AN EVALUATION OF THE DISSOLUTION BEHAVIOUR OF POORLY SOLUBLE ACIDIC AND BASIC DRUGS IN BIORELEVANT MEDIA

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ABSTRACT

The utility of the dissolution test has broadened from a simple quality control tool to have a more developmental and predictive role. Consequently, it has been realised that compendial dissolution media may not adequately represent the environment in the gastro-intestinal tract (GIT), resulting in poor *in vitro-in vivo* correlations (IVIVC). Biorelevant media are being developed in which oral dosage forms may be studied. Of particular interest is the effect of biorelevant media on poorly soluble and highly permeable drugs (Biopharmaceutics Classification System Class II), which may suffer from dissolution rate-limited bioavailability. In addition to pH and secretory changes between the fasted and fed state, the composition of the ingested meal may influence drug dissolution through surfactant, complexation and micellization effects.

The dissolution behaviour of the acidic drugs ibuprofen and naproxen was investigated in compendial media, in 50/50 milk/0.1N HCl mixtures and, where appropriate, in mono component media based on milk (4.8% w/v lactose, 1.475% w/v casein). The dissolution of the basic drug loratadine was investigated in a pH-shift experiment (pH 1.2 – 7.3) using buffer media and in Fasted state simulated intestinal fluid (FaSSIF) and Fed state simulated intestinal fluid (FeSSIF). Loratadine solubility was assessed in a range of surfactant media in an attempt to develop less expensive replacement media for FaSSIF and FeSSIF. Consequently dissolution tests were carried out using varying concentrations of sodium dodecyl sulphate (SDS) and hexadecyltrimethyl ammonium bromide (CTAB) media to match profiles obtained from the biorelevant media. The dissolution of a second basic drug, trimethoprim, was investigated in phosphate buffer pH 6.8.

Tablet dissolution tests were performed using the USP 28 Apparatus 2 under test conditions of 37°C, 50 rpm and 500mL (gastric) or 1000mL (intestinal) volume. Intrinsic dissolution rate (IDR) studies were carried out for ibuprofen and naproxen using the USP28 Rotating Disk Method fitted with 10 mm dies. 24 h solubility studies were completed for all the drugs in the relevant media and disintegration tests were performed on ibuprofen, naproxen and loratadine tablets. All samples were analysed by HPLC.

The solubility of ibuprofen and naproxen was improved in the milk media. Casein, the major bovine milk protein, was identified as a food component that increased the release of ibuprofen and naproxen from tablets through interactions with the excipients. The amount of loratadine dissolved in the dissolution vessel decreased when the pH was increased from 1.2. to 7.3. The dissolution profiles of three loratadine tablet brands (A, B and C) containing the same excipients differed significantly in FeSSIF over 1h and in FaSSIF over 4h. The neutral and zwitterionic surfactants tested were poor solubilisers of loratadine. The dissolution profiles in FaSSIF and in FeSSIF were matched for one product (Brand B) by using SDS and CTAB media but the profile similarity was not maintained when another product (Brand C) was tested in the same media. Variations in the manufacturing formulae and processes of these brands may be implicated in the differences observed. Despite being placed in BCS Class II, trimethoprim dissolution in phosphate buffer pH 6.8 was high (100% in 15min).

The use of milk as a biorelevant gastric medium highlighted a food effect on drug solubility and dissolution from tablets for ibuprofen and naproxen. Loratadine solubilisation in both biological and synthetic surfactant systems appears to be sensitive to formulation factors. The BCS criteria for solubility classification are inappropriate for trimethoprim in the physiologically relevant pH range 1.2-6.8.

"It is the mark of an educated mind to be able to entertain a thought without accepting it."

Aristotle (384BC – 322BC)

This thesis is dedicated to my family:

Upen, Nirupa, Niraj and Snehal

for their continuous love, support and encouragement and for the difficult years apart.

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LIST OF ABBREVIATIONS

%	percent
w/v	weight per volume
w/w	weight per weight
°C	degree Celsius
®	Registered Tradename
≥	greater than or equal to
≤	less than or equal to
≈	approximately
ρ	density
Π	pi
γ	surface tension
$\gamma_{S/A}$	solid surface tension
$\gamma_{S/L}$	solid-liquid interfacial tension
$\gamma_{L/A}$	liquid surface tension
θ	contact angle
ΔH_s	enthalpy of solution
ΔG	Gibbs free energy
ΔS	change in entropy
υ	kinematic viscosity
ω	angular velocity
A	surface area
ADME	absorption, distribution, metabolism and excretion
ANDA	abbreviated New Drug Application
BA	bioavailability
BE	bioequivalence
BP	British Pharmacopoeia
BCS	Biopharmaceutics Classification System
C	concentration

CCD	charge-coupled device
cm	centimetre
cmc	critical micelle concentration
C_o	intrinsic solubility
C_s	saturation solubility
CDs	Cyclodextrins
CTAB	hexadecyltrimethyl ammonium bromide
dm/dt	change in mass with time
D	diffusion coefficient.
DSC	differential scanning calorimetry
EMEA	European Medicines Agency
FaSSIF	Fasted state simulated intestinal fluid
FeSSIF	Fed state simulated intestinal fluid
FDA	Food and Drug Administration
g	gram
GI	gastro-intestinal
GIT	gastro-intestinal tract
h_l	thickness of boundary layer
h	hour
HCl	hydrochloric acid
HPLC	high performance liquid chromatography
HPMC	hydroxypropyl methylcellulose
ICH	International Conference on Harmonisation
IDR	intrinsic dissolution rate
IR	immediate-release
IVIVC	*in vitro-in vivo* correlation
k	intrinsic dissolution rate constant
k_1	effective interfacial rate constant
k_2	cube root dissolution rate constant
K_a	ionisation constant
kilogram	kg
pH	negative log of the hydrogen ion concentration
pK_a	negative log of the ionisation constant
JP	Japanese Pharmacopoeia

L	litre
LOD	limit of detection
M	Molar
MCC	microcrystalline cellulose
µL	microlitre
µm	micrometer
mg	milligram
min	minute
mL	millilitre
MLGM	milk lipid globule membrane
mm	millimetre
mOsm	milliOsmol
mM	millimole
mN/m	milliNewtons per metre
N	Normal
NaSIF	sodium based simulated intestinal fluid
NaTC	sodium taurocholate
NDA	New Drug Application
nm	nanometer
NMR	nuclear magnetic resonance
NSAID	non-steroidal anti-inflammatory drug
PVP	polyvinylpyrrollidone
Q	the minimum percentage-dissolved at time stipulated in monograph.
QC	quality control
q.s.	*quantum sufficit* (as much as suffices)
R&D	research and development
rpm	revolutions per minute
s	second
S	mean rate of production of fresh surface
SANS	small-angle neutron scattering
SDS	sodium dodecyl sulphate
SIF	simulated intestinal fluid
SUPAC	scale up and post approval changes

T	temperature
TRFQ	time-resolved fluorescence quenching
$t_{x\%}$	time taken for x % to dissolve
USP	United States Pharmacopoeia
UV	ultraviolet
w_0	initial weight of a particle
w	weight of a particle

1. INTRODUCTION

The *process* of dissolution and the *methods* of dissolution testing are fundamental topics that need to be appreciated in order to further probe dissolution phenomena occurring in pharmaceutical compounds. Scientific and technological advances in discovery and development programmes in the industry have instigated a need to characterise dissolution behaviour earlier in the product development lifecycle. Such information could lead to the synthesis of more soluble compounds or the formulation of more soluble products to improve bioavailability. From a viewpoint of dissolution, the aims are to assess changes in the dissolution behaviour and hence potential bioavailability of candidate drugs, to formulate a drug product from which drug absorption is not dissolution rate limited, and further, to be able to predict *in vivo* drug dissolution performance using *in vitro* methods. This chapter introduces the basic concepts and theory of dissolution and dissolution testing, together with the factors that affect them and related issues, leading to the aims and objectives of this research project.

1.1. Drug dissolution

1.1.1. Solubility and dissolution

Unless a drug is administered directly into the blood stream intravenously, it must be absorbed into the systemic circulation from its site of administration. On reaching the systemic circulation a drug may partition between erythrocytes and blood plasma. Furthermore, the drug in blood plasma may bind to plasma protein or remain in plasma water. The unbound drug is free to be distributed to tissues and sites of action (Proudfoot, 1988a). The absorption, and thus bioavailability, of drugs from oral dosage forms depend on a variety of drug and formulation factors as well as the physiological condition of the gastro-intestinal tract (GIT). Essentially, for a drug to be absorbed into the blood stream it must first dissolve in the gastric or intestinal fluid and then cross the mucosal membrane via para- or trans-cellular

mechanisms that range from passive diffusion to complex carrier mediated transport. Thus, the dissolution rate of a drug in these fluids can be the rate-limiting step to absorption, especially for poorly water-soluble drugs.

Before considering the factors that affect dissolution, it is important to clarify what is meant by solubility and to understand the dissolution process itself. Solubility can be defined as the number of molecules or ions that move from the solid state into solution until equilibrium is reached under a given set of experimental conditions. The process of transfer is termed dissolution. Carstensen (2001) confirmed concerns about the definition of solubility, assuming that when solubility is referred to, it is meant to be the equilibrium solubility. This is when a solid and solvent are agitated together for a sufficiently long period of time for the concentration of solute in the solvent to have reached a constant level.

Solubility is temperature dependent and small fluctuations in the experimental temperature can affect the solubility results for a compound. Also, solubility may be a function of the particle size (surface area increases with decreasing particle size) (Atkinson *et al.*, 1962; Fincher, 1968) and the existence of polymorphs and amorphates (Shaw *et al.*, 1973). Literature also refers to degrees of solubility e.g. poorly soluble. What does this mean? The United States Pharmacopoeia 28 (2005) describes some of these subjective terms (Table 1.1).

Table 1.1. Quantitative description of commonly used solubility terms

Solubility term	weight of solvent (g) required to dissolve 1 g of solute
Very soluble	<1
Soluble	10-30
Sparingly soluble	30-100
Slightly soluble	100-1000
Very slightly soluble	1000-10 000
Practically insoluble	>10 000

Aqueous solubility is a function of the ability of a non-ionic compound to form hydrogen bonds with water molecules and generally is directly proportional to the number of bonds that can be formed with water (Martinez *et al.*, 2002).

Dissolution requires bond-breaking and bond-forming processes to allow drug molecules to move away from the solid structure and interact with the solvent. The energy changes associated with such processes are the thermodynamic basis of dissolution.

1.1.2. Thermodynamics of dissolution

Heat is usually absorbed when a solid dissolves. This is called the enthalpy of solution (ΔH_s) and is defined as the heat absorbed at constant temperature and pressure when 1 mole of solid dissolves in a very large amount of solvent.

$$\Delta H_s = \text{lattice enthalpy} + \text{enthalpy of solvation} \qquad \{Eq.1.1\}$$

The lattice enthalpy is the heat absorbed (at constant temperature and pressure) to separate completely the molecules or ions of 1 mole of crystalline solid from their mutual attraction in the crystal lattice and is an endothermic process. The enthalpy of solvation is the heat absorbed (at constant temperature and pressure) when 1 mole of the separated molecules or ions become immersed and interact with a very large volume of solvent, i.e. water. This is an exothermic process. Solubility usually increases with temperature because ΔH_s is positive since the endothermic lattice enthalpy is higher than the exothermic enthalpy of solvation. In rare cases where the affinity of the solute for solvent is so great that the enthalpy of solvation is higher than the lattice enthalpy e.g. anhydrous sodium sulphate in water, increasing temperature will decrease solubility according to Le Chatelier's principle (Wallwork & Grant, 1977).

For dissolution to occur spontaneously, there must be a decrease in the free energy of the system, known as Gibbs free energy (ΔG). Gibbs free energy is a measure of

the energy available in a system to do work (Richards, 1988). In this case it would be the work done to overcome the lattice energy of a solid,

$$\Delta G = \Delta H_s - T\Delta S \qquad \{Eq.1.2\}$$

where T is the thermodynamic temperature and ΔS is the change in entropy. Entropy is a measure of the randomness of the system and for a process like dissolution, where molecules are no longer held in a solid structure, the randomness increases. It follows then that if ΔG has to be negative for spontaneity and ΔS is positive for dissolution then ΔH_s must be negative, zero or very slightly positive (Aulton, 2002).

At a molecular level, if a solute is to be soluble in a solvent, the intermolecular attractive forces between solute and solvent must be equivalent to or stronger than the attractive forces between solute-solute and solvent-solvent molecules themselves. Ionic compounds and salts dissolve in polar solvents because the dielectric constant of the solvent weakens the attractive forces between oppositely charged solute ions. Furthermore, the permanent dipole of the solvent results in the introduction of new electrostatic attractions such that the ions will be surrounded by interacting solvent molecules. In cases where non-ionic compounds have shown aqueous solubility it is the result of hydrogen bonding of the -OH, $-NH_2$ and -COOH groups with the solvent with the possibility of dipole-dipole attractions at other parts of the molecule (Wallwork & Grant, 1977).

1.1.3. Mechanisms of dissolution

Dissolution experiments were first cited in the work by Noyes and Whitney (1897) who expressed the law : "The rate at which a solid substance dissolves in its own solution is proportional to the difference between the concentration of that solution and the concentration of the saturated solution."

Further investigations by various workers led to the theories of dissolution described below.

Film Theory (Nernst & Brunner, 1904). This is a widely utilised theory in literature. It states that if a solid is immersed in an agitated liquid and allowed to dissolve, bulk liquid will move past the solid at a certain velocity but a layer of liquid, with thickness h_l, will surround the solid (Figure 1.1). This boundary layer is static due to intermolecular forces at the solid/liquid interface. The concentration in the boundary layer adjacent to the solid is the saturation solubility C_s and that in the bulk solution is C. Hence, there is a concentration gradient across this layer, which obeys Fick's first law of diffusion. This theory is the basis of the Nernst-Brunner equation that has been extensively used in dissolution rate studies and which is described later in this section.

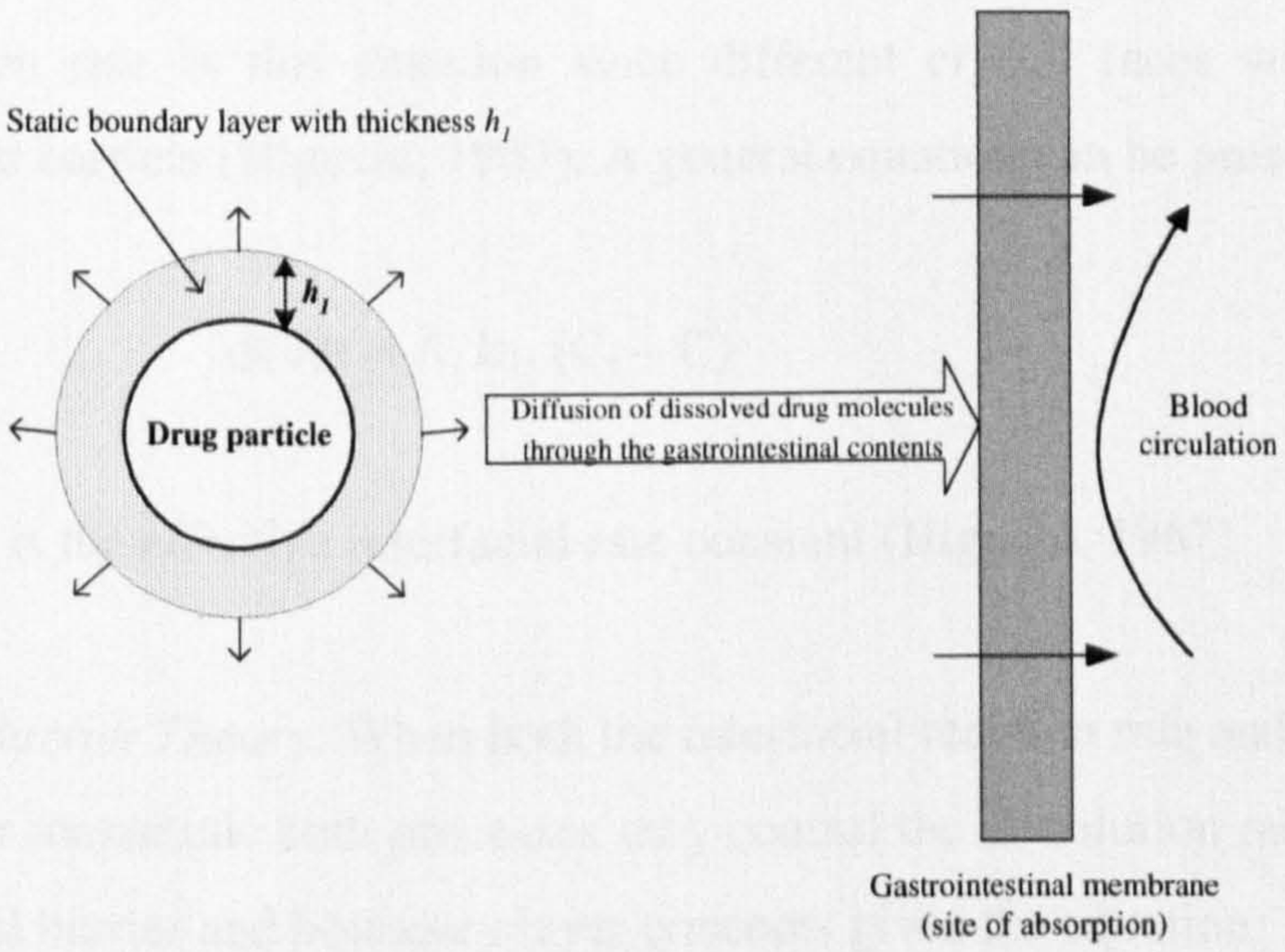


Figure 1.1. Schematic illustration of the dissolution of a drug particle in gastrointestinal fluids

Surface Renewal Theory/ Penetration Theory (Danckwerts, 1951). In the event that the reaction rate at the solid surface is the rate-limiting step to dissolution, then the concentration at the solid/liquid interface will actually be less than the C_s. This led Danckwerts (1951) to disregard the existence of a stagnant boundary layer and propose that turbulence reaches the solid surface, which is then continually being replaced with fresh liquid. Carstensen (1974) suggested that this renewal could be looked at as solvent molecules from the bulk diffusing through the boundary layer

as being the rate-limiting step. The Danckwerts equation equivalent to the Nernst-Brunner equation is:

$$dC/dt = A.\ S^{½}.D^{½}.(C_s\text{-}C) \qquad \{Eq.1.3\}$$

where dC/dt is the change in concentration with time; A is the surface area of the solid; S is the mean rate of production of fresh surface and D is the diffusion coefficient (Higuchi, 1967).

Interfacial Barrier Theory. In the case where the interfacial reaction at the solid surface is much slower than the transport process to the bulk solution, the dissolution rate is 'surface controlled'. It is difficult to derive a relationship for the dissolution rate in this situation since different crystal faces will have different interfacial barriers (Higuchi, 1967). A general equation can be presented as:

$$dC/dt = A.\ k_1.\ (C_s - C) \qquad \{Eq.1.4\}$$

where k_1 is the effective interfacial rate constant (Higuchi, 1967)

Double Barrier Theory. When both the interfacial reaction rate and transport rate are of similar magnitude both processes may control the dissolution rate. Combining the interfacial barrier and boundary layer concepts gives the equation:

$$dC/dt = \frac{A.\ D.\ (C_s\text{-}C)}{h_1.[1 + D/h_1k_1]} \qquad \{Eq.1.5\}$$

where h_1 is the boundary layer thickness (Higuchi, 1967)

Combining the interfacial barrier and surface penetration concepts gives the equation:

$$dC/dt = \frac{S^{½}.\ D^{½}.\ (Cs\text{-}C)}{[1 + (S^{½}.D^{½})/k_1]} \qquad \{Eq.1.6\}$$

Returning to the film theory, the Nernst-Brunner equation (Nernst & Brunner, 1904) can be used to describe the diffusion-controlled dissolution of a solid:

$$\frac{dC}{dt} = \frac{D.A\,(C_s - C)}{h_1} \qquad \{Eq.1.7\}$$

It follows then, that the factors which influence the terms of this equation will consequently affect the dissolution rate.

During dissolution the surface area of dissolving particles will continuously change. Hixson and Crowell (1931) derived a law which related the dissolution rate to surface area and concentration. They expressed the particle surface as proportional to weight and introduced the Hixson-Crowell cube root law equation (Hixson & Crowell, 1931). For a spherical particle under sink conditions (see below) the equation is written as:

$$k_2 t = w_0^{1/3} - w^{1/3} \qquad \{Eq.1.8\}$$

where w_0 is the initial weight of the particle, w is the weight of the particle at time t and k_2 is the cube root dissolution rate constant.

Plotting the cube root difference ($w_0^{1/3} - w^{1/3}$) against time shows a linear relationship, the slope of which gives the constant k_2. For non- spherical particles the assumptions are:

a) The dissolution characteristics of all the crystal faces are identical i.e. particles dissolve in an isotropic fashion

b) Particles are isometric, which implies that the shape factor (α) of a particle is independent of time.

If the bulk concentration (C) is less than 10 % of the saturated concentration i.e. when the solute is removed from the dissolution medium at a faster rate than it passes into solution or when the dissolution volume is sufficiently large, then 'sink'

conditions are said to operate (Richards, 1988). The Nernst-Brunner equation (Equation 1.7) can then be approximated to:

$$\frac{dC}{dt} = \frac{D.A.C_s}{h_l} \qquad \{Eq.1.9\}$$

However, during the dissolution of tablets, disintegration, deaggregation and dissolution causes the effective surface area to continuously change therefore the dissolution rate is measured as the total amount of drug dissolved per unit time (mg/min) (Wells & Aulton, 1988).

1.1.4. Intrinsic Dissolution Rate

The intrinsic dissolution rate (IDR) of a pure substance is the rate at which it dissolves from a constant surface area whilst the temperature, agitation, pH and ionic strength of the dissolution medium are kept constant. Thus, for a drug substance, the IDR is independent of formulation factors and measures the inherent dissolution rate of the drug in the dissolution medium. IDR determinations can be used to characterise bulk drug substances and excipients and to test the chemical equivalence of active pharmaceutical ingredients synthesised by different processes. They can also provide an important insight into the dissolution behaviour of a drug in physiological conditions or distinguish whether changes in the dissolution profile of a drug product in various biorelevant media are due to interactions between the medium and formulation excipients or medium and drug substance or both. Subsequently, this test has a place in the screening of drug candidates for further development. Yu *et al* (2004) discussed the feasibility of using IDR (as opposed to saturation solubility data) to place drugs in a Biopharmaceutics Classification System (BCS) class (*see* Section 1.5) since *in vivo* drug dissolution is a dynamic rate controlled process rather than an equilibrium process.

The IDR is a key indicator of the potential bioavailability of a candidate drug before formulation, where an IDR $\geq$1.0mg/min/cm^2 suggests that drug dissolution <u>will not</u> be the rate-limiting step to absorption whilst an IDR $\leq$0.1mg/min/cm^2 suggests that

drug dissolution will be the rate-limiting step to absorption. An intermediate value suggests that drug dissolution may be the rate-limiting step to absorption. This guide is based on stirring at 50 rpm, 37°C, pH 1-8 under sink conditions (Kaplan, 1974).

Maintenance of a constant surface is a critical requirement for IDR studies. Levy and Sahli (1962) fixed a flat disk perpendicular to a rotating shaft exposing a single surface to the dissolution medium. An improved rotating disk apparatus was introduced by Wood *et al* (1965) and is described in Section 1.1.4.2.1.

The transport of solute in a moving liquid is governed by molecular diffusion due to concentration differences and also by entrainment of solute molecules in the moving liquid (Levich, 1962). Combination of the two mechanisms is termed convective diffusion of a solute in a liquid. Levich (1962) proposed a convective diffusion theory for the rate of mass transport to and from the face of a rotating disk. Fluid in the dissolution vessel moves towards the rotating disk and a thin layer adjacent to the disk surface acquires a rotating motion with angular velocity equal to that of the rotating disk. Also, the thickness of the diffusion boundary layer is constant over the disk surface such that transport of matter to and from any point on the disk is identical (Levich, 1962). The Levich equation for the IDR is:

$$IDR = \frac{0.62.\, D^{2/3}.\, \omega^{1/2}.\, C_s}{\upsilon^{1/6}} \qquad \{Eq.1.10\}$$

where ω is the angular velocity of the rotating disk and υ is the kinematic viscosity of the medium.

1.1.4.1. Factors affecting the intrinsic dissolution rate

The IDR assumes a constant surface area and boundary layer thickness. Hence, the Nernst-Brunner equation (Equation 1.7) simplifies to:

$$IDR = D.C_s \qquad \{Eq.1.11\}$$

Therefore, the properties of the dissolution medium that influence diffusivity and C_s will determine the IDR.

It is well established that the conditions in the GIT such as pH, buffer capacity, surfactant concentration, viscosity, etc. affect the dissolution rate of drugs and changes to these conditions including the presence of food consequently affect absorption of a drug. Pharmacopoeial dissolution tests are not realistic representations of the *in vivo* environment and there has been a move towards the development of biorelevant media for use in dissolution testing to improve *in vitro-in vivo* correlations (IVIVC) (Dressman *et al.*, 1998; Galia *et al.*, 1998). This is discussed further in Section 1.3.5. The factors that influence the IDR of drugs based on the film theory of dissolution are discussed below.

1.1.4.1.1. *Polymorphism and solvation*

The existence of different crystal lattices for a particular drug (polymorphism) can have important consequences on bioavailability, especially if the drug is poorly water-soluble. In a monotropic system, only one polymorph is stable below the melting point of the drug, therefore reversible transitions between polymorphic forms are not observed below the melting point. Conversely in an enantiotropic system, reversible transitions occur at a specific transition temperature below the melting point, because different polymorphic forms are the most stable above and below the transition temperature (Grant, 1999). For an enantiotropic system, the most stable polymorph at room temperature usually has the lowest solubility and slowest dissolution rate, however, when little energy is required to convert from one form to another it is likely that these will interconvert *in vivo* such that administration of either form will not impact upon the bioavailability of the drug (Florence & Atwood, 1988).

An appropriate metastable polymorph with suitable solubility can be acquired by manipulation of the recrystallisation process and choice of solvent but it must be emphasised that an enhanced dissolution rate does not necessarily translate to improved bioavailibility (Haleblian *et al.*, 1971) . A major problem with the use of metastable polymorphs is the risk of interconversion during manufacture and storage (Horter & Dressman, 2001). Manufacturing processes such as recrytallisation, comminution, granulation, drying and compaction can change the

crystal structure of a drug and its solubility (Brittain & Fiese, 1999). For example, milling digoxin increases the surface area and the drug becomes amorphous, both of which increase the saturation solubility of the drug. (Florence & Salole, 1976).

Anhydrous crystals show greater solubility and increased dissolution rates compared to hydrates because in the hydrate the drug has already interacted intimately with water and the energy released for crystal break-up is less when the hydrate is in contact with the water (Florence & Atwood, 1988). Non-aqueous solvates tend to be more soluble in water than non-solvates e.g. the n-amyl alcohol solvate of fludrocortisone is five times more soluble than the parent compound (Shefter & Higuchi, 1963).

1.1.4.1.2. *Wetting*

Prior to dissolution the solvent must wet the surface of solid particles. When the solid is first immersed in the liquid the initial wetting is referred to as 'immersional wetting'. The extent of this wetting is measured by the contact angle between the solid and liquid (Figure 1.2). For complete wetting the contact angle will be zero. This occurs when the attractive forces between solid and solvent are equal to or greater than the attractive forces between the solvent and solvent (Florence & Atwood, 1988). The attractive surface forces are referred to as surface tensions. The solid surface tension ($\gamma_{S/A}$) in Figure 1.2 will tend to spread the liquid but this will be opposed by the solid-liquid interfacial tension ($\gamma_{S/L}$) and the surface tension of the liquid in the plane of the solid ($\gamma_{L/A}$).

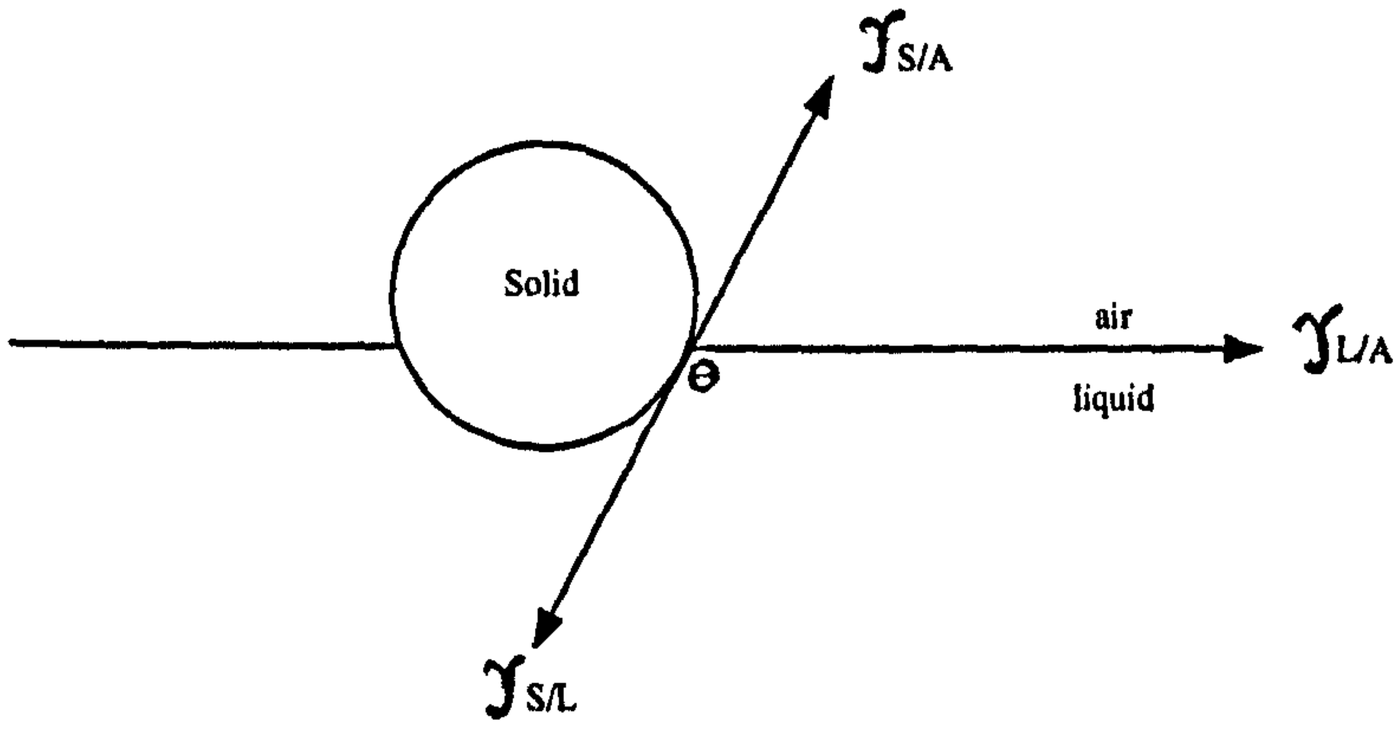


Figure 1.2. Illustration of the forces acting on a solid partially immersed in liquid.

This gives rise to Young's equation:

$$\gamma_{S/A} = \gamma_{S/L} + \gamma_{L/A} \cos\theta \qquad \{Eq.1.12\}$$

where θ is the contact angle.

According to Florence and Attwood (1988) once the solid is submerged in the solvent 'spreading wetting' becomes important, during which the liquid will spread over the solid.

Surfactants are important wetting agents that can increase the solubility of drugs. They work by lowering the surface tension of the liquid and also by adsorbing onto the powder surface thus reducing the surface tension of the solid. Both these events reduce the contact angle (Florence & Atwood, 1988).

According to the Nernst-Brunner equation a decrease in particle size and the subsequent increase in surface area will result in an increase in the dissolution rate. However, this may not be the case if the drug is hydrophobic and/or the dissolution medium has poor wetting characteristics. In such situations a decrease in particle size causes a decrease in effective surface area (Finholt, 1974). Air is adsorbed onto the hydrophobic surface of a drug powder, which then remains floating on the top of the dissolution medium. If the surface tension of the dissolution medium is reduced by the presence of surface-active components in the GIT or by the addition of synthetic surfactants then the contact angle between the drug particle and liquid medium is reduced and the drug particle is more readily wetted. Finholt and Solvang (1968) illustrated an increase in the dissolution rate of paracetamol using polysorbate 80 as a surfactant. They found that increasing polysorbate 80 concentration from 0.001 to 0.1% w/v had little effect on the solubility of paracetamol. The improved dissolution rate was mainly due to the ability of polysorbate 80 to reduce the interfacial tension between the solid and liquid and due only to a small extent to the solubilising power of the surfactant. For hydrophilic drugs (e.g. aspirin) addition of a surfactant to the dissolution medium only increased the dissolution rate when the particle size was small (0.2-0.3mm) (Finholt, 1974).

For larger particles (0.71-1.00mm) no significant change in dissolution rate was observed because the hydrophobic properties were so insignificant that a decrease in surface tension was of little importance except when the specific surface area was large.

For IVIVC, an important dissolution medium to consider is gastric fluid because it is the first *in vivo* fluid the drug comes into contact with. Finholt and Solvang (1968) and Efentakis and Dressman (1998) confirmed the surface tension of fasted human gastric fluid (35- 45 mN/m) is lower than water (≈ 70mN/m) and that surface tension is independent of pH. Various surfactants have been used to lower the surface tension of water to this range in dissolution testing e.g. Tween 80, dioctyl sulfosuccinate and Triton X-100R amongst others (Luner *et al.*, 1996). Dressman *et al* (1998) suggested an aqueous solution of sodium dodecyl sulphate as being physiologically representative of gastric fluid.

1.1.4.1.3. *Solubilisation*

There has often been debate as to whether the increased dissolution rate of drugs by surfactants has been due to wetting or solubilisation. The two processes are related. When the surfactant concentration is high enough to reach the critical micelle concentration (cmc) then the solubility of the drug can be enhanced by its incorporation into micelles in the dissolution medium. This is solubilisation.

Physiologically, the presence of bile can increase the absorption of poorly water-soluble drugs by increasing their solubility or dissolution rate. Bile is amphiphilic and reduces the surface tension of the dissolution medium allowing better wetting of drug particles and a greater effective surface area for dissolution. Naylor *et al* (1993) proposed that the dissolution of hydrocortisone was improved by wetting in simple sodium taurocholate (NaTC) micelles and by solubilisation in NaTC/lecithin mixed micellar systems. Furthermore, the concept of wetting versus solubilisation by bile salts has been reported to be compound specific (Horter & Dressman, 2001) leading to a lack of predictability of the solubility/dissolution behaviour of poorly water-soluble drugs.

1.1.4.1.4. *Complexation*

This is a method used to enhance the solubility/dissolution rate of poorly soluble drugs (Truelove *et al.*, 1984; Tinwalla *et al.*, 1993). The question that arises here is at what stage of the dissolution process do these different complexing agents take effect. In terms of the intrinsic dissolution rate, complexing agents that actively remove molecules from the solid surface and take them into the bulk solution are of particular interest. Unfortunately there is little differentiation as to whether the interactions proposed are occurring between the complexing agent and solid surface, such that there is a direct effect on the intrinsic solubility (C_o), or between complexing agent and drug particles in the boundary layer, such that there is an increase in Cs. Two types of complexes, Π donor/ Π acceptor and cyclodextrin complexes, are discussed below.

Π donor/ Π acceptor complexes

These are charge transfer interactions where one molecule is usually aromatic and the other has a lone pair of electrons (donor atom) or an acidic hydrogen (acceptor atom). Nicotinamide, a Π acceptor, increased the solubility of poorly soluble diazepam and progesterone via a Π donor/ Π acceptor complex (Rasool *et al.*, 1991). The greater solubilisation of benzoic acid by caffeine compared to theophylline implicated the role of the methylated imidazole nucleus to form hydrophobic bonds with the other component as a factor in the interaction (Higuchi & Zuck, 1952, 1953).

Cyclodextrin complexes

Cyclodextrins (CDs) have received a great deal of attention over the last 20 years (Tokumura *et al.*, 1986; Okada *et al.*, 1988; Woodcock *et al.*, 1993; Loftsson, 2002; Magnusdottir *et al.*, 2002; Loftsson *et al.*, 2003; Loftsson & Masson, 2004). The parent compounds consist of glucose molecules arranged in a α-1,4-linked toroid, where the hydrophilic hydroxyl groups are arranged around the outside of the molecule whilst the internal cavity is essentially hydrophobic. Cyclodextrins form inclusion complexes with poorly soluble drug by taking up a guest molecule into the central cavity (Loftsson & Brewster, 1996; Horter & Dressman, 2001). The drug-

CD complexation does not involve breaking or forming any covalent bonds. Instead the driving force for complexation is a combination of removal of enthalpy-rich water molecules from within the CD cavity, van der Waals interactions, hydrogen bonding, hydrophobic interactions, release of ring strain in the CD molecule and changes in the solvent-surface tensions (Loftsson & Brewster, 1996).

α, β and γ-cyclodextrin contain 6, 7 and 8 glucose units respectively. The internal diameter of α-CD is too small for the inclusion of pharmaceutical compounds and β-CD has poor water solubility due to intramolecular hydrogen bonding (Loftsson & Brewster, 1996).γ-CD has been well documented as being able to significantly increase the rate of dissolution but recently the derivative hydroxypropyl β-CD (HP-β-CD) has won favour because of its higher aqueous solubility and lower toxicity compared to methylated derivatives, e.g. in the solubilisation of thiazolobenzimadole (Tinwalla *et al.*, 1993). The aqueous solubility of compounds such as the fat soluble vitamins may be enhanced further by substituting β-CD with maltosyl or glucosyl groups (Okada *et al.*, 1990). Also, maltosylated CDs are less toxic than unsubstituted CDs. CDs increase the rate of dissolution of solid drugs but their effect on bioavailability depends on whether dissolution is the rate-limiting step to absorption.

1.1.4.1.5. *The effect of pK_a, pH and buffer capacity*

75% of all drugs are weak bases, 20% are weak acids and 5% are non-ionic or amphoteric (Wells & Aulton, 1988). Thus the majority are ionisable and their aqueous solubility will depend on their ionisation constant (K_a). It has been established that the IDR is dependent on C_s of the drug in the dissolution medium. In turn, the C_s is dependent on the intrinsic solubility of the drug (C_o), which is defined as the solubility of a compound in its free acid or base form. For a weak acid, C_o is approximated as the solubility at more than 1 pH unit below its pK_a (Horter & Dressman, 2001). The pH at the surface of the dissolving solid will influence the dissolution rate of ionisable drugs. It follows that the pH of the boundary layer and the pK_a of the drug are significant in determining its C_s. The pH in the bulk solution

can be considerably different to that in the boundary layer depending on the C_o and pK_a of the drug and the buffer capacity of the bulk solution.

Ozturk *et al* (1988) demonstrated that the pH at the surface of dissolving drug particles was considerably different to the pH in the bulk solution using the three weak acids indomethacin, 2-napthoic acid and benzoic acid in unbuffered media. An increase in the C_o of the drug (weak acid in this case) suppresses the pH at the surface of the dissolving drug.

Horter and Dressman (2001) commented that intestinal juice contains such a significant amount of bicarbonate buffer that *in-vitro* testing in unbuffered or weakly buffered medium does not reflect *in-vivo* dissolution of ionisable drugs. An increase in the buffer concentration would increase the solubility of weakly acidic drugs because the pH at the drug surface would be nearer to the bulk lumenal pH. McNamara *et al* (2003) were able to maintain stable bicarbonate buffer media at pH 5.0 and 6.8 by continuous gas sparging of CO_2 into the media

1.1.4.1.6. *Salt form of the drug*

The pH in the boundary layer (microclimate pH) around drug particles is affected by

a) the pK_a and C_o of the drug

b) the pK_a and C_o of the buffers in the bulk solvent/GI fluids

It is also known that the dissolution rate of weakly acidic drugs in the gastric fluid will be relatively low (Proudfoot, 1988b). If the pH in the boundary layer were increased then C_s and dissolution rate would increase. This can be done by changing the weakly acidic drug from the free acid to basic salt e.g. Na^+ or K^+ salts, which have a strong neutralising effect and would increase the pH to 5-6. However, as the drug diffuses through the boundary layer and arrives at the bulk solution of lower pH, it may precipitate (Proudfoot, 1988b). The extent of this precipitation depends on the C_o of the drug. The precipitated free acid drug usually consists of very fine wetted particles that aid rapid re-dissolution as soon as more gastric fluid is available. This occurs when:

a) drug is absorbed, therefore the gastric fluid is no longer saturated

b) precipitated particles move into the intestine

c) additional fluid enters the stomach

For weakly basic drugs, a salt of a strongly acidic anion can be made e.g. Cl^-. This ensures dissolution occurs prior to gastric emptying. If absorption is fast enough, precipitation in the intestine will not occur and bioavailability will not be affected. Unfortunately, the Cl^- ions already present in the stomach may cause a common ion effect, competing for hydration by the gastric fluid therefore suppressing drug dissolution.

1.1.4.1.7. *Electrolytes in the dissolution medium and the common ion effect*

Electrolytes in the dissolution medium can impact on various aspects of the ionic dissolution process (Wells & Aulton, 1988). They may alter the pH of the medium, which as discussed earlier, might affect the solubility of weak acids and bases based on the drugs' pKa values and the buffer capacity of the medium. Electrolytes can also reduce the solubility of salts through the common ion effect. This drives the dissociation equilibrium of the salt in the opposite direction such that precipitation (salting out) or reduced solubility occurs. Various inorganic ions can be arranged in a series based on their ability to 'salt out' different proteins which depends on ion characteristics such as size, hydration and charge. For univalent cations the series order was $K^+ < Na^+ < Li^+$ and common anions followed the order $CNS^- < I^- < Br^- < NO_3^- < Cl^- < SO_4^- < PO_4^{3-}$ (Heyman *et al.*, 1938)

1.1.4.1.8. *Experimental variations*

The IDR will be affected by experimental parameters such as temperature, speed of agitation and turbulence. For IVIVC, these variables need to mimic the GI conditions as closely as possible.

1.1.4.1.9. *Physiological factors affecting IDR*

Brief references have been made to physiological conditions that will affect the IDR. Considerations for IVIVC include the changing pH down the GIT and the effect of co-ingested food on transit time, pH and solubilisation capacity of the stomach and intestine. Drugs whose physicochemical properties are affected by these changes will result in changes in the rate and extent of absorption. The effect of age and disease states on the gastric secretions also needs to be considered.

1.1.4.2. Methods of determining the IDR

1.1.4.2.1. *Intrinsic Dissolution Method (USP <1087>)*

Two variations of the IDR apparatus exist – the rotating disk apparatus (Woods Apparatus, Figure 1.3) and the stationary disk apparatus (Figure 1.4). Both types of apparatus employ a compacted pellet of pure drug, produced by compressing an aliquot of powder in a stainless steel punch & die (Figure 1.5). The die is mounted onto a smooth polished base plate and the punch is driven into the die using a hydraulic press. The United States Pharmacopoeia states a compression dwell time of 1 min at the minimum compression force required to form a non-disintegrating compacted pellet. The base plate is then detached to expose a smooth and constant pellet surface at the face of the die and it is this pellet surface that is subjected to the dissolution test.

In the rotating disk apparatus the die is then inverted and screwed onto a customised shaft on a dissolution tester, which is then lowered into the dissolution medium until the face pellet is 2.54 cm from the bottom of the vessel (Figure 1.3). The shaft is then rotated in the same way as the USP apparatus 1 and 2. In the stationary disk apparatus the die containing compressed pellet is placed face up into a flat-bottomed dissolution-vessel (Figure 1.4), prefilled with the appropriate volume of dissolution medium. The medium is stirred by means of a rotating paddle (e.g. USP apparatus 2) positioned 6 mm above the pellet surface.

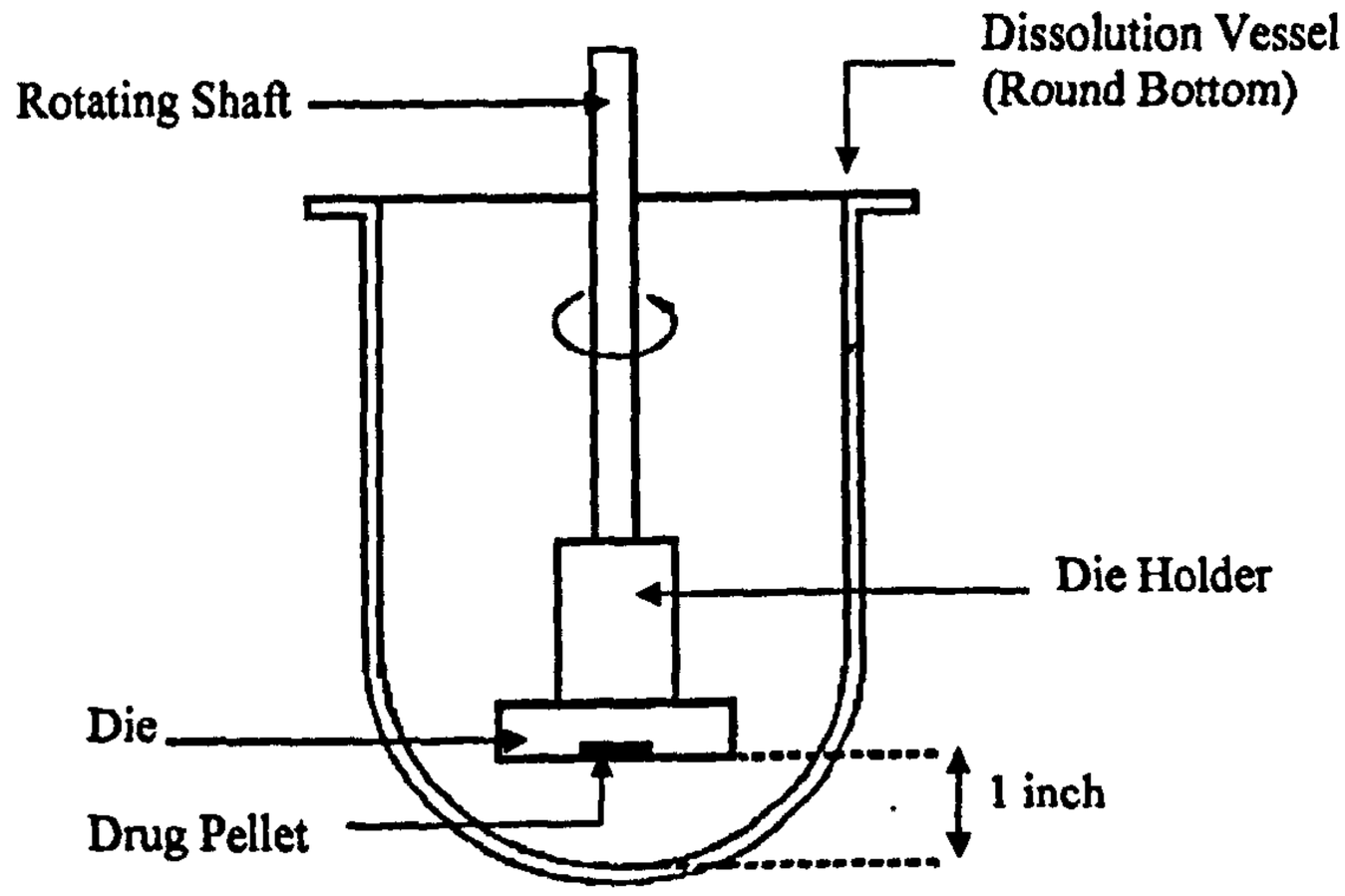


Figure 1.3. Schematic of rotating disk IDR apparatus (Woods apparatus).

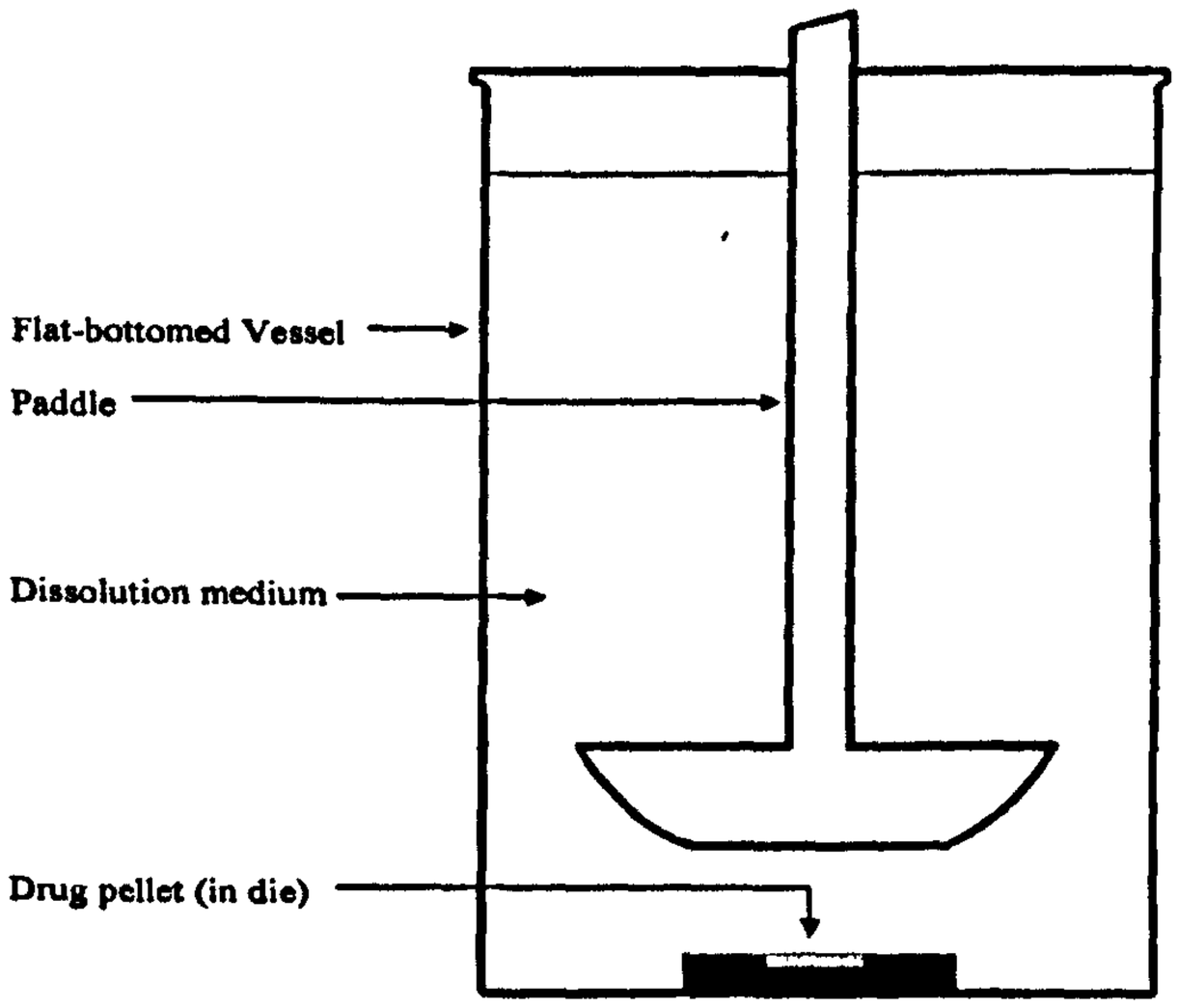


Figure 1.4 Schematic of a stationary disk IDR apparatus

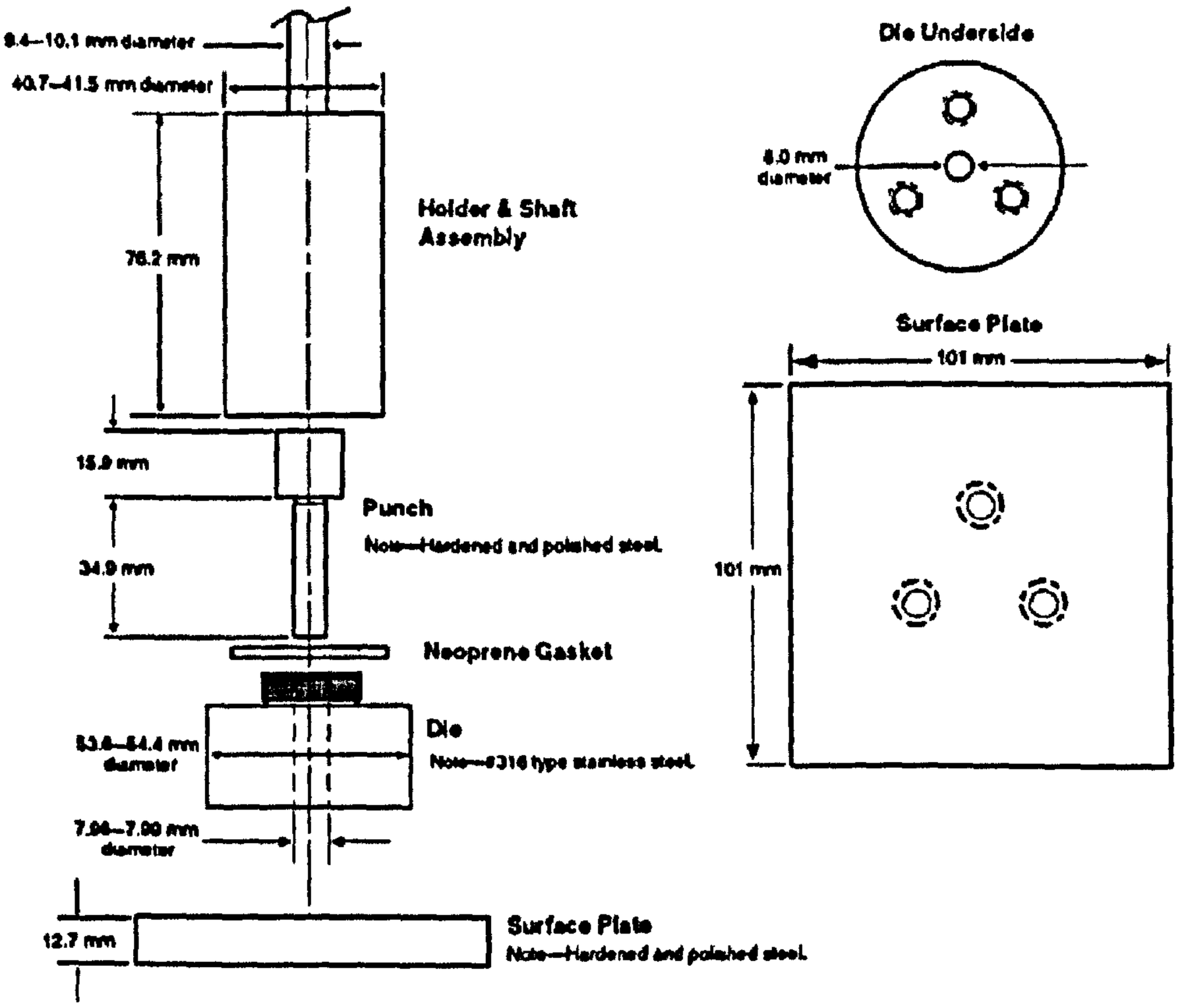


Figure 1.5. Tooling used to produce disks for IDR testing. *From* United States Pharmacopoeia 28 (USP, 2005)

Disadvantages of the rotating disk apparatus include the risk of air bubbles forming on the surface of pellet, which could affect the dissolution rate, and heat loss of approximately 2°C through the shafts when the dies are first lowered into the dissolution medium. Using the stationary disk apparatus significantly reduces the formation of air bubbles, whilst heat losses are eliminated since the dies are totally submerged in the dissolution medium (Viegas *et al.*, 2001).

The IDR is calculated by plotting the cumulative amount of substance dissolved per unit area of the exposed pellet surface against time until 10% of the drug pellet has dissolved (limit of sink conditions). Linear regression should be applied to data points up to this point and the slope of the regression line gives the IDR of the substance under test in mg/min/cm^2 (United States Pharmacopoeia 28, 2005). However, this compendial calculation may prove difficult to apply to poorly soluble compounds where 10% dissolution may not be achieved. In these instances, it may

be practical to use an analytical method with sufficiently high sensitivity to be able to plot 6-8 data points before 10% dissolution and apply linear region.

1.2. Disintegration as a prerequisite for dissolution from solid oral dosage forms

The IDR of a drug substance is a measure of the inherent drug solubility and does not take into account formulation factors that may affect dissolution (Section 1.1.4). During the determination of the IDR, maintenance of a constant surface of the exposed disk is required. Conversely, for solid oral dosage forms such as tablets and capsules, drug exposure to the dissolution medium requires the collapse of the unit. The disintegration of tablets and the disintegration test method are described in this section.

1.2.1. Tablet disintegration

The disintegration of a tablet is a fundamental requirement for release of the drug from the dosage form and to promote dissolution. When immersed in liquid, the solid surface is wetted and liquid penetrates the tablet pores causing a break up of the dosage form. In practice, this process is usually aided by the addition of a disintegrating agent to the formulation, which performs its function by swelling, capillary action or generation of a gas (Lowenthal, 1973). The initial fragments produced may be large agglomerates that disintegrate further into primary drug particles. Deaggregation into these primary drug particles provides the largest surface area for dissolution (Wagner, 1969; Wells & Rubinstein, 1976). The mechanism is related to dissolution diagrammatically in Figure 1.6.

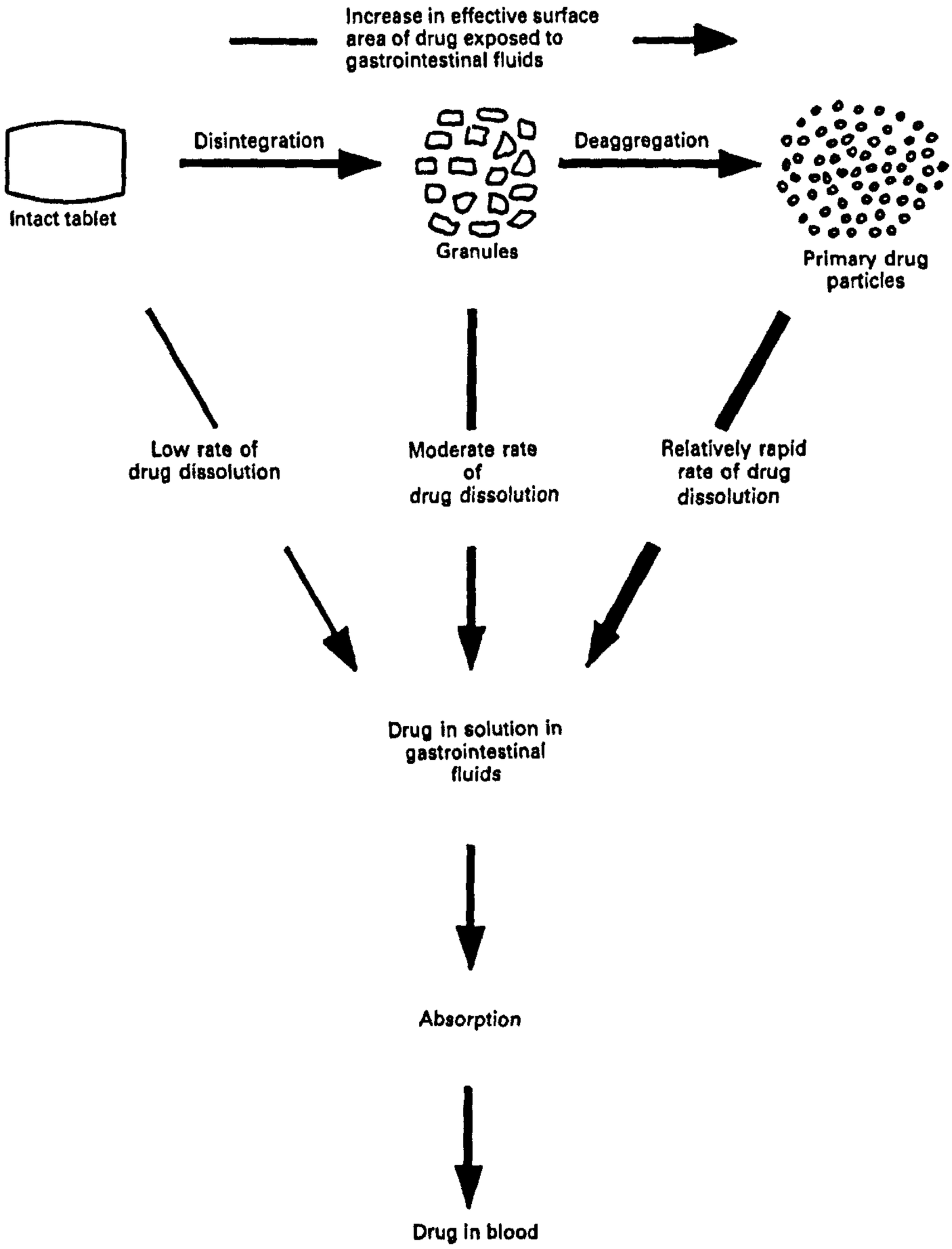


Figure 1.6. Mechanism of the drug release process from a tablet by disintegration and dissolution. *From* Rubinstein (1988)

1.2.2. Disintegration Testing

The pharmacopoeial disintegration test for tablets and capsules determines whether these dosage forms disintegrate within the time prescribed in the individual monograph. The test is not intended for sustained release and chewable dosage forms. The United States Pharmacopoeia 28 and British Pharmacopoeia (2004)

describe a similar apparatus for disintegration testing. The description below is taken from the British Pharmacopoeia 2004.

The apparatus consists of a basket-rack assembly (Figure 1.7) holding six cylindrical transparent tubes 75.0 to 80.0 mm long, 21.5 mm in internal diameter and with a wall thickness of about 2 mm. The tubes are held vertically by two separate and superimposed rigid plastic plates 90 mm in diameter and 6 mm thick, perforated by six holes. The holes are equidistant from the centre of the plate and are equally spaced from one another. Attached to the under side of the lower plate is a piece of woven gauze made from stainless steel wire 0.635 mm in diameter and having mesh apertures of 2.00 mm. The plates are held rigidly in position and 77.5 mm apart by vertical metal rods at the periphery and a metal rod is also fixed to the centre of the upper plate to enable the assembly to be attached to a mechanical device capable of raising and lowering it smoothly through a distance of 50 to 60 mm at a constant frequency of between 29 and 32 cycles per minute. The assembly is suspended in the specified liquid medium in a 1000-ml beaker. The volume of liquid is such that when the assembly is in the highest position the wire mesh is at least 15 mm below the surface of the liquid and when the assembly is in the lowest position the wire mesh is at least 25 mm above the bottom of the beaker and the upper open ends of the tubes remain above the surface of the liquid. The temperature of the liquid is maintained at 35° to 39°. The design of the basket-rack assembly may be varied provided that the specifications for the tubes and wire mesh are maintained. Where stated in the monograph, a plastic disk may be added to each tube to aid immersion of floating dosage forms. The disk specifications are described in the pharmacopoeias.

To run a test, one tablet or capsule is placed into each of the six tubes, the assembly is suspended in the beaker containing the specified liquid and the apparatus operated for the specified time. The tablets or capsules pass the test if all six have disintegrated. The United States Pharmacopoeia 28 defines complete disintegration as:

"that state in which any residue of the unit, except fragments of insoluble coating or capsule shell, remaining on the screen of the test apparatus is a soft mass having no palpably firm core."

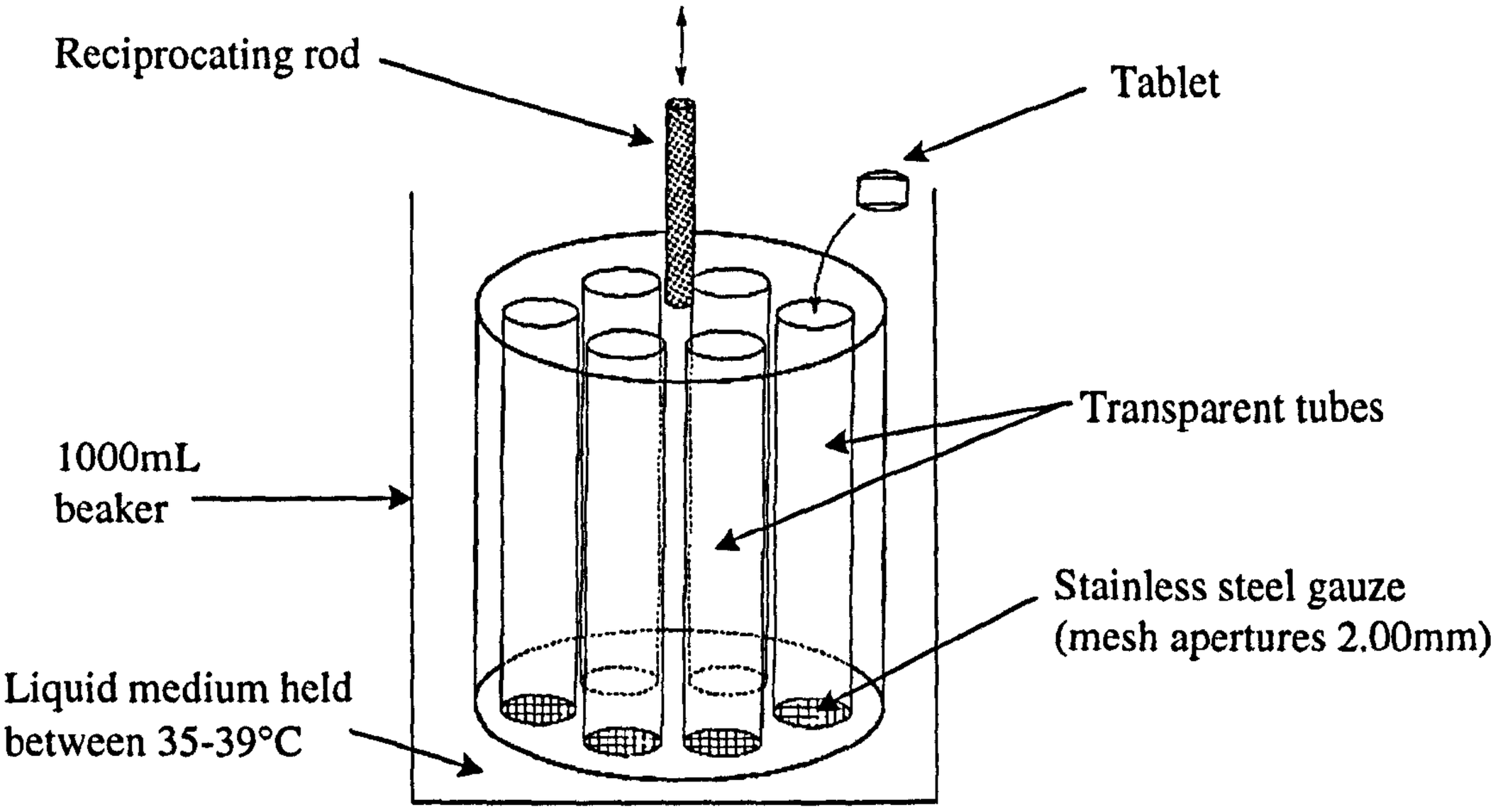


Figure 1.7. Schematic diagram of the basket rack assembly of the Disintegration Test apparatus.

1.3. Dissolution Testing

Dissolution tests are one of the most commonly used tests in the characterization and quality control (QC) of oral dosage forms. They are vital if dissolution is the rate-limiting step in drug absorption, e.g. in rapidly disintegrating tablets or capsules. From a QC perspective, dissolution testing is mainly used to confirm batch-to-batch consistency, site and manufacturer variation, product stability and to identify good and bad formulations (Hanson, 1991a). Dissolution tests are used to confirm compliance with compendial specifications and are therefore needed as part of a marketing authorisation. Additionally, they are used during product development and stability testing as part of the development specification for the product. Critically, from an R&D perspective, there is the potential to correlate *in vitro* dissolution data with *in vivo* bioavailability, which would greatly facilitate product development.

1.3.1. Compendial Testing Methods

The general principle of dissolution tests is that the powder or solid dosage form is tested under uniform agitation, which is accomplished by either passing the medium over the sample or by agitating the sample in the medium. Two general methods, using the basket or paddle apparatus, are currently included in the United States Pharmacopoeia 28 and the British Pharmacopoeia 2004 to measure dissolution from immediate-release oral tablets and capsules whilst there are several variants used in the testing or modified-release oral dosage forms and other, non-oral types of dosage form. The compendial dissolution tests for oral dosage forms are described below.

1.3.1.1. *Basket Apparatus (USP Apparatus 1)*

Essentially, in this method, the dosage form is placed in a basket that is lowered into the dissolution medium and rotated at a specified speed.

The apparatus consists of a motor, a metallic drive shaft, a cylindrical basket and a covered vessel made of glass or other inert transparent material. The latter should be made of materials that do not sorb or react with the sample tested. The contents are held at 37 ± 0.5°C. There should be no significant motion, agitation, or vibration caused by anything other than the smoothly rotating stirring element. Ideally, the apparatus should provide observation of the stirring element and sample. The vessel is cylindrical with a hemispherical bottom and sides that are flanged at the top. It is 160 to 175 mm high and has an inside diameter of 98 to 106 mm, and a nominal capacity of 1000 mL. A fitted cover may be used to retard evaporation but should provide sufficient openings to allow ready insertion of a thermometer and allow withdrawal of samples for analysis. The shaft is so positioned that its axis is no more than 2 mm at any point from the vertical axis of the vessel and should rotate smoothly, without significant wobble. The shaft rotation speed should be maintained within ± 4% of the rate specified in the individual monograph. The shaft and basket should be constructed of stainless steel, type 316 or equivalent. Other specifications for the basket apparatus are given in Figure 1.8. A 2.5 μm thick gold coating on the basket may be used for acidic media. For testing, a dosage unit is

placed in a dry basket at the beginning of each test. The distance between the inside bottom of the vessel and the basket is 25 ± 2 mm (Ford & Rajabi-Siahboomi, 2002).

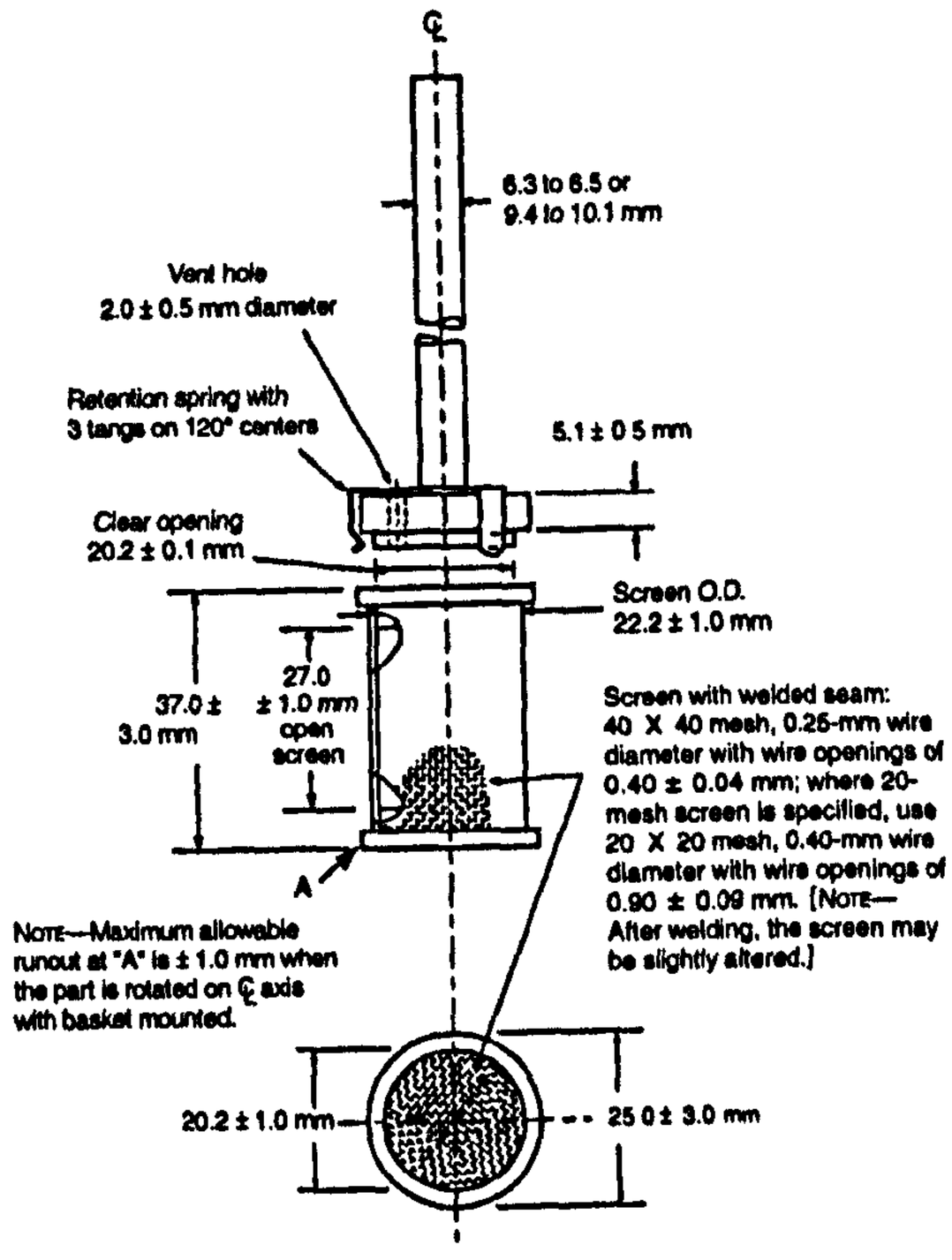


Figure 1.8. The basket stirring element of USP 28 (Apparatus 1)

Apart from apparatus design, certain practical issues can potentially be problematic. For example, corrosion of the wire basket when placed in acidic media; obstruction of fluid flow through the basket mesh due to adhering substances; variation in agitation conditions produced by the rotating basket leading to poor reproducibility and the risk of particles falling from the basket to the bottom of the vessel and consequently being subjected to different agitation conditions to those within the basket (Hanson, 1991b; Ford & Rajabi-Siahboomi, 2002). Finally, there is the possibility of dissolution being accelerated due to abrasion of the surface of the dosage form as it rubs against the basket mesh, the so-called 'cheesegrater effect'.

1.3.1.2. *Paddle Apparatus (USP Apparatus 2)*

In Apparatus 2, the paddle apparatus, a paddle is used as the source of agitation. As with the basket apparatus, the shaft should position no more than 2 mm at any point from the vertical axis of the vessel and rotate without significant wobble. Figure 1.9 gives the shaft specification. The paddle should be rotated at a fixed depth of 25 ± 2 mm from the bottom of the vessel throughout the test. The metallic blade and shaft may be coated with a protective inert coating to prevent corrosion. The dosage form is dropped into the vessel and the paddle rotated when the dosage form has reached the bottom of the vessel.

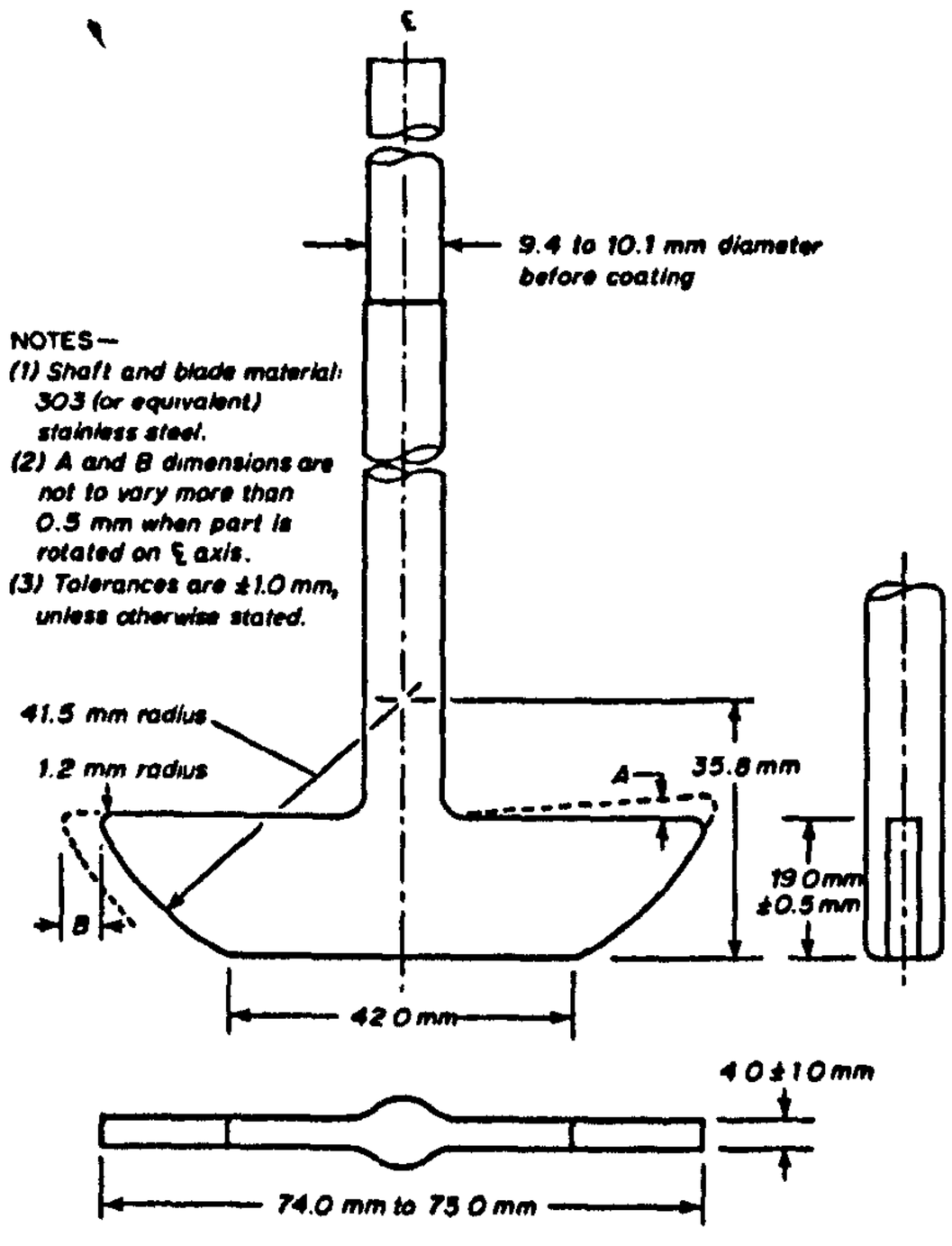
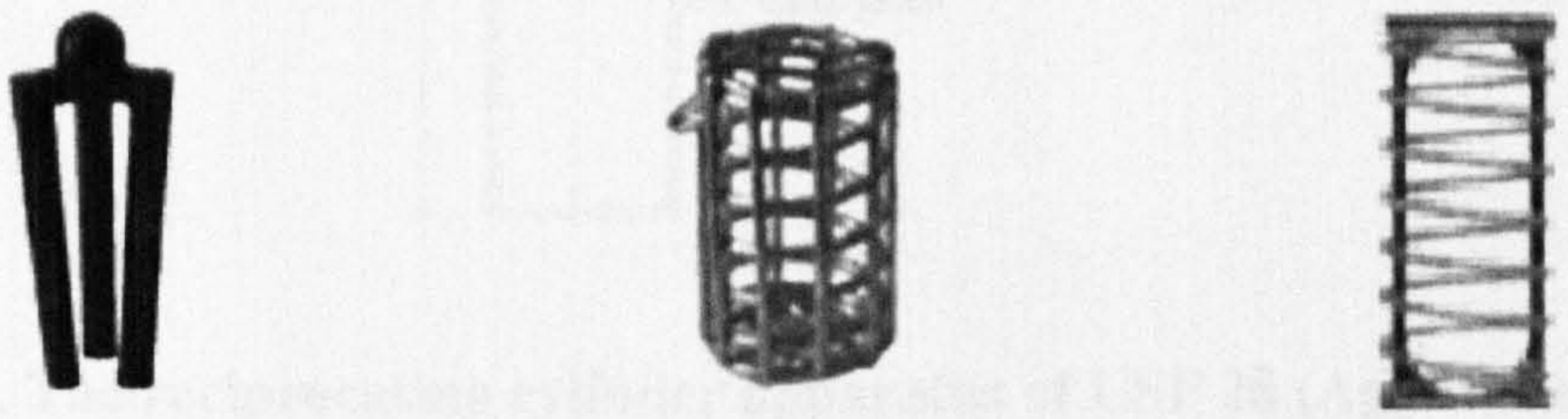


Figure 1.9. The paddle stirring element of USP 28 (Apparatus 2)

In the case of hard-gelatin capsules and other floating dosage forms, a 'sinker' is required to weight the sample down until it disintegrates and releases its contents at the bottom of the vessel. The sinker has to hold the capsule in a reproducible and stable position directly below the paddle, but it needs to be constructed in such a fashion that it does not significantly affect hydrodynamic flow within the vessel nor should it appreciably reduce the surface area of the capsule available to the

dissolution medium. Two designs have predominated, the three-fingered clip and the helical spring. The former comprises a small circular disc with three short, parallel rods sticking out from it, into which the capsule is wedged. The device is typically plastic, but the disc contains metal, which gives it the necessary weight to fall to the bottom of the vessel. The latter is a stainless steel or plastic-coated stainless steel helix (coil, spring) down the middle of which the capsule is inserted. However, with this design, as the thickness of the wire used and the number of turns in the spring increase, the available surface area of the capsule decreases, leading to a concomitant decrease in the observed rate of dissolution (Soltero *et al.*, 1989; Avgoustakis *et al.*, 1992). The United States Pharmacopoeia allows for 'a small, loose piece of non-reactive material such as not more than a few turns of wire helix…' whilst the Japanese Pharmacopoeia (JP) actually prescribes a specific sinker (Figure 1.10).



(a) 3-Prong sinker (b) JP Basket Sinker (c) Helical-spring sinker

Figure 1.10. Typical sinker designs. (a) 3-prong sinker, (b) Japanese Pharmacopoeia basket sinker, (c) helical-spring sinker

1.3.1.3. *Reciprocating Cylinder Apparatus (USP Apparatus 3)*

An apparatus comprising vertically reciprocating tubes, sealed with mesh discs at each end to restrain the dosage form is official in United States Pharmacopoeia 28 as the Reciprocating Cylinder Apparatus. This has been commercially developed as the Bio-Dis apparatus® which allows tubes containing the sample to be plunged up and down in a small vessel containing the dissolution medium (Figure 1.11). It has been designed to allow the tubes to be dipped sequentially in up to six different

media vessels, using programmes that vary the speed and duration of immersion. It allows automated testing for up to 6 days and the manufacturers advocate its use in the testing of extended-release dosage forms (Dyas & Shah, 2006).

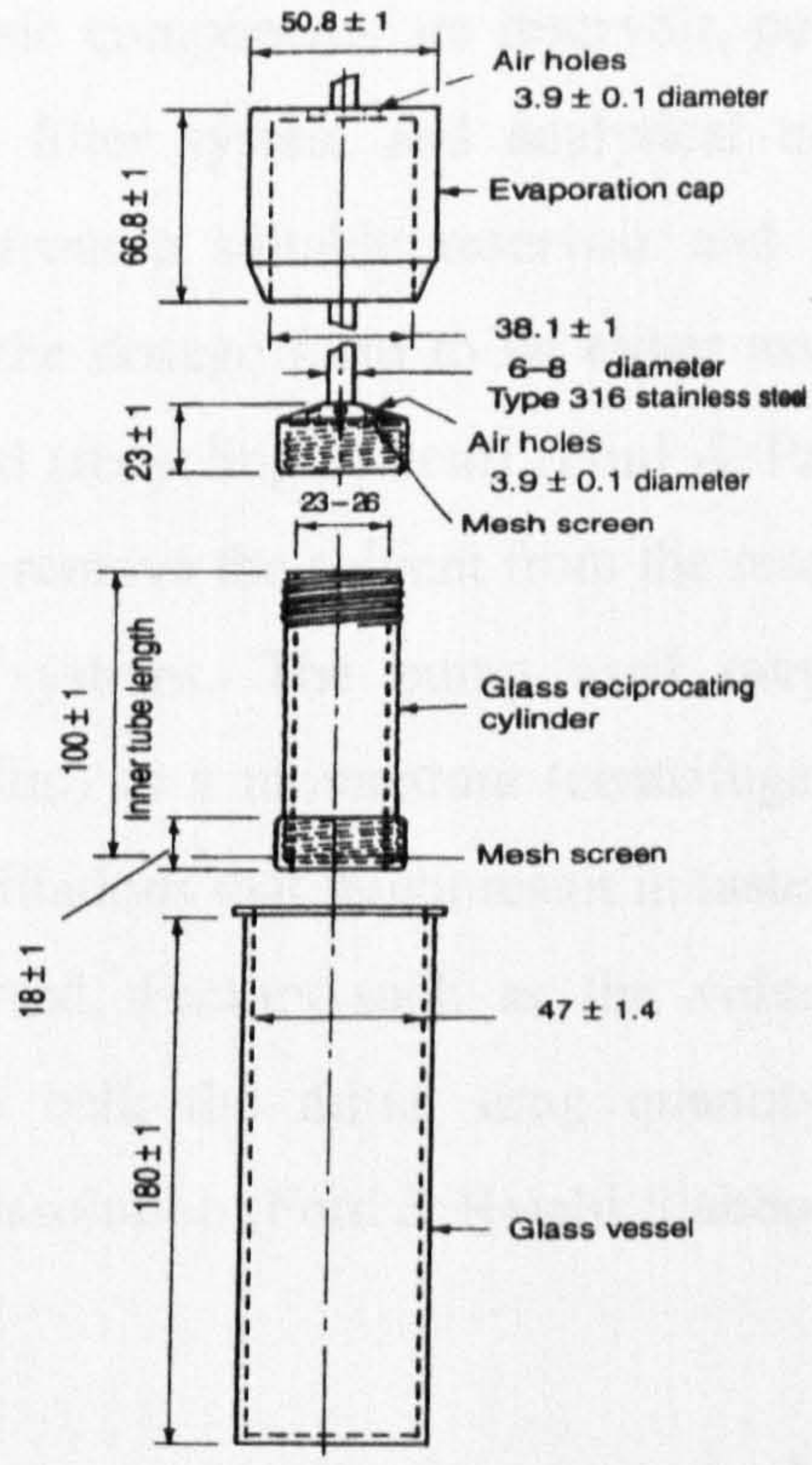


Figure 1.11. The reciprocating cylinder apparatus of USP 28 (Apparatus 3)

1.3.1.4. *Flow-through Cell Apparatus (USP Apparatus 4)*

The previously described limited-volume apparati operate under non-sink conditions, which can restrict the study of poorly soluble drugs. A flow-through system and reservoir may be used to provide sink conditions by continually removing solvent and replacing it with fresh solvent. Alternatively, continuous re-circulation may be used when sink conditions are not required. The disadvantages of limited-volume apparati (Tingstad & Riegelman, 1970) are [1] lack of flexibility, [2] lack of homogeneity, [3] the establishment of concentration gradients, [4] their semi-quantitative agitation, [5] the obscuring of details of the dissolution processes and [6] their variable shear.

Consequently, the flow-through apparatus has been developed which features a dissolution cell of low volume (often less than 30 mL) and a reservoir to provide fresh solvent. The apparatus is now official in the United States Pharmacopoeia as USP Apparatus 4 (Figure 1.12), where it is prescribed for testing extended-release dosage forms. The basic components are reservoir, pump, heat exchanger, column (cell), tablet support, filter system and analytical method. The systems enable solvent to be taken from a suitable reservoir and passed straight through the apparatus containing the dosage form to be either assayed and removed (effluent system) or recirculated (recycling system) (Ford & Rajabi-Siahboomi, 2002). The design of the pump to remove the solvent from the reservoir is crucial to the results obtained from such systems. The pump used may be either a displacement (oscillating or peristaltic) or a momentum (centrifugal) type. However, peristaltic pumps may create oscillations that might result in faster dissolution rates than might otherwise have occurred. Factors such as the volumetric flow rate, the cross-sectional area of the cell, the initial drug quantity, liquid velocity and drug concentration affect dissolution (Ford & Rajabi-Siahboomi, 2002) .

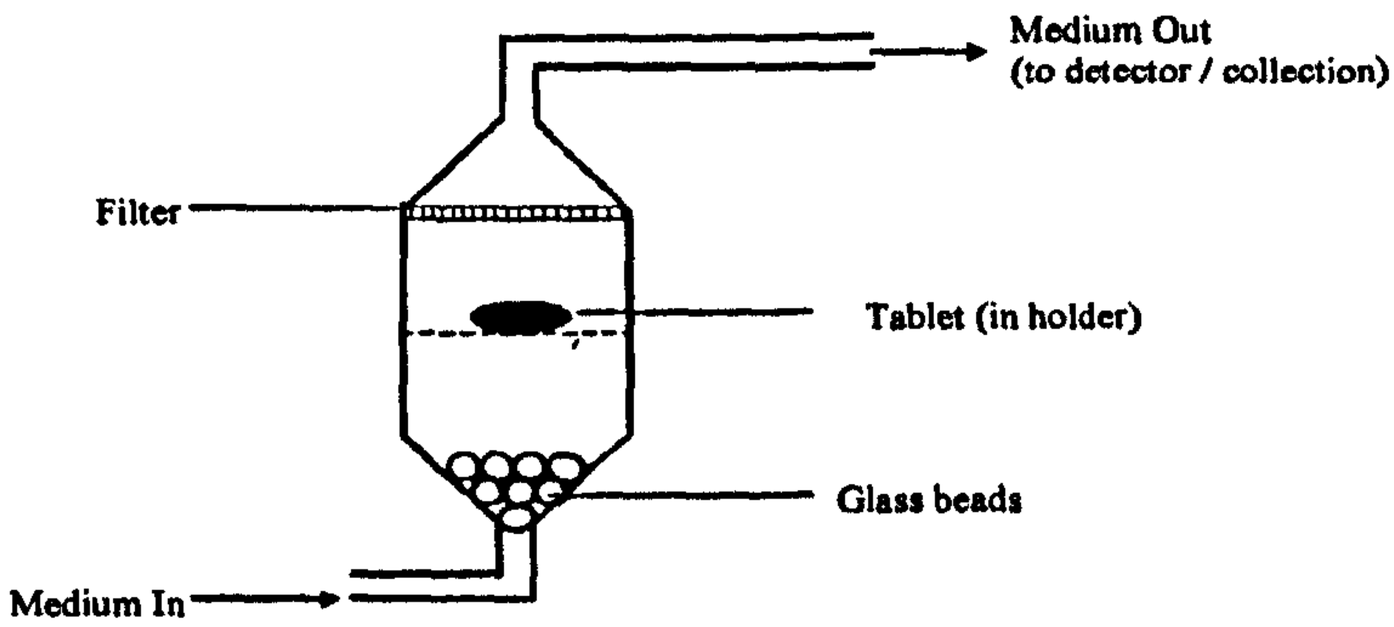


Figure 1.12. Schematic diagram of a flow-through dissolution cell. *From* Ford and Rajabi-Siahboomi (2002)

The maintenance of a controlled flow is crucial to column methods and can be influenced by the inlet system. Glass beads are often inserted at the bottom of the cell to ensure laminar flow of the solvent and allow similar disintegration of all the surfaces of the dosage form. However, attrition of the dosage form by the glass beads may influence disintegration and give erroneous dissolution rates. In order to generate reproducible results the tablet must be supported and positioned in the

fluid flow in a consistent manner. Consequently, attempts have been made to set the tablet in glass wool or glass beads.

Laminar flow conditions are typically used for tablets, hard gelatin capsules, powders and granules. Suppositories and soft gelatin capsules are placed in the cell without beads for turbulent flow. Nicolaides *et al* (2000) reported differences in the dissolution rate of a poorly soluble drug, tiaglitazone, from an immediate release tablet formulation based on the presence or absence of a tablet holder and/or beads in the flow through cell.

Flow-through facilities can be constructed from cylindrical chromatographic columns with flow-rates as low as 1 mL/min. Ascending flow minimises the problems associated with air bubbles and allows laminar flow of the solvent and ascending columns are the most common types of flow-through apparatus. The columns may be short with tapered inlet and outlet sections but generally are long sections of straight-sided tubing to provide hydrodynamic stability to the liquid flow. The material under test is placed in the vertically mounted dissolution cell which permits solvent at 37° ± 0.5 °C to be pumped in from the bottom. The cell type selected is dependent on the dosage form being tested. Standard cells used for tablets and capsules have an internal diameter of 12mm but may show a higher dissolution rate when compared to 26mm cells due to higher flow velocity. For testing powders and granules a modified cell containing 2 screen plates is used where the sample is placed between the screen plates.

The flow rate of the dissolution medium through the cell must be specified for each product. The USP recommends a flow rate between 4-16mL/min with an allowance of ±5%. Manual operation and sampling for this type of test can be tedious and the system can be automated to control the pump, heat exchanger and test procedure and deliver samples to a fraction collector. The system can be programmed to switch between different media at predetermined time points to allow pH changes during the test.

Further advantages of the flow through method (Looney, 1996) include [1] selection of laminar or turbulent solvent flow conditions, [2] simple manipulation of medium pH to match physiological conditions, [3] application to a wide range of dosage forms e.g. tablets, hard and soft gelatin capsules, powders, granules, implants and suppositories.

Whilst the applicability of the flow through apparatus to biorelevant dissolution tests still requires further investigation and optimisation, a Level A IVIVC has been reported with this apparatus using physiologically relevant media and a flow rate of 8 mL/min (Sunesen *et al.*, 2005).

1.3.1.5. *Reciprocating Holder Apparatus (USP Apparatus 7)*

There are several variants to this apparatus, which is based on a sample holder that oscillates up and down in the medium vessel. The sample holder may take the form of a disk, cylinder or a spring on the end of a stainless steel or acrylic rod, or it may simply be the rod alone. The sample is attached to the outside of the sample holder either by virtue of being self-adhesive (e.g transdermal delivery system) or is glued in place using a suitable adhesive. This apparatus may be used for transdermal products, coated drug delivery systems or other suitable products (e.g osmotic pump devices). It is prescribed for the drug-release testing of Psuedoephedrine hydrochloride extended-release tablets USP where the tablets are enclosed in a 5 cm x 5 cm square of nylon, which is then attached to the rod (Dyas & Shah, 2006).

1.3.2. Sample Collection and Analysis

1.3.2.1. Sample collection

The United States Pharmacopoeia 28 and the British Pharmacopoeia 2004 both state that samples should be drawn from a region equidistant between the surface of the dissolution medium and the top of the rotating basket or paddle, not less than 1 cm from the vessel wall. The volume of sample withdrawn should be replaced with an

equal volume of fresh medium or the reduced volume should be accounted for in the dissolution calculation. Samples taken should be filtered using appropriate inert filters that do not adsorb the drug nor liberate materials that could interfere with the analytical procedures. The filter pore size should not be greater than 1 μm. When capsule shells interfere with the analysis, the contents of a minimum of six capsules should be removed as completely as possible and the shells dissolved in the dissolution medium. This allows calculation of correction factors, which should be no greater than 25% of the labelled content.

The advent of sophisticated, programmable autosamplers allows samples to be removed automatically into tubes or vials for off-line spectroscopic or chromatographic analysis. However, care has to be taken to ensure in-line filters do not become blocked after repeated use. Autosamplers that can pump liquid samples from the dissolution vessel through a spectrophotometer and back into the vessel offer combined on-line sampling and analysis. Again, care has to be taken to ensure particles do not enter the tubing system and cause blockage. Also, excessive lengths of tubing can lead to a significant lag-time before the sample reaches the spectrophotometer or pools of cool, stagnant fluid causing precipitation of dissolved solute. A typical on-line UV dissolution system is depicted in Figure 1.13.

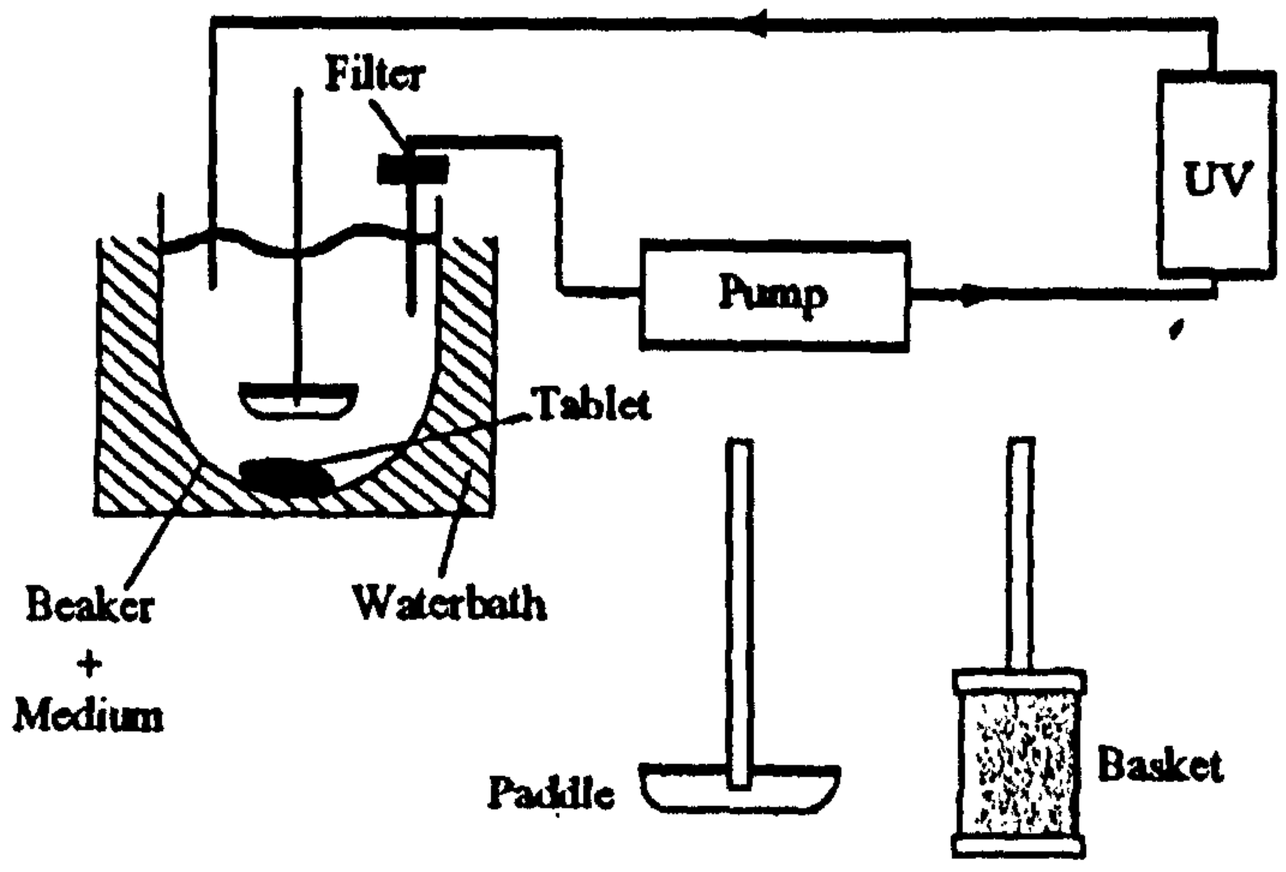


Figure 1.13. Typical apparatus set-up for on-line UV measurement of dissolution. *From* Ford and Rajabi-Siahboomi (2002)

1.3.2.2. Assay methods

Whatever assay technique is chosen, it must be specific to the drug being studied. For single-drug entities, UV spectroscopy is often the method of choice as it is quick, simple and easily lends itself to on-line automation. The use of UV photodiode array spectrophotometry has become popular in the testing of multidrug dosage forms. Where analytical sensitivity or selectivity is an issue, HPLC assays are commonly resorted-to as these allow low-level quantitation of high-potency (low-strength) drugs. In the case of multidrug dosage forms, more complicated assay techniques may be required, which makes HPLC useful since more than one drug can be analysed in the same chromatogram. Whilst HPLC can be automated it is essentially off-line and on-line strategies are often preferred.

1.3.2.2.1. *Fibre-optic technology*

Fibre optic technology for *in-situ* UV assay has received particular interest as it offers significant labour, time and cost savings by eliminating the sampling and filtering stages of the dissolution test (Martin, 2003). A fibre optic probe is positioned in each vessel in the same fashion as a sampling probe. Light is then transmitted from a linked spectrophotometer into an open-sided reflectance cell in the vessel, reflecting back to the detector where absorbance is measured (Bynum & Kraft, 1999). The dissolution profiles are calculated in real time allowing the direct scrutiny of product performance. This technology is also capable of rapid measurements of a series of vessels allowing intervals of less than 1 min between sampling time points during a dissolution run. A multiplexing arrangement is required to selectively transmit the signal from each vessel, in turn, to the spectrophotometer.

Proposals for a new general chapter on dissolution in the USP (Gray *et al.*, 2001) have highlighted the use of this technology and a regulatory perspective has also been published (Gray, 2003). These developments suggest that fibre optic technology is likely to emerge as a common analytical tool as long as the following considerations are addressed:

1. The spectrum of each component of the formulation needs to be evaluated to ensure there is no excipient interference. Spectra should be determined in the dissolution media to be used and at concentrations expected in the dissolution vessel. (Minor interference can be corrected for using software algorithms.)

2. The absorbance of the active ingredient at 100% release from the dosage form being tested must be below the photometric range maxima since sample dilutions are not possible, A shorter path-length cell will be required if the absorbance is too high.

3. The limit-of-detection for fibre optics is higher than for conventional spectrophotometry because the loss of signal down the fibre decreases the Signal:Noise ratio. This loss of sensitivity may be overcome with a longer path length cell.

4. Turbidity and light scattering caused by undissolved drug or excipients interfere with spectral measurement and increases noise (Martin, 2003). The interference should be limited to 2-3% and if this is not achievable, conventional discrete sampling and off-line measurement may be required.

5. Formation of air bubbles on the fibre optic probe can affect the accuracy of the analysis (Lu *et al.*, 2003). The dissolution medium should always be properly degassed prior to the test.

Furthermore, prolonged transmission of far-UV radiation leads to degradation of the fibre and increasing opacity whilst fouling of the external surface of the fibre by some media components e.g. enzymes, surfactants etc. can be a problem in some cases.

1.3.3. Data Presentation and Interpretation

The data generated from dissolution tests are usually presented as dissolution profiles of the amount released plotted as a function of time. Drug release is

monitored at several time-points or, where possible, continuously until 100% of the dose is dissolved. Values equivalent to the times for 10, 50, 70 or 85% drug release are often cited as $t_{10\%}$, $t_{50\%}$, $t_{70\%}$, or $t_{85\%}$ (Figure 1.14). The dissolution profiles from tablets and capsules are often sigmoidal in shape.

Whilst pharmacopoeias do not require a dissolution profile to be generated, they do state the amount of drug that must dissolve (or not dissolve in the case of enteric-coated products) within a specified time. When a single-time specification is stated, the test can be stopped earlier if the requirement for a minimum amount dissolved is met. If two or more times are specified, then the samples should be withdrawn with a tolerance of ± 2% of the stated time.

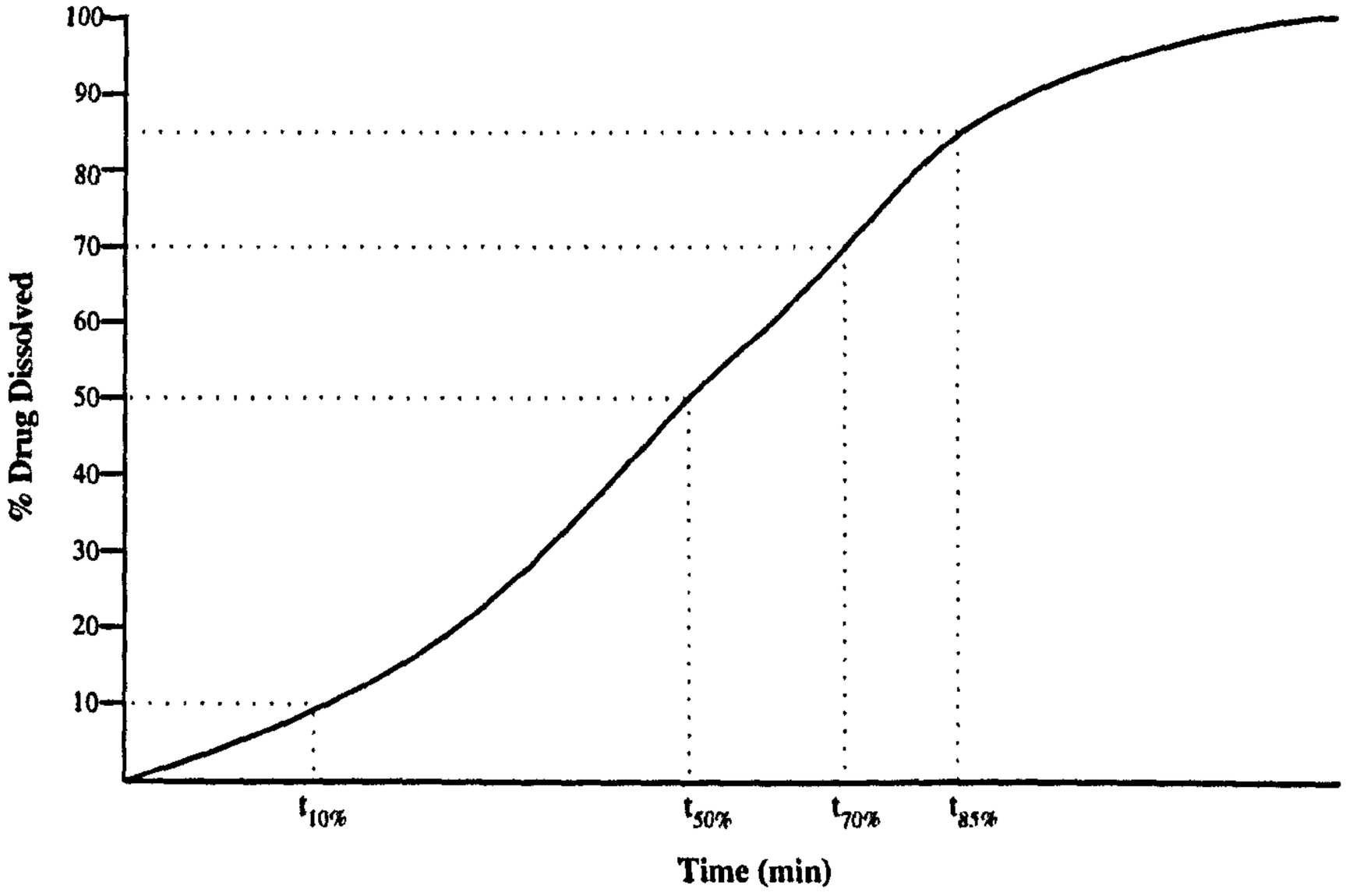


Figure 1.14. Hypothetical dissolution profile showing the determination of $t_{10\%}$ $t_{50\%}$, $t_{70\%}$ and $t_{85\%}$

The United States Pharmacopoeia 28 assesses dissolution in a three–stage series of tests with the amount of drug dissolved after a specified time being expressed as a percentage of the nominal content of the dosage form. The time at which the sample is to be tested is specified in the monograph as is the so-called Q-value, the minimum percentage-dissolved at that time. In the first stage (S1), six units are tested and the pass criteria are that the amount of drug dissolved from each unit at the specified time should be no less than Q + 5%. Failure at S1 requires a second

stage test (S2) to be performed on an additional six units. To pass the test at this stage the average content dissolved, from the combined two stages (i.e. 12 units) should be equal to or greater than Q with no unit being less than Q-15%. Failure leads to stage 3 where a further 12 units are tested. These results are combined with the results from the previous stages. The average of the total of the 24 units thus tested should be equal to or greater than Q. No more than two units should be less than Q - 15% and no unit should be less than Q - 25% (USP, 2005)

In the testing of delayed-release products there is a similar three-tiered approach for both the acid-dissolution (A1, A2 & A3) and buffer-dissolution (B1, B2 & B3) stages. For extended-release the levels are denoted L1, L2 and L3.

1.3.4. Operational factors that may affect the dissolution test

The British Pharmacopoeia 2004 and the United States Pharmacopoeia 28 each give considerable guidance about external factors that may affect dissolution. The more significant factors are referred to in this section.

1.3.4.1. Vibration

Vibration is a source of energy that is transmitted to the dissolution medium and can therefore significantly affect dissolution data. It was Beyer and Smith (1971) who first reported a six fold increase in the dissolution rate of tolbutamide tablets due to increased vibration of the dissolution vessel. Therefore, to minimize the effects of vibration, the distance from the stirring drive motor to the stand supporting it and the distance from the rotating basket to the point connecting the shaft to the dissolution motor should be specified. Vibrations may arise from various sources including equipment such as the water bath and pump. There is anecdotal evidence that vibration transmitted through building fabric (floors, walls) and furnishings (benches) from more distant sources such as lifting-gear, air-conditioning and heating systems may also have an effect. The net result is that equipment should be heavily insulated and clamped into position to effectively increase the mass of the vessels and thereby decrease vibration.

1.3.4.2. Alignment of the Stirrer Shaft

Misalignment of the stirring element can be the cause of variation in the dissolution rate of a drug. According to compendial limits, the shaft should be positioned such that its axis is no more than 2 mm at any point from the vertical axis of the vessel. Significant wobble should also be eliminated. Bending of the stirring rod has been reduced by increasing the acceptable diameter from 6.0 to 6.5 mm to approximately 10 mm. Minor changes in physical alignment of the paddle may produce large variations in results.

1.3.4.3. Vessel Design and Construction

Rosalia *et al* (1972) discovered that flask shape affected the hydrodynamics of the system through differences observed in the dissolution rate of hydrochlorthiazide tablets using flasks from two manufacturers. The use of a hemispherical flask allows some variation in construction. Minor changes in vessel shape may considerably alter the dissolution rates determined by the paddle method (Cox *et al.*, 1982) due to changes in the hydrodynamics created by the different shapes. Plastic vessels provide closer dimensional tolerances than glass vessels (Cox & Furman, 1984) and should be preferred to glass vessels so long as drug does not sorb to them and that the dissolution fluid does not interact with the plastic (Cox *et al.*, 1982). However, plastic vessels also have practical disadvantages. Plastic has a lower thermal conductivity than glass, therefore, the warm up time for the medium is longer and the temperature of the medium may be difficult to maintain as heat may radiate faster from the surface of the medium than from the vessel wall (Hanson, 1991b). During dissolution, some tablets may become more centred in glass vessels than in plastic vessels, which is probably attributable to friction between the tablet and the wetted vessel surface being greater for plastic than for glass. Even the method of cleaning the vessel may result in different dissolution properties. Differences have been obtained from glass containers when cleaned with soap and water or 95% ethanol, the former method leaving a residual film (Cox & Furman, 1984).

1.3.4.4. Sampling Procedures

Flow-through facilities usually allow on-line ultraviolet (UV) analysis of drug dissolved or collection of samples for subsequent analysis. The sample must be filtered prior to analysis to reduce turbidity problems caused by undissolved drug and excipients and to help eliminate false results caused by particles dissolving following removal.

A sampling probe used for continuous sampling must not disturb the hydrodynamics of the system. When continuous monitoring is accomplished by flow-through systems, the flow rate should not be too high or some of the suspended drug will adhere to the filter, resulting in a percolation effect and artificially high estimates of the amount of drug released (Ford & Rajabi-Siahboomi, 2002). The sampling probe should be as small as possible because the displacement volume of the probe may modify dissolution rates. Savage and Wells (1982) described an apparatus that allows removal of the probes giving a maximum of 40 s immersion in the dissolution fluids. Capillary sampling probes of 1.5 mm diameter were used, their size being reduced to minimize their influence on fluid hydrodynamics.

Adsorption of the drug onto the filter should be avoided when sampling and the release of iso-octylphenoxypolyethoxyethanol, which is often included in membrane filters as a wetting agent, may be a potential problem as this surfactant has significant absorption in the range 220 to 240 nm. Hence, selection of an appropriate filter material is important to avoid selective loss of drug through adsorption. Selection of a suitable pore size is also important, as it should be small enough to retain undissolved particles of a significant size but without restricting flow and impeding sample withdrawal. Replacement of sampled fluid must be by fluid of the same temperature as that inside the dissolution vessels.

1.3.4.5. Temperature Control

The Unites States Pharmacopoeia directs that the thermometer should be removed before the test and that the temperature should be checked periodically. The dissolution fluids should be maintained at 37 ± 0.5°C as even slight temperature variations may have a significant effect on tablet dissolution (Hanson, 1991c). It is important, therefore, to prevent evaporation of the medium both to reduce heat loss and maintain the volume of the liquid for dissolution. This is accomplished through the use of plastic covers, placed over the opening of the vessels.

1.3.4.6. Deaeration of the Dissolution Medium

Cox *et al* (1983) investigated the dissolution of prednisolone tablets in water presaturated with air. These authors found that the higher air content in the medium resulted in more drug being dissolved using the paddle method. In water presaturated with air, air bubbles attached to particles of the disintegrating tablets owing to sorption of air. Instead of sinking to the bottom of the vessel, the particles were suspended by the air bubbles and thus subjected to greater conditions of agitation.

Failure to deaerate media used with the basket apparatus results in the clogging of the pores of the basket by air, causing different flow characteristics (Cartwright, 1979) and often a decrease in dissolution rates. This has even greater influence when gel forming extended release dosage forms are being tested (Ford & Rajabi-Siahboomi, 2002). Bubbles attached to tablets or capsules may cause the dosage form to relocate near to the top of the basket, thereby reducing dissolution rates or amounts dissolved (Cartwright, 1979). The basket is intended to hold the tablet in a fixed position. Disintegration of the dosage form will occur in the basket and fluid must flow through the basket sufficiently to disperse the tablets and sweep dissolved drug into the bulk of the dissolution fluid (Ford & Rajabi-Siahboomi, 2002).

The United States Pharmacopoeia 28 and the British Pharmacopoeia 2004 each state that media should be deaerated before use. The United States Pharmacopoeia recommended method of heating the medium and filtering under vacuum is labour intensive and time consuming and other validated methods may be used. Inert gas purging is a popular alternative. Current media preparation stations available can dispense heated and deaerated medium based on the vacuum principle. They also reduce foaming problems encountered during deaeration when surfactants are used in the media. Degenhardt *et al* (2004) compared these methods of deaeration and found helium sparging to be the most effective and efficient. They recommended that media be sparged for 30 s per litre of container volume at a helium flow rate of 40 mL/s, using an inlet filter pore size between 2 and 10 μm.

However, there is a risk of re-aeration when the medium is transferred to the dissolution vessels. A 275% increase in the level of oxygen has been reported after deaeration and dispensing of media into dissolution vessels (Diebold & Dressman, 1998). An alternative approach to deaeration would be to sparge the medium *in-situ* with helium. Dissolution testers with an on-line deaeration facility are available commercially.

1.3.4.7. Variation in Speed of Agitation

Agitation speed must be kept constant during the test. The compendia specify a speed tolerance of ± 4%. Technological advancements have led to electronic control of motors that allows speed to be checked throughout the test period by means of a digital display.

1.3.5. Standardisation and Calibration

All apparati must be calibrated and the variables standardized and known. Variation in dissolution data between individual vessels and between experimental runs can be determined through appropriately designed experiments. Errors in experimental set-up are minimised by using mean dissolution times and partial balancing.

Standardization of equipment and experimental conditions should remove all variable factors.

The United States Pharmacopoeia 28 specifies apparatus suitability tests, based on the operating conditions, involving the USP Dissolution Calibrator, Disintegrating Type or the USP Dissolution Calibrator, Non-disintegrating Type. The apparatus is deemed suitable if the obtained results are within the accepted range stated for that type of calibrator.

Equipment problems such as chain looseness, tilting of stirrer motor, excessive vibration, or misalignment of the flasks with the stirrers have been identified using calibrators (Hanson, 1991d). Non-disintegrating calibrators are composed of 300 mg salicylic acid, whereas disintegrating calibrators contain 10 mg prednisone. The dissolution rates obtained from the calibrants should fall within established ranges at both 50 and 100 rpm.

There has been a growing realisation of late that modern dissolution testers are high-performance instruments, which if subjected to a regular programme of maintenance and mechanical calibration, can perform very accurately and precisely. This has led to a growing body of opinion that appropriate electromechanical calibration regimes may offer far more confidence than chemical calibrators (Shah, 2004).

1.3.6. Other Variables in Compendial Methods

The British Pharmacopoeia 2004 usually specifies 900 mL of dissolution fluid, but for digoxin tablets the volume is 600 mL. Variation in the volume of dissolution medium to be used is more prevalent in the United States Pharmacopoeia 28, for example, 500 mL for alprazolam tablets, 750 mL for metyrosine capsules, 900 mL for ampicillin capsules and 1000 mL for dicumarol tablets. In addition, different volumes may be specified for various strengths of the same product, as in the case of phentermine hydrochloride capsules, where 500 mL of medium is used if the strength is 15 mg or less, but 900 mL is used for preparations containing in excess

of 15 mg. The same volumes are specified for prednisone tablets where the cut-off point is 10 mg and for cinoxacin capsules where 500 mL is used for capsules containing 250 mg or less but 1000 mL is used for capsules containing in excess of 250 mg.

1.3.7. Dissolution Media

The selection of an appropriate dissolution medium is a critical stage of the dissolution test. As previously described the dissolution test has numerous roles that must be taken into account during media selection. For the quality control of a drug product, the chosen medium must be able to distinguish between batch-to-batch variability, scale up and post approval changes (SUPAC) and stability issues. This discriminating ability is built in during dissolution test method development. In general, physiological media are preferred to water-organic solvent mixtures or solutions incorporating surfactants. For a New Drug Application (NDA) it is recommended that, during dissolution method development, dissolution profiles in at least 3 media should be generated i.e. pH 1.2, 4.5 and 6.8. Water can be used as an additional medium. If the drug is poorly soluble, appropriate concentrations of surfactants are recommended (CDER, 2003). All the *in vitro* and *in vivo* data generated can then be used to select a dissolution medium (and agitation speed) with adequate discriminating ability.

Ideally, a dissolution medium should be formulated as close as possible to that pH anticipated in *in vivo* fluids; for example, dissolution media based on 0.1N HCl are used to mimic gastric pH. Simulated gastric fluid is similarly used. Food can increase the gastric pH to as high as 3 to 5. Many compendial dissolution fluids are at a pH near neutral despite the fact that tablets, when swallowed, will meet a lower gastric pH. Both the United States Pharmacopoeia 28 and the British Pharmacopoeia 2004 indicate that the pH of dissolution fluid should be within 0.05 of that specified in the relevant monograph. The use of surfactants and enzymes may also be a coarse approximation of the intestinal fluids, although surfactants may be included to increase drug solubility by solubilization into micelles.

Complex biorelevant media are being used more frequently during the drug development stages to mimic the physicochemical characteristics of the gastrointestinal tract in the fasted and fed states. The aims are to highlight potential bioavailibility issues and attempt to achieve IVIVC that would aid candidate selection and predict product performance without resorting to *in vivo* studies. Thus far, it has not proved viable to extend their utility to quality control applications. Klein *et al* (2004) characterised the physicochemical properties of homogenised standard breakfasts administered during pharmacokinetic studies and compared them to several commercially available liquid meals in an attempt to propose a biorelevant medium. Examples of biorelevant media include fasted state and fed state simulated intestinal fluids (FaSSIF and FeSSIF) consisting of bile salt-phospholipid mixed micelle systems (Tables 1.2.a and 1.2.b), which significantly increase the cost of the test. Bovine milk has also been investigated (Macheras *et al.*, 1987; Galia *et al.*, 1998). Diluted Intralipid® emulsions have been suggested as an alternative to milk because they are not subjected to biological variation. Aiache *et al* (1989), suggested that drug release from controlled-release formulations should be studied following a 2 h contact with peanut oil by a dissolution test involving increases in pH (peanut oil resembles a heavy fatty food).

Table 1.2a. Typical FaSSIF Medium. *From* Vertzoni *et al* (2004)

Component	Content
Sodium taurocholate	3 mM
Lecithin	0.75 mM
NaOH (pellets)	0.174 g
$NaH_2PO_4.H_2O$	1.977 g
NaCl	3.093 g
Purified water q.s.	500 mL

Medium pH = of 6.50; osmolality ≈ 270 mOsm/kg

Table 1.2b. Typical FeSSIF Medium. *From* Vertzoni *et al* (2004)

Component	Content
Sodium taurocholate	15 mM
Lecithin	3.75 mM
NaOH (pellets)	4.04 g
Glacial acetic acid	8.65 g
NaCl	11.874 g
Purified water q.s.	1000 mL

Medium pH = 5.00; osmolality ≈ 635 mOsm/kg

1.3.8. Dissolution Testing of Selected Dosage Forms

1.3.8.1. Dissolution Testing versus Drug Release Testing

The United States Pharmacopoeia distinguishes dissolution from drug-release. Dissolution testing is applied to dosage forms where the drug content is considered to be readily available for dissolution, i.e. the dosage forms have not been formulated to retard the passage of the drug into solution in any substantial way. In contrast to this, products that have been formulated to retard the dissolution in specific media or for a pre-determined period of time are considered to be modified release products and are subjected to drug release testing. Modified release products include extended-release (sustained or slow-release) products and delayed-release (enteric-coated) products. The former are described as allowing at least a twofold reduction in the dosing frequency as compared with that drug presented as a conventional dosage form. A delayed-release product is one that releases the drug at a time other than promptly after administration.

1.3.8.2. Immediate-release Tablets & Capsules

For immediate-release tablets and capsules, the only barrier to drug release is a simple disintegration or erosion stage, which is generally accomplished in less than

1 h. For such dosage forms the dissolution test is based on the straightforward basket or paddle apparatus with a single sample being taken before quantitation. The United States Pharmacopoeia test specification is typically Q = 75% in 45 min although this may vary. For example, for Calcium Carbonate Tablets USP the time specificed is 30 min but for Calcium Gluconate Tablets USP it is 45 min. Nitrofurantoin Capsules USP present a special case where capsules containing macrocrystals dissolve slowly (to reduce GI irritation) and Q at 1, 3 and 8 h is between 20-60%, ≥ 45% and ≥60% respectively. Capsules containing both macrocrystals and the monohydrate form are subjected to a two phase test in an acidic then basic medium as described in the relevant monograph (United States Pharmacopoeia 28, 2005).

For immediate-release formulations of highly water-soluble drugs (e.g. BCS Class I) the United States Pharmacopoeia monograph may omit the dissolution test and rely solely on disintegration testing. This is the case for a number of well-established preparations such as Apomorphine Hydrochloride Tablets USP. Otherwise, the only practical modification that may be required for immediate-release drugs is for floating dosage forms where a sinker is required (Figure 1.10)

1.3.8.3. Delayed-release (Enteric-coated) Tablets & Capsules

For enteric-coated (gastroresistant) dosage forms designed not to release their drug content in an acidic environment, a two-stage test is prescribed in both the United States Pharmacopoeia and British Pharmacopoeia, which can take one of two forms. In Method A, the dosage form is tested using the basket or paddle apparatus initially containing 750 ml of 0.1 N hydrochloric. After 2 h exposure a sample is removed for analysis and 250 ml of phosphate buffer is immediately added and the mixed contents of the dissolution vessel adjusted to a pH of 6.8 ± 0.05. The test is continued for 45 min, or whatever time is prescribed in the relevant monograph before a further sample is taken for analysis.

In Method B, the dosage form is tested using the basket or paddle apparatus containing 1000 ml of 0.01 N hydrochloric acid. After 2 h exposure a sample is removed for analysis and the remaining contents of the vessel are discarded and 1000 ml of a phosphate buffer having a pH of 6.8 ± 0.05 added. The test is

continued for 45 min, or whatever time is prescribed in the relevant monograph before a further sample is taken for analysis.

In both instances, six units are initially tested and no more than 10% of the labelled content of drug should be released after 2 h. If these conditions are not met, a further six units are examined; the average released for the 12 units tested should not exceed 10% with no individual unit being greater than 25%. Failure leads to testing of a further 12 units; the average of the 24 units tested should not exceed 10% released and no unit should exceed 25%. For the studies at pH 6.8, none of the initial six units tested should release less than Q + 5% after the prescribed time; failure leads to testing a further six units where the average of the 12 tested is equal to or greater than Q and no unit is less than Q - 15%. Failure leads to the evaluation of another 12 units; of the 24 units examined, the average must be equal to or greater than Q; no more than 2 units can be less than Q - 15%, and no unit can be less than Q - 25%. Here Q is defined as the total amount of active ingredient dissolved in both the acid and buffer stages expressed as a percentage of labelled content.

The official tolerances for delayed-release products have been given above and commercial manufacturers will have their own in-house specifications. Standards for enteric-coated forms ensure the integrity of the coat, but consideration should be given to a pH change method for evaluating extended-release medications. This evaluation should ensure that dose dumping does not occur at a specific pH, as might be a problem with certain delayed-release dosage forms, for example those using hydroxypropylmethylcellulose phthalate as a coat.

1.3.8.4. Drug Release From Modified-release Dosage Forms

Various methods have been used to study the influence of pH changes on drug dissolution, e.g. media change from 0.1 N HCl to pH of 7.5 or a progressive rise from pH 1.5, 4.5, 6.9, 7.2 to 7.5 over 22 h. Such methods are aimed at mimicking the pH changes that a dosage form is subjected to during transit through the gastrointestinal tract. Skelly *et al* (1986) developed topographic profiles of the matrix tablets to determine the dissolution profiles at a variety of pHs where an

apparent three-dimensional profile of amount released versus time versus pH was produced. Such information may help to identify the pH range over which the drug dissolution rate is faster than intended, thus avoiding the problem of dose dumping at a particular narrow pH range. Additionally, they lead to the suitable single pH for testing; for example, phosphate buffer pH 5.4 was most meaningful for quinidine gluconate controlled-released products

Apart from being used for QC purposes, dissolution testing can also be used as a predictive tool during the development of modified release dosage forms to discriminate the *in vivo* effect of good and bad formulations. Therefore, one needs to understand the mechanism of drug release from the dosage form in order to utilise the dissolution testing effectively. For extended-release reservoir formulations, where the drug is dissolved and released by diffusion through a barrier coat, factors such as the influence of medium on solubility of the drug needs to be established (Ford & Rajabi-Siahboomi, 2002).

Drug release from hydrophilic matrix sustained release formulations occurs via diffusion through and erosion of the hydrated viscous surface polymer (Alderman, 1984; Ford *et al.*, 1987). Factors that hinder these processes or provide additional support to the surface polymer must be minimised to avoid false results. For example, dissolution testing of a large swelling matrix using the basket apparatus may be inappropriate because the basket wall may support the matrix, which, when combined with a slow speed of rotation, leads to unrealistically slow drug release (Ford & Rajabi-Siahboomi, 2002). Conversely *in vivo,* faster drug release may occur due to gut contractions and the presence of food enhancing matrix erosion. A second example involves matrix-sustained release tablets sticking to the walls of the dissolution vessel, resulting in a reduction in the surface area exposed to the dissolution medium. Hence, the choice of apparatus is critical and a discriminating dissolution test would aid the accomplishment of the desired *in vivo* drug release profile, through manipulation of the formulation variables.

Osmotic pump devices, where a microscopic orifice is laser-drilled into the surface of the tablet coating, are not suitable for testing by USP dissolution Apparati 1 – 4 as the tablet may lie in such an orientation as to obscure the hole. In this case, drug

release is determined using USP Apparatus 7 with the tablet glued onto the end of a reciprocating rod.

Pellets, beads and other particulate systems intended to provide prolonged release are probably best tested by column methods that facilitate changes in pH or dissolution fluids. With compendial apparati, problems arise; for instance, it is difficult to capture dispersed particles when the paddle method is used, and small particles may pass through the mesh of the basket in the basket method (Ford & Rajabi-Siahboomi, 2002).

Moore and Flanner (1996) described a simple model using mathematical indices to define a similarity factor f_2, to compare dissolution profiles as in Equation 1.8:

$$f_2 = 50 \log \{[1+1/n \sum_t{}^{n=1} (R_t - T_t)^2]^{-0.5} \times 100\} \qquad \{Eq.1.13\}$$

where R_t and T_t are percent dissolved at each time point for the reference product and the test product, respectively. Using the f_2 values, dissolution profiles are considered dissimilar if these values were less than 50 with average difference between any dissolution samples not being greater than 50%. The similarity factor and similarity testing have been recommended for dissolution profile comparison in the FDA's Guidance for Industry (CDER, 1995, 1997a, b).

1.3.8.5. Granules & Suspensions

Release of drug from particles in granules and suspensions is usually intended to occur rapidly *in vivo* and testing of such dosage forms is not common. However, United States Pharmacopoeia 28 includes dissolution testing of Flunixin Meglumine Granules (Apparatus 2, Q = 75% in 60 min), Cefuroxime Axetil for Oral suspension (Apparatus 2, Q = 60% in 30 min) and Indomethacin Oral Suspension (Apparatus 2, Q = 80% in 20 min). The flunixin granules are tipped into the filled vessel, the indomethacin suspension is simply poured onto the surface of the dissolution medium whilst the cefuroxime powder is reconstituted before being poured onto the medium.

1.3.9. Robotics and Automation

Dissolution testing by manual methods is very labour-intensive and time-consuming, particularly during critical stages of product development, such as stability studies or plant trials and also when modified or sustained-release dosage forms are involved. It is also costly to implement on a 24 hour-a-day, 7 day-a-week basis. Therefore, computerised systems have been developed which control many features of the test procedure, from sample introduction, through instrument operation to data capture and reporting. The additional cost of such systems is easily justified where the test may take up to 24 h. The use of robotic systems is particularly valuable in busy development laboratories where the robot can also remove samples for off-line testing. Complicated samples may require off-line clean-up by liquid-liquid extraction or solid-phase extraction followed by chromatographic analysis. Rapid insertion and withdrawal of a small sample probe controlled robotically, produces minimum disturbance of the hydrodynamic conditions, with a sampling time as low as 40 s. The use of robotics increases the speed of assay, the throughput and the productivity of dissolution testing although the speed of assay may then be the rate-limiting step to productivity. Consequently, short (2 – 5 cm) HPLC columns may be used to increase assay speed, increasing productivity by as much as 75%.

1.4. *In Vitro – In Vivo* Correlation

The QC functions of dissolution tests were established in Section 1.3. In addition, one may consider the *in vitro* dissolution test as a prognostic tool for *in vivo* performance during the drug development stages. In this respect, dissolution tests are used at preformulation stages to characterise candidate drug properties and select suitable excipients to optimise drug release. Dissolution testing is then applied to candidate formulations to determine which formulation produces the most desirable and reproducible dissolution profile (Dressman *et al.*, 1998). Later in the development life cycle dissolution test data are used to establish *in vitro – in vivo* correlations (IVIVC) between drug release from the dosage form and absorption. In such studies the dissolution test conditions must be physiologically

relevant otherwise the prediction of which drug and which dosage form will provide the most favourable release profile *in vivo* will be invalid (Dressman *et al.*, 1998). In situations where in vitro results do not adequately predict in vivo performance of a drug product, the size and number of clinical trials have to be increased, at significant cost.

For poorly soluble drugs where dissolution is the rate-limiting step to absorption, the presence of food may improve bioavailability. However, current pharmacopeial dissolution tests do not account for this. The dissolution test should mimic the conditions in the GIT as closely as possible to be meaningful (Anon., 1997). In relation, a crucial factor that needs to be considered is the composition of the dissolution medium and its biorelevance (*see* Section 1.3.7).

For some drugs, simple test conditions have allowed dissolution rate to be related to bioavailability. Kingsford (1984) showed a linear relationship between the percentage frusemide dissolved from tablets in 30 min and drug bioavailability relative to an oral solution when frusemide was examined using the rotating basket apparatus and buffer at pH 5.0. Therefore, in certain cases, it may be appropriate to apply dissolution testing data to evaluate biopharmaceutical implications of a product change rather than an automatic bioequivalence study (CDER, 1995).

1.5. Biopharmaceutics Classification System (BCS)

Biopharmaceutics is the study of how the physico-chemical properties of drugs and formulations and the physiology of the route of administration affect the rate and extent of drug absorption. *Bioavailibility* and *bioequivalence* are two elementary terms used in biopharmaceutics.

Bioavailablity can be defined as *"the rate and extent to which the active drug ingredient or therapeutic moiety is absorbed from a drug product and becomes available at the site of drug action"* (Lobenberg & Amidon, 2000), or *" ...the extent*

and the rate to which a substance or its therapeutic moiety is delivered from a pharmaceutical form into the general circulation" (EMEA).

Bioequivalence is *"the absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutically equivalent dosage forms becomes available at the site of action when administered at the same molar dose under similar conditions in an appropriately designed study in human subjects"* (Kanfer, 2002), or *"two medical products are considered to be bioequivalent when their concentration vs. time profiles, from the same molar dose, are so similar that they are unlikely to produce clinically relevant differences in therapeutic and/or adverse effects"* (Anon, 1994).

As part of the safety and efficacy assessment of a generic drug product, regulatory agencies require a bioavalability (BA) study and a bioequivalence (BE) study versus the innovator product whose safety and efficacy will have been established through expensive clinical trials. Such studies require *in vivo* comparisons of the plasma drug concentration in healthy human subjects between the test and reference products. As a result, the BCS was introduced with guidance from the FDA. The regulatory aim of the BCS is to make the drug development and review process more efficient by recommending a strategy for replacing certain BE studies with surrogate *in vitro* dissolution tests. In addition, biowavers that exempt a product from *in vivo* BA and BE studies may be granted for lower strengths of immediate release (IR) and modified release (MR) drug products, based on formulation proportionality and dissolution profile comparison (CDER, 2000a).

The three major factors that determine the rate and extent of drug absorption from solid oral dosage forms are solubility, dissolution rate and permeability. Solubility and permeability are recognised in the BCS, which categorises drugs into four classes based on their aqueous solubility and intestinal permeability (Table 1.3). Dissolution rate is recognised in the BCS biowaver criteria.

Table 1.3. The Biopharmaceutics Classification System

BCS CLASS	DRUG SOLUBILITY	DRUG PERMEABILITY
I	High	High
II	Low	High
III	High	Low
IV	Low	Low

The following ranges are required when estimating the drug solubility and permeability classification as high or low in Table 1.3 (CDER, 2000b):

- A drug substance is considered HIGHLY SOLUBLE when the highest dose strength is soluble in ≤ 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 ≥ 90% of an administered dose, based on mass-balance or in comparison to an intravenous reference dose.

At present, biowavers for *in vivo* BA and BE studies can be requested if the drug falls into BCS Class 1, is formulated as an IR solid oral dosage form and shows rapid and comparable dissolution (f_2 similarity factor > 50) in pH 1.2, 4.5, 6.8 with the innovator product (Lennernas & Abrahamsson, 2005). The BCS definition for 'rapid' dissolution is as follows (CDER, 2000b):

- A drug product is considered to be RAPIDLY DISSOLVING when ≥ 85% of the labelled amount of drug substance dissolves within 30 min using USP apparatus 1 (100rpm) or apparatus 2 (50rpm) in a volume ≤ 900ml of 0.1N HCl or simulated gastric fluid, pH 4.5 buffer and pH 6.8 buffer or simulated intestinal fluid.

However, certain restrictions are applied when applying for a biowaiver:

1. The drug must have a wide therapeutic index;

2. The drug must be chemically stable in the gastrointestinal tract;

3. Excipients used must have previously been used in FDA approved IR dosage forms and must not have a significant effect on the rate and extent of oral drug absorption;

4. The product must not be designed for absorption from the buccal cavity.

1.5.1. The Impact of the BCS Solubility and Dissolution Rate Criteria on Drug Discovery and Development

The BCS provides criteria for determining the rate-limiting factor for drug absorption from oral dosage forms. Therefore, it may influence the choice of drug candidate(s) for further development, the prediction and elucidation of food interactions, the choice of formulation and IVIVC in the dissolution testing of oral dosage forms.

Furthermore, pharmacokinetic factors such as absorption, distribution, metabolism and excretion (ADME) affect drug bioavailability, efficacy and safety and thus are vital considerations in the selection process of oral drug candidates in development pipelines. Since solubility, permeability and the fraction of dose absorbed are fundamental BCS parameters that affect ADME, their knowledge should prove useful in drug discovery and development. In particular, the classification can be used to make the development process more efficient (Yazdanian *et al.*, 2004). For example, in the case of a drug placed in BCS Class II where dissolution is the rate-limiting step to absorption, formulation principles such as polymorph selection, salt selection, complex formation and particle size reduction (i.e. nanoparticles) could be applied earlier in development to improve bioavailability.

However, it must be acknowledged that the current BCS class boundaries are too conservative in certain aspects, which could lead to the loss of promising compounds in early development stages or prevent biowavers from being granted

for drugs that exhibit Class I behaviour in physiologically relevant conditions (Polli, 2004).

Suggestions have been made to further improve the applicability of the BCS in industry and were summarised in a BCS workshop report by Polli *et al* (2004). Of particular importance to the topic of this thesis are the suggestions that are likely to affect the future solubility and dissolution classifications of the BCS. They are as follows:

1. The pH range for solubility studies should be limited to only include pH 1.2, 4.5 and 6.8.

2. The solubility of amphoteric compounds should be determined at the isoelectric point if it occurs between pH 1.2 and 6.8.

3. An intermediate solubility class should be introduced given the tendency of many acids and bases to be highly soluble at pH 6.8 and 1.2 respectively.

4. The dose:solubility ratio for determining the solubility class boundary should be increased from 250 mL to 500 mL, particularly at pH values of 4.5 and 6.8 which are representative of the small intestine where the fluid volume is greater than in the stomach.

5. The dissolution classification should be broadened from at least 85% dissolved in 30 min to at least 85% dissolved in 60 min.

The implementation of BCS guidance to utilise *in vitro* dissolution tests as a surrogate for *in vivo* BE studies and the incorporation of the BCS into drug development strategies exemplifies the rapidly evolving function of the dissolution test.

1.6. Regulatory Developments

The previously contrasting views of dissolution testing held by pharmaceutical scientists and regulators led to constant development and review of the technology. The industrial drive has been to improve the IVIVC, so speeding up the identification and development of new therapeutic products and elimination of unworkable drug candidates, whilst the regulatory focus was traditionally on the use of dissolution testing as a quality control tool to confirm product safety & efficacy. However, regulatory perspectives are now changing, signalled by the emergence of the dissolution test as a surrogate *in vitro* bioequivalence test. Several aspects of dissolution testing are receiving particular attention in various industry-government *fora* (Williams *et al.*, 2004)

1.7. Harmonisation

Discussions within the Pharmacopoeial Discussion Group under the auspices of the International Conference on Harmonisation (ICH) process have led to some harmonization of compendial approaches. There is an agreement by all to adopt the United States Pharmacopoeia three-tiered system of testing and specification where appropriate. However, some differences are further from resolution. For example, the Japanese Pharmacopoeia does not recognise USP Apparatus 3 and maintains a different approach to delayed release products. The European Pharmacopoeia (2001) prescribes three apparatuses for oral solid dosage forms, the basket, the paddle and the flow-through along with a flow-through device for suppositories and three systems for testing transdermals which equate to the USP paddle-over-disk, diffusion cell and rotating cylinder.

1.8. Aim and Objectives

The overall aim of this project is to gain a better understanding of the effect of the dissolution medium on the drug release profile *in vitro*, so that *in vitro* dissolution testing can be used more effectively in the early stages of formulation development.

This aim is to be achieved through *in vitro* dissolution testing of drugs using media that are a closer representation of the gastric and intestinal environment than compendial media. Consequently, insight into possible interactions between drugs and/or excipients and biorelevant media would be useful to understand some of the physicochemical factors affecting dissolution into these complex media. Hence, a more accurate prediction of *in vivo* dissolution performance would be provided, progressing to the concept of achieving IVIVC.

In order to discriminate between differences in drug dissolution in various media, the model drugs selected in this project have to be poorly soluble. Also, to investigate drug dissolution as a barrier to drug absorption the chosen drugs must be highly permeable for absorption to be dissolution rate limited. According to the BCS these criteria would fall under Class II. Drugs that are acids will be poorly soluble in an acidic environment such as the stomach but on movement into the intestinal region, where the pH is higher, rapid dissolution is likely occur. Conversely, basic drugs will be more readily soluble in the stomach with the possibility of precipitation in the fed state or as the stomach contents empty into the small intestine.

The Objectives of this project are to:

- Investigate the dissolution of two acidic BCS Class II drugs in milk, which is a more realistic representation of the fed state stomach, in comparison to compendial media
- Devise and conduct a pH shift experiment (from acidic to neutral) using simple media to assess the extent of any reprecipitation of a basic drug on exposure to intestinal pH and the utility of such a test
- Investigate the dissolution of two basic BCS Class II drugs in the proposed FaSSIF and FeSSIF media and their constituents in comparison to compendial media
- Compose and assess the use of alternative, less expensive, surfactant based media to replace FaSSIF and FeSSIF media.

2. MATERIALS AND GENERAL METHODS

2.1. Materials

A list of the materials and suppliers used throughout the project is provided below. Where necessary, specific details are described in the relevant chapter.

2.1.1. Drug substances

As stated in Section 1.8, the model drugs chosen for investigation in this project would have to be poorly soluble so that differences in the dissolution behaviour of each drug in various media could be distinguished. Also, for drug dissolution to be the rate-limiting step to absorption, drug permeability through the gut wall would have to be high. Consequently, two acidic drugs (ibuprofen and naproxen) and two basic drugs (loratadine and trimethoprim) were selected (Table 2.1.). All four drugs are categorised in BCS Class II, i.e. they are poorly soluble and highly permeable.

Table 2.1. Drug substances used.

Drug	Source	Batch Number
Ibuprofen (particle size 25 μm)	Knoll Pharma Chemicals, Nottingham, U.K.	453408
Loratadine (particle size 10 μm)	Kindly donated by Ranbaxy Laboratories Ltd, Hamachal Pradesh, India.	1459651
Naproxen (particle size 20 μm)	Sigma-Aldrich Chemie GmbH, Steinheim, Germany.	02230LB-024
Trimethoprim (particle size 50 μm)	Kindly donated by Alpharma Ltd, Barnstaple, U.K.	39423

2.1.2. Marketed drug products

Tablets are the most common and preferred oral dosage form. It was decided to investigate a marketed tablet formulation of each drug for its dissolution behaviour in various media. Brand selection was random. However, for loratadine, poor disintegration of the selected product (Brand A) in biorelevant media led to the investigation of two further products (Brand B and C). The products selected are listed in Table 2.2.

Table 2.2. Marketed drug products used.

Marketed Product	Source	Batch Number
Ibuprofen 400mg tablets	The Boots Company PLC, Nottingham, U.K.	1SS
Loratadine 10mg tablets (Brand A)	Ranbaxy (UK) Ltd, London, U.K.	140 9665
Loratadine 10mg tablets (Brand B)	APS Ltd, Eastbourne, U.K.	4T27LT
Loratadine 10mg tablets (Brand C)	Boots Brand, PL Holder Schering-Plough Ltd, Hertfordshire, U.K.	05B1607
Naproxen 250mg tablets (Naprosyn®)	Roche Products Ltd, Hertfordshire, U.K.	E1171
Trimethoprim 200mg tablets	Alpharma Ltd, Barnstaple, U.K.	TG 424

2.1.3. Ingredients used in solubility screens and dissolution media

All the materials used in the preparation of the dissolution media and the surfactants investigated for their solubilising capacity for loratadine are listed in Table 2.3 below.

Table 2.3. List of materials used.

Material	Source	Batch Number
Brij® 35. Polyoxyethylene 23 lauryl ether.	Sigma-Aldrich Chemie GmbH, Steinheim, Germany.	025K0055
Casein (Technical grade)	Sigma-Aldrich Chemie GmbH, Steinheim, Germany.	013KO162
CHAPSO. 3 - [(3-Cholamidopropyl) dimethylammonio] – 2 – hydroxyl – 1 – propanesulfonate (98%)	Sigma-Aldrich Chemie GmbH, Steinheim, Germany.	015K5315
Cremophor EL®. Castor oil polyoxyethylene ether	Sigma-Aldrich Chemie GmbH, Steinheim, Germany.	103K2521
CTAB. Hexadecyltrimethyl ammonium bromide.	Sigma-Aldrich Chemie GmbH, Steinheim, Germany.	113K0092
DDAO. N,N-Dimethyldodecylamine N-Oxide.	Sigma-Aldrich Chemie GmbH, Steinheim, Germany.	063K2607
Glacial acetic acid (~100%, AnalaR®)	BDH, Poole, U.K.	K23898917
Hydrochloric Acid (GPR®)	BDH, Poole U.K.	K32540451 343
Lactose (Lactopress®, spray dried monohydrate, USP)	Borculo Domo Ingredients, Chester, U.K.	204022

Continued.....

Table 2.3 (contd). List of materials used.

Material	Source	Batch Number
Lecithin. Egg phosphatidylcholine. (EPC S)	Lipoid GmbH, Ludwigshafen, Germany	108015-1/88; 108015-1/124; 108015-1/911
Potassium dihydrogen phosphate	Rectapur®, Prolabo, Bois, France.	J188
Skimmed, Semi-Skimmed and Whole Milk	ASDA, Liverpool, U.K.	---
Sodium acetate (AnalaR®)	BDH, Poole, U.K.	K91214705 339
Sodium chloride	Rectapur®, Prolabo, Bois, France.	N020
Sodium dihydrogen orthophosphate (AnalaR®)	BDH, Poole, U.K.	A856721
Sodium dodecyl sulphate	BDH, Poole, U.K.	6744110
Sodium hydroxide pellets	GPR®, BDH, Poole, U.K.	260494H16S
Sodium Taurocholate (95%)	Sigma-Aldrich Chemie GmbH, Steinheim, Germany.	084K5301
Sodium Taurocholate hydrate (97%)	Alfa Aesar, Lancaster, U.K.	10109329
Triton® X100. Polyethylene glycol tert-octylphenyl ether (Sigma Ultra)	Sigma-Aldrich Chemie GmbH, Steinheim, Germany.	024K0025
*Tween®*80 Polyoxyethylenesorbitan Monooleate (Sigma Ultra)	Sigma-Aldrich Chemie GmbH, Steinheim, Germany.	052K0144

2.1.4. Drug extraction and mobile phase solvents

Organic solvents used to extract drug from milk-based media and in the preparation of mobile phases fro HPLC analyses are listed in Table 2.4.

Table 2.4. Drug extraction and mobile phase solvents used.

Solvent	Source
Acetonitrile (HiPerSolv for HPLC®)	BDH, Poole, U.K.
Cyclohexane (HiPerSolv for HPLC®)	BDH, Poole, U.K
Ethyl acetate (HiPerSolv for HPLC®)	BDH, Poole, U.K
Methanol (HiPerSolv for HPLC®)	BDH, Poole, U.K.
Orthophosphoric acid (85%)	J.T. Baker, Deventer, Holland.
Sodium perchlorate	BDH, Poole, U.K.
Water (HiPerSolv for HPLC®)	BDH, Poole, U.K.

2.1.5. Filters

Samples collected during solubility, dissolution and intrinsic dissolution rate experiments were passed through the appropriate filters listed in Table 2.5.

Table 2.5. Filters used for sample collection procedures.

Filter size and type	Source
0.45µm syringe filters Millex®-LCR, PTFE, 25mm	Millipore Corp, Bedford, U.S.A
20µm cannula filters Erweka style, UHMW polyethylene	Science Marketing International Ltd, Maisemore, U.K.

2.2. General Experimental Methods

2.2.1. Solubility determinations

2.2.1.1. Ibuprofen and naproxen

10 mg of drug substance was placed into a 20 x 42 mm screw cap vial followed by 6 mL of medium. The vial was then capped and agitated in a heated shaker (Model AM89B, Dynex Technologies Ltd, Worthing, U.K) for 24 h at 37°C. At 1, 5 and 24 h 1.5 mL of the bulk suspension was sampled and passed through a 0.45 μm filter, into a 2 mL glass autosampler vial. For solubility studies in milk media, the samples were excessively particulate for filtration and therefore, 1.5 mL of sample was transferred to a 2 mL Eppendorf tube instead and spun at 14, 000 rpm (G force = 9878 g) for 1 h in a mini centrifuge (Model Z160M, Hermle Labortechnik, Wehingen, Germany). 1 mL of the supernatant was then removed and added to 1 mL of cylohexane (ibuprofen) or to 1 mL of ethylacetate (naproxen) in a test tube. This mixture was then shaken for 20 s before 500 μL of the organic layer was removed, mixed with 1 mL of phosphate buffer pH 10 and shaken for 20 s. 800 μL of the phosphate buffer was then transferred to a 2 mL glass autosampler vial for analysis. The pH of the bulk suspension was measured at 1, 5 and 24 h using a digital pH meter (Delta 350, Mettler Toledo, Leicester, U.K.).

2.2.1.2. Loratadine

10 mg of drug substance was placed into a 20 x 42 mm screw cap vial followed by 2 mL of medium. A magnetic stirrer bar was then inserted into the vial containing the bulk suspension and the vial capped before being placed in a Variomag Telemodul 40CT heated stirrer (H+P Labortechnik GmbH, Oberschleissheim, Germany) at 37°C for 24 h. 600 μL samples were withdrawn from the vial at 1, 5 and 24 h and transferred to an Eppendorf tube using an auto pipette and spun at 40, 000 rpm (G force = 80, 640 g) for 15 min in a Beckman Optima TL ultracentrifuge (Beckman Coulter Inc, California, USA). 450 μL of the supernatant was transferred to a second Eppendorf tube and spun again at 40, 000 rpm for a further 15min. 300μL of the

supernatant was then transferred to a 2 mL glass autosampler vial for dilution or direct analysis. The pH of the bulk suspension was measured at 1, 5 and 24 h using a digital pH meter.

2.2.1.3. Trimethoprim

100 mg of drug substance was placed into a 20 x 42 mm screw cap vial followed by 4 mL of medium. The vial was then capped and a similar procedure as ibuprofen and naproxen was used except that 1 mL samples were removed from the bulk mixture instead of 1.5 mL and no extractions were required, as milk media were not used.

The samples were assayed as described in Section 2.3.

N.B. Whilst samples were assayed at 1, 5 and 24h, where drug 'solubility' is referred to throughout this thesis it is with reference to 24h data.

2.2.2. Disintegration testing

A Pharma Test PTZ (Pharma Test Apparatebau GmbH, Hainburg, Germany) disintegration tester was used throughout. Three tablets were placed in the basket rack assembly without disks (see Section 1.2.2) and lowered into the relevant medium held at 37°C. The tester was operated and the time taken for complete disintegration of all the tablets was noted.

For ibuprofen and naproxen, tablet disintegration tests were carried out in 0.1N HCl, 0.003N HCl containing 0.017%v/w sodium dodecyl sulphate, the filtrate of 1.475% w/v dispersion of casein in 0.01N HCl and in 50/50 mixtures of 0.1N HCl with whole, semi-skimmed or skimmed milk (see Section 4.2.2 for media preparation).

For loratadine, tablet disintegration tests were carried out in 0.1N HCl, FaSSIF blank and FeSSIF blank (see Section 6.2.2 for media preparation).

Disintegration tests were not conducted for trimethoprim for reasons explained in Chapter 7.

2.2.3. Dissolution testing

Experiments were conducted in a Pharmatest PTW S2C (Pharma Test Apparatebau GmbH, Hainburg, Germany) or a Distek 2100C (Distek Inc, New Jersey, USA) dissolution tester using Apparatus 2 (paddle method) described in General Chapter <711> of the United States Pharmacopoeia 28 (see Section 1.3.1.2). Six tablets were tested for each experiment. 500 mL or 1000 mL of dissolution medium was placed in each of six vessels, the paddles lowered to 25 mm from the base of the vessels and the vessels covered with plastic lids. After lowering, the paddles were rotated at 50 rpm to agitate the dissolution medium. The dissolution medium was heated to 37°C by a water bath. Each tablet was then dropped into a separate vessel containing the dissolution medium. 5 mL samples were taken at 15, 30, 45, 60, 90 and 120 min or designated time points using 0.45 μm syringe filters for non- particulate media and 20 μm cannula filters for milk media. The pH of the medium was measured at the start and end of the test using a digital pH meter (Delta 350, Mettler Toledo, Leicester, U.K.).

2.2.4. Intrinsic dissolution rate determinations

IDR was measured by the rotating disk method of the United States Pharmacopoeia 28, using the Woods apparatus (Figure 1.3). The test preparation was as follows:

Each stainless steel die (10 mm diameter) was bolted to a base plate made from hardened polished steel. 200 mg of ibuprofen or 400 mg of naproxen drug substance was weighed and added to each die. The drug powder was then compressed into a disk using a hydraulic press (Model M-30, Research and Industrial Instrument Company, London, England) and hardened steel punch (Figure 1.5) at a

compression force of $8x10^6$ N/m^2 (80 bar). The base plate was removed after compression to expose a smooth drug surface. Screw threads on the top of the die allowed attachment to a specially designed shaft that could be interchanged with the paddle on the Pharmatest PTW S2C dissolution apparatus. Experimental conditions and sampling times were identical to those used for tablet dissolution tests (see Section 2.2.3).

2.2.5. Procedures for drug extraction from milk based media

A significant challenge to dissolution testing in food-based media was the extraction of dissolved drug into a suitable matrix for analysis, which can be both time and labour intensive. For HPLC, the dissolved drug had to be extracted from the various milk media (50/50 mixtures of whole, semi-skimmed or skimmed milk with 0.1N HCl) into an aqueous medium that could be injected directly onto the column. A back extraction method was developed and applied to each drug as described below.

2.2.5.1. Extraction of ibuprofen

Dissolution and solubility studies: After removal and filtering, 2 mL of the sample solution was mixed with 2 mL of cyclohexane and the mixture was shaken for 20 s. The mixture was allowed to settle before 1 mL of the (upper) cyclohexane layer was transferred to a second tube and 2 mL of phosphate buffer pH 10 added. This mixture was shaken for 20 s, allowed to settle and 1 mL of the (lower) aqueous layer transferred to a 2 mL glass autosampler vial for analysis. Method validation studies using each of the three milk-HCl media spiked with an ibuprofen standard (n = 6) indicated this process recovered >90% of the available ibuprofen in the sample. For solubility studies, a similar procedure was used except that it was scaled down such that half of all the volumes stated above were used.

IDR studies: After removal and filtering, 2 mL of the sample solution was mixed with 2 mL of cyclohexane and the mixture was shaken for 20 s. The mixture was allowed to settle before 1 mL of the (upper) cyclohexane layer was transferred to a second tube and 1 mL of phosphate buffer pH 10 added. This mixture was shaken

for 20 s, allowed to settle and 800 µL of the (lower) aqueous layer transferred to a 2 mL glass autosampler vial for analysis.

2.2.5.2. Extraction of naproxen

Dissolution and solubility studies: After removal and filtering, 2 mL of the sample solution was mixed with 2 mL of ethyl acetate and the mixture was shaken for 20 s. This mixture was allowed to settle before 500 µL of the (upper) ethyl acetate layer was transferred to a second tube and 2 mL of phosphate buffer pH 10 added. This was shaken for 20 s, allowed to settle and 1 mL of the (lower) aqueous layer transferred to a 2mL glass autosampler vial for analysis. Method validation studies using each of the three milk-HCl media spiked with a naproxen standard (n=6) indicated this process recovered >85% of the available naproxen in the sample. For solubility studies, a similar procedure was used except that it was scaled down such that half of all the volumes stated above were used.

IDR studies: After removal and filtering, 2 mL of the sample solution was mixed with 2 mL of ethyl acetate and the mixture was shaken for 20 s. The mixture was allowed to settle before 500 µL of the (upper) ethyl acetate layer was transferred to a second tube and 1 mL of phosphate buffer pH 10 added. This mixture was shaken for 20 s, allowed to settle and 800 µL of the (lower) aqueous layer transferred to a 2 mL glass autosampler vial for analysis.

2.3. Analytical Method

2.3.1. HPLC

2.3.1.1 Ibuprofen

For dissolution and solubility studies, quantitation was carried out using a Hewlett Packard HP1090 liquid chromatograph (Agilent Technologies U.K. Ltd, Stockport, U.K.). 25 µL of sample for assay was injected into a mobile phase consisting of

0.3% v/v orthophosphoric acid, 24.7% v/v water and 75% v/v methanol flowing through the column at a rate of 1 mL/min. A 5µm Nucleosil C18 column (25 cm x 4.6 mm) (Hichrom Ltd, Reading, U.K.) was used and eluting peaks were detected at a wavelength of 264 nm. Quantitation was based on the use of an external ibuprofen calibration standard and peak area measurement.

For IDR studies, quantitation was carried out using a Waters 2695 liquid chromatograph connected to a Waters 474 fluorescence detector (Waters S.A.S., Cedex, France). The injection volume, mobile phase and flow rate were unchanged from the dissolution and solubility studies. A 5 µm Hypersil C18 column (10 cm x 4.6 mm) (Hichrom Ltd, Reading U.K.) was used fitted with a Phenomenex C18 guard cartridge (4 mm x 3 mm) (Macclesfield, U.K.). Eluting peaks were detected at excitation and emission wavelengths of 222 nm and 285 nm respectively. Quantitation was based on the use of an external ibuprofen calibration standard and peak area measurement.

2.3.1.2. Loratadine

Quantitation was carried out using either a Hewlett Packard HP1090 or Agilent 1100 (Agilent Technologies U.K. Ltd, Stockport, U.K.) liquid chromatograph. 10 µL of sample was injected into a mobile phase consisting of 1% v/v orthophosphoric acid, 8%v/v potassium dihydrogen phosphate (0.5M), 44%v/v acetonitrile and 48% v/v water flowing through the column at a rate of 1.8 mL/min. A 5 µm Hypersil BDS C18 column (15 cm x 4.6 mm) (Hichrom Ltd, Reading, U.K.) fitted with a Phenomenex C18 guard cartridge (4 mm x 3 mm) was used and eluting peaks were detected at a wavelength of 200 nm. Quantitation was based on the use of an external loratadine calibration standard and peak area measurement.

2.3.1.3. Naproxen

Dissolution samples were analysed using a Hewlett Packard HP1090 liquid chromatograph whilst solubility and IDR samples were analysed using an Agilent

1100 liquid chromatograph. 25 µL of sample was injected into a mobile phase consisting of 32% v/v orthophosphoric acid (0.01M) and 68% v/v methanol flowing through the column at a rate of 1.5 mL/min. A 5 µm Spherisorb C18 column (10 cm x 4.6 mm) (Hichrom Ltd, Reading, U.K.) fitted with a Phenomenex C18 guard cartridge (4 mm x 3 mm) was used and eluting peaks were detected at a wavelength of 262 nm. For IDR studies, the injection volume was increased to 50 µL for all media tested except 0.1M HCl where the injection volume was increased to 100 µL. Quantitation was based on the use of an external naproxen calibration standard and peak area measurement.

2.3.1.4. Trimethoprim

Dissolution samples were analysed using a Hewlett Packard HP1090 liquid chromatograph whilst solubility samples were analysed using an Agilent 1100 liquid chromatograph. 5µl of sample was injected into a mobile phase consisting of 30% v/v methanol and 70%v/v sodium perchlorate (0.01M), adjusted to pH 3.6 with phosphoric acid, flowing through the column at a rate of 1.3 mL/min. A 5 µm Hypersil BDS C18 column (15 cm x 4.6 mm) (Hichrom Ltd, Reading, U.K.) was used and eluting peaks were detected at a wavelength of 280 nm. Quantitation was based on the use of an external trimethoprim calibration standard and peak area measurement.

3. DETERMINATION OF THE SURFACE TENSIONS OF THE DISSOLUTION MEDIA INVESTIGATED

3.1. Introduction

As previously explained in Section 1.1.4.1, the surface tension of the solvent/dissolution medium can affect the dissolution of a drug particle through its influence on the wetting phenomena at the solid surface. Hence, it is an important parameter to consider when comparing the effect of different media on drug dissolution. For example, Chen *et al* (2003) found that decreasing the surface tension of 0.1N HCl by addition of Tween 80 reduced the IDR of the hydrophobic drug, CI-1041. The lower surface tension promoted nucleation of the insoluble chloride salt onto the disk surface causing the reduction in IDR. Also, the dissolution rate of the poorly soluble drug, griseofulvin, from a solid dispersion was correlated to the surface tensions of carrier aqueous solutions (Saito *et al.*, 2002). An assessment of the wetting properties of physiological concentrations of bile salt solutions versus compendial media was conducted by Luner (2000). The study revealed that the bile salt solutions had lower surface tensions and contact angles compared to compendial media and that individual bile salts differed in surface tension lowering effect and contact angles. These findings are important in establishing an IVIVC for drug dissolution because they illustrate that drug wetting may differ between compendial media and physiological fluids.

In terms of tablet formulations, wetting of the tablet surface is an elementary requirement for disintegration, therefore, the surface tension of the medium can influence the drug release rate. Anwar *et al* (2005) investigated tablet disintegration in relation to the liquid penetration rate, which is dependent on the surface tension and viscosity of the liquid, the contact angle and the tablet pore size.

The purpose of this chapter is to obtain definitive in-house surface tension data for the variety of dissolution media used throughout the project. No literature values

were available for some of the unique media used whilst those reported for other media were inconsistent. Also, by using the same method and conditions for all the media, more accurate and meaningful comparisons of surface tensions were possible through reduced experimental variability.

3.2. Methods

3.2.1. Density measurements

For the method used to determine surface tension, the densities of the dissolution media were required to resolve their surface tensions. The densities of the all the dissolution media were determined using a Paar Model DMA 55 density meter (Anton Parr Ltd, Hertford, U.K.). The measurement is based on the oscillating U-tube principle whereby the resonant frequency of the sample is inversely proportional to the square root of its mass. Since the volume of the U-tube is standardised, the resonant frequency measured is used to automatically calculate and display the density (ρ) of the sample. The instrument was first calibrated using two fluids of accurately known densities at the same temperature and pressure to be used for the unknown samples. 17.53% w/v sodium chloride solution (ρ = 1.1058 g/cm^3) and methanol (ρ = 0.7866 g/cm^3) were used for this purpose. After calibration, a sample of medium was injected into the U-tube and the density at 25°C recorded. The mean of three determinations was used for the surface tension calculation.

3.2.2. Surface tension measurements

The drop shape technique was used to determine surface tension (Satherley *et al.*, 1990; Miller *et al.*, 2001; Miqueu *et al.*, 2001). Fundamentally, the opposing forces of surface tension and gravity determine the shape assumed by a pendant drop of liquid. Surface tension makes the drop spherical while gravitational forces elongate the drop. Advances in video digital techniques have allowed highly accurate

detection of the droplet edge enabling drop profile coordinates to be mapped by computer programmes. Hence working backwards from the acquired droplet shape and known force of gravity, the surface tension can be determined.

A pendant drop of the sample medium was formed at the tip of a capillary positioned inside a glass cell with optically flat windows (Figure 3.1). Zoom lens and a silicon charge-coupled device (CCD) camera were used to capture an image of the drop and a digital image processor captured the profile coordinates of the drop. Axisymmetric drop shape diagnostic software utilised the Laplace-Young equation (Equation.3.1) and the measured sample density to compute the surface tension:

$$\Delta\rho\, g\, h = \gamma\,(1/R_1 + 1/R_2) \qquad \{Eq.3.1\}$$

where $\Delta\rho$ is density difference between the droplet and substance surrounding it, g is the acceleration due to gravity, h is the height in the drop measured from its apex, γ is the surface tension and R_1 and R_2 are radii of curvature determined from the drop profile coordinates.

A schematic diagram of the apparatus is shown in Figure 3.1. For each medium tested, three drops were analysed and the results reported in Table 3.1. For each drop, the software reported the mean data from 10 simultaneously captured images.

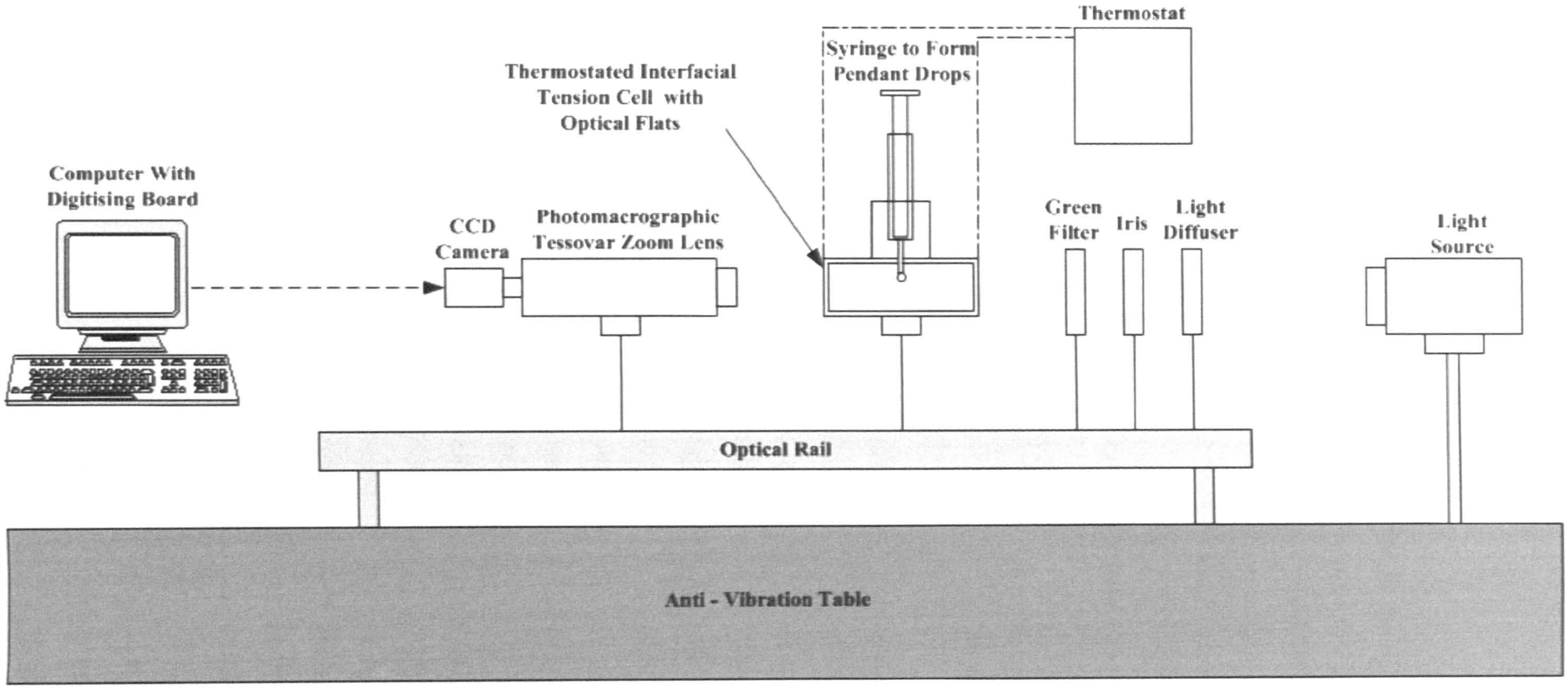


Figure 3.1. Schematic diagram of pendant drop shape apparatus to measure surface tension

3.3. Results

The density and surface tension measurements for the media used are presented in Table 3.1 below. Numerous SDS concentrations were used in the project. 2.5 mg/mL SDS was used to lower the surface tension of 0.1M HCl to that of gastric fluid. 0.17 mg/mL SDS was used to lower the surface tension of 0.003M HCl to match that of the 50/50 milk/HCl mixtures (Chapter 4 and 5) but as determined in

Table 3.1. Density and surface tension data for dissolution media investigated.

Medium	Density (g/cm^3) @ 25°C	Mean Surface tension (mN/m) *n=3*	± S.D. (mN/m)
Purified water (Milli Q®)	0.99262	66.02	0.13
Distilled water	0.99268	68.08	0.40
0.1M HCl	0.99424	68.48	2.99
0.1M HCl + 2.5 mg/mL SDS	0.99461	32.03	0.16
0.003M HCl	0.99230	67.43	0.33
0.003M HCl + 0.17 mg/mL SDS	0.99256	40.35	1.26
Simulated Intestinal Fluid (SIF)	0.9980	63.35	0.22
50/50 Whole milk/0.1M HCl	1.00670	47.80	0.83
50/50 Semi-skimmed milk/0.1M HCl	1.00800	47.94	0.27
50/50 Skimmed milk/0.1M HCl	1.00873	47.52	0.74
Casein filtrate in 0.01M HCl	0.99358	48.95	0.78
FaSSIF Blank	0.99957	66.98	0.74
FaSSIF	0.99984	49.28	0.53
FeSSIF Blank	1.00491	65.95	0.99
FeSSIF	1.00683	46.12	0.49
CTAB 1.82 mg/mL	1.00493	33.42	0.11
CTAB 0.1365 mg/mL	0.99965	35.29	0.12
SDS 1 mg/mL	1.00543	27.66	0.10
SDS 0.225 mg/mL	0.99982	31.90	0.94

Table 3.1 the surface tension of this medium was lower than the 50/50 milk/HCl media.The 1 mg/mL and 0.225 mg/mL SDS media at the bottom of Table 3.1 were used to match loratadine dissolution profiles in FeSSIF and FaSSIF respectively (Chapter 6).

3.4. Discussion

The surface tension of pure water is reported as 72 mN/m at 25°C (Weast, 1976). Repeated determinations using 2 different sources of Milli-Q® purified water (John Moores University and University of Liverpool) gave similar results (≈ 66mN/m). Satherley, J (personal communication) revealed that his group saw the same discrepancy. It is believed that there is some impurity passing through the Milli-Q® filtration system that is responsible for the reduced surface tension, and it is not an error in the experimental procedure. The surface tension recorded for distilled water was slightly higher at 68 mN/m. i.e. the disparity in surface tension with literature values is related to the source of the water used.

Table 3.1. shows that the milk and casein filtrate media had similar surface tensions to each other and therefore differences in drug dissolution between these media should not be attributed to this factor. It must be noted that complete wetting of solid particles may not be governed solely by the surface tension of the liquid phase. Fell and Mohammad (1995) and Luner *et al* (1996) demonstrated differences in the wetting of solid surfaces by different media at similar surface tensions. The nature of the surface-active components and their interaction at the liquid-vapour and solid-liquid interfaces may control wetting as opposed to surface tension itself (Luner & VanDer Kamp, 2001)

FaSSIF and FeSSIF blanks (i.e. without bile salt and lecithin) had surface tensions approximately 19mN/m higher than the complete FaSSIF and FeSSIF media. The difference can be explained by the addition of the surface-active components

sodium taurocholate (NaTC) and lecithin to the complete media. Interestingly, there was only a small difference in surface tension between FaSSIF and FeSSIF (Table 3.1.) despite large differences in concentration of NaTC and lecithin between the two media. The critical micelle concentrations (cmcs) of NaTC and lecithin are 1.6 mg/mL and 0.076 ng/mL respectively (Leng *et al.*, 2003). Lecithin does not form micelles easily in aqueous media and floats on the liquid surface until it can form mixed micelles. This implies that at the concentrations of NaTC and lecithin used mixed miceller systems were formed. Consequently, dissolution enhancements from tablets observed in these media are probably micelle solubilisation effects as opposed to improved wetting effects.

CTAB and SDS-containing media had lower surface tensions than FaSSIF and FeSSIF media. For the SDS media, the two concentrations used were well below the cmc of SDS, which is 2.37 mg/mL. For the CTAB media (cmc = 0.36 mg/mL) only the higher of two concentrations used was above the cmc. The fact that these synthetic surfactant media were able to match dissolution profiles generated by FaSSIF and FeSSIF suggests that profile matching was the result of improved wetting of the drug. In support of this, Luner and VanDerkamp (2001) found that 0.25% w/v SDS and 0.1% w/v Triton X-100 were significantly better wetting agents for a model surface, poly(methyl methacrylate), than various physiologically representative media.

The data generated show there were no significant surface tension differences between the aqueous media without surfactants i.e. Water, HCl, SIF, FaSSIF blank and FeSSIF blank. Hence it can be postulated that factors other than surface tension, such as pH and other variables, may account for any differences observed in drug dissolution using these media.

4. IBUPROFEN

4.1. Introduction

Ibuprofen ((RS)-2-(4 isobutylphenyl) propionic acid) is a widely used non-steroidal anti-inflammatory drug (NSAID) with analgesic and antipyretic properties (Figure 4.1). It used in the clinical management of mild to moderate pain, in the treatment of arthritic and soft tissue disorders and in primary dysmenorrhoea.

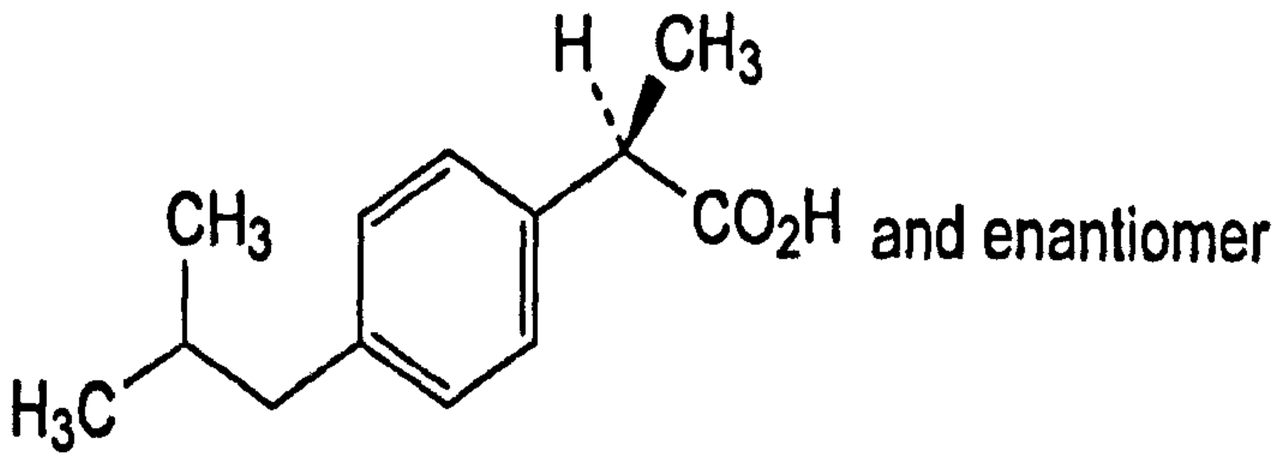


Figure 4.1. The chemical structure of ibuprofen

Ibuprofen is a poorly soluble acidic drug with a pKa of 4.4 (Clarke, 1986) and Log P of 3.50 (Fini *et al.*, 1995). The intrinsic solubility of the drug is 21 μg/mL at 37°C (Table 4.2). It is considered highly permeable based on oral bioavailability being > 90% compared to a reference intravenous dose (Martin *et al.*, 1990). Ibuprofen has been classified as a BCS Class II drug (Lindenberg *et al.*, 2004). The drug is usually administered as the free acid form. It was chosen as a model drug to investigate because the low stomach pH in the fasted state (approx pH 1.2 – 2.0) is likely to hinder the solubility of ibuprofen in the upper GI-tract and hence result in dissolution rate-limited bioavailability in this region. The time to peak plasma levels of 1-2h (Davies, 1998) and scintigraphic monitoring of an orally administered formulation (Parr *et al.*, 1987) show that ibuprofen absorption occurs in this region. Levine *et al* (1992) showed that the presence of food increased the absorption rate of ibuprofen, probably due to increased *in vivo* dissolution.

Thus it can be expected that *in vitro* dissolution tests using simple compendial media may not accurately predict the *in vivo* behaviour. The aim of these investigations was to carry out *in vitro* dissolution testing in more realistic media to assess the physicochemical factors affecting drug dissolution in the presence of meal components. This would aid the predictive nature of *in vitro* dissolution testing prior to expensive *in vivo* studies to ascertain an IVIVC.

Dissolution tests on ibuprofen 400mg tablets were initially carried out in 0.1N HCl pH1.2, simulated intestinal fluid (SIF) pH 6.8 and 0.1N HCl + 0.25%w/v sodium dodecyl sulphate (HCl+SDS). This provided baseline dissolution profiles for subsequent comparison with more complex media. For the HCl+SDS experiment, 0.25%w/v SDS was added to bulk HCl to lower the surface tension to that of gastric fluid (≈35mN/m) (Finholt & Solvang, 1968; Dressman *et al.*, 1998; Efentakis & Dressman, 1998). Various media have been advocated to mimic the fed state conditions in the GIT (Dressman *et al.*, 1998; Galia *et al.*, 1998). Milk was put forward as being nutritionally balanced (appropriate ratio of fats: proteins: carbohydrates) and therefore a relevant dissolution medium (Dressman *et al.*, 1998). Ibuprofen is also known to be a GI irritant and is commonly taken with milk. Hence, a mixture of 50% milk and 50% 0.1N HCl was used as a 'realistic' medium to mimic the fed state stomach. The fat content of the medium was varied by using skimmed, semi-skimmed and whole milk. Lactose and casein based media were also investigated given that they are significant components of milk. IDR, solubility and disintegration tests were carried out in the specified media and the results discussed in light of the dissolution data.

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.

4.2.2. Methods

Media preparation

SIF was prepared in accordance with United States Pharmacopoeia 28 (2005) in a 5 L volumetric flask.

HCl + SDS (0.25% w/v) was prepared by addition of 12.5 g of SDS to a 5 L volumetric flask and making up to volume with 0.1N HCl. The pH of the medium was 1.2.

For the milk media, 250 mL of the specified milk was mixed with 250 mL 0.1N HCl in the dissolution vessel. The pHs of the media were approximately 2.7.

The lactose medium was composed of 4.8% w/v lactose monohydrate in 0.003N HCl adjusted to pH 2.7. The medium was prepared by adding 240g of lactose monohydrate to a 5 L volumetric flask and making up to volume with 0.003N HCl.

The casein medium was a dispersion of 1.475%w/v bovine casein in 0.01N HCl (pH 2.7) to match the casein concentration in the 50/50 milk/HCl mixtures (*see* Section 4.3.2.4.). 7.375 g of casein was accurately weighed and transferred to each dissolution vessel and 500 mL of 0.01N HCl added separately.

A further medium (casein filtrate) was prepared by filtering 5 L of a 1.475% w/v dispersion of casein in 0.01N HCl, pH 2.7, under vaccum using the buchner apparatus fitted with a Whatman No.2 filter paper (Whatman International Ltd, Maidstone, England).

0.003N HCl containing 0.017%w/v of the surfactant sodium dodecyl sulphate (0.017% w/v SDS), pH 2.7, was used as a medium to match the surface tension and pH of the milk mixtures. The medium was prepared by addition of 850 mg of SDS to a 5 L volumetic flask and making up to volume with 0.003N HCl.

Dissolution testing

The dissolution tests were performed according to the method described in Section 2.2.3 using 500 mL of dissolution medium.

Intrinsic dissolution rate determinations

See Section 2.2.4.

Solubility determinations

See Section 2.2.1.

Disintegration testing

See Section 2.2.2.

4.3. Results and Discussion

4.3.1. Dissolution in compendial media

The dissolution of ibuprofen tablets in 500mL 0.1N HCl at pH 1.2 (non-sink conditions) did not meet the typical compendial criteria for immediate release (IR) dosage forms of 70% in 45 min (BP) or 75% in 45 min (USP) as the medium became saturated with drug (Figure 4.2). At more than 1 pH unit below its pKa, an acidic drug is less than 10% ionised leading to a pH dependent solubility issue. The addition of SDS as surfactant to the dissolution medium, to match the surface tension of gastric fluid, increased ibuprofen dissolution but saturation was reached at 60 min and the amount of drug dissolved was still below the required amount for IR dosage forms. Conversely, 94% ibuprofen dissolved within 15 min from tablets exposed to 500 mL of SIF at pH 6.8, where the drug is fully ionised. As anticipated, these results confirm that the presence of surfactant and medium pH affect the dissolution of an acidic drug such as ibuprofen. The United States Pharmacopoeia 28 (2005) criteria for the dissolution of ibuprofen tablets state that not less than 80% ibuprofen should dissolve in 60 min using apparatus 2 at 50 rpm. But the specified

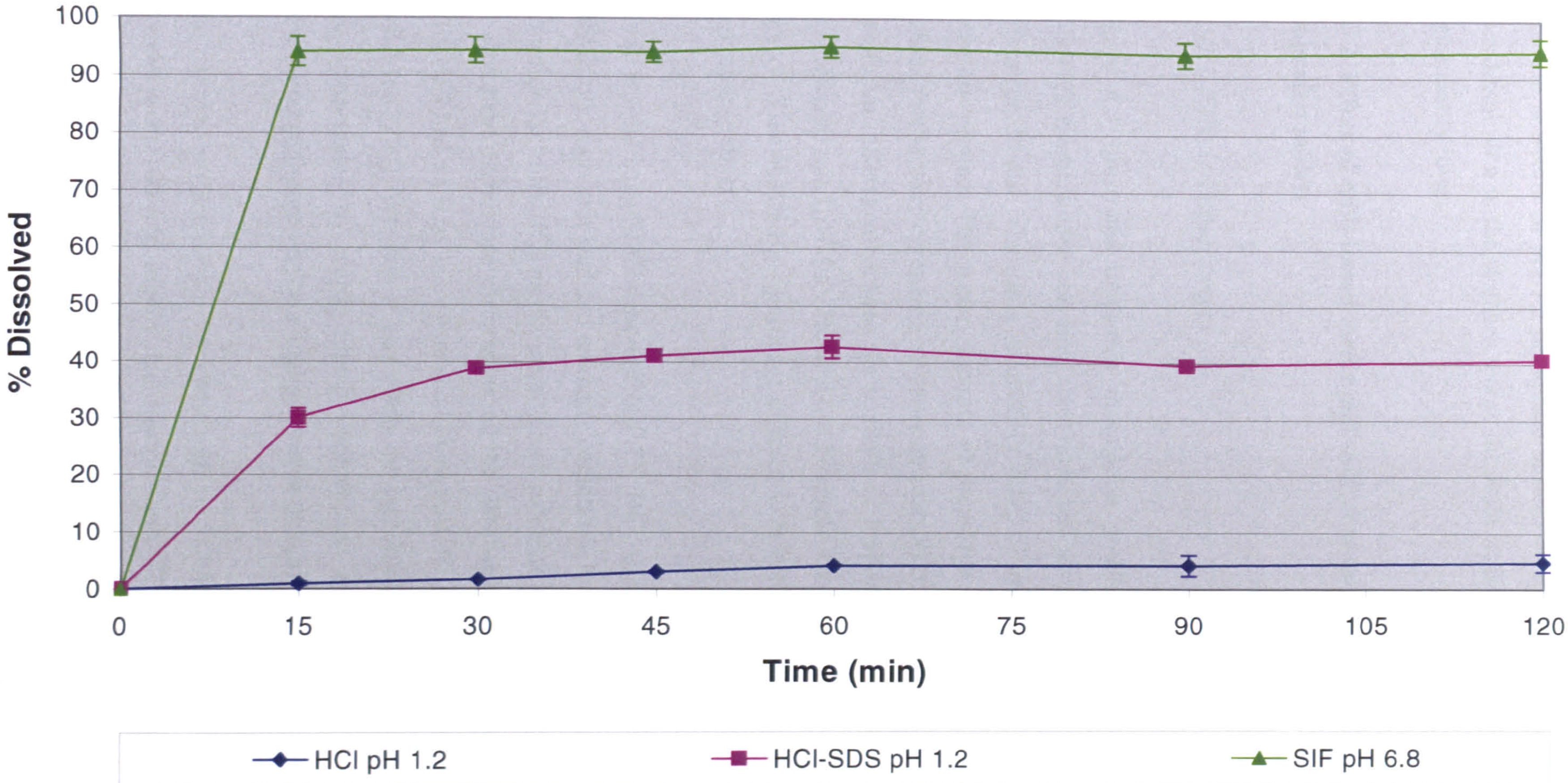


Figure 4.2. The effect of three typical dissolution media (HCl, HCl-SDS and SIF) on the dissolution of ibuprofen 400mg tablets (*n=6*)

medium is 900ml of pH 7.2 phosphate buffer, which may not accurately reflect *in vivo* dissolution in the stomach where the fluid volume, pH and surface tension are significantly lower.

4.3.2. Effect of milk-based media and milk components on the dissolution of ibuprofen tablets

4.3.2.1. Effect of lipid content

Figure 4.3 illustrates ibuprofen dissolution profiles in 500mL of the various milk/HCl media under non-sink conditions but media saturation was not reached within the 2h test. The mean amounts (± S.D.) of ibuprofen dissolved at each time point are plotted. The mean amounts of ibuprofen dissolved after 60 min in the various media were compared. A one-way analysis of variance showed there was no significant difference between the amounts of ibuprofen dissolved in the 3 types of milk after 60 min ($p>0.05$). However, a 2-sample T-test between 0.1N HCl and each type of milk showed a significant difference in mean dissolution after 60 min in each case ($p<0.05$).

These results suggest that the use of 0.1N HCl as a dissolution medium may not adequately represent real fed stomach conditions for the dissolution of ibuprofen. Although the milk/HCl media were coarse representations of the fed state stomach environment significant differences in the dissolution profiles were evident. The 0.1N HCl had a pH of 1.2 whereas all three milk-HCl mixtures had pH values close to 2.5 (at 37°C). Whether it was solely the increased pH that improved dissolution was investigated by using diluted hydrochloric acid adjusted to pH 2.5. The dissolution of ibuprofen was slightly lower at this pH.

The reported surface tension of whole milk of 52N/m (Fox & McSweeney, 1998b) is more than 100-fold higher than gastric fluid. Theoretically, mixing milk with gastric fluid in the stomach may alter the overall surface tension of the stomach contents, further influencing the dissolution of ibuprofen compared to 0.1N HCl. Indeed, Table 3.1 shows that the surface tension of all 3 milk/HCl mixtures was approximately 48mN/m, some 20mN/m lower than that of 0.1N HCl.

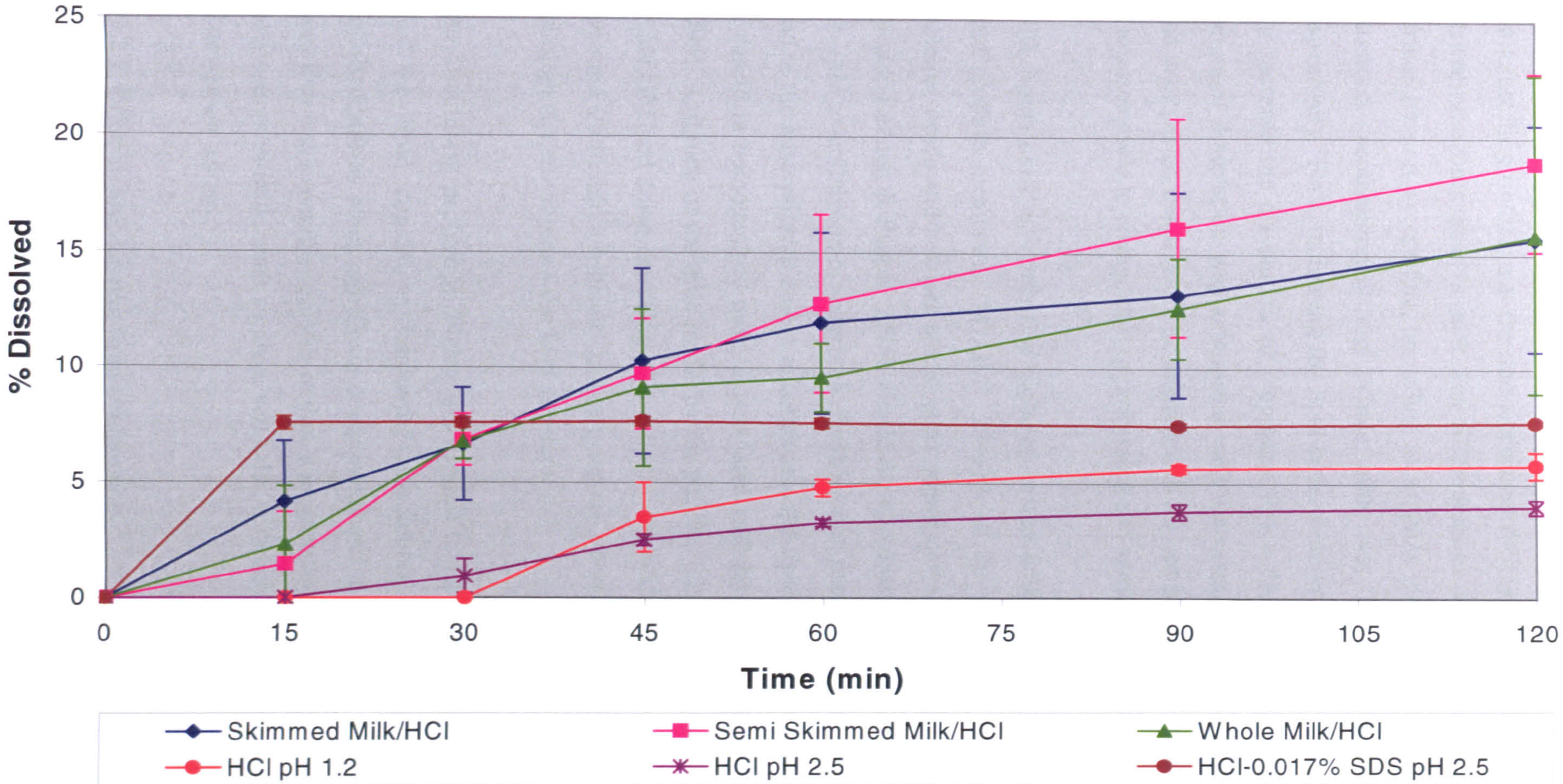


Figure 4.3. The effect of 50/50 milk/HCl mixtures, HCl pH 1.2, HCl pH 2.5 and HCl-0.017% w/v SDS (pH 2.5) media on the dissolution of ibuprofen 400mg tablets (*n=6*)

An interesting finding was the non-significant difference in dissolution after 60 min between the three types of milk. The different milks were similar in composition and surface tension except that, according to supplier information, the lipid content in skimmed, semi-skimmed and whole milk was 0.1%, 1.7% and 3.6% w/v respectively. Thus, increasing the lipid concentration in the dissolution medium did not increase the dissolution of ibuprofen. For a drug with poor water solubility, increased dissolution was anticipated with increasing lipophilicity of the medium. The unexpected result could be attributed to the complex structure of milk, which is also susceptible to change during processing and storage.

Milk lipids exist as emulsified globules coated with a membrane called the milk lipid globule membrane (MLGM). 25-60% of the membrane weight consists of proteins, lipids and hexoses whilst other minor constituents make up the remaining weight (Keenan & Patton, 1995). Hence the composition of the MLGM may hinder the lipid solubilisation of ibuprofen. Furthermore, processes such as homogenisation lead to the adsorption of casein micelles onto the lipid globules. Approximately 30% of casein in homogenised milk is associated with lipid globules (Keenan & Patton, 1995). Agitation of the milk media by the paddles may cause random additional loss of MLGM and some denuding of the lipid core, which could account for the large variation in dissolution results between the six vessels at each sampling time point although test conditions were kept constant.

The dissolution profiles in Figure 4.3 suggest that some constituent of milk other than the lipid content is improving dissolution. Milk contains proteins and carbohydrates as other significant components and their effect on the dissolution of ibuprofen was investigated. Prior to this, a gross assessment of the effect of the other components in milk, such as dissolved salts, minerals and enzymes was carried out as described below.

4.3.2.2. Effect of soluble non-lipid components

To assess the effect of the other components present in milk, further dissolution tests on ibuprofen tablets were attempted using skimmed milk, which is virtually fat free.

The experiment involved increasing the milk content of the dissolution medium from 50% to 100% to avoid dilution of the soluble components while maintaining a pH of 2.5 by acidifying the medium with hydrochloric acid. The experiment was unsuccessful due to significant precipitation of protein that separated the milk into an aqueous whey layer with a heavy precipitate settling to the bottom of the vessel that clogged the sampling filters.

An alternative experiment was to assess the effect of diluting skimmed milk on the dissolution profile of ibuprofen tablets. The pH of 100% skimmed milk was 6.8 at 37°C. The effect of dilution was investigated using 50% skimmed milk and 50% water as a medium (pH 6.7 at 37°C). The dissolution profiles of ibuprofen 400 mg tablets in the various media are compared in Figure 4.4. Again, non-sink conditions caused media saturation with drug by 15 min at pH 6.8.

The clear improvement in the initial dissolution profile of the skimmed milk/water medium compared to the skimmed milk/HCl medium (Figure 4.4) may be attributed to a more favourable pH for ionisation (pKa of ibuprofen = 4.4.). However, at similar pH values, milk concentration did not appear to have a significant effect on dissolution ($p>0.05$). This suggests that the component(s) in milk that improved ibuprofen dissolution is/are capable of a similar extent of reaction at half their original concentration, such that the mechanism of interaction is almost saturated at 50% dilution of the reactant(s) in milk. In terms of IVIVC, if skimmed milk was to be used as a biorelevant medium, dilution with water in the GIT is not likely to hinder the effect of the milk on ibuprofen tablets. The rapid dissolution (94% in 15 min) in SIF may be due to potassium and sodium salt formation in the phosphate buffer. The strong neutralising effect of Na^+ and K^+ cations raises the microclimate pH around the acidic drug particles thus aiding dissolution. This result should caution against the possible positive deviation from true intestinal dissolution of acidic drugs when using SIF as an *in vitro* dissolution medium.

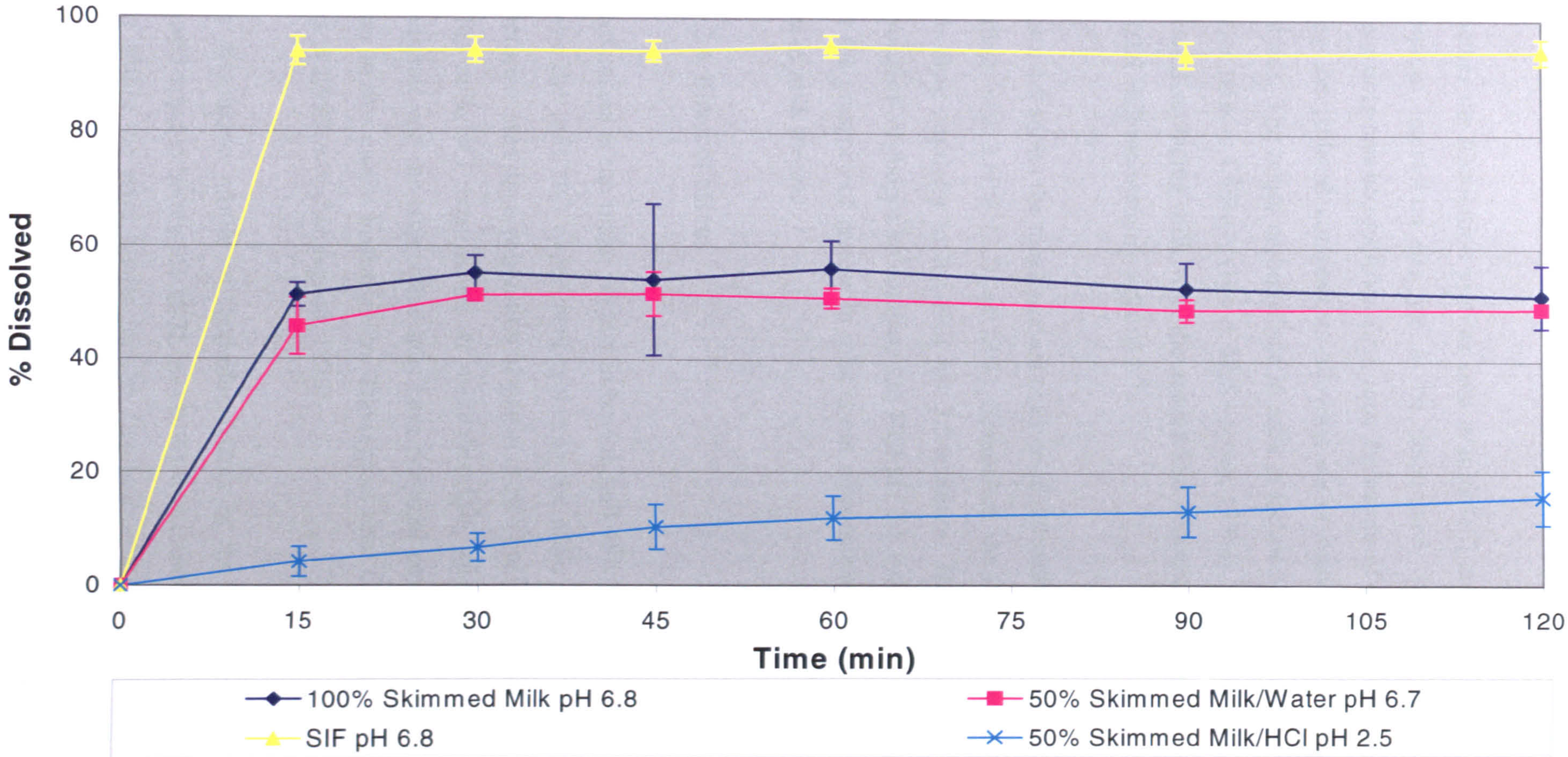


Figure 4.4. The effect of skimmed milk, 50/50 skimmed milk/water, 50/50 skimmed milk/HCl pH 2.5 and SIF on the dissolution of ibuprofen 400mg tablets (*n=6*)

4.3.2.3. Effect of lactose

Whether the dissolution profiles seen in milk/HCl are due to a drug interaction or excipient interaction has yet to be determined. If the component(s) in milk responsible can be identified first, then the nature of the interaction can be studied using the isolated components allowing easier manipulation of experiments and analyses. The major carbohydrate in milk is lactose. The typical lactose content of bovine milk is 4.8%w/v (Fox & McSweeney, 1998a). The milk/HCl experiment was repeated replacing milk with the appropriate concentration of lactose dissolved in 0.003N HCl maintaining a pH of 2.7.

Figure 4.5 shows the mean amount of ibuprofen dissolved after 60 min in the lactose medium was 3.60% (S.D. ±0.24%). Thereafter, the lactose medium was saturated with drug similarly to the HCl pH 2.5 medium. The non-significant increase in dissolution of ibuprofen compared to HCl pH 2.5 ($p>0.05$) up to 60 min suggests that the presence of lactose in milk is not responsible for the improved dissolution seen in milk media.

4.3.2.4. Effect of casein

Since casein is the major protein in bovine milk (Swaisgood, 1995), it was used to mimic the milk/HCl experiment replacing the milk. A potential limitation to this experiment was the precipitation of casein in HCl but protein was seen to precipitate in the milk/HCl experiments as well, therefore, any interaction between protein precipitate and the tablets that may have occurred previously would be expected to occur in this casein investigation. The typical total casein concentration in bovine milk is 2.95% w/v (Swaisgood, 1995).

The dissolution profile of ibuprofen tablets in the casein medium (Figure 4.5) showed approximately 9% (S.D. ± 0.84%) was dissolved after 60 min. This result falls within the error ranges for the dissolution data from milk/HCl experiments indicating that casein plays a role in the improved dissolution seen in milk media. Casein in bovine milk exists as micelles that are colloidal complexes of proteins and salt, mainly calcium (Farrell *et al.*, 1990). In the absence of calcium as in this

experiment, protein submicelles are still produced. These subunits form large spherical casein micelles via hydrophobic interactions (Jensen *et al.*, 1995). A possible explanation for the increased dissolution of ibuprofen is the solubilisation of the unionised drug in the casein micelles.

The possibility that the enhanced dissolution arose from a physical erosion of the tablet by suspended particles of undissolved casein (casein is poorly soluble at pH 2.5) was assessed by filtering the medium prior to use. Paradoxically, using the casein filtrate resulted in an increase in the rate and amount of ibuprofen dissolved over 30 min (Figure 4.5). This phenomenon remains unexplained. The dissolution profile plateau in the casein filtrate at 30 min suggests saturation was reached whereas the lower dissolution and later plateau at 90 min in the unfiltered casein medium suggests limited ibuprofen release from the tablets in the latter medium.

Having identified casein as the likely component in milk improving the dissolution of the ibuprofen tablets used, the next stage of the investigation was to determine whether the interaction was one with the drug or formulation excipients or both.

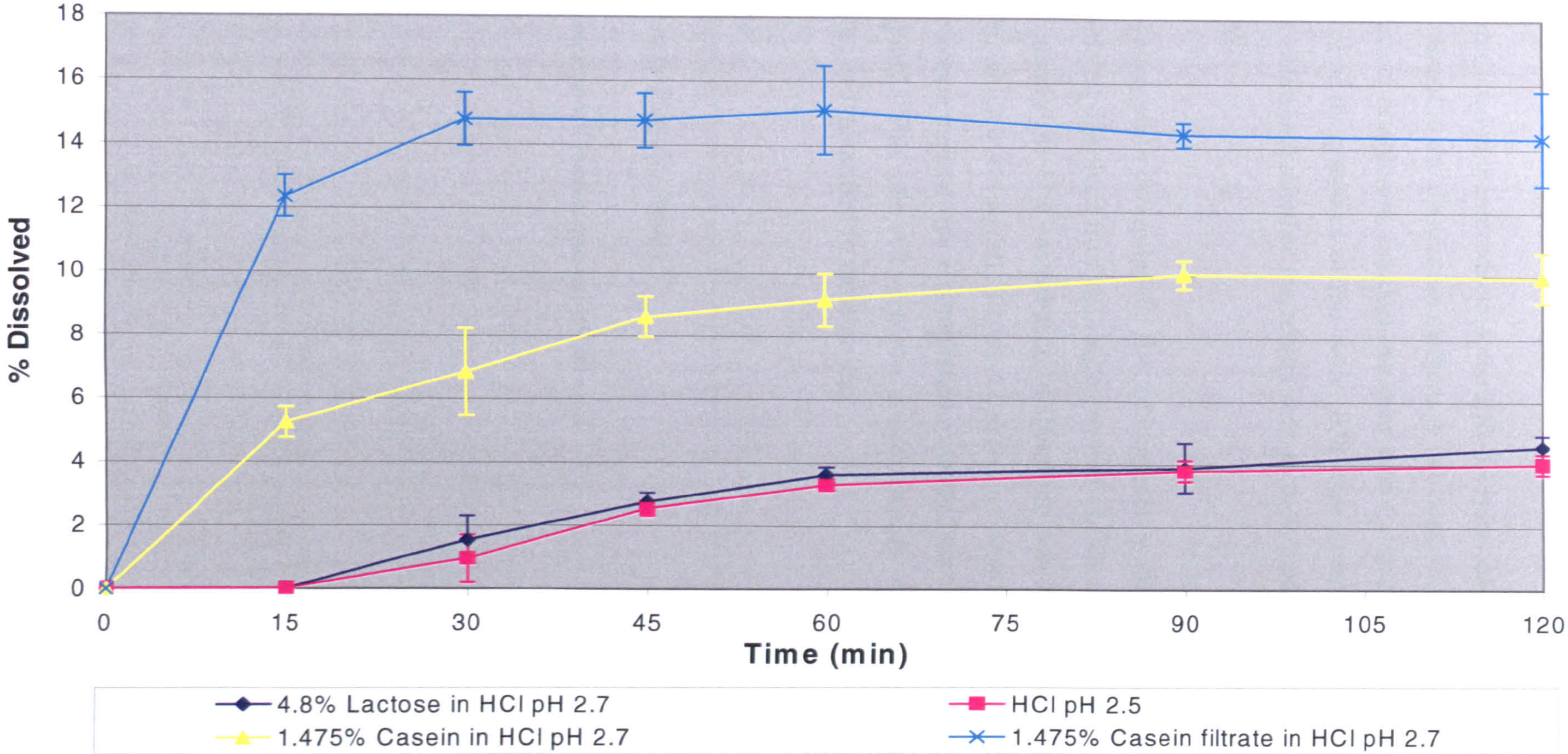


Figure 4.5. The effect of HCl pH 2.5, 4.8% w/v lactose, 1.475% w/v casein and 1.475%w/v casein filtrate in HCl pH 2.7 on the dissolution of ibuprofen 400mg tablets (*n=6*)

4.3.3. Intrinsic dissolution rate, solubility and disintegration studies

As mentioned in the introduction, the IDR measures the dissolution properties of the drug related to the dissolution medium. It is independent of formulation factors that may affect dissolution and thus has been used for many years to characterise solid drugs. For the purposes of this work, IDR experiments on ibuprofen were carried out to determine whether the results seen in milk media were drug or formulation related.

Initial attempts to determine the IDR of ibuprofen in these media were unsuccessful. The amount dissolved from the single exposed surface of the disks was very low and consequently the U.V. absorbance of ibuprofen was below the limit of detection (LOD), which was 4 μg/mL. Figure 4.6 shows a 91-fold increase in the sensitivity of the detection method was accomplished by using fluorescence spectroscopy (LOD = 47 ng/mL). This allowed very low concentrations of dissolved ibuprofen to be detected and quantified in these media.

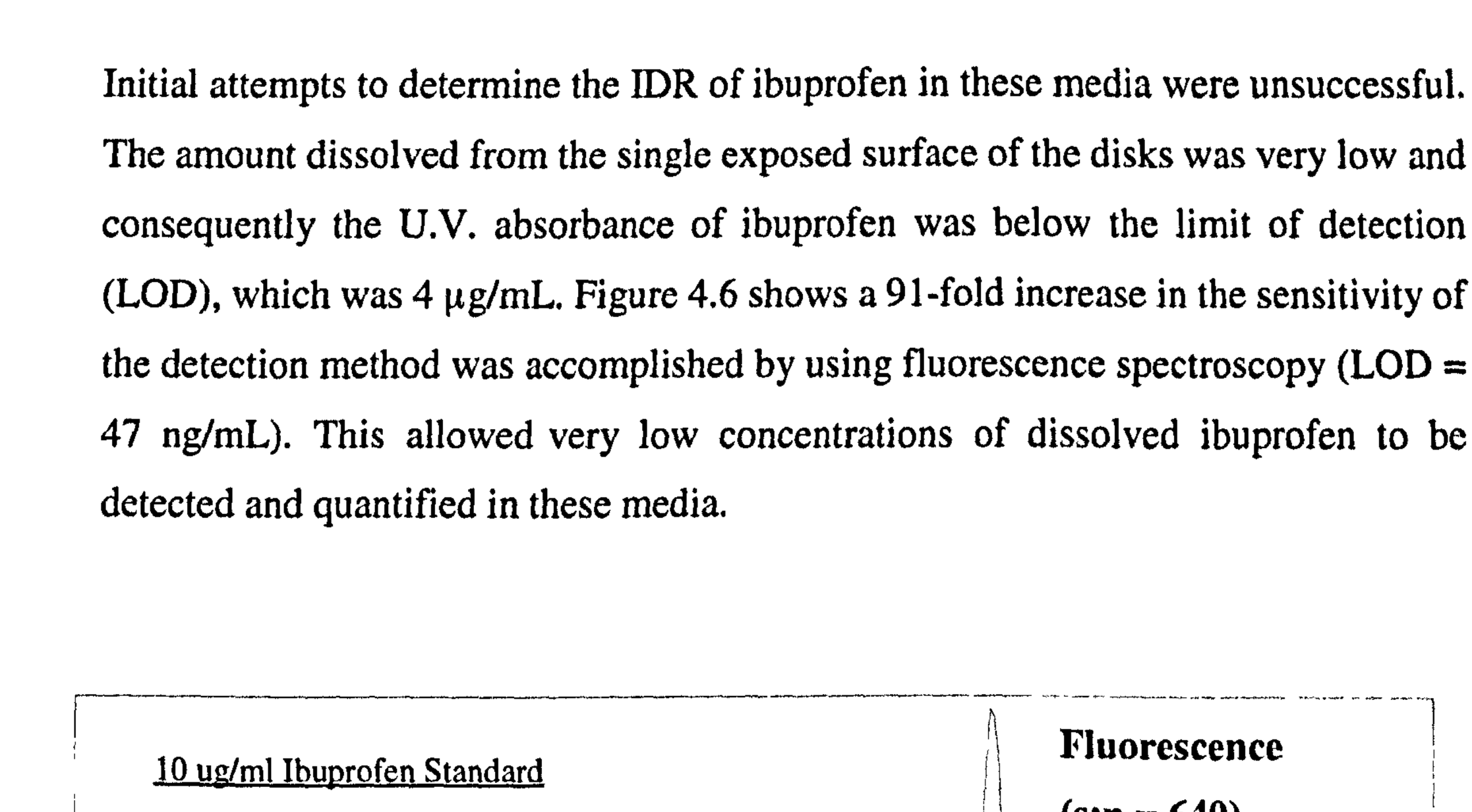


Figure 4.6. Comparison of fluorescence and UV detection in the liquid chromatographic determination of Ibuprofen

Ibuprofen release was expressed as the amount of ibuprofen dissolved per unit area plotted versus time in Figure 4.7. In addition, linear regression analysis was applied to the dissolution profiles between 15 and 90 min to determine the IDRs in Table 4.1, calculated as the slope of the regression line. The data generated enabled the effects of the various media on the dissolution of drug substance to be evaluated.

The dissolution from the disks during the first 15 min was not considered in the IDR determinations because only a single sample was taken at 15 min. Also the initial ibuprofen dissolution rate in the whole milk/HCl medium was >10 fold higher than in the other media. However, the higher initial dissolution in the whole milk/HCl medium did not impact upon the IDR between 15 and 90 min, which was comparable to the IDR in the other milk media shown in Table 4.1. Perhaps, the viscosity of the whole milk and a heavier precipitate was responsible for the initial 'burst' dissolution seen, via a greater erosive effect on the disk surface.

Table 4.2 shows the solubility determinations of ibuprofen in the various media. The change in pHs of the bulk suspensions at the 1, 5 and 24h sampling times were < 0.1 pH unit. The equilibrium solubilities of ibuprofen (24h data) in all three milk based media were at least 6 fold higher than the 0.1N HCl, 0.017% w/v SDS and the casein filtrate media. However, Table 4.1 shows that the IDR in the HCl and SDS media between 15-90 min was higher than in the milk and casein media, which was unexpected based on dissolution and solubility data. This could be the result of variable occlusion of the exposed disk surface by precipitated fragments of milk media as the experiment progressed. In the casein medium, ibuprofen diffusivity could have been lowered by a combination of microenvironmental pH change and increased viscosity of the diffusion layer at the solid-liquid interface caused by the dissolved casein in contact with the drug. This concept was put forward by Millar and Corrigan (1993) for the release of drug from ibuprofen-casein compacts into phosphate buffer media.

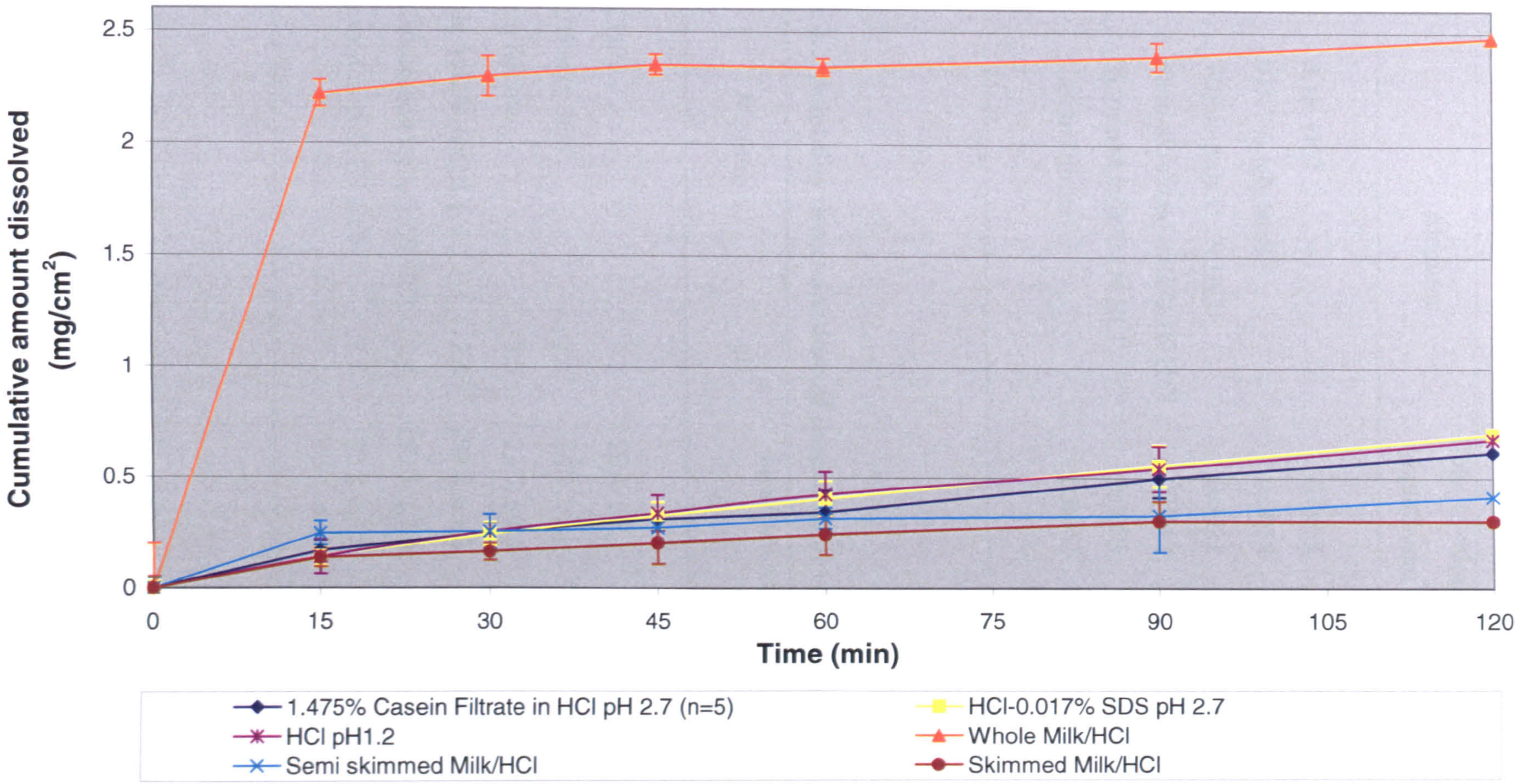


Figure 4.7. The effect of 50/50 milk/HCl mixtures, 1.475% w/v casein filtrate in HCl pH 2.7, HCl-0.017% w/v SDS pH 2.7 and HCl pH 1.2 on the dissolution of ibuprofen from compressed disks (*n=6*)

Table 4.1. IDR of ibuprofen in various media

Medium	IDR: 15-90min ($mg/min/cm^2$)	r^2 value
0.1N HCl	0.0053	0.9829
0.017% w/v SDS	0.0055	0.9965
Whole milk/0.1N HCl	0.0021	0.9181
Semi-skimmed milk/0.1N HCl	0.0012	0.9085
Skimmed milk/0.1N HCl	0.0023	0.9968
Casein filtrate	0.0042	0.9879

Table 4.2. Solubility determinations of ibuprofen in various media at 37°C.

Medium	pH	Mean amount dissolved (µg/ml) ± S.D. *n*=2		
		1h	5h	24h
0.1N HCl	1.2	20.4 ± 2.1	25.7 ± 2.4	21.1 ± 3.2
0.017% w/v SDS	2.5	34.2 ± 2.9	30.3 ± 0.9	24.7 ± 3.3
Casein filtrate	2.5	23.7 ± 1.6	23.3 ± 0.1	24.7 ± 10.9
Whole milk/0.1N HCl	2.4	103.8 ± 1.2	105.5 ± 3.4	132.0 ± 0.7
Semi-skimmed milk/0.1N HCl	2.4	153.7 ± 2.4	193.6 ± 6.6	219.8 ± 17.4
Skimmed milk/0.1N HCl	2.4	132.8 ± 3.5	157.9 ± 4.8	124.0 ± 2.4

Overall, the IDR data suggest that the dissolution enhancements seen with tablets may be due to a formulation-related effect of casein on the tablets. However, the solubility data revealed improved ibuprofen solubility in milk media. From the dissolution results in Figure 4.5 it can be seen that the profile for the casein filtrate

medium reached a plateau by 30 min (≈ 15% ibuprofen dissolved) whereas the milk media profiles (Figure 4.3) indicated steadily increasing drug dissolution up to 120 min (≈ 16% ibuprofen dissolved). This suggests that casein is somehow involved in improving the release of ibuprofen from the formulation, the drug is then available for dissolution, which is aided by higher solubility in milk, but in the casein medium saturation is reached accounting for the early plateau. The data for the solubility in casein filtrate medium do not support the previous hypothesis of solubilisation of ibuprofen into casein micelles.

Disintegration studies on the tablet formulation were performed in the various media to further evaluate the nature of the interaction seen with the tablets. The results are shown in Table 4.3.

Table 4.3. Disintegration times for ibuprofen 400mg tablets in various media.

Medium	pH	Mean Disintegration Time ± S.D. (min) *n=3*
0.1N HCl	1.2	2.91 ± 0.70
0.017% w/v SDS	2.6	3.47 ± 0.06
Casein filtrate	2.5	3.53 ± 0.48
Whole milk/0.1N HCl	2.5	3.95 ± 0.44
Semi-skimmed milk/0.1N HCl	2.5	3.12 ± 1.03
Skimmed milk/0.1N HCl	2.5	2.59 ± 0.53

The tablets disintegrated in all media in less than 4 min – an acceptable time according to industrial perspectives for IR dosage forms. Furthermore, there were no significant differences in disintegration times between the various media to warrant a possible binder performance issue for this dosage form in the different media. These findings differ from those of Abrahamsson *et al* (2004), who reported a delay in the disintegration time of several tablet formulations immersed in a multi-

component nutritional drink, caused by the precipitation of a casein film around the tablet surface.

The question that remains unanswered is that what is casein interacting with to improve the drug dissolution from the tablet? Although the tablets in this study disintegrated in all the media to a sufficient extent to pass through the standard compendial mesh size, further breakdown of the agglomerates to primary particles still needs to occur for the dissolution process. Perhaps this is where casein exerts its influence by promoting desorption of drug from drug-excipient agglomerates. It is believed that at low pH values unionised drug is immobilised onto excipient by adsorption or complexation and casein interferes with this effect.

The commercial ibuprofen tablet formulation used listed the following excipients in the ingredients (their functions are stated in brackets): microcrystalline cellulose (diluent); lactose (diluent); croscarmellose sodium (disintegrant); hydroxypropyl methylcellulose (binder); SDS (wetting agent); magnesium stearate (lubricant); French chalk (glidant/lubricant); colloidal silicon dioxide (glidant) and titanium dioxide (opacifier).

The quantitative manufacturing formula was not obtainable. However, from the functions of the ingredients included and the typical percentages used in practice, the excipients likely to be involved in drug interactions can be identified. Lactose is a highly soluble bulking agent and not likely to affect drug dissolution. Microcrystalline cellulose (MCC), croscarmellose sodium, Hydroxypropyl methylcellulose (HPMC) and magnesium stearate may be implicated in drug-excipient interactions. The disintegration data exclude casein interaction with croscaremellose sodium and HPMC since no major differences in disintegration times were observed between casein-containing and casein-free media.

Of several tablet formulations investigated, Abrahamsson *et al* (2004) found that a tablet formulation with 50% MCC exhibited the strongest food effect, taking 70 min to disintegrate in a fed state medium. Mixing of the individual excipients with the fed state medium produced a precipitate, which was shown to include caseinate by IR spectroscopy. Hence, it is plausible that although disintegration was not delayed

by casein containing media in this study, this surface-active component may interact with the MCC surface, either displacing or competing with ibuprofen for interaction at the excipient surface.

Magnesium stearate is incorporated in low levels (0.25-5% w/w) as a tablet lubricant therefore unlikely to have a significant interaction with casein but it cannot be ruled out. Further investigations of the possible drug-excipient interactions in this study are required to elucidate the cause of the higher ibuprofen dissolution from the tablet formulation.

4.4. Conclusion

The dissolution of ibuprofen tablets in milk/HCl media was significantly higher than in typical compendial media. The bovine milk protein, casein, appeared to play a role in the increased dissolution seen. Lactose had no effect. Whilst the solubility of ibuprofen in milk/HCl media was higher than in the other media, it is not believed to be directly responsible for the effect seen. The improved ibuprofen dissolution in milk/HCl media may be the result of casein influencing the availability of ibuprofen for dissolution from a drug- excipient complex coupled by the higher drug solubility in these media. The interaction does not affect the disintegration time of the tablets in the various media.

5. NAPROXEN

5.1. Introduction

Like ibuprofen, naproxen ((2*S*)-2-(6-methoxynaphthalen-2-yl)propionic acid) is an NSAID with analgesic and antipyretic properties. It is indicated to treat similar conditions i.e. mild to moderate pain, rheumatoid and osteoarthritis, tendonitis etc. It is restricted to prescription supply only in the U.K. and therefore is not used by the general population as extensively as ibuprofen.

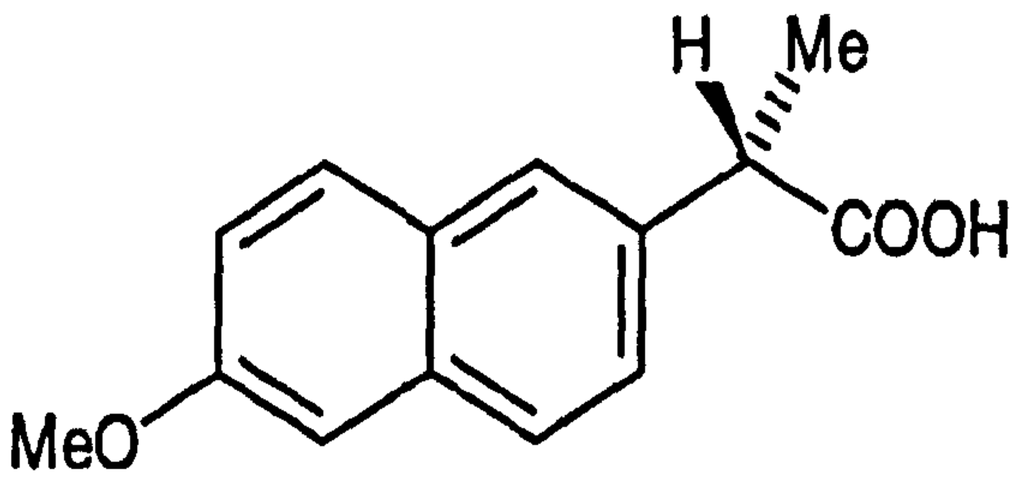


Figure 5.1. The chemical structure of naproxen

Naproxen differs structurally from ibuprofen in that it is a naphthyl rather than a benzyl derivative and also has a methoxy group incorporated into the molecule (Figure 5.1). However this does not improve its lipophilicity based on its log P value of 3.18 compared to a log P value of 3.50 for ibuprofen (Fini *et al.*, 1995). It has a lower intrinsic solubility of 12μg/mL (Table 5.1) at 37°C than ibuprofen (21μg/mL in Table 4.2). Naproxen has a pKa of 4.2 and is administered as the free acid or sodium salt. It is considered highly permeable based on oral absorption compared to an intravenous dose (Davies & Anderson, 1997). Charles and Mogg (1994) reported peak plasma levels of the free acid within 1-1.5h, which suggests absorption from the upper GI-tract. Food reduces the rate but not extent of absorption (Parfitt, 1999).

This NSAID was selected as a second acidic BCS Class II drug to study in order to compare its solubility and IDR in milk based media to that of ibuprofen. This would allow comparison of the dissolution behaviour of two propionic acid-derived NSAIDs in order to identify drug-specific or class-specific behaviour. Disintegration and dissolution tests were also carried out on a marketed tablet product to assess the effect of the medium on drug release from the formulation. The media used for the investigations included 0.1N HCl, 50/50 mixtures of whole, semi-skimmed or skimmed milk with 0.1N HCl, 0.017% w/v SDS and casein filtrate.

5.2. Materials and Methods

5.2.1. Materials

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

5.2.2. Methods

Media preparation

See Section 4.2.2.

Solubility determinations

See Section 2.2.1.

Intrinsic dissolution rate determinations

See Section 2.2.4.

Disintegration testing

See Section 2.2.2.

Dissolution testing

See Section 2.2.3. The volume of dissolution medium used was 500 mL.

5.3. Results and Discussion

5.3.1. Solubility Data

The solubility of naproxen drug substance in all the dissolution media was lower than that of ibuprofen. At 24h, the solubility of naproxen in all the media was approximately half that of ibuprofen except in semi-skimmed milk/0.1N HCl where it was approximately one third (Table 5.1). These results are reflected in the dissolution profiles (Figure 5.4), where maximum dissolution was reached for naproxen in milk-based media at about 16% from a 250 mg dose tablet in a 500 mL dissolution volume (i.e. 80µg/mL). As a result the dissolution began to plateau after about 60 min. In comparison, dissolution profiles for ibuprofen in milk based media (Figure 4.3) continued to rise until the last time point of 120 min. At this point, 15% of the drug had dissolved from a 400 mg tablet into 500mL of dissolution medium giving a concentration of 120µg/mL, just about reaching the saturation concentration for ibuprofen in the skimmed milk/0.1N HCl medium.

Table 5.1. Solubility determinations of naproxen in various media at 37°C.

Medium	pH	Mean amount dissolved (µg/ml) ± S.D. *n*=2		
		1h	5h	24h
0.1N HCl	1.2	12.0 ± 2.2	10.9 ± 0.7	12.0 ± 0.3
0.017% w/v SDS	2.5	12.6 ± 0.2	11.4 ± 0.1	10.1 ± 0.3
Casein filtrate	2.5	15.0 ± 0.7	13.4 ± 0.7	11.1 ± 0.7
Whole milk/0.1N HCl	2.4	67.0 ± 3.5	65.6 ± 0.8	61.0 ± 0.4
Semi-skimmed milk/0.1N HCl	2.4	68.0 ± 3.4	63.6 ± 0.6	69.6 ± 4.7
Skimmed milk/0.1N HCl	2.4	70.1 ± 1.8	72.5 ± 17.2	66.3 ± 0.4

It is worth noting from Table 5.1 that the solubility of naproxen in casein filtrate was not much higher than in the 0.1N HCl and 0.017% w/v SDS media. The same was true for ibuprofen solubility in these media (Table 4.2) and so, it appears that casein does not affect the solubility of these two propionic acid derived NSAIDs in acidic conditions below pH 3.

The solubility of naproxen in all three milk-based media was in the region of 6-fold higher than in the casein, SDS and HCl media. The difference in solubility between the three milk-based media was within a 10 μg/mL range. The skimmed milk medium showed a higher solubility than the whole milk, undermining the argument that the lipid content in milk could be responsible for the 6-fold increase in solubility compared to the non-milk based media.

Interestingly, for both ibuprofen and naproxen, the highest solubilities were recorded in the semi-skimmed milk/0.1N HCl medium. It was previously mentioned in the ibuprofen discussion (Section 4.3.2), that in whole milk, lipid-mediated drug solubilisation may be limited by the MLGM. In skimmed milk the negligible lipid content would be unable to exert an effect in any case. However, during the processing procedure to produce semi-skimmed milk, partial removal of the MLGM and some denuding of lipid globules and other structural changes to the composition of milk may enhance the lipid-mediated solubilisation of the drugs. As seen for ibuprofen, this improved solubility in semi-skimmed milk did not translate to an improved tablet dissolution profile compared to the whole and skimmed milk media as an excipient interaction was thought to have an overriding effect.

5.3.2. IDR Determinations

Table 5.2 shows the calculated IDR values and coefficients of determination (r^2) for naproxen in the various media. Six further sampling time points in the range 0-15 min were included in this study to monitor more accurately the initial dissolution rate. O.1N HCl, 0.017%w/v SDS and semi-skimmed milk/0.1NHCl showed linear dissolution (Figure 5.2) whereas the casein filtrate, whole and skimmed milk/0.1N HCl had more erratic release form the disk surface over the first 15 min. Hence two

IDR values are reported in Table 5.2, the second of which does not include the results of the first 15 min, omitted to improve the linear regression fit. However, the regression fits were not appreciably altered by using only the 15-120 min data as indicated by the coefficients of determination for the two IDR values in each medium (Table 5.2). Figure 5.3 shows examples of the linear regression fit applied to dissolution data from the naproxen disks.

The IDR in the 0.1N HCl medium was slightly higher than the SDS medium, such that the addition of SDS had little effect on the wetting of the disk surface and consequent dissolution.

Table 5.2. IDR of naproxen in various media

Medium	IDR: 0-120min ($mg/min/cm^2$)	R^2 value 0-120 min	IDR: 15-120min ($mg/min/cm^2$)	R^2 value 15-120 min
0.1N HCl	0.0038	0.9932	0.0036	0.9934
0.017% w/v SDS	0.0030	0.9838	0.0028	0.9809
Whole milk/0.1N HCl	0.0036	0.9008	0.0040	0.9391
Semi-skimmed milk/0.1N HCl	0.0046	0.9951	0.0045	0.9973
Skimmed milk/0.1N HCl	0.0008	0.3324	0.0004	0.2568
Casein filtrate	0.0077	0.8893	0.0076	0.9413

Despite the higher solubility of naproxen in the whole milk/0.1N HCl medium, the IDR was similar to that in 0.1N HCl. The rate of drug dissolution from the disk surface in 0.1N HCl and in semi-skimmed milk/0.1N HCl was constant and showed the best linearity (r^2 >0.99 for both calculations).

Dissolution of naproxen into the skimmed milk medium was limited and thus a credible IDR could not be calculated from the data. This was unexpected from the naproxen solubility data (Table 5.1). This anomalous result requires further

investigation. Naproxen showed the highest IDR in the casein filtrate although its solubility in this medium was similar to that in 0.1N HCl and SDS media. Sink conditions existed in all the media. What appears to be a high naproxen dissolution rate in the first minute in the casein and milk media (Figure 5.2) is possibly an interference from the naproxen assay at the low amounts dissolved.

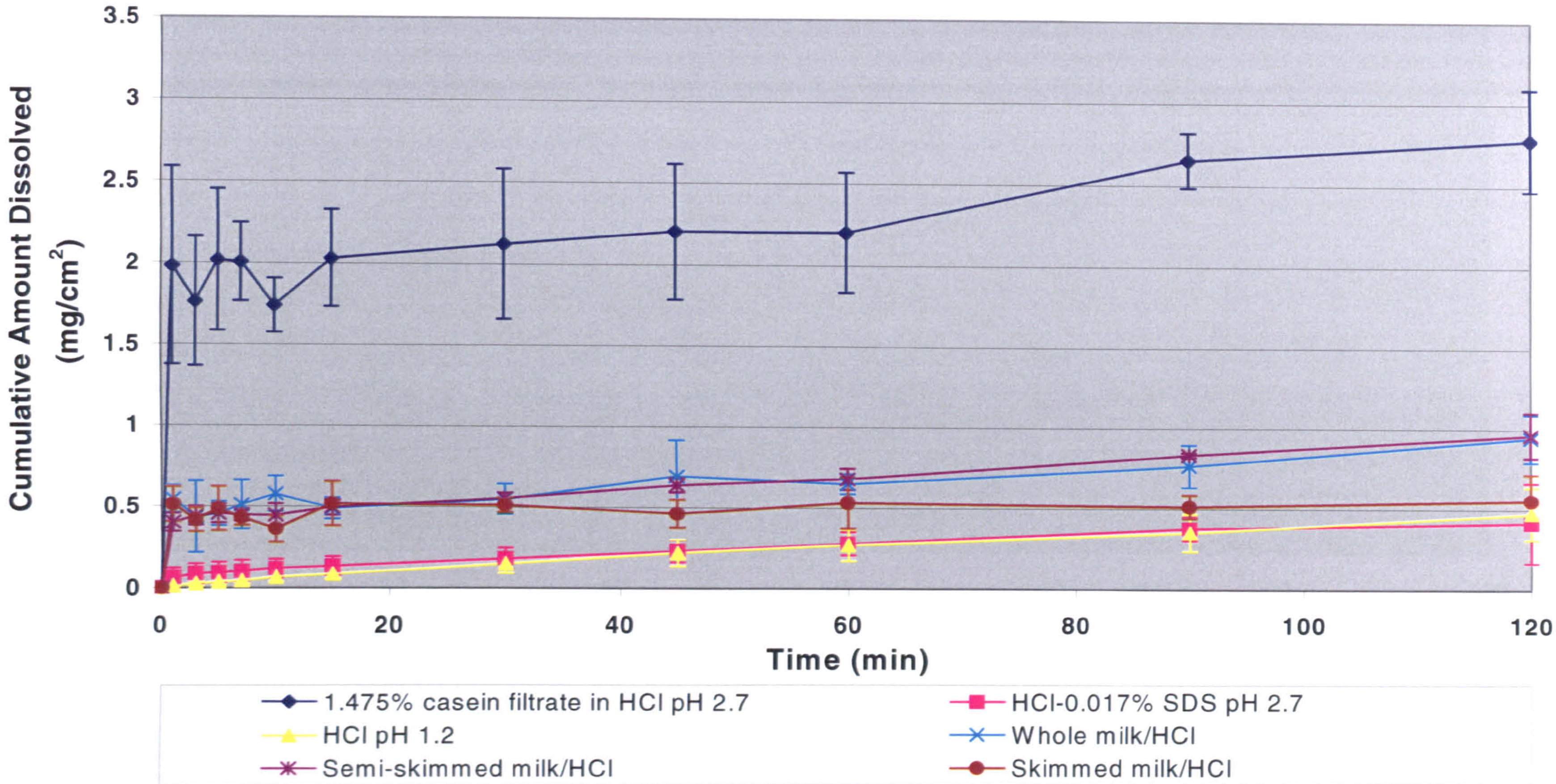


Figure 5.2. The effect of 50/50 milk/HCl mixtures, 1.475% w/v casein filtrate in HCl pH 2.7, HCl-0.017% w/v SDS pH 2.7 and HCl pH 1.2 media on the dissolution of naproxen from compressed disks (*n=6*)

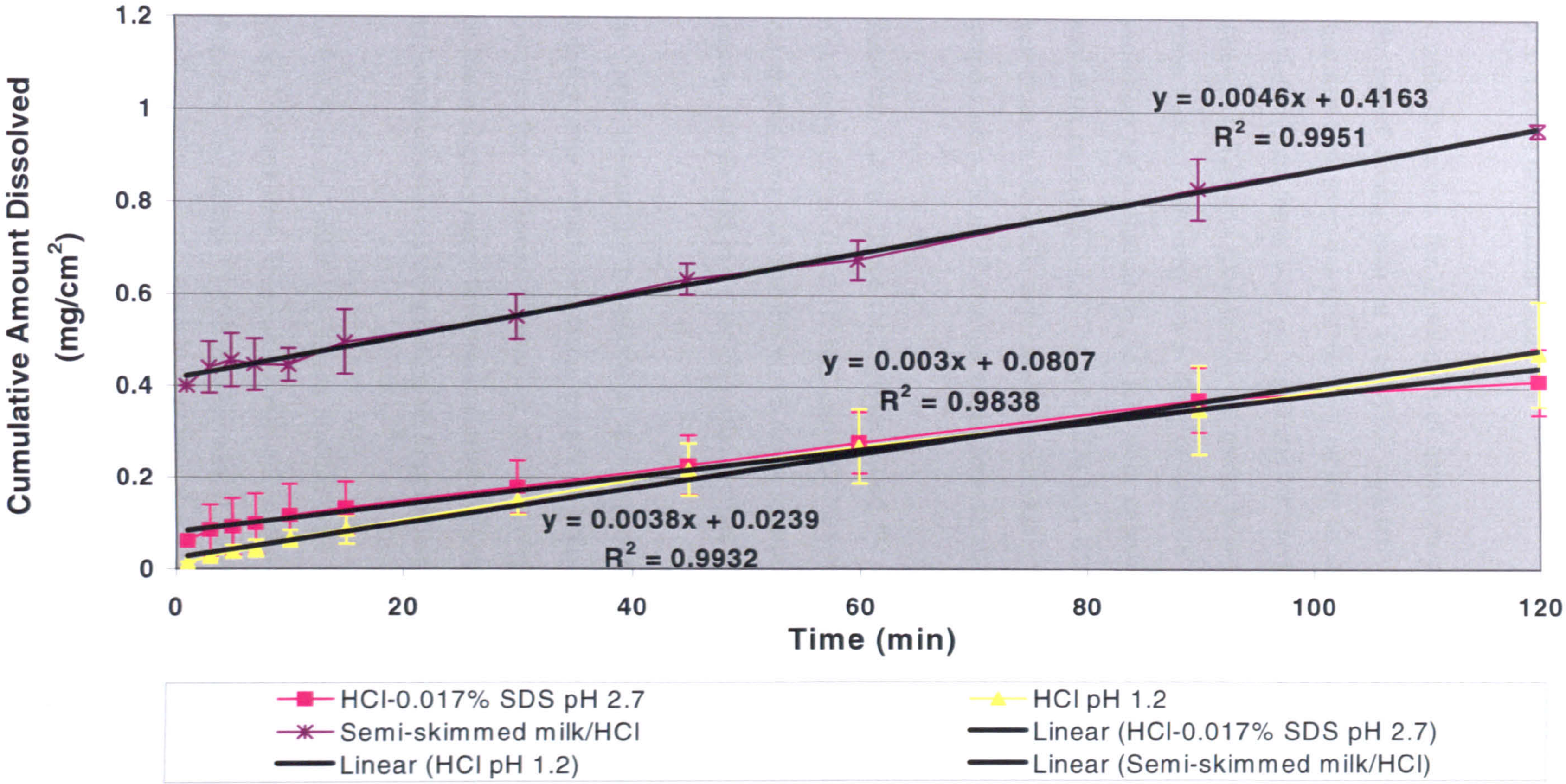


Figure 5.3. Example illustrations of the linear regression fit applied to dissolution data from rotating disks of naproxen in HCl-0.017% w/v SDS pH 2.7, HCl pH 1.2 and 50/50 semi-skimmed milk/HCl to calculate the IDR

5.3.3. Tablet dissolution studies

As with ibuprofen, the dissolution of naproxen tablets was significantly greater in milk based media than in 0.1N HCl and 0.017% w/v SDS media. Figure 5.4 illustrates that over the first 60 min tablets in skimmed milk showed the highest dissolution but there was no significant difference between the 3 types of milk between 60-120 min ($p>0.05$) as the media became saturated. Tablets in the casein filtrate medium showed lower drug dissolution than in the milk media, and the dissolution hit a plateau by 45 min with approximately 8% dissolved. Naproxen dissolution in the HCl-0.017% w/v SDS and HCl pH 1.2 media was significantly lower than in the milk and casein media, again with dissolution being limited to approximately 5% at 15 min due to media saturation with drug.

Whilst the casein filtrate medium increased the dissolution of naproxen from the tablets compared to the 0.1N HCl and 0.017% w/v SDS media, its effect was not as great as that seen with the ibuprofen tablet formulation where the dissolution profile in casein filtrate was in the region of the profiles in milk media.

The lower dissolution compared to dissolution in milk media is in line with the solubility data, which illustrates poorer solubility of naproxen in casein filtrate. However, the enhanced dissolution profile compared to 0.017%w/v SDS (which also had a slightly more favourable surface tension) did not agree with the solubility data, suggesting an effect of casein on drug release from the tablet formulation.

The trends in the tablet dissolution profiles were not as clearly reflected in the IDR profiles. Nonetheless, dissolution rate calculations showed differences in the IDR values over the period 15-120 min between 0.1N HCl and the milk-based media.

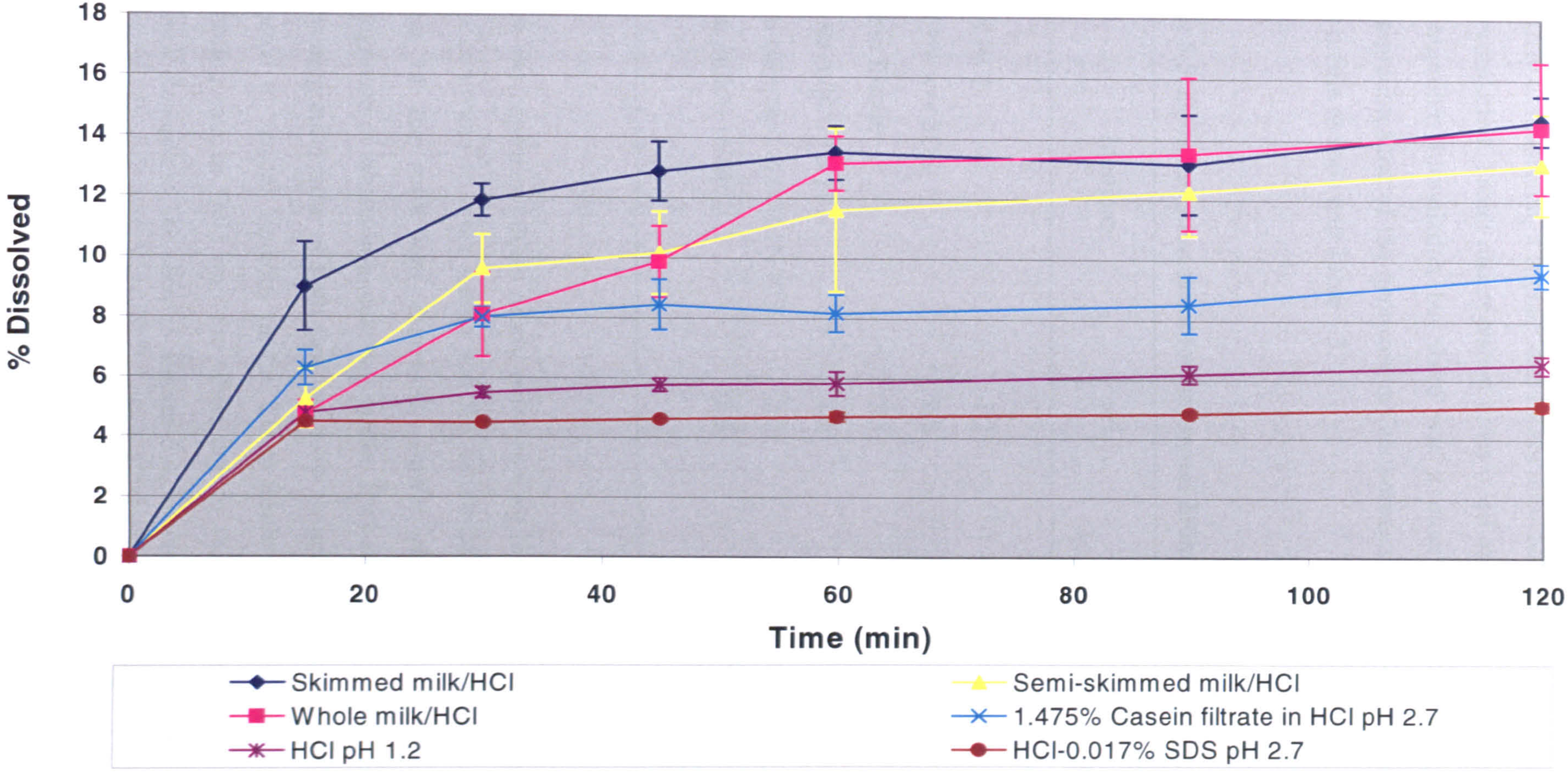


Figure 5.4. Effect of 50/50 milk/HCl mixtures, 1.475% w/v casein filtrate in HCl pH 2.7, HCl-0.017% w/v SDS and HCl pH 1.2 media on the dissolution of Naproxen 250mg tablets (*n=6*)

Similarly to ibuprofen, it appears that casein influences the release of naproxen from the formulation. Tablet disintegration times in all media were below 4 min as with the ibuprofen formulation but there was a more discernible difference in the disintegration time of the naproxen tablets between the milk-based media and the other media as shown in Table 5.3.

Table 5.3. Disintegration times for naproxen 250mg tablets in various media.

Medium	pH	Mean Disintegration Time ± S.D. (min) *n=3*
0.1N HCl	1.2	0.97 ± 0.51
0.017% w/v SDS	2.6	1.63 ± 0.55
Casein filtrate	2.5	1.50 ± 0.69
Whole milk/0.1N HCl	2.5	3.52 ± 0.44
Semi skimmed milk/0.1N HCl	2.5	2.98 ± 1.03
Skim milk/0.1N HCl	2.5	2.47 ± 0.64

It took the naproxen tablets nearly twice as long to disintegrate in the milk-based media compared to other media and the disintegration time decreased as the fat content of the milk was decreased. Anwar *et al* (2005) investigated the disintegration of tablets in biorelevant media. They reported a significant increase in the disintegration time of both fast and slow disintegrating tablets in whole milk compared to various aqueous media. These results were attributed to the slower penetration rate of fluid into the tablet due to the higher viscosity of the milk medium. Regardless, as seen in Figure 5.4, disintegration time did not have a bearing on the dissolution of naproxen into the milk-based media.

The marketed naproxen tablet brand used in this study contained the following inactive ingredients: polyvinylpyrrollidone (PVP) (diluent/binder), croscarmellose sodium (disintegrant), magnesium stearate (lubricant) and iron oxide (E172) (colourant).

It was previously observed (Bettinetti *et al.*, 1992) that mixing higher grades of PVP with naproxen increased the melting point of naproxen through the formation of crystalline microaggregates of the drug dispersed within the polymer matrix. Does casein somehow disrupt this sold state interaction and hence influence the release of naproxen from this dispersion? Indeed it has already been demonstrated that water soluble polymers such as PVP do form complexes with proteins (Matsudo *et al.*, 2003). Such protein complexes can cause change in the conformation of the polymer chain due to stearic repulsion, electrostatic repulsion of the charged bound proteins or local collapse of the surface polymer segment onto which protein is bound (Matsudo *et al.*, 2003). Whilst only speculative at this stage, the possible formation and nature of casein–PVP complexes needs to be further investigated.

Furthermore, magnesium stearate is hydrophobic and is reported to have retarded drug dissolution from oral dosage forms (Levy & Gumtow, 1963; Ganderton, 1969; Lerk *et al.*, 1982; Hussain *et al.*, 1992). However, little work has been done to assess such a hindrance in the dissolution of oral dosage forms in biorelevant media. Thus the effect of low levels (0.25-5% w/w) of magnesium stearate on the solubility and dissolution of naproxen in the casein medium needs to be assessed.

Overall, whatever the nature of the interaction between casein and the formulation excipients, it does not override the solubility influence of the milk-based media on dissolution (unlike ibuprofen, where dissolution in casein filtrate and milk-based media was similar). In this case, once the naproxen is mobilised from the formulation for dissolution (e.g. via a desorption process), its higher solubility in milk-based media is clearly demonstrated in the higher tablet dissolution profiles compared to casein filtrate.

5.4. Conclusion

The solubility of naproxen in the 50/50 milk/0.1N HCl media was significantly greater than in the other media tested. This was reflected in the enhanced tablet dissolution profiles in the milk-based media. The highest IDR of naproxen in the casein filtrate is probably the result of an erosive effect of the medium. Casein appears to enhance the dissolution of naproxen from the tablet studied through an interaction with some excipient. The possible casein–excipient interaction requires further investigation. Overall, the improved dissolution of naproxen from the marketed tablet brand studied was the result of a combined effect of enhanced solubility and excipient interaction.

6. LORATADINE

6.1. Introduction

Loratadine (ethyl 4-(8-chloro-5,6-dihydro-11H-benzo[5,6]-cyclohepta[1,2-b]pyridine-11-ylidene)-1-piperidinecarboxylic acid ethyl ester) is a non-sedating antihistamine used in the symptomatic treatment of allergic disorders such as hayfever, pruritus, urticaria and insect bites and stings.

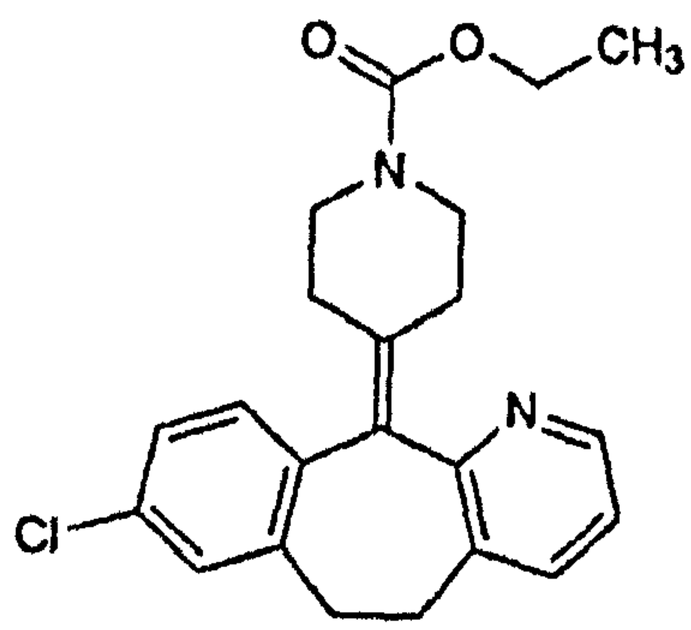


Figure 6.1. The chemical structure of loratadine

Loratadine is a poorly soluble weak base with a pKa of 5.0 and a Log P of 5.2. (Clarke, 1986). It has an intrinisic solubility of 1.9 µg/mL at 37°C (Table 6.3a). Loratadine has been characterized as a highly permeable drug based on its apparent permeability coefficient (4.8 x 10^{-5} cm/s) across CaCo-2 cell monolayers and has been classified as a BCS Class II drug (Khan *et al.*, 2004). After oral administration, the drug is rapidly absorbed under fasting conditions with peak plasma levels being reached within 1-1.5h (Simons, 2002), although there is a high inter-subject variability.

One objective of this study was to investigate, *in vitro*, the dissolution of any unabsorbed drug leaving the stomach and passing into the intestine. The aim was to

mimic, *in situ* in a dissolution vessel, the change in pH that occurs during the gastro-intestinal transit of a tablet using a common compendial dissolution apparatus (USP apparatus 2). This would involve a 2h dissolution run in acidic conditions followed by an immediate increase in volume and pH whilst the test is continued for a further 4h. The second objective was to use loratadine as a model to study the behaviour of a poorly soluble basic drug (BCS Class II) in proposed intestinal biorelevant media and attempt to develop more economical surfactant based media to replace them.

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 Section 2.1.

6.2.2. Methods

Media preparation

The pH of all the media was checked after preparation using a digital pH meter (Delta 350, Mettler Toledo, Leicester, U.K.)

0.017% w/v sodium dodecyl sulphate in 0.1N HCl (HCl-0.017% SDS pH 1.2) was prepared by addition of 850 mg of SDS to a 5 L volumetric flask and making up to volume with 0.1N HCl.

0.017% w/v sodium dodecyl sulphate in 0.003N HCl (HCl-0.017% SDS pH 2.5) was prepared by addition of 850 mg of SDS to a 5 L volumetric flask and making up to volume with 0.003N HCl.

0.25% w/v sodium dodecyl sulphate in 0.1N HCl (HCl-0.25% SDS pH 1.2) was prepared by addition of 12.5 g of SDS to a 5 L volumetric flask and making up to volume with 0.1N HCl.

Sodium-based simulated intestinal fluid (NaSIF) consisted of phosphate buffer pH 6.8 except that NaH_2PO_4 was used instead of KH_2PO_4 as suggested by Vertzoni *et al* (2004) to mimic better the cationic environment in the small intestine. Also, FaSSIF includes NaH_2PO_4 as the buffering agent.

8 L of both Fasted state simulated intestinal fluid blank (FaSSIF blank) and Fed state simulated intestinal fluid blank (FeSSIF blank) were prepared as per Table 1.2 without sodium taurocholate (NaTC) and lecithin, to be used as controls and for the preparation of FaSSIF and FeSSIF.

8 L of both FaSSIF and FeSSIF were constituted as per the formulae in Table 1.2. To obtain clear micellar solutions, 8 L of FaSSIF and FeSSIF blanks were first prepared and the correct quantities of NaTC and lecithin (Table1.2) were mixed with 1.6 L of the corresponding blank medium to produce concentrates. These concentrates were then stirred overnight (at room temperature) using magnetic stirrers and the clear concentrates made up to volume with 6.4 L of the corresponding blank medium.

6 L of 0.059% w/v lecithin was prepared by addition of 3.54 g of lecithin to a container followed by 6 L of FaSSIF blank. 6L of 0.295% w/v lecithin was prepared by addition of 17.7g of lecithin to a container followed by 6 L of FeSSIF blank.

6 L of 0.165% w/v NaTC was prepared by addition of 9.9 g of NaTC to a container followed by 6 L of FaSSIF blank. 6 L of 0.825% w/v NaTC was prepared by addition of 49.5 g of NaTC to a container followed by 6 L of FeSSIF blank.

For surfactant-based media used in the solubility screen, 20 mL of each medium was prepared in a volumetric flask (except for Triton X 100 and C12AO where 200 mL

were prepared) according to the concentration stated in Table 6.1 in both FaSSIF and FeSSIF blanks.

Table 6.1. Concentrations of the surfactants used in the loratadine solubility study and their cmc values.

Surfactant	cmc[1,2] (mg/ml)	Concentration used for solubility study (mg/ml)
CTAB	0.36	0.73
SDS	2.37	4.73
Brij 35	0.13	0.26
Triton X 100	0.15	0.30
Cremophor EL	0.09	0.18
Tween 80	0.02	0.04
CHAPSO	5.05	10.10
C12AO	0.00046	0.0092

[1] Values quoted at 20-25°C in water
[2] Source: Sigma Aldrich. (*Detergent Properties and Applications Table*)

Solubility determinations

See section 2.2.1.

Dissolution testing

See section 2.2.3. The volume of dissolution medium used was 1000 mL.

pH-shift method:

The dissolution test was carried out in 500 mL of an acidic medium (0.1N HCl, HCl-0.25% SDS pH 1.2, HCl-0.017% SDS pH 1.2 or HCl-0.017% SDS pH 2.5) for 2h as normal, with 5ml samples being taken at 15, 30, 45, 60, 90 and 120 min. After sampling at the 2h time point, 50 mL of 1N NaOH, which was already at 37°C, was immediately

added to each of the six dissolution vessels. This was followed by the addition of 450ml of phosphate buffer pH 7.4 (also at 37°C) to each vessel. The final medium pH was measured as 7.3. The dissolution run was continued during this pH shift and for a further 4h. 5ml samples were taken at 2h 15min, 3h, 4h, 5h and 6h. The pH was re-measured at the end of the run.

Disintegration testing

See section 2.2.2.

6.3. Results and Discussion

6.3.1. pH-solubility data and pH shift experiments

The pH-solubility data for loratadine (Table 6.2) show the rapidly diminishing solubility of the drug between pH 1.2 (36.7 mg/mL) and pH 2.5 (639 µg/mL). Thereafter the solubility continued to decrease with increasing pH. The lowest solubility was 1.3 µg/ml at pH 6.8.

Table 6.2. pH-related solubility determinations of loratadine at 37°C

		Mean amount dissolved (µg/ml) ± S.D. *n*=2		
Medium	pH	1h	5h	24h
0.1N HCl	1.2	$3.76 \times 10^4 \pm 1.1 \times 10^3$	$3.62 \times 10^4 \pm 43.6$	$3.67 \times 10^4 \pm 112.5$
0.003N HCl	2.5	679.9 ± 7.9	670.5 ± 25.5	639.0 ± 1.1
Acetate buffer	4.5	10.0 ± 0.1	10.9 ± 0.1	10.1 ± 0.1
Phosphate buffer	6.8	1.7 ± 0.1	1.7 ± 0.04	1.3 ± 0.1

The drastic reduction in solubility between pH 1.2 and 2.5 may have implications on drug absorption from the stomach, depending not only on individual pH variability but also on whether the individual is in the fed or fasted state. The solubility data suggest that dissolution and consequent absorption of loratadine will be hindered in the fed stomach due to the raised pH (2.5-4.5) in this state, if pH alone controls absorption.

In relation to pH dependent solubility, the dissolution of a commercially available brand of loratadine 10mg tablets (Brand A) was investigated in various simple media (Figure 6.2). The effects of pH were evident in the differences in drug dissolution after 2h between HCl-0.017% SDS at pH 1.2 and HCl-0.017% SDS at pH 2.5 and in NaSIF pH 6.8 (103.51%, 31.81% and 0.09% drug dissolved respectively). However, with pH shift, the addition of 1N NaOH and phosphate buffer to 0.25%w/v SDS pH 1.2 in the dissolution vessel (final pH 7.3) did not cause precipitation of the dissolved drug (Figure 6.2). This dissolution profile in the HCl-0.25% SDS pH 1.2 – pH 7.3 shift experiment shows 109% loratadine dissolved at 30 min. This high percentage of drug dissolution can be attributed to weight variation of the tablets leading to dose variations (accepted regulatory variation is 90-110% of the stated dose) and slight analytical bias. When the pH shift experiment was repeated without using SDS in the medium, drug precipitation on pH shift was evident from the dissolution profile.

The presence of surfactant in the medium prevented precipitation as the pH was raised. At a pH above 6 almost all of the loratadine would have been unionized, thus, the drug was probably solubilised within the hydrophobic micellar core of the surfactant. This result demonstrated that the incorporation of SDS to the gastric phase of this dissolution test to match physiological surface tension had a consequence on the prediction of the consecutive intestinal dissolution phase, highlighting a limitation of such an *in situ* test. To determine the extent to which physiological surfactants forming micelles in the small intestine protect basic drugs from precipitation during transit requires pH shift experiments to FaSSIF and FeSSIF media.

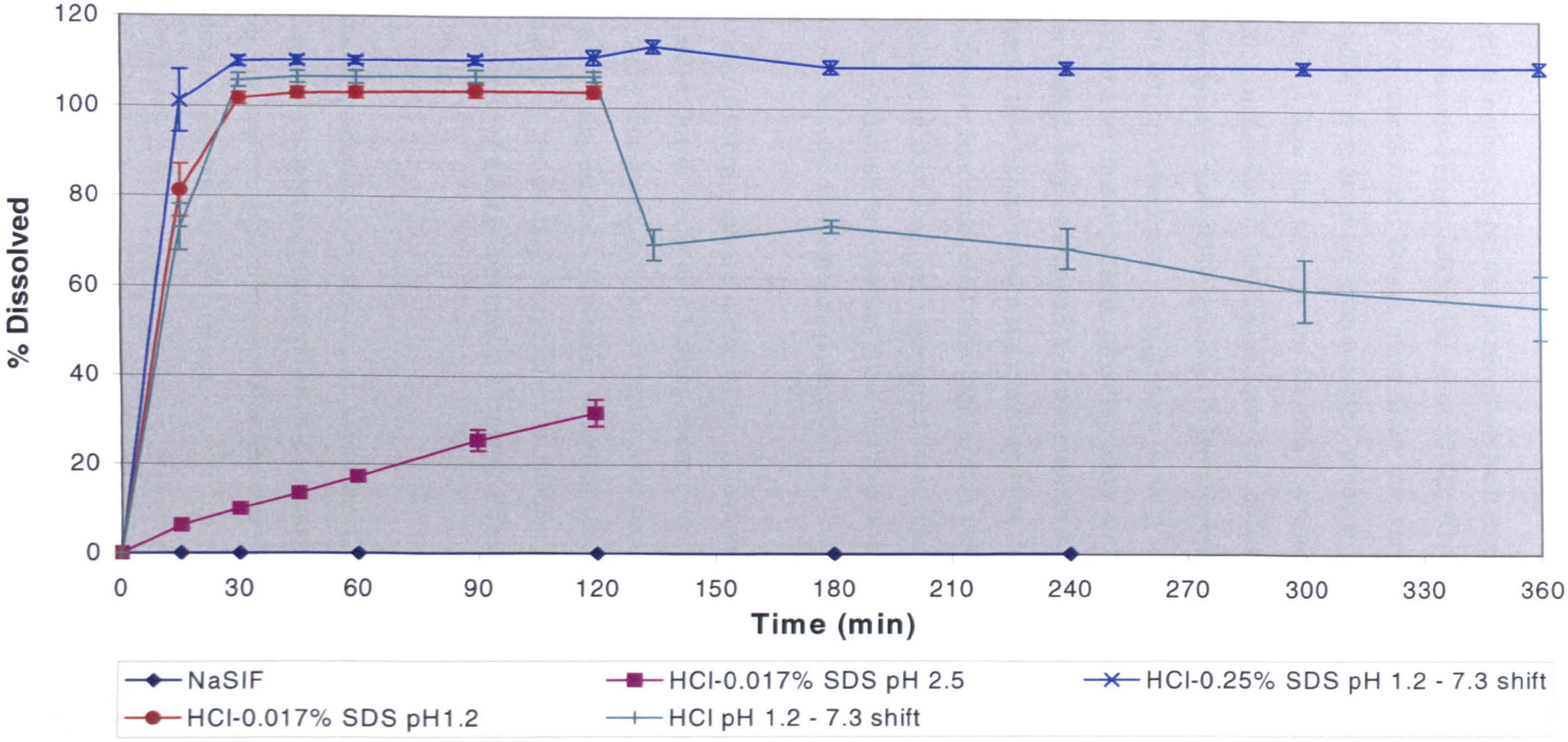


Figure 6.2. Effect of NaSIF, HCl-0.017% w/v SDS pH 1.2 and 2.5, HCl (with pH shifted from 1.2 to 7.3) and HCl-0.25% w/v SDS (with pH shifted from 1.2.to 7.3) on the dissolution of Loratadine 10mg tablets (Brand A) (*n=6*)

Alternative methods of pH shift experiments have recently been investigated (Qiu *et al.*, 2003; Kostewicz *et al.*, 2004; Gu *et al.*, 2005; Kreutzwald *et al.*, 2005) that avoid having to transfer the dosage form from one medium to another, which is the compendial procedure for enteric-coated products (Section 1.3.8.3). The transfer procedure is also the basis of the Bio-Dis® system (USP apparatus 3) that allows sequential immersion of the dosage form into different media (Section 1.3.1.3). The flow through apparatus (USP apparatus 4) has also been suggested to mimic the *in vivo* pH shift during dissolution investigations (Looney, 1997) by switching the medium reservoir at a set time or addition of buffer. Some of these methods suffer from the fact that the dosage form is exposed to fresh medium instead of allowing mixing of the medium of first exposure with a second medium. *In vivo*, all the stomach contents empty into the duodenum over time. Hence, such methods do not allow monitoring of precipitation of dissolved but unabsorbed basic drug caused by the pH shift during gastric emptying into the intestine. Also, methods involving transfer of the dosage form are not practical for disintegrating dosage forms.

The *in situ* pH shift addresses these issues. Although the pH shift experiments in this study utilize simple media as a starting point, if viable the method can be developed further using biorelevant dissolution media, food components, enzymes and include an absorption compartment. However, sampling and drug extraction may prove challenging as the medium becomes more complex. The effect of additives on the buffer capacity of the medium may also make it difficult to maintain a constant pH over several hours.

In terms of practicality for this set of experiments, the increased number of stages during testing compared to compendial tests increases the risk of human error during the test. For routine application, operator and inter-lab variability may be increased depending on the complexity of the test.

With regard to predicting the dissolution behaviour of a drug during transit through the GIT, such a test can lend insight into the physicochemical properties of the medium

affecting drug dissolution. Of course, this hinges on how closely the medium resembles the respective *in vivo* environments through gastro-intestinal transit. Also, experimental factors such as agitation speed can affect the result. The true utility of such a test needs to be determined through correlation with *in vivo* studies.

6.3.2. Solubility and dissolution in FaSSIF, FeSSIF and constituents

Table 6.3a shows that the solubility of loratadine in FaSSIF was much higher than in the individual constituent and blank media – approximately 5 times higher than lecithin and 7 times higher than NaTC and FaSSIF blank at 24h. Loratadine showed the lowest solubility in FaSSIF blank (pH 6.4), which was similar to that in the phosphate buffer pH 6.8 (Section 6.3.1). Comparison of the data in Table 6.3a to Table 6.3b shows that drug solubility in the fed state media was higher. This is attributed to pH difference and the higher concentration of bile salt (NaTC) and lecithin compared to the fasted state media.

The inclusion of the NaTC and lecithin as solubilisers is of particular interest. At the higher pH of 6.5 little difference in loratadine solubility was seen between the lecithin and NaTC media. This was a combined effect of the low but still physiologically relevant concentrations of lecithin and NaTC used (0.059% w/v and 0.165% w/v respectively) and the higher pH. At a relatively more favourable pH and at much higher concentrations of lecithin and NaTC (0.295% w/v and 0.825% w/v respectively), matching those reported in the fed state human intestine, solubility in NaTC (≈ 64 µg/mL) was at least 4 times higher than in lecithin (Table 6.3b). However the higher solubility in the NaTC medium was still appreciably lower than in the complete FeSSIF medium containing both components. This synergistic effect when both components were present was attributed to the formation of mixed micelles. Incorporation of lecithin into the bile salt micelle decreases the cmc, increases the micelle size and enhances the solubilisation capacity (Charman *et al.*, 1997; Horter & Dressman, 2001). Thus the omission of lecithin from these proposed biorelevant dissolution media to

make them more cost effective may potentially compromise the prediction of *in vivo* product performance.

Two readily available suppliers of NaTC were also compared to test the variability in solubilizing behaviour. No discernable differences were noted at the low NaTC concentration (fasted state). However, at the higher NaTC concentration loratadine solubility was approximately 10% higher in the NaTC obtained from Lancaster (97% purity) than in the NaTC obtained from Sigma (95% purity).

Table 6.3a. Solubility determination of loratadine in fasted state media at 37°C

Medium	pH	Mean amount dissolved (µg/ml) ± S.D. *n=2*		
		1h	5h	24h
FaSSIF	6.4	9.1 ± 0.3	15.2 ± 0.01	14.0 ± 0.2
FaSSIF blank	6.4	1.8 ± 0.04	1.8 ± 0.1	1.9 ± 0.2
0.059% w/v lecithin	6.5	2.3 ± 0.3	3.1 ± 0.1	3.3 ± 1.0
0.165% w/v NaTC (Sigma)	6.4	2.1 ± 0.01	2.2 ± 0.02	2.2 ± 0.03
0.165% w/v NaTC (Lancaster)	6.5	2.1 ± 0.03	2.2 ± 0.1	2.2 ± 0.03

Table 6.3b. Solubility determination of loratadine in fed state media at 37°C

Medium	pH	Mean amount dissolved (µg/ml) ± S.D. *n=2*		
		1h	5h	24h
FeSSIF	5.0	144.6 ± 0.8	146.6 ± 1.7	155.1 ± 6.5
FeSSIF blank	5.0	5.8 ± 0.1	6.2 ± 0.2	5.2 ± 0.6
0.295% w/v lecithin	5.0	6.6 ± 0.2	10.7 ± 1.2	15.6 ± 3.6
0.825% w/v NaTC (Sigma)	5.0	61.6 ± 0.1	63.2 ± 0.04	63.8 ± 0.6
0.825% w/v NaTC (Lancaster)	5.0	66.1 ± 0.2	68.1 ± 0.5	68.7 ± 0.3

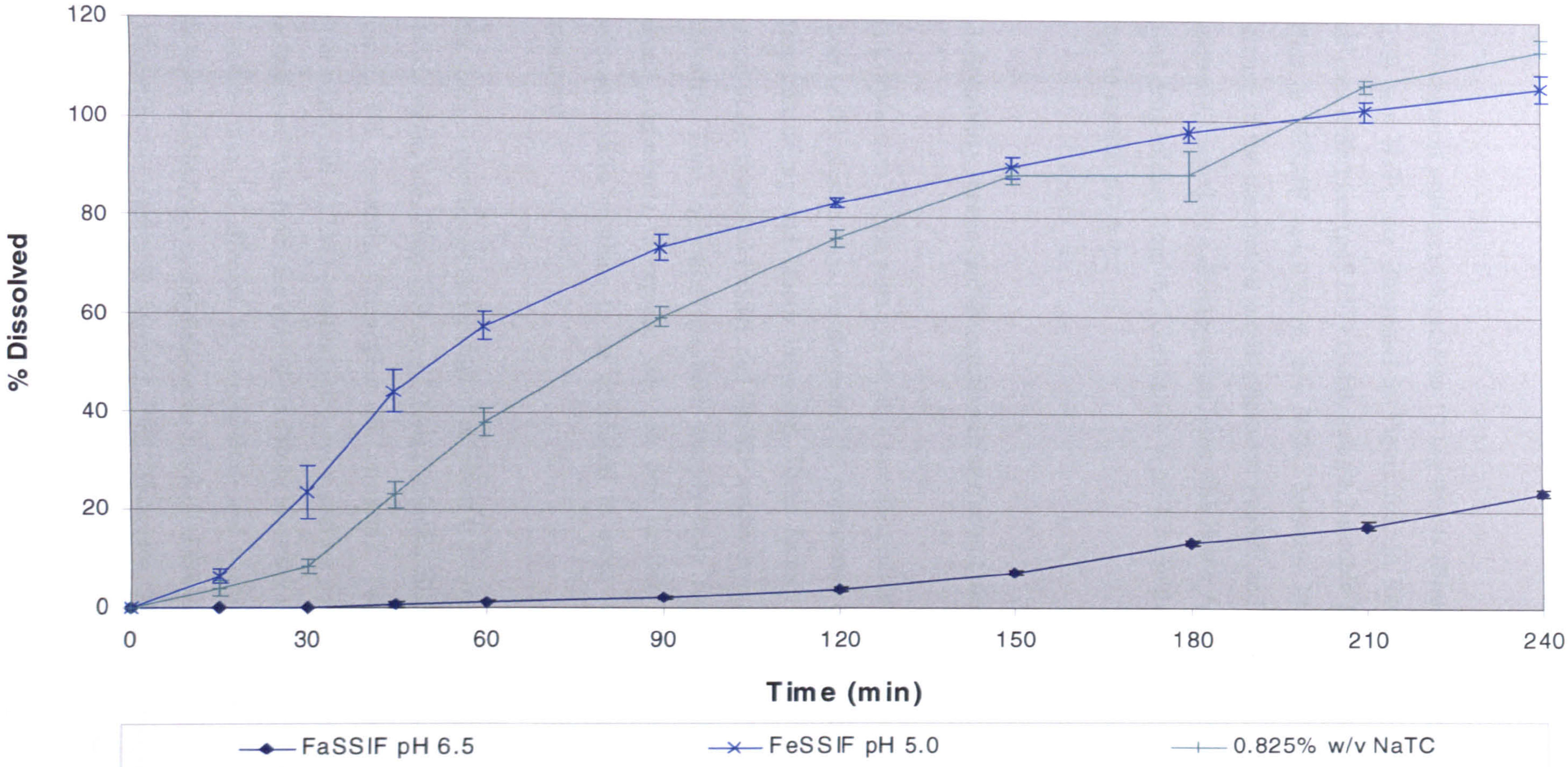


Figure 6.3. The effect of Fasted state simulated intestinal fluid (FaSSIF), Fed state simulated intestinal fluid (FeSSIF) and 0.825% w/v sodium taurocholate (NaTC) media on the dissolution of loratadine 10mg tablets (Brand A) (*n=6*)

The dissolution profiles of loratadine 10 mg tablets (Brand A) in FaSSIF, FeSSIF and 0.825% w/v NaTC are illustrated in Figure 6.3. In line with the solubility data, a significant difference ($p<0.05$) existed, using a 2-sample T-test between the dissolution profiles in FESSIF and 0.825% w/v NaTC between 30-120 min. The extent of the difference in the dissolution profiles between FaSSIF and FeSSIF seen in Figure 6.3 is an anomaly, caused by a disintegration issue with Brand A loratadine tablets that further delayed drug release from the formulation at pH 6.5 (Table 6.6). This issue is discussed further in section 6.3.4.

6.3.3. Solubility screen and dissolution in surfactant based media

The incorporation of bile salts and lecithin into dissolution media significantly increases the cost of dissolution tests. Furthermore, the preparation of these more complex media is time consuming and labour intensive. These factors have limited the utility and adoption of the proposed FaSSIF and FeSSIF media during product development stages in industry. Thus, attention has been already been focused on finding alternative synthetic surfactants to replace bile salt and lecithin whilst these relatively new biorelevant media continue to be developed to more accurately match the *in vivo* environment.

In this study, a number of surfactants of different type were screened for their ability to solubilise loratadine. They included CTAB (cationic), SDS (anionic), Brij 35 (neutral), Triton X 100 (neutral), Cremophor EL (neutral), Tween 80 (neutral), CHAPSO (zwitterionic) and C12AO (zwitterionic). As a starting point, the concentration of each surfactant used was twice the quoted cmc from literature (Table 6.1). It is accepted that cmc values may be affected by temperature and electrolytes. In the case of ionic surfactants, electrolytes in the dissolution medium decrease the cmc whilst temperature has little effect (Florence & Atwood, 1988; Malmsten, 2002) . Conversely for neutral surfactants, electrolytes have little effect on the cmc whilst raising the temperature of

the dissolution medium decreases the cmc (Florence & Atwood, 1988; Malmsten, 2002). Hence, for the purposes of this study, using surfactants at twice their cmcs was considered appropriate to form micellar solutions. The results of the solubility experiments are given in Tables 6.4a and 6.4b. The enhancement in loratadine solubility in synthetic surfactant media relative to NaTC for SDS, CTAB and CHAPSO was 586, 46 and 32 fold respectively in FaSSIF blank and 25, 1.8 and 1.3 fold respectively in FeSSIF blank. The other synthetic surfactants used did not significantly enhance loratadine solubility relative to NaTC. The decrease in magnitude of drug solubility enhancements in the synthetic surfactants when moving from FaSSIF blank to FeSSIF blank was due to the higher concentration of NaTC in the FeSSIF medium.

Table 6.4a. Solubility determination of loratadine in FaSSIF blank- surfactant media.

Medium	pH	Mean amount dissolved (µg/ml) ± S.D. *n=2*		
		1h	5h	24h
SDS	6.7	1194.4 ± 12.9	1186.9 ± 15.9	1290.0 ± 41.3
CTAB	6.6	102.4 ± 0.04	102.5 ± 0.1	101.4 ± 0.3
CHAPSO	6.7	68.7 ± 0.4	71.6 ± 0.5	71.2 ± 0.3
Cremophor EL	6.7	5.5 ± 0.1	5.3 ± 0.1	3.4 ± 0.5
Brij 35	6.7	5.3 ± 0.04	5.5 ± 0.04	4.5 ± 0.1
Triton X 100	6.7	3.2 ± 0.2	3.2 ± 0.1	1.5 ± 0.1
Tween 80	6.7	1.8 ± 0.02	1.7 ± 0.1	1.7 ± 0.2
C12AO	6.7	1.7 ± 0.04	1.6 ± 0.1	1.5 ± 0.1

Table 6.4b. Solubility of loratadine in FeSSIF blank- surfactant media.

Medium	pH	Mean Solubility (µg/ml) ± S.D. *n=2*		
		1h	5h	24h
SDS	5.0	1467.3 ± 10.0	1550.0 ± 67.1	1637.3 ± 124.8
CTAB	4.9	107.9 ± 7.3	107.6 ± 3.3	119.0 ± 7.1
CHAPSO	5.0	80.7 ± 3.2	81.1 ± 1.9	83.2 ± 1.5
Brij 35	4.9	10.8 ± 0.2	12.8 ± 2.3	10.9 ± 0.1
Triton X 100	5.0	10.3 ± 0.6	12.4 ± 2.3	9.4 ± 0.04
Cremophor EL	5.0	10.0 ± 0.6	10.0 ± 0.4	9.1 ± 0.8
C12AO	5.0	5.6 ± 1.5	5.1 ± 0.1	4.8 ± 0.04
Tween 80	5.0	4.9 ± 0.2	5.2 ± 0.6	4.8 ± 0.1

The solubilising capacity of a micelle is defined as the number of moles of solubilisate per mole of micellised surfactant (i.e. C – cmc) (Rosen, 1989). The moles of loratadine solubilised in the synthetic surfactants were calculated from the 24h data in Table 6.4a and 6.4b. The solubilising capacities of the synthetic surfactants are shown in Table 6.5. From Table 6.5 it can clearly be seen that SDS was the best at solubilising loratadine. CTAB showed the second highest solubilising capacity. The significantly lower loratadine solubility in the neutral surfactant media at higher pH (Table 6.4a) and their respective capacities (or lack of) in Table 6.5 showed that these surfactants were poor solubilisers of the drug. Where solubilising capacities were not calculated in Table 6.5 it was because loratadine solubilities in the synthetic surfactants were similar to that in the FaSSIF and FeSSIF blanks i.e surfactant mediated solubility enhancement did not occur. The higher solubilising capacities at pH 5 compared to pH 6.7 may be attributed to electrolyte effects enhancing micelle formation in FeSSIF.

Table 6.5. Solubilising capacity of synthetic surfactants for loratadine at 37°C based on 24h solubility data.

Surfactant	Solubility[1] (mole drug/mole surfactant) pH 5.0	pH 6.7
SDS	0.52	0.41
CTAB	0.29	0.25
Cremophor EL	0.28	0.11
Brij 35	0.14	0.07
Triton X 100	0.05	---
CHAPSO	0.03	0.02
Tween 80	---	---
C12AO	---	---

[1]Data corrected for non-micellar loratadine solubility by subtracting the solubility of loratadine in FaSSIF and FeSSIF blanks at 24h (Tables 6.3a and 6.3b respectively) from the solubility in the surfactant media at 24h (Tables 6.4a and 6.4b)

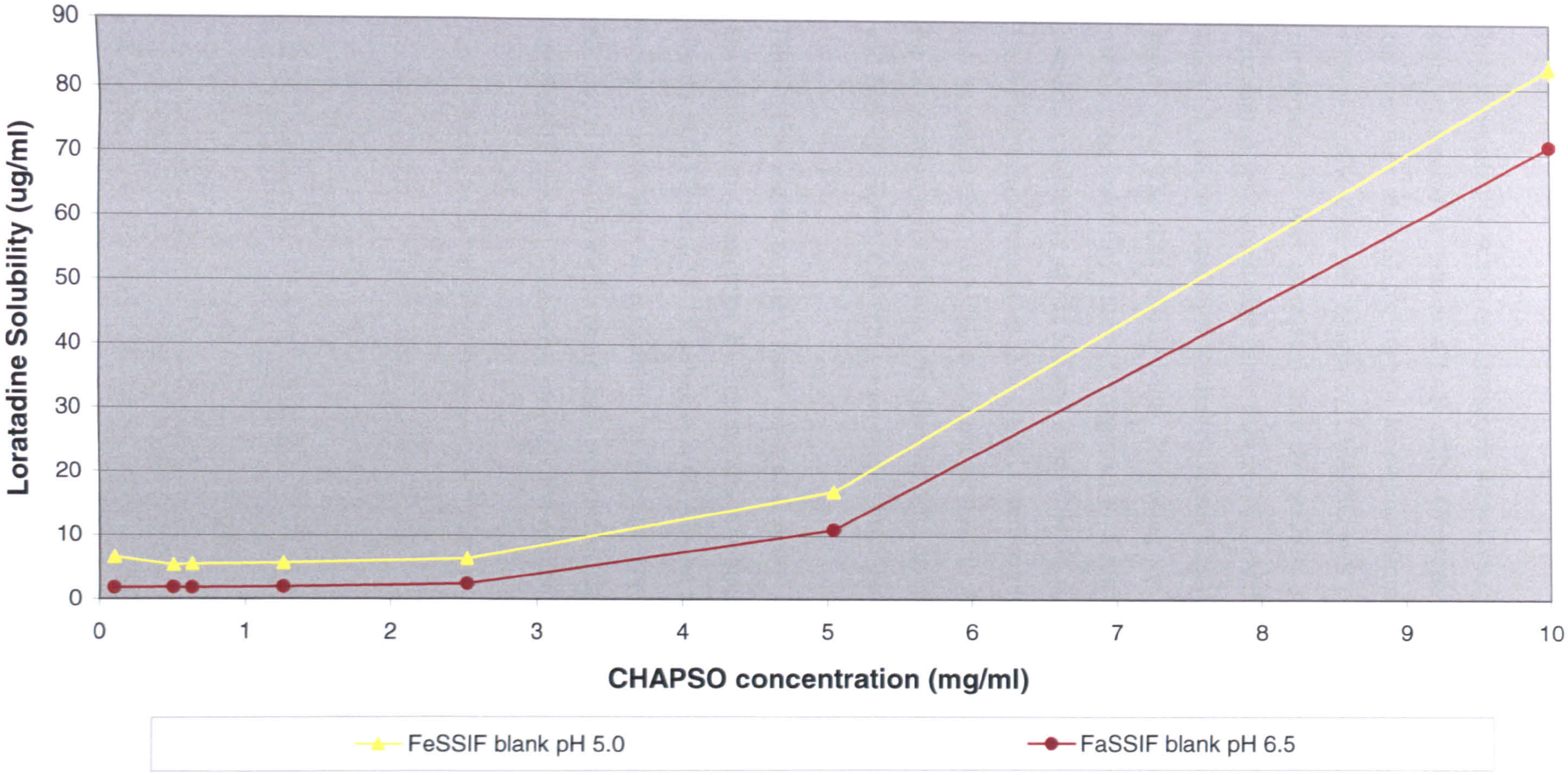


Figure 6.4. Effect of CHAPSO concentration on the solubility of loratadine in FeSSIF blank (pH 5.0) and FaSSIF blank (pH6.5) (***n=2,*** error bars are within the data points)

It was decided to select one surfactant from each class for further study in dissolution tests of loratadine tablets. CTAB, SDS, Cremophor EL and CHAPSO were chosen because they showed the highest loratadine solubility at the concentrations selected. However, the cost per weight of CHAPSO was considerably greater than the other surfactants. It was calculated that the quantity required for a standard six-vessel dissolution test would cost more than NaTC, which would defeat the objective of the study. A plot of loratadine solubility versus CHAPSO concentration is illustrated in Figure 6.4. The data revealed that decreasing the CHAPSO concentration from that used in the solubility study (10 mg/mL) to 5 mg/mL caused a five-fold decrease in loratadine solubility at pH 5.0 and seven-fold decrease at pH 6.5. Further reduction in CHAPSO concentration to 2.5 mg/mL further reduced loratadine solubility. These results meant that CHAPSO could not be used in lower, cost effective, concentrations for dissolution tests without affecting the drug dissolution profile compared to FASSIF and FeSSIF. Hence, CHAPSO was omitted from the dissolution study.

Initial dissolution profiles were generated using Brand A loratadine 10mg tablets. Dissolution in Cremophor EL media was considerably lower than in other media at similar pH (Figure 6.5). Dissolution in CTAB and SDS media was more promising in terms of matching the dissolution profiles generated in FaSSIF and FeSSIF. It was decided to develop media using these two surfactants in an attempt to match the FaSSIF and FeSSIF derived dissolution profiles. The results are discussed in Section 6.3.5.

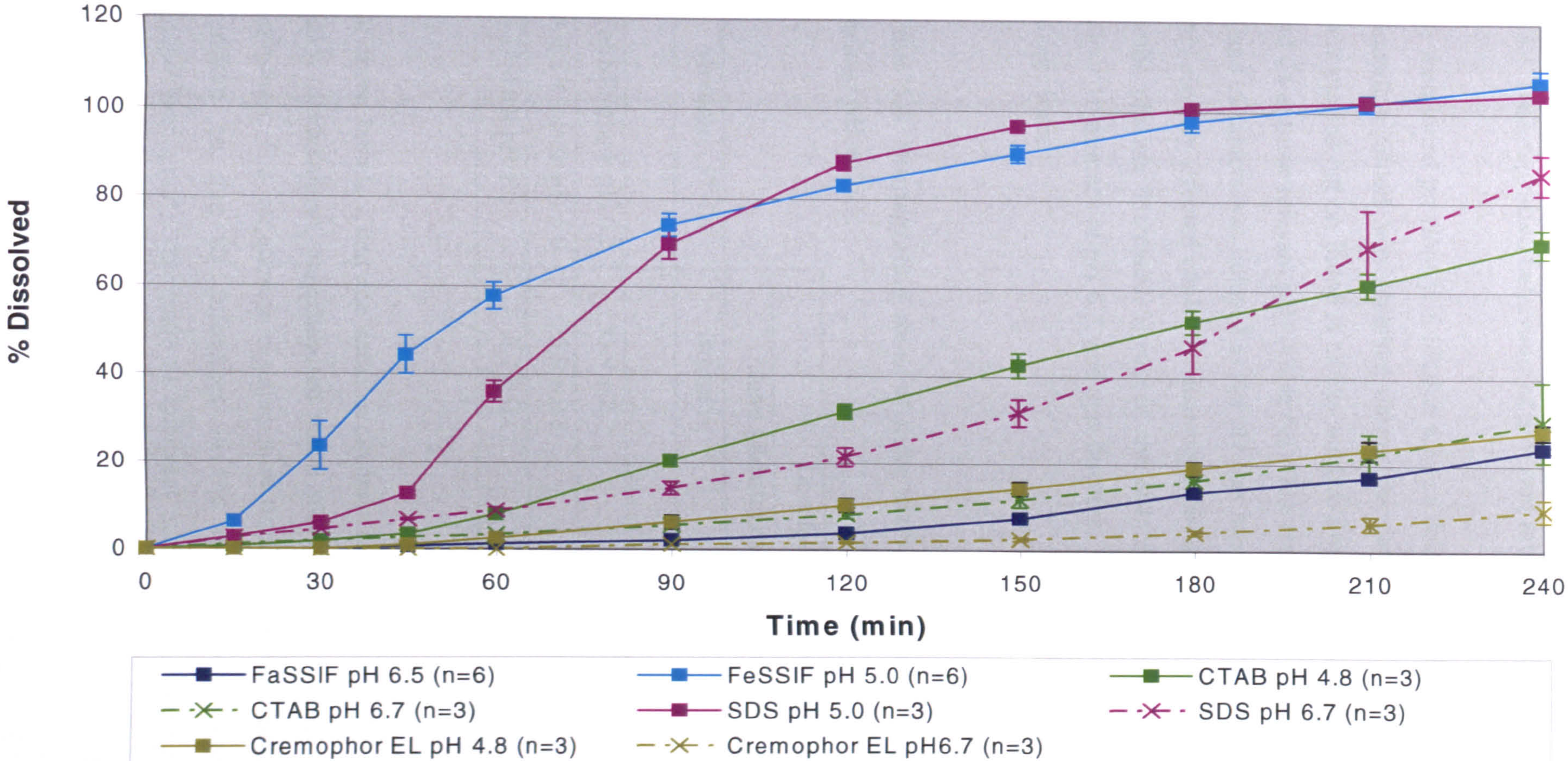


Figure 6.5. The effect of proposed biorelevant media (FaSSIF and FeSSIF) and synthetic surfactant media (CTAB, SDS and Cremophor EL) on the dissolution of loratadine 10 mg tablets (Brand A)

6.3.4. Disintegration behaviour of different tablet brands

As mentioned previously in Section 6.3.2, a delay in the disintegration of Brand A loratadine tablets was observed during the dissolution tests. Disintegration tests were carried out on Brand A tablets and two further brands of loratadine (Brands B and C) at three relevant pHs (Table 6.6). The results show that the disintegration of Brand A tablets was delayed as the pH was increased to 5.0 and 6.5. Brand B was only slightly affected whilst the disintegration time of Brand C was almost doubled as the pH was increased. However, the disintegration time of Brand C was still below 10 minutes at pH 6.5, and was therefore allowable for an immediate release dosage form.

Table 6.6. Disntegration times of three marketed loratadine tablet brands at various pH values.

	Loratadine Tablet Brand		
	Mean Disintegration Time (min) ± S.D. *n=3*		
Medium (37°C)	**Brand A**	**Brand B**	**Brand C**
FaSSIF blank pH 6.5	53.9 ± 2.2	3.3 ± 0.1	6.4 ± 0.9
FeSSIF blank pH 5.0	25.9 ± 0.8	2.0 ± 0.4	6.0 ± 0.5
0.1N HCl pH 1.2	6.2 ± 0.1	1.2 ± 0.1	3.5 ± 0.4

All three brands listed the same excipients: lactose monohydrate (filler), maize starch (disintegrant), pregelatinised maize starch (binder) and magnesium stearate (lubricant). The variation in disintegration time between the three products must therefore be due to differences in the quantity of excipient used (e.g. binder/disintegrant), the grade of excipient and/or manufacturing processes (e.g. mixing time/ drying time).

6.3.5 Dissolution profile matching using loratadine brands B and C

Loratadine dissolution profiles were generated in FaSSIF and FeSSIF using Brand B and C tablets and the profiles compared to those previously obtained for Brand A in Figure 6.6. A pronounced difference in *in vitro* dissolution between the three marketed products was evident for the first hour in FeSSIF and throughout the test in FaSSIF. The data suggest that if FaSSIF and FeSSIF are biorelevant, then dissolution testing in these media during *in vitro* bioequivalence studies would be more discriminating than compendial media towards product performance. Furthermore, bio-data needs to be sought for the three loratadine products from the manufacturers or regulatory authorities to determine whether the three products are in effect bioinequivalent or whether the FaSSIF and FeSSIF media were not biorelevant in this case.

Further dissolution tests were carried out using Brands B and C only. In an attempt to match the FaSSIF and FeSSIF profiles generated for Brand B using CTAB and SDS, the concentrations of CTAB and SDS were adjusted until the best fit was obtained. The matched profiles are illustrated in Figure 6.7 and 6.8 for CTAB and SDS respectively. The cost reductions through the use of the synthetic surfactants are shown in Table 6.7.

Table 6.7. Comparison of the cost of dissolution testing media[1]

FaSSIF		Replacement for FaSSIF	
		CTAB	SDS
		(0.1365 mg/mL)	(0.225 mg/mL)
£85	⇨	**£0.10**	**£0.60**
FeSSIF		**Replacement for FeSSIF**	
		CTAB	SDS
		(1.82 mg/mL)	(1 mg/mL)
£410	⇨	**£1.10**	**£2.60**

[1]Figures for typical test: n=6, dissolution volume 1000ml

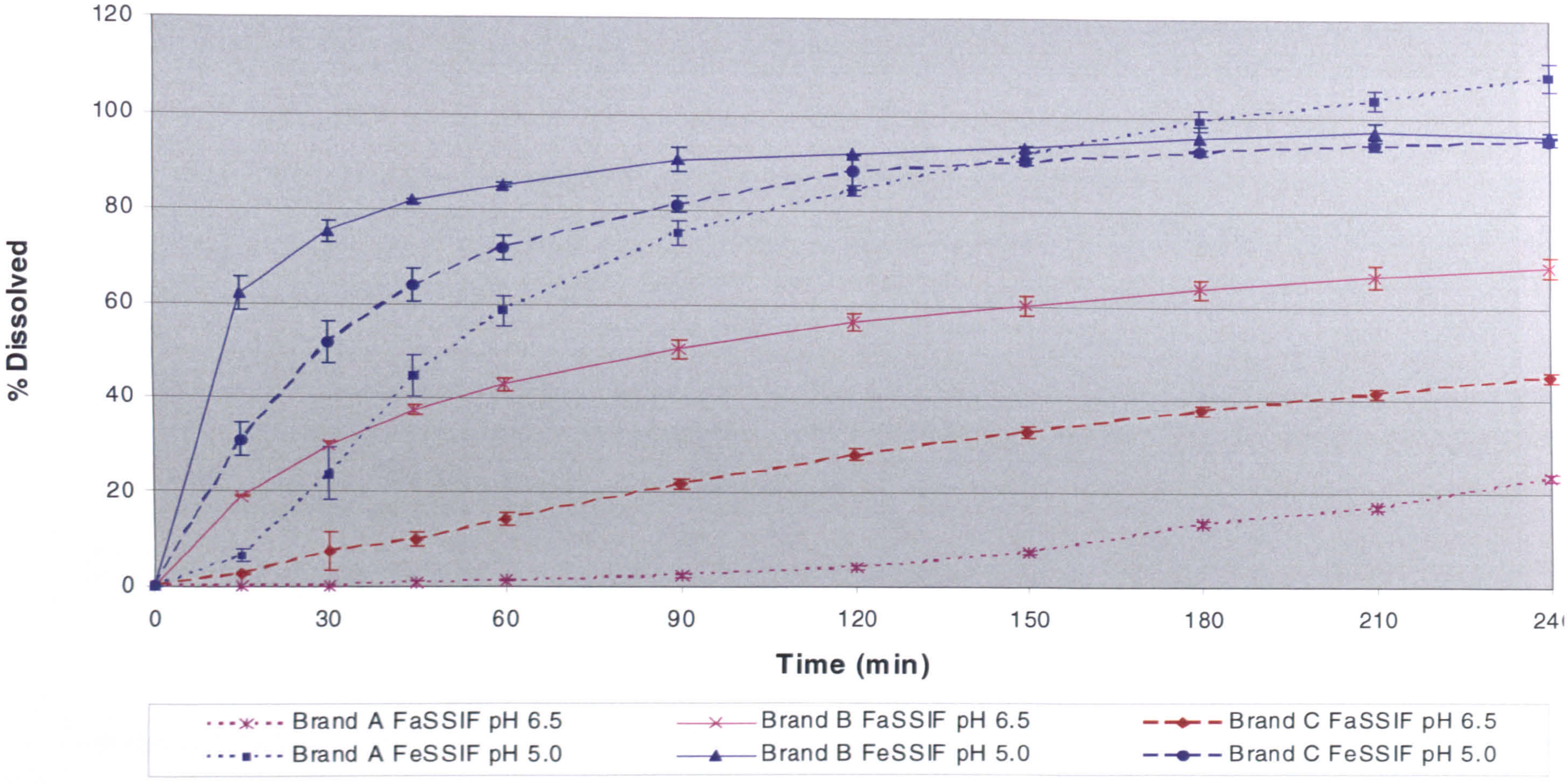


Figure 6.6. Effect of FaSSIF and FeSSIF media on the dissolution of three loratadine 10 mg tablet brands (A, B and C) (*n=3*)

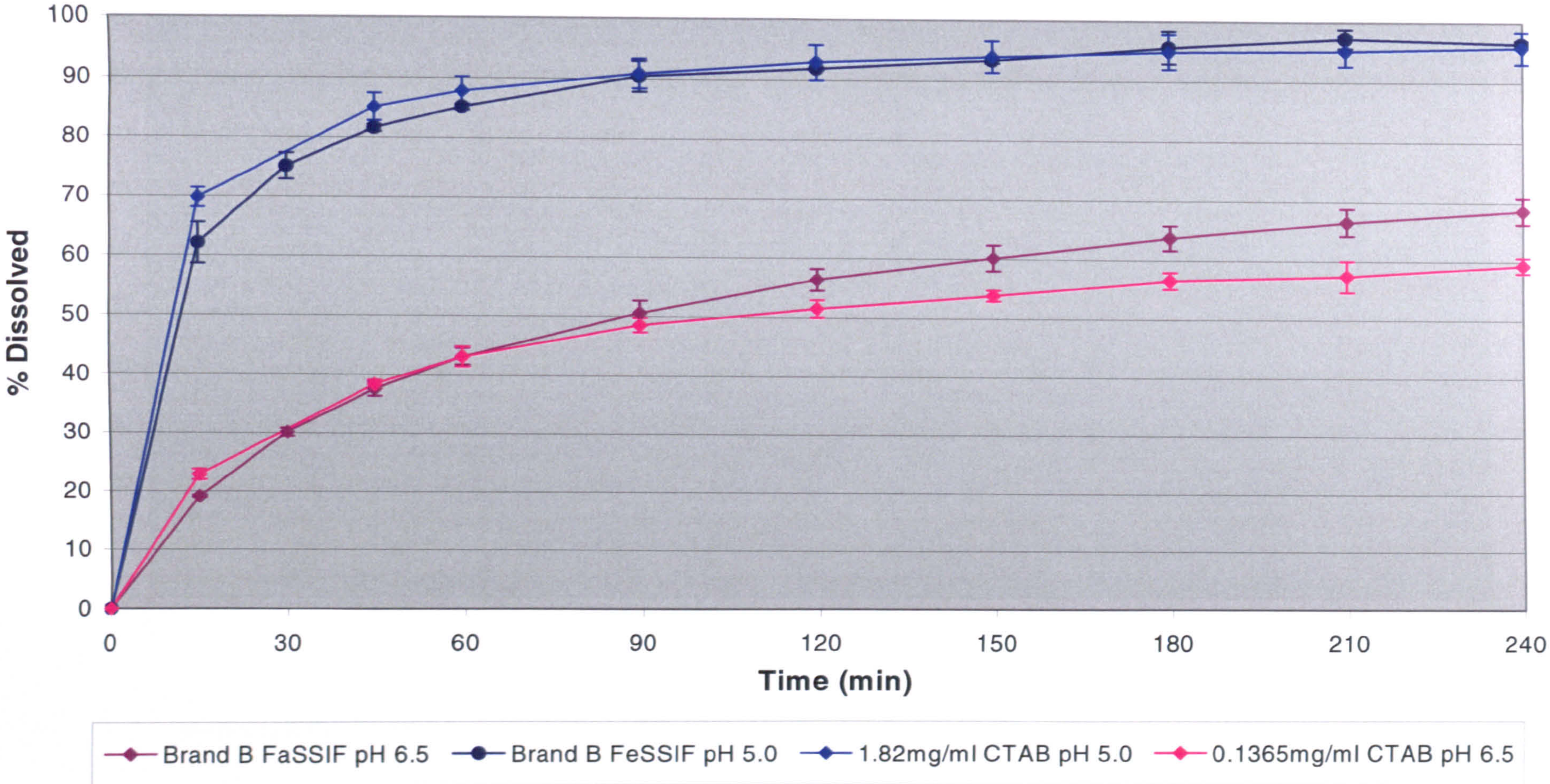


Figure 6.7. Effect of FaSSIF, FeSSIF and CTAB media on the dissolution of Brand B loratadine 10 mg tablets (*n=3*)

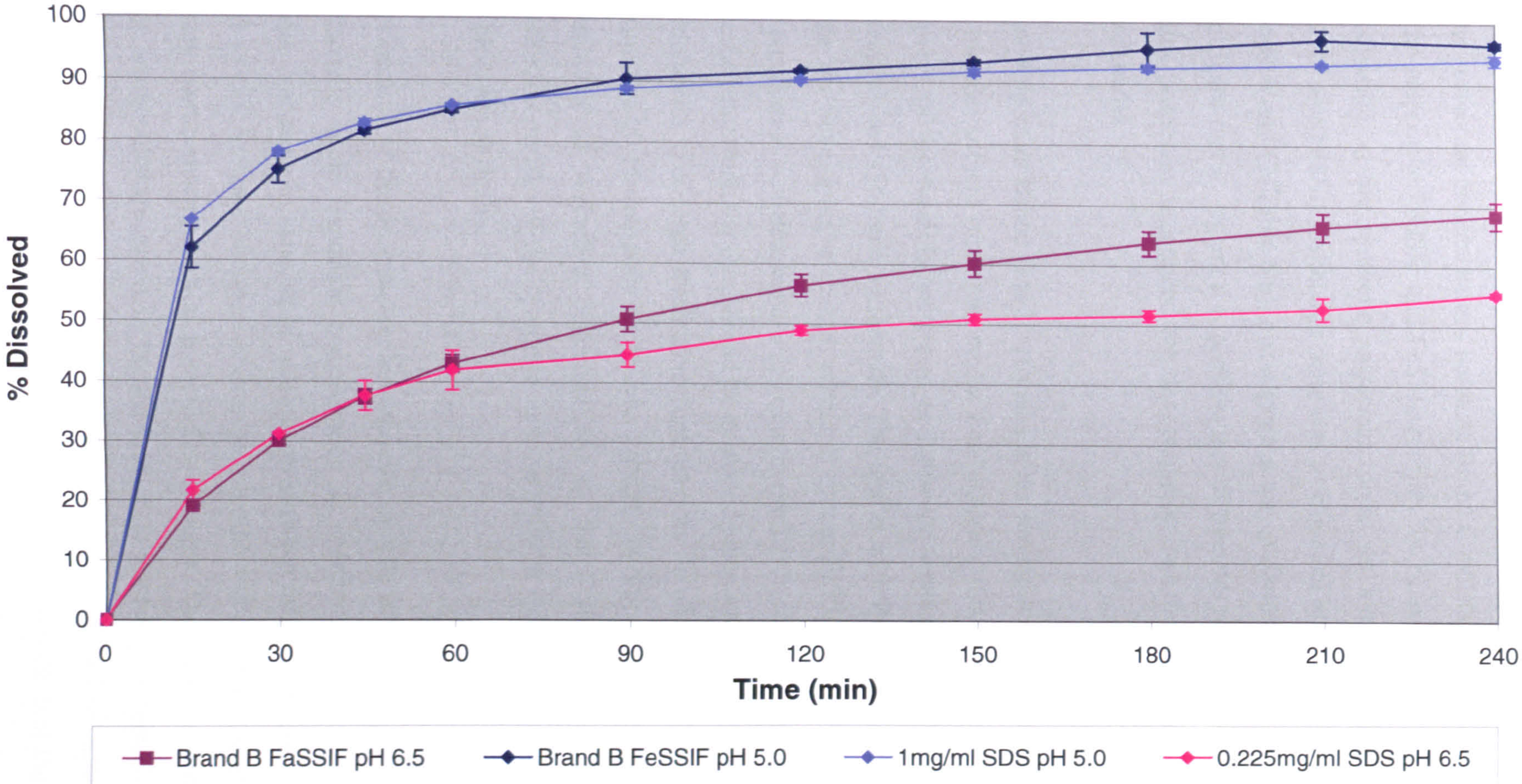


Figure 6.8. Effect of FaSSIF, FeSSIF and SDS media on the dissolution of Brand B loratadine 10 mg tablets (*n=3*)

After finding the CTAB and SDS concentrations required to match loratadine dissolution in the biorelevant media, dissolution tests were repeated using these same surfactant concentrations but with Brand C tablets. The results were compared to the respective FaSSIF and FeSSIF profiles for Brand C in Figures 6.9 and 6.10. Dissolution in the synthetic surfactant media no longer matched dissolution in FaSSIF and FeSSIF. Although there was a difference in disintegration time between brands B and C (Table 6.6), disintegration was sufficiently rapid for this not to be a significant factor. Again, it is possible that differences in the manufacturing formula and/or processes influenced the deaggregation and thus the drug dissolution into these media.

In moving from Brand B to C, changes in the loratadine dissolution profile between the biorelevant and surfactant media were observed that need to be explained. Some property of the formulation seems to influence loratadine solubilisation into both biological and synthetic surfactants. The results with Brand B indicate that there is scope for developing surfactant media to mimic FaSSIF/FeSSIF media. However, the correlation between *in vitro* dissolution profiles in the different media is product specific, therefore, caution should be exercised when investigating "similar" generic formulations (i.e. same qualitative formulae) as dissolution differences have been demonstrated. Formulation or process changes during development would require adjustment of surfactant concentrations to match the affected FaSSIF/FeSSIF dissolution profiles if the synthetic surfactant media were to be used as a surrogate for biorelevant media.

The location of the drug molecule within the micelle of the biological and synthetic surfactants is an important consideration in searching for surrogate dissolution media. Variation in location of solubilisate between different micellar systems may lead to discriminate changes in drug dissolution observed in these media under certain circumstances such as formulation changes. The chemical structures of the ionic surfactants are given in Figure 6.11. It is generally accepted that for ionic micelles the solubilisate would be located in the hydrocarbon core (Florence & Atwood, 1988). A polar solubilisate would be oriented such that the polar groups would be amongst the micellar charged head groups and the non-polar groups would extend into the hydrophobic micellar core. Neutral polyoxyethylated

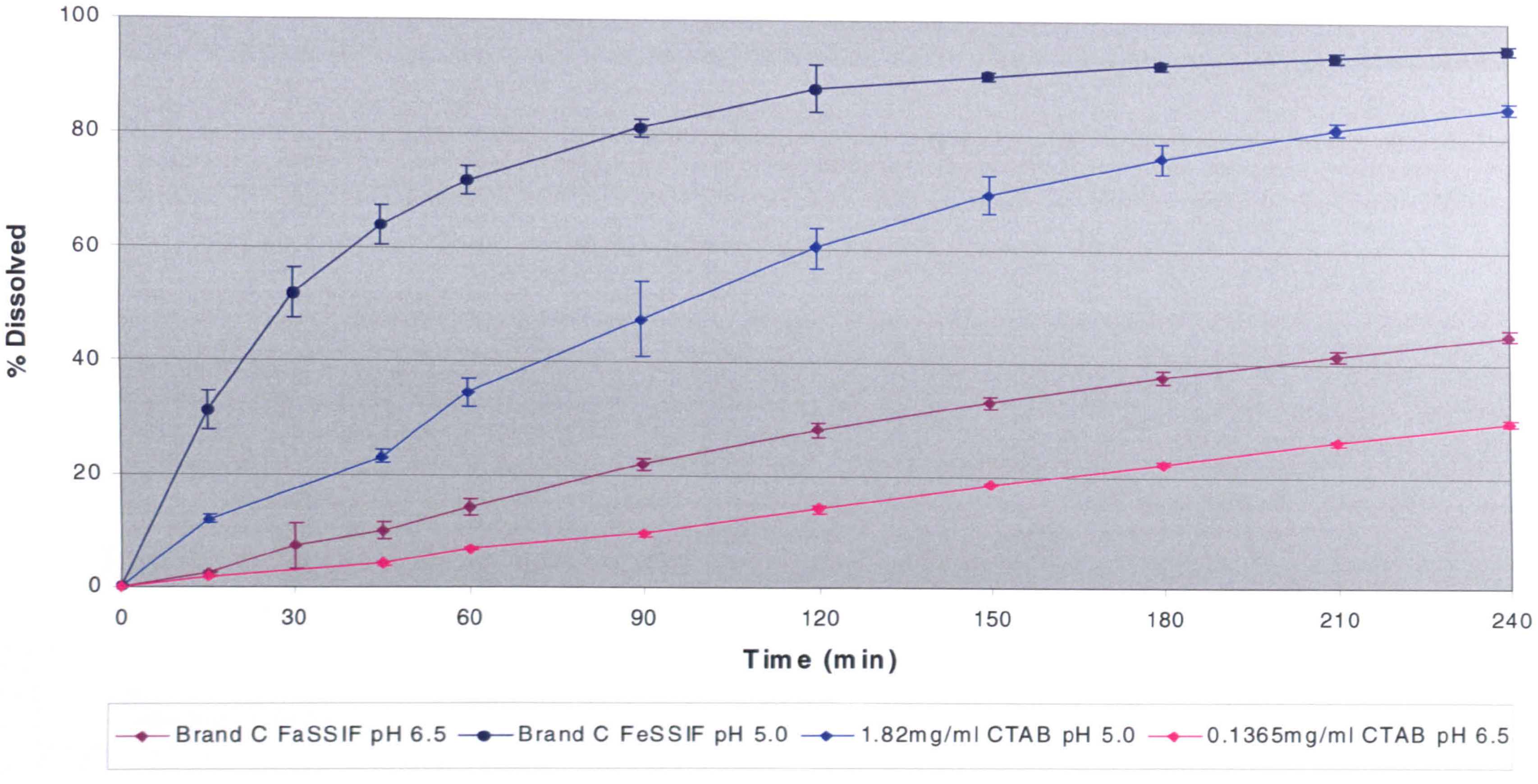


Figure 6.9. Effect of FaSSIF, FeSSIF and CTAB media on the dissolution of Brand C loratadine 10 mg tablets (*n=3*)

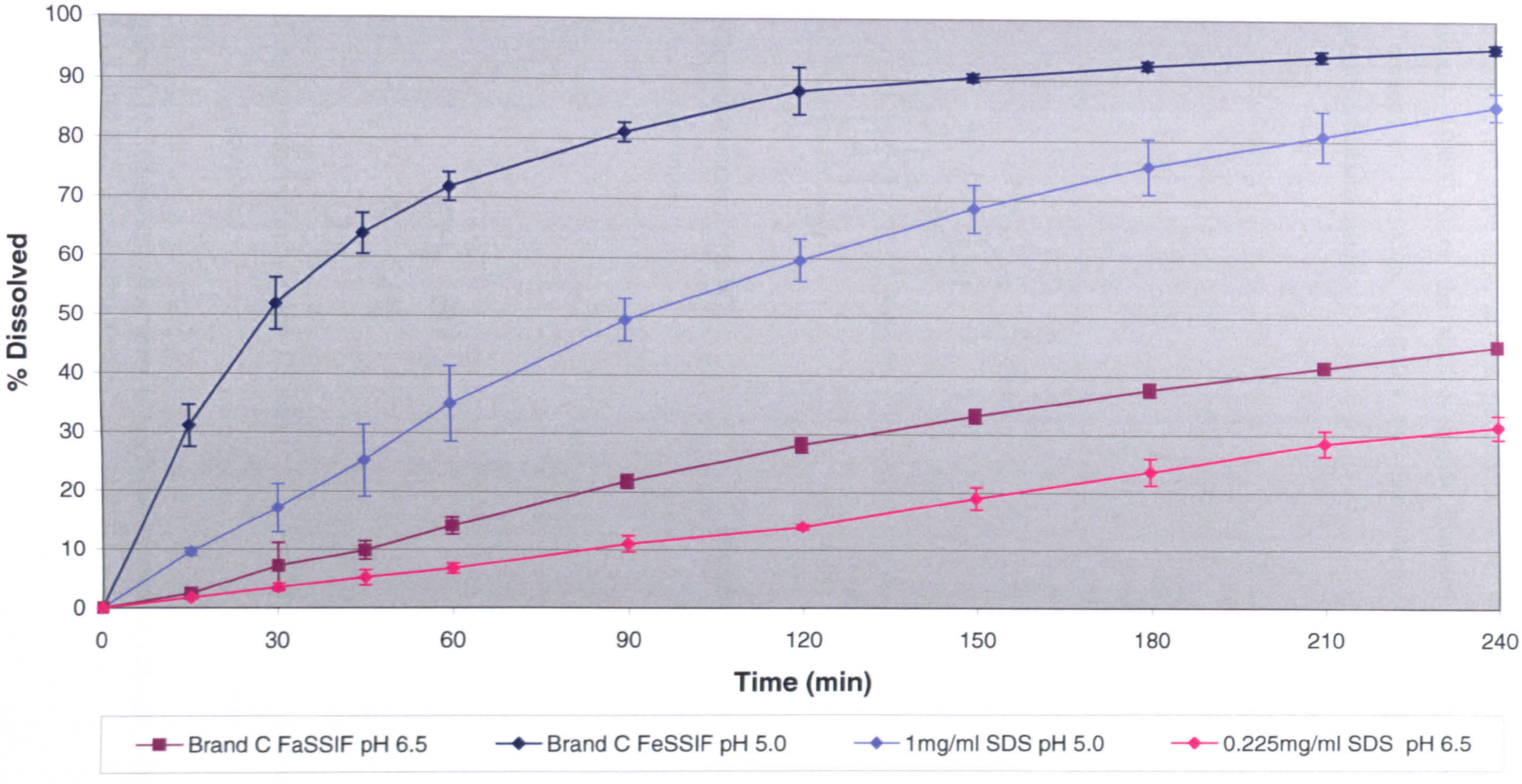
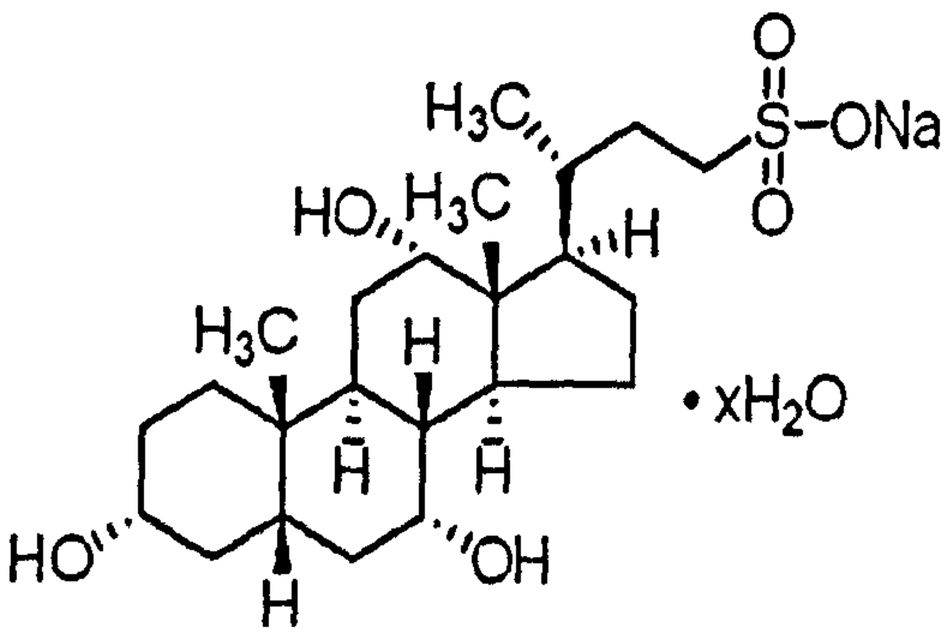


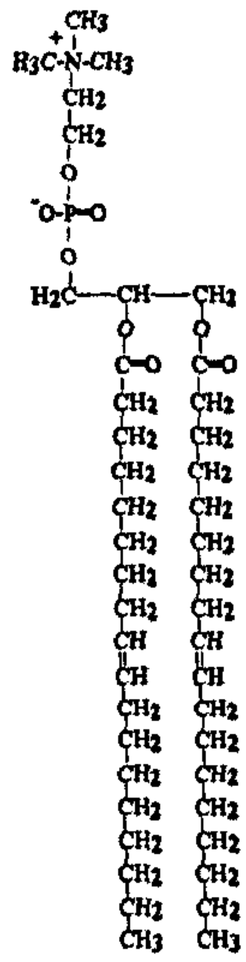
Figure 6.10. Effect of FaSSIF, FeSSIF and SDS media on the dissolution of Brand C loratadine 10 mg tablets (*n=3*)

surfactants (i.e. Brij 35, Cremophor EL and Tween 80) solubilise polar molecules in the polyoxyethylene shell around the micelle rather than in the core (Florence & Atwood, 1988). Since the shell is highly hydrated this may account for the differences in loratadine solubility and dissolution seen between the ionic and neutral surfactants tested.



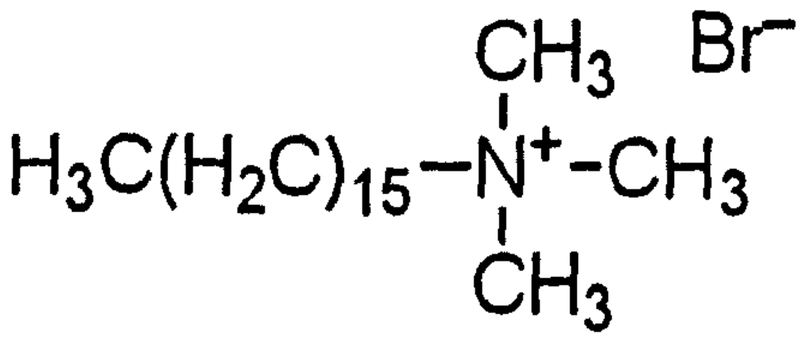
Sodium taurocholate (NaTC)

(anionic)



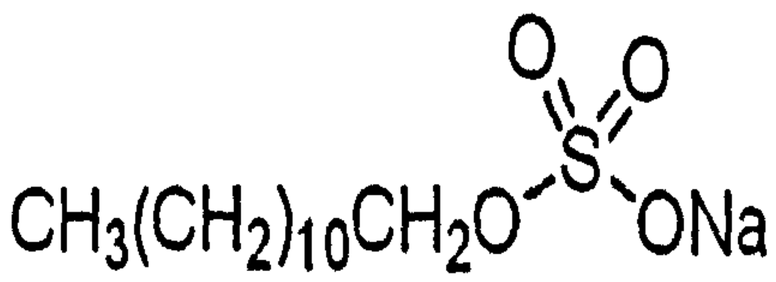
Phosphatidylcholine (Lecithin)

(zwitterionic)



Cetyltrimethylammonium bromide (CTAB)

(cationic)



Sodium dodecyl sulphate (SDS)

(anionic)

Figure 6.11. Chemical structures of surfactants used in FaSSIF, FeSSIF, CTAB and SDS dissolution media.

Confirmation of the inclusion of unionized loratadine within the hydrophobic core of the biological and synthetic surfactants could be attained through studying changes in the size and aggregation number of micelles before and after addition of the drug to the surfactant solutions. Given that a change in the size and shape of micelles results in changes in optical density, light scattering or turbidity measurements can be used to detect drug solubilisation or formation of mixed micelles between bile salt and phospholipids (Andrieux *et al.*, 2004). Micelle size has been measured by static and dynamic light scattering methods (Chaibundit *et al.*, 2002; Andrieux *et al.*, 2004). Aggregation number determinations commonly involve time-resolved fluorescence quenching (TRFQ) studies (Benrraou *et al.*, 2003; Griffiths *et al.*, 2004). Recently, small-angle neutron scattering (SANS) studies using appropriate models, have been used to study the morphology and interaction of aggregates formed in mixed surfactant solutions (Arleth *et al.*, 2003; Griffiths *et al.*, 2004; Penfold *et al.*, 2005).

6.4. Conclusion

The precipitation of a poorly soluble basic drug, loratadine, on transit from the acidic stomach to the intestine of higher pH, has been simply demonstrated by *in vitro* dissolution testing using an *in situ* pH-shift method and compendial apparatus. The addition of the synthetic surfactant SDS, commonly used in dissolution media can mask the precipitation effect.

The neutral and zwitterionic synthetic surfactants investigated were found to have poor solubilising capacities for loratadine and thus were not suitable for replacing NaTC and lecithin. SDS and CTAB were more promising in this respect and for one brand of loratadine tablet, FaSSIF and FeSSIF dissolution profiles were adequately matched by media based on these surfactants. Further work is required to elucidate formulation-related or process-related differences affecting the nature of micelles and drug solubilisation in both biological and synthetic surfactant systems.

7. TRIMETHOPRIM

7.1. Introduction

Trimethoprim (5-[(3,4,5-trimethoxyphenyl)methyl]-2-4-pyrimidinediamine) is an antibacterial drug used primarily in the treatment and prophylaxis of urinary tract infections. Trimethoprim is also used to treat gastro-enteritis, respiratory tract infections and malaria.

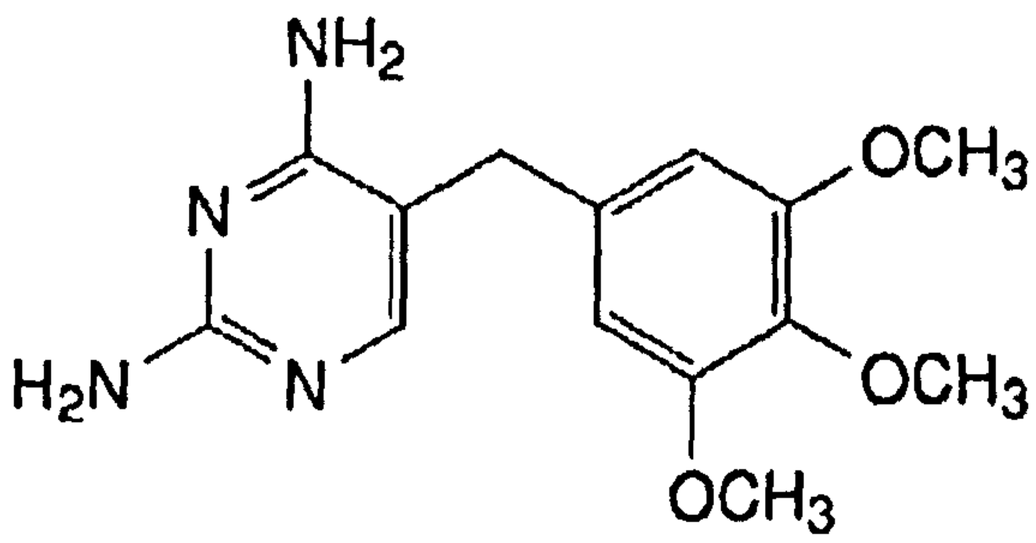


Figure 7.1. The chemical structure of trimethoprim

Trimethoprim is described as a 'very slightly soluble' (1 in 2500 parts water) weak base with a pKa of 7.2 and a Log P value of 0.91 (Clarke, 1986). It has an intrinsic solubility of 0.4 mg/mL at 37°C (Table 7.1) and is completely absorbed from the GIT after an oral dose with peak plasma concentrations being reached between 1-4h after administration (Parfitt, 1999). High drug permeability was maintained in the bio-data of Klepser *et al* (1996) who reported a mean oral bioavailability of 102% compared to a reference intravenous dose. Trimethoprim has been placed in BCS Class II in a classification of the World Health Organisation's Model list of Essential Medicines (Lindenberg *et al.*, 2004).

It was intended to use this drug as a second basic model compound to monitor precipitation on pH shift and dissolution behaviour in biorelevant and synthetic surfactant based media.

7.2. Materials and Methods

7.2.1. Materials

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

7.2.2. Methods

Solubility determinations

See Section 2.2.1.

Dissolution testing

See Section 2.2.3. The volume of dissolution medium used was 1000 mL.

7.3. Results and Discussion

Preliminary trimethoprim dissolution data in simulated intestinal fluid (SIF) pH 6.8 (Figure 7.2) were unexpectedly high. Therefore, the BCS solubility classification of trimethoprim was investigated to address the dissolution results. The findings and their implications are discussed below.

Solubility studies of trimethoprim were carried out at pH 1.2, 5.0, 6.2, 6.8, 7.2, 7.5 and 8.2 in appropriate buffers as per the FDA BCS guidance for determining the drug substance solubility class (CDER, 2000). The results are shown in Table 7.1. According to the guidance, the solubility class is determined by calculating the volume of aqueous medium sufficient to dissolve the highest dose strength (200 mg in this case) in the pH range 1-7.5 (the dose:solubility ratio). For a drug substance

to be classified as 'highly soluble' it must have a dose:solubility ratio ≤ 250 mL over the pH range 1-7.5.

Table 7.1. pH related solubility determination of trimethoprim at 37°C.

Medium	pH	Mean amount dissolved (mg/mL) ± S.D. (*n=3*) 1h	5h	24h	Dose:Solubility ratio (mL)
0.1N HCl	1.2	8.2 ± 0.2	8.5 ± 0.1	6.7 ± 0.3	30
Acetate buffer	5.0	3.7 ± 0.2	3.9 ± 0.01	4.1 ± 0.1	49
Phosphate buffer	6.2	2.6 ± 0.1	2.7 ± 0.02	3.7 ± 0.6	54
Phosphate buffer	6.8	1.6 ± 0.01	1.6 ± 0.003	1.9 ± 0.1	108
Phosphate buffer	7.2	1.0 ± 0.01	1.0 ± 0.02	1.1 ± 0.1	184
Phosphate buffer	7.5	0.8 ± 0.01	0.7 ± 0.01	0.6 ± 0.03	303
Phosphate buffer	8.2	0.5 ± 0.01	0.5 ± 0.02	0.4 ± 0.01	476

From the results it can be seen that trimethoprim exceeds the solubility class boundary at pH 7.5 with a dose:solubility ratio of 303 mL. This explains its 'poor solubility' classification according to the BCS. Consequently, the dissolution of trimethoprim was higher than one would expect for a basic BCS Class II compound at intestinal pH. Approximately 100% of the drug dissolved from a tablet in 15 min and 80% of powdered drug substance (50 μm) dissolved in 1 h in 1000 mL phosphate buffer at pH 6.8 (Figure 7.2). The 115% dissolution at 30 min from the tablets can be explained by the fact that tablet products can contain a maximum of 110% of the stated drug dose and excipient interference in the analytical method. The dissolution volume and pH used are typically used to mimic intestinal conditions.

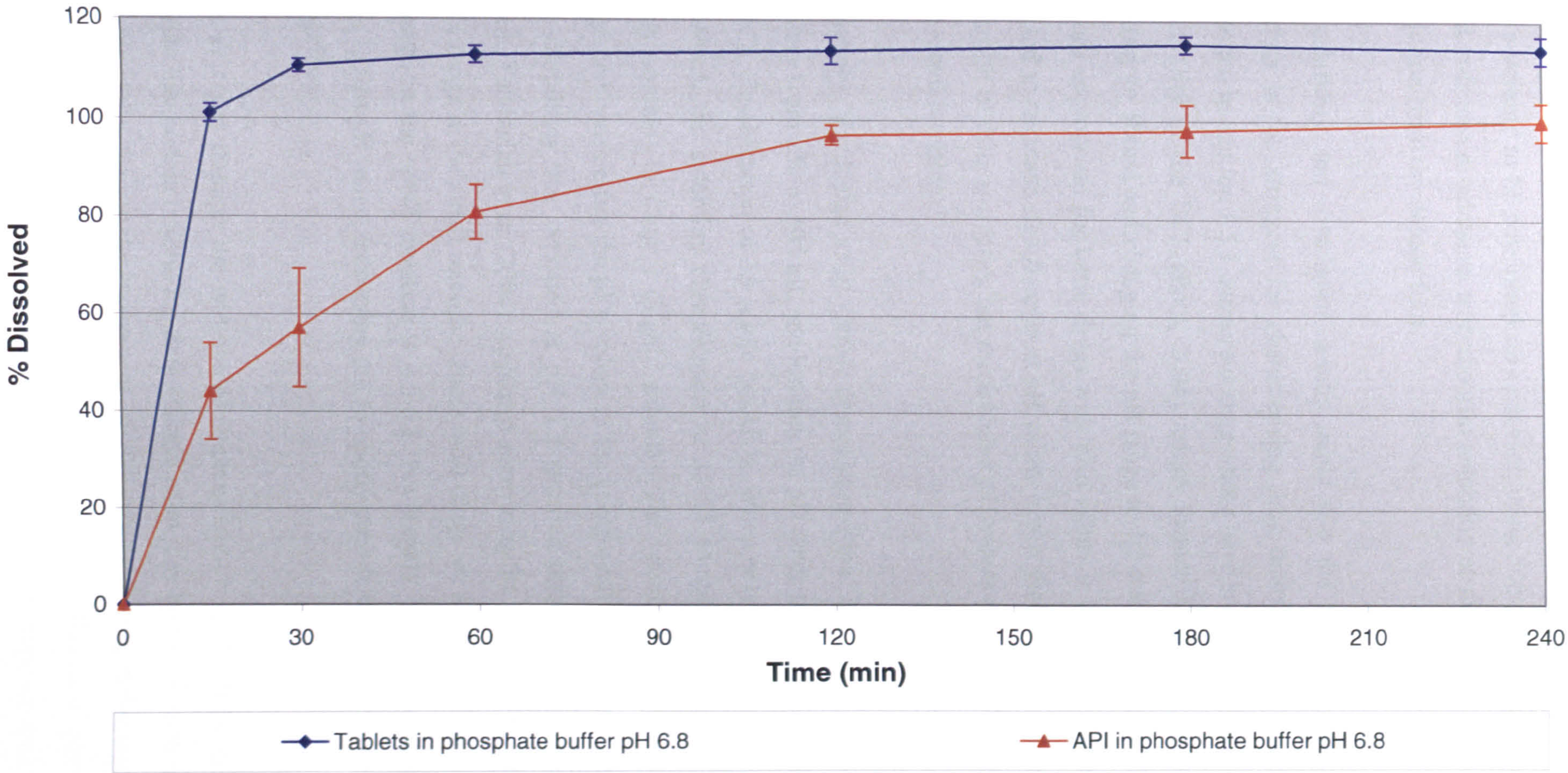


Figure 7.2. Effect of phosphate buffer (pH 6.8) on the dissolution of trimethoprim from a 200mg tablet brand and 200mg of drug substance (API) (*n=6*)

Based on the dose:solubility ratios (Table 7.1), if it was argued that 1000 mL was too large a volume to be physiologically relevant, using 500 mL as the dissolution volume would still ensure adequate trimethoprim solubility at pH 6.8 so as not to limit drug dissolution from the dosage form.

The solubility and dissolution results imply that the BCS solubility classification boundary is too stringent for characterising drug dissolution behaviour. As already mentioned in Section 1.5.1, numerous suggestions have been put forward to improve the applicability of the BCS (Polli, 2004), which include increasing the solubility class boundary to 500ml and changing the pH range to 1.2-6.8. A narrower pH range would be more appropriate for basic drugs with pKa values ≥ 7.0. Drug solubility would be expected to decrease significantly at pH values higher than the pKa when the unionised proportion of drug increases, but these higher pH values are unlikely to be experienced in the small intestine.

Conversely, Yazdanian *et al* (2004) demonstrated that increasing the solubility class boundary from 250 to 500 mL and limiting the pH range to 1.2.-6.8 had little impact on the BCS classification of 15 acidic NSAIDs. When only the solubility at pH 7.4 was considered, the solubility classification of all 15 compounds changed from Class II (poorly soluble) to Class I (highly soluble). The results of these workers were not unexpected considering the pKa values of the compounds studied were ≥ 3.5. The poor drug solubilities that persisted at pH 1.2 required dissolution volumes > 500 mL. Instead, Yazdanian *et al* (2004) suggested narrowing the pH range to 5.0-7.4, which is the pH range in the small intestine where most drugs are absorbed. The widely accepted pH range in the small intestine is 4.5-6.8. For weakly basic compounds having pKa values in this pH range, increasing the solubility class boundary to 500 mL may still be appropriate.

It has emerged through the widespread implementation of the BCS in pharmaceutics research, that certain criteria for drug classification may be too restrictive resulting in a physiologically inaccurate assessment of the predicted drug behaviour *in vivo*. The increasing amount of experimental data to exemplify this and general consensus amongst pharmaceutical scientists lends support for future proposed amendments by the

FDA to extend the applicability of the BCS through scientifically justified changes to the criteria. This will facilitate regulatory implementation of the BCS in the pharmaceutical industry to apply for biowaivers after SUPAC and for new generic products, which at present is not preferred to *in vivo* bioequivalence studies due to lack of certainty in regulatory outcome.

7.4. Conclusion

It was not feasible to continue the study with trimethoprim owing to its high solubility resulting in a low discriminating power for dissolution differences in various media. The classification of trimethoprim as a BCS Class II compound is inappropriate based on its solubility and dissolution behaviour at physiologically relevant pHs. Recognition should be given to this during solubility class determinations of basic drugs with pKa values ≥ 7. Increasing the dose:solubility ratio from 250 mL to 500 mL for the solubility class boundary and/or narrowing the pH range from 1.2-6.8 may provide a more accurate classification for these types of compounds.

8. GENERAL DISCUSSION

8.1. Dissolution testing in physiological conditions

Dissolution testing has received a lot of attention in recent years as its role has shifted from a simple quality control tool to a more predictive test for *in vivo* performance. Drug dissolution from oral dosage forms depends not only on the inherent drug and excipient/formulation properties but also on the physiological conditions in the GIT such as the presence of food, gastric motility, transit time, pH, buffer capacity, osmolality, surface tension, viscosity and liquid volume. These combined physiological factors are assessed during pharmacokinetic studies in fasted and in fed healthy human subjects. However, if an IVIVC could be established between drug dissolution from the dosage form and absorption, it would reduce the size, number and therefore cost of human studies required to select an appropriate candidate drug product through more accurate preclinical *in vitro* dissolution tests. A crucial factor to correctly predict *in vivo* performance using dissolution tests is to employ a physiologically relevant dissolution medium.

Various workers have investigated a range of dissolution media such as milk (Macheras *et al.*, 1986; Macheras *et al.*, 1987; Macheras *et al.*, 1989; Galia *et al.*, 1998), nutritional liquid meals and lipid emulsions (Ashby *et al.*, 1989; Buckton *et al.*, 1989) and fasted and fed state simulated intestinal fluids (Dressman *et al.*, 1998) in an attempt to identify those which were more physiologically relevant. This evolution of biorelevant *in vitro* dissolution testing media was the main driver for the investigations of this research project. The aim was to extend the development of such media by evaluating more fully the variables affecting in vitro dissolution of oral dosage forms such as surface tension, presence of meal (milk), reduced volume and pH. These effects were investigated to evaluate whether they originated from an interaction between the medium components and the drug and/or the formulation excipients.

The need to develop biorelevant dissolution test conditions was further emphasised by the regulatory introduction of the BCS and the biowaver concept for SUPAC and generic products (ANDAs), where *in vitro* dissolution tests could be used as a surrogate for *in vivo* bioequivalence studies for rapidly dissolving Class I drugs. Although the current BCS biowaver criteria require dissolution data in media having pHs 1.2, 4.5 and 6.8, the use of more accurate biorelevant media is a future prospect, particularly if biowavers were to be extended to Class II drugs. In consideration of proposed biorelevant media, the *in vitro* dissolution discriminating ability of FaSSIF for three loratadine tablet products was demonstrated in this work (Figure 6.6), of which the *in vivo* significance requires bio-data.

8.2. Surface tension considerations of dissolution media

A key variable that needs to be measured when comparing different dissolution media is the surface tension. Differences in surface tension between media can potentially be the cause of changes in drug dissolution through altered wetting phenomena and result in inaccurate deductions regarding the effect of media components. Thus the surface tension of the media investigated in this project were measured in Chapter 3 and were borne in mind during interpretations of the data generated.

The surface tensions of FaSSIF and FeSSIF (46-49mN/m) were considerably higher than that of the comparable synthetic surfactant media (27-35mN/m). Except for the CTAB 1.82mg/ml medium, the concentrations of these synthetic surfactant media were below their cmc values. Consequently, drug dissolution into these media was attributed to improved wetting rather than solubilisation as is considered the case in FaSSIF and FeSSIF. This has important implications in trying to find cheaper synthetic alternatives to the proposed biorelevant media containing bile salts and lecithin. If drug dissolution is influenced by a different process (i.e. wetting versus solubilisation) in the replacement media, then the altered media may not accurately represent *in vivo* behaviour or correctly discriminate between differences in product

performance. This issue could possibly be addressed if the synthetic surfactants utilised had similar structures and micelle forming characteristics to NaTC and lecithin.

8.3. Dissolution of ibuprofen and naproxen tablets in milk-based media

The use of milk-based media to investigate the dissolution of poorly soluble acidic drugs (Chapter 4 and 5) revealed some interesting phenomena. The work highlighted that the effect of a milk protein, casein, on the dissolution performance of the ibuprofen and naproxen tablets tested was associated with an interaction with the formulation excipients. For naproxen, enhanced drug solubility in milk media also played a role.

Most excipients for oral dose formulations are characterised according to their applicable physical and chemical properties, stability and incompatibilities in the relevant literature (for example, Rowe *et al.*, 2003). Formulators can also consult the FDA's Inactive Ingredient Database, which lists the maximum amounts of inactive substances permitted for a particular dosage form and route based on FDA approved drug products. However, there is limited data available regarding food-excipient interactions, particularly for excipients typically used in IR oral dosage forms. For this reason, excipients need to be screened not only for toxicity and drug interaction but also for their interaction with major food components (e.g. dietary proteins, lipids and carbohydrates) and physiological secretions (e.g. bile salts, phospholipids and enzymes) if *in vitro* dissolution testing is to be used as a predictive tool for *in vivo* product performance.

8.4. Loratadine dissolution

Anecdotal evidence suggests it is not uncommon for patients to complain about the lack of effectiveness of loratadine tablets purchased over the counter in relieving allergic symptoms. Whether this is a pharmacokinetic or pharmacodynamic issue is a point of debate. The pH-solubility profile for loratadine (Chapter 6) suggests that a raised stomach pH may hinder drug dissolution and absorption. The pH in the stomach may be raised due to individual variation, administration of drugs or the presence of food. In the latter case, drug absorption may be improved by taking loratadine in the fasted state.

At present there is no recommendation to patients to take loratadine on an empty stomach, obviously based on the pharmacokinetic data submitted to regulatory authorities prior to granting a product licence. Nevertheless, Simons (2002) and Khan *et al* (2004) have shown high inter-subject variability during pharmacokinetic studies. Consequently, for a proportion of the population it is possible that loratadine bioavailability will be poor. For poorly soluble basic drugs with rapidly diminishing pH-solubility profiles and a risk of precipitation on intestinal transfer, it may improve the treatment outcome if manufacturers recommended a conservative measure of fasted state dosing.

The differences in the disintegration times and dissolution profiles of the three brands of loratadine tablets (Chapter 6) emphasize that caution should be exercised if using 'similar' generic products for these types of investigations because of the variability in manufacturing formulae and process controls, both of which are not often released by the manufacturer. In such a situation, it can prove difficult to isolate formulation variables responsible for certain effects seen during *in vitro* testing. More insight into formulation based dissolution differences in biorelevant media would be acquired if the dosage form were manufactured in-house. Being able to alter controllably the excipients, concentrations and processes in response to dissolution data would enable the identification of problem excipients that may interact with food and/or physiological components and allow the formulation of more robust products.

8.5. Development of biorelevant intestinal dissolution media

The higher loratadine solubility in FaSSIF and FeSSIF in Chapter 6, compared to the individual component media, illustrated the importance of phospholipids in forming mixed micelles with NaTC and the higher solubilising capacity of these mixed micelles. However, FeSSIF is not truly representative of the fed state environment although it contains physiological concentrations of bile salt and lecithin, which are believed to be the major components affecting drug dissolution through solubilisation. To represent the fed state more accurately, meal components such as digested lipids, proteins and carbohydrates need to be included in FeSSIF.

In addition, it is important to note that lecithin-bile salt mixed micelles are involved in dietary lipid solubilisation. Thus, in the presence of digested lipid in FeSSIF, the proportion of the formed mixed micelles available for drug solubilisation may change. Also, the presence of meal components in FeSSIF may affect the composition, nature and solubilising capacity of the mixed micelles or the kinetics of their formation, resulting in changes in the *in vitro* dissolution profile, which may actually be a closer correlation to the *in vivo* occurrence.

8.6. Solubility categorization of drugs in the BCS

Based on the trimethoprim discussion in Chapter 7 and references therein, the BCS solubility classification criteria should be used with appropriate caution if adopted in the drug development stages so as not to exclude promising candidates erroneously. The BCS was essentially developed as a regulatory tool to aid the review process and accordingly incorporates stringent specifications. Nonetheless, it can be argued that the criteria for placing drugs in a solubility class are not justified for the prediction of *in vivo* performance.

It is well known for ionisable drugs that the pH of the medium and the pKa of the drug will influence solubility. At present the BCS requires solubility data throughout

the pH range 1.2-7.5 and dissolution data at pH values of 1.2, 4.5, and 6.8, regardless of whether the drug is acidic or basic. For biorelevance, increasing the solubility class boundary to 500ml and changing the pH range to 1.2-6.8 may be appropriate for basic drugs. For acidic drugs, increasing the solubility class boundary to 500 ml and changing the pH range to 4.5-6.8 may be appropriate if it can be justified that the drug in question has the most significant absorption in the intestine and dissolution and absorption in the stomach is not critical to the pharmacokinetics. Hence, it is proposed that two separate solubility and dissolution criteria should be applicable in the BCS for acidic and basic ionisable drugs reflecting *in vivo* drug dissolution more realistically.

9. GENERAL CONCLUSIONS AND FUTURE WORK

9.1. General Conclusions

The utilisation of milk-0.1N HCL mixtures as biorelevant gastric dissolution media led to some interesting findings during the solubility, disintegration, dissolution and IDR investigations of ibuprofen and naproxen. This work has identified casein, the major bovine milk protein, as a food component that may increase the dissolution of ibuprofen and naproxen from tablet formulations during residence in a fed state stomach environment. The effect was attributed to an interaction of casein with the formulation excipients, which requires further elucidation. These findings have demonstrated that the use of more complex, alternative dissolution media to represent realistic gut conditions has the potential to reveal changes in product dissolution performance that may occur *in vivo* and alter the previously predicted bioavailibility. Hence the elucidation of interactions between food constituents and formulation excipients will enable the development of more robust oral dose products. Furthermore, the inclusion of meal components into *in vitro* dissolution testing media will provide more accurate dissolution data and improve IVIVCs. The physicochemical properties of the medium such as pH, buffer capacity, osmolality, surface tension and viscosity will also influence drug dissolution from oral dosage forms and must be representative of physiological conditions.

Oral absorption of poorly soluble basic drugs can potentially be problematic if precipitation occurs on transit to the small intestine- a major site for drug absorption. pH-solubility profiles and *in vitro* pH-shift dissolution experiments can be useful to predict such problems if the media used are physiologically representative. At present simple buffer systems are used and more biorelevant conditions need to be applied to account for factors such as *in vivo* solubilisation by bile salts. However, the inclusion of synthetic surfactants in the gastric phase of a pH-shift experiment may affect the results.

Anionic (SDS) and cationic (CTAB) synthetic surfactants were found to be better solubilisers of loratadine than the neutral and zwitterionic surfactants for the future development of more cost effective media to replace the proposed biorelevant media. Loratadine dissolution profiles in FaSSIF and FeSSIF were successfully matched using SDS and CTAB based media for one tablet brand but the correlation did not hold when the tablet brand was replaced. The solubilisation behaviour of FaSSIF, FeSSIF, SDS and CTAB media was highly specific for the product under test suggesting that drug solubilisation was sensitive to formulation factors. Hence, if these surfactant media were to be used to replace biorelevant media, any formulation and process changes would require adjustment of the surfactant concentrations to re-match the dissolution profiles in the biorelevant media.

Whilst FeSSIF is representative of the physiological bile salt and phospholipid concentrations it needs to be developed further to mimic the fed state. In particular, it lacks food constituents that could affect drug dissolution and solubilisation. The addition of monoglycerides, digested proteins, carbohydrates and pancreatin would be appropriate.

The solubility criteria for categorization of drugs into the BCS have received considerable comment in literature and in this project. The FDA is reviewing suggestions from industry and future changes to the classification are imminent.

9.2. Recommendations for Future Work

- The present research highlighted interactions of casein with the excipients used in the ibuprofen and naproxen tablets. These interactions require further investigation through analysis of solid and liquid mixtures of casein with the individual excipients using methods such as DSC and NMR

- Ensure Plus® with 0.45% pectin has been reported to adequately match fed state stomach after administration of the FDA standard breakfast (Klein *et al.*, 2004). It would be useful to investigate excipient and drug interactions with this medium that could affect drug release and dissolution in clinical trial subjects. Taking into account that the FDA breakfast has been criticised for not being representative of the daily breakfast consumed by many populations, the utility of media matched to this breakfast may be compromised. Although milk composition can suffer from natural variability, the general population consumes it whereas only specific patient groups use nutritional liquid meals such as Ensure Plus. It is advocated to develop a biorelevant milk medium by addition of physiological concentrations of pepsin, bile salts refluxed from the intestine and pectin to adjust the viscosity of the medium. Other physicochemical factors such as pH and surface tension should also be considered.

- The pH-shift dissolution experiments for poorly soluble basic drugs carried out thus far usually involve simple buffer media. The use of more biorelevant media in both the gastric and intestinal phases of such a test would provide a more accurate prediction of *in vivo* dissolution behaviour.

- None of the synthetic surfactants commonly used in the pharmaceutical industry have structures similar to those of bile salts. Hence, the nature and extent of interactions between bile salts and drugs/excipients may not be adequately represented by surrogate surfactant media. For this reason, less expensive compounds with similar structures to taurocholate need to be screened for their use in biorelevant media. Furthermore, micelle formation and drug solubilisation phenomena in these physiological and synthetic surfactant environments need to be explored using light scattering methods, NMR, TRFQ and SANS.

- The dissolution data acquired in FeSSIF needs to be compared to dissolution data in a modified FeSSIF medium containing monoglycerides, digested proteins, carbohydrates and pancreatin.

- Overall, the *in vitro* dissolution tests suggested above need to be assessed for their biorelevance through *in vivo* studies where the pharmacokinetic data could be used to derive IVIVCs.

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APPENDICES

Appendix 1: Attendance at Conferences and Seminars

1. **British Pharmaceutical Conference.**
   September 2002. Manchester, U.K.

2. **British Pharmaceutical Conference.**
   September 2003. Harrogate, U.K.

3. **BioInternational 2003: Towards harmonisation in bioavailibility and bioequivalence.**
   October 2003. London, U.K.

4. **Faculty of Science Post graduate Research Seminars and Poster Session.** ***(Chairperson)***
   March 2004. Liverpool John Moores University, U.K.

5. **European Conference on Drug Delivery and Pharmaceutical Technology.**
   May 2004. Seville, Spain.

6. **British Pharmaceutical Conference.**
   September 2004. Manchester, U.K.

7. **Biopharmaceutics Considerations for Oral CR/MR Formulations.**
   February 2005. London, U.K.

8. **AstraZeneca Annual PhD Seminar Session.**
   September 2005. Alderley Edge, U.K.

9. **British Pharmaceutical Conference.**
   September 2005. Manchester, U.K.

Appendix 2: Publications

1. Shah, U.U., Dyas, A.M., Ford, J.L., Kirk, G.G., Sharma-Singh, G. (2004). The effect of milk on the dissolution behaviour of ibuprofen and ibuprofen tablets. *Journal of Pharmacy and Pharmacology* **56 (Suppl.),** S-76.

2. Shah, U.U., Dyas, A.M., Ford, J.L., Kirk, G.G., Sharma-Singh, G. (2005). Determination of the intrinsic dissolution rate of a poorly soluble drug using fluorescence spectroscopy. *Journal of Pharmacy and Pharmacology* **57 (Suppl.),** S-103.

3. Dyas, A.M. and Shah, U.U. (2006) Dissolution and dissolution testing. In *Encyclopaedia of Pharmaceutical Technology.* Taylor and Francis Group, New York. *In press.*

Appendix 3: Poster and Oral Presentations

1. The effect of milk on the dissolution of ibuprofen tablets.
   Shah, U.U., Dyas, A.M., Ford, J.L., Kirk, G.G., Sharma-Singh, G.
   Poster Presentation. European Conference on Drug Delivery and Pharmaceutical Technology. *May 2004, Seville, Spain.*

2. The effect of milk on the dissolution behaviour of ibuprofen and ibuprofen tablets.
   Shah, U.U., Dyas, A.M., Ford, J.L., Kirk, G.G., Sharma-Singh, G.
   Poster Presentation. British Pharmaceutical Conference. *September 2004, Manchester, U.K.*

3. Determination of the intrinsic dissolution rate of a poorly soluble drug using fluorescence spectroscopy.
   Shah, U.U., Dyas, A.M., Ford, J.L., Kirk, G.G., Sharma-Singh, G.
   Poster Presentation. British Pharmaceutical Conference. *September 2005, Manchester, U.K.*

4. Matching the dissolution of poorly soluble basic drugs in biorelevant media using synthetic surfactant media.
   Oral Presentation. AstraZeneca Annual PhD Seminar Session. *September 2005, Alderley Edge, U.K.*