (Vertzoni et al., 2007). Nevertheless, Persson et al. (2005) found good correlation in respect to solubility and intrinsic drug dissolution between fed dog intestinal fluid (DIF) and fed HIF but only when subjects were given small meals. Kalantzi et al. (2006b) demonstrated DIF was a good surrogate to HIF at fasting conditions with regard to the solubility of two basic drugs, dipyridamole and ketoconazole.

The lack of ingested lipids which originate from lipid digestion in FeSSIF may lead to underestimation of dissolution for highly lipophilic drugs (Nicolaides et al., 1999). Therefore, fatty acids and monoglycerides were added to FeSSIF to better mimic the intestinal fluid (Sunesen et al., 2005) which in turn led to better IVIVC for danazole dosage forms compared to FeSSIF. Furthermore, FeSSIF was found to include greater amount of bile salts than the human aspirate fluid (Kalantzi et al., 2006b).

A recent study updated the composition of FeSSIF with the aim to better mimic the intestinal fluid at fed state (Jantratid et al., 2008b). Three media were developed to reflect changes in the luminal fluids according to the phases of digestion; early, middle and late. Thereafter, a new version of FeSSIF, (pH 5.8) containing monoolein and oleic acid in addition to bile salts and phospholipids, was recommended as a standard simulated intestinal medium for the general assessment of drug dissolution.

1.10 Azole anti-fungal drugs

1.10.1 Introduction

Azole anti-fungal drugs are used in the treatment of a variety of fungal infections. They have a fungistatic effect when taken in low concentrations and are fungicidal at higher concentrations. A weakly basic imidazole or 1,2,4 triazole moiety substituted at N-1 with a lipophilic root are essential for the antifungal activity of this group. These compounds usually contain five or six membered rings (Thomas, 2000).

Ketoconazole was synthesized in 1977 and it possessed a broad spectrum of antifungal activity (Fromtling, 1988). Thereafter, there was the introduction of the first-generation of triazoles, such as itraconazole and fluconazole, which represented a more advanced fungal treatment and showed promise as systemic antifungal drugs. In general, the triazole compounds exhibited greater potency and less toxicity than the imidazole-derivatives. Subsequently, a second-generation of triazoles was developed, to overcome problems raised with first generation such as pathogenic resistance.

Itraconazole and ketoconazole were chosen from the azole antifungal family for the present investigation due to their poor water-solubility and good permeability which make them BCS Class II drugs (Six et al., 2004). Moreover, they are widely used antifungal agents, available for oral administration and, most importantly, their bioavailability is food-correlated. Itraconazole previously showed variability in the absorption and plasma drug concentrations when taken as capsules which was problematic for systematic application (Boogaerts and Maertens, 2001).

Itraconazole may be given orally or intravenously. It is marketed in the UK as either capsules containing 100mg of itraconazole coated on sugar spheres or as a 10mg ml^{-1} oral solution in 40% hydroxypropyl-β-CD. The drug is dosed as 100-400mg and 200mg via the oral and parenteral routes, respectively. Ketoconazole is available as tablets (200mg) and a topical agent (2% cream) with a maximum dose of 7.5mg kg^{1} via the oral route (Sweetman, 2009a).

Although fluconazole and miconazole are widely used azole antifungal agents, they were not suitable candidates for the present study. Fluconazole is classified as class I because of its high water solubility (8mg ml^{-1} at 37°C) and good permeability (Lindenberg et al., 2004). Its rate and extent of GI absorption are not affected by food or changes in the GI environment (Zimmermann et al., 1994). Miconazole, an example of an imidazole anti-fungal compound, is slightly soluble in water (1μg ml^{-1} at 37°C) and classified as class II. Although it is a highly lipophilic compound, it is incompletely absorbed after oral administration as the solubility to dose ratio is small (Zhao et al., 2002) so it is primarily used topically. The main purpose of the study was investigating the effect of GI–relevant media on solubility and dissolution of poorly soluble drugs and hence miconazole was not a candidate for this study.

1.10.2 Physicochemical properties

1.10.2.1 Itraconazole

Itraconazole has the molecular formula $C_{35}H_{38}Cl_2N_8O_4$ (Figure 1.8) with a molecular weight of 706. It can be characterized as a highly lipophilic compound and a very poorly soluble, weak base. Its pKa is 3.6 with an octanol-aqueous buffer (pH 6) partition coefficient (log P) > 5 and a melting range of 165–169 °C. It is insoluble in water, very slightly soluble in alcohols and freely soluble in dichloromethane (Sweetman, 2009a).

Itraconazole is composed of two triazole rings, piperazin, dioxolan, dichlorophenyl and two phenyl rings. Four of the nitrogens are potentially ionisable (Peeters et al., 2002). As postulated from proton and carbon nuclear magnetic resonance (NMR) analysis of itraconazole (dissolved in deuterated chloroform, methanol and water), by adding one equivalent of deuterated hydrochloric acid to itraconazole, the nitrogen N_{26} of the piperazine ring (Figure 1.8) was ionised proving that this nitrogen has the highest proton affinity. By adding another equivalent of acid, the nitrogen N_{11} in the triazole ring was protonated. Then an excess of the acid was added which resulted in the ionisation of the entire triazole ring. Peeters et al. (2002) calculated the pKa values of these groups and determined that pKa = 4 was associated with the protonation at N_{26} of the piperazine ring. An estimated value of 1.5–2 was assigned to the ionisation constant of N_{11} in the triazole ring. The other two ionisation constants were assigned to N_9 and N_{13} in the triazole ring and they are only ionised at very low pH < 2. Consequently, itraconazole is only fully ionised under very acidic conditions such as that of gastric fluid in fasting conditions.

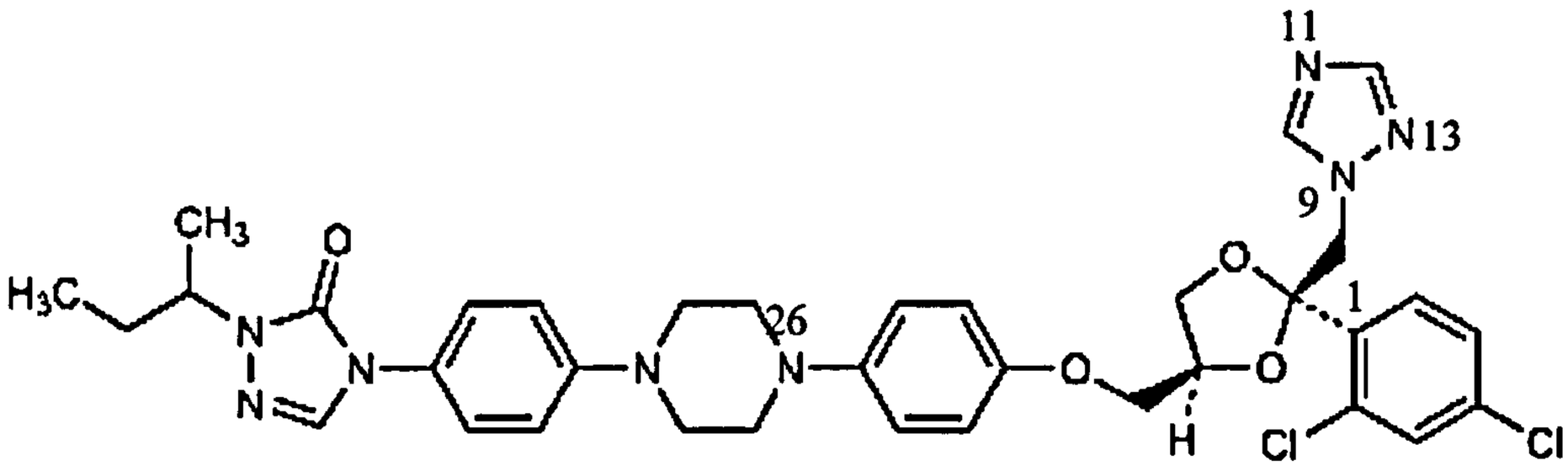


Figure 1.8 Chemical structure of itraconazole : 4-[4-[4-[4-[[cis-2-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazin-1-yl]phenyl]-2-[(1RS)-1-methylpropyl]-2,4- dihydro-3H-1,2,4-triazol-3-one

1.10.2.2 Ketoconazole

Ketoconazole has the molecular formula $C_{26}H_{28}C_{l2}N_4O_4$ (Figure 1.9) and a molecular weight of 531. It is practically insoluble in water and freely soluble in methanol and diluted hydrochloric acid (Sweetman, 2009a). Its melting point is 146°C and log P is 3.73.

The drug is a weak dibasic compound as its structure presents two basic groups, a piperazine ring and an imidazole ring, with pKa values of 2.94 and 6.51, respectively. Thus, there are two basic centres for protonation in ketoconazole structure, N_{26} of the piperazine ring and N_{11} of the imidazole ring.

The percentage of protonation on the piperazine ring mostly determines drug solubility and the *in vitro* antimicotic activity of the drug (Minagawa et al., 1983). Thus, at pH = pKa_2= 6.5, the imidazole ring will be 50% ionised while the piperazine ring will be virtually unionised. At pH = pKa_1 = 3 all the imidazole ring and 50% of the piperazine ring will be ionised under acidic conditions. By further acidifying the medium both rings will be fully protonated which explains the importance of an acidic gastric environment to dissolve the drug.

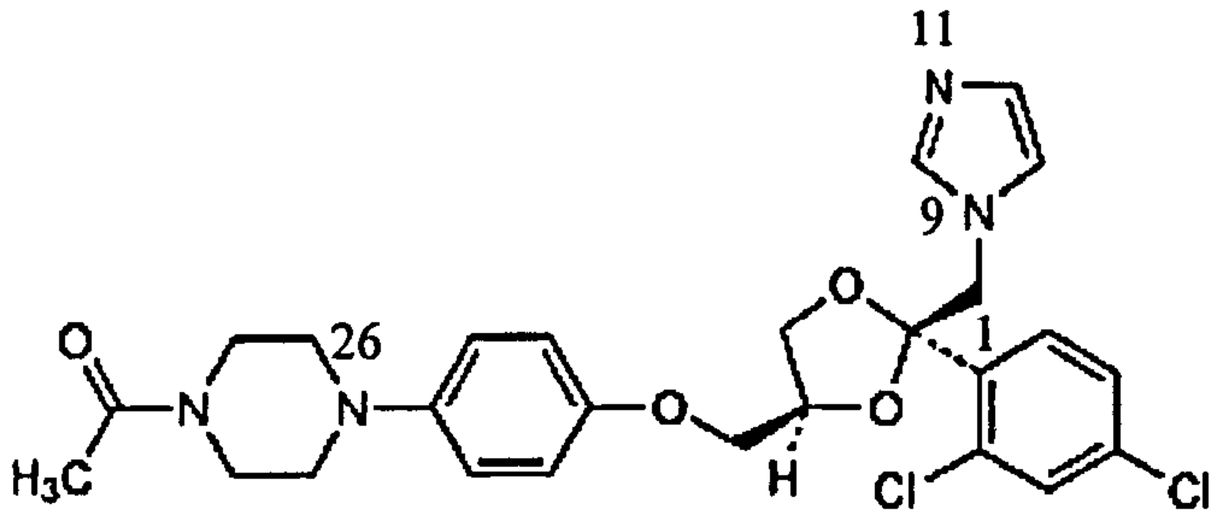


Figure 1.9 Chemical structure of ketoconazole: 1-acetyl-4-[4-[[(2RS,4SR)-2-(2,4-dichlorophenyl)-2-(1H-imidazol-1-ylmethyl)-1,3-dioxolan-4 yl]methoxy]phenyl]piperazine

Ketoconazole is not as poorly soluble as itraconazole; however, both compounds exhibit strong pH dependent solubility. Due to the poor solubility and good absorption of both drugs, they are classified in the BCS as class II drugs (Amidon et al., 1995). The poor solubility of the drugs, which is insufficient for the whole dose to be dissolved in the gastro-intestinal fluid under normal conditions means the drugs have dissolution-limited oral absorption.

1.10.3 Mechanism of action

Both categories of the azole drugs, imidazole and triazoles, have the same mechanism of action inhibiting the biosynthesis of ergosterol, a major component of the cell

membrane of yeast and fungal cells. The free azole nitrogens (position 11 of imidazole and position 11 of triazole ring) are believed to be the active sites. These nitrogens compete for oxygen at the catalytic haem iron atom of cytochrome P-450 enzymes, thereby blocking the action of this enzyme (Saag and Dismukes, 1988). Itraconazole has a broader spectrum of antifungal activity, more effective and better tolerated than ketoconazole. This is due to a higher affinity for fungal cytochrome P-450 than ketoconazole, but a low affinity for mammalian cytochrome P-450, and because its main metabolite (hydroxy-itraconazole) has also considerable antifungal activity.

1.10.4 Pharmacokinetics

Both drugs are highly protein bound (≥ 99%) and widely distributed in the body (Hoesley and Dismukes, 1997). The schematic diagram in Figure 1.10 displays the pharmacokinetics of itraconazole administered orally. Dissolution takes place in the stomach and subsequently the drug is absorbed in the intestine and passes into the blood stream. The drug is extensively metabolized in the liver producing a large number of different metabolites, the major active metabolite being hydroxyl-itraconazole (De Beule, 1996).

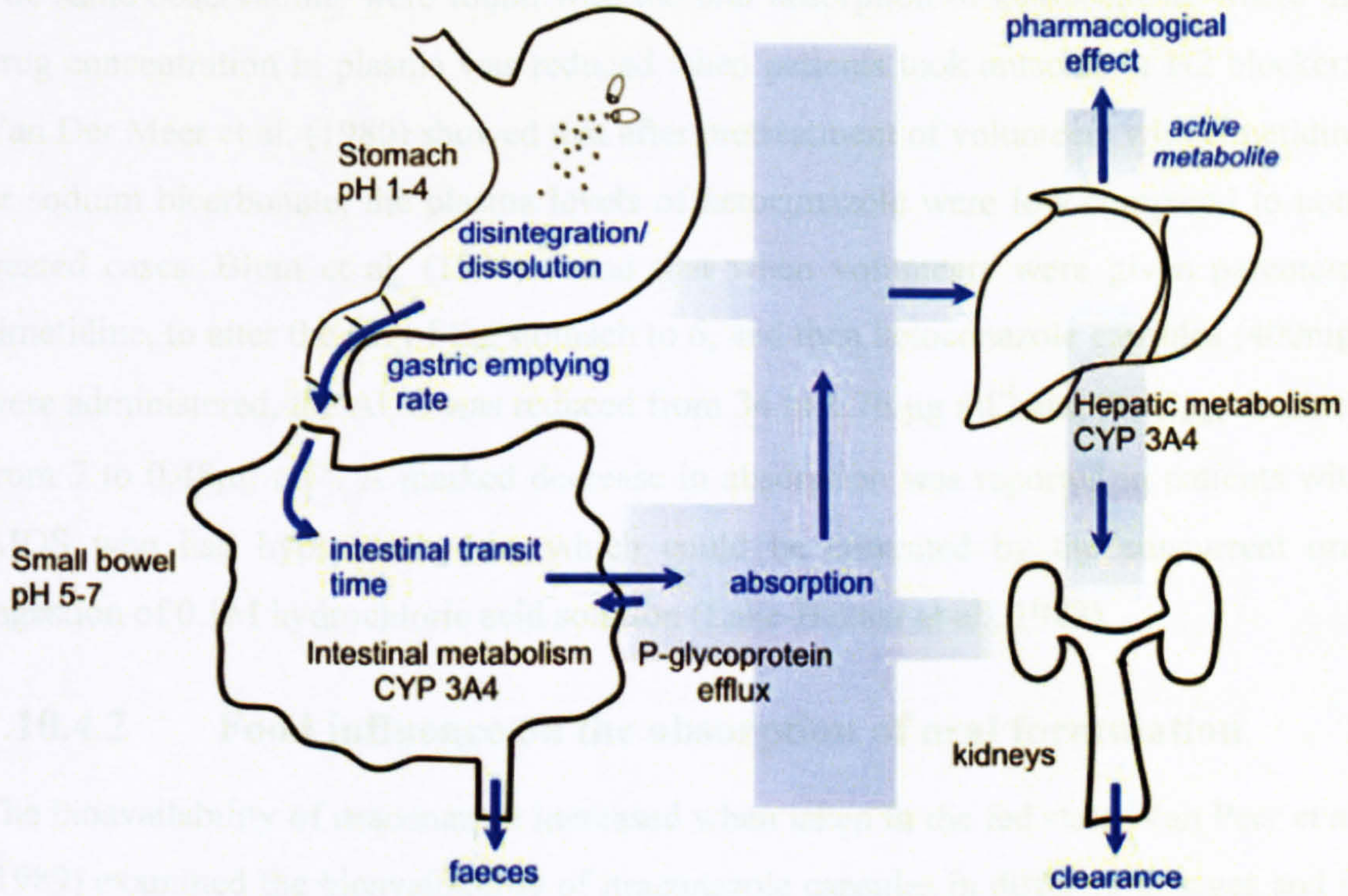


Figure 1.10 Dissolution, absorption and metabolism of itraconazole, from Lewis (2002)

1.10.4.1 The effect of gastric acidity on bioavailability

The absorption of itraconazole under fasted conditions is related to gastric acidity. The bioavailability of the drug was reduced in individuals suffering from relative or absolute achlorhydria. Lohitnavy et al. (2005) demonstrated that when gastric acidity was reduced by co-administration of itraconazole capsules with an antacid suspension (aluminum hydroxide and magnesium hydroxide) 5min prior to itraconazole administration, a decrease in the rate and extent of itraconazole absorption was observed. The time to reach the maximum concentration, t_{max}, increased from 3 to 5.1h while the peak plasma concentration (C_{max}) was reduced from 146 to 43.6ng ml^{-1}. Lange et al. (1997) noticed that when subjects were pretreated with H2 blockers (ranitidine) to increase the pH of the stomach to 5 a decline in C_{max} and area under curve (AUC) of 40-50% was estimated. To compensate for the effect of ranitidine on gastric acidity an acidic beverage of cola (pH 2.27) was co-administrated with itraconazole. The pharmacokinetic parameters were approximately the same as when itraconazole was given alone. Finally, hypochlorhydria is a common complication in acquired immune deficiency syndrome (AIDS) patients and it was found that the serum concentration of itraconazole was less than that recorded in normal subjects (Cartledge et al., 1997).

The same observations were found with the oral absorption of ketoconazole where the drug concentration in plasma was reduced when patients took antacids or H2 blockers. Van Der Meer et al. (1980) showed that after pretreatment of volunteers with cimetidine or sodium bicarbonate, the plasma levels of ketoconazole were low compared to non-treated cases. Blum et al. (1991) found that when volunteers were given parenteral cimetidine, to alter the pH of the stomach to 6, and then ketoconazole capsules (400mg) were administered, the AUC was reduced from 34 to 1.7h μg ml^{-1} and the C_{max} declined from 7 to 0.48μg ml^{-1}. A marked decrease in absorption was reported in patients with AIDS who had hypochlorhydria, which could be corrected by the concurrent oral ingestion of 0.1M hydrochloric acid solution (Lake-Bakaar et al., 1988).

1.10.4.2 Food influence on the absorption of oral formulation

The bioavailability of itraconazole increased when taken in the fed state, Van Peer et al. (1989) examined the bioavailability of itraconazole capsules in different dosages and in solution in the fast and fed states. To simulate the fed condition, the capsules were given directly after a meal of standard breakfast comprising bread, ham, cheese, butter, jam and coffee. Food increased the C_{max} by 3.4-fold and the AUC by 2.6-fold.

When itraconazole capsules were given after the ingestion of a standard breakfast, an increase in the systemic availability of the drug was noticed. The composition of the standard breakfast was orange juice, egg, bacon, toast, butter, jam, whole milk and banana. The AUC increased under postprandial conditions by 1.6-fold and the C_{max} elevated by 1.7-fold (Barone et al., 1993).

Zimmermann et al. (1994) conducted a comparative study investigating the effect of food on the absorption of itraconazole capsules. The ingested meal was divided into different patterns, namely a light meal composed of bread, jam, and decaffeinated coffee, and a heavy meal composed of bread, butter, fried eggs, sausages, bacon and decaffeinated coffee. Bioavailability increased when drug was administered under postprandial conditions. The AUC measured after heavy meal ingestion was the greatest where the bioavailability were 54% in fasting conditions and 86% after a light meal, considering heavy meal conditions as 100%. This increase in absorption was attributed to a long gastric retention time, high fat content of the coadministered meal and the low gastric pH that was reported in their investigation.

Regarding the effect of food on ketoconazole bioavailability, some contradictions appear in the literature. When a 200mg dose of ketoconazole was given with a breakfast meal to 30 patients, the drug concentration was higher after 1-2h of dose administration compared to fasting conditions (Gascoigne et al., 1981). However, Daneshmend et al., (1984) reported no significant effect of food on the bioavailability of the ketoconazole when given with breakfast food. This study showed that there was an increase in AUC upon ingestion with food but that this was not significant. Mannisto et al. (1982) performed the test on subjects who were given ketoconazole after a standardized meal or 300ml of orange juice. Contrary to others finding, food ingestion led to a decrease in AUC and C_{max} of approximately 1.6. Co-administration with orange juice did not significantly change the pharmacokinetic parameters from the fasting state apart from a decrease was observed with the t_{max}. The authors explained their contradictory results by the type of the standard breakfast administered as their meal was rich in carbohydrate and low in fat while other studies used a meal with a richer fat content.

1.11 Aims and objectives of this study

It is desirable in drug discovery and formulation development to forecast drug dissolution in the gastrointestinal tract since dissolution is a prerequisite for drug absorption and subsequent bioavailability.

Dissolution is an important issue for poorly soluble drugs in particular as it can be the rate-limiting step for absorption. Therefore, two poorly soluble drugs (itraconazole and ketoconazole) were chosen for this investigation. These two drugs are azole orally administered antifungal agents classified as Class II according to the BCS due to their poor solubility and good permeability and their bioavailability is affected by food ingestion. Furthermore, most of the innovative drugs are poorly soluble basic drugs which made the two drugs good candidates to examine the effect of biorelevant media on their solubility and dissolution characteristics.

The main objectives of this thesis were to gain an understanding of the *in vitro* dissolution of poorly soluble drugs in biorelevant media and thereby improve the ability to predict the behaviour of the drugs *in vivo*. To achieve this goal biorelevant media were developed based on the fact that in the gastrointestinal tract drugs are exposed to a medium of partially digested food, comprising mixtures of fat, protein and carbohydrate. Thus, the intentions of this thesis were to:

- Investigate the solubility and intrinsic dissolution behaviour of itraconazole and ketoconazole in simple compendial media, with or without enzymes, and understand the effect of pH on these properties.
- Study food-induced effects on drug behaviour in simulated gastric fluids by determining the solubility and dissolution (IDR) in the proposed dietary media in comparison to compendial media. The dietary additives were:
    1. Milk with different fat contents.
    2. Proteins: albumin, casein, gluten and gelatin.
    3. Carbohydrates: glucose, lactose and starch.
    4. Amino acids: lysine, glycine, alanine and aspartic acid.
    5. Fat emulsions: Soybean oil emulsions.
- Investigate the effect of simulated intestinal fluids containing natural surfactants, bile salts and phospholipids (sodium taurocholate and lecithin), representing key endogenous digestive materials, on the dissolution of the drugs.

- Investigate the possibility of utilizing synthetic surfactants to develop simplified simulated intestinal fluids which may replace the expensive bile salts media.
- Evaluate the dissolution of the drugs in fasted state-simulated gastric fluids containing synthetic and natural surfactants using formulations proposed in literature.
- Assess the effect of the viscosity and surface tension of the investigated media on the dissolution of the drugs. Furthermore, to evaluate the effect of the dissolution media on the wettability of itraconazole and ketoconazole compact surfaces.

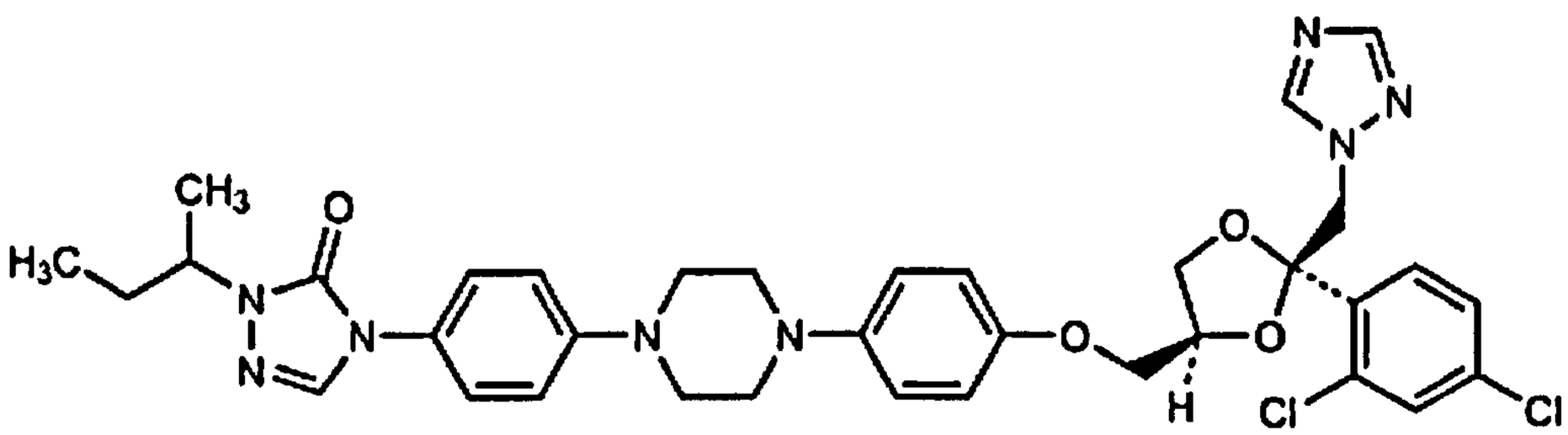
Chapter 2: Materials & General Methods

2.1 Materials

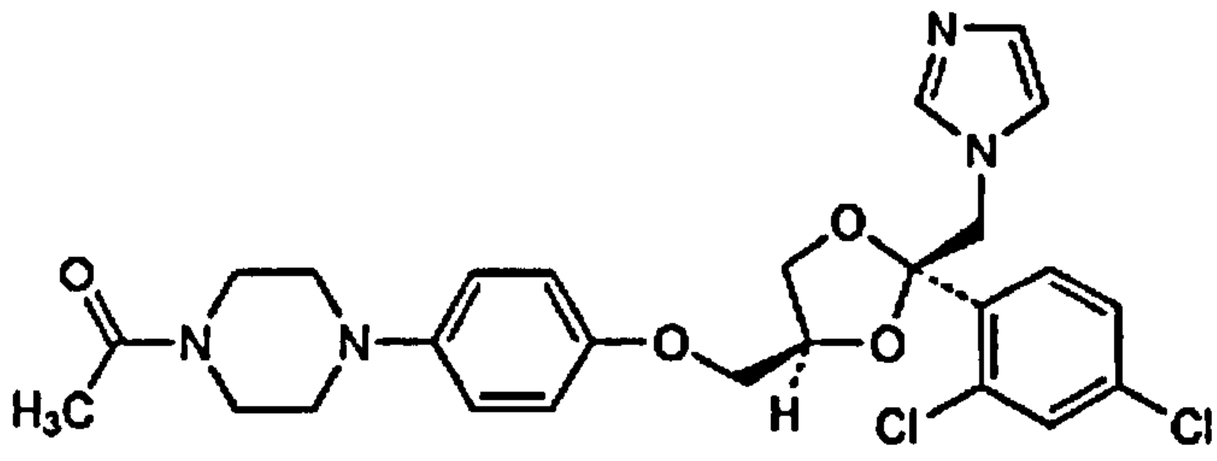
A list of the materials used throughout the project is provided below.

2.1.1 Drug substances

Itraconazole powder (batch number 20050303) and ketoconazole powder (batch number 060910) were purchased from Medichem, China Group Company (Shenzhen, Guangdong, China). Itraconazole donated by Jansssen Pharmaceutica was used for the initial studies. Figure 2.1 shows the chemical structure of the drugs studied.



Itraconazole



Ketoconazole

Figure 2.1 The chemical structures of itraconazole and ketoconazole

2.1.2 Dissolution media

A list of materials used in the preparation of dissolution media and their suppliers is given in Table 2.1

Table 2.1 Materials used in the preparation of dissolution media

Material	Source	Batch number
ß-Alanine	Sigma-Aldrich Chemie, Steinheim, Germany	1259810
Albumin (from hen egg white)	Fluka, Sigma-Aldrich Chemie, Steinheim, Germany	1143891
DL-Aspartic acid	Sigma-Aldrich Chemie, Steinheim, Germany	075K0705
Casein from bovine milk (Technical grade)	Sigma-Aldrich Chemie, Steinheim, Germany.	103k0680
Fresh pasteurised bovine milk containing different fat contents	Express dairies, Liverpool, UK	The fat contents: Skimmed milk (0.1%) semi-Skimmed (1.7%) whole fat milk (3.6%)
Gelatine from bovine skin, Type B ~75 bloom	Sigma-Aldrich Chemie, Steinheim, Germany	015k0221
Glacial acetic acid	BDH, Poole, UK	Anala R, K23898917
Glucose	BDH, Poole, UK	Anal R, K33805414
Gluten from wheat	Sigma-Aldrich Chemie, Steinheim, Germany	114k0077
Glycine	Sigma-Aldrich Chemie, Steinheim, Germany	034k0166
Hexadecyltrimethyl ammonium bromide (CTAB)	Sigma-Aldrich Chemie, Steinheim, Germany.	113k0092
Hydrochloric acid (d 1.18, 36.5 - 38%)	BDH, Poole, UK	GPR, K32540451 343
L-Lysine	Sigma-Aldrich Chemie, Steinheim, Germany.	1269151
Lactose, monohydrate N.F spray dried	Foremost, Baraboo, Wisconsin, USA	3RF908
Lecithin Egg phosphatidylcholine (Lipoid E P C, 96%)	Lipoid GmbH, Ludwigshafen, Germany	Donated by Lipoid GmbH (lot 108024-2)
DL-Leucine	Sigma-Aldrich Chemie, Steinheim, Germany	AnalaR, A651725

Table 2.1 continued from previous page:

Pancreatin powder	Acros Organics, New Jersey USA	A014159201
Pepsin powder	Acros Organics, New Jersey USA	A017535901
Potassium dihydrogen phosphate	BDH, Poole, UK	AnalaR, 102034B
Sodium acetate	BDH, Poole, UK	AnalaR, K91214705339
Sodium chloride	Fluka Analytical-Sigma-Aldrich Chemie, Steinheim, Germany	1360238
Sodium dihydrogen orthophosphate 1-hydrate	BDH, Poole, UK	AnalaR, A856721
Sodium hydroxide pellets	BDH, Poole, UK	B449148 481
Sodium lauryl phosphate	BDH, Poole, UK	30175
Sodium taurocholate hydrate, 97%	Alfa Aesar, Lancaster, UK	10111536
Soybean oil	Sigma-Aldrich Chemie, Steinheim, Germany	115k0061
Starch soluble	BDH, Poole, UK	1160402
Triton X-100	Sigma-Aldrich Chemie, Steinheim, Germany	066K0089
Tween 20	Sigma-Aldrich Chemie, Steinheim, Germany	045K0082

2.1.3

2.1.4 Solvents

The solvents used for sample extraction and as mobile phases for HPLC analysis are given in Table 2.2.

Table 2.2 Solvents used in these studies

Solvent	Source	Grade
Acetonitrile	BDH, (Chromanorm grade) Prolabo, Poole, UK	HPLC
n-Heptane	Poole, BDH, UK	HPLC (Hipersolv)
Isoamyl alcohol	Poole, BDH, UK	GPR
Dimethylformamide	Fisons, Loughborough, UK	Analytical grade
1-Cholorobutane	Fluka, Sigma-Aldrich, Steinheim, Germany	purum≥99.0%GC
Water	Poole, BDH, UK	HPLC (Hipersolv)

2.1.5 Filters

The type and size of filters used in the solubility and dissolution studies and the specifications of the dialysis membrane used in the dialysis studies are listed in Table 2.3.

Table 2.3 Filters and dialysis membrane used in solubility, dissolution and dialysis experiments

Filter type and size	Source
0.2µm poly-vinylidene fluoride (PVDF) filters, 12 mm syringe filter with tube tip	Whatman , Maidstone, UK
0.45µm PVDF filters, 25mm syringe, Acrodisc filters	Gelman Sciences, Northampton, UK
1.0 µm glass fibre 25mm syringe, Glass Acrodisc filters	Gelman Sciences, Northampton, UK
5µ acrylic polymer, 25mm syringe filters, Acrodisc Versapor	Gelman Sciences, Northampton, UK
0.45µm (poly-tetrafluoroethylene) PTFE, 25mm syringe filters	Millipore Millex LCR, Watford, UK
Cannula filters- 20 µm, Erweka style, UHMW Polyethylene micron filters	Quality Lab Accessories, New Jersey, USA
Filter papers, type 1 and 3	Whatman, Maidstone, UK
0.2 µm polyamide filter	Sartorius, Gottingen, Germany
Dialysis tubing, size 10, diameter 31.7mm, molecular weight cut off 12000-14000 Daltons	Visking-Medicell International, London, UK

2.2 Solubility and dissolution determinations

2.2.1 Media preparation

Media were prepared according to the method specified in each chapter using deionised water which was prepared using a HP 340 water deioniser (Purite, UK). The pH of the media was measured and adjusted to the desired value using a digital pH meter (Delta 350, UK). The pH meter was calibrated prior to use with three standard pH buffer solutions (4, 7 and 10) (BDH, Poole, UK). Specific procedures for each medium are described in the relevant chapters.

2.2.2 Solubility determination

The solubilities of itraconazole and ketoconazole in each dissolution medium were determined using a thermodynamic solubility test, the modified 'shake-flask method'. This method was adopted by Sunesen et al. (2005) for studying the solubility of poorly soluble drug in complex biorelevant media. Each experiment was performed at least in triplicate.

Medium (10ml) and drug powder (approximately 30mg for itraconazole and 300mg for ketoconazole) were transferred into closed-cap vials and shaken gently for 24h at 37°C in a shaking incubator (Model AM89B, Dynex Technologies Ltd, Worthing, U.K). After this time, the suspension was centrifuged at 4000rev min^{-1} (2325×gravity (g)) for 10min (Centaur 2, MSE, Fisons, England).

The supernatants were collected and filtered through the appropriate filter with the first portion of each filtrate being discarded to circumvent the effect of the initial adsorption of the drugs to the filters. Generally, filtration was performed using 0.2μm PVDF syringe filters discarding the first 1ml of each filtrate. For particulate biorelevant dissolution media, the use of filters with bigger pore size was required (Nicolaides et al., 1999) and details are specified in the relevant chapters.

Itraconazole filtrates were then diluted, where appropriate, with the mobile phase used for HPLC analysis, which was acetonitrile-0.02M potassium dihydrogen phosphate (60:40, v/v). Collected samples were analysed using HPLC to determine the concentration of drug in solutions as described in Section 2.2.6.1.

Ketoconazole filtrates were diluted as required with a mixture of acetonitrile-water (50:50, v/v). Collected samples were analyzed using HPLC as described in Section 2.2.6.2

2.2.3 Disk preparation

Intrinsic Dissolution Rate (IDR) testing was performed using the stationary disk apparatus. The equipment was manufactured by the Engineering workshop in Liverpool John Moores University (Liverpool, UK). A hardened polished steel plate was attached to the steel die. Drug powder (150mg of itraconazole or 200mg of ketoconazole) was inserted into the cavity of the steel die (9.5mm diameter) (Figure 2.2). A hardened-steel punch was inserted into the die cavity and then the whole arrangement was transferred

to a hydraulic compressor (Model M-30, Research and Industrial Instrument Company, London, England) (Figure 2.3). Drug substance was compressed at 1000p.s.i. (6.89Mpa) for 30s forming a smooth face with a circular surface area of 70.9mm^2. The steel plate was detached and the die containing the compressed powder was blown with compressed air to remove any loose particles.

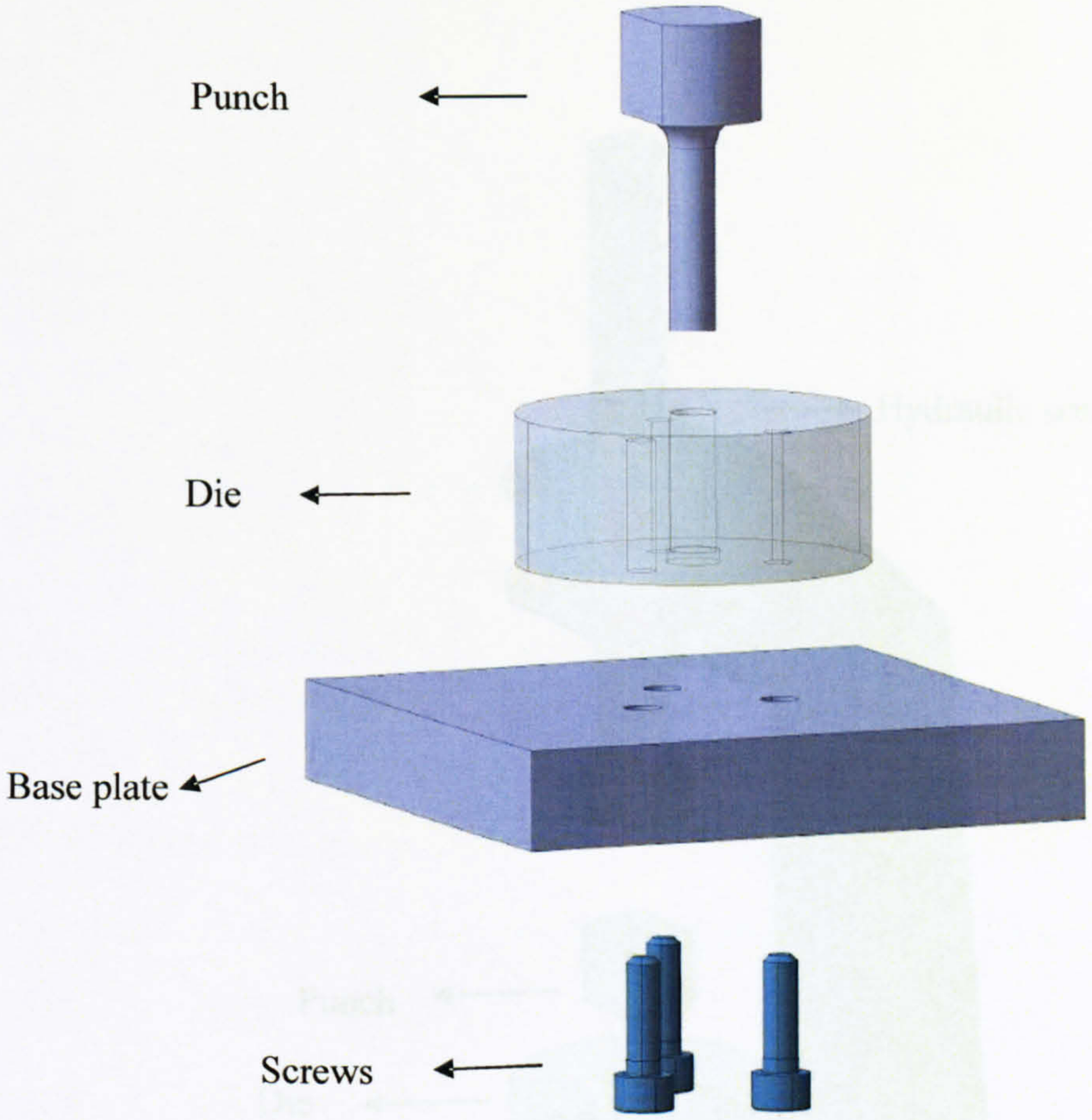


Figure 2.2 Schematic diagram of intrinsic dissolution disk assembly used for the preparation of drug compact disks

2.2.4 Intrinsic dissolution studies

Dissolution studies were carried out using USP apparatus II (paddle) (Pharmatest PTW S3C, Pharmatest GmbH, Hainburg, Germany). The die, containing the compressed drug powder was positioned disk-up at the bottom of a flat-bottom dissolution vessel containing 500ml of the dissolution medium (Figure 2.4). The temperature was set at 37 ± 0.5°C and a rotation speed of 100rev min^{-1} was used. The paddles were lowered to a position where the distance of the bottom of the paddles from the die upper surface was approximately 1.7cm. The vessels were covered with plastic lids to reduce evaporation.

Samples (3ml) were withdrawn periodically from the dissolution vessels through filters with the first 1.5ml being discarded. Itraconazole samples were filtered using 5μm Versapor filters and ketoconazole samples were filtered using 0.45μm PVDF filters. Unless particulate complex dissolution media were used, itraconazole and ketoconazole samples were withdrawn through 20μm Cannula filters.

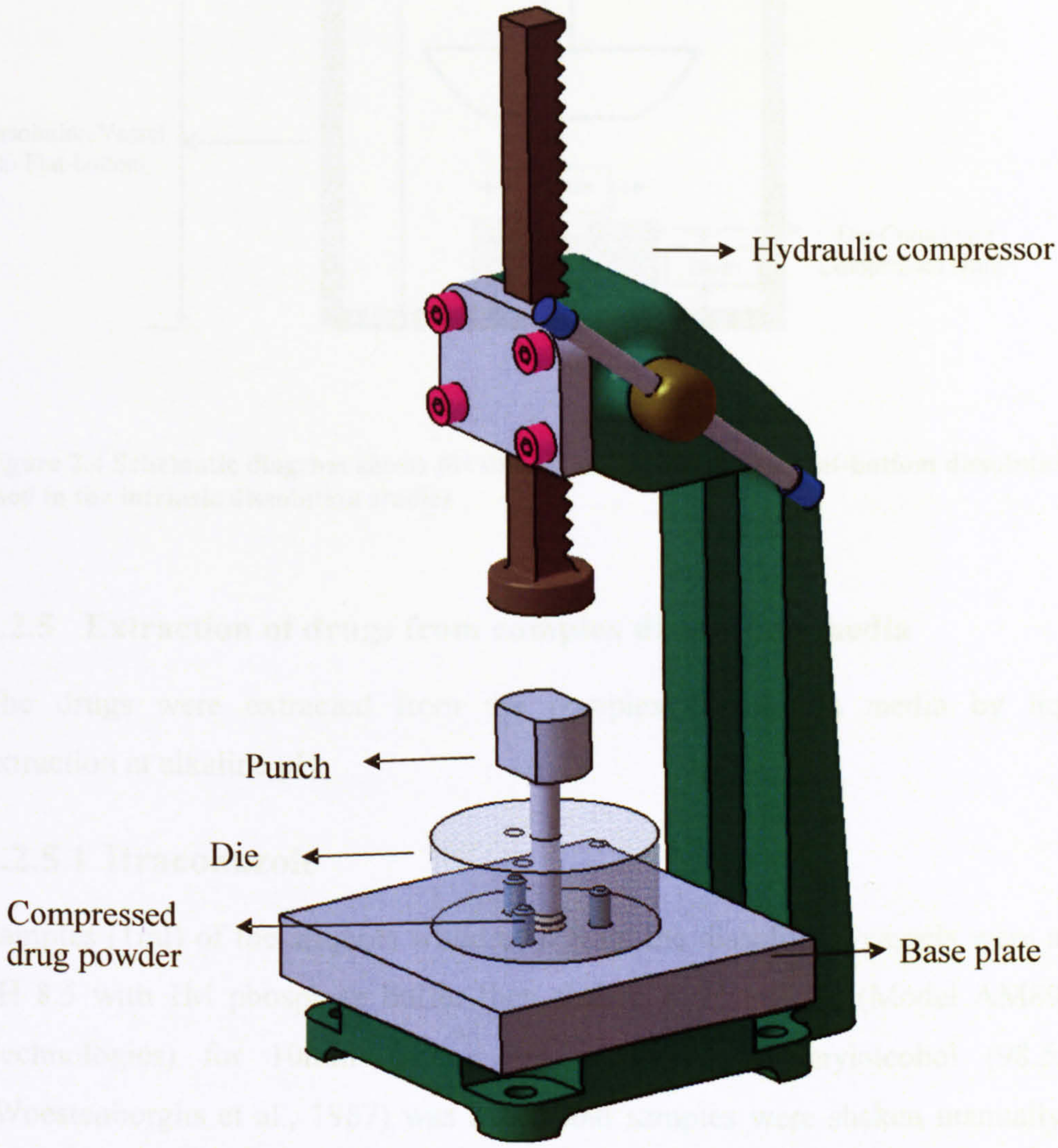


Figure 2.3 Schematic diagram of the settings used to prepare intrinsic dissolution disks; it shows the disks assembly and hydraulic compressor

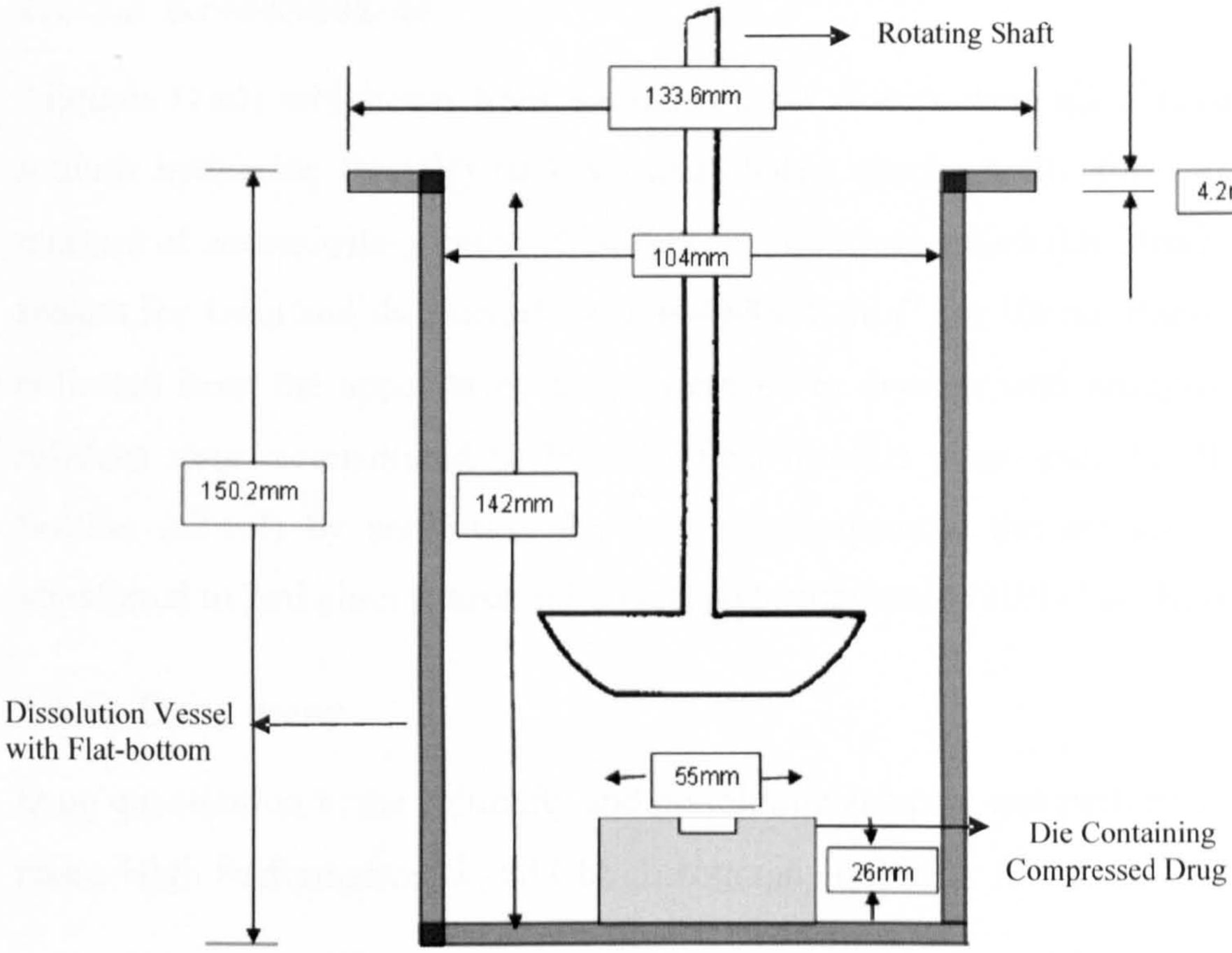


Figure 2.4 Schematic diagram shows the dimensions of die and the flat-bottom dissolution vessel used in the intrinsic dissolution studies

2.2.5 Extraction of drugs from complex dissolution media

The drugs were extracted from the complex dissolution media by liquid-liquid extraction at alkaline pH.

2.2.5.1 Itraconazole

Samples (1ml) of the aliquots withdrawn from the dissolution vessels were adjusted to pH 8.5 with 1M phosphate buffer then shaken mechanically (Model AM89B, Dynex Technologies) for 10min. Then, 5ml of heptane-isoamylalcohol (98.5:1.5, v/v) (Woestenborghs et al., 1987) was added and samples were shaken manually for 1min and centrifuged at 4000rev min^{-1} for 10min. Portions (2ml) were removed from the upper organic layer and dried under a stream of nitrogen at 60°C. Thereafter, 2ml of eluent (mobile phase used for itraconazole HPLC analysis, Section 2.2.6.1) was added to reconstitute the dried residue and sonication was applied for 2min (Kerry Ultrasonics, UK). Subsequently, the resultant samples were transferred to 2ml glass autosampler vials and submitted to HPLC analysis.

2.2.5.2 Ketoconazole

Aliquots (1ml) withdrawn from the dissolution vessels were alkalinised with 1ml of sodium hydroxide (NaOH) (0.05M) and shaken mechanically for 10min. 5ml of a mixture of acetonitrile–n-butyl chloride (1:4, v/v) was added (De Bruijn et al., 2001), shaken for 1min and then centrifuged at 4000rev min^{-1} for 10min. Portions (2ml) were collected from the upper layer and evaporated to dryness with nitrogen at 60°C. The residues were reconstituted in 2ml of eluent (mobile phase used for HPLC analysis, Section 2.2.6.2) by sonication for 2min. Subsequently, the resultant samples were transferred to 2ml glass autosampler vials and submitted to HPLC analysis.

2.2.6 Drug assay

Drug quantitation in the solubility and dissolution samples was performed using reverse phase-High Performance Liquid Chromatography (HPLC)

2.2.6.1 Itraconazole

Itraconazole was assayed with a modified HPLC method proposed by Wong et al. (2003). The same method was applied for itraconazole dissolution and solubility studies.

The column used was a LUNA® C18 (2) (250 x 4.60mm, 5µm) (Phenomenex, USA) and the mobile phase was a mixture of acetonitrile and 0.02M potassium dihydrogen phosphate (60:40, v/v) adjusted to pH 3.0 with 5M HCl. This mixture was vacuum filtered using 0.2µm polyamide filters in a Millipore vacuum filtration assembly and then degassed with helium-sparging. Chromatography was performed at a flow rate of 1.3ml min^{-1} held at ambient temperature. The injection volume was 50µl and the injection needle was washed with acetonitrile between injections to prevent any carry over. Eluting peaks were detected using a Waters 474 fluorescence detector (Waters, UK) with the gain set at 100 and the attenuation at 32. The equipment was controlled and the data were handled using Millennium software (Version 3.05).

Due to the extremely poor solubility of itraconazole, a stock solution (100µg ml^{-1}) was prepared by dissolving 10mg of itraconazole in 3ml of dimethylformamide then the volume was taken up to 100ml with the mobile phase. Subsequent standard solutions were prepared in a range from 0.025 to 5µg ml^{-1} by diluting the stock solution with the mobile phase as appropriate. The method of external standards was used to convert the

measured peak areas to concentration units. The data acquired following the HPLC analysis were transferred to Excel (Microsoft Office) for subsequent data analysis.

2.2.6.2 Ketoconazole

Drug quantitation was carried out using a HPLC separation module Waters Alliance 2695 chromatograph (Waters, UK). The same method was applied for ketoconazole dissolution and solubility studies. Detection and quantitation were carried out using a Waters 996 Photodiode Array Detector (PAD) (Waters, UK).

Ketoconazole samples (20-30µl) were eluted using a mobile phase consisting of acetonitrile, water and triethylamine (50:50:0.1 v/v), with a flow rate of 1.1ml min^{-1}. A 5µm Hypersil BDS (Base Deactivated Silica) C18 column (150 x 4.6mm) (Thermo Electron Corporation, USA) fitted with a Phenomenex C18 guard cartridge (4mm x 3mm) was used and eluting peaks were detected at a wavelength of 254nm. In spite of using the BDS column, which has hydroxylated silanol in the stationary phase, the addition of triethylamine was necessary for peak symmetry to compensate for the acidity of the silanol groups which led to tailing of the peak and a longer retention time (Vertzoni et al., 2006).

A Fluorescence detector (Waters 474, UK) operating at 251nm for the excitation wavelength and at 376nm for the emission wavelength was employed when the dissolution media had a pH ≥ 5.

2.3 Method validation

2.3.1 Filter tests

2.3.1.1 Introduction

Filtration was essential in the solubility studies to remove the residual undissolved drug particles. Moreover, filtration was required for samples collected from the dissolution media to avoid any potential transference of undissolved particles from the media to the sample. Therefore, it was important to choose the appropriate type of filter to avoid adsorption of drug on the filters.

A set of filters of different materials and pore sizes were tested for possible adsorption. The amount of each drug retained by the filter was determined by assaying a drug solution with and without passage through the filters and consequently the adsorptive losses to filters were calculated (Lindenberg et al., 2005).

2.3.1.2 Procedures

A stock solution of itraconazole (1.5μg ml^{-1}) was prepared in simulated gastric fluid (SGF) pH 1.2. A stock solution of ketoconazole (30μg ml^{-1}) was prepared in SGF pH 3. SGF solutions were prepared as described in Section 3.2.3.1.

Three samples of each drug stock solution were drawn up into a 5ml plastic syringe (concentric luer tip syringes, Becton Dickinson) and filtered immediately. The drug concentration in the filtrate was determined by HPLC analysis (Section 2.2.6). The experiments were conducted in triplicate and a new filter was used for each experiment.

As a reference, the stock solutions of the drugs were withdrawn through the syringes without filters and the concentration of the drug was determined using HPLC. Drug recovery was calculated by comparing the concentrations of the corresponding filtrates and references using Eq. (2-1) (Lindenberg et al., 2005).

% Recovery = (Concentration of filtrate / Concentration of reference) x 100 Eq. (2.1)

Additionally, the effect of discarding the first portion of the filtrate was evaluated by determining the recovery after the first 1.5ml of the filtrate was discarded. 1ml was discarded only when using PVDF filters of smaller diameter (13mm).

2.3.2 Assay validation

Analytical parameters used in the validation of the drug assay methods were: linearity, precision, limit of detection (L.O.D) and limit of quantification (L.O.Q).

Calibration curves were obtained for standard solutions of both drugs. The solutions were injected to the HPLC system according to the chromatographic conditions in Section 2.2.6. The linearity was evaluated by the least square regression method.

The L.O.Q was defined as the lowest concentration determined with acceptable precision and accuracy (ICH, 2005). L.O.Q of the analyte in the sample was calculated as the concentration yielding a signal-to-noise (S/N) ratio of 10:1.

The L.O.D referred to the lowest amount of the analyte that could be reliably detected and not necessarily quantified in an exact amount (ICH, 2005). It was calculated in the present investigation as S/N of 3:1.

The precision of the assay (repeatability) was computed as the percent relevant standard deviation (%R.S.D) over a range of concentrations greater than L.O.Q (ICH, 2005). The average of triplicate analysis of each sample was evaluated.

To compare the sensitivity of UV absorbance detection with fluorescence detection, L.O.Q of itraconazole was evaluated in both cases as described above.

2.3.3 Stability tests

The stability of itraconazole and ketoconazole was assessed in the dissolution media under dissolution testing conditions. The drugs were evaluated for their stability over 12h in acidic medium (pH 3) under dissolution testing conditions. Itraconazole (0.016μg ml^{-1}) and ketoconazole (0.10mg ml^{-1}) solutions in SGF were incubated at 37°C and injected onto the HPLC system at certain time points over 12h. The chromatograms obtained by the HPLC were verified with regard to peak area and possible formation of degradation products.

2.3.4 Disk surface morphology

Scanning electron microscopy SEM (Model JSM-840, Jeol Technics Ltd, Tokyo, Japan) was employed to investigate the morphology of itraconazole compact surfaces. The surface of the sample needed to be conductive to reduce electric charge build-up on the surface that might change its morphology. Therefore, itraconazole disks were coated with a thin layer of gold (sputter coating) (Crowder et al., 2003). Images were obtained for fresh disks of itraconazole substance to check the smoothness of the surface. Simple techniques were applied to enhance the smoothness of the surface, re-polishing the plate surface by surface finishing processes (fine grinding) and covering the plate with aluminium foil. Images of the produced disks were taken thereafter. Furthermore, images were taken of itraconazole disks after they had been through the dissolution process in chosen media and details are specified in the relevant chapter.

2.4 Results of method validation

2.4.1 Filter tests

2.4.1.1 Itraconazole

Table 2.4 shows the percentage recovery after the filtration of itraconazole solutions, including the first and second aliquots. The second aliquot always had a higher drug recovery. PVDF filters (0.2μm, 13mm) gave the highest recovery for itraconazole second aliquot, which was greater than 95%. Since the criterion for filter acceptance is 95% drug recovery in the filtrate (Lindenberg et al., 2005), this type of filter material was used for most of the solubility studies with the first aliquot (1ml) being discarded. The Acrylic polymer also gave acceptable results so it was used for most of itraconazole dissolution studies.

Table 2.4 Itraconazole recovery after filtration for two aliquots. Each data point represents the mean ± standard deviation (S.D.) of 3 measurements

Product name	Material	Mesh width (μm)	Itraconazole recovery (%)	
			1st aliquot	2nd aliquot
Millipore	PTFE	0.45	81.4 ± 1.9	92.1 ± 2.2
Whatman	PVDF (13mm)	0.2	72.9 ± 1.6	99.6 ± 0.9
Gelman	PVDF (25mm)	0.45	73.9 ± 1.6	94.1 ± 3.7
Gelman	Glass fibre	1	64.7 ± 4.7	93.1 ± 2.7
Acrodisc Versapor	Acrylic polymer	5	83.2 ± 1.1	97.4 ± 0.8
Nalgen	Cellulose acetate	0.45	75.2 ± 3.1	79.1 ± 5.4
Whatman, mini-filters-centrifuge	Regenerated cellulose	0.45	64.2 ± 1.6	65.9 ± 2.8

2.4.1.2 Ketoconazole

The highest recovery of ketoconazole was from filters with PVDF membrane (0.2μm -0.45μm) where the recovery in the second aliquot was within the accepted value (>95%) (Table 2.5). Therefore, filters with PVDF membranes were used for most of the solubility and dissolution studies.

Table 2.5 Ketoconazole recovery after filtration for two aliquots. Each data point represents the mean ± S.D. of 3 measurements

Product name	Material	Mesh width (μm)	Ketoconazole recovery (%)	
			1st aliquot	2nd aliquot
Millipore	PTFE	0.45	60.9 ± 3.0	91.2 ± 2.8
Whatman	PVDF (13mm)	0.2	88.1 ± 3.2	97.2 ± 1.9
Gelman	PVDF (25mm)	0.45	91.6 ± 2.1	96.7 ± 2.1
Gelman	Glass fibre	1	72.5 ± 5.3	94.6 ± 2.5
Acrodisc Versapor	Acrylic polymer	5	91.9 ± 3.4	95.3 ± 1.0

In general, discarding the first portion of filtrate allowed saturation of the filters and so produced better recovery agreeing with the findings of Kiehm and Dressman (2008). Filters which gave the highest recovery were used thereafter except when the dissolution media were complex biorelevant particulate media. The composition of which did not allow the use of these filters which had a small pore size so a compromise between filtering recovery and pore size was considered.

2.4.2 Assay validation

Validation of the analytical methods was necessary to demonstrate the methods were suitable for the required analysis.

2.4.2.1 Itraconazole

Since the solubility of itraconazole was very low, it was necessary to apply a sensitive method that could detect and quantify it at very low concentrations so fluorescence spectroscopy was chosen as the detection method. The triazole and piperazine rings provide itraconazole with the capacity for fluorescence emission. The excitation and emission peaks were determined using a fluorescence spectrophotometer, Varian Cary Eclipse (Varian, USA), operated via the Cary Eclipse Advanced Reads Application Version 1.1 (132) software. The maximum excitation and emission wavelengths were recorded at 252nm and 360nm, respectively, for itraconazole dissolved in the mobile phase (Figure 2.5).

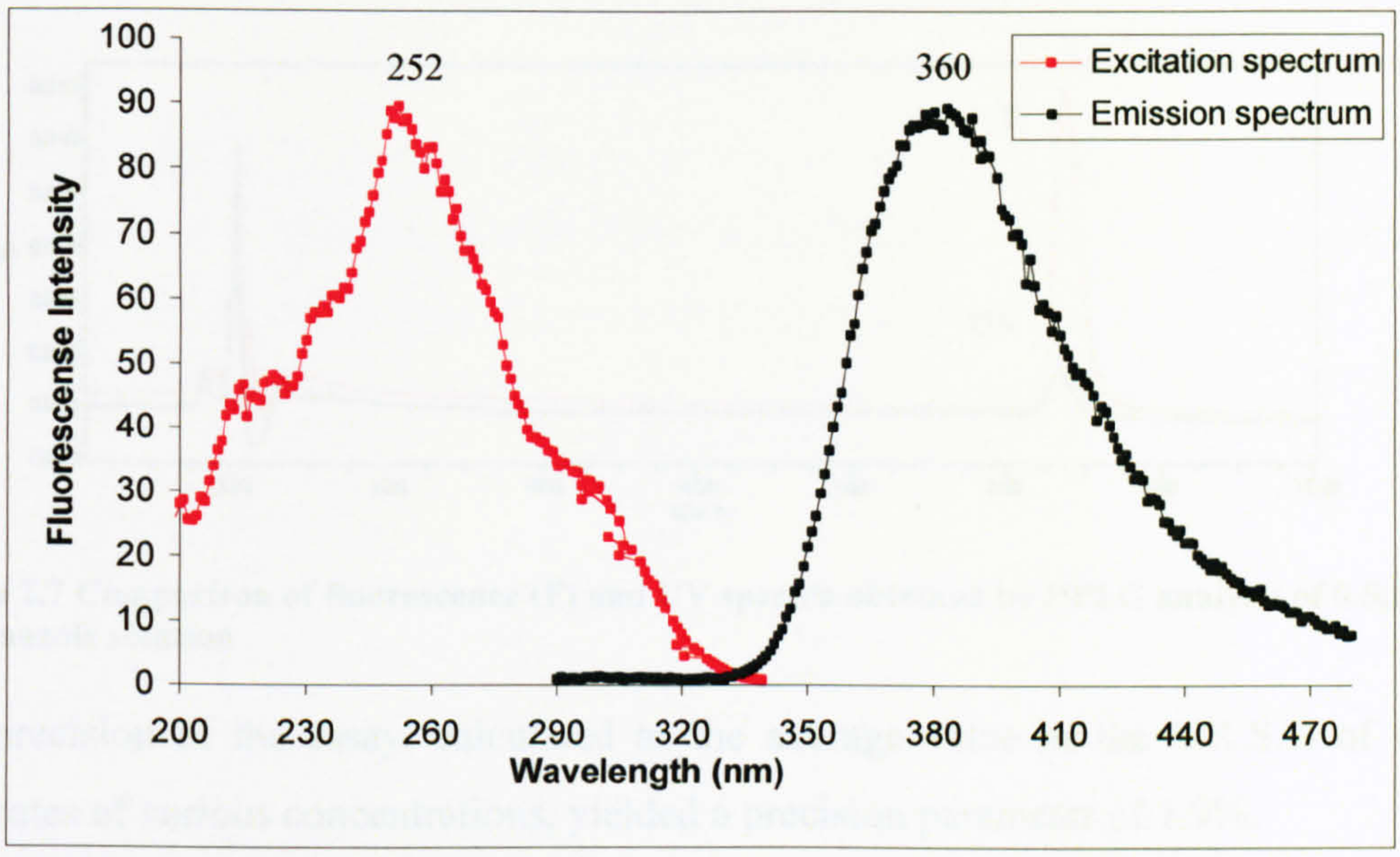


Figure 2.5 Emission and excitation wavelength scans for 1μg ml^{-1} itraconazole solution: emission wavelength scans generated by excitation at 252nm and excitation wavelength scans generated by emission at 360nm

The calibration curve was linear over the range of concentrations studied with a high correlation coefficient (Figure 2.6).

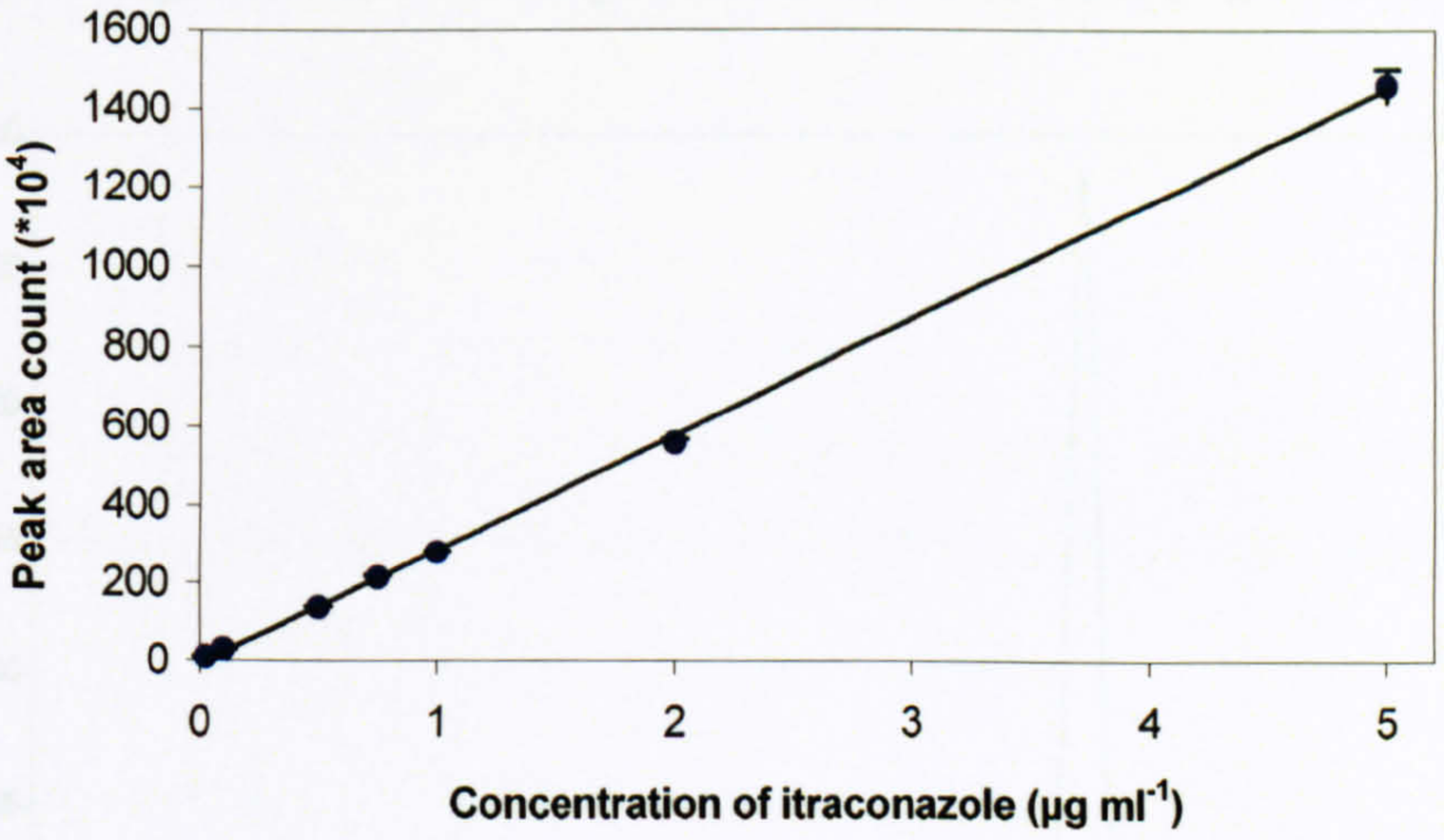


Figure 2.6 Calibration curve of itraconazole obtained by HPLC analysis using fluorescence detection, range from 0.03 to 5μg ml^{-1}, r^2> 0.99 equation is y=291.5x -6.0. Each data point represents the mean ± S.D. of 3 measurements

The L.O.Q of itraconazole was 6ng ml^{-1} and the L.O.D was 1.8ng ml^{-1}, whereas the L.O.Q of itraconazole using UV detection at 254nm was found to be 30ng ml^{-1}. This indicated that using fluorescence detection increased the sensitivity of the analysis 5 times compared to UV detection (Figure 2.7).

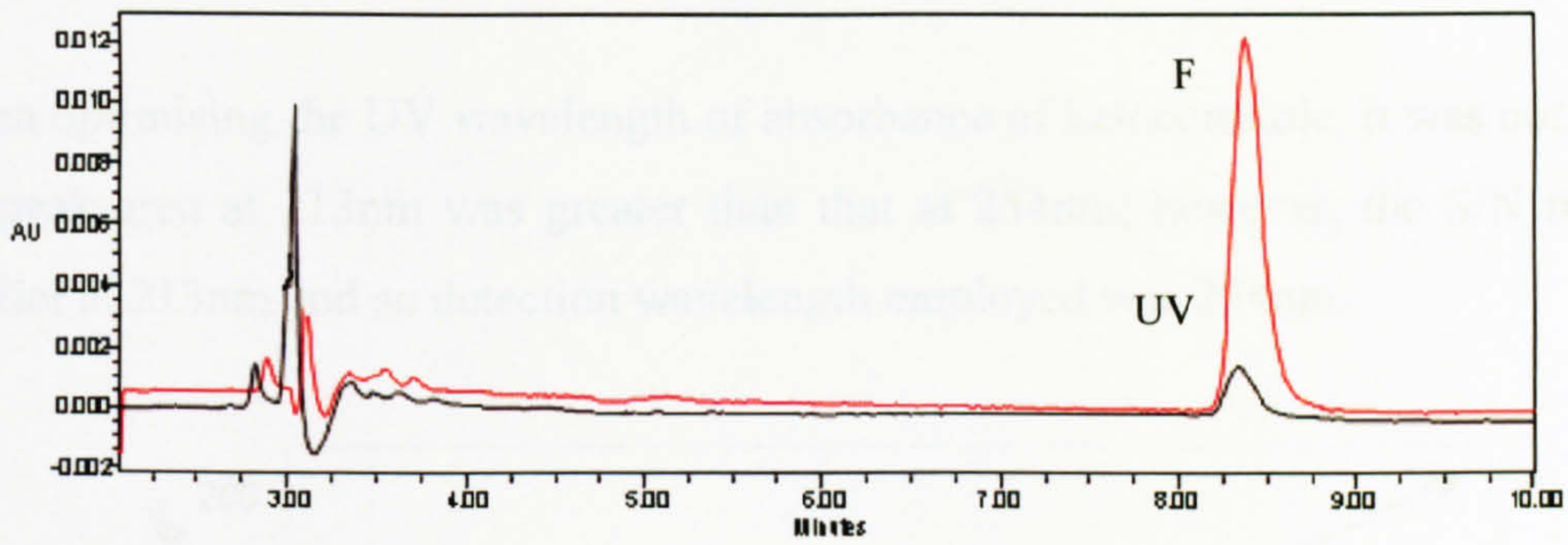


Figure 2.7 Comparison of fluorescence (F) and UV spectra obtained by HPLC analysis of 0.5μg ml^{-1} itraconazole solution

The precision of the assay, calculated as the average value of the %R.S.D of seven triplicates of various concentrations, yielded a precision parameter of 1.9%.

2.4.2.2 Ketoconazole

Figure 2.8 shows a typical chromatogram of ketoconazole where the retention time of the drug was 6.8min. A linear calibration curve was obtained throughout the concentration range studied with a high correlation coefficient (Figure 2.9).

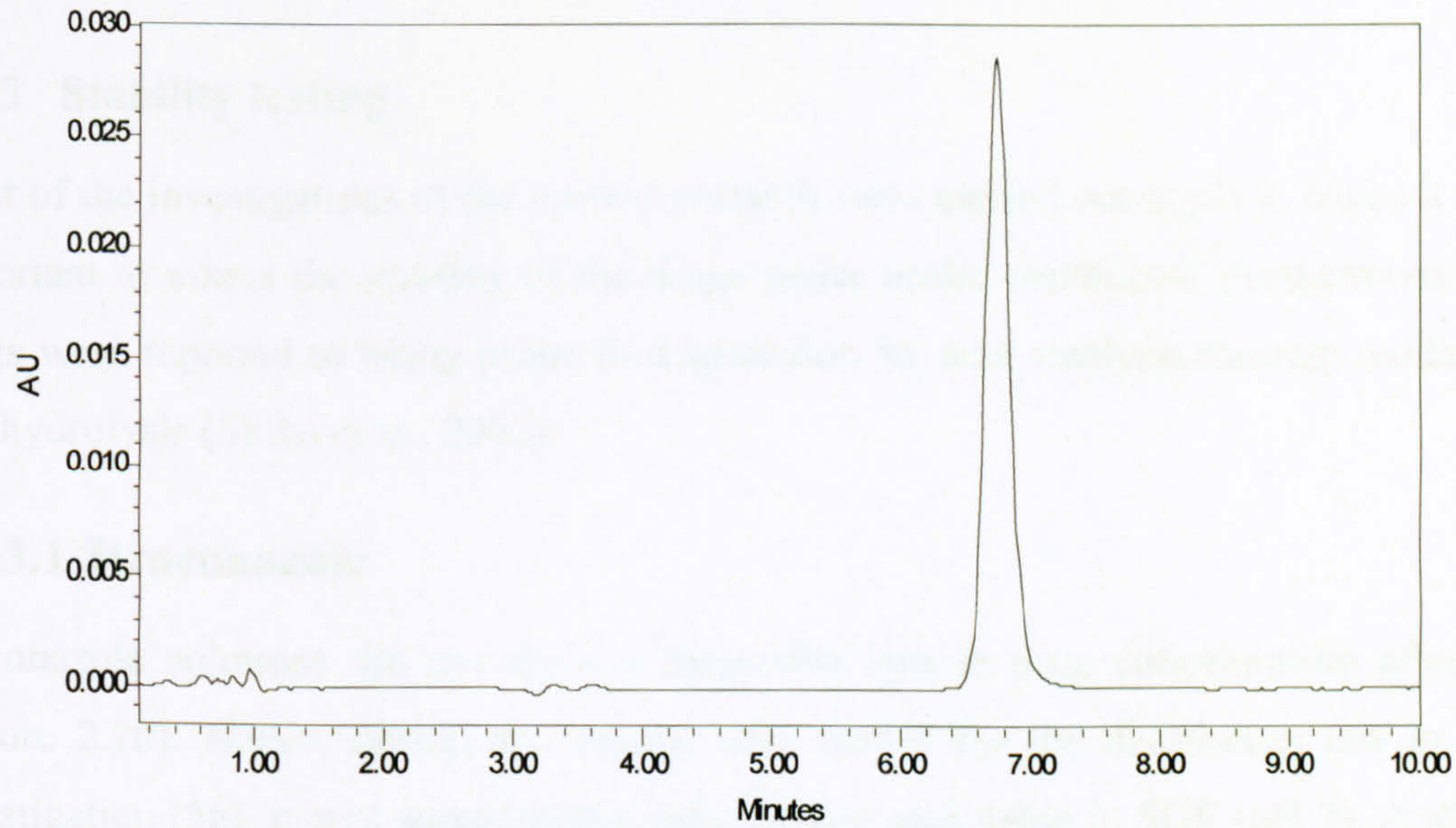


Figure 2.8 Typical HPLC Chromatogram of ketoconazole solution in mobile phase using UV detection at 254nm, injection volume 20μl

The L.O.D was 0.03μg ml^{-1} and the L.O.Q was 0.1μg ml^{-1}. The precision parameter of the assay, calculated as %R.S.D. of seven triplicates of various concentrations, was 2.4%.

When optimising the UV wavelength of absorbance of ketoconazole, it was noticed that the peak area at 213nm was greater than that at 254nm; however, the S/N ratio was smaller at 213nm and so detection wavelength employed was 254nm.

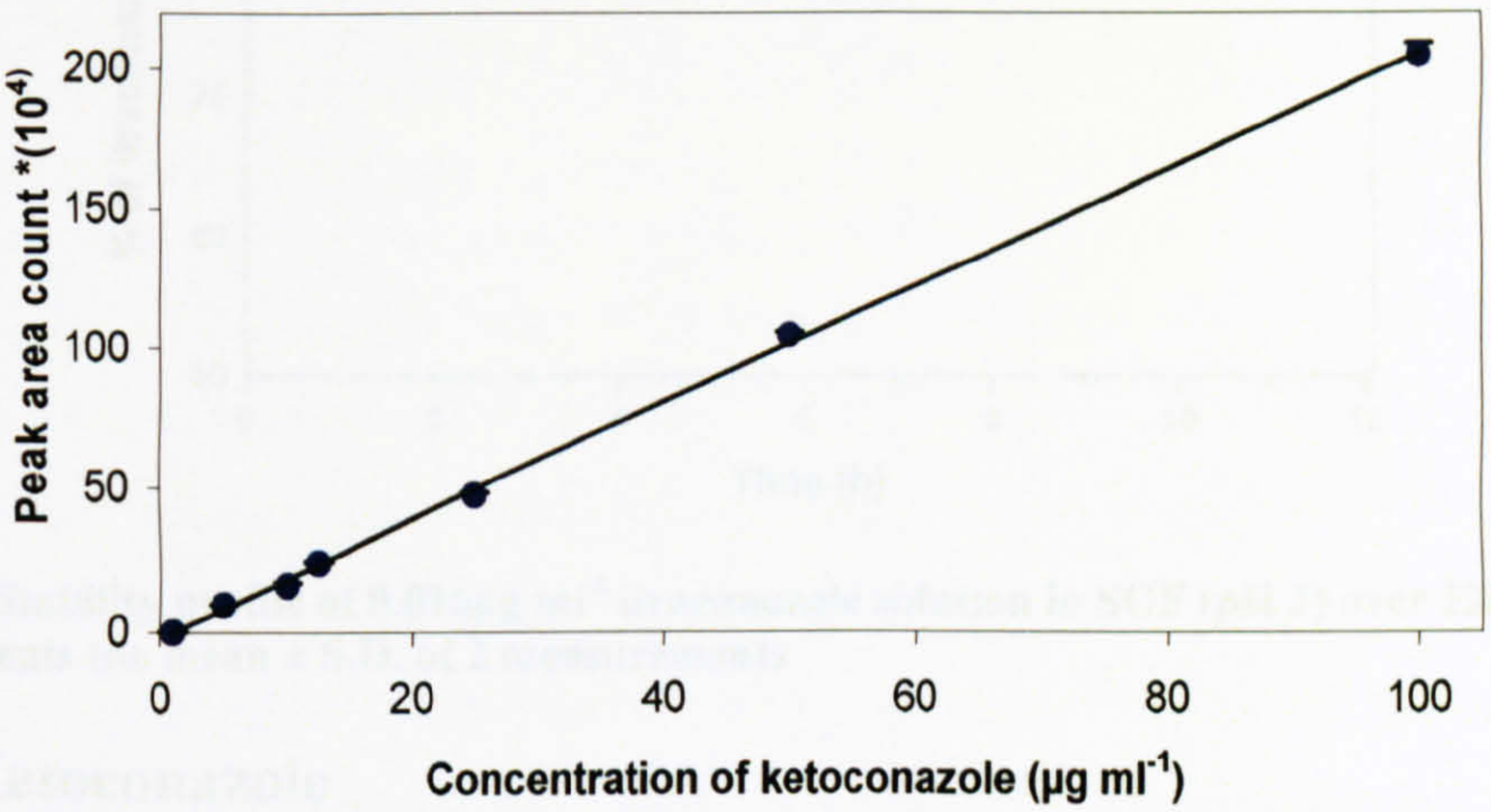


Figure 2.9 Calibration curve of ketoconazole obtained by HPLC analysis using UV detection, concentrations range from 1 to 100µg ml^{-1}, r2> 0.99, equation is y = 2.08x - 2.07. Each data point represents the mean ± S.D. of 3 measurements

2.4.3 Stability testing

Most of the investigations of the current research were carried out at pH 3, hence it was important to assess the stability of the drugs under acidic conditions. Furthermore, the drugs were reported to being prone to degradation by acid catalysis through oxidation and hydrolysis (Skiba et al., 2000).

2.4.3.1 Itraconazole

Itraconazole solutions did not show a detectable loss in drug concentration after 5h (Figure 2.10). Hence, during the normal time period for the dissolution test in this investigation (5h), it was apparent that itraconazole was stable in SGF (pH 3). A slight decrease in itraconazole concentration was observed after 12h where the recovery was 97.9 ± 1.7% but no degradation peaks were observed.

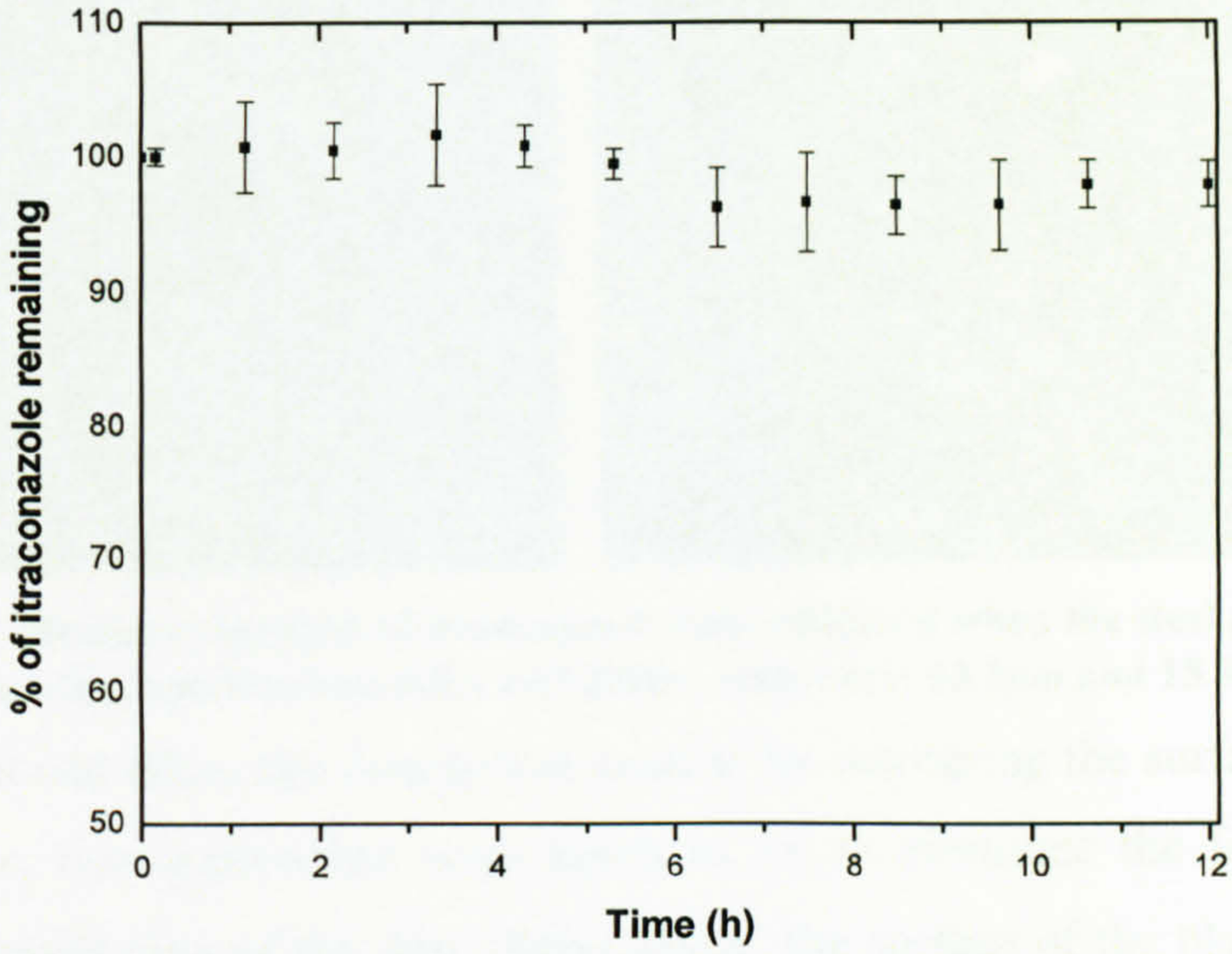


Figure 2.10 Stability profile of 0.016μg ml^{-1} itraconazole solution in SGF (pH 3) over 12h. Each data point represents the mean ± S.D. of 2 measurements

2.4.3.2 Ketoconazole

The concentration of ketoconazole (0.10mg ml^{-1}) did not drop significantly over 12h as drug recovery measured 98.6 ± 1.7%, n=2. This indicated that the ketoconazole solution in SGF (pH 3) was stable at the experimental conditions throughout this period.

2.4.4 Disk morphology

A principal requirement for determining the IDR of a drug is that dissolution takes place from a compressed sample of the drug having a constant surface area (USP, 2008). Any unevenness in the disk surface arising from cracks, produced during the compression process, may be exacerbated as the test proceeds leading to a gradually changing surface area (Levy, 1963). Therefore, it was necessary to confirm that the IDR disk surface was intact and so ensure dissolution would take place evenly across the entire surface. The surfaces of a number of disks were evaluated prior to performing the dissolution to check the smoothness of the drug compact surface.

Micrographs of freshly-compressed disks (Figure 2.11) exhibited a striated surface with regular, raised ridges which arose from a roughness of the base-plate against which the drug powder had been compacted and were due to the fabrication procedure of the base-plate itself.

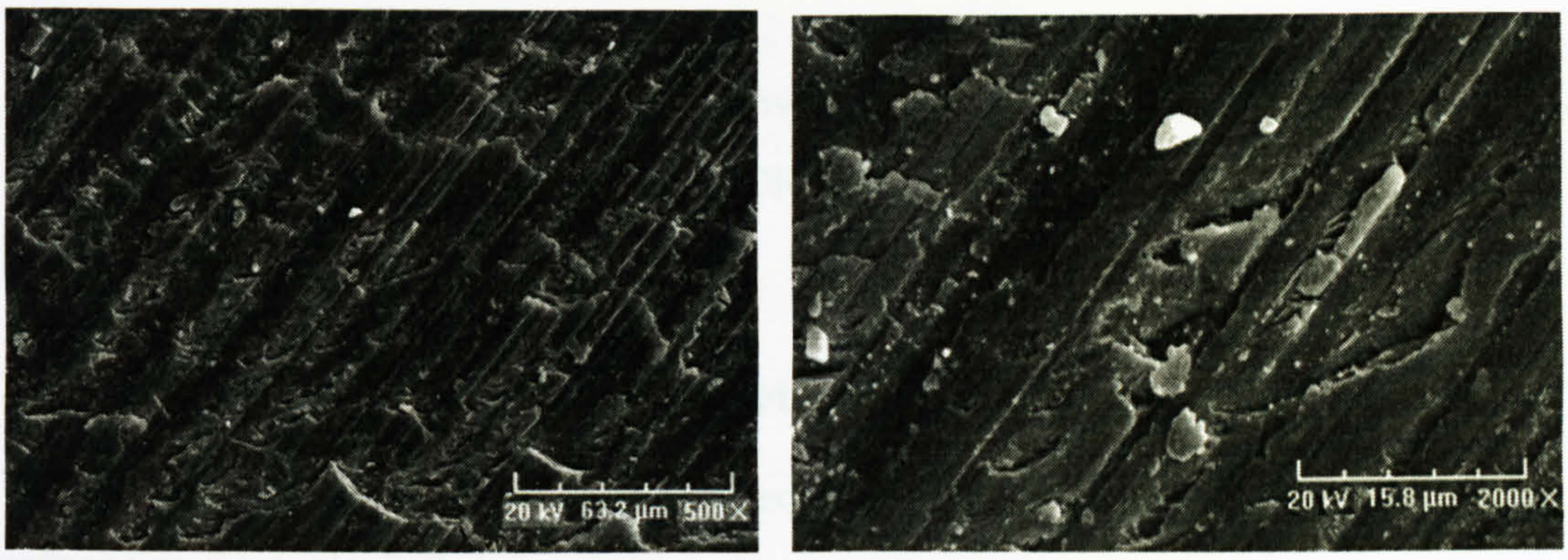


Figure 2.11 SEM photomicrographs of itraconazole disks obtained when the steel plate was unpolished, taken at two magnifications 500x and 2000x, scale bars: 63.2µm and 15.8µm

These striations can affect the dissolution process by increasing the surface area of drug disk. Therefore, two approaches were taken to try to eliminate the formation of the ridges during production of the drug disks; firstly, the surface of the plate was polished in an attempt to make it smoother. Secondly, aluminium foil was placed onto the surface of the plate in order to present a smoother surface against which to compress the drug.

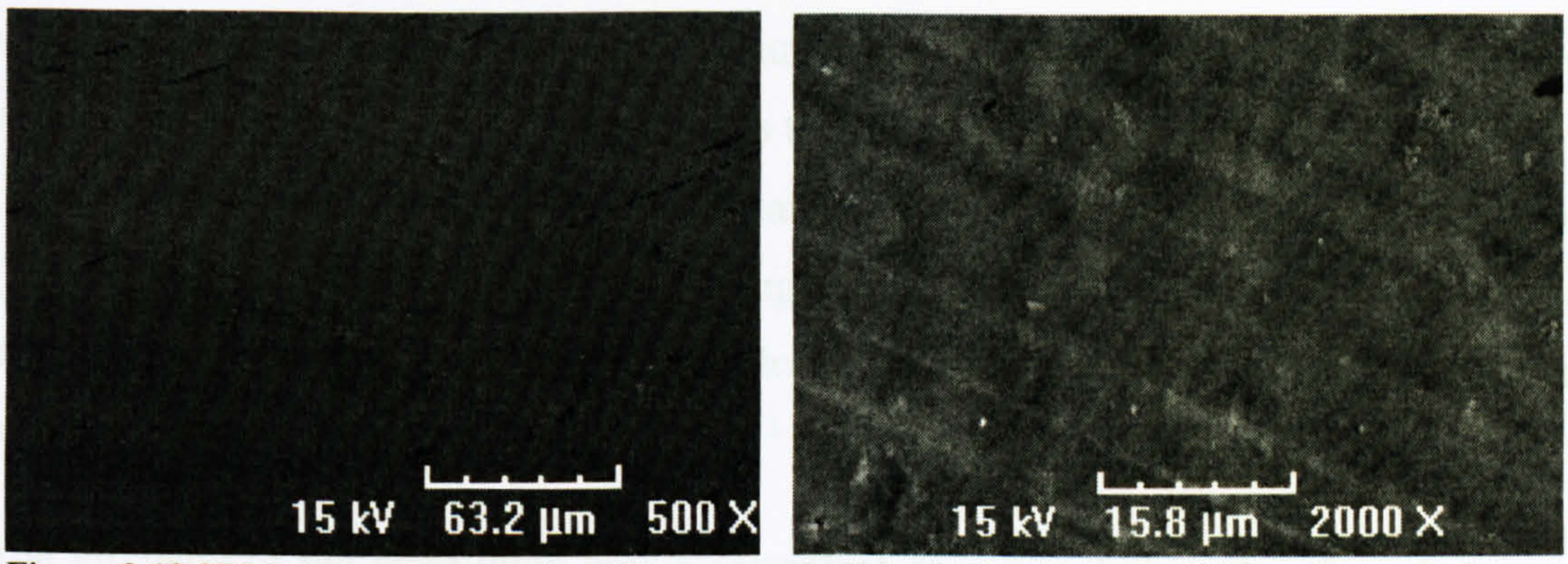


Figure 2.12 SEM photomicrographs of itraconazole disk obtained after polishing the steel plate, taken at two magnifications 500x and 2000x, scale bars: 63.2µm and 15.8µm

Micrographs produced by polishing (Figure 2.12) indicated that re-polishing the plate was seen to reduce the striations. However, covering the plate with aluminium foil produced an even smoother surface (Figure 2.13).

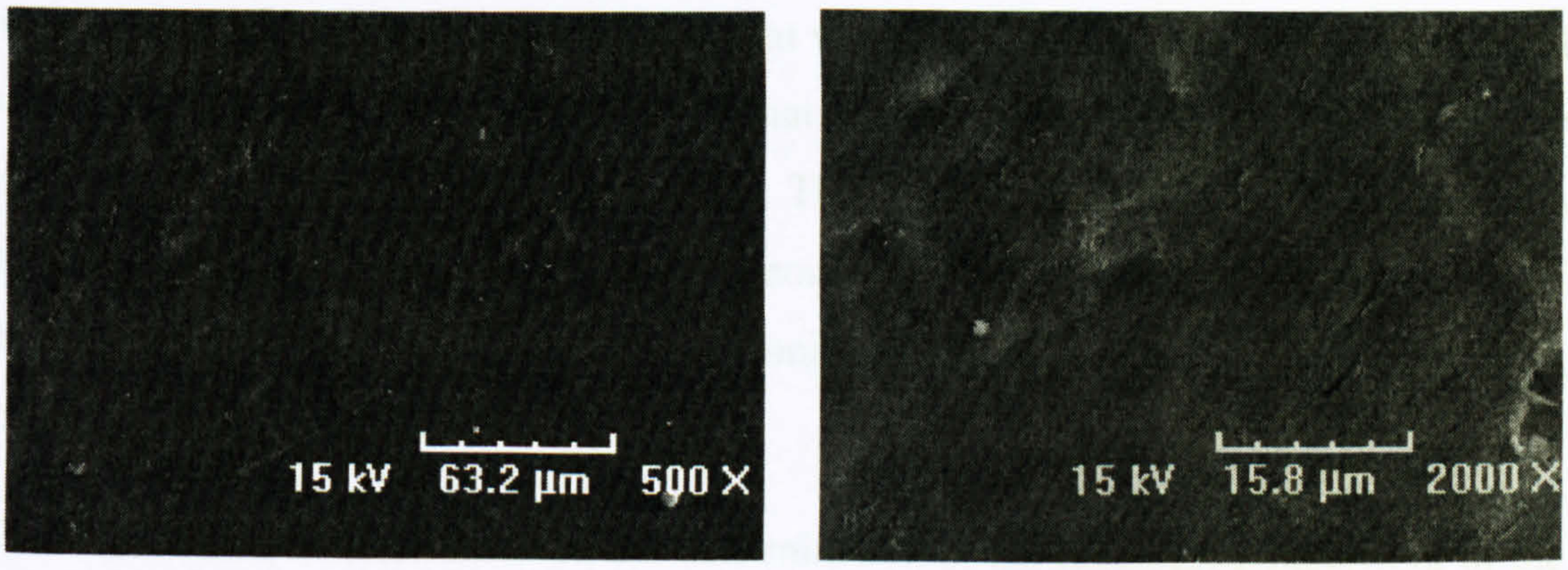


Figure 2.13 SEM photomicrographs of itraconazole disk obtained after covering the steel plate with aluminium foil, taken at two magnifications 500x and 2000x, scale bars: 63.2µm and 15.8µm

Consequently, the simple technique of covering the steel plate with aluminium foil was adopted for the preparation of all the IDR disks, and furthermore, the plates were re-polished occasionally.

2.5 Media characterisation & wettability assessment

The investigated media were characterised in term of viscosity and surface tension in order to evaluate the influence of these parameters on the dissolution of the drugs.

2.5.1 Viscosity

2.5.1.1 Introduction

The viscosity of a fluid is described as its resistance to flow. Viscosity can be demonstrated by suspending two horizontal, parallel plates in a liquid, keeping the bottom plate stationary whilst setting the upper plate in motion at constant velocity without changing the distance to the stationary plate. The difference in velocity between adjacent layers, i.e. the velocity gradient is termed shear rate and the force per unit area required to create the flow is the shearing stress. The unit of viscosity, poise (100mpascal.second), is defined according to the Newtonian liquid concept as the shearing force required to produce a velocity of 1cm s^{-1} between two parallel flat surfaces of liquid each with an area of $1cm^2$ and separated by 1cm (Martin, 1993b).

The viscosity of the dissolution media can affect drug dissolution; therefore, it was an important parameter to consider and to investigate its influence on dissolution (Section 1.6.1.3).

A rotational rheometer was initially employed to measure the viscosity of deionised water but it did not provide values consistent with the literature value and demonstrated poor repeatability. Thus, this indicated that this apparatus was not appropriate for measuring the viscosity of aqueous media. Thereafter, the viscosity of the dissolution media was measured using a vibrational rheometer which allowed the determination of the viscosity of Newtonian and non-Newtonian fluids with good repeatability (2.1%, measured as average of % RSD).

The rotational viscometer allowed the determination of the rheological behaviour of the media and their classification as Newtonian or non-Newtonian by plotting the viscosity versus shear rate, (more details are given in Section 4.3.1.1). Obtaining such

information gives an insight into the change of viscosity of the fluid according to change in the shear rate, which is produced by the stirring in the dissolution tester. Furthermore, the viscosity of non-Newtonian fluids will be different in different positions in the dissolution vessels according to the shear rate which in turn may affect the flow of the fluid (Kukura et al., 2004).

2.5.1.2 Vibrational rheometer

A Viscolite VL700-T15 viscometer (Hydramotion, UK), a vibrational or resonant viscometer, was used for measurements of media whose viscosity was similar to water (Hydramotion, Viscolite VL700). This type of viscometer works by creating waves and only shear waves are utilized for measurements. When the sensor is immersed in fluids, high frequency waves (3200 - 3300 Hertz) emanating from its surface shear through the fluid and the energy is lost in the fluid. This loss in energy is measured by microprocessor-controlled electronics and is expressed as viscosity through programmed calculations. The dissipated energy is related to liquid viscosity as high viscous solutions cause more loss in energy (Kress-Rogers and Brimelow, 2001).

The apparatus creates a high shear rate and so forces the viscosity measurement to a flat part of the viscosity versus shear rate curve, allowing the determination of the viscosity of both Newtonian and non-Newtonian fluids.

Procedures

Samples were warmed to 37°C prior to measurement using a water bath (Clifton, UK) and this temperature was maintained during the measurements by putting the samples on a holder placed over a heating plate. The solid stainless steel sensor of the rheometer was immersed into the fluid (approximately 25ml). The measured viscosity was displayed on the read-out unit as soon as the probe was immersed in the sample. The sensor was washed with deionised water and wiped between measurements to prevent carryover.

2.5.1.3 Rotational rheometer

The rotational rheometer measures fluid viscosity under controlled stress conditions. The test fluid is sheared between rotating cones, plates or cylinders. The system consists of four parts: (i) a measurement tool with a well-defined geometry, (ii) a device to produce a constant rotation speed to the tool, (iii) a device to measure the stress or shear rate response and (iv) a means of temperature control.

A CarriMed CSL2 100 rheometer (TA Instruments Ltd., USA) was used in the current investigation, employing a cone/plate geometry system with a 6 cm diameter and cone angle of approximately 1° (Figure 2.14). A large diameter-geometry was chosen for its sensitivity to stress changes, being more suitable for the investigated fluids which were not highly viscous.

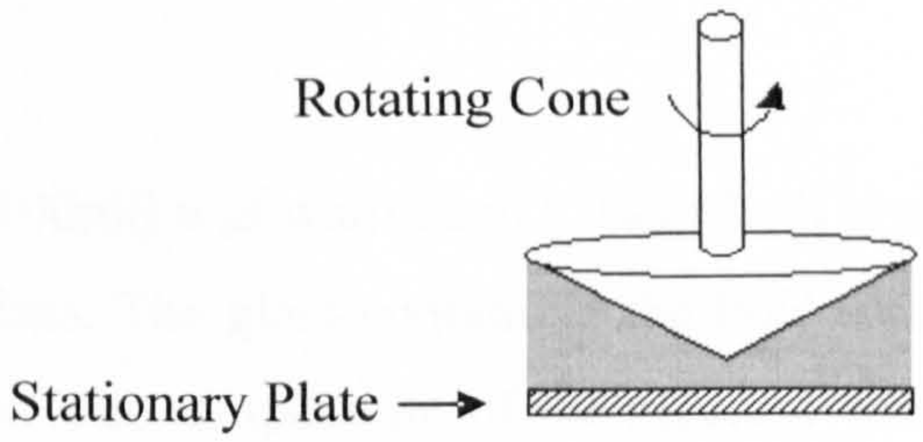


Figure 2.14 Schematic diagram of a cone and plate viscometer

Procedures

The procedure was composed of three steps; pre-experimental where solutions were pre-sheared for 1min and the temperature equilibrated at 37°C; then a flow step where the shear rate was increased over 3min from 0 to 500 s^{-1}; finally, the shear rate was decreased from 500 to 0 s^{-1} over 3min. The sample was placed at the centre of the plate which was raised up to the correct position. Data were transferred to software where a plot of shear rate versus shear stress was obtained. The rheological behaviour of media containing milk or proteins was determined using this technique.

2.5.2 Surface tension

2.5.2.1 Introduction

The surface tension of all dissolution media was measured to determine its influence on drug dissolution. Although the values of surface tension for some of the media used already exist in literature, it was still necessary to determine the surface tension using the starting materials utilized in the current investigations, because of the possible existence of impurities in these materials which may have an impact on the surface tension (Crison et al., 1997). Furthermore, measuring the surface tension of all media using the same technique and conditions would be more appropriate for comparative purposes.

2.5.2.2 Method

The surface tension of the dissolution media was measured using a Wilhelmy plate tensiometer Camtel CDCA model 100 (Camtel Ltd. UK). The method utilized the interaction of a platinum plate with the liquid interface being tested using the static mode. The length, width and thickness of the platinum plate were 40.4, 20 and 0.2 mm, respectively.

The medium (100ml) was warmed in a water bath to approximately 37° and then poured into a Pyrex glass. The glass containing the fluid was placed on the motorized stage of the tensiometer. The temperature of the medium was maintained at 37 ± 0.5°C by the use of a circulating water bath and samples left for 10min before starting the experiment for the temperature to equilibrate. The stage was lifted up automatically towards the Wilhelmy plate at a speed of 0.2mm s^{-1} until the plate was 0.2mm immersed in the fluid. Then, the plate perimeter was wetted and its total mass increased to a maximum, proportional to the surface tension of the fluid. The apparatus was interfaced by a computer for control and data acquisition.

The contact angle between the plate and the lower liquid must be zero and hence $\cos \theta = 1$ (Figure 2.15). Therefore, the surface of the plate was carefully cleaned with distilled water and roughened by flaming using a Bunson burner.

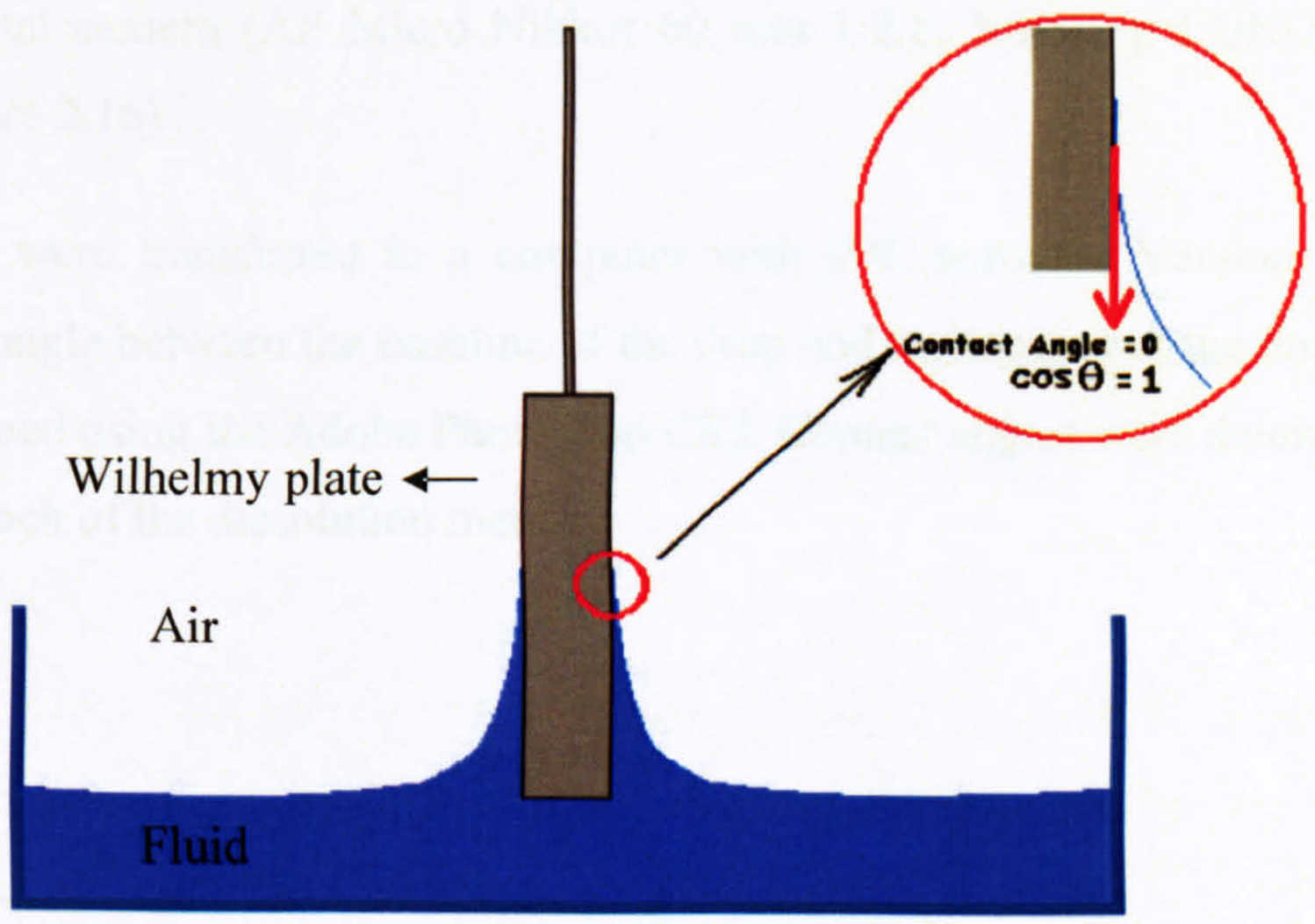


Figure 2.15 Schematic diagram of Wilhelmy plate method, from Moules (1998)

Extra care was taken to avoid any carry over. All glassware used for the surface tension measurements was thoroughly cleaned by soaking with ammonium persulphate in sulphuric acid, washed with distilled water and then dried in hot air oven.

2.5.3 Wettability assessment

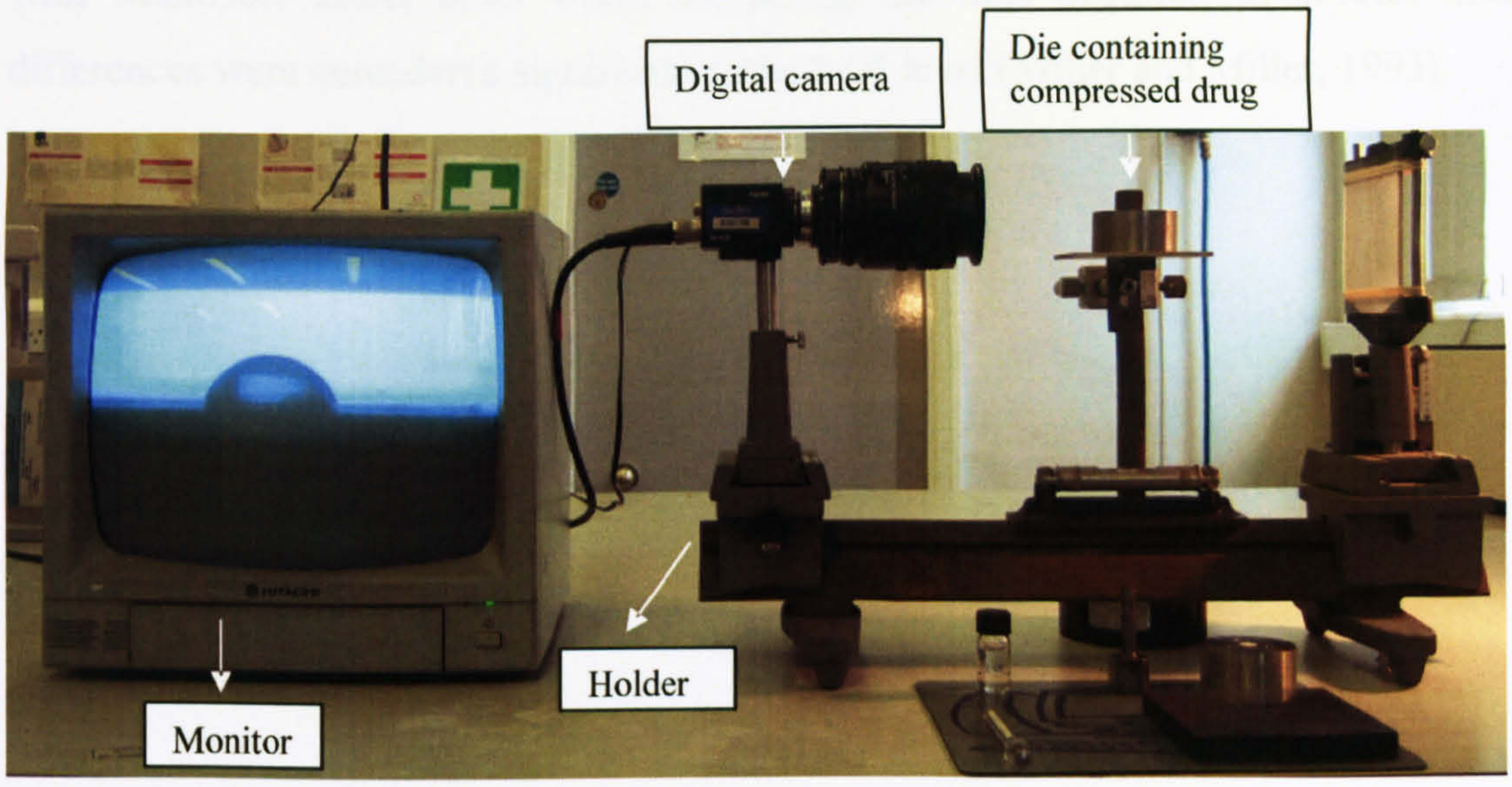
2.5.3.1 Introduction

The contact angle describes the shape of a liquid droplet resting on a solid surface and it is defined as the angle between the tangent line that the drop forms with the solid surface (Fell, 2002). The ability of a liquid to wet the surface and displace the air phase from that surface leads to an increase in the effective surface area subjected to the dissolution fluid.

2.5.3.2 Method

The Sessile drop method was adopted for measuring the contact angle on drug compacts prepared following same procedures for IDR disks, Section 2.2.3. The contact angle between the disk and the dissolution medium was determined by placing one drop (4µl) of each medium onto the surface of an intact compact using a 10µl glass syringe (Hamilton co., Nevada, USA) (Crowder et al., 2003). Photos were taken immediately after the medium was placed on the compact and every 5s for 5min at room temperature using a digital camera (AF Micro Nikkor 60 mm 1:2.8, Nikon, p LUNIX TM-6CN, Japan) (Figure 2.16) .

The images were transferred to a computer with IDL software Version 6.0 win 32 (x 86). The angle between the baseline of the drop and the tangent at the drop boundary was determined using the Adobe Photoshop CS2. Contact angles were determined twice at least for each of the dissolution media.



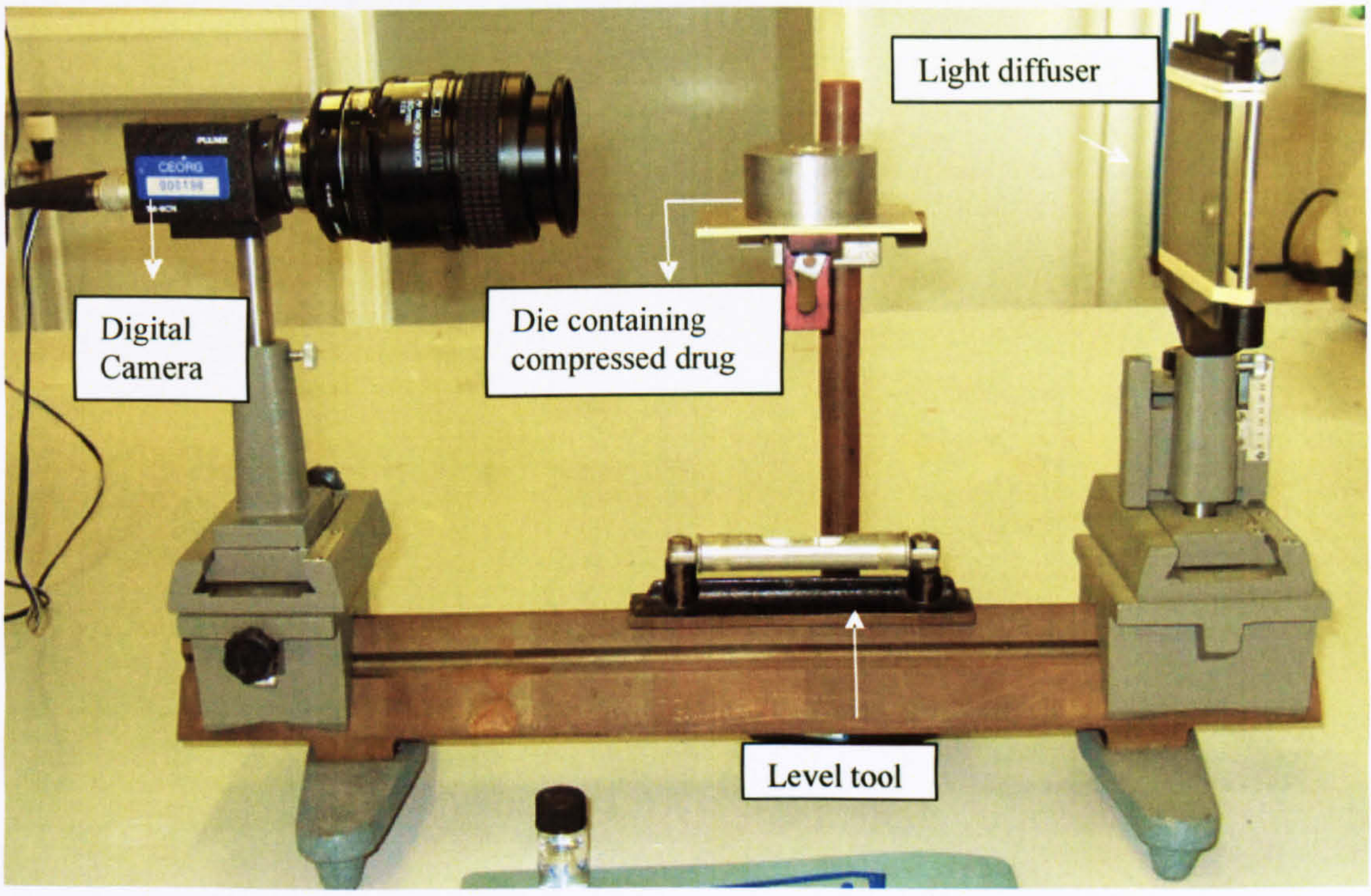


Figure 2.16 Images of arrangement used for contact angle determination

The measured parameters of viscosity, surface tension of the dissolution media and the contact angle formed between the dissolution media and the compact surfaces of the drugs are reported and discussed in the relevant chapters.

2.6 Data analysis

Differences in solubility, dissolution data and physical parameters of various media compared to corresponding blank solutions were statistically evaluated using a t-test at

the probability level of 0.05. A one-way analysis of variance (ANOVA) was employed with Microsoft Excel 2003 when comparing the data acquired in several media, differences were considered significant at the 0.05 level (Miller and Miller, 1993).

Chapter 3: Initial studies: characterisation of itraconazole and ketoconazole

3.1 Introduction

The dissolution tests of itraconazole and ketoconazole were performed using the intrinsic dissolution rate technique which required compaction of the drug powder. Therefore, characterisation of the solid-state form of itraconazole and ketoconazole compressed disks was performed prior to the dissolution studies to detect if any solid-state changes occurred due to compression.

Initial studies were performed to evaluate the dissolution and solubility of itraconazole and ketoconazole in compendial media at various pHs, with or without enzymes. Thus, the aim was to explore the behaviour of both the drugs at physiologically relevant pH values. Therefore, the pH of the media was varied over the range typically encountered in the GIT; pH 1.2 was considered a simulation for the fasted stomach (pH: 1.2), pH 3 a simulation for the fed stomach (pH: 3-5) and pH 6.8 a simulation for the small intestine fluid (pH: 5 -7.5), (Section 1.6.2.2). These data were then employed for the subsequent comparison with the dissolution profiles in more complex media.

3.2 Materials and methods

3.2.1 Materials

Details of all materials used in the thermal analysis, preparation of the dissolution media and in performing the experiments are listed in Section 2.1.

3.2.2 Solid phase characterisation

In the present investigation, studies were carried out to determine whether compression of the drug led to any polymorphic changes using differential scanning calorimetry, infra-red spectroscopy and powder x-ray diffractometry (Brittain, 1999c).

3.2.2.1 Principles of solid phase analysis

3.2.2.1.1 Differential scanning calorimetry

Differential scanning calorimetry (DSC) measures the amount of energy absorbed or evolved by a sample when heated, cooled or kept at constant temperature. This energy represents the heat flow required to maintain equal temperature in the sample and a reference. Thus, by scanning through a range of temperatures, while heating and/or

cooling, phase transitions such as melting, crystallization or glass transition can be determined (Brittain, 1999c).

If the drug is present in the crystalline form, an endothermic peak in the plot corresponding to the melting point (m.p.) of the crystals will be usually observed. If the sample is transformed to a less stable crystalline form or it is composed of mixture of polymorphs, three possible DSC curves could characterise this phenomena (Figure 3.1), as reported by Giron (1995):

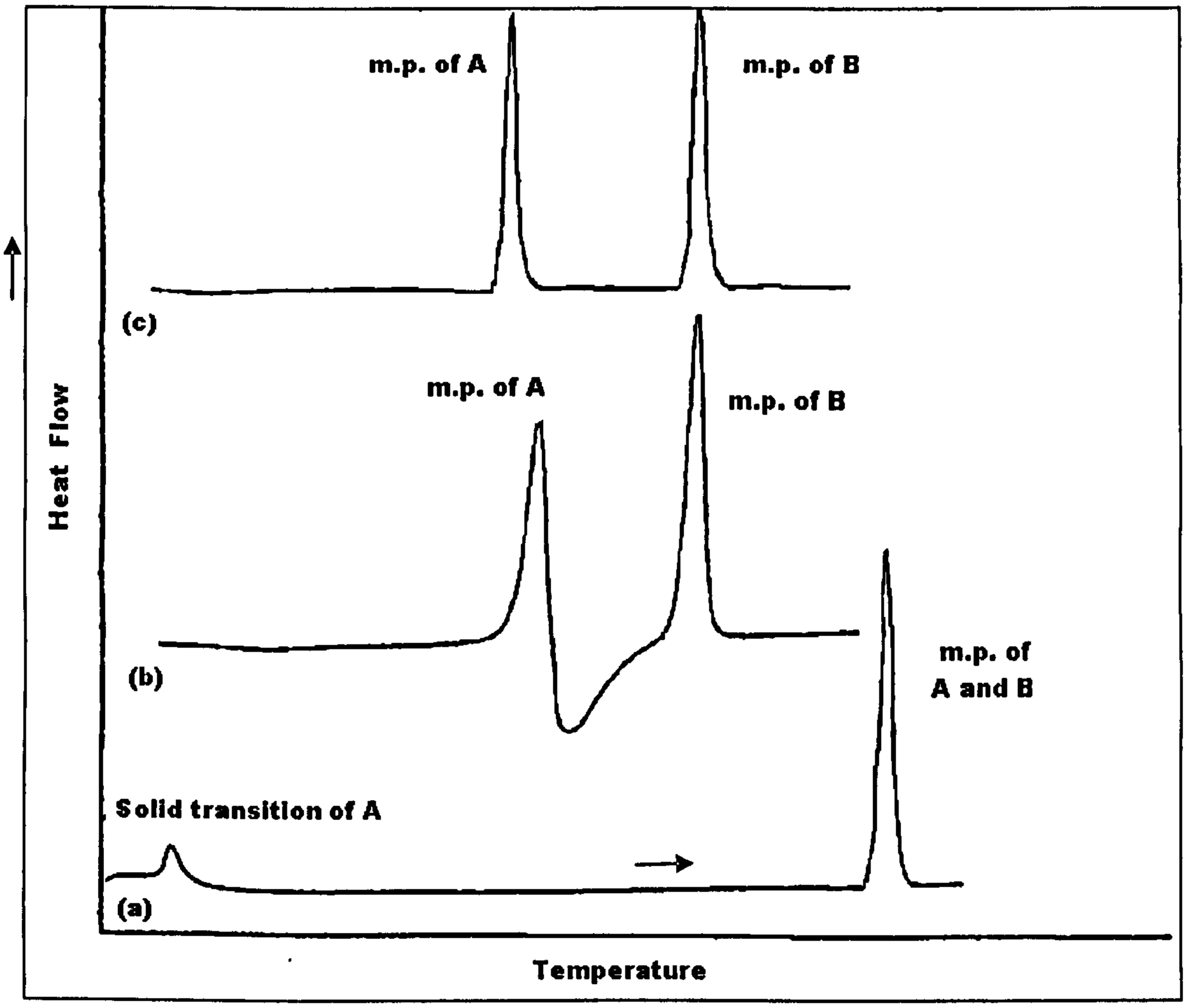


Figure 3.1 Examples of DSC curves obtained for two polymorphs A and B, a) Solid transition of A then one melting peak, b) Melting of A then recrystallisation and subsequent melting of B and c) Two crystalline forms, each has its own m.p.

a) A solid-solid transition occurs before the melting point of the high m.p. form. By this transition the less stable form is converted to the stable one which then melts at the characteristic m.p.

b) After the melting of the form with the lower m.p., another melting peak appears in the DSC curve attributed to the formation of crystals that grow from the melt of the first form (recrystallisation). Thus, the DSC graph shows two melting peaks.

c) Each form has a melting peak and no transformation occurs between the forms.

If the drug is present in an amorphous state, it may undergo a glass transition corresponding to a decrease in heat capacity, followed by crystallisation and melting.

Previous DSC studies described the thermal behaviour of amorphous itraconazole and ketoconazole. When amorphous itraconazole was heated, it showed the following possible stages: transition at 59°C, 74 °C and 90°C and then a possible re-crystallization at 120°C (Six et al., 2001). Amorphous ketoconazole went through a glass transition at 44.5°C, followed by a possible exothermic crystallization at 120°C (Van den Mooter et al., 2001).

3.2.2.1.2 Powder X-ray Diffraction

Powder X-ray diffraction (PXRD) is one of the most reliable techniques for solid phase differentiation (Rodriguez-Spong et al., 2004). A beam of X-rays passed through a sample of randomly-oriented microcrystals produces a diffraction pattern of rings on a distant screen (Brittain, 1999c). The position of the peaks corresponds to the periodic spacing between the atoms. Polymorphs usually have different diffraction patterns because different lattice constants lead to different peak positions (Rodriguez-Spong et al., 2004). The amorphous compound often does not show a pattern, or possibly shows only one or two broad peaks.

3.2.2.1.3 Infrared spectroscopy

Infrared spectroscopy provides information about the structure and conformation of the solid state and allows the distinction between polymorphs by probing the vibration of the atoms. A change in crystal morphology and a lack of crystallinity often affects hydrogen bonding and weak interactions between functional groups in polymorphs. Consequently, a change in the vibration of the atoms occurs leading to a shift in the IR absorbance of these bands (Rodriguez-Spong et al., 2004).

3.2.2.2 Sample handling

Itraconazole and ketoconazole disks were prepared as detailed in Section 2.2.3. The disks were broken by knocking the disks smoothly using a small stainless steel spatula and the pieces were collected on a weighing boat. Samples of the compressed materials analysed were not fine powder but pieces of compressed disk. The reason for not grinding the compressed samples was to avoid applying energy through the grinding process that could possibly change the lattice structure of the drug and so form a different solid form. Grinding may also convert the crystal form to an amorphous substance (Brittain, 1999b).

3.2.2.3 Differential scanning calorimetry

Thermal analysis of itraconazole and ketoconazole samples was performed using a Perkin-Elmer Pyris DSC calorimeter (Norwalk, USA). The calorimeter was calibrated for temperature against the melting point of pure indium (158.6°C and the onset 157.4°C when heated at 10°C min^{-1}). Samples (2-3mg) were placed in aluminium pans and crimped in position; a similar empty pan was used as a reference. Samples were heated at 1, 10 and 100°C min^{-1} from 20°C to 200°C.

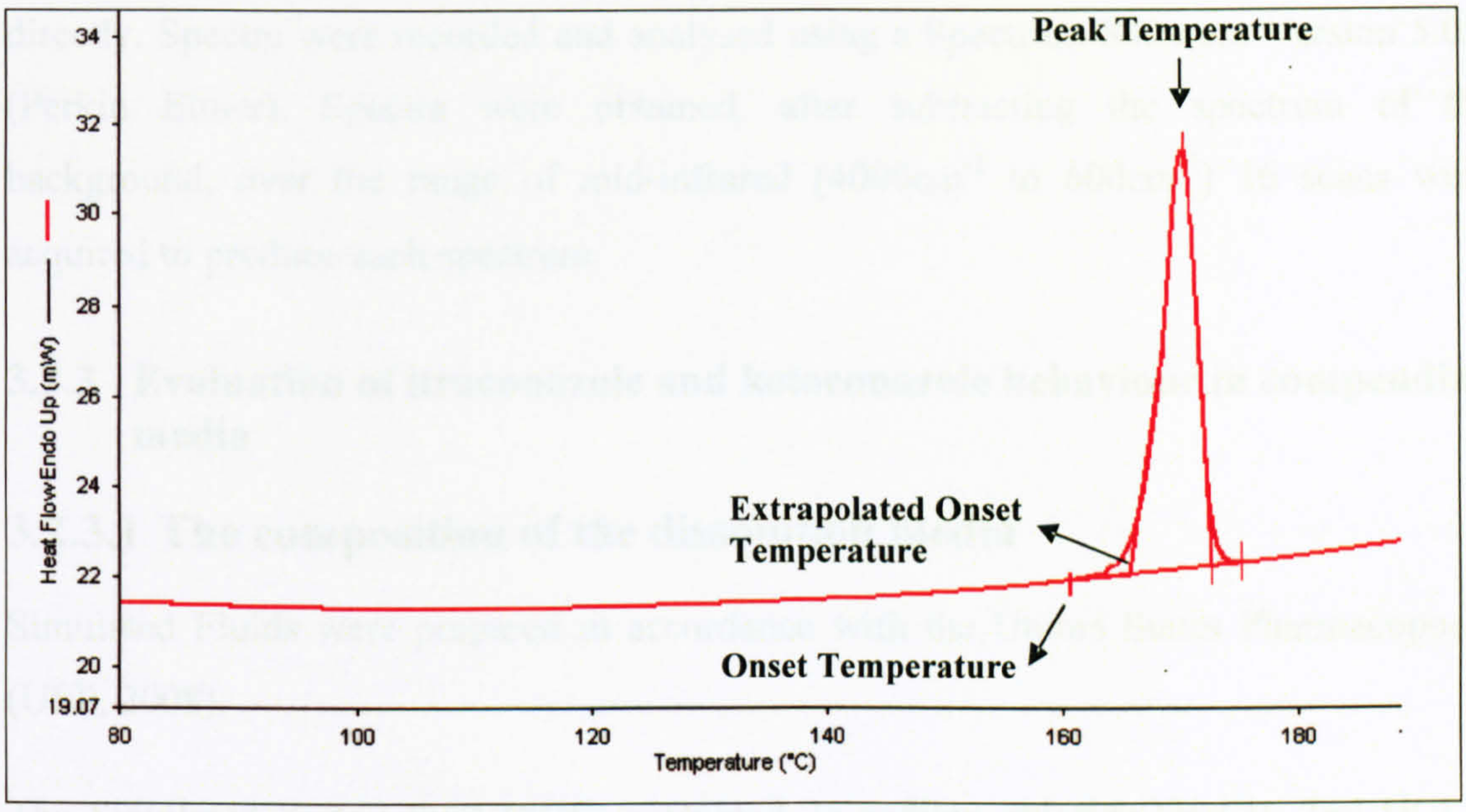


Figure 3.2 DSC scan for itraconazole at 10°C min^{-1} showing the positions of onset temperature, extrapolated onset temperature and peak temperature

The DSC data were presented as curves of the heat flow as a function of temperature where the area under a DSC peak was proportional to the heat absorbed by the thermal event. The peak temperature was defined as the temperature corresponding to the maximum of the endotherm (Ford and Timmins, 1989b). The extrapolated onset temperature represented the intersection of the extrapolated leading edge of the peak to the baseline. The onset temperature represented the temperature where the transition started, i.e. just left the baseline. Figure 3.2 shows an illustration of a typical DSC scan. The parameters: extrapolated onset peak, enthalpy values (ΔH) and peak area were determined using the provided software (Pyris 1^{TM} DSC).

3.2.2.4 Powder X-ray diffraction

X-ray diffraction patterns for each of the un-compressed and compressed itraconazole and ketoconazole samples were recorded using an X-ray diffractometer (Miniflex, Rigaku Corporation, Tokyo, Japan) with a kb-filtered Cu/Kα line as the source of

radiation, operated at 30kV voltages and a current of 15mA. The angular range 5-60° 2θ was scanned at a speed of 5° min^{-1} at room temperature.

3.2.2.5 FT-IR spectroscopy

Infrared spectra for compressed and non-compressed samples were recorded using a Perkin Elmer BX FT-IR system (Perkin-Elmer Corporation, UK). Samples did not require any pre-treatment as a MIRacle attenuated total reflectance (ATR) accessory was used which allowed powder and small piece of compressed sample to be analysed directly. Spectra were recorded and analysed using a Spectrum Software Version 5.0.1 (Perkin Elmer). Spectra were obtained, after subtracting the spectrum of the background, over the range of mid-infrared (4000cm^{-1} to 600cm^{-1}) 16 scans were acquired to produce each spectrum.

3.2.3 Evaluation of itraconazole and ketoconazole behaviour in compendial media

3.2.3.1 The composition of the dissolution media

Simulated Fluids were prepared in accordance with the United States Pharmacopoeia (USP, 2008).

The Simulated gastric fluid (SGF) contained 2g sodium chloride (NaCl), 7ml HCl to make the pH 1.2 and deionised water up to 1L

SGF with enzyme contained 2g NaCl and 3.2g pepsin in 7ml HCl and volume was taken to 1L by deionised water, the pH of the media was 1.2.

SGF (pH 3) contained 2g NaCl and 0.1M HCl (to make the pH) and the volume was taken to 1L by deionised water.

SGF (pH 3) with enzymes had same composition as SGF (pH 3) but with the addition of 3.2g pepsin.

Simulated intestinal fluid (SIF) with enzymes contained 10g of pancreatin, 6.8g of monobasic potassium phosphate (KH_2PO_4), 0.2M sodium hydroxide (NaOH) and deionised water up to 1L. Potassium phosphate was dissolved in 250ml of water then 77ml of 0.2M NaOH and pancreatin were added. The solution was mixed, the volume was completed to 1L with water and the final pH was adjusted with either 0.2M NaOH or 0.2M HCl to 6.8.

SIF (pH 6.8) was prepared as for SIF with enzymes but without the addition of pancreatin.

Phosphate buffer 0.05M (pH 7.5) contained 27.2g of KH_2PO_4, 40ml of 30%, w/v of potassium hydroxide solution and deionised water up t o 1L (BP, 2008).

Acetate buffer (pH 5) contained 13.6g of sodium acetate, 6ml of glacial acetic acid and sufficient deionised water up to 1L (BP, 2008).

3.2.3.2 Characterisation of the media

The viscosity and surface tension have important effects on drug dissolution (Section 1.6.1). Therefore, it was essential to evaluate and consider these parameters when comparing the effect of different media on drug dissolution.

3.2.3.2.1 Viscosity

The viscosities of the media were evaluated using the vibrational viscometer (Section 2.5.1.2). The probe of the apparatus was immersed in the media and measurements were taken directly from the read-out unit of the apparatus. Measurements were taken in triplicate.

3.2.3.2.2 Surface tension

The surface tension was measured using the Wilhelmy plate technique in the static mode (Section 2.5.2). The liquid was raised up and allowed to touch the plate and so the force acting on the plate was measured automatically and transferred to the interfaced computer. Measurements were taken at 37 ± 0.5°C in triplicate.

3.2.3.3 Solubility studies in compendial media

The solubility of itraconazole and ketoconazole was determined as a function of the pH in the following media: SGF (pH 1.2), SGF (pH 3), acetate buffer (pH 5), SIF (pH 6.8) and phosphate buffer (pH 7.5).

The modified shake-flask method was applied (Section 2.2.2). Drug substances (approximately 30mg for itraconazole and 300mg for ketoconazole) were added in excess to closed vials containing 10ml of dissolution medium and shaken for 24h at 37°C. The amount of ketoconazole was increased to 400mg when studying the solubility in SGF (pH 1.2). Undissolved material was separated by centrifugation followed by filtration and the resultant filtrates were quantified using HPLC.

Prior to analysis itraconazole samples in highly acidic media were diluted two-fold with the mobile phase used for HPLC analysis (acetonitrile and 0.02M potassium dihydrogen phosphate (60:40, v/v) adjusted to pH 3.0 with 5M HCl). No dilution was applied to other samples due to the low concentration of the drug in these fluids. The temperature of the samples in the HPLC compartments was maintained at 35 ± 2°C, to avoid any possible precipitation in the HPLC vials.

Filtrates of ketoconazole were diluted, if required, with a mixture of water: acetonitrile (50:50). Samples in SGF (pH 1.2) were diluted 10-fold.

3.2.3.4 Dissolution tests in compendial media

Dissolution studies were carried out using the stationary disk method (Section 2.2.3). Itraconazole and ketoconazole powders were compressed to form circular compacts. The dies containing the drug compacts were then placed in the dissolution vessels with a single face exposed to 500ml of dissolution media (37°C). Samples were withdrawn at different time points through syringe filters (5μm Versapor filters for itraconazole and 0.45μm PVDF filters for ketoconazole). The dissolution tests were performed for 5h, except the dissolution of itraconazole in SGF (pH 1.2) which was further monitored for a longer period of 2 days.

Collected samples of itraconazole and ketoconazole aliquots were injected onto the HPLC system via autosampler without pre-treatment. Only when SGF (pH 1.2) with enzymes was used as dissolution medium for itraconazole, the collected samples were diluted 50:50 with the mobile phase. Ketoconazole samples collected from media containing enzymes were diluted 50:50 with acetonitrile-water (50:50).

3.2.3.5 Drug Analysis

HPLC was used for the analysis of solubility and dissolution samples of both drugs, (Section 2.2.6). For itraconazole analysis, a fluorescence detector was used with an injection volume of 50μl.

For ketoconazole analysis, the UV detector wavelength was set at 254nm with an injection volume of 20μl. However, for samples collected from SIF media, the fluorescence detector (λ_{Exc}=251nm, λ_{Emm}=376nm) was employed instead.

3.2.3.6 Disk surface morphology

The change in the morphology of itraconazole disks during the dissolution process was monitored using SEM according to the method described in Section 2.4.4. SEM images were taken for itraconazole disks after exposure to the dissolution process in SGF (pH 1.2) after 5, 24 and 48h and in SIF after 5h.

3.3 Results and discussion

3.3.1 Solid phase characterisation

The morphology of solids can affect the solubility and dissolution rate of materials (Section 1.6.1.1). Polymorphs behave differently when they are in the solid state but once in solution behave the same. Compression is a high energy process which may affect the crystallinity of drugs by inducing dislocational strains in crystal which facilitate nucleation. Chan and Doelker (1985) reported that compression of drugs, which exist in different polymorphic states, induced polymorphic transformations towards the more stable forms. In addition, the compaction process can destroy the crystal structure leading to the formation of amorphous material which usually has higher solubility (Connors, 2002). Therefore, an assessment of solid state is a pre-requisite for IDR studies from disks of compact drug powder. For example, if the dissolution experiment was carried out on a metastable form which has higher solubility compared to the stable form, then the liquid will be supersaturated with respect to metastable form leading to possible precipitation. The production of amorphism was reported by Persson et al. (2005) whilst preparing IDR disks of griseofulvin and cyclosporine, where signs of minor amorphism were observed on the surface of the disks.

3.3.1.1 Differential scanning calorimetry

3.3.1.1.1 Itraconazole

DSC scans taken before and after compression showed one single endothermic peak corresponding to the melting point of itraconazole (169-170.4°C) and did not exhibit any thermal event before melting (Figure 3.3). This suggests that the drug did not undergo any solid-state alterations indicating the samples contained only one crystal form of itraconazole. However, the compressed sample displayed a decrease in peak parameters, approximately computed as: the extrapolated onset m.p. (1.7°C), the onset

m.p. (2.9°C), ΔH (6.5J g^{-1}) and the peak area (16m J), compared to non-compressed itraconazole powder (Table 3.1).

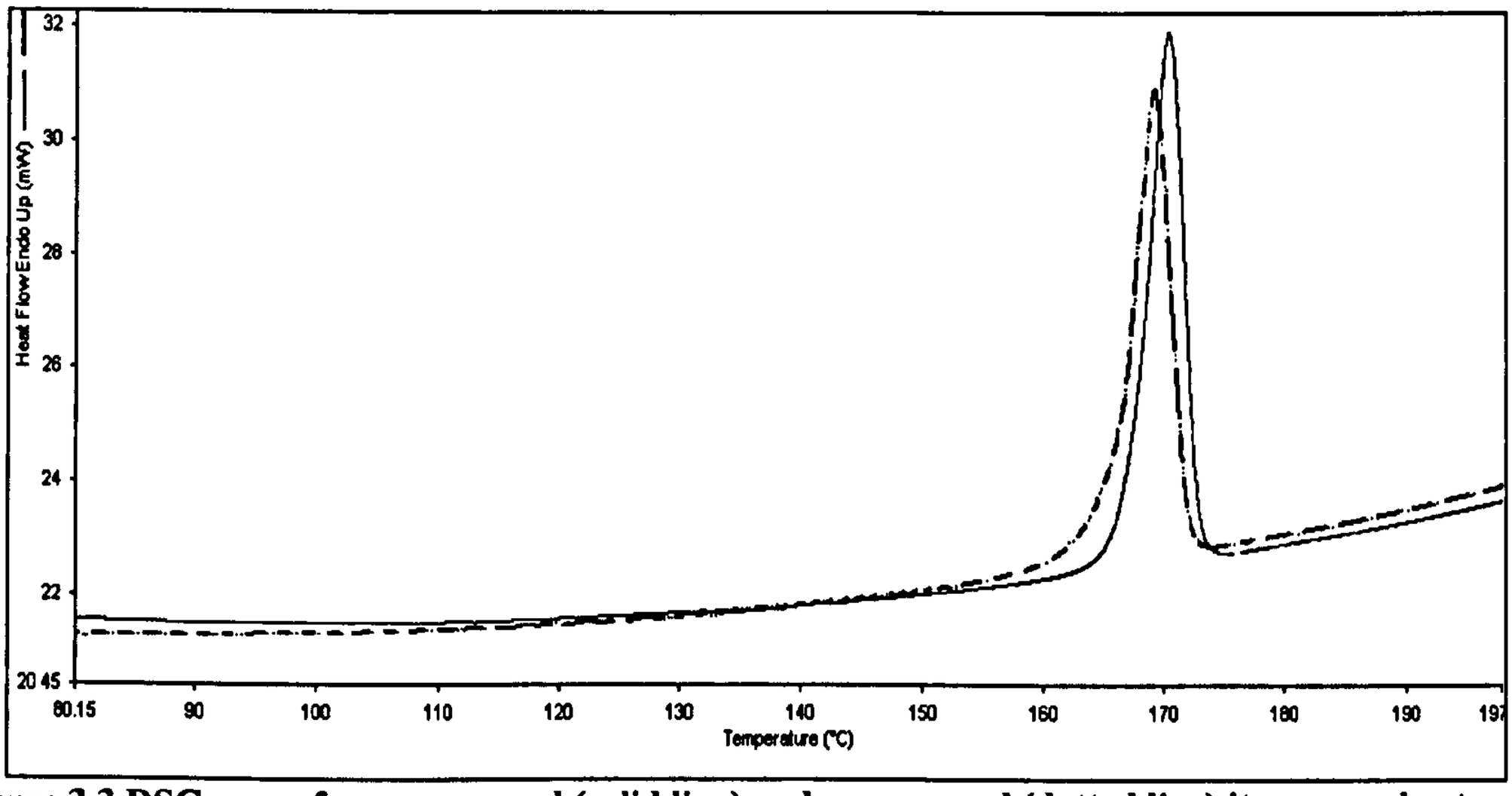


Figure 3.3 DSC scan of uncompressed (solid line) and compressed (dotted line) itraconazole at a heating rate of 10°C min^{-1}

To find out the reason for the decrease in these values, different heating rates were applied with the aim of differentiating any thermal transition events that occurred (Giron, 1995). Consequently, samples were scanned at slow (1°C min^{-1}) and fast heating rates (100°C min^{-1}). One endothermic peak appeared in each DSC scan and the same observations were found regarding the decrease in the onset peak (Table 3.1). A decrease in the extrapolated onset, peak area and ΔH was recorded also but was not significant (t-test at 0.05 level). An increase in the onset interval, defined as extrapolated onset minus onset peak, was noticed.

Table 3.1 DSC data for itraconazole compressed and un-compressed, the values presented are average of two experiments ± S.D.

Heating rate (°C min^{-1})	Itraconazole	1	10	100
Extrapolated onset peak (°C)	Non-compressed	164.0 ± 0.6	167.6 ± 0.5	172.1 ± 0.2
	Compressed	163.4 ± 0.7	165.9 ± 0.6	169.8 ± 1.3
Onset peak (°C)	Non-compressed	161.8 ± 0.3	165.6 ± 0.2	166.7 ± 1.1
	Compressed	160.2 ± 0.2	162.7 ± 0.5	162.9 ± 1.5
Peak area (m J)	Non-compressed	204.9 ± 9.4	227.5 ± 2.2	218.7 ± 7.4
	Compressed	173.4 ± 13.3	211.5 ± 3.5	203.9 ± 12.8
ΔH (J g^{-1})	Non-compressed	81.7 ± 6.1	83.1 ± 0.3	87.6 ± 5.7
	Compressed	71.0 ± 5.6	76.6 ± 0.9	77.6 ± 3.8

3.3.1.1.2 Ketoconazole

One endotherm peak appeared in the DSC scan at 146.1-148.5°C representing the melting point of ketoconazole with no other transitions observed (Figure 3.4). This suggested the presence of ketoconazole in one crystal form.

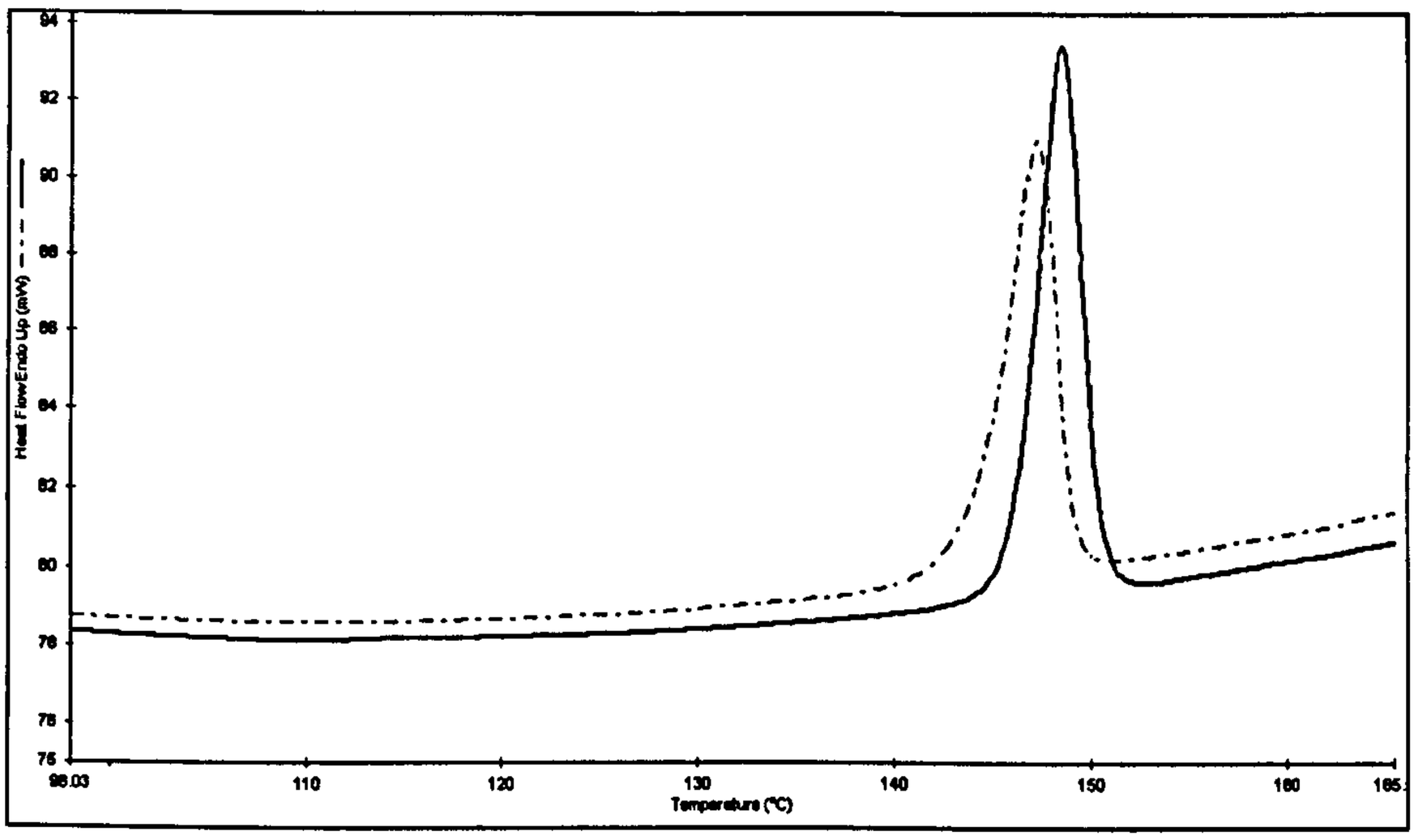


Figure 3.4 DSC scan of uncompressed (solid line) and compressed (dotted line) ketoconazole at a heating rate of 10°C min^{-1}

However, there was a decrease in the peak parameters of the compressed samples. When samples were heated at 10°C min^{-1}, the decrease was computed as approximately: the onset peak (3°C), extrapolated onset peak (1.6°C), ΔH (12.8J g^{-1}) and peak area (25m J), compared to non-compressed sample (Table 3.2). Similar observations were found with slow (1°C min^{-1}) and fast heating rates (100°C min^{-1}) where a reduction in DSC peak parameters of the compressed samples was apparent although not all the differences observed, such as the peak areas, were significant. An increase in the peak onset interval (extrapolated onset minus onset) was also noted.

The decrease in peak parameters could be attributed to the size and the shape of the samples. Uncompressed samples were powder composed of fine particles, whereas the compressed samples were small pieces of broken disks of the compact drug powder which had a larger particle size. It was reported previously that the size and shape of particle can have an important effect on thermal properties (Ford and Timmins, 1989a).

Table 3.2 DSC data for ketoconazole compressed and un-compressed, the values presented are average of two experiments ± S.D.

Heating rate (°C min^{-1})	Ketoconazole	1	10	100
Extrapolated onset peak (°C)	Non-compressed	146.7 ± 0.2	146.0 ± 0.2	147.9 ± 0.1
	Compressed	145.1 ± 0.1	144.5 ± 0.1	146.6 ± 0.2
Onset peak (°C)	Non-compressed	143.8 ± 1.3	143.8 ± 0.9	144.1 ± 0.9
	Compressed	142.1 ± 1.5	140.9 ± 0.9	142.1 ± 1.1
Peak area (m J)	Non-compressed	208.7 ± 8.9	235.5 ± 2.1	213.8 ± 3.5
	Compressed	195.1 ± 8.6	210.5 ± 6.4	195.8 ± 7.7
ΔH (J g^{-1})	Non-compressed	90.1 ± 3.2	102.4 ± 0.9	87.4 ± 2.5
	Compressed	82.3 ± 1.0	89.6 ± 2.8	85.7 ± 5.2

Van Dooren (1982) found that larger particles of adipic acid have a lower onset melting peak, lower ΔH and greater peak onset interval than the smaller particles. This shift in the values was ascribed to less heat transfer occurring between the bigger particles so the hotter parts of the particles may reach a higher temperature and melt before releasing the energy to the colder parts. This resulted in a decrease in the thermal effect and a smaller peak area with an earlier melting onset.

3.3.1.2 Powder X-ray diffraction

The representative X-ray diffraction patterns for compressed and non-compressed itraconazole (Figure 3.5) exhibited the characteristic crystalline peaks which were at the same position for both samples (12.71°, 14.78°, 19.16° and 20.25°). This represented a slight shift from literature values recorded for both samples (Lee et al., 2005).

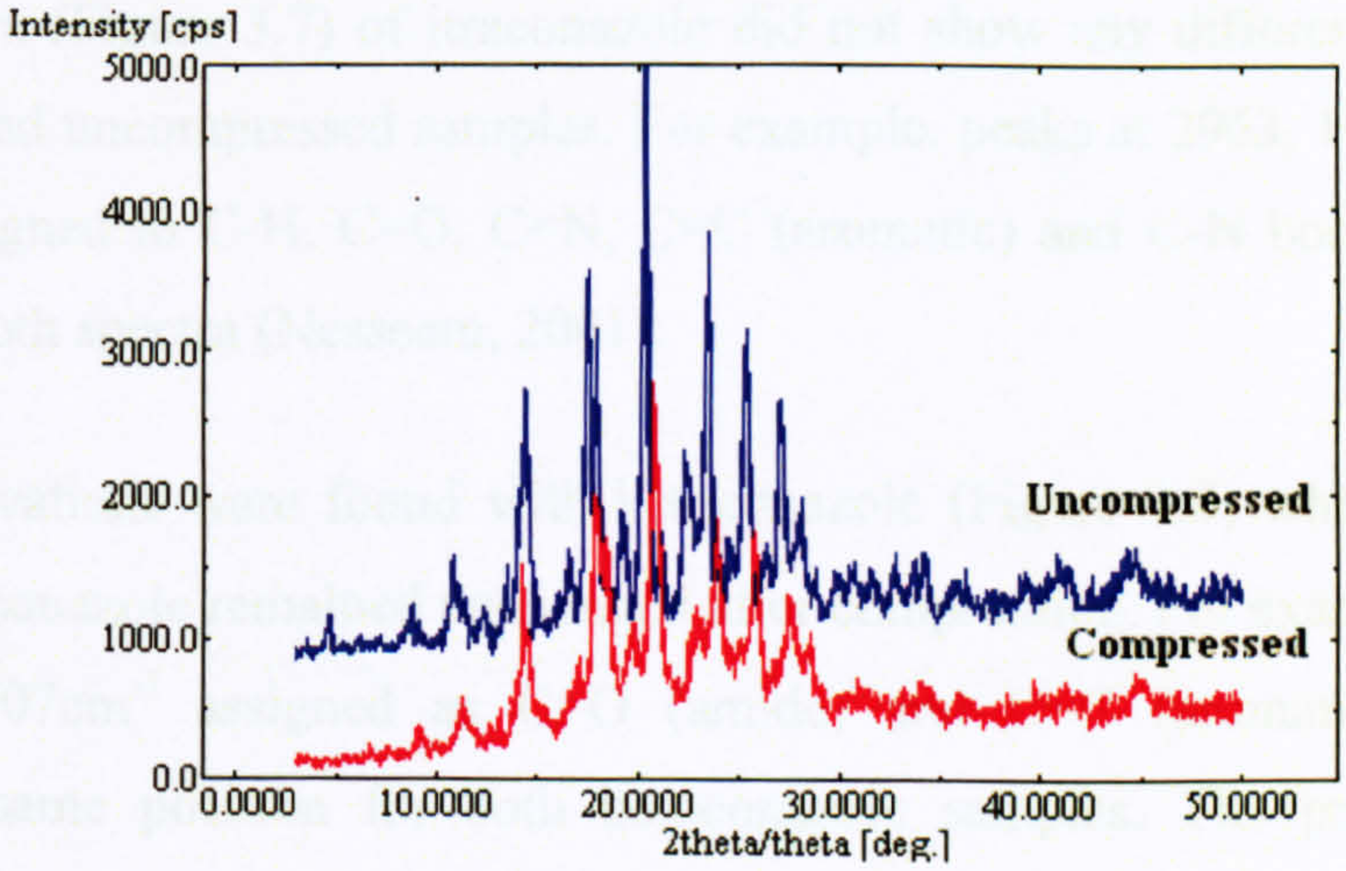


Figure 3.5 Powder X-ray diffraction patterns of compressed and non-compressed itraconazole

The same observations were recorded for ketoconazole as the characteristic diffraction peaks (6.8° and 22.3°) were apparent at the same position for uncompressed and compressed samples (Figure 3.6).

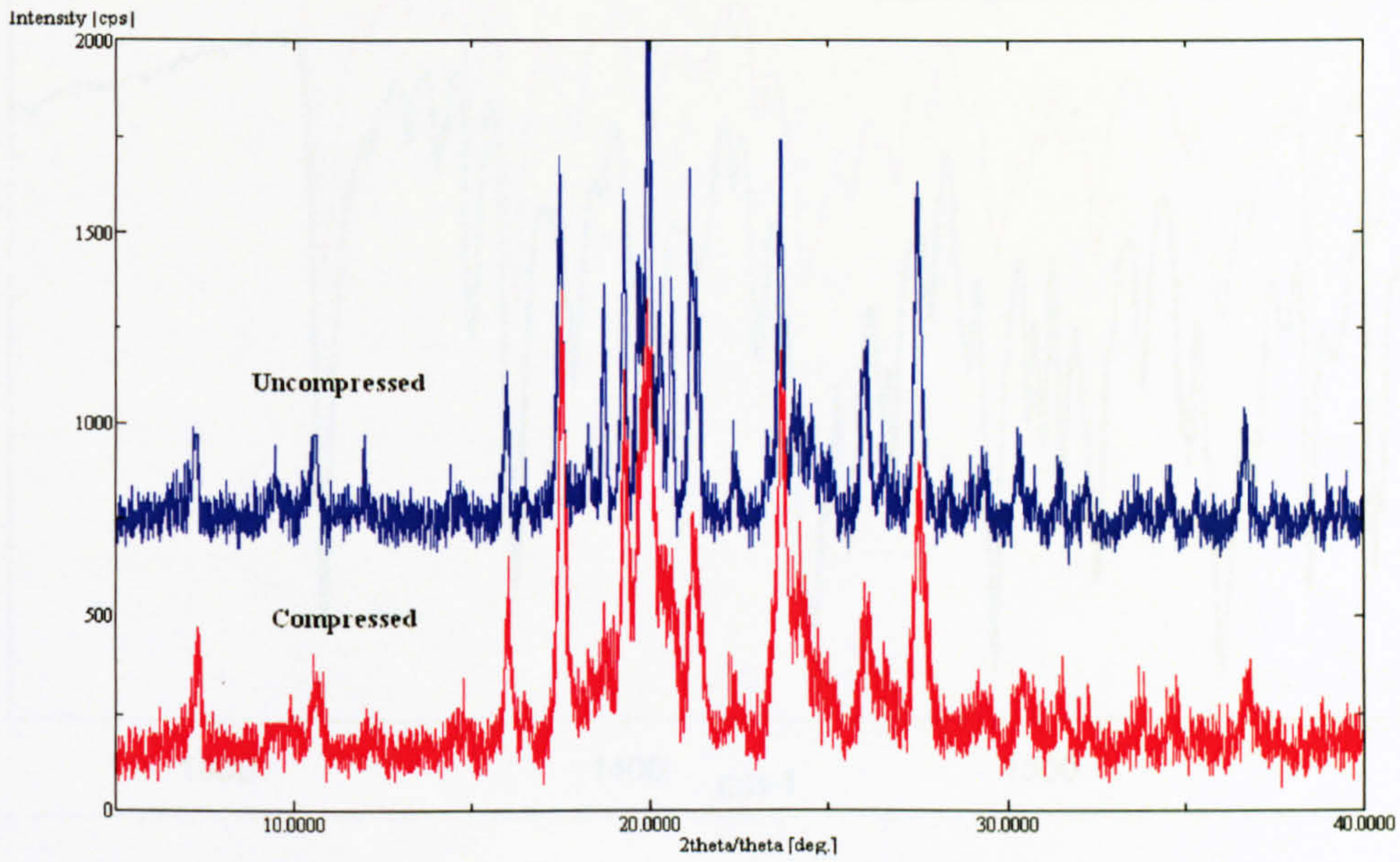


Figure 3.6 Powder X-ray diffraction patterns of compressed and non-compressed ketoconazole

Thus, the results did not show any evidence of crystal lattice modification of itraconazole and ketoconazole with compression. However, the diffraction patterns of the compressed sample of both drugs had a noisier baseline and wider and distorted peaks. It is believed that this change in peak shape was due to differences in sample particle size.

3.3.1.3 FT-IR analysis

The IR spectra (Figure 3.7) of itraconazole did not show any differences between the compressed and uncompressed samples. For example, peaks at 2963, 1697, 1609, 1510, 1425cm^{-1} assigned to C-H, C=O, C=N, C=C (aromatic) and C-N bonds, respectively, appeared in both spectra (Nesseem, 2001).

Similar observations were found with ketoconazole (Figure 3.8) where characteristic peaks of ketoconazole remained unchanged after compression. For example the bands at 1643 and 1507cm^{-1} assigned as C=O (amide) and C=C (aromatic), respectively, appeared at same position for both ketoconazole samples. The provided software indicated a correlation of 0.9931 for itraconazole and 0.9991 for ketoconazole, when spectra of compressed and non-compressed samples were overlaid. Thus, this indicated that no polymorphic transformation occurred.

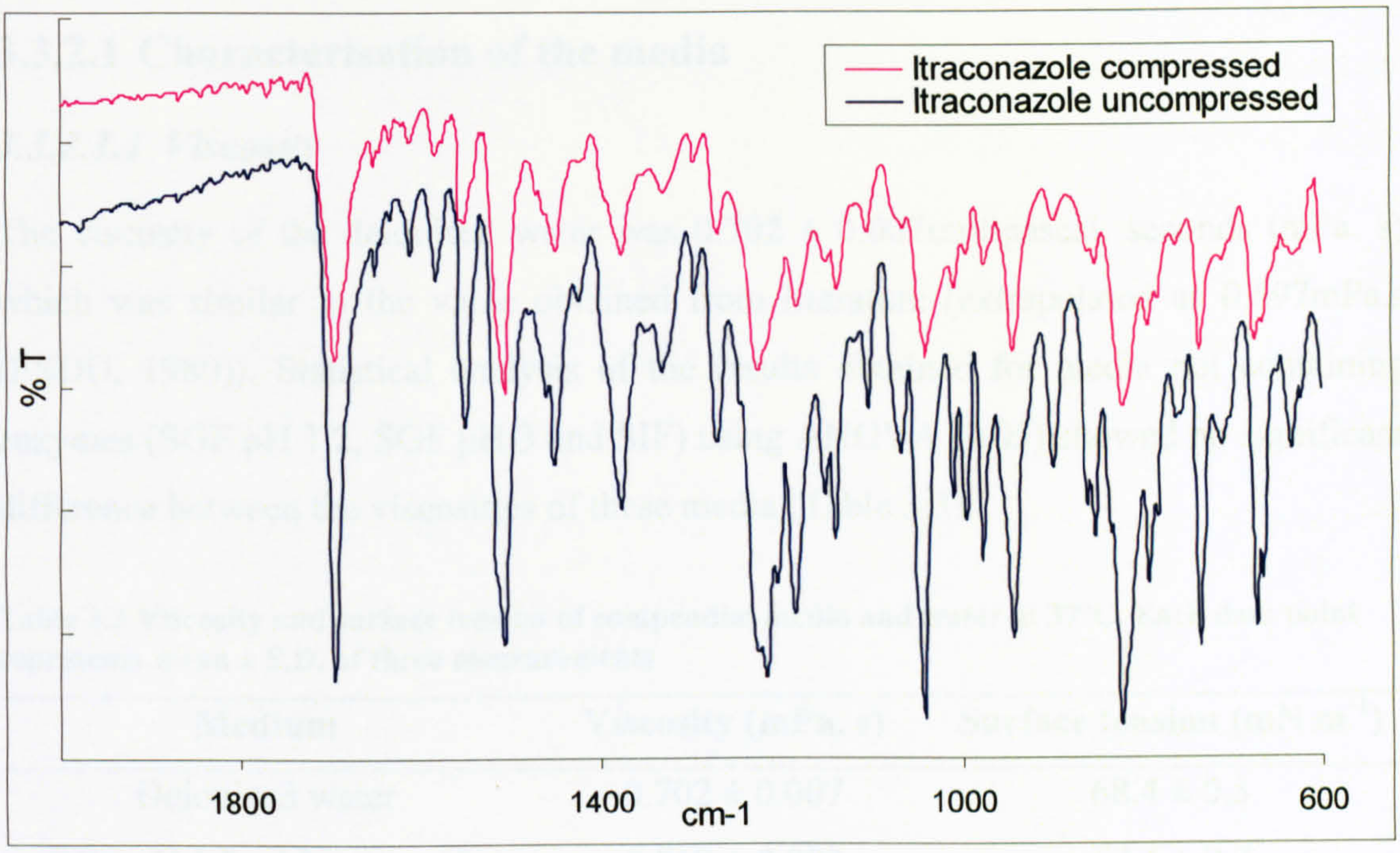


Figure 3.7 FT-IR spectra of uncompressed and compressed itraconazole

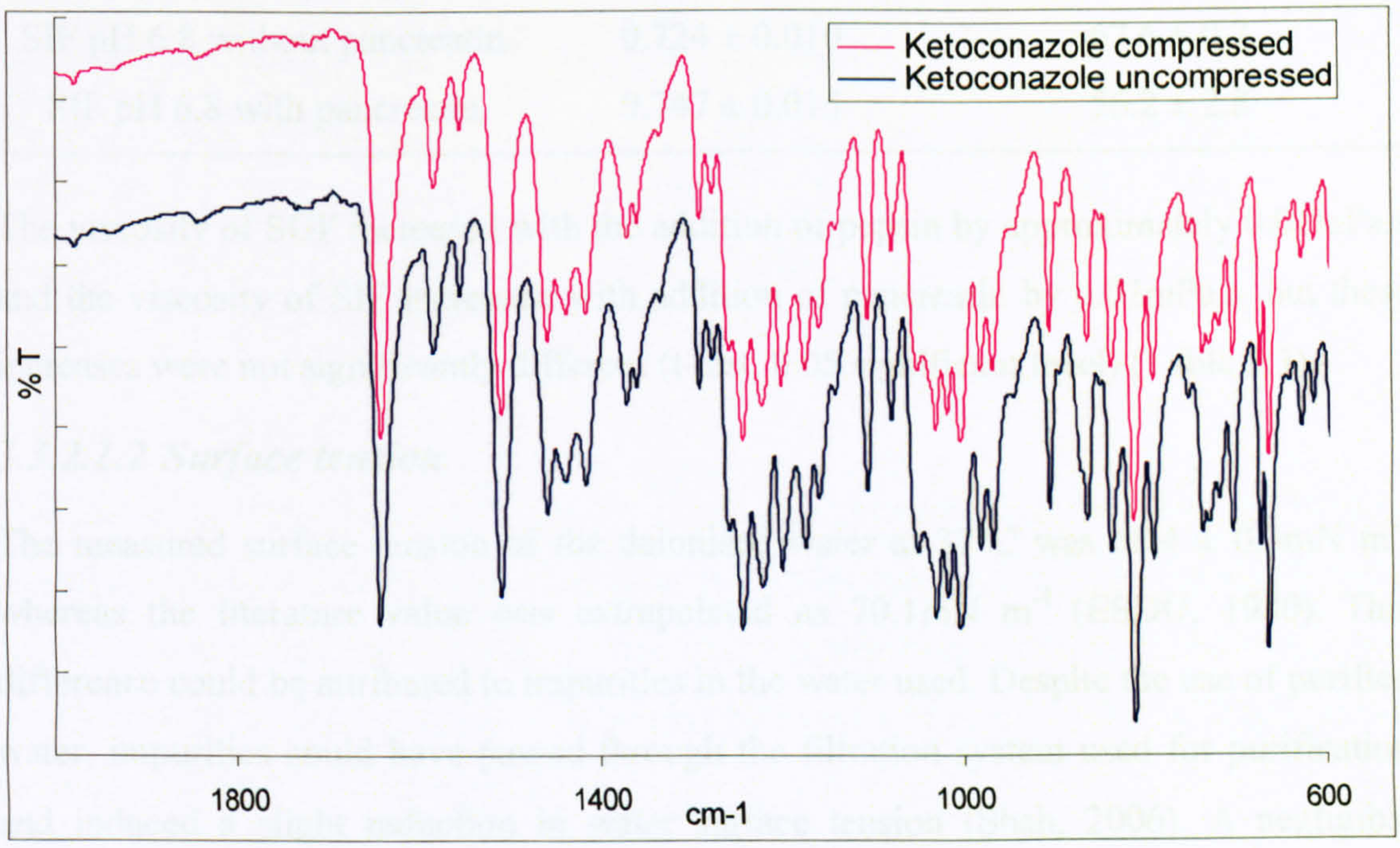


Figure 3.8 FT-IR spectra of uncompressed and compressed ketoconazole

3.3.2 Evaluation of itraconazole and ketoconazole behaviour in compendial media

3.3.2.1 Characterisation of the media

3.3.2.1.1 Viscosity

The viscosity of the deionised water was 0.702 ± 0.007millipascal. seconds (mPa. s) which was similar to the value obtained from literature (extrapolated as 0.697mPa.s (ESDU, 1980)). Statistical analysis of the results obtained for media not containing enzymes (SGF pH 1.2, SGF pH 3 and SIF) using ANOVA (0.05) showed no significant difference between the viscosities of these media (Table 3.3).

Table 3.3 Viscosity and surface tension of compendial media and water at 37°C. Each data point represents mean ± S.D. of three measurements

Medium	Viscosity (mPa. s)	Surface tension (mN m^{-1})
Deionised water	0.702 ± 0.007	68.4 ± 0.3
SGF pH 1.2 without pepsin	0.719 ± 0.009	67.4 ± 0.7
SGF pH 1.2 with pepsin	0.731 ± 0.014	57.5 ± 1.4
SGF pH 3	0.714 ± 0.010	66.4± 1.6
SIF pH 6.8 without pancreatin	0.724 ± 0.010	67.6 ± 0.7
SIF pH 6.8 with pancreatin	0.747 ± 0.015	56.2 ± 2.8

The viscosity of SGF increased with the addition of pepsin by approximately 0.01mPa.s and the viscosity of SIF increased with addition of pancreatin by 0.02mPa.s, but these increases were not significantly different (t-test, 0.05 significant level) (Table 3.3).

3.3.2.1.2 Surface tension

The measured surface tension of the deionised water at 37°C was 68.4 ± 0.3mN m^{-1} whereas the literature value was extrapolated as 70.1mN m^{-1} (ESDU, 1980). This difference could be attributed to impurities in the water used. Despite the use of purified water, impurities could have passed through the filtration system used for purification and induced a slight reduction in water surface tension (Shah, 2006). A negligible difference was observed between the surface tension values determined for water and the aqueous media (SGF pH 1.2, SGF pH 3 and SIF pH 6.8). However the additions of pepsin or pancreatin resulted in a significant decrease in the surface tension of SGF and SIF by approximately 10mN $m^{-1,}$ (Table 3.3).

3.3.2.2 Solubility determinations

The solubility of both drugs was assessed in various media of different compositions over a pH range from 1.2 to 7.5, the composition of the media and the solubility method were described in Sections 3.2.3.1 and 3.2.3.3. Evidence of the poor aqueous solubility of the two drugs is presented in Table 3.4. Itraconazole proved to be approximately 2700 times less soluble than ketoconazole. These results also indicated that the solubility of both drugs is highly pH dependent and requires an acidic environment for maximum solubility.

Table 3.4 Solubility of itraconazole and ketoconazole at 37°C in SGF (with and without pepsin), SIF (with and without pancreatin), acetate buffer, phosphate buffer and deionised water. Each data point represents the mean ± SD of 3 measurements

Medium	Itraconazole Solubility ($\mu g\ ml^{-1}$)	Ketoconazole Solubility ($mg\ ml^{-1}$)
Deionised water	~ 0.002	0.0054 ± 0.0005
SGF (pH 1.2)	3.9 ± 0.7	20.33 ± 3.26
SGF (pH 1.2) with pepsin	8.1 ± 1.15	23.42 ± 2.41
SGF (pH 3)	0.035 ± 0.003	0.43 ± 0.05
Acetate buffer (pH 5)	0.011 ± 0.004	0.1 ± 0.009
SIF (pH 6.8)	~ 0.003	0.007 ± 0.001
SIF (pH 6.8) with pancreatin	0.076 ± 0.01	0.010 ± 0.003
Phosphate buffer (pH 7.5)	~ 0.003	0.006 ± 0.001

3.3.2.2.1 Itraconazole

The solubility of itraconazole in water and SIF was extremely low, less than the limit of quantification of the analysis method (6ng ml^{-1}) (Section 2.4.2.1). The values obtained for aqueous solubility were in accordance with the solubility (0.00152 $\mu g\ ml^{-1}$) reported by Glomme et al. (2005), which was calculated based on log P and the melting point of the drug.

Approximately a 100-fold decrease in itraconazole solubility was noticed when the pH of the medium increased from 1.2 to 3 (Table 3.4). The solubility of the free base is much less than that of the protonated forms. Itraconazole is weakly-basic drug with a pKa of 3.6 and four ionisable nitrogens in its structure, full protonation of the four nitrogens occurs only at low pH of less than 2 (Peeters et al., 2002) (Section 1.10.2). This explains the drop in solubility noticed between pH 1.2 and 3.

3.3.2.2.2 Ketoconazole

The aqueous solubility of ketoconazole was 5.4μg ml^{-1}, which was in accordance with the literature values of 4.5μg ml^{-1} (Galia et al., 1998) and 5.98μg ml^{-1} (Glomme et al., 2005). Ketoconazole solubility was also highly dependent on the pH of the medium. Table 3.4 shows that solubility dramatically decreased 47 times when the pH of SGF increased from 1.2 to 3 and further dropped 62 times in SIF compared to SGF pH 3. Ketoconazole is a dibasic drug with two ionisable species, the imidazole and piperazine (Section 1.10.2). At pH 3 all imidazole moieties were ionised (pKa_1 = 6.5) and nearly 50% of piperazine moieties (pKa_2 = 2.9) were protonated. So by lowering the pH further to 1.2, both base moieties were protonated leading to an increase in its solubility under more acidic conditions.

3.3.2.2.3 The effect of enzymes on solubility

The media containing enzymes were turbid and developed signs of precipitation during the solubility and dissolution experiments. This was observed in particular with ketoconazole samples in SIF-pancreatin where the solubility recorded was higher than the solubility in SIF but the difference was not significant. Qazi et al. (2003) studied the solubility of the antiviral drug (stampidine) in SIF with enzyme; however the drug could not be detected on HPLC after performing the solubility test and filtrations. Authors presumed that the association of the drug with the precipitated enzymes led to separation of the drug upon filtration.

The presence of pepsin in the SGF medium led to a 2-fold increase in itraconazole solubility however, a less pronounced effect was seen with ketoconazole. The effect of enzymes is believed to be due to their solubilising effect as indicated by the low surface tension of their media compared to the corresponding media without enzymes (Table 3.3).

3.3.2.3 Intrinsic dissolution studies

The dissolution of itraconazole and ketoconazole was evaluated at 37°C in compendial media with and without enzymes (SGF and SIF) using the IDR method (Section 2.2.4).

3.3.2.3.1 Itraconazole

Due to the low solubility of itraconazole, all the dissolution experiments were performed under non-sink conditions. The accumulation of the drug in the media soon

affected the dissolution process so the linear portion of each curve was brief as saturation solubility was quickly approached. The initial dissolution was considered during the first 15min. A plot of the amount dissolved per unit area versus time was constructed and the slope of the fitted linear regression represented the IDR. The zero point was considered to be when samples were taken from dissolution vessels before immersing the disks. Thereafter, for comparative purposes and in addition to the initial IDR, data for the amounts dissolved per unit area after one hour was also utilized.

It was evident that dissolution was proportional to the pH of the media (Figure 3.9), as under the more acidic conditions (pH 1.2) a greater extent and rate of dissolution was observed. The initial dissolution rate was 12 times greater than that at pH 3.

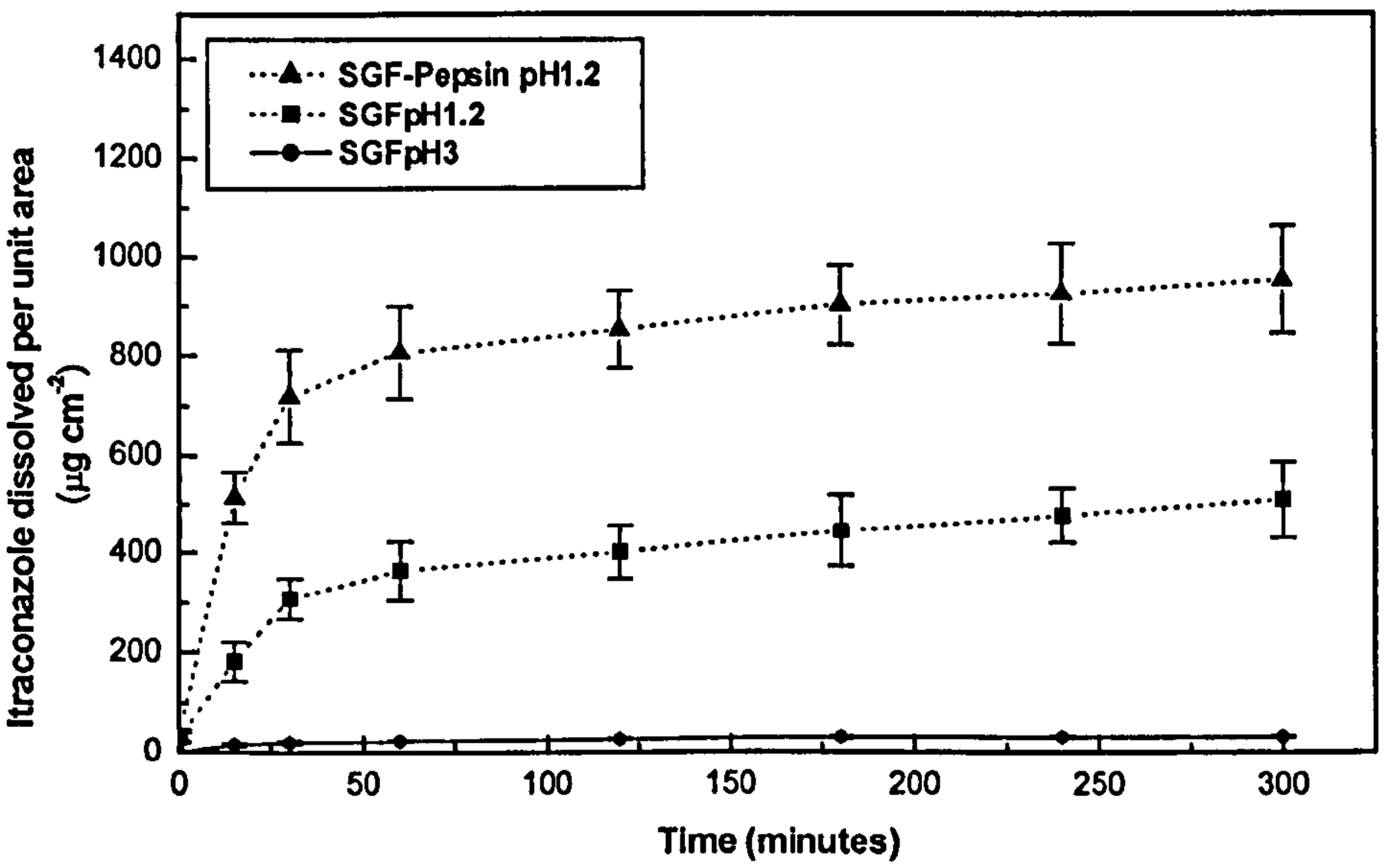


Figure 3.9 Intrinsic dissolution profile of itraconazole at 37°C in SGF at pH 1.2 (with and without pepsin) (n=3) and in SGF pH 3 (n=6). Each data point represents the mean ± S.D.

The dissolution in SGF (pH 3) reached a plateau by 3h since the amount dissolved reached the saturation solubility. The dissolution of itraconazole in the more acidic media (pH 1.2) still increased gradually after 5h. Consequently, the dissolution at pH 1.2 was further monitored for a longer period of 48h (Figure 3.10). This profile exhibited a steady rise throughout the course of the experiment. The amount of itraconazole dissolved after 48h was approximately the same as the solubility indicating an equilibrium state had been reached in the dissolution medium.

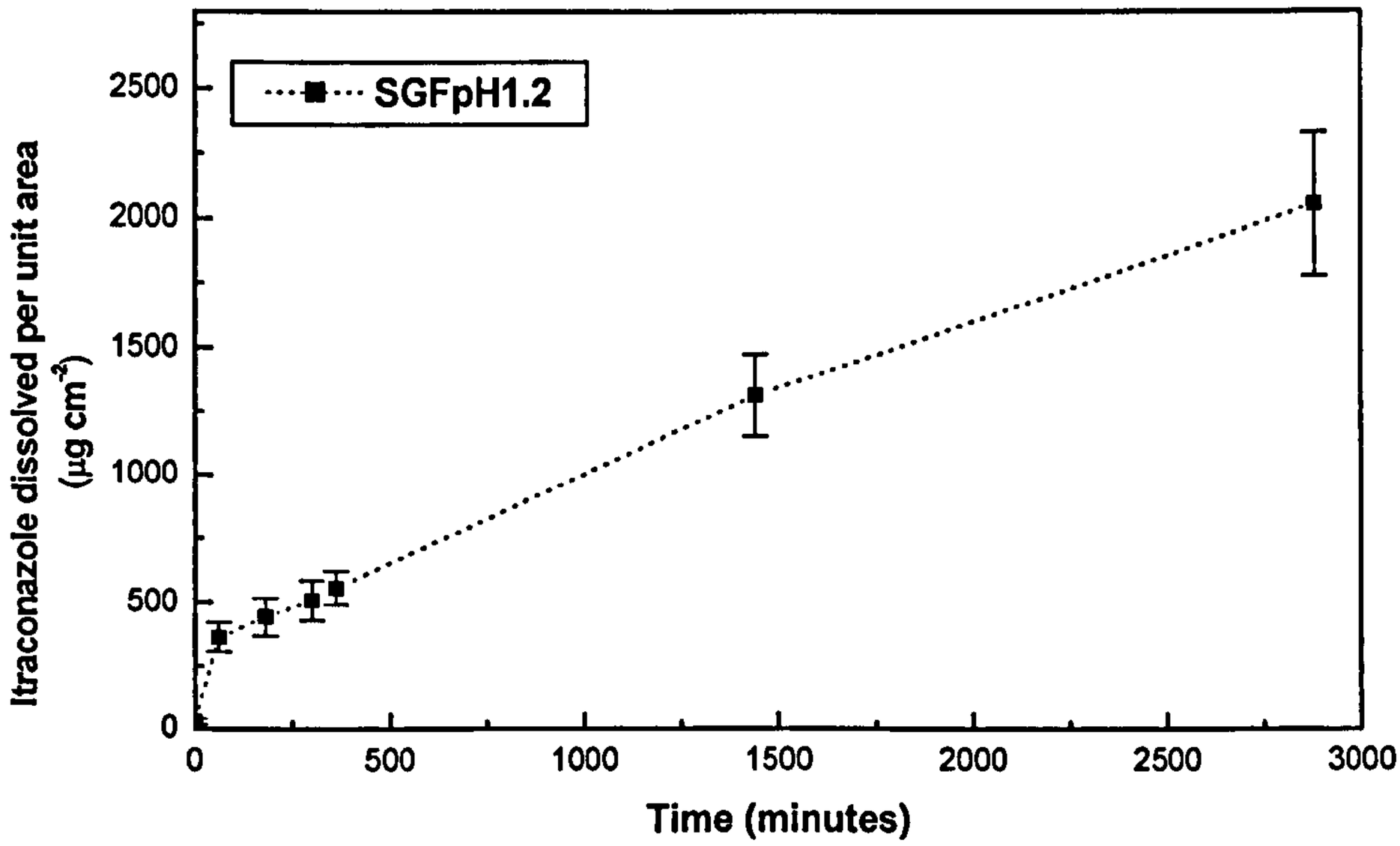


Figure 3.10 Intrinsic dissolution profile of itraconazole at 37°C in SGF at pH 1.2 over 48h. Each data point represents the mean of 3 measurements ± S.D.

The inclusion of pepsin in the medium increased the dissolution rate significantly with an initial burst at the beginning. The initial IDR was three times higher than the value in the non-enzyme solution (Table 3.5). Hence, this increase mirrored the trend in solubility and was attributed to the solubilisation effect of the enzymes that enhanced the dissolution of itraconazole.

Table 3.5 IDR data of itraconazole at 37°C in SGF (pH 1.2) with and without pepsin (n=3) and in SGF (pH 3) (n=6). Each data point represents the mean ± S.D.

Medium	Initial IDR (0-15min) ($\mu g\ min^{-1}\ cm^{-2}$)
SGF pH 1.2	12.1 ± 2.5
SGF pH 1.2 with pepsin	34.3 ± 5.2
SGF pH 3	1.1 ± 0.3

3.3.2.3.2 Ketoconazole

The dissolution of ketoconazole could not be performed at pH 1.2 due to its high solubility at that pH whereby the disks entirely dissolved within 15min. Dissolution of ketoconazole was performed in SGF (pH 3) over a period of 4h. A change in the disk surface was observed after 4h indicating that disk integrity was not maintained after this period which made the IDR determination invalid after that time point.

The dissolution profiles displayed in Figure 3.11 show the profound effect of the pH of the medium on the rate and extent of drug dissolution. The amount dissolved in SGF

(pH 3) was 10 times greater than in SIF (pH 6.8) within one hour, and became 26 times greater within 4h.

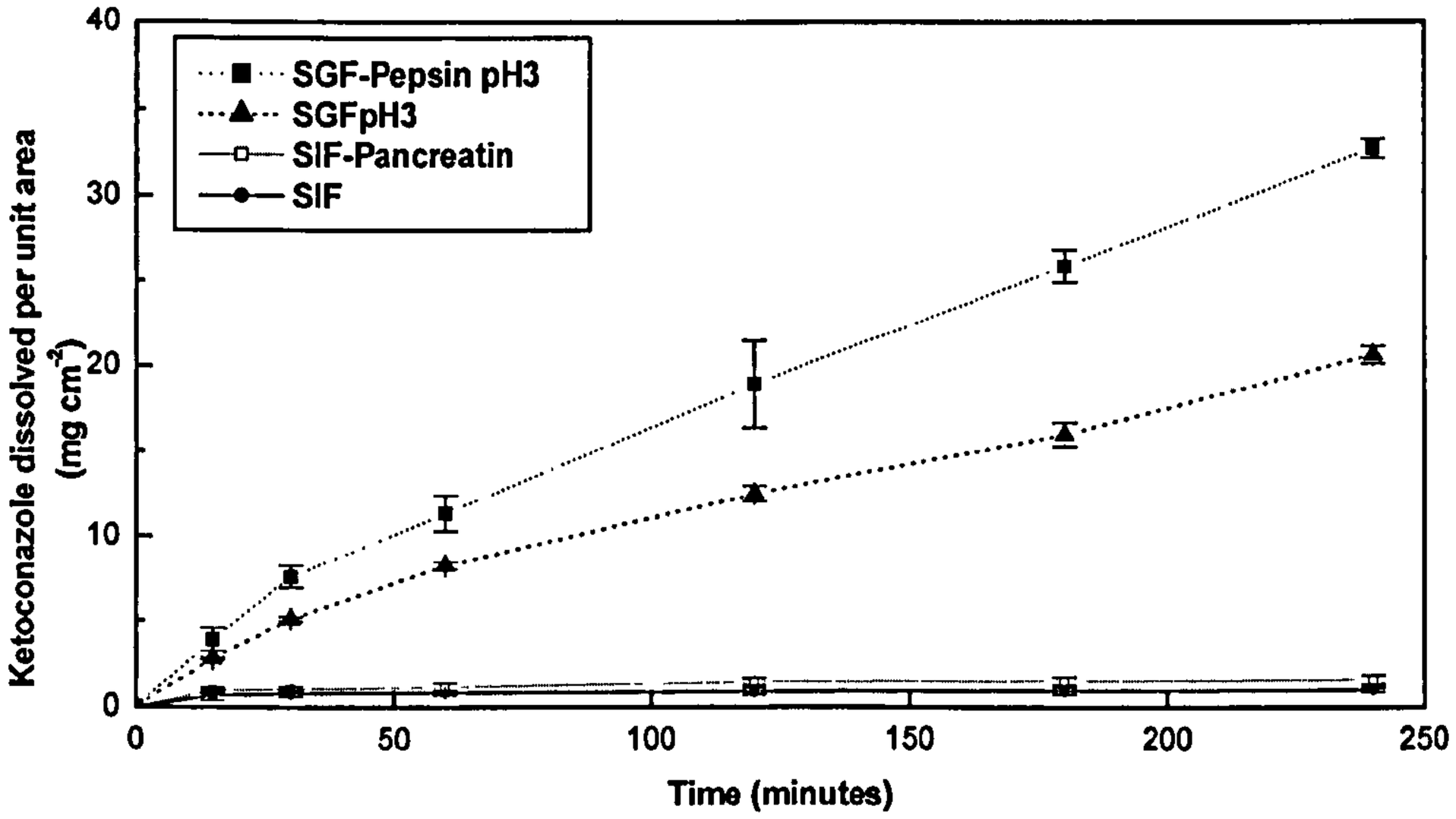


Figure 3.11 The intrinsic dissolution profiles of ketoconazole at 37°C in SGF pH 3 (with and without pepsin) and SIF pH 6.8 (with and without pancreatin). Each data point represents the mean ± S.D. of 3 measurements

The dissolution profiles of ketoconazole were divided into two stages, the initial dissolution profile from time zero to 15min and the subsequent stage up to 240min. The profiles in SGF with and without enzymes were well fitted by a linear function (coefficient of determination, $r^2 > 0.99$). In all cases, the initial dissolution stage (0–15min) was steeper than the final segment indicating a more rapid dissolution in the first 15min (Table 3.6). The addition of pepsin to SGF enhanced the rate of dissolution by 1.4 and 1.7 during the two stages, respectively.

Table 3.6 IDR data of ketoconazole at 37°C in SGF (pH 3) with and without pepsin. Each data point represents the mean ± S.D., n=3

Medium	Initial IDR (0-15min) ($\mu g\ min^{-1}\ cm^{-2}$)	IDR (15-240min) ($\mu g\ min^{-1}\ cm^{-2}$)
SGF pH 3	187.5 ± 27.3	75.4 ± 3.5
SGF pH 3 with pepsin	266.4 ± 13.0	124.3 ± 4.1

On the other hand, the dissolution profiles in the simulated intestinal media were not linear. This non-linearity could be attributed to approaching sink conditions which led to a slowing of the dissolution process. The dissolution in the enzyme-containing medium appeared slightly higher than in the medium without enzyme. However, by applying a t-test (at significance level of 0.05) for the cumulative amounts dissolved at each time point no significant difference between the two graphs was observed. Furthermore, for comparing the dissolution profiles in SIF with and without enzymes

the similarity factor f_2 (Eq.(3.1)) was calculated where T_1 and T_2 are the percentage of accumulative amount dissolved in each dissolution media at each of the sampling time points (n).

$$f2 = 50.\log\left\{\left[1+(1/n)\sum_{t=1}^{n}(T_1-T_2)^2\right]^{-0.5}.100\right\} \qquad \textbf{Eq. (3.1)}$$

This factor was originally employed for comparing the dissolution profiles of solid dosage forms however it was used by Viegas (2001) for comparing IDR profiles. If dissolution profiles were equivalent, the f_2 value would lie between 50 and 100 (Shah et al., 1998). In the current investigation, when comparing the dissolution in SIFs, the calculated f_2 value was 99 which indicated a similarity between these two profiles. Although SIF with pancreatin had a solubilisation activity and was expected to increase drug dissolution this was not observed probably due to the precipitation which led to lower dissolution rates.

3.3.2.4 Surface morphology of itraconazole disks

To gain a deeper understanding of the dissolution process of itraconazole, the surface morphology of the disks was monitored using SEM as a visualizing method. Images were taken after exposure to SGF pH 1.2 at three time points 5, 24 and 48h, and in SIF only after 5h as the IDR dissolution did not show any change after that time point. The displayed images were taken at 500, 1000 and 2000X magnifications.

Although it was expected that dissolution would take place evenly across the surface of the disks, the signs of superficial disintegration started to appear after 5h as if gradual erosion was occurring (Figure 3.12). These observations were more pronounced after 24h (Figure 3.13) where the smoothness of the disk started to diminish. After 48h (Figure 3.14) a marked change was noticed as if a plume was being formed over the disk surface during the dissolution process and the surface appeared to become more porous.

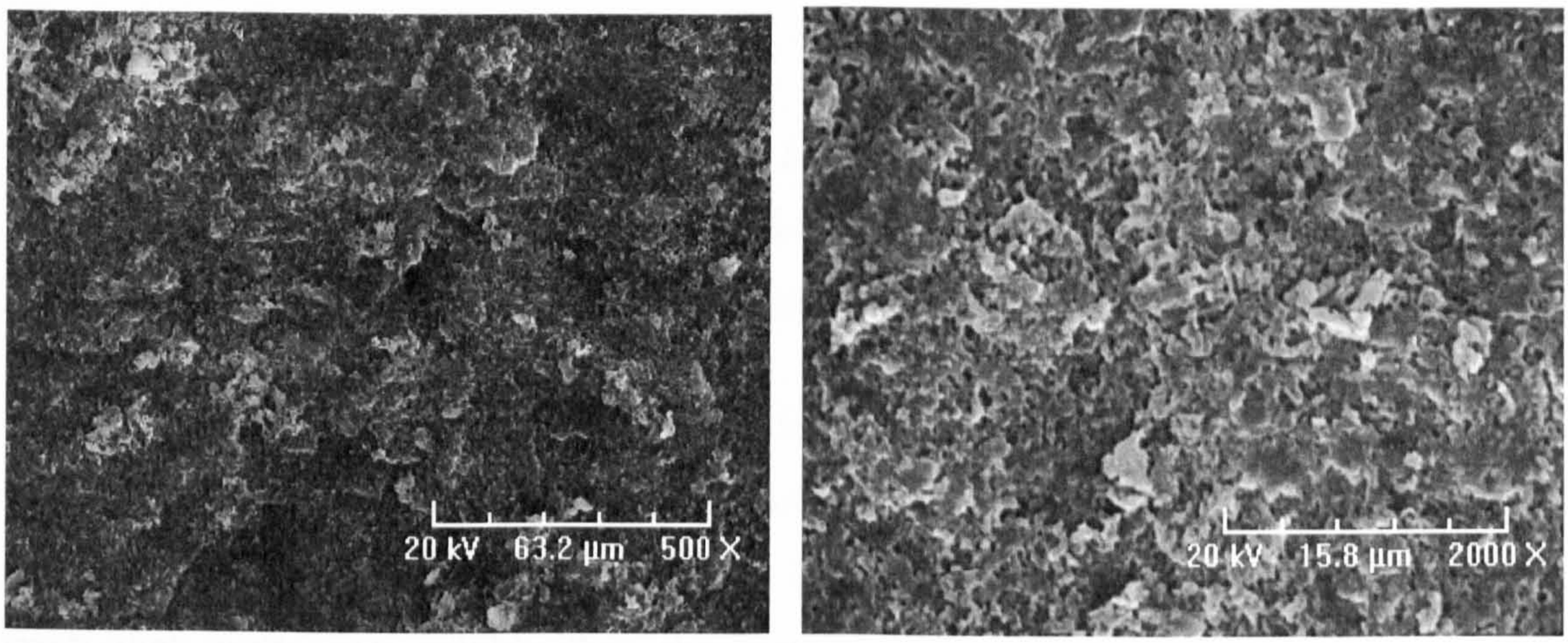


Figure 3.12 SEM photomicrographs for itraconazole disks after exposure to SGF for 5h, taken at two magnifications 500x and 2000x, scale bars: 63.2 µm and 15.8µm

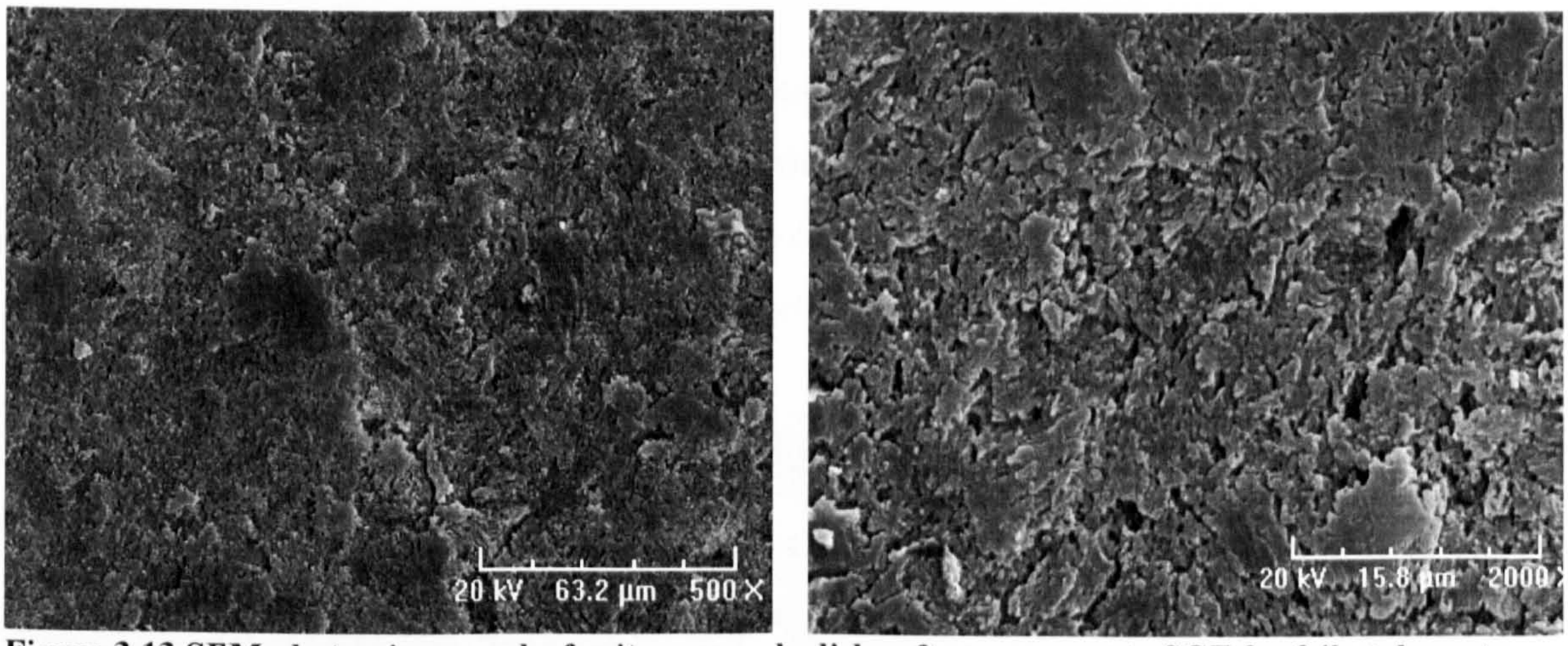


Figure 3.13 SEM photomicrographs for itraconazole disks after exposure to SGF for 24h, taken at two magnifications 500x and 2000x, scale bars: 63.2µm and 15.8µm

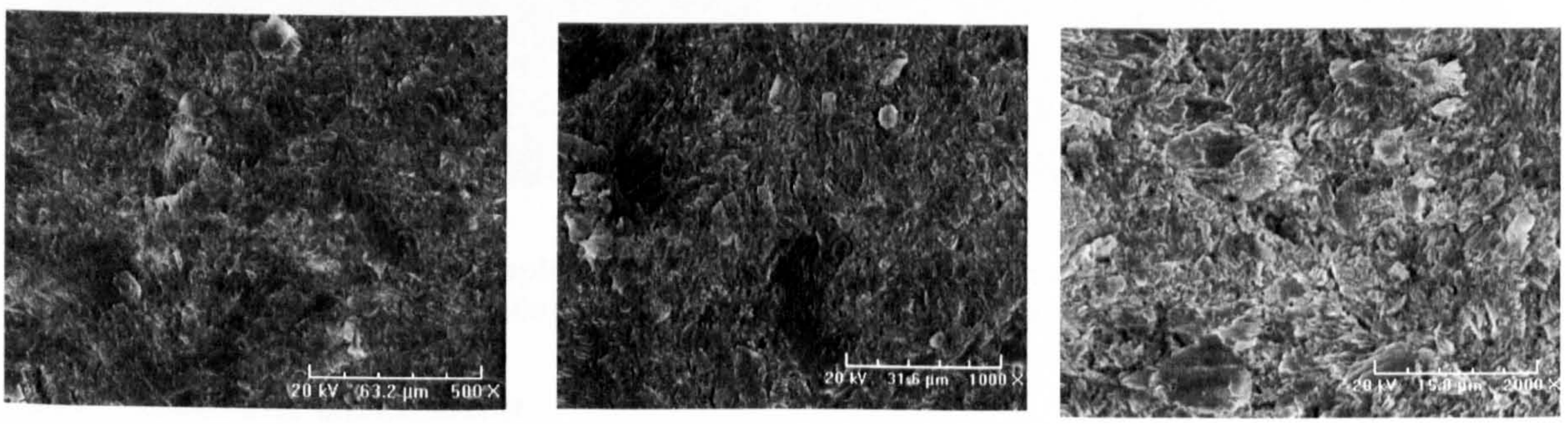


Figure 3.14 SEM photomicrographs for itraconazole disks after exposure to SGF for 48h, taken at three magnifications 500x, 1000x and 2000x, scale bars: 63.2µm, 31.6µm and 15.8µm

This irregularity in the dissolution of the disk could be attributed to the hydrodynamics of the fluid and the direction of fluid flow on the dissolution surface. Previous studies showed that when using the static disk configuration, the fluid flows from the surface of the disk radially and sweeps inwards to the centre of the disk. Then fluid moves axially upwards towards the centre of the paddles, forming a plume of the dissolved material

over the disk. Consequently, this suggested that not all the disk surface was equally accessible to dissolution (Smith and Colton, 1972; Mauger et al., 2003).

Another possibility behind the irregularity in the surface of the disks is that although much effort was applied to smooth the surface of the disk some striations, resulting from the compression of the surface, may still exist leading to this unevenness.

Finally, although investigations to characterise the solid phase of the drug did not show any evidence of amorphism, minor transformations may have occurred which were less than the detection of the applied procedures. Such transformations could create localised spots that were more soluble than the remaining crystalline material (Hancock and Parks, 2000).

In SIF, there was a minimal change in the surface morphology (Figure 3.15), with the surface remaining relatively flat throughout the 5h. This reflects the dissolution profile acquired in that medium where the amount of itraconazole dissolved was less than the limit of quantification of the analytical method.

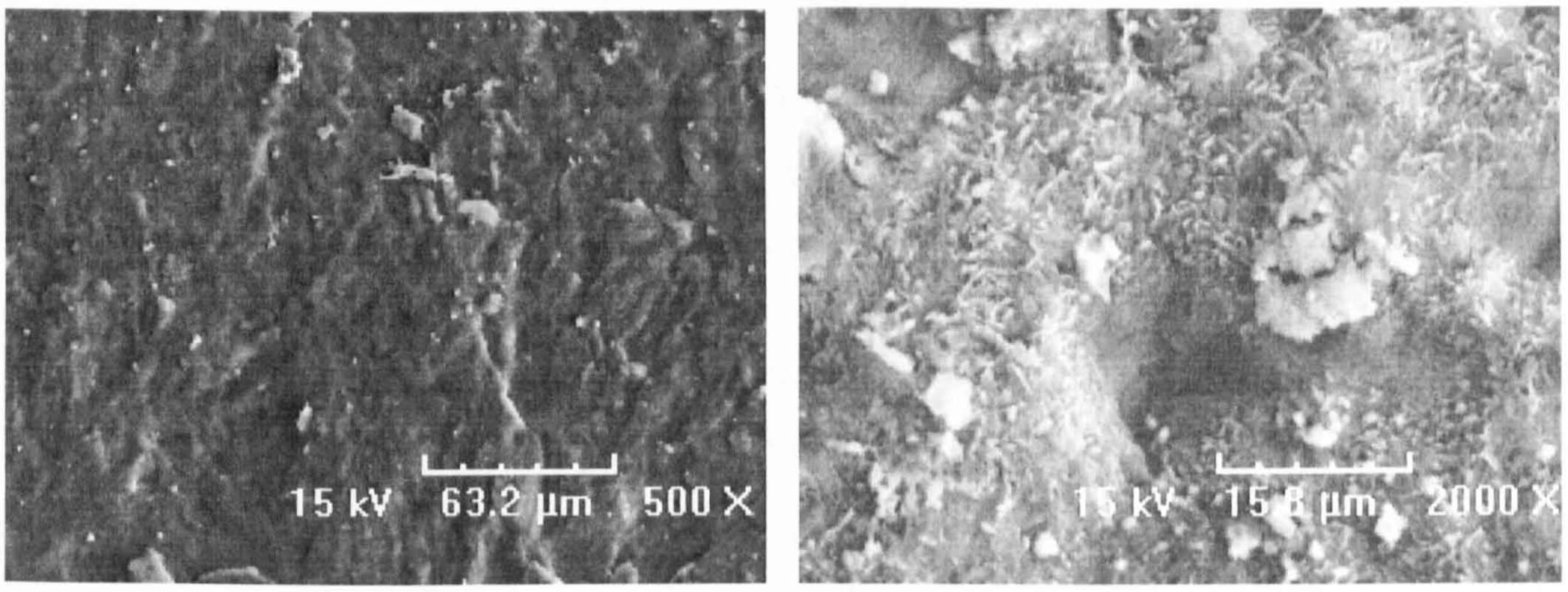


Figure 3.15 SEM photomicrographs for itraconazole disks after exposure to SIF for 5h, taken at two magnifications 500 x and 2000x, scale bars: 63.2µm and 15.8µm

3.4 General discussion

Due to the low solubility of itraconazole, it was difficult to maintain sink conditions in the dissolution experiments. Although it is desirable to keep sink conditions throughout the dissolution process (CDER/FDA, 1997), these conditions may not prevail in the GIT. Attaining these conditions *in vivo* depends upon the permeability, volume and composition of luminal fluids (Dressman and Reppas, 2000). Since itraconazole has a solubility of less than 1µg ml^{-1}, the volume of the GI fluids is not large enough to dissolve a typical dose of 100mg, 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 the intestinal mucosa (Dressman et al., 1998). So 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.

The acquired dissolution profiles of ketoconazole were compared to the literature data on ketoconazole dissolution from various formulations. For example, Galia et al. (1998) studied the dissolution of ketoconazole tablets (Nizoral ™, 200 mg) in 500ml SGF (pH 1.2) in USP II apparatus stirred at 100rpm and found that all tablets dissolved within 30min. Zhou et al. (2005) found that 90% of ketoconazole dissolved from ketoconazole USP tablets (200 mg) within 20min in 0.1N HCl (pH 1.2) stirred at 50rpm. While at pH 6.8 only 4% of ketoconazole dissolved within one hour. This provides an approximation for drug behaviour from intrinsic disks and dosage forms with a slightly slower release from the formulations that could be attributed to interactions from formulation constituents and different experimental conditions.

The same comparison was not valid in the evaluation of the dissolution of itraconazole marketed capsules (Sporanox™) as in this dosage form the drug exists in an amorphous form while in the present investigation the itraconazole powder was crystalline and it is known that the solid state affects the dissolution and the solubility of the entities.

Upon dissolving the ionisable basic drugs an increase in the pH of the dissolving media was expected, however, a final pH adjustment was not performed. Some investigators previously recommended adjusting the final pH when determining the solubility of ionised compounds (Alsenz and Kansy, 2007). However, this procedure might be physiologically irrelevant (Vertzoni et al., 2007). With highly dosed drugs, such as in the present case, where doses are 100-200mg a pH shift may also occur during dissolution in the GIT, and this is more likely at fasting conditions where the buffer capacity is at its lowest level (Kalantzi et al., 2006a). Hence, this suggests that to achieve a better IVIVC there is no need to alter the final pH of the media.

3.5 Conclusion

The data acquired from the techniques employed to detect changes in the solid state of itraconazole and ketoconazole did not provide any clear evidence of polymorphism changes due to compression. The decrease in the thermal parameters observed in the DSC scans and the noisier PXRD patterns of the compressed samples compared to the

uncompressed samples were probably due to the bigger particle size of the compressed material. Consequently, drug compact disks were prepared and employed in the current investigation to assess the intrinsic dissolution of the drugs.

To predict the *in vivo* performance of a drug, it is important to run the *in vitro* dissolution test under conditions that are likely to simulate *in vivo* fluids. This initial dissolution investigation was concerned with mimicking the pH values of the GIT in the fed and fasting states. The two drugs showed a strongly pH dependent solubility which increased markedly when the pH dropped to 1.2. The dissolution profiles mirrored the solubility studies. This clearly underpins the importance of elevated gastric acidity on the dissolution of those two basic drugs *in vivo*.

Studies concerned with the influence of gastric pH on the bioavailability of itraconazole (Section 1.10.4) showed that when the drug was co-administrated to subjects with an antacid suspension or subjects were pre-treated with H2 blockers, itraconazole bioavailability decreased (Lange et al., 1997; Lohitnavy et al., 2005). The same observations were found with ketoconazole as when it was administered in acidified solution its bioavailability increased compared to administering the drug alone, whereas it decreased when ketoconazole was giving with H2 blockers (Van Der Meer et al., 1980).

Food components have a buffering effect which increases the gastric pH, this is supposed to suppress the bioavailability of itraconazole and ketoconazole, however, an increase in the bioavailability of the drugs was reported. This raised the question of whether food is affecting the dissolution and so improving the bioavailability of the examined drugs. Consequently, further investigations of the dissolution of itraconazole and ketoconazole in the presence of dietary constituents were carried out.

Chapter 4: The effect of food components on the dissolution of itraconazole and ketoconazole

4.1 Introduction

Drugs in the gastrointestinal tract are exposed to a medium of partially digested food, comprising mixtures of fat, protein and carbohydrate. The effect of food on the bioavailability of drugs can be determined and predicted by studying the dissolution of drugs in simulated *in vivo* conditions. The dissolution behaviours of itraconazole and ketoconazole were evaluated in biorelevant media which were developed to take the effect of food into account. Consequently, the objective of this work was to understand the impact of food on the solubility and dissolution of itraconazole and ketoconazole. Since the composition of the fed stomach is variable according to the type of the meal consumed, a milk mixture was used as an example of a balanced diet. Furthermore, dissolution was also studied in less complex media composed of specific food types; protein (albumin, casein, gluten and gelatin), carbohydrates (glucose, lactose and starch) and amino acids (lysine, glycine, alanine, leucin and aspartic acid) to mimic a digested meal.

4.2 Materials and methods

4.2.1 Materials

Details of all materials used in the preparation of the dissolution media and in performing the experiments are listed in Section 2.1.

Materials used for the protein assay were Bradford reagent obtained from Sigma-aldrich Chemie (Steinheim, Germany) batch number 046K4374 and bovine serum albumin obtained from BDH (Poole, UK) with a batch number BAH62-630

4.2.2 Preparation of dissolution media

Simulated gastric fluids SGF (pH 1.2) and SGF (pH 3) were prepared as described in 3.2.3.1. Generally, the dissolution media were prepared by dissolving or dispersing food substances in SGF pH 3 and then the final pH was adjusted to 3 with diluted HCl. Specific procedures for each medium are described below:

4.2.2.1 Milk-containing media

Media containing milk were prepared by mixing an equal volume of the specified milk with SGF pH 1.2. The pH of the resulting solutions were variable (pH 2 to 4) depending on the type of the milk added and batch to batch variations. Therefore, for consistency, the final pH was adjusted to 3 using either 0.1M HCl or 0.1M NaOH. This allowed investigation of the effect of one variable; fat content, on the dissolution of the drugs.

4.2.2.2 Albumin-containing media

Albumin from hen egg white was used at concentrations of 0.1, 1, 2, 3 and 4%, w/v and for the phase solubility studies additional dilutions were prepared. Addition of albumin to SGF raised the pH because of the buffering effect of albumin. For instance, 1%, w/v albumin in SGF had a pH of 5.3. Therefore, the pH of the solutions and dispersions were all adjusted to 3 using 1M HCl as required.

4.2.2.3 Gelatin-containing media

Gelatin from bovine skin was dissolved in SGF at concentrations of 0.5, 1, and 2%, w/v. For solubility determinations further dilutions were prepared. An increase in pH was also observed due to adding gelatin. For instance, 1%, w/v gelatin solution had a pH of 4.9. Therefore, the final pH was reduced to 3 with 0.1M HCl.

4.2.2.4 Casein- and gluten-containing media

Casein from bovine milk (2%, w/v) and gluten from wheat (2%, w/v) were dispersed in SGF. Saturated solutions of the two proteins were prepared as follows: an excess amount of the protein was added to water and the resulting dispersion was stirred overnight and then filtered under vacuum using the Buchner apparatus and filter paper (Whatman, type 1). Sodium chloride (2g L^{-1}) was added and the pH of the resultant solution was adjusted to pH 3 with 0.1M HCl.

4.2.2.5 Amino acid-containing media

The amino acids lysine (LYS), glycine (GLY), leucin (LEU) and alanine (ALA) (1%, w/v) were dissolved in SGF and the final pH was taken to 3 with 1M HCl.

Media containing the same molar concentrations (133mM) of the amino acids GLY, LYS and ALA were prepared by dissolving the appropriate amount of the amino acid in SGF. The final pH was taken to 3 with 1M HCl.

SIF containing 1%, w/v GLY was prepared by dissolving GLY in SIF (SIF composition is listed in Section 3.2.3.1). The final pH was checked as 6.8.

When aspartic acid (ASP) was added to SGF, it formed a dispersion rather than a solution due to the low solubility of the acidic ASP in SGF. Therefore, the suspension was filtered through Whatman filter paper type 1 and then the final pH was checked. The concentration of ASP in the aqueous saturated solution (pH 3) was extrapolated theoretically using the Henderson–Hasselbalch equation for ampholyte compounds (Eq. (4.1)) (Avdeef, 2007). S denotes the solubility of ASP at pH 3 and S_0 the intrinsic solubility (minimum solubility) which was computed as 1.5×10^{-5} M.

$$\log S = \log S_0 + \log\left(10^{-pKa3-pKa2+2pH} + 10^{-pKa2+pH} + 10^{+pKa1-pH} + 1\right) \qquad \textbf{Eq. (4.1)}$$

pKa_1=1.9, pKa_2=3.7, pKa_3=9.6 (The Merck Index, 2006).

4.2.2.6 Sugar-containing media

Sugar solutions, composed of glucose, lactose and starch in SGF pH 3 (1%, w/v), were prepared by dissolving the appropriate amount of the sugar in SGF pH 3 and the final pH was adjusted to 3 with a few drops of 0.1M HCl.

4.2.3 Media characterisation

4.2.3.1 Viscosity

The viscosity of each medium was measured in triplicate using a Hydramotion vibrational sensor viscometer (Viscolite VL700-T15) (Section 2.5.1.2). The sensor was immersed in 30ml of the medium and measurements were recorded.

Another technique, controlled stress rheometery (Carri-Med model CSL^2-100) was employed to investigate the flow behaviour of media containing milk, albumin, casein, gluten and gelatin, using the method described in Section 2.5.1.3. The flow properties of the fluids were measured in a shear stress range of 0 to $500s^{-1}$ in up and down curves. Accordingly, the results were represented as shear rate versus shear stress using Rheology Advantage Data Analysis Software (TA Instruments Ltd.).

4.2.3.2 Surface tension

Surface tension was determined in triplicate using a Wilhelmy plate tensiometer, static mode, at 37°C (Section 2.5.2). Data points for each medium were collected by an interfaced computer every 7s up to 600s.

4.2.4 Contact angle measurements

The wettability of itraconazole and ketoconazole compact surfaces with the media containing proteins and milk were assessed in triplicate using the Sessile drop method. Photographs were taken immediately after placing a drop of fluid on drug surface and every 5s thereafter for a total period of 5min (Section 2.5.3).

4.2.5 Solubility studies

Solubility studies were performed according to the phase solubility studies reported by Higuchi and Connors (1965) to investigate the effect of the additives on the solubility of the drugs.

Excess amounts of itraconazole or ketoconazole (30mg or 300mg, respectively) were added to screw capped vials containing 10ml of media (SGF) containing various quantities of food constituents. The vials were shaken at 37°C for 24h. The suspensions were centrifuged and filtered through 0.2μm PVDF syringe filters (Section 2.2.2). Media which contained particulate matter (milk and albumin containing-media) clogged these filters which required the use of filters with a bigger pore size (Nicolaides et al., 1999). For the albumin-containing samples, 1μm glass filters (Gelman Sciences) were used and 5μm Acrodisc Versapor filters (Gelman Sciences, medium: acrylic polymer) were used for the milk-containing samples. Filter validation was performed as described in Section 2.4.1.

Itraconazole samples were diluted 2-fold with the mobile phase which was a mixture of acetonitrile-0.02M potassium dihydrogen phosphate (60:40, v/v) except samples collected from media containing carbohydrates or amino acids. All ketoconazole samples were diluted as appropriate (from 2 to 16-fold) with water-acetonitrile (50:50).

4.2.6 Dissolution tests

In parallel, intrinsic dissolution studies in the media were performed using the stationary disk method under the same conditions described in Section 2.2.4. Samples collected from complex dissolution media containing milk and protein were pre-treated by

extraction (Section 2.2.5). No extraction was performed with samples collected from media containing carbohydrates or amino acids where ketoconazole samples were diluted with a mixture of water-acetonitrile (50:50) as appropriate and itraconazole samples were not diluted due to the low solubility of the drug in these media.

4.2.7 Drug analysis

Assays were preformed by HPLC using fluorescence detection for itraconazole and UV detection for ketoconazole (Section 2.2.6).

4.2.8 Casein solutions quantitation: Bradford assay

The concentration of casein in the filtered saturated solution was determined using the Bradford protein assay (Bradford, 1976). The principle of the assay is when an acidic solution of Coomassie Brilliant Blue G-250 binds to arginine and aromatic residues present in the protein, a shift in the UV absorbance occurs. The anionic (bound form) has absorbance maximum at 595nm whereas the cationic form (unbound form) has and absorbance maximum at 470nm. The increase of absorbance at 595nm is proportional to the amount of bound dye and so to the amount of the protein in the sample.

Standard solutions of bovine serum albumin ranging from 100 to 200μg ml^{-1} (5 points) were prepared by dissolving the protein in 50mM tris-buffer (hydroxymethyl) aminomethane-HCl (pH 8). Each standard solution (0.1ml) was added into a plastic cuvette containing 3ml of Bradford reagent. Cuvettes were covered with paraffin film, gently inverted a few times to mix and then incubated for 5min prior to taking the measurements.The same procedures were followed with casein solutions which were diluted with tris-HCl buffer. Casein solutions (0.1ml) were added to 3ml of Bradford reagent in plastic cuvettes with parafilm, inverted and incubated for 5min. The absorbance of all casein samples and albumin standards were measured at 595nm using a Thermo Electron spectrophotometer.

4.3 Results

4.3.1 Media characterisation

4.3.1.1 Viscosity

4.3.1.1.1 Rotational rheometer

The rheological behaviour of the media containing milk and protein was determined using the provided data analysis software and the type of flow was based on minimizing

the standard error of the fitting model. SGF and SGF containing gelatin exhibited Newtonian behaviour (Figure 4.1) whereas milk and the other protein-containing media were non-Newtonian.

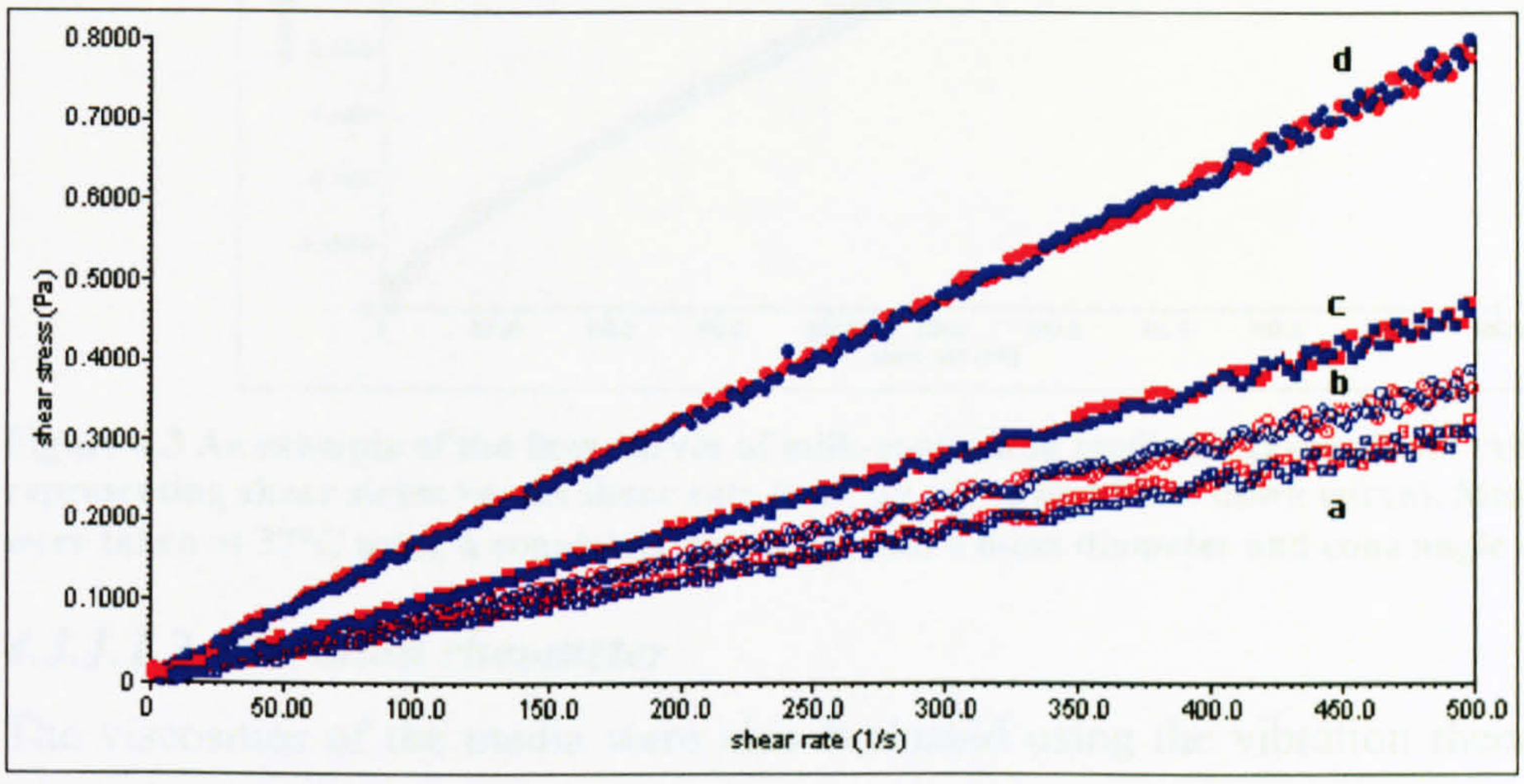


Figure 4.1 Shear stress versus shear rate of SGF containing gelatin (a) 0.5%, (b) 1%, (c) 2% and (d) 4%, w/v (red: up curve and blue: down curve). Measurements were taken at 37°C using a cone /plate geometry with a 6-cm diameter and cone angle of ~ 1°

In Newtonian fluids the viscosity (η) does not change with the shear rate ($\dot{\gamma}$), σ denotes the shear stress, while the viscosity of non-Newtonian fluids varies with shear rate, (Figure 4.2).

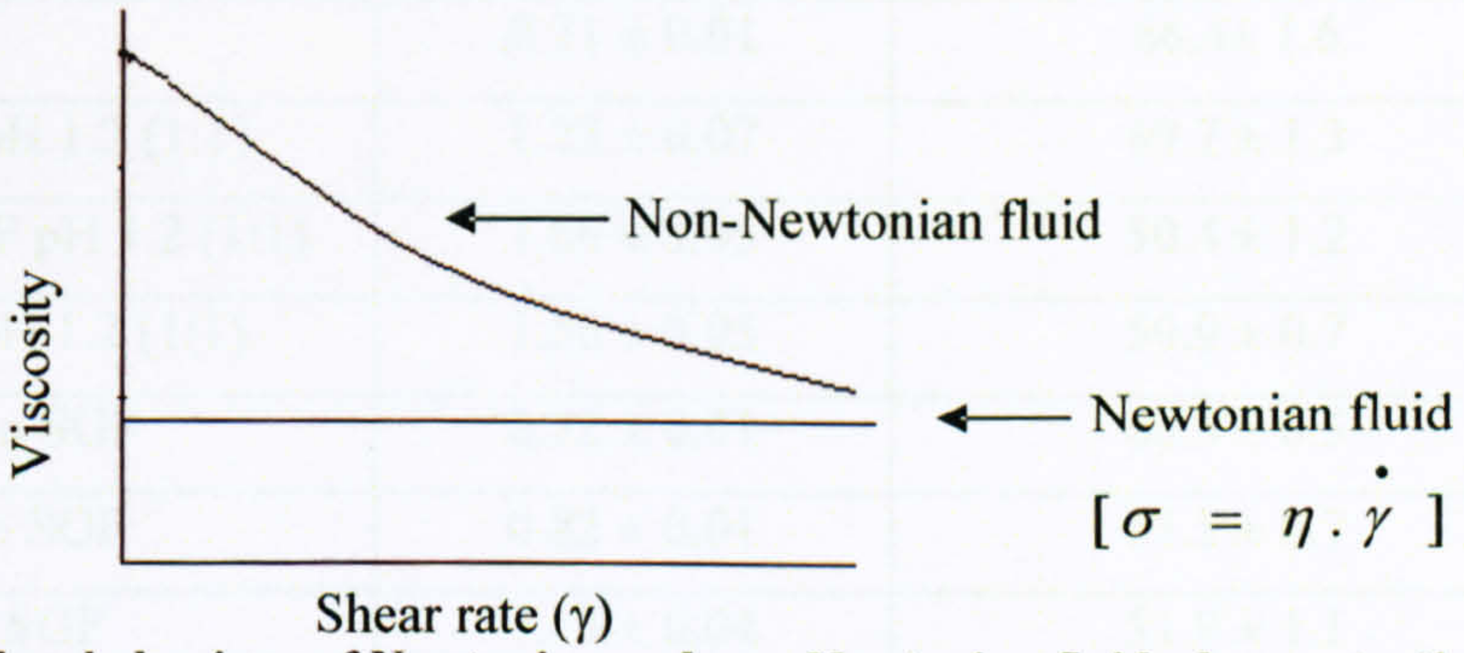


Figure 4.2 Example of the flow behaviour of Newtonian and non-Newtonian fluids demonstrating the relationship of viscosity to shear rate

A 1 %, w/v albumin in SGF demonstrated non-Newtonian behaviour, whereas the flow of 0.1%, w/v albumin in SGF was near-Newtonian. The media containing the three types of milk, casein or gluten exhibited non-Newtonian behaviour throughout the applied conditions (Figure 4.3).

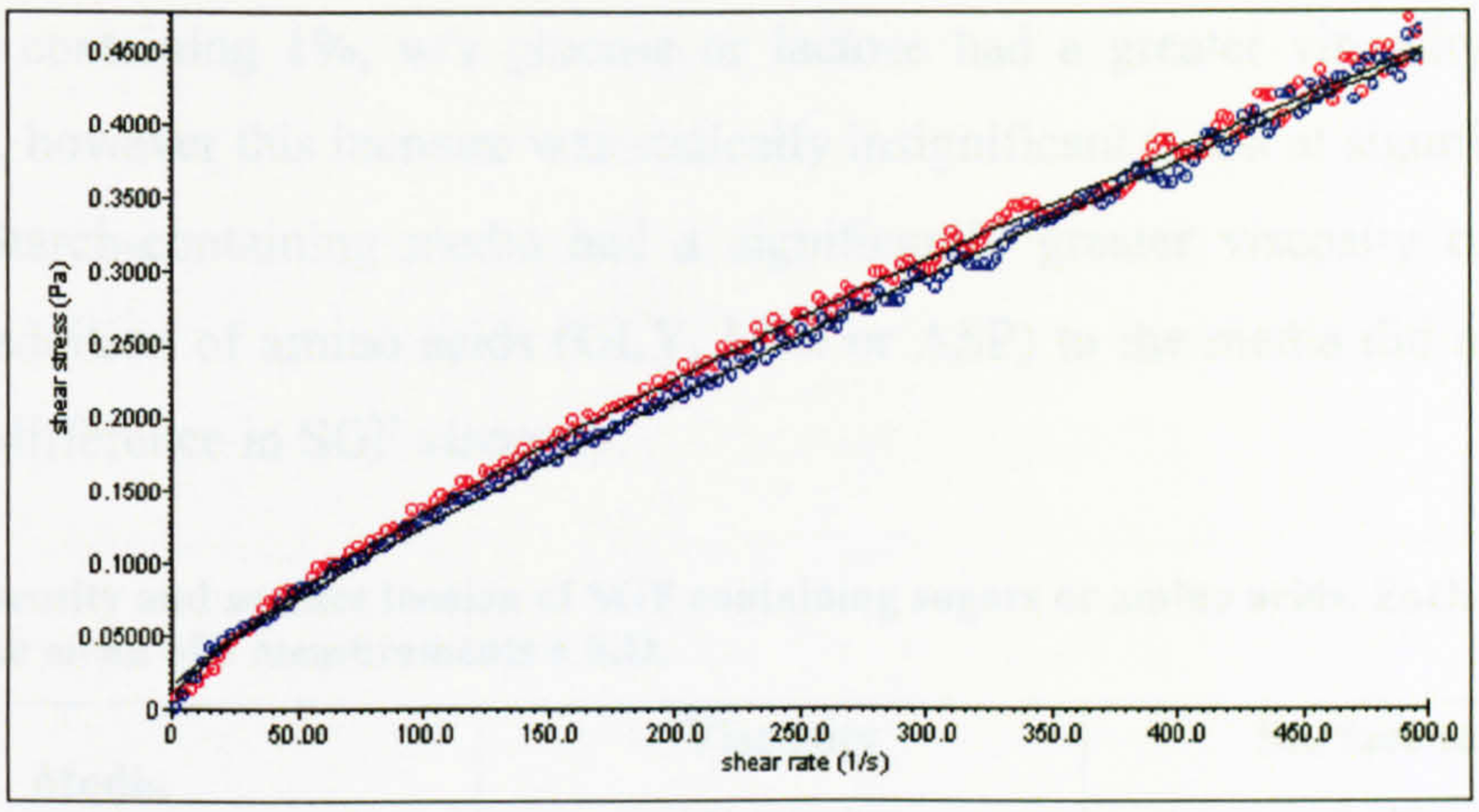


Figure 4.3 An example of the flow curves of milk-containing media (SGF-whole fat milk) representing shear stress versus shear rate (red: up curve and blue: down curve). Measurements were taken at 37°C using a cone/plate geometry with a 6-cm diameter and cone angle of ~ 1°

4.3.1.1.2 Vibration rheometer

The viscosities of the media were also evaluated using the vibration rheometer which allowed the determination of the viscosity of both Newtonian and non-Newtonian fluids. Table 4.1 shows the mean viscosities of media containing milk or protein. The viscosity of the media increased in the presence of food additives and the more viscous media were milk and gelatin-containing media.

Table 4.1 Viscosity and surface tension of SGF containing milk, albumin, casein or gelatin. Each data point represents the mean of 3 measurements ± S.D.

Medium	Viscosity (mpa.s)	Surface tension (mN m^{-1})
SGF pH 3	0.71 ± 0.01	66.4± 1.6
Whole fat milk-SGF pH 1.2 (1:1)	1.72 ± 0.07	49.7 ± 1.3
Semi-skimmed milk-SGF pH 1.2 (1:1)	1.66 ± 0.05	50.4 ± 1.2
Skimmed milk-SGF pH 1.2 (1:1)	1.56 ± 0.05	50.9 ± 0.7
Albumin 0.01% in SGF	0.72 ± 0.01	60.3 ± 0.5
Albumin 0.1% in SGF	0.82 ± 0.01	55.5 ± 0.7
Albumin 1% in SGF	1.15 ± 0.04	51.9 ± 1.1
Albumin 2% in SGF	1.28 ± 0.03	49.6 ± 1.2
Casein filtered solution in SGF	1.04 ± 0.03	48.4 ± 0.6
Casein solution-SGF (3:1)	1.01 ± 0.01	50.0 ± 1.0
Casein diluted solution-SGF (1:1)	0.94 ± 0.01	50.2 ± 0.7
Casein diluted solution-SGF (1:3)	0.90 ± 0.04	51.4 ± 1.0
Gluten filtered solution in SGF	0.84 ± 0.02	50.2 ± 1.1
Gelatin 0.5% in SGF	0.87 ± 0.01	51.9 ± 0.5
Gelatin 1% in SGF	1.15 ± 0.01	50.5 ± 0.9
Gelatin 2% in SGF	1.43 ± 0.04	45.2 ± 0.6
Gelatin 4% in SGF	1.98 ± 0.05	45.1 ± 0.9

The media containing 1%, w/v glucose or lactose had a greater viscosity than SGF (Table 4.2), however this increase was statically insignificant (t-test at significance level of 0.05). Starch-containing media had a significantly greater viscosity compared to SGF. The addition of amino acids (GLY, LYS or ASP) to the media did not induce a significant difference in SGF viscosity.

Table 4.2 Viscosity and surface tension of SGF containing sugars or amino acids. Each data point represents the mean of 3 measurements ± S.D.

Media	Viscosity (mpa.s)	Surface tension ($mN\ m^{-1}$)
1% glucose in SGF	0.72 ± 0.01	68.5 ± 0.5
1% lactose in SGF	0.73 ± 0.01	61.1± 0.8
1% starch in SGF	0.76 ± 0.01	67.7± 0.6
1% GLY in SGF	0.73 ± 0.02	65.6 ± 1.7
1% LYS in SGF	0.70 ± 0.03	60.2 ± 1.0
ASP saturated solution in SGF	0.71 ± 0.01	65.0 ± 1.1

4.3.1.2 Surface tension

The media containing milk or proteins had significantly lower surface tensions compared to SGF (Table 4.1). Only slight variations were observed between the media containing sugars or amino acids (Table 4.2).

4.3.1.3 Casein quantitation

The concentration of casein in the diluted casein-containing media was determined in duplicate; variations were not more than 2%. The concentrations of casein in the media were 0.005, 0.0038, 0.0025 and 0.0013%, w/v.

4.3.2 Contact angle measurements

Since wetting is the first step for dissolution, it was important to assess the wettability of drug disks with the biorelevant media.

4.3.2.1 Itraconazole

For comparison, the contact angle formed between a drop of the dissolution medium and an itraconazole disk surface at 300s was considered. The contact angles formed between the itraconazole surface and the majority of the tested biorelevant media were smaller than those observed with SGF (Figure 4.4). This indicated that the media tested had the ability to enhance the wetting of the drug.

Figure 4.4 shows the mean contact angles of protein- and milk-containing media on itraconazole disks. SGF formed a contact angle of 65° and with the inclusion of food additives the angle was reduced by approximately 11°. Slight differences were observed among the contact angles of the different biorelevant media with the itraconazole surface. Contact angles for milk-containing media decreased by about 10°, compared to SGF. This indicated that addition of milk increased the wettability of the drug but the difference was not significant between the three types of milk. The inclusion of gelatin in the media decreased the contact angle by 12° but with no marked variations observed with an increase in the concentration of gelatin in the media. Similar observations were found with gluten-containing medium where the contact angle was reduced by 13°.

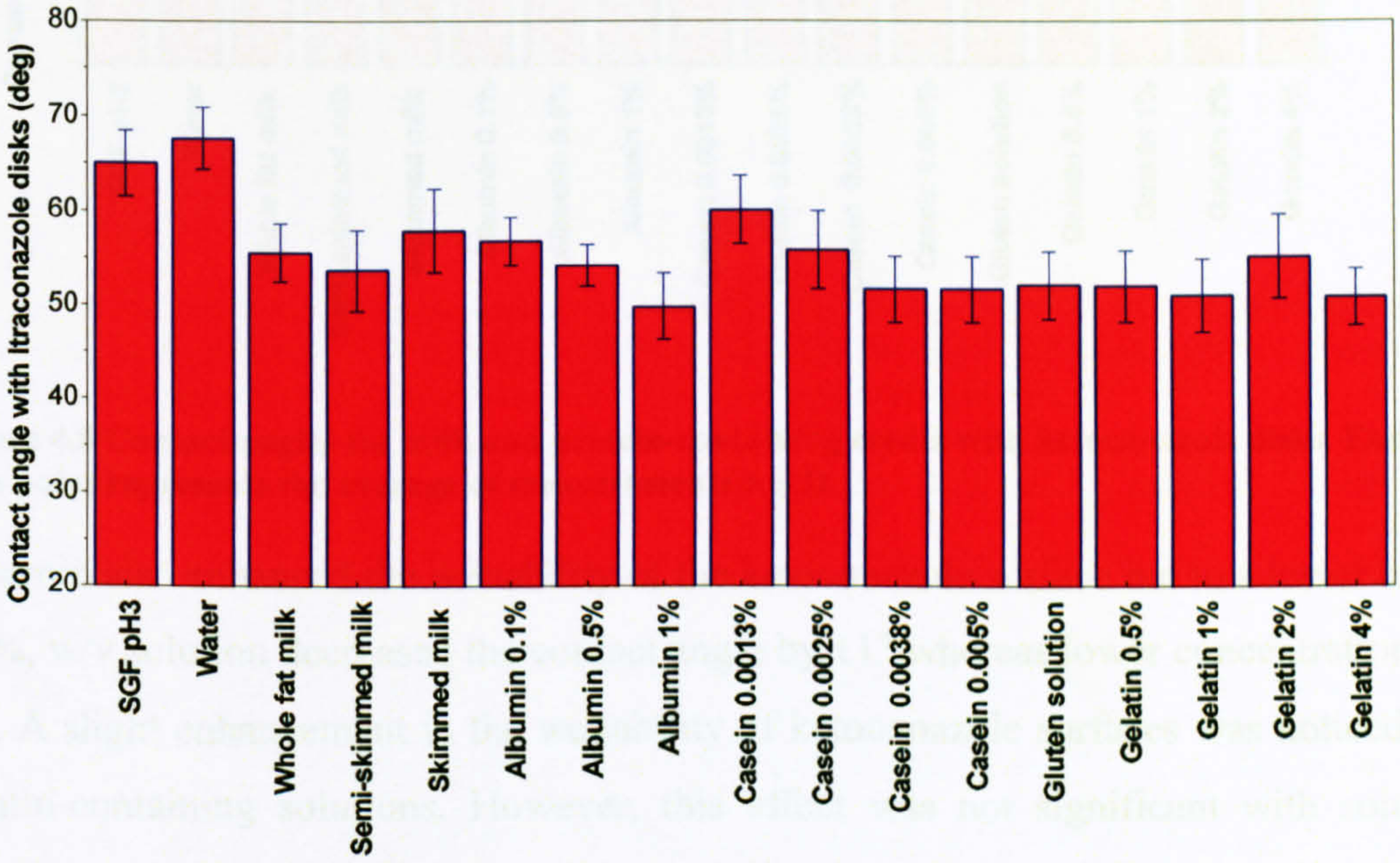


Figure 4.4 Contact angles for milk- and proteins-containing media with itraconazole disks. Each data point represents the mean of 3 measurements ± S.D.

The enhancement in the wettability was more pronounced with the inclusion of albumin in the media, where a 1%, w/v albumin-containing medium reduced the contact angle by approximately 15°. Three of the casein-containing solutions induced a decrease in the contact angle of approximately 10° while the more diluted casein solution (0.0013% w/v) did not markedly affect the wetting.

4.3.2.2 Ketoconazole

To compare the contact angles formed on ketoconazole surfaces, it was more appropriate to consider the measurements taken after 150s of placing the liquid drop on the drug surface since most of the contact angles at 300s were small and variable (S.D $\geq 5°$).

Milk-containing media also resulted in a decrease in the contact angle with ketoconazole of approximately 14° compared to SGF (Figure 4.5) with no significant variations between the milk-containing media (ANOVA at 0.05 level of significance).

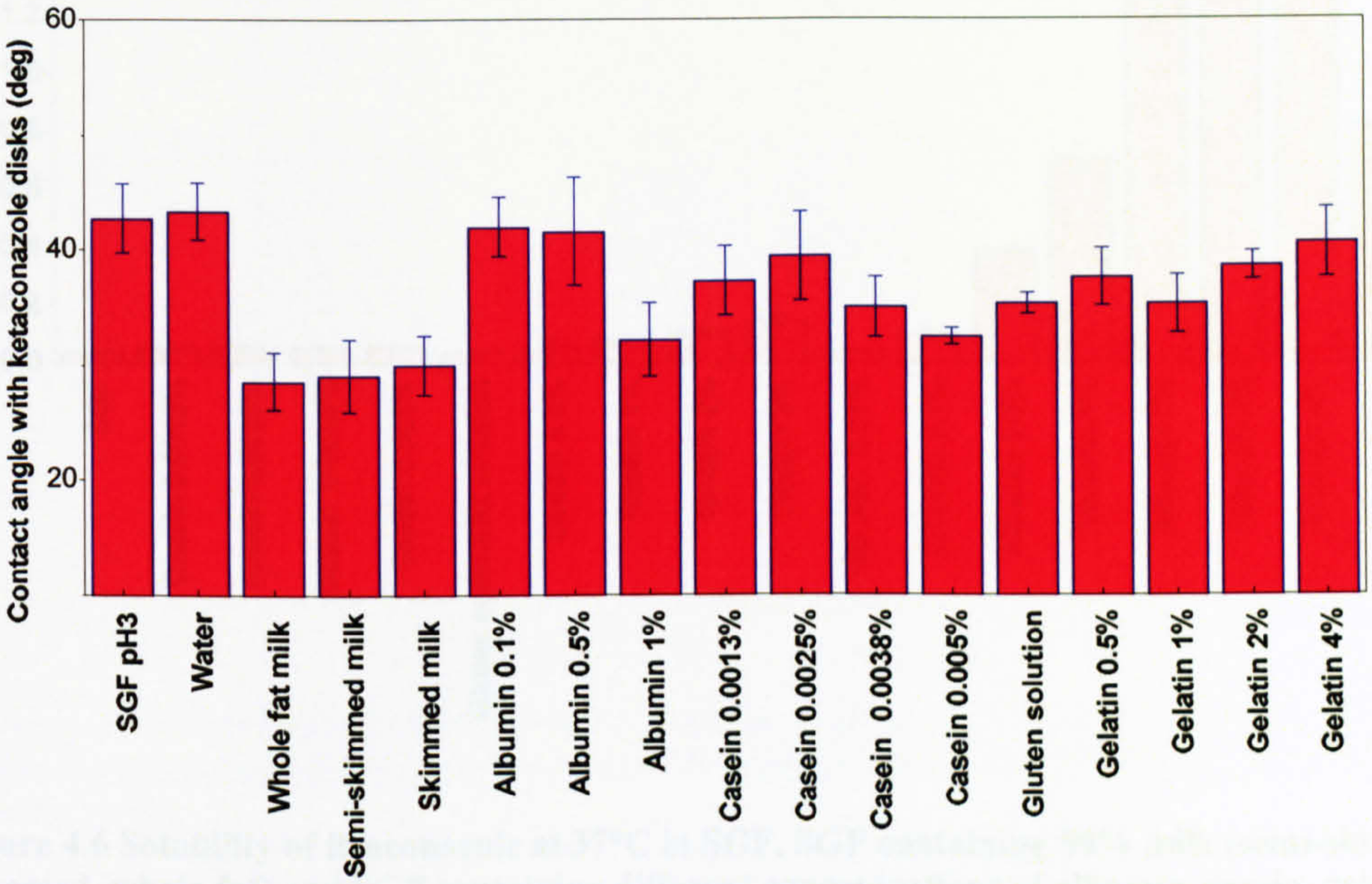


Figure 4.5 Contact angles for milk and protein-containing media with ketoconazole disks. Each data point represents the average of measurements ± S.D.

Albumin also enhanced the wettability of the ketoconazole surface but to a lesser extent; a 1%, w/v solution decreased the contact angle by 11° whereas lower concentrations did not. A slight enhancement in the wettability of ketoconazole surfaces was noticed with gelatin-containing solutions. However, this effect was not significant with solutions containing a high amount (t-test, 0.05), most likely due to the high viscosity of gelatin-containing media which led to poor spreading and penetration. Gluten and casein-containing solutions at the higher concentrations (0.0038, 0.005%. w/v) lowered the contact angle by 7°-10°, compared to SGF.

4.3.3 Solubility and dissolution studies in dietary media

4.3.3.1 Milk-containing media

Milk contains the three basic nutritious constituents; proteins, carbohydrates and fat so milk mixtures were considered a physiologically relevant dissolution media to investigate the dissolution of the drugs *in vitro*.

4.3.3.1.1 Itraconazole

The saturation solubilities for itraconazole media containing whole fat, semi-skimmed and skimmed milk increased approximately 24 times from that in SGF (Figure 4.6).

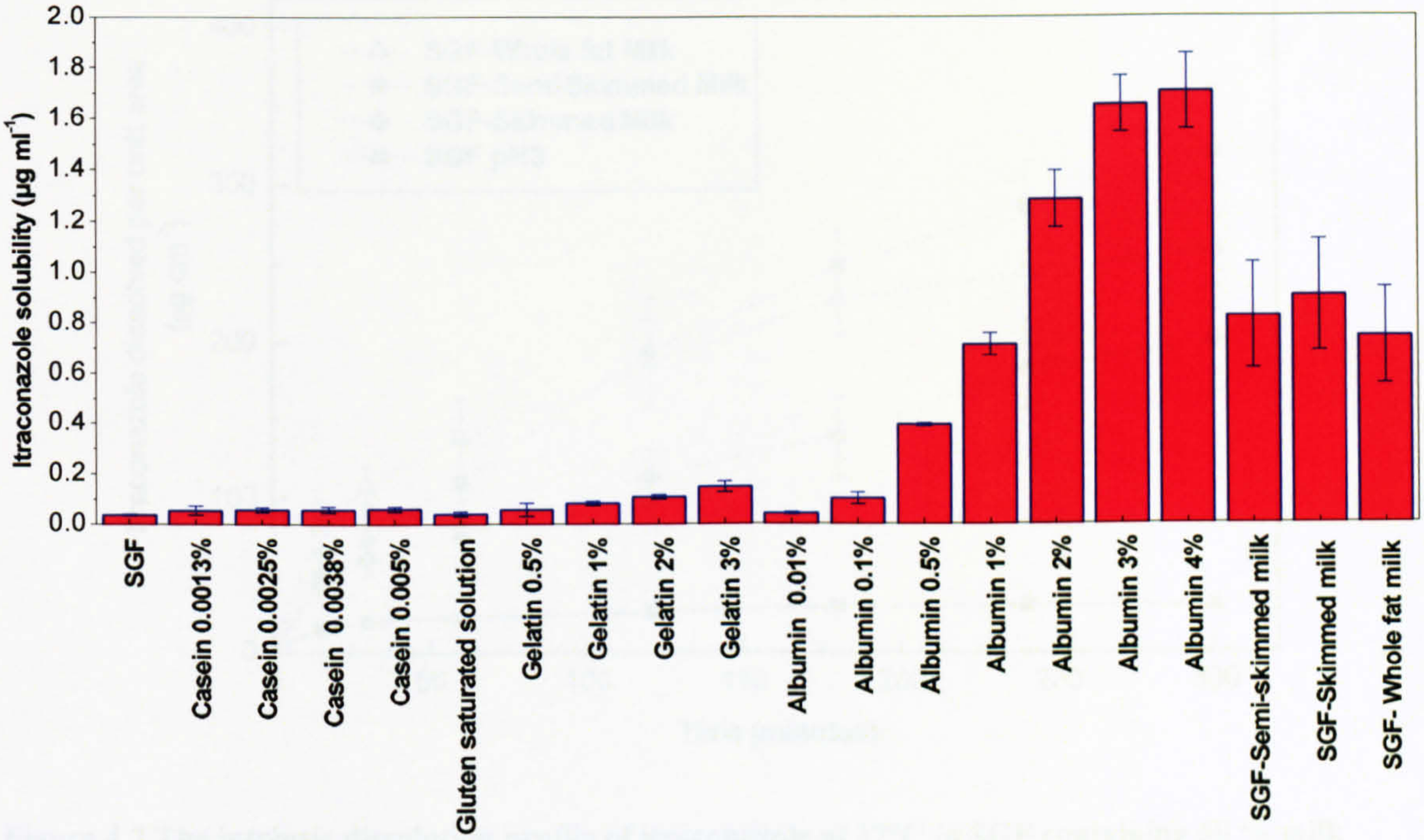


Figure 4.6 Solubility of itraconazole at 37°C in SGF, SGF containing 50% milk (semi-skimmed, skimmed, whole fat) and SGF containing different concentrations of albumin, casein, gelatin and gluten. Each data point represents the mean ± S.D. of 3 measurements

Statistical analysis of the results indicated that there was no significant difference in solubility between the different types of milk (ANOVA at 0.05 level of significance).

An increase in dissolution was apparent in milk-containing media compared to SGF (Figure 4.7). By comparing the amount of itraconazole dissolved in the three milk-containing media, it was noticed that the amount dissolved in whole fat milk-containing media within one hour was the greatest, whilst after 3h the amount dissolved into semi-skimmed milk-containing medium exceeded the corresponding value in whole fat milk-containing medium. ANOVA analysis of the IDR data at one and three hours indicated a significant difference among the three media.

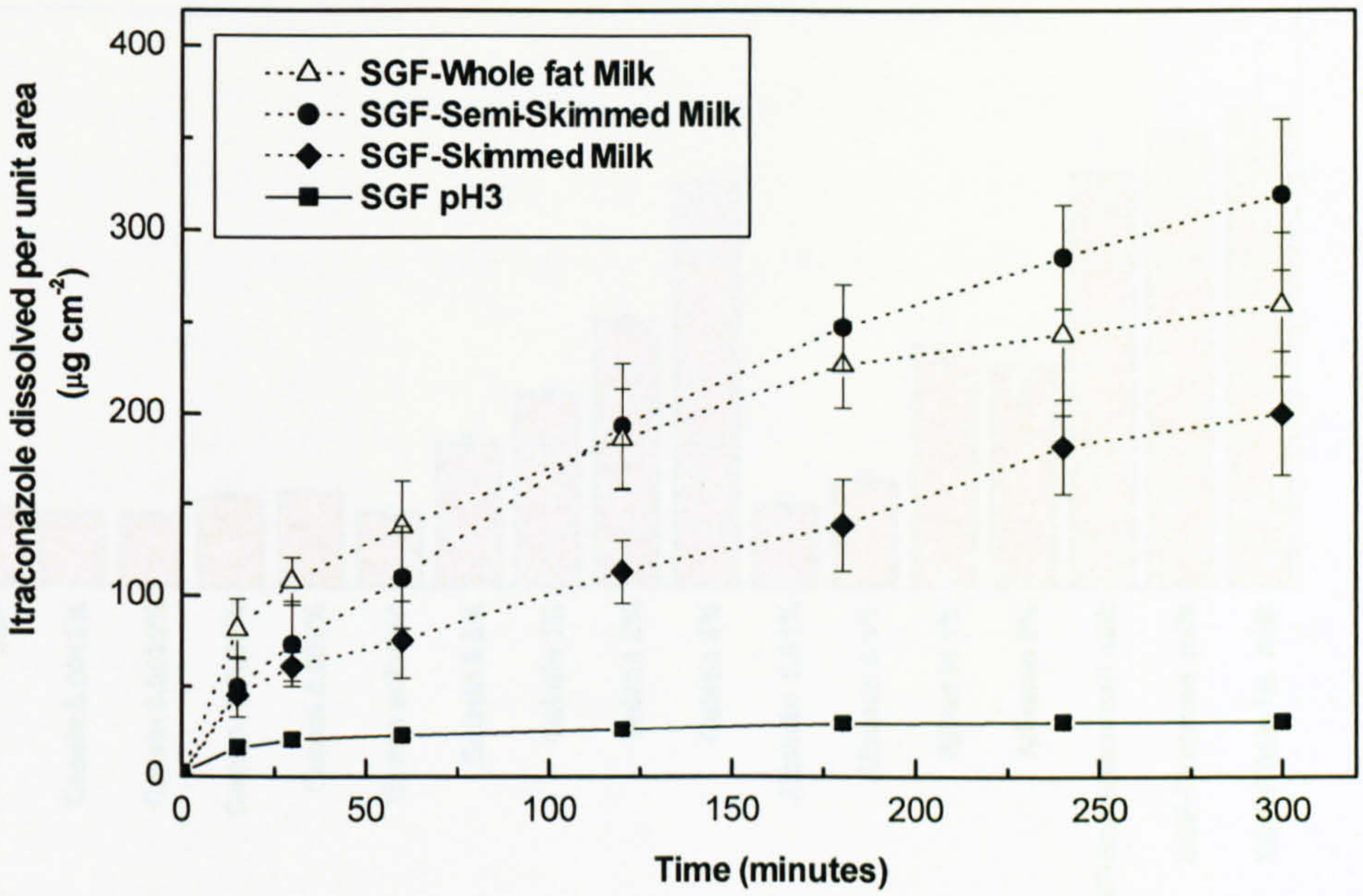


Figure 4.7 The intrinsic dissolution profile of itraconazole at 37°C in SGF containing 50 % milk (whole fat, semi-skimmed or skimmed). Each data point represents the mean ± S.D. of (at least 4) measurements

The initial IDR (Table 4.3) in whole fat milk-containing medium was the highest, probably due to the greater amount of lipid (3.5%) which could have affected the dissolution of the lipophilic drug. However, with time, the dissolution in the whole fat milk-containing medium started to slow down. The relative decline in the dissolution in that medium could be due to stability problems of the milk which were more pronounced in the whole fat milk.

These results provided clear evidence of an increase in both dissolution rate and solubility in milk containing-media. The increase in the initial dissolution rate was up to 6 times greater whereas the solubility increased 24 times, compared to SGF. Consequently, the increase in solubility was greater than the increase in dissolution.

4.3.3.1.2 Ketoconazole

The solubility of ketoconazole in milk-containing media was approximately 6 times greater than the solubility in SGF with no significant difference between the solubility values for the three different milk-containing media (Figure 4.8).

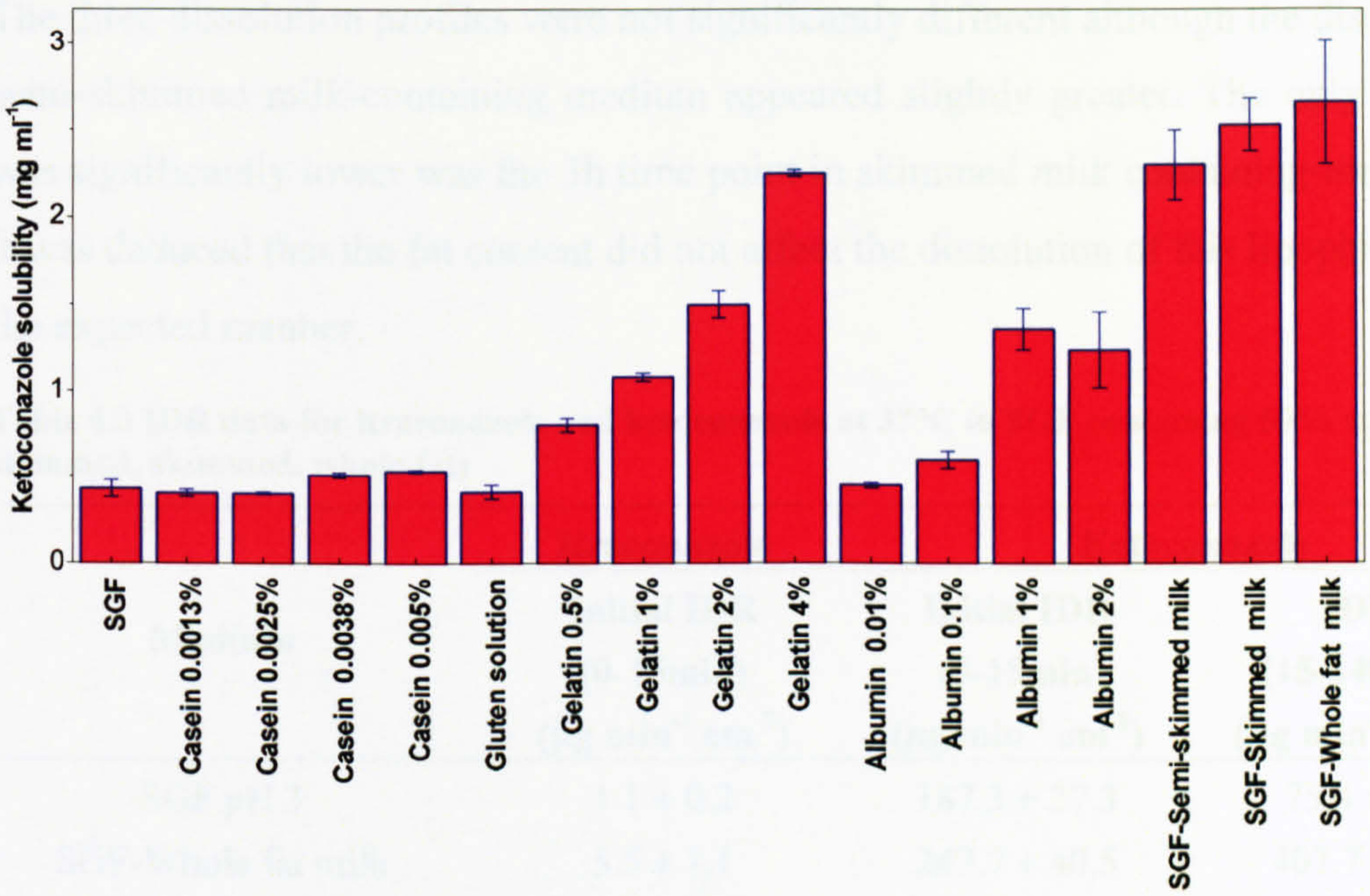


Figure 4.8 Solubility of ketoconazole at 37°C in SGF, SGF containing 50% milk (semi-skimmed, skimmed, whole fat) and (SGF) containing different concentrations of albumin, casein, gelatin and gluten. Each data point represents the mean ± S.D. of 3 measurements

The initial IDR in whole fat milk-containing medium was 1.3-fold greater than that in SGF and it was slightly higher than in the two milk-containing media (Table 4.3). A greater increase was observed in the IDR representing the subsequent time period (15-240min), of around 5.4-fold compared to SGF (Figure 4.9).

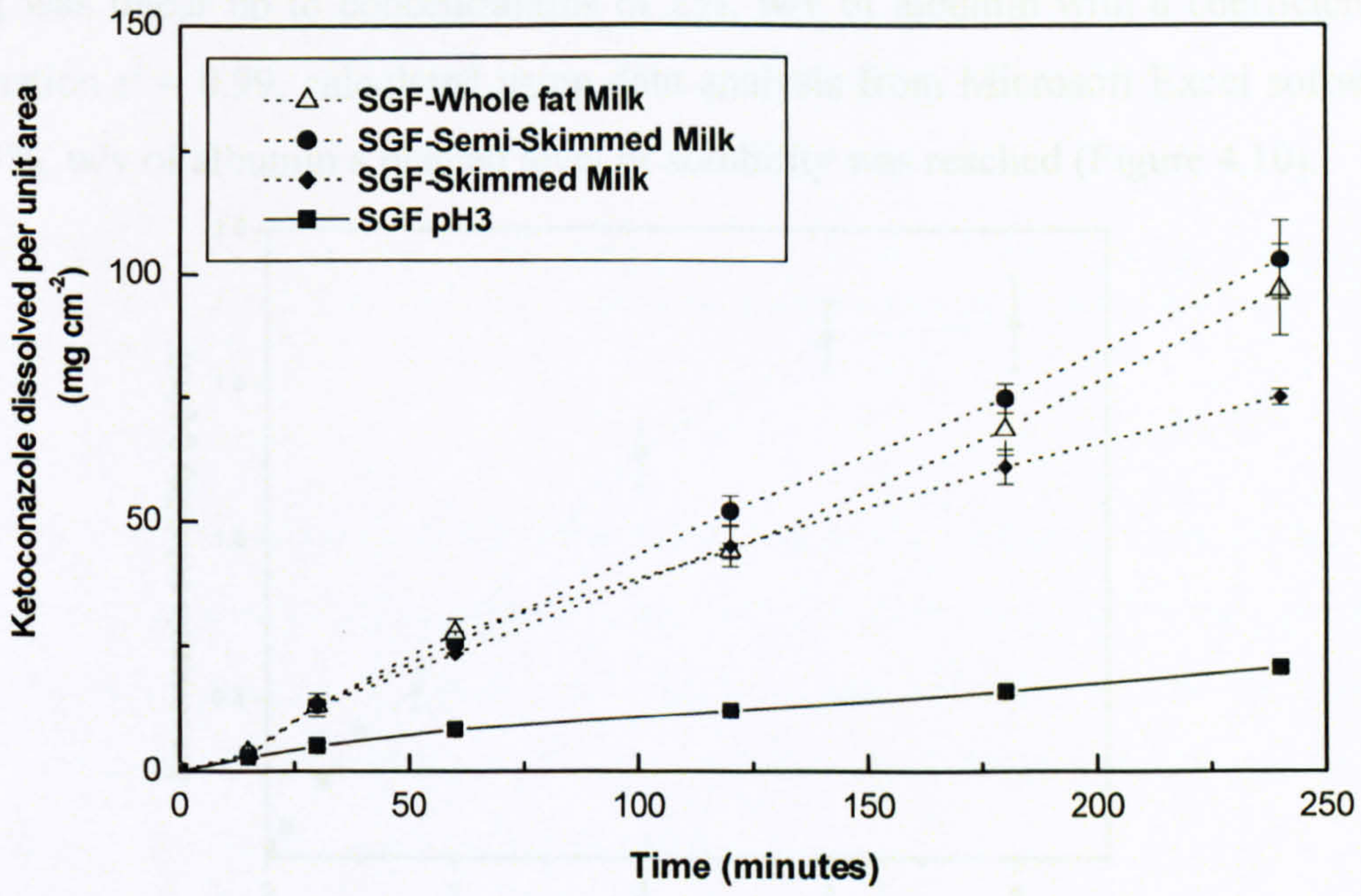


Figure 4.9 The intrinsic dissolution profile of ketoconazole at 37°C in SGF containing 50 % milk (whole fat semi-skimmed or skimmed). Each data point represents the mean ± S.D. of 3 measurements

The three dissolution profiles were not significantly different although the dissolution in semi-skimmed milk-containing medium appeared slightly greater. The only point that was significantly lower was the 3h time point in skimmed milk containing-media. Thus, it was deduced that the fat content did not affect the dissolution of this lipophilic drug in the expected manner.

Table 4.3 IDR data for itraconazole and ketoconazole at 37°C in SGF containing 50% milk (semi-skimmed, skimmed, whole fat)

Medium	Itraconazole	Ketoconazole	
	Initial IDR (0-15min) ($\mu g\ min^{-1}\ cm^{-2}$)	Initial IDR (0-15min) ($\mu g\ min^{-1}\ cm^{-2}$)	IDR (15-240min) ($\mu g\ min^{-1}\ cm^{-2}$)
SGF pH 3	1.1 ± 0.2	187.3 ± 27.3	75.4 ± 3.5
SGF-Whole fat milk	5.5 ± 1.1	247.7 ± 40.5	407.7 ± 37.5
SGF-Semi-skimmed milk	3.3 ± 1.1	242.7 ± 39.8	433.7 ± 58.5
SGF-Skimmed milk	3.0 ± 0.4	206.4 ± 13.7	309.5 ± 6.8

4.3.3.2 Albumin-containing media

4.3.3.2.1 Itraconazole

The inclusion of albumin in SGF increased the solubility and dissolution of itraconazole with a quantitative relationship between concentration and solubility. The solubility of the drug was linear up to concentrations of 2%, w/v of albumin with a coefficient of determination $r^2 = 0.99$, calculated using data analysis from Microsoft Excel software. Above 3%, w/v of albumin a plateau level of solubility was reached (Figure 4.10).

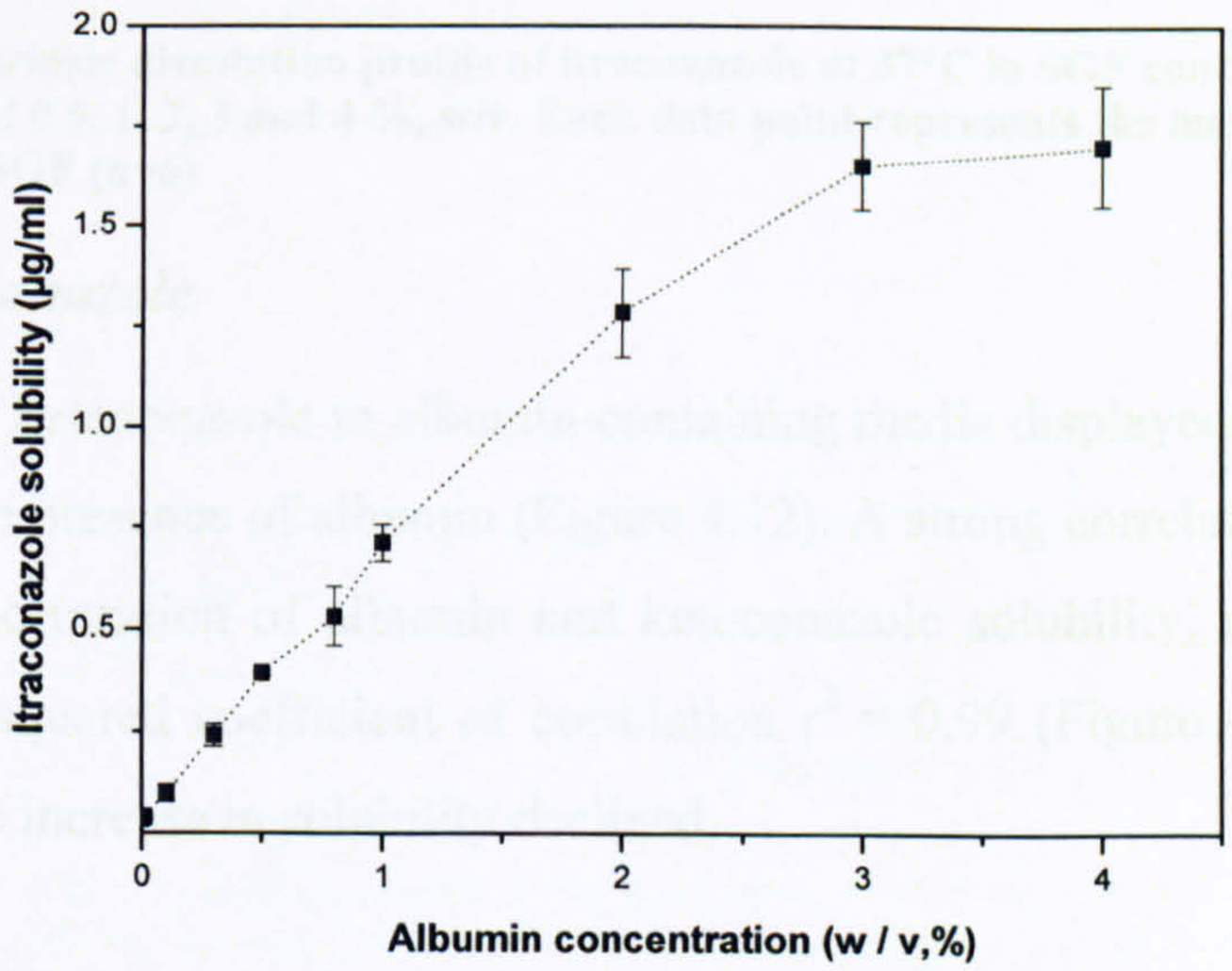


Figure 4.10 Effect of egg albumin on the solubility of itraconazole at 37°C in SGF. The amount of albumin added varied from 0.01% to 4%, w/v. Each data point represents the mean ± S.D. of 3 measurements

The dissolution of the drug increased with an increase in albumin concentration (Figure 4.11). For example, in media containing 1, 2 and 4%, w/v of albumin the amount dissolved per unit area after one hour were 5.1-, 6.7- and 7.8-fold (respectively) greater than that recorded in SGF.

The solubility in 1%, w/v albumin-containing medium was 20-fold greater than that in SGF whereas the initial IDR increased only 4 times. At a low concentration of albumin (0.5%, w/v) the increase in solubility was 11 times and the initial IDR was 1.7 times greater than in SGF. Accordingly, the increase in the solubility was greater than the increase in the dissolution.

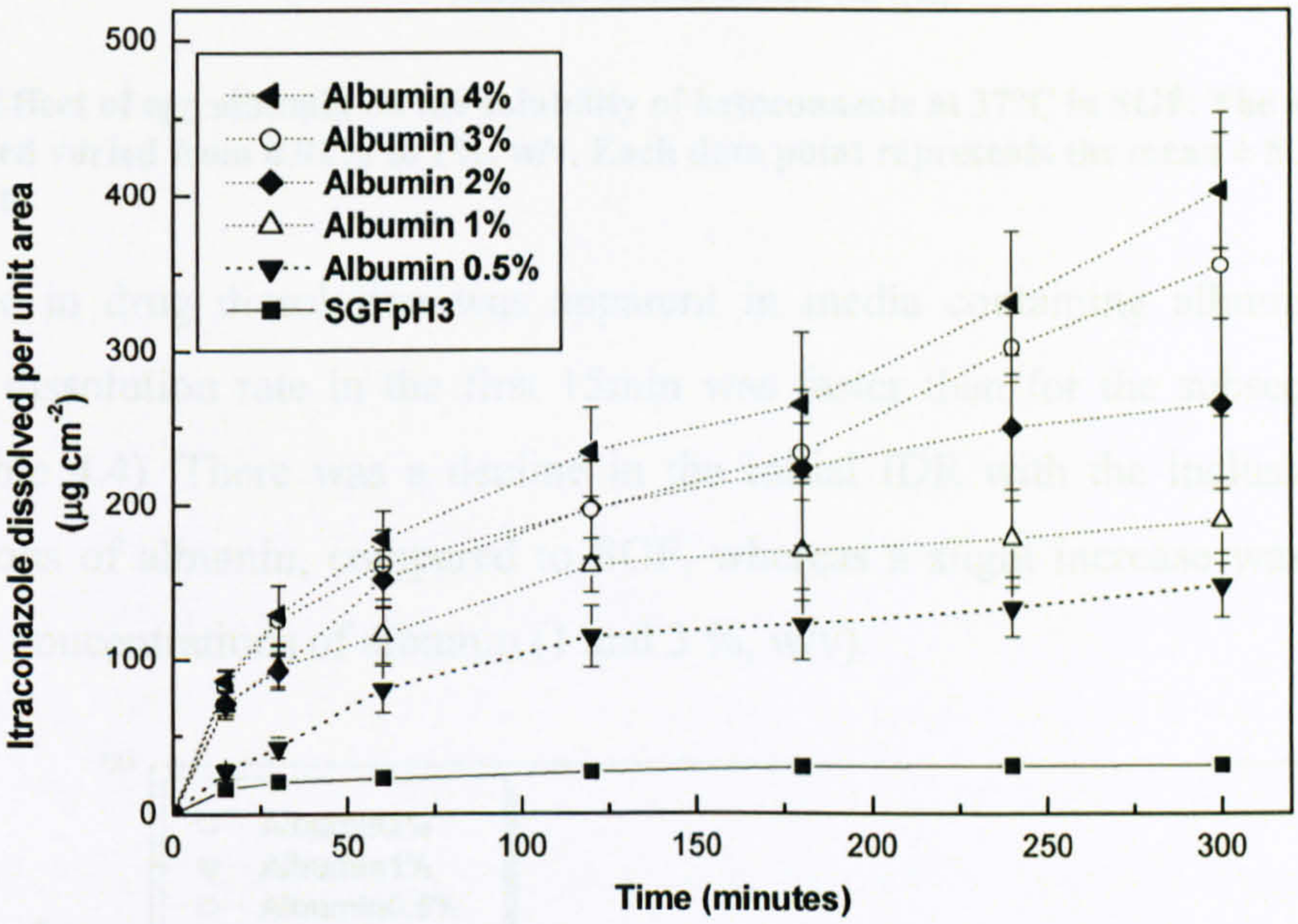


Figure 4.11 The intrinsic dissolution profile of itraconazole at 37°C in SGF containing egg albumin in concentrations of 0.5, 1, 2, 3 and 4 %, w/v. Each data point represents the mean ± S.D. of 5 measurements, in SGF (n=6)

4.3.3.2.2 Ketoconazole

The solubility of ketoconazole in albumin-containing media displayed clear evidence of an increase in the presence of albumin (Figure 4.12). A strong correlation was observed between the concentration of albumin and ketoconazole solubility, up to 1%, w/v of albumin with a squared coefficient of correlation $r^2 = 0.99$ (Figure 4.12). Above this concentration the increase in solubility declined.

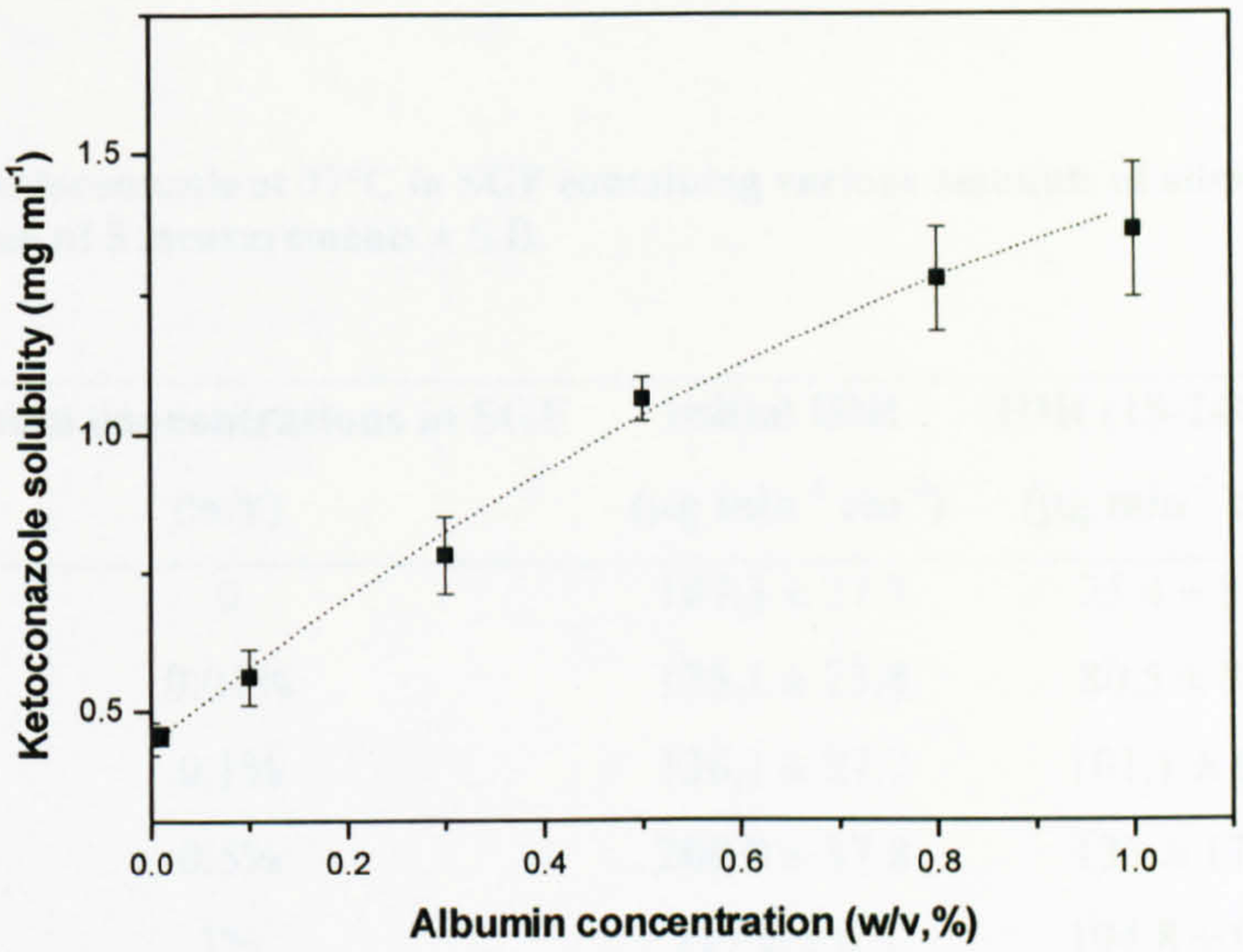


Figure 4.12 Effect of egg albumin on the solubility of ketoconazole at 37°C in SGF. The amount of albumin added varied from 0.01% to 1%, w/v. Each data point represents the mean ± S.D. of 3 measurements

An increase in drug dissolution was apparent in media containing albumin (Figure 4.13). The dissolution rate in the first 15min was faster than for the subsequent time period (Table 4.4). There was a decline in the initial IDR with the inclusion of low concentrations of albumin, compared to SGF, whereas a slight increase was observed with higher concentrations of albumin (1 and 3 %, w/v).

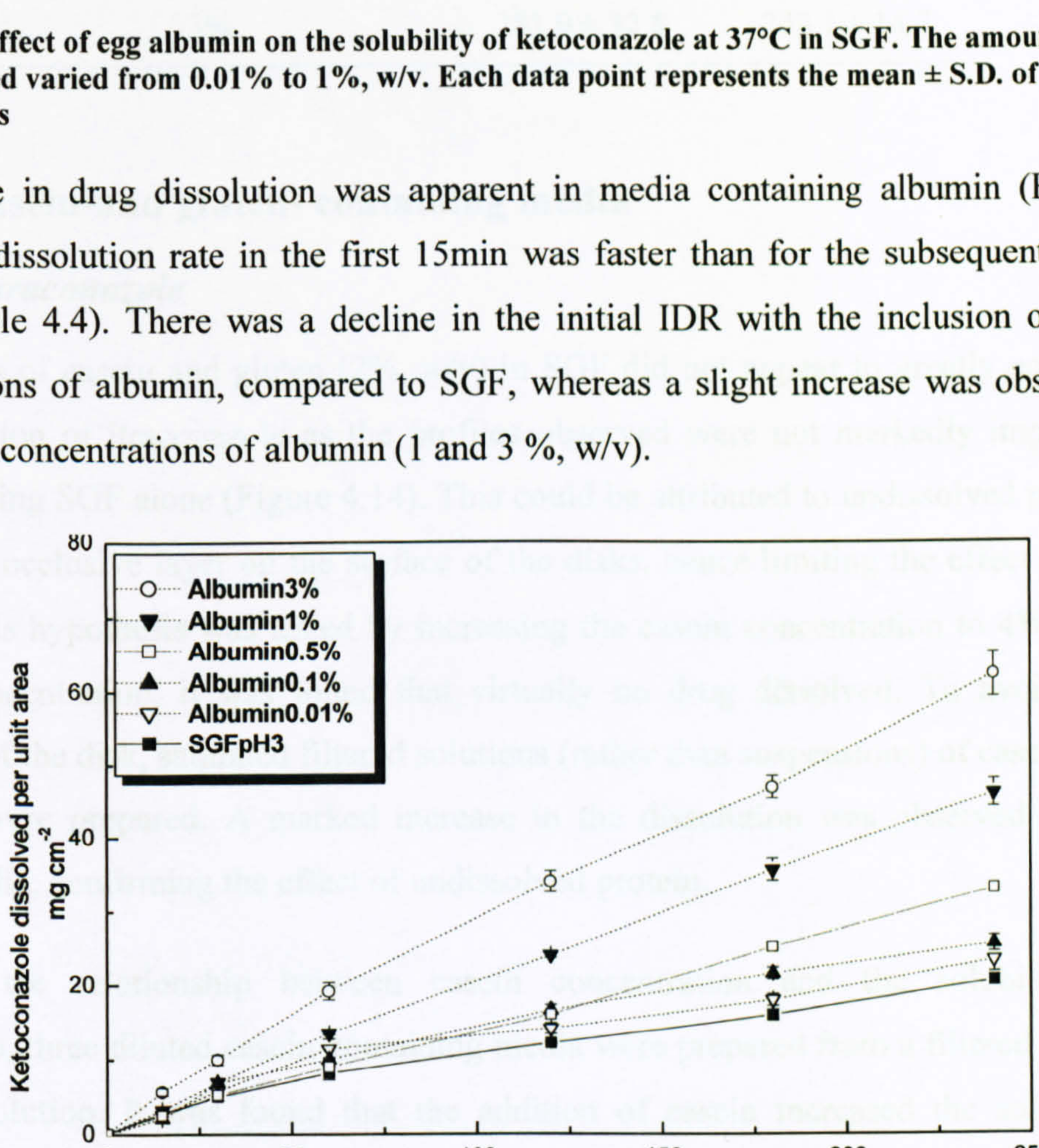


Figure 4.13 The intrinsic dissolution profile of ketoconazole at 37°C in SGF containing egg albumin in a concentrations of 0.01, 0.1, 0.5, 1 and 3%, w/v. Each data point represents the mean ± S.D. of 3 measurements

The solubility increased 3-fold in 1%, w/v albumin-containing medium compared to SGF but the initial dissolution of the drug only increased by a factor of 1.2 and the IDR for the subsequent step (15-240min) increased by a factor of 2.6.

Table 4.4 IDR of ketoconazole at 37°C in SGF containing various amounts of albumin. Each data represents the mean of 3 measurements ± S.D.

Albumin concentrations in SGF (w/v)	Initial IDR ($\mu g\ min^{-1}\ cm^{-2}$)	IDR (15-240min) ($\mu g\ min^{-1}\ cm^{-2}$)
0	187.3 ± 27.3	75.4 ± 3.5
0.01%	175.1 ± 23.8	80.5 ± 8.6
0.1%	136.1 ± 27.2	101.1 ± 6.7
0.5%	200.0 ± 37.8	133 ± 17.0
1%	217.6 ± 6.3	194.8 ± 9.3
3%	381.0 ± 32.8	248.1 ± 14.7

4.3.3.3 Casein-and gluten- containing media

4.3.3.3.1 Itraconazole

Suspensions of casein and gluten (2%, w/v) in SGF did not appear to greatly enhance the dissolution of itraconazole as the profiles observed were not markedly improved over that using SGF alone (Figure 4.14). This could be attributed to undissolved protein forming an occlusive layer on the surface of the disks, hence 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 filtered solutions (rather than suspensions) of casein and of gluten were prepared. A marked increase in the dissolution was observed in the filtered media, confirming the effect of undissolved protein.

To study the relationship between casein concentration and the solubility of itraconazole, three diluted casein-containing media were prepared from a filtered casein saturated solution. It was found that the addition of casein increased the solubility approximately 1.6-fold (Figure 4.6) and a statistical evaluation of these data (ANOVA, 0.05) showed no significant difference between the solubilities observed with the four different casein solutions. Similar observations were found with the dissolution studies where no obvious differences were apparent between the dissolution profiles in the casein-containing media. Hence, there was no quantitative relationship observed between casein concentration and solubility over the range tested. The initial IDR was

enhanced 2.4-fold in 0.005% casein and the amount dissolved increased 5-fold compared to SGF.

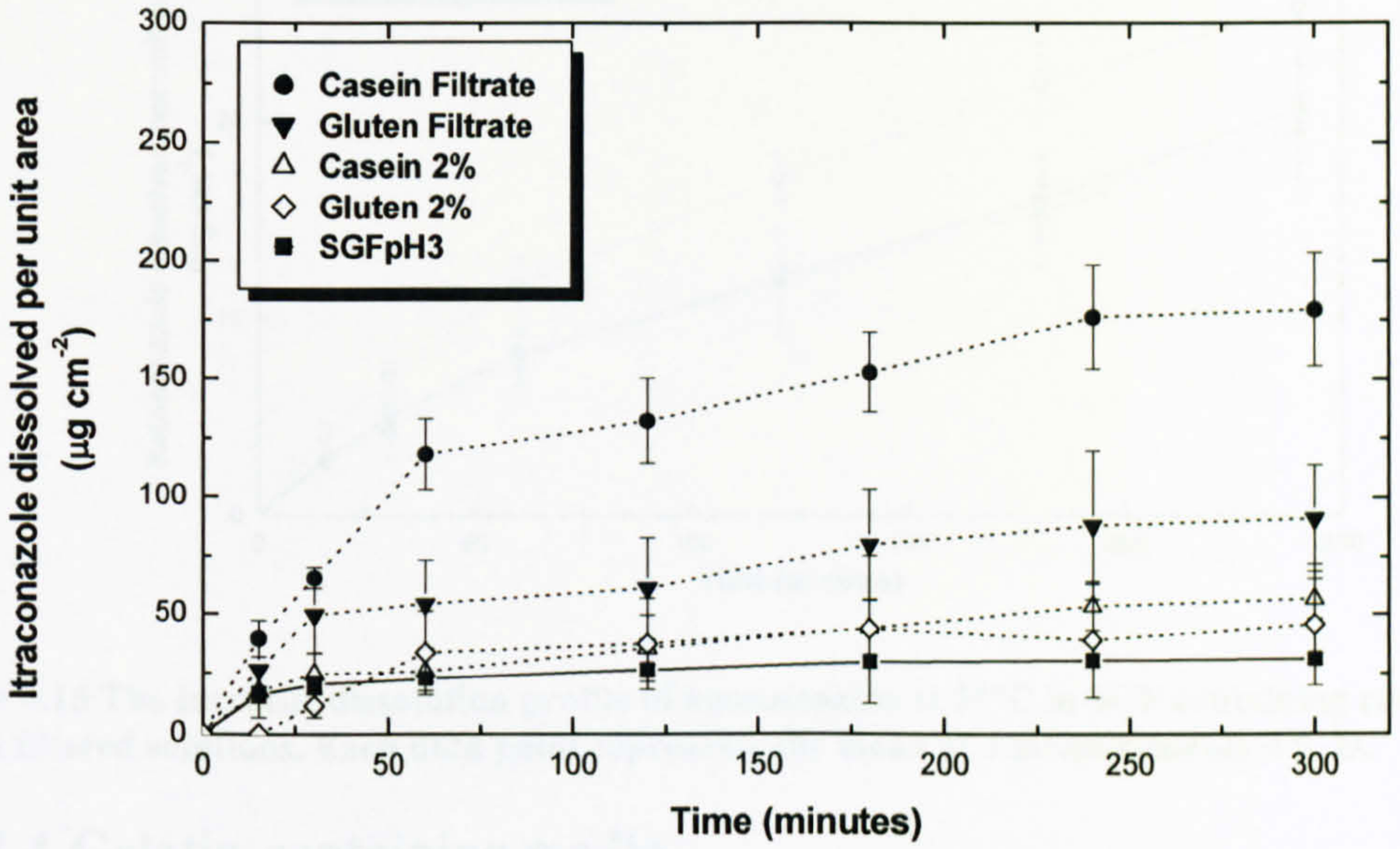


Figure 4.14 The Intrinsic dissolution profile of itraconazole at 37°C in 2%, w/v casein in SGF, casein saturated filtered solution, 2%, w/v gluten in SGF and gluten saturated filtered solution. Each data point represents the mean ± S.D. of 3 measurements, SGF (n=6)

Although the gluten-containing solution appeared to increase the dissolution rate, this increase was not statistically significant compared to the SGF because the data were widely distributed.

4.3.3.3.2 Ketoconazole

The solubility studies indicated that there was an enhancement of ketoconazole solubility of approximately 1.2-fold in 0.0038 and 0.005%, w/v casein solutions whereas the more diluted solutions of casein did not induce a significant effect (Figure 4.8). The data presented in Figure 4.15 shows that there was an increase in dissolution of ketoconazole in casein saturated solution compared to the SGF. The IDR increased by a factor of 1.2 to 1.5 in the presence of casein.

The inclusion of gluten in the media did not induce a discernible effect on either the solubility or the dissolution of ketoconazole.

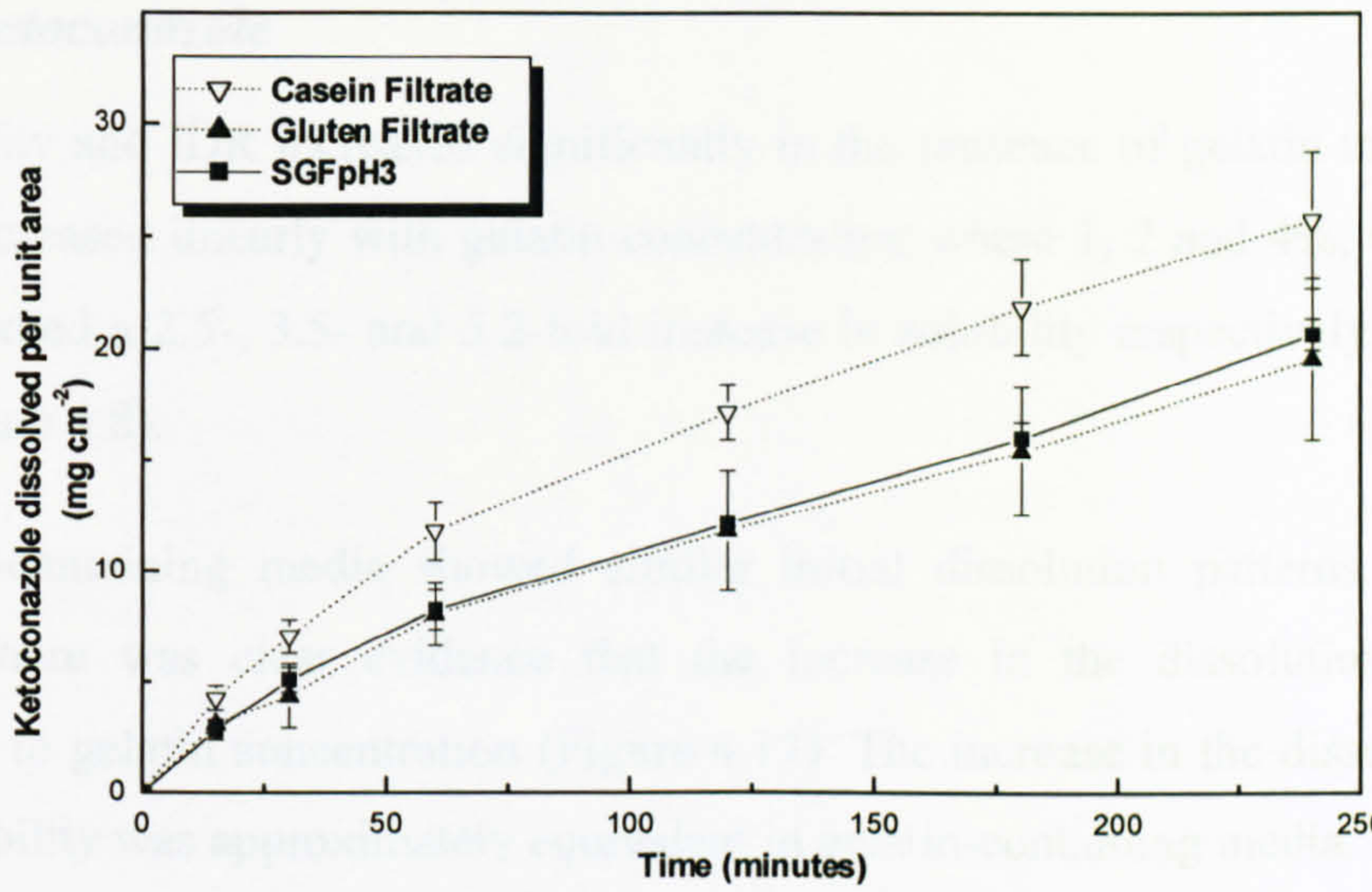


Figure 4.15 The Intrinsic dissolution profile of ketoconazole at 37°C in SGF containing casein and gluten filtered solutions. Each data point represents the mean of 3 measurements ± S. D.

4.3.3.4 Gelatin-containing media

4.3.3.4.1 Itraconazole

The solubility of itraconazole in gelatin containing media depicted an increase in itraconazole solubility (2- to 4-fold) with an increase in the concentration of gelatin (Figure 4.6).

The solubility and the amount dissolved in one hour per unit area in 0.5%, w/v gelatin media did not increase significantly from that in SGF but with media containing higher concentrations of gelatin a marked increase was recorded (Figure 4.16).

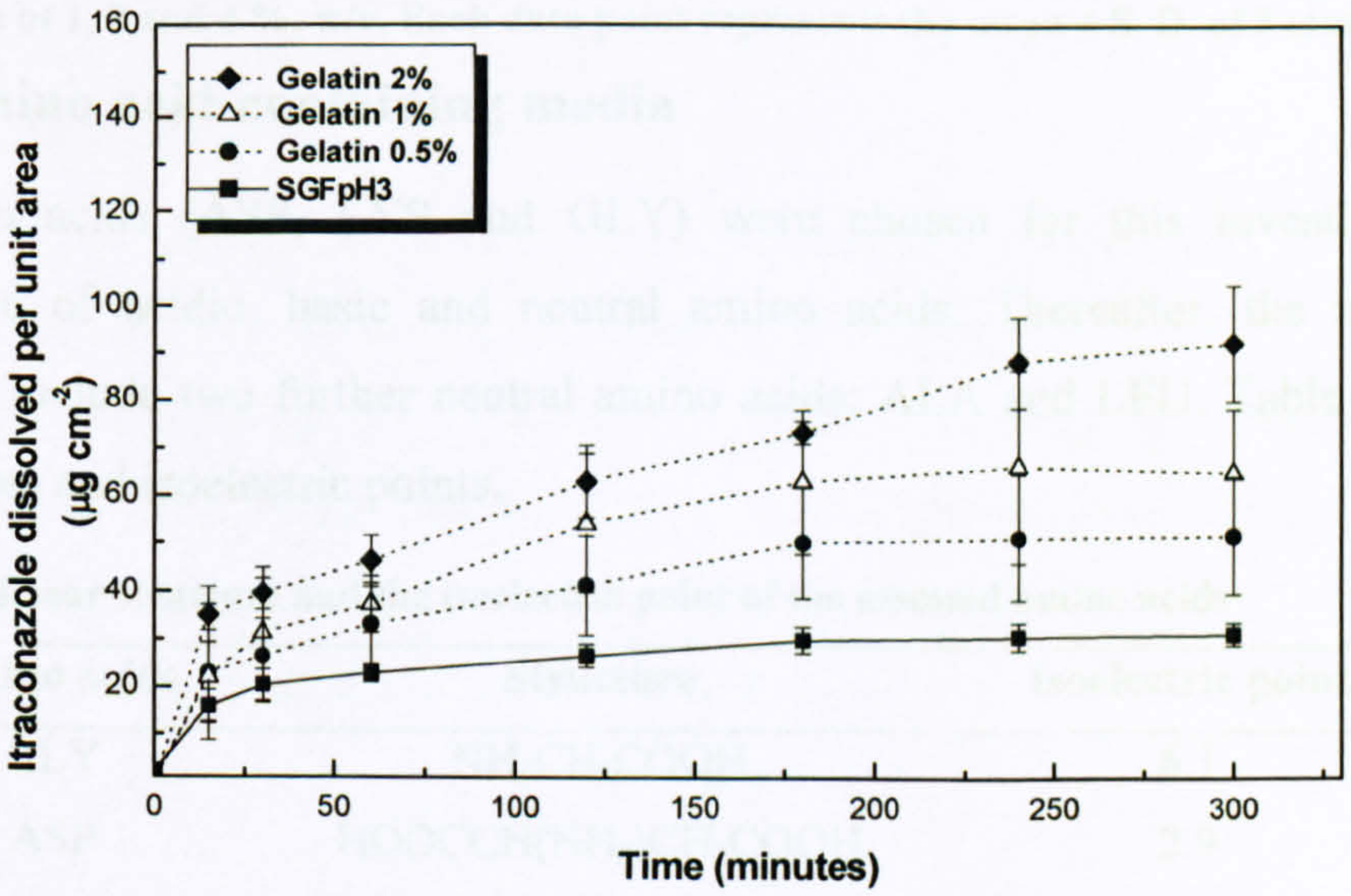


Figure 4.16 The intrinsic dissolution profile of itraconazole at 37°C in SGF (pH 3) containing gelatin in concentrations of 0.5, 1 and 2 %, w/v. Each data point represents the mean ± S.D of 3 measurements, in SGF (n=6)

4.3.3.4.2 Ketoconazole

Both solubility and IDR increased significantly in the presence of gelatin in SGF. The solubility increased linearly with gelatin concentration where 1, 2 and 4%, w/v gelatin in SGF recorded a 2.5-, 3.5- and 5.2-fold increase in solubility respectively, compared to SGF (Figure 4.8).

The gelatin-containing media showed similar initial dissolution patterns. However, within 3h there was clear evidence that the increase in the dissolution rate was proportional to gelatin concentration (Figure 4.17). The increase in the dissolution rate and the solubility was approximately equivalent in gelatin-containing media.

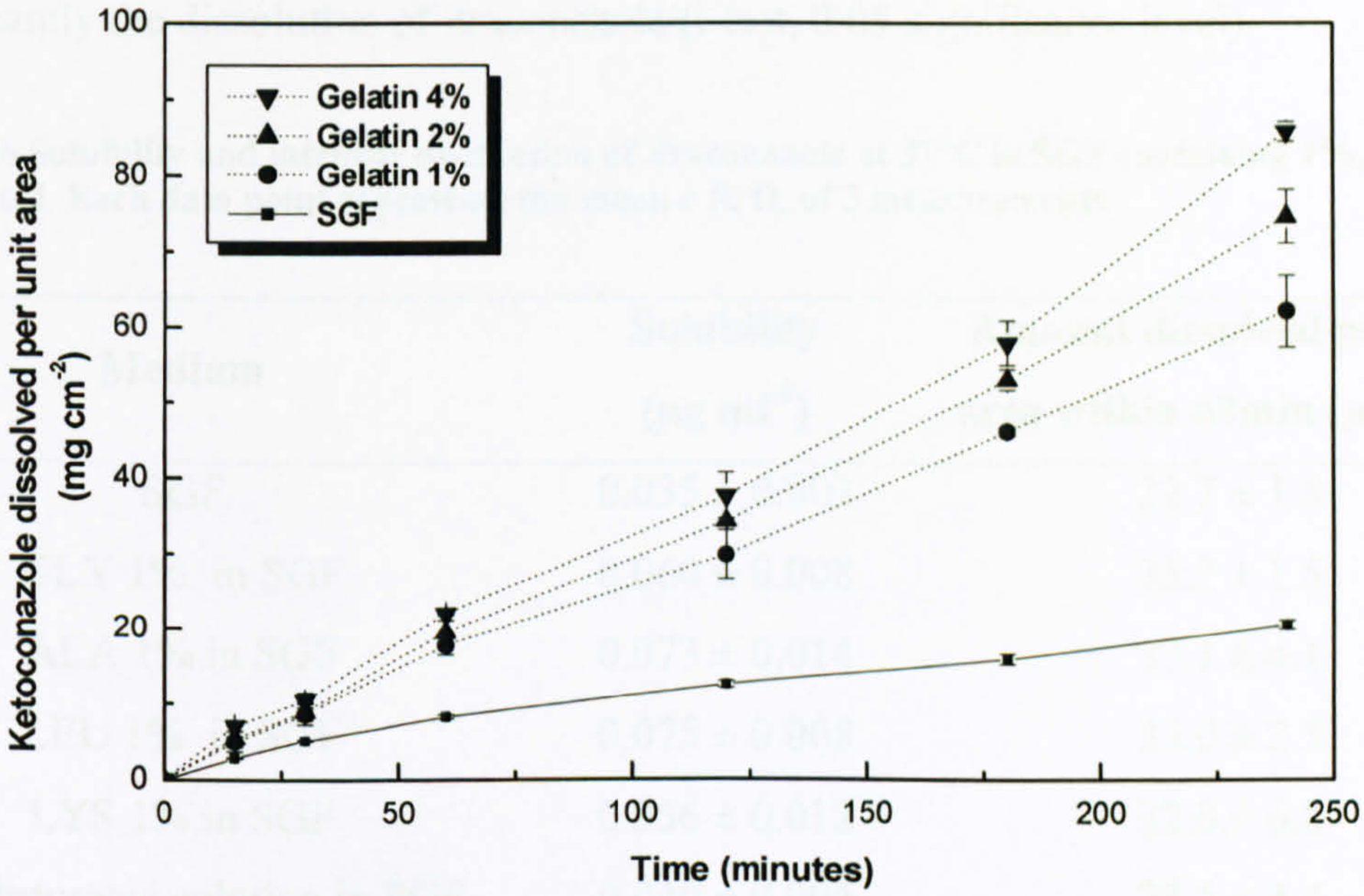


Figure 4.17 The intrinsic dissolution profile of ketoconazole at 37°C in SGF containing gelatin in concentrations of 1, 2 and 4 %, w/v. Each data point represents the mean ± S. D. of 3 measurements

4.3.3.5 Amino acid-containing media

Three amino acids (ASP, LYS and GLY) were chosen for this investigation as representative of acidic, basic and neutral amino acids. Thereafter, the study was expanded to include two further neutral amino acids: ALA and LEU. Table 4.5 gives their structures and isoelectric points.

Table 4.5 The linear structure and the isoelectric point of the assessed amino acids

Amino acids	Structure	Isoelectric point
GLY	NH_2CH_2COOH	6.1
ASP	$HOOCCH(NH_2)CH_2COOH$	2.9
LYS	$HOOCCH(NH_2)(CH_2)_4NH_2$	9.6
ALA	$CH_3CH(NH_2)COOH$	6.0
LEU	$HOOCCH(NH_2)CH_2CH(CH_3)_2$	6.0

4.3.3.5.1 Itraconazole

Media containing amino acids showed a slight increase in both dissolution and saturation solubility (Table 4.6). The solubility was greater in GLY, ALA and LEU-containing media. LYS-containing medium induced an enhancement in the solubility but this was not significant. ASP did not affect the solubility which could be explained by its low concentration in the medium. The solubility of ASP at pH 3 was computed as 0.0003 %, based on its aqueous solubility which is 0.4%, w/v.

The amount of itraconazole dissolved within 1h was slightly higher in media-containing GLY, ALA and LEU and increased further by 3h. LYS and ASP inclusion did not affect significantly the dissolution of itraconazole (t-test, 0.05 significance level).

Table 4.6 Solubility and intrinsic dissolution of itraconazole at 37°C in SGF containing 1%, w/v amino acid. Each data point represents the mean ± S. D. of 3 measurements

Medium	Solubility ($\mu g\ ml^{-1}$)	Amount dissolved per unit area within 60min ($\mu g\ cm^{-2}$)
SGF	0.035 ± 0.003	22.7 ± 1.8
GLY 1% in SGF	0.060 ± 0.008	35.7 ± 1.5
ALA 1% in SGF	0.073 ± 0.014	37.1 ± 4.1
LEU 1% in SGF	0.075 ± 0.008	35.0 ± 2.5
LYS 1% in SGF	0.056 ± 0.012	32.0 ± 6.8
ASP saturated solution in SGF	0.040 ± 0.004	23.5 ± 3.4

4.3.3.5.2 Ketoconazole

GLY inclusion in the medium increased the solubility 11-fold whereas LYS increased the solubility 6-fold. ASP did not induce a discernible effect. Consequently, the effect of further neutral amino acids was investigated. Media containing ALA and LEU were assessed. Each neutral amino acid-containing media showed considerable solubility enhancement of ketoconazole (Figure 4.18). A parallel increase in the dissolution rate of ketoconazole was also observed in these media (Figure 4.19).

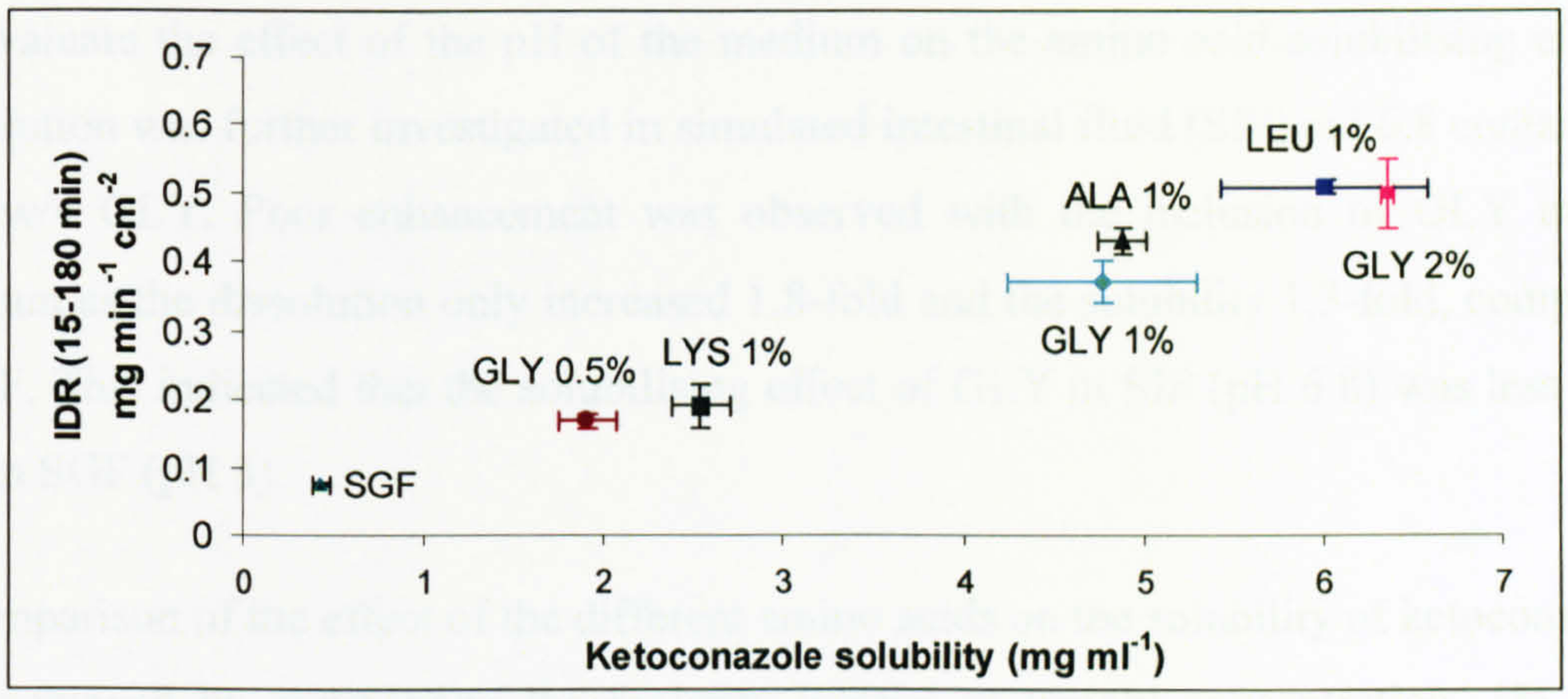


Figure 4.18 Solubility and IDR of ketoconazole at 37°C in amino acid-containing SGF (pH 3). Each data point represents the mean ± S.D. of 3 measurements

Visual observations of the disks during the dissolution process showed that holes formed at the surface of the disks after 3h. Therefore the results after this time point were invalid for IDR calculations due to the change in the disk surface area. This phenomenon was due to the high solubility of the drug in these media which led to fast dissolution.

The IDR (15-180min) increased by approximately 4.8-fold in solutions containing 1%, w/v GLY and 5.6-fold in 1%, w/v ALA, whereas a greater increase of 6.6-fold was observed in media containing 1%, w/v LEU.

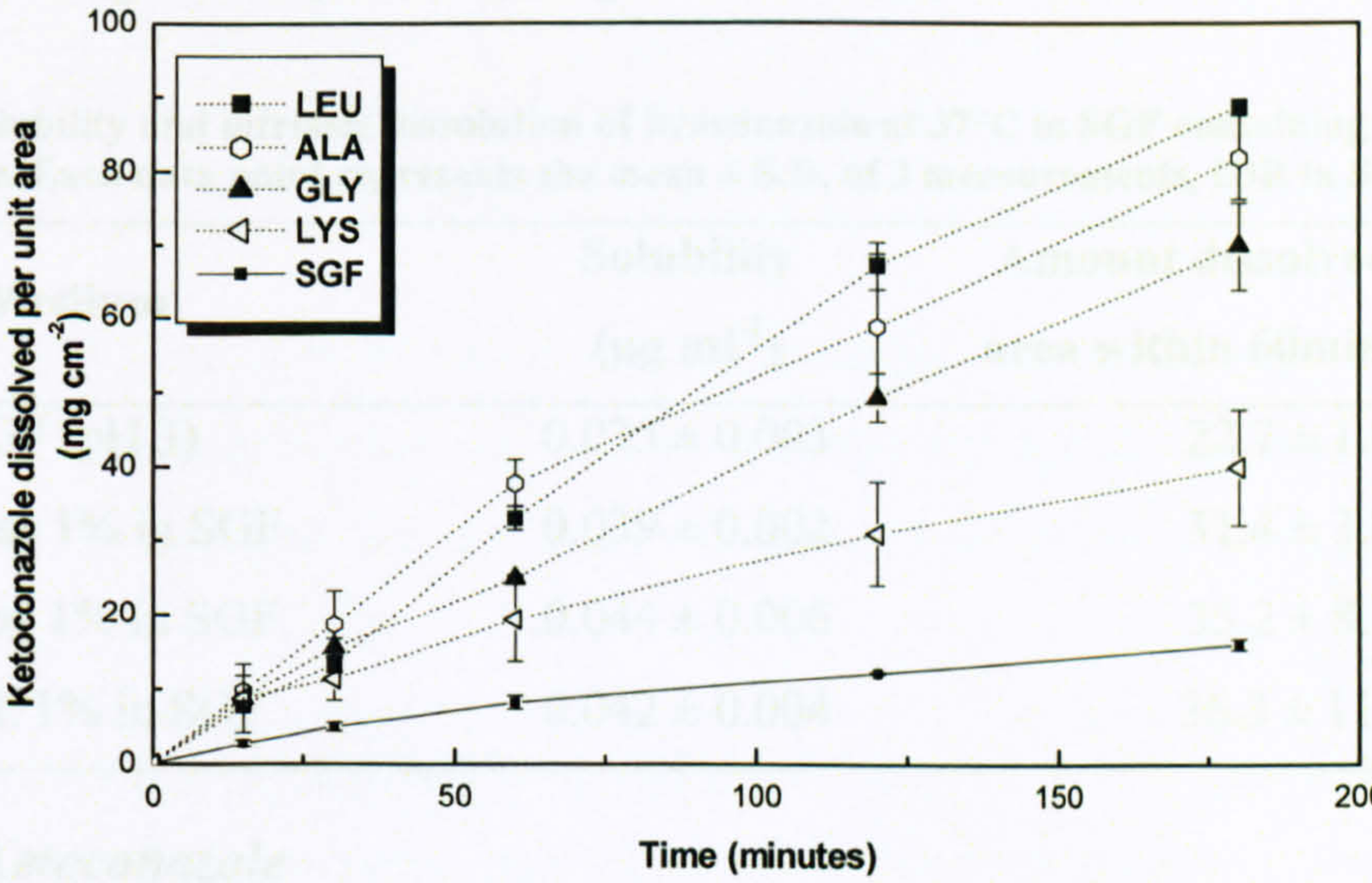


Figure 4.19 The intrinsic dissolution profile of ketoconazole at 37°C in SGF containing 1%, w/v amino acids (LYS, GLY, ALA and LEU). Each data point represents the mean ± S.D. of 3 measurements

To evaluate the effect of the pH of the medium on the amino acid-solubilising effect, dissolution was further investigated in simulated intestinal fluid (SIF) pH 6.8 containing 1%, w/v GLY. Poor enhancement was observed with the inclusion of GLY in the medium as the dissolution only increased 1.8-fold and the solubility 1.3-fold, compared to SIF. This indicated that the solubilising effect of GLY in SIF (pH 6.8) was less than that in SGF (pH 3).

A comparison of the effect of the different amino acids on the solubility of ketoconazole was achieved by considering the molarity instead of weight concentration. 1%, w/v GLY was equivalent to 133mM so equimolar solutions of LYS (1.9%, w/v) and ALA (1.2%, w/v) were prepared. It was found that ALA induced the greatest enhancement in solubility of 11.6-fold whereas the increase in LYS-containing media was 7.8-fold.

4.3.3.6 Sugar-containing media

4.3.3.6.1 Itraconazole

Three different carbohydrates were chosen to represent examples of mono-saccharides (glucose), disaccharides (lactose) and polysaccharides (starch). The solubility and dissolution values of itraconazole in these media exhibited a slight increase compared to SGF. However, statistical studies for the amount dissolved in one hour showed this increase was insignificant (t-test, 0.05 significant level) (Table 4.7).

Table 4.7. Solubility and intrinsic dissolution of itraconazole at 37°C in SGF containing 1%, w/v carbohydrate. Each data point represents the mean ± S.D. of 3 measurements, IDR in SGF (n=6)

Medium	Solubility ($\mu g\ ml^{-1}$)	Amount dissolved per unit area within 60min ($\mu g\ cm^{-2}$)
SGF (pH 3)	0.035 ± 0.003	22.7 ± 1.8
Glucose 1% in SGF	0.039 ± 0.002	31.4 ± 3.8
Lactose 1% in SGF	0.044 ± 0.006	35.2 ± 8.1
Starch 1% in SGF	0.042 ± 0.004	36.3 ± 11.0

4.3.3.6.2 Ketoconazole

Glucose and starch inclusion did not induce an effect on ketoconazole solubility or dissolution (Figure 4.20). A slight increase in dissolution was seen with lactose-containing medium.

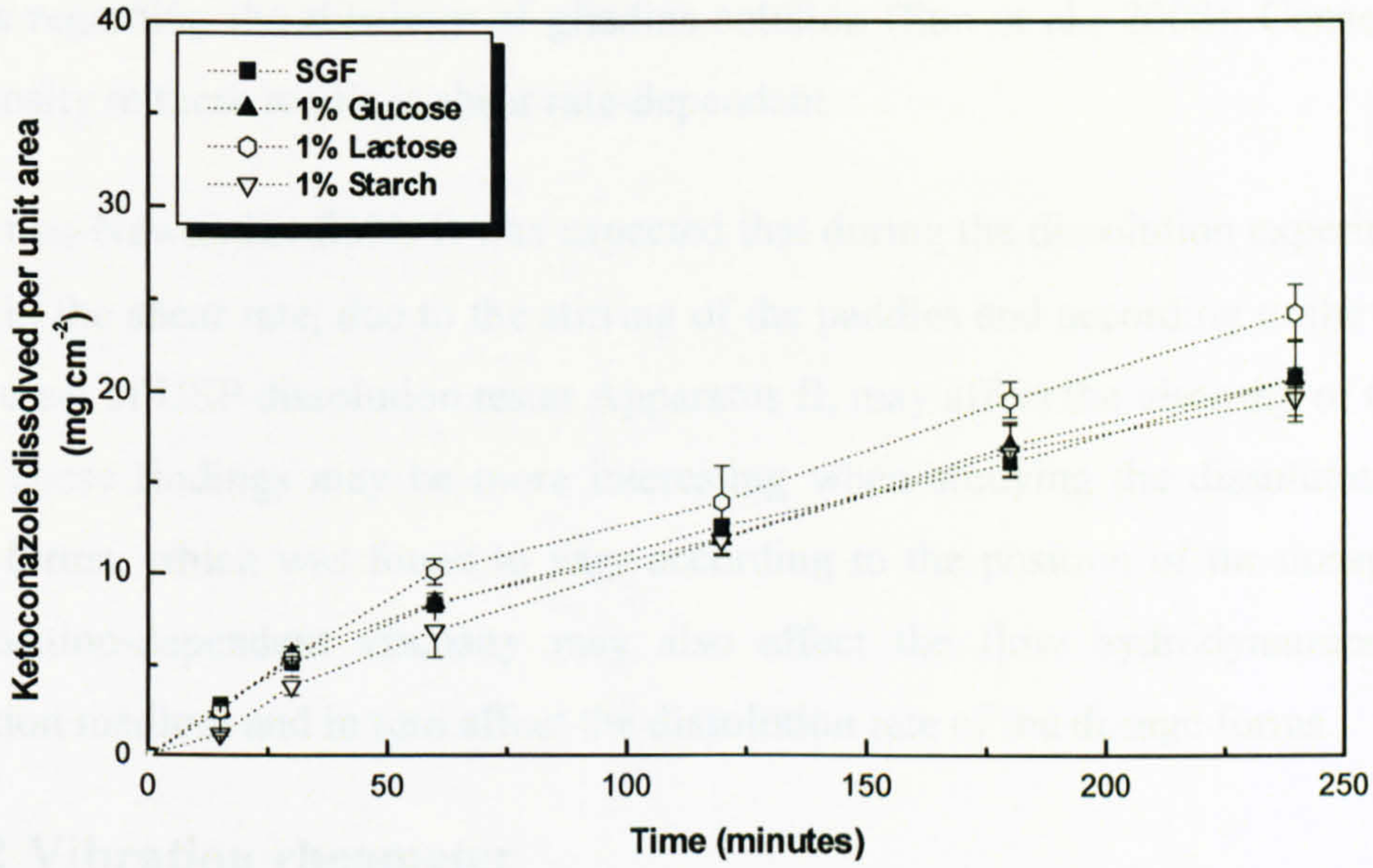


Figure 4.20 The intrinsic dissolution profile of ketoconazole at 37°C in SGF containing 1%, w/v carbohydrate (glucose, lactose and starch). Each data point represents the mean ± S.D. of 3 measurements

4.4 Discussions

4.4.1 Viscosity

4.4.1.1 Rheology studies

The purpose of studying the rheology of the media was to investigate probable changes in viscosity due to the rotation of the paddle in the dissolution tester. This creates a shear rate in the liquid inside the dissolution vessels and the shear rate will vary according to the position in the USP II dissolution vessels (Kukura et al., 2004).

It was found that viscosity of gelatin-containing media was independent of the shear rate. This Newtonian behaviour was consistent in the four gelatin-containing solutions (0.5, 1, 2, 4%, w/v) (Figure 4.1) and was in agreement with previous findings (Marcotte et al., 2001).

On the other hand, milk- and casein-containing media were non-Newtonian. Skimmed and whole fat milk were previously found to be non-Newtonian (Kristensen et al., 1997). Medium with a higher concentration of albumin (1%, w/v) was non-Newtonian whereas the more diluted solution was Newtonian (Singh et al., 2005).

Gluten is composed of two proteins, glutenins and gliadins. The former contributes to the elasticity and the latter to the viscosity. Gluten flow was fitted to the power law model suggesting non-Newtonian behaviour, which comes in agreement with previous

findings regarding the rheology of gliadins solution (Sun et al., 2008). Consequently, the viscosity in these media is shear rate-dependant.

For the non-Newtonian fluids it was expected that during the dissolution experiment the change in the shear rate, due to the stirring of the paddles and according to the location in the vessel of USP dissolution tester Apparatus II, may affect the viscosity of the these media. These findings may be more interesting when studying the dissolution of the dosage forms, which was found to vary according to the position of the dosage form. This position-dependent viscosity may also affect the flow hydrodynamics of the dissolution medium and in turn affect the dissolution rate of the dosage forms.

4.4.1.2 Vibration rheometer

The viscosity of a protein-containing solution depends on its intrinsic properties, such as molecular weight, size, volume, surface charge and protein-protein interactions (Nandi, 1997). Viscosity is also influenced by environmental factors such as pH, temperature and ionic strength. Protein molecules absorb water and swell affecting the flow behaviour of their solution. The viscosity of albumin and gelatin-containing media showed an increase proportional to the concentration of the protein. Less increase in the viscosity was noticed in gluten-containing medium.

Milk-SGF media had a higher viscosity than SGF and the greater the fat content of milk the higher the viscosity (Table 4.1). Casein is the main protein in milk as it represents about 80% of milk protein. Casein molecules do not exist as monomers at physiological conditions as they tend to associate with each other and with calcium phosphate to form spherical structures called casein micelles. The hydrophobic fractions are mainly in the core and the hydrophilic fractions are on the surface of the micelle (Liu and Guo, 2008). These micelles are the main contributors to the viscosity of milk (Walstra et al., 2006b). The fat content also contributes to the viscosity but to a lesser extent. An increase in the viscosity of SGF containing casein was observed, compared to SGF. This was attributed to the flexible open structure of casein and the relatively high capacity to bind water which led to the elevation in viscosity of the media (Fox and McSweeney, 1998a).

Carbohydrates have numerous hydroxyl groups which enable them to bind water molecules through hydrogen bindings and which lead to an increase in the viscosity of their aqueous solutions. Glucose or lactose solution induced a slight increase in the viscosity, compared to SGF (Table 4.2) which is in accordance with literature values

obtained at 20°C. A 1%, w/v glucose or lactose solution in water had a viscosity of 1.021 and 1.026mPa.s, respectively, whereas the viscosity of water was 1.002 mPa.s (Lide, 2002). The presence of starch in the medium induced more increase in viscosity. It is composed of two components; amylose and amylopectin, the former is a straight chain molecule which has the ability to bind water and swell, and in turn increase the viscosity of starch-containing media (Niba, 2006).

Although amino acids form hydrogen bonds with water molecules through their carboxylic acid and amino groups, the viscosity of media containing amino acids did not change considerably (Table 4.2).

4.4.2 Surface tension

In general, protein molecules exhibit surface tension activity due to their amphiphilic nature. Proteins are adsorbed to the interface between two phases: liquid/gas or oil/water, causing a distinct reduction in interfacial tension. Differences in surface activity among proteins arise mainly from differences in their structures, size, stability and hydrophobicity (Magdassi and Kamyshny, 1996).

The surface tension of the milk-containing media was relatively low due to the surface active components of milk which are proteins, casein micelles and whey protein, phospholipids, free fatty acids and monoglycerides (Walstra et al., 2006a). These components can adsorb on the air/liquid interfacial surface and reduce the surface tension. The milk media with a higher fat content had slightly lower surface tension. The mean values observed for the whole fat milk-containing medium were in good agreement with data for simulated fed gastric fluid composed of milk and buffer (1:1), obtained by Jantratid et al. (2008b).

The presence of albumin lowered the surface tension of the media proportionally up to a concentration of 2% albumin. The medium of pH 3 led to denaturing of the protein which was shifted from its isoelectric point (PI: 4.8). Egg albumin exhibits a surface activity; the denaturing phenomena combined with the probable effect of salts resulted in a further drop in the surface tension of albumin-containing media (Koseki et al., 1988). A 1%, w/v solution of albumin in SGF exhibited a 15mN m^{-1} decrease in the surface tension, relative to SGF.

Gelatin-containing solutions also had a mild surface activity which was proportional to protein concentration (Sato and Ueberreiter, 1979). However, the acidity of the media

combined with the presence of an electrolyte (NaCl) in the media led to a marked decrease in the surface tension of gelatin media. The inclusion of 1%, w/v gelatin in SGF lowered the surface tension by 16mN m^{-1}, compared to SGF.

Casein containing solutions also had low surface tensions. The values dropped with an increase in casein concentration suggesting casein micelles were not formed. However, the drop in surface tension was not significant. If casein micelles were formed, then no further drop in the surface tension would be expected by increasing the concentration of the protein, i.e. the surface tension would remain constant after reaching the CMC. Thus, these results suggested that CMC for casein was not reached but because the variations were not significant this could not be confirmed.

The inclusion of gluten in the SGF resulted in a 16mN m^{-1} drop in the surface tension. This protein is composed of glutenins and gliadins and the latter is responsible for the surface activity. This agrees with a previous report that gluten solutions had low surface tensions under acidic conditions (Takeda et al., 2001).

Sugars can cause an increase in the surface tension of water due to an increase in the hydrogen bond driven cohesion among the sugar solute molecules (Docoslis et al., 2000). The measured surface tension of glucose-containing medium increased compared to SGF (2mN m^{-1}). However, the lactose solution had a significantly lower surface tension than SGF. This was probably due to the presence of impurities in lactose which led to these lower surface tension measurements (Table 4.2).

In a similar way amino acids were expected to induce a rise in the surface tension due to an increase in the cohesive forces due to hydrogen bonding combined with electrostatic attractions among the solute molecules (Docoslis et al., 2000). However this was not observed.

4.4.3 Dissolution and solubility

The two azole compounds had a significantly higher solubility and dissolution rate in the presence of most of the assessed food components, compared to the reference that did not contain these additives, which was SGF.

4.4.3.1 Milk-containing media

Although itraconazole and ketoconazole are lipophilic molecules, the increased fat content of milk was expected to aid solubility but the different fat content of the three types of milk did not show the expected systematic effect on drug behaviour.

The composition of the three types of milk was identical, except for the fat content (according to supplier information). Furthermore, only minor variations in the surface tension of the three types of milk containing-media were noticed. A slight difference in viscosity was noted, however such small variations were not expected to significantly affect the dissolution (Macheras and Reppas, 1987). Although it was expected that a higher fat content would induce more dissolution, this did not occur.

It is possible that some disruption of the milk emulsion occurred during the dissolution experiment which led to separation of the fat, which would then float to the surface (Fox and McSweeney, 1998b), making it unavailable to the drug 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-containing media could also explain why the itraconazole results acquired were variable.

With ketoconazole, which is a relatively less lipophilic drug, variations in the dissolution behaviour in the three types of milk containing-media were not obvious and the three profiles were nearly identical, except at the later time point. In a previous study about drug dissolution in milk mixtures (Shah, 2006), the author reported that the dissolution of ibuprofen did not vary according to the fat content of the milk-containing media and this was attributed to the structure of the lipids in milk. Nearly all of the fat in milk exists in separate small globules coated with a globule membrane which acts as a barrier between the fat and the milk plasma and prevents fat globules from coalescence (Walstra et al., 2006a). Thus, the composition of the membrane may physically hinder the fat solubilising effect onto the lipopholic drugs. However, the membrane is fragile and the stability of the fat globules decreases linearly with increasing fat content in milk. Thus, the mechanical effect of the stirring during the dissolution experiment may affect the stability of milk fat globules and release the fat to the media causing variable effects, particularly with the whole fat milk-containing medium (Fox and McSweeney, 1998b).

Milk-containing media had a lower surface tension, higher viscosity and relatively smaller contact angle with both drug surfaces than SGF. The mechanism by which milk enhanced the dissolution of the drugs was through solubilisation effect in addition to enhancing the wettability of drug surfaces.

From the Noyes and Whitney equation (Eq. (1-3)) the dissolution increased through increasing the saturation solubility. Furthermore, drug-milk complex formation led to a decline in the concentration of free drug in the dissolution medium which in turn increased the gradient of the concentration (C_s-C_t) leading to an increase in the dissolution rate. Consequently, more diffusion of the drug from the disk into the bulk solution occurred. Thus, the additives to the dissolution media played the role of a carrier by transporting the solutes from the solid surface to the liquid phase. Thus, although milk-containing media had a higher viscosity compared to SGF, which is normally expected to slow the dissolution process (Section 1.6.1.3.), this effect was not seen as the complexation effect was more dominant.

Macheras et al., (1990) reported that the log P value is the most important predictor for the effect of milk on drug dissolution as the higher the log P value, the more distinct the increase in solubility. These present findings support this since itraconazole was more solubilised by milk components than ketoconazole due to its higher lipophilicity.

4.4.3.2 Albumin-containing media

The presence of albumin in the media induced an increase in the solubility and the dissolution of the two drugs but the effect was more pronounced with itraconazole (6 times greater) than with ketoconazole. The increase in solubility was greater than the increase in the dissolution throughout the time of the experiment.

Albumin-containing media had a relatively low surface tension, high viscosity and achieved better wettability for drugs' surfaces. The mechanism affecting drug behaviour in albumin-containing media is believed to be protein-drug binding.

Egg albumin induced an interesting solubilising effect which was proportional to the concentration of the protein and this effect was more apparent with itraconazole than ketoconazole. This interaction is probably through hydrophobic, electrostatic interactions and hydrogen bonding. This is similar to the case with human serum albumin where itraconazole and ketoconazole exhibited high protein binding *in vivo*, 99.8% and 99%, respectively (Heykants et al., 1989).

Previous studies based on thermodynamic and spectroscopic studies also found that the mechanisms of the interaction between ketoconazole and serum albumin were electrostatic interaction and hydrophobic interactions (Guo et al., 2008). Figure 4.21 illustrates possible types of interaction between drug and protein on the molecular level.

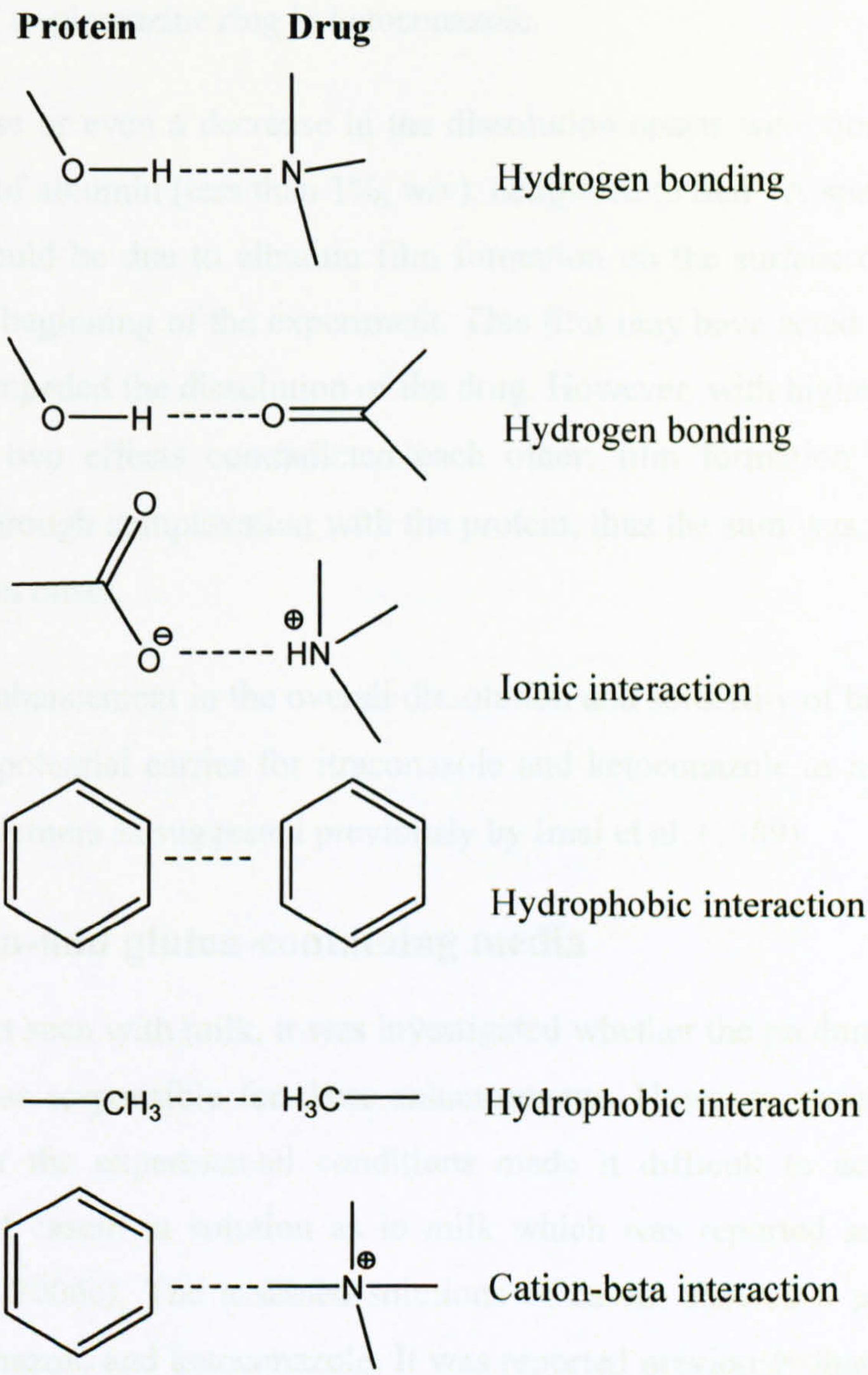


Figure 4.21 Possible interactions between protein and drug molecules

The hydrophobic interaction seems to contribute essentially to the association of the drug with protein molecules. The possible sites of hydrophobic interactions on itraconazole are the two phenyl rings, 2, 4-dichloro-phenyl ring and 2-butyl moieties of itraconazole. While the hydrophobic sites on ketoconazole are the aromatic ring and 2, 4-dichloro-phenyl ring. Ketoconazole held more positive charges than itraconazole at the pH of the investigation (pH 3); and so the more ionised moieties had less affinity to

the protein hydrophobic cavities. Therefore, this may explain the more profound solubilising effect of albumin on itraconazole compared to its effect on ketoconazole.

The possible multi-hydrogen bonding sites in the drug molecules were: the unionised nitrogens, carbonyl groups attached to the triazole ring in itraconazole and the carbonyl groups adjacent to piperazine ring in ketoconazole.

A slight increase or even a decrease in the dissolution onsets were observed with low concentrations of albumin (less than 1%, w/v), compared to SGF. A speculation for this phenomenon could be due to albumin film formation on the surface of the disks that occurred at the beginning of the experiment. This film may have acted as an interfacial barrier and so impeded the dissolution of the drug. However, with higher concentrations of the protein two effects contradicted each other: film formation and more drug solubilisation through complexation with the protein, thus the sum was a slight increase in the dissolution onset.

The observed enhancement in the overall dissolution and solubility of both drugs makes egg albumin a potential carrier for itraconazole and ketoconazole as a safe alternative for synthetic polymers as suggested previously by Imai et al. (1989).

4.4.3.3 Casein-and gluten-containing media

Due to the effect seen with milk, it was investigated whether the predominant protein in milk, casein, was responsible for these enhancements. However, casein with its poor solubility under the experimental conditions made it difficult to achieve the same concentration of casein in solution as in milk which was reported as 1.7-3.5%, w/v (Walstra et al., 2006c). The assessed solutions of casein showed a poor solubilising effect on itraconazole and ketoconazole. It was reported previously that the mechanism by which casein affected drug dissolution was via micelle formation (Macheras and Reppas, 1987).

Casein molecules have a strong tendency to self-assemble into micelles because of their amphiphilic nature in aqueous solution. Various models of the assembly and structure of the casein have been suggested in the literature of which sub-unit model for casein aggregation was the most widely accepted. This model suggests that 15–20 molecules of casein aggregate via hydrophobic interactions and form sub-units where the hydrophobic core is surrounded by a polar portion. These sub-units form the building units of the micelles (McMahon and Rodney, 1984). Casein monomers and sub-units

exist in the solution when the casein concentration is below the CMC, whereas above the CMC casein micelles are combined with monomers and sub-micelles.

Based on this structure, casein is able to encapsulate hydrophobic compounds into the hydrophobic core even at the sub-micellar level. Consequently, itraconazole and ketoconazole could be solubilised through these sub-units and the position of the drug would vary according to the polarity of the drug.

Previous studies showed that the CMC of casein solution at pH 7 was 0.1%, w/v (Liu and Guo, 2008), however, the existence of salt and the pH of the solution can affect the micelle formation. Considering casein is like a surfactant, if the CMC was exceeded, a linear increase in the solubility of the drug would be expected as more micelle formation leads to more drug solubilisation. An increase in the solubility of both drugs was observed with an increase in casein concentration suggesting micelles were likely formed but because the increase was not significant this phenomenon could not proved. The surface tension measurements of casein solutions (Table 4.1) suggested that the CMC was not reached but this also could not be confirmed because the variations were not significant. Consequently, it is uncertain whether micelles were formed under the present experimental conditions or not, however, casein with its pre-micellar aggregation demonstrated the ability of casein to solubilise the drugs.

Although gluten-containing media had a low surface tension, it did not affect the solubility or the dissolution of the drugs significantly indicating this protein could not solubilise the investigated drugs.

4.4.3.4 Gelatin-containing media

Gelatin inclusion in the media increased the solubility and dissolution of both drugs. The effect of gelatin may be attributed to the surface activity of gelatin (Acarturk et al., 1992) and the ability of gelatin-containing solutions to enhance the wettability of the drug compact surfaces. This would allow the gelatin solutions to penetrate faster into the drug particles and so induce more solubilising influence. Despite an increase in the viscosity of gelatin solutions, the solubilising effect increased proportionally with protein concentration. A slightly higher effect was seen with ketoconazole suggesting the effect of gelatin increased with the less lipophilic drug.

Kallinteri and Antimisiaris (2001) found that gelatin increased the solubility of drugs and this effect was more pronounced with drugs of high lipophilicity and low aqueous

solubility (Kallinteri and Antimisiaris, 2001). Since both drugs investigated here fall into this category, the present results support their findings.

4.4.3.5 Amino acid-containing media

The presence of neutral amino acids enhanced both the dissolution and solubility of both drugs. The effect was greater on ketoconazole than itraconazole. Although the differences in the dissolution profiles of ketoconazole with the type of the neutral amino acids were not very large, it could be said that the greatest effect was seen with LEU-then ALA- and finally GLY-containing media. Since these amino acids have identical isoelectric points, this suggested the increase in solubility and dissolution was proportional to the length of side chain of the amino acids and consequently their hydrophobicity.

Ketoconazole is a dibasic drug with two pKa values: 6.51 and 2.94. At pH 3, ketoconazole molecules coexist in the two ionised forms: $H_2(keto)^{+2}$ and $H(keto)^{+1}$. Itraconazole has four pKa values: 4, 1.5-2 and two other pKa values which are lower than 1 (not precisely specified) (Peeters et al., 2002). Consequently, itraconazole exists mainly in the mono-ionised form $H(itra)^{+1}$ at pH 3, since the other protonated species exist at low pH (< 2). The acidic group of the amino acids has a pKa_1 of 2.3 and consequently at pH 3, 83% of these acidic moieties are deprotonated. Consequently, ionic interactions may occur between the negatively charged carboxylic acid of the amino acid and the positively charged drug molecules. This interaction could lead to the formation of a soluble complex.

The limited effect of GLY on the dissolution of ketoconazole at pH 6.8 supported this assumption as the drug has lost most of its positive charge at this pH. Furthermore, hydrogen bonding could occur between the uncharged nitrogen and carbonyl groups of the drugs and the carboxylic acid group of the amino acid.

In addition, since the solubility of the drugs increased with the increase in the hydrophobic character of the amino acid in the dissolution medium; this suggested that hydrophobic interactions could be an important force in this association.

The effect of the basic amino acid LYS on the behaviour of itraconazole was not significant (t-test, 0.05 significant level). However, the inclusion of LYS in the medium induced a marked increase in ketoconazole dissolution and solubility.

The solubilising effect of LYS was less than that of the neutral amino acids, probably because the side chain of LYS, amine group with pKa=10.5, was protonated at pH 3 and so it acted as a competitive binding site to the cationic drug entities.

ASP has an isoelectric point of 2.9. Thus at pH 3 its solubility was at a minimum level. In addition, ASP molecules have a net charge of zero at this pH which may have limited the effect of possible interaction between ASP molecules and the drugs.

The effect of amino acid was an interesting phenomenon with a marked increase in the solubility and dissolution of ketoconazole. So contrary to the albumin solubilisation effect, ketoconazole was more solubilised in these media than itraconazole. The suggested mechanism was via the formation of soluble complexes through ionic interactions and since ketoconazole was highly ionised at pH 3, a more pronounced effect was seen with ketoconazole.

4.4.3.6 Sugar-containing media

A mechanism by which carbohydrates increased the dissolution of the drug could also be through soluble complex formation. Hydrogen bonds can be formed between hydroxyl groups in sugars and nitrogen or carbonyl groups in drug entities leading to solubilisation of the drugs.

4.5 Conclusion

The increase in itraconazole solubility varied with a maximum increase of approximately 24-fold in milk and 20-fold in albumin (1%, w/v) containing media. The maximum solubilising effect of ketoconazole was seen in media containing 1%, w/v neutral amino acid and milk where an approximate increase of a factor of 14 and 6, respectively, was recorded. The dissolution rate also increased but not to the same extent as the solubility. This relatively slow dissolution could be ascribed to the formation of larger complexes or micelles which made their diffusion to the bulk solution slower than the free drug (Macheras and Reppas, 1987).

It is evident from this study that changes in media composition significantly influenced the behaviours of the assessed azole antifungal drugs. Since food additives had a significant impact on the dissolution, this revealed the importance of simulating gastric fluids at fed state in order to develop a more predictive IVIVC model. Thus, the study indicated that itraconazole and ketoconazole co-ingestion with food can increase the

bioavailability of the drugs and the extent of this effect may vary according to the type of the meal. Accordingly, it would be interesting to investigate how these components affected drug dissolution and in particular in the media that induced the maximum effect, namely; milk- and albumin-containing media.

Chapter 5: Itraconazole and ketoconazole binding to milk and proteins

5.1 Introduction

Media containing milk, egg albumin, casein and gelatin enhanced the solubility and dissolution of itraconazole and ketoconazole (Chapter 4). The mechanism of how these components could possibly affect the behaviour of the drugs was investigated. It is believed that this effect was through the interactions of the drugs with proteins or milk components in the media. Therefore, a conventional method for detecting protein-ligand interaction was employed, namely, the dynamic dialysis method (Martin, 1993a). Ketoconazole binding to egg albumin was further explored using spectroscopic techniques.

5.2 Materials and methods

5.2.1 Materials

Details of all materials used for the dialysis and spectroscopy studies are listed in 2.1.

5.2.2 Dialysis studies

5.2.2.1 Preparation of drug sample

A stock solution (0.5mg ml^{-1}) of itraconazole was prepared by dissolving the drug powder in dimethylformamide because of its poor aqueous solubility. Appropriate dilutions of the stock solution with simulated gastric fluid (SGF) pH 1.2, were made to generate drug solutions in concentrations of 5-10μg ml^{-1}.

Ketoconazole was dissolved directly in SGF pH 3 in concentrations of 100-200μg ml^{-1}. SGF at pH 1.2 and SGF at pH 3 were prepared as described in 3.3.1.1.

5.2.2.2 Preparation of drug-protein and drug-milk mixtures

10ml of drug solution (itraconazole 10μg ml^{-1}, ketoconazole 200μg ml^{-1}) was added to 10ml of whole fat milk, skimmed and semi-skimmed milk and saturated filtered casein solution.

Albumin or gelatin powders were added in quantities of 0.02-0.4g to 20ml to the drug solutions (itraconazole 5μg ml^{-1}, ketoconazole 100μg ml^{-1}). The concentrations of the albumin in the resultant mixtures were 0.1 and 1%, w/v. The concentrations of the

gelatin in the resultant mixtures were 1 and 2%, w/v. The mixtures of drug-protein or drug-milk were stirred for 10min and warmed up to around 37°C.

5.2.2.3 Preparation of the reference solutions

Reference solutions were prepared in the same manner without the addition of protein or milk to the drug solutions. Thus, for milk and casein experiments, drug solutions (itraconazole 10μg $ml^{-1,}$ ketoconazole 200μg ml^{-1}) were diluted 50:50 with SGF. For gelatin and albumin experiments, the reference solution was drug solution in SGF (itraconazole 5μg ml^{-1}, ketoconazole 100μg ml^{-1}).

5.2.2.4 Dialysis settings

Visking cellouse tubing with a molecular weight cut-off of 3500 Daltons was cut into 10cm long strips (5cm width) and soaked in distilled water. 10ml of drug-protein or drug-milk mixture was transferred accurately into a piece of the tubing which had been tied at one end. The amount of itraconazole and ketoconazole in the bags were 50μg and 1mg, respectively.

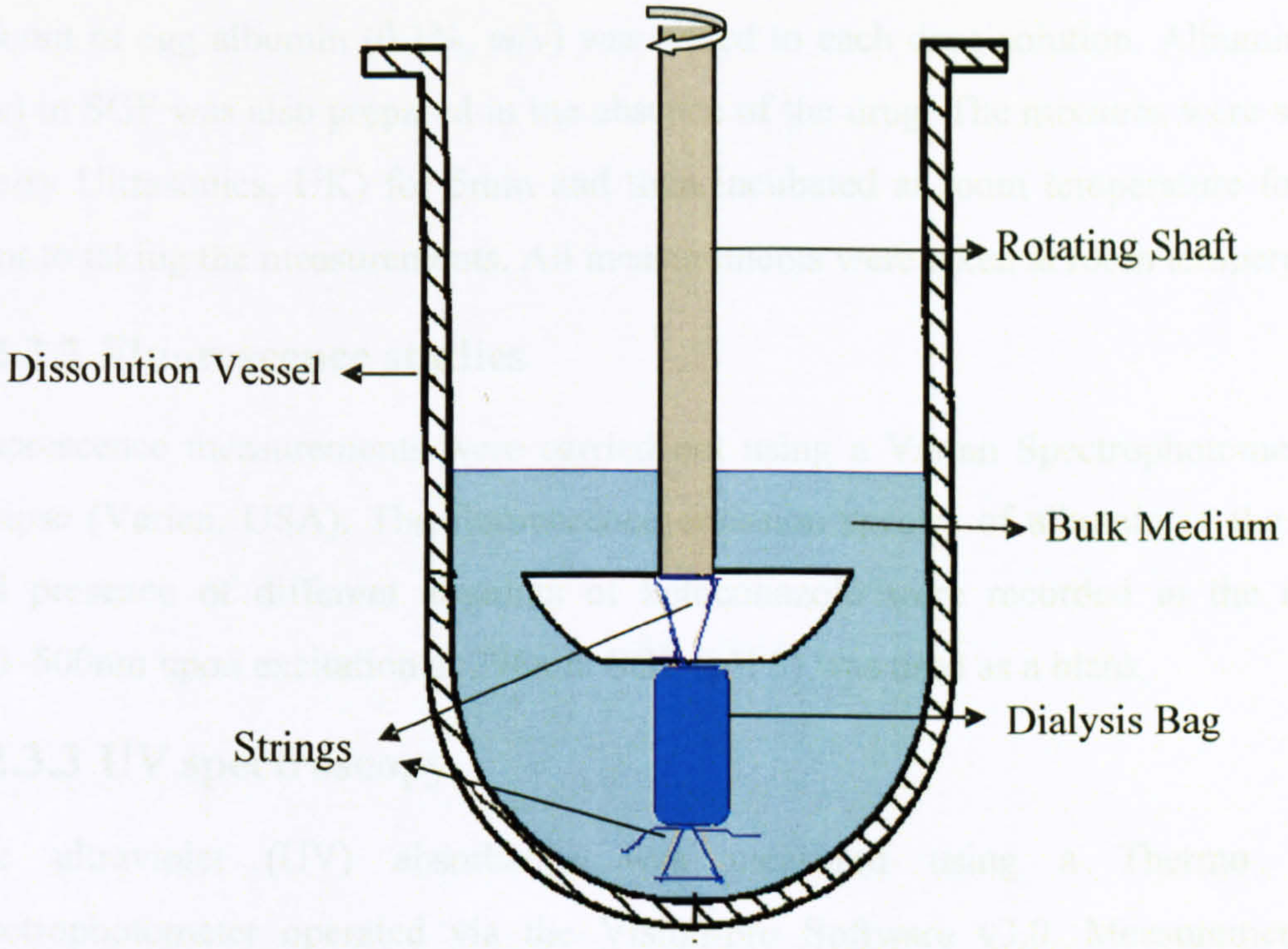


Figure 5.1 Schematic diagram illustrating the dialysis experiment, it shows the USP II dissolution tester and dialysis bag attached to the shaft (contained milk-drug, protein-drug mixtures or only drug solutions)

The dissolution tester USP II (Pharmatest Type PTW S3C) was employed to simulate the conditions of the dissolution test, in term of temperature and rotation. The tubes

were tied and attached to the flat, wide part of the paddles (Figure 5.1). The vessels of the dissolution tester were filled with SGF (250ml for itraconazole and 500ml for ketoconazole studies) and warmed to 37 ± 0.5°C. Stirring of the paddles was applied at 100 rpm and so the dialysis bags were also rotated.

5.2.2.5 Sampling and analysis

Aliquots of the external solution (2ml) were collected from the vessels periodically through sampling Cannulae and subsequently transferred to HPLC glass vials. The drug concentrations were quantified using the previously described HPLC method (Section 2.2.6). The binding of the drugs to the different protein and milk-containing media was estimated from the difference in the concentration of free drug released from the dialysis bag of the reference (in the absence of milk or protein) and the drug released from dialysis bags containing drug-protein or drug-milk mixtures.

5.2.3 Spectroscopic studies of albumin- ketoconazole mixtures

5.2.3.1 Preparation of ketoconazole-albumin mixtures

Ketoconazole was dissolved in SGF (pH 3) (10, 50, 100 and 200μg ml^{-1}). A fixed amount of egg albumin (0.1%, w/v) was added to each drug solution. Albumin (0.1%, w/v) in SGF was also prepared in the absence of the drug. The mixtures were sonicated (Kerry Ultrasonics, UK) for 5min and then incubated at room temperature for 30min prior to taking the measurements. All measurements were taken at room temperature.

5.2.3.2 Fluorescence studies

Fluorescence measurements were carried out using a Varian Spectrophotometer Cary Eclipse (Varian, USA). The fluorescence emission spectra of albumin in the absence and presence of different amounts of ketoconazole were recorded in the range of 300–500nm upon excitation at 296nm. SGF (pH 3) was used as a blank.

5.2.3.3 UV spectroscopy

The ultraviolet (UV) absorbance was measured using a Thermo Electron spectrophotometer operated via the Vision-pro Software v3.0. Measurements were taken for egg albumin in the presence and absence of ketoconazole in the range of 240–350 nm.

5.3 Results

5.3.1 Dynamic dialysis

Dynamic dialysis was performed to assess protein-drug binding. This is based on the determination of the drug disappearance from a dialysis bag which contained a mixture of drug-protein or drug-milk by quantifying the amount of the unbound drug that passed through the membrane (Martin, 1993a). The amount of the drug in the bulk medium, outside of the dialysis bag, was divided by the total initial amount of drug that was in the dialysis bag at the beginning of the experiment and expressed as percentage.

5.3.1.1 Milk-containing media

5.3.1.1.1 Itraconazole

The release of itraconazole from the dialysis bags containing milk-drug mixtures was less, in rate and extent, than the release from the reference solution that did not include milk (Figure 5.2).

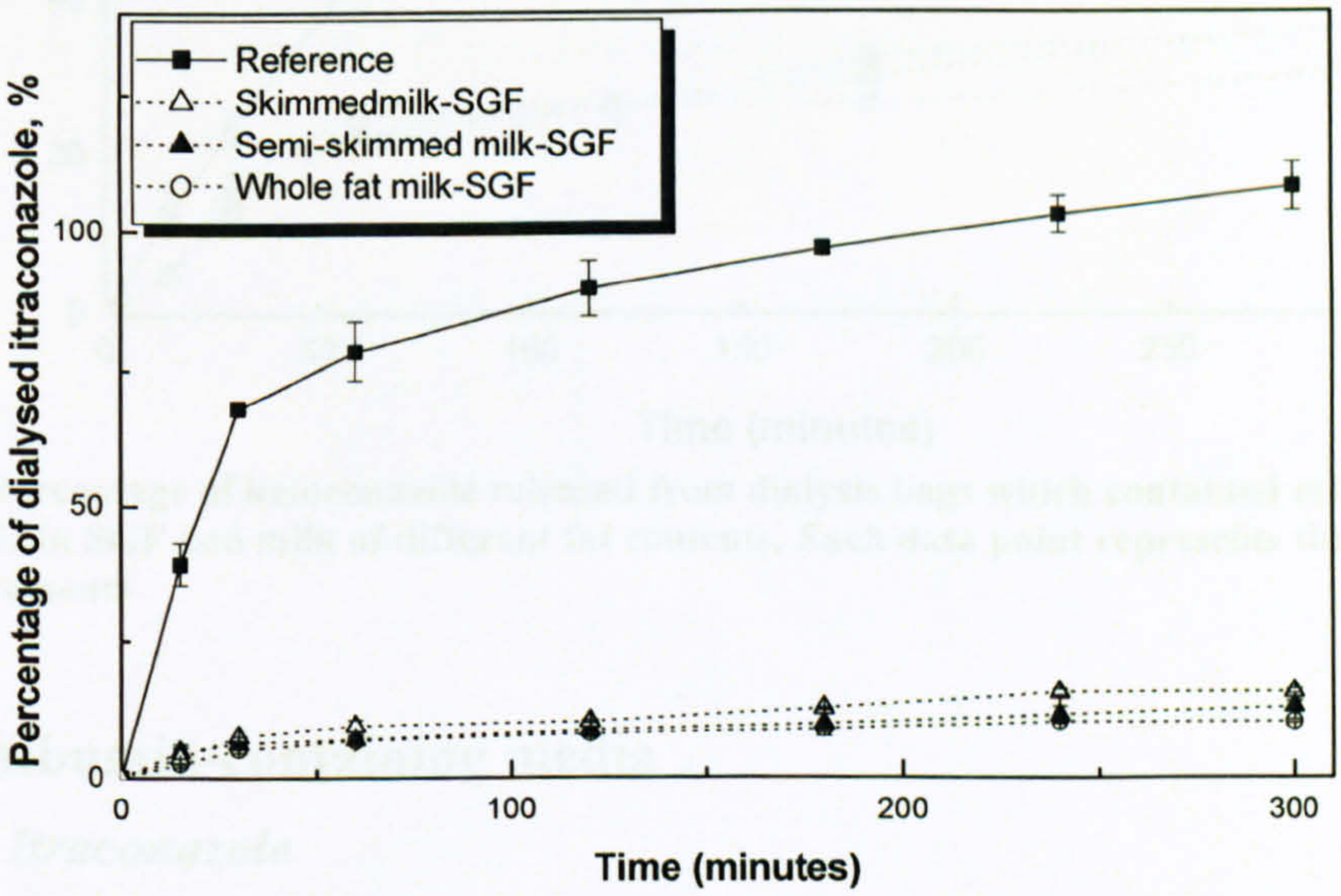


Figure 5.2 Percentage of itraconazole released from dialysis bags which contained mixtures of itraconazole in SGF and milk of different fat contents. Each data point represents the mean ± S.D. of 3 measurements

No obvious difference between the release profiles of itraconazole from the dialysis bags containing whole fat milk, semi-skimmed milk and skimmed milk were observed throughout the first 4h of the dialysis experiment. However, within 24h, the skimmed milk-containing media showed a higher release of itraconazole (22.4 ± 1.3 %) and the minimum release (12.9 ± 0.9 %) was observed in the whole fat milk-containing medium.

5.3.1.1.2 Ketoconazole

Ketoconazole dialysis in milk-containing media against SGF was carried out for 5h (Figure 5.3). The concentration of the released drug from the dialysis bag containing milk into the bulk media was less than that when the dialysis bag contained the reference solution. At the end of the run, 31.2 ± 0.3 %, 36.6 ± 1.4 % and 37.2 ± 0.3 % of the drug was released from the dialysis bags that contained mixtures of SGF and milk: whole fat milk, semi-skimmed milk and skimmed milk, respectively.

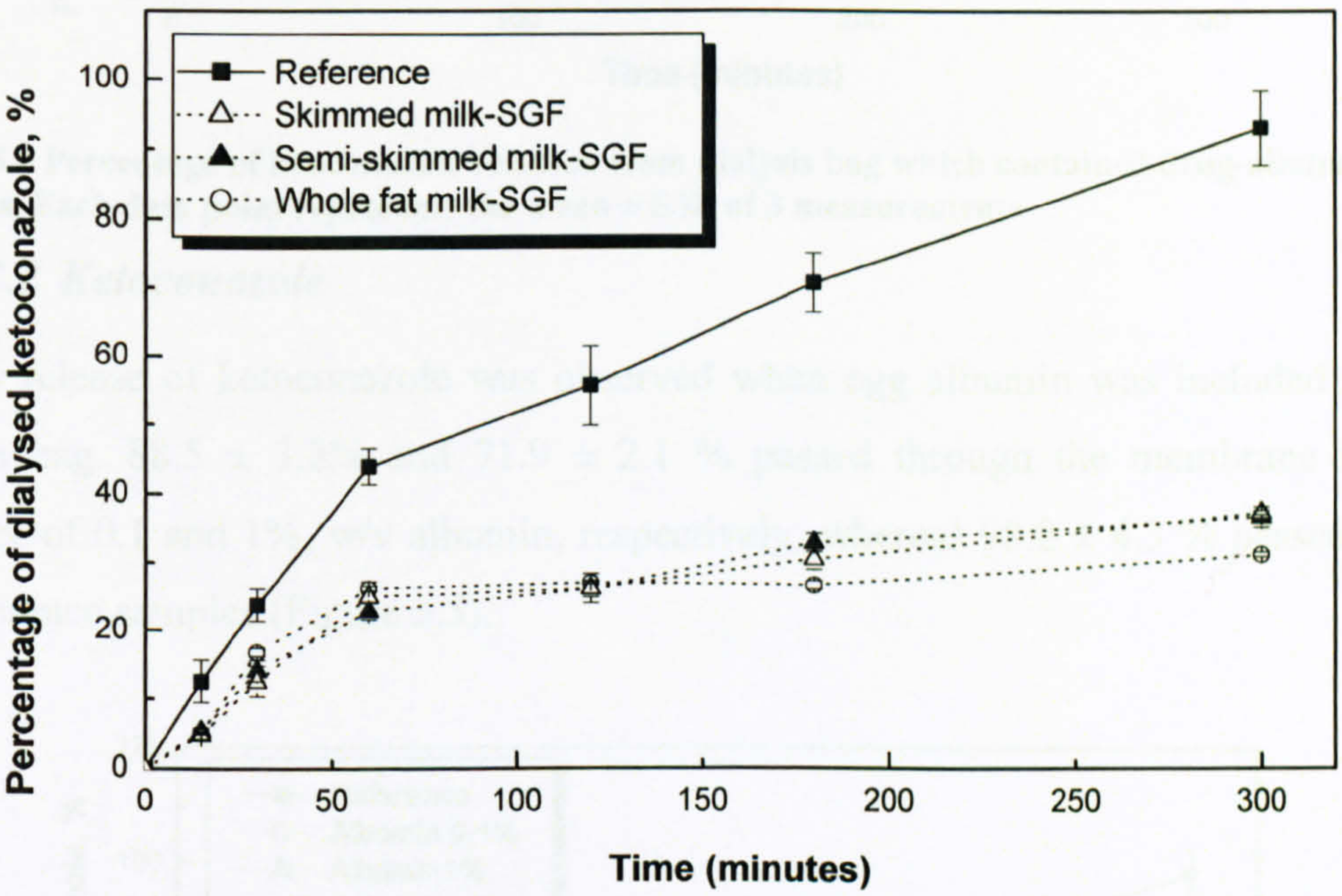


Figure 5.3 Percentage of ketoconazole released from dialysis bags which contained mixtures of ketoconazole in SGF and milk of different fat contents. Each data point represents the mean ± S.D. of 2 measurements

5.3.1.2 Albumin-containing media

5.3.1.2.1 Itraconazole

When albumin was included in the dialysis bag, a slow release for itraconazole was apparent and a higher concentration of albumin led to even slower release. The percentage of itraconazole that passed across the membrane over 5h was 65.1 ± 3.4 % with albumin 1%, w/v whereas with the reference solution an average of 100.7 ± 2.9 % was released (Figure 5.4).

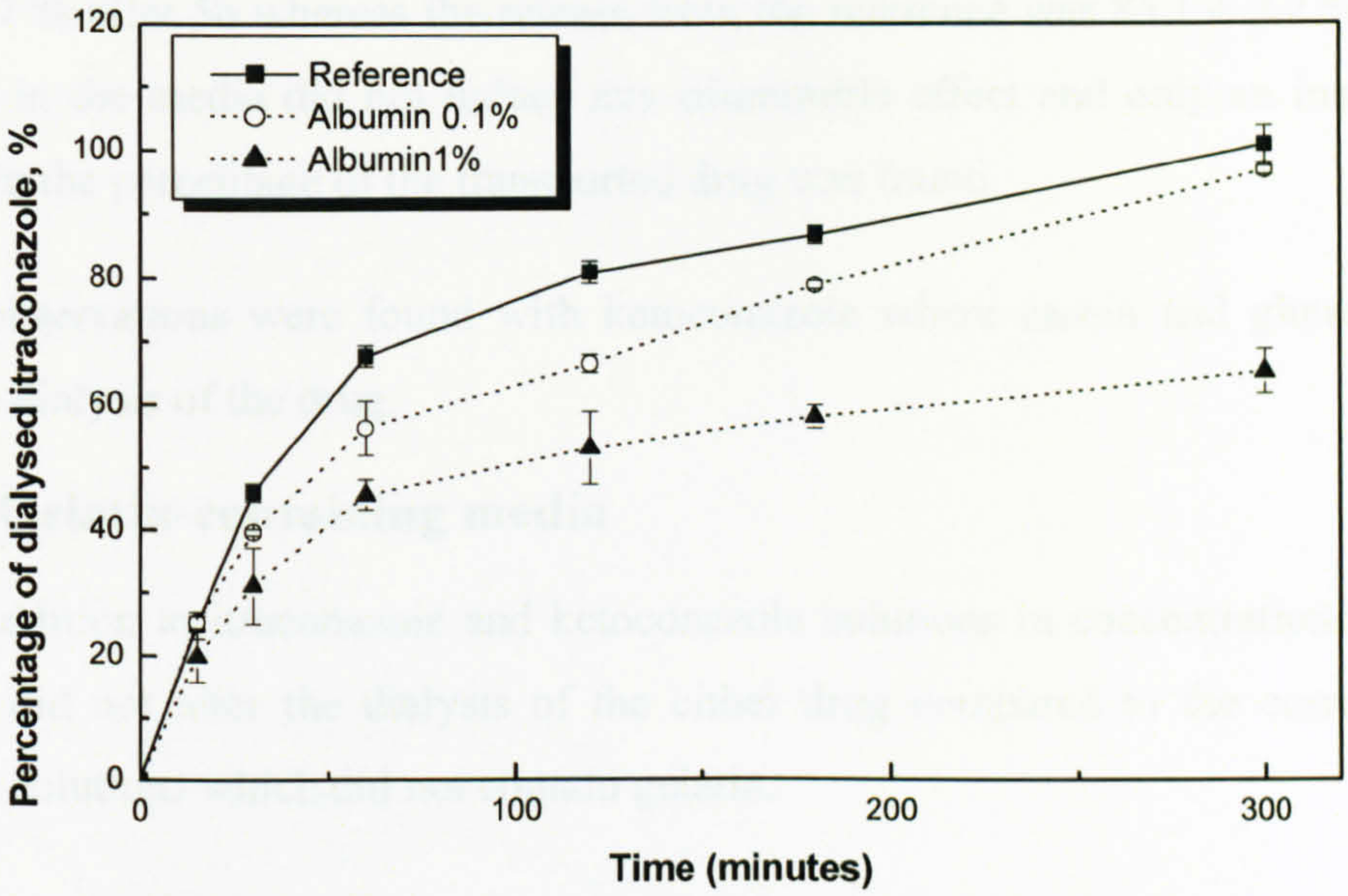


Figure 5.4 Percentage of itraconazole released from dialysis bag which contained drug-albumin mixtures. Each data point represents the mean ± S.D. of 3 measurements

5.3.1.2.2 Ketoconazole

A slow release of ketoconazole was observed when egg albumin was included in the dialysis bag. 88.5 ± 3.2% and 71.9 ± 2.1 % passed through the membrane in the presence of 0.1 and 1%, w/v albumin, respectively, whereas 98.2 ± 4.3 % passed from the reference samples (Figure 5.5).

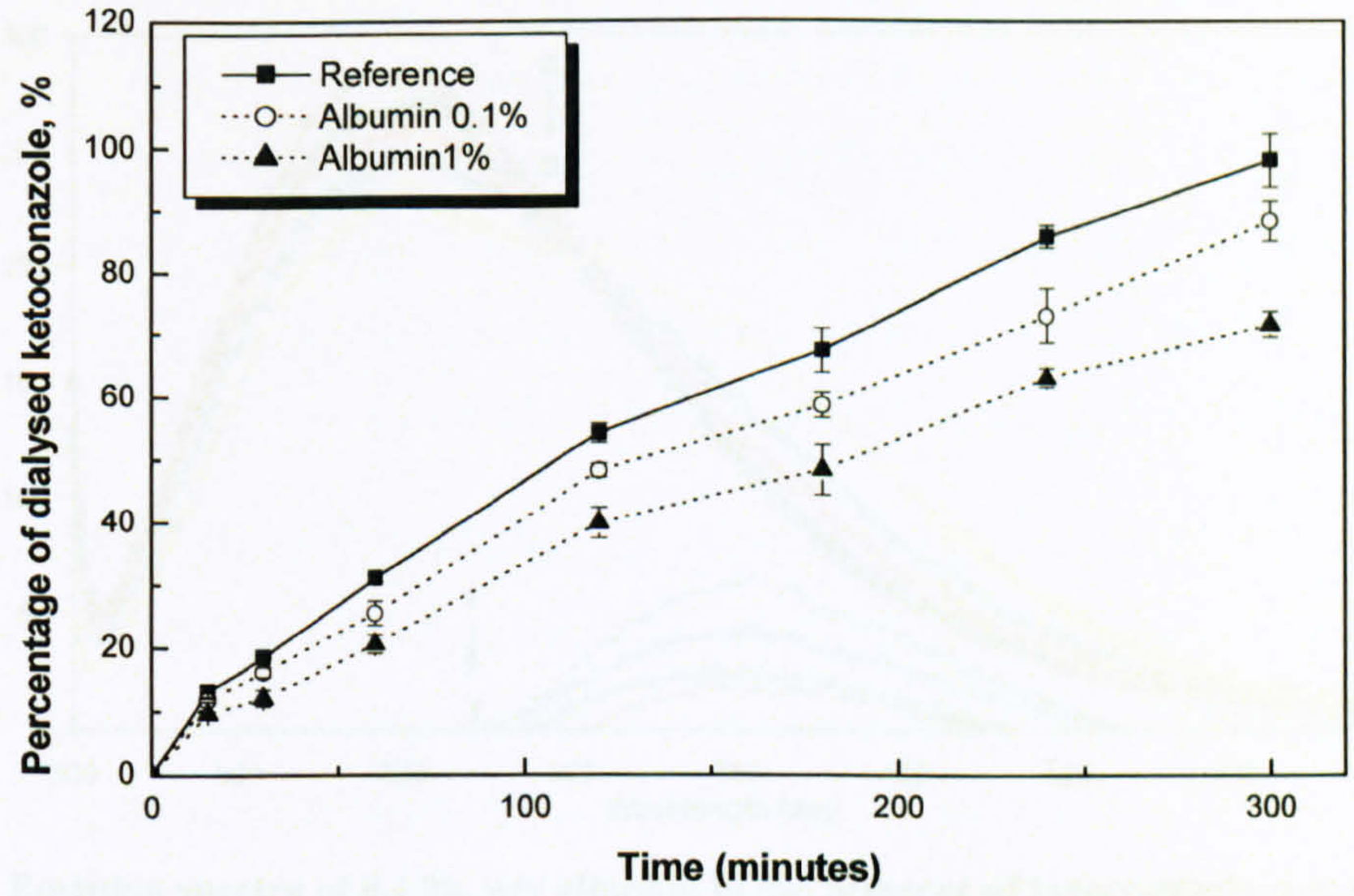


Figure 5.5 Percentage of ketoconazole released from dialysis bags which contained drug-albumin mixtures. Each data point represents the mean ± S.D. of 2 measurements

5.3.1.3 Casein-and gluten-containing media

Casein inclusion with itraconazole solutions inside the dialysis bag did not lead to a significant change (t-test, significance level 0.05) in drug release, which was

87.0 ± 3.7 % after 5h whereas the release from the reference was 85.1 ± 2.2 %. Gluten inclusion in the media did not induce any discernible effect and only an insignificant increase in the percentage of the transported drug was found.

Similar observations were found with ketoconazole where casein and gluten did not affect the dialysis of the drug.

5.3.1.4 Gelatin-containing media

Gelatin addition to itraconazole and ketoconazole solutions in concentrations of 1 and 2%, w/v did not alter the dialysis of the either drug compared to the corresponding reference solutions which did not contain gelatin.

5.3.2 Spectroscopic studies of albumin- ketoconazole mixtures

5.3.2.1 Fluorescence studies

The fluorescence spectra of albumin in the presence of different concentrations of ketoconazole are displayed in Figure 5.6. Egg albumin had a strong fluorescence emission with a peak at 340nm upon excitation at 296nm. The fluorescence intensity of albumin decreased slightly in the presence of increasing amounts of ketoconazole without changing the emission maximum wavelength.

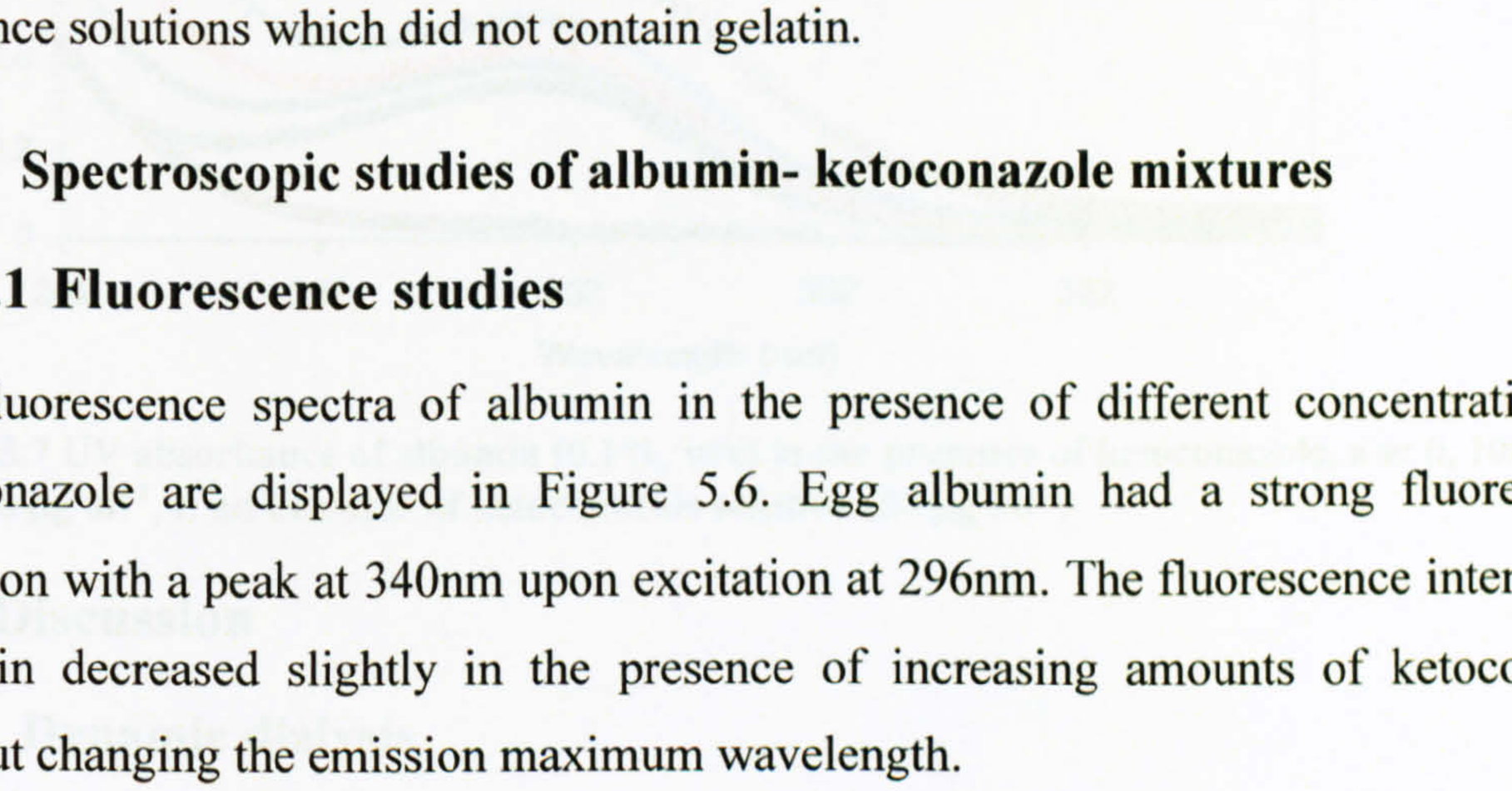


Figure 5.6 Emission spectra of 0.1 %, w/v albumin in the presence of ketoconazole, a-e: 0, 10, 50, 100, 200 μg ml^{-1}, f-i: emission spectra of ketoconazole 10, 50, 100, 200 μg ml^{-1}, emission at 340nm and excitation at 296nm

5.3.2.2 UV spectroscopy

The UV absorbance intensity of the albumin increased in the range between 240nm and 340nm with the increasing ketoconazole concentrations (Figure 5.7). Furthermore, a

very slight red shift (towards longer wavelength) of the maximum peak position was noticed indicating possible interactions between ketoconazole and egg albumin.

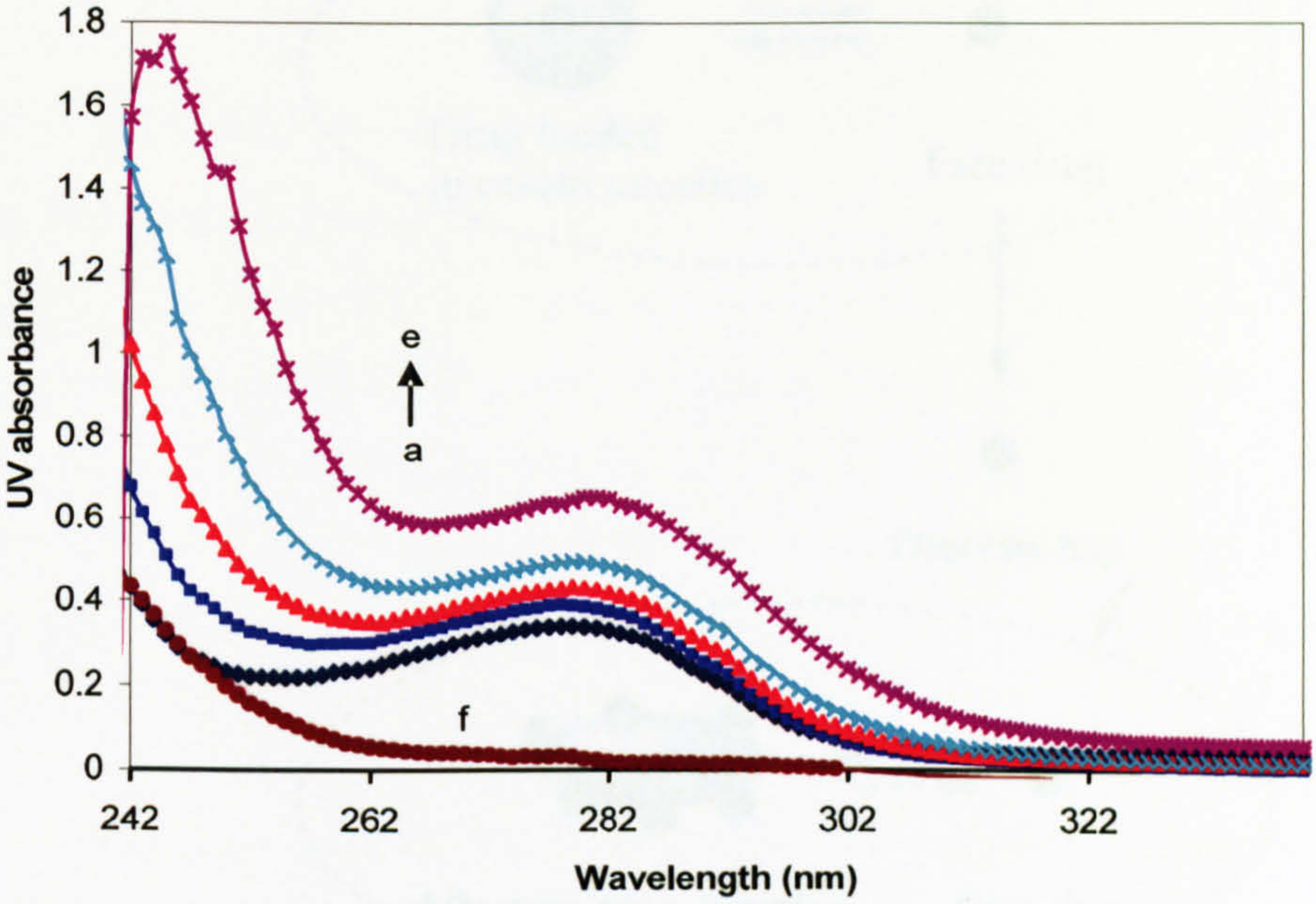


Figure 5.7 UV absorbance of albumin (0.1%, w/v) in the presence of ketoconazole, a-e: 0, 10, 50, 100, 200 μg ml^{-1}, f: an example of ketoconazole solution (50 μg ml^{-1})

5.4 Discussion

5.4.1 Dynamic dialysis

In the present investigation, preliminary studies were performed for the purpose of monitoring possible changes in drug transport due to the presence of additives compared to a reference. When milk was included in the media a slower rate of drug release from the dialysis bags was observed, compared to a reference that did not contain milk. Thus, this indicated that drug entities may be entrapped within milk particles due to binding to milk components. The fat content of the milk did not affect drug binding throughout 5h.

The dialysis data indicated that casein did not bind to the drugs. However, this did not exclude the possibility of protein binding. Macheras and Reppas (1987) stated that during the dialysis studies when casein was included in the media no transport change was noticed. This was attributed to the ability of casein to form micelles and so it acted as a reservoir for the drug. Hence, the micelles were delivering the free drug to be transported which kept the dialysis rate unaltered (Figure 5.8). Furthermore, casein micelles or sub-units had porous structures which allowed the release of the entrapped drug molecules.

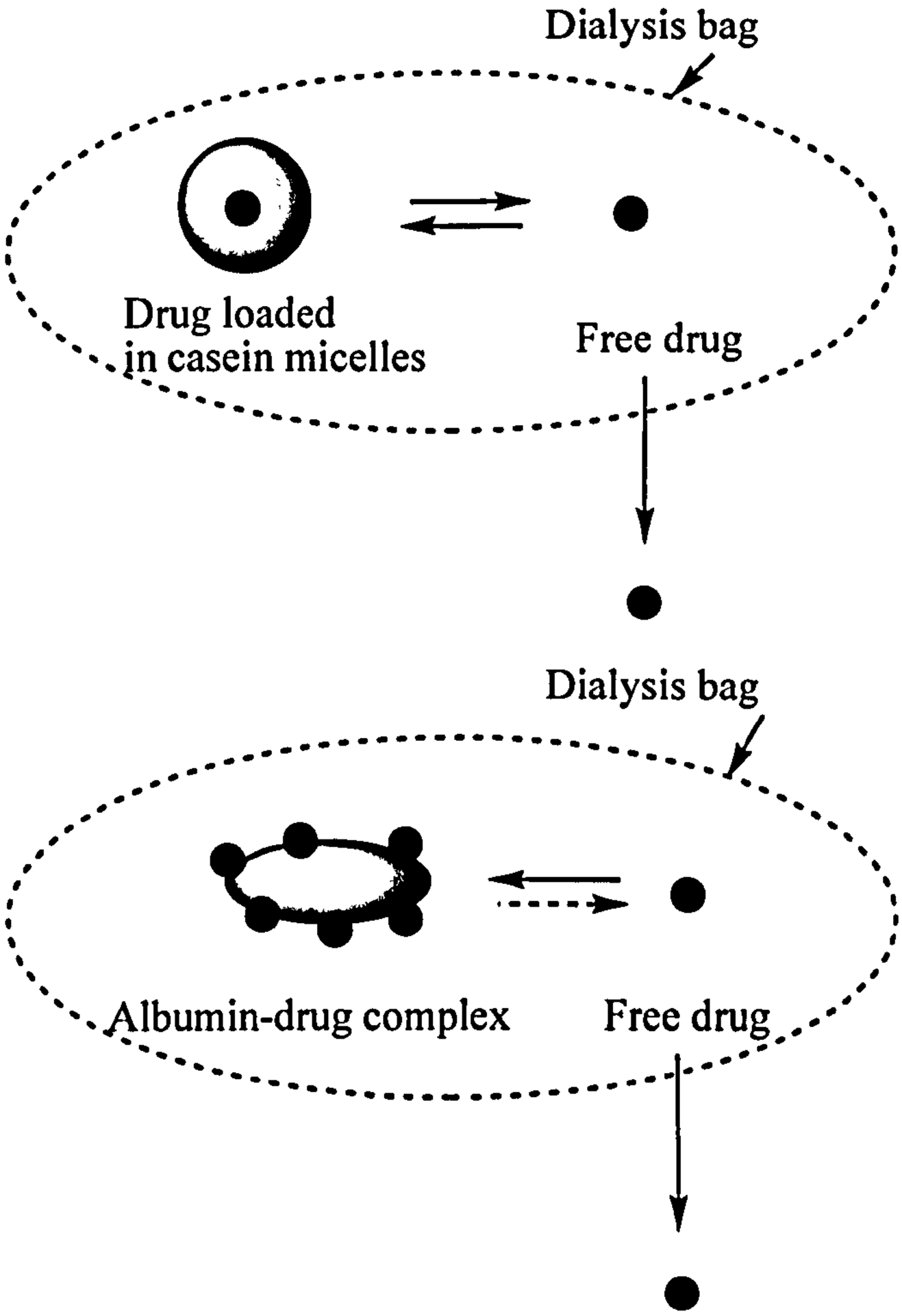


Figure 5.8 Schematic diagram for the transport of drug from the dialysis bag containing albumin- or casein-drug mixtures to the bulk solution, modified from Macheras and Reppas (1987)

The solubility and dissolution of itraconazole and ketoconazole increased in the presence of milk in the media (Section 4.3.3.1) and the dialysis results indicated there was drug binding to the milk. Since casein is the major protein in milk and it did not show any evidence of the ability to bind the drugs, the solubilization of itraconazole and ketoconazole in milk was not due to specific protein binding but to the entire complex structure of milk. This included binding to milk components, casein micelles, ionic interactions with the amino acids and partitioning into the lipid contents. This agrees with the finding of Macheras et al. (1989) who also attributed drug solubilisation to the whole structure of milk.

The presence of egg albumin in the dialysis bags also slowed the release of itraconazole and ketoconazole, which suggests that albumin had the ability to form complexes with the drugs (Figure 5.8). The binding is possibly through interaction on the molecular level such as hydrogen bonding and hydrophobic interaction or physical entrapment through van der Waals forces. Thus, protein binding and complex formation appears responsible for drug solubilisation effect (Section 4.3.3.2).

The presence of gluten and gelatin in the media did not affect the dialysis process of itraconazole and ketoconazole which indicated poor binding of the drugs to these proteins.

5.4.2 Spectroscopic studies of albumin- ketoconazole mixtures

The fluorescence intensity of a compound can be decreased by molecular interactions and conformational changes. Such changes may occur upon complex formation because the intramolecular forces involved in keeping the secondary structure are altered. This decrease in fluorescence intensity is called quenching. Tryptophan residues are mainly responsible for the fluorescence of albumin and this fluorescence is very sensitive to changes such as ligand binding leading to a change in the emission intensity (Möller and Denicola, 2002).

It was reported that human and bovine serum albumin had the ability to form complexes with ketoconazole and such interaction led to fluorescence quenching of albumin (Guo et al., 2008). UV absorption measurements also allowed the investigation of structural changes and complex formation between ketoconazole and serum albumin (Guo et al., 2008). Consequently, the current findings of the fluorescence quenching and slight shift in the UV spectra indicated complex formation between ketoconazole and egg albumin.

5.5 Conclusion

The solubilising effect of itraconazole and ketoconazole by proteins and milk was attributed to different types of interactions. These *in vitro* findings can provide a useful insight into the effect of food proteins on the bioavailability of itraconazole and ketoconazole. Thus, it was found that food components increased the dissolution of the investigated drugs through various mechanisms such as drug binding, solubilisation into sub-micelles units or wetting enhancement. However, further studies need to be carried out to further explain these interactions on the molecular level using techniques such as Circular Dichroism and to monitor heat changes that accompany the interactions using microcalorimetry method. In addition, food ingestion can also induce important physiological changes that may affect drug dissolution such as change in the pH of the intraluminal fluids and stimulation of bile and lipid secretions. Accordingly, the impact of the bile salts and phospholipids on the dissolution of both drugs was investigated.

Chapter 6: The effect of synthetic and natural surfactants on the dissolution of itraconazole and ketoconazole

6.1 Introduction

In the gastrointestinal fluid, various surfactants are present such as bile salts, lecithin, cholesterol and its esters, so luminal fluids have a low surface tension that varies according to the prandial state (Kalantzi et al., 2006a) (Section 1.6.2.1). Despite this, the compendial dissolution media used for dissolution testing (SGF, SIF and water) do not contain surfactants and therefore fail to estimate the possibility of solubilisation and the wetting effects of the luminal fluids. Simulated gastric and intestinal media containing relevant surfactants have been suggested. For example, Galia et al. (1999) developed media containing sodium taurocholate and phospholipids in relevant concentrations to mimic fasting and fed conditions.

In the current investigation, the aim was to explore itraconazole and ketoconazole dissolution in the presence of natural surfactants which can allow prediction of the drug behaviour *in vivo*. Furthermore, the effect of natural surfactants on the dissolution of both drugs was compared with the effect of synthetic surfactants.

The impact of the following media on the dissolution and solubility of itraconazole and ketoconazole was assessed:

1) Simulated gastric fluids containing either synthetic or natural surfactants.
2) Simulated intestinal media containing only a single type of bile salt, sodium taurocholate.
3) Biorelevant media simulating intestinal fluids in fed and fasting states containing bile salts and phospholipids.
4) Simplified simulated intestinal media containing sodium dodecyl sulfate (SDS) as an anionic surfactant and cetyltrimethylammonium bromide (CTAB) as a cationic surfactant.
5) An oil in water (o/w) emulsion, composed of Soybean oil emulsified in SGF (pH 3) using Tween 20.

6.2 Materials and methods

6.2.1 Materials

Details of all materials used in the preparation of the dissolution media and in performing the experiments are listed in 2.1.

6.2.2 Media preparation

All media were prepared on the day of the dissolution experiment and the pH was measured using a calibrated pH meter as described in Section 2.2.1.

6.2.2.1 Simulated fasting gastric media

Fasted state simulated gastric fluid (FaSSGF) consisted of SGF pH 1.2 (0.1M HCl and 34.2mM NaCl) containing 80μM NaTC and 20μM lecithin.

Simulated gastric fluids containing synthetic surfactants were prepared by the addition of appropriate amount of surfactants to SGF pH 1.2. The assessed media were: SGF containing sodium dodecyl sulfate (SDS) (0.25%, w/v), Triton X-100 (0.1%, w/v) or Tween 20 (0.02%, w/v). The final pH was adjusted to 1.2 when required.

6.2.2.2 Simulated intestinal media

The compositions of the biorelevant media; fasted state simulated intestinal fluid (FaSSIF) and fed state simulated intestinal fluid (FeSSIF) are listed in Table 6.1. The two media were prepared according to Marques (2004). Blank simulated fluids refer to buffer solutions without the addition of NaTC or lecithin.

Table 6.1 Composition of FaSSIF and FeSSIF as listed by Vertzoni et al. (2004)

Components	FaSSIF	FeSSIF
Sodium taurocholate	3 mM	15 mM
Lecithin	0.75mM	3.75 mM
NaH_2PO_4	28.66mM	-
NaCl	106mM	173mM
Acetic acid	-	144mM
NaOH pellets	13.8mM	101mM
Deionised water	to 1L	to 1L

6.2.2.2.1 Fasted state simulated intestinal fluid

Blank FaSSIF was prepared by dissolving 0.55g of NaOH, 3.44g of NaH_2PO_4 and 6.19g of NaCl in 1L of deionised water. The pH was adjusted to 6.5 using 1M NaOH or 1M HCl.

FaSSIF was prepared as follows: 1.65g of sodium taurocholate (NaTC) was dissolved in approximately 250ml of the blank FaSSIF. 0.57g of lecithin was dissolved in 5.7ml of dichloromethane and added to the NaTC solution. Dichloromethane was evaporated under vacuum using a Rotavap (Heidolph, Germany) at a temperature of 40°C for about 30min until no noticeable odor of dichloromethane was noticed indicating complete removal of the organic solvent. Finally, the volume was made up to 1L with blank FaSSIF.

6.2.2.2.2 Fed state simulated intestinal fluid

Blank FeSSIF was prepared by dissolving 4.04g of NaOH, 8.64g of glacial acetic acid, and 10.11g of NaCl in 1L of purified water. The pH was adjusted to 5 using 1M NaOH or 1M HCl.

FeSSIF was prepared by dissolving 8.06g NaTC in 1L of blank FeSSIF. Then, 2.87g lecithin was dissolved in 28.7ml of dichloromethane and added to the NaTC solution, resulting in an emulsion. Likewise to FaSSIF, the solvent was evaporated and the resulting emulsion was slightly hazy with no noticeable smell of dichloromethane. The volume was made up to 1L with blank FeSSIF.

6.2.2.3 Simulated intestinal media containing sodium taurocholate only

Media containing NaTC with various concentrations of NaTC were prepared by dissolving 1.1, 4.0, 8.1 and 16.1g of NaTC in 1L of blank FeSSIF to prepare solutions of 2, 7.5, 15 and 30mM, respectively. The final pH of each media was adjusted to 5 when required.

6.2.2.4 Simplified intestinal media

Simplified simulated intestinal media containing two types of surfactants: anionic surfactants (SDS) and cationic surfactants (CTAB) in various concentrations were developed. The media were prepared by dissolving the required amounts of the surfactant in blank FeSSIF (pH 5) or simulated intestinal fluid (SIF) (pH 6.8). The final

pH was adjusted to 5 for fed-simulating intestinal media and to 6.8 for fasted-simulating intestinal media.

6.2.2.5 Emulsion of Soybean oil in simulated gastric fluid

An o/w emulsion was composed of 10%, w/v Soybean oil in SGF (pH 3) emulsified using Tween 20 (Hsu and Nacu, 2003). The emulsion was prepared by mixing 15%, w/w Tween 20 with Soybean oil and the resultant mixture was preheated to 60°C. Subsequently, the oil phase was introduced gradually into SGF medium, which was previously brought to 60°C. Further homogenization was performed with a mixer (Silverson Model L4 RT, England) at 3500rev min^{-1} for 5min. To assess the effect of the fat content on drug dissolution, the resultant emulsion was diluted with SGF to prepare two solutions containing 20% or 50% of the original concentration.

6.2.3 Media characterisation

Viscosities of the media were measured using a vibrational rheometer according to the method described in Section 2.5.1.2.

The surface tension of the dissolution media was measured using a Wilhelmy plate tensiometer (Section 2.5.2).

The critical micelle concentration (CMC) of SIF containing SDS or CTAB was determined by measuring the surface tension profile of solutions with various concentrations of the surfactants. The surfactant-containing solutions exhibited changes in surface tension with time and therefore, the measurements were taken after 30min. The surface tension values were plotted versus log concentration and the intersection of the two linear portions of the surface tension indicated the value of the CMC.

6.2.4 Contact angle measurements

The contact angles between the media and itraconazole and ketoconazole surfaces were evaluated using the Sessile drop method (Section 2.5.3). A drop of the fluid (4µl) was placed on the surface of compacts of the drugs and images were taken over a period of 5min.

6.2.5 Solubility determination

The solubility of the drugs in the media was determined according to the method described in Section 2.2.2. An excess amount of the drug was added to the media and

the suspensions were shaken for 24h at 37°C. The solid was separated by centrifugation followed by filtration.

6.2.6 Intrinsic dissolution studies

The dissolution of the drugs in the media was assessed using stationary disk methodology. Itraconazole and ketoconazole were compressed to prepare disks with constant surface area. The die containing drug compact was placed at the bottom of flat vessel in USP Apparatus II. Stirring was applied at 37°C and aliquots were withdrawn from the bulk solution periodically and analysed. Details of disk preparation and dissolution conditions are given in Sections 2.2.3 and 2.2.4.

6.2.7 Sample treatment and drug analysis

6.2.7.1 Itraconazole

Samples collected from simulated gastric and intestinal media were diluted (50:50) with the mobile phase, whereas no dilution was required for samples collected from blank simulated media. Then, samples were submitted to HPLC analysis.

Quantitation of itraconazole in samples collected from dissolution and solubility studies was performed using HPLC with fluorescence detection (Section 2.2.6.1).

For samples collected from dissolution media containing Soybean oil emulsion, itraconazole was first extracted using solvent extraction according to the method in Section 2.2.5.1. The drug was then assayed using HPLC.

6.2.7.2 Ketoconazole

Samples collected from solubility tests in simulated gastric media were diluted as appropriate (10 to 16-fold) with a mixture of acetonitrile-water (50:50). For experiments in simulated intestinal fluids, samples were diluted twice. No dilution was required for samples collected from the solubility test in blank simulated intestinal fluids.

Samples collected from acidic media were analyzed by HPLC using UV detection whereas samples collected from simulated intestinal fluids were analyzed using HPLC with fluorescence detection (Section 2.2.6.2).

Aliquots withdrawn from the dissolution media containing an emulsion were extracted according to the general method specified in Section 2.2.5.2. Ketoconazole was deprotonated using 1ml of 1M NaOH and then extracted with a mixture of acetonitrile–

n-butyl chloride (1:4, v/v). The residues were reconstituted and subsequently analysed with HPLC with UV detection.

6.3 Results

6.3.1 Media Characterisation

6.3.1.1 Viscosity

Slight differences were observed in the viscosities of the simulated gastric fluids (Table 6.2). However, by applying ANOVA test (0.05) it was found that these variations were not significant. This indicated that the addition of the surfactants in the stated amounts did not lead to an increase in the viscosity of the media.

The viscosities of media containing bile salts were slightly higher than the corresponding blank buffer. The increase in NaTC concentration led to an increase in viscosity. The addition of lecithin to FaSSIF and FeSSIF induced further increases in viscosity.

Table 6.2 Viscosity of gastric and intestinal simulated media at 37°C. Each data point represents the mean ± S.D. of 3 measurements

Medium	Viscosity (mpa.s)
SGF pH 1.2	0.719 ± 0.009
FaSSGF pH 1.2	0.719 ± 0.008
0.25%, w/v SDS in SGF pH 1.2	0.721 ± 0.009
0.1%, w/v Triton-X in SGF pH 1.2	0.724 ± 0.006
0.02%, w/v Tween 20 in SGF pH 1.2	0.717 ± 0.007
Blank FaSSIF	0.713 ± 0.004
Blank FeSSIF	0.716 ± 0.005
FaSSIF	0.734 ± 0.009
FeSSIF	0.754 ± 0.009
2mM NaTC in blank FeSSIF	0.715 ± 0.005
7.5mM NaTC in blank FeSSIF	0.733 ± 0.003
15mM NaTC in blank FeSSIF	0.746 ± 0.005
30mM NaTC in blank FeSSIF	0.753 ± 0.009

6.3.1.2 Surface tension

The surface tensions of the investigated simulated media and their respective blank solutions are displayed in Figure 6.1. The inclusion of synthetic surfactants to SGF

induced a marked decrease in the surface tension, even when the surfactant was added in a small amount, such as 0.02%, w/v Tween 20. The maximum decrease was observed in media containing Triton X-100 and SDS where the measured values were 31-32mN m^{-1}. The surface tension of FaSSGF was higher than the other gastric simulated media.

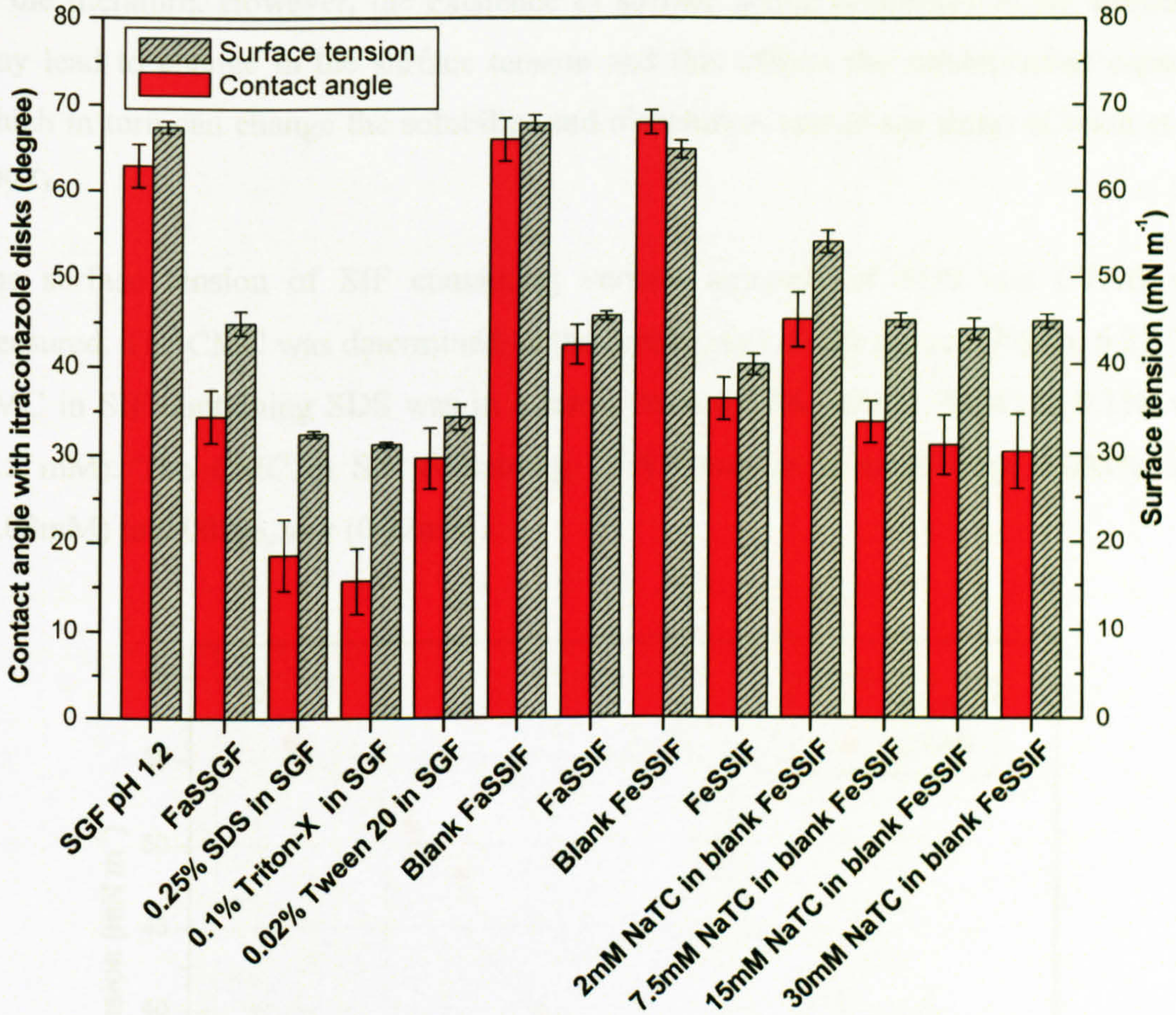


Figure 6.1 Surface tension and contact angles for simulated gastric and intestinal media with itraconazole disks. Each data point represents the mean ± S.D. of 2 measurements

FaSSIF and FeSSIF had surface tensions substantially lower than the corresponding blanks. A 2mM NaTC solution lowered the surface tension to 54.1mN m^{-1} and inclusion of 7.5mM NaTC induced a further decrease. However, with higher concentrations of NaTC the surface tension did not drop further. This indicated that the aggregation of NaTC to form micelles occurred at some point between 2 and 7.5mM. The literature value for CMC of NaTC in solution at 37°C with 0.1-0.15M ionic strength was reported as 3-4mM which indicated compliance with the present findings (Carey and Small, 1972; Mithani et al., 1996).

The inclusion of lecithin led to a further increase in the surface activity and by comparing the surface tension of medium containing 15mM NaTC only with that of FeSSIF, it was found that the latter had a lower surface tension by 5mN m^{-1}.

It is important to note, that the surface tensions of many of these assessed media appear in the literature. However, the existence of surface active impurities in the surfactant may lead to change in the surface tension and this affects the solubilisation capacity which in turn can change the solubility and dissolution rate of the drugs (Crison et al., 1997).

The surface tension of SIF containing various amounts of SDS and CTAB was measured. The CMC was determined as the break point on the curves (Figure 6.2). The CMC in SIF containing SDS was in a range from 0.05%, w/v (1.7mM) to 0.1%, w/v (3.5 mM). The CMC in SIF containing CTAB was in a range from 0.003%, w/v (0.08mM) to 0.008%, w/v (0.22mM).

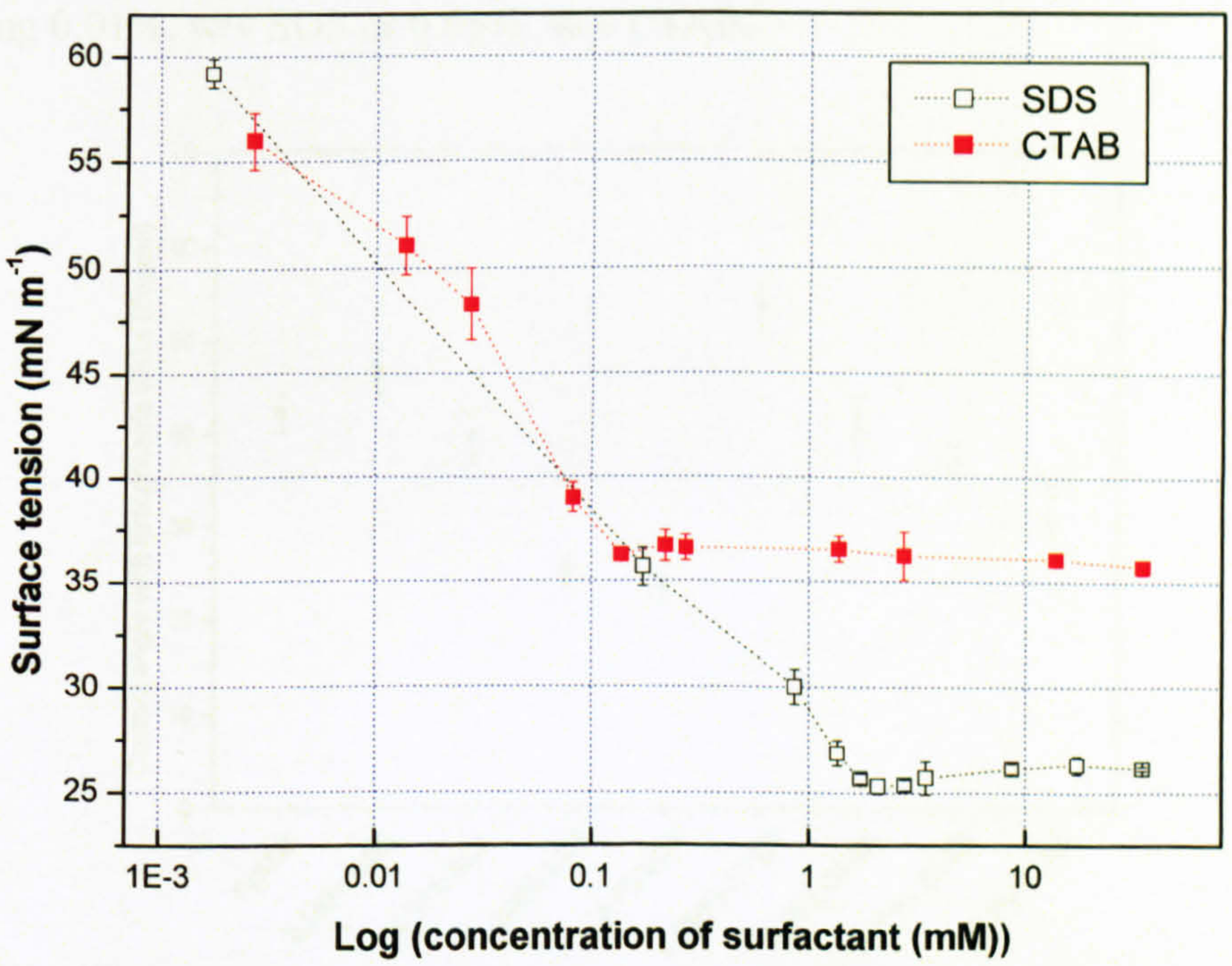


Figure 6.2 Surface tension versus log surfactant concentration (SDS, CTAB) at 37°C in simulated intestinal fluid (SIF). Each data point represents the mean ± S.D. of 2 measurements

6.3.2 Contact angle measurements

6.3.2.1 Itraconazole

The contact angles measured on itraconazole disks at 300s are presented in Figure 6.1. The smallest contact angle was found with SGF containing the synthetic surfactants,

SDS and Triton X-100. This reflected the surface tension values as both media showed the maximum surface activity. Less wettability was observed by FaSSGF. No significant difference was found between the values for FaSSGF and medium containing Tween 20.

Better wettability was observed with FaSSIF and FeSSIF compared to their corresponding blank solutions. Media containing only NaTC induced a decline in the contact angle, however, no significant variations were found among the contact angles of 7.5, 15 and 30mM NaTC-containing media suggesting that a plateau was reached. This confirmed that both the interface liquid/air and the bulk liquid were saturated with NaTC monomers which led to the formation of micelles. Consequently, no further enhancement in the wettability occurred with the higher concentrations of NaTC.

The contact angle between the itraconazole surface and simulated media containing SDS or CTAB decreased with an increase in the amount of the surfactant (Figure 6.3). No significant difference in the contact angle was observed between FaSSIF and SIF containing 0.01%, w/v SDS or 0.05%, w/v CTAB.

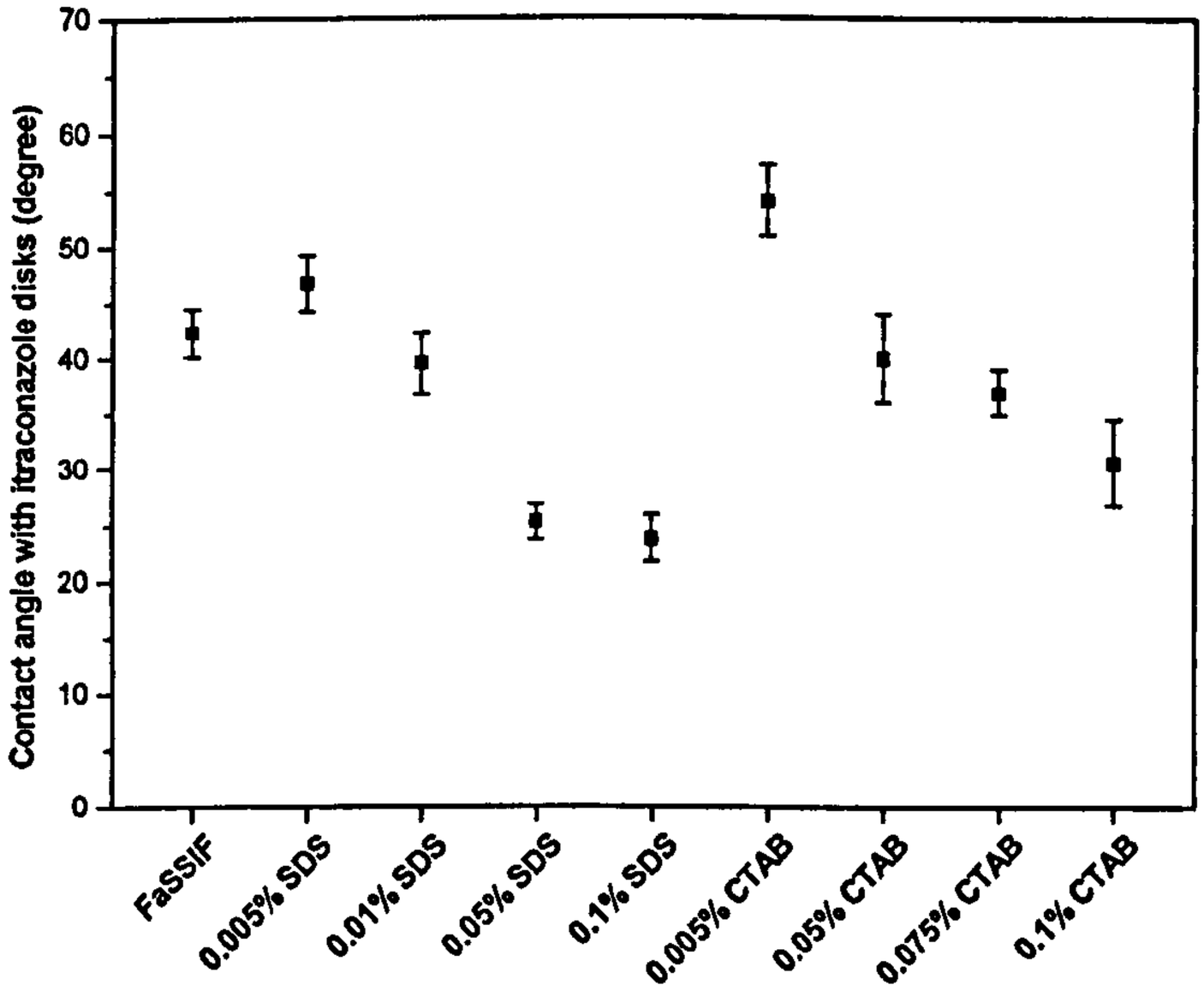


Figure 6.3 Contact angles for simplified simulated intestinal media containing various amounts of surfactants: SDS (n=2) or CTAB (n=3) with itraconazole disks. Each data point represents the mean ± S.D. of 2 measurements at least

6.3.2.2 Ketoconazole

Contact angles could not be determined on ketoconazole disks using simulated gastric media due to the fast wetting of the drug surface. The contact angles measured at time 0 were in the range of 10-20° and subsequently, within a few seconds, the angle nearly declined to zero which indicated full wetting of the drug surface.

The contact angles formed between ketoconazole disks and simulated intestinal fluids were measured at 150s of placing the liquid drops on the drug compact surface (Figure 6.4). The standard deviations for the measurements on the ketoconazole surface were high. FaSSIF and FaSSIF enhanced the wettability of ketoconazole compared to their corresponding blank fluids. A decline in the contact angle was also observed with the inclusion of 2mM NaTC and a further drop was observed with an increase in NaTC concentration indicating that wetting continued to increase beyond the CMC. No significant difference was found between the wettability of solutions containing 15mM and 30mM NaTC.

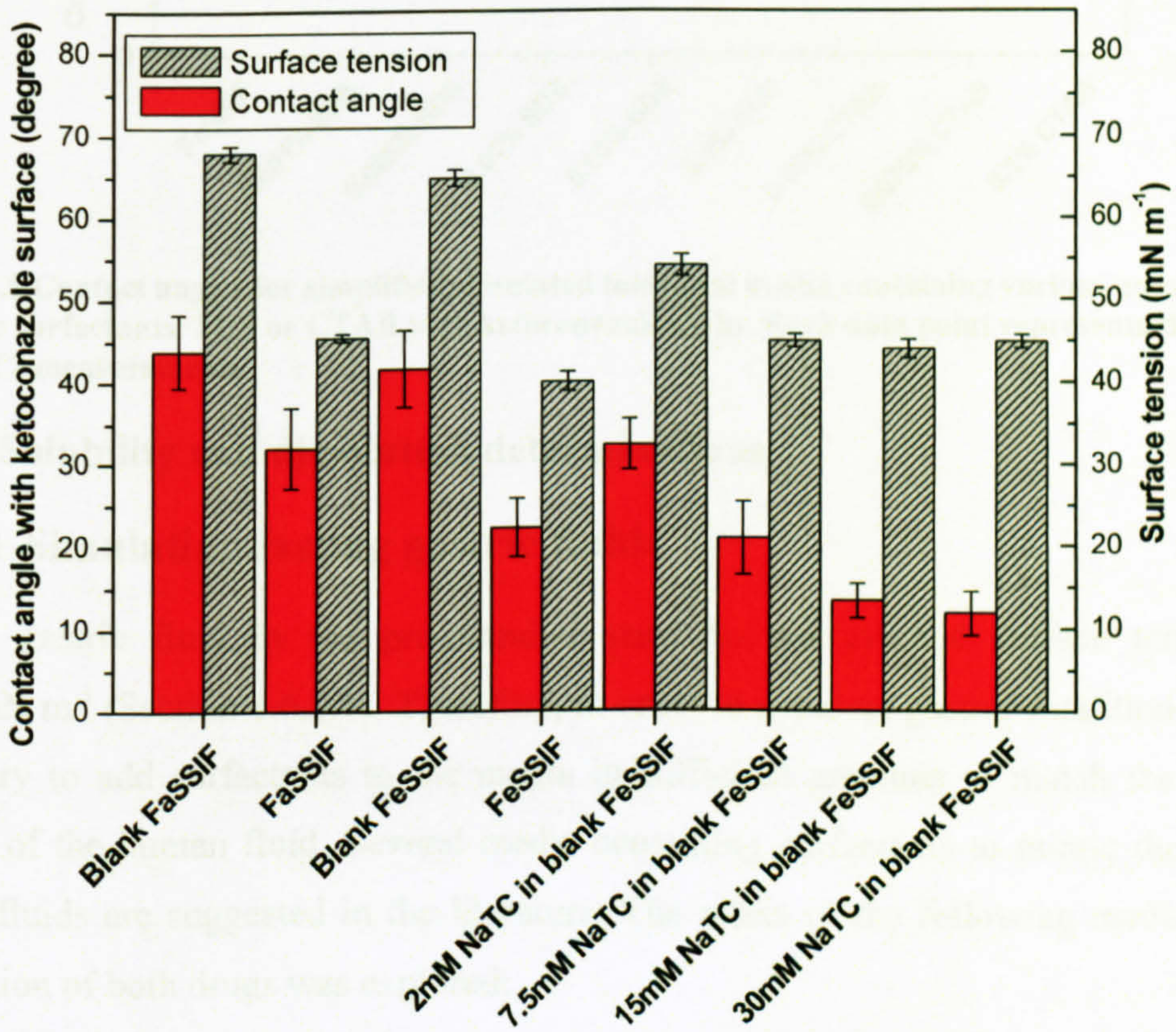


Figure 6.4 Contact angles for intestinal simulated media with ketoconazole disks. Each data point represents the mean ± S.D. of 2 measurements

Simplified media containing SDS or CTAB induced a decrease in the contact angles which was proportional to the increase in the surfactant concentration despite exceeding the relevant CMC value (Figure 6.5). Wettability with FaSSIF did not vary significantly from that with SIF containing 0.05-0.1%, w/v SDS, whereas solutions containing CTAB induced less wettability compared to FaSSIF.

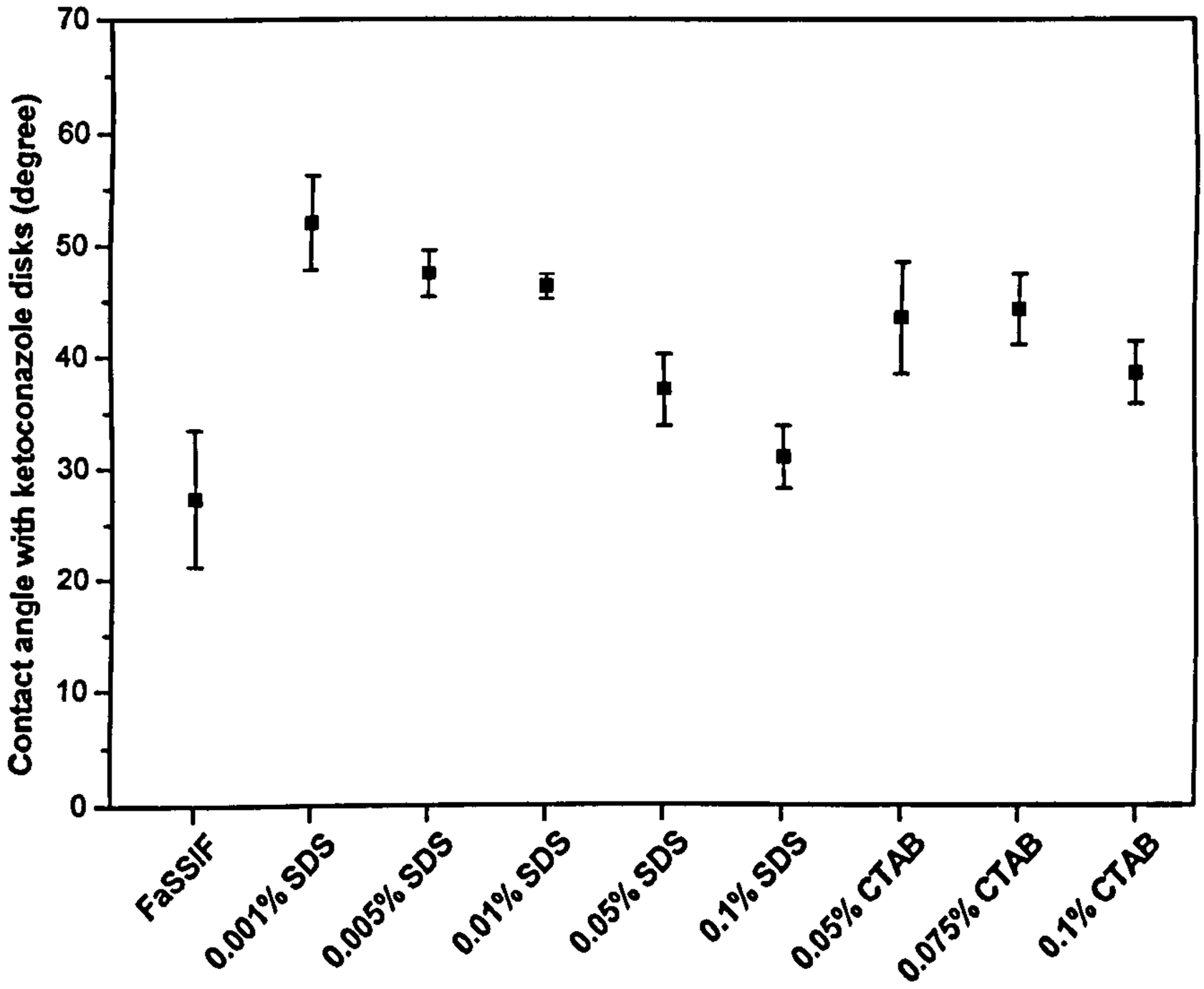


Figure 6.5 Contact angles for simplified simulated intestinal media containing various amounts of synthetic surfactants: SDS or CTAB with ketoconazole disks. Each data point represents the mean ± S.D. of 2 measurements

6.3.3 Solubility and dissolution determinations

6.3.3.1 Simulating fasting gastric fluids

Human gastric fluid in the pre-prandial state has an average surface tension of 35-45mN m^{-1} (Section 1.6.2.1). Therefore, in order to simulate gastric conditions, it was necessary to add surfactants to the media in sufficient amounts to match the surface tension of the human fluid. Several media containing surfactants to mimic the fasting gastric fluids are suggested in the literature. The effect of the following media on the dissolution of both drugs was explored:

1) 0.25%, w/v of SDS (anionic surfactant) in SGF (Galia et al., 1999).

2) 0.1%, w/v of Triton X-100 (non-ionic surfactant) in SGF (Dressman et al., 1998).

3) FaSSGF, a medium containing physiologically-relevant amounts of bile salt, namely; NaTC, and lecithin developed by Vertzoni et al. (2005). The formulation of FaSSGF was modified by making the pH 1.2 instead of 1.6 to match the pH of the previously investigated simulated fasting gastric media. Furthermore, the gastric enzyme, pepsin, was excluded to avoid the complexity and stability problem of pepsin in the medium (Aburub et al., 2008).

4) 0.02%, w/v Tween 20 (non-ionic surfactant) in SGF was developed as this medium had a surface tension equivalent to the simulated gastric media.

All these SGF media had a similar pH of 1.2.

6.3.3.1.1 Itraconazole

Each surfactant-containing medium enhanced both the solubility and the dissolution of the drug (Table 6.3). The maximum increase in solubility was seen in SGF containing 0.25%, w/v SDS.

Table 6.3 The solubility and the IDR data of itraconazole at 37°C and the solubility of ketoconazole at 37°C in SGF containing 0.25%, w/v SDS, 0.1%, w/v Triton X-100, 0.02%, w/v Tween 20 and in FaSSGF. Each data point represents the mean ± SD of at least 3 measurements

Medium	Itraconazole		Ketoconazole
	Solubility ($\mu g\ ml^{-1}$)	Initial IDR ($\mu g\ min^{-1}\ cm^{-2}$) (0-15min)	Solubility ($mg\ ml^{-1}$)
FaSSGF	6.8 ± 0.1	60.7 ± 11.8	26.8 ± 0.4
0.25%, w/v SDS in SGF	8.3 ± 1.9	13.9 ± 1.0	33.1 ± 1.3
0.1%, w/v Triton X-100 in SGF	4.9 ± 0.8	13.3 ± 1.2	29.4 ± 2.3
0.02%, w/v Tween 20 in SGF	7.7 ± 0.3	13.0 ± 0.9	30.8 ± 0.6

The dissolution in FaSSGF was greater than that in SGF with a significant initial burst, the initial IDR was 5 times greater compared to SGF (Figure 6.6). The medium containing SDS showed a different pattern of dissolution where a steadier increase in the amount dissolved versus time was observed. Since SDS induced the highest solubility, this may explain the steady rise in the dissolution in SDS medium, as the amount dissolved within 5h had not yet reached saturation solubility.

The inclusion of 0.1%, w/v Triton X-100 induced a slight increase in the dissolution. The initial IDR in SGF containing 0.02%, w/v Tween 20 did not increase significantly compared to SGF but after one hour an increase in the dissolution was apparent (Figure 6.6).

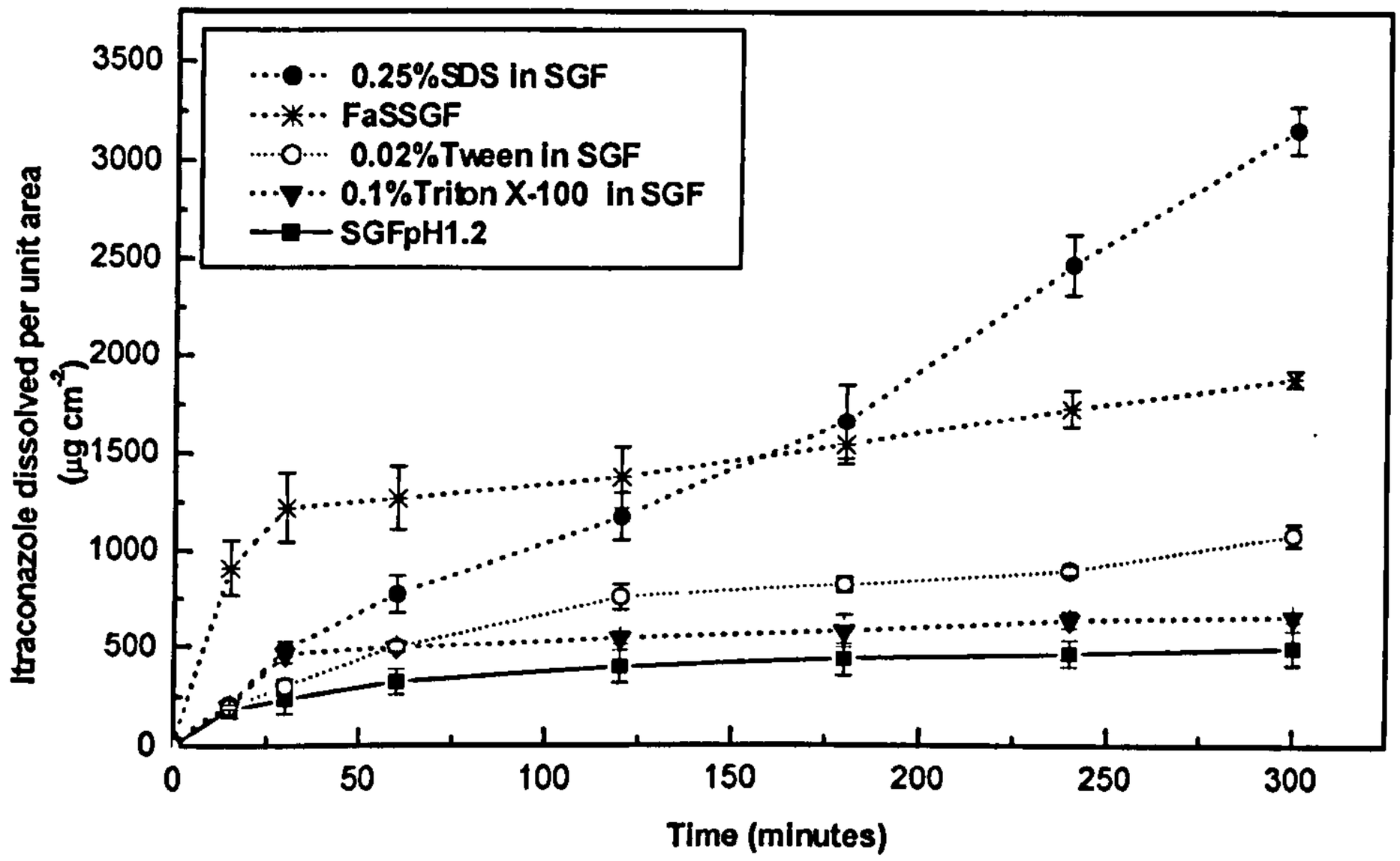


Figure 6.6 The intrinsic dissolution profile of itraconazole at 37°C in SGF (pH 1.2) and in SGF containing 0.25%, w/v SDS, 0.1%, w/v Triton X-100, 0.02%, w/v Tween 20 and in FaSSGF. Each data point represents the mean ± S.D. of 3 measurements

The data collected for the initial IDR were normalised for solubility (C_s) according to Eq. (6.1). J denotes the IDR or the mass flux of the drug, S is the surface area of drug disks and V is the volume of the dissolution medium.

$$normalised(J) = \frac{J.S}{VC_s} \qquad \textbf{Eq. (6.1)}$$

Itraconazole had a similar normalized value of 0.002min^{-1} in media containing SDS and Tween 20. A higher value of 0.004min^{-1} was found in Triton X 100-containing media. Meanwhile the normalized value was greater in FaSSGF (0.013min^{-1}). These figures indicated that it was not the solubilization effect that was responsible for the relatively higher initial IDR in FaSSGF and Triton X 100-containing medium, otherwise similar values for the normalized IDR would be obtained.

Another two important factors can affect the dissolution process, namely, diffusion and wetting. The effect of Triton-containing solution was mainly due to wetting enhancement as the contact angle with itraconazole disk was the smallest (Figure 6.1). The contact angle with FaSSGF was the greatest among the gastric media indicating less wettability for the bile salt and lecithin. Consequently, differences other than changes due to solubility and wetting were the reason for the high initial IDR in FaSSGF, which were more likely related to the greater diffusivity of the drug in this medium.

6.3.3.1.2 Ketoconazole

The dissolution studies of ketoconazole at pH 1.2 could not be performed because of the high solubility of ketoconazole at this pH (Section 3.3.2.2). Nevertheless, saturation solubility was determined in these media (Table 6.3). The greatest increase in solubility (1.6-fold) compared to SGF was recorded in SGF containing 0.25%, w/v SDS. A smaller increase was observed in FaSSGF (1.3-fold). The solubility of ketoconazole in SGF containing SDS was consistent with the literature value which was ~32mg ml^{-1} (Vertzoni et al., 2007).

6.3.3.2 Simulated intestinal fluid

The behaviour of the drugs was investigated in media containing NaTC in various concentrations. NaTC was chosen as a representative for bile since it is prevalent in the human bile and its micelle aggregation number (number of monomers per micelles) is less sensitive to the changes in the pH and ionic strength of the media (Wiedmann and Kamel, 2002).

Typical concentrations of bile salts in intestinal fluids are 4-6 mM in the fasted state and 10-20 mM in the fed state (Section 1.6.2.1). Consequently, the range of NaTC concentrations was chosen in the current investigation to represent the fasting and fed states. In order to predict the degree of solubilisation by bile salts, solubilities of the drug were constructed as a function of NaTC concentrations. As aforementioned the CMC of the NaTC-containing solution at 37°C was found to be 3-4mM. Accordingly, concentrations of 2mM of NaTC represented sub-CMC levels, whereas higher concentrations (7.5, 15 and 30mM) exceeded the CMC. The dissolution was further explored in more complex media containing NaTC and a phospholipid, namely, lecithin.

6.3.3.2.1 Itraconazole

Both the solubility and the dissolution rate were significantly higher in media representing the fed state. In the media containing only NaTC, the solubility of itraconazole was enhanced by increasing NaTC concentration.

A straight line relationship was found between the concentration of NaTC and drug solubility. Table 6.4 illustrates that the solubility improved in a range from 2- to 28-fold in media containing NaTC, compared to the corresponding blank FeSSIF which did not

contain NaTC. Likewise, the dissolution increased in the presence of NaTC, however, the amount dissolved quickly levelled off. Figure 6.7 shows the amounts dissolved per unit area plotted versus 3h.

Table 6.4 Solubility and IDR data of itraconazole at 37°C in FeSSIF, FaSSIF, blank FeSSIF, blank FaSSIF and in blank FeSSIF containing various amounts of NaTC. Each data point represents the mean ± S.D. of 3 measurements

Medium	Solubility ($\mu g\ ml^{-1}$)	Initial IDR (0-15min) ($\mu g\ min^{-1}\ cm^{-2}$)
Blank FaSSIF	~ 0.003	-
FaSSIF	0.070 ± 0.005	1.85 ± 0.47
Blank FeSSIF	0.011 ± 0.004	0.39 ± 0.08
FeSSIF	0.201 ± 0.019	7.28 ± 1.04
2mM NaTC in blank FeSSIF	0.027 ± 0.004	0.71 ± 0.13
7.5mM NaTC in blank FeSSIF	0.095 ± 0.010	2.94 ± 0.32
15mM NaTC in blank FeSSIF	0.158 ± 0.003	4.23 ± 0.29
30mM NaTC in blank FeSSIF	0.311 ± 0.004	8.21 ± 1.02

The initial dissolution rates were normalized by the solubility (Eq. (6.1). In medium containing 2mM NaTC, the normalized dissolution was calculated as $0.037 min^{-1}$, the greatest value ($0.044 min^{-1}$) was found in 7.5mM NaTC medium. However, a slight decrease in the normalized value was noticed at higher concentrations of NaTC.

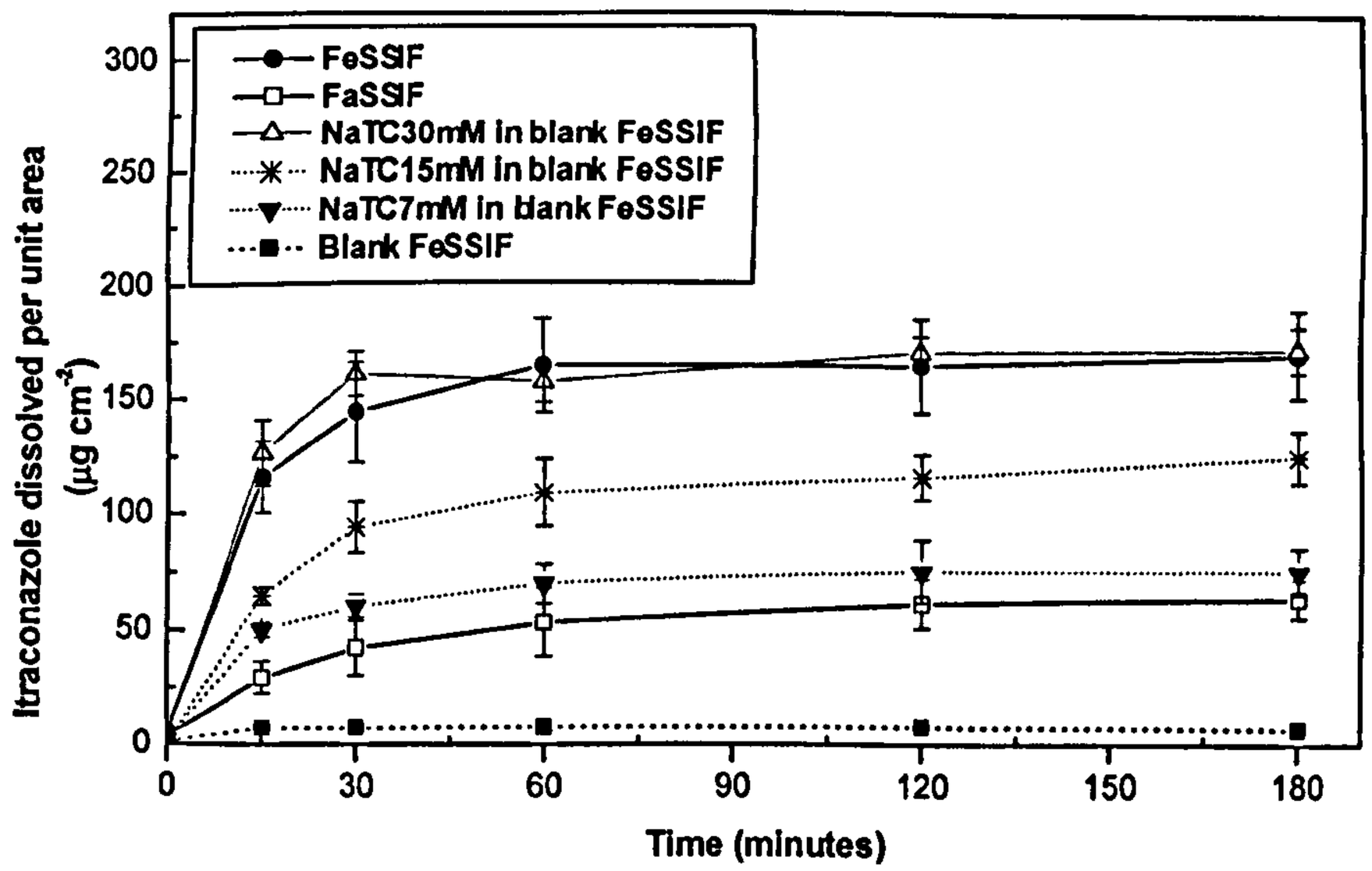


Figure 6.7 The intrinsic dissolution profile of itraconazole at 37°C in FeSSIF, FaSSIF, blank FeSSIF, blank FaSSIF and in blank FeSSIF containing various amounts of sodium taurocholate (NaTC). Each data point represents the mean ± S.D. of 3 measurements

At these micellar concentrations, 15mM and 30mM of NaTC, most of the drug molecules were likely associated with micelles resulting in slower diffusion of the drug-

loaded in micelles. At the sub-micellar concentrations, the slight enhancement in dissolution was explained by an increase in wetting as the contact angle dropped significantly compared to the blank buffer.

The dissolution of itraconazole in blank FaSSIF could not be determined due to the extremely low solubility of the drug at this pH. An increase in the solubility and dissolution of itraconazole in FaSSIF and FeSSIF was observed compared to the corresponding blank media (Table 6.4). In FeSSIF, the solubility and dissolution enhanced around three times relative to FaSSIF. By comparing FeSSIF to 15mM NaTC-containing medium, which contained the same amount of the bile salt, it was noticed that the solubility and dissolution in FeSSIF were greater.

6.3.3.2.2 Ketoconazole

The increase in ketoconazole solubility was insignificant in medium containing 2mM NaTC, which represented a concentration below the CMC. At concentrations above the CMC, the solubilities increased linearly as a function of bile salt concentration (Table 6.5).

Table 6.5 Solubility and IDR data of ketoconazole at 37°C in FeSSIF, FaSSIF, blank FeSSIF, blank FaSSIF and in blank FeSSIF containing various amounts of NaTC. Each data point represents the mean ± S.D. of 3 measurements

Medium	Ketoconazole solubility (mg ml^{-1})
Blank FaSSIF	0.008 ± 0.001
FaSSIF	0.020 ± 0.004
Blank FeSSIF	0.111 ± 0.008
FeSSIF	0.517 ± 0.050
2mM NaTC in Blank FeSSIF	0.116 ± 0.042
7.5mM NaTC in Blank FeSSIF	0.122 ± 0.008
15mM NaTC in Blank FeSSIF	0.238 ± 0.032
30mM NaTC in Blank FeSSIF	0.568 ± 0.050

An increase in the dissolution rate was observed with the inclusions of NaTC (Figure 6.8). The IDR in 30mM NaTC-containing media could not be determined due to the poor linearity where r^2 was 0.89.

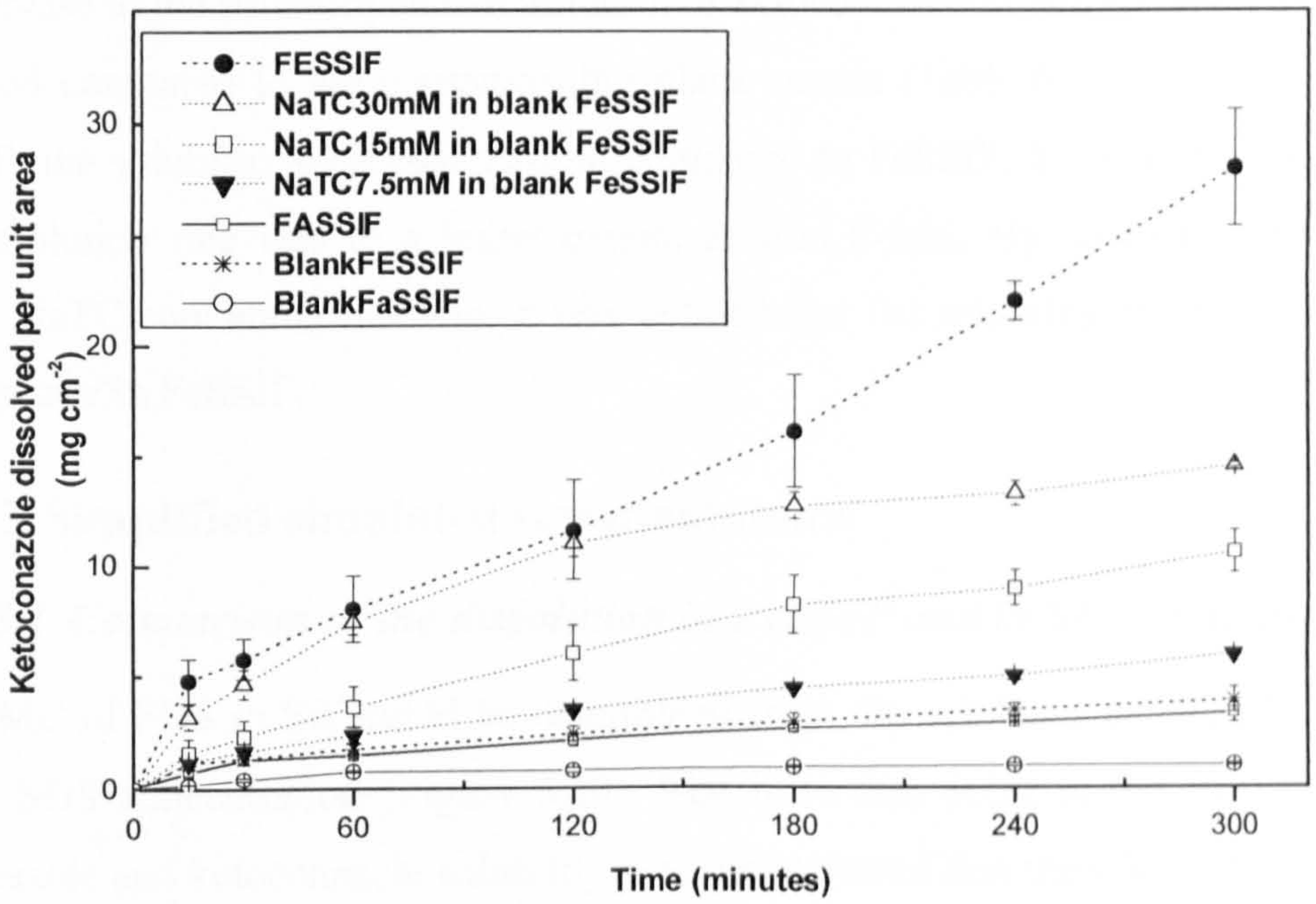


Figure 6.8 The intrinsic dissolution profile of ketoconazole at 37°C in FeSSIF, FaSSIF, blank FeSSIF, blank FaSSIF and in blank FeSSIF containing various amounts of NaTC. Each data point represents the mean ± S.D. of 3 measurements

The initial intrinsic dissolution rates (0-15min) of ketoconazole in media containing NaTC were normalized by the solubility values (Figure 6.9). Since the obtained data were not similar, this suggested that a mechanism other than solubilisation accounted for the increase in dissolution (Bakatselou et al., 1991). A drop in the normalized dissolution with the two high concentrations of NaTC was observed suggesting low diffusion coefficients in these media. The sub-micellar solution increased the dissolution through increasing the wettability of the drug surface.

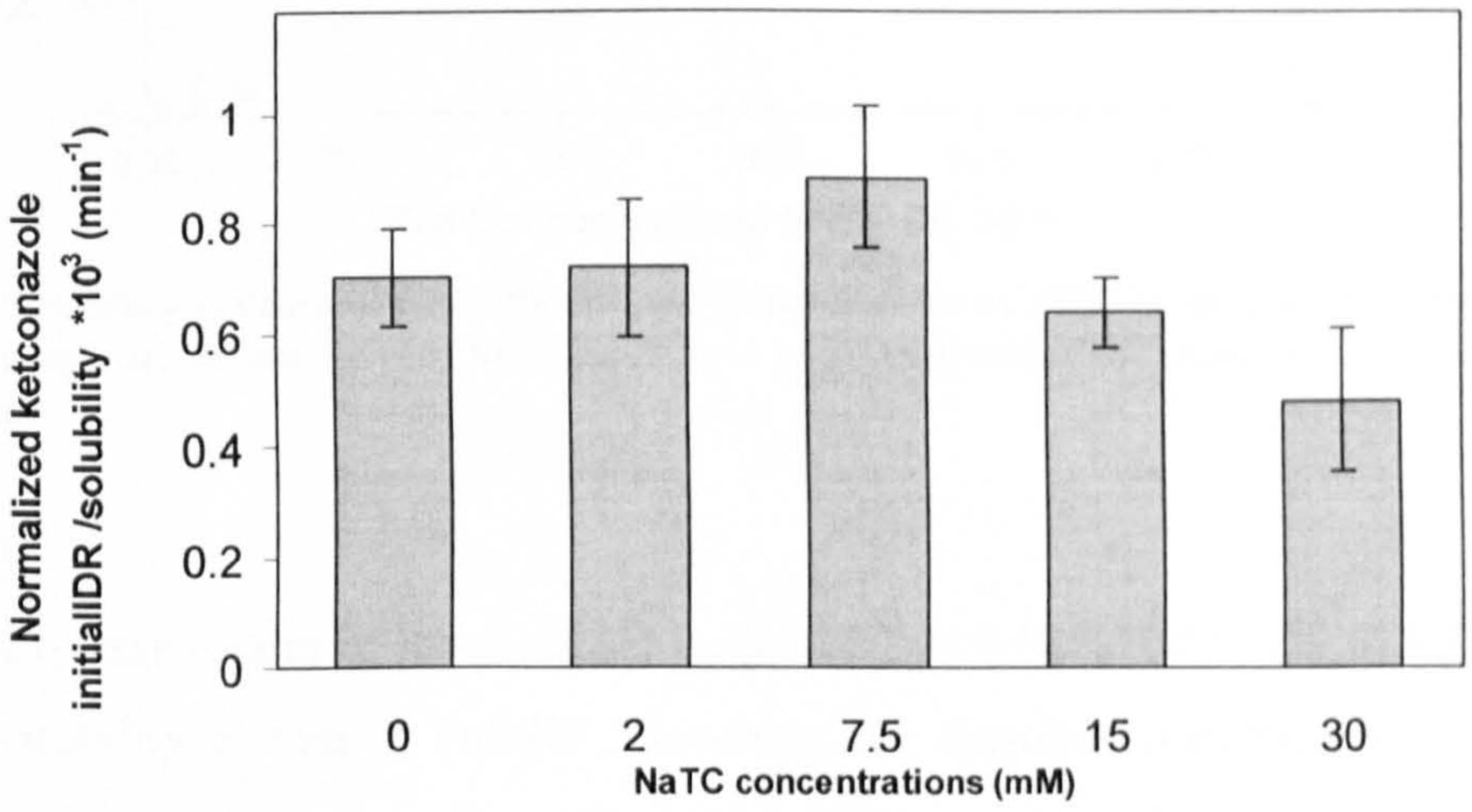


Figure 6.9 The initial dissolution rates of ketoconazole normalized for solubility as a function of NaTC concentration in blank FeSSIF. Each data point represents the mean ± S.D. of 2 measurements

An increase in the solubility and dissolution of ketoconazole in FaSSIF and FeSSIF was observed compared to the corresponding blank media (Table 6.5 and Figure 6.8). In FeSSIF, the solubility increased 25-fold compared to FaSSIF, however the increase in the dissolution rate was to a lesser extent, around 8-fold. By comparing FeSSIF to 15mM NaTC-containing medium, it was noticed that the solubility and the dissolution were greater in FeSSIF.

6.3.3.3 Simplified simulated intestinal fluids

6.3.3.3.1 Comparison of the dissolution in FaSSIF and in SIF containing SDS

The CMC of SDS in SIF could be determined from the solubility profile of the drugs versus SDS concentration (Figure 6.10). The inflection point in the curves of both itraconazole and ketoconazole solubility profiles indicated that the CMC of SDS in SIF was around 0.01%, w/v (0.35mM).

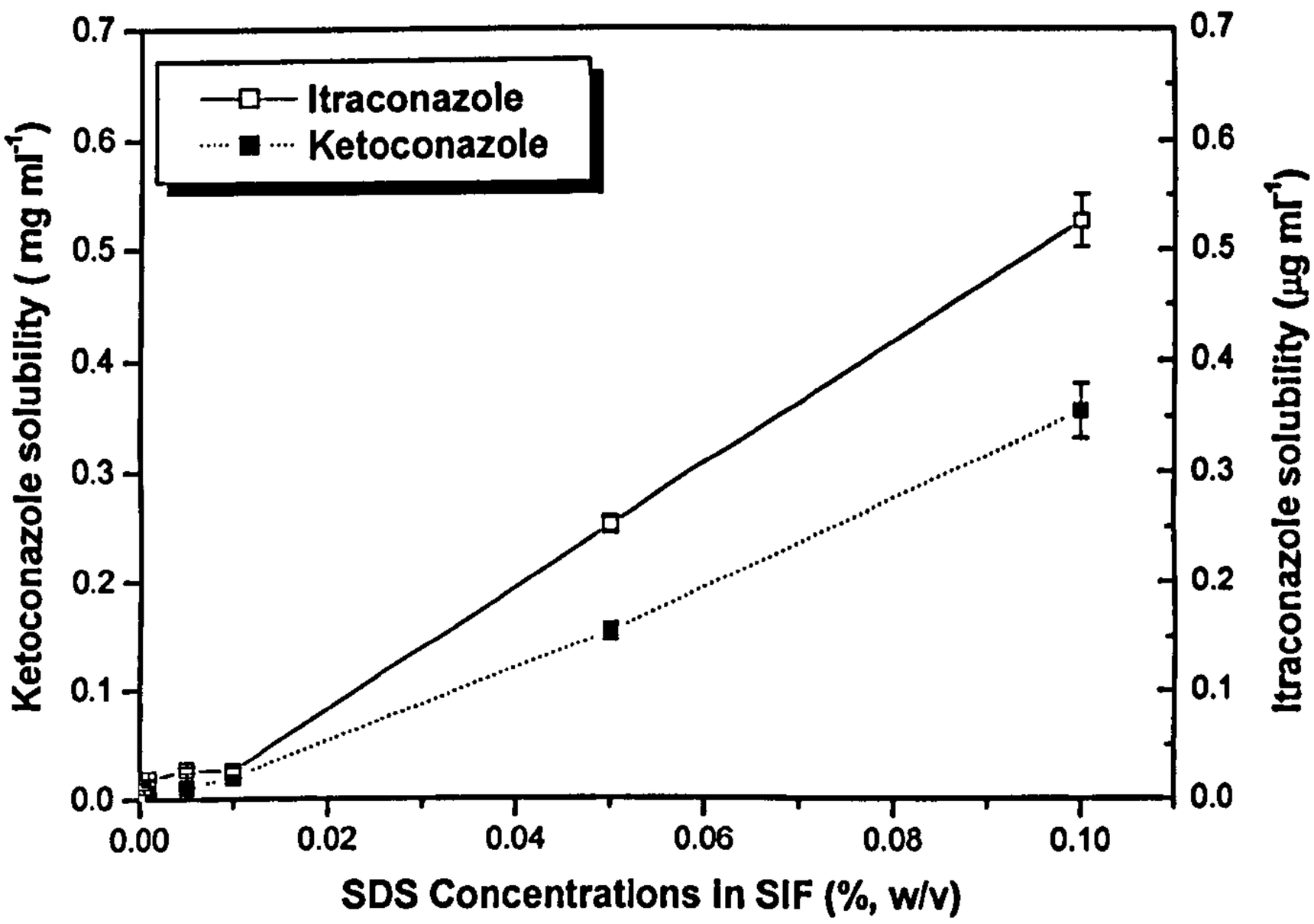


Figure 6.10 Solubility profiles of itraconazole and ketoconazole at 37°C in simulated intestinal fluid (SIF) containing various amounts of SDS. Each data point represents the mean ± S.D. of at least 2 measurements

Itraconazole

The solubility assessment of itraconazole indicated that 0.02%, w/v SDS in SIF induced a similar solubility to that in FaSSIF. However, the dissolution profiles (Figure 6.11) were different in these two media as the initial IDR was significantly greater in 0.02%, w/v SDS medium.

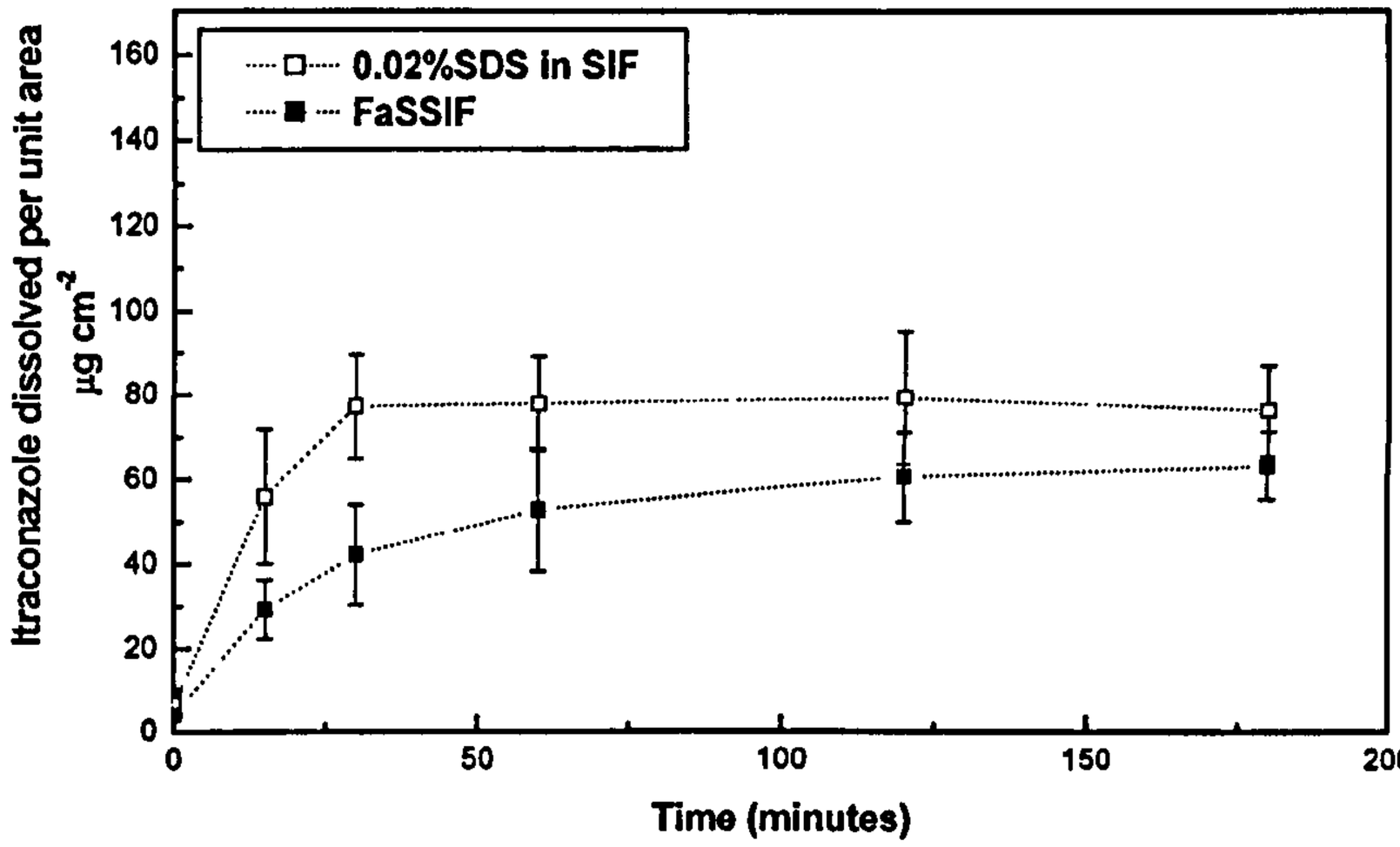


Figure 6.11 Comparison of the intrinsic dissolution profiles of itraconazole at 37°C in 0.02%, w/v SDS in SIF and in FaSSIF. Each data point represents the mean of 2 measurements ± S.D.

The dissolution of itraconazole was further assessed in SIF containing amounts of SDS above the CMC. The initial dissolution rates increased linearly with an increase in SDS concentration (Figure 6.12). The data for 0.05%, w/v SDS in SIF were employed for comparison instead of that in SIF alone as itraconazole was extremely poorly soluble in SIF and consequently the initial IDR could not be determined. Both the initial dissolution rate and the solubility values increased at the same ratio in media containing 0.05% or 0.1% of SDS. However in media containing higher concentrations of SDS a greater increase in the solubility was observed. In media containing 0.25, 0.5 and 1%, w/v SDS the initial IDR increased 2.3-, 4.4- and 6.8-fold, whereas the solubility increased 41-, 74- and 206-fold, respectively.

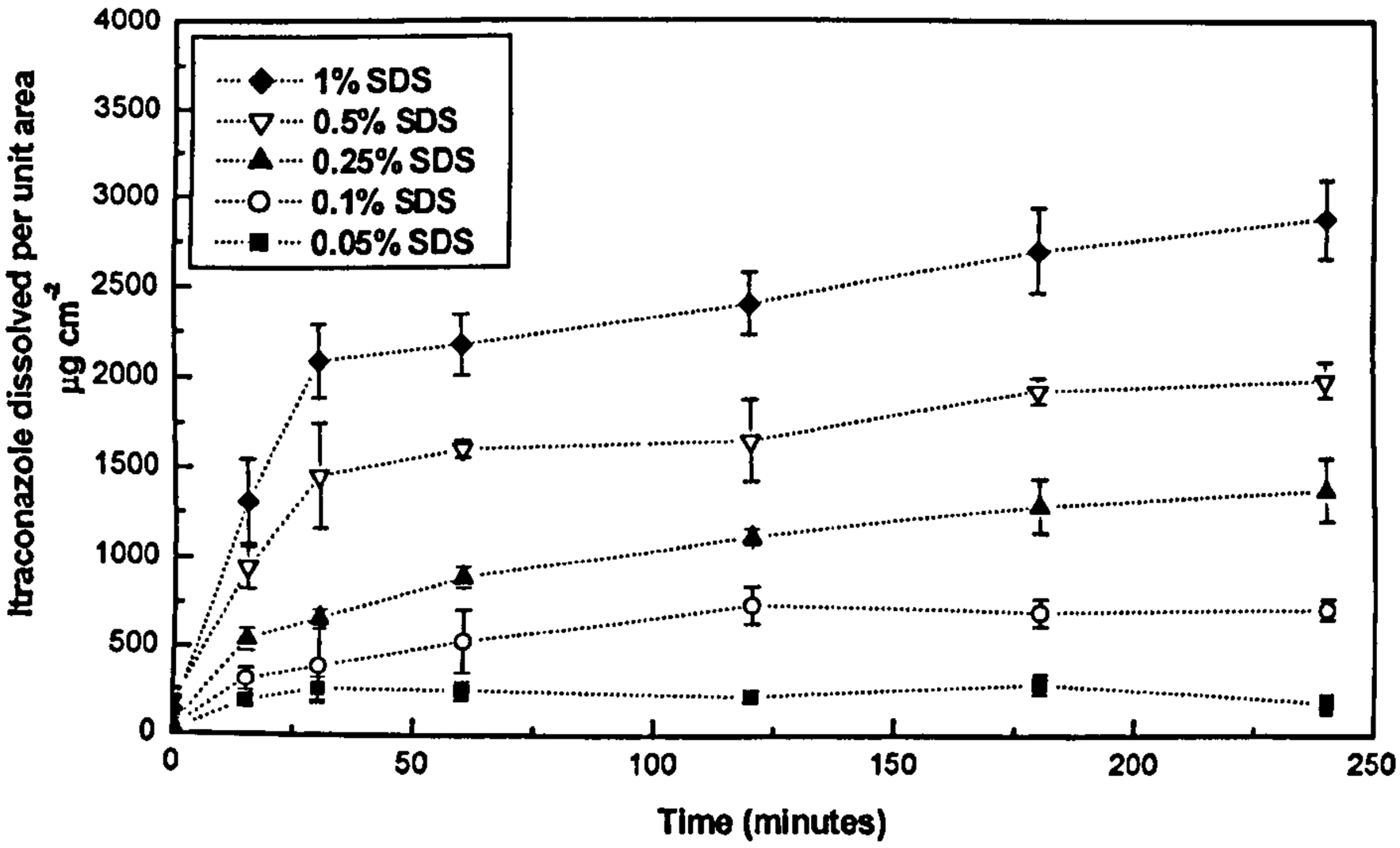


Figure 6.12 The intrinsic dissolution profile of itraconazole at 37°C in SIF containing various amounts of SDS (pH 6.8). Each data point represents the mean ± S.D. of 3 measurements

Ketoconazole

The solubility of ketoconazole in 0.01%, w/v SDS in SIF was similar to that in FaSSIF but the dissolution rates in FaSSIF were greater (Figure 6.13). Therefore, the amount of SDS in SIF was increased to 0.02%, w/v to achieve a better simulation; nevertheless the initial IDR was still greater in FaSSIF.

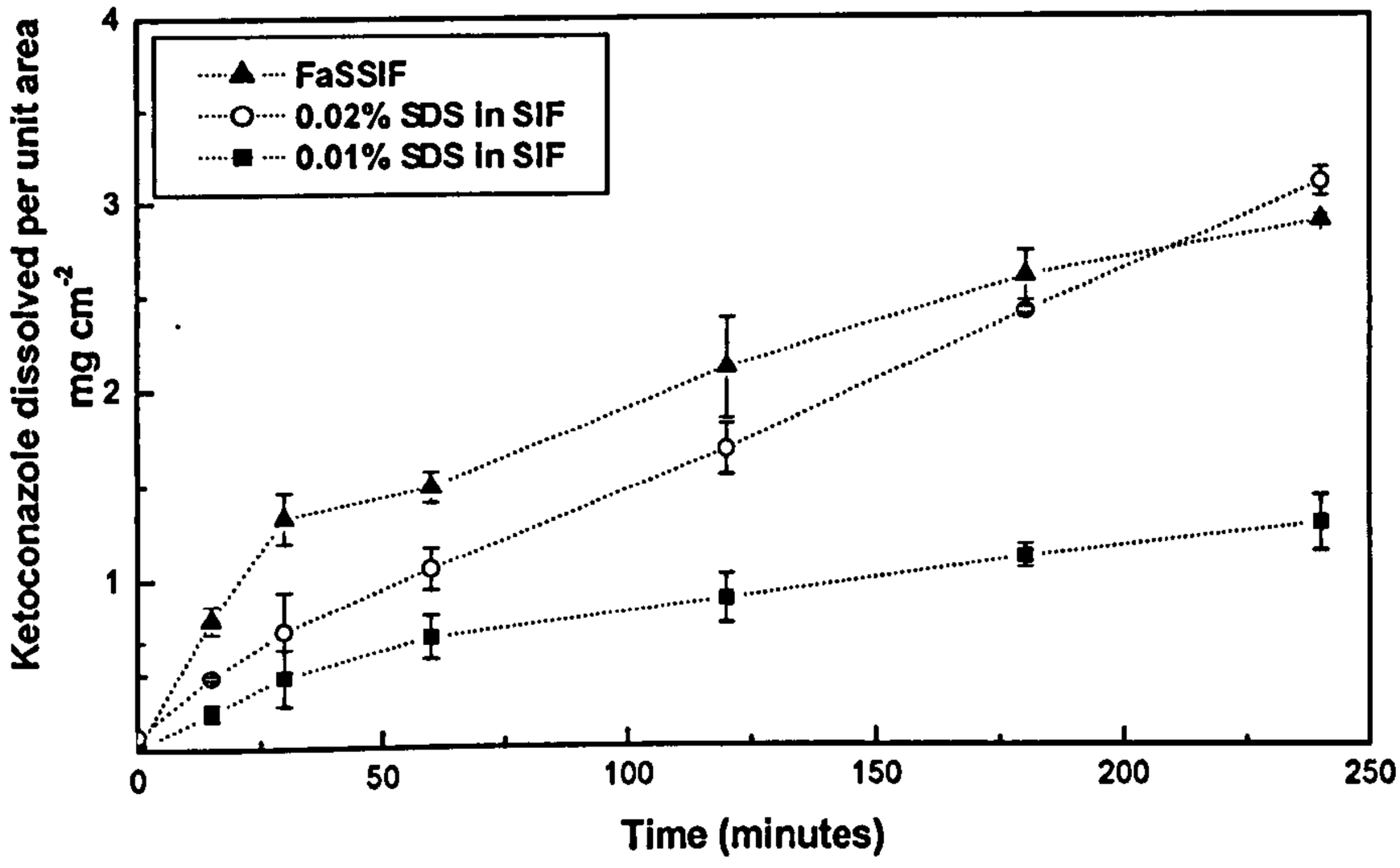


Figure 6.13 Comparison of the intrinsic dissolution profiles of ketoconazole at 37°C in FaSSIF and in SIF containing 0.01%-0.02% SDS (pH 6.8). Each data point in SDS-containing media represents the mean of 2 measurements ± S.D.

6.3.3.3.2 Comparison of the dissolution in FaSSIF and SIF containing CTAB

The solubility profiles of itraconazole and ketoconazole in SIF containing CTAB (Figure 6.14) revealed that the CMC of CTAB was around 0.005%, w/v (0.14mM).

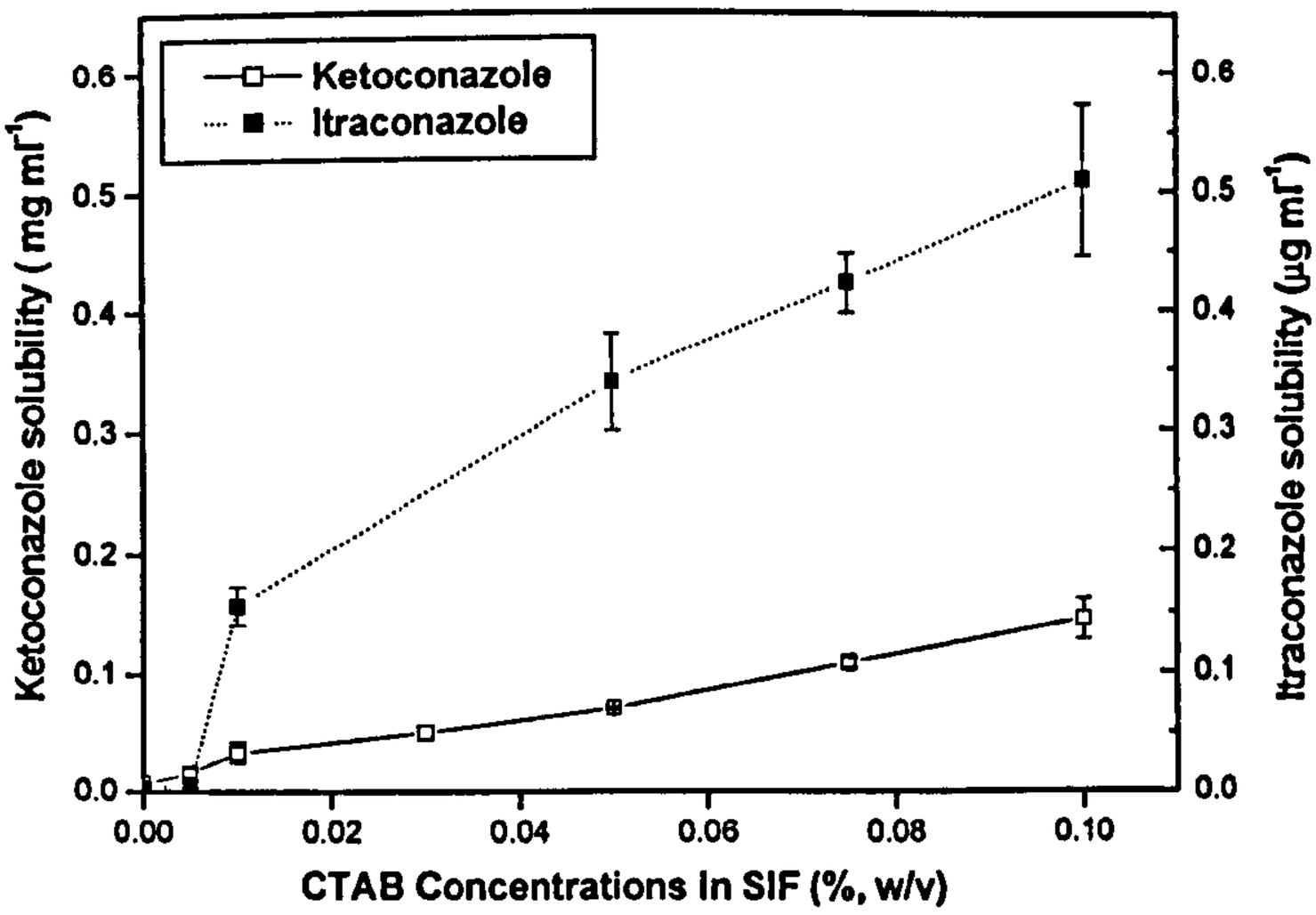


Figure 6.14 Solubility profiles of itraconazole and ketoconazole at 37°C in SIF containing various amounts of CTAB. Each data point represents the mean ± S.D. of 2 measurements

Itraconazole

The solubility of itraconazole in FaSSIF was similar to the solubility in SIF containing CTAB in a range of 0.005-0.01%, w/v. The dissolution of itraconazole was investigated in SIF containing various amounts of CTAB and it was found that dissolution in 0.005%, w/v CTAB was similar to the dissolution in FaSSIF (Figure 6.15).

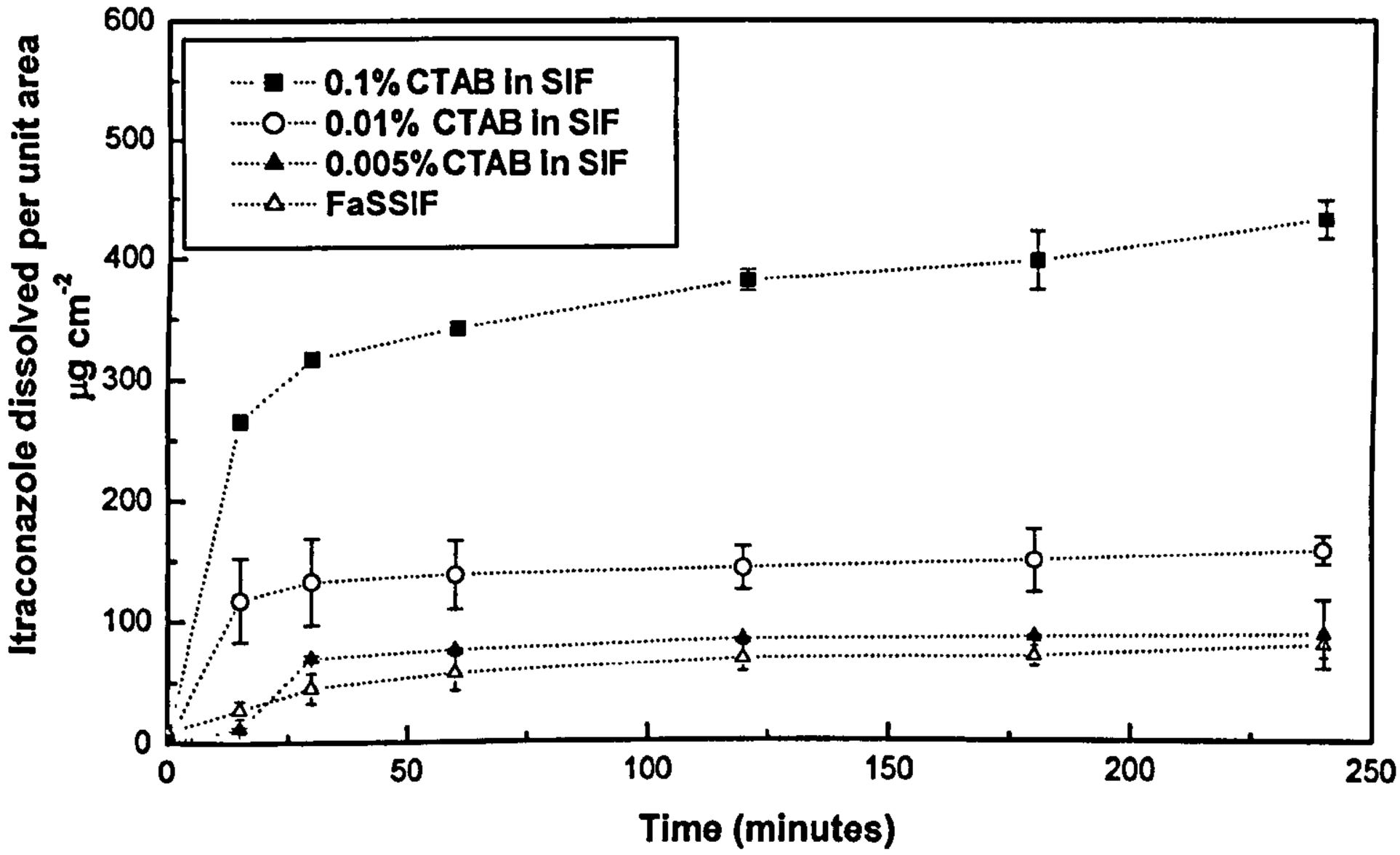


Figure 6.15 Comparison of the intrinsic dissolution profiles of itraconazole at 37°C in FaSSIF (pH 6.5) and in SIF containing 0.005, 0.01 and 0.1%, w/v CTAB (pH 6.8). Each data point in CTAB-containing media represents the mean of 2 measurements ± S.D.

Ketoconazole

Although concentrations of 0.005-0.01%, w/v CTAB in SIF generated similar solubility of ketoconazole to that in FaSSIF, higher concentration of CTAB, 0.05%, w/v was needed to approximate the dissolution in FaSSIF (Figure 6.16).

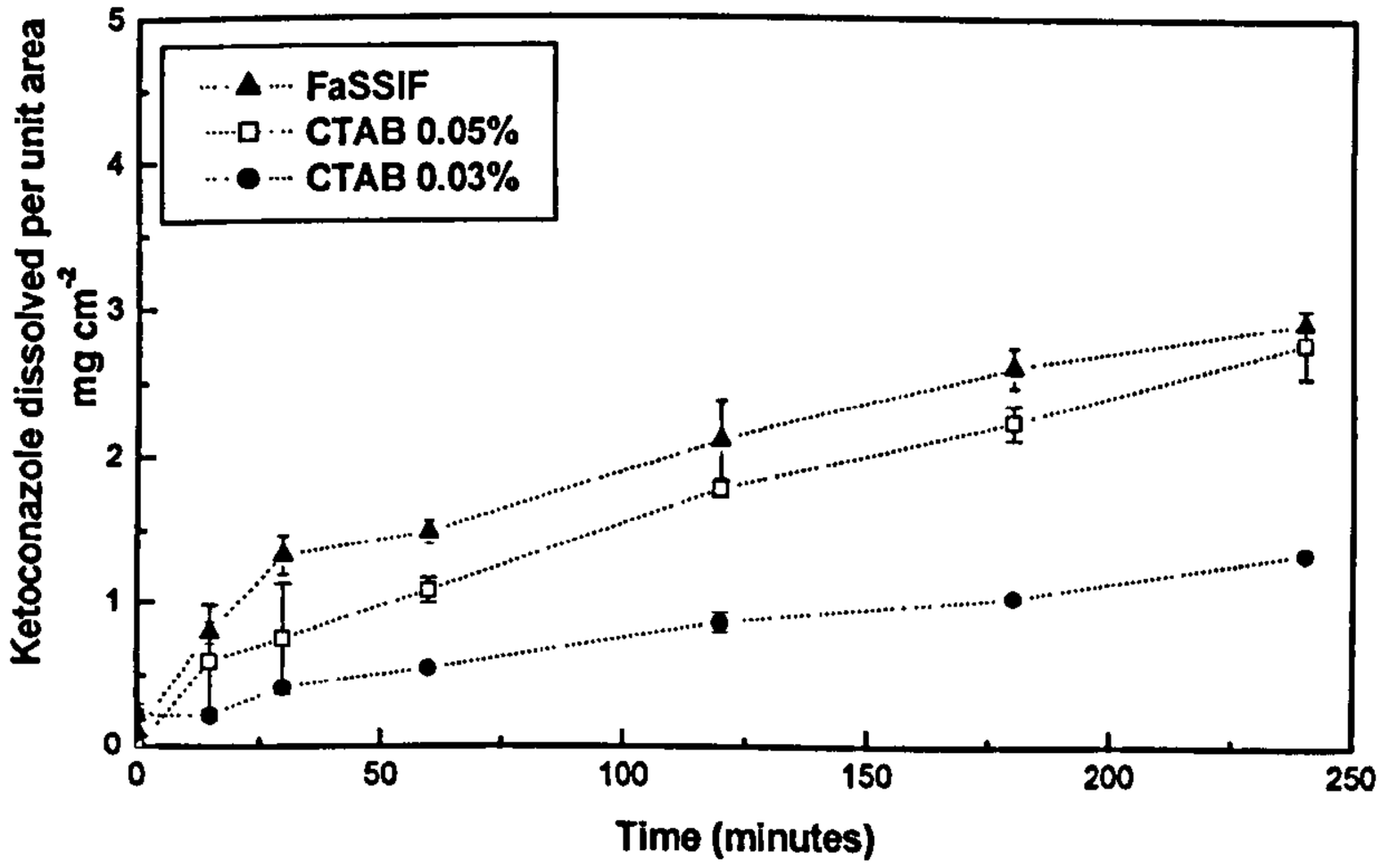


Figure 6.16 Comparison of the intrinsic dissolution profiles of ketoconazole at 37°C in FaSSIF (pH 6.5) and in SIF containing 0.05-0.1 %, w/v CTAB (pH 6.8). Each data point in CTAB-containing media represents the mean of 2 measurements ± S.D.

6.3.3.3.3 Comparison of the dissolution in FeSSIF and in blank FeSSIF containing SDS

The effect of SDS on the dissolution of ketoconazole was further investigated in medium at pH 5 which represented simulation of the intestinal fluid at fed state.

The solubility of ketoconazole in FeSSIF was similar to the solubility in blank FeSSIF containing SDS in a range of 0.05-0.1%, w/v. The dissolution data mirrored the solubility (Figure 6.17) where the dissolution of ketoconazole in FeSSIF was similar to that in blank FeSSIF containing 0.05-0.1%, w/v SDS. However, the dissolution profile was not completely identical to any of the investigated SDS-containing media.

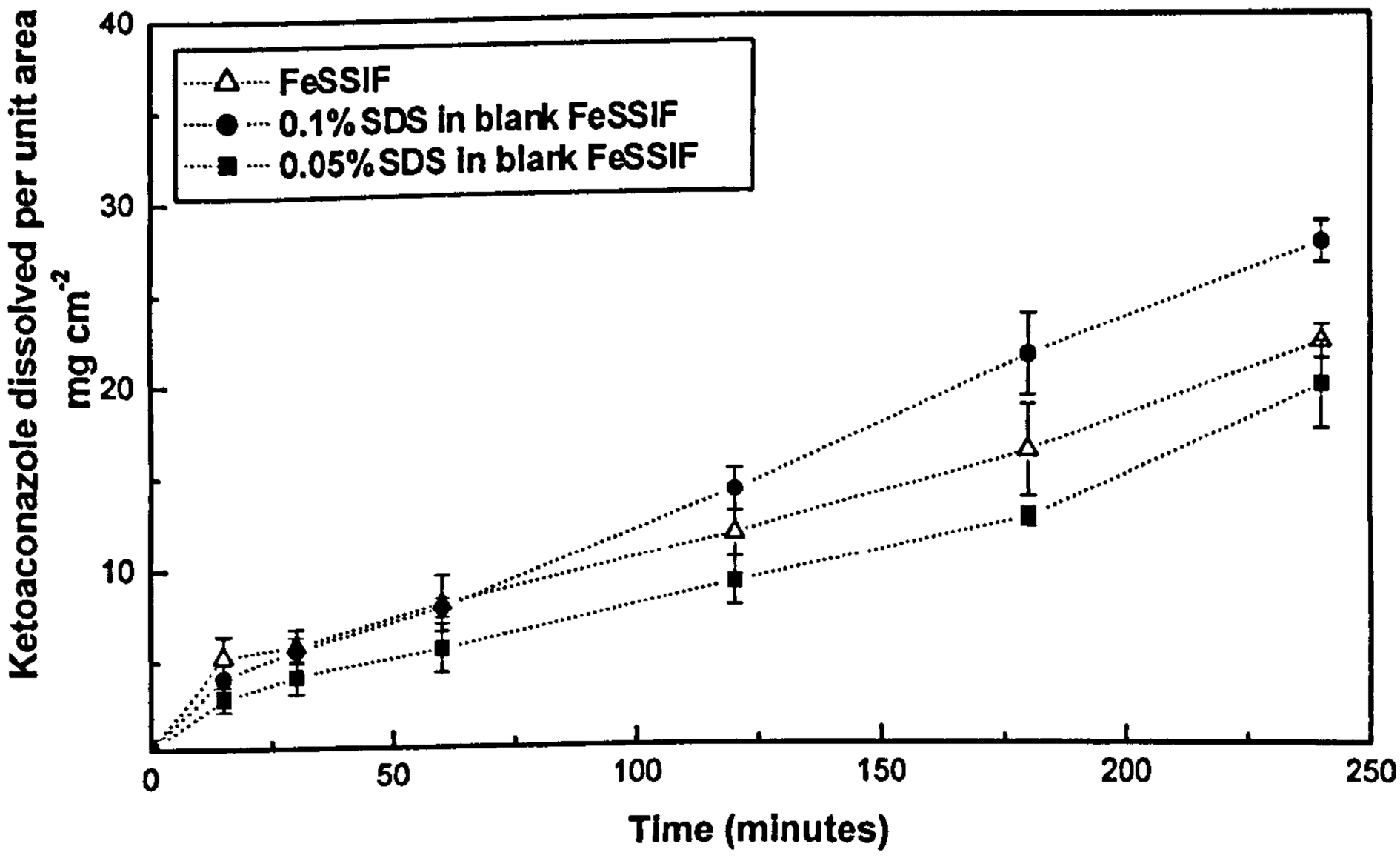


Figure 6.17 Comparison of the intrinsic dissolution profiles of ketoconazole at 37°C in fed state simulated intestinal fluid (FeSSIF) and in blank FeSSIF containing 0.01%-0.05%, w/v SDS (pH 5). Each measurement in SDS-containing media represents the mean of 2 measurements ± S.D.

6.3.3.4 Emulsion of Soybean oil in simulated gastric fluid

The solubility and dissolution of itraconazole and ketoconazole were assessed in media containing emulsified fat; the pH of the media was 3 to mimic the fed stomach acidity. The dissolution tests were carried out in media containing only Tween 20 at concentrations equivalent to that in the emulsions.

The solubilities of both drugs in the media containing diluted emulsions were slightly greater than in the corresponding media containing Tween 20 however the variations were not significant (t-test, 0.05 significant level) (Table 6.6).

Table 6.6 Solubility of itraconazole and ketoconazole at 37°C in media containing either emulsions based on Soybean oil and Tween 20 or only Tween 20 (pH 3). Each data point represents the mean ± S.D. of 3 measurements

Medium	Itraconazole solubility ($\mu g\ ml^{-1}$)	Ketoconazole solubility ($mg\ ml^{-1}$)
Emulsion (4.25% fat, 0.75% Tween 20)	0.53 ± 0.08	0.80 ± 0.03
0.75% Tween 20 in SGF	0.48 ± 0.08	0.76 ± 0.06
Emulsion (1.7% fat, 0.3% Tween 20)	0.47 ± 0.06	0.61 ± 0.06
0.3% Tween 20 in SGF	0.44 ± 0.04	0.59 ± 0.07

6.3.3.4.1 Itraconazole

The extent of dissolution of itraconazole in media containing emulsions and media containing equal amounts of Tween 20 were similar (Figure 6.18). The amounts dissolved within 4h approximated to the saturation solubility of itraconazole in the corresponding media. However, the initial IDR were greater in the absence of fat.

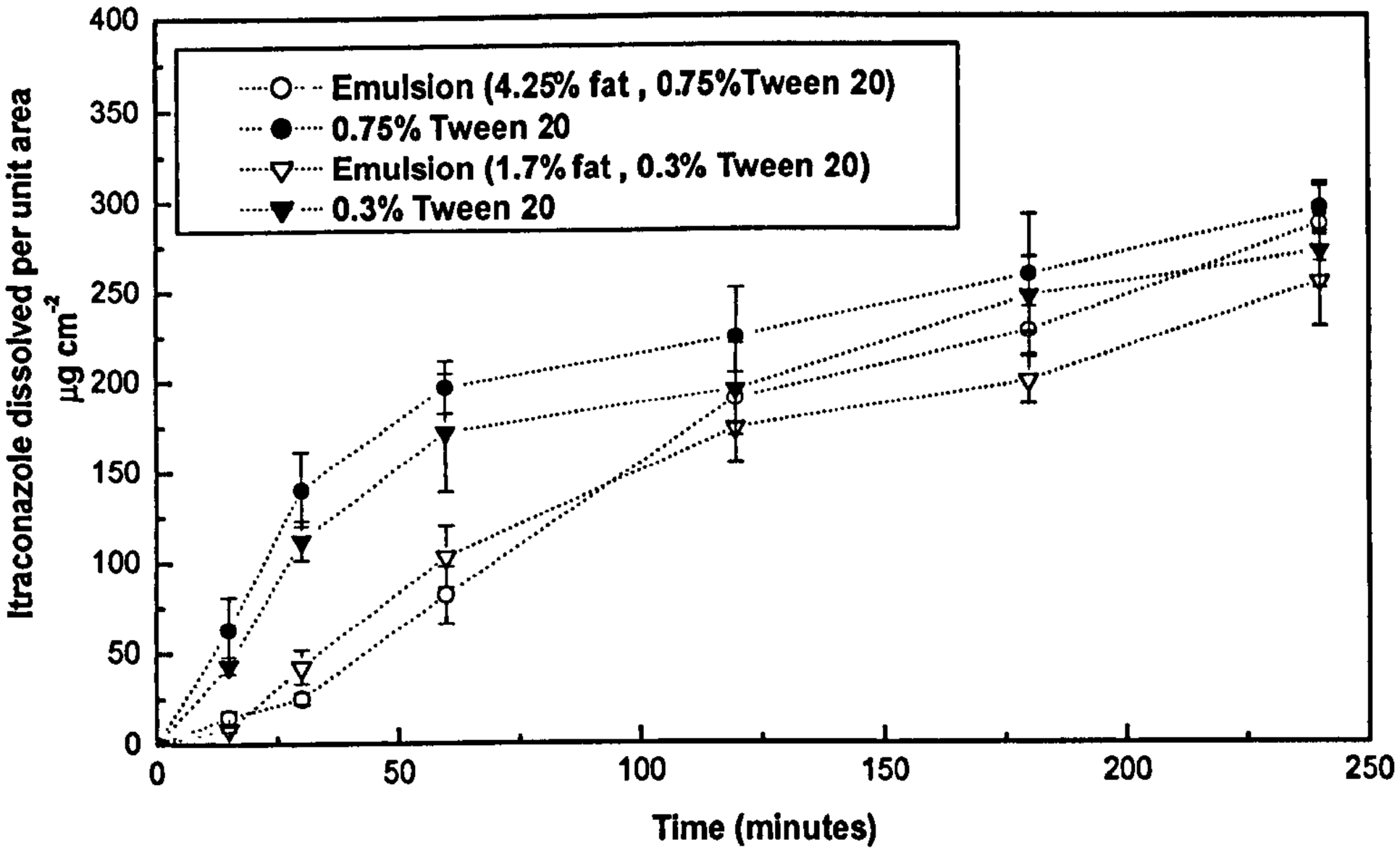


Figure 6.18 Intrinsic dissolution profiles of itraconazole at 37°C in media containing either emulsions based on Soybean oil and Tween 20 or in SGF containing equal amounts of Tween 20 (pH 3). Each data point represents the mean ± S.D. of 3 measurements

6.3.3.4.2 Ketoconazole

The dissolution of ketoconazole was monitored over 3h as the surface of the disks started to erode forming holes after that time point. Slightly slower dissolution rates were observed in media containing emulsion compared to media of equivalent amounts

of Tween 20 (Figure 6.19). The extent of the amount dissolved throughout 3h in media containing more fat, 4.25 %, w/v were less than in the medium containing 1.7 %, w/v fat.

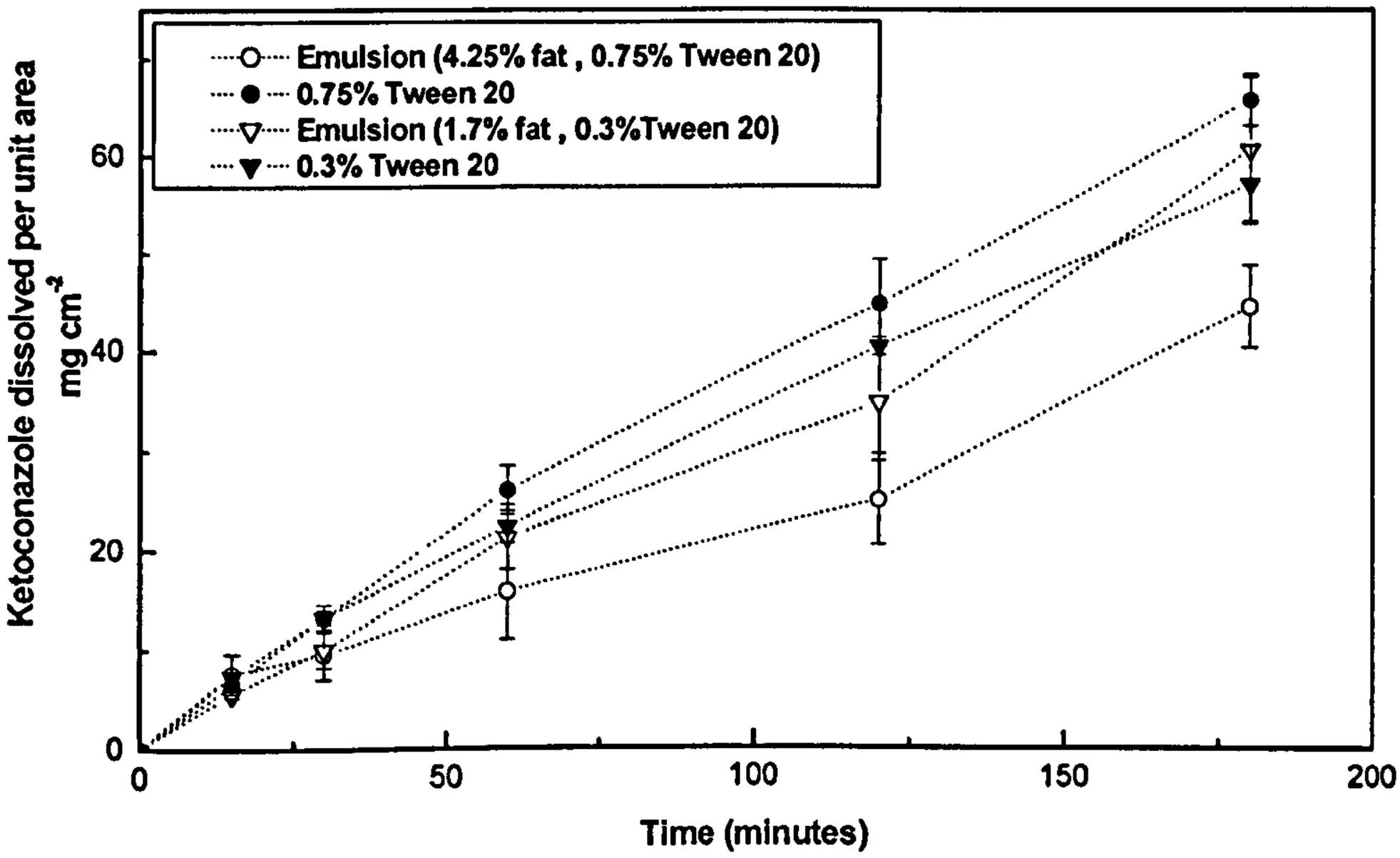


Figure 6.19 Intrinsic dissolution profiles of ketoconazole at 37°C in media (pH 3) containing either emulsions based on Soybean oil and Tween 20 or in SGF containing equal amounts of Tween 20. Each data point represents the mean ± S.D. of 3 measurements

6.4 Discussion

6.4.1 Simulating fasting gastric fluids

The CMC of SDS in SGF was reported as 0.05%, w/v at 37°C (Aburub et al., 2008). Triton's CMC in water was in the range 0.01 to 0.015%, w/v (Sharmin et al., 2006) at 20°C. The CMC of Tween 20 was reported as 0.006%, w/v in water (Luner et al., 1996). Since the CMC is dependant on the experimental conditions such pH, ionic strength of the medium and the temperature of the measurement, a drop in the CMC of these surfactants is expected under the present experimental conditions of 37°C in acetate buffer (pH 5). Consequently, this suggests that the concentrations of the synthetic surfactants in simulated gastric media were well above their CMC.

The CMC of NaTC was reported to be 3-4mM and with the addition of lecithin in a ratio (4:1), it dropped to 0.25mM in simulating intestinal fluid (pH 5.5) at an ionic strength of 0.1M at 25°C (Naylor et al., 1993). The CMC of FaSSGF containing 80μM NaTC and 20μM lecithin was predicted to be less than the CMC of NaTC (Aburub et al., 2008). Thus, the media with synthetic surfactants contained them in concentrations

exceeding their CMC while the concentration of endogenous surfactants in FaSSGF was below the CMC. This suggested that the media containing synthetic surfactants had a different solubilising mechanism and wetting effect to that of FaSSGF.

When molecules are associated with the micelles, their diffusivity is expected to decrease due to the bigger size of the drug-loaded micelles compared to individual drug molecules (Balakrishnan et al., 2004). Consequently, this may explain why the initial dissolution rate was greater in FaSSGF where no micelles were formed and the diffusion was mainly of the free drug molecules.

FaSSGF was recommended as an appropriate dissolution medium that reflects the fasted state by Jantratid et al. (2008a). The distinct differences between FaSSGF, the medium containing physiologically relevant components, and the artificial surfactant-containing SGF could call into doubt the relevance of using an artificial component in the medium.

Tween 20 (0.02%, w/v) in SGF provided a more reasonable simulation for FaSSGF in terms of solubility although the dissolution profiles were different. This suggested that this medium could be considered an alternative for FaSSGF for solubility determination.

6.4.2 Simulated intestinal media containing only sodium taurocholate

The linear increase in the solubility of the hydrophobic drugs observed in the presence of NaTC in micellar concentrations was attributed to the parallel increase in the number of micelles leading to more drug solubilisation.

Micelles can solubilise poorly soluble non-polar molecules within the micelle core, while polar molecules are adsorbed on the micelle surface or between the hydrophilic heads whereas semi-polar substances are distributed along surfactant molecules in intermediate locations (Corrigan and Healy, 2007). Since the pKa of itraconazole is 3.6, nearly all the drug moieties were unionised at the pH of the investigation (pH 5). Thus, it would be expected for the drug to be solubilised in the hydrophobic core of micelles or deeply in the palisades layer. On the other hand, the pKa of the imidazole ring in ketoconazole is 6.5 so the ring is mostly ionised at pH 5, (Section 1.10.2.2). Thus, ketoconazole was partly protonated at pH 5 and so more probably positioned at the surface of the micelles or between the hydrophilic head groups (hydroxyl) of NaTC. The side chains of NaTC (pKa 2) held a negative charge at pH 5 so ionic interactions are expected with ketoconazole however with itraconazole which present mainly in the neutral form, such ionic attraction is precluded.

The total solubility of a drug (C_{tot}) in an aqueous surfactant system equals the solubility of the drug (C_s) in the absence of surfactant added to the micellar solubility of the drug (C_{dm}).

The solubilization process in concentrations exceeding the CMC was considered as a simple partition phenomenon between an aqueous and a micellar phase. Thus the relationship between surfactant concentration (C_m) and drug solubility (C_{tot}) was given by Eq. (6.2), where P is the distribution coefficient of drug between the micelle and aqueous phase (Corrigan and Healy, 2007).

$$C_{tot} = C_s + PC_sC_m \qquad \textbf{Eq. (6.2)}$$

The molar solubilities of the itraconazole and ketoconazole in media containing NaTC were plotted as a function of NaTC molar concentrations in these media. The slope of the linear curve ($P.C_s$) represented the molar solubilisation ratio of the micelles which was computed as 1×10^{-5} for itraconazole and 0.04 for ketoconazole and P was 641 and $181M^{-1}$, respectively. Thus, the proportion of itraconazole in the micellar phase was higher than the proportion of ketoconazole. However, the solubilisation capacity was greater with ketoconazole. This could be attributed to the variations in drug hydrophobicity and affinity to bile salt micelles.

Although an increase in the solubility of both drugs with the increase in NaTC was observed, the increase in the dissolution rate of both drugs was smaller. This was probably due to slow diffusion for the drug incorporated in the micelles.

The Noyes-Whitney equation (Eq. (1.3)) showed that the dissolution rate was linearly proportional to the solubility under perfect sink conditions. However, Higuchi (Higuchi, 1964) developed a theoretical model that predicted that the effect of the solubility on the dissolution would be less than that predicted by the Noyes-Whitney equation in surfactant solutions. Hence, the dissolution in surfactant system is the result of two combined effects; solubilisation by the surfactant combined with slow diffusion of the drug loaded-micelles.

6.4.3 Simulated intestinal media containing NaTC and lecithin

Itraconazole and ketoconazole had significantly higher solubilities in the fed state fluid than in the fasted fluid. This was attributed to the greater bile salt and phospholipid content combined with the lower pH of FeSSIF (pH 5), compared to FaSSIF (pH 6.5).

The dissolution rates of the drugs also increased but with ketoconazole the increase in the dissolution rate was to a lesser extent than the increase in solubility.

A greater solubilisation in FeSSIF was observed when compared to media containing the same amount of NaTC only (15mM). The concentration of NaTC in FaSSIF and FeSSIF exceeded the CMC due to the incorporation of lecithin into the bile salt micelle. Consequently, the formation of mixed micelle systems with lower CMC led to an increase in the number of the micelles and exhibited greater drug solubilisation than the corresponding simple micelle containing NaTC only. This agrees with the findings of Naylor et al. (1993) who studied the dissolution of hydrocortisone in similar media.

The enhancement in the dissolution of the drugs in the simulated intestinal media was likely due to the combined effects of solubilisation and wetting enhancement.

It is worthy of note that the composition of FeSSIF lacks the lipolytic products. Consequently, this medium was recently modified to better reflect the physiological conditions (Jantratid et al., 2008b) (Section 1.9.2). Thus, it would be interesting to investigate the dissolution in the presence of these components which are expected to enhance the dissolution of poorly soluble drugs.

6.4.4 Simplified simulated intestinal fluid

The reported CMCs of SDS and CTAB in water at 25°C are 8.3mM and 1.0mM, respectively. The obtained values for the CMC of SDS and CTAB in SIF (from drugs solubility studies) were approximately 0.01%, w/v (0.35 mM) and 0.005%, w/v (0.14mM), respectively. Thus, a drop in the CMC from the literature values was probably due to the experimental conditions as the temperature of the current investigation was 37°C and the presence of phosphate buffer increased the ionic strength of the medium. With ionic surfactants, the addition of electrolytes to a surfactant solution neutralizes the charge of the ionic head groups and so decreases the electrostatic repulsion between these groups which leads to a decrease in the CMC from that in pure water (Rangel-Yagui et al., 2005).

The solubilisation capacity of itraconazole by SDS above the micellar concentration was found to be 0.0002 (mole drug per mole SDS) and the distribution coefficient *P* was $47000M^{-1}$. In contrast, the solubilisation capacity of ketoconazole by SDS was 0.2 and the distribution coefficient *P* was $15171M^{-1}$. This suggested good solubilisation capacity by SDS for both drugs which was more pronounced with ketoconazole.

A similar solubilisation capacity of itraconazole by SDS and CTAB systems was noticed. The solubilisation capacity of ketoconazole by CTAB in SIF was 0.09 (mole drug per mole CTAB) and the distribution coefficient *P* was $6523M^{-1}$. Hence, CTAB had less solubilisation capacity for ketoconazole than SDS.

The CMC value of SDS in SIF acquired from solubilities of the drugs versus the concentration of SDS was lower than that determined from surface tensions (Figure 6.2). Drug molecules frequently exhibit surface activity due to their amphiphilic structure; this could be the reason why the lower CMC was in the presence of the drugs through the formation of complex micelles. For example, Naylor et al. (1993) noted that the CMC of a NaTC containing-solution was lower in the presence of saturated hydrocortisone.

The concentration of SDS (0.02%, w/v) required to induce similar dissolution for itraconazole and ketoconazole to that in FaSSIF was in a region corresponding to the CMC of SDS. Similar findings were noticed with the dissolution of itraconazole in CTAB-containing medium where the concentration of CTAB (0.005%, w/v) in SIF required for matching the dissolution in FaSSIF was around the CMC of CTAB. In contrast, the surfactant concentration in FaSSIF (3mM NaTC) was well above its CMC.

Solutions containing SDS and CTAB which induced similar dissolution of itraconazole and ketoconazole to the dissolution in FaSSIF exhibited lower surface tension and different wettability of drug surfaces, compared to FaSSIF. This suggested that there were different mechanisms for the increase in drug dissolution in the presence of synthetic and natural surfactants.

It was reported that the inclusion of 0.5%, w/v Tween 60 in blank FaSSIF produced similar dissolution of ketoconazole tablets (Nizoral^{TM}) to the dissolution in FaSSIF (Zoeller and Klein, 2007). The latter medium had a similar surface tension to FaSSIF which indicated that matching the physical properties of bile salts-containing media was an important issue for the simulation.

Thus, the difference in the physical and chemical properties between the simulated intestinal media containing endogenous surfactants and the simplified media containing synthetic surfactants, in addition to different solubilisation mechanisms of the surfactants made matching the dissolution profiles unfeasible. However, the proposed media may be used for initial screening as an alternative to the bile salts media which

are expensive, their preparation is time consuming process and furthermore they need to be freshly prepared.

6.4.5 Emulsion of Soybean oil in simulated gastric fluid

This part of the work investigated the effect of lipid emulsions based on Soybean oil and Tween 20, on the dissolution of the drugs. The emulsion system induced a slight increase in the solubility of both drugs. However, the dissolution rate was slightly slower compared to the surfactant solution possibly due to the slow transfer of the drugs into the fat droplets of the emulsion. The concentration of Tween 20 in all media was greater than the reported value of CMC in water.

Drug solubilisation in emulsions is through drug transfer into fat droplets and/or through surfactant micelles (Lobenberg and Amidon, 2000). Thus, the dissolution in the emulsion is similar to that in the micelle system in respect to the partition of the drug into emulsion droplets depending on the affinity of the drug to the interior phase which is Soybean oil in the present case (McNamara et al., 2000).

Although the dissolution in the emulsion system is similar to dissolution in micelles, the size of the fat droplets is usually bigger than the size of the micelles resulting in different mass transfer mechanism in each system (McNamara et al., 2000). Consequently, the drug loaded in the fat droplets of the emulsion will have slower dissolution compared to the surfactant solution.

The micellar concentration in the emulsion is likely to be less than that in a surfactant solution which contains an identical amount of the surfactant because part of the surfactant surrounds the fat droplets. Therefore, the dissolution comparison with medium containing surfactant alone is probably not very accurate and an underestimation of the effect of fat on drug solubilisation.

6.5 Conclusion

The endogenous surfactants employed in biorelevant concentrations increased the dissolution of the assessed drug significantly. Such an effect was more pronounced in media representing the fed state than the fasting state which is in accordance with clinical studies (Zimmermann et al., 1994). Hence, the obtained results enforce the importance of the fed state in dissolving the poorly soluble drugs due to higher levels of bile secretion and lipids and increase the pH of the intestinal fluid.

A synergistic solubilizing effect for itraconazole and ketoconazole could possibly be generated using a certain amount of synthetic surfactants as an alternative to the bile components. The proposed media based on SDS and CTAB allowed a considerable simplification since they were easily prepared and based on the use of an inexpensive surfactant. Although these media did not provide identical dissolution to that in the frequently used simulated intestinal fluid containing natural surfactants, they could provide quantification of drug behaviour in simulated intraluminal fluids. These studies need to be expanded to include the dissolution of drug formulations in the proposed media which contained synthetic surfactants to see whether they could substitute FaSSIF and FeSSIF so these media can possibly be used in routine quality control tests and substitute the costly bile components based media.

Chapter 7: General discussion & conclusion

7.1 Discussion

Dissolution of drugs is the rate-limiting step for absorption of Class II drugs; therefore, it is an important factor to consider when developing new drug entities and during subsequent stages of formulation. The *in vivo* relevance to *in vitro* dissolution could be achieved by simulating the conditions prevailing in the GI tract. Compendial media are still simple media and so are not expected to distinguish between the fed and fasting conditions. Therefore, the use of biorelevant media that simulate post and pre-prandial condition is essential.

7.1.1 Solubility versus dissolution

The solubility of itraconazole and ketoconazole in various media was found to be the most important determinant of the dissolution rate. Sink conditions could not be maintained in itraconazole dissolution experiments which explains the general profile obtained for itraconazole dissolution, starting with a relatively fast dissolution which soon levelled off indicating that saturation solubility was reached. On the other hand, ketoconazole dissolution profiles were likely to be linear, in particular in acidic media, due to the higher solubility of ketoconazole at low pH.

In cases of micelle-facilitated and emulsion-facilitated dissolution, the dissolution was not driven by solubility since a more pronounced enhancement in the solubility was observed, compared to the increase in the dissolution. These findings came in accordance with previous studies when the increase in dissolution in complex media of human and dog intestinal fluids (Persson et al., 2005) or in media containing synthetic surfactants (Balakrishnan et al., 2004) was less than that predicted from solubility. This shows the value in determining both parameters: solubility and dissolution rate, as the determination of the solubility alone may not allow accurate speculation of the dissolution rate. To further prove this point, evaluation of the diffusion constants for the free drug and drug loaded in micelles are required for each experiment.

7.1.2 Simulated gastric media

The dissolution of both drugs increased when the pH of the medium was highly acidic (pH 1.2) and a further increase was observed in the presence of pepsin. This could be

correlated to the pharmacokinetic data where drug bioavailability decreased by reducing the stomach acidity (Section 1.10.4.1). For the pre-prandial stomach, the simulation was based on mimicking the surface tension of the stomach using bile salt and lecithin or media containing synthetic surfactants. Both cases exhibited a significant enhancement in the dissolution of the drugs. FaSSGF was previously found to be a good surrogate for the gastric medium representing fasting conditions (Jantratid et al., 2008a) and subsequently the dissolution in this medium was expected to be reasonably similar to gastric medium in pre-prandial conditions. The use of simulating gastric simplified formulations containing 0.25% SDS, 0.1% Triton X-100 and 0.02% Tween 20 (w/v) in SGF did not provide similar dissolution profiles to FaSSGF rendering these media inappropriate for the simulation of gastric fluid.

For fed stomach simulation, the composition of gastric fluid varies according to the ingested meal (Nicolaides et al., 1999). Hence, there was no specific medium that could mimic the fed stomach fluid. Therefore, a selection of media containing food components was assessed. Milk mixed with SGF (pH 1.2) (50:50) was considered as a reasonable surrogate to common food as it contains all the main nutrients. The solubility and dissolution of both drugs was enhanced in milk-containing media. The mechanism of this influence was attributed to the entire complex structure of milk since the main components of milk; casein and lactose did not induce the same discernible effect. The mechanism of the solubilising effect of milk was proposed to be through drug binding as indicated by the dialysis data. This supported the previous findings of Macheras et al. (1990) where milk was found to bind to drugs.

The second direction of the studies was towards evaluating the effect of simpler media composed of a single protein. Consequently, the dissolution in media containing egg albumin in various concentrations was evaluated. The inclusion of albumin in the media induced an important effect on the solubility and dissolution of both drugs. This effect was attributed to drug-albumin complex formation as indicated by protein binding studies including dialysis and spectroscopic analysis. Gelatin presence in the medium also increased the solubility and dissolution of both drugs; however, no evidence of protein binding was obtained. The effect of gelatin-containing solutions was possibly due to its moderate surface activity which enhanced the wettability of the drug surfaces. Gluten inclusion did not induce a significant effect whereas casein slightly increased the dissolution. The effect of casein on drug dissolution was probably due to the decrease in the contact angle with the drug surface and, with its specific structure of sub-micelles

units, it could solubilise drug molecules. Thus, it was concluded that protein-containing media enhanced both solubility and dissolution of itraconazole and ketoconazole. Many factors accounted for this effect: complex formation, increase in the wettability and solubilisation into casein units.

The effect of the inclusion of simpler additives such as sugars and amino acids on the dissolution of drugs was studied. Media containing carbohydrates, namely, glucose, lactose and starch did not exert any distinct effect on dissolution. The slight effect observed was probably due to interactions through hydrogen bonds. Amino acids slightly increased the dissolution of itraconazole, and in particular, neutral amino acids such as GLY and ALA. However, more interesting results were acquired with ketoconazole as the dissolution increased markedly with the inclusion of 1%, w/v neutral amino acids.

The effect of emulsions on the dissolution of the drugs was evaluated in media of diluted Soybean emulsions using Tween 20 as an emulsifier. The dissolution in the emulsion was similar to that observed in media containing Tween 20 alone at the same concentrations. This suggested that emulsified fat did not affect the dissolution. However, this comparison did not seem very important due to the different solubilising capacity of Tween 20 in the presence and absence of fat. It could be concluded from the study in media containing emulsion or the three types of milk (varied in fat content) that there was no clear evidence of fat affecting drug solubility and dissolution.

The data acquired from dissolution in the presence of food components revealed the importance of dissolution tests in biorelevant media in predicting the effect of food. Thus, a speculation based on the *in vitro* results obtained would be if a patient takes his medication with a glass of milk and/or with protein and amino acid-rich diet such as eggs, then the dissolution of the drug *in the stomach* will increase subsequently leading to more absorption and greater bioavailability. This means that patients may get variable bioavailability depending on their diet.

These findings need to be taken further to investigate whether the dissolution of oral dosage formulations of itraconazole and ketoconazole will be similarly affected by food components. With the formulations, excipients can affect the dissolution process of the dosage forms and probably interfere with the additives in the dissolution medium which may in turn limit or increase the effect of the additives on the dissolution of the drugs. For instance, Shah (2006) reported that the dissolution of ibuprofen dosage forms

increased in the presence of casein however the IDR of the ibuprofen remained unaffected. Accordingly, the effect of casein was attributed to interactions between casein and excipients in the formulation.

The salt and crystal form of the drug in the dosage form can affect the dissolution and so the presence of food may influence the dissolution of the formulated drug differently from the intrinsic pure drug.

7.1.3 Intestinal fluid simulation

The solubility of itraconazole and ketoconazole increased in the fed state compared to the fasted state due to an increase in bile and phospholipids levels. Dissolution in media containing NaTC alone at sub-micellar levels revealed that the mechanism of increase in dissolution was due to an increase in wettability, whereas above the CMC both solubilisation and wettability accounted for the enhancement in dissolution. Dissolution did not increase to same extent as solubility in media containing surfactants in concentrations above the CMC. This was probably due to the low diffusivity of drug-loaded in micelles.

Dissolution in FaSSIF and FeSSIF reported good IVIVC (Nicolaides et al., 1999), however, the preparation of these media is costly and time consuming. SDS and CTAB could possibly be used to replace natural surfactants. The proposed media in the current investigation were: 0.02%, w/v SDS in SIF (pH 6.8), 0.005-0.05%, w/v CTAB in SIF (pH 6.8) to mimic FaSSIF (pH 6.5) and (0.05-0.1%), w/v SDS in blank FeSSIF (pH 5) to mimic FeSSIF. These media did not provide identical dissolution profiles for itraconazole and ketoconazole to that found in FaSSIF and FeSSIF. However, approximate simulation was achieved. Thus, these simplified media can be used to predict drug dissolution in intestinal fluids as alternatives to bile salt and lecithin containing-media. Further investigations need to be conducted on drug formulations in these media and if the dissolution of formulations complies with IDR data, the use of simplified media could be generalized to quality tests, lot-to-lot uniformity and bio-equivalency.

The pharmacokinetic studies conducted in humans found that the bioavailability of itraconazole and ketoconazole increased in the post-prandial state. For example, the bioavailability of itraconazole capsules was reduced by 40% when it was administered under the fasting state (Heykants et al., 1989). Ketoconazole bioavailability increased

when tablets were taken with a standard breakfast (Gascoigne et al., 1981). 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 postprandial stage due to longer gastric retention and the high fat content of the co-ingested meal. The data presented here support 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 extending gastric residence time with food co-ingestion, the potential solubilisation of the lipophilic drugs by the fat in the food combined with the increase in bile secretion, the proteins in the diet might also promote the dissolution of the drugs and consequently their rate and extent of absorption

7.1.4 Itraconazole versus ketoconazole

Although both drugs have azole structures, differences were observed in their dissolution behaviours. The different physiochemical properties of the drugs, in particular, pKa and log P had an important impact on their solubility. Itraconazole is more lipophilic, extremely poorly soluble and can be ionised at pH < 4.6 where as ketoconazole is relatively more soluble and can be ionised over a wider range of pH values < 7.5. Accordingly, these variations led to different solubilities and interactions with the additives and consequently different dissolution patterns of the two drugs. In general, the same positive effect of the dissolution media containing food or surfactants on the dissolution of itraconazole and ketoconazole was observed, however, the extent of the effect varied between the two drugs.

7.1.5 Physical properties of the dissolution media

The change in the composition of the media led to a change in their measured physical properties. In general, the viscosity of the assessed media did not show important variations, apart from media containing milk, albumin, and gelatin. Although a negative correlation was expected between the viscosities of the media and initial dissolution rates of the drug, this was not apparent. This could be explained by the higher solubilising capacity of the media with higher viscosity, represented by the protein-containing media, which in turn compensated for the increase in the viscosity.

Surface tension was an important determinate of dissolution and solubility. Media with high surface activity exhibited high solubilising capacity of the drugs in addition to enhancing the wettability.

This investigation was concerned with evaluating the viscosity and surface tension. However, further factors could also have an impact on the dissolution rate such as buffer capacity and osmolality. Therefore, the effect of these factors on the dissolution needs to be determined in future work.

7.2 Concluding remarks

The increased dissolution of the two assessed azole anti-fungal drugs in the presence of the investigated dietary and bile components more than likely accounted for the reported increases in their bioavailability when co-administered with meals. Consequently, biorelevant media based on food constituents and endogenous surfactants are recommended for the prediction of the behaviour of the drugs in the GIT as they appear as more realistically representative of the intraluminal fluids than the simple compendial dissolution media. Such media have the potential to reflect changes in the performance of BCS Class II drugs due to fed and fasting conditions and, even more specifically, due to the type of the meal which in turn can affect drug bioavailability. Clinicians can benefit from such information when prescribing drugs to achieve the desired bioavailability and therapeutic efficacy.

The investigating of the mechanism contributing to the effect of food additives suggested the interactions between additives and drug as a possible reason for drug solubilisation. Other factors also contributed like the increase in the wettability of the drug surface. These studies need to be further expanded to elucidate the mechanism on a molecular level and include all the components added to the dissolution media in the present investigation.

The replacement of the bile components with the synthetic surfactants did not provide identical dissolution of the drugs but indicated that these media can be employed within the proposed concentrations to give an insight to the effect of the endogenous surfactants.

7.3 Recommendations for future work

The following recommendations are suggested for future work:

- This research was concerned with studying the intrinsic dissolution of pure itraconazole and ketoconazole however it is also important to study the dissolution of dosage forms in biorelevant media containing food constituents. If the media induced the same effect observed with the pure drug then it is of value to expand the studies to the clinical level to evaluate drug absorption and bioavailability when co-ingested with meals or drinks containing the studied additives. Such information is useful for drug prescription regarding the time and type of the administrated meal.

- It is worthy to extend the study to include further azole anti-fungal drugs and moreover, include drug substances of different physical and chemical properties such as basic, acidic and neutral drugs of different solubilities and of various BCS Classes.

- The dissolution in conditions more akin to the *in vivo* environment is expected to provide better IVIVC. Literature showed some differences between the FeSSIF and human intestinal fluid such as the lack of lipolytic products in the former, which could affect the dissolution of lipophilic drugs. Consequently, the dissolution and solubility of the drugs need to be assessed in modified media containing fatty acids and monoglycerides. Furthermore, to provide an even more accurate picture about the dissolution *in vivo*, human aspirate fluids representing fed and fasting states could be employed and so a comparison with dissolution in the simulated media could be carried out.

- Studies performed to investigate the mechanism of drug solubilisation need to be further expanded using different techniques. For example, Circular Dichroism can allow the monitoring of protein conformational changes due to drug binding on the molecular level. Micro-calorimetery may detect the thermodynamic differences that occur due to the interactions between food components and drug. Solid dispersions of drug-protein can be prepared and analysed using thermal analysis and PXRD to elucidate of the mechanism of the interactions. Light scattering methods can be employed to detect the formation of casein micelles.

- Trials to develop simplified dissolution media based on synthetic surfactants as alternatives to the biorelevant media containing endogenous surfactants can be expanded to include other surfactants. Simulation of the physicochemical properties is a vital issue that needs to be considered.

- Since food additives such as milk, egg albumin and gelatin increased the solubility of itraconazole and ketoconazole, it might be useful to employ these substances in developing drug formulations. The use of such natural vehicles in appropriately designed formulations may enhance the solubility of the poorly soluble drugs and provide safe and cheap alternative for the synthetic polymers. *In vitro* and *in vivo* studies need to be performed to validate the potential formulations.

REFERENCES

Abdou, H. M. (1989a). Effect of the test parameters on dissolution rate, In *Dissolution, Bioavailability and Bioequivalence*, Mack Publishing Co., Easton, PA, pp. 145-170.

Abdou, H. M. (1989b). Theory of dissolution, In *Dissolution, Bioavailability and Bioequivalence*, Mack Publishing Co., Easton, PA, pp. 11-36.

Abrahamsson, B., Albery, T., Eriksson, A., Gustafsson, I. and Sjoberg, M., (2004). Food effects on tablet disintegration, *European Journal of Pharmaceutical Sciences,* 22, 165-172.

Aburub, A., Risley, D. S. and Mishra, D., (2008). A critical evaluation of fasted state simulating gastric fluid (FaSSGF) that contains sodium lauryl sulfate and proposal of a modified recipe, *International Journal of Pharmaceutics,* 347, 16-22.

Acarturk, F., Kislal, O. and Celebi, N., (1992). The effect of some natural polymers on the solubility and dissolution characteristics of nifedipine, *International Journal of Pharmaceutics,* 85, 1-6.

Aiache, J. M., Aoyagi, N., Blume, H., Dressman, J., Friedel, H. B. and Grady, I. T., (1997). FIP guidelines for dissolution testing of solid oral products, *Dissolution Technologies,* 4, 5–13.

Alsenz, J. and Kansy, M., (2007). High throughput solubility measurement in drug discovery and development, *Advanced Drug Delivery Reviews,* 59, 546-567.

Amidon, G. L., Lennernaes, H., Shah, V. P. and Crison, J. R., (1995). A theoretical basis for a biopharmaceutic drug classification: the correlation of *in vitro* drug product dissolution and *in vivo* bioavailability, *Pharmaceutical Research,* 12, 413-420.

Anwar, S., Fell, J. T. and Dickinson, P. A., (2005). An investigation of the disintegration of tablets in biorelevant media, *International Journal of Pharmaceutics,* 290, 121-127.

Ashby, L. J., Beezer, A. E. and Buckton, G., (1989). *In vitro* dissolution testing of oral controlled release preparations in the presence of artificial foodstuffs. I: Exploration of alternative methodology: microcalorimetry, *International Journal of Pharmaceutics,* 51, 245-251.

Aulton, M. E. (2002). Dissolution and solubility, In *Pharmaceutics: The Science of Dosage Form Design*, (Ed, Aulton, M. E.), Churchill Livingstone, Edinburgh, pp. 15-32.

Avdeef, A., (2007). Solubility of sparingly-soluble ionizable drugs, *Advanced Drug Delivery Reviews,* 59, 568-590.

Bakatselou, V., Oppenheim, R. C. and Dressman, J., (1991). Solubilization and wetting effects of bile salts on the dissolution of steroids, *Pharmaceutical Research,* 8, 1461-1469.

Balakrishnan, A., Rege, B., Amidon, G. L. and Polli, J. E., (2004). Surfactant-mediated dissolution: contributions of solubility enhancement and relatively low micelle diffusivity, *Journal of Pharmaceutical Sciences,* 93, 2064-2075.

Banakar, U. V. (1992a). Dissolution testing devices, In *Pharmaceutical Dissolution Testing*, Marcel Dekker, New York, USA, pp. 53-105.

Banakar, U. V. (1992b). Factors that influence dissolution testing, In *Pharmaceutical Dissolution Testing*, Marcel Dekker, New York, USA, pp. 133-187.

Barone, J. A., Koh, J. G., Bierman, R. H. and Colaizzi, J. L., (1993). Food interaction and steady-state pharmacokinetics of itraconazole capsules in healthy male volunteers, *Antimicrobial Agents and Chemotherapy,* 37, 778-784.

Barrett, K. E. (2006a). Intestinal secretory functions, In *Gastrointestinal Physiology*, Lange Medical Books/McGraw-Hill, New York, pp. 37-118.

Barrett, K. E. (2006b). Motility, In *Gastrointestinal Physiology*, Lange Medical Books/ McGraw-Hill, New York, pp. 120-166.

Blum, R. A., D'Andrea, D. T., Florentino, B. M., Wilton, J. H., Hilligoss, D. M., Gardner, M. J., Henry, E. B., Goldstein, H. and Schentag, J. J., (1991). Increased gastric pH and the bioavailability of fluconazole and ketoconazole, *Annals of Internal Medicine,* 114, 755-757.

Boni, J. E., Brickl, R. S. and Dressman, J. B., (2007). Is bicarbonate buffer suitable as a dissolution medium?, *Journal of Pharmacy and Pharmacology,* 59, 1375-1382.

Boogaerts, M. and Maertens, J., (2001). Clinical experience with itraconazole in systemic fungal infections, *Drugs,* 61, 39-47.

BP, (2008). British Pharmacopoeia 2008, The Stationary Office, London, http://www.pharmacopoeia.co.uk/, accessed on March 2008.

Bradford, M. M., (1976). A rapid and sensitive for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, *Analytical Biochemistry,* 72, 248-254.

Brewster, M., Neeskens, P. and Peeters, J., (2007). Solubilization of itraconazole as a function of cyclodextrin structural space, *Journal of Inclusion Phenomena and Macrocyclic Chemistry,* 57, 561-566.

Brittain, H. G. (1999a). Effects of polymorphism and solid-state solvation on solubility and dissolution rate, In *Polymorphism in Pharmaceutical Solids*, (Ed, Brittain, H. G.), Marcel Dekker, Inc., New York, pp. 280-325.

Brittain, H. G. (1999b). Grinding, In *Polymorphism in Pharmaceutical Solids*, (Ed, Brittain, H. G.), Marcel Dekker, Inc., New York, pp. 202.

Brittain, H. G. (1999c). Methods for the characterization of polymorphs and solvates, In *Polymorphism in Pharmaceutical Solids*, (Ed, Brittain, H. G.), Marcel Dekker, Inc., New York, pp. 228-278.

Brittain, H. G. (2007). Ionic equilibria and the pH dependence of solubility, In *Solvent Systems and Their Selection in Pharmaceutics and Biopharmaceutics*, (Eds, Augustijns, P. and Brewster, M. E.), Springer Science & Business Media, New York, pp. 29-53.

Bruner, L. and Tolloczko, S., (1900). On the velocity of solution of solid bodies, *Zeitschrift für Physikalische Chemie,* 35, 283–290.

Buckton, G., Beezer, A. E., Chatham, S. M. and Patel, K. K., (1989). In vitro dissolution testing of oral controlled release preparations in the presence of artificial foodstuffs. II. Probing drug/food interactions using microcalorimetry, *International Journal of Pharmaceutics,* 56, 151-157.

Campbell, J. A., Chapman, D. G. and Chatten, L. G., (1957). Timed disintegration medication—Need for more quantitative criteria, *Canadian Medical Association Journal,* 77, 602-603.

Carey, M. and Small, D., (1972). Micelle formation by bile salts: Physical chemical and thermodynamic considerations, *Archive of Internal Medicine,* 130, 506-533.

Cartledge, J. D., Midgely, J. and Gazzard, B. G., (1997). Itraconazole solution: higher serum drug concentrations and better clinical response rates than the capsule formulation in acquired immunodeficiency syndrome patients with candidosis, *Journal of Clinical Pathology,* 50, 477–480.

Carver, P. L., Fleisher, D., Zhou, S. Y., Kaul, D., Kazanjian, P. and Li, C., (1999). Meal composition effects on the oral bioavailability of Indinavir in HIV-infected patients, *Pharmaceutical Research,* 16, 718-724.

CDER/FDA, (1997). Dissolution testing of immediate release solid dosage forms, FDA/Centre for Drug Evaluation and Research, USA. http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070237.pdf, accessed on January 2005.

Challa, R., Ahuja, A., Javed, A. and Khar, R. K., (2005). Cyclodextrins in drug delivery: an updated review, *AAPS PharmSciTech,* 6, E329-357.

Chan, H. K. and Doelker, E., (1985). Polymorphic transformation of some drugs under compression, *Drug Development and Industrial Pharmacy,* 11, 315 - 332.

Charman, W. N., Porter, C. J. H., Mithani, S. and Dressman, J. B., (1997). Physicochemical and physiological mechanisms for the effects of food on drug absorption: the role of lipids and pH, *Journal of Pharmaceutical Sciences,* 86, 269-282.

Chen, A. X., Zito, S. W. and Nash, R. A., (1994). Solubility enhancement of nucleosides and structurally related compounds by complex formation, *Pharmaceutical Research,* 11, 398-401.

Chen, L. R., Wesley, J. A., Bhattachar, S., Ruiz, B., Bahash, K. and Babu, S. R., (2003). Dissolution behavior of a poorly water soluble compound in the presence of Tween 80, *Pharmaceutical Research,* 20, 797-801.

Christensen, J. Ø., Kristensena, G. H. and Mullertz, A., (2004). Solubilisation of poorly water-soluble drugs during *in vitro* lipolysis of medium- and long-chain triacylglycerols, *European Journal of Pharmaceutical Sciences,* 23, 287-296.

Colton, C. and Smith, K., (1972). Mass transfer to a rotating fluid II. Transport from a base of an agitated cylindrical tank, *AIChE Journal,* 18, 958-967.

Connors, K. A. (2002). Phase transformations, In *Thermodynamics of Pharmaceutical Systems: An Introduction for Students of Pharmacy*, (Ed, Connors, K. A.), John Wiley & Sons, Inc., New Jersey, pp. 69-72.

Corrigan, O. I. and Healy, A. M., (2007). Surfactants in pharmaceutical products and systems, *Encyclopedia of Pharmaceutical Technology*, (Ed, Swarbrick, J.), Informa Healthcare, New York, 3583-3596.

Crison, J. R., Weiner, N. D. and Amidon, G. L., (1997). Dissolution media for *in vitro* testing of water-insoluble drugs: Effect of surfactant purity and electrolyte on *in vitro* dissolution of carbamazepine in aqueous solutions of sodium lauryl sulfate, *Journal of Pharmaceutical Sciences,* 86, 384-388.

Crowder, T. M., Hickey, A. J., Louey, M. D. and Orr, N. (2003). Scanning electron microscopy, In *A Guide to Pharmaceutical Particulate Sciences*, Interpharm / CRC Press, Florida, pp. 131-132.

Danckwerts, P. V., (1951). Significance of liquid-film coefficients in gas absorption, *Industrial and Engineering Chemistry,* 43, 1460-1467.

Daneshmend, T. K., Warnock, D. W., Ene, M. D., Johnson, E. M., Potten, M. R., Richardson, M. D. and Williamson, P. J., (1984). Influence of food on the pharmacokinetics of ketoconazole, *Antimicrobial Agents and Chemotherapy,* 25, 1-3.

Davis, S. S., Hardy, J. G. and Fara, J. W., (1986). Transit of pharmaceutical dosage forms through the small intestine, *Gut,* 27, 886-892.

De Beule, K., (1996). Itraconazole: pharmacology, clinical experience and future development, *International Journal of Antimicrobial Agents,* 6, 175-181.

De Bruijn, P., Kehrer, D. F. S., Verweij, P. and Sparreboom, A., (2001). Liquid chromatographic determination of ketoconazole, a potent inhibitor of CYP3A4-mediated metabolism, *Journal of Chromatography B*, 395-400.

Dinora, G.-E., Julio, R., Nelly, C., Lilian, Y.-M. and Cook, H. J., (2005). *In vitro* characterization of some biopharmaceutical properties of praziquantel, *International Journal of Pharmaceutics,* 295, 93-99.

Docoslis, A., Giese, R. F. and van Oss, C. J., (2000). Influence of the water-air interface on the apparent surface tension of aqueous solutions of hydrophilic solutes, *Colloids and Surfaces B: Biointerfaces,* 19, 147-162.

Dokoumetzidis, A. and Macheras, P., (2006). A century of dissolution research: From Noyes and Whitney to the Biopharmaceutics Classification System, *International Journal of Pharmaceutics,* 321, 1-11.

Van Dooren, A. A., (1982). Effects of heating rates and particle sizes on DSC peaks, *Analytical Proceedings,* 19, 554-556.

Dressman, J. B., Amidon, G. L., Reppas, C. and Shah, V. P., (1998). Dissolution testing as a prognostic tool for oral drug absorption: immediate release dosage forms, *Pharmaceutical Research,* 15, 11-22.

Dressman, J. B., Berardi, R. R., Dermentzoglou, L. C., Russell, T. L., Schmaltz, S. P., Barnett, J. L. and Jarvenpaa, K. M., (1990). Upper gastrointestinal GI pH in young, healthy men and women, *Pharmaceutical Research,* 7, 756-761.

Dressman, J. B. and Reppas, C., (2000). *In vitro - in vivo* correlations for lipophilic, poorly water-soluble drugs, *European Journal of Pharmaceutical Sciences,* 11(suppl. 2), 73-80.

Dressman, J. B., Vertzoni, M., Goumas, K. and Reppas, C., (2007). Estimating drug solubility in the gastrointestinal tract, *Advanced Drug Delivery Reviews,* 59, 591-602.

Dyas, A. M. and Shah, U. U. (2007). Dissolution and dissolution testing, In *Encyclopedia of Pharmaceutical Technology*, (Ed, Swarbrick, J.), Informa Healthcare, New York, pp. 908 - 928.

Efentakis, M. and Dressman, J. B., (1998). Gastric juice as a dissolution medium: Surface tension and pH, *European Journal of Drug Metabolism and Pharmacokinetics,* 23, 97-102.

El-Arini, S. K., Shiu, G. K. and Skelly, J. P., (1989). An *in vitro* study of possible food-drug interactions of the controlled-release propranolol products, *International Journal of Pharmaceutics,* 55, 25-30.

El-Arini, S. K., Shiu, G. K. and Skelly, J. P., (1990). Theophylline-controlled release preparations and fatty food: an *in vitro* study using the rotating dialysis cell method, *Pharmaceutical Research,* 7, 1134-1140.

ESDU, (1980). Thermophysical properties of heat pipe working fluids. Data Item No.80017, *ESDU International plc.*, 1-25.

Farraj, N., F., Davis, S., S., Parr, G., D. and Stevens, H. N. E., (1989). Modification of the aqueous solubility and stability of progabide, *International Journal of Pharmaceutics,* 52, 11-18.

FDA, (2000). Guidance for industry, waiver of *in vivo* bioavailability and bioequivalence studies for immediate release solid oral dosage forms based on a Biopharmaceutics Classification System, FDA/Centre for Drug Evaluation and Research.http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoy Information/Guidances/ucm070246.pdf, accessed on January 2005.

FDA, (2003). Guidance for industry, bioavailability and bioequivalence studies for orally administered drug products—general considerations, FDA/Centre for

Drug Evaluation and Research. Accessed on line: http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm070124.pdf, accessed on January 2005.

Fell, J. (2002). Surface tension and surface free energy, In *Pharmaceutics: The Science of Dosage Form Design*, (Ed, Aulton, M. E.), Churchill Livingstone, Edinburgh, pp. 59-65.

Filleborn, V. M., (1948). A new approach to tablet disintegration testing, *American Journal of Pharmacy and the Sciences Supporting Public Health,* 120, 233-255.

Finholt, P. and Solvang, S., (1968). Dissolution kinetics of drugs in human gastric juice-the role of surface tension, *Journal of Pharmaceutical Sciences,* 57, 1322-1335.

Fleisher, D., Li, C., Zhou, Y., Pao, L. H. and Karim, A., (1999). Drug, meal and formulation interactions influencing drug absorption after oral interactions administration: clinical implications, *Clinical Pharmacokinetics,* 36, 233-254.

Ford, J. L. and Timmins, P. (1989a). Factors influencing DSC and DTA scans, In *Pharmaceutical Thermal Analysis: Techniques and Applications*, Ellis Horwood, Chichester, pp. 76-82.

Ford, J. L. and Timmins, P. (1989b). Information derived from thermal analytical data, In *Pharmaceutical Thermal Analysis: Techniques and Applications*, Ellis Horwood, Chichester, pp. 25-27.

Fox, P. F. and McSweeney, P. L. H. (1998a). Functional (physiochemical) properties of caseins, In *Dairy Chemistry and Biochemistry*, Kluwer Academic/ Plenum Publishers, New York, pp. 218-219.

Fox, P. F. and McSweeney, P. L. H. (1998b). Stability of the milk fat emulsion, In *Dairy Chemistry and Biochemistry*, Kluwer Academic / Plenum Publishers, New York, pp. 104-108.

Fromtling, R. A., (1988). Overview of medically important antifungal azole derivatives, *Clinical Microbiology Reviews,* 1, 187-217.

Galia, E., Horton, J. and Dressman, J. B., (1999). Albendazole generics - a comparative *in vitro* study, *Pharmaceutical Research,* 16, 1871-1875.

Galia, E., Nicolaides, E., Hoerter, D., Loebenberg, R., Reppas, C. and Dressman, J. B., (1998). Evaluation of various dissolution media for predicting *in vivo* performance of class I and II drugs, *Pharmaceutical Research,* 15, 698-705.

Gascoigne, E. W., Barton, G. J., Michaels, M. and Meuldermans, W., (1981). The kinetics of ketoconazole in animals and man, *Clinical Research Reviews,* 1, 177-187.

Giron, D., (1995). Thermal analysis and calorimetric methods in the characterisation of polymorphs and solvates, *Thermochimica Acta*, 248, 1-59.

Glomme, A., Marz, J. and Dressman, J. B., (2005). Comparison of a miniaturized shake-flask solubility method with automated potentiometric acid/base titrations and calculated solubilities, *Journal of Pharmaceutical Sciences,* 94, 1-16.

Gong, Y., Grant, D. J. and Brittain, H. G. (2007). Solubility of substances in various solid-state forms, In *Solvent Systems and Their Selection in Pharmaceutics and Biopharmaceutics*, (Eds, Augustijns, P. and Brewster, M. E.), Springer Science & Business Media, New York, pp. 16-19.

Grant, D. J. W. (1999). Theory and origin of polymorphism, In *Polymorphism in Pharmaceutical Solids*, (Ed, Brittain, H. G.), Marcel Dekker, Inc., New York, pp. 1-34.

Gray, V., Kelly, G., Xia, M., Butler, C., Thomas, S. and Mayock, S., (2009). The science of USP 1 and 2 dissolution: present challenges and future relevance, *Pharmaceutical Research*, 26, 1289-1302.

Guo, Q.-L., Li, R., Zhou, X. and Liu, Y., (2008). Study on the interaction of ketoconazole with human and bovine serum albumins by fluorescence Spectroscopy, *Chinese Journal of Chemistry*, 26, 2207-2215.

Hancock, B. C. and Parks, M., (2000). What is the true solubility advantage for amorphous pharmaceuticals?, *Pharmaceutical Research*, 17, 397-404.

Hanson, W. A. (1991). Solving practical problems, In *Handbook of Dissolution Testing*, Pharmaceutical Technology Publishing, Oregon, pp. 111-123.

Heykants, J., Van Peer, A., Van de Velde, V., Van Rooy, P., Meuldermans, W., Lavrijson, K., Woestenborghs, R., Van Cutsem, J. and Gauwenbergh, G., (1989). The clinical pharmacokinetics of itraconazole: an overview, *Mycoses*, 32 (suppl 1), 67-87.

Higuchi, T. and Connors, K. A., (1965). Phase-solubility techniques, *Advanced in Analytical Chemistry and Instrumentation*, 4, 117–212.

Higuchi, W., (1967). Diffusional models useful in biopharmaceutics, *Journal of Pharmaceutical Sciences*, 56, 315-324.

Higuchi, W. I., (1964). Effects of interacting colloids on transport rates, *Journal of Pharmaceutical Sciences*, 53, 532-535.

Hixson, A. W. and Crowell, J. H., (1931). Dependence of reaction velocity upon surface and agitation, *Industrial Engineering Chemistry Research*, 23, 923-931.

Hoesley, C. and Dismukes, W. E., (1997). Overview of oral azole drugs as systemic antifungal therapy, *Seminars in Respiratory Critical Care Medicine*, 18, 301-309.

Horter, D. and Dressman, J. B., (2001). Influence of physicochemical properties on dissolution of drugs in the gastrointestinal tract, *Advanced Drug Delivery Reviews*, 46, 75-87.

Hsu, J.-P. and Nacu, A., (2003). Behavior of soybean oil-in-water emulsion stabilized by nonionic surfactant, *Journal of Colloid and Interface Science*, 259, 374-381.

Hunter, E., Fell, J. T. and Sharma, H., (1982). The gastric emptying of pellets contained in hard gelatin capsules, *Drug Development and Industrial Pharmacy*, 8, 751—757.

ICH, (2005). International Conference on Harmonisation. Harmonized Tripartite Guideline ICH Q2 (R1). Validation of analytical procedures: text and methodology, http://www.ich.org/LOB/media/MEDIA417.pdf, accessed on September 2008.

Imai, T., Nishiyama, T., Ueno, M. and Otagiri, M., (1989a). Enhancement of the dissolution rates of poorly water-soluble drugs by water-soluble gelatin, *Chemical and Pharmaceutical Bulletin,* 37, 2251-2252.

Imai, T., Nodomi, K., Shameem, M., Matsumoto, H., Satoh, T. and Otagiri, M., (1997). Mutual effect of egg albumin and fatty acids on bioavailability of dl-[alpha]-tocopherol, *International Journal of Pharmaceutics,* 155, 45-52.

Imai, T., Nohdomi, k., Acartürk, F. and Otagiri, M., (1991). Enhancement of dissolution and absorption of mefenamic acid by egg albumin, *Journal of Pharmaceutical Sciences,* 80, 484-487.

Imai, T., Saito, Y., Matsumoto, H., Satoh, T. and Otagiri, M., (1989b). Influence of egg albumin on dissolution of several drugs, *International Journal of Pharmaceutics,* 53, 7-12.

Jamzad, S. and Fassihi, R., (2006). Role of surfactant and pH on dissolution properties of fenofibrate and glipizide—A technical note, *AAPS PharmSciTech*, E1-E6.

Jantratid, E., Janssen, N., Chokshi, H., Tang, K. and Dressman, J. B., (2008a). Designing biorelevant dissolution tests for lipid formulations: Case example - Lipid suspension of RZ-50, *European Journal of Pharmaceutics and Biopharmaceutics,* 69, 776-785.

Jantratid, E., Janssen, N., Reppas, C. and Dressman, J., (2008b). Dissolution media simulating conditions in the proximal human gastrointestinal tract: an update, *Pharmaceutical Research,* 25, 1663-1676.

Johnson, B. F., Lindenbaum, J., Budnitz, E. and Marwaha, J., (1986). Variability of steady-state digoxin kinetics during administration of tablets or capsules, *Clinical Pharmacology and Therapeutics,* 39, 306-312.

Junginger, H. E., Verhoeven, J. and Peschier, J. C., (1990). A new *in vitro* model to detect interactions between controlled release dosage forms and food, *Acta Pharm Technol,* 36, 155-160.

Kalantzi, L., Goumas, K., Kalioras, V., Abrahamsson, B., Dressman, J. and Reppas, C., (2006a). Characterization of the human upper gastrointestinal contents under conditions simulating bioavailability/bioequivalence studies, *Pharmaceutical Research,* 23, 165-176.

Kalantzi, L., Persson, E., Polentarutti, B., Abrahamsson, B., Goumas, K., Dressman, J. and Reppas, C., (2006b). Canine intestinal contents vs. simulated media for the assessment of solubility of two weak bases in the human small intestinal contents, *Pharmaceutical Research,* 23, 1373-1381.

Kalantzi, L., Reppas, C., Dressman, J. B., Amidon, G. L., Junginger, H. E., Midha, K. K., Shah, V. P., Stavchansky, S. A. and Barends, D. M., (2006c). Biowaiver

monographs for immediate release solid oral dosage forms: acetaminophen (paracetamol). *Journal of Pharmaceutical Sciences,* 95, 4-14.

Kallinteri, P. and Antimisiaris, S., (2001). Solubility of drugs in the presence of gelatin: effect of drug lipophilicity and degree of ionization, *International Journal of Pharmaceutics,* 221, 219-226.

Kaplan, S. A. (1974). Biological implications of *in vitro* dissolution testing, In *Dissolution technology*, (Eds, Leeson, L. and Carstensen, J.), Academy of Pharmaceutical Sciences, Washington, pp. 163-186.

Khoury, N., Mauger, J. W. and Howard, S., (1988). Dissolution rate studies from a stationary disk/rotating fluid system, *Pharmaceutical Research,* 5, 495-500.

Kiehm, K. and Dressman, J. B., (2008). Evaluation of drug adsorption to membrane filters under biowaiver test conditions, *Dissolution Technologies,* 15, 13-17.

Klein, S., Butler, J., Hempenstall, J. M., Reppas, C. and Dressman, J. B., (2004). Media to simulate the postprandial stomach I. Matching the physicochemical characteristics of standard breakfasts, *Journal of Pharmacy and Pharmacology,* 56, 605-610.

Koseki, T., Kitabatake, N. and Doi, E., (1988). Conformational changes in ovalbumin at acid pH, *The Journal of Biochemistry,* 103, 425-430.

Kostewicz, E. S., Brauns, U., Becker, R. and Dressman, J. B., (2002). Forecasting the oral absorption behavior of poorly soluble weak bases using solubility and dissolution studies in biorelevant media, *Pharmaceutical Research,* 19, 345-349.

Kostewicz, E. S., Wunderlich, M., Brauns, U., Becker, R., Bock, T. and Dressman, J. B., (2004). Predicting the precipitation of poorly soluble weak bases upon entry in the small intestine, *Journal of Pharmacy and Pharmacology,* 56, 43-51.

Kramer, J., Grady, L. T. and Gajendran, J. (2005). Historical development of dissolution testing, In *Pharmaceutical dissolution testing*, (Eds, Dressman, J. and Kramer, J.), Taylor & Francis Group, Florida, pp. 1-37.

Kress-Rogers, E. and Brimelow, C. J. B. (2001). Vibrational viscometers, In *Instrumentation and Sensors for the Food Industry*, (Eds, Kress-Rogers, E. and Brimelow, C. J. B.), Woodhead Publishing, Cambridge, pp. 416-418.

Kristensen, D., Jensen, P. Y., Madsen, F. and Bird, K. S., (1997). Rheology and surface tension of selected processed dairy fluids: Influence of temperature, *Journal of Dairy Sciences,* 80, 2282-2290.

Kukura, J., Baxter, J. L. and Muzzio, F. J., (2004). Shear distribution and variability in the USP Apparatus 2 under turbulent conditions, *International Journal of Pharmaceutics,* 279, 9-17.

Lake-Bakaar, G., Tom, W., Gupta, N., Lake-Bakaar, D., Beidas, S., Elsakr, M. and Strauss, E. W., (1988). AIDS gastropathy: reversible ketoconazole malabsorption, *Gastroenterology,* 94, 247.

Lange, D., Pavao, J. H., Wu, J. and Klausner, M., (1997). Effect of a cola beverage on the bioavailability of itraconazole in the presence of H2 blockers, *Journal of Clinical Pharmacology,* 37, 535-540.

Lauwo, J. A. K., (1985). Effect of Particle Size and excipients on the dissolution rate of metronidazole from solid dosage forms: I, *Drug Development and Industrial Pharmacy,* 11, 1565-1576.

Lee, S. H., Nam, K., Kim, M., Wook Jun, S., Park, J., Woo, J. and Hwang, S., (2005). Preparation and characterization of solid dispersions of itraconazole by using aerosol solvent extraction system for improvement in drug solubility and bioavailability, *Archives of Pharmacal Research,* 28, 866-874.

Lennernas, H. and Abrahamsson, B., (2005). The use of biopharmaceutic classification of drugs in drug discovery and development: current status and future extension, *Journal of Pharmacy and Pharmacology,* 57, 273-285.

Levich, V. G., (1962). *Physicochemcial Hydrodynamics,* Prentice Hall, Englewood Cliffs, pp. 57-78.

Levy, G., (1963). Effect of certain tablet formulation factors on dissolution rate of the active ingredient I. Importance of using appropriate agitation intensities for *in vitro* dissolution rate measurements to reflect *in vivo* conditions, *Journal of Pharmaceutical Sciences,* 52, 1039-1046.

Levy, G. and Hayes, B. A., (1960). Physicochemical basis of the buffered acetylsalicylic acid controversy, *The New England Journal of Medicine,* 262, 1053-1058.

Levy, G. and Sahli, B. A., (1962). Comparison of the gastrointestinal absorption aluminum acetylsalicylate and acetylsalicylic acid in man, *Journal of Pharmaceutical Sciences,* 51, 58-62.

Lewis, R. E. (2002). Pharmacokinetic optimization of itraconazole therapy, In *The Aspergillus Website*, Fungal research trust, www.aspergillus.org.uk/secure/articles/itraconRLewis/Itra_PK_revised, accessed on January 2007.

Li, S., Wong, S., Sethia, S., Almoazen, H., Joshi, Y. M. and Serajuddin, A. M., (2005). Investigation of solubility and dissolution of a free base and two different salt forms as a function of pH, *Pharmaceutical Research,* 22, 628-635.

Lide, D. R. (2002). In *Handbook of Chemistry and Physics*, CRC Press, Boca Raton, pp. 8(64-66).

Lim, L.-Y. and Go, M.-L., (2000). Caffeine and nicotinamide enhances the aqueous solubility of the antimalarial agent halofantrine, *European Journal of Pharmaceutical Sciences,* 10, 17-28.

Lindenberg, M., Kopp, S. and Dressman, J. B., (2004). Classification of orally administered drugs on the World Health Organization model list of essential medicines according to the biopharmaceutics classification system, *European Journal of Pharmaceutics and Biopharmaceutics,* 58, 265-278.

Lindenberg, M., Wiegand, C. and Dressman, J. B., (2005). Comparison of the adsorption of several drugs to typical filter materials, *Dissolution Technologies*, 22-25.

Liu, Y. and Guo, R., (2008). pH-dependent structures and properties of casein micelles, *Biophysical Chemistry*, 136, 67-73.

Lobenberg, R. and Amidon, G. L., (2000). Modern bioavailability, bioequivalence and biopharmaceutics classification system. New scientific approaches to international regulatory standards, *European Journal of Pharmaceutics and Biopharmaceutics*, 50, 3-12.

Lohitnavy, M., Lohitnavy, O., Thangkeattiyanon, O. and Srichai, W., (2005). Reduced oral itraconazole bioavailability by antacid suspension, *Journal of Clinical Pharmacy and Therapeutics*, 30, 201-206.

Luner, P. E., Babu, S. R. and Mehta, S. C., (1996). Wettability of a hydrophobic drug by surfactant solutions, *International Journal of Pharmaceutics*, 128, 29-44.

Luner, P. E. and VanDer Kamp, D., (2001a). Wetting behavior of bile salt-lipid dispersions and dissolution media patterned after intestinal fluids, *Journal of Pharmaceutical Sciences*, 90, 348-359.

Luner, P. E. and VanDer Kamp, D., (2001b). Wetting characteristics of media emulating gastric fluids, *International Journal of Pharmaceutics*, 212, 81 - 91.

Macheras, P., Koupparis, M. and Antimisiaris, S., (1989). Effect of temperature and fat content on the solubility of hydrochlorothiazide and chlorothiazide in milk, *Journal of Pharmaceutical Sciences*, 78, 933-936.

Macheras, P., Koupparis, M. and Tsaprounis, C., (1986). Drug dissolution studies in milk using the automated flow injection serial dynamic dialysis technique, *International Journal of Pharmaceutics*, 33, 125-136.

Macheras, P. and Reppas, C., (1986). Studies on drug-milk freeze-dried formulations I: Bioavailability of sulfamethizole and dicumarol formulations, *Journal of Pharmaceutical Sciences*, 75, 692-696.

Macheras, P. and Reppas, C., (1987). Dissolution and *in vitro* permeation behaviours of dicumarol, nitrofurantoin and sulfamethizole in the presence of protein, *International Journal of Pharmaceutics*, 37, 103-112.

Macheras, P. E., Koupparis, M. A. and Antimisiaris, S. G., (1990). Drug binding and solubility in milk, *Pharmaceutical Research*, 7, 537-541.

Magdassi, S. and Kamyshny, A. (1996). Introduction: surface activity and functional properties of proteins, In *Surface Activity of Proteins: Chemical and Physicochemical Modifications*, (Ed, Magdassi, S.), Marcd Dekker, Inc., New York, pp. 1-3.

Malmsten, M. (2002). Introduction to surfactants, In *Surfactants and Polymers in Drug Delivery*, Marcel Dekker, New York, pp. 2-8.

Mannisto, P. T., Mantyla, R., Nykanen, S., Lamminsivu, U. and Ottoila, P., (1982). Impairing effect of food on ketoconazole absorption, *Antimicrobial Agents and Chemotherapy,* 21, 730-733.

Marcotte, M., Taherian Hoshahili, A. R. and Ramaswamy, H. S., (2001). Rheological properties of selected hydrocolloids as a function of concentration and temperature, *Food Research International,* 34, 695-703.

Marques, M., (2004). Dissolution media simulating fasted and fed states, *Dissolution Technologies,* 11, 16.

Martin, A. (1993a). Protein binding, In *Physical Pharmacy: Physical Chemical Principles in the Pharmaceutical Sciences, 4th ed.*, Lippincott Williams & Wilkins, Philadelphia, pp. 268-274.

Martin, A. (1993b). Rheology, In *Physical Pharmacy: Physical Chemical Principles in the Pharmaceutical Sciences, 4th ed.*, Lippincott Williams & Wilkins, Philadelphia, pp. 453-457.

Martin, A. (1993c). Solubility and distribution phenomena, In *Physical Pharmacy: Physical Chemical Principles in the Pharmaceutical Sciences, 4th ed.*, Lippincott Williams & Wilkins, Philadelphia, pp. 212-213.

Martinez, M., Augsburger, L., Johnston, T. and Jones, W. W., (2002). Applying the Biopharmaceutics Classification System to veterinary pharmaceutical products, *Advanced Drug Delivery Reviews,* 54, 805-824.

Mauger, J., Ballard, J., Brockson, R., Sinjan, D., Gray, V. and Robinson, D., (2003). Intrinsic dissolution performance testing of the USP dissolution apparatus 2 (rotating paddle) using modified salicylic acid calibrator tablets: proof of principle, *Dissolution Technologies,* 10, 6-15.

McMahon, D. J. and Rodney, J. B., (1984). Composition, structure, and integrity of casein micelles: A review, *Journal of dairy science,* 67, 499-512.

McNamara, D. P., Vieira, M. L. and Crison, J. R. (2000). Dissolution of pharmaceuticals in simple and complex systems, In *Transport Processes in Pharmaceutical Systems*, (Eds, Amidon, G. L., Lee, P. I. and Topp, E. M.), Marcel Dekker, New York, pp. 109-146.

Miller, J. C. and Miller, J. N. (1993). Significance tests, In *Statistics for Analytical Chemistry*, Ellis Horwood Ltd., Chichester, pp. 53-77.

Milosovich, G., (1964). Determination of solubility of a metastable polymorph, *Journal of Pharmaceutical Sciences,* 53, 484-487.

Minagawa, H., Kitaura, K. and Nakamizo, N., (1983). Effects of pH on the activity of ketoconazole against Candida albicans, *Antimicrobial Agents and Chemotherapy,* 23, 105-107.

Mithani, S. D., Bakatselou, V., TenHoor, C. N. and Dressman, J. B., (1996). Estimation of the increase in solubility of drugs as a function of bile salt concentration, *Pharmaceutical Research,* 13, 163-167.

Moules, C., (1998). Static and dynamic surface tension of coatings, Camtel Ltd., http://www.esum.com.cn/updata/biaomian3.pdf, accessed on January 2007.

Möller, M. and Denicola, A., (2002). Protein tryptophan accessibility studied by fluorescence quenching, *Biochemistry and Molecular Biology Education,* 30, 175-178.

Mullertz, A. (2007). Biorelevant dissolution media, In *Solvent Systems and Their Selection in Pharmaceutics and Biopharmaceutics*, (Eds, Augustijns, P. and Brewster, M.), Springer Science & Business Media, New York, pp. 151-177.

Nandi, P. K. (1997). Chemical and physical methods for the characterization of proteins, In *Food Proteins and Their Applications*, (Eds, Damodaran, S. and Paraf, A.), Marcel Dekker, New York, pp. 598-603.

Naylor, L. J., Bakatselou, V. and Dressman, J. B., (1993). Comparison of the mechanism of dissolution of hydrocortisone in simple and mixed micelle systems, *Pharmaceutical Research,* 10, 865-870.

Nernst, W., (1904). Theory of reaction velocity in heterogeneous systems, *Zeitschrift für Physikalische Chemie,* 47, 52-55.

Nesseem, D. I., (2001). Formulation and evaluation of itraconazole via liquid crystal for topical delivery system, *Journal of Pharmaceutical and Biomedical Analysis,* 26, 387-399.

Niba, L. (2006). Carbohydrates: Starch, In *Handbook of Food Science, Technology, and Engineering*, (Ed, Hui, Y. H.), Taylor & Francis, Boca Raton, pp. 3 (2-3).

Nicolaides, E., Galia, E., Efthymiopoulos, C., Dressman, J. B. and Reppas, C., (1999). Forecasting the *in vivo* performance of four low solubility drugs from their *in vitro* dissolution data, *Pharmaceutical Research,* 16, 1876-1882.

Noyes, A. A. and Whitney, W. R., (1897). The rate of solution of solid substances in their own solutions, *Journal of American Chemical Society,* 19, 930–934.

O'Driscoll, K. M. and Corrigan, O. I., (1982). Chlorothiazide-Polyvinylpyrrolidone (PVP) interactions: influence on membrane permeation (everted rat intestine) and dissolution, *Drug Development and Industrial Pharmacy,* 8, 547 - 564.

Ozturk, S. S., Palsson, B. O. and Dressman, J. B., (1988). Dissolution of ionizable drugs in buffered and unbuffered solutions, *Pharmaceutical Research,* 5, 272-282.

Parojcic, J., Vasiljevic, D., Ibric, S. and Djuric, Z., (2008). Tablet disintegration and drug dissolution in viscous media: paracetamol IR tablets, *International Journal of Pharmaceutics,* 355, 93-99.

Pedersen, B. L., Brondsted, H., Lennernas, H., Christensen, F. N., Müllertz, A. and Kristensen, H. G., (2000a). Dissolution of hydrocortisone in human and simulated intestinal fluids, *Pharmaceutical Research,* 17, 183-189.

Pedersen, B. L., Müllertz, A., Brondsted, H. and Kristensen, H. G., (2000b). A Comparison of the solubility of danazol in human and simulated gastrointestinal fluids, *Pharmaceutical Research,* 17, 891-894.

Peeters, J., Neeskens, P., Tollenaere, J. P., Van Remoortere, P. and Brewster, M. E., (2002). Characterization of the interaction of 2-hydroxypropyl-beta-cyclodextrin with itraconazole at pH 2, 4, and 7, *Journal of Pharmaceutical Sciences,* 91, 1414-1422.

Persson, E. M., Baumann, K., Sundelf, L., Lindberg, W., Sokolowski, A. and Pettersson, C., (2008). Design and characterization of a new miniaturized rotating disk equipment for *in vitro* dissolution rate studies, *Journal of Pharmaceutical Sciences,* 97, 3344-3355.

Persson, E. M., Gustafsson, A. S., Carlsson, A. S., Nilsson, R. G., Knutson, L., Forsell, P., Hanisch, G., Lennernas, H. and Abrahamsson, B., (2005). The effects of food on the dissolution of poorly soluble drugs in human and in model small intestinal fluids, *Pharmaceutical Research,* 22, 2141-2151.

Persson, E. M., Nilsson, R. G., Hansson, G., Lofgren, L., Liback, F., Knutson, L., Abrahamsson, B. and Lennernaes, H., (2006). A clinical single-pass perfusion investigation of the dynamic *in vivo* secretory response to a dietary meal in human proximal small intestine, *Pharmaceutical Research,* 23, 742-751.

Qazi, S., Samuel, N. K. P., Venkatachalam, T. K. and Uckun, F. M., (2003). Evaluating dissolution profiles of an anti-HIV agent using ANOVA and non-linear regression models in JMP software, *International Journal of Pharmaceutics,* 252, 27-39.

Rangel-Yagui, C. O., A., P. J. and Tavares, L. C., (2005). Micellar solubilization of drugs, *Journal of Pharmacy and Pharmaceutical sciences,* 8, 147-163.

Rautureau, M., Bisalli, A. and Rimbaud, J., (1981). Etude de la phase aqueuse inter-jejunale des sels biliaires et des lipides au cours de la digestion d'un repas standard chez le sujet normal, *Gastroenterol Clinical Biology,* 5, 417-425.

Reppas, C., Eleftheriou, G., Macheras, P., Symillides, M. and Dressman, J. B., (1998). Effect of elevated viscosity in the upper gastrointestinal tract on drug absorption in dogs, *European Journal of Pharmaceutical Sciences,* 6, 131-139.

Rodriguez-Spong, B., Price, C. P., Jayasankar, A., Matzger, A. J. and Rodriguez-Hornedo, N., (2004). General principles of pharmaceutical solid polymorphism - a supramolecular perspective, *Advanced Drug Delivery Reviews,* 56, 241-274.

Rosen, A. and Macheras, P., (1984). The effect of protein on the dissolution of phenytoin, *Journal of Pharmacy and Pharmacology,* 36, 723-727.

Saag, M. S. and Dismukes, W. E., (1988). Azole antifungal agents: emphasis on new triazoles, *Antimicrobial Agents and Chemotherapy,* 32, 1-8.

Sato, H. and Ueberreiter, K., (1979). Surface tension of aqueous gelatin solutions, 1. Concentration dependence, *Die Makromolekulare Chemie,* 180, 829-835.

Shah, U., (2006). Ph.D. Thesis. An evaluation of the dissolution behaviour of poorly soluble acidic and basic drugs in biorelevant media, School of Pharmacy and Chemistry, Liverpool John Moores University, Liverpool.

Shah, V. P., Tsong, Y., Sathe, P. and Liu, J. P., (1998). *In vitro* dissolution profile comparison - statistics and analysis of the similarity factor, f2, *Pharmaceutical Research,* 15, 889-896.

Sharmin, R., Ioannidis, M. A. and Legge, R. L., (2006). Effect of nonionic surfactant partitioning on the dissolution kinetics of residual perchloroethylene in a model porous medium, *Journal of Contaminant Hydrology,* 82, 145-164.

Shaw, T. R. D., Raymond, K., Howard, M. R. and Hamer, J., (1973). Therapeutic non-equivalence of digoxin tablets in United Kingdom: correlation with tablet dissolution rate, *British Medical Journal,* 29, 763-766.

Singh, M., Chand, H. and Gupta, K. C., (2005). The studies of density, apparent molar volume, and viscosity of bovine serum albumin, egg albumin, and lysozyme in aqueous and RbI, CsI, and DTAB aqueous solutions at 303.15 K, *Chemistry and Biodiversity,* 2, 809-824.

Six, K., Verreck, G., Peeters, J., Binnemans, K., Berghmans, H., Augustijns, P., Kinget, R. and Van den Mooter, G., (2001). Investigation of thermal properties of glassy itraconazole: identification of a monotropic mesophase, *Thermochimica Acta,* 376, 175-181.

Six, K., Verreck, G., Peeters, J., Brewster, M. and Van den Mooter, G., (2004). Increased physical stability and improved dissolution properties of itraconazole, a class II drug, by solid dispersions that combine fast- and slow-dissolving polymers, *Journal of Pharmaceutical Sciences,* 93, 124-131.

Skiba, M., Skiba-Lahiani, M., Marchais, H., Duclos, R. and Arnaud, P., (2000). Stability assessment of ketoconazole in aqueous formulations, *International Journal of Pharmaceutics,* 198, 1-6.

Smith, K. and Colton, C., (1972). Mass transfer to a rotating fluid. Part I. Transport from stationary disk to a fluid Bodewadt flow tank, *AIChE Journal,* 18, 949-958.

Sun, S., Song, Y. and Zheng, Q., (2008). pH-induced rheological changes for semi-dilute solutions of wheat gliadins, *Food Hydrocolloids,* 22, 1090-1096.

Sunesen, V. H., Pedersen, B. L., Kristensen, H. G. and Mullertz, A., (2005). *In vivo in vitro* correlations for a poorly soluble drug, danazol, using the flow-through dissolution method with biorelevant dissolution media, *European Journal of Pharmaceutical Sciences,* 24, 305-313.

Sweetman, S. C. (2009a). Antifungals, In *Martindale: The Complete Drug Reference,* 36th Ed., (Ed, Sweetman, S. C.), The Pharmaceutical Press, London, http://www.medicinescomplete.com, accessed on June 2009.

Sweetman, S. C. (2009b). Tetracycline, In *Martindale: The Complete Drug Reference,* 36th ED., (Ed, Sweetman, S. C.), Pharmaceutical Press, London, http://www.medicinescomplete.com, accessed on June 2009.

Takeda, K., Matsumura, Y. and Shimizu, M., (2001). Emulsifying and surface properties of wheat gluten under acidic conditions, *Journal of Food Science,* 66, 393-399.

The Merck Index, (2006). The Merck Index, Merck & Co., Inc, Whitehouse Station, NJ, USA. http://www.medicinescomplete.com, accessed on April 2009.

Thomas, G. (2000). Antifungal agents, In *Medicinal Chemistry: An Introduction.*, John Wiley & Sons, Ltd, Chichester, pp. 156-158.

Tian, F., Sandler, N., Aaltonen, J., Lang, C., Saville, D. J., Gordon, C. K., Strachan, C. J., Rantanen, J. and Rades, T., (2007). Influence of polymorphic form, morphology, and excipient interactions on the dissolution of carbamazepine compacts, *Journal of Pharmaceutical Sciences,* 96, 584-594.

Topp, E. M. (2000). Diffusion, In *Transport Processes in Pharmaceutical Systems*, (Eds, Amidon, G. L., Lee, P. I. and Topp, E. M.), Marcel Dekker, New York, pp. 12-15.

Ueda, H., Naambu, N. and Nagai, T., (1984). Dissolution behavior of chlorpropamide polymorphs, *Chemical and pharmaceutical bulletin,* 32, 244-250.

Underwood, T. and Cadwallader, D., (1978). Automated poteniometric procedure for studying dissolution kinetics of acidic drugs under sink conditions, *Journal of Pharmaceutical Sciences,* 67, 1163-1167.

USP, (2007). United States Pharmacopeia 30. The US Pharmacopeial Convention, Rockville, MD.

USP, (2008). United States Pharmacopeia 31. The US Pharmacopeial Convention, Rockville, MD.

Van den Mooter, G., Wuyts, M., Blaton, N., Busson, R., Grobet, P., Augustijns, P. and Kinget, R., (2001). Physical stabilisation of amorphous ketoconazole in solid dispersions with polyvinylpyrrolidone K25, *European Journal of Pharmaceutical Sciences,* 12, 261-269.

Van Der Meer, J. W. M., Keuning, J. J., Scheijgrond, H. W., Heykants, J., Van Cutsem, J. and Brugmans, J., (1980). The influence of gastric acidity on the bio-availability of ketoconazole, *Journal of Antimicrobial Chemotherapy,* 6, 552-554.

Van Peer, A., Woestenborghs, R., Heykants, J., Gasparini, R. and Gauwenbergh, G., (1989). The effects of food and dose on the oral systemic availability of itraconazole in healthy subjects, *European Journal of Clinical Pharmacology,* 36, 423-426.

Vertzoni, M., Dressman, J., Butler, J., Hempenstall, J. and Reppas, C., (2005). Simulation of fasting gastric conditions and its importance for the *in vivo* dissolution of lipophilic compounds, *European Journal of Pharmaceutics and Biopharmaceutics,* 60, 413-417.

Vertzoni, M., Fotki, N., Kostewicz, E., Stippler, E., Leuner, C., Nicolaides, E., Dressman, J. and Reppas, C., (2004). Dissolution media simulating the intralumenal composition of the small intestine: physiological issues and practical aspects, *Journal of Pharmacy and Pharmacology,* 56, 453-462.

Vertzoni, M., Pastelli, E., Psachoulias, D., Kalantzi, L. and Reppas, C., (2007). Estimation of intragastric solubility of drugs: in what medium?, *Pharmaceutical Research,* 24, 909-917.

Vertzoni, M. V., Reppas, C. and Archontaki, H. A., (2006). Optimization and validation of a high-performance liquid chromatographic method with UV detection for the determination of ketoconazole in canine plasma, *Journal of Chromatography B,* 839, 62-67.

Viegas, T. X. C., R.U. VanWinkle, L. L. Brinker, G., (2001). Intrinsic drug dissolution testing using the stationary disk system, *Dissolution Technologies,* 8, 19-23.

Viness, P. and Reza, F., (1999). Unconventional dissolution methodologies, *Journal of Pharmaceutical Sciences,* 88, 843-851.

Viscolite 700, Hydramotion-Ltd, http://www.hydramotion.com/portable.html, accessed on September 2007.

Vogt, M., Derendorf, H., Kramer, J., Junginger, H. E., Midha, K. K., Shah, V. P., Stavchansky, S., Dressman, J. B. and Barends, D. M., (2007). Biowaiver monographs for immediate release solid oral dosage forms: prednisolone, *Journal of Pharmaceutical Sciences,* 96, 27-37.

Wagner, J. G. (1971). Introduction to rate of dissolution *in vitro and in vivo*, In *Biopharmaceutics and relevant pharmacokinetics*, Drug Intelligence Publications, Hamilton, Illinois, pp. 98-147.

Wall, B. P., Parkin, J. E. and Sunderland, V. B., (1985). Intrinsic dissolution of lithium carbonate in aqueous solutions, *Journal of Pharmacy and Pharmacology,* 37, 338-340.

Walstra, P., Wouters, J. M. and Geurts, T. J. (2006a). Colloidal particles of milk, In *Dairy Science and Technology*, Taylor & Francis, Boca Raton, pp. 109-157.

Walstra, P., Wouters, J. M. and Geurts, T. J. (2006b). Liquid milk products, In *Dairy Science and Technology*, Taylor & Francis, Boca Raton, pp. 173-174.

Walstra, P., Wouters, J. M. and Geurts, T. J. (2006c). Milk: main characteristics, In *Dairy Science and Technology,* Taylor & Francis, Boca Raton, pp. 3-16.

Wei, H. and Lobenberg, R., (2006). Biorelevant dissolution media as a predictive tool for glyburide a class II drug, *European Journal of Pharmaceutical Sciences,* 29, 45-52.

Wiedmann, T. S. and Kamel, L., (2002). Examination of the solubilization of drugs by bile salt micelles, *Journal of Pharmaceutical Sciences,* 91, 1743-1764.

Woestenborghs, R., Lorreyne, W. and Heykants, J., (1987). Determination of itraconazole in plasma and animal tissues by high-performance liquid chromatography, *Journal of Chromatography,* 413, 332-337.

Wong, J. W., Nisar, U. R. and Yuen, K. H., (2003). Liquid chromatographic method for the determination of plasma itraconazole and its hydroxy metabolite in

pharmacokinetic/bioavailability studies, *Journal of Chromatography B,* 798, 355-360.

Wood, J. H., Syarto, J. E. and Letterman, H., (1965). Improved holder for intrinsic dissolution rate studies, *Journal of Pharmaceutical Sciences,* 54, 1068-1068.

Yu, L. X., Carlin, A. S., Amidon, G. L. and Hussain, A. S., (2004). Feasibility studies of utilizing disk intrinsic dissolution rate to classify drugs, *International Journal of Pharmaceutics,* 270, 221-227.

Zhao, F., Malayev, V., Rao, V. and Hussain, M., (2004). Effect of sodium lauryl sulfate in dissolution media on dissolution of hard gelatin capsule shells, *Pharmaceutical Research,* 21, 144-148.

Zhao, Y. H., Abraham, M. H., Le, J., Hersey, A., Luscombe, C. N., Beck, G., Sherborne, B. and Cooper, I., (2002). Rate-limited steps of human oral absorption and QSAR studies, *Pharmaceutical Research,* 19, 1446-1457.

Zhou, R., Moench, P., Heran, C., Lu, X., Mathias, N., Faria, T. N., Wall, D. A., Hussain, M. A., Smith, R. L. and Sun, D., (2005). pH-dependent dissolution *in vitro* and absorption *in vivo* of weakly basic drugs: development of a canine model, *Pharmaceutical Research,* 22, 188-192.

Zimmermann, T., Yeates, R. A., Laufen, H., Pfaff, G. and Wildfeuer, A., (1994). Influence of concomitant food intake on the oral absorption of two triazole antifungal agents, itraconazole and fluconazole, *European Journal of Clinical Pharmacology,* 46, 147-150.

Zoeller, T. and Klein, S., (2007). Simplified biorelevant media for screening dissolution performance of poorly soluble drugs, *Dissolution Technologies*, 14, 8-13.

APPENDIX I

Publications

Ghazal, H. S., Dyas, A. M., Ford, J. L. and Hutcheon, G. A., (2009). *In vitro* evaluation of the dissolution behaviour of itraconazole in bio-relevant media, *International Journal of Pharmaceutics,* 366, 117-123.

Ghazal, H., Dyas, A. M., Ford, J. L. and Hutcheon, G. A., (2007). The intrinsic dissolution rate of ketoconazole in proteinaceous biorelevant media, *Journal of Pharmacy and Pharmacology,* 59; SUPP, 124.

Ghazal, H., Ford, J. L. and Dyas, A. M., (2006). Morphological changes observed during intrinsic dissolution rate testing of itraconazole, *Journal of Pharmacy and Pharmacology,* 58; SUPP, 138.

Ghazal, H., (2004). Master of Philosophy (M.Phil) thesis. Evaluation of PAMAM dendrimers as drug carriers, School of Pharmacy and Pharmaceutical Sciences. The University of Manchester, Manchester.

Poster and Oral Presentations

The intrinsic dissolution rate of ketoconazole in proteinaceous biorelevant media Ghazal, H., Dyas, A. M., Ford, J. L. and Hutcheon, G. A. Poster presentation. British Pharmaceutical Conference. September 2007, Manchester, U.K.

Study of the effects of various food components on the in vitro dissolution of the anti-fungal drug itraconazole. Poster presentation. Ghazal, H. Ford, J. L. Dyas, A. M. Hutcheon, G.A. Pharmaceutical Sciences World Congress. April 2007, Amsterdam, the Netherlands.

A study of the effects of proteinaceous media on the *in vitro* dissolution of itraconazole. Ghazal, H., Ford, J. L. Dyas, A. M. and Hutcheon, G. A. Poster presentation. Postgraduate Researchers in Science Medicine conference. July 2007, Chester, UK.

Morphological changes observed during intrinsic dissolution rate testing of itraconazole. Ghazal, H., Dyas, A. M. and Ford, J. L. Poster presentation. British Pharmaceutical Conference. September 2006, Manchester, UK.

Postgraduate Research Seminars and Poster Sessions. Faculty of Science, Liverpool John Moores University. Posters and oral presentations. May 2005, 2006 and 2007, Liverpool, UK.