

**BRIDGING *IN VITRO* DISSOLUTION TESTS TO *IN VIVO* DISSOLUTION
FOR POORLY SOLUBLE ACIDIC DRUGS**

by

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DEDICATION

To my parents and my husband

With Love and gratitude

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ABSTRACT

BRIDGING IN VITRO DISSOLUTION TESTS TO IN VIVO DISSOLUTION FOR POORLY SOLUBLE ACIDIC DRUGS

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Co-chairs: Gordon L. Amidon and Steven P. Schwendeman

Developing meaningful *in vitro* dissolution methods is critical for evaluating the drug *in vivo* performance and providing a better standard for biowaiver tests. For Biopharmaceutical Classification System (BCS) Class II poorly soluble drugs, the dissolution tests are especially important because *in vivo* dissolution is the rate-limiting process in oral absorption of drugs. There are many factors affecting the *in vivo* dissolution processes that may have not been fully considered when designing the *in vitro* dissolution tests.

In this dissertation, in order to bridge the gaps between the *in vitro* dissolution tests with the *in vivo* dissolution, the human intestinal fluid (HIF) was characterized in terms of buffering species and buffer effect on dissolution of acidic drugs using a miniature rotating disk dissolution apparatus; Mathematical models such as film and reaction plane models were utilized and refined to study the dissolution media factors

such as pH, buffering species, and buffer strength on the dissolution of poorly soluble acidic drug with known physicochemical properties; Other factors such as CO₂ partial pressure, the effect of enzymatic reaction in the case of bicarbonate buffer were also investigated and discussed.

The bicarbonate buffer contributes up to 74% to the buffer capacity in human intestinal fluid and dissolution of the model drug, ibuprofen, in HIF decreased by 48% when HCO₃⁻/CO₂ was depleted from *ex vivo* human intestinal fluid. The two mathematical models were in reasonable good agreement of the buffer effect on dissolution of ibuprofen. Physiological bicarbonate buffer has been compared with United States Pharmacopeia (USP) acetate buffer. With physiochemical properties of the drug known, and dissolution buffer can be equated to USP buffer species of proper buffer strength to reflect the *in vivo* dissolution. Other factors, especially partial pressure of CO₂ and enzyme like carbonic anhydrase have also been shown to affect the dissolution through their effect on bicarbonate buffer system. The results provide important information and a valuable approach for developing *in vitro* dissolution test for poorly soluble acidic drugs for better *in vitro*- *in vivo* correlation (IVIVC) and scientific basis for setting biowaiver test standards.

**CHAPTER I. DESIGNING THE *IN VITRO* DISSOLUTION TESTS
EVALUATING THE DRUG PERFORMANCE *IN VIVO*:
FACTORS TO BE CONSIDERED.**

The application of *in vitro* dissolution tests

Dissolution tests have been widely used in pharmaceutical industry to characterize the release of drug from dosage forms. It is needed in many stages in drug development life cycle.

Quality Control: Dissolution was introduced more than 30 years as the quality control tools to assure product uniformity and detect batch-to-batch differences of drug products occurring during the manufacture processes(1). It also provides the possibility to test large number of batches, which is otherwise impossible to test clinically. It continues to play an important role in stability tests such as finding out the possible product changes during manufacture and storage period affected by temperature and humidity. (2)

Bioequivalence and Biowaiver: Dissolution tests are simple, reliable, highly reproducible methods that allow monitoring the product quality efficiently and could be used to establish the equivalence between formulations at different stage of development and also different sites or scale for manufacturing. By using carefully designed dissolution tests , which considered the multidimensional combinations and interactions of both the drug and material attributes and process parameters, the

quality could be assured from the early stage of development.(3)

Formulations development and *in vitro*- *in vivo* correlation (IVIVC): In recent years, dissolution tests have received increasing attention for its function of serving the surrogate for *in vivo* availability. The relationships between *in vitro* dissolution and *in vivo* input rate have been categorized into four different levels, A, B, C and multiple level C correlations. A level A correlation represents a point-to-point relationship between *in vitro* dissolution and the *in vivo* input rate, which is the considered the most informative and also very useful from a regulatory viewpoint. Generally, these correlations are linear; however non-linear correlations are also acceptable. A level B *IVIVC* uses the principles of statistical moment analysis, correlating the mean *in vitro* dissolution time to either the mean residence time or the mean *in vivo* dissolution time. The level B uses the information from the whole *in vitro* and *in vivo* data, but it is not uniquely reflect the actual *in vivo* plasma level curve. A level C correlation establishes a single point relationship between a dissolution parameter (e.g. $T_{50\%}$ or percent dissolved in 4 h) and a pharmacokinetic parameter (e.g. AUC or C_{max}). A multiple level C correlation relates one or several pharmacokinetic parameters of interest to the amount of drug dissolved at several time points of the dissolution profile (4-6). For novel drug delivery systems and modified release products, the establishment of *IVIVC* helps in establishing dissolution specifications and allows the waiver for *in vivo* bioequivalence study (7-12). For novel drug products, the dissolution is the essential screening tool to select the formulation for clinical trials. The dissolution

tests help to reduce the cost, assess product performance more directly and avoid the complex and variable pharmacokinetic profiles which are caused by the complications such as post-absorption, enteroheptic recycling and highly variable drugs, which are defined as drugs with within-subject variability (WSV) of the maximum concentration (Cmax) equals or exceeds 30% (13, 14). It also offers benefits in terms of ethical consideration and speeds up the development decision making process.(15)

Biopharmaceutical classification system and BCS II drugs

Biopharmaceutical classification has provided the scientific basis for the correlation of *in vitro* drug product dissolution and *in vivo* bioavailability and has been adopted by FDA as the guidance for the waiver of *in vivo* bioavailability and bioequivalence studies for immediate release solid oral dosage forms (16, 17). Based on the model analysis on dissolution and absorption(18), drugs are classified into four high/low solubility-permeability classes with the expectations of their *in vitro-in vivo* correlations more clearly stated in Table 1.1.

[\(Table 1.1\)](#)

For BCS II drugs of low solubility and high permeability, the absorption number = An

$$= \frac{P_{eff}}{R} \cdot t_{res} \text{ is high, and the dissolution number } Dn = \frac{DC_s}{r_0} \cdot \frac{4\pi r_0^2}{\frac{4}{3}\pi r_0^3 \rho} \cdot t_{res} =$$

$t_{res} \cdot 3DC_s / \rho r_0^2 = t_{res} / t_{Diss}$ is low. Here, the P_{eff} is the effective permeability of the

drug, C_s is solubility of the drug, R is the intestine, t_{res} and t_{Diss} is the mean residence time and the time required for a particle of drug to dissolve. r_o is the initial particle radius. The dissolution *in vivo* is then the rate limiting step in drug absorption except for very high dose number ($D_0 = \frac{M_0/V_0}{C_s}$). Dissolution profile will determine the concentration profile along the intestine for a much great period of time when intestinal luminal contents, intestinal membrane change along the intestine and much more of the intestinal content is exposed to the intestine. Figure 1.1 shows the for high permeability drugs, the estimated fraction dose absorbed depends on the dose number D_0 , and dissolution number D_n . This work stated the possibility of the *IVIVC* for Class II drugs. Correspondingly, the *in vitro* dissolution profile should also be determined at several physiological conditions with 4-6 time points and with at least 85% dissolved.(16)

[\(Figure 1.1\)](#)

Since most new chemical entities developed as drug candidates are poorly soluble compounds (19, 20). Therefore, a lot of efforts are being made to overcome the difficulties of low solubility for developing new drugs as oral dosage forms. In which, the *IVIVC* is useful in helping to select and make desired formulations, but it is not easy to achieve especially when dissolution methods are not developed to reflect the *in vivo* dissolution kinetics.

Physiological factors contributing to the in vivo dissolution

The in vivo dissolution rate is not only affected by the physiochemical properties of the drug, but also the gastrointestinal (GI) physiological factors: the pH, the composition and hydrodynamics of the GI fluid caused from the motility of GI, the secretions of the glands and mucosa, food and drug intake, etc. All these factors interact with and result from each other and also with the drug properties determining the final level of drug dissolution and further absorption of the drug into the systemic circulation and amount available at the sites of actions.

Gastrointestinal pH

The pH gradients existing all along the gastrointestinal tract has been studied from early in 1960s using the telemetering capsule or electrode and later multichannel and online measurements (21-25). It is affected and in reverse affected by gastric emptying, the secretion of the glands and mucosa, bactericidal effects(26) and disease states or treatment (27-32), enzymes and transporters along GI(29) and also drug administration and food feed (33, 34). In health subjects, the intraluminal pH is rapidly changed from highly acidic of 1-3 in the stomach (fasted pH 1.3 (1.1-1.6) versus fed state 4.9(3.9-5.5) to about pH 5-7 in the duodenum (fasted duodenal pH, 6.5 (6.2-6.7); and duodenal pH during the meal, 6.5 (6.4-6.7)), then gradually increases in the small intestine from pH 6 to about pH 7.4 in the terminal ileum. Finally, pH drops to 5.7 in the caecum, but again gradually increases, reaching pH 6.7

in the rectum.(29,35,36). The pH gradients from studies along normal human subjects are list in Table 1.2.

(Table 1.2)

For acidic solubility limited drugs, studies have shown that drug dissolutions in buffer solutions are much higher than their intrinsic dissolution rates (37, 38). The results suggest that the poorly water soluble BCS Class II weak acid NSAIDs would have higher solubility because of highly ionized in intestinal environment, thus the dissolution process will also be faster.

Gastrointestinal motility

The gastrointestinal responses to food and drug intake are characterized by two patterns, inter-digestive pattern and the digestive motility pattern, with different upper GI motility and secretions which are regulated by the nervous system and the hormones (39-43). The inter-digestive pattern or the recurring cycles of intense contractile activity is termed as the migrating motor complex (MMC)(44) and been divided into four different phases. Phase I is a quiescent period. Phase II is a period with intermittent and irregular contractions with increasing strength, its contractile activity accumulates in a short period. Phase III is called the gastrointestinal interdigestive housekeeper and its contractile activity usually initiates from antrum and duodenum, also proximal jejunum(45), then migrates along the small intestine distally. It is also known as the interdigestive migrating myoelectric complex (IMMC).

The human MMC activity varies widely between individuals and within the same individual on separate days(46). The IMMC cycle is typically 90-120 minutes but could vary from 15min to 3 hours.(45, 47, 48) The pH of the duodenum is also believed to affect the IMMC in humans. Phase IV are sometime referred as the short period transition from phase III to Phase I.(49)

The motility plays an important role in the gastrointestinal environment interacting with different factors such as gastric emptying, luminal fluid volume, flow rates(47), pH(50), luminal metabolism, blood flow and their interactions with the drugs will also affect the drug *in vivo* dissolution and absorption. The transit time could also affected by gastrointestinal symptoms and also drug treatment (30, 51, 52)

Composition of gastrointestinal fluids

Gastrointestinal fluids are the media that oral drugs directly encounter after administration. Its composition therefore is very important for study the *in vivo* dissolution process. Besides the exogenous liquids, foods, drugs and their metabolites such as lecithin, monooleins, long chain fatty acids and/or triglycerides(53), the GI fluids are constituted of physiological buffers, bile salts, enzymes.

Bicarbonate is secreted from pancreas, gall bladder and duodenum mucosa into the duodenal chyme as the primary protection from the gastric acid (54, 55). Pancreatic fluid has a bicarbonate concentration of between the basal 25 mmol/l and 150 mmol/l

postprandial and up to 200 mmol in a 24 hour period. The bile from the gallbladder has a bicarbonate concentration of about 40 mmol/l. The duodenal mucosa also generates and secretes bicarbonate. Jejunum secretes small amount of proton and ileum secretes bicarbonate, and ileum could secret bicarbonate over 200 mmol per day into intestinal lumen. Most of the bicarbonate is consumed by organic anions from the acids which are the metabolites by bacteria.(56)

The intubation studies early in 1935 already showed that bicarbonate concentration ranged from 4.0-21.1 mM, jejunum 5.8 mM, upper ileum 2.3mM, middle ileum 3.9 to 39.8mM lower ileum 10.4-17.0mM(57). McNamara reviewed the bicarbonate luminal concentrations listed in Table 1.3(58).

[\(Table 1.3\)](#)

More recent research reported the buffer capacities of human jejunal fluids are 2.4-2.8 mmol L⁻¹ pH unit⁻¹ in fasted state with pH 7.5 and 13.2-14.6 mmol L⁻¹ pH unit⁻¹ with pH 6.1 in fed state, the corresponding bicarbonate buffer concentration calculated as 16.5-19.3mM for fasted and 25.4-27.7 mM for fed state, and 5.6 mmol L⁻¹ pH unit⁻¹ for fasted duodenum converted to bicarbonate of 10mM at pH6.2 (59, 60)

The ionic composition of the dissolution buffer could greatly affect the dissolution of ionizable drugs and is critical for solubility limited ionizable drugs even at the same pH (61-64). Currently, most of the dissolution buffers in applications are with

non-physiological buffer species and may not reflect the real *in vivo* dissolution profiles of BCSII drugs. So it is important to decide the methods including the buffers that should be used in the *in vitro* dissolution test to predict more accurately the *in vivo* dissolution.

Dissolutions of drug in products

Since drugs are always given in certain dosage forms, the formulation factors, the manufacture procedures and the physiochemical properties of the drugs will all influence the *in vivo* dissolution. The formulation factors includes the granule size, the particle size distribution, homogeneity and polymorphisms of the materials, and drug-excipient interactions; Attention should also be paid to the factors in manufacture process such as compression force, temperature, moisture and storage of the dosage forms; The physiochemical properties of drug itself, such as solubility, pka, diffusion coefficient, partition coefficient, chemical stability and dose are also essential. All of these factors interact with the physiological factors, thus as a result, could change the actual *in vivo* dissolution process of the drug products simultaneously.

Bridging the *in vitro* tests to *in vivo* dissolution process and dissolution media consideration

Investigating the *in vivo* physiological and pathological situations

To bridge the discrepancy between the *in vivo* dissolution tests to the *in vivo* dissolution process, it is critical to investigate the *in vivo* physiological and pathological situations. The more pieces of information could allow us to have a more complete and clear picture of what the key factors are limiting the dissolution.

Mathematical Models describing the dissolution tests

Mathematical models have been developed to provide a better way to understand the drug dissolution process or the release from the formulations quantitatively and qualitatively. In 1897, Noyes and Whitney published that the solid substance dissolution rate would be affected by their own solution concentration(65). The classic Noyes-Whitney equation is:

$$\frac{dM}{dt} = k(C_s - C_i) .$$

where dM/dt is the dissolution rate, dM is the amount of drug dissolved in unit time t , C_s is the solubility of the drug, C_i is the concentration of the dissolved drug, k is dissolution rate constant.

Nernst and Brunner modified the original Noyes-Whitney equation, consider the dissolution as diffusion limited two-step process:

$$\frac{dM}{dt} = \frac{D S}{V h} (C_s - C_i)$$

where dM/dt is the dissolution rate, dM is the amount of drug dissolved in unit time t , D is the diffusion coefficient, V is the volume of dissolution media, S is the surface area, h is the thickness of diffusion layer, C_s is the solubility of the drug, C_i is the concentration of the dissolved drug(66, 67).

The empirical model, Weibull model based on the distribution were proposed and applied to dissolution curves successfully(68, 69).

$$M = 1 - \exp(-\alpha t^\beta),$$

where M is the fraction of accumulated drug in solution at time t , α , β is a scale factor and shape factor respectively. For sparingly soluble, suspended drugs, diffusion transport plays an important role in the dissolution kinetics and the apparent diffusion distance(70) is

$$h_{APP} = DC_s/G$$

where D is the diffusion coefficient, C_s is the equilibrium solubility, and G is the surface specific dissolution rate.

Based on the film model of Olander (71, 72), Mooney et al. (64, 73) investigated the pH and buffer effect on the solubility and dissolution of ionizable drugs. McNamara et al also developed a convection diffusion model, reaction plane model, taking into account the irreversible reaction of the dissolved species with buffer in the hydrodynamic boundary layer. (62, 74)

Combination of experimental and theoretical approaches reflecting the *in vivo* process

Although developing the *in vitro* dissolution methods that could reflect the *in vivo* dissolution process is complicated with all above factors involved. We will first consider the dissolution media which plays an important role in the process and get started to understand the *in vivo* dissolution conditions. The contribution of bicarbonate buffer in human intestinal fluid to buffer capacity and its effect on the dissolution of model drug, ibuprofen (Figure 1.2 lists the structure and properties of Ibuprofen) will be investigated. Since physiological bicarbonate buffer is hard to be prepared and maintained due to its complicated dissociation and equilibrium with the $\text{CO}_2(75)$, the buffer effect on the dissolution of model compound, ibuprofen will be compared in compendia acetate buffer and physiological bicarbonate buffer. The comparison will be done both experimentally by the intrinsic dissolution and theoretically with film model and reaction plane model. The effect of CO_2 partial pressure and carbonic anhydrase enzymatic effect on the bicarbonate dissolution buffer will also be explored.

. ([Figure 1.2](#))

Table1.1 In Vitro – in vivo (IVIV) correlation Expectations for Immediate Release Products Based on Biopharmaceutical Class (reproduced from (16))

Class	Solubility	Permeability	IVIV correlation Expectation*
I	High	High	IVIV correlation if dissolution rate is slower than gastric emptying rate, otherwise limited or no correlation
II	Low	High	IVIV correlation expected if <i>in vitro</i> dissolution rate is similar to <i>in vivo</i> dissolution rate, unless dose is very high.
III	High	Low	Absorption (permeability) is rate determining and limited or no IVIV correlation with dissolution rate.
IV	Low	Low	Limited or no IVIV correlation expected.

*A limited correlation means that the dissolution rate while not rate controlling may be similar to the absorption rate and the extent of correlation will depend on the relative rates.

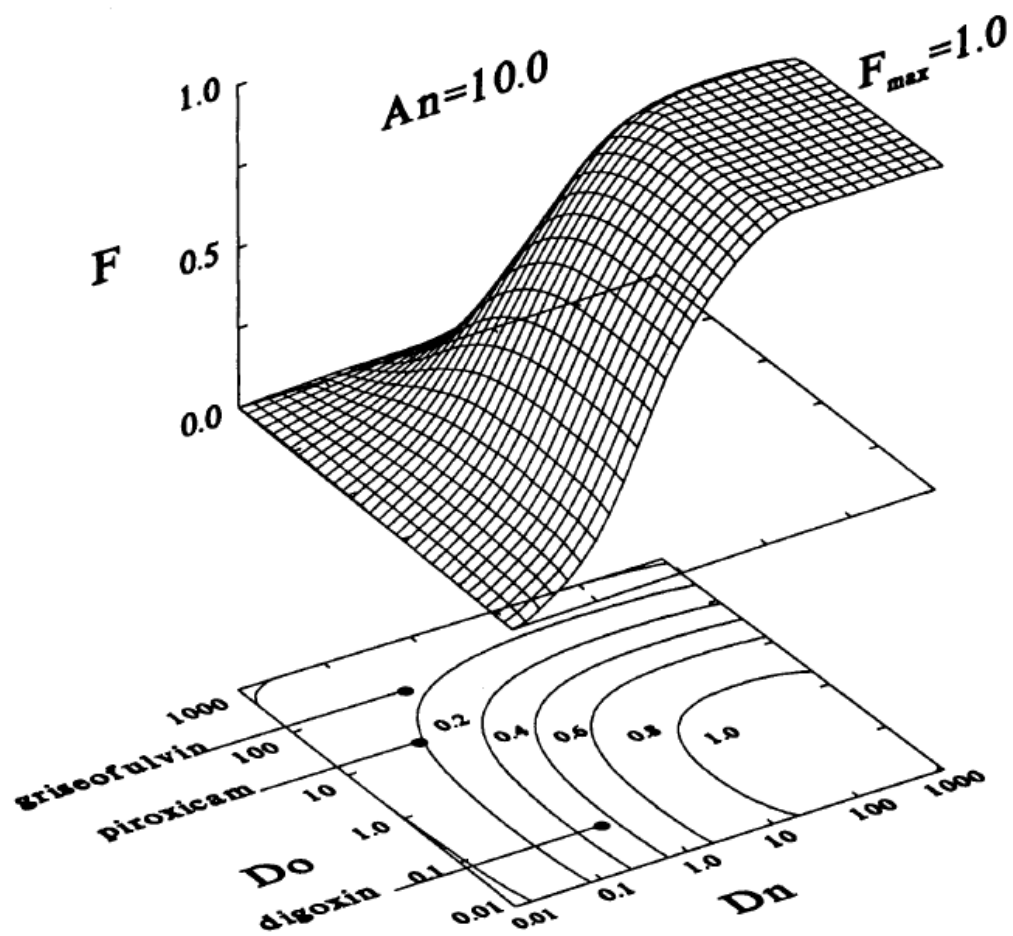


Figure 1.1 Graph of estimated fraction dose absorbed vs Dissolution Number, D_n , and Dose Number, D_o , for a high permeability drug. $A_n = 10$ corresponds to a drug with a permeability approximately that of glucose.

Table1.2 Median or mean gastrointestinal pH levels of normal, adult human subjects measured by pH- sensitive radiotransmitting capsules.(reproduced from (29)).

References	N	Stomach	Duodenum	Proximal SI	Mid SI	Distal SI	Caecum	Asc.c.	Transv.c.	Right c.	Desc.c.	Sigm/reci	Left c.
Arulani et al. 1967 (27)	9	1.7	5.66	6.49	7.06	7.52	7.7	7.71	8.1 6	-	8. 66	8.6 6	-
Maxwell et al, 1971 (99)	10	-	-	5.5- 6.5	-	-	-	-	-	-	-	-	-
Bown et al. 1974 (101)	11	-	-	5.9	6.8	7.5	-	-	-	6.0	-	-	6.8
Evans et al. 1986 (102)	23	-	-	-	-	-	-	-	-	6.2	-	-	6.7 5
Patil et al. 1988 (103)	6	-	-	-	-	7.51	-	-	-	6.5 1	-	6.5 3	-
Pye et al, 1987 (104)	8	-	-	-	-	-	-	-	-	6.5	-	-	7.3
Gilbert et al, 1988 (35)	42	-	-	6.61	-	7.50	6.37	-	-	-	-	-	-
Evans et al. 1988 (35)	66	1.75	-	6.63	7.41	7.49	-	-	-	6.3 7	-	-	7.0
Fallingborg et al, 1989 (11)	33	1.4	6.4	6.6	7.0	7.3	5.7	5.6	5.7	-	6. 6	6.6	-
Mojaverian et al, 1989 (45)	6	-	-	-	-	7.3	-	-	-	-	-	-	-
Raimundo et al, 1992 (107)	7	-	5.8	6.6	-	7.4	-	-	-	6.7	-	-	-
Wyeth et al. 1992 (107)	8	-	-	-	-	-	-	-	-	5.7	-	-	6.0 1
Fallingborg et al, 1994 (34)	13	-	6.22	6.06	-	-	-	-	-	-	-	-	-
Press et al, 1996 (48)	12	-	-	6.7	-	7.4	6.0	-	-	6.0	-	-	6.2
Sasaki et al, 1997 (37)	4	1.4	-	6.8	-	7.7	-	-	-	6.8	-	-	7.2
Fallingborg et al, 1998 (15)	13	1.4	-	6.4	7.1	7.4	-	-	-	5.8	-	-	-

Table1.3 Summary of reported bicarbonate luminal concentrations(Range or Mean Values)from(58)

GI location	[HCO ₃ ⁻] (mEq/L)	Species
Stomach	5-33	Dog ⁹
	7.3	Man ¹⁰
Duodenum	9-20	Man ¹¹
	14-22	Dog ⁹
	2.7	Man ¹²
	6.7	Man ¹³
	10	Man (fed) ¹⁴
Jejunum	15	Man ¹⁵
	1-4	Dog ¹⁶
	14	Dog ¹⁷
	5-30	Dog ⁹
	2-20	Man ¹⁸
	5-10	Man ¹⁹
	6-20	Man ²⁰
	17	Man ¹²
	30	Man ¹⁵
	30	Man ²¹
Ileum	9-37	Dog ¹⁶
	67	Dog ¹⁷
	70-114	Dog ⁹
	40	Man ²⁰
	50	Man ²²
	70	Man ¹⁵
	74	Man ²³
75	Man ²¹	

CHAPTER II. DISSOLUTION OF ACIDIC DRUGS: THE ROLE OF BICARBONATE IN HUMAN INTESTINAL FLUIDS

Abstract

Drug dissolution rate can be significantly affected by the buffer species in dissolution media. Bicarbonate has been recognized as the predominant buffer system in human GI tract for a long time. Therefore, quantitatively determining bicarbonate buffer contribution to the buffer capacity in *ex vivo* human intestinal fluid (HIF), and its effect on acidic drug intrinsic dissolution, are important for predicting the *in vivo* dissolution of acidic drugs.

Human intestinal fluids with $\text{HCO}_3^-/\text{CO}_2$ as collected and depleted were titrated from pH 7.0 to pH 3.0 to determine the carbonate contribution to the buffer capacity. The $\text{HCO}_3^-/\text{CO}_2$ species were also determined using ion chromatography (IC). The dissolution of the model compound, ibuprofen (weak acid, pKa 4.4), was conducted at pH 6 in HIF with $\text{HCO}_3^-/\text{CO}_2$ as collected or depleted using a miniaturized intrinsic dissolution release apparatus.

The overall buffer capacity from original HIF varied from 2.9 to 5.8 mM H^+ /(L HIF* pH unit), to which the bicarbonate contribution was 18% at pH 3.0 to 59% at pH 6.5, with the highest 74% at pH 5.5. The absolute concentration of $\text{HCO}_3^-/\text{CO}_2$ buffer determined by titration was 4.5 mM, which was consistent with the IC results of 4.3 mM for bicarbonate and only 0.62 mM for phosphate. Further, at pH 6.0, the

bicarbonate contributed of 57% to the total buffer capacity in HIF, and resulted in a 48% decrease of ibuprofen flux in HIF depleted of $\text{HCO}_3^-/\text{CO}_2$ compared with that of non $\text{HCO}_3^-/\text{CO}_2$ depleted HIF.

The results of the present work demonstrated that bicarbonate buffer contributed significantly to the buffer capacity in HIF, and plays an important role in the dissolution of acidic drugs *in vivo*. Since in most cases, aspirated intestinal fluids may be partially depleted of $\text{HCO}_3^-/\text{CO}_2$, the impact of bicarbonate on acidic drug dissolution is likely underestimated. The information of buffer species and strength obtained by characterizing HIF will aid in the design of dissolution media that are more closely representative of *in vivo* fluid dissolution, especially for the ionisable compounds.

Introduction

Understanding the gastrointestinal (GI) physiology is extremely important in developing an *in vitro* dissolution method to help formulation screening or control drug product *in vivo* quality. Many factors have been studied over the past years, such as GI fluid pH, volume, composition, transit time, motility, also effect of food, disease state, gender and age(76, 77). Buffer species and capacity to be used in the *in vitro* dissolution test have been showed influencing the dissolution rate of ionisable drugs (58, 63, 78).

For acidic drug taken orally, after experiencing the gastric low pH, the drug is emptied into upper intestinal tract, where the pH is higher and variable. It is in this region of the GI tract where the dissolution of drug from formulation plays a critical role in determining the bioavailability. Bicarbonate secretion has been recognized as the first line defense to protect intestinal mucosa from gastric acid and pepsin. HCO_3^- is secreted by pancreas, liver and epithelial cells to neutralize the gastric hydrochloride acid and help to establish the pH gradient in mucus gel (79, 80). Pancreatic fluid has a 25 mmol/l basal HCO_3^- concentration and postprandial concentration can reach 150 mmol/l. The total pancreatic bicarbonate secretion is about 200 mmol in one day. Bile from gall bladder has HCO_3^- concentration about 40 mmol/l. Basal duodenal mucosal output of HCO_3^- is $0.355 \text{ mmol/cm} \cdot \text{h}^{-1}$ (81). Overall, in spite of lacking of firm data, the net bicarbonate secretion in excess of proton secretion into the gut lumen is about 20 to 40 mmol/day (56). HCO_3^- is also in reversible reaction with CO_2 dissolved in water which is further in equilibrium with CO_2 in gas.

The use of bicarbonate buffers which reflecting the *in vivo* buffer environment has showed obvious advantage over non physiological buffer species. There are attempts made to using bicarbonate buffers system to discriminate different formulations (82, 83), also different methods are discussed to maintain the stable bicarbonate systems (78). The efforts have also been made to develop compendial buffer system in which drugs has the equivalent flux as in bicarbonate (63). It is still important to find out the bicarbonate strength of in human intestinal fluid and its effect on drug dissolution directly.

In this study, we used a special designed differential titration method and determined the bicarbonate buffer capacity in fasted human intestinal fluid (HIF) directly. The results are compared with those from ion-exchange column experiments on the same HIF. The dissolution of the model compound, ibuprofen (weak acid, pKa 4.4), was conducted at pH 6 in HIF with $\text{HCO}_3^-/\text{CO}_2$ as collected or depleted using a miniaturized intrinsic dissolution release apparatus.

The objective is to determine the contribution of bicarbonate in buffer capacity of HIF and its effect on drug dissolution.

Material and methods:

Collection of Human Intestinal Fluid

Human small intestinal fluid was collected using Loc-I-Gut technique from proximal jejunum (84, 85). Subjects are fasted 10 hours before collection, samples are pooled from 11 men and 6 women aged from 23 to 43. pH of fluid was measured right after collection to be 7.1(n=2, 7.0 and 7.1). Samples of fluid are frozen quickly to be used.

Determine bicarbonate capacity in Human Intestinal Fluid by differential titration

For determining the bicarbonate capacity in HIF, a differential titration method is designed to determine the whole buffer capacity of HIF as collected and that of the

HIF depleted $\text{HCO}_3^-/\text{CO}_2$, therefore, the difference of those two buffer capacities is the portion contributed by $\text{HCO}_3^-/\text{CO}_2$ in HIF.

Measure the whole HIF buffer capacity

1 mL HIF was transferred into a scintillation bottle, and then covered with a layer of paraffin oil to prevent the further CO_2 dissolving and CO_2 evaporation. The bottle was placed in 37C water bath with circulating water. pH was recorded with a pH meter(fisher scientific accument AB15). HIF was titrated with 1mol/l HCl using a microsyringe while the mini magnetic bar stirring at the bottom, pH was recorded till it reached down to pH 3 when adding every 2 μL of HCl.

Measure the whole HIF depleted of $\text{HCO}_3^-/\text{CO}_2$ buffer capacity

1 mL HIF was transferred into a scintillation bottle and kept in 37C water bath and pH of HIF was recorded. 1 mol/l HCl was used to adjust pH to 6.1, which is the pKa of carbonic acid at 37C. Moistened nitrogen gas was purged through the samples under maximal stirring for 1 hr and pH is recorded again. If pH increases due to CO_2 loss, adjusted it to pH6.1 using 1 mol/l HCl and repeated the step of purging the sample with nitrogen, till the pH of the sample is stable in the range of 0.1 pH unit for at least 1 hr. Recorded the final pH. Bottles are weighed before and after these step to make sure no significant moisture lost. Covered HIF with paraffin oil and adjust pH to original pH with 1mol/l NaOH. The bottle was again kept in 37C water bath, 1mol/l HCl was used to titrate the fluid with the same method as titrated the whole HIF buffer capacity till pH below 3.

Anion Exchange chromatography determining the carbonate and phosphate strength in HIF

The total carbonate strength and phosphate strength in HIF were also investigated using ion exchange chromatography (IC) equipped with ASRS 4 mm suppressor and a Dionex IonPac AS11 column (4 x 250 mm) (Dionex, Sunnyvale, CA). 100 μ L HIF was mixed well with 100 μ L acetonitrile, centrifuged with 10,000 x g for 15 min at 37 C, and the supernatant was diluted 20 times and analyzed via IC. The mobile phase was isocratic 5mM KOH, Suppressor current was 13mA, and flow rate was 1mL/min. Standards were prepared using Na_2HCO_3 from 0.05mM to 1mM and Na_2HPO_4 0 to 0.1mM.

Intrinsic Dissolution in Human intestinal Fluid

Mini-scale intrinsic dissolution apparatus

A mini-scale intrinsic rotating disk dissolution apparatus was designed for using small volume HIF. The drug tablet was also scaled down from the traditional and a load cell (0-500lbs) (transducer techniques, Temucula, CA) was attached to carver compressor to monitor the pressure applied when making mini-tablets. The apparatus was validated by comparing the results from the intrinsic dissolutions in the conventional rotating disk apparatus with in the new mini-scale apparatus using model compound, benzoic acid in 0.1M HCl dissolution media.

Dissolution of Model compound Ibuprofen in HIF

Dissolution tests were conducted with mini-scale rotating disk dissolution apparatus. HIF was used as dissolution media, 5mL HIF purged with CO₂ and reached pH6.0. The same experiments were also conducted in 5 mL HIF depleted of HCO₃⁻/CO₂ by using the moistened nitrogen with the same method as described earlier and adjusted pH to 6.0 before starting the experiments. The temperatures of the fluid were kept at 37C and the disk rotating speeds were 100rpm. 10 µL samples were taken at 1, 3, 5,10,15,20 min. The samples were mixed with equal volume of acetonitrile, centrifuged with 10,000 x g for 15 min at 37 C, and the supernatant was analyzed using high performance liquid chromatography (HPLC). Standards were prepared with HIF spiked with Ibuprofen from 0.66µg/mL to 3.32µg/mL. Each experiment is done in duplicates.

Recovery experiment and Assay

Blank HIF was spiked with ibuprofen of 2,3,5,8,10 µL of 33.16 µg/mL ibuprofen solution in mobile phase. A standard of 100% recovery was prepared by diluting 33.16 µg/mL ibuprofen solution in mobile phase respectively. The recovery percentage was calculated by ratios of areas of the peaks obtained from HIF samples with the areas obtained from the recovery standards.

Dissolution of Ibuprofen in bicarbonate buffer in different concentrations at pH

6.0

Dissolution studies of Ibuprofen were also tried in traditional IDR setting with different concentrations of bicarbonate buffers at 37C purged with CO₂ and maintain the pH 6.0 by adjusting the flow of CO₂. The experimental conditions were the same with the experiments with HIF. Each experiment was done in triplicates.

Results

Bicarbonate capacity in Human Intestinal Fluid

Determining HIF buffer capacity:

The representative titration curves are showed on Figure 2.1.

[\(Figure 2.1\)](#)

The curves illustrated the pH changes of *ex vivo* HIFs with original CO₂/HCO₃⁻ and with CO₂/HCO₃⁻ depleted during the titrations. Each type of titration was run in duplicates and the results were consistent between different runs. The slope of the curve shows the pH change due to the added acid. At where the slope is steep, the fluid has smaller buffer capacity, because, for certain unit pH change, only smaller amount of acid is needed. The buffer capacities of both were calculated according to

$$\beta(\text{buffer capacity}) = \frac{dn}{d(\text{pH})}$$

where dn is the amount of added acid and d(pH) is the resulting change in pH.

In each experiment, the slopes of the lines in 0.5 pH intervals between 7.0 and 3.0 were calculated. The results are showed in Figure 2.2.

[\(Figure 2.2\)](#)

The buffer capacity ranged from 0.95 to 4.73 mM H⁺/(L HIF * pH unit) for HIF depleted of CO₂/HCO₃⁻ and from 2.80 to 5.77 H⁺/(L HIF * pH unit) for *ex vivo* HIF with CO₂/HCO₃⁻ for pH 5.5 to pH 3.0. The later was consistent with fasted state *ex vivo* HIF capacity reported by Persson et al and Perez de la Cruz Moreno et al (59, 86). With the CO₂/HCO₃⁻ being depleted, the buffer capacities of HIF are significantly reduced in the whole pH range, the difference was contributed by HCO₃⁻/CO₂.

The contribution of HCO₃⁻/CO₂ in whole *ex vivo* HIF was also determined by the ratio of this difference in buffer capacity of these two HIFs to the whole HIF. The results are showed in Figure 2. 3.

[\(Figure 2.3\)](#)

The contribution of bicarbonate to the overall buffer capacity in the *ex vivo* HIF was pH dependent, ranging from 18% at pH 3.0 to 59% at pH 6.5 with the highest 74% at pH 5.5. When pH was higher than 5, the contribution of bicarbonate was mostly larger than 50%, confirming bicarbonate is dominant in fasted state *ex vivo* human intestinal fluid.

Since the buffer capacity could be determined by the following equation(87):

$$\beta = 2.303C \frac{Ka[H^+]}{(Ka+[H^+])^2}$$

Where the C is the total buffer concentration, Ka is the dissociation constant; [H⁺] is the proton concentration. The total carbonate concentration could be calculated from

$$C = \frac{\beta(Ka+[H^+])^2}{2.303Ka[H^+]}$$

Where, β had been determined from the experimental results.

The bicarbonate buffer concentration was calculated to be 4.5mM at pH6.0, since carbonate pka is 10.3 which is 4 unit above pH6.0, CO_3^{2-} should be negligible comparing to HCO_3^- .

Anion Exchange chromatography determining the carbonate and phosphate strength in HIF:

The concentration bicarbonate determined by Anion exchange chromatography was 4.30 ± 0.43 mM (n=2), which was pretty close to the bicarbonate concentration determined by the differential titration. The phosphate ion in HIF was only 0.62mM although phosphate is used as compendial buffer species.

Intrinsic Dissolution in Human intestinal Fluid

Mini-scale intrinsic dissolution apparatus

A miniature rotating disk apparatus was designed to scale down the drug and fluid samples needed from the traditional apparatus. The blueprint of the disk and module is showed in Figure 2.4. The mini tablets were made by carver laboratory presser (Summit, NJ) attached with a transducer loading cell (transducer techniques, Temucula, CA) measuring the pressure on the tablet. The mini-scale intrinsic dissolution apparatus was successfully validated using benzoic acid as testing

compound. The comparison of the key parameters of the miniature rotating disk apparatus to the traditional one was listed in Table 2.1. Much less active pharmaceutical ingredient (API) and dissolution media are needed as compared, which served the purpose of using HIF as the dissolution media.

[\(Figure 2.4\)](#)

[\(Table 2.1\)](#)

Table 2.2 shows the flux escaped from the solid surface when benzoic acid was used as a testing compound in both traditional and miniature dissolution apparatus. The ratio of these two flux values is 0.9973, which indicated that the new dissolution apparatus was suitable for dissolution study with HIF.

[\(Table 2.2\)](#)

Dissolution of Model compound Ibuprofen in HIF

The pH changes during the dissolution experiments were monitored to be less than 0.1 unit in HIF depleted $\text{CO}_2/\text{HCO}_3^-$. The pH value remained almost constant for experiments in the whole HIF with $\text{CO}_2/\text{HCO}_3^-$ as collected. The IDR dissolution profiles were obtained and the drug fluxes from the surface of the drug disk were calculated and listed in Table 2.3. Recovery of the ibuprofen from HIF ranged 92% to 100% at different concentrations.

[\(Table 2.3\)](#)

Dissolution of Ibuprofen in bicarbonate buffer in different concentrations at pH

6.0

When purging CO₂ to 30mM and 25mM sodium bicarbonate buffer at 37C in open container, after 1 hour, the pH could only reach to 6.18 and 6.09. So the dissolution studies of Ibuprofen were performed with 10mM and 20mM sodium bicarbonate buffers. The results are showed in Table 2.4.

[\(Table 2.4\)](#)

Discussion

The bicarbonate impact on the buffer capacity and dissolution of weak acid drug

Our studies showed that the bicarbonate was contributing more than 50% to the buffer capacity of fasted *ex vivo* intestinal fluid in pH 5.0 to 7.0 range. At pH 6.0, the bicarbonate contributed to 57% of the total buffer capacity in HIF, which resulted in an about 50% decrease of ibuprofen flux in HIF depleted of HCO₃⁻/CO₂ compared with that of non HCO₃⁻/CO₂ depleted HIF. These showed that the bicarbonate contributed significantly to the dissolution of weak acid drug *in vivo* even with relatively low concentration. The 20mM bicarbonate buffer with total HCO₃⁻/CO₂ of 45.18mM had about the equivalent intrinsic dissolution rate to the non-bicarbonate portion of HIF at pH6.0. These results suggested that the effect of bicarbonate buffer strength on acidic drug dissolution rate was not simply linear additive.

Partial pressure effect on the $\text{HCO}_3^-/\text{CO}_2$ in HIF

The $\text{HCO}_3^-/\text{CO}_2$ concentration in *ex vivo* human intestinal fluid was only 4.3 or 4.5 mM. However, in $\text{HCO}_3^-/\text{CO}_2$ system, the partial pressure of CO_2 will affect the HCO_3^- in buffers. At normal atmosphere, partial pressure of CO_2 (P_{CO_2}) is 3.5×10^{-4} atm. The concentration of HCO_3^- , H_2CO_3 and total concentrations were calculated in Table 2.5. (88)

[\(Table 2.5\)](#)

So the $\text{HCO}_3^-/\text{CO}_2$ concentration in *ex vivo* human intestinal fluid is 100 times higher than that resulting just from dissolving CO_2 under normal atmosphere. While P_{CO_2} in normal atmosphere is only 0.03% atm, the resting P_{CO_2} in lumen is 38mmHg (5% atm), which is comparable to P_{CO_2} in arterial blood. Postprandial P_{CO_2} could increase to 280mmHg (37%atm) or even higher (58, 89, 90). Table 2.6 calculated the HCO_3^- at these conditions.

[\(Table 2.6\)](#)

The $[\text{HCO}_3^-]$ calculated are in the same range with the experimental measured $[\text{HCO}_3^-]$ (58). Since in most cases, aspirated intestinal fluids may be already partially depleted of HCO_3^- during the collection, storage and handling, the impact of bicarbonate on acidic drug dissolution is likely to be underestimated here.

Interplay of different factors on HCO_3^- strength

Bicarbonate is unique in physiological buffers because the system is open- ended and

the continuous removal of acids is made possible by inhalation of CO₂. It is the most powerful extracellular buffer in the body, and buffers up to 90% hydrogen ions in extracellular fluid(91). The lumen HCO₃⁻ concentration is in equilibrium with H⁺, H₂CO₃, CO₂ (aq) and CO₂ (gas) with the facilitation of carbonic anhydrase as showed in Figure 2.5. The HCO₃⁻ secretion in collaboration with mucus plays important role in mucosal protection through two ways: one is neutralization of luminal acid directly by secreted HCO₃⁻ in the lumen and mucus gel; the other is the establishment of pH gradient in the mucus gel with the aid of the physicochemical property of mucus(79). Our studies provided a clearer description of HCO₃⁻ in fasted state GI fluid and its effect on weak acid drug dissolution. More investigations are needed in determination of the actual *in vivo* HCO₃⁻ buffer strength, thus further determining the proper *in vitro* buffer strength for this group of drugs is possible.

[\(Figure 2.5\)](#)

pH, mucosal secretion, bacteria,enzymes,transporters,disease change effects

Sham-feeding and the presence of acids in upper small intestinal trigger the P_{CO2} changes and secret HCO₃⁻ mediated by E- prostaglandins and vasoactive intestinal peptide(VIP), to maintain the pH and protect the mucosa from the damage of the acids. This process is also regulated by transporters (NHE,AE, CFTR) (81, 92-94), enzymes(carbonic anhydrase) (95) and neurohumoral process(96) (97-100). The secretion of HCO₃⁻ is also closely related to the activity of intestinal alkaline phosphatase(101), the development of ulcers(102) and also H. pylori infection.(103)

Smoking and NSAIDS like indomethacin also inhibit stimulation of HCO_3^- secretion by luminal acid.(104)

In physiological condition, HCO_3^- is secreted to response to food and luminal acid. In the disease state, the physiological secretion may be disturbed and the normal balance or activities will be disrupted when the different factors regulating bicarbonate involved. When drugs are taken orally to treat the disease locally or systemically, they add another dimension to the complexity of whole the system. It is very hard to understand the interactions of all these factors in GI bicarbonate system, but the capturing of the characteristics of *in vivo* gastrointestinal the bicarbonate buffer strength at different stages of digestive cycle and its effect on acidic drug dissolution will provide a baseline to develop the dissolution methods that could meet the needs of both quality control and *in vivo* simulations.

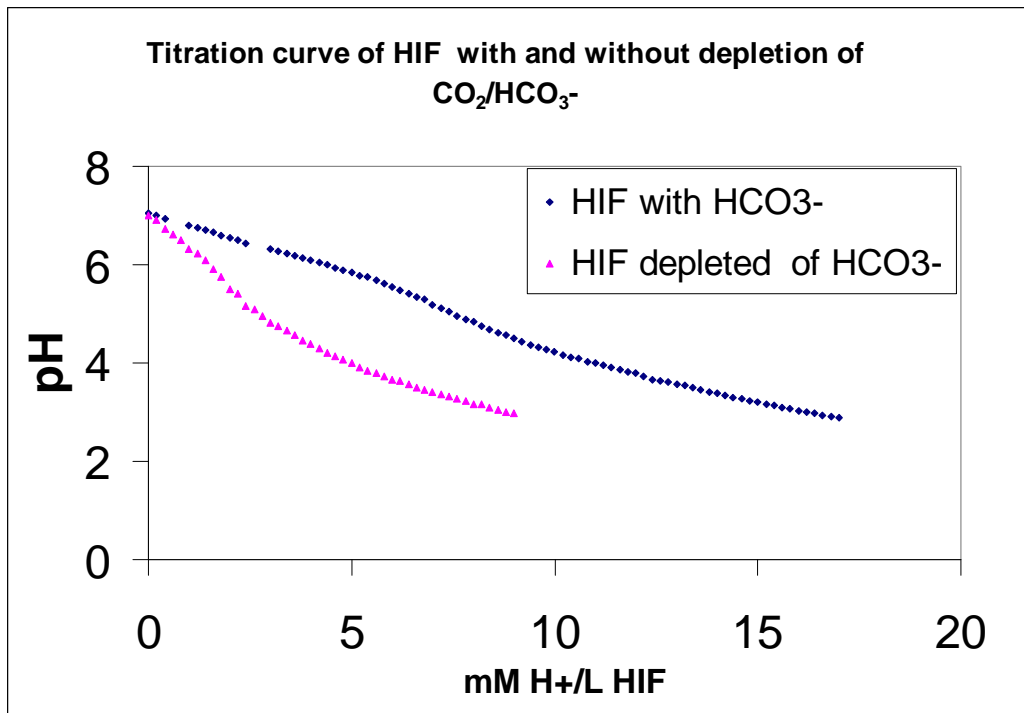


Figure 2. 1 Representative titration curves of *ex vivo* HIF. The diamond dotted line shows the original HIF containing $\text{CO}_2/\text{HCO}_3^-$ titration curves of *ex vivo* HIF, and the triangle dotted line shows the HIF depleted of $\text{CO}_2/\text{HCO}_3^-$.

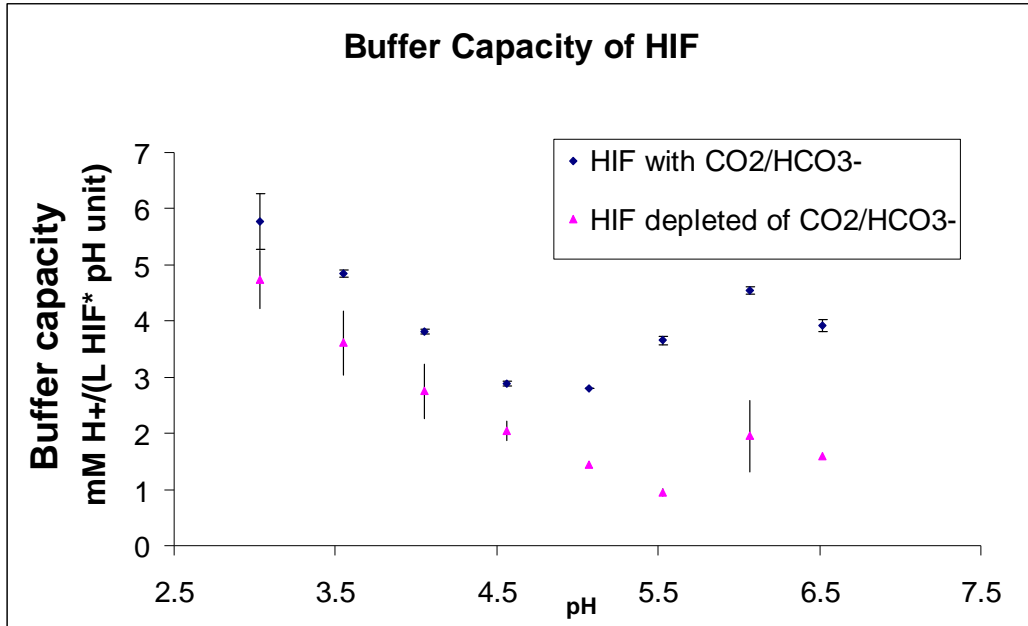


Figure 2. 2 Illustrates the buffer capacities of ex vivo HIF with CO₂/HCO₃⁻ and with CO₂/HCO₃⁻ depleted. The diamond dots show the original HIF containing CO₂/HCO₃⁻, the triangle dots show the HIF depleted of CO₂/HCO₃⁻ at different pHs. Standard deviations from duplicate runs are showed with the error bar (n=2). End point pH reported.

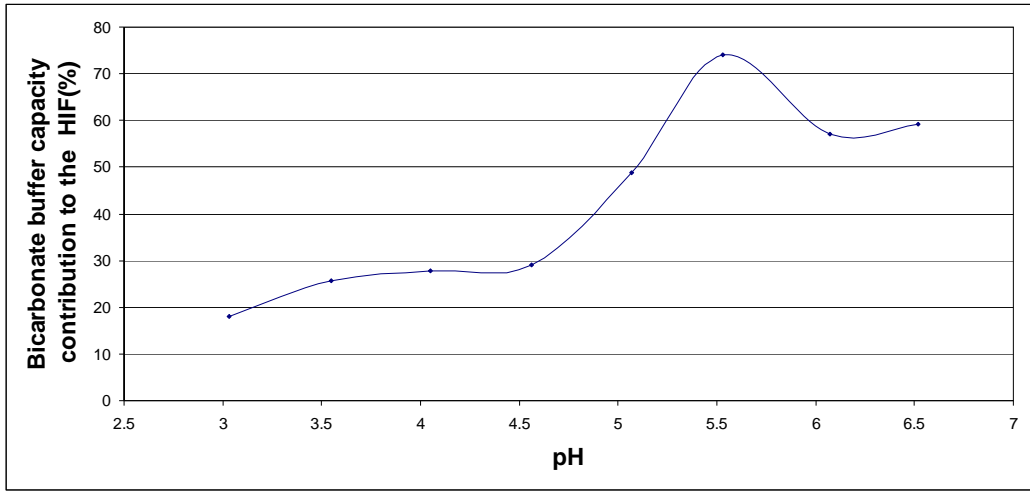
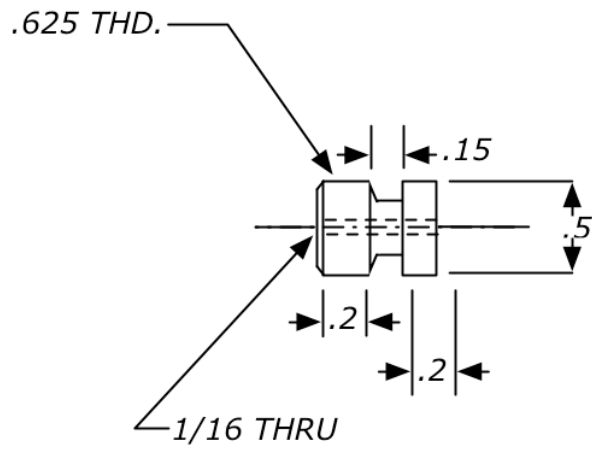
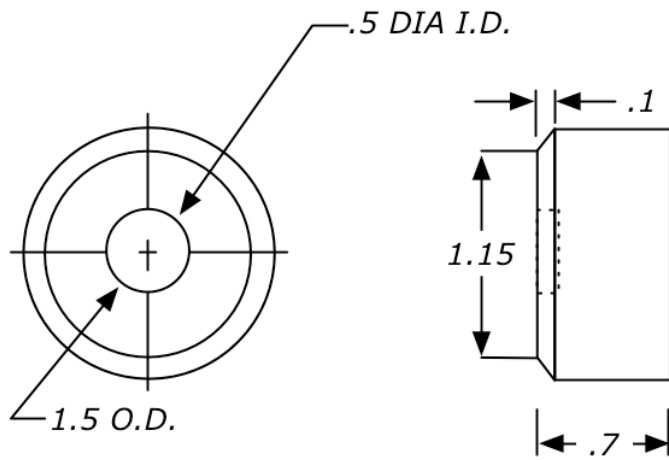


Figure 2. 3 The percentage contribution of bicarbonate to the whole ex vivo human intestinal fluid.



1 inch Long 1/16 O.D.



Figure 2. 4 The drawing the mini-scale rotating disk apparatus.

	Traditional	Miniature
Tablet Diameter (cm)	0.90	0.16
Tablet surface(cm ²)	0.64	0.020
Compression force (lbs)	~2000	~100
API needed (mg)	200	5
Media needed(ml)	200	5

Table 2.1 Comparison of the key parameters of the miniature rotating disk apparatus to the traditional apparatus.

	Traditional(n=3)	Miniature(n=3)
Flux from the surface (mg/cm ² /min)	0.7418 ±0.0032	0.7393±0.0018

Table 2.2 Comparison IDR tests of benzoic acid in 0.1M HCl using traditional and miniature apparatuses.

	Drug flux (j(mg/cm ² /min))	Std Dev.(n=2)
pH6.0 HIF with CO ₂ / HCO ₃ ⁻	0.1552	0.0012
pH6.0 HIF with CO ₂ / HCO ₃ ⁻ depleted	0.0805	0.0053

Table 2.3 The drug flux from the surface of the drug disk in pH 6.0 HIF with and without CO₂/ HCO₃⁻.

Concentration of HCO ₃ ⁻ (mM)	Total concentration of CO ₂ / HCO ₃ ⁻ (mM)	Drug flux ±s.d. (mg/(cm ² *min))
10	22.59	0.06736 ±0.002542
20	45.18	0.08377 ±0.007569

Table 2.4 The intrinsic dissolution of ibuprofen in different concentrations of bicarbonate buffers.

pH	5	6	6.8
[H ⁺](Mol/L)	1.00E-05	1.00E-06	1.58E-07
[H ₂ CO ₃](Mol/L)	1.18E-05	1.18E-05	1.18E-05
[HCO ₃ ⁻](Mol/L)	5.13E-07	5.13E-06	3.24E-05
Total [H ₂ CO ₃]+[HCO ₃ ⁻](Mol/L)	1.23E-05	1.69E-05	4.42E-05

Table 2.5 Concentration of buffer species in carbonic acid system under normal atmosphere condition ($P_{CO_2}=3.5 \times 10^{-4}$ atm).

	Normal atmosphere P _{CO2} (0.03% atm/0.228mmHg/0.030 kPa)	Normal GI lumen P _{CO2} (5% atm/38mmHg/5.07 kPa)	Postprandial GI lumen P _{CO2} (37%atm/280mmHg/ 37.49 kPa)
pH	[HCO ₃ ⁻] (mM)	[HCO ₃ ⁻] (mM)	[HCO ₃ ⁻] (mM)
5.0	5.13E-04	7.33E-02	5.40E-01
5.5	1.62E-03	2,32E-01	1.71E00
6.0	5.13E-03	7.33E-01	5.40E00
6.8	3.24E-02	4.63E00	3.41E01

Table 2.6 [HCO₃⁻] at different physiological relevant partial pressures.

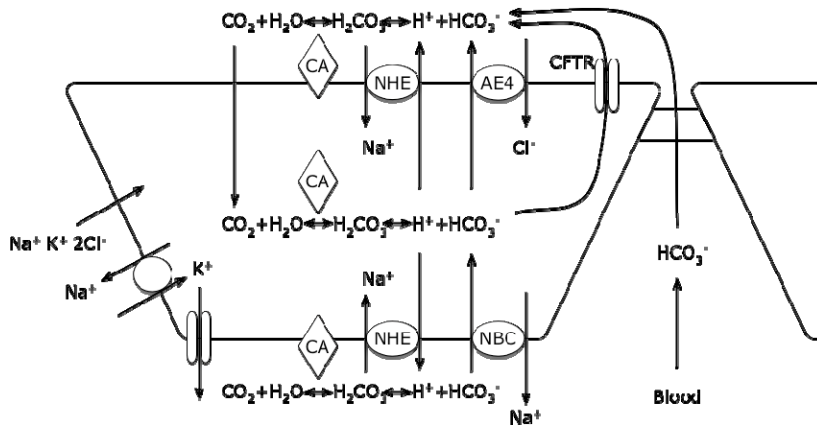


Figure 2.5 Transport of bicarbonate at gastrointestinal epithelial cell summarized from (96) (100, 105)

CHAPTER III. CHOICE OF BUFFER FOR PHYSIOLOGICAL RELEVANT DISSOLUTION OF NSAIDS: IMPLICATIONS FOR BIOWAIVER

Abstract

Developing proper *in vitro* dissolution tests for weak acid drugs are important for establishing *in vitro- in vivo* correlations and better bioequivalence tests. Factors from both dissolution media and drug properties are important and interact with each other in determining the drug release from the dosage form. This study investigates the effect of buffer factors and drug properties on the intrinsic dissolution test to improve the *in vitro* dissolution media with the aim of more closely reflecting physiological dissolution.

Intrinsic dissolution tests of model NSAIDS drug, ibuprofen, have been performed in buffers with different pHs, buffer strengths in physiological bicarbonate buffer and USP acetate buffer. Two mass transport models, reaction plane model and film model were developed using Mathematica® and Matlab® to simulate the buffer and drug species effects on dissolution. Simulations were performed with buffer parameters changing including buffer strength, buffer species, pH. The effects of drug properties such as solubility, pKa and diffusion coefficient were also determined and compared with experimental intrinsic dissolution tests.

The two models agreed well and can be used to predict the intrinsic dissolution rate of

the drugs. Buffer factors, including buffer strength, species and pH, affected the rate of dissolution when being changed. Both Models were sensitive to drug properties especially drug pKa.

Buffer species can significantly impact the dissolution rate of weak acid drugs. Comparing the different buffers with bicarbonate buffer, we could establish a correspondence between buffers that can be used in *in vitro* testing and suggest a more physiological relevant dissolution methodology.

Introduction

With the implementation of FDA guidance of “Waiver of *In Vivo* Bioavailability and Bioequivalence Studies for Immediate Release Solid Oral Dosage Forms based on biopharmaceutical class”(16, 17), developing an proper *in-vitro* dissolution test became more critical for reflecting *in vivo* dissolution the drug product, further evaluating the drug performance in human body. The *in vivo* dissolution process is an interaction of drug properties including physiochemical, dosage factors with gastrointestinal fluid and its hydrodynamics. The drug properties are usually extensively studies during development while the gastrointestinal fluid and its proper surrogate media are still under investigation. The determination of simple buffer that could be used for dissolution test is not only useful for bioequivalence standard but also plays an important role earlier in development for selecting of formulations.

For BCS I and III drugs, with their high solubility, the bioavailability are not reply on formulation characteristics. For BCS II drugs, however, the drug absorption is dissolution limited, thus to establish an meaningful *in vivo- in vitro* correlation could be expected and the key is to develop an *in vitro* dissolution method reflecting the *in vivo* situation. The failure of developing IVIVC for nonsteroidal anti-inflammatory drugs (NSAIDs), could due to the dissolution media used for dissolution testing having large discrepancy with human intestinal fluid. NSAIDs are commonly recommended to be taken after meals. The current used dissolution media at postprandial pH are listed in Table 3.1. There are also continuous research on developing the FeSSIF-V2 combining the postprandial changes at different time scale.(106)

[\(Table 3.1\)](#)

When using *in vitro* testing to predict the *in vivo* situation, a lot of factors that involving in translating *in vivo* to *in vitro* need to be considered. The direct using the human intestinal fluid could predict the *in vivo* drug dissolution more precisely(59). However, the huge cost and complicated procedure associated with the collection of fluid make it unrealistic use in industrial setting. Even when taking the human intestinal fluid from *in vivo* to *ex vivo*, there are factors changing and information missing that may affect the correct predication of drug performance physiologically.

In this study, we investigated the dissolution of BCS II weak acidic drug, using

ibuprofen as a model NSAIDs drug, in USP acetate buffers and physiological bicarbonate buffers. Two mathematical models, buffered diffusion film model and reaction plane model were also utilized and refined to explain pH, the buffer strength and species difference interactions with drug physiochemical properties including pKa, solubility and diffusivity. With these efforts, the possibility of developing a physiological equivalent simple buffer system for drugs with known physiochemical properties will be discussed.

[\(Figure 3.1\)](#)

Experimental studies:

Materials: ibuprofen was purchased from Acros Organics (Morris Plains, NJ), sodium chloride, sodium bicarbonate, sodium acetate and other chemicals of analytical grade were purchased from Sigma (St.Louis, MO). Distilled/deionized water was prepared using Milli-Q water (Millipore, Bedford, MA). 100% dried CO₂ was purchased from lifeGas (Ann Arbor, MI).

Establishing saturated bicarbonate buffer at normal atmosphere

Different concentrations of sodium bicarbonate buffers were prepared at normal atmosphere and room temperature, and adjusted to isotonic using sodium chloride. Compressed CO₂ was continuous purged into the buffers and the lowest pHs that the buffer could reach were recorded.

pH effect on ibuprofen intrinsic dissolution rate in bicarbonate buffer

The rotating disk apparatus was based on Levich method(107) as in Figure 3.2, and consisted of a jacketed reactor maintained at 37°C. 1mM bicarbonate buffer was prepared isotonic with sodium chloride. CO₂ was purged into buffer and reached the pH desired before experiments and maintained pH during experiments at pH 5.0, 6.0, 6.8 by adjusting the CO₂ flow rate. The 200mL buffer was placed into the reactor and a pH electrode was placed into the buffer. The drug disks were prepared with 200mg of bulk drug and compressed with 2000 lbs pressure for 60 seconds. The drug disk was attached to a shaft driven by a motor. The rotating speed was set at 100rpm. The disk was immersed into the medium when experiment started. The Agilent UV spectrometer (Santa Clara, CA) was set to measure the bulk buffer ibuprofen concentrations at 220nm through flow cell circulating the medium from the reactor. The intrinsic dissolutions of ibuprofen were performed at different pHs. Each experiment was done in triplicates and the initial drug fluxes from the disk surface were calculated.

[\(Figure 3.2\)](#)

Acetate buffer strength effect on ibuprofen intrinsic dissolution rate

Simulated gastric fluids (SGF) of pH 1.2 without pepsin were prepared following the USP standards. Sodium acetate buffers were prepared isotonic with sodium chloride from 0mM to 50mM. pH of the buffers were adjusted to 5.0 using hydrogen chloride acid. The intrinsic dissolution tests of ibuprofen were performed in 200mL buffer at

37°C with disk rotating speed of 100rpm. The disk were prepared in the same conditions as in bicarbonate buffers and the initial drug fluxes are calculated.

Theoretical Analysis:

Two mathematical models, reaction plane model and film model were utilized to model the dissolution of model drug, ibuprofen in buffers of different buffer strengths at different pHs.

Reaction Plane Model:

Reaction plane model is convective diffusion model different than film model in separating the fast reaction process in a thin “reaction plane” in boundary layer from the rate limiting process, thus the reaction process could be introduced as the flux boundary conditions of diffusive and convection process (62, 74, 108). In this study, the model was further refined to more accurately describe the buffer effect on acidic drug dissolution. The general continuity equation Eqn.1 is reduced to Eqn.2 when describing the mass transfer in the rotating disk system.

$$\frac{\partial c_i}{\partial t} = D_i \nabla^2 c_i - v \nabla c_i + R_i \text{ -----Eqn.1}$$

$D_i \nabla^2 c_i$ ---diffusive flux of species i ,

$v \nabla c_i$ ---convective amount of species i

R_i ---production of species I from reaction

$$\frac{\partial c_i}{\partial t} = D_i \frac{d^2 c_i}{dz^2} - v \frac{dc_i}{dz} \text{-----Eqn.2}$$

where c_i is the molar concentration of the species i, t is the time, D_i is the diffusion coefficient of species i, v is the fluid velocity. In Eqn.2, the mass transfer in rotating disk system could be simplified as one-dimension diffusion and convection along axial z.

Specifically when assuming the reaction plane is at the surface of the disk, at steady state, $\frac{\partial c_i}{\partial t} = 0$. Litt and Serad(109) showed that Eqn.2 could be further simplified into an ordinary differential equation Eqn.3 by introducing the dimensionless distance variable n for the axial distance z and defining the dimensionless dependent variables as following:

$$n = z \sqrt{\frac{\omega}{\nu}}$$

Where ω is the angular velocity of the disk (rad/s), ν is the kinetic viscosity of the fluid (cm^2/s)

$$C_{in}(n) = \frac{C_{in} - C_{ib}}{C_{i0} - C_{ib}}$$

$$V(n) = \frac{v_z}{\sqrt{\nu \omega}}$$

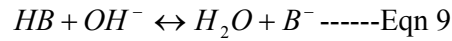
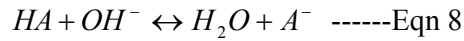
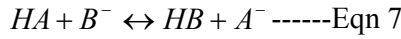
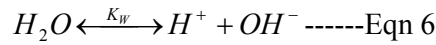
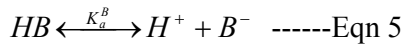
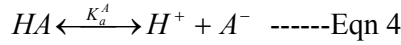
$$Sc_i = \frac{\nu}{D_i}$$

C_{ib} and C_{i0} are the molar concentration of species i in the bulk solution and at the solid-liquid interface, $C_{in}(n)$ is the dimensionless concentration of species i at position n, v_z is the axial velocity of the fluid that could be expended using the first

term of $[-(v\omega)^{1/2}(0.510n^2 - 0.333n^3 + \dots)]$. $V(n)$ is the dimensionless velocity of the fluid, Sc_i is the dimensionless Schmidt number.

$$\frac{d^2c_i}{dn^2} - VSc_i \frac{dc_i}{dn} = 0 \text{ -----Eqn.3}$$

At the surface of the disk ($n=0$), the reversible reactions occur as below:



Three of them are independent, the mathematical expressions could be represented by

Eqn 3 with $i = HA, A^-, H^+, HB$,

Applying the boundary conditions at $n=0$ (surface): $C_{HA}=1, C_H=1, C_A=1, C_{HB}=1$

and the boundary condition at $n=\infty$ (bulk solution): $C_{HA}=0, C_H=0, C_A=0, C_{HB}=0$

The solution to Eqn.3 is

$$C_i(n) = 1 - \int_0^n \exp(Sc_i \int_0^n V dn) / \int_0^\infty \exp(Sc_i \int_0^n V dn) dn \text{ -----Eqn 10.}$$

Differentiating Eqn 10 and using first term of the velocity series expansion as Levich showed(107), at $n=0$ (surface), the result is

$$\frac{dc_i}{dn} = -0.62Sc_i^{1/3}$$

In pH region of $\text{pH} \leq \text{p}K_a^A - 1$ and $\text{pH} \leq \text{p}K_a^B - 1$, ionization of HA and HB and the reaction of HA with B^- and OH^- are limited. So the total molar flux from the surface is

$$N_0 = J_{HA} = -\sqrt{\frac{\omega}{\nu}} D_{HA} [HA]_0 (Sc_{HA})^{1/3} \text{ -----Eqn 11}$$

With the pH increases, the ionization and reactions become significant at the surface, the total drug flux

$$N = J_{HA} + J_A = -\sqrt{\frac{\omega}{\nu}} (D_{HA} [HA]_0 (Sc_{HA})^{1/3} + D_A \frac{K_a^A [HA]_0}{C_{H0}} (Sc_A)^{1/3}) \text{ -----Eqn 12}$$

The flux ratio

$$N/N_0 = 1 + \frac{K_a^A}{C_{H0}} \left(\frac{D_{HA}}{D_A}\right)^{2/3} = 1 + \frac{K_a^A}{C_{H0}} \alpha \text{ -----Eqn 13}$$

allows the comparison of drug flux in different buffers once the C_{H0} is solved. $\alpha = 1$ when diffusion coefficient of HA and A^- are equal.

From the Eqn 4-6, the following flux equation holds since for every molar H^+ produced, there is either a molar of A^- , B^- or OH^- produced. Also A^- , B^- and OH^- conserve in between either two of them in Eqn 7-9.

So,

$$N_H = N_A + N_B + N_{OH} \text{ -----Eqn 14}$$

Where N_H is the molar flux of H^+ , N_A is the molar flux of A^- , N_B is the molar flux of B^- , N_{OH} is the molar flux of OH^- .

$$D_H (C_{H0} - C_{Hb}) \frac{dC_H}{dn} = D_A C_{A0} \frac{dC_A}{dn} + D_B (C_{B0} - C_{Bb}) \frac{dC_B}{dn} + D_{OH} (C_{OH0} - C_{OHb}) \frac{dC_{OH}}{dn} \text{ -----Eqn 15}$$

The total concentration of buffer is $C_T = C_{Bn} + C_{HBn}$. Assuming Levich gradients for all species and substitute all possible terms with C_{H0} , the Eqn 15 could be rewritten into:

$$K_a^A [HA]_0 (Sc_A)^{1/3} / C_{H0} - Sc_A (Sc_H)^{-2/3} (C_{H0} - C_{Hb}) - Sc_A (Sc_B)^{-2/3} \frac{K_a^B C_T (C_{H0} - C_{Hb})}{(C_{H0} + K_a^B)(C_{Hb} + K_a^B)} - Sc_A (Sc_{OH})^{-2/3} \left(\frac{K_w}{C_{H0}} - \frac{K_w}{C_{Hb}} \right) = 0 \text{ -----Eqn 16}$$

In Eqn 16, the ionization constant of drug, K_a^A and buffer K_a^B are known, Schmidt number of A , HA, H^+, B^-, OH^- could be calculated from their diffusion coefficient in buffer, intrinsic solubility of the drug $[HA]_0$ is known for specific drug and total buffer concentration C_T and pH (C_{Hb}) are also known in certain buffer system. So the Equation could be solved using Mathematica ® with the only unknown C_{H0} . Then, N/N_0 could be determined using Eqn 13 to see the buffer effect on drug dissolution.

Film Model:

The first model is a homogeneous chemico-diffusion film model developed and applied to drug dissolution describing the drug diffusing and reacting with other ion species from bulk buffer throughout the stagnant film adjacent to the solid-liquid surface (64, 72, 73, 110). In our system, the weak acidic drug dissolves and dissociates into ionized form when pH is higher at the solid-liquid surface and diffuses and react with buffer species all across the boundary layer. At steady state, the reactions described in Eqn 4-9 occur in the boundary layer, similarly Eqn 14 preserves. Also, when closely observing the reactions in Eqn 4-9 the following relationships could be established:

$$N_H + N_{HA} = N_{OH} + N_B \text{ -----Eqn 17}$$

Because from Eqn 5 and 6, for every molar H^+ generated, there is either a molar B^- or OH^- generated along with it. Similarly from Eqn 7 and 8, we see the same situation for every molar of HA . Eqn 4 and 9 describe that terms in either end of Eqn 17 are in equilibrium between themselves.

By comparing Eqn 14 and Eqn 17, we get

$$N_{HA} = -N_A \text{-----Eqn 18}$$

Eqn 18 presents that the weak acid drug is either in unionized form or ionized form.

Similarly for buffer species, we have

$$N_B = -N_{HB} \text{-----Eqn 19}$$

Substitute $N_i = -\frac{\partial c_i}{\partial t} = -D_i \frac{d^2 c_i}{dz^2}$ to each term of Eqn 17-19, and apply boundary

conditions:

$$\text{at } z=0, C_{HA} = C_{HA0} = [HA]_0, C_H = C_{H0}, C_A = C_{A0}, C_{HB} = C_{HB0}, C_B = C_{B0}, C_{OH} = C_{OH0}$$

and the boundary condition at $z=h$ (bulk solution): $C_{HA} = C_{HAh} \approx 0, C_H = C_{Hh}, C_A = C_{Ah} \approx 0,$

$$C_{HB} = C_{HBh}, C_B = C_{Bh}, C_{OH} = C_{OHh}.$$

The concentrations at the bulk solution and the intrinsic solubility of HA is known, others are unknown.

The final equation could be reduce to a single equation with only C_{H0} as unknown similar to Eq 51 in (64):

$$pC_{H0}^3 + qC_{H0}^2 + rC_{H0} + s = 0 \text{-----Eqn 20}$$

Where

$$p = D_H D_{HB}$$

$$q = D_H D_B K_a^B + D_{HB} a$$

$$r = D_B K_a^B (a - b) - D_A D_{HB} K_a^A [HA]_0 - D_{OH} D_{HB} K_W$$

$$a = D_{OH} C_{OHh} + D_B C_{Bh} - D_H C_{Hh}$$

$$b = D_B C_{Bh} + D_{HB} C_{HBh}$$

Once C_{H0} could also be solved using Mathematica ®, the other unknowns could also be easily calculated.

The negative total acid flux at $z=0$ is the drug dissolution rate

$$\begin{aligned} N &= (N_A N_{HA^+}) = (N_{HA} + N_H - N_B - N_{OH}) \\ &= 1/h [D_{HA} [HA]_0 + D_H (C_{H0} - C_{Hh}) - D_B (C_{B0} - C_{Bh}) - D_{OH} (C_{OH0} - C_{OHh})] \text{-----Eqn 21} \end{aligned}$$

Similarly with reaction plane model, we take ratio of this drug flux with the drug flux in low pH where only drug flux is in unionized form:

$$N_0 = 1/h [D_{HA} [HA]_0]$$

$$N/N_0 = 1 + [D_H (C_{H0} - C_{Hh}) - D_B (C_{B0} - C_{Bh}) - D_{OH} (C_{OH0} - C_{OHh})] / D_{HA} [HA]_0$$

The assumption here is no difference in the boundary layer thickness h the same drug.

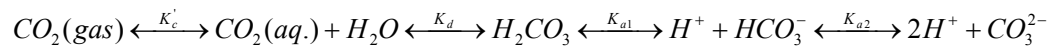
$$h = 1.612 D^{1/3} \nu^{1/6} \omega^{-1/2}$$

Model parameters selection:

The parameters selected to be used in the reaction plane model and film model are listed in Table 3.2, including drug properties such as diffusion coefficient, pKa and intrinsic solubility, also, buffer species properties like diffusion coefficient of ionized and unionized forms, pKa.

([Table 3.2](#))

The carbonic acid buffer system could be more accurately described as below (111-115):



The equilibrium constant is

$$K_c' = 1 / K_h = [CO_2] / PCO_2,$$

where $K_h = 29.41L \cdot atm / mol$ is Henry's law constant for CO₂ at 25C. $K_d = 1.6 \times 10^{-3}$, $K_{a1} = 2.72 \times 10^{-4}$ and $K_{a2} = 5.61 \times 10^{-11}$ at 25C with corresponding $pK_{a1} = 3.57$ $pK_{a2} = 10.25$. At the experimental conditions, pH 5-6.8, carbonate presented only in trace amount, only pK_{a1} is considered. $K_a = K_d * K_{a1} = 4.30 \times 10^{-7}$ gives the apparent pKa of carbonic acid 6.36. However, in our experimental conditions, the bicarbonate buffer is purged with CO₂ before experiments and keep pH at certain pH by adjust the CO₂ flow rate, the step with K_d is negligible and the step with K_{a1} is the controlling step and the dissociation constant is more close to K_{a1} which is used for model.

RESULTS:

The saturated bicarbonate buffer could be established at pH5.0 only at low concentration of 1mM buffer strength at normal atmosphere pressure. The lowest pHs that bicarbonate buffer could reach at the different concentrations are listed in Table (3.3).

[\(Table 3.3\)](#)

pH effect on ibuprofen intrinsic disk dissolution rate in bicarbonate buffer

With the pH of the bulk 1mM isotonic bicarbonate buffer increased from 5 to 6.8, the intrinsic dissolution rates of ibuprofen gradually increased. The results are showed in Figure 3.3.

[\(Figure 3.3\)](#)

The reaction plane model and film model discussed were tested over the same pH range in bicarbonate buffers. The initial drug flux ratios in buffers and in pH1.2 SGF, N/N_0 are plotted in Figure 3.4. The agreement of the observed and predicted initial dissolution rates was good over the pH range of the bulk solution buffer. When $D_A = D_{HA} = 0.92 \times 10^{-5} \text{ cm}^2/\text{s}$, $\alpha = 1$ was used, film model predicted more accurate at higher pH ends and reaction plane model predicted better at the lower pH end. In the situation where $D_A \neq D_{HA}$, D_A was estimated using the equation of harmonic average

with D_H and D_{HA} , $\alpha = 1.54$ and in both models, the predicting curves shifted towards the experimental data, and the reaction plane model was more close to experimental data at higher pH end and film model performed better at lower pH end. So the combination of these models would provide a best picture of the drug dissolution increased in bicarbonate buffers.

([Figure 3.4](#))

Acetate buffer strength effect on ibuprofen intrinsic dissolution rate

Initial dissolution flux ratios predicted from models were compared with the experimental data in acetate buffers of different buffer strength. The results are showed in Figure 3.5. In Model 1, it was the assuming that $D_A = D_{HA}$ and diffusion coefficient was constant in the experimental acetate concentrations. Film model and reaction plane model agreed well with each other and were very close to the experimental data. The models underestimated the flux ratio by just 7% at 50mM concentration of acetate buffer, and overestimated the flux ratio at 0mM isotonic solution by 28% due to the low absolute value.

([Figure 3.5](#))

Experimentally, acetate buffer of concentration 1.75mM were showed to have the equivalent drug dissolution flux ratio as 1.0mM bicarbonate buffer at pH5.0. The results are showed in Table 3.4.

[\(Table 3.4\)](#)

Model sensitivity analysis:

To better provide suggestion of USP acetate buffer which could be equated to bicarbonate buffer, the sensitivity of the drug physiochemical properties were tested in 50mM acetate buffer. The drug intrinsic solubility of 10^{-2} to 10^{-8} M, the drug pKa from 10^{-3} to 10^{-6} , and the diffusion coefficient from 10^{-6} to 10^{-5} cm²/s were set to test. The drug pKa and intrinsic solubility effects on drug dissolution with the drug diffusion coefficient = 0.5×10^{-5} cm²/s were shown in Figure 3.6.

[\(Figure 3.6\)](#)

The buffer effect could increase the flux by 100 times compared with in SGF solution at solubility of 10^{-8} and pKa of 3. The increase dependence intrinsic solubility was larger at pKa 3.0 than 6.0, also the dependence on pKa was larger at lower than at higher intrinsic solubility.

Similar test was done on drug diffusion coefficient and pKa with fixed intrinsic solubility of 1×10^{-4} cm²/s, also, on drug solubility and diffusion coefficient with fixed pKa = 4.0. The results were showed in Figure 3.7 and Figure 3.8. When drug intrinsic solubility was 10^{-4} M, the drug flux ratio was increased from 1.1 when pKa is 6.0 to the highest of 94.3 when pKa is 3.0 and diffusion coefficient was 1×10^{-6} cm²/s.

[\(Figure 3.7\)](#)

With $pK_a = 4.0$, changing of intrinsic solubility and diffusion coefficient of drug, the flux ratio could increase from 3.34 at the high solubility and high diffusion coefficient end to 11 when solubility is $10^{-8}M$.

[\(Figure 3.8\)](#)

Conclusions:

Buffer species can significantly impact the dissolution rate of weak acid drugs. The dissolution process could be well described by mathematical models such as reaction plane model and film model. Comparing USP acetate buffer with physiological bicarbonate buffer species, we could establish correspondence between buffers using models and the results were verified by experiments. The effect of drug physiochemical properties on the dissolution was also analyzed and could be utilized to develop the computational tools which providing the suggestions of the proper buffer strength to do the biorelevant dissolution test.

For the best biowaiver test, the *in vitro* bioequivalence has to be designed to predict the drug products performance *in vivo*; the dissolution media has to reflect the *in vivo* gastrointestinal fluid to be predictive. The combination of theoretical work and experimental work here demonstrated a useful approach for a rational design of

dissolution media in terms of pH and buffer strength. The USP buffers which are more widely used in industry could be used and equated to physiological bicarbonate buffer when drug physiochemical properties are known.

With an *in vivo* reflecting dissolution methodology, the biowaiver could be considered for BCS II poorly soluble acidic drugs.

Dissolution Media	pH	Buffer composition
United State Pharmacopeias (USP) buffer⁽¹¹⁶⁾	5.0	50 mM sodium acetate buffer
European Pharmacopeias(EP) buffer⁽¹¹⁷⁾	5.0	12mM potassium acetate buffer
International Pharmacopeias(IP) buffer⁽¹¹⁸⁾	4.5	50mM potassium dihydrogen phosphate
Fed State Simulated Intestinal Fluid (FeSSIF)⁽¹⁰⁶⁾	5.0	144mM acetate buffer: Sodium taurocholate 15 mM Lecithin 3.75 mM NaOH (pellets) 4.04 g Glacial Acetic Acid 8.65 g NaCl 11.874 g Purified water qs. 1000 mL osmolality of about 670 mOsmol/kg.

Table 3.1 The current used dissolution media at postprandial pH.

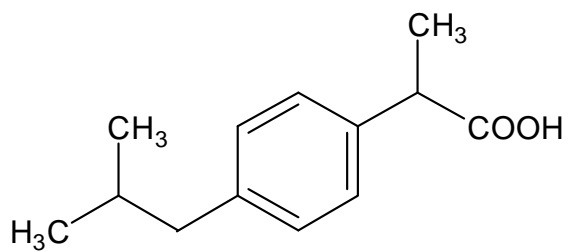


Figure 3.1 The structure of ibuprofen

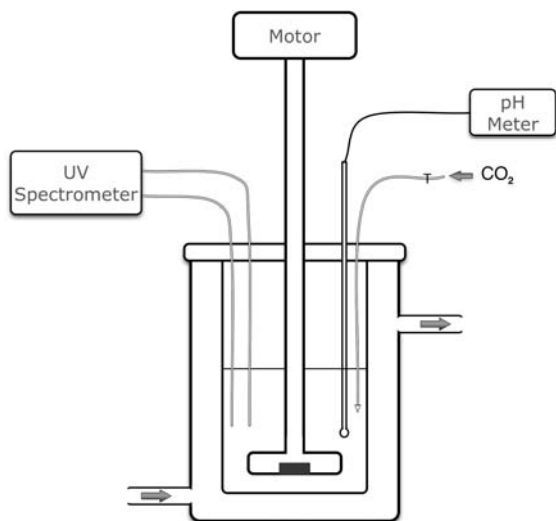


Figure 3.2 Rotating disk apparatus studying bicarbonate buffers.

substance	D (x10 ⁵) cm ² /s	pKa	MW(g/mol)	Intrinsic solubility (M)
ibuprofen	HA 0.92 ^a /A ⁻ 0.48 ^b	4.42 ⁽¹¹⁹⁾	206.28	2.38 x 10 ⁻⁴ (119)
CH ₃ COOH	1.26 ^d	4.60 ⁽⁶⁴⁾	60.05	
CH ₃ COO ⁻	1.39 ^c			
H ₂ CO ₃	1.99 ^c	3.57 ^(114, 115)	62.03	
HCO ₃ ⁻	1.25 ⁽¹¹⁰⁾			
H ⁺	9.68 ^c			
OH ⁻	5.49 ^c			

Table 3.2 Parameters used in theoretical analysis

a. calculated by ADMET predictor (Simulations Plus, Lancaster)(120).b. calculated using harmonic average equation with D_{HA} and D_{H⁺} c. data from (121) and corrected to 37C using Stokes-Einstein equation. d. using conductance data in (122) HCO₃⁻ 44.5 cm²/Ω/equiv. calculated using $D=2.662 \cdot 10^{-6} \lambda_i / Z_i$ (121) and corrected to 37C with Stokes-Einstein equation

Bicarbonate concentration (mM)	Lowest pH reached (RT,1 atm)
20	5.95
15	5.85
10	5.75
5	5.36
2	5.07
1	4.71

Table 3.3 The pH of CO₂ saturated bicarbonate buffer at normal atmosphere and the room temperature.

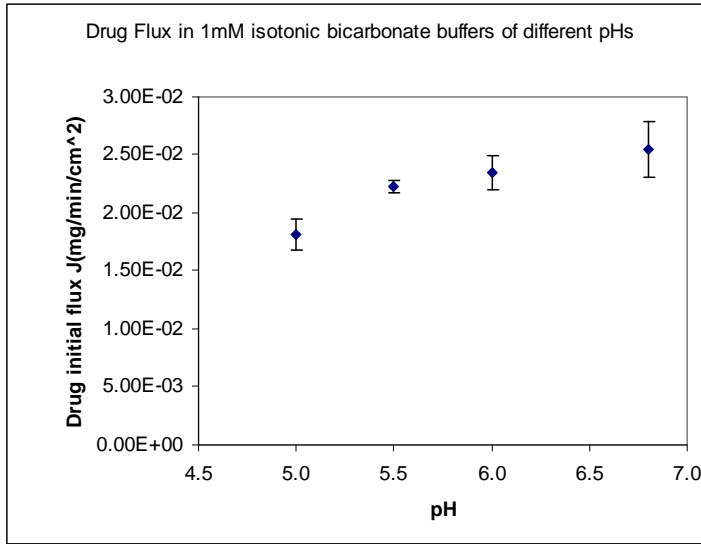
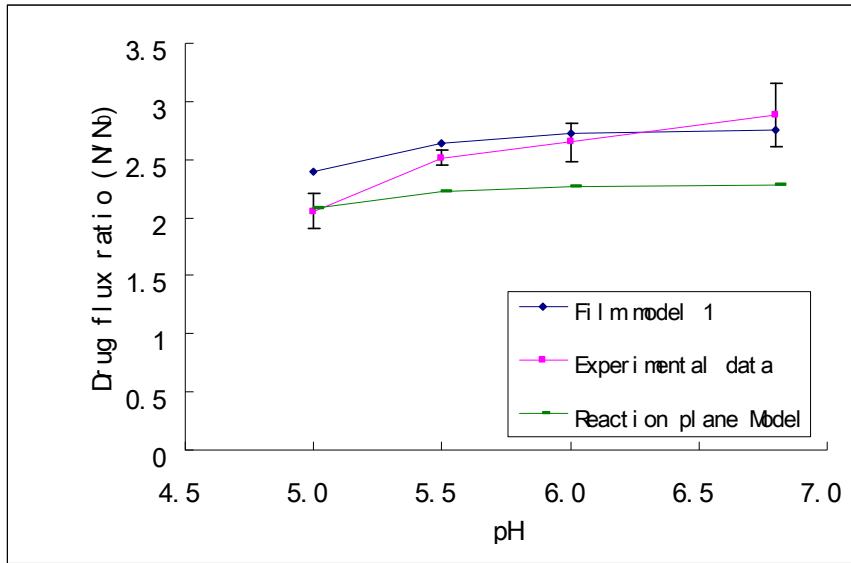


Figure 3.3 The Initial ibuprofen dissolution rate in 1mM bicarbonate buffer with pH 5.0, 5.5, 6.0, 6.8

(a)



(b)

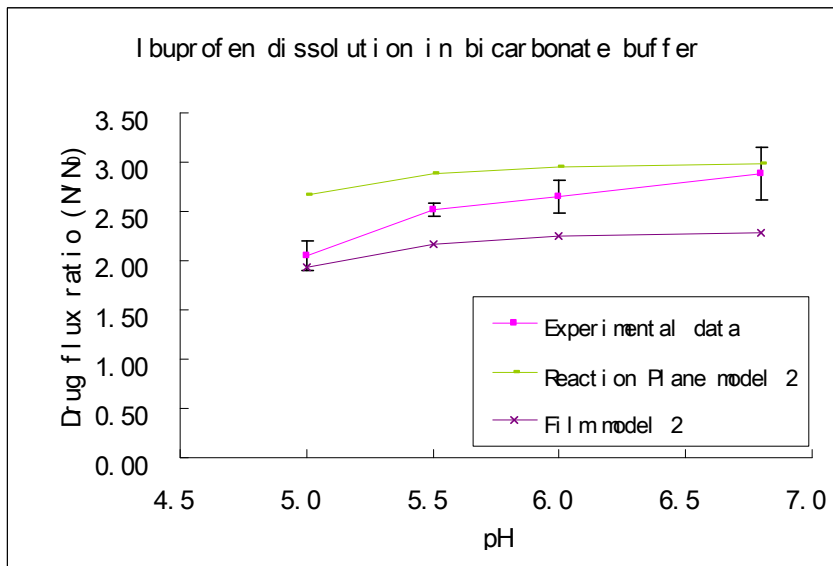


Figure 3.4 Initial dissolution flux ratios predicted from models compare with the experimental data in bicarbonate buffer of different pHs. (a) Models 1 , $DA = DHA = 0.92 \times 10^{-5} \text{ cm}^2/\text{s}$, $\alpha = 1$ was used. (b) Models 2, $\alpha = (DA / DHA)^{2/3} = (0.92 \times 10^{-5} \text{ cm}^2/\text{s} / 0.48 \times 10^{-5} \text{ cm}^2/\text{s})^{2/3} = 1.54$ was used.

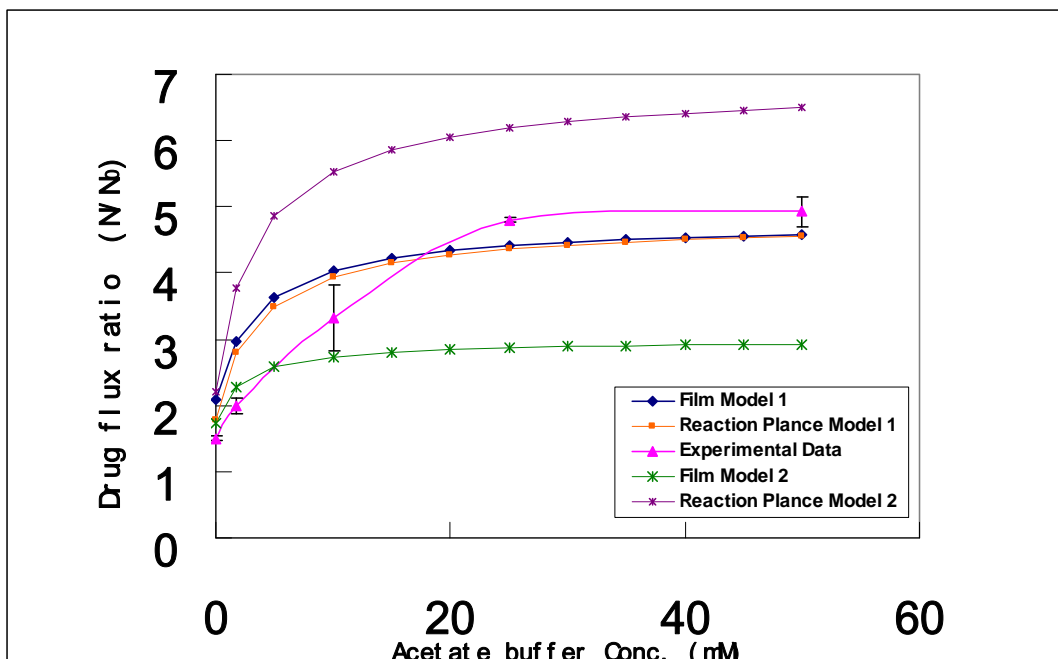


Figure 3.5 Initial dissolution flux ratios predicted from models compare with the experimental data in acetate buffer of different buffer strength. (a) Models 1, $D_A = D_{HA} = 0.92 \times 10^{-5} \text{ cm}^2/\text{s}$, $\alpha = 1$ was used. (b) Models 2, $\alpha = (D_A / D_{HA})^{2/3} = (0.92 \times 10^{-5} \text{ cm}^2/\text{s} / 0.48 \times 10^{-5} \text{ cm}^2/\text{s})^{2/3} = 1.54$ was used.

Buffer Species	pH	Mean Flux ($\times 10^{-2}$ mg/cm²/min) (s.d.)	Experimental Ntotal/N0
1 mM Sodium Bicarbonate	5.0	1.9190(0.13)	2.0563
1.75mM Sodium Acetate	5.0	1.7667(0.10)	1.9971
SGF, 0.1N HCl	1.2	0.8846 (0.05)	1.0000

Table 3.4 The acetate buffer equivalent to 1mM bicarbonate buffer at pH5.0.

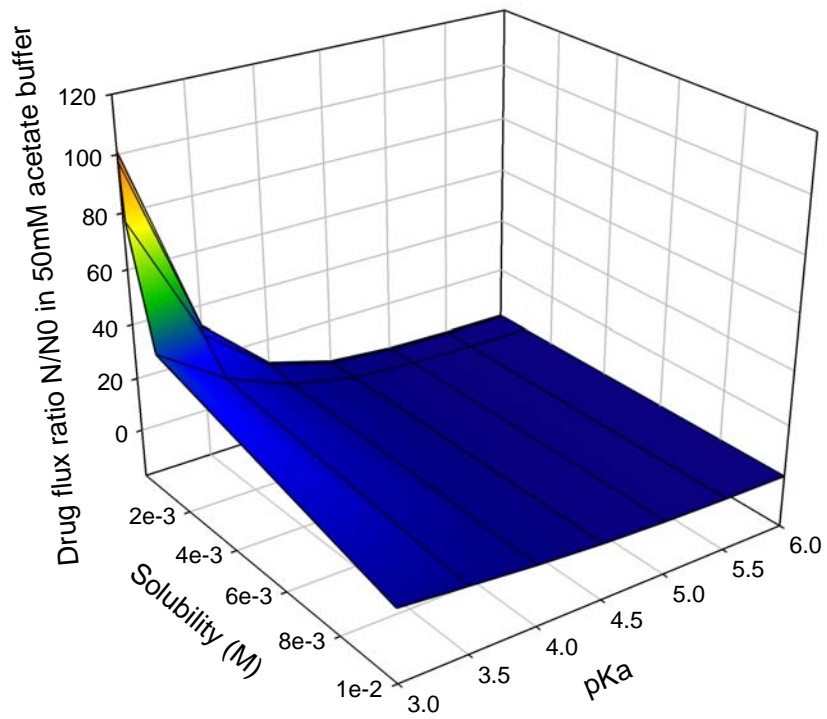


Figure 3.6 When drug diffusion coefficient is $0.5 \times 10^{-5} \text{ cm}^2/\text{s}$, the drug pKa and intrinsic solubility effects on drug dissolution rate in 50mM acetate buffer.

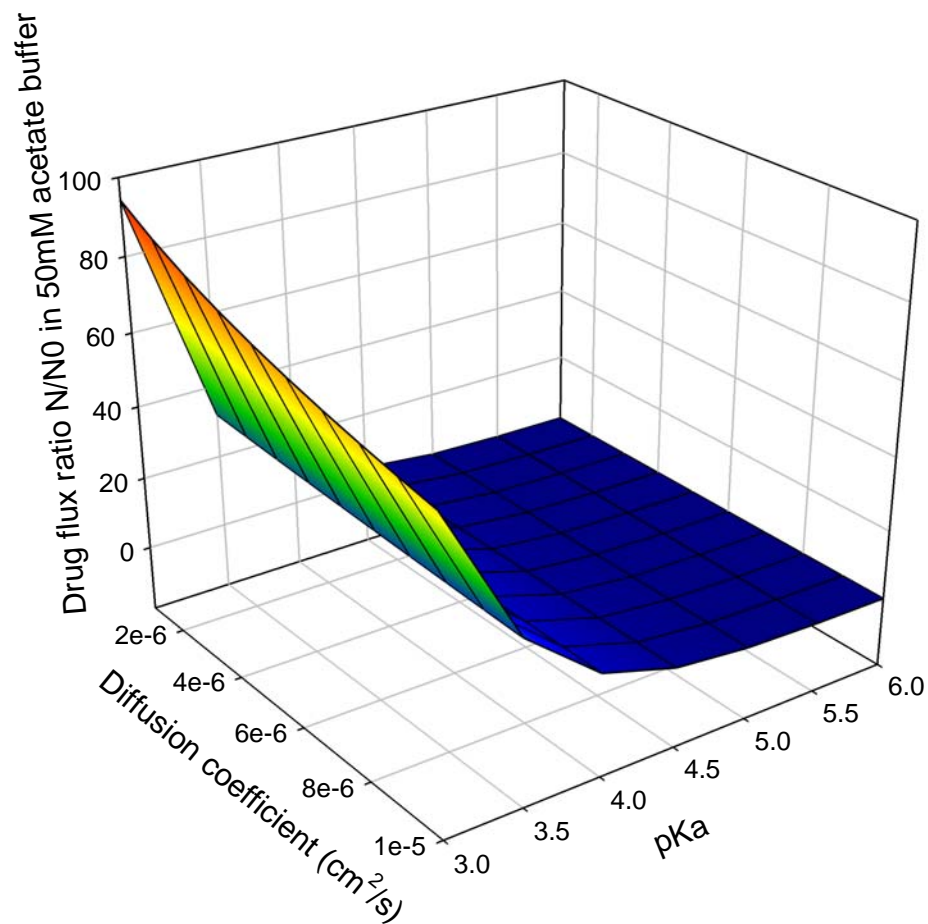


Figure 3.7 When drug intrinsic solubility = 1×10^{-4} cm²/s, the drug pKa and diffusion coefficient effects on drug dissolution rate in 50mM acetate buffer.

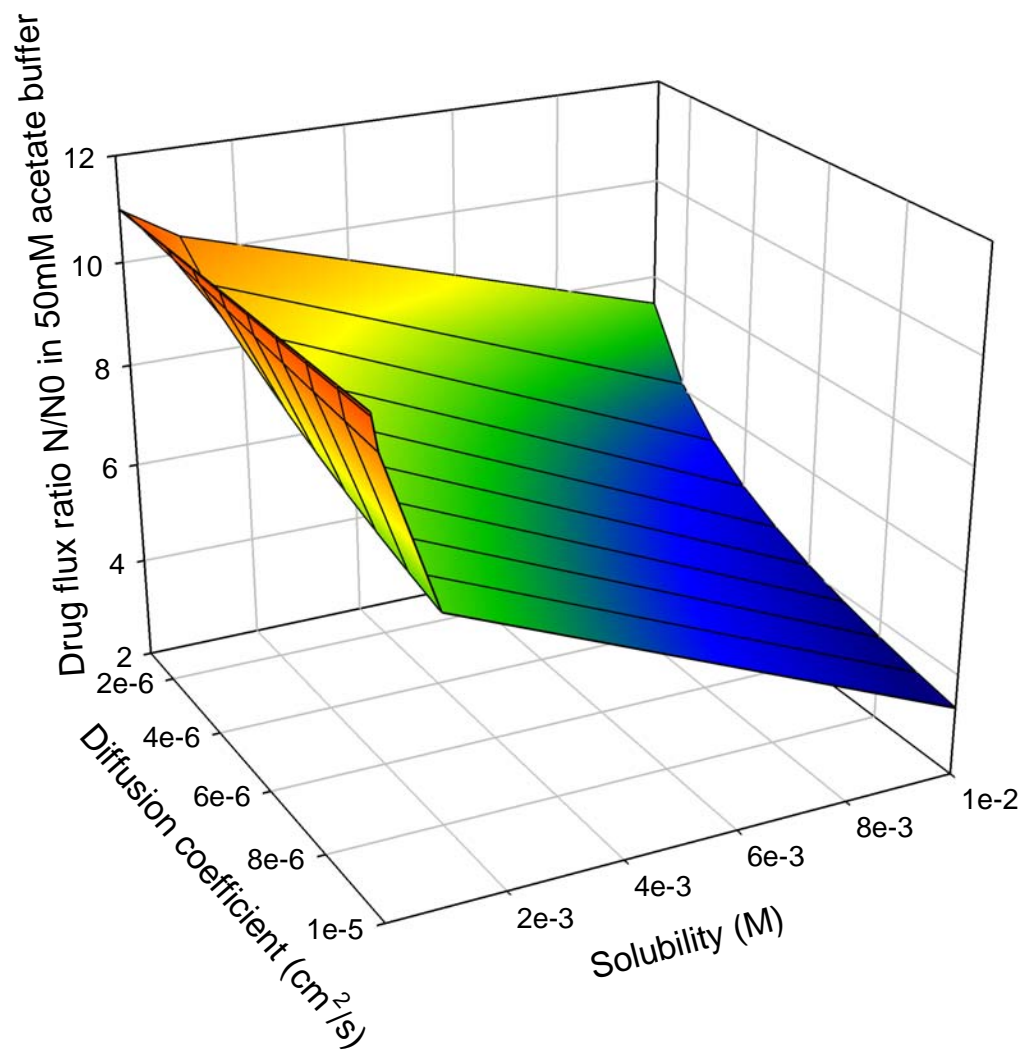


Figure 3.8 When drug pKa = 4.0, the drug intrinsic solubility and diffusion coefficient effects on drug dissolution rate in 50mM acetate buffer.

CHAPTER IV. THE EFFECT OF PHYSIOLOGICAL FACTORS ON BICARBONATE DISSOLUTION BUFFER

Abstract

Bicarbonate has been determined to be the dominant buffer species in human intestinal fluid and has a large effect on the drug dissolution. However, the bicarbonate buffer system is a much more complicated system than other buffer systems since the bicarbonate ions are always in equilibrium with carbonic acid, further with CO₂ dissolved and water. The carbon dioxide dissolved in water is affected by the partial pressure of it in gas state which changes with different physiological and pathological status in gastrointestinal lumen. The formation of carbonic acid equilibrium is catalyzed by carbonic anhydrase which plays a central role in bicarbonate equilibrium in the gastrointestinal tract. These factors influence the bicarbonate buffer system and through it affect the dissolution of the ionizable acidic drugs. The intrinsic dissolution study of the model drug, ibuprofen is conducted with the bovine carbonic anhydrase in the bicarbonate buffers in the gastrointestinal physiological pH ranged from 5-6.8, and the results indicated the significant increase of dissolution rate. The effect of acidic drug dissolution in bicarbonate buffer in equilibrium with different partial pressure was also simulated by the mathematical models, reaction plane model and film model, to demonstrate that the partial pressure of carbon dioxide can also affect the dissolution.

Introduction

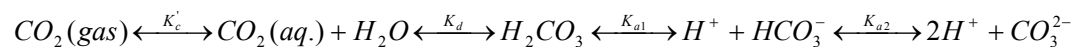
Bicarbonate buffer system is the major buffer in biological system regulating the acid-base balance. It is also the dominant buffer in gastrointestinal tract. There are many physiological factors could affect the dissolution of acidic drug through their effect on the buffer system. Carbonic anhydrase and partial pressure of CO₂ are major factors involving in the bicarbonate equilibrium system.

Carbonic anhydrase (CA) accelerate the reaction of CO₂ hydration reversibly. It has the highest turnover number of molecules among all known enzymes. The carbonic anhydrase family has been divided into cytosolic CAs(CA I, CA II, CA VII, CA XIII), mitochondrial CAs(CA-VA, CA-VB), and membrane associated CAs(CA IV, CA IX, CA XII, CA XIV, and CA XV) (123-126). There are three additional CA isoforms (CA VIII, CA X, CA XI) whose function are unknown yet. Carbonic anhydrase II is the monomeric with molecular weight over 30KD. Since it is lack of side chain of cysteine, it requires no external cofactors and relatively stable against the oxidation and inhibition of heavy metals. Its solution could be extremely stable and retain enzymatic activity for weeks, also its mobilized form on solid matrix used in chemical reactors could allow the operation temperature close to 50 °C (127).

Carbonic anhydrase has been shown widely distributed and has activities in various

segments of gastrointestinal tract (128, 129). The stomach and the colon showed high carbonic anhydrase activity, the jejunum had intermediate activity, and the ileum had low activity. There is also evidence showing the CA IV abundant available at the brush border in human GI. CA VI has been shown by radioimmunoassay secreted in to saliva and tissue (130-132). Carbonic anhydrase facilitates the secretion of bicarbonate to protect the GI tract(133), thus could also involving the bicarbonate equilibrium in intestinal fluid and affect the acidic drug dissolution process.

Since bicarbonate system is open ended equilibrium with CO₂. The partial pressure of CO₂ in the head space of the bicarbonate could affect the system and push the equilibrium



to the right end, and thus affect the acidic drug dissolution through the buffer system.

In vivo, the resting P_{CO₂} in lumen is 38mmHg (5 % atm), which is comparable to P_{CO₂} in arterial blood. Postprandial P_{CO₂} could increase to 280mmHg (37%atm) with the extreme of 502mmHg (66% atm) (58, 89, 90, 134). In the duodenal ulcer patients, the partial pressure is even higher with average of 480mmHg, and some patient has 700mmHg the reading from the plot of results (89). With higher physiological and pathological CO₂ partial pressure, the concentration of bicarbonate is also expected to be higher than that under the normal atmosphere. P_{CO₂} is likely to affect the acidic drug dissolution through its effect on the bicarbonate equilibrium.

In this section, intrinsic dissolution of ibuprofen with CA and mathematical models, film model and reaction plane model are used to illustrate the carbonic anhydrase enzymatic effect and the effect of partial pressure of CO₂ on acidic drug dissolution.

Experimental studies:

Materials:

ibuprofen was purchased from Acros Organics (Morris Plains, NJ), sodium chloride, sodium bicarbonate, sodium acetate and other chemicals of analytical grade were purchased from Sigma (St.Louis, MO). Distilled/deionized water was prepared using Milli-Q water (Millipore, Bedford, MA). 100% dried CO₂ was purchased from lifeGas (Ann Arbor, MI). Carbonic anhydrase from bovine erythrocytes was purchased from MPbiomedicals (solon,OH)

Methods:

Rotating disk dissolution of ibuprofen with Carbonic anhydrase in bicarbonate buffer

1mM NaHCO₃ buffer was prepared isotonic with sodium chloride. CO₂ was purged into 200 mL buffer in the rotating disk jacket beaker and reached the pH5.0, 5.5, 6.0, 6.8 before experiments. 5mg Carbonic anhydrase (enzymatic activity: 4580 u/mg solid) was added to the buffer until dissolved. The drug disks were prepared with 200mg of bulk drug and compressed with 2000 lbs pressure for 60 seconds. The drug disk was attached to a shaft driven by a motor, speed was set at 100rpm. The disk was

immersed into the medium when the experiment started. The Agilent UV spectrometer (Santa Clara, CA) was set measuring the bulk buffer ibuprofen concentration at 220nm through flow cell circulating the medium from the reactor, blank was taken before the disk was immersed. Each experiment was run for 20min, and measurements were taken every one minute. The intrinsic dissolutions of ibuprofen were performed at different pH. Each experiment was done in triplicates and the initial drug fluxes from the disk surface were calculated. This is referred as “Group A experiments with CA and fixed pH”.

For pH5.0, the same experiment was perform with carbonic anhydrase but only the initial pH was controlled, during the dissolution there was no CO₂ sparing to maintain the pH, with the acidic drug dissolving pH increased during experimental process, but final pH recorded was no higher than 0.3 unit above the starting pH.

“Group B experiments with CA, starting pH”.

Rotating disk dissolution of ibuprofen without Carbonic anhydrase in bicarbonate buffer

The same experiment with group A was done except without the step of adding carbonic anhydrase. The experiments were also done at pH5.0, 5.5, 6.0 and 6.8. Every experiment was run for 20min, and measurements are taken every one minute. Each experiment was done in triplicates and the initial drug fluxes from the disk surface were calculated. This is referred as “Group C experiments without CA and fixed pH”.

The controlled experiments of the group B were also done with no carbonic anhydrase added, and without CO₂ sparing to maintain the pH and this group is referred as “Group D experiments without CA, starting pH”. The pH increased during the experiments was no larger than 0.38 pH unit from pH5.0.

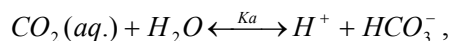
Theoretical studies:

The partial pressure of CO₂ on bicarbonate buffer was integrated in to the film model and reaction plane model discussed. Its effect on the dissolution model drug, ibuprofen was simulated and the ratio of drug flux in CO₂ influenced bicarbonate buffer to that in the non-buffered solution is calculated.

The partial pressure of CO₂ values from the pressure in normal atmosphere to the possible highest reported value were used to calculate against the physiological GI pH 5.0-6.8 using Henderson–Hasselbalch equation.

$$\frac{[CO_2]_{aq}}{P_{CO_2}} = \frac{1}{K'_c},$$

$K'_c = 29.76 \text{ atm}/(\text{mol/L})$ is the henry's constant at 25 °C.



$K_a = 6.1$ at 37 °C.

Figure 4.1 shows that the effect of pH and P_{CO₂} on the concentration of bicarbonate in

physiological and pathological values reported.

[\(Figure 4.1\)](#)

The bicarbonate concentration at resting P_{CO_2} could be 0.13mM to 8.42 mM, postprandial bicarbonate would increase from 0.9mM to 62mM with pH varies from pH5.0 to pH6.8, with the extreme incident of 1.76mM to 111mM. In duodenum ulcer patients, the bicarbonate could be very high with the average of 1.69mM to 106mM across physiology pH and 2.46mM to 155mM under the highest CO_2 partial pressure incident.

Results

The experiments showed that the effects of carbonic anhydrase in the bicarbonate buffer solution on the dissolution rate of acidic drug were significant across the different fixed pHs. The results are showed in Figure 4.2.

[\(Figure 4.2\)](#)

At pH5.0, both groups (A, B) with carbonic anhydrase showed the increased drug flux from the disk surface compared with the groups(C, D). But, since the absolute values were small due to the low concentration and pH, it was hard to differentiate the fluxes from group A, C, and D. In group B with carbonic anhydrase but no CO_2 sparing, the effect of increasing weak acid drug dissolution rate was most significant. Although, since pH in group B was also increasing, the increased flux could partially be caused by the pH effect, the flux could still be larger than the flux of that in higher initial pH

buffer but without carbonic anhydrase. At this condition, without purging CO₂ to control pH and with the enzymatic catalysis, the variation of experiment results was larger than at other conditions.

In the equilibrium of $CO_2(aq.) + H_2O \xrightleftharpoons{CA} H_2CO_3 \xrightleftharpoons{K_{a1}} H^+ + HCO_3^-$

Since carbonic anhydrase catalyzed the hydration reaction of CO₂, with CO₂ purging supply, according to LeChatelier's principle, the reaction is pushed towards the generation of bicarbonate. Since the effect of purging CO₂ is also pushing the reaction towards the same direction, the enzymatic effect is more significant without the supply of CO₂. Although the pH increased about 0.3 units, the flux of the drug release from the tablet increased is larger than that increased just by the pH effect.

At higher pH, with the CO₂ sparging to maintain the pH, the fluxes were increased by the adding of the carbonic anhydrase. When pH is 6.8, the increase was the most significant and almost doubled the rate without CA. Since it is reported that the enzymatic activity of CA is higher in pH 7.5 compared with 5.5(135), also at alkaline pH higher pH accompanied by higher activity (136), the increased activity of the flux may also be explained by the increased CA activity.

The effect of bicarbonate buffers under the effect of physiological and pathological PCO₂ on the dissolution of ibuprofen was simulated by reaction plane model and film model. The ibuprofen dissolution flux ratios in buffered verse unbuffered bicarbonate

solutions were listed in Table 4.1 and 4.2. At duodenal resting PCO_2 , the drug flux in bicarbonate buffer increase about 2-4 times compared with non-buffered solution across the pH5.0-6.8. At postprandial duodenal PCO_2 , the drug flux increased from about 2 times at pH5.0 to about 8 or 10 times at pH6.8 compared non-buffered solution.

([Table 4.1](#))

([Table 4.2](#))

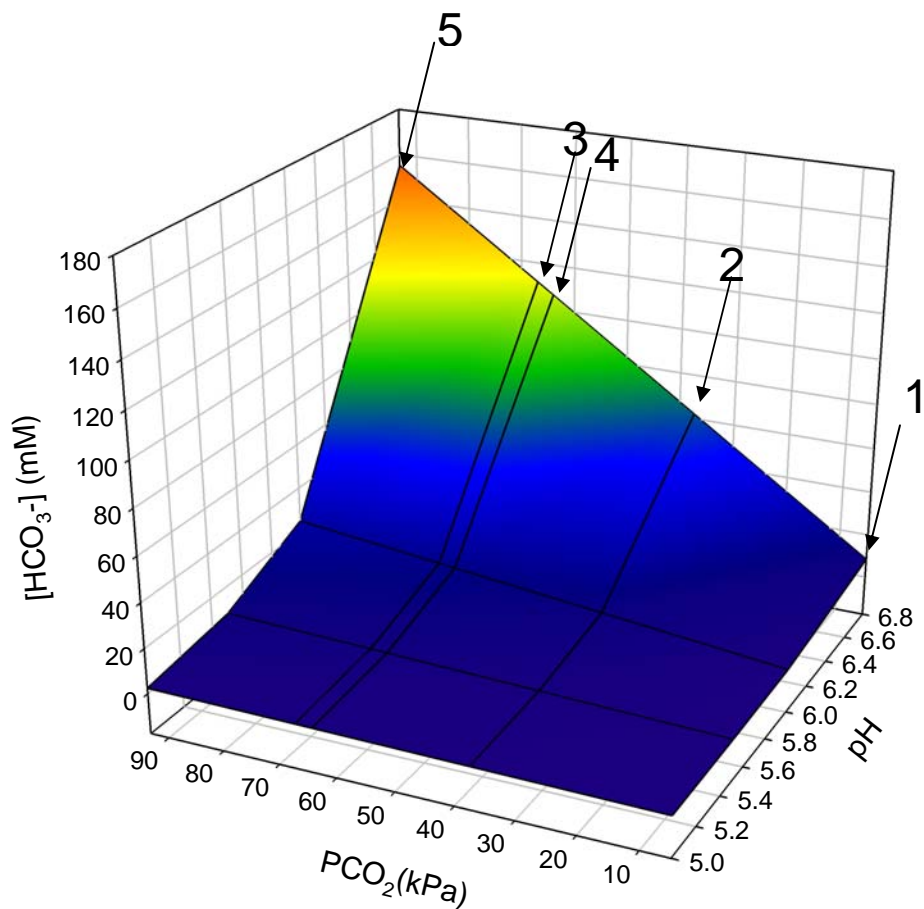
Discussions

The consideration of the physiological and pathological factors in gastrointestinal tract is essential for *in vivo-in vitro* correlation for the drug absorption. These factors could affect the dissolution process directly, or through their effects on the drug dosage forms, and also could be involved in the dissolution media, since the dissolution process is ultimately the drug molecule mass transfer between delivery system and the biological fluid under the physiological and/or pathological hydrodynamics.

The key factors involved in bicarbonate system equilibrium was investigated and demonstrated to affect the acidic drug dissolution. The pH, buffer species, partial pressure of CO_2 , and also carbonic anhydrase constitute a complicated buffer system itself without other factors like bile salts, protein, etc. More knowledge is needed to fully understand this system such as the input rate of bicarbonate or CO_2 that is

physiologically relevant, the amount of carbonic anhydrase that functions in the bicarbonate buffer system and so on. However, capturing the key parameters in this bicarbonate system with the aid of computational method would lead us to more understanding and further utilizing this system as dissolution media for testing drug *in vivo* performance.

There are also pathological conditions that could result in different level of absorption of poorly soluble drug. In clinical, for duodenal ulcer patients, NSAIDs are suggested to be avoided because its GI irritation side effect. From our study, we can see pathological PCO_2 would affect the poorly soluble ionisable drugs including NSAIDs, so serious considerations should be given when considering the use of drug under pathological GI conditions.



1. Duodenal resting (fasted) CO_2 partial pressure.
2. Duodenal postprandial (fed) CO_2 partial pressure.
3. Extremely high duodenal postprandial CO_2 partial pressure.
4. Duodenal ulcer CO_2 partial pressure.
5. Extremely high duodenal ulcer CO_2 partial pressure

Figure 4.1. Bicarbonate concentrations under physiological/pathological pH and P_{CO_2}

Dissolution of Ibuprofen in 1mM isotonic NaHCO₃ solution

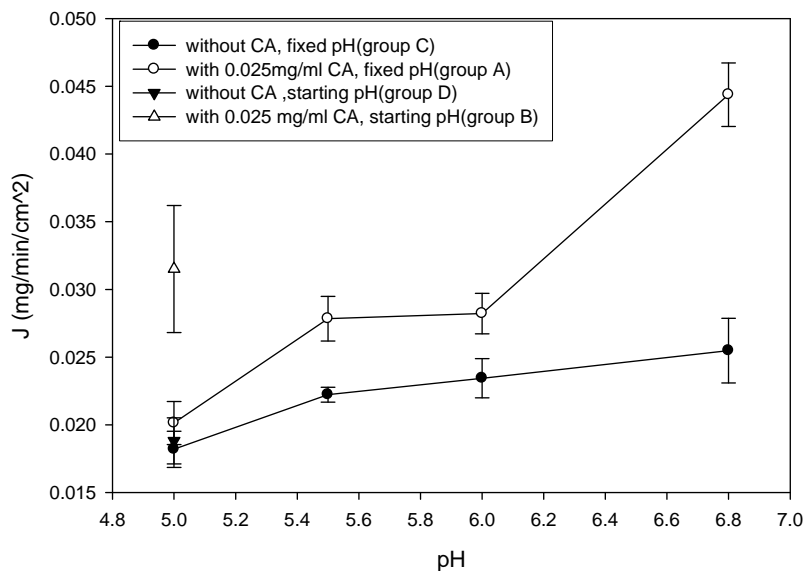


Figure 4.2 Enzymatic effect of carbonic anhydrase on the dissolution of acidic drug in bicarbonate buffers.

P _{CO2} (kPa) N/N0 pH	Duodenal resting	Duodenal postprandial	Duodenal ulcer	Duodenal postprandial extreme incident	Duodenal ulcer extreme incident
	5.07	37.33	63.99	66.93	93.33
5	1.83	2.10	2.26	2.27	2.41
5.5	2.03	2.80	3.23	3.27	3.60
6	2.40	4.14	5.03	5.10	5.78
6.8	4.03	8.97	11.36	11.59	13.47

Table 4.1 Reaction plane model simulated ibuprofen dissolution flux ratios in bicarbonate buffer under physiological and pathological P_{CO2}

P _{CO2} (kPa) N/N0 pH	Duodenal resting	Duodenal postprandial	Duodenal ulcer	Duodenal postprandial extreme incident	Duodenal ulcer extreme incident
	5.07	37.33	63.99	66.93	93.33
5	2.14	2.35	2.50	2.51	2.63
5.5	2.39	3.13	3.57	3.61	3.96
6	2.77	4.61	5.58	5.66	6.41
6.8	4.52	10.04	12.74	12.95	15.12

Table 4.2 Film model simulated ibuprofen dissolution flux ratios in bicarbonate buffer under physiological and pathological P_{CO2}

CHAPTER V. SUMMARY

The *in vitro* dissolution test is important for quality control, formulation development and for bioequivalence tests. However, designing the proper dissolution test to closely reflect the *in vivo* dissolution process is difficult because the complexity of gastrointestinal physiological and logical factors, the drug physiochemical factors, the factors involved making the drug into final product and their interactions with each other. The developing of the dissolution methods could be advanced when more information about above factors becoming available. The key factors then could be identified and utilized in the refining of the methodology. The dissolution media is one of the most important issues among all the factors and is studied here.

The research in this dissertation provides more information on the physiological buffer species, bicarbonate buffer in real human intestinal fluid in terms of its buffer capacity and its effect on dissolution of acidic drugs. The results showed that at physiological pH range 5.0-7.0, bicarbonate contribution to the buffer capacity of fasted *ex vivo* whole human intestinal fluid was larger than 50%. The intrinsic dissolution rate of BCS II acidic drug in human intestinal fluid reduced 48% when bicarbonate buffer been depleted from the fluid; The concentration of $\text{HCO}_3^-/\text{CO}_2$ buffer determined by titration was 4.5 mM, which was consistent with the IC results of 4.3 mM for bicarbonate and only 0.62 mM for phosphate. These studies suggested the importance of the physiological *in vivo* buffer, bicarbonate buffer, when

considering the choice of buffer species for the *in vitro* dissolution test. Furthermore, a miniature rotating disk apparatus has been demonstrated to be useful when the dissolution media or active pharmaceutical ingredient is limited.

Since the pharmacopeial buffers have been widely used in pharmaceutical industry with different types of dissolution apparatus, it is meaningful to determine the physiological equivalent compendial buffer. Through the analysis of reaction plane and film models in our work, the relationships among different buffer species and strength effect on the dissolution of a BCS II acidic drug was predicted and has been verified by experimental results; Models built in mathematica® and Matlab® can also be developed into a tool to provide suggestions on compendial buffer strength with the drugs of known physiochemical properties.

The other physiological factors including partial pressure of CO₂ and carbonic anhydrase would also affect the *in vivo* dissolution process through their effect on the physiological buffer media. In this work, carbonic anhydrase and partial pressure of CO₂ were investigated since they both play critical roles in the equilibrium in the bicarbonate systems. With enzymatic effect of carbonic anhydrase, the dissolution rate of BCS II acidic drug, ibuprofen increased significantly at pH5.0-6.8. The reaction plane and film models showed that the increase of partial pressure of CO₂ at physiological and pathological range would increase the dissolution of BCS II acidic drug. The theoretical approach can assist us to analyze the *in vivo* situation

more closely when the experimental conditions are difficult to set.

While bridging the *in vitro* to *in vivo* dissolution is a desirable goal, there are many gaps in our knowledge that need to be filled to completely understand and develop media reflecting the *in vivo* situation as showed in Figure 5.1.

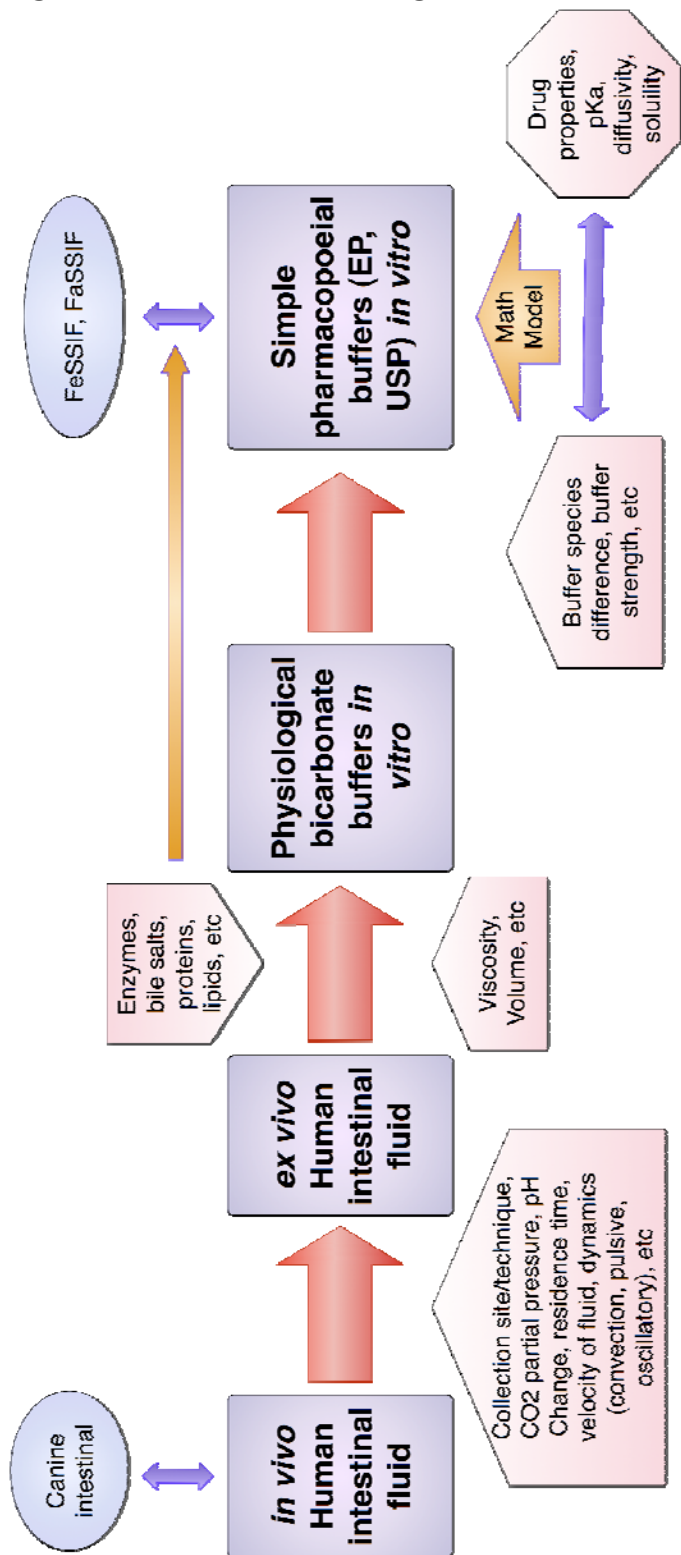
[\(Figure 5.1\)](#)

1. From the *in vivo* human intestinal fluid to *ex vivo* human intestinal fluid: the collection site in different segments along the intestinal tract; the technique used will generate differences in between human intestinal fluids *in vivo* and *ex vivo*. Since experimental interference and partial pressure over the fluid is changed once the fluid is outside the body, the pH increase as CO₂ evaporates from the fluid. The flow velocity of the fluid, the transit time, temperature, also the gastrointestinal dynamics information are lost in between the *in vivo* and *ex vivo* human intestinal fluids. There are also studies using canine intestinal fluid as the substitute of humans, but there are many species differences to be considered(25).
2. From the *ex vivo* human intestinal fluid to physiological bicarbonate buffer, there are a lot of factors should be considered to overcome the gap in between these fluids, such as viscosity, volume, surface tension of the dissolution media, also exogenous and endogenous substances like enzymes, bile salts, protein, lipids in the fluid. The biorelevant FaSSIF and FeSSIF buffers proposed by Dressman et.al (106, 116, 137, 138) are in the correct route of thinking in this aspect.
3. When correlating the physiological bicarbonate buffer to simple pharmacopoeial

buffers *in vitro*, the buffer species and strength differences could be considered together with the drug physiochemical properties such as pKa, diffusion coefficient, solubility etc.

The research presented here focused on several of above gaps and made a solid step in the rational design of *in vitro* dissolution methods. With the combination of the experimental and theoretical considerations, we are in the process of identifying the essential parameters in the *in vivo* process of dissolution of BCS II acidic drugs and developing the proper *in vitro* dissolution tests that will reflect the *in vivo* circumstance better.

Figure 5.1 Factors in Translating *in vivo* to *in vitro* dissolution.



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