Ibuprofen and its interaction with glucosamine

TING DU

Master of Philosophy

THE UNIVERSITY OF ASTON IN BIRMINGHAM

THE UNIVERSITY OF ASTON IN BIRMINGHAM

THE INTERACTION OF IBUPROFEN AND GLUCOSAMINE

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Summary: Ibuprofen is a non-steroidal anti-inflammatory drug (NSAID) with analgesic, anti-inflammatory and antipyretic actions. In addition, ibuprofen is well absorbed from the gastrointestinal tract following oral or rectal administration. For these reasons, ibuprofen is recommended for the symptomatic relief of certain acute arthritis, nonrheumatic inflammations, fever and dysmenorrehea. Although ibuprofen is well absorbed from the gastrointestinal tract, it has a low aqueous solubility. Recently using surfactants or compounding with another compound has been used to improve the solubility and efficacy of ibuprofen. Glucosamine is effective for relieving the symptoms of osteoarthritis. In this project, 2%Tween80 aqueous solution was used as a surfactant, studies were: determination of the purity, solubility of ibuprofen, and comparisons of the dissolution rate, the diffusion rate, and the binding of ibuprofen in the presence of glucosamine. An HPLC method was established and used throughout to measure ibuprofen and ibuprofen combined with glucosamine at different ratios. Fenoprofen was used as internal standard. This method is simple, sensitive, precise and rapid, and Carbon-14 radio-labeling was also used in the binding study. Glucosamine increased solubility of ibuprofen in 2% Tween80 aqueous solution, and at the ratio between ibuprofen and glucosamine as 1:1, the solubility of ibuprofen in 2% Tween80 reached 2.73g/L. Glucosamine also had a synergetic effect on ibuprofen dissolution and diffusion. However glucosamine had no significant effect on the ibuprofen binding in our studies.

Key Word: Ibuprofen, Glucosamine, Tween80, HPLC, solubility, dissolution, diffusion, binding.

To my family, especially to Mum and Dad

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CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

2-APA	2-arylpropionic acid
AP-1	activator protein-1
ATP	adenosine triphosphate
Ar	aromatic ring
AUF	absorbance units corresponding to full scale deflection on the recorder
COX-1	cyclooxygenase 1
cmc	critical micelle concentration
ECM	extensive extracellular matrix
FGF-2	fibroblast growth factor
GAGs	glycosaminoglycans
GC	gas chromatography
G1cN	glucosamine
G1cAT-I	glucuronosyltransferase I
GFAT	fructose-6-phosphate amidotransferase
GLUT-4	glucose transporter
HLB	hydrophile-lipophile balance
HPLC	high performance liquid chromatography
HSA	human serum albumin
Hsc	haemopoietic stem cell
HSF	heat shock factors
IL-IR1	the IL-1 receptor type 1
LC	liquid chromatography
MAPK	mitogen-activated protein kinase
NIK	NFκB inducing kinase
NSAID	non-steroidal anti-inflammatory drug
NFκB	nuclear factor κB

OA	osteoarthritis
ODS	octadecylsilane
RA	rheumatoid arthritis
ROS	reactive oxygen species
SD	standard deviation of the mean
TRAF-6	tumor necrosis factor receptor molecule-associated factor-6
TGF-α	transforming growth factor-α
TGF-β	transforming growth factor-β
VSMC	vascular smooth muscle cell

CHAPTER ONE

INTRODUCTION

1.1 High Performance Liquid Chromatography (HPLC)

1.1.1 The basic principles of HPLC

High performance liquid chromatography (HPLC) is a technique that has arisen from the application to liquid chromatography (LC) of theories and instrumentation that were originally developed for gas chromatography (GC). It was known from gas chromatographic theory that efficiency could be improved if the particle size of the stationary phase materials used in LC could be reduced. As HPLC has developed, the particle size of the stationary phase materials used in LC has become progressively smaller. The stationary phases used today are called micro-particulate columns or irregular shape, and have nominal diameters of 10, 5 or 3μm. The different separation mechanisms can be realized by the bonding of different chemical groups to the surface of the silica particle, to produce what are called bonded phases. Almost 75% of the work in HPLC at the moment is carried out using a bonded phase in which C-18 alkyl groups are attached to the surface of the silica particles. These types are called ODS (octadecylsilane) bonded phases, and this mode of HPLC is called reversed-phase liquid chromatography[1].

Reversed-phase liquid chromatography is the most popular modes of chromatography because of its high sensitivity, precision, and specificity (selectivity) used in analytical and preparative separation of compounds of interest in the chemical, biological, pharmaceutical and biomedical sciesces[2]. The popularity of reversed-phase liquid chromatography derives, is in part due to the fact that it requires aqueous mobile

phases. Therefore, it is generally compatible with most aqueous samples, which can often be injected directly onto the column without pretreatment. If pretreatment of the samples is required, it is often relatively simple. Another important advantage is that the analysis is conducted under mild conditions, whereby the samples are not subjected to high temperatures[3].

The term reversed-phase liquid chromatography derives from the fact that the mobile phase is more polar than the stationary phase, which is the opposite of normal-phase chromatography. The reversal of the polarities of the mobile phase and the stationary phase compared with normal-phase chromatography also reverses the order of elution of analytes. If one thinks of a reversed phase separation as being a partitioning process in which solutes are distributed between a non-polar stationary phase and a polar mobile phase, then the non-polar solutes will be soluble in stationary phase and will travel through the system more slowly than the polar solutes, which favour the mobile phase. The distribution can be altered by changing the polarity of the mobile phase[4]. A typical reversed-phase system employs a hydrocarbonaeous phase covalently bonded to silica gel and a hydro-organic mobile phase such as a mixture of water and methanol. In addition to silica-based stationary phases, a range of polymeric supports such as polystyrene-divinylbenzene copolymer has been developed for reversed-phase liquid chromatography. The main advantage of the polymeric supports is the lack of residual silicanol groups and the greater chemical stability in acidic and basic solution compared with silica gel. The polymeric supports are chemically stable over the pH

range of 1-13 compared with a pH range of 2.5-7.5 for silica-based supports[5].

1.1.2 Basic Instrumentation of HPLC

An HPLC instrument requires a high pressure pump and a supply of mobile phase, a column containing a high efficiency stationary phase, an injection unit for introducing samples on to the column, an in-line detector and some method of displaying the detector signal. **Fig. 1.1** is a block diagram showing the way in which these different components are arranged to form a high performance liquid chromatography instrument.

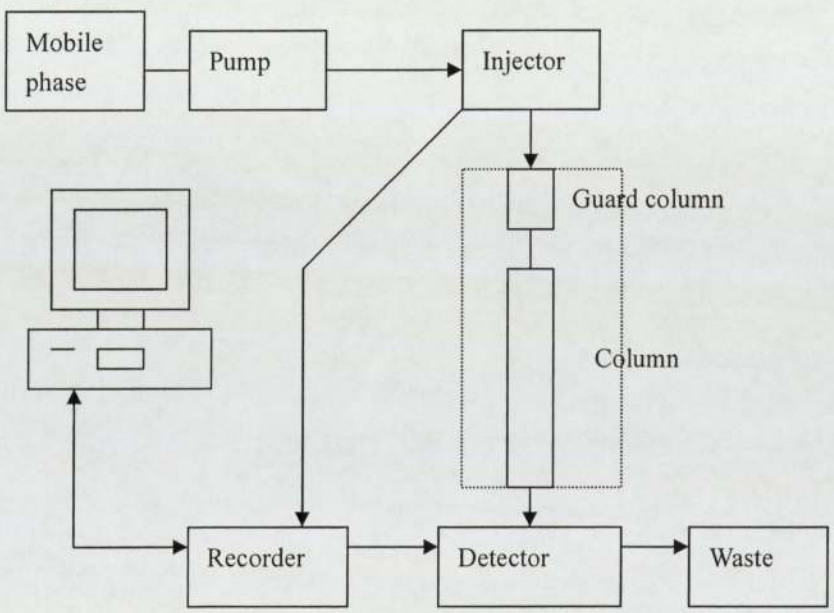


Fig. 1.1 Basic instrumentation of HPLC

1.1.3 The quantitative analysis method in HPLC (Relative assays)

For accurate results, the purity of a compound or the % content of a compound in a product is determined by an assay in which a sample of unknown purity or content is compared against a pure reference standard. The only exception to this is when a compound has just been discovered and no suitable reference standard is yet available.

1.1.3.1 External standard method

In the external standard method, the detector response for pure compounds is found by determining the peak area per unit concentration of pure compound by obtaining chromatograms from injections of solutions of the reference standard at known concentrations and measuring peak areas. This detector response may then be used to calculate how much pure compound is in a solution of a sample by considering the peak area obtained from the injection of a sample solution of known concentration.

The concentration of a sample (Ci) is calculated by using the equation $\mathbf{C_i/A_i=C_s/A_s}$, where A_i stands for the peak area of the sample, C_s is the concentration of the reference standard and A_s stands for the peak area of the reference standard.

The method involves the usual sources of error such as those made in measuring weights and volumes. Two key potential sources of error are those occurring through poor repeatability of injection volumes and through inconsistent loss of sample in any sample preparation step.

1.1.3.2 Internal standard method

The internal standard method is similar to the external standard method in that the solution of the reference standard is compared with solution of the sample. The key difference is that, prior to any sample pre-treatment, all solutions are mixed with the same amount of a compound called the internal standard. For the method to work well, it is important to choose a suitable internal standard. Ideally, an internal standard should have very similar physicochemical properties to those of the analyte, yet not so similar that it cannot be resolved from the analyte in the HPLC chromatographic system. Because the internal standard method eliminates the injection error of standard and samples found in the external standard method, it does not automatically follow that the internal standard method should always be used.

1.2 Ibuprofen

Ibuprofen is a chiral non-steroidal anti-inflammatory drug (NSAID) of the 2-arylpropionic acid (2-APA) class. It was developed as an anti-rheumatic drug in the 1960s and has been widely used as a prescription drug in Great Britain since 1967 and since 1974 in the US. Ibuprofen became available without a prescription in the US and the UK in 1984[6].

1.2.1 Molecular Structure

The empirical formula is $C_{13}H_{18}O_2$ and the structural formula of ibuprofen is shown in **Fig. 1.2**.

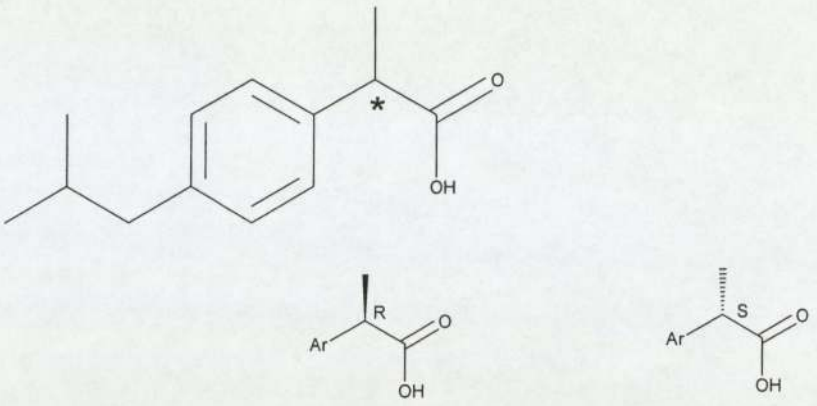


Fig. 1.2 Ibuprofen and its R, S forms (Ar stands for aromatic ring).

It has one asymmetric center. The S-ibuprofen exhibits pharmacological effects but the R-form is inactive. A unidirectional inversion from the R- to S-form occurs during metabolism[7]. This inversion was shown to occur both pre-systemically in the gastrointestinal tract[8] and systemically in circulation[9]. Chiral inversion is a unique metabolic pathway which influences the disposition of ibuprofen and other 2-arylpropionic acid NSAIDs, including fenoprofen and ketoprofen. This pathway is of great interest because in most species it involves a unidirectional conversion of the inactive R-ibuprofen to active S-ibuprofen. The mechanism behind this inversion has been studied in depth. It was proposed that the inversion process was linked to enzymes involved in lipid metabolism, and required the substrates Co-enzyme A (CoA) and ATP for initiation. The liver mitochondria were also required in the first step of the chiral inversion[10], the formation of an ibuprofenyl-adenylate complex.

1.2.2 Physical Properties

Ibuprofen is a white or almost white, powder or crystals, with a melting point of

75-77.5°C, a characteristic odour and a slight taste and a molecular weight of 206.3. It is practically insoluble in water (approximately 0.004% m/v), soluble 1 in 1.5 of alcohol, 1 in 1 of chloroform, 1 in 2 ether and 1 in 1.5 acetone; it is soluble in alkaline hydroxide and bicarbonate solution[11]. It is freely soluble in dichloromethane. Ibuprofen is a weak organic acid with measured partition coefficient of between 52.0-20.8 over the pH range 1.2-7.5[12] and a pKa between 4.4-5.3[13]. A profile of ibuprofen solubility in water versus pH for the pH range 1.2 to 7.5 has been presented by Herzfeld and Kümmel.[14] Solubility increased rapidly at pH values higher than the pKa. The approximate solubility at pH4, pH6, and pH7 were given as 1 in 35000, 1 in 1900, and 1 in 410 respectively[14] in aqueous buffer solution.

1.2.3 Pharmacological Effect of Ibuprofen

1.2.3.1 Basic Pharmacology of Ibuprofen

Ibuprofen is widely used as one of the best tolerated non-steroidal anti-inflammatory drugs available for the treatment of rheumatoid arthritis, osteoarthritis, and mild to moderate pains. At doses of 0.6-1.2g per day, ibuprofen is well tolerated for the treatment of fever pain, migraine and dysmenorrhoea. At the doses between 1.6 to 2.4g per day, ibuprofen is effective for the treatment of chronic painful arthritic conditions. In contrast to aspirin, ibuprofen only has weak antithrombotic activity[15]. It does not exhibit any anticoagulant effect and also it is a weak inhibitor of platelet aggregation in humans[16]. The prolonged, irreversible inhibition of platelet cyclooxygenase I (COX-1) results in sustained inhibition of thromboxane production

observed with aspirin[17]. This difference is partly responsible for the relatively low incidence of gastrointestinal bleeding and ulceration observed with ibuprofen in comparison with aspirin. The major actions of ibuprofen centre on the effects of S-enantiomer in inhibiting cyclooxygenase (COX). In inflammation and in the spinal pathway mediating pain, the effect of the drug is to inhibit the production of prostaglandins by blocking the activity of the inducible COX-2 isoenzyme. Ibuprofen is also involved in leukocyte accumulation and activation, both R-and S-isomer exhibit their effects in this case. Ibuprofen has a better safety profile than any other NSAIDs, this is because ibuprofen has relative low gastrointestinal (GI) ulcerogenicity and renal toxicity. The pharmacokinetics of ibuprofen also account for this higher safety. The mean value of peak serum concentration (C_{max}) for S- and R-ibuprofen are 17.5 and 16.3 μg/ml respectively and the (C_{max}) of both enantiomers are achieved within 3.0h.[18] Compared to aspirin, ibuprofen is 16-32 times more potent in the UV erthema assay[19], and 10 times more potent in treatment of carrageenan oedema.[20]

1.2.3.2 Other Actions of Ibuprofen

As described in section 1.2.3.1, NSAIDs are a class of pharmacological agents that are traditionally used for their anti-cyclooxygenase properties in the treatment of inflammation and other associated illnesses. However, recently, it has been reported that some NSAIDs, including ibuprofen, exhibit anti-inflammatory as well as anti-proliferative effects independent of cyclooxygenase activity. NSAIDs affect

multiple intracellular signalling pathways that: (1) inhibit the induction of NF-κB[21]; (2) induce injury-response genes in plants;[22] and (3) sensitize tumours to the cytotoxicity of ionising radiation[23]. Interestingly, the concentrations of NSAIDs needed to initiate these processes are similar and much greater than those required for the inhibition of cyclooxygenase activity, which suggests that it is a unique cyclooxygenase-independent pharmaco-dynamic effect of NSAIDs on these processes. The mechanisms of these actions remains unknown, however, since NSAIDs induce some aspect of the heat shock response, a hypothesis was given that they may also induce Hsc70 nuclear translocation.[24] Hsc70 is a cytoplasmic chaperone protein involved in folding and trafficking of client proteins to different subcellular compartments and plays an important role in signalling transduction and apoptosis processes, and translocates to the nucleus following exposure to heat shock.

The cellular stress induced by exposure to elevated temperature, known as hyperthermia, also has profound effects on many aspects of cellular biochemistry, morphology and physiology. This effect is called heat shock that is primarily mediated at the signal transduction level by pre-existing transcriptional activators known as heat shock factors (HSFs) [25]. Similar to thermal stress, some NSAIDs also initiate some aspects of the HSF-1 DNA binding complex in certain mammalian cell types[26]. Moreover, pre-treatment with NSAIDs such as indomethacin decreases the threshold thermal dose to induce HSF-1 activation as well as hyperthermic radiosensitization, which suggests the overlap between heat shock and exposure to high NSAIDs

concentrations.[27] Interestingly, the fact that many of the intracellular processes influenced by the Hsc70, a known protein regulating the heat shock, are also altered by exposure to high concentrations of particular NSAIDs increases the possibility that Hsc70 may also be a potential target for the cyclooxygenase-independent effects of some NSAIDs.

1.2.3.3 Possible protective role of ibuprofen in age-related diseases

There is growing evidence that ibuprofen and its metabolites may play a protective role in the aging and age-related diseases such as cataract formation[28] and Alzheimer's disease.[29] Epidemiological studies established an association between ibuprofen and reduced risk of cataracts. In addition, ibuprofen showed protective effects to diabetic rats against cataractogenesis[30], and was shown to prevent glycation[31] and carbamylation of lens proteins *in vitro*. The mode of action of ibuprofen and the possible involvement of its anti-inflammatory activities is yet not known. However, it has to been emphasised that cataract formation is a multifactorial disease and the therapeutic effects may be brought about in several ways, including the protection of lens enzymes. Ibuprofen and its metabolites may play a role in its anti-cataract effect by protecting lenticular enzymes such as catalase and fumarase. It is reported that ibuprofen, or its metabolites, protected catalase against fructose-, cyanate-, and prednisolone induced inactivation, while a low dose ibuprofen or its metabolites protected fumarase against fructose- and cyanate-induced inactivation by up to 26%, but had no effect on prednisolone-induced inactivation[32]. Two main metabolites of

ibuprofen are shown in **Fig. 1.3**

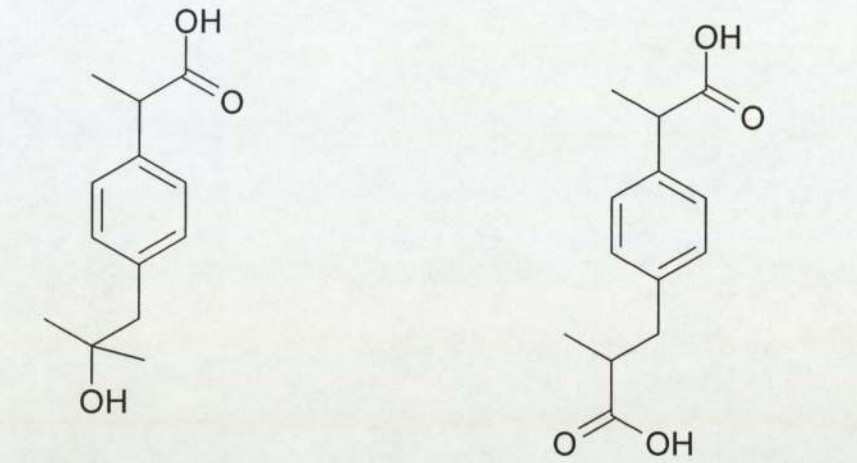


Fig. 1.3 Structures of ibuprofen metabolites.

1.3 Surfactants in pharmaceutical applications

Some compounds, because of their chemical structure, have the ability to accumulate at the boundary between two phases. Such compounds are termed amphiphiles, surface-active agents, or surfactants. Adsorption of these kinds of surfactants results in the changes in the nature of the surface, which is of great importance in pharmaceutical practice. For example, surfactants can: lower the interfacial tension between water and oil to facilitate emulsion formation; stabilize a suspension by adsorbing on the insoluble particle and more importantly, form micelles to increase the solubility of insoluble compounds.

Surface-active compounds are characterised by the possession of two distinct regions

in their chemical structures, termed hydrophilic and hydrophobic regions. The hydrophobic regions are usually saturated or unsaturated hydrocarbon chains or, less commonly, heterocyclic or aromatic ring systems. The hydrophilic regions can be anionic, cationic or non-ionic. The surfactants are generally classified by their hydrophilic region.

1.3.1 Classification of surfactants

There are four main categories of surfactants, depending on their ionisation in aqueous solutions: anionic, cationic, non-ionic and amphoteric. Anionic surfactants dissociate in water to form negatively charged anions that are responsible for the surface-active property. This class of surfactants are widely used because their low price, but because of their toxicity are only used for externally applied preparations. This category contains soaps of alkali metals, ammonium salts, amine soaps and sulphate and sulphonated compounds. Cationic surfactants form a positive charge in aqueous solution and are widely used for their disinfectant and preservative effects. Because of toxicity, cationic surfactants are also limited to external use only. Non-ionic surfactants are particularly widely used due to their low toxicity and low irritancy; some can therefore be used for oral and parenteral administration. They also have a greater degree of compatibility with other materials than the anionic or cationic surfactants, and are less sensitive to pH changes and addition of electrolytes. The non-ionic surfactant could be glycol and glycol esters; sorbitan esters; fatty alcohol polyglycol esters; fatty acid polyglycol esters; poloxalkols; higher fatty alcohols and

the non-ionic surfactants used in these experiments, polysorbates. The structure of polysorbates is given in **Fig. 1.4,** where R represents a fatty acid chain.

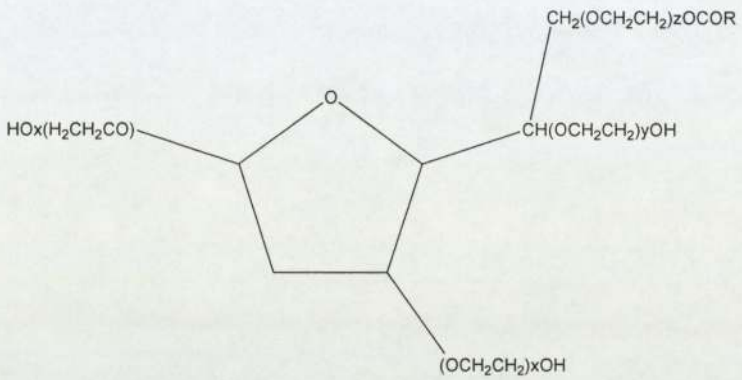


Fig. 1.4 Structure of Polysorbates.

1.3.2 Micellisation and Solubilisation

The surface tension of a surfactant solution decreases progressively with increasing concentration as more surfactant molecules enter the surface or interfacial layer. However, at a certain concentration this layer becomes saturated and an alternative means of shielding the hydrophobic group of the surfactant from the aqueous environment occurs through the formation of aggregates (usually spherical) of colloidal dimensions, called micelles. The concentration which micelles first form at in solution is termed the critical micelle concentration (cmc).

The basic reason for micelle formation is the attainment of a state of minimum free energy. At low concentrations, surfactants can achieve enough decrease in the over-all

free energy by accumulating at the surface of the interface. In this way, the hydrophobic region is removed from the aqueous environment, but when the concentration is increased, this method of free energy reduction becomes inadequate and the monomers forms micelles to shield the hydrophobic groups from the water. Two classes of micelles can be formed depending on the type of surfactants. Ionic micelles are formed by ionic surfactants, which have the spherical or near spherical shape at concentrations close to the cmc. The hydrophobic part of the amphiphile is located in the core and around this core is a concentric shell of hydrophilic head groups together with (1-a) *N* counterions (a stands for the degree of ionisation), where for most ionic micelles is between 0.2 and 0.3. That is, 70-80% of the counterions may be considered to be bound to the micelles. The structure of ionic micelles is shown in **Fig. 1.5**.

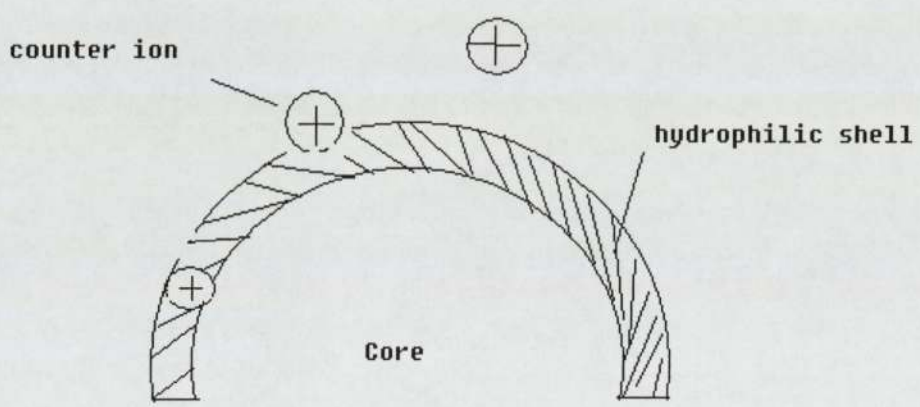


Fig. 1.5 Ionic micelles and counterions

The non-ionic micelles formed by non-ionic surfactants are generally larger than their ionic counterparts. The reason for this is clearly attributable to the removal of

electrical work which must be done when a monomer of an ionic surfactant is added to existing charged micelles. As a consequence of the larger size, the non-ionic micelles are normally not symmetrical. Non-ionic micelles have a hydrophobic core surrounded by a shell of oxyethylene chains which is often termed the palisade layer. Besides the water molecules which are hydrogen bonded to the oxyethylene chains, this layer is also capable of mechanically entrapping a considerable number of water molecules. Non-ionic micelles are therefore, as a consequence, highly hydrated. The structure of a non-ionic micelle is shown in **Fig. 1.6 (c)**.

As previously outlined, formation of the micelles can help bring water-insoluble substances into solution. This process is termed solubilisation. The site of solubilisation within the ionic micelles is closely related to the chemical nature of solubilisate. It is generally accepted that non-polar solubilisates are dissolved in the hydrocarbon core; water-insoluble compounds containing polar groups are orientated with the polar group at the surface of the ionic micelle among the micellar charged head groups, and the hydrophobic part is hidden inside the micelle core. It is suggested that some compounds are absorbed on the surface of the micelle. Solubilisation in non-ionic micelles can occur in the palisade layer, the polyoxyethylated shell surrounding the core. The solubilisation sites in both ionic and non-ionic micelles are shown in **Fig. 1.6.**

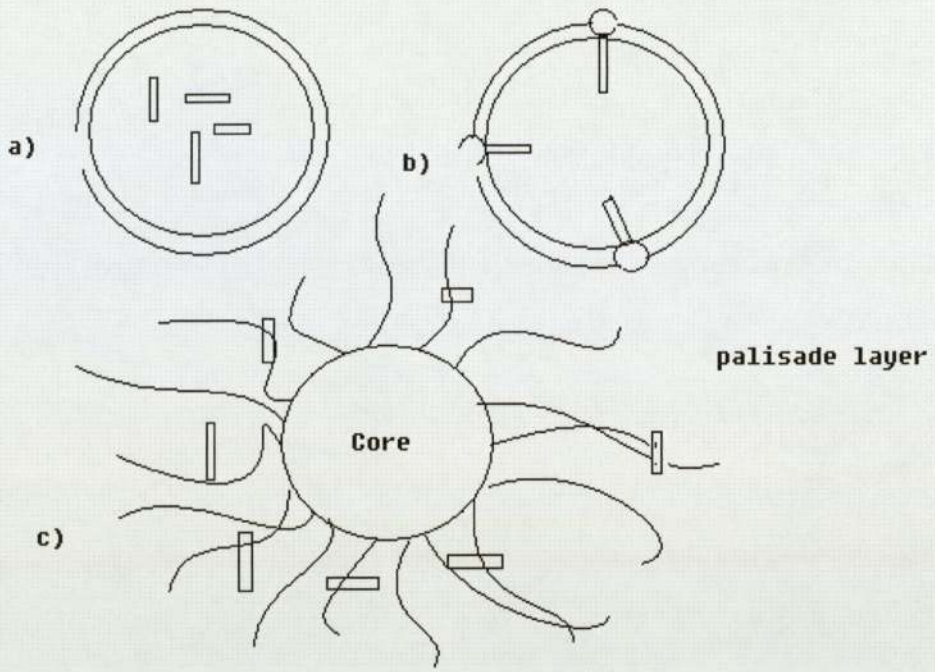


Fig. 1.6: Non-ionic micelles: a) Non-polar solubilisate in the core; b) Water insoluble compounds containing a polar group were oriented in the micelle; c) solubilisate in the palisade layer

In our experiments, the property of solubilisation was used to bring poorly water soluble ibuprofen into aqueous solution. The ideal surfactants in this case need to have high HLB value (hydrophile-lipophile balance) and a relatively low cmc. The higher the HLB number, the more hydrophilic the surfactant, which means better solubility in aqueous solution. A low cmc value makes sure only small amount of surfactants are used in the solvent system which in turn minimizes the toxicity caused by the surfactants. Importantly, in order to reduce the toxicity, only non-ionic surfactants are used in this experiment. A list of common commercially available non-ionic surfactants is shown in **Table 1**.

Table 1.1 HLB and cmc values of some non-ionic surfactants[33].

Commercial name	HLB	cmc
Tween 20	16.7	0.06
Tween 40	15.6	0.031
Tween 60	14.9	0.028
Tween 80	**15.0**	**0.014**
Span(20-85)	Less than 10	----------

According to the table above, Tween80, which has a quite high HLB number and lowest cmc, was used in our experiments.

1.4 Glucosamine

Glucosamine (G1cN) is an important intermediate in the hexosamine biosynthesis pathway. It can be converted into uridine diphosphate-N-acetyl-glucosamines which is used for the O-linked glycosylation of several important proteins, including RNA polymerase, transcription factors and nuclear pore proteins.[34] Glucosamine is also suggested to play a role in leading to the insulin resistance.[35] Incubation of kidney cells with glucosamine produces a transcriptional upregulation of transforming growth factor β, which suggests a possible involvement of the hexosamine pathway in diabetic nephropathy.[36] G1cN also introduces platelet-derived growth factor activated DNA synthesis in rat aortic smooth muscle cells, suggesting that it may be involved in

the development of atherosclerosis.[37] GlcN is important in the metabolism of all glycoproteins including those in the cartilage, where it is required for the formation of the glycosaminoglycans. In associated with collagen fibers, these molecules are responsible for the resilience of the cartilage to deformation. In addition to its effect on cartilage metabolism, glucosamine has been reported to have anti-inflammatory activity in rat models of inflammation, in kaolin arthritis and in adjuvant arthritis.[38]

1.4.1 Glucosamine and Osteoarthritis

Arthritis can be differentiated into rheumatoid arthritis (RA) and osteoarthritis (OA). RA is a systemic inflammatory disease while OA is a degenerative joint disease. The common consequences of both diseases are destruction of articular cartilage leading to bone deformity and loss of joint function. Articular cartilage is a hydrated (75-80% water by weight) extensive extracellular matrix comprising a small number of chondrocytes (less than 5%), type II collagen and large aggregates of proteoglycans or aggrecans (20-25%).[39] Chemically, glucosamine provides the building block for the O-linked and N-linked glycosaminoglycans (GAGs). Once glucosamine is taken up into the chondrocytes of cartilage, it is incorporated into GAGs. Proteoglycans, made up of 95 percent of polysaccharides and 5 percent GAGs, are then secreted into the extensive extracellular matrix (ECM) and bind cations and water to form a viscous, elastic matrix which helps to lubricate the joint where artilage is found. Decreased synthesis or increased degradation of this matrix will cause articular damage or OA.

Osteoarthritis is a major cause of disability and is among the most frequent forms of musculoskeletal disorders. Traditional treatment is accomplished by the use of non-steroidal anti-inflammatory drugs. However, NSAIDs provide symptomatic relief but fail to modify the degenerative processes underlying the damage occurring in the joint space between the bones. In long-term treatment, they could even worsen the progress. A growing number of studies in humans and animals demonstrate that glucosamine may slow the progress of and even reverse some structural abnormalities associated with arthritis.[40] Glucosamine was reported to be able to reverse the decrease in proteoglycan synthesis induced by IL-1β in rat chondrocytes and increase proteoglycan synthesis dose-dependently in human chondrocytes derived from osteoarthritic cartilage.[41] Cartilage damage in OA is known to be largely mediated by interleukin 1β (IL-1β), a cytokine that initiates a number of events leading to cartilage destruction, which includes the inhibition of matrix macromolecule biosynthesis and the increase of catabolic pathways. IL-1β stimulates the transcription of many genes through the activation of different transcription factors such as nuclear factor κB (NF-κB) or activator protein-1 (AP-1)[42]. NF-κB binding sites are present in the promoter regions of many genes involved in the pathophysiology of joint inflammation and tissue destruction. IL-1 binds to the IL-1 receptor type I (IL-IRI) that activates tumor necrosis factor receptor molecule-associated factor-6 (TRAF-6), leading to the activation of NF-κB-inducing kinase (NIK). [43] In this way, NF-κB can enter the nucleus and activate target genes by binding with high affinity to κB response element. Alternatively, TRAF-6 can also activate mitogen-activated protein kinase (MAPK).

The MAPK pathways promote phosphorylation of other substrates, such as c-Jun N-terminal kinase and the Jun and Fos family, all of which are associated with a transcriptional activity of AP-1.[43]

As previously described, glucosamine has been shown to be effective in relieving the symptoms of OA, although the mechanisms of the beneficial effect of glucosamine on cartilage disease are still not known. In some reports, it was demonstrated that glucosamine (4.5 g/l) prevented the IL-1β-mediated decrease in PG synthesis.[44] This process was related, in part, to a decrease in the expression of UDP-glucuronosyltransferase I (GlcAT-I), which is involved in the biosynthesis of glycosaminoglycans.[45] Research has also suggested that glucosamine can act through the scavenging of free radicals, although this action has never been proved[45]. Glucosamine can also modulate the expression of inflammatory enzymes (inducible NO synthase) and the production of NO.[46] Altogether, these results suggest that the pharmacological effects of the glucosamine in the treatment of OA would involve a modulation action of this amino sugar at some stages of IL-1 signaling events. There are many potential ways that glucosamine could modify IL-1 signaling pathway directly or indirectly. The protective effects have been considered at three different levels: (i) towards the effects induced by radicals, (ii) the variation of mRNA encoding the expression of the decoy receptor IL-IRII and the receptor antagonist IL-1ra, and (iii) the activation of two transcriptional nuclear factors, NF-κB and AP-1, known to be involved in inflammation. IL-1 could induce a cellular stress that can

produce reactive oxygen species (ROS), which in turn could activate the NF-κB pathway by interacting with NIK.

Two types of IL-1 receptors have been reported. The inflammatory effects of IL-1 require signaling through the cytoplasmic domain of IL-IRI. The decoy IL-IRII can also interact with IL-1. However as a truncated protein, its action with the cytokine is unable to generate signaling. By trapping part of IL-1, the decoy receptor can modulate its concentration and effects. This property makes IL-IRII a potential target in order to block the IL-1β signaling pathways.[47] Many studies suggest that IL-1RII plays an important pharmacological role in the treatment of inflammation.[48] Some drugs, such as aspirin, could increase this decoy receptor, thus decreasing inflammation by preventing the binding of 1L-1β to target cells.[49] Glucosamine was suggested to significantly increase the mRNA expression of IL-1RII in chondrocytes, but only when the cells were treated with IL-1β. By increasing the IL-1RII mRNA expression, glucosamine is able to reduce the binding of IL-1β with its receptor and therefore modulates its effect. When IL-1β binds to the receptor, the signaling is transmitted within the cell through a cascade of signaling proteins that can be potential targets of glucosamine. Glucosamine significantly decrease the efficiency of the IL-1β signaling pathway by antagonizing NF-κB activation and moreover, glucosamine also blocks the activation if NF-κB induced by ROS (reactive oxygen species). The anti-inflammatory effect of glucosamine could be explained, at least in part, via an inhibition of the binding of NF-κB to its response element, because the

cyclooxygenase 2 and inducible NO syntheses are known to be induced by NF-κB.[50]

1.4.2 Glucosamine and Insulin resistance

Insulin resistance is associated with diseases such as diabetes, obesity and hypertension. Although genetics contributes significantly to insulin resistance, it was reported that hyperglycemia was also, partially a cause of insulin resistance.[51] One potential mechanism by which hyperglycemia may attribute to insulin resistance is to increase the flux of glucose through the hexosamine biosynthetic pathway. A current hypothesis is that high extracellular glucose concentrations, and the consequent increased glycolytic flux, result in greater diversion of fructose-6-phosphate to glucosamine-6-phosphate in a reaction catalyzed by glutamine: fructose-6-phosphate amidotransferase (GFAT). Further metabolism of glucosamine metabolites, which could accumulate during hyperglycaemia, that may be responsible for the desensitisation of the glucose transport system to insulin, for example, a decreased rate of insulin-simulated glucose transport.[52] Stimulation of glucose uptake by insulin in classical targets such as muscle and fat is mediated by recruitment of glucose transporters (primarily GLUT4) to the cell in an insulin dose-dependent manner. It was reported that incubation of adipose cells or muscle with insulin and glucosamine, a substrate of the hexosamine biosynthetic pathway downstream from GFAT, also causes insulin resistance. Treatment of adipose cells with a combination of either glucose, insulin and glutamine or glucosamine and insulin cause an increase in the level of hexamine products with a concomitant decrease in GFAT activity.[53] Further

-more, overexpression of GFAT in fibroblasts caused decreased insulin sensitivity with respect to glycogen synthase activity. This evidence resulted in a hypothesis that GFAT plays an important role in the process of insulin resistance and the GAFT could be the main cause of the glucose transport system desensitisation. However this hypothesis was disproved by Hui Chen and his colleagues[54]. It was reported that transient over-expression of GFAT in adipose cells in primary culture did not significantly impair insulin stimulated translocation of GLUT4, and acute treatment of glucosamine did not decrease the translocation of GLUT4 to the cell surface. However, long-term treatment of glucosamine in the plasma-membrane-enriched fraction will cause a decrease in GLUT4 in the cell surface. The conclusion drawn is that glucosamine could cause insulin resistance via the action of the key enzyme GFAT in the hexosamine biosynthetic pathway. Acute administration of glucosamine exerts its effect on insulin resistance by altering the intrinsic activity of GLUT4 in some unknown mechanism, while a long-term effect of glucosamine could contribute to chronically changing the amount GLUT4 at the cell surface.

1.4.3 Other Effects of Glucosamine

Apart from the well known effect on OA and insulin resistance, glucosamine is also known to be a transcriptional stimulator of transforming growth factor-α (TGF-α), transforming growth factor-β (TGF-β) and fibroblast growth factor (FGF-2) in some VSMCs (vascular smooth muscle cells).[55] It is known that VSMCs play a key role in the development of atherosclerosis in diabetes. Glucosamine therefore claims its

effect on the atherosclerosis.

1.5 Aims and Objectives

Glucosamine compounds exhibit weak anti-inflammatory activity, but no analgesic activity. However, when combined with some NSAIDs or other analgesic compounds in a particular ratio, the combination exhibits an analgesic efficacy at least equal to the analgesic efficacy of the analgesic compounds alone, and in many instances may produce an unexpected synergistic increase in the analgesic efficacy of the analgesic compound or compounds employed in the combination. Glucosamine has also been used to improve the solubility of NSAIDs by combining a glucosamine with an NSAID in certain ratios to form a glucosamine salt or complex with the NSAID[56].

Therefore, the interaction between ibuprofen and glucosamine will be studied in this project. The aims of this study are as follows: the first was to investigate the interaction of glucosamine and ibuprofen in different solutions; the second was to determine the solubility of ibuprofen in water, 2% aqueous tween80 and ibuprofen containing different ratios of glucosamine; the third was to carry out a dissolution study of ibuprofen (2.78 g/L) and ibuprofen (2.78 g/L) containing different ratios of glucosamine (at the ratio of 2:1, 1:1, 1:2) in water and 2% tween80; the fourth was to use Franz diffusion cells to investigate the diffusion of ibuprofen in the presence of glucosamine; the last one was to establish whether glucosamine affects the degree of plasma binding for ibuprofen.

CHAPTER TWO

GENERAL METHODS

2.1 Materials

Ibuprofen was supplied by GlaxoSmithKline Company. Methanol and acetonitrile were purchased from Fisher Scientific. Fenoprofen (internal standard), glucosamine hydrochloride salt (99% pure, equivalent to 83% m/v glucosamine, so all studies were corrected for the parent compound), ortho-phosphoric acid, NaH_2PO_3, and Na_2HPO_3 (both NaH_2PO_3 and Na_2HPO_3 were used to prepare the buffer solution) were supplied by Sigma Chemicals (Dorset, UK). Tween80 was purchased from Aldrich (Dorset, UK). 0.45μm syringe filters were supplied by Chromos Express. Normal dialysis membrane and Silicone sheeting membrane (0.05μm) were supplied by Dow Corning (US). Human Serum Albumin (HSA), dialysis tubes (2 ml volume), and carbon-14 labeled ibuprofen were also purchased from ICN Biomedicals (New York).

All materials were of pharmaceutical, analytical or HPLC grade as appropriate. Double-distilled water was generated using fison's fi-stream still and used throughout for the preparation of all solutions. Human blood (50 ml) was supplied by a volunteer (Aston University UK). Pooled mouse blood (40 ml) was obtained from Balb-C mice (Biomedical Research Unit, Aston University, UK).

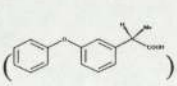
2.2 Equipment

2.2.1 High Performance Liquid Chromatography (HPLC) System

HPLC is a liquid chromatography method for separating molecular mixtures that depends on the differential affinities of the solutes between two immiscible phases.

One of the phases is fixed bed of large surface area (stationary phase), while the other is fluid (mobile phase), which moves through, or over the surface of the fixed phase. In HPLC method, the mobile phase is forced through the packed column under high pressure[57].

In this study, an HPLC method was used throughout the whole analysis process, purity assay of ibuprofen, confirming the solubility of ibuprofen in different solvents, the dissolution of ibuprofen and the diffusion of ibuprofen through a membrane. The HPLC system in this study consisted of the following components: a Waters 501 HPLC pump, Waters 710B (Water Intelligent Sample Processor) auto-injector system, a SA6503 Programmable Absorbance Detector, and a computer for data acquisition, recorder software JC6000. A reversed-phase HPLC mode was used for analyzing ibuprofen where the stationary phase was non-polar, a hydrocarbon, and the mobile phase was polar. The mobile phase consisted of acetonitrile-water (50:50) mixture using phosphate buffer solution to adjust the pH at the range of 2.70-3.00. An ODS analysis column (octadecylsilane, C18, 5μm, 150mm x 4.6mm ID) was selected as it has a high carbon loading which will result in a greater degree of end-capping and consequently, less peak-tailing.

An internal standard is required because it compensates for possible errors in the injection step. Fenoprofen () was chosen as the internal standard as it could be adequately separate and detected under the HPLC conditions employed for

ibuprofen. The peak area ratio of ibuprofen and fenoprofen in each sample was defined as responses. Response was used describing the quantification of sample peak, and also used for the data in the calibration graph.

2.2.2 Dissolution Testing System

The dissolution testing system comprised the ValiData@, Hanson SR2 dissolution apparatus. The ValiData@ controls the speed of the paddles and the water-bath temperature of a Hanson SR2 dissolution test stand. It displays speed, temperature, and elapsed time. Dissolution studies were carried out at 37 ºC and the paddle speed used was 50 rpm.

2.2.3 Diffusion Cells

Six Franz cells were connected with H_2O pump which controlled the temperature at 37°C, and were also set on a magnetic IKA-WERKE stirrer. The diffusion system was used to mimic a gastrointestinal absorption environment, in order to model ibuprofen absorption in gastrointestinal membrane.

There are different types of diffusion cells. Basic variations include orientation (horizontal and vertical) and receptor compartments (static or flow-through). Coldman[58] designed the first vertical cell and other workers modified this design later. Franz[59] studied the *in vitro* diffusion of various compounds from thin solids through human skin. Data obtained were compared with those previously obtained in *in vivo.*

Good agreement was reported between the two sets of data.

In this study, vertical cells (Franz cells) were used throughout the experiment. A schematic representation of a vertical diffusion cell is shown in **Fig. 2.1**. Vertical cells (Franz cells) comprise two chambers; the top one (donor) holds the formulation under investigation and the bottom part contains the receptor solution that can be a buffer solution or pure water. Between them the average diffusion surface area was 1.65 cm^2, and the receptor volume was approximately 29 ml. The receptor compartment has a sampling port for taking samples for analysis. It is continuously agitated using a magnetic stirrer bar and maintained at a constant temperature via a water jacket. The membrane is mounted horizontally between two halves of the cell. This kind of orientation of the membrane means that the donor chamber might be exposed to ambient temperature and humidity, while the lower chamber could be maintained at physiological temperature, simulating *in vivo* conditions for intestinal membrane.

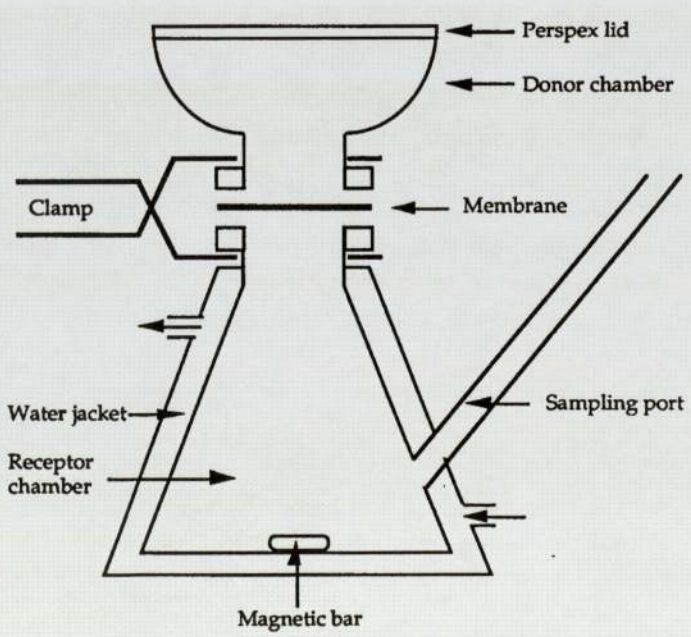


Fig. 2.1 Franz diffusion cell

In the ibuprofen diffusion study, dialysis membrane was used between the donor and the receptor; 20 ml of solution was added to the donor chamber; pH 6.8 phosphoric buffer was used as the receptor solution; 2 ml sample were taken and 2ml pH 6.8 buffer was added to keep the same volume in the receptor.

2.2.4 Carbon-14 labeling Method

It is known that ibuprofen strongly bind with plasma serum. In the section of ibuprofen binding study, 5μl radio-labeled ^{14}C-ibuprofen was added to each ibuprofen sample, and then binding with different plasma. The quantity of free ibuprofen was determined by using a 1600TR Liquid Scintillation Analyzer to detect the radio activity of labeled ibuprofen. More details will be discussed in chapter six.

2.3 Ibuprofen impurities assay by HPLC

2.3.1 Sample preparation

Approximate 0.1 g of ibuprofen was dissolved in 20 ml of acetonitrile. All samples were filtered through 0.45μm syringe filters before use.

2.3.2 Chromatographic conditions

The mobile phase (acetonitrile:water:ortho-phosphoric acid 340:660:0.5) was filtered and then degassed in an ultrasonic bath. The flow rate was 2.0 ml/min at room temperature. The runtime was 7 minutes. And the samples were injected at 10μl. The UV detector was set at wavelength 214nm, and the sensitivity was set at 0.02 AUF

(AUF- absorbance units corresponding to full scale deflection on the recorder).

2.3.3 Results and discussion

The method which was used to determine the purity of ibuprofen is to calculate the percentage of an impurity peak area against the main peak area. If the peak area is equivalent to or beyond 0.07% of the main peak area, it could be detected as an impurity; otherwise it was attributed associated with the detected method.[60]

The total number of peaks in each sample (n=3) and the number of impurity peaks compared to the standard percentage (0.07%) are presented in **Table 2.1**, and a typical chromatograph of ibuprofen is shown in **Fig.2.2**

Table 2.1 The number of peaks from assay to determine impurities in ibuprofen.

Number of peaks	Sample 1	Sample 2	Sample 3
Impurity peak	9	11	11
Main peak	1	1	1
Peaks less than 0.07%	9	11	11
Peaks equivalent or more than 0.07%	0	0	0

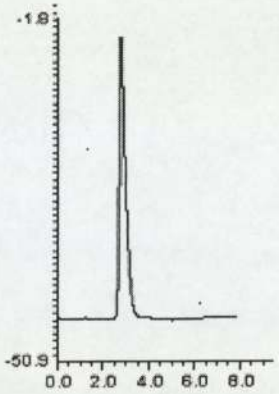


Fig. 2.2 A typical ibuprofen chromatograph in the impurity study.

From **Table 2.1**, there is no impurity peak which the percentage of peak area against the main peak is equivalent or beyond 0.07%.

2.3.4 Conclusion

The ibuprofen which supplied was of adequate purely for our study.

CHAPTER THREE

THE SOLUBILITY OF IBUPROFEN

3.1 Introduction

3.1.1 Solubility and factors affecting solubility

The solution produced when equilibrium is established between undissolved drug and dissolved solute in a dissolution process is termed a saturated solution. The amount of substance that passes into solution in order to establish the equilibrium at constant temperature and pressure and so produce a saturated solution is known as the solubility of the substance.[61]

Ibuprofen is very poorly soluble in distilled water (<0.1 w/v at ambient temperature), sparingly soluble in hexane and freely soluble in ethanol, octanol, and acetone.[62]

Factors affecting the solubility of solid in liquid as follows:

Temperature A rise in temperature will lead to an increase in the solubility of a solid with a positive heat of solution. Conversely, in the case of the less commonly occurring systems that exhibit exothermic dissolution, an increase in temperature will give rise to a decrease in solubility. The solubility of ibuprofen will increase when temperature rises in both aqueous solution and organic solvents.[63] Thus, in this study the temperature was maintained at 25 °C or 37 °C.

Molecular structure of solute It should appreciated from the previous comments on the prediction of solubility that the nature of the solute and the solvent will be of paramount importance in determining the solubility of a solid in a liquid. It should

also be realised that even a small change in the molecular structure of a compound can have a marked effect on its solubility in a given liquid. Thus, ibuprofen is kept in a well-closed container to prevent degradation and its purity measured.

Nature of solvent: cosolvents Some mixtures of solvents may be employed as the co-solvents. Such mixtures are often used in pharmaceutical practice to obtain aqueous-based systems that contain solutes in excess of their solubility in pure water. In this study the co-solvents (acetronitrile-water) are used to improve the solubility of ibuprofen.

Particle size of the solid The changes in interfacial free energy that accompany the dissolution particle of varying sizes cause the solubility of a substance to increase with decreasing particle size.

pH If the pH of a solution of either a weakly acidic drug or a salt of such a drug is reduced then the proportion of unionized acid molecules in the solution increases. Precipitation may therefore occur because the solubility of the unionized species is less that that of ionized form. Conversely, in the case of solutions of weakly basic drugs or their salts precipitation is favored by an increase in pH.

The relationship between pH and the solubility of ionized solutes is extremely important with respect to the ionization of weakly acidic and basic drugs as they pass

through the gastrointestinal tract and experience pH changes between about 1 and 8. The relationship between pH and the solubility and pK_a value of an acidic drug is given by **Eqn 3.1**[61]:

$$pH = pK_a + \log ((S-S_u)/S_o) \qquad (3.1)$$

where S is the overall solubility of the drug ; S_u is the solubility of its unionized form, i.e. $S=S_o+$ solubility of ionized form (S_I); and S_o is the intrinsic solubility. If the pH of the solution is known then **Eqn 3.1** may be used to calculate the solubility of an acidic drug at that pH.

Solubilising agents The agents are capable of forming large aggregates or micelles in solution when their concentrations exceed certain values. In aqueous solution, the centre of these aggregates resembles a separate organic phase and organic solutes may be taken up by the aggregates, thus producing an apparent increase on their solubility in water. This phenomenon is known as solubilisation. A similar phenomenon occurs in organic solvents containing dissolved solubilising agents, because the centre of aggregates in these systems constitutes a more polar region than the bulk of the organic solvent. If polar solutes are taken up into these regions their apparent solubilities in the organic solvents are increased.

Other factors are common ion effect, effects of indifferent electrolytes on the solubility product, effect of non-electrolytes on the solubility of electrolytes, and

effect of electrolytes on the solubility of non-electrolytes.

3.1.2 The aim of solubility studies

Solubility determinations were performed for those solvent systems employed during the dissolution and penetration experiments. The solubility of ibuprofen alone and its solubility in the presence of different percentages of glucosamine were determined in (a) acetronitrile-water (50:50); (b) distilled water; (c) 2% Tween80 aqueous solution. The selection of Tween80 as a solubilising agent was due to the fact that Tween80 is a pharmacological dispersant[56], and has no chemical reaction with either ibuprofen or glucosamine.

3.1.3 Concentration Units – weight concentration

Weight concentration, which is a traditional system, is used in this study for concentration. Concentration is often expressed as a weight of solute in a unit volume of solution; for example, gL^{-1}, or %w/v which is the number of grams of solute in 100 ml of solution. The disadvantage is that it is not an exact method when working as a range of temperatures since the volume of the solution is temperature dependent and hence the weight concentration also changes with temperature. But in this study, all the samples were prepared in the same temperature, so the weight concentration unit can be used throughout.

3.2 Ibuprofen dissolved in acetonitrile-water (50:50) solution and assayed by HPLC

3.2.1 Solution and sample preparation

3.2.1.1 Standard stock solution

Acetonitrile-water (50:50) was used to prepare the standard stock solutions of ibuprofen, glucosamine and fenoprofen (internal standard). The reasons of selecting acetonitrile as a co-solvent were that a) ibuprofen, fenoprofen, and glucosamine are soluble in acetonitrile-water solution; b) acetonitrile-water solution also used in the mobile phase of HPLC analysis, thus avoiding the generation of solution peak in the chromatogram.

3.2.1.2 Sample preparation

Appropriate volumes of the stock ibuprofen solution (0.5 mg/ml) and 0.5 ml of the fenoprofen stock solution (0.6 mg/ml) were transferred precisely into 10 ml volumetric flasks, glucosamine was added, and made up to volume using acetonitrile-water (50:50). The concentrations of samples used are in **table 3.1**.

Table 3.1 Concentrations of ibuprofen and glucosamine samples used in this study

Ibuprofen (μg/ml)	Ibuprofen:Glucosamine (2:1) (μg/ml)	Ibuprofen:Glucosamine (1:1) (μg/ml)	Ibuprofen:Glucosamine (1:2) (μg/ml)
30	30:15	30:30	30:60
40	40:20	40:40	40:80
50	50:25	50:50	50:100
60	60:30	60:60	60:120
90	90:45	90:90	90:180
100	100:50	100:100	100:200

3.2.2 Chromatographic conditions

The mobile phase (acetonitrile:water:ortho-phosphoric acid 500:500:10) was filtered and then degassed using an ultrasonic bath. The mobile phase flow rate was 1.0 ml/min at room temperature. The runtime was 11 minutes and the samples were injected at 20μL. Ibuprofen was determined at a wavelength 225 nm, and the sensitivity at 0.1 AUF.

3.2.3 Results

The HPLC method was established for comparing the solubility of ibuprofen and ibuprofen combined with different ratios of glucosamine. Under the chromatographic conditions in section 3.2.2, ibuprofen and the internal standard (fenoprofen) were completely separated. The retention times were 9.20 min and 6.60 min respectively. A typical chromatograph is shown in **Fig.3.1**. Quantification was based on the least-squares linear regression analysis of response (peak-area ratio (y)) versus concentration (x). The calibration curve displayed good linearity over the range examined. The linear regression equation was $y=0.0252x+0.0878$ ($R^2=0.9973$). The solubility of ibuprofen in water-acetonitrile and in glucosamine solution is shown in **Table 3.2** and **Fig. 3.2**.

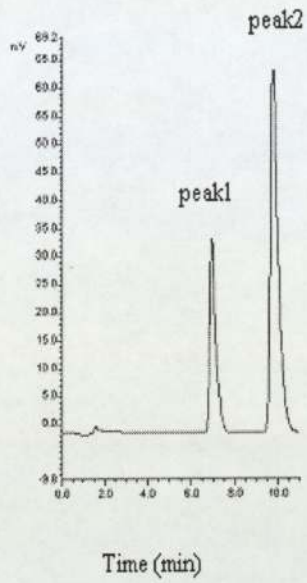


Fig.3.1 Example of typical ibuprofen chromatogram (peak 2) with fenoprofen (peak 1) as internal standard.

Table 3.2 Ibuprofen and glucosamine analysis by HPLC in acetonitrile-water (50:50) (n=3; mean±s.d.)

Concentration of ibuprofen (μg/ml)	Ibuprofen Only (Response)	Ibuprofen : glucosamine (2:1) (Response)	Ibuprofen : glucosamine (1:1) (Response)	Ibuprofen : glucosamine (1:2) (Response)
30	0.8177±0.0086	0.8269±0.0070	0.8676±0.0016	0.8142±0.0013
40	1.1109±0.0003	1.1101±0.0007	1.0681±0.0006	1.0602±0.0009
50	1.3477±0.0018	1.3440±0.0012	1.3438±0.0021	1.3318±0.0022
60	1.5997±0.0432	1.5744±0.0038	1.6028±0.0016	1.6083±0.0011
90	2.4075±0.0069	2.4396±0.0032	2.4637±0.1630	2.4588±0.0106
100	2.5524±0.0070	2.6777±0.0017	2.6859±0.0159	2.7646±0.0074

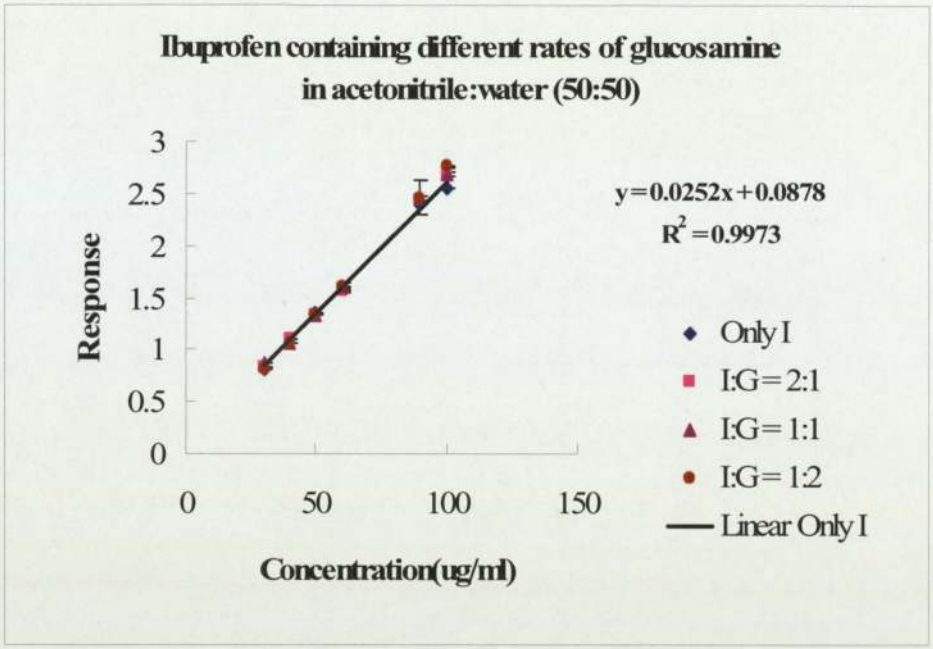


Fig. 3.2 A HPLC calibration for ibuprofen in acetonitrile-water (50:50) and ibuprofen with different percentages of glucosamine (I= ibuprofen, G= glucosamine)

3.2.4 Discussion

In **Fig. 3.2**, calibration graphs for ibuprofen alone and in the presence of glucosamine, suggest that there is no difference on the response generated. Table 3.2 shows similar responses for ibuprofen over the range 30-100 μg/ml in the presence of increasing amounts of glucosamine.

3.2.5 Conclusion

The presence of glucosamine has no identifiable effect on the HPLC analysis of ibuprofen when ibuprofen is in solution. That means glucosamine does not react with or change the ionization state of ibuprofen in the given solvent and temperature in our experiments.

3.3 Determination of the solubility of ibuprofen in water and 2%(v/v) Tween80 aqueous solution

3.3.1 Sample preparation

Ibuprofen is very sparingly soluble in distilled water (the saturated solubility is approximately 0.004%w/v[56]), it is somewhat unexpected that that addition of 2% (v/v) Tween80 would increase the solubility to 0.278% w/v as claimed by reference 68. In this study, the saturated solubility of ibuprofen in distilled water and 2% Tween80 aqueous solution were used as the boundary. For example, 0.00326% ibuprofen water solution (37 °C, shaking 24 hours) was used as standard stock solution to prepare a series of solutions with a range of concentrations from 6.5 to 31 μg/ml as HPLC samples, and assayed by HPLC to produce a calibration of ibuprofen dissolved in distilled water. Saturated solutions were prepared by adding excess ibuprofen, shaking at 37 °C for 24 hours, then filtered using a 0.45 μm syringe filter, and assayed by HPLC. Samples of ibuprofen in 2% Tween80 (v/v) were prepared similarly. 0.1%w/v ibuprofen 2% Tween80 aqueous solutions were used to prepare the calibration solution. According to the conclusion in section 3.2.5, the solubility of ibuprofen in the presence of glucosamine can also be determined using the same method.

3.3.2 Chromatographic conditions

The conditions were as detailed in section 3.2.2.

3.3.3 Results

3.3.3.1 The solubility of ibuprofen in distilled water

A calibration curve was produced for ibuprofen in water using HPLC under the chromatographic conditions in the section 3.2.2. Ibuprofen and the internal standard (fenoprofen) were completely separated (R>1.5). The retention times were 8.30 min and 6.0 min respectively. Quantification was based on the least-squares linear regression analysis of response (peak-area ratio (y)) versus concentration (x). The data is shown on **Table 3.3**. The calibration curve displayed good linearity over the range examined, y=0.0349x-0.0778 (r=0.9987), **Fig. 3.3**. The standard deviations are too small to be visible.

The sample X was diluted from the ibuprofen water saturated solution. From **Table 3.3**, the response value (Y=0.69122) was obtained from HPLC assay, and corresponded to 22.0350 μg/ml. Then the saturated solubility of ibuprofen in distilled water can be calculated correcting for dilution at 0.0044%w/v.

Table 3.3 The calibration data (HPLC responses) of ibuprofen dissolved in distilled water assayed by HPLC

Concentration (μg/ml)	Response1	Response2	Response3	Mean	s.d.
6.5	0.1605	0.1694	0.1621	0.1640	0.0048
10	0.2548	0.2578	0.2685	0.2604	0.0072
15	0.4376	0.4389	0.4419	0.4395	0.0022
21	0.6396	0.6414	0.6404	0.6405	0.0009
26	0.8456	0.8409	0.8422	0.8429	0.0024
31	1.1472	1.1496	1.1475	1.1481	0.0013
SampleX	0.6943	0.6875	0.6919	0.6912	0.0035

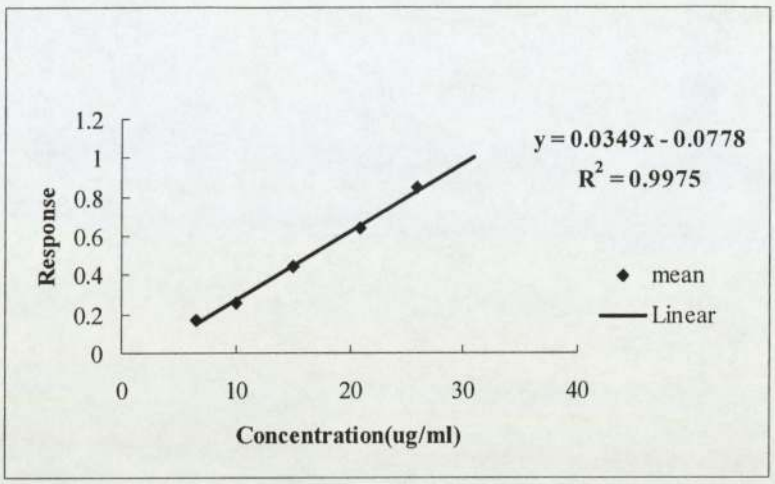


Fig. 3.3 A typical HPLC calibration graph for ibuprofen dissolved in distilled water (n=3; mean±s.d.)

3.3.3.2 The solubility of ibuprofen in glucosamine and 2%Tween80 aqueous solution

The solubility of ibuprofen in 2% Tween80 solution was examined. 1 g, 2.78 g, and 5 g ibuprofen were added into 1L 2%Tween80 aqueous solution separately, the solutions kept shaking in at 37 °C in a water bath for 24 hours. The solutions were filtered and the concentration of ibuprofen measured using HPLC.

Visually, the results are described below:

1g ibuprofen in 1L 2%Tween80 solution → dissolved completely

2.78g ibuprofen in 1L 2%Tween80 solution → dissolved, but slightly turbid

5g ibuprofen in 1L 2%Tween80 solution → excess solid

When these solutions were stored under ambient conditions, ibuprofen was

precipitated from the 2.78 g/L ibuprofen 2% Tween80 solution. It means the solubility of ibuprofen in 2% Tween80 is close to 0.278% w/v in this study. Ibuprofen solutions containing 1 g/L were used as stock solution for calibration in order to determine the solubility of ibuprofen and ibuprofen mixed with glucosamine in 2%Tween80 aqueous solution.

As discussed in secion 3.2.4, when ibuprofen was dissolved in acetonitrile-water, glucosamine did not affect the HPLC analysis of ibuprofen. The same experiment was carried out with the 1 g ibuprofen in 1L 2%Tween 80 solution. The result is shown in **Table 3.4** and **Fig. 3.4**. The presence of glucosamine has no effect on the responses produced by ibuprofen.

Table 3.4 HPLC responses of ibuprofen (1 g/L) in 2% Tween80 aqueous solution and in the presence of glucosamine (n=3; mean±s.d.)

Concentration of ibuprofen (μg/ml)	Ibuprofen Only Response	Ibuprofen: glucosamine (2:1) Response	Ibuprofen: glucosamine (1:1) Response	Ibuprofen: glucosamine (1:2) Response
10	0.2643±0.0014	0.2558±0.0014	0.2684±0.0013	0.2637±0.0018
20	0.5332±0.0015	0.5301±0.0012	0.5158±0.0019	0.5503±0.0030
30	0.7946±0.0066	0.7757±0.0009	0.7515±0.0034	0.7622±0.0028
50	1.3114±0.0057	1.2909±0.0023	1.2795±0.0025	1.2846±0.0022
80	2.1380±0.0020	2.0598±0.0085	2.0387±0.0045	2.0930±0.0029
100	2.6504±0.0079	2.5162±0.0028	2.5258±0.0020	2.5844±0.0038

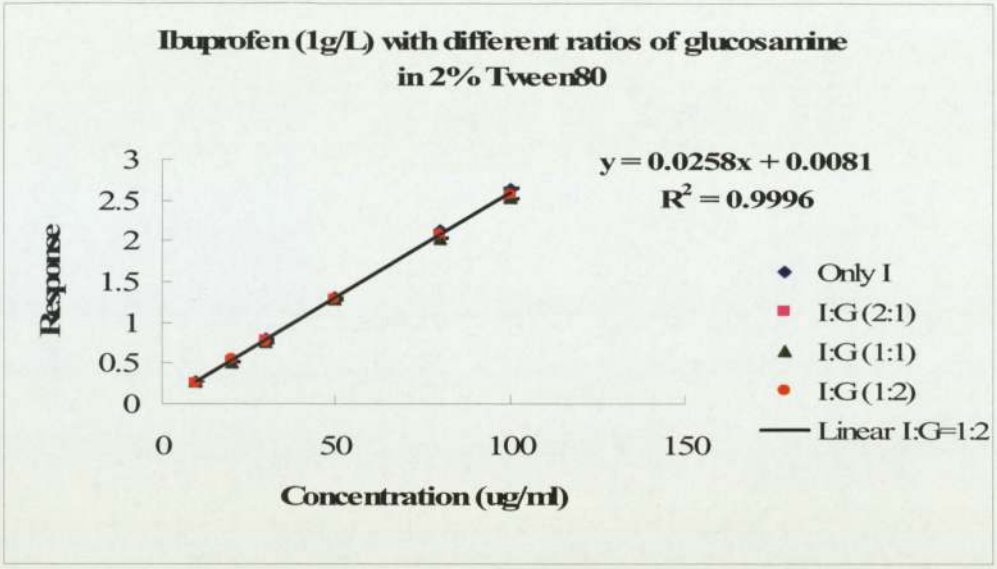


Fig. 3.4 A typical HPLC calibration for ibuprofen (1g/L) in 2%Tween80 aqueous solution and ibuprofen in the presence of glucosamine (I= ibuprofen; G= glucosamine)

The same method as section 3.3.3.1 was used to determine the solubility of ibuprofen in 2% Tween80 with increasing concentration of glucosamine. The equation y=0.0258x + 0.0081 (R=0.9998) was used to calculate the solubility of ibuprofen. The results are shown in **Table 3.5**.

Table 3.5 The solubility results of ibuprofen and with glucosamine in 2%Tween80 (mean, n=3)

	Response (y)	Concentration(x μg/ml)	Solubility (g/L)
Only Ibuprofen	0.83048	31.8752	**2.65627**
Ibuprofen : Glucosamine (1:2)	0.84567	32.464	**2.70533**
Ibuprofen : Glucosamine (1:1)	0.85235	32.7229	**2.72691**
Ibuprofen : Glucosamine (2:1)	0.85214	32.7147	**2.72623**

§The solubility group was analysis by ANOVA. Significantly different between the solubility of ibuprofen only and any of ibuprofen in the presence of different ratios glucosamine ($P<0.05$, ANOVA).

3.3.4 Discussion

Tween80 was used as a solubilizing agent in this study. Tween80 is miscible with water, as reflected in its higher HLB (hydrophile-lipophile balance; the HLB of Tween80 is 15.0). Tween80 also has a low cmc (0.014g/L), so it readily forms micelles. It is suggested in our experiments that the presence of Tween80 increases the solubility of ibuprofen in water over 50 times from 0.044 g/L (0.0044% w/v) to 2.656 g/L. The applications of Tween80 for dissolution will be discussed in chapter four.

The solubility of ibuprofen was definitely increased when glucosamine was added to the system. A maximum solubility of 2.73g/L was obtained at the ratio of Ibuprofen: glucosamine 1:1. Further increases of glucosamine had no further increases in solubility in our study.

3.3.5 Conclusion

In this study, the solubility of ibuprofen in different solvents was determined by HPLC assay. Ibuprofen has very poor solubility in distilled water (0.0044% w/v). This was increased to 0.2656% w/v in 2% Tween80 aqueous solution. When glucosamine was added, the solubility of ibuprofen was further increased (0.2705% w/v (I:G = 2:1), 0.2727% w/v (I:G = 1:1), 0.2726% w/v (I:G = 1:2); I=ibuprofen, G= glucosamine). The explanation will be presented in chapter four section 4.2.2.4.2.

CHAPTER FOUR

THE DISSOLUTION OF IBUPROFEN

4.1 Introduction

Solutes are dispersed as molecules or ions throughout the solvents, i.e. they are said to be dissolved in the solvent. The transfer of molecules or ions from a solid state in solution is known as dissolution.

4.1.1 Dissolution mechanisms

The dissolution of a solid in a liquid may be regarded as being composed of two consecutive stages.

1. First is an interfacial reaction that results in the liberation of solute molecules from solid phase. This involves a phase change, so that molecules of solid become molecules of solute in the solvent in which the crystal is dissolving. The solution in contact with the solid will be saturated (because it is in direct contact with undissolved solid).

2. After this, the solute molecules must migrate through the boundary layers surrounding the crystal to the bulk of the solution. This step involves the transport of these molecules away from the solid-liquid interface into the bulk of the liquid phase under the influence of diffusion or convection. Boundary layers are static or slow-moving layers of liquid that surround all wetted solid surfaces. Mass transfer takes place more slowly through these static or slow-moving layers, which inhibit the movement of solute molecules from the surface of the solid to the bulk of solution.

Like any reaction that involves consecutive stages, the overall rate of dissolution normally depends on whichever of these steps is slowest (the rate-determining or rate-limiting step). In dissolution the interfacial step (1) is virtually instantaneous and so the rate of dissolution will be determined by the rate of the slower step (2), of diffusion of dissolved solute across the static boundary layer of liquid that exists at a solid-liquid interface.

4.1.2 Measurement of dissolution rates

Beaker method The methodology of Levy and Hays forms the basis of this technique. In their initial work they used a 400 cm^3 beaker containing 250 cm^3 of dissolution medium, which was agitated by means of a three-bladed polyethylene stirrer with a diameter of 50 mm. The stirrer was immersed to the depth of 27 mm into the dissolution medium and rotated at 60 rpm. Tablets were dropped into the beaker and samples of the liquid were removed at known times, filtered and assayed.[61]

Flask-stirrer method This is similar to the beaker method except that a round-bottomed flask is used instead of a beaker. The use of a round-bottomed container helps to avoid the problems that may arise from the formation of 'mounds' of particles in different position on the flat bottom of beaker.

Rotating basket method this method is described in most pharmacopoeias for the determination of the dissolution rates of drugs from tablets and capsules. This method

involves placing the tablet or capsule inside a stainless steel wire basket, which is rotated at a fixed speed while immersed in the dissolution medium, which is contained in a wide-mouthed cylindrical vessel, the bottom of which is either flat or spherical. Samples of the dissolution medium are removed at specified times, filtered and assayed.

Paddle method This is another official method. The dissolution vessel described in the rotating basket method, i.e. the cylindrical vessel with the spherical bottom, is also used in this method. Agitation is provided by a rotating paddle and the dosage form is allowed to sink to the bottom of dissolution vessel before agitation is commenced.

4.1.3 The aims of dissolution study

To investigate the process of ibuprofen dissolution in distilled water and 2% Tween80 aqueous solution, and in the presence of glucosamine, to monitor the pH changes during the ibuprofen dissolution. The paddle method was used throughout the experiments.

4.2 Experimental details

4.2.1 Comparison of the ibuprofen dissolution in distilled water and 2%Tween80 aqueous solution

4.2.1.1 Sample preparation

1g ibuprofen was added to 1L spherical bottom dissolution flask (n=3), containing

distilled water and 2%Tween80 aqueous solution separately. The dissolution conditions were 50.0 RPM at 37 °C for 120 minutes (min). 5 ml samples were taken at 5, 10, 15, 20, 25, 30, 60, 90, and 120 min after the dissolution experiment beginning, and then filtered using 0.45μm syringe filters, diluted with mobile phase to 10 ml and assayed by HPLC.

4.2.1.2 Chromatographic conditions

The mobile phase was actonitrile:water (500:500), using phosphoric buffer solution to adjust the pH in the range of 2.6-3.0. The mobile phase was filtered and degassed prior to use. The flow-rate was set at 1.0 ml/min at room temperature. The runtime was 10 minutes, and sample injection volume was 15μl. The UV absorbance of the column eluent was monitored at 222nm,[64] the sensitivity at 0.1 AUF.

4.2.1.3 Results and discussion

The dissolution of ibuprofen in 2%Tween80 aqueous solution and distilled water is shown in **Fig. 4.1** and the data is shown in **Table 4.1**. In this graph, the response was direct proportion with the concentration of ibuprofen; the time was carried out for 120 minutes. In **Table 4.1**, the data for ibuprofen dissolved in 2% Tween80 is corrected for dilution.

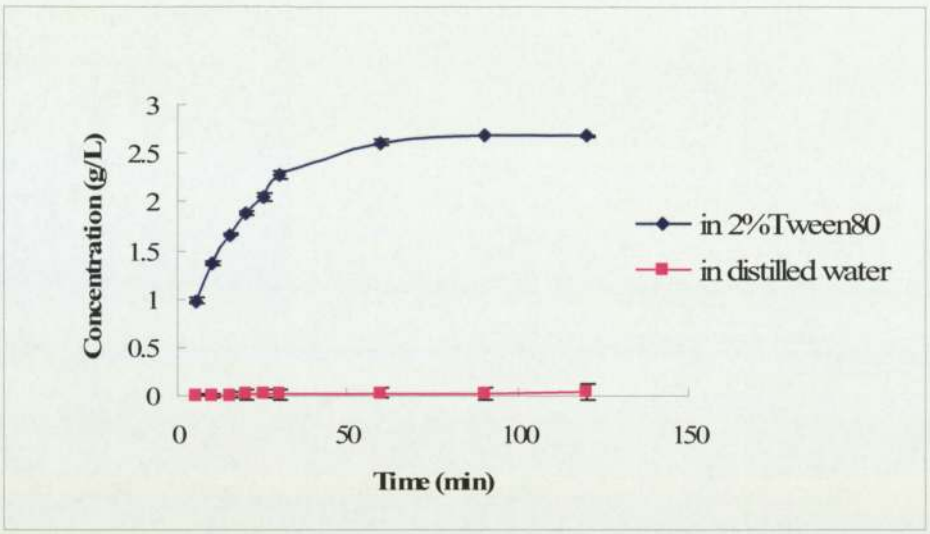


Fig. 4.1 The dissolution profile of ibuprofen in 2%Tween80 and distilled water

Table 4.1 Ibuprofen dissolution data in 2%Tween80 and water (n=3; mean±s.d.)

Time (min)	Concentration (g/L) in 2%Tween80	Concentration (10^{-3} g/L) in water
5	0.9807±0.0362	3.9532±0.0076
10	1.3611±0.0239	5.7568±0.0180
15	1.6647±0.0050	7.9988±0.0328
20	1.8769±0.0236	10.8299±0.0433
25	2.0450±0.0435	13.5525±0.0393
30	2.2796±0.0450	15.8930±0.0488
60	2.6168±0.0244	23.6455±0.0539
90	2.7063±0.0089	29.4159±0.0611
120	2.6862±0.0124	35.8608±0.0813

From this graph, there was a large difference in 1 g ibuprofen dissolved in 1L 2%Tween80 aqueous solution and distilled water. Ibuprofen dissolved much more rapidly and to a great extent in 2% Tween80 aqueous solution than in distilled water. This graph also showed the equilibrium for ibuprofen dissolved was around 90 minutes. The conditions when ibuprofen dissolved in 2%Tween80 aqueous solutions containing glucosamine were investigated in future experiments.

.4.2.2 Dissolution of ibuprofen in the presence of glucosamine in 2%Tween80 aqueous solution

4.2.2.1 Sample preparation

2.78 g ibuprofen and different ratios of glucosamine were added to 1L spherical bottom dissolution flask (n=3). The dissolution medium was 2% Tween80 aqueous solution. The experiment was carried out as described in section 4.2.1.1. The pH of samples was monitored in this experiment using a sartonius pH meter.

4.2.2.2 Chromatographic conditions

A new Jones Chromatography HPLC column was used to this study. It was the same packaging material as the column used in the past experiment, however because the efficiency of the column improved, chromatographic conditions were adjusted accordingly.

The mobile phase was acetonitrile: water (600:400), and 0.2 mmole/L hexanesulfonic acid (from Sigma Chemicals) was added to the mobile phase for further reduction of peak tailing. The pH was adjusted by ortho-phosphoric acid in a range of 2.63-2.65. The mobile phase was filtered and then degassed using an ultrasonic bath prior to use. The flow rate was 1.5 ml/min at room temperature, and the run time was 5 minutes. The injection volume was 5µl; ibuprofen was detected at 222nm, and the sensitivity at 0.1AUF.

4.2.2.3 Results

Ibuprofen and the internal standard (fenoprofen) were completely separated under the chromatography conditions in section 4.2.2.2. The retention times were 2.98 min and 4.06 min respectively. A typical chromatographic trace is shown in **Fig. 4.2**.

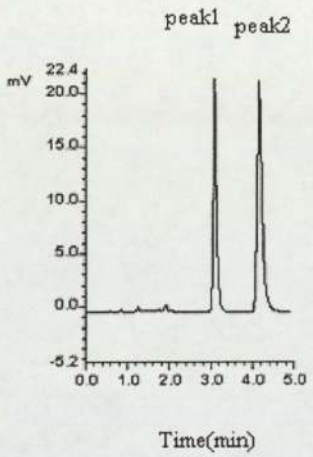


Fig.4.2 Example of typical ibuprofen chromatogram (peak 2) with fenoprofen (peak 1) as internal standard.

Quantification was based on the least-squares linear regression analysis of response (peak-area ration (y)) versus concentration (x), the data displayed in **Table 4.2**. A good linearity calibration is shown in **Fig. 4.3**. The equation y= 0.0248x-0.0103 (R=0.9996) was used for calculation of ibuprofen concentration.

Table 4.2 HPLC responses for ibuprofen calibration in 2% Tween80 solution

Concentration (μg/ml)	Response 1	Response 2	Response 3	Mean	s.d.
16.2	0.4146	0.4154	0.4146	0.4148	0.0004
32.4	0.7693	0.7671	0.7681	0.7682	0.0011
54.0	1.3464	1.3479	1.3461	1.3468	0.0010
64.8	1.5685	1.5663	1.5651	1.5666	0.0018
108	2.6662	2.6868	2.6862	2.6797	0.0117

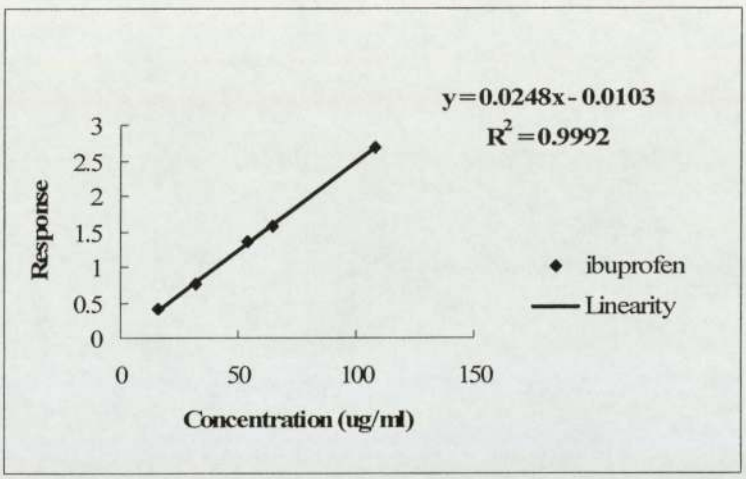


Fig. 4.3 A typical HPLC calibration curve for ibuprofen in 2% Tween80 aqueous solution (n=3; mean±s.d.)

As introduced in section 3.1.1.1, pH is one of the important factors during the dissolution process, especially for ibuprofen, because it is a weak acid. The pH of the dissolution medium was observed in this dissolution study, the results are shown in **Table 4.3** and **Fig. 4.4**. Standard deviations are too small to be visible.

Table 4.3 pH data for ibuprofen dissolution in 2%Tween80 aqueous solution

(n=3; mean±s.d. ; I= ibuprofen, G= glucosamine)

Time (min)	pH mean ± s.d. I Only	pH mean ± s.d. (I:G = 2:1)	pH mean ± s.d. (I:G = 1:1)	pH mean ± s.d. (I:G = 1:2)
5	4.6167±0.0152	4.6067±0.0208	4.5267±0.0058	4.3800±0.0300
10	4.5233±0.0058	4.4867±0.0153	4.3400±0.0200	4.2633±0.0153
15	4.4500±0.0100	4.4330±0.0153	4.2700±0.0265	4.2100±0.0173
20	4.4067±0.0058	4.3333±0.0208	4.2133±0.0153	4.1600±0.0173
25	4.3800±0.0000	4.3033±0.0115	4.1933±0.0231	4.1300±0.0100
30	4.3533±0.0058	4.2767±0.0058	4.1767±0.0289	4.1233±0.0058
60	4.3267±0.0115	4.2367±0.0058	4.1600±0.0265	4.1100±0.0000
90	4.3000±0.0000	4.2133±0.0058	4.1567±0.0289	4.1000±0.0000
120	4.3033±0.0058	4.2200±0.0000	4.1700±0.0346	4.1067±0.0058
180	4.3300±0.0100	4.2233±0.0058	4.1800±0.0346	4.1300±0.0000
240	4.3433±0.0058	4.2300±0.0100	4.1800±0.0246	4.1333±0.0058

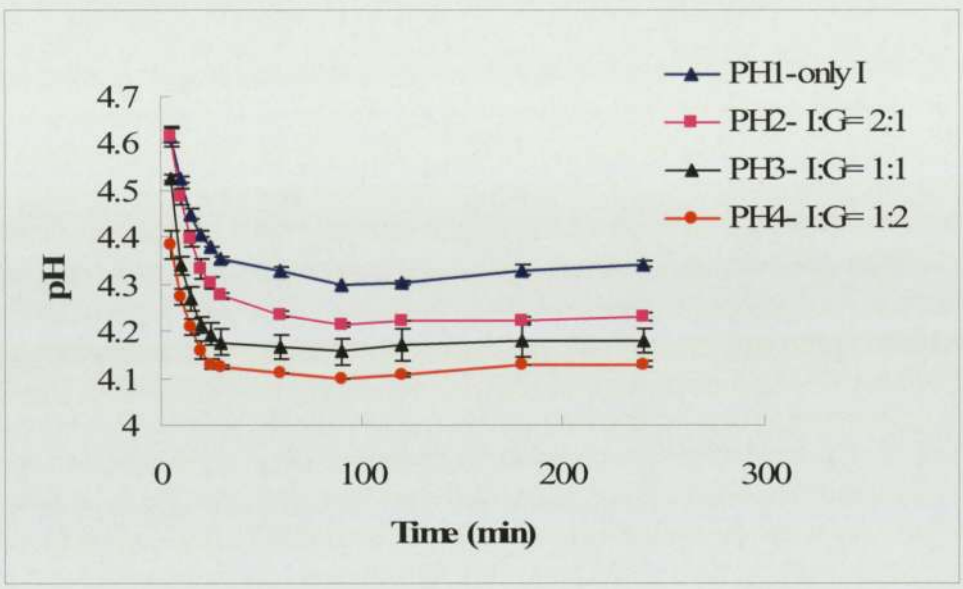


Fig. 4.4 pH changes with time for ibuprofen dissolution in 2%Tween80 aqueous solution containing glucosamine (n=3; mean±s.d.)

The results for ibuprofen dissolution in the presence of glucosamine in 2%Tween80 aqueous solution assayed by HPLC are shown in **Table 4.4** and **Fig. 4.5**. The concentrations of ibuprofen in each time point were presented in **Table 4.5**.

Table 4.4 Ibuprofen dissolution data assayed by HPLC (n=3; mean±s.d. ; I= ibuprofen, G= glucosamine)

Time (min)	Response ± s.d. I Only	Response± s.d. (I:G = 2:1)	Response± s.d. (I:G = 1:1)	Response± s.d. (I:G = 1:2)
5	0.4761±0.0272	0.6083±0.0252	0.4668±0.0171	0.5019±0.0141
10	0.6648±0.0234	0.8939±0.0153	0.7623±0.0261	0.6440±0.0148
15	0.8154±0.0258	1.0810±0.0264	1.0525±0.0099	0.9117±0.0365
20	0.9207±0.0281	1.1861±0.0100	1.0873±0.0259	1.0714±0.0171
25	1.0040±0.0035	1.2059±0.0152	1.1515±0.0133	1.1336±0.0212
30	1.1204±0.0086	1.2616±0.0183	1.2096±0.0063	1.1642±0.0164
60	1.2877±0.0198	1.2961±0.0106	1.2908±0.0225	1.2607±0.0083
90	1.3234±0.0022	1.3035±0.0241	1.3188±0.0032	1.3029±0.0138
120	1.3204±0.0108	1.3017±0.0162	1.3230±0.0066	1.3246±0.0017
180	1.3320±0.0102	1.2937±0.0250	1.3347±0.0004	1.3370±0.0157
240	1.3220±0.0119	1.3235±0.0218	1.3368±0.0085	1.3368±0.0120

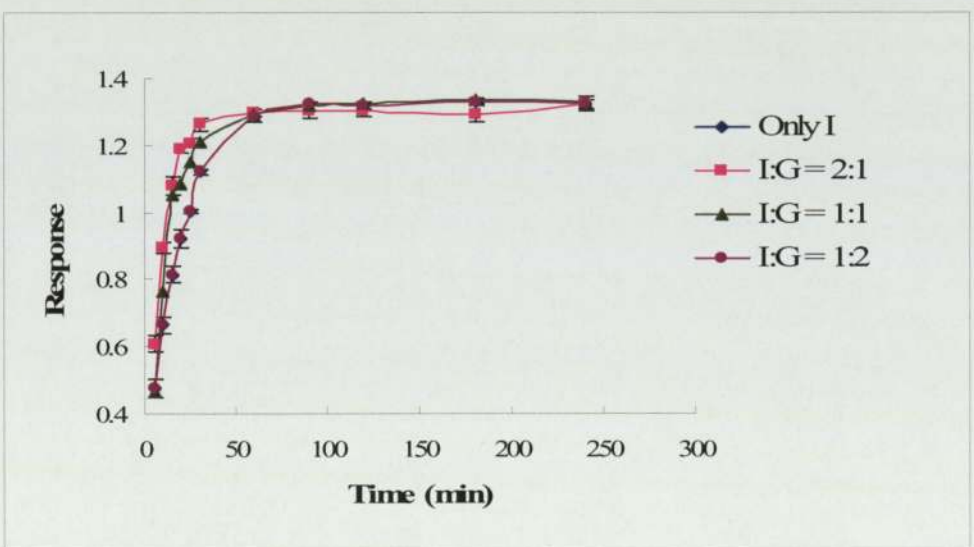


Fig. 4.5 Ibuprofen dissolving in 2%Tween80 aqueous solution, containing glucosamine, assayed by HPLC (n=3; mean±s.d.)

According to the HPLC assay result, the concentration of ibuprofen dissolved in 2%Tween80 solution was calculated through the equation y= 0.0248x-0.0103 (R=0.9996). The quantity of ibuprofen dissolving is shown in **Table 4.5**.

Table 4.5 The concentration of ibuprofen in 2% Tween80 calculated by the equation y=0.0248x-0.0103 (R^2=0.9992); in water using the equation y=0.0349x-0.0778 (R^2=0.9975)

Time (min)	Concentration (g/L) Only I	Concentration (g/L) I:G=2:1	Concentration (g/L) I:G=1:1	Concentration (g/L) I:G=1:2	Ibuprofen in water ($g/Lx10^{-3}$)
5	0.9807	1.2472	0.9618	1.0326	3.9532
10	1.3611	1.8231	1.5576	1.3191	5.7569
15	1.6647	2.2001	2.1428	1.8588	7.9988
20	1.8769	2.4123	2.2130	2.1808	10.8300
25	2.0450	2.4521	2.3423	2.3063	13.5525
30	2.2796	2.5643	2.4596	2.3678	15.8930
60	2.6168	2.6339	2.6233	2.5625	23.6455
90	2.6889	2.6488	2.6796	2.6475	29.4159
120	2.6829	2.6452	2.6882	2.6913	35.8608
180	2.7063	2.6289	2.7116	2.7163	------
240	2.6862	2.6892	2.7160	2.7158	------

4.2.2.4 Discussion

4.2.2.4.1 The predicted effect of pH on solubility

Fig. 4.4 shows that the pH of each sample decreased as the ibuprofen dissolved, and when the quantity of glucosamine increased, the pH decreased further. Using the **Eqn 3.1** ($pH= pK_a + \log ((S-S_u)/S_o)$), the relationship between pH and solubility can be calculated. Because ibuprofen is a weak acid, the relationship of S and pH in ibuprofen solution can be described as **Eqn 4.1**.

$$pH = pK_a + \log ((S - S_o)/S_o) \qquad \textbf{Eqn 4.1}$$

where S is also the overall solubility of ibuprofen and S_o is the intrinsic solubility of ibuprofen. In this ibuprofen dissolution study, the pK_a and S_o were fixed, so the relationship between pH and S should be model by the equation. When the pH decreases, the solubility should decrease. Therefore as glucosamineHCl is added, pH decreases (**Fig. 4.4**), however the dissolution of ibuprofen in the resultant solution increases. This enhancement is strong enough to overcome any changes in pH.

4.2.2.4.2 The interaction between ibuprofen and glucosamine in the dissolution study

From **Table 4.4** and **Fig. 4.5**, there were slight differences in the dissolution process in the presence of glucosamine. From the graph, the effect of glucosamine on ibuprofen dissolution is clearly shown. Glucosamine can increase the dissolution rate and the quantity dissolved, and the ratio of ibuprofen: glucosamine 2:1 had the greatest effect.

As discussed in section 4.2.2.4.1, when glucosamine was added as the hydrochloride salt, the more glucosamine was added, the lower pH in the dissolution process. This means that the quantity of ibuprofen dissolving should decrease with increasing glucosamine. However, glucosamine could still increase the dissolution rate and amount of ibuprofen dissolved. Thus glucosamine has a stronger effect on increasing

the dissolution rate.

4.2.2.4.3 Ibuprofen dissolution in 2% Tween80 solution

In the dissolution study, the same phenomenon described in section 3.3.3.3 was observed. After 3 hours dissolution, the solutions still seemed turbid. When the temperature decreased and agitation was stopped, it crystallized very quickly. The data from **Table 4.5** also showed, the solubility did not reach 2.78 g/L even with glucosamine in this study.

4.2.2.5 Conclusion

Glucosamine can improve the dissolution rate of ibuprofen in 2% Tween80 aqueous solution. Among these ratios, ibuprofen and glucosamine at 2:1 ratio gave the greatest effect on the dissolute rate. Although addition of glucosamine HCl decreased the pH, the dissolution of ibuprofen was enhanced.

CHAPTER FIVE

DIFFUSION OF IBUPROFEN ACROSS SYNTHETIC MEMBRANES

5.1 Introduction

5.1.1 Mechanism of transport across membranes

Diffusion may be defined as the spontaneous transference of a component from a region in the system where it has a high chemical potential into one where its chemical potential is lower.

There are two main mechanisms of drug transport across the gastrointestinal epithelium: transcellular, i.e. across the cells, and paracelluar, i.e. between the cells. The transcellular pathway is further divided in to simple passive diffusion, carrier-mediated transport (active transport and facilitated diffusion) and endocytosis.

In this diffusion study, the passive diffusion mechanism was investigated. This is the preferred route of transport for relatively small lipophilic molecules and thus many drugs. In this process, drug molecules pass across the membrane via passive diffusion from a region of high concentration in the donor to a region of lower concentration in the receptor. The rate of transport is determined by the physicochemical properties of the drug, the nature of the membrane and the concentration gradient of the drug across the membrane.

5.1.2 Absorption of ibuprofen

Ibuprofen is usually given as its acid form, although salt and ester formulations are available in some countries[65]. For routine clinical use, the oral route is mostly used for administration of ibuprofen, but ibuprofen has also been administered topically, intraocular, intravenously, intramuscularly and rectally. Ibuprofen is rapidly absorbed, with peak plasma or serum drug concentrations observed within 3 hours post-drug administration.[66]

The main absorption site of oral drug is small intestine. The small intestine is the longest and most convoluted part of the gastrointestinal tract, and its main functions are digestion (the process of enzymatic digestion, which begin in the stomach, is completed in the small intestine) and absorption (the small intestine is the region where most nutrients and other materials are absorbed). The luminal pH of the small intestine is between about 6 and 7.5 .[61]

5.1.3 The aims

Diffusion studies can provide information about the ability of a system to diffuse in or across the gastrointestinal epithelium. In this study, the absorption of ibuprofen in small intestine was imitated by using Franz-type diffusion cells in order to investigate the quantity of ibuprofen absorbed in 3 hours, and the interaction between ibuprofen

and glucosamine in 2% Tween80 aqueous solution.

5.2 Experimental details

5.2.1 Diffusion cells set-up

In each Franz cell (n=6), the dialysis membrane was mounted between the two halves of the diffusion cell and parafilm was used to help produce a seal between the membrane and the two compartments. Phosphate buffer solution (pH=6.8) was used as the receptor phase, and ibuprofen in different solvents or ibuprofen containing glucosamine solutions were used as the donor phase. Air bubbles were carefully removed by tipping the cell. A teflon-coated stirring bar was used in the receptor chamber to provide efficient mixing. Each cell was then placed over a magnetic stirrer. The receptor cell was maintained at 37 °C by a thermostatic water-pump which circulated water through a jacket surrounding the cell body. Then 20 ml of ibuprofen solution was pipetted into the donor chamber and, at appropriate intervals, samples of the receptor phase (2 ml) were removed and assayed by HPLC. At the same time, 2 ml fresh buffer solution was added to restore the original volume.

5.2.2 Sample preparation

Phosphate buffer (pH=6.8) was prepared by 0.60 g NaH_2PO_3 and 0.71 g Na_2HPO_3 per 100 ml distilled water.

A $1.39x10^{-2}$ g/L ibuprofen solution which was diluted in pH 6.8 phosphate buffer solution was the external standard.

Diffusion samples were taken 2 ml in every time point (5, 10, 15, 20, 25, 30, 60, 90, 120, and 180) from the sampling port, then directly assayed by HPLC (no dilutions were performed as the ibuprofen concentrations were low).

5.2.3 Method and chromatographic conditions

5.2.3.1 An external standardization method for HPLC assay

External standardization method is another analysis method for HPLC data. It does not need an internal standard, and it is considered easier to determine sample concentrations. All the data were transformed using **Eqn. 5.1**

$$Ci/Ai = (Ci)s/ (Ai)s \qquad \textbf{Eqn 5.1}$$

Where Ci is the sample concentration (unknown), Ai is the sample peak area (detected by HPLC), (Ci)s is the standard concentration (1.39 μg/ml), and (Ai)s is the peak area of the standard (measured by HPLC, n=3). A coefficient called Ks in this relationship can be introduced by Ks= (Ci)s/(Ai)s, **Eqn 5.1** can be rewritten to **Eqn 5.2**.

$$Ci = Ks \times Ai \qquad \textbf{Eqn 5.2}$$

After HPLC assay, the Ks value of this experiment was calculated as 0.000127. All the sample concentrations were then calculated using **Eqn 5.2**, and the quantity of ibuprofen determined following correction of dilution resulting from sampling.

5.2.3.2 Chromatographic conditions

Chromatographic conditions were as described in section 4.2.2.2, but with a sample injection volume of 10 μl.

5.3 Results and Discussion

5.3.1 Selection of a surface membrane

There were two kinds of membranes to be considered in this diffusion study. One was a chemical dialysis membrane (membrane 1); the other was a silicone sheeting membrane (0.05 μm) (membrane 2). Using ibuprofen saturated aqueous solution and 1g/L ibuprofen in 2% Tween80 aqueous solution in the donor, and pH 6.8 buffer solutions in the receptor, ibuprofen diffusion through the two membranes was compared. The results are shown on **Table 5.1** and **Fig. 5.1**. This was a development study and was carried out once only.

Table 5.1 Ibuprofen diffusion through two membranes: (1) dialysis membrane, (2) silicone sheeting membrane

Time(min)	Ibuprofen in water (1) (10^{-3} mg/ml)	Ibuprofen in water (2) (10^{-3} mg/ml)	Ibuprofen in 2%Tween80 (1) (10^{-3} mg/ml)	Ibuprofen in 2%Tween80 (2) (10^{-3} mg/ml)
5	0.0038	0.2033	1.3943	1.0395
10	0.1561	0.2255	2.3067	3.0239
15	0.3285	0.2973	3.3946	4.6298
20	0.5764	0.4018	3.8303	6.6078
25	0.8867	0.5161	4.4079	7.8380
30	1.1642	0.6789	5.0674	9.2742
60	1.6123	2.2214	10.9567	19.7839
90	3.2737	4.4524	15.3708	30.7580
120	4.9826	6.2267	19.6897	41.0358
180	5.3150	7.2544	30.7295	55.8831

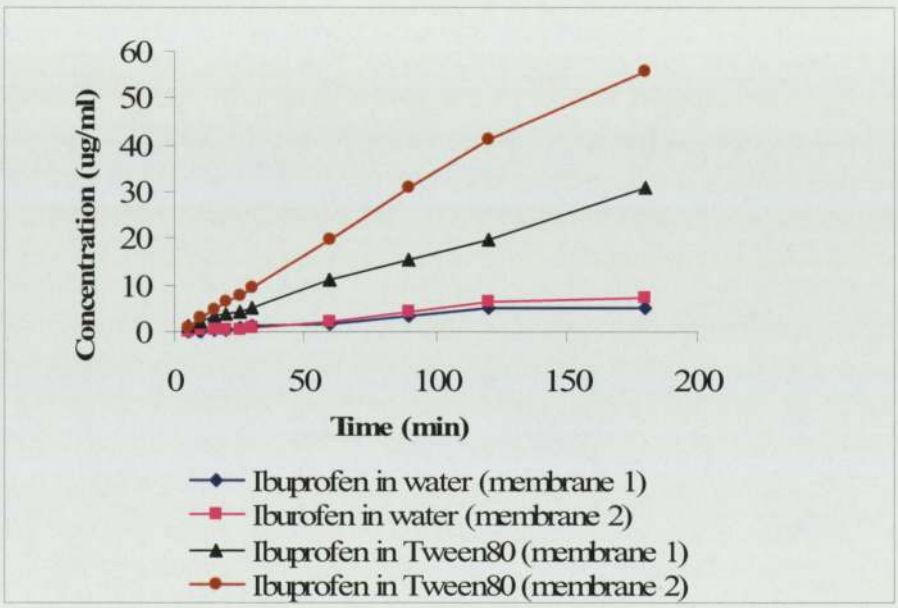


Fig. 5.1 The diffusion of ibuprofen through different membranes

From the data and the graph above, the rate of ibuprofen diffusion was faster through silicone sheeting membrane (2) than the dialysis membrane (1), especially for

ibuprofen in 2% Tween80. Diffusion through the dialysis membrane was less variable and this was selected for further study.

5.3.2 The diffusion of ibuprofen from different media including glucosamine

Table 5.2 and **Fig. 5.2** show the diffusion of ibuprofen from different solvents across dialysis membrane. The solutions used were ibuprofen saturated solutions in distilled water, pH 6.8 phosphate buffer solution and 2%Tween aqueous solution. Samples were taken at every time point (in section 5.2.2, n=3), and assayed by HPLC.

Table 5.2 Diffusion of ibuprofen from different donor media (n=3; mean±s.d.)

Time (min)	water (mg/ml)	pH 6.8 buffer solution (mg/ml)	2% Tween80 solution (mg/ml)
5	0.0156±0.0007	0.0111±0.0009	0.0303±0.0049
10	0.0173±0.0013	0.0172±0.0032	0.0537±0.0066
15	0.0197±0.0018	0.0188±0.0010	0.0776±0.0081
20	0.0211±0.0018	0.0222±0.0006	0.0939±0.0060
25	0.0224±0.0011	0.0330±0.0010	0.1115±0.0022
30	0.0242±0.0020	0.0417±0.0010	0.1274±0.0055
60	0.0369±0.0022	0.1442±0.0118	0.2687±0.0076
90	0.0629±0.0042	0.2604±0.0199	0.3995±0.0150
120	0.0663±0.0045	0.3829±0.0376	0.4964±0.0117
180	0.0913±0.0011	0.6133±0.0743	0.7819±0.0124

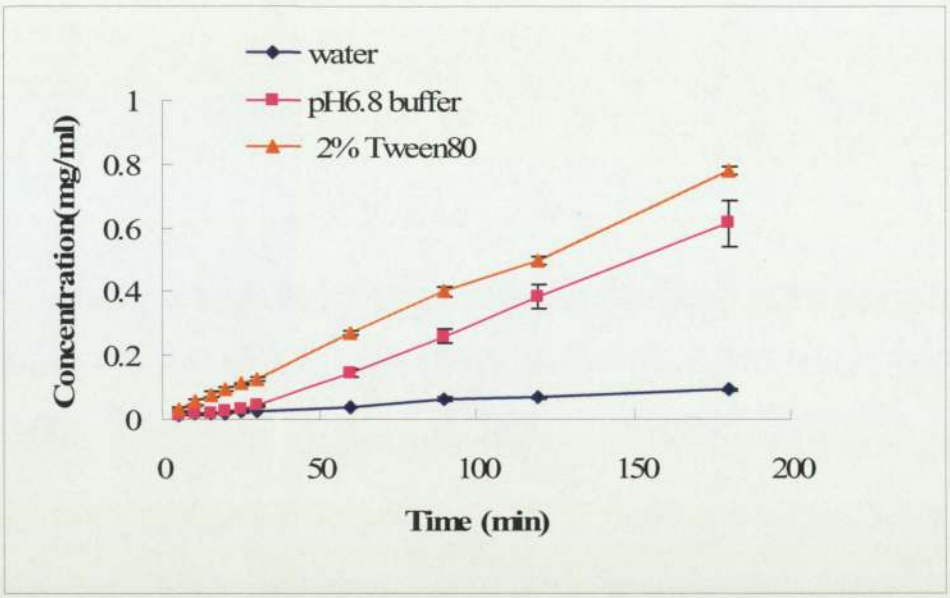


Fig. 5.2 The diffusion of ibuprofen from different donor solutions through dialysis membrane (n=3; mean±s.d.)

From **Fig. 5.2**, it can be seen that ibuprofen in 2% Tween80 resulted in faster diffusion than the other solutions. The main factor is that the solubility of ibuprofen in 2% Tween80 is higher than in other two solvents. The pH of water used in the experiment was pH 5.3. Thus when pH 6.8 buffer solution was used, diffusion was enhanced due to the greater solubility of ibuprofen. Because ibuprofen is a weak acid, its solubility will be higher in a pH higher solution.

5.3.3 Diffusion of ibuprofen from 2%Tween80 solution containing glucosamine

The donor solution was made from 2.78 mg/ml ibuprofen stock solution containing different ratios of glucosamine. Samples were taken at every time point (n=3),

assayed by HPLC. The results are shown in **Table 5.3** and **Fig. 5.3**.

Table 5.3 Diffusion of ibuprofen from 2% Tween80 aqueous solutions containing glucosamine (n=3; mean±s.d.)

Time(min)	Only I (mg/ml)	I:G = 2:1 (mg/ml)	I:G = 1:1 (mg/ml)	I:G = 1:2 (mg/ml)
5	0.0303±0.0049	0.0501±0.0001	0.0257±0.0020	0.0532±0.0017
10	0.0537±0.0066	0.0633±0.0024	0.0507±0.0032	0.0863±0.0021
15	0.0776±0.0081	0.0858±0.0054	0.0729±0.0051	0.1064±0.0053
20	0.0939±0.0060	0.1047±0.0115	0.0911±0.0110	0.1295±0.0052
25	0.1115±0.0022	0.1110±0.0079	0.1124±.00086	0.1438±0.0017
30	0.1274±0.0055	0.1362±0.0156	0.1299±0.0056	0.1651±0.0048
60	0.2687±0.0076	0.2653±0.0046	0.3014±0.0143	0.3133±0.0100
90	0.3995±0.0150	0.3884±0.0173	0.4765±0.0105	0.4693±0.0034
120	0.4964±0.0117	0.4912±0.0157	0.6280±0.0245	0.6298±0.0234
180	0.7819±0.0124	0.7277±0.0308	0.9863±0.0378	0.8940±0.0198

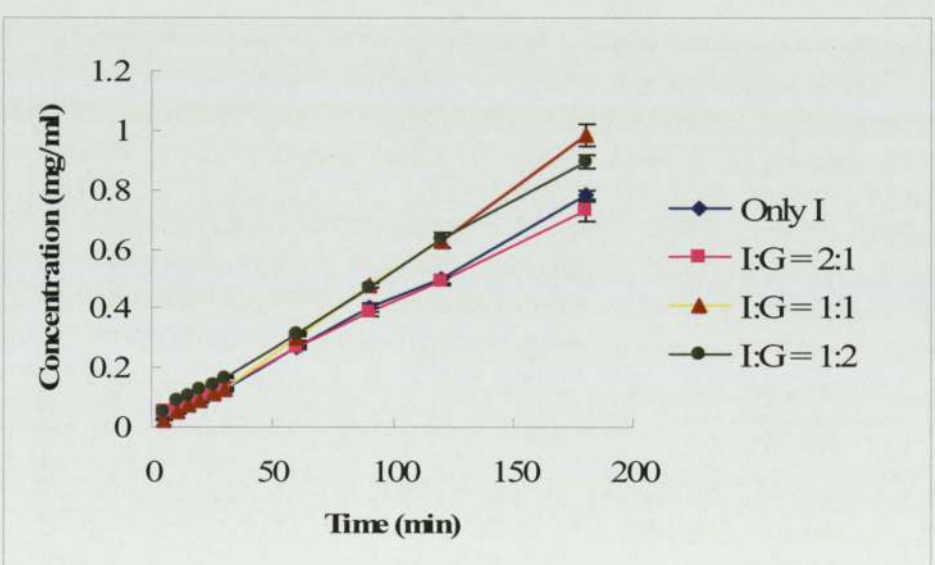


Fig. 5.3 Ibuprofen diffusion from 2% Tween80 aqueous solution containing glucosamine (n=3; mean±s.d.)

As discussed in the section 3.3.4, glucosamine in the ratio of 1:1 and 1:2 can slightly increase the dissolution of ibuprofen from 2% Tween80 aqueous solution. In this study, the ibuprofen saturate solutions were used in the donor chamber, and they contained different quantities of glucosamine. The concentration of ibuprofen in the presence of glucosamine should be different as discussed in section 3.3.4. When glucosamine was added, the diffusion of ibuprofen was affected. Some ratios increased the diffusion rate of ibuprofen (I: G = 1:1, I: G = 2:1); however, when the rate of ibuprofen and glucosamine was 2:1, the diffusion rate of ibuprofen was not significantly different to ibuprofen in 2% Tween80 with no glucosamine. Thus in the diffusion study, glucosamine had different effects on the diffusion of ibuprofen as the ratio of glucosamine changed. When ibuprofen and glucosamine were 1:1, the diffusion was maximal.

During the diffusion process, pH was monitored, but there was almost no change in the whole process. As only relatively low amount of ibuprofen can diffuse to the receptor, the buffer is strong enough to adjust the pH. Therefore there was no pH effect in the diffusion study, and on the other hand, it proved glucosamine could increase the solubility of ibuprofen in a certain ratio. Thus, the increased diffusion is due to the greater activity of ibuprofen in the presence of glucosamine.

5.4 Conclusion

In the diffusion study, the drug concentration was an important factor. The rate is increased as the concentration of ibuprofen increases. Glucosamine can also affect the diffusion of ibuprofen, and the ibuprofen: glucosamine ratio 1:1 gave a the fastest diffusion rate of ibuprofen.

CHAPTER SIX

PROTEIN BINDING OF IBUPROFEN

IN THE PRESENCE OF GLUCOSAMINE

6.1 Introduction

The interactions of proteins with various ligands can create the basis of an interlocked set of dynamic processes providing a communication and regulation pathway within, and between, different structures of a living organism. Drug binding to specific plasma transport proteins [albumin (HSA), α_1-acid glycoprotein (AAG), lipoprotein, etc.], is an integral part of many other types of intermolecular interactions within a cellular environment. Different aspects of drug-protein interactions have been reviewed recently, including their molecular nature, biological function, pharmacological significance as well as the methodological approaches applied in investigations and their potential shortcomings.[67-70]

According to Oravcová[71], the techniques used *in vitro* are usually based on one of the following procedures: 1. separation of free and protein-bound fractions of ligand, i.e. determination of the concentration of free ligand; 2. detection of a change in a physicochemical property of the complex ligand; 3. detection of a change in a physicochemical behavior of the binding protein.

Ibuprofen is widely used in the treatment of rheumatoid arthritis and related conditions[72] and it is strongly bound to plasma proteins, particularly albuium.[73] As it is commonly believed that only unbound drug is available for disposition and responsible for pharmacological activity, [74] a considerable attention has been given to the binding of highly bound drugs.

Similarly to other NSAIDs, ibuprofen displays extensive (99%) binding to plasma proteins.[75] The association constant for the binding of ibuprofen to purified human serum albumin (HSA) has been reported to be about 10^6 M^{-1} [76] and is primarily bound to site II of the albumin molecule.[77] **(Fig. 6.1)**

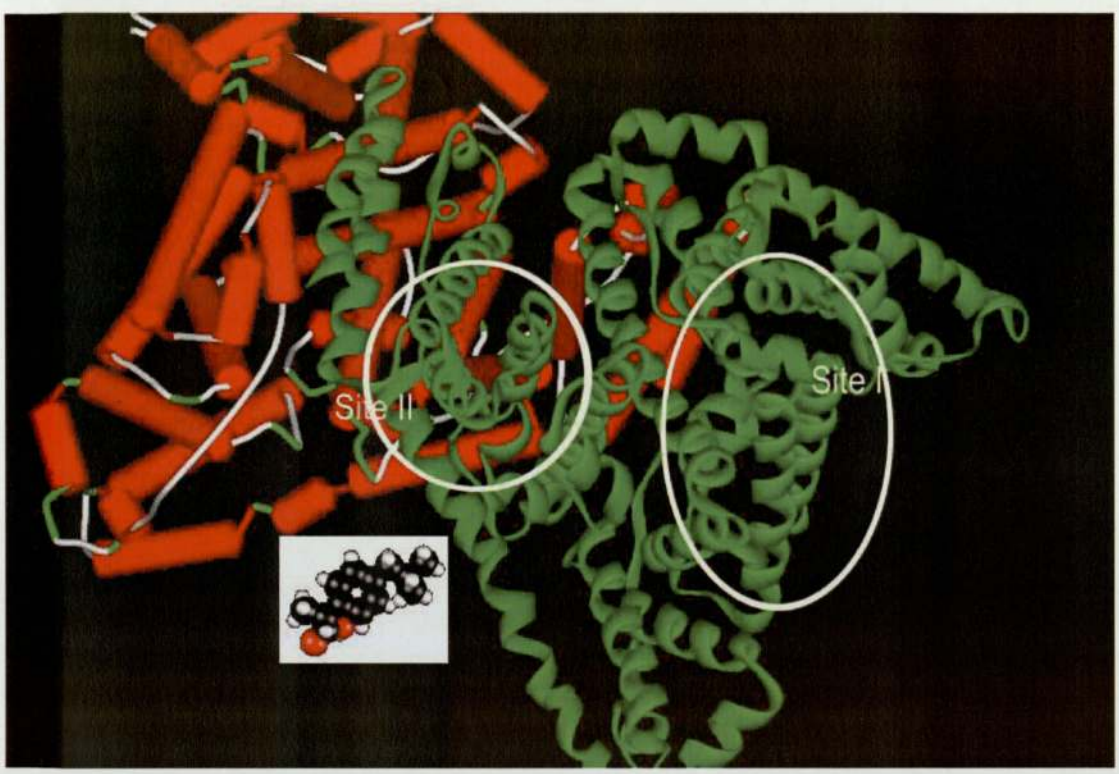


Fig. 6.1 Ibuprofen binding sites (Data obtained from protein data bank, modeled using software viewlite)

In this study, the binding of ibuprofen, in the presence of glucosamine, to human serum albumin (HSA), human plasma, and mouse plasma was studied. The interaction between ibuprofen and glucosamine, and the comparison of ibuprofen binding with different plasma were investigated.

6.2 Sample preparation

HSA and ibuprofen were dissolved in pH 7.4 phosphate buffer (a mixture of 19.6 ml KH_2PO_4 and 80.4 ml Na_2HPO_4 per 100 ml).

50 ml human plasma collected from a healthy volunteer and 40 ml mouse plasma were used. Anticoagulant was added into the plasma (i.e. sodium citrate 1:9 v/v). All the blood samples were centrifuged at 4000 rpm for 20 minutes. The serum was transferred by pipette to 2 ml microcentrifuge tubes and frozen at -70 °C until used.

The samples in the dialysis tubes consisted of 40 mg/ml HAS (or 1ml blood serum), 50 mg/L (or100 mg/L) ibuprofen, and 5µl ^{14}C-labelled ibuprofen. The calibration of ibuprofen diffusion from the dialysis tube was carried before the binding experiment.

6.3 Method- ^{14}C radiolabeled method

One of the problems in accurately measuring the binding of ibuprofen is the sensitivity of the assay method. Since ibuprofen is an extremely highly bound drug, its unbound concentration in plasma is very low[78]. It is quite difficult to measure very low concentrations of free ibuprofen. Using a radiolabel method was the simplest method to determine the trace quantity concentrations of drug. ^{14}C-ibuprofen with a

special radioactivity was mixed with "cold" ibuprofen and the counts per minute measured for the starting samples and together with the unbound ibuprofen. 1600TR Liquid Scintillation Analyzer (from Packard A Canberra Company) was used to determine the radio activity of free ibuprofen.

Fig. 6.2 shows the experimental set up. The dialysis tube was placed in 50 ml cylinders, using pH 7.4 phosphate buffer solutions as the receptor solution. 1 ml samples were taken every hour (n=3), and detected by the scintillation counting, and the amount of ibuprofen in the dialysis tube was then determined.

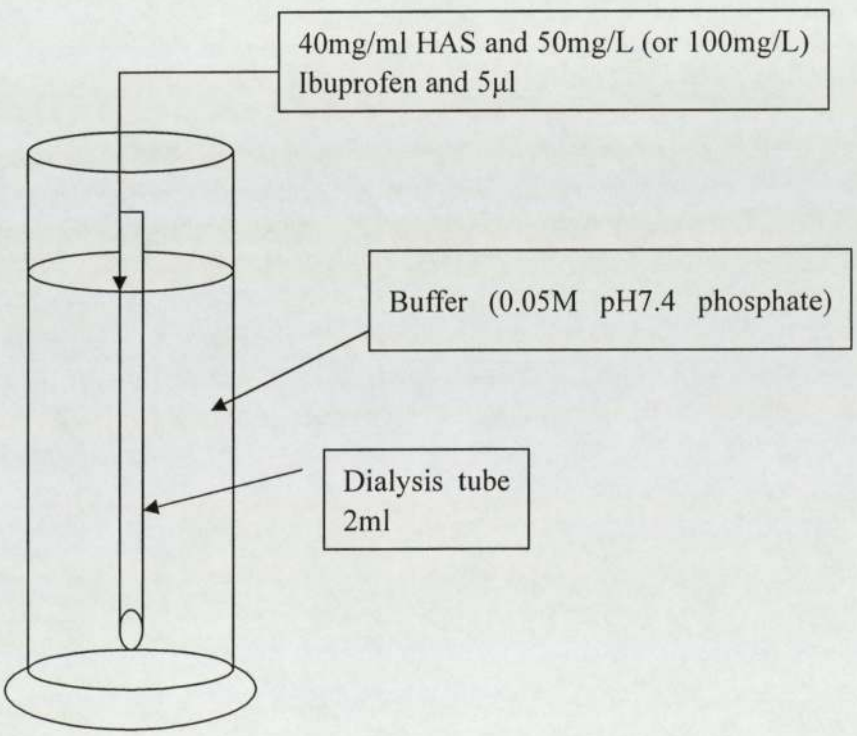


Fig. 6.2 Experimental setup for protein-binding studies

6.4 Results and discussion

6.4.1 Ibuprofen binding with Human serum albumin (HSA)

Using ibuprofen concentrations of 50 mg/L or 100 mg/L, and containing glucosamine (I:G=1:2), the results are shown on **Table 6.1**, **6.2** and **Fig. 6.3**, **6.4**. When the concentration of ibuprofen was 50 mg/L, ibuprofen was very strongly bound to HSA, between 98%-99%. As glucosamine was added, there were no significant changes in the binding (**Fig. 6.3**).

However, when the concentration of ibuprofen used was 100 mg/L, HSA binding was 94%-99%, and the presence of glucosamine further reduced the binding to 94.50% after 12 hours. When glucosamine was included, the ibuprofen binding decreased around 1%. Thus glucosamine can affect binding of ibuprofen to HSA. However, this was only detected at high levels of ibuprofen.

Table 6.1 HSA-binding of 50 mg/L ibuprofen with glucosamine (1:2) (n=3; mean±s.d.)

Time (hour)	Ibuprofen (%)	I: G = 1:2 (%)
1	98.71±0.0035	98.54±0.0076
2	98.88±0.0021	98.35±0.0108
3	98.89±0.0018	98.65±0.0055
4	98.68±0.0017	98.56±0.0040
5	98.59±0.0023	98.58±0.0040
6	98.53±0.0024	98.42±0.0049
7	98.30±0.0032	98.39±0.0048
8	98.19±0.0030	98.40±0.0048
9	98.23±0.0026	98.41±0.0047
10	98.05±0.0031	98.42±0.0037
11	98.32±0.0024	98.29±0.0026
12	98.06±0.0044	98.55±0.0026

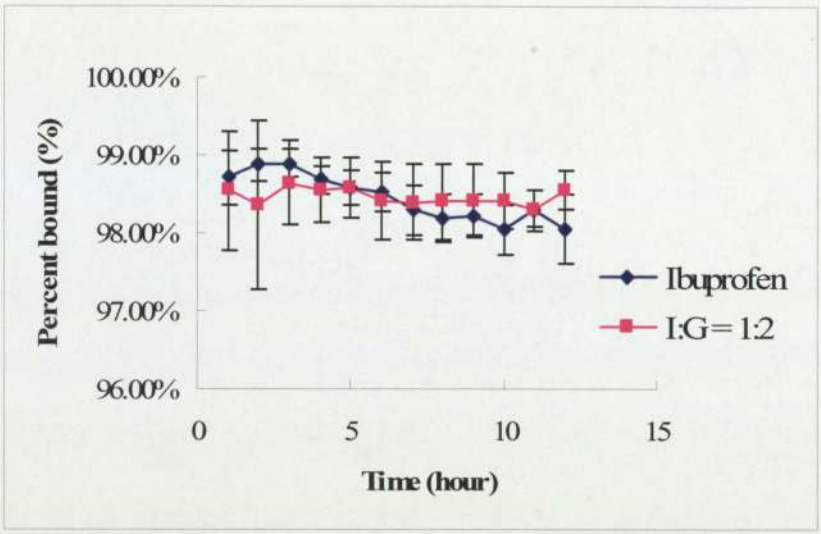


Fig. 6.3 HSA-binding of ibuprofen (50 mg/L) in the presence of glucosamine (1:2) (n=3; mean±s.d.)

Table 6.2 HSA-binding of ibuprofen (100 mg/L) in presence of glucosamine (1:2) (n=3; mean±s.d.)

Time (hour)	Ibuprofen (%)	I: G = 1:2 (%)
1	98.88±0.0099	98.10±0.0038
2	97.58±0.0235	97.51±0.0032
3	97.34±0.0057	97.66±0.0033
4	97.37±0.0011	97.17±0.0048
5	97.04±0.0007	96.54±0.0059
6	97.02±0.0033	96.15±0.0066
7	96.85±0.0052	95.61±0.0090
8	96.62±0.0059	95.63±0.0075
9	96.62±0.0060	94.58±0.0299
10	96.75±0.0057	94.95±0.0085
11	96.21±0.0057	94.68±0.0106
12	96.05±0.0020	94.47±0.0084

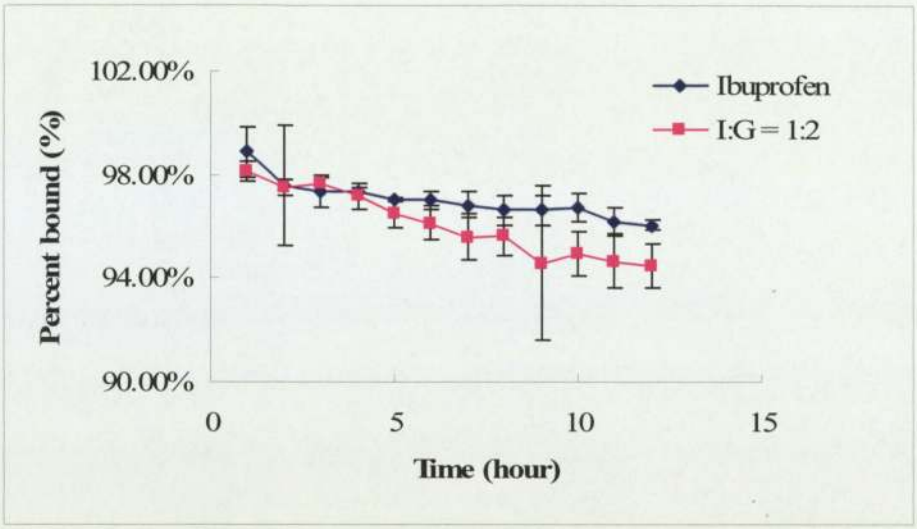


Fig. 6.4 HSA-binding of ibuprofen (100 mg/L) in the presence of glucosamine (1:2) (n=3; mean±s.d.)

6.4.2 Ibuprofen binding with normal human plasma protein

The experiment was developed using human blood instead of HSA. The fraction of ibuprofen bound to plasma protein decreased significantly (from 98%-99% for HSA to 95%-97% for human serum). The data for 50 mg/L and 100 mg/L ibuprofen binding to human plasma protein are shown in **Tables 6.3**, **6.4**; and the curve shown in **Figs. 6.5**, **6.6**. When the concentration of ibuprofen increased to 100 mg/L, the binding rate decreased rapidly, after 12 hours it was only around 90%. Another important factor was the properties of the dialysis tubes. Because the whole body of the dialysis tubes consists of dialysis membranes, it gave a direct effect on the results. Normally diffusion between the membranes would reach the equilibrium after 4-5 hours that was why the data in the first several hours fluctuated.

Table 6.3 Human plasma-binding of 50 mg/L ibuprofen in the presence of glucosamine (1:2) (n=3; mean±s.d.)

Time (hour)	Ibuprofen (%)	I: G = 1:2 (%)
1	96.44±0.0106	97.48±0.0082
2	96.11±0.0419	96.79±0.0024
3	96.07±0.0101	96.22±0.0077
4	96.22±0.0085	96.43±0.0066
5	96.57±0.0075	96.12±0.0033
6	96.21±0.0117	95.97±0.0038
7	96.05±0.0096	96.02±0.0037
8	95.84±0.0083	95.92±0.0054
9	95.77±0.0067	96.14±0.0054
10	95.74±0.0066	95.93±0.0060
11	95.82±0.0142	95.82±0.0050
12	95.31±0.0012	95.07±0.0238

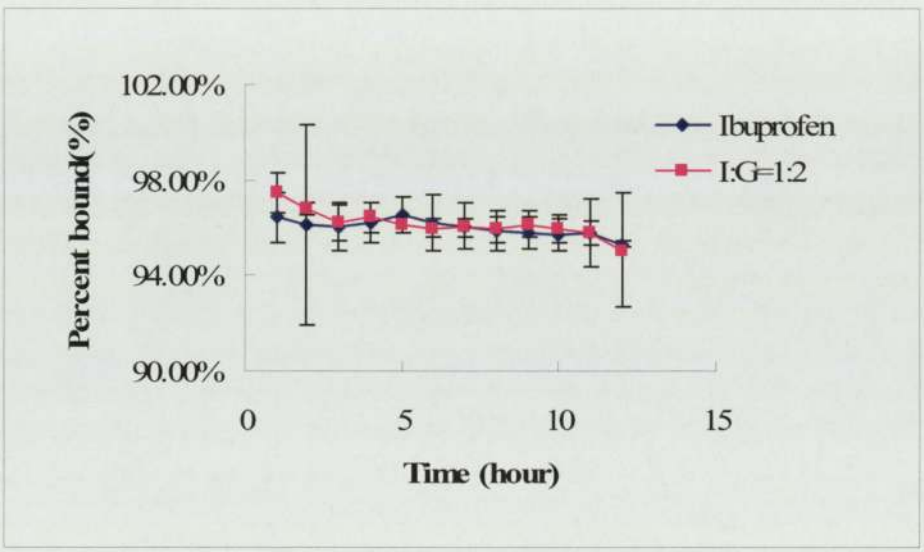


Fig. 6.5 Human plasma-binding of ibuprofen (50 mg/L) in the presence of glucosamine (1:2) (n=3; mean±s.d.)

Table 6.4 Human plasma-binding of ibuprofen (100mg/L) in the presence of glucosamine (1:2) (n=3; mean±s.d.)

Time (hour)	Ibuprofen (%)	I: G = 1:2 (%)
1	99.92±0.0007	100.00±0.000
2	95.46±0.0191	97.59±0.0058
3	94.31±0.0312	95.94±0.0320
4	93.92±0.0037	93.86±0.0130
5	93.15±0.0109	92.92±0.0088
6	92.45±0.0128	92.47±0.0197
7	92.70±0.0129	92.45±0.0158
8	92.12±0.0141	92.55±0.0282
9	91.84±0.0123	91.89±0.0196
10	90.94±0.0130	91.51±0.0192
11	89.91±0.0122	91.62±0.0202
12	89.94±0.0181	91.58±0.0146

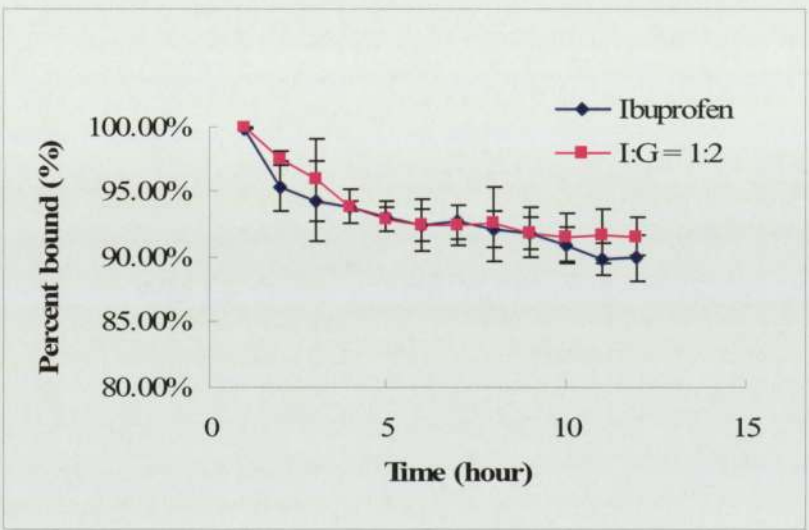


Fig 6.6 Human plasma-binding of ibuprofen (100mg/L) in the presence of glucosamine (1:2) (n=3; mean±s.d.)

In this section, ibuprofen binding with normal human blood plasma, there was no significant difference when glucosamine was present. Therefore, it appears that glucosamine does not affect binding of ibuprofen to human plasma under the

conditions studied.

6.4.3 Ibuprofen binding with mouse plasma protein

The experiments were repeated using blood from Balb-C mice. The results are shown in **Tables 6.5**, **6.6** and **Figs. 6.7**, **6.8**. The ibuprofen to mouse plasma protein was much lower than the other two samples, the lowest binding was only 80%. A possible problem is that there was not enough anti-coagulant in the plasma sample, and some serum coagulated before samples were centrifuged and frozen.

However, clear differences are seen with glucosamine and ibuprofen. Binding of both 50 and 100 mg/L ibuprofen was affected by glucosamine. Furthermore, as the concentration of ibuprofen increased, glucosamine had a greater effect.

Table 6.5 Mouse plasma-binding of ibuprofen (50mg/L) in the presence of glucosamine (1:2) (n=3; mean±s.d.)

Time (hour)	Ibuprofen (%)	I: G = 1:2 (%)
1	99.55±0.0002	99.57±0.0001
2	94.07±0.0284	85.94±0.0095
3	90.31±0.0196	85.85±0.0292
4	87.13±0.0151	85.83±0.0240
5	84.60±0.0062	81.13±0.0299
6	81.77±0.0148	74.90±0.0260
7	81.40±0.0065	76.07±0.0099
8	83.07±0.0228	75.51±0.0046
9	84.05±0.0097	76.25±0.0091
10	85.10±0.0188	77.12±0.0152
11	86.91±0.0100	77.72±0.0150
12	91.00±0.0196	79.57±0.0113

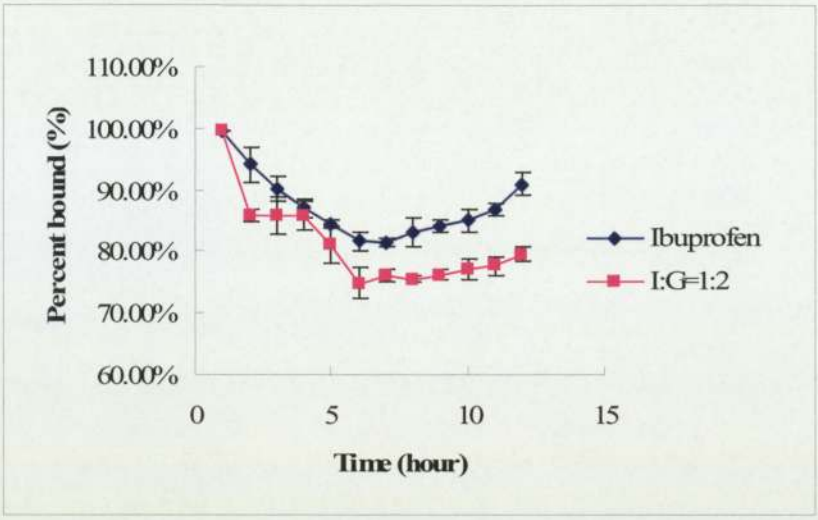


Fig. 6.7 Mouse plasma-binding of ibuprofen (50 mg/L) in the presence of glucosamine (1:2) (n=3; mean±s.d.)

Table 6.6 Mouse plasma-binding of ibuprofen (100 mg/L) in the presence of glucosamine (1:2) (n=3; mean±s.d.)

Time (hour)	Ibuprofen (%)	I: G = 1:2 (%)
1	99.75±0.0002	99.61±0.0004
2	96.75±0.0153	97.83±0.0125
3	95.61±0.0188	92.31±0.0170
4	95.00±0.0069	90.62±0.0123
5	92.79±0.0150	89.94±0.0103
6	92.31±0.0162	88.79±0.0079
7	91.37±0.0079	88.39±0.0064
8	89.70±0.0153	88.24±0.0065
9	89.56±0.0168	87.31±0.0058
10	88.48±0.0262	85.46±0.0060
11	87.78±0.0182	84.49±0.0071
12	87.08±0.0250	84.05±0.0099

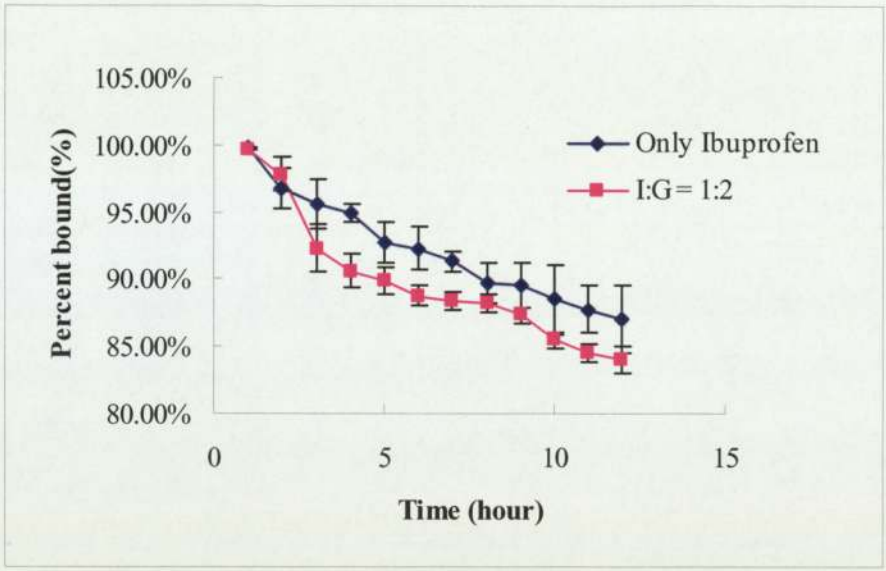


Fig. 6.8 Mouse plasma-binding of ibuprofen (100 mg/L) in the presence of glucosamine (1:2) (n=3; mean±s.d.)

6.5 Summary

Radiolabeled drug was used in this study, in order to detect the very low levels of free ibuprofen. Binding of ibuprofen (or with glucosamine) was studied in three different protein samples. The free ibuprofen was more bound with human plasma serum and mouse plasma serum than with HSA. When glucosamine was present, it reduced binding with HSA and mouse plasma at both ibuprofen concentrations; but no significant changes were found with 50 mg/L ibuprofen in HSA, and normal human plasma protein.

CHAPTER SEVEN

GENERAL SUMMARY

Ibuprofen is a non-steroidal anti-inflammatory (NSAID), analgesic and antipyretic drug that is used extensively in the treatment of several forms of arthritis and additionally in the treatment of mild to moderate pain and for antipyresis[79]. It has poor solubility in distilled water and high binding with plasma protein.

Glucosamine is an essential intermediate in the biosynthetic pathway of proteoglycans, which are the primary building blocks of connective tissue and cartilage. It is also widely used in many research fields. The important reason in this project is it can enhance the analgesic efficacy and solubility of ibuprofen. The aim of this project was to explore interaction between ibuprofen and glucosamine.

In this study, 2% Tween80 aqueous solution was used to increase the solubility of ibuprofen and ibuprofen containing different ratios of glucosamine. The solubility study discussed in chapter 3 showed that 2% Tween80 can greatly increase the solubility of ibuprofen from 0.044 mg/ml in distilled water to 2.656 mg/ml. When glucosamine was added, this was further increased to 2.727 mg/ml for the ratio of ibuprofen: glucosamine 1:1.

Further investigation of ibuprofen dissolution is discussed in chapter 4. During the process of ibuprofen dissolution, pH will decrease as ibuprofen dissolves. When glucosamine was added in the form of the hydrochloride salt, pH will further decrease,

and the pH was shown to decrease with the amount of glucosamine added. Therefore, ibuprofen dissolution was affected by the presence of glucosamine with dissolution increasing as pH fell.

In order to investigate the permeation of ibuprofen in small intestine, diffusion experiment was established in our study. Ibuprofen was dissolved in different media to produce saturated solutions. It was found that ibuprofen in 2% Tween80 aqueous solution diffused more and faster than from any other solutions as shown in **Fig. 5.2**. This phenomenon was because that the concentration of the ibuprofen in 2% Tween80 was higher. When glucosamine was present in the donor, the diffusion rate and amount were slightly further increased, and the best result was given when the ratio of ibuprofen and glucosamine was 1:1. There was no evidence of any complexation altering diffusion of ibuprofen in the presence of glucosamine.

In chapter six, plasma proteins from three different sources were used to investigate the effects of glucosamine on ibuprofen binding. The same concentration of ibuprofen and glucosamine was used to repeat the experiment with different plasma proteins. The results showed that there was no significant difference in HSA and human blood serum binding, but the extent binding of was reduced in binding with mouse plasma in present of glucosamine. This was probably due to the error of the counting machine and also could be possible because the concentration of free ibuprofen was too low to

detect accurately.

In conclusion, this study has examined the interaction between ibuprofen and glucosamine in solubility, diffusion, dissolution and plasma binding. Glucosamine can affect some properties of ibuprofen. However, whether glucosamine can enhance the function of ibuprofen also need further investigation.

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