THE IN VIVO AND IN VITRO STUDIES OF DRUG MILK: PLASMA DISTRIBUTION AND ASSESSING THE RISK TO INFANT

HU YULAN

DEPARTMENT OF PHARMACY

NATIONAL UNIVERSITY OF SINGAPORE

2005

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HU YULAN
(M.Sc., NUS)

A THESIS SUBMITTED
FOR THE DEGREE OF MASTER OF SCIENCE
DEPARTMENT OF PHARMACY
NATIONAL UNIVERSITY OF SINGAPORE
2005

ACKNOWLEDGEMENTS

I would like to express my profound gratitude to my supervisor, Associate Professor Eli Chan Wing Yuen, for his warm encouragement, excellent guidance and constructive suggestion during the course of this project. I wish to thank Associate Professor Paul WS Heng, Head of the Department of Pharmacy and Associate Professor Go Mei Lin, previous head of the department, for their constant support. I am also very grateful to the National University of Singapore for providing me with the research scholarship and excellent research facilities for my study.

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SUMMARY

Depression commonly affects women, particularly during their childbearing year and after childbirth. The rate of new psychiatric episodes in women increased markedly in the first few months after childbirth with 10 % of mothers experiencing postnatal depression. The issue of prescribing antidepressant drugs during lactating is clinically important, but also complex, because the decision on medication during the postpartum period is a difficult balancing act between maternal and infant safety. Data for some drugs are completely lacking and that for other drugs, information is only available for single dose or short-term studies or case reports. Therefore, the in vitro prediction of drug milk-to-plasma concentration ratio (M/P) will be of great value.

The present study was thus carried out to examine the relationship between in vitro and in vivo drug distribution, using sertraline and bupropion as the two model antidepressant drugs, and rabbit as an in vivo model, with the main objectives as follows. Firstly, to explore possible factor(s) that may affect in vitro drug milk distribution of sertraline and bupropion (BUP), and secondly, to study the effect of different stages of lactating period on in vivo and in vitro milk plasma distribution.

A stability-indicating High Performance Liquid Chromatograph (HPLC) assay for BUP was developed to determine the milk and plasma concentrations of BUP in the presence of its degradation products. The method was validated to be specific and accurate, and was successfully applied to in vivo pharmacokinetic study, in vitro milk: plasma distribution, and in vitro protein binding determination.

A new relationship between log milk:lipid partition coefficient (log k_f) and physicochemical parameters, such as molecular weight hydrophilic-lipophilic balance (MW_HLB), volumetric hydrophilic-lipophilic balance (V_HLB), percent hydrophilic surface area (HSA), was developed and validated with regard to M/P ratio prediction using 55 selected drugs and relevant literature data. When compared to the conventional relationship of log k_f and LogP (log octanol:water apparent partition coefficient). The prediction of M/P ratio based on the former appeared to perform better than that based the latter.

The in vivo experimental (M/P ratio) and in vitro data obtained in the present study suggest that the stage of lactating period has a great effect on the drug milk:plasma distribution. Thus it is better to avoid breastfeeding in early lactating period by mothers who are on medication and have a higher M/P ratio during the colostrum period.

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CHAPTER *1*

INTRODUCTION

1.1. General Introduction

Depression is one of the most common forms of mental disorder in the general population, affecting about 15% of the general population and accounting for approximately 10% in primary care[1].

In any given one-year period, 9.5 percent of the population, or about 18.8 million American adults, suffer from a depression[2]. The economic cost for this disorder is high, but the cost in human suffering cannot be estimated.

Diagnostic and Statistical Manual IV (DSM IV)[3], published by the American Psychiatric Association, was adopted for the diagnosis of depression. It describes 3 unipolar disorders including major depressive disorder, dysthymic disorder and depressive disorder not otherwise specified.

For a diagnosis of a major depression, five (or more) of the following symptoms must have been present nearly every day during the same two-week period and represent a change from previous functioning. At least one of the symptoms is either depressed mood or loss of interest or pleasure in daily activities[3].

1. Depressed mood most of the day.
2. Markedly diminished interest or pleasure in (almost) all activities most of the day.

3. Significant weight loss (when not dieting) or gain (eg, greater than 5% of body weight in a month), or change in appetite.

4. Insomnia or hypersomnia.

5. Psychomotor agitation or retardation (observable by others).

6. Fatigue or loss of energy.

7. Feelings of worthlessness or inappropriate guilt.

8. Diminished ability to think or concentrate, or indecisiveness.

9. Recurrent thoughts of death (not just fear of dying), recurrent suicidal ideation, a specific suicidal plan, or suicide attempt.

In addition, the symptoms should:

1. Not meet the criteria for a mixed mood episode.

2. Cause clinically significant distress or impairment in social, occupational, or other important areas of functioning.

3. Not be due to the direct physiologic effects of a substance (eg, an abused drug, a medication) or a general medical condition.

4. Not be better accounted for by bereavement (i.e., a diagnosis of depression can be considered if, after the loss of a loved one, symptoms persist for longer than two months or are characterized by marked functional impairment, morbid preoccupation with worthlessness, suicidal ideation, psychotic symptoms, or psychomotor retardation).

Depression is often a correlation of physical illness or can be a direct response to illness. It can also be the result of the changes in life circumstances, interpersonal disorder or loss, and intrapsychic conflict. Very often, a combination of genetic, psychological, and

environmental factors is involved in the onset of a depressive disorder[4]. Depression strikes women twice as often as it does men[5].There is also a higher incidence of depression in married women with children than in childless married women[6]. Many hormonal factors may contribute to the increased rate of depression. In women particularly such factors as menstrual cycle changes, pregnancy, miscarriage, postpartum period, pre-menopause, and menopause. It has long been recognized that women are more liable to become depressed during the postpartum period[7]. The hormonal and physical changes, as well as the added responsibility of a new life, can be factors that lead to postpartum depression in some women.

1.2. Postpartum depression (PPD)

At least one in ten women experiences depression in the weeks or months after the birth of a baby. While many recover spontaneously within a few months, one-third to one-half still have features of depression 6 months after delivery, and some go on to develop a chronic or recurrent mood disorder[8,9]. Depression in the early postpartum months can have important effects on the mother and her baby, and on other family relationships[10].

1.2.1. Classification

Three types of postpartum disorder have been defined: posrpartum blues (also known as maternal or baby blues), postpartum neurotic depression (also known as puerperal neurosis) and puerperal psychoses.

Postpartum blues occur in about 50% to 70% of puerperal women[11,12]. The syndrome is transitory, resolving spontaneously within a few hours to 2 weeks[13]. It is characterized by

intermittent mild fatigue, crying, anxiety, difficulty of thinking clearly and sleep disturbances.

Postpartum depression begins within the first four weeks postpartum. It is said to affect 10% of all childbearing women[14,15]. The syndromes disable the patient for more than 2 weeks and is characterized by a depressed mood and difficulty coping, particularly within the infant[16].

Psychoses occur in 1 to 2 per 1000 postpartum women; they may present as schizophrenic or affective disorders or as confusional state[17]. Due to the relatively rare occurrence of this disorder and the absence of specific symptoms, puerperal affective psychosis is treated similar to non puerperal affective psychosis[18].

1.2.2. Etiology

There are many factors that may contribute to the increase in pregnancy-associated affective syndromes. Hormonal factors play a major role in influencing central nervous functioning. Women who develop PPD may be particularly sensitive to the marked hormonal changes associated with the pregnancy. Of course, other factors are also important such as genetics, socioeconomic issues, stress, and emotional support system for the new mother[19,20].

1.2.3. Diagnosis

The DSM-IV-TR[4] does not classify postpartum psychiatric disorders as diagnostic categories but allows the specifier "with postpartum onset" to be applied to major depressive disorder, bipolar disorder (type I or II) and brief psychotic disorder if the onset

of symptoms occurs within the four weeks following childbirth. The DSM-IV-TR does not allow the specifier to be applied to other psychiatric disorders.

1.2.4. Treatment

Postpartum depression is successfully treated with medications, psychotherapy, or a combination of both[21,22]. Pharmacologic treatment is preferred to psychotherapeutic intervention in patients with more severe or chronic symptoms, prior episodes or family histories, or a prior response to treatment. Medications have the advantage of being less costly and time-consuming.

There are several types of antidepressant medications used to treat depressive disorders. These include newer medications chiefly the selective serotonin reuptake inhibitors (SSRIs), the tricyclics, and the monoamine oxidase inhibitors (MAOIs). The SSRIs and other newer medications that affect neurotransmitters such as dopamine or norepinephrine generally have fewer side effects than tricyclics[23].

Pharmacologic treatment of depressive disorders during the postpartum period creates a dilemma for women who are breast-feeding. The safety of antidepressant therapy in this population is not completely known partly because infants are not allowed to breastfeed in many studies evaluating drug excretion in breast milk[24].

1.3. Antidepressant drugs

The vulnerable for psychiatric illness during the 3 months after delivery raises the possibility that psychotropic medications will be administrated[25,26]. In postpartum

depression, antidepressant drugs are frequently prescribed for lactating women yet this poses problems for those mothers who wish to breast-feed their babies[27,28]. These drugs (as listed in Table 1.1) and often their metabolites, especially if pharmacologically active, are lipid-soluble and are excreted into breast milk[29] . So it is of importance to monitor those drug concentrations in breast milk. Almost all the antidepressant studies have been found in breast milk and the milk-to-plasma ratio is typically $\geqslant 1$[30]. However, for the use of antidepressant drugs in breast-feeding women, the current data do not warranty any absolute recommendation. The Committee on Drugs of the American Academy of Pediatrics classifies antidepressant as "drug whose effect on nursing infants is unknown but may be of concern"[31]. Thus, the decision to treat a breast-feeding woman with antidepressants must be a case-specific risk-benefit assessment pending the accumulation of experience and data.

Table 1.1. Antidepressant class

Class	Tricyclic antidepressants	Selective serotonin Reuptake inhibitors	Others
Drugs	Amitriptyline Nortriptyline Imipramine Desipramine Clomipramin Doxepin	Fluoxetine Fluvoxamine Paroxetine Sertraline Citalopram	Bupropion Mianserin Venlafaxine

SSRIs are often used as the first-line antidepressant due to their favorable side effect profile, ease of use and proven efficacy[23]. Most of SSRIs, such as fluoxetine, fluvoxamine, paroxetine and citalopram, have been extensively studied in our laboratory, with respect to their physiochemical properties and in-vitro milk plasma distribution. Therefore, sertraline,

one of the remaining SSIRs yet to be scrutinized, was studied in this project. Another antidepressant drug, bupropion, as a unique antidepressant of the aminoketon class, was also chosen as a model drug to study the various factors that may affect the milk plasma distribution.

1.3.1. Sertraline

Sertraline (1S-cis)-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-N-methyl-1-naphthalenamine; C17H17Cl2N) is a 1-aminotetrahydronaphthalene that selective inhibits serotonin uptake into presynaptic nerve sites and is used in the treatment of depression[32].

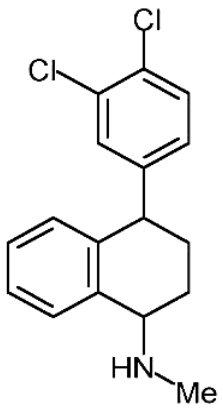


Figure 1.1. Chemical structure of sertraline.

It is highly bound to plasma proteins particularly albumin α1 acid glycoprotein; levels of the latter protein are increased in depression[33]. Following absorption, sertraline undergoes extensive metabolism. Partial demethylation occurs to form the primary metabolite, demethyl-sertraline, a clinically inactive compound. It was reported that multiple forms of CYP, including CYP2B6, CYP2C9, CYP2C19, CYP2D6, and CYP3A4, are involvled in the sertraline N-demethylation in human liver microsomes. The amine is further

metabolized to the α-hydroxy ketone[34]. Conjugated metabolites, sertraline carbamoyl-O-glucuronide, are then excreted via the urine, and unconjugated metabolites via bile[32]. Its physicochemical properties and pharmacokinetic parameters are listed in Table 1.2.

Table 1.2. The physiochemical properties and pharmacokinetic parameters of sertraline

Physiochemical properties						
Character	Molecular Formula	pKa	Molecular Weight	Solubility	LogP	Ref.
Weak base	C17H17Cl2N	8.9	342.7	In water: slightly soluble	high	[32]
Pharmacokinetic parameters						
T_{max}[a] (hr)	Vd/F[b] (l/kg)	Protein binding (%)	Cl/F[c] (L/hr)	Clinical plasma level (ng/ml)	Half-life[d] ($t_{1/2}$, hr)	Ref.
6-8	20	99	96	10-60	25-26	[35]

Notes:
a. time to reach peak drug concentration
b. volume of distribution
c. total body clearance
d. elimination half life

There have been several reports published with regard to the use of sertraline during breastfeeding[36-43] (see Table 1.3). Some studies have shown that sertraline (Zoloft) could not even be found in breast milk[44]. We now know that the drug is present in tiny amounts. The highest concentrations are found in hindmilk (the high-fat milk that follows the initial foremilk, which contains more water) 7-10 hours after taking the tablet[45]. The lowest amounts are found in an hour prior to taking the Zoloft, which is usually a once-a-day medication. Overall, nursing infants receive less than 0.3 percent of mothers' dose, even after adjusting for their weight[46]. No adverse events have been reported. Where studied,

developmental milestones have proceeded on course, although one baby has been found who had blood concentrations of Zoloft at half its mother's levels[47].

Table 1.3. Reported plasma/serum levels of sertraline in breast-feeding mothers and their infants

No. of cases	Dose (mg/d)	Infant age at dose (weeks)	Time[a] (days)	Concentration (ng/ml)		Maximum Observed milk Con. (ng/ml)	M/P[b]	Infant plasma or serum conc. (ng/ml)	Comments and References.
				Milk	Plasma				
1	100	3,7	1	30[c]	48	43	0.65	4.5	No detectable or very low concentration in infant plasma [48]
11	25-150	4-141	>14	S 17-173 DS 22-294	S 25.3[c] DS 62	S 173 DS 294	S 2.3 DS 1.4	S: <3.0 DS: <10.0	[49]
9	50-200	4-22	19 (7-48)		S 77[c] DS 118			S: 2 DS: 2-3	Metabolite was found in infants' plasma and have a mean concentration of 3ng/ml [50]
8	1.05 mg/kg/d	23 (8-61)	9 (5.7-18.2)			S 65[d] DS 85	S: 1.93[e] DS: 1.64 (AUC)		No drug or its metabolite were detected in infant plasma. [51]
10	150		>14	47 (7.3-207)	90 (17-62.7)	207	1.76[c]		The dose of drug to which the infant was exposed was calculated, which was less than 2% of maternal dose. [52]
14	25-200	26.3[c]	6-16 weeks		S: 30.7[c] DS: 45.3			S: <2.5 DS: <5.0	[53]

Notes: SER, S: Sertraline; DS: Desmethylsertraline;
a: time after treatment when sampling; b: M:P ratio; c: average data; d: estimated data; e: AUC based value

1.3.2. Bupropion

Bupropion ((R,S)-1-(3-Chlorophenyl)-2-[(1,1-dimethylehty)amino]-1-propanone hydrochloride) is a unique antidepressant of the aminoketon class. It differs from other depressant drugs that it is not a monamine oxidase inhibitor and exerts little or no inhibition of reuptake of norepinephrine or serotonin in rat brain synaptosomes[54]. It appears to be particularly effective in hypersomniac, hyperphagic unipolar depression and in bipolar depression[41]. The drug has 3 metabolites: hydroxybupropion, threobupropion and erythrobupropion. The first 2 metabolites are active, with 50% of the activity of the parent drug[55]. The physiochemical properties and pharmacokinetic parameters of bupropion are summarized in Table 1.4.

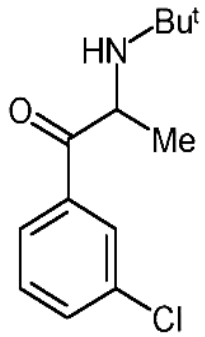


Figure 1. 2. Chemical structures of bupropion

Briggs and colleagues[56] reported bupropion excretion into breast milk in a 37-year-old woman receiving 100mg three times daily (see Table 1.5). Simultaneously milk and maternal plasma samples obtained after dose administration demonstrated that bupropion was consistently present in greater amount in milk as compared to plasma, with a M/P ratio ranging from 2.51 to 8.58. Bupropion accumulates in human breast milk, more than other antidepressants, with concentration more than twice that found in mother's blood. Buproprion has not been detected in the infant's blood, though it accumulates in small amounts there, if any.

Table 1.4. The physiochemical properties and pharmacokinetic parameters of Bupropion

Physiochemical properties						
Character	Molecular Formula	pKa	Molecular weight	Solubility	LogP	Ref.
Weak base	C13H18CINO·HCl	7.9	276.2	In water: < 1 in 3	high	[32]
Pharmacokinetic parameters						
Oral bioavailability (F)	T_{max}[a] (hr)	Vd/F[b] (l/kg)	Protein binding (%)	Clinical plasma level (ng/ml)	Half-life[C] ($t_{1/2}$, hr)	Ref.
>95%	1-3	27-63	75-85	10-50	14 (8-24)	[32]

Notes:
a. time to reach peak drug concentration
b. volume of distribution
c. elimination half life

Table 1.5. Reported plasma/serum levels of bupropion in breast-feeding mothers and their infants

No. of cases	Dose (mg/d)	Infant age at dose	Time[a] (days)	Concentration (ng/ml)		Maximum Observed Milk Concentration (ng/ml)	M/P[b]	Comments and References.
1	300	14 months	14	26.9[c]	121.8[c]	0.189	B: 2.5-8.58 HB: 0.09-0.11 TB: 1.23-1.57	No drug or metabolite found in the infant's plasma. [57]

Notes: BUP, B: Burpopion; HB: hydroxybupropion; TB: threohydrobupropion
a: time after treatment when sampling; b: M:P ratio; c: average data

1.4. Benefits of breast feeding

Breast-feeding is an essential physiologic process that provides nutrition to the infant. It is beneficial to both mother and infant[58-63]

The usefulness of breast milk offers not only essential nutrition for the infant, but also protection against infection and other immunology disorder[61]. The American Academy of Pediatrics endorses breast milk as the best and only source of nutrition necessary for the infant during the first 6 months of life[25]. Breast-feeding also appears to provide protection against sudden infant death syndrome, the development of food allergies

and some chronic diseases including childhood-onset diabetes mellitus, lymphoma, ulcerative colitis, and Crohn's diseases[25,62,64].

Women who breast-feed their infants experience a number of health benefits such as less postpartum blood loss and more rapid uterine involution, earlier return to prepregnancy weight, concurrent fertility reduction, lower risks of breast and ovarian cancer, and probable protect against osteoporosis and adult-onset obesity[25,62].

In addition, breast-feeding has been shown to enhance mother-infant bonding and may enhance both maternal self-esteem and self-efficacy by allowing a mother to provide very personal and optimal nourishment to her infant[60].

Although breastfeeding is now widely accepted for those reasons, it is often contraindicated in case of maternal drug use in the lactating period. In many cases, concentrations of drug in breast milk have not been measured and M: P ratio may vary due to the changes of milk composition in different lactation period. Thus, it is often difficult to assess the risk to the infant of exposure to these agents through nursing.

1.5. Milk Composition

The composition of milk is very complex. In addition to nutrients such as proteins, fats, sugars, minerals, and vitamins, there are antibodies, hormones and growth factors. The major components, which affect drug distribution into milk, are the aqueous itself, the lipid content and the proteins[63].

The fat component of milk is composed of a complex mixture of lipids. Triglycerides are the major type of lipid in milk fat[65]. Milk lipid content increases during the time course of lactation from around 2.9 % in early milk to 5.4 % in mature milk. Lipid composition also changes during breast-feeding [65]. These effects can alter M/P ratio, but the extent of these appears to be minimal.

Proteins account for approximately 75 % of the nitrogen-containing compounds in breastmilk. The proteins of breastmilk can be divided into two categories: micellar caseins and aqueous whey proteins, present in the ratio of about 40:60. The predominant casein of human milk is β-casein, which forms micelles of relatively small volume and produces a soft, flocculent curd in the infant's stomach. The major whey proteins are α-lactalbumin, lactoferrin, secretory IgA, and serum albumin with a large number of other proteins present in smaller amounts[66].

The composition of breastmilk is not uniform, and the concentrations of many of its constituents change during the lactation period and differ between individual mothers. There are several factors that are known to influence the concentration of breastmilk constituents in predictable ways[67]. These include stage of lactation, breastfeeding routine, parity, age, and other maternal characteristics, regional differences, and in some situations, season of the year and maternal diet.

Human lactation can be divided into 3 identifiable stages that differ in the composition and volume of milk produced: colostrums, transitional, mature[68]. Colostrums, a milk-like fluid produced during first few days of lactation, is significantly different from mature milk that it contains 2-4 times more protein but has relatively low

concentrations of fat, lactose, and vitamin B1[69]. The milk produced between colostrum and mature stages is transitional milk, which is approximately from 7 to 10 days postpartum to 2 weeks postpartum. Its content gradually changes. The concentration of immunoglobulins and total protein decreases, whereas the lactose, fat and total caloric content increases[70]. Mature milk is produced from approximately 15 days after delivery up until the termination of the breastfeeding. Mature breastmilk composition also changes during the course of lactation, although not as markedly as in the early weeks[71-73]. Mature milk has a greater amount of carbohydrate and fat and less protein than colostrum. For example, the total protein content of mature milk is 9-12 g/l, but 35 g/l in colostrums[74]. It is thinner and watery and can be divided into hindmilk and foremilk. The milk, which comes at the start of the feed, is called foremilk. Foremilk, which is watery and bluish in color, has a low level of fat and is high in lactose, sugar, protein, vitamins, minerals and water. Hindmilk, the milk which comes later in a feed, is richer in fat and this extra fat makes it look whiter than foremilk[75]. Table 1.6 lists the milk composition variances during postpartum period[60,76].

Table 1.6. Milk composition variances during postpartum period

	Colostrum (first 5 days post partum)	Transitional milk (6-10 days post partum)	Mature milk (15 days to 15 months post partum)	Reference
pH	7.26±0.22	7.05±0.14	7.08±0.10	[76]
Protein (total, g/dl)	1.84±0.38	1.52±0.15	1.22±0.20	[76]
Casein (g/dl)	0.369±0.175	0.344±0.116	0.238±0.085	[76]
Whey (g/dl)	1.47±0.27	1.18±0.21	0.98±0.21	[76]
Creamatocrit, %	6.47±2.01	7.01±1.62	6.83±1.64	[76]
Serum albumin (g/l)				
Fat (g/dl)	2.9	3.6	3.8	[60]
Lipid (g/dl)	3.16	3.49	4.14	[60]

Breastmilk contains a unique combination of ingredients, differing from the milks of other mammals in both the concentration and the nature of its many components. In common with the milk of other primates, human milk has low energy and nutrient density compared to the milks of most other mammals, except for a high density of carbohydrates[77]. In addition, the daily output of the major nutrients in milk relative to the size of the mother is lower in humans than in other mammals, especially dairy and laboratory species[78].

The composition of rabbit's milk, which we used in our in vivo experiment, is different from that of human milk as shown in Table 1.7[72,79]. In comparison with the composition of human milk, rabbit milk is quite concentrated, high in fat and protein,

and very low in sugar. For example, human milk contains 1.2 % protein, minus 0.3 % non-protein nitrogen, which leaves 0.9 %, while rabbit's milk contains 10 times as much protein. Due to this reasons, the absolute M/P ratios determined in rabbit may differ greatly from those in humans. However, the rabbit model we used in this study was to examine the changes in physiological factors during the lactating period, which may affect the transfer of drug into milk. This *in vivo* study will allow to correlate *in vitro* with in vivo data and thus to validate the *in vitro* M: P ratio prediction method.

Table 1.7. Average milk compositions of human and rabbit

Constituent	Percent composition	
	Human	Rabbit
Water (g/dl)	87.6	67.2
Solids (g/dl)	12.4	40.8
Protein (g/dl)	1.0	13.9
Casein (g/dl)	0.4	19.7
Whey (g/dl)	0.6	4.0
Lipids (g/dl)	3.8	18.3
Fat (g/dl)	3.8	13.9
Carbohydrates (g/dl)	7.0	2.1
Ash (g/dl)	0.2	1.8
pH	7.0	NA

NA: data not available

1.6. Factors affecting drug transfer into human milk and exposure to infants

The mechanisms by which medications are transferred into breastmilk are no different from those governing passage into any other maternal body fluid or organ system. Most drugs are transferred across membranes by passive diffusion, reaching concentration equilibrium with the concentration in the blood[80,81].

Several factors affect drug excretion in breast milk and the dose consumed by infant: maternal factors, physiochemical properties of the drug, milk factors and drug disposition in the sucking infant (Figure. 1.4)[63,82-88].

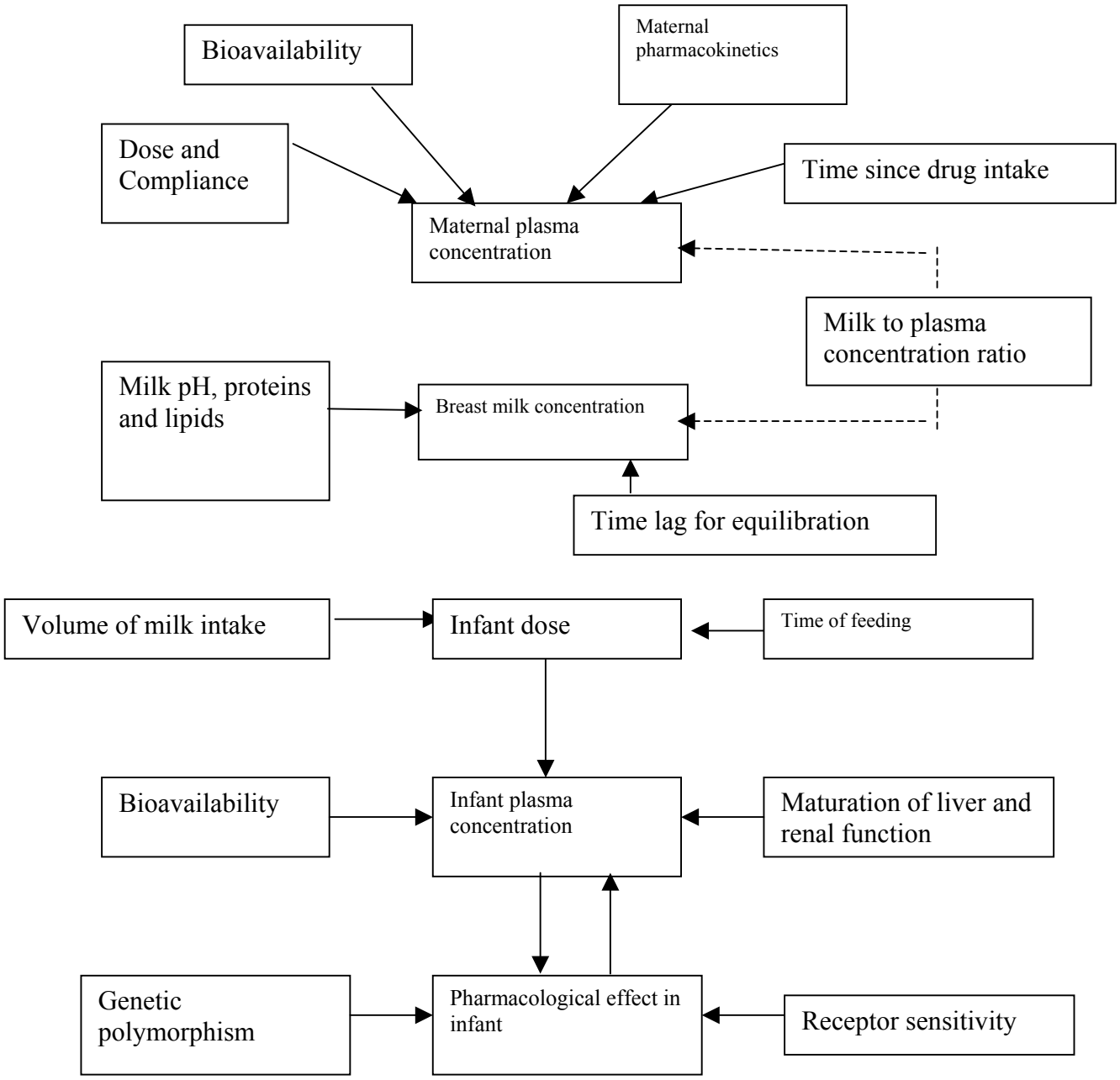


Figure 1.3. Schematic presentation of determinants of drug concentration in milk and potential exposure of the infant

1.6.1. Maternal factors

Compliance, bioavailability in mother, the dosage, and frequency as well as route of drug administration affects the magnitude and duration of drug passage into breast milk. After maternal intake, the pharmacokinetic principles of absorption, distribution, metabolism and excretion of drugs will play a role in the determination of drug levels in the milk[83].

1.6.2. Drug factors

The most important factors influencing drug excretion into milk are the physiochemical features of the compound[89,90]. These factors include the drug's molecular weight, the degree to which the drug is bound to plasma and milk proteins, its solubility in lipids and in water, degree of ionization, its pH factor, its half-life and its milk/plasma ratio (Figure 1.3)[85].

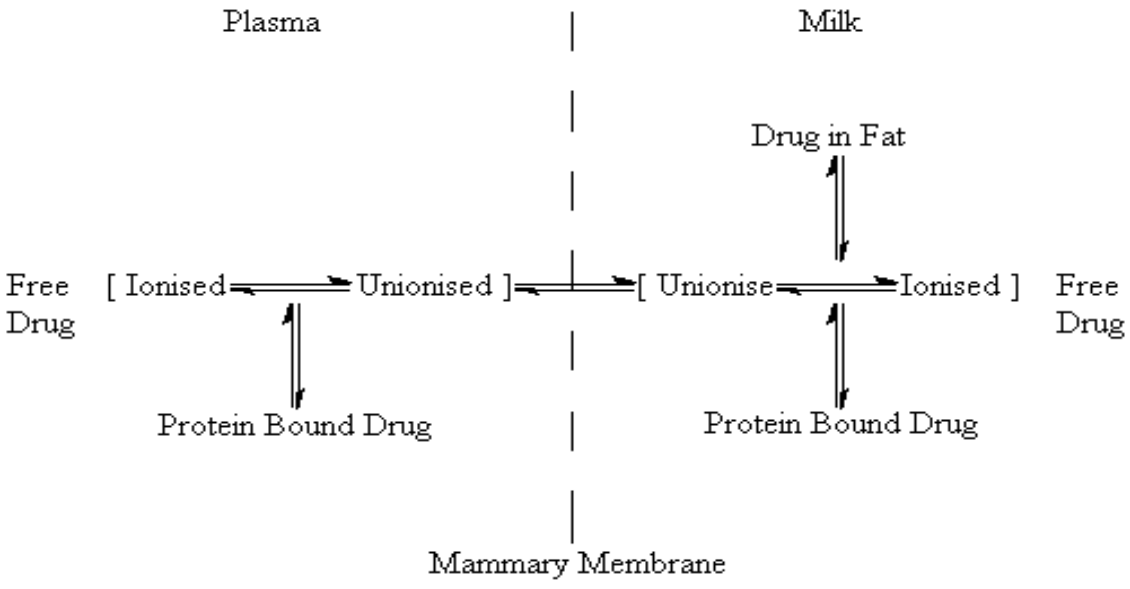


Figure 1.4. Drug transfer between plasma and milk

Following are the general guidelines apply to drug factors[85]:

1. The lower the drug's molecular weight, the more easily it passes into a mother's milk. Drug with a high molecular weight (>200) are restricted from passing into human milk.

2. The more a drug bind to plasma protein, the less likely it freely diffuses through the alveolar membranes into breastmilk.

3. The more lipid-soluble of a drug, the greater the quantity transferred and the faster it transfers into breastmilk.

4. The greater the proportions of the drug in a nonionized form, the more readily it diffuses across the lipid cellular membrane and into milk.

5. Drugs that are weak bases tend to concentrate more in breastmilk. This is because human milk is usually more acid (pH7.0-7.4) than is plasma (pH7.4).

6. The longer the half-life of the drug, the greater the risk of accumulation in the mother and in the infant.

7. The higher Mu/Pu ratio, the greater the amount of the drug found in milk The Mu/Pu ratio refers to the concentration of the protein-free fractions in milk and in plasma.

1.6.3. Milk factors

The composition of breastmilk changes greatly from the initial colostrum to mature milk. For example, lipophilic drugs such as diazepam are likely to be present in greater quantity in the milk of women who have been breastfeeding for several months than in the milk of women who have recently given birth. Protein concentration, however, is greater in colostrum than it is in mature milk[87,88].

Even during a single breastfeeding session, milk composition will vary, with milk expressed towards the end of a feeding having a greater fat content. Blood flow to the breast as well as differences between milk and blood pH affect drug transfer and cause "trapping" of drugs that are weak bases within the milk already produced[81].

1.6.4. Infant factors

The age and maturity of the beast-feeding infant are important in light of the risks and benefits of maternal ingestion of a drug. Also, the sucking patterns, duration of feeding and volume consumed play a role in determining the amount of drug ingested. Once ingested, the drug must cross into the infant's bloodstream to exert systemic toxicity[91-94].

1.7. Theoretical models

Excretion of drug in milk depends on plasma protein binding, ionization, molecular weight and pharmacokinetics of the drug. Passive diffusion is the most common mechanism by which drugs pass from bloodstream in to milk. Only unbound, nonionized drug can cross biological membrane. Its concentration difference across the lipid membrane regulates the amount of drug excreted in to milk. Therefore, the drug binding to plasma and milk proteins and solubility in milk fat determine its milk-to-plasma drug concentration[63]. Most drugs have a milk-to-plasma drug ratio of 1:1 or less, while some 25 percent of ratios lie between 1:1 and 2:1.

1.7.1. *Unbound drug distribution model*

The stead-state distribution of unbound drug between milk and plasma (Mu/Pu ratio) may be predicted using a rearrangement of the Henderson-Hasselbach equation.

For acid drugs,

$$\frac{M_u}{P_u} = \frac{1+10^{(pHm-pKa)}}{1+10^{(pHp-pKa)}} \qquad \text{(Eq. 1.1)}$$

and

For basic drugs,

$$\frac{M_u}{P_u} = \frac{1+10^{(pKa-pHm)}}{1+10^{(pKa-pHp)}} \qquad \text{(Eq. 1.2)}$$

Where pHm and pHp refer to the pH of milk and plasma, respectively. These equations predict that the Mu/Pu ratio will be <1 for acid drugs and >1 for basic drugs and equal to 1 for neutral drugs[86].

This equation adequately predicts the experimental ratio of unbound drug in milk to unbound drug in plasma, but it does not predict the M/P ratio for most drugs. The M/P ratio is more clinically relevant, since this value can be used to determine the infant drug dose administered via nursing.

1.7.2. *Phase distribution model*

Fleishaker et al[76] propose to predict the M/P ratio of a drug based on the above mentioned principles by using the following phase distribution model:

$$\frac{M}{P} = \frac{f_{u,p} f_p^{un}}{f_{u,m} f_m^{un} (S/M)} \qquad \text{(Eq. 1.3)}$$

The S/M ratio is calculated using the following equation:

$$\frac{S}{M} = \frac{1}{1+ct(f_{u,m}k_f - 1)} \qquad \text{(Eq. 1.4)}$$

while the milk:lipid partition coefficient, k_f is given by:

$$k_f = \frac{C_{mf}}{f_{u,m} C_{SM}} \qquad \text{(Eq. 1.5)}$$

Where f_u is the fraction of unbound drug, f^{un} is the fraction of unionised drug, S/M is the skim milk-to-whole milk ratio, *ct* is the creamatocrit ratio, C_{mf} is the concentration in milk fat, and C_{sm} the concentration in skim milk. This model reflects the M/P ratio under steady state conditions such as multiple oral dosing.

1.7.3. *Membrane diffusional model*

Meskin and Lien[88] extended the consideration of the pH difference between milk and plasma and the p*Ka* of the compound to include two other factors which can affect the ability of a molecule to traverse a lipid bilayer: molecular weight (*M*) and the octanol-water partition coefficient, or log *P*. Using regression analysis of M/P ratios and physicochemical parameters for 20 acidic and 15 basic drugs, the following regression equations were found to explain 65 % and 64 % of the variance, respectively:

For acidic drugs,

$$\log \frac{M}{P} = 2.068 - 0.162\sqrt{MW} - 0.185 \log P \qquad \text{(Eq. 1.6a)}$$

For basic drugs,

$$\log \frac{M}{P} = 0.265 - 0.153 \log P - 0.128 \log \frac{U}{D} \qquad \text{(Eq. 1.6b)}$$

Where U/D is the ratio of undissociated (unionized) to dissociated (ionized) drug. The reasonable predictions in this case may be due to the fact that log *P* and molecular weight have been shown to affect protein binding and would be likely to affect partitioning into milk fat.

1.7.4. *log-transformed phase distribution model*

For comparison of the phase distribution model (equation 1), the log-transformed phase distribution model was proposed by Atkinson and Begg[94]. Rather than used the measurements of protein binding in skim milk and partitioning into milk fat, they employed the relationships between milk and plasma protein binding[91] and between the milk: ultrafiltrate partition coefficient and log P[30]. The resulting equation was:

$$\frac{M}{P} = \frac{f_{u,p} M_u}{P_u} \times \left[\left(0.955 \Big/ f_{u,m} \right) + 0.045 \times k_f \right] \qquad \text{(Eq. 1.7)}$$

Where $f_{u,m}$ and $f_{u,p}$ refer to the unbound fractions in milk and plasma, Mu/Pu is the ratio of unbound concentrations in milk and plasma predicted from the Henderson-Hasselbach equation, and k_f is milk: lipid partition coefficient[92].

$$\log k_f = 1.29 \log p - 0.88 \qquad \text{(Eq. 1.8)}$$

The phase distribution model resulted in slight overestimates of the M/P ratio for acidic drugs and underestimation of M/P ratios for basic drugs.

Regression analysis was conducted for acidic and basic drugs separately, with the following equations:

For acid drugs,

$$\ln M/P = -0.405 + 9.36 \ln(M_u/P_u) - 0.69 \ln f_{u,p} - 1.54 \ln K \qquad \text{(Eq. 1.9a)}$$

For basic drugs,

$$\ln M/P = 0.025 + 2.3 \ln(M_u/P_u) + 0.9 \ln f_{u,p} + 0.5 \ln K \qquad \text{(Eq. 1.9b)}$$

Where K is defined as: $K = \left(0.955 / f_{u,m} \right) + 0.045 k_f$. (Eq. 1.10)

1.7.5. *Structural models*

Agatonovic-Kustrin et al.[93] employed an artificial neural network to allow prediction of the M/P ratio based on the basic structural, chemical, and physical properties of a molecule. Data from 60 compounds were used in this analysis. Sets of data for 50 compounds were used in training and testing the network outputs; 10 compounds were used for external validation. Compared with the log transformed phase distribution model. The neural network showed less predictive error and method bias.

Both the log phase distribution model and the artificial neural network perform fairly well in predicting M/P in humans in the absence of experimental data. However, they cannot account for deviations from expected behavior due to active transport processes. In addition, they do provide insight into the physiological processes that affect drug transfer into milk. Thus, some combination of *in vitro* experiments, *in vivo* studies, and modeling approaches will be necessary to describe a drug's M/P ratio in humans.

1.7.6. *Exposure models*

The ultimate goal in determining the M/P ratio is to predict the exposure of a human infant to drugs via breast milk. The dose in human milk may be calculated as

$$Dose_{\inf} = C_p^{ss} \times M / P \times V_{milk} \qquad \text{(Eq. 1.11)}$$

Where C_p^{ss} is the average concentration at steady state in maternal plasma. M/P is the M/P ratio based on AUC values in both fluids, and V_{milk} is the volume of milk ingested by the infant, which is approximately 150 ml/kg/day[94].

If clearance in the infant (C_{inf}) *is* known, the average systemic exposure to the infant may be calculated as:

$$C_{\text{inf}} = \frac{Dose_{\text{int}} \times F_{\text{inf}}}{Cl_{\text{inf}}} \qquad \text{(Eq.1.12)}$$

$$RD = \frac{Dose_{\text{inf}}}{Dose_{mat}} \times 100\% \qquad \text{(Eq. 1.13)}$$

Where C_{inf} is infant drug concentration in plasma, F_{inf} is the infant oral bioavailability and Cl_{inf} is the infant total drug clearance.

Prediction of the exact infant dose and exposure is difficult, as nursing times in relation to maternal dosing will vary, as will the volume of milk be consumed at any particular feeding.

Over the past decades, our knowledge about the factors that affect the milk plasma distribution was increased. However, for most drugs, its milk plasma distribution ratios are unknown. Various *in vitro,* and *in vivo* and predictive models have been established to estimate M/P ratio, but no model is accurate enough in predicting the amount of drug transfer into milk. For this, a combination of *in vitro* experiments and *in vivo* studies in animal models should be conducted to determine whether a particular compound would show high milk to plasma ratio in human milk, and thus be a potential risk to the nursing infant.

CHAPTER *2*

AIM and OBJECTIVES

The postnatal period is a time of increased onset and relapse of depression. It poses a clinical dilemma, as many mothers requiring medication will also choose to breast feed their infants. Any decision to institute treatment for depression must weigh the benefits of maternal treatment against the potential harm to the breastfeeding mother of withholding medication which may improve her illness. For the neonate, one must balance the risk of medication exposure against the benefit of receiving breast milk.

The amount of free drug available for transport depends on the drug physiochemical properties, degrees of plasma and milk protein binding, the plasma and milk pH[24]. Another factor affecting excretion of drugs is the time when breast-feeding occurs, as the milk composition and its pH value change at different lactating period. The basic drugs with low plasma, but high milk, protein binding tends to concentrate more in milk. In the same way, the milk with lower pH and higher protein content can trap more basic drugs. Therefore, the potential risk to infant may be greater when the drug is administrated in transitional stage, whose milk pH value is lower than that in colostrum stage. As currently reported data on drug milk plasma distribution was not obtained from experiments carried out under controlled conditions and did not take the different stage of lactation period into account. We can assume that the conclusion made about whether the drug exposed to infants is safe or not is quite arbitrarily. Hence, a prediction of drug milk plasma distribution and the infant exposure with respect to different lactating period will be helpful in the determination of the safety of the drug in infant.

There are various methods for predicting M/P, which involve *in vitro* experiments in mammary cell monolayer, assessment of drug binding to plasma and milk protein and lipid, *in vivo* experiments in animals, and regression models based on a compound's physicochemical characteristics[94]. The *in vitro* predictive models, although consider the change of milk pH and protein content, can not mimic the *in vivo* dynamic behaviour, and hence lack the accuracy in the prediction of milk/plasma distribution. Thus, a correlation of *in vivo* drug milk:plama distribution studies in animal models with data obtained from *in vitro* experiments can be established and may enable to determine whether a particular compound will show a high milk to plasma ratio in human milk, and thus be a potential risk to the nursing infant.

The aim of this work was to correlate the M/P ratio obtained by using revised regression model and *in vitro* experiment with that obtained by conducting the *in vivo* studies in lactation rabbit. Also, as the milk protein, lipid content and eliminating capacity in mothers vary within lactation period; assessment was made to evaluate the relative risks to infant taking antidepressant via breastfeeding at different stages of lactation period. Two antidepressant drugs, sertraline and bupropion, were selected as model drugs as they are two commonly used antidepressants for women who encountered depression. For the regression model studies, the prediction was made by finding drug's physiochemical properties and the pH of milk and plasma, as well as the drug protein binding data. Prediction performance was evaluated by comparing the M/P ratio obtained by Atkinson's model and our proposed made of 50 drugs. Various factors, such as milk pH, protein content and protein composition, which may affect the transfer of antidepressant, were studied by *in vitro* experiments. In *in vivo* studies, the lactation rabbit was used to compare the M/P ratio obtained in colostrum and

mature stages. Furthermore, an attempt was made to correlate the M/P ratio with changes of milk protein, pH and lipid content both *in vivo* and *in vitro*.

CHAPTER *3*

A NOVEL METHOD

FOR DRUG MILK-TO-PLASMA RATIO PREDICTION

3.1. Introduction

Breastfeeding provides important benefits to mother and infant and should be strongly encouraged as the optimal infant feeding choice for most infants[95]. However, prescription and nonprescription medications are commonly used by women who breast-feed their infants. Drug ingested by a lactating mother would be expected in human milk to some extent and be ingested by a breast-feeding infant. The concentration in the milk is related to the maternal plasma concentration, reflected in the often-quoted milk to plasma concentration (M/P) ratio. This ratio is reliable when it comes from studies where areas under the concentration-time profiles have been measured over a whole dose interval. Unfortunately, there are many drugs for which the M/P ratio is not known. Therefore, the ability to predict the approximate amount of drug that might be present in milk from the drug structure would be very useful in the clinical setting.

Phase distribution models are proposed by Fleishaker[96] [Eq. 1.3, 1.4] to predict the M/P ratio when steady-state plasma concentration achieves. These models are based on the assumption that only the unbound and unionized form of the drugs, which are located in the aqueous phase of the plasma and milk, can diffuse across mammary membranes.

The fraction unbound in plasma (f_p) can be calculated from the protein binding (PB) [Eq. 3.1] and fraction unbound in milk (f_m) may be calculated by the established

relationship[92] [Eq. 3.2]. The milk:lipid partition coefficient (k_f), which can be calculated from the oil/water partition coefficient (LogP) [Eq. 3.3].

f_p=1-(%PB/100) (Eq.3.1)

$$f_m = \frac{f_p^{0.45}}{(6.94\times10^{-4})^{0.45} + f_p^{0.45}} \quad \text{(Eq.3.2)}$$

$$\log k_f = 1.29LogP - 0.88 \quad \text{(Eq.3.3)}$$

This equation [Eq. 3.3] is derived from experimental data of 16 drugs by linear regression[92]. The LogP values of these 16 drugs range from 0.29 to 3.11. However, for those drugs with logP values more than 3.11, the equation may not be effectively in prediction their $\log k_f$ value, thus the prediction of the M/P ratio will be negatively affected. Evaluation of the model by comparison of the predicted M/P value with literature milk:plasma area under the curve (AUC) ratios indicated that this method tends to over predict those drugs whose LogP values are more than 3.11. Moreover, the logP data are obtained only through literature search. However, there are still some drugs whose LogP data are unknown and must be obtained experimentally. In this study, we propose a new method, which not only enable us to calculate LogP values based on its structure but also makes the prediction of M/P more simply and accurately.

The growth in drug discovery has increased the demand for rapid and efficient methods to estimate M/P ratio and other physiochemical properties from molecular structure. The physical and chemical properties of a drug are a function of its molecular structure.

Finding one or more molecular descriptors to explain variation in the physical or chemical properties of a group of analogues develops quantitative structure-property relationship (QSPR) and quantitative structure-activity relationship studies (QSAR)[97]. A relationship, once quantified, can be used to estimate the properties of other

molecules simply from their structure and without the need for experimental determinations or synthesis. A number of commercial software products for physical property prediction exist. Experimental determination of such properties can be time consuming and in some cases, being subject to experimental variation and errors. These methods have successfully been used to model physicochemical[98-101], physiological[102], spectroscopic[103] and toxicity[104-106] properties of organic compounds.

A goal in this study design was to develop alternative physiochemical factors other than LogP to be incorporated into the phase distribution model to enable better prediction of the milk:plasma distribution (M/P) ratio.

3.2. Methods

3.2.1. Equipment

The computational works were performed on a Pentium PC running the Windows 2000 operating system. Statistical analyses were done using SPSS 10.0 for windows (SPSS Inc., USA) for model building. WindowChem Software ChemSW (Physical Properties! Pro) was used for calculating relevant physical drug properties from molecular structures.

3.2.2. Sources of Data

Data for drug M/P ratio and pharmacokinetic parameters were obtained from the literature and used as the basis for comparison of calculated M/P_{pre} (predicted M/P value) with M/P_{obs} (observed M/P value). Those drugs that are likely to excrete into milk and to be administrated by lactating women are selected. Drug structures were drawn using WindowChem Software ChemSW. The structure was then optimized by geometry minimizing. Upon minimizing, their physiochemical properties were

calculated. For those basic drugs with milk: lipid partition coefficient ($\mathrm{Log}\,k_f$) and skim to whole milk (S/M) ratio data, their physiochemical properties: LogP, molecular weight hydrophilic-lipophilic balance (MW_HLB), volumetric hydrophilic-lipophilic balance (V_HLB), percent hydrophilic surface area (HSA), water solubility (WS) and solubility parameter (SP), were calculated.

3.2.3. Log k_f -Physiochemical Relationship

Correlation analyses were performed between $\log k_f$ and physiochemical parameters including pKa, LogP, V_HLB, MW_HLB, WS and SP. The correlation coefficients obtained were compared.

3.2.3. M/P Prediction

M/P ratio predictions were made using the phase distribution model based on the reported plasma protein binding and pKa, as well as Log p, V_HLB, MW_HLB, WS or SP, assuming that pH=7.4 in plasma, pH=7.1 in milk and creamatocrit=0.088. The f_p values were used to calculate the corresponding f_m values, using equation 3.2.

3.2.4. Data Analysis

Prediction performance was assessed in terms of accuracy (mean prediction error, MPE) and precision (root mean square prediction error, RMSE) by comparing predicted M/P values of 55 basic drugs with the respective reported M/P values. Accuracy, or mean prediction error, was assessed as follows:

$$\text{Accuracy}=1/N\sum_{i=1}^{N}\text{prediction error} \qquad (3.4)$$

Precision, or mean square prediction error, was assessed as follows:

$$\text{Precision}=[1/N\sum_{i=1}^{N}(\text{prediction error})^2]^{1/2} \quad (3.5)$$

3.3. Results

Eight basic drugs were studied to derive relationships between $\text{Log}\,k_f$ and their physiochemical properties: LogP, molecular weight hydrophilic-lipophilic balance (MW_HLB), volumetric hydrophilic-lipophilic balance (V_HLB), percent hydrophilic surface area (HSA), water solubility (WS) and solubility parameter (SP). (Table 3.1)

Table 3.1. $\text{Log}\,k_f$ and calculated physiochemical property values of 8 selected basic model drugs

Drug	$\text{Log}\,k_f$	LogP	MW	MW HLB	V HLB	%HSA	Solubility(g/l)	SP
Diazepam	1.638[107,a]	2.82	284.800	6.608	2.900	27.403	8.13E-05	24.330
Propranolol	1.173[108,a]	1.45	259.348	8.723	6.974	36.548	4.79E-01	22.686
Atenolol	0.581[92]	0.16	266.340	12.703	10.924	51.669	2.21E+01	23.691
Verapamil	2.020[92]	2.22	454.600	8.804	6.175	26.449	1.13E-03	20.772
Imipramine	2.240[92]	2.43	280.400	4.568	2.312	10.897	1.25E-04	21.510
Pirenzepine	-1.460[92]	-0.47	351.400	17.376	17.311	77.030	1.97E-04	24.722
Fluphenazine	2.320[92]	2.58	437.529	7.094	6.979	38.812	3.96E-07	24.986
Chlorpromazine	2.930[92]	2.92	318.870	4.017	2.296	10.949	6.59E-05	24.217

a. The values were calculated using the following Eq.

$$\frac{S}{M}=\frac{1}{1+ct(f_{u,m}k_f-1)}$$

Bivariate correlations were used to derive Pearson's correlation coefficient, assuming that each pair of variables is a bivariate normal distribution. Two-tailed $p<0.05$ was used for statistical significance. Table 3.2 summarized the correlations of $\text{Log}\,k_f$ with

LogP, MW_HLB, volumetric V_HLB, HSA, WS and SP. It was showed that among all the physiochemical parameters, LogP, MW_HLB**,** V_HLB and HSA were found well correlated with Log k_f (r^2=0.926, 0.954, 0.921, 0.925 respectively)**, w**ith their respective relationships as described by Eqs. (3.6)-(3.9). The range values of LogP, MW_HLB**,** V_HLB and HAS obtained were from -0.47-2.92, 4.568-17.376, 2.296-10.924 and 10.949-77.030 respectively.

Table 3.2. Correlations between Log k_f and other physiochemical values obtained by SPSS

		LogP	MW	HLB_MW	HLB_V	HSA	SOLUBLE	SP	Log k_f
LOG p	Pearson Correlation	1.000	.186	-.945**	-.931**	-.888**	-.511	-.175	.926**
	Sig. (2-tailed)	.	.660	.000	.001	.003	.196	.678	.001
	N	8	8	8	8	8	8	8	8
MW	Pearson Correlation	.186	1.000	.051	.123	.075	-.353	-.064	.154
	Sig. (2-tailed)	.660	.	.905	.772	.860	.391	.880	.717
	N	8	8	8	8	8	8	8	8
HLB_MW	Pearson Correlation	-.945**	.051	1.000	.978**	.962**	.363	.246	-.954**
	Sig. (2-tailed)	.000	.905	.	.000	.000	.377	.557	.000
	N	8	8	8	8	8	8	8	8
HLB_V	Pearson Correlation	-.931**	.123	.978**	1.000	.973**	.312	.326	-.921**
	Sig. (2-tailed)	.001	.772	.000	.	.000	.452	.431	.001
	N	8	8	8	8	8	8	8	8
HSA	Pearson Correlation	-.888**	.075	.962**	.973**	1.000	.310	.449	-.925**
	Sig. (2-tailed)	.003	.860	.000	.000	.	.455	.265	.001
	N	8	8	8	8	8	8	8	8
SOLUBLE	Pearson Correlation	-.511	-.353	.363	.312	.310	1.000	.082	-.252
	Sig. (2-tailed)	.196	.391	.377	.452	.455	.	.848	.547
	N	8	8	8	8	8	8	8	8
SP	Pearson Correlation	-.175	-.064	.246	.326	.449	.082	1.000	-.277
	Sig. (2-tailed)	.678	.880	.557	.431	.265	.848	.	.507
	N	8	8	8	8	8	8	8	8
Log k_f	Pearson Correlation	.926**	.154	-.954**	-.921**	-.925**	-.252	-.277	1.000
	Sig. (2-tailed)	.001	.717	.000	.001	.001	.547	.507	.
	N	8	8	8	8	8	8	8	8

** Correlation was significant at the 0.01 level (2-tailed).

* Correlation was significant at the 0.05 level (2-tailed).

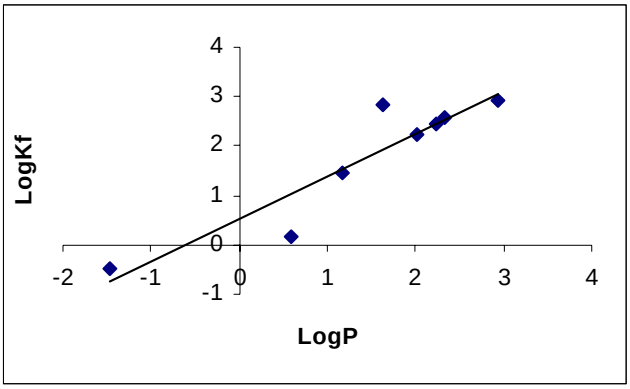


Figure 3.1. Plot of $\mathrm{Log}\,k_f$ *vs* calculated LogP value. Equation of best-fit line was as follows: $\mathrm{Log}\,k_f$ =0.996xLogP-0.327 (r^2=0.926) (3.6)

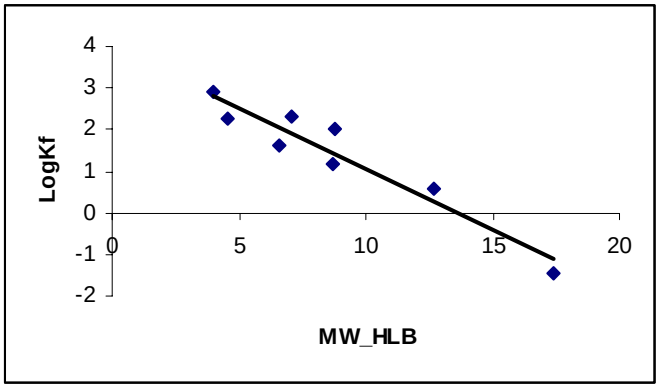


Figure 3.2. Plot of $\mathrm{Log}\,k_f$ *vs* calculated MW_HLB value. Equation of best-fit line was as follows: $\mathrm{Log}\,k_f$ =4.018-0.296xMW_HLB (r^2=0.954) (3.7)

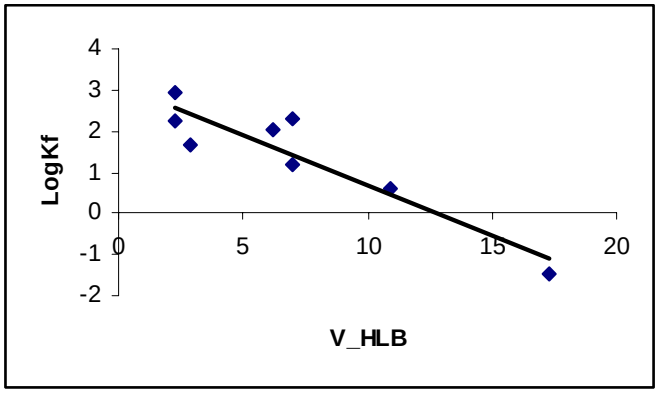


Figure 3.3. Plot of $\mathrm{Log}\,k_f$ *vs* calculated V_HLB value. Equation of best fit line was as follows: $\mathrm{Log}\,k_f$ =3.157-0.247xV_HLB (r^2=0.921) (3.8)

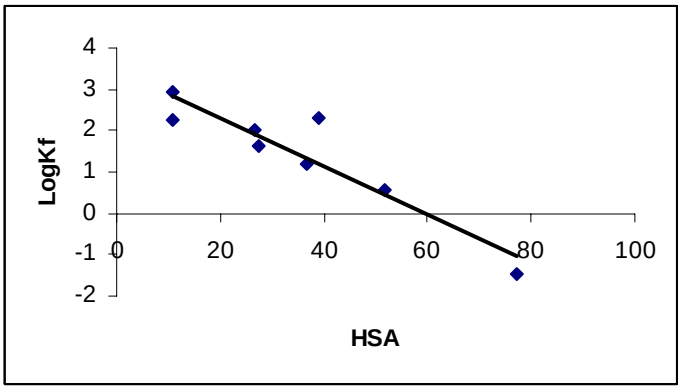


Figure 3.4. Plot of Log k_f *vs* calculated HSA value. Equation of best fit line was as follows: Log k_f =3.463-0.058xHSA (r^2=0.925) (3.9)

Table 3.3 listed various values of 55 selected basic drugs and predictor variables used in M/P ratio prediction. These 55 basic drugs included anticonvulsant, antidepressant, antiarrhythmic and antihistamine drugs, with the known reported M/P ratios. Table 3.4 summarized the calculated M/P values compared to the observed M/P data of 55 basic drugs using LogP, MW_HLB**,** V_HLB and HSA methods (see Figures 3.5-3.9). The MPE and RMSE of M/P prediction values obtained were showed in Table 3.5.

Table 3.3. Drugs and predictor variables used in M/P ratio predictions.

Drugs	Pka*	P.B.*	f_p	f_m	LogP	MW_HLB	V_ HLB	HSA
Analgesics & antipyretics								
Codeine	8.2	0.07	0.93	0.96	1.14	6.62	4.01	22.79
Morphine	7.93	0.30	0.70	0.96	1.28	9.61	7.64	36.06
Tolmetin	3.5	0.997	0.003	0.66	1.9	12.52	11.28	52.54
Paracetamol	9.5	0.20	0.80	0.96	1.09	12.71	10.85	49.68
Antibiotics								
Metronidazole	2.62	0.10	0.90	0.96	0.3	18.26	17.90	83.58
Praziquantel	3	0.80	0.20	0.93	2.41	8.91	6.99	38.22
Dapsone	1.3	0.50	0.50	0.95	2.7	11.61	10.04	53.27
Pyriomethamine	7.3	0.87	0.13	0.91	2.72	7.73	6.63	37.22
Rifampin	1.7	0.70	0.30	0.94	2.94	14.74	13.33	60.70
Bronchodilators								
Theophylline	8.8	0.56	0.44	0.95	-0.32	20.00	21.07	100
Terbutaline	8.8	0.20	0.80	0.96	1.39	16.77	15.61	67.09
Cardiovascular drugs								
Atenolol	9.6	0.05	0.95	0.96	0.73	12.70	10.92	51.67
Metoprolol	9.5	0.12	0.88	0.96	1.79	10.56	8.30	39.06
Mexiletine	8.4	0.65	0.35	0.94	2.35	6.59	4.69	29.49
minoxidil	4.6	0.00	1.00	0.96	2.84	13.30	10.91	52.94
Nadolol	9.67	0.30	0.70	0.96	0.49	10.42	8.85	46.94
Oxprenolol	9.5	0.75	0.25	0.93	2.28	11.54	9.10	40.36
Propranolol	9.5	0.90	0.10	0.90	2.8	8.72	6.97	36.55

Miscellaneous 'social' drugs								
Caffeine	13.9	0.35	0.65	0.96	-0.52	19.69	19.94	89.10
Nicotine	3.2	0.05	0.95	0.96	1.82	9.38	7.02	26.91
Antithyroid agents								
Propylthiouracil	7.5	0.80	0.20	0.93	0.99	14.94	12.68	59.98
Antiepileptic drug								
Carbamazepine	13.94	0.75	0.25	0.93	2.45	6.95	4.99	29.30
Clobazam	6	0.65	0.35	0.94	2.20	9.12	7.87	42.13
Clonazepam	1.5	0.85	0.15	0.92	3.54	8.25	6.70	41.86
Diazepam	3.3	0.97	0.03	0.84	4.18	6.61	4.94	27.40
Lorazepam	1.3	0.90	0.10	0.90	3.90	6.92	565	32.93
Nitrazepam	3.2	0.87	0.13	0.91	3.02	10.10	8.03	46.56
Lamotrigine	5.5	0.55	0.45	0.95	2.22	8.69	8.74	48.61
Zonisamide	10.2	0.45	0.55	0.95	1.50	12.83	11.39	63.17
Antidepressant drugs								
Amitriptyline	9.4	0.95	0.05	0.87	4.52	3.61	2.02	10.86
Nortriptyline	9.5	0.92	0.08	0.89	4.16	3.19	2.18	18.7.
Imipramine	9.7	0.85	0.15	0.92	4.00	4.57	2.31	10.90
Desipramine	9.8	0.90	0.10	0.90	3.64	4.21	2.49	48.61
Clomipramine	9.9	0.975	0.03	0.93	2.09	4.96	3.57	17.31
Doxepin	9.1	0.83	0.17	0.92	2.86	4.84	2.97	16.27
Fluoxetine	8.7	0.87	0.13	0.91	4.19	5.44	4.68	27.62
Fluvoxamine	8.7	0.77	0.23	0.93	3.11	6.41	5.55	32.84
Paroxetine	9.9	0.95	0.05	0.87	3.25	11.30	10.33	45.44
Sertraline	8.9	0.98	0.02	0.82	4.49	1.96	1.17	16.51
Citalopram	9.5	0.75	0.25	0.93	3.37	6.42	5.02	24.59
Bupropion	7.9	0.80	0.20	0.93	2.87	4.68	2.50	17.21
Mianserin	7.05	0.96	0.04	0.86	3.99	5.91	4.01	18.70
Venlafaxin	9.4	0.27	0.73	0.96	3.86	5.12	2.26	14.98
Trazadone	4.6	0.93	0.07	0.89	3.95	12.06	11.12	47.04
Antiarrhythmic drugs								
Quinidine	4.2	0.83	0.17	0.92	1.34	9.01	6.60	29.85
Procainamide	9.2	0.15	0.85	0.96	3.11	11.66	9.70	44.81
Disopyramide	8.4	0.58	0.42	0.95	3.11	11.02	8.90	41.34
Mexiletine	8.4	0.65	0.35	0.94	3.31	6.59	4.69	29.51
Flecainide	9.3	0.61	0.39	0.94	3.40	10.33	10.41	42.93
Verapamil	8.6	0.90	0.10	0.90	3.44	8.80	6.18	26.45
Diltiazem	7.7	0.85	0.15	0.92	0.27	10.48	9.08	41.37
Histamine H 2 -receptor antagonist								
Cimetidine	7.1	0.19	0.81	0.96	0.14	18.49	17.91	78.91
Ranitidine	2.3	0.15	0.85	0.96	1.07	16.20	15.22	68.72
Famotidine	7.1	0.17	0.83	0.96	0.14	18.99	19.17	88.62
Nizatidine	2.1	0.30	0.70	0.96	2.30	16.20	15.23	68.72

Data are from[109] Therapeutic Drugs, Dollery C, New York; Edinburgh: Churchill Livingstone, 1999

Table 3.4. Observed and predicted M/P values using LogP (Eqs.3.5 & 3.6), MW_HLB (Eq.3.7)**,** V_HLB (Eq.3.8) and HSA (Eq.3.9) methods

M/P / Drugs	Observed M/P	Predicted by Eq. 3.5	Predicted by Eq.3.6	Predicted by Eq. 3.7	Predicted by Eq.3.8	Predicted By Eq.3.9
Analgesics & antipyretics						
Codeine	2.16 [110]	1.69	0.21	1.51	1.90	1.70
Morphine	2.46 [110]	1.48	2.15	2.80	3.20	3.74
Tolmetin	0.0055 [111]	0.014	0.01	0.0047	0.0048	0.0048
Paracetamol	0.81[110]	1.53	2.31	1.76	1.93	2.05
Antibiotics						
Metronidazole	0.98[112]	1.85	0.93	0.86	0.86	0.86
Praziquantel	0.28[113]	0.60	2.28	0.62	0.67	0.51

Dapsone	0.35[114]	3.36	10.61	0.65	0.69	0.58
Pyriomethamine	5.46[115]	0.52	4.16	1.07	0.73	0.52
Rifampin	0.2[116]	10.02	10.85	0.30	0.31	0.31
Bronchodilators						
Theophylline	0.76[117]	0.43	0.85	0.83	0.83	0.83
Terbutaline	1.04 [118]	1.41	3.06	1.50	1.52	1.54
Cardiovascular drugs						
Atenolol	2.32, 4.5 [119,120]	56.03	2.21	2.09	2.27	2.28
Metoprolol	2.81, 3.6 [119,120]	9.21	6.06	2.86	3.62	4.08
Mexiletine	1.48 [121]	3.07	6.70	7.47	6.47	3.96
Minoxidil	0.76 [122]	2.94	28.93	1.05	1.02	1.17
Nadolol	4.6 [123]	6.12	1.51	2.38	2.47	2.00
Oxprenolol	0.29,0.45 [124,125]	0.93	4.33	0.66	0.84	1.06
Propranolol	0.32,0.76 [126,127]	1.97	5.27	0.68	0.67	0.59
Miscellaneous 'social' drugs						
Caffeine	0.51[128]	23.89	1.25	1.24	1.24	1.24
Nicotine	2.92[129]	24.05	3.46	2.36	3.11	7.58
Antithyroid agents						
Propylthiouracil	0.23[130]	0.37	0.43	0.32	0.33	0.33
Antiepileptic drug						
Carbamazepine	0.41[131]	11.79	6.12	4.49	4.17	3.03
Clobazam	0.13[132]	4.04	2.70	1.02	0.87	0.69
Clonazepam	0.3[133]	38.12	21.00	0.64	0.57	0.29
Diazepam	0.16[134]	8.36	18.08	0.34	0.26	0.23
Lorazepam	0.22[135]	45.59	31.90	0.92	0.61	0.42
Nitrazepam	0.27[136]	5.40	5.64	0.25	0.30	0.20
Lamotrigine	0.68 [137]	5.03	3.53	1.55	0.83	0.61
Zonisamide	0.93[138]	5.80	2.37	1.21	1.26	1.11
Antidepressant drugs						
Amitriptyline	1.07-1.62 [139]	61.74	130.37	7.88	4.09	6.06
Nortriptyline	0.68-0.88 [140]	54.52	91.24	16.67	5.98	3.51
Imipramine	1 [141]	75.90	121.29	12.44	10.42	18.10
Desipramine	1.2 [142]	31.09	35.43	10.54	6.31	4.44
Clomipramine	1.2 [143]	0.93	0.30	1.61	0.88	1.31
Doxepin	1.08 [144]	15.20	10.09	11.70	8.16	10.11
Fluoxetine	0.28[145]	85.21	157.97	5.94	2.48	1.87
Fluvoxamine	0.30 [146]	28.38	23.92	5.61	2.84	1.86
Paroxetine	0.39[147]	9.42	7.21	0.15	0.14	0.16
Sertraline	1.93 [148]	26.21	47.97	9.49	2.60	1.15
Citalopram	1.8 [149]	46.33	47.47	6.21	4.09	5.24
Bupropion	8.58 [150]	14.77	10.95	13.62	11.05	9.35
Mianserin	2.2 [151]	11.94	20.64	0.91	0.73	1.15
Venlafaxine	2.5[152]	248.52	424.44	41.79	52.11	51.49
Trazodone	0.14[153]	12.48	25.07	0.09	0.09	0.11
Antiarrhythmic drugs						
Quinidine	0.71[154]	0.57	0.32	0.50	0.67	0.98
Procainamide	4.3 [155]	92.11	88.29	2.14	2.45	2.68
Disopyramide	0.4 [156]	46.09	41.99	1.17	1.41	1.59
Mexiletine	1.48[157]	53.39	55.97	7.47	6.47	3.96
Flecainide	0.891 [158]	71.43	78.85	1.36	1.01	1.39
Verapamil	0.6[159]	21.93	21.70	0.63	0.92	1.64
Diltiazem	0.98 [160]	0.23	0.27	0.43	0.43	0.50

Histamine H 2 -receptor antagonist						
Cimetidine	1.7 [161]	0.63	1.09	1.03	1.03	1.03
Ranitidine	0.25-7[162]	1.57	1.21	0.82	0.83	0.83
Famotidine	0.14-1.78 [162]	0.65	1.11	1.05	1.05	1.05
Nizatidine	1.0-4.9[83]	8.22	6.34	0.68	0.68	0.69

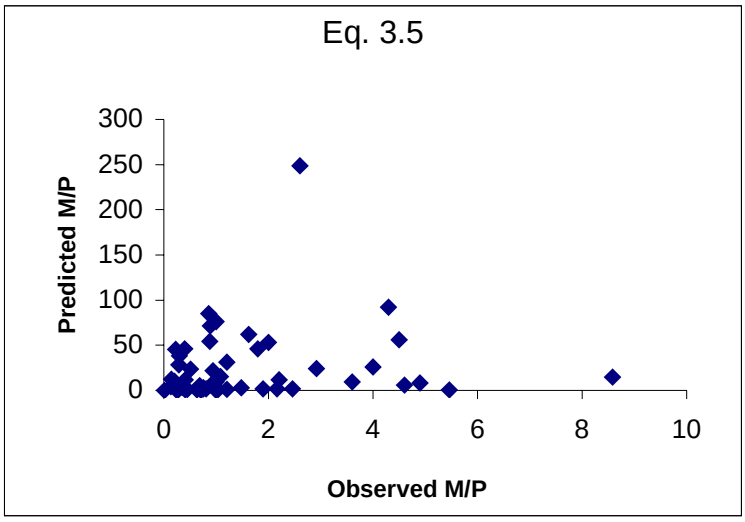


Figure 3.5. Plot of predicted M/P ratios (by LogP method-using reported Eq) *vs* observed M/P ratios

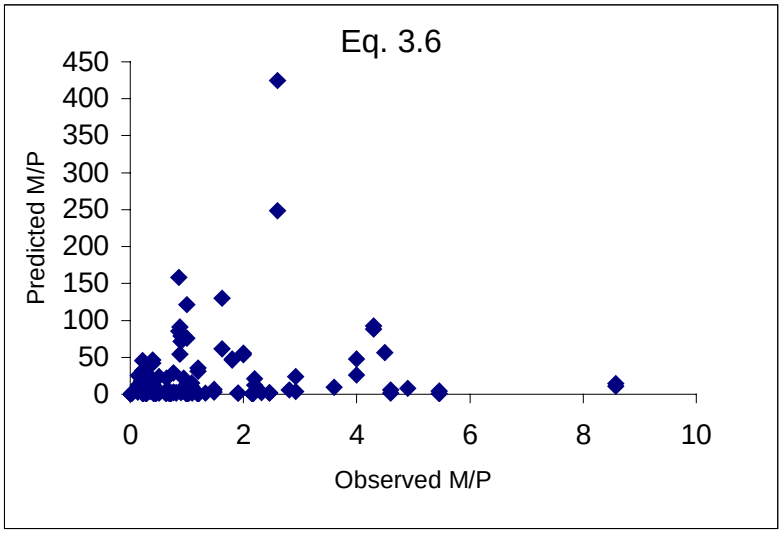


Figure 3.6. Plot of predicted M/P ratios (by LogP method-using newly derived Eq.) *vs* observed M/P ratios

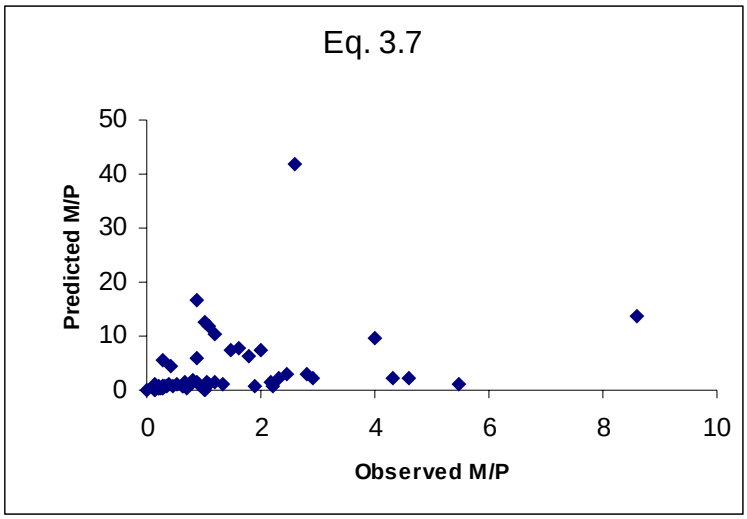


Figure 3.7. Plot of predicted M/P ratios (by MW_HLB method) *vs* observed M/P ratios

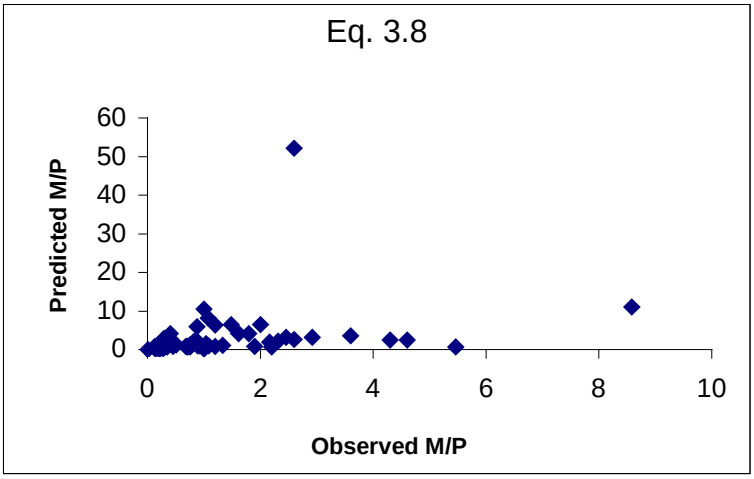


Figure 3.8. Plot of predicted M/P ratios (by V_HLB method) *vs* observed M/P ratios

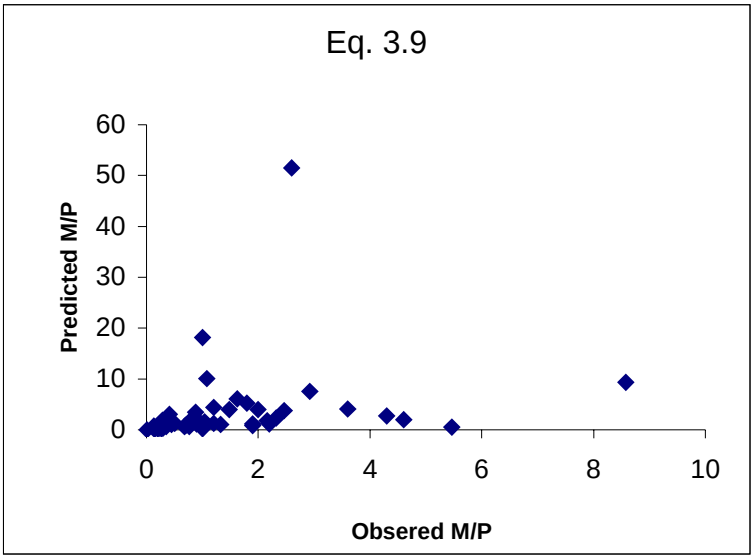


Figure 3.9. Plot of predicted M/P ratios (by HAS method) *vs* observed M/P ratios

Table 3.5. MPE and RMSE for predicted M/P ratios using LogP (Eqs.3.5 & 3.6), MW_HLB (Eq.3.7)**,** V_HLB (Eq.3.8) and HSA (Eq.3.9) methods

	Eq. 3.5	**Eq.3.6**	**Eq. 3.7**	**Eq.3.8**	**Eq.3.9**
MPE	27.3±11.08	32.6±13.1	2.61±1.17	1.86±0.88	1.81±0.94
RMSE	49.7±1732.3	59.0±2051.8	5.1±18.9	3.79±13.92	3.97±16.28

3.4. Discussion

The drugs studied are widely varied with their structures and respective physiochemical properties. The LogP values computed are comparable with literature values. For most drugs, the milk: lipid partition coefficient ($\mathrm{Log}\,k_f$) are not known but can be predicted from the LogP[92].

Due to the pH gradient between plasma and milk, weakly acidic drugs are less concentrated than weak basic (alkaline) drugs in milk. Weakly basic drugs can become ion-trapped in milk as physicochemical structure of the drug changes (extensive ionization), and prevents its passive diffusion back into the maternal circulation.

The acid drugs such as ampicillin (M/P 0.04)[163], carbenicillin (M/P 0.02)[164] are not included in this study to derive relationship because the pH of milk is relatively low (pH6.8-7.1) and only small amount of acid drugs can be excreted into milk The predicted drug concentration for such drugs in milk lipid would be negligible.

LogP, HLB and HSA are a measure of drug lipophilicity or hydrophobicity properties. Lipophilicity is approximately correlated to passive transport across cell membranes and the ability of a compound to partition through a membrane. Drugs are expected to partition into milk in accordance with their lipid characteristics. High lipid solubility favors drug partitioning into milk fat from plasma, reducing the amount of drug in milk available for its diffusion back into plasma. Therefore, as HLB and HSA increase, the log M/P tends to increase.

Drug molecular weight is a measure of a molecular size. Medications with a low molecular weight that are unionized and liphophilic will be excreted into breast milk to a higher extent.

Comparison of MPE and RMSE values indicates that prediction of M/P ratio based on either V_HLB, MW_HLB or HSA performs better than that based on Log p in the 55 drugs examined. However, the proposed methods still cannot predict well in some drugs. Sometimes, the difference is significant. This may be due to the drugs' physiochemical values that are not within the range of MW_HLB**,** V_HLB or HSA of the 8 selected basic model drugs, from which we used to derive the relationship. Nevertheless, for drugs with different physiochemical properties, prediction of the M/P values based on V_HLB, MW_HLB or HSA is still pretty well compared to that based

on Log p. The prerequisite is that one of its physiochemical properties should be similar to that of the model drugs. Each of MW_HLB**,** V_HLB or HSA equations has its own fitting range and may complement each other to predict drug M/P ratio and get better results. The prediction of drug M/P ratio should follow the following steps: (I) obtain the drug's chemical structure, (II) draw structure by using software ChemSW, (III) calculate various physiochemical parameters, (IV) check the range of the different parameters and find the proper equation, and (V) calculate the M/P value.

Unlike the previously reported method for M/P prediction, the novel method described here does not require experimental parameters and could provide a quick assessment of potential risk associated with breast-feeding for drugs with unknown M/P ratios.

3.5. Conclusion

We have established relationships of $\log k_f$ with V_HLB, MW_HLB and HSA values. The corresponding equations derived allow estimation of milk:lipid partition coefficient based on drug's V_HLB, MW_HLB or HSA value, and either one of which incorporates with the phase distribution model of Fleishaker[165], enables estimation of M/P ratio. Unlike previously reported models, the model described here does not require experimental parameters and could potentially provide a useful prediction of M/P ratio of new drugs. The improved model for M/P prediction that takes into account other factors like V_HLB, MW_HLB or HSA values does help to increase the accuracy of predictions. However, the relationship derived based on the 8 model compounds is most likely not sufficient to describe drug transfer into breast milk by all possible routes. Furthermore, uncritical use of data sets compiled from the literature is associated with the risk of possibly erroneous values.

CHAPTER *4*

IN VITRO STUDY OF SERTRALINE AND BUPROPION BREAST MILK DISTRIBUTION

4.1. Introduction

Many women will experience depression during pregnancy or postpartum and the administration of antidepressant drugs while breast feeding is of great concern to both mothers and physicians, because this requires the knowledge of the extent to which drugs are excreted into breast milk. The milk to plasma concentration ratio (M/P) is used as an index of the extent of drug excretion in milk and thus is crucial in estimating the dose ingested by the infant so that infant exposure and the potential for adverse effects can be assessed. Most antidepressant drugs pass into breast milk to some extent through passive diffusion[166]. The amount of drug excreted into breast milk depends on the characteristics of the drug, such as plasma protein binding, ionization, and lipophilicity[167]. The *in vivo* M/P data are generally lacking due to ethical and experimental constraint, and very often, the available data are based on the taking of opportunistic samples, with consequent compromise in experimental design and quality of data obtained. Such lack of information may lead to the inaccuracy in assessing the risk of drug exposure to infant. Hence, the *in vitro* determination of drug transfer to milk will be a useful tool in *in vivo* M/P ratio prediction.

Sertraline (SER) and Bupropion (BUP) are two antidepressant drugs selected in this study as model drugs. As women in their postpartum period are liable to suffer from depression, they are likely to be treated by antidepressant drugs. SER, a SSRI, is one of the first-line drugs used for the treatment of depressive illness, while BUP is a novel

antidepressant that offers a similar side effect profile without sexual dysfunction compared to SSRIs. The present *in vitro* study was thus carried out on M/P distribution of SER and BUP. The effect of different stages of a lactating period on M/P ratio was examined. Perhaps, the information obtained would be helpful to both patients and doctors in evaluating drug safety on suckling infant.

4.2. Materials

4.2.1. Chemicals and Reagents

Sertraline hydrochloride was obtained as a free sample from Pfizer Company (USA). Bupropion hydrochloride was purchased from Sigma-Aldrich (Singapore). Imipramin and Tradozone used as internal standards for sertraline and bupropion, respectively, were obtained from Sigma-Aldrich (Singapore). Acetonitrile (HPLC grade) was purchased from Mallinckrodt Baker, Inc (Paris, Kentucky). Methanol, n-heptane, ethylacetate, phosphoric acid and hydrochloride (37 %), all of analytical reagent grade, were obtained from Lab-Scan Analytical Science (Dublin, Ireland).

Plasma was obtained from healthy volunteers. Human breast milk was obtained from healthy nursing mother according to the protocol approved by the ethical committee of the National University of Singapore. They were stored in aliquots at –20°C before being used in experiments. Human albumin used was a 4.5 % solution (Zenalb ®, Bio Products Laboratory, Herts, UK) purchased from National University Hospital Pharmacy (Singapore). Whey and casein were purchased from Sigma Chemical Co. (St. Louis, MO, USA), which were extracted from bovine milk.

4.2.2. Apparatus

The HPLC analysis was performed on a Shimadzu LC-10AT gradient liquid chromatography connected to a Shimadzu SPD-10AVP detector set at a wavelength of 254nm. (Shimadzu Corporation, Japan). A Waters XTerra® Phenyl 5µm column (4.6 x 150mm) (Waters Corporation, Milford, Massachusetts), with a guard column and in-line filter, was used to separate the compounds. Ultrafiltration was performed using Microcon® YM-3 Centrifugal Filter Devices with a nominal molecular weight limit of 3000 (Millipore Corporation, Bedford, USA) in a Beckman Avanti J-18.1 centrifuge. Equilibrium dialyses were performed using a five 1-ml semi-microcells equilibrium dialyser (Spectrum Medical Industries Inc., Los Angeles, USA) consisting of five Semi-Micro Teflon dialysis cells, stoppers, six stainless steel spacers, three knurled nuts, a clear plastic water bath, a base plate and Spectra/Por 3 membrane with a molecular cut-off of 3500.

4.3. Methods

4.3.1. HPLC assay method for Sertraline and Bupropion

4.3.1.1. HPLC assay method for Sertraline

The high performance liquid chromatography with UV detector was used for determination of sertraline in biological fluids. The mobile phase for the separation of sertraline and imipramine (as IS) consisted of acetonitrile-30mM sodium phosphate buffer (40:60), which was adjusted to pH 3.00$\pm$0.05 with 85 % phosphoric acid. The mobile phase was filtered with a 0.20µm filter membrane (Nylon, 47mm, phenomenex, USA) prior to its use. The pump was set at a flow rate of 1ml/min. The peak was detected at the wavelength of 225nm.

4.3.1.2. HPLC assay method for Bupropion

The high performance liquid chromatography with UV detector was employed for quantifying bupropion in biological fluids. The mobile phase for the separation of bupropion and tradozone (as IS) consisted of acetonitrile-50mM sodium phosphate buffer (21:79), which was adjusted to pH 3.00±0.05 with 85 % phosphoric acid. The mobile phase was filtered with a 0.20μm filter membrane (Nylon, 47mm, phenomenex, USA) prior to its use. The pump was set at a flow rate of 1.2ml/min. The peak was detected at wavelength of 248nm.

4.3.2. Calibrations of sertraline and bupropion in plasma, skim milk, plasma ultrafiltrate and skim milk ultrafiltrate.

Standard calibrations were performed in plasma, skim milk, plasma ultrafiltrate and skim milk ultrafiltrate. Appropriate volumes of 1 to 20 μg/ml drug and 20 μl of 10 μg/ml internal standard working solution were added to 100 μl of ultrafiltrate, or 200 μl plasma or skim milk to give a desired range of standard concentration. After subject to extraction procedure, the samples were analyzed and the inter-day and intra-day precision was estimated. The limit of detection was determined as well.

4.3.3. Protein binding study

Protein binding study was carried out by ultrafiltration, with the ultrafiltration device consisting of 2 reservoirs that allows lower molecular weight compounds like plasma water and drug to pass through but retains larger molecules like plasma proteins. The ultrafiltrate of plasma or skim milk was obtained by pipetting 450ul of plasma or skim milk sample (with drug spiked) or blank (without drug spiked) into the upper compartment of each Amicon YM3 ultrafiltration unit (Amicon, USA, molecular weight cut-off 3000). The unit was centrifuged at 7000 *g* for 45 minutes at 37°C. The

ultrafiltrate obtained was then subject to the assay procedure. The blank ultrafiltrate was used as a baseline control.

4.3.4. Sample preparation

4.3.4.1. Plasma and Albumin

For plasma binding studies, 10mM phosphate buffer of pH7.4 was prepared. Plasma was diluted with the buffer to give protein concentration ranging from 6.25 % to 100 % (6.25 %, 12.5 %, 25 %, 50 %, 100 %) of the original plasma. The blank plasma or albumin was spiked with drug (sertraline or bupropion) to give a concentration of 0.1 μg/ml. Ultrafiltration was carried out by pipetting 450 μl of spiked plasma or albumin into the upper compartment of each Amicon YM3 ultrafiltration unit and then centrifuged at 7000g at 37˚C for 45 minutes. The ultrifitrate in the lower reservoir was collected and analyzed.

4.3.4.2. Skim milk

Skim milk was prepared by centrifuging the whole milk at 20000g for 20 minutes at 20˚C. After removing the creamed fat layer, the remaining part was then divided into two portions. The pH of each portion was adjusted to 6.4 or 7.4, using 0.01M HCL or NaOH. The skim milk was then diluted with the phosphate buffer to yield a concentration of 50 % and 100 % of the original skim milk. The blank skim milk was spiked with sertraline or bupropion to give a concentration of 0.1 μg/ml. Ultracentrifuge was carried out by pipetting 450 μl of spiked milk into the upper compartment of each Amicon YM3 ultrafiltration unit and then centrifuged at 7000g at 37˚C for 45 minutes. The ultrifitrate in the lower reservoir was collected and analyzed.

4.3.4.3. Whey and casein

The averaged total protein concentration in milk was found to be 10.3 g/L[168]. As whey accounts for 60 % to 80 % of total milk proteins[168], whey solutions containing protein concentrations representing 60 %, 70 % and 80 % of total milk protein were prepared by dissolving suitable amount of whey in milli-Q water. As casein accounts for the remaining total milk proteins, casein solutions containing protein concentrations representing 20 %, 30 % and 40 % of total milk protein were prepared by dissolving suitable amount of casein in pH 6.4 or pH 7 4 10mM phosphate buffer. Both whey and casein solutions were adjusted to pH 6.4 and pH 7.4, using 0.1M NaOH and 0.1M HCl. The blank whey and casein solutions were then spiked with drug (sertraline or bupropion) to give an expected concentration of 1.0 μg/ml.

4.3.4.4. Mixture of albumin, whey and casein

Suitable amounts of albumin, whey and casein were dissolved in phosphate buffer of pH 6.4 or pH 7.4 to represent protein concentrations during the 3 stages of a lactation period, i.e., colostrum, transitional and mature stages[26]. The protein mixtures were spiked with drug (sertraline or bupropion) to give a desired concentration of 1.0 μg/ml and adjusted to pH 6.4 and pH 7.4, using 0.1M NaOH and 0.1M HCl.

4.3.5. Determination of S/M ratio

Whole milk was divided into 2 portions and the pH of each was adjusted to pH 6.4 or pH 7.4 using 0.1M NaOH and 0.1M HCl. The pH-adjusted whole milk was then diluted with phosphate buffers of pH 6.4 or pH 7.4 to give 50 % or 100 % of the original concentration. The blank whole milk specimens were spiked with drug

(sertraline or bupropion) to give a desired concentration. The spiked whole milk samples were then subject to centrifugation at 20000g for 20 minutes at 20^{o}C to separate the creamed fat from the skim milk. Creamatocrit ratio (ct) was determined by the ratio of the volume of milk fat to the volume of skim milk. The skim milk layer was subject to HPLC analysis. S/M ratio was calculated by taking the ratio of the drug concentration in skim milk to the drug concentration in whole milk.

4.3.6. Equilibrium dialysis

The cut pieces of Spectra Por/3 membranes were soaked in distilled water for at least 15 minutes, followed by soaking in 30 % ethanol for a further 30 minutes. After rinsing with distilled water, the membranes were soaked in phosphate buffer solution for 40 minutes. Plasma was spiked with drug (sertraline or bupropion) to give a desired concentration. Plasma to skim milk dialysis was carried out by setting up the dialyzer. Each dialysis cell containing two reservoirs was separated by a semi-permeable membrane. 1ml of skim milk (at pH7.4 or 6.4) and spiked plasma were introduced into their respective reservoirs. The cells were incubated in water bath of 37˚C and rotated for 5 hours.

4.4. Results

4.4.1. HPLC assay of sertraline and bupropion in different biological specimens

HPLC permitted a relatively fast and reliable determination of sertraline and bupropion. Both intra-day and inter-day variations of the drugs were less than 10 % (Table 4.1). The limit of detection was calculated using the equation, LOD = 3.3δ/S, where δ represents the standard deviation of the response (residual δ of the regression line) and S denotes the slope of the calibration curve. The LOD for sertraline and bupropion was

2.34ng/ml and 15.86ng/ml in plasma, while in skim milk they are 1.19ng/ml and 9.46ng/ml respectively. The method developed in the study was adequate to quantify sertraline and bupropion in all biological specimens.

4.4.2. Protein binding studies

4.4.2.1. Plasma and albumin

Five dilutions of plasma and albumin with buffer to give protein concentration of 6.25 %, 12.5 %, 25 %, 50 % and 100 % of the original plasma protein and albumin concentration were assessed for the fraction of unbound sertraline and bupropion. As shown in Table 4.2, the results showed that the fraction of unbound drug decreased with an increase in protein concentration, and the fraction of unbound drug appeared to be inversely proportional to the natural logarithm of plasma protein and albumin concentration (Figures 4.1).

4.4.2.2. Skim milk, whey and casein

Skim milk was adjusted to pH 6.4 and pH 7.4 and subsequently diluted with buffer to give skim milk concentration of 50 % and 100 %. As shown in Table 4.3, at a lower pH of 6.4, fraction of unbound drug was higher. Fraction of unbound drug was also higher when skim milk was diluted to 50 % of its original concentration. Fraction of unbound drug increased as concentration of whey or casein decreased. A lower milk pH also resulted in higher fraction of unbound drug

4.4.2.3. Mixture of albumin, whey and casein

Milk protein concentration is the highest in colostrum, and then declines rapidly over 15 days postpartum to reach the relatively constant levels of mature milk. The main

changes are in the concentrations of proteins specific to milk, namely whey and casein, while albumin content remains relatively constant throughout lactation[82]. Fraction of unbound drug was the highest in mature milk and the lowest in colostrum because colotrum milk contains more protein than mature milk. There was lower binding to the milk proteins at the lower milk pH (Table 4.4).

4.4.2.4. Determination of S/M ratio

The S/M ratio was found to be lower at milk pH of 7.4 compared to pH 6.4 (Table 4.6). As more drugs were unionized at the higher pH, it resulted in more drugs partitioning into the milk lipid layer. Thus, milk lipid partition coefficient values were higher at milk pH 7.4. As the concentration of whole milk was halved, milk lipid partition coefficient values decreased while S/M ratio increased.

4.4.2.5. Transfer of sertraline and bupropion from plasma to skim milk by equilibrium dialysis

The results of dialysis of drugs containing plasma against skim milk are shown in Table 4.5. The post-dialysis plasma and skim milk samples were assessed for the amount of drugs. The mean Sm/P ratios were higher when skim milk concentration was 100 %. Fraction of unbound drugs in plasma and skim milk was higher at pH 6.4 compared to pH 7.4. It appeared that more drugs were transferred into milk from plasma at pH6.4 compared to pH7.4 studied.

4.5. Discussion

Breast-feeding was widely accepted as it offers variaty benefits to both infants and mother. However, it may pose a risk to infant when mother was given some

medications while breast feeding. Basic drugs, such as antidepressants, tend to concentrate in milk compare to acidic drugs. Hence, the knowledge of the extent to which antidepressant drugs are excreted into milk is necessary so that the infants' exposure to drug and the potential for its adverse effects can be assessed. As both milk composition and pH differ in different lactating period, the study of the factors affecting the transfer of basic drugs to breast milk will be helpful in the determination of the amount of drugs in milk and estimate the safety of drug ingestion in different lactation time.

For the protein binding studies, the mean fraction of sertraline and bupropion unbound in plasma was determined to be 5.34 % and 12.7 % respectively. These indicate that the plasma protein binding of sertraline and bupropion in about 94.66 and 87.3 %, which are comparable to the literature report[32]. The major binding protein in plasma appeared to be albumin, judging from the albumin binding values.

Sertraline and bupropion binding in skim milk also changed considerably with the change of pH and dilution (Table 3.3). At a lower pH of 6.4, fractions of unbound drugs were higher. This could be due to the total contribution of ionisable groups in the protein molecule. As the acidic groups (glutamic acid, tyrosine and aspartic acid)[168] present in the protein molecule are responsible for the binding of basic drugs. These groups become less ionized at a lower pH, thus binding of an ionized basic drug to them by electrostatic forces decreased, resulting in an increase in the fraction of unbound drug. At 50 % protein binding was lower at both pH due to a lower number of available binding sites.

Compared to the protein binding of drugs in plasma, the binding in human milk was much lower because skim milk contains less protein (10.3 g/L) is less than in plasma (74.6 g/L). This was supported by the experimental findings where fractions of unbound drugs were higher in milk than in plasma. Fraction of unbound drugs also increased with decreasing skim milk, whey and casein concentrations.

Equilibrium dialysis was carried out to determine the in-vitro M/P ratio at milk pH of 6.4 and 7.4, skim milk concentrations of 100 % and 50 %, and at different drug concentration levels. M/P ratios determined at skim milk pH 6.4 which was higher compared to at milk pH 7.4. This could be explained using the pH partitioning theory. Sertraline and bupropion are weak base drugs, which were more unionized in pH 7.4 in plasma, resulting in more drugs partitioning into breast milk. The lower pH of 6.4 in milk caused the drug to be ionized to a greater extent than when milk pH was 7.4. As the ionized form was less lipophilic compared to the unionized form, those weak base drugs could not pass through the mammary alveolar membrane easily and were trapped in the milk, resulting in higher M/P ratio.

M/P ratio decreased with the dilution of skim milk. Fraction of unbound sertraline and bupropion were higher when skim milk concentration was 50 %, resulting in more free basic drugs. Only free drugs exert diffusion pressure across the mammary alveolar membrane, with free drug in milk existing in equilibrium with free drug in plasma. Since fewer drugs were bound to milk proteins at 50 % skim milk, total amount of drugs present in skim milk would be lower, resulting in a lower M/P ratio.

The in vitro M/P ratio was compared with the in vivo data and the ratio predicted by Fleishaker's model (Table 4.7). It was found that the in vitro M/P values were quite close to the in vivo value.

4.6. Conclusion

We have studied different factors that may affect the drug milk plasma distribution. A relationship between free protein concentration and fraction of unbound drug was established. It is determined that milk pH, protein concentration, as well as protein composition will affect the M/P ratio.

As basic drugs are known to have a higher potential to concentrate in breast milk, the study was carried out mainly for basic drugs. Consequently, the M/P ratio determined has its own pitfall. As it is only a ratio, it does not provide adequate information for the actual concentration of drug in milk. Thus, a careful monitor the clinical status of infant who is breast-fed by mother taking antidepressant will be necessary.

An in vivo study, combined with in vitro investigation, will be helpful in increasing the predictability for future clinical application.

Table 4.1. Validation of HPLC assay

(a) Validation of HPLC assay of sertraline extracted from plasma

Conc. (µg/ml)	Intra-day		Inter-day	
	n	CV (%)	n	CV (%)
0.2	5	0.33	3	3.00
0.8	5	3.40	3	3.89
1.5	5	2.29	3	4.97
Mean	5	2.0	3	3.95

(b) Validation of HPLC assay of sertraline extracted from skim milk

Conc. (µg/ml)	Intra-day		Inter-day	
	n	CV (%)	n	CV (%)
0.2	5	0.81	3	1.33
0.8	5	0.38	3	1.92
1.5	5	7.20	3	6.00
Mean	5	2.79	3	3.08

(c) Validation of HPLC assay of bupropion extracted from plasma

Conc. (µg/ml)	Intra-day		Inter-day	
	n	CV (%)	n	CV (%)
0.2	5	3.81	3	6.06
0.8	5	5.36	3	10.6
2.0	5	1.36	3	3.82
Mean	5	3.51	3	6.82

(d) Validation of HPLC assay of bupropion extracted from skim milk

Conc. (µg/ml)	Intra-day		Inter-day	
	n	CV (%)	n	CV (%)
0.2	5	1.58	3	1.67
0.8	5	10.1	3	1.84
2.0	5	5.46	3	7.41
Mean	5	5.71	3	3.64

Table 4.2. Protein binding study in plasma and albumin

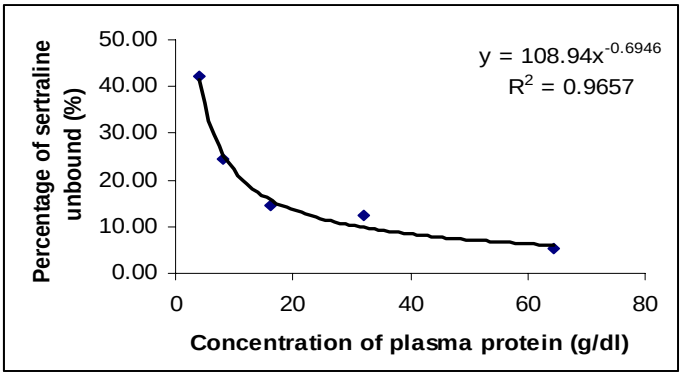
(a) Protein binding of Sertraline in plasma and albumin

Plasma (g/l)	Albumin (g/l)	Fraction unbound	
		Plasma	Albumin
64.4	50	0.0534	0.101
32.2	25	0.124	0.134
16.1	12.5	0.144	0.264
8.05	6.25	0.246	0.519
4.025	3.125	0.421	0.848

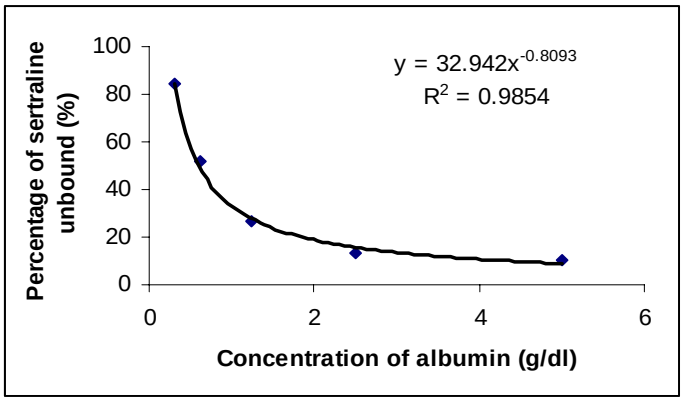
(b) Protein binding of bupropion in plasma and albumin

Plasma (g/l)	Albumin (g/l)	Fraction unbound	
		Plasma	Albumin
64.4	50	0.127	0.427
32.2	25	0.210	0.561
16.1	12.5	0.343	0.680
8.05	6.25	0.577	0.773
4.025	3.125	0.878	0.939

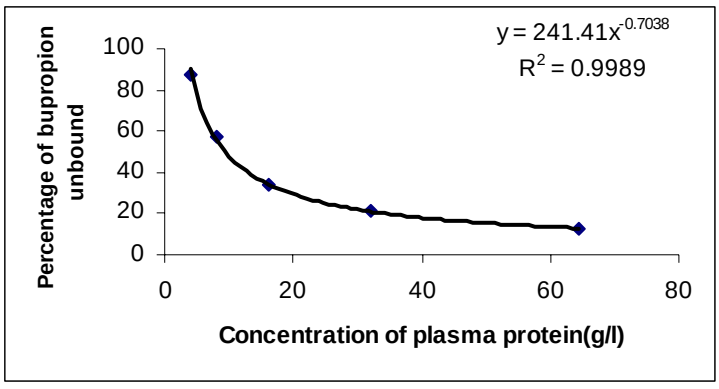
Figure 4.1. Relationship between fraction of unbound drugs and plasma protein concentration



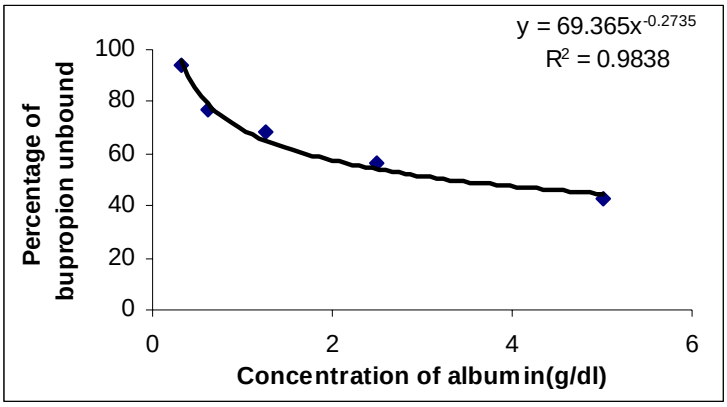
(a) Relationship between fraction of unbound SER and plasma protein concentration



(b) Relationship between fraction of unbound sertraline and albumin concentration



(c) Relationship between fractions of unbound bupropion and plasma protein concentration



(d) Relationship between fraction of unbound bupropion and albumin concentration

Table 4.3. Protein binding in skim milk, whey and casein

(a) Protein binding of sertraline in skim milk

pH	n	Dilution (% original)	Fraction unbound ($f_{u,m}$) (mean ± SD)
7.4	2	100	0.408 ± 0.038
	2	50	0.489 ± 0.016
6.4	2	100	0.537 ± 0.027
	2	50	0.736 ± 0.105

(b) Protein binding of sertraline in whey and casein

Protein	pH	n	Conc. (g/l)	Fraction unbound (mean±SD)
Whey	7.4	2	6.18	0.604 ± 0.052
		2	7.21	0.518 ± 0.023
		2	8.24	0.435 ± 0.020
	6.4	2	6.18	0.771 ± 0.032
		2	7.21	0.604 ± 0.021
		2	8.24	0.479 ± 0.038
Casein	7.4	2	2.06	0.517 ± 0.003
		2	3.09	0.415 ± 0.044
		2	4.12	0.355 ± 0.040
	6.4	2	2.06	0.535 ± 0.057
		2	3.09	0.445 ± 0.015
		2	4.12	0.396 ± 0.038

(c) Protein binding of Bupropion in skim milk

pH	n	Dilution (% original)	Fraction unbound ($f_{u,m}$) (mean±SD)
7.4	2	100	0.323 ± 0.005
	2	50	0.479 ± 0.031
6.4	2	100	0.660 ± 0.043
	2	50	0.843 ± 0.055

(d) Protein binding of bupropion in whey and casein

Protein	pH	n	Conc. (g/l)	Fraction unbound (mean ± SD)
Whey	7.4	2	6.18	0.815 ± 0.033
		2	7.21	0.699 ± 0.036
		2	8.24	0.578 ± 0.037
	6.4	2	6.18	0.920 ± 0.004
		2	7.21	0.813 ± 0.074
		2	8.24	0.734 ± 0.014
Casein	7.4	2	2.06	0.792 ± 0.021
		2	3.09	0.733 ± 0.041
		2	4.12	0.623 ± 0.056
	6.4	2	2.06	0.946 ± 0.018
		2	3.09	0.840 ± 0.030
		2	4.12	0.788 ± 0.041

Table 4.4. Protein binding study at different stages in vitro

(a) Protein binding of sertraline in different lactational periods in vitro

Lactational period	Protein composition			pH	Fraction unbound (mean ± SD) (n=2)
	Casein (g/dl)	Whey (g/dl)	Albumin (g/dl)		
Colostrum	0.380	1.335	0.05	7.4	0.054 ± 0.009
				6.4	0.0983 ± 0.005
Transitional	0.344	1.18	0.05	7.4	0.114 ± 0.012
				6.4	0.144 ± 0.025
Mature	0.238	0.98	0.05	7.4	0.206 ± 0.010
				6.4	0.246 ± 0.006

(b) Protein binding of bupropion in the different lactational periods in vitro

Protein	Protein composition			Protein composition	Protein composition (mean ± SD) (n=2)
	Casein (g/dl)	Casein (g/dl)	Casein (g/dl)		
Colostrum	0.380	0.380	0.380	7.4	0.487 ± 0.032
				6.4	0.626 ± 0.113
Transitional	0.344	0.344	0.344	7.4	0.556 ± 0.014
				6.4	0.694 ± 0.019
Mature	0.238	0.238	0.238	7.4	0.669 ± 0.002
				6.4	0.755 ± 0.069

Table 4.5. Plasma to Milk Partition studies: Effects of pH and skim milk concentrations (% of normal) on the observed in-vitro M/P ratio

(a) Observed in-vitro M/P ratio of sertraline (0.087ug/ml)

Spiked plasma conc (μg/ml)	Diluted	Skim milk pH	Post-dialysis conc (μg/ml)		Sm/P	Fraction unbound		Observed M/P[a]
			Plasma	Skim milk		Plasma	Skim	
0.087	100 %	6.4	0.0292	0.0615	0.474	0.021	0.325	0.588
			0.0304	0.0613	0.496	(0.0005)	(0.0919)	0.584
		7.4	0.0287	0.0583	0.406	0.018	0.22	0.575
			0.0221	0.0653	0.338	(0.0014)		0.478
			0.0220	0.0697	0.315			0.446
0.087	50 %	6.4	0.0157	0.0656	0.239		0.545	0.254
			0.0187	0.0712	0.262		(0.0919)	0.278
		7.4	0.0158	0.0784	0.201		0.37	0.233
			0.0148	0.0686	0.206		(0.0424)	0.239
			0.0129	0.0753	0.172			0.199

(b) Observed in-vitro M/P ratio of sertraline (0.021ug/ml)

Spiked plasma conc (μg/ml)	Diluted	Skim milk pH	Post-dialysis conc (μg/ml)		Sm/P	Observed M/P[a]
			Plasma	Skim milk		
0.021	100 %	6.4	0.0071	0.0151	0.473	0.558
			0.0076	0.0161	0.471	0.555
			0.0065	0.0154	0.423	0.598
		7.4	0.0071	0.0192	0.368	0.520
			0.0067	0.0178	0.377	0.533
0.021	50 %	6.4	0.0059	0.0215	0.275	0.292
			0.0071	0.0187	0.378	0.401
		7.4	0.0043	0.0234	0.184	0.212
			0.0045	0.0205	0.218	0.353
			0.0051	0.0189	0.268	0.311

(c) Observed in-vitro M/P ratio of bupropion (0.14ug/ml)

Spiked plasma conc (μg/ml)	Diluted	Skim milk pH	Post-dialysis conc (μg/ml)		Sm/P	Fraction unbound		Observed M/P[a]
			Plasma	Skim milk		Plasma	Skim	
0.14	100 %	6.4	0.0370	0.0382	1.031	0.23	0.695	2.014
			0.0388	0.0418	0.928		(0.0778)	1.812
		7.4	0.0345	0.0442	0.780	0.17	0.465	1.599
			0.0334	0.0407	0.820		(0.0636)	1.681
			0.0327	0.0377	0.867			1.777
0.14	50 %	6.4	0.0299	0.0528	0.565		0.84	0.897
			0.0265	0.0562	0.472			0.749
		7.4	0.02	0.0489	0.409		0.550	0.762
			0.0188	0.0632	0.298		(0.127)	0.554
			0.0171	0.0554	0.309			0.575

(d) Observed in-vitro M/P ratio of bupropion (0.0744ug/ml)

Spiked plasma conc (μg/ml)	Diluted	Skim milk pH	Post-dialysis conc (μg/ml)		Sm/P	Observed M/P[a]
			Plasma	Skim milk		
0.0744	100 %	6.4	0.025	0.027	0.920	1.797
			0.019	0.021	0.904	1.767
		7.4	0.022	0.027	0.814	1.668
			0.026	0.035	0.759	1.556
			0.023	0.032	0.739	1.515
0.0744	50 %	6.4	0.0152	0.0294	0.518	0.822
			0.0187	0.0349	0.535	0.850
		7.4	0.0107	0.0328	0.326	0.607
			0.0095	0.0346	0.275	0.511
			0.0109	0.0364	0.300	0.559

a: calculated by taking the ratio of Sm/P to S/M

Table 4. 6. Fat partitioning of drugs in human whole milk (n=2)

(a) Fat partition studies of sertraline

pH	[Whole milk] (% of normal)	ct[a]	[SER] in SM (mean ± SD)	S/M (mean ± SD)	$f_{u,m}$[b]	k_f[c]
6.4	100	0.092	0.849±0.177	0.849±0.177	0.537±0.027	0.331
	50	0.060	0.942±0.130	0.942±0.130	0.736±0.105	0.084
7.4	100	0.088	0.707±0.136	0.707±0.136	0.408±0.038	1.016
	50	0.054	0.863±0.096	0.863±0.096	0.489±0.016	0.325

(b) Fat partition studies of bupropion

pH	[Whole milk] (% of normal)	ct[a]	[BUP] in SM (mean ± SD)	S/M (mean ± SD)	$f_{u,m}$[b]	k_f[c]
6.4	100	0.092	0.512± 0.012	0.512± 0.012	0.660±0.043	2.951
	50	0.058	0.63±0.003	0.63±0.003	0.843±0.055	1.226
7.4	100	0.087	0.488±0.005	0.488±0.005	0.323±0.005	1.590
	50	0.054	0.537±0.042	0.537±0.042	0.479±0.031	1.023

ct = creamatocrit, [SER] = sertraline concentration in μg/ml, [BUP] = bupropion concentration in μg/ml, SM = skim milk, S/M = skim milk-whole-milk ratio, $f_{u,m}$ = fraction unbound in skim milk, k_f = milk lipid partition coefficient

a: calculated using:

$$ct = \frac{Vol\ of\ milk\ fat}{Vol\ of\ whole\ milk}$$

b: values obtained from skim milk protein binding studies (Table 3.3)

c: calculated using:

$$k_f = \frac{C_{mf}}{f_{u,m} C_{SM}}$$

where C_{mf} = concentration in milk fat, C_{sm} = concentration in skim milk

Table 4.7. Prediction of M/P ratio using Fleishaker's and our proposed model

(a) Prediction of M/P ratio of sertraline

pH	Concentration of milk (% of normal)	In-vitro M/P ratio[a]	Predicted M/P using Fleishaker's model[b]	Reported in-vivo M/P ratio
6.4	100	0.577	2.11	
	50	0.304	1.39	0.65-1.93
7.4	100	0.510	0.34	[169,170]
	50	0.258	0.23	

(b) Prediction of M/P ratio of bupropion

pH	Concentration of milk (% of normal)	In-vitro M/P ratio[a]	Predicted M/P using Fleishaker's model[b]	Reported in-vivo M/P ratio
6.4	100	1.848	4.61	
	50	0.830	2.93	2.5-8.58
7.4	100	1.636	1.26	[171]
	50	0.595	0.77	

a: values obtained from Plasma to Milk Partition studies (Table 4.5)
b: calculated using equations 2.1-2.5

CHAPTER *5*

DEGRADATION KINETIC STUDY

AND STABILITY-INDICATING ASSAY OF BUPROPION

5.1. Introduction

Bupropion (BUP), *dl*-2-tert-butylamino-3'-chloropropiophenone, is a second-generation clinically efficacious antidepressant agent[172]. The structure formula was shown in figure 1.2. It is a structurally novel, nontricyclic antidepressant and appears to be particularly effective in hypersomniac, hyperphagic unipolar depression and in bipolar depression[173].

BUP is a highly metabolized compound in humans. Its three major basic metabolites are erythroamino alcohol (EB), threoamino alcohol (TB), and the hydroxy (HB] metabolites[174].

Several methods were described for the estimation of BUP in human plasma or serum. These include HPLC with dual-wavelength ultraviolet detection[175], radioimmunoassay[176], gas chromatography using nitrogen phosphorous detection[177], gas chromatography combined with mass spectrometry[178].

No evaluation has yet been demonstrated about the stability indicating characteristics of the cited HPLC methods for the determination of BUP in the presence of its degradation products. And no detailed studies have been performed to study the stability of bupropion except for one paper which reported the stability of bupropion in plasma[179]. The published data[179] consistent with our findings when we conducted equilibrium dialysis in our lab. The reported degradation studies, however, appear to

be quite preliminary, because the stability of BUP was studies only in plasma and the degradation kinetic has not been fully investigated. Thus, we further investigated the stability of BUP in plasma and milk specimens under different temperature and studied its degradation kinetics.

5.2. Materials and Methods

5.2.1. Materials

Bupropion hydrochloride was purchased from Sigma Chemicals (Singapore). Acetonitrile (HPLC grade) was purchased from Mallinckrodt Baker, Inc (Paris, Kentucky). Methanol, n-heptane, ethylacetate, phosphoric acid and hydrochloride (37 %), all of analytical reagent, were obtained from Lab-Scan Analytical Science (Dublin, Ireland)

5.2.2. HPLC assay method for Bupropion

HPLC analysis were performed using an isocratic high-performance liquid chromatograph (Shimadzu 2010A, Japan) equipped with an autosampler (Shimadzu, Japan) and UV detector. The chromatographic analysis was performed in a 5cm Ultrasphere ODS column (25mm x 4.6mm i.d.) from Algilent. The mobile phase consisted of acetonitrile-50mM sodium phosphate buffer (21:79), which was adjusted to pH 3.00±0.05 with 85 % phosphoric acid. The mobile phase was filtered with 0.20 μm filter membrane (Nylon, 47mm, phenomenex, USA). The pump was set at a rate of 1.2 ml/ml. The detect wavelength was set at 248nm.

5.2.3. Preparation of stock solution

An accurate weight of BUP power 1mg was transferred into a 10-ml volumetric flask, dissolved and diluted to volume with distilled water (0.1mg/ml). The solution was freshly prepared for the study.

5.2.4. Calibration curve of BUP

Aliquots of 10-100 μg/ml of the stock solution were transferred into 1 ml vials and diluted with distilled water. An accurate volume (20 μl) of each solution was injected into HPLC using autosampler and analyzed under he described chromatographic conditions. The peak area (PA) of BUP was calculated by the instrument software. Calibration curves were constructed by plotting PA values versus concentrations of BUP.

5.2.5. Stability study of BUP in aqueous media.

The degradation of BUP HCl was studied under different conditions as follows

5.2.5.1. Effect of temperature

Solutions of 1μg/ml of BUP was prepared and sealed in the glass tubes, which were kept at a thermostatically controlled water bath at 23 ºC, 37ºC, 60ºC, and 80ºC±0.2ºC for the appropriate period of time. 20 μl of solutions were taken out at 0, 1, 3, 5, 11hr and analyzed using HPLC for an estimation of the remaining amount of bupropion.

5.2.5.2. Effect of pH

The standard solution was diluted with 0.01N HCl, 0.01NaOH or distilled water in order to make acidic, basic or neutral solution, respectively, with a concentration of

1μg/ml. The acid, basic and neutral solution were sealed in glass tubes and incubated at 25ºC ±0.2ºC and subjected to HPLC analyses at different time point.

5.2.5.3. Effect of light

Solutions of 1μg/ml of BUP was prepared and sealed in the glass tubes. The tube were exposed to the light. Half of the tube were wrapped with aluminum foil to protect the solution from light and were used at the control group. The study was conducted at 25ºC ±0.2ºC.

5.2.5.4. Effect of Ionic strength

The effect of the ionic strength on the stability of BUP was investigated by adding 1 % (W/V) to the 0.01M phosphate buffer solutions and adjusted pH to 12.0.

5.2.6. Stability study of BUP in plasma and milk

The degradation of BUP HCl was studied in plasma and milk at room temperature (25ºC ±0.2ºC) and 37ºC ±0.2ºC respectively. The plasma and skim milk were spiked with bupropion to give a concentration of 1μg/ml and 1ml of plasma and milk sample was added to an 2 ml plastic vials respectively. Some of the vials were sealed and placed in a thermostatically controlled water bath, which set temperature at 37ºC ±0.2ºC . The others were kept at room temperature (25ºC ±0.2ºC). An aliquot of 100μl sample was removed at each predetermined checkpoint. The remaining bupropion in the solution was assayed with the established stability-indicating HPLC assay method.

5.3 Results

5.3.1 High-performance Liquid chromatographic analysis

The HPLC procedure was optimized to develop a stability method. The chromatograph showing the stability indicating nature of HPLC were demonstrated by forcibly degrading 1μg/ml BUP solutions (temperature 37 ºC, 60 ºC, 80 ºC). After incubated in high temperature, the peak area of BUP decreased without apparent interference from the degradation products. As the degradation process was fast, the peak area of the predominant peak of BUP was rapidly diminishing while the putative product was progressively increased (Figure 5.1). The retention time of BUP was 8.24 min. The peak purity of BUP was examined by a photodiode array UV-Vis detector by comparing the UV spectra with that of the pure drug. It is showed that there are no overlaps between the peak of degradation products and the peak of the sample. The linearity of calibration curve of peak area versus BUP concentration was demonstrated by an excellent correction coefficient (γ^2=0.9993) (Y=36949X-9194.5). The intra-day and inter-day precision (n=5) of the HPLC method were shown in Table 5.1. The limit of detections in plasma and skim milk were 19.5 and 9.5ng/ml, respectively.

5.3.2. Degradation of BUP

5.3.2.1. Bupropion stability at different pH

The stability of bupropion was investigated under different pH values. It is shown that in acidic and neutral aqueous solutions, there is no apparent degradation of BUP under room temperature. While under basic condition, BUP degraded rapidly (Figure 5.2.). We can conclude that bupropion was more stable in acidic environment than in pH7.0 or basic condition (pH2>pH7>pH12). This finding may also be helpful in the formulation of bupropion tablet. The acidic environment provided in the tablet can improve stability and further minimizing degradation of bupropion hydrochloride.

5.3.2.2. Light

There are no significant differences between the light-protected and light-exposed BUP solution (P>0.05). No apparent degradation of the light-exposed BUP solution was observed under the room temperature.

5.3.2.3. Ionic strength effect

The effect of ionic strength on the degradation of bupropion was shown on Table 4.2. It is observed that there are no statistically significant differences between two groups (P>0.05), which indicate that ionic strength have no significant effect on the degradation of BUP.

5.3.3. Stability kinetics studies of BUP in water

The temperature dependence degradation was examined at the temperature range 23ºC to 80ºC. This study was intended to validate the proposed HPLC method as a stability indicating method and to obtain useful information about the degradation kinetics of BUP. At constant temperature, the overall degradation constant was calculated from the linear decrease of the logarithm of BUP concentration with time. The concentration versus time plots at various temperatures for BUP is shown in Figure 5.3.

Arrhenius plot of log (rate constant) versus the reciprocal of absolute temperature (in K) is show in Figure 5.4. The linearity (γ^2=0.9538) of the plot had a good indication of invariant activation energy for the degradation of BUP in the temperature range of 23ºC-80ºC.The activation energy of degradation was 19.03kal/mol from the slope of this plot. If the activation energy maintained constant at the temperature range from 23ºC to 80ºC, the degradation energy maintained constant of BUP in aqueous solution

at room temperature could be estimated as $0.0015h^{-1}$, which gave an estimation of a half-life of 19.46 days.

5.3.4. The degradation of BUP in plasma and skim milk

The degradation of bupropion was investigated in plasma and skim milk at room temperature (25ºC±0.2ºC) and 37ºC. It was found that the half life of bupropion in plasma pH 7.68 was 32.5 hours for 25 degree and 12.4 hours for 37 degree.

5.4. Discussion

A selective high-performance liquid chromatographic for the stability-indicating determination of bupropion in the presence of its degradation products is demonstrated. The developed method was specific, accurate and reproducible. The stability of bupropion was investigated as a function of pH, temperature, light intensity and ionic strength. Bupropion was found to undergo fast degradation under high temperature, and it is more sensitive in basic conditions, but it is stable in acidic medium. The kinetic study of the degradation follows an apparent first-order reaction.

Furthermore, the stability of bupropion was investigated in plasma, skim milk, skim milk (adjusted to pH6.4) and skim milk (adjusted to pH7.4). The degradation kinetics was studied in these biologic samples. From the comparison of the degradation rate of BUP in pH6.4 skim milk and pH7.4 skim milk, we can conclude the BUP was more stable in environment of slight acidity than in alkalized environment (Table 5.3). Therefore, the increase in pH value with increase in storage time and temperature would have promoted the temperature-dependent degradation of BUP.

Laizure et al[179] compared the stability of bupropion in different pH plasma samples and reported that BUP half-life in pH 7.4 plasma stored at 22 and 37 degrees was 54.2 and 11.4 h, respectively. These findings consistent with our results, which indicate the half-life in plasma pH 7.68 was 32.5 hours for 25 degree and 12.4 hours for 37 degree. The current studies have expanded upon this finding and have shown that basic medium can markedly accelerate the degradation of bupropion.

The knowledge of the stability of BUP is essential for the pharmacokinetic study, sample storage and dosage form design. As in the equilibrium study of BUP, the plasma sample should be incubated in water bath maintaining temperature at 37ºC for several hours. From current study, the degradation of BUP would be considered under such condition. For the clinical pharmacokinetics studies, the blood sample should be collected, centrifuge and the plasma frozen immediately after collection. In addition, the information provided in this study would be also helpful in the dosage form design and development. Some stabilizer can be used to provide an acid environment for the compound to increase its storage time.

Another finding is that Bupropion degraded more rapidly in biological samples than in water under same pH values. From this, we can make an assumption that other factors, besides pH, may control the degradation of bupropion. Unfortunately, the underline mechanisms are not fully discovered.

5.5 Conclusions

The stability of BUP was investigated using a stability-indicating HPLC procedure. This method permits detection and quantification of BUP in the presence of its

degradation products. The kinetic studies indicate that BUP undergoes fast degradation under high temperature. It's degradation followed first-order rates, fitting Arrhenius kinetics. And it is sensitive to basic environment than to acidic condition. The degradation rate of BUP in plasma and milk was much higher than in water under same pH and temperature. Therefore, the low pH and temperature favor the stability of BUP.

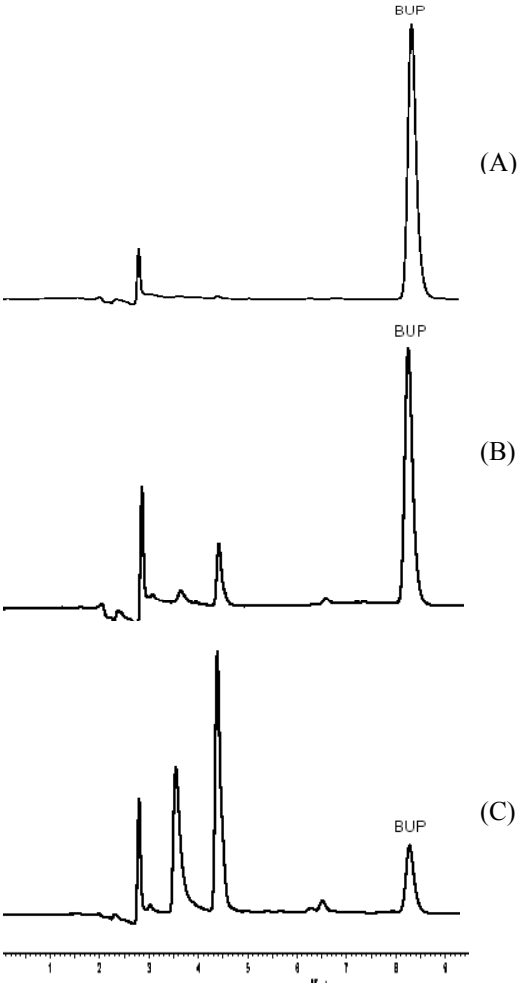


Figure 5.1. HPLC chromatograms of BUP at 80˚C at (A) zero time; (B) 3hr; (C) 5hr

Table 5.1. Validation of HPLC assay of bupropion in biological samples

Specimen	LOD (ng/ml)	LOQ (ng/ml)	Recovery (%)		Intra-day precision (CV) (%)	Inter-day precision (CV) (%)
			Bup	Tra		
Plasma	15.9	46.7	64.7	65.6	3.51	6.82
Skim milk	9.5	28.5	77.7	75.2	5.71	3.64

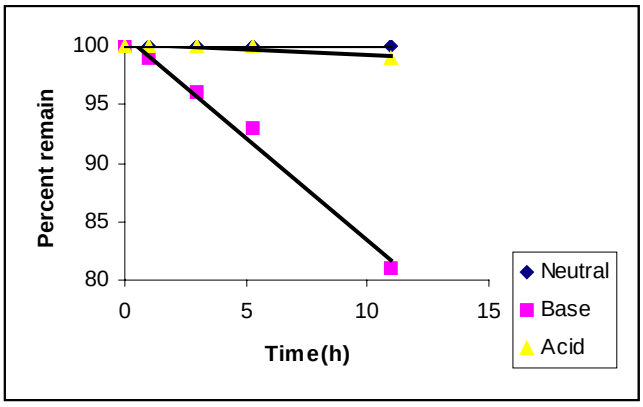


Figure 5.2. Stability of BUP under acidic, neutral and basic condition

Table 5.2. Effect of ionic strength on the stability of BUP in pH12 phosphate buffer

Time (h)	Percent remaining	
	0.01M phosphate buffer	0.01M phosphate buffer + 10 % NaCL
0	100	100
1	90.3 ± 0.63	91.2 ±1.28
3	80.9 ± 0.41	74.0 ± 3.6
6	29.3 ± 1.70	24.6 ± 1.2
10	23.1 ± 1.09	17.4 ± 2.48

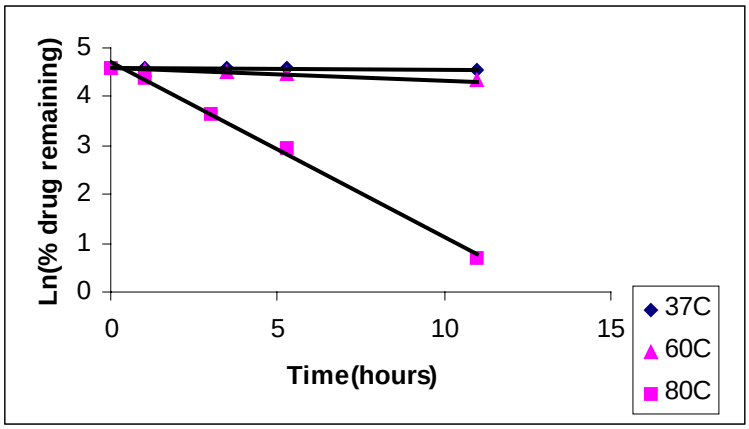


Figure 5.3. Apparent first-order reaction of BUP in aqueous solution at different temperature

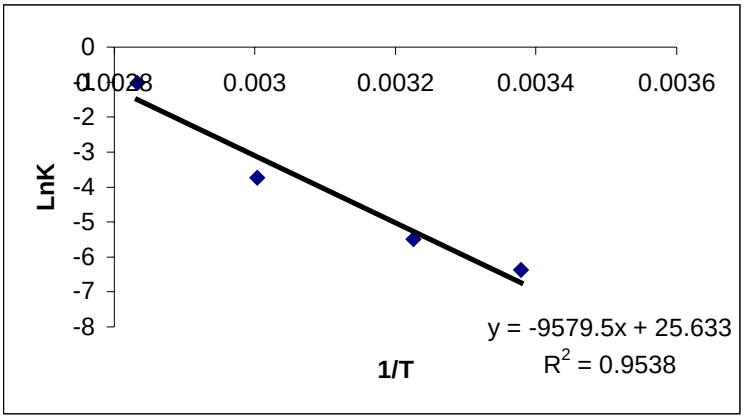


Figure 5.4. Arrhenius plot of log (rate constant) versus the reciprocal of absolute temperature

Table 5.3. Observed degradation rate and $t_{1/2}$ of bupropion in human plasma and skim milk at 25 and 37°C

	Room (25°C)		Water bath (37°C)	
	k (h^{-1})×10^{-2}	$t_{1/2}$ (h)	k (h^{-1}) ×10^{-2}	$t_{1/2}$ (h)
Water (pH6.26)	0.17	407.6	0.41	169.0
Plasma (pH7.68)	2.13	32.5	5.45	12.7
Skim milk (pH6.06)	1.36	51.0	4.67	14.8
Skim milk (pH6.4)	0.97	71.4	5.18	13.4
Skim milk (pH7.4)	1.06	65.4	6.49	10.7

Table 5.4. The activation energy (Ea) of bupropion in different biological medium

	Water (pH6.26)	Plasma (pH7.68)	Skim milk (pH6.06)	Skim milk (pH6.4)	Skim milk (pH7.4)
Ea(kal.mol^{-1})	19.03	60.13	78.96	107.2	116.0

CHAPTER *6*

IN VIVO STUDY OF BUPROPION DISTRIBUTION INTO RABBIT MILK

6.1. Introduction

Bupropion (BUP) is one of the effective drugs for the treatment of depression[180]. Its low side-effect profile suggests that it will have important clinical advantages over tricyclic antidepressants[181,182]. As a consequence, it may be necessary to treat nursing women with bupropion.

The milk distribution of bupropion was not fully discovered, in fact, there is only one reference in the literature about the use of bupropion in a nursing mother. The data shows that bupropion is freely diffusible in milk. However, the information was based on only one case report and lacks the ability to predict the risk to infant under all circumstances[183].

It is suggested that no model can accurately predict the M/P ratio for every compound by itself. Some combination of those approaches might be useful for the prediction of drug transfer into milk. For this, the combination of in vivo data with in vitro model established may enable the accurate prediction the M/P ratio, hence the infants' dose.

6. 2. Materials and Methods

6.2.1. Materials

Bupropion hydrochloride was extracted from tablet (Zyban, 50mg/tablet). Trazodone was purchased from Sigma Chemicals (Singapore).

6.2.2. Animals

Pregnant New Zealand White rabbits (Body weight 3.0-4.0kg) were purchased from Laboratory Animal Center, Singapore. All procedures involving animals were approved by the Institution Animal Care and Use Committee of this center. Animals were acclimatized in our laboratory 7 days before delivery. During this period, does were housed separately in cages with controlled light cycle (12/12h).

6.2.3. Protein binding Determination

In vitro protein binding of bupropion in rabbit plasma and skim milk is measured by ultrafiltration as described by Aramayona et al[184].

6.2.4. Skimmed-to whole (S/M) milk ratio determination

The milk specimens in different stages were spiked with drugs to give an expected concentration of 1μg/ml. The spiked whole milk samples were then subjected to centrifugation at 20000g for 20 minutes at 20^{o}C to separate the creamed fat from the skim milk. Creamatocrit ratio (*ct*) was determined by the ratio of the volume of milk fat to the volume of skim milk. The skim milk layer was subjected to HPLC analysis. S/M ratio was calculated by taking the ratio of the drug concentration in skim milk to the drug concentration in whole milk.

6.2.5. Blood and milk pH determination

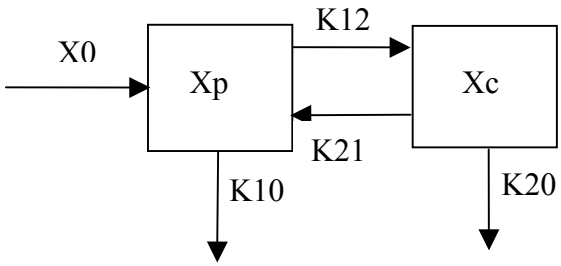
Plasma and milk pH were measured using Beckman Φ110 ISFET pH meter (Beckman Instruments, INC, USA).

6.2.6. Pharmacokinetic Study

Milk and blood sampling was carried out on the 3rd (colostrums) and 15th (mature) day of lactating. The drug (30mg/kg) was administered as i.v. bolus dose via marginal ear vein. Serial blood samples (0.5ml) was drawn into heparinised syringe before starting the kinetic study and at 0.125, 0.25, 0.5, 1, 2, 3, 4 and 6 and 8 hrs after drug administration and are always replaced with an equal volume of saline solution. Serial milk samples will be drawn before starting the kinetic study and will also be taken at the sampling time described above after drug administration by manual expression into a centrifuge tube connected to a vacuum system. At each time, the gland is emptied as completely as possible. The blood and milk samples were stored frozen at -20°C until analysis.

6.2.7. Data Analysis

The pharmacokinetic profile of bupropion in lactating rabbits following intravenous administration is described in the form of two-compartment model.



The area under the plasma concentration-time curve (AUC) was calculated by trapezoidal rule with extrapolation to infinity.

The observed milk-to-plasma ratio(M/P) was calculated using equation 5.1

M/Pobs=AUCm/AUCp. (Eq. 6.1)

6.3. Results

6.3.1. Physiology properties of plasma and milk in rabbit

Table 5.1 summarized the different values of pH, protein content, creamatocrit and S/M in rabbit plasma and milk. It is found that in rabbit, the pH value increased from colostrum to mature period. This trend agrees with the findings in human breast milk, whose pH also increase from colostrum to mature.

6.3.2. In vivo studies

The pharmacokinetic parameters of BUP plasma concentration-time curve after iv administration in lactating rabbits were derived using non linear regression analysis and single iv bolus two-compartment modeling (WinNonlin, v1.1, Scientific Consulting Inc., Apex, NC, USA).). The time course of plasma concentrations and milk concentration of BUP following a single iv bolus dose of 30 mg/kg (n=2) is illustrated in Figure 5.1a-5.1d.

The volume of distribution, calculated by use of the area method, ranged from 1.54 to 4.43 $l \cdot kg^{-1}$ (Table 5.2). BUP was 74.6 % bound in the plasma of the lactating doe (Table 6.1). The AUC found in milk was almost ten times higher than that described for plasma in colostrum stage; while in mature period, the milk-to-plasma ratio is less than two. S/W calculated for BUP indicated that more than half of the drug remained in skimmed milk.

The milk pharmacokinetic parameters can be observed in Table 6.3. The maximal concentration was 18.4ng/ml and was observed within two hours. After that time, the linear phase of elimination from milk began, and was found not to be significantly different from the elimination rate constant calculated in plasma (Figure 6.1).

6.4. Discussion

Studies of drug transfer into milk are hampered by the wide species variability in the protein and lipid content of milk. Laboratory animals, such as rabbits, produce milk with much higher lipid and protein content than human milk (Table 2.6). Therefore, the M/P ratios measured in rabbit may differ greatly from those in humans. However, the rabbit model used in this study was to study the effect of different lactating periods, as well as the change of physiology conditions, in the milk transfer and prediction the infant exposure.

When BUP was administered to the lactating rabbit as a bolus dose of 30mg/kg body weight, the drug was just detected in milk at the first sample time and the reached its maximal concentration in milk within two hours. The results of the present study agree well with the only other available data, which relate to the concentrations in milk and serum following the treatment of a lactating woman with 100mg BUP three times daily[185].

The diffusional model for distribution of antidepressant across biological membranes has drawn attention in recent years in an attempt to predict milk using easy and inexpensive in vitro experiments, which have been conducted in our lab. Thus, the diffusional model incorporated the pH partition theory, milk and plasma protein binding, and drug partitioning into milk fat as control factors in the M/P ratio was used to predict the milk-to-plasma ratio[186]. However, the prediction was based on theoretical study and does not correlate with the in vivo data as the in vivo data obtained was always based on case report and lack the experiment control. For example, the changing of lactating period may influence the milk plasma distribution which can be

predicted by phase distribution model. However, the prediction may not accurate because the drug action in body is a dynamic process. For this, an in vivo and in vitro correlation must be established to enable better prediction of drug milk plasma distribution.

It have been demonstrated[187] that BUP is metabolized in many tissues to HB (hydroxy metabolite) or TB (Threohydro metabolite), so it is possible that BUP may also be metabolized in milk or in the mammary gland. For this reason, complementary studies on BUP metabolism in these tissues should be also carried out in the future study.

The M/P ratio determined in colostrum period was about 6 times higher than that in mature stage [Table 6.4], this may due to the increase of pH from colostrum to mature period. It is suggested that the administration of bupropion in early lactation period may pose more risk to infant than the later stage.

In conclusion, BUP seems to diffusion use freely into milk after its i.v. administration to the lactating rabbit, so this drug should not be given to nursing mammals because of potential risk to their offspring. It is shown that M:P ratio in early lactation stage was much higher then later stage. However, neonatal exposure to BUP via suckling will be influenced by several factors such as the nursing schedule relative to maternal dosing schedule, type of elimination pattern in dams, neonatal bioavailability and the neonatal ability to remove drug from its body. Thus, the high M/P ratio does not guarantee the high drug concentration in infant. Nevertheless, the drug should be used with caution during lactation because of the potential for adverse effect.

Table 6.1. Parameters in rabbit milk and plasma

		Rabbit		
		1	2	Mean
Plasma	pH	7.47	7.45	7.46±0.014
	Protein content(g/dl)	5.84	5.55	5.70±0.205
	fu	25.5	25.2	25.35±0.212
Colostrum	pH	6.94	6.99	6.97±0.035
	fu	20.5	19.7	20.1±0.566
	S/M	0.288	0.302	0.30±0.010
	Cr	13.2	13.0	13.1±0.141
Mature	pH	7.42	7.30	7.36±0.085
	fu	24.1	24.7	24.4±0.424
	S/M	0.435	0.498	0.467±0.045
	Cr	12.2	12.2	12.2

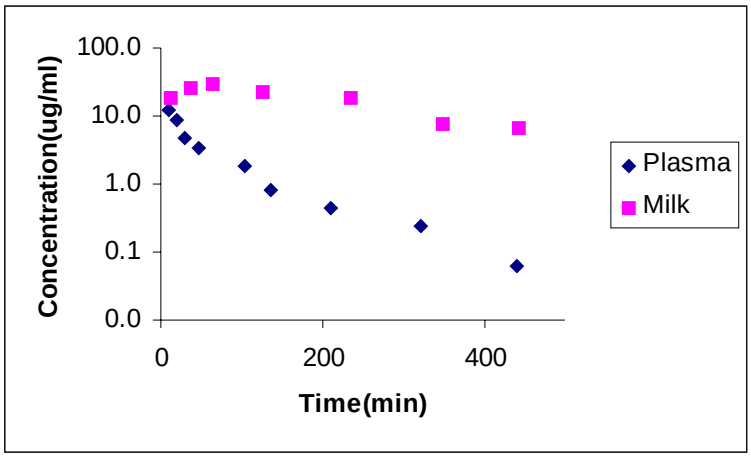


Figure 6.1a. Semilogarithmic plot of concentration vs. time for bupropion in milk and plasma after intraveneous administration of bupropion (30mg/kg) to lactating rabbit (I) in colostrum stage.

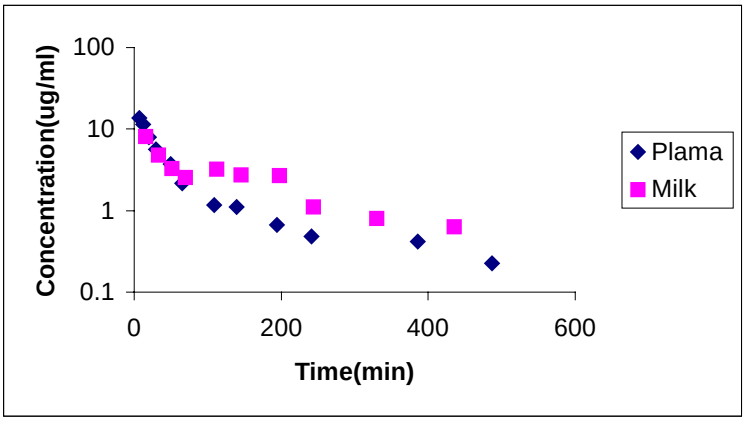


Figure 6.1b. Semilogarithmic plot of concentration vs. time for bupropion in milk and plasma after intraveneous administration of bupropion (30mg/kg) to lactating rabbit (I) in mature stage.

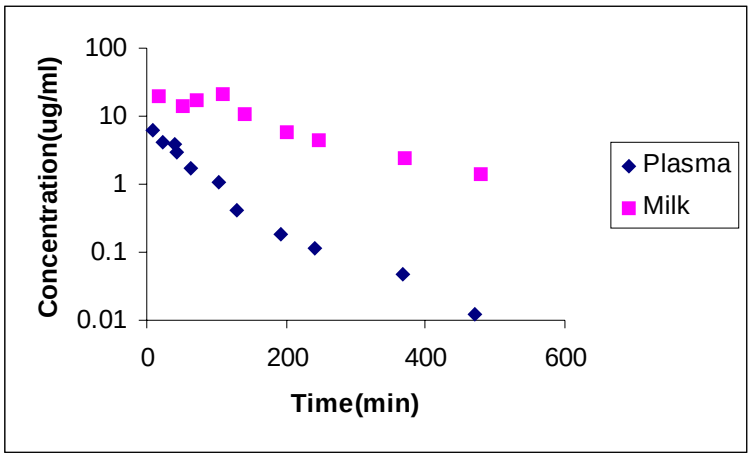


Figure 6.1c. Semilogarithmic plot of concentration vs. time for bupropion in milk and plasma after intraveneous administration of bupropion (30mg/kg) to lactating rabbit (II) in colostrum stage.

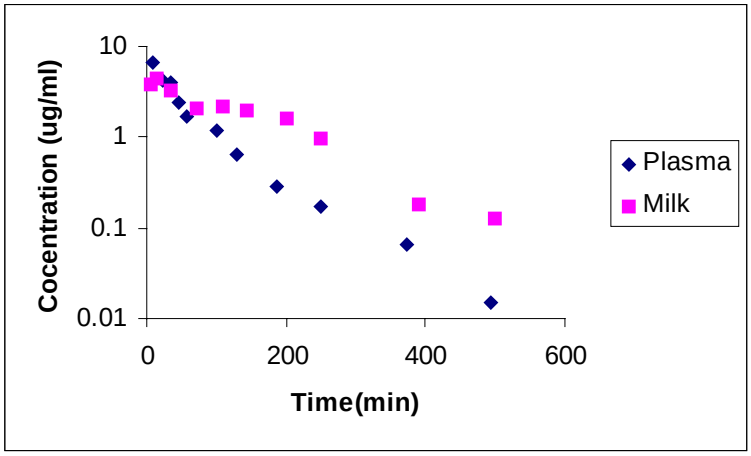


Figure 6.1d. Semilogarithmic plot of concentration vs. time for bupropion in milk and plasma after intraveneous administration of bupropion (30mg/kg) to lactating rabbit (II) in Mature stage.

Table 6.2. Individual and mean pharmacokinetic parameters in plasma determined after iv. bonus of 30mg/kg BUP.

(a) Pharmacokinetic parameters determined for 2 rabbits in colostrum stage

	Rabbit			
	1	2	Mean	SD
C_0 (μg/ml)	19.4	7.11	13.3	8.69
α(min^{-1})	0.0547	0.0218	0.038	0.023
β(min^{-1})	0.0018	0.0032	0.0025	0.00099
$t_{1/2}$α (min)	12.7	31.77	22.2	13.5
$t_{1/2}$β (min)	83.7	218.1	150.9	95.0
AUC (μg/ml^{-1}min)	684.9	384.5	534.7	212.4
CL (ml/minkg)	43.8	78.0	60.9	24.2
*V*d (l • kg^{-1})	1.55	4.34	2.95	1.97

(b) Pharmacokinetic parameters determined for 2 rabbits in mature stage

	Rabbit			
	1	2	Mean	SD
C0 (μg/ml)	18.0	7.48	12.7	7.43
α(min-1)	0.0489	0.047	0.048	0.0013
β(min-1)	0.0062	0.012	0.0091	0.0041
t1/2α (min)	14.2	14.7	14.4	0.38
t1/2β (min)	112.2	57.8	85.0	38.4
AUC (μg/ml^{-1}min)	726.9	396.7	561.8	233.5
CL (ml/minkg)	41.3	75.6	58.5	24.3
Vd (l•kg^{-1})	1.67	4.12	2.90	1.73

α and β are the exponents that were fitted by winonlin, t1/2α= distribution half-life; t1/2β = elimination half-life; Vd= apparent volume of distribution; AUC= area under the plasma concentration vs. time curve; and CL= body clearance

Table 6.3. Individual and mean pharmacokinetic parameters in milk determined after iv. bonus of 30mg/kg BUP

(a) Pharmacokinetic parameters determined for 2 rabbits in colostrum stage

	Rabbit			
	1	2	Mean	SD
Cmax(μg/ml)	15.9	20.9	18.4	3.5
tmax(min)	37-125	71-140	/	/
λmilk(min^{-1})	0.004	0.0052	0.0046	0.0008
$t_{1/2}$λmilk (min)	173.3	133	153.15	28.5
AUC (μg/ml^{-1}min)	7594	3871	5733	2633

(b) Pharmacokinetic parameters determined for 2 rabbits in mature stage

	Rabbit			
	1	2	Mean	SD
Cmax(μg/ml)	11.4	4.4	7.9	4.9
tmax(min)	25-35	7-35	/	/
λmilk(min^{-1})	0.0015	0.009	0.0053	0.005
$t_{1/2}$λmilk (min)	462	77	270	272
AUC (μg/ml^{-1}min)	1408	646	1027	539

Cmax = maximum experimental milk concentration; tmax = the time where maximum verapamil concentration was observed; λmilk = slope of the elimination phase of the verapamil milk concentration vs. time curve; t1/2λmilk = elimination half-life from milk; AUC= area under the curve of the BUP milk concentration vs. time curve

Table 6.4. The in vivo values of M/P for bupropion

M/P	1	2	Mean	SD
Colostrum	11.08775	14.90897	12.99836	2.702012
Mature	1.936993	1.628435	1.782714	0.218184

CHAPTER *7*

THE EFFECT OF LACTATION STAGE ON THE MILK-TO-PLASMA RATIO AND THE PREDICTION OF INFANT EXPOSURE: AN IN VITRO AND IN VIVO EVALUATION

Drugs pass from plasma to milk despite the biological filtration system and are bioavailable to the infant, presenting a potential danger[188,189]. To determine the magnitude of the risk, it is necessary to know the amount of drug excreted into milk. The drug milk plasma distribution ratio is affected by drug dosage, proportion bound in plasma[190], maternal clearance rate, half-life of the drug, molecular weight, lipid solubility, degree of ionization, pH difference between plasma and milk composition[162]. Due to the pH gradient between plasma and milk (the mean pH of milk is lower than that of plasma), weak basic drugs, such as antidepressant, are more ionized in milk and can become ion-trapped in milk.

Breast milk is a dynamic body fluid whose composition changes throughout lactation. Hence, the M/P ratio may affected by the changing of milk composition[191], as milk pH, fat and protein concentration are affected by the time of day, diet, the stage during a single feeding, and possibly which breast was milked[191]. More dramatic changes in milk composition occur in the first several weeks postpartum (Table 7.1). The alternation in milk composition may affect antidepressant drug concentration in breast milk, thereby placing the infant at increased risk of exposure to these drugs.

7.1. Prediction of M/P ratio for sertraline and bupropion during different lactation periods

To determine the effect of lactation stage on M/P ratio, five lactation periods were chosen to calculate the M/P ratio by using parameters obtained from literature and experiment (Table 7.1).

The phase distribution model was used to calculate the M/P ratio (Eq. 1.1). The S/M ratio is calculated using equation 1.2.

As protein binding of most drugs in plasma is known while protein binding in breast milk is not known, a predictive relationship between the two would enable the estimation of the appropriate protein binding of any drug in milk. Thus, in our proposed relationship, total protein concentration in plasma and milk, and 2 correction factors, f_{pH} and f_{cor} are incorporated in the calculation of fraction unbound in milk to account for the differences in total milk concentration and milk pH. The equation is given by:

$$f_{u,m} = \frac{1}{1 + \frac{1 - f_{u,p}}{f_{u,p}} \left(\frac{p_{t,m} f_{pH}}{p_{t,p} f_{cor}} \right)} \qquad \text{(Eq. 7.1)}$$

where $P_{t,m}$ is the total protein concentration in milk, and $P_{t,p}$ is the total protein concentration in plasma.

The f_{cor} and f_{pH} values for basic drugs are predicted using the following relationships:

$$\log f_{cor} = 0.42 \log p - 0.12 \qquad \text{(Eq. 7.2)}$$

As f_{pH} was found to correlate well with P_{app}, ($r^2 = 0.9991$, n = 6, Figure 6.1)) the following equation is used to predict f_{pH} :

$$f_{pH} = 0.033 Papp + 1.0688 \qquad \text{(Eq. 7.3)}$$

while log k_f is predicted using:

$$\log k_f = 1.29 \text{Log p} - 0.88 \qquad \text{(Eq. 7.4)}$$

As shown from Table 6.2, M/P ratio decreased with the change of lactation period, which consistent with our in vio findings.

From our prediction of M/P ratio for bupropion and the case report [], it may safely be concluded that it is better to avoid bupropion administrated in the early lactation period, i.e., the first two weeks.

7.2. Prediction of relative dose exposure to infants

The ingestion of drugs via milk feeding must be considered as a potential hazard for neonates[192]. The elimination of drugs from neonates seems to be lower than in adults, and thus, neonatal clearance could have clinical importance. Furthermore, most drug-metabolizing enzymes are either not present or have only limited activity at birth, developing at different stages of life depending on the species[193]. Indeed, deficient development of microsomal mixed-function oxidase activity has been reported during the first month of life in rabbits[194]. These facts suggest that the diminished elimination capacity of newborns could result in greater exposure than that predicted from milk values alone[195]. Thus, it is necessary to consider increasing age of the infant in estimating the consequences of drug intake through breast milk.

The potential risk of sertraline and bupropion to infant was accessed using using M/P ratios predicted using our proposed model. The M/P ratio and likely dose in three stages of lactation in both pre-term and full-term infants were predicted. It is shown that M/P ratio decrease with the change of lactation stage, which agrees with our findings in lactating rabbit. The M/P ratio was 13.0 in colostrum stage, while it decreased to 1.78 in mature stage (Table 7.3).

The three stages of lactation coincide with the infant's stage of development. For instance, in the early stage of lactation (the first 60 days), the clearance in a full-term infant is about 33 % of maternal's clearance, while it is approximately equal to the maternal's clearance in the late stage of lactation (greater than 180 days). Thus, infants exposed after the first five to six months may be better able to clear drugs ingested via breast milk. This was reflected in Table 6.5, where the average drug concentration in the infant of a few days old was more than 10times higher than when he was more than 180 days old. Predicted infant dose varies from 15.3-102.9μg/kg for bupropion and 3.49-81.6μg/kg for sertraline. These data demonstrate that with increasing infant age, infant dose and plasma concentrations decrease, leading to lesser risk to infants. From these estimations, it is apparent that mother should not breast feeding their baby during early postpartum period, especially in colostrum stage. However, There are significant differences in the functioning of drug metabolism pathways in the neonatal and young infant[16]. Also, drug delivery to the infant may also be altered by change in the intestinal tract, especially in the maturation of the gut epithelium [17]. In addition, there are development changes in bile salt formation and gastric pH [18]. These factors may influence the amount of drug absorbed, as well as the ultimate plasma concentration. The existing data on drug excretion in breast milk are valuable but require critical analysis and careful interpretation to get any clinical significance for nursing infants and neonates.

Our estimation, based on predicted M/P ratios and infants' pharmacokinetic parameters, is an attempt to assess the excretion of drugs into infants from the early stage of post partum to the later. All of infant drug concentrations are well below the therapeutic

range and the relative dose estimated in rabbit baby is also small (Table 7.3), which indicates that the amount of drug ingested by the infant could be insignificant.

Postnatal maturation of pharmacokinetic processes has significant implications with respect to systemic exposure levels and the safety and/or efficacy of a drug in the newborn and young infant. Functional immaturity of absorption, distribution, metabolism and excretion processes contribute to the different responses observed between newborns, infants and adults[196]. The anatomical and functional immaturity of the organs and other biochemical and physiological processes of infants poses a potential risk to themselves when ingested some antidepressant drugs. An assessment of the therapeutic efficacy or toxicant susceptibility of a newborn to antidepressnant drugs will require a careful consideration of the development of pharmacokinetic processes. However, the effects of age-related changes in each pharmacokinetic process on plasma levels of a compound are poorly understood. Clinical antidepressant pharmacokintics/pharmacodynamic studies in infant will help to establish establish more effective guidelines to predict the toxicity in a newborn or young infant. .

In conclusion, decision to treat nursing mothers with antidepressants should be based on a risk to benefit assessment for each patient. Substantial risk of untreated depression on both mother and infant must be balanced against the risk of commonly prescribed antidepressants on nursing infants.

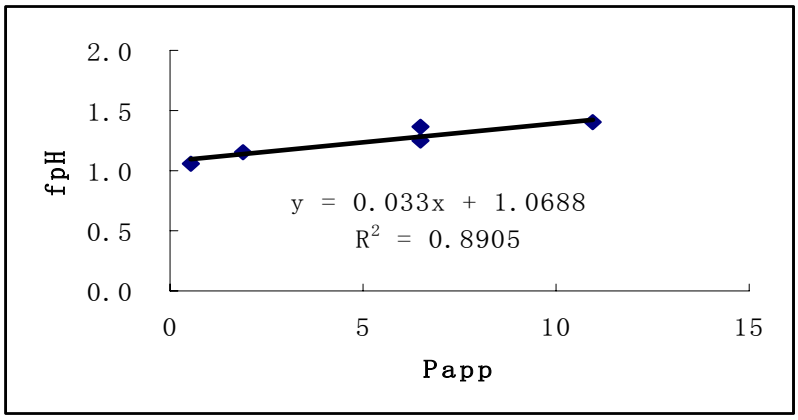


Figure 7. 1. Relationship between fpH and Papp for 5 basic drugs at pH 6.4

Table 7. 1. Composition of breast milk in the different lactational periods

Lactational period [91,197,198]	pH [199,200]	Protein concentration (g/l) [198,201]	Fat concentration (g/l) [202]	*ct* [76,198]
Early *Colostrum* (1-4 days)	6.4 – 6.63	15 – 60	39 – 43	0.035
Transitional (5-14 days)	6.63 – 6.72	12 – 14	43 – 47	0.045
Mature (15-60 days)	6.72 – 6.78	6 – 12	47 – 51	0.055
Full (60-180 days)	6.78 – 6.97	6 – 12	51 – 58	0.058
Late (>180 days)	6.97 – 7.6	8 – 10	58 – 77	0.0701

Table 7. 2. Estimation of CL_{inf} [89]

Infant post-conceptual age	CL
28-34 weeks	0.1 x CL_{mat}
34-40 weeks	0.33 x CL_{mat}
40-44 weeks	0.5 x CL_{mat}
44-68 weeks	0.66 x CL_{mat}
> 68 weeks	CL_{mat}

Table 7.3. Estimation of likely rabbit baby exposure in the 2 stages of lactation in rabbit baby

Stage	M/P[a]	Cmat (ng/ml)	Rabbit baby Dose (μg/day)	RD%[f]
Colostrum	13.0	342.1[b]	22.24[d]	0.022
Mature	1.78	356.1[c]	12.28[e]	0.012

a: get from experiment data
b: Dose=30mg/kg, CL/F=12.4l/h
c: Dose=30mg/kg, CL/F=12.3l/h
d: Assuming that the volume of milk taken by rabbit baby is 5ml/day
e: Assuming that the volume of milk taken by rabbit baby is 20ml/day
f: Calculated using equation 1.13.

Table 7.4. Prediction of M/P ratio in the different stages of lactation
(a) Prediction of M/P ratio of sertraline

[6, 7, 8]	pH_m [8, 9]	$P_{t,m}$(g/l) [7, 8]	*ct* [7, 10]	$f^{un\ a}$ ($\times 10^{-3}$)	$LogP_{app}{}^{b}$	$f_{pH}{}^{c}$	$f_{cor}{}^{d}$	$Logk_f{}^{e}$	$f_{u,m}{}^{f}$	M/P
Early *Colostrum* (1-4 days)	6.4	15	0.035	3.15	1.98	4.28	5.19	1.69	0.110	2.04
		60							0.030	6.62
	6.63	15		5.34	2.21	6.51	6.48	1.98	0.092	1.59
		60							0.025	4.89
Transitional (5-14 days)	6.63	12	0.045	5.34	2.21	6.51	6.48	1.98	0.112	1.47
		14							0.097	1.62
	6.72	12		6.56	2.30	7.76	7.06	2.10	0.103	1.39
		14							0.090	1.52
Mature (15-60 days)	6.72	6	0.055	6.56	2.30	7.76	7.06	2.10	0.188	1.11
		12							0.103	1.49
	6.78	6		7.53	2.36	8.74	7.48	2.17	0.178	1.10
		12							0.098	1.45
Full (60-180 days)	6.78	6	0.058	7.53	2.36	8/74	7.48	2.17	0.178	1.13
		12							0.097	1.49
	6.97	6		11.6	2.55	12.91	8.98	2.41	0.150	1.13
		12							0.081	1.41
	6.97	8	0.0701	11.6	2.55	12.91	8.98	2.41	0.117	1.38
		10							0.096	1.48
Late (>180 days)	7.6	8		47.7	3.17	49.7	16.25	3.21	0.059	1.66
		10							0.047	1.70

(b) Prediction of M/P ratio of bupropion

[6, 7, 8]	pH_m [8, 9]	$P_{t,m}$(g/l) [7, 8]	*ct* [7, 10]	$f^{un\ a}$ ($\times 10^{-2}$)	$LogP_{app}{}^{b}$	$f_{pH}{}^{c}$	$f_{cor}{}^{d}$	$Logk_f{}^{e}$	$f_{u,m}{}^{f}$	M/P
Early *Colostrum* (1-4 days)	6.4	15	0.035	3.07	1.36	1.83	2.82	0.87	0.658	2.70
		60							0.325	5.06
	6.63	15		5.10	1.58	2.32	3.49	1.15	0.652	1.87
		60							0.319	3.32
Transitional (5-14 days)	6.63	12	0.045	5.10	1.58	2.32	3.49	1.15	0.700	1.89
		14							0.667	1.95
	6.72	12		6.20	1.66	2.58	3.79	1.26	0.695	1.71
		14							0.661	1.76
Mature (15-60 days)	6.72	6	0.055	6.20	1.66	2.58	3.79	1.26	0.820	1.68
		12							0.695	1.84
	6.78	6		7.05	1.72	2.79	4.00	1.34	0.816	1.60
		12							0.690	1.75
Full (60-180 days)	6.78	6	0.058	7.05	1.72	2.79	4.00	1.34	0.816	1.64
		12							0.690	1.79
	6.97	6		10.5	1.89	3.64	4.73	1.56	0.801	1.50
		12							0.669	1.61
	6.97	8	0.0701	10.5	1.89	3.64	4.73	1.56	0.752	1.73
Late (>180 days)		10							0.708	1.76
	7.6	8		33.4	2.39	9.24	7.68	2.21	0.660	1.83
		10							0.608	1.85

pH_m = pH of milk, Total plasma protein = 74.6g/l

a: calculated using: $f^{un} = 1/(1 + 10^{pKa-pH})$

b: calculated using: $P_{o/w,app} = P_{o/w,\ true}$ x f_{un}

c: calculated using: $f_{pH} = 0.0703P_{app} - 0.0335$

d: calculated using: $\log f_{cor} = 0.4132\ \log P - 0.1192$

e: calculated using: $\log k_f = 1.29 LogP_{app} - 0.88$

f: calculated using: $$f_{u,m} = \frac{1}{1 + \frac{1 - f_{u,p}}{f_{u,p}} \left(\frac{p_{t,m}\, f_{pH}}{p_{t,p}\, f_{cor}} \right)}$$

Table 7.5. Estimation of likely infant exposure in the 3 stages of lactation in pre-term and full-term infants

(a) Estimation of likely infant exposure in pre-term and full-term infants when maternal dosage is 50mg and 200mg for sertraline

Lactational Period	M/P	Pre-term Maternal dose (50mg/d)		Pre-term Maternal dose (200mg/d)		Full-term Maternal dose (50mg/d)		Full-term Maternal dose (200mg/d)	
		Infant dose[a] (mg/kg/day)	C_{inf}[b] (μg/l)	Infant dose[a] (mg/kg/day)	C_{inf}[b] (μg/l)	Infant dose[a] (mg/kg/day)	C_{inf}[b] (μg/l)	Infant dose[a] (mg/kg/day)	C_{inf}[b] (μg/l)
Early[c]									
Colostrum	2.04	6.30	0.091	25.2	0.36	6.30	0.027	25.2	0.110
(1-4 days)	6.62	20.40	0.293	81.6	1.17	20.40	0.089	81.6	0.355
	1.59	4.91	0.071	19.6	0.28	4.91	0.021	19.6	0.086
	4.89	15.05	0.216	60.2	0.87	15.05	0.066	60.2	0.262
Transitional	1.47	4.98	0.072	18.1	0.26	4.54	0.020	18.1	0.079
(5-14 days)	1.62	4.27	0.061	19.9	0.29	4.98	0.022	19.9	0.087
	1.39	4.67	0.067	17.1	0.25	4.27	0.019	17.1	0.074
	1.52	3.42	0.049	18.7	0.27	4.67	0.020	18.7	0.081
Mature	1.11	3.38	0.049	13.7	0.20	3.42	0.015	13.7	0.060
(15-60 days)	1.49	4.48	0.064	18.4	0.26	4.60	0.020	18.4	0.080
	1.10	3.49	0.050	13.5	0.19	3.38	0.015	13.5	0.059
	1.45	4.58	0.066	17.9	0.26	4.48	0.020	17.9	0.078
Full[d]	1.13	4.35	0.013	14.0	0.04	3.49	0.008	14.0	0.030
(60-180 days)	1.49	4.26	0.012	18.3	0.05	4.58	0.010	18.3	0.040
	1.13	4.55	0.013	13.9	0.04	3.48	0.008	13.9	0.030
	1.41	5.10	0.015	17.4	0.05	4.35	0.009	17.4	0.038
Late[e]	1.38	4.26	0.006	17.1	0.02	4.26	0.006	17.1	0.025
(>180 days)	1.48	4.55	0.007	18.2	0.03	4.55	0.007	18.2	0.026
	1.66	5.10	0.007	20.4	0.03	5.10	0.007	20.4	0.029
	1.70	5.25	0.008	21.0	0.03	5.25	0.008	21.0	0.030

(b) Estimation of likely infant exposure in pre-term and full term infants when maternal dosage is 200mg and 800mg for bupropion

Lactational Period	M/P	Pre-term Maternal dose (200mg/d)		Pre-term Maternal dose (800mg/d)		Full-term Maternal dose (200mg/d)		Full-term Maternal dose (800mg/d)	
		Infant dose[f] (mg/kg/day)	C_{inf}[g] (mg/l)	Infant dose[f] (mg/kg/day)	C_{inf}[g] (mg/l)	Infant dose[f] (mg/kg/day)	C_{inf}[g] (mg/l)	Infant dose[f] (mg/kg/day)	C_{inf}[g] (mg/l)
Early[h] *Colostrum* (1-4 days)	2.70	25.6	0.29	57.5	0.66	25.6	0.088	57.5	0.199
	5.06	45.7	0.52	102.9	1.17	45.7	0.158	102.9	0.356
	1.87	19.0	0.22	42.7	0.49	19.0	0.066	42.7	0.148
	3.32	35.6	0.41	80.0	0.91	35.6	0.123	80.0	0.277
Transitional (5-14 days)	1.89	19.1	0.22	43.0	0.49	19.1	0.066	43.0	0.149
	1.95	19.9	0.23	44.7	0.51	19.9	0.069	44.7	0.155
	1.71	17.5	0.20	39.4	0.45	17.5	0.061	39.4	0.136
	1.76	18.2	0.21	41.0	0.47	18.2	0.063	41.0	0.142
Mature (15-60 days)	1.68	16.8	0.19	37.9	0.43	16.8	0.058	37.9	0.131
	1.84	18.8	0.21	42.3	0.48	18.8	0.065	42.3	0.146
	1.60	16.2	0.18	36.3	0.42	16.2	0.056	36.3	0.126
	1.75	18.0	0.21	40.5	0.46	18.0	0.062	40.5	0.140
Full[i] (60-180 days)	1.64	16.6	0.19	37.2	0.09	16.6	0.029	37.2	0.064
	1.79	18.5	0.21	41.6	0.10	18.5	0.032	41.6	0.072
	1.50	15.3	0.17	34.4	0.08	15.3	0.026	34.4	0.059
	1.61	16.8	0.19	37.9	0.09	16.8	0.029	37.9	0.066
Late[j] (>180 days)	1.73	17.7	0.20	39.9	0.046	17.7	0.031	39.9	0.069
	1.76	18.2	0.21	41.0	0.047	18.2	0.032	41.0	0.071
	1.83	18.6	0.21	41.9	0.048	18.6	0.032	41.9	0.072
	1.85	18.9	0.22	42.5	0.049	18.9	0.033	42.5	0.074

a: calculated using equations 1.11 where, CL_{mat} /F= 101.4 l/h, assuming that the volume of milk taken by infant is 150ml/kg/day
b: calculated using equation 1.12, the therapeutic concentration of sertraline in maternal plasma is: 10-60ng/ml.
c: $CL_{inf} = 0.1 \times CL_{mat}$ corresponding to infant post-conceptual age of 28-34 weeks
d: $CL_{inf} = 0.5 \times CL_{mat}$ corresponding to infant post-conceptual age of 40-44 weeks
e: $CL_{inf} = CL_{mat}$ corresponding to infant post-conceptual age of > 68 week
f: calculated using equations 1.11 where, CL_{mat} /F= 127.7 l/h, assuming that the volume of milk taken by infant is 150ml/kg/day
g: calculated using equation 1.12, the therapeutic concentration of bupropion in maternal plasma is: 10-50ng/ml.
h: $CL_{inf} = 0.33 \times CL_{mat}$ corresponding to infant post-conceptual age of 34-40 weeks
i: $CL_{inf} = 0.66 \times CL_{mat}$ corresponding to infant post-conceptual age of 44-68 weeks
j: $CL_{inf} = CL_{mat}$ corresponding to infant post-conceptual age of > 68 weeks

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