ASIAN JOURNAL OF PHARMACEUTICAL SCIENCES 11 (2016) 722–734

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Original Research Paper

Pharmaceutical salts: Theory, use in solid dosage forms and *in situ* preparation in an aerosol

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ARTICLE INFO

Article history:
Received 22 April 2016
Received in revised form 20 May 2016
Accepted 7 July 2016
Available online 16 July 2016

Keywords:
Solubility
Salt
Aerosol
Inhalation
Solubility product

ABSTRACT

In this article, the theoretical foundation for salts is given with an emphasis on the amount of drug in solution. Consideration is given for the solubility of the non-ionized form, acid dissociation constant and solubility product, which are the limiting constraints. For dissolution of nonionized drugs, the surface pH differs from the bulk pH, giving rise to a lower than expected rate. For salts, theoretical considerations are relatively complex, and an experimental approach to estimating the surface pH is more likely to be of value in predicting the dissolution rate. General guidelines are described for screening, preparing and characterizing drugs as salts, which critically depend on the goal of the product development. Thereafter, our work involving the preparation of salts as a means to generate aerosols from a solution is provided. The solubility of six structurally related compounds was determined in four acids. Thereafter, the amount of the compound in solution was determined as a function of pH, using the acid that provided the highest solubility. Because the pH required to achieve the needed concentration for aerosol generation was low, ammonia vapor was introduced into the air stream to neutralize aerosol droplets. Solvent was then removed from the aerosol by a silica column. The resulting aerosol had a concentration of 96 μg/l and a mass median particle size of 1.8 μm. The reported pharmacokinetic study substantiated the feasibility of evaluating its safety and efficacy of inhalation administration in the rat model.

1. Introduction

1.1. *Prevalence and importance of salts*

The formation of salts is invaluable for the preparation of safe and effective dosage forms of many drugs [1–3]. Whether the drug products are solutions or solids, the use of a salt provides a higher concentration in solution than the free acid or free base (nonionized forms). Typically, salts readily undergo crystallization, and the resulting material facilitates subsequent processing. Thus, the salt is often the preferred form for isolating and purifying the drug. Historically, the number of available salts was rather limited; however, today there is a wide

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Peer review under responsibility of Shenyang Pharmaceutical University.
http://dx.doi.org/10.1016/j.ajps.2016.07.002

Table 1 – List of compounds available for preparing salts [4].

Cations		Anions	
Aluminum	Acetate	Glutamate	Mucate
Arginine	Aspartate	Glycolate	Napsylate
Benzathine	Benzenesulfonate	Glycollylarsanilate	Nitrate
Calcium	Benzoate	Hexanoate	Octanoate
Chloroprocaine	Besylate	Hexylresorcinate	Oleate
Choline	Bicarbonate	Hydrabamine	Pamoate
Diethanolamine	Bitartrate	Hydroxynaphthoate	Pantothenate
Ethanolamine	Bromide	Iodide	Phosphate
Ethylenediamine	Camsylate	Isethionate	Polygalacturonate
Histidine	Carbonate	Isethionate	Propionate
Lithium	Chloride	Lactate	Salicylate
Lysine	Citrate	Lactobionate	Stearate
Magnesium	Decanoate	Malate	Subacetate
Meglumine	Edetate	Maleate	Succinate
Potassium	Estolate	Mandelate	Sulfate
Procaine	Esylate	Mesylate	Tartrate
Sodium	Fumarate	Methylbromide	Teoclate
Triethylamine	Gluceptate	Methylnitrate	Tosylate
Zinc	Gluconate	Methylsulfate	Triethiodide

range of chemical entities that are recognized as being safe, which can be used in the preparation of drug products (cf Table 1) [4,5].

In addition to solubility and manufacturing, the salt is typically a more stable form of the drug. This too is an important advantage for developing a product with a long shelf-life. Although non-ionized drugs often exist in multiple polymorphic forms, the number for salts appears limited. This may be an inherent property of the ionic bond, but it should also be recognized that relatively little effort has been expended in the search for different polymorphic forms of salts [6]. As such, there may be an untapped potential, because as it has been noted, the number of polymorphic forms appears to be function of the time expended in searching for them.

In this article, the theoretical foundation for salts is given with an emphasis on the observed increased amount of drug in solution. Here, consideration is given for the solubility of the non-ionized form, acid dissociation constant and solubility product, which are the limiting constraints. The salient features of the pH dependence of the dissolution of nonionized and ionized drugs and their salts are given. Some general guidelines are reviewed for screening and characterizing drugs as salts for development of products. Thereafter, our work involving the preparation of salts as a means to generate aerosols from a solution is provided. Here, *in situ* neutralization was needed to allow the drug to be evaluated for safety and efficacy in a rat model of a respiratory disease.

1.2. *Definitions*

In 1923, Johannes Nicolaus Brønsted (Denmark) and independently Martin Lowry (England) formulated a definition of an acid and a base [7]; an acid (generically HA) gives up or donates a proton (hydrogen ion, H^+) and a base (B) accepts a proton. This may be written as:

Acid: $HA \rightleftharpoons H^+ + A^-$

Base: $B + H^+ \rightleftharpoons BH^+$

It can be noted that A^- acts as a base in the reverse reaction and is thus called a conjugate base, just as BH^+ acts as an acid and is referred to as a conjugate acid. These form a conjugate acid–base pair. That is:

$$\text{Acid} \rightleftharpoons \text{Base} + H^+$$

Water is amphoteric, acting as both an acid and a base, and is often the source of the hydrogen ions as well as hydroxide ions in pharmaceutical systems. The Brønsted–Lowry model explains the dissociation of water into hydronium and hydroxide ions:

$$H_2O + H_2O \rightleftharpoons H_3O^+ + OH^-$$

This is a type of disproportionation reaction, in which identical components react to form two different species. The corresponding equilibrium constants follow the usual convention and are as follows with the assumption of ideality:

Acid dissociation constant, $K_a = [H^+][A^-]/[HA]$

Base dissociation constant, $K_b = [BH^+][OH^-]/[B]$

For water,

$$K_w = [H^+][OH^-]$$

In these expressions, the concentration of water is assumed constant and incorporated into K_w, K_a, and K_b. Finally, for a given conjugate acid–base pair, it is noted that:

$$K_w = K_a K_b$$

and introducing the convention that the operator, p(x) = – log(x), then

$$pK_w = pK_a + pK_b$$

The value of pK_w is 14.00 at 25 °C, while the value of the acid dissociation constant of a weak acid or conjugate acid of a weak base is governed by the chemical interaction between the solute and the solvent [8].

A salt is an ionic compound that results from a reaction of an acid and a base. In general, this is written as:

$$mB^{n+} + nA^{m-} \rightleftharpoons B_m A_n(s)$$

where m moles of the base with a valence of n^+ combine with n moles of an acid of valence m^- to form a solid (s) salt with the given stoichiometry to achieve electro-neutrality.

1.3. *Solubility and solubility product*

The thermodynamic definition of a solution is a mixture of two or more substances, which forms a stable, molecular dispersion [8]. The process of solution involves a change in the system from an initial state comprising a solid and a pure liquid phase and the final state comprising a drug in solution. The change in Gibbs free energy is related to the amount of solute in solution. The solubility is the maximum amount of material (e.g. solid) that will dissolve in a given volume of solvent. This is an equilibrium property, and there are no degrees of freedom with specifying the two components (solute and solvent) and the temperature and pressure. For equilibrium to exist, the solid form of the solute must be in the lowest energy state. As is well known, solids often have multiple polymorphic forms, which exist in higher energy, metastable states or can incorporate water (hydrates) or other solvents (solvates) into the crystalline lattice. In addition, a solid need not have a crystalline structure but can exist in an amorphous form. In this discussion, these aspects will not be considered as they have been recently reviewed [6].

The solubility of a salt is more complicated, because dissociation introduces another component and thereby an additional degree of freedom [1]. Here, the definition of the solubility product, K_{sp}, stems from the chemical equation used for the salt:

$$K_{sp} = [B^{n+}]^m [A^{m-}]^n$$

where the solid salt is taken as having unit activity. For a concrete example, ephedrine can be used, where 25 different salts were made [9]. The solubility product of the HCl salt is written as follows:

$$K_{sp} = [\text{Ephedrine-H}^+][Cl^-]$$

In essence, there are now three components: ephedrine, chloride, and the solvent, water. As K_{sp} is a constant, the amount of ephedrine in solution depends on the concentration of chloride ions.

For the other salt forms, each will have a unique solubility product that determines the amount of ephedrine in solution along with the concentration of the counter ions. Moreover in solution, ephedrine must necessarily exist both as a base and conjugate acid; the distribution is governed by the acid dissociation constant and the pH of the solution. Thus, along with specifying the temperature and pressure, the additional parameters of the pH, $[Cl^-]$, and pKa must be defined to ascribe a unique value for the maximum amount of ephedrine in solution. A cautionary note is in order as often an apparent solubility product is reported. That is, rather than determining the concentration of the ionized form, the total concentration is determined [10]. This apparent solubility product, $K_{sp,app}$, is given by

$$K_{sp,app} = [C_{tot}][\text{counter ion}]$$

$$\text{Weak acid,} \quad K_{sp,app} = \{[HA] + [A^-]\}[M^+]$$

$$\text{Weak base,} \quad K_{sp,app} = \{[B] + [BH^+]\}[X^-]$$

Perhaps the most dramatic example of the influence of the solubility product on the total amount of drug in solution is chlorhexidine (CHX) [11,12]. Three possible salt forms are the dihydrochloride, diacetate, and digluconate. The corresponding solubility products yield CHX concentrations in the ug/ml, mg/ml, and the gluconate readily forms a 40 wt% solution. The latter observation has been related to the self-association of CHX in the presence of gluconate ions. This represents another possibility with additional theoretical implications. That is,

$$\text{CHX-gluconate}_2 \rightleftharpoons CHX^{2+} + 2\text{gluconate}^-$$

$$CHX^{2+} + CHX^{2+} \rightleftharpoons (CHX^{2+})_2$$

$$(CHX^{2+})_2 + CHX^{2+} \rightleftharpoons (CHX^{2+})_3$$

…

where the stepwise self-association may be described by multiple equilibria.

1.4. *Ion pairing*

A related phenomenon is when the salt is dissolved but does not undergo complete dissociation. When the counter ion remains spatially close to the parent compound, the association is written chemically as:

$$B_m A_n(s) \rightleftharpoons (B_m{}^{n+} A_n{}^{m-})_{aq}$$

Bjerrum [8] initially suggested that the term, ion pair, be used to describe two ions that have a separation distance less than q, which is given by:

$$q = \frac{|z_i z_j| e^2}{8\pi\epsilon_o \varepsilon_r kT}$$

where z is the valence, e is the electronic charge, ε_o is the permittivity of a vacuum, ε_r is the relative permittivity of the solution, and kT is the thermal energy given by the product

of Boltzmann's constant and the temperature in Kelvin. In essence, this equation provides the distance at which the Columbic attractive energy is equal to the thermal energy.

1.5. *Complexation*

The word complex is used in a number of pharmaceutical contexts. Complexation is defined as the reversible, non-covalent interaction between m molecules of a drug with n molecules of a ligand species. Generally, it refers to a type of binding that involves strong attractive intermolecular interactions or equivalently, high affinity, and therefore has a large association constant. In chemistry, it typically refers to association with metals, although IUPAC recommends the term, coordination entity [8]. This involves a metal center plus ligands. Complexes can be positively charged, neutral, or negatively charged. The overall charge on the complex depends on the oxidation state of the metal and the charges brought by the ligands. The ligand may have a single bond or single coordination number (unidentate = literally, one tooth) or multiple bonds (multiple coordination number or multidentate). In the latter case, the term chelate (claw) is used, which implies encirclement of the metal ion by the ligand. Ethylenediamine tetraacetic acid (EDTA) with metal ions is the well-known pharmaceutical example of a coordination entity.

1.6. *pH-solubility*

The primary purpose of forming a salt is to increase the amount of drug in solution [1–3]. Because the vast majority of approved drugs are weak acids or bases, the latter of which is much more common, it is instructive to address the pH dependence of the amount of drug in solution. The usual assumptions are that the ionized form has an infinite solubility and the solubility of the non-ionized form, C_s, is independent of pH. This allows derivation of the following expressions for the total amount of drug in solution, C_{tot} [2,13,14]:

Weak acid: $C_{tot} = C_s\left[1 + 10^{(pH-pKa)}\right]$

Weak base: $C_{tot} = C_s\left[1 + 10^{(pKa-pH)}\right]$

In Fig. 1, the curved lines are given for the theoretical plot of the total concentration of a weak base and weak acid, both with a pK_a of 5. The total concentration rises with a decrease in pH for the weak base, whereas it rises with an increase in pH for the weak acid.

Despite the assumption, the solubility of the ionized form cannot be infinite as it is constrained by the solubility product. Thus, an observed maximum solubility at a specified pH, that is, pH_{max}, is predicted [2]. In the case when HA forms a salt of M^+A^-, the solubility product is:

$$K_{sp} = [M^+][A^-] = [A^-]^2$$

assuming the only source of the cation arises from the salt. The total amount in solution is then given by:

$$C_{tot} = [HA] + K_{sp}^{1/2} = \left(1 + [H^+]/K_a\right)K_{sp}^{1/2}$$

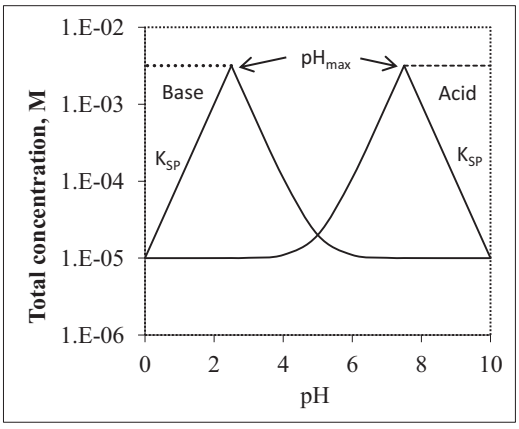

Fig. 1 – Total concentration of weak acid and weak base in solution as a function of pH reflecting the effect of ionization and solubility product.

The corresponding equation for a weak base is:

$$C_{tot} = [B] + K_{sp}^{1/2} = \left(1 + K_a/[H^+]\right)K_{sp}^{1/2}$$

These are shown as horizontal dotted lines in Fig. 1, which are independent of pH when the amount in solution is determined by the solubility product of the salt. This may be observed by preparing the salt as a saturated solution and controlling the pH with a buffer that neither contains the counter ion in the salt nor appreciably affects the observed amount of salt in solution.

A more common approach to measure the solubility as a function of pH is to place excess nonionized form into the solvent and vary the amount of acid, HX, (or base, MOH) to yield a range of pH values [2]. Chemically, this is given as:

$$HA + MOH \rightleftharpoons M^+ + A^- + H_2O$$

$$B + HX \rightleftharpoons BH^+ + X^-$$

The observed total amount of drug in solution plotted as a function of pH will increase, reach a maximum, and then decrease. The decrease arises from the solubility product, which is also shown in Fig. 1 as straight lines, decreasing in the opposite direction to the effect of ionization. That is, the addition of an acid (or base) must necessarily involve the addition of counter ions, M^+ (or X^-), which causes the drug concentration to fall in proportion to the added counter ion. This is known as the common ion effect.

Thus, there are the two relationships for the total amount of weak acid in solution; one constrained by the pK_a at low pH and the other by the K_{sp} at high pH. The intersection of the two relationships yields the maximum possible total concentration. Equating these expressions and solving for $[H^+]_{max}$ yields the pH at which this occurs, pH_{max} [2],

Weak acid:	$[H+]_{max} = K_a[HA]_o/K_{sp}^{1/2}$
	$pH_{max} = pK_a + 0.5pK_{sp} - \log[HA]$
Weak base:	$[H+]_{max} = K_aK_{sp}^{1/2}/[B]$
	$pH_{max} = pK_a - 0.5pK_{sp} + \log[B]_o$

where $[HA]_o$ and $[B]_o$ represent the solubilities of the nonionized forms of the weak acid and weak base, respectively. In these expressions, the maximum in the amount of drug in solution will be displaced from the pK_a by the value of the pK_{sp} and solubility of the nonionized from. That is, for a weak acid the pH_{max} is moved to a value greater than the pK_a as pK_{sp} becomes larger. For a weak base, an analogous shift is seen, but here it is to a lower value of the pK_a. pK_{sp} appears as the square root due to the assumption that the counter ion concentration is equal to the concentration of the ionized form; $K_{sp} = [A^-]^2$ or $[BH^+]^2$. The solubility of the nonionized form, either [HA] or [B], offsets the effect of pK_{sp} and shifts the pH_{max} closer to the pKa.

1.7. *Nonionized drug dissolution*

The use of salts to generate a drug with high water solubility may represent the end goal for preparation of a solution formulation. However, it is much more commonly exploited in solid dosage forms, where the goal is to yield a drug product with a high dissolution rate [2,13–16]. The value in having a high dissolution rate is that dissolution and the subsequent membrane transport can be completed in the allotted gastrointestinal transit time.

The Noyes–Whitney equation provides the empirical relationship for the rate of dissolution, dm/dt, and is given as [1]:

$$dm/dt = (DS/h)(C_s - C_b)$$

where D is the diffusion coefficient, S is the surface area, C_s and C_b are the concentrations at the surface and the bulk, respectively. The parameter, h, is the diffusional boundary layer thickness (DBL) or sometimes referred to as the unstirred or stagnant layer. The DBL appears to be the distance over which diffusion occurs but in reality involves both diffusive and convective contributions [13,17]. Moreover, these contributions vary with the distance from the solid surface. This is shown in Fig. 2.

An exact, analytical expression for dissolution, which separates the diffusive and convective contributions, is available provided the contribution from convection is controlled. The rotating disk method is an example for which the equation for the DBL was derived by Levich [18] and is:

$$h = 1.61 D^{1/3} \nu^{1/6} \omega^{-1/2}$$

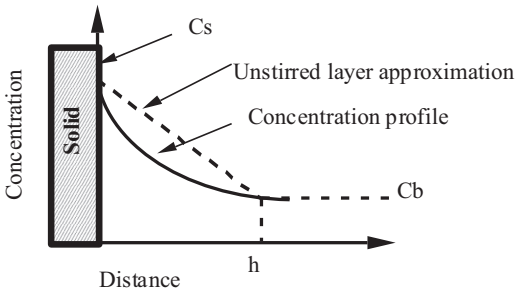

Fig. 2 – Schematic diagram of dissolution of drug depicting actual concentration (solid line) and assumption of linearity (dotted line).

where ν is the kinematic viscosity and ω is the angular velocity (rad/s). With the use of a dissolution apparatus (e.g. USP Dissolution Apparatus I or II), the hydrodynamic conditions are reproducibly controlled but do not afford an analytical solution for the DBL.

1.8. *Dissolution of weak acids and bases*

The above expression is appropriate for the dissolution of a free acid or base, when ionization can be neglected. For dissolution of a species in which ionization occurs, the situation is more complicated as there is more than one component undergoing diffusion [2,13,15–17]. At the interface, HA (or B) will undergo ionization to form A^- (or BH^+), the distribution is determined by the pK_a and pH. If the assumption is initially made that the pH is uniform and the diffusion coefficients of the ionized and nonionized forms are the same, then the rate of dissolution should increase in proportion to the increase in the ionized concentration. However, due to the dissociation, the interfacial pH for the weak acid will be lower than the bulk. For the base, the interfacial pH will be higher as OH^- is formed with ionization of B to BH^+. The difference in pH between the interface and bulk will be a function of the relative transport rates of the drug away from the surface and the diffusion of H^+ and drug solubility. Moreover, despite the relatively rapid diffusion of protons in water, the pH difference can be significant.

Mooney et al. [13,15] performed the seminal work involving the dissolution of benzoic acid as a function of pH in unbuffered solutions. In excellent agreement with theoretical predictions, the surface pH was similar to the bulk pH at low pH values where little dissociation took place. However, as the bulk pH was raised through the pK_a, the surface pH was found to be much lower than the bulk, a result of the self-buffering capacity of benzoic acid. That is, benzoic acid dissolves in water and dissociates into the benzoate anion and a proton. The proton will diffuse away from the surface, but the time dependence of this transport step maintains the pH at the solid interface at a lower value than the bulk. Fig. 3A shows the surface pH as a function of bulk pH for four different weak acids that vary with respect to pK_a. This self-buffering affects the flux as shown in Fig. 3B, which reveals a similar plateau in the data indicating a lower than expected flux given the high bulk pH.

Studies were also conducted with naphthoic acid [15]. Here, there was also a buffering effect, but it was smaller. This can be understood by the lower solubility of naphthoic acid and hence the slower dissolution rate, which will result in a smaller rate of production of protons for maintaining a low pH at the surface. At much higher pH values, this effect was diminished, but the surface pH still remained lower than the bulk pH by more than six units. The effect of the intrinsic solubility on the surface pH is shown in Fig. 3C. The surface pH is seen to be lower as the solubility of the weak acid is increased, due to the greater release of protons. A more general equation was derived by McNamarra and Amidon [17], which followed the approach of Levich. This is more rigorous but also more cumbersome for calculations.

1.9. *Dissolution of salts*

In Fig. 4A and B, schematic diagrams are given for the dissolution of a salt of weak acid, MA, and a salt of weak base, BHX

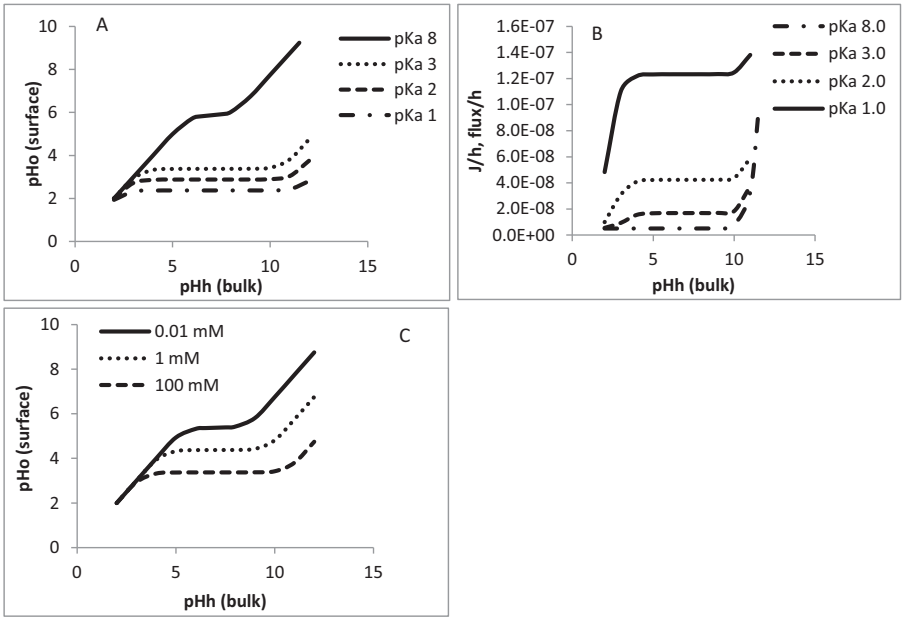

Fig. 3 – (A) Surface pH as a function of bulk pH for solutes with different pKa's, (B) flux arising from dissolution as a function of bulk pH for drugs with different pKa's, and (C) surface pH as a function of bulk pH for drugs with different intrinsic solubilities.

[10,19]. The acid–base reactions of the drugs at the surface are further complicated by the presence of the counter ions, M^+ and X^-. For the simple case, when the bulk pH is set to a value where the drugs are largely present in the ionized form, the dissolution rate is proportional to the concentration of the ionized form, A^- or BH^+, as given by the K_{sp}. For pH values that approach the pK_a, MA (or BHX) will dissolve and undergo dissociation into M^+ and A^- (or $BH^+ + X^-$), generally assumed to be complete. HA will form in the presence of H^+ in the solution (and BH^+ formed with water); the extent is determined by the pK_a and the pH at the interface. That is:

$$MA \rightleftharpoons M^+ + A^-; \quad K_{sp} = [M^+][A^-]$$

$$A^- + H_2O \rightleftharpoons HA + OH^-; \quad K_b = [HA][OH^-]/[A^-]$$

The equilibrium values, pH, log[HA], and log[A^-] are shown as a function of log(K_{sp}) in Fig. 5 for a weak acid with a pK_a of 7.0. It can be seen that as the K_{sp} increases from 10^{-14} to 10^{-4} or ten orders of magnitude, [A^-] increases five orders of magnitude as $[A^-] = K_{sp}^{1/2}$. The pH increases from just below 7 to near 9.5 in a similar manner as [HA], which increases from 10^{-7} to $10^{-4.5}$.

For dissolution of a salt at pH values where acid–base reactions will occur, there is simultaneous transport of both forms

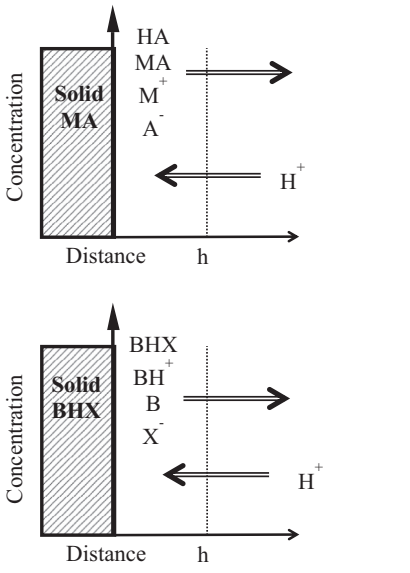

Fig. 4 – Schematic diagram of dissolution of a salt of a (A) weak acid and (B) weak base.

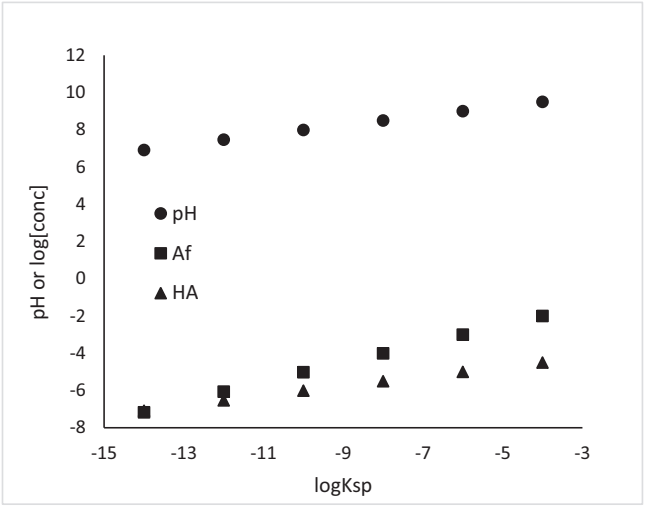

Fig. 5 – The equilibrium values, pH, log[HA], and log[A^-] given as a function of log(K_{sp}).

of the drug, HA and A^- (and B and BH^+) from the surface [10,16,19]. In addition, OH^- will diffuse into the bulk. However, in these cases, the drug counter ion, M^+ or X^-, is present, which too is transported away from the surface due to its high interfacial concentration. The presence of the counter ions affects the transport of H^+, and therefore the pH at the interface (microenvironment pH) as well.

At this point, it is of value to comment about the relative diffusion rates of H^+ and OH^-. Because of the extensive hydrogen bonding and rapid exchange of protons among water molecules, there is not typically physical movement of a proton, but rather a formation and associated cleavage of the O—H bond in the neighboring water molecules as shown in Fig. 6 [20]. This is known as the Grotthuss mechanism, where proton-hopping occurs among the main forms of solvated hydrogen ions of $H_9O_4^+$ (Eigen cation) and $H_5O_2^+$ (Zundel cation). It can also be appreciated that proton movement in a specified direction necessary involves OH^- movement in the opposite direction, and thus the equal diffusivities of H^+ and OH^- become unremarkable.

Returning to the consideration of the dissolution rate of a salt, the key experimental parameter in determining the rate is the interfacial pH as this determines the extent of dissolution. Rather than use a theoretical calculation of the interfacial pH, Serradajin et al. [10,19] developed an experimental approach. Excess salt was dispersed into water, and the pH of the solution was determined, which was argued to be representative of the interfacial pH. They found that the rate of dissolution correlated much better with the total concentration of solute using the measured pH in comparison to the bulk pH. In examining the equations, the initial concentration of the salt will be determined by the solubility product,

$$K_{sp} = [A^-][M^+] = [A^-]^2$$

However, A^- will react with water to form HA. This has two effects: one, the pH will be increased by the formation of OH^-, and two, additional MA will go into solution to raise the concentration such that the K_{sp} is satisfied. When this occurs, $[A^-] < [M^+]$, because A^- was consumed in the formation of HA. The governing equation becomes

$$K_{sp} = ([A^-] - [HA] + x) * ([M^+]_o + x)$$

where x represents the additional concentration of salt that will dissolve to satisfy the solubility product, and

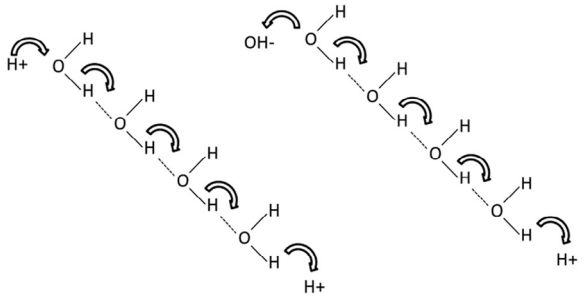

Fig. 6 – Grotthuss mechanism of hydrogen ion and hydroxide ion diffusion.

$$K_a = [H^+][A^-]/[HA]$$

Intuitively, as the pH difference between the interface and bulk becomes larger, there is a favorable effect on the extent of ionization. Thus, the total concentration is higher than expected based on bulk pH calculations, and thereby the dissolution rate is also higher.

The above analysis considered only conditions where the species remained in solution despite being unstable. In conducting dissolution experiments, metastable solutions can undergo conversion to the more stable state, characterized by precipitation [1,2,10,21,22]. This is analogous to the dissolution experiments conducted with a metastable polymorphic form or amorphous form of a nonionized drug. With the dissolution of salts, the precipitated solid may be composed of the nonionized form of the drug or the salt, depending on the pH. The location of the precipitation is also variable, because it can occur in the bulk solution or at the surface of the solid, again depending of the pH (dictating the degree of supersaturation) and nucleation tendency. There are a number of examples cited in the literature, which were explained according to appropriate theoretical considerations [2,22]. However, the complexity of the dissolution process in regard to the distant dependent pH coupled with the difficulty in predicting nucleation and crystal growth of solids makes *a priori* prediction of what will precipitate (free acid or base, or salt) and where it will precipitate (bulk or surface) intractable. In essence, experimentation is required to ascertain the outcome.

1.10. *Salt screening for conventional dosage forms*

The complexity of the dissolution of salts and its dependence on pH poses a dilemma for the drug development process but also creates opportunities. That is, for the dilemma, development of a new chemical entity into a marketable drug product requires extensive experimentation, even if just limited to salt selection [23–27]. The opportunity arises from the fact that the experimental parameter space is large; too large to be exhaustively examined in the initial commercialization. Thus, following marketing, it is not only possible but perhaps probable, that a new drug product can be developed, which comprises a different salt or polymorphic form and also has superior performance in terms of dissolution rate or chemical stability. In this section, the general approach to screening NCEs for salt selection is provided along with the factors to be considered and the methods of characterization.

For the development of dosage forms, the physical state of the active ingredient has a critical role in the observed performance [23,24,28–33]. The first consideration is the dosage form, for which there are many possibilities [4,6]. Thereafter, solubility, chemical stability, degree of crystallinity, and the mechanical properties are important factors. Limiting the discussion to drugs that must be in solution or solid state, the solubility is critical. For liquids, an adequate amount of drug must be in solution so that the entire dose is contained in a reasonable volume for convenient dosing, with preference often for a smaller volume. For drugs in the solid state, as present in tablets and capsules, the solubility is also important, because

this in turn has a profound effect on the dissolution rate, which can affect the rate and extent of absorption.

Another critical property is stability. Here, there is an obvious preference to prepare a very stable dosage form. Even though solubility may be the end goal, the significance of stability is such that it should be the first criterion used in selecting the form of the drug. Related to stability is the purity and uniformity of the material. For example, preference is given to those compounds that readily form highly crystalline solids and can be prepared easily and economically. In the development of solid dosage forms, the interaction of the drug with water, whether it is the hygroscopicity or deliquescence behavior, is of concern. Also specific for solid drugs are the mechanical properties. Although a listing of the important properties for tableting is beyond the scope of this overview, the reader can readily appreciate that the ease in consolidating and compressing drug particles with excipients into a functional tablet will often depend on the crystal structure of the drug.

The above properties are well-recognized, but presently there are no reliable methods to guide the formulation scientist in selecting or even preparing the form of the drug that will best meet the criteria. In fact, most often, no one drug form best meets all of the criteria. Thus, devoid of theoretical prediction, it remains a process of screening to identify the optimal form among competing interests. At the outset, there must be a clear goal for the screening process. The initial screen for an NCE is carried out often with little material available, and the goal is to determine the propensity for polymorphism. When more material becomes available, additional screening is carried out to identify the stable form. Thereafter, confirmation is needed that the selected form can be prepared with GMP material and then identify the best form for product development. Lastly, an extensive screen can be conducted in an attempt to identify all possible forms. The purpose here is to have expansive patent protection. Alternatively, screening may also be conducted with a drug that is in a marketed product. Here, the goal is to identify a form that has superior properties and possibly can be considered new intellectual property.

The screening process begins with selecting possible counterions to prepare the salts [23–25,30,32,33]. Table 1 was provided in the context that there is a benefit from the wide range of possibilities, but in the context of screening, it represents an extremely large experimental space. It is unrealistic to screen all possible counter ions in the preparation of salts, and fortunately, there are some broad guidelines to limit the selection. It goes without saying that a negatively charged counter ion is needed for a drug that is a weak base and positively charged counter is needed for a weak acid. The second guideline is that the drug must be completely ionized and in a single state of ionization in order to allow salt formation. With incomplete ionization, there is a risk of precipitating the nonionized form; and with multiple ionization states, there is a risk of forming a mixture of salts. The third limiting guideline is that the pK_a of the base and the acid should differ minimally by a factor of two, although there are exceptions, where the difference is less. It should also be noted that the pK_a difference must be maintained in the solvent system used for crystallization. The point here is that often non-aqueous or mixed solvent systems are used in the crystallization, which can dramatically affect the observed pK_a.

1.11. *Preparation methods of salts*

With identification of the counter ions that will be used, the next task is to select the methods for preparing salts [25–27,34–36]. There are four main methods used for salt preparation: thermal, anti-solvent, evaporation, and slurry conversion. In each of these methods, the outcome is dependent on several experimental variables, given below; however, all methods will be sensitive to the additive type and concentration, pH, and ionic strength. Moreover, the goal here is not to identify the process for salt preparation but to obtain seed crystals that can be subjected to preliminary evaluation.

The thermal method exploits the temperature dependence of the solubility, where a solution is prepared at a high temperature that is then cooled to cause precipitation. The outcome can depend on the heating rate, cooling rate, maximum temperature, and incubation temperature and time. The anti-solvent approach involves the addition of a solvent to a solution that induces precipitation, because of the lower solubility. Here, it is helpful to be aware of the dielectric constant of each of the solvents to limit the possibilities. In addition, the yield of precipitated material can depend on the anti-solvent and the rate at which it is added and the temperature and time of the addition. Evaporation relies on the selective removal of the solvent to increase the solution concentration to the point where the solubility is exceeded resulting in precipitation. The important processing parameters are the rate and time of evaporation, carrier gas, and the surface to volume ratio of the solution container. Finally, slurry conversion involves a solvent mediated polymorphic transition from a metastable solid form through a supersaturated solution and ultimately formation of a stable solid form. It is sensitive to mixing rate, impeller and crystallization vessel design, and the solvent.

Other less common methods include the use of capillary crystallization, binary melts, grinding, salt exchange, vapor diffusion or bubbling (volatile acids or bases; e.g. HCl, ammonia), ion exchange resins, varying pH as in a phase solubility study, and selective precipitation of unwanted counter ion (e.g. use of silver salts, where the unwanted silver is removed as silver iodide) [24,35]. The traditional approach of preparing individual samples has given way to high throughput screens that are conducted in 96-well plates [26]. This allows preparation of a large number of samples, but tends to be limited to solvent based methods with fewer conditions allowed for crystallization.

1.12. *Characterization methods for salts*

With material in hand, the next step is to characterize the samples [4,6,24,28,30,32]. X-ray powder diffraction is the definitive tool for characterizing crystalline samples. The pattern observed is unique to the polymorphic form and allows verification that the material is crystalline, as amorphous samples will yield a featureless halo. The appearance of the peaks also provides information on the uniformity, where broadened peaks indicate a lack of crystallinity, provided the particle size is sufficiently large. Complementary spectroscopic methods are infrared (IR), Raman, and nuclear magnetic resonance (NMR). IR and Raman spectroscopy are most readily available and can be used to assess interactions in the solid state, particularly

between the parent compound and the counter ion. NMR spectroscopy, particularly solid state, is much more cumbersome to use and typically requires >10–50 mg for a sample. It can be quantitative, but usually requires an experienced individual to avoid pitfalls.

Thermal methods, including differential scanning calorimetry (DSC), thermogravimetry (TG), and hot stage microscopy, are readily available, easy to perform, and do not require a large sample size. DSC can be used to determine the melting point and enthalpy of fusion. Neither are specifically required properties, but they are extremely helpful in predicting solubility and stability. The sharpness of the melting endotherm also gives an indication of the purity and crystallinity of the sample. Complex thermograms reveal polymorphism, which can be indispensable in the search for the most stable form. Finally, hydrates and solvates can generally be distinguished from anhydrous forms due to the nature of the endotherm associated with their loss of volatile components from the sample. TG provides a measure of the weight change either at a constant temperature or as a function of the changing temperature. As such, it can provide information as to the amount of adsorbed moisture for hygroscopic samples as well as the stoichiometry of hydrates/solvates. Hot stage microscopy provides visual evidence to confirm the nature of the thermal changes, whether it be melting, phase change, or decomposition. Other routine characterization based on microscopy would be visual characterization of the particle size and morphology.

1.13. *Inhalation formulation*

Our objective was to develop an inhalation formulation of a drug from several possible candidates to evaluate the safety and efficacy in a rat animal model [36]. At this early stage, it is desirable to administer pure drug as an aerosol without extensive formulation development to establish the feasibility of inhalation drug delivery. Our approach was to prepare a solution of drug that may be atomized with a nebulizer, after which the solvent is removed from the aerosol. It then comprises pure drug in air with an appropriate particle size distribution for deposition in the rat lung [37]. The advantage is that the atomization and drying processes are largely independent of the properties of the drug, and thus with the initial set up, several compounds can be sequentially tested to identify the optimal in terms of safety and efficacy [38]. Atomization of liquids readily conforms to a continuous process, which allows the dose (inhaled and deposited mass) to be adjusted by the exposure time. In addition, multiple animals may be simultaneously exposed.

The compounds for evaluation were all weak bases with relatively low, but quantitatively unknown, pK_a's. The nonionized forms had very low aqueous solubilities but had good solubility in DMSO; however, removal of DMSO from aerosol droplets is very difficult, due to its high boiling point/low vapor pressure. The solubility in ethanol was inadequate to generate a concentrated aerosol of drug. Thus, the approach was to ionize these compounds with acid such that they would have sufficient solubility in water for nebulization. It was discovered during the preliminary studies that the pH of the solution would need to be very low. This in turn raised a concern of toxicity should such acidic particles deposit in the lung lining. As a solution, a novel *in situ* neutralization of the aerosol was used where aerosol droplets were combined with ammonium vapor. Following neutralization, the particles were dried. With this approach, successful administration of the compounds by inhalation was achieved.

1.14. In situ *salt formation in aerosols*

For salt preparation in a flowing aerosol, there are two equilibria in operation. One is the equilibrium distribution of ammonia (NH_3) between the vapor and liquid, and the other is the acid/base equilibrium. For the first, the expression is:

$$NH_3\,(\text{vapor}) \rightleftharpoons NH_3\,(\text{solution})$$

Experimentally, air is initially saturated with ammonia as it is passed through a bubbler containing ammonium hydroxide. The vapor concentration may be estimated from reported values in the literature. It is important to note that the vapor pressure is a strong function of temperature, so thermal control of the bubbler is essential.

In the next step, the air with a given concentration of ammonia is combined with the aerosol. In this step, ammonia vapor will condense into the liquid droplets in essentially a reverse process of the bubbler. However, the acid–base chemistry within the droplet must be considered. Thus,

$$NH_3 + H_2O \rightleftharpoons NH_4OH + H^+$$

and in the presence of the divalent conjugate acid, BH_2^{2+}

$$BH_2^{2+} + 2X^- + 2NH_3 \rightleftharpoons B + 2NH_4^+ + 2X^-$$

where the ammonia is converted to the quaternary ammonium ion by protonation from the acid. With an increase in pH, the drug is neutralized and may lead to precipitation in the droplet.

$$BH_2^{2+} + 2X^- + 2NH_3 \rightleftharpoons 2NH_4^+ + 2X^- + B(\text{ppt})$$

Alternatively, it may remain kinetically stable in the drop and precipitate on drying as the salt.

$$BH_2^{2+} + 2X^- + 2NH_3 \rightleftharpoons [BH_2X_2](\text{ppt}) + NH_3$$

2. Materials and methods

2.1. *Materials*

Compounds I–VI were obtained from the Principal Investigator per contract through an SBIR grant from NIH. The acids used, hydrochloric acid, sulfuric acid, mesylic acid, tosylic acid, and phosphoric acid, and ammonium hydroxide were analytical/reagent grade.

2.2. *Solubility measurements*

The solubility of the non-ionized form of compounds I–VI was determined as follows. For I and II, which were received as HCl

salts, the material was dissolved in 0.1 M HCl and titrated with additional concentrated HCl until a solution was obtained. A volume of sodium hydroxide (ca 24 mM) was added such that there was an excess of hydroxide ions relative to the estimated hydrogen ions in the sample. A precipitate (free base) was formed that was washed in a basic solution by alternating between centrifugation and resuspension. A final rinse of pH 8 water was used to wash the pellet. The wet pellet was transferred to 1.5 ml plastic centrifuge tubes and dried. For compounds III–V approximately 10 mg was accurately weighed and placed into centrifuge tubes with water. The tubes were stored at room temperature (22 °C) for a minimum of 3 days with periodic mixing.

After centrifugation, an aliquot was taken, typically 10–20 µl, and placed into a 1 ml auto sample vial for HPLC. It was diluted with mobile phase (80/20 methanol/water) and then run on the HPLC. The HPLC system included a Shimadzu LC-10AT solvent delivery module, SIL-10AD auto injector, RF-10A XL UV/Vis detector, C-R5A Chromatopac integrator (Shimadzu Corp., Tokyo, Japan) and a C18 Agilent column (250 mm × 4 mm, 5 µm) (Agilent Technologies, Inc. Santa Clara, California). The mobile phase consisted of 80/20 methanol/water. The UV absorption was measured at 280 nm. If the resulting peak height fell outside the standard curve, the solution was further diluted with mobile phase to yield an appropriate concentration for assay.

HPLC standards were prepared by accurately weighing approximately 10 mg of the compounds and placing into 25 ml volumetric flasks. The compound was dissolved in 0.1 M HCl or DMSO (compounds III and IV). Standard curves were prepared by plotting the observed peak height as a function of concentration in the standard. Peak areas were also examined but found to be less reproducible.

The amount of compound in solution in the presence of various acids was determined as follows. Concentrated acids were diluted with water to yield an approximate concentration of 0.1 M assuming the stock concentration was 98% for H_2SO_4, 37% for HCl, and the remaining acids were assumed to be 100%. To centrifuge tubes, 200 µl was added, the dispersion was vortexed. If a solution was obtained, additional powder was added. The samples were stored in the refrigerator for 1 day and then at room temperature for several hours. The samples were periodically vortexed and then vortexed immediately before centrifuging. If the volume of the supernatant was insufficient for taking an aliquot or measuring the pH, more acid was added. In these cases, the sample was allowed to equilibrate again. The pH was measured using a micro electrode (Cole-Parmer, EW-55500-40, 3 mm × 38 mm BNC) that was carefully inserted into the supernatant of the centrifuge tube. The meter was unable to measure below a pH of zero, and these are indicated as such.

2.3. *Aerosolization, neutralization, and drying*

In preparing the solution for the aerosol experiment, 51 mg of II was weighed and 0.1 M HCl was added to a final weight of 1.995 g. To yield a solution, 0.234 g of concentrated HCl was added. In this way, a 22.9 mg/g solution of compound II was obtained. No evidence of decomposition was evident neither in this solution nor in assaying the compound on the filters and cascade impactor.

The solution was placed into a PARI nebulizer operated at a pressure of 12.5 psi, which was the critical pressure for the maximum flow rate of 1.7 l/min. Air was also directed into a flask with a bubbler containing 0.3% ammonium hydroxide solution, and the outflow rate was adjusted to 0.3 l/min. These flows were combined to neutralize the particles, which were then directed into a drying column. The 3″ (id) Plexiglas column had a stainless steel mesh fashioned into an annular ring, which contained silica beads, and an open diameter of 2″ and a 24″ length. At the exit of the column, measurements were made of the output rate and particle size distribution. For the inhalation study in rats, the column was connected to a custom built, nose-only exposure system [36].

Aerosol exiting the column was collected for a fixed period of time on filter paper, which was extracted with 0.1 M HCl. The extract was then assayed by HPLC, and the output rate is given as mass/time. The aerosol concentration was calculated by dividing the output rate by the total airflow rate. The particle size distribution of the aerosol was determined by a low flow rate cascade impactor (Intox) operating at 0.5 l/min. Aerosol particles were also collected with Anderson cascade impactor (filter paper collection affected the pH measurement) and then dissolved in distilled water, and the pH of the solution was measured.

3. Results and discussion

3.1. *Solubility measurements*

The results from the measurement of the solubility (mg/ml) of the free base and the total amount in solution in 0.1 M sulfuric, mesylic, tosylic, and phosphoric acids are given in Table 2 for the six structurally related compounds. The solubilities of the nonionized parent bases were very low and in the microgram per milliliter range. With the addition of the different acids, the total amount of base in solution increased. Sulfuric acid was most effective for increasing the concentration of compound I and III, and phosphoric acid was most effective for compounds II, IV, V and VI. However, the effect was relatively modest for III, IV and VI.

Based on the results obtained for the 0.1 M acids, the solubility was measured as a function of acid concentration using the acid that yielded the highest concentration. The observed total concentration was plotted as a function of pH (Fig. 7). The solid lines represent simulations, which were calculated for the two ionizable groups using the

Table 2 – Concentration (mg/ml) in solution in the presence of the indicated acid at a concentration of 0.1 M for structurally related compounds, I, II, III, IV, V, and VI.

	I	II	III	IV	V	VI
Base	0.0013	0.03	0.01	0.0025	0.04	0.08
H_2SO_4	26.	4.5	0.34	0.49	0.46	0.54
Mesylic acid	6.9	5.3	0.05	0.04	0.20	0.1o6
Tosylic acid	18	2.0	0.09	0.02	43	0.11
H_3PO_4	2.0	11.	0.30	3.0	240	1.2

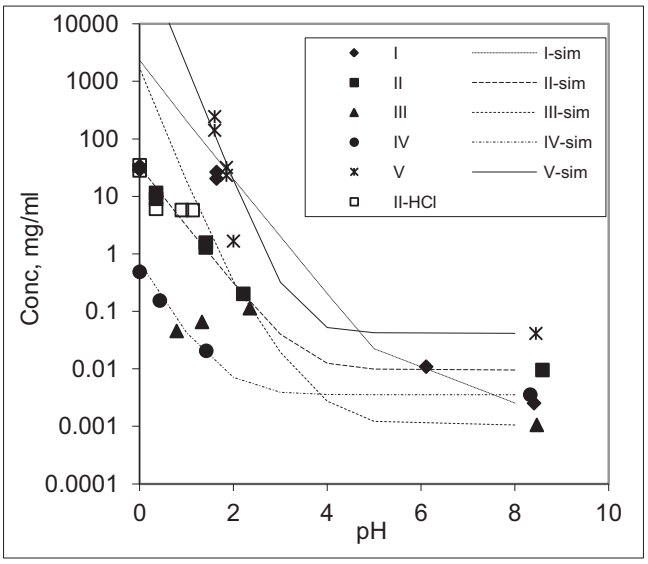

Fig. 7 – Concentration of compound (I and IV in sulfuric acid; II and V in phosphoric acid, and III in mesylic acid) in solution as a function of pH, and the solid lines representing simulated concentrations based on the observed nonionized concentration and the Henderson–Hasselbalch relationship for the dibasic compounds.

Henderson–Hasselbalch expression. The concentration of the nonionized free base was taken from Table 2, and it was assumed that the charged form had an infinite solubility.

Theoretically, the logarithm of the amount of compound in solution should increase with decreasing pH near the pKa. There are two evident linear portions in most cases reflecting the presence of two ionizable groups. In this simulation, the acid dissociation constants were varied to obtain a good fit based on the visual appearance. For compound II, the data were well fit. Those compounds that had lower solubility at low pH than the simulated values are expected to be limited by solubility product. Compound V is unusual in that the amount in solution was much higher than theoretically expected. It is speculated that self-association may be occurring, which was not considered in the simulations.

When it became apparent that phosphoric acid could not be neutralized by ammonium vapor, additional studies of the solubility as function of acid were carried out. In essence, the pKa of phosphoric acid is not separated from the pK_a of the drugs by the required two units and thus the captured aerosol particles remained at a pH of 2.5.

3.2. *Aerosol properties*

In preliminary neutralization studies, the observed drug output rate with filter collection was 2.13 ± 0.94 mg/min. The total output rate of II determined gravimetrically from the change in mass of the nebulizer was 3.0 mg/min, yielding a column efficiency of 72%. The mass fraction as a function of cut point of the cascade impactor is given in Fig. 8. The mass median aerodynamic diameter was 1.8 μm and the geometric standard deviation was 1.7.

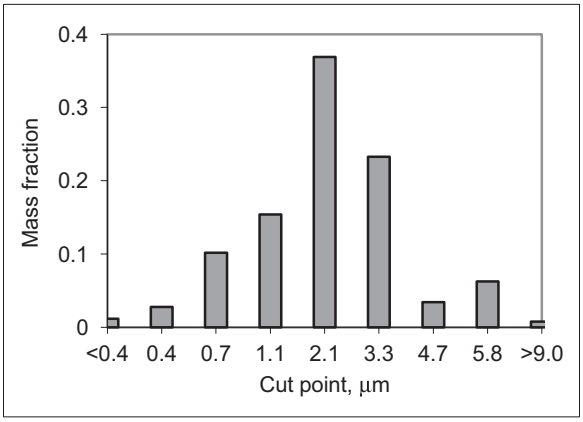

Fig. 8 – Mass fraction as a function of cut point of the cascade impactor.

From the measured pH of the nebulization solution and output rate of solution (SOPRaer in ml/min), the moles of acid in unit time in the flowing aerosol were determined. This dictates the moles of ammonia that are required in unit time for neutralization, which are generated from bubbling air through a solution of ammonium hydroxide. That is:

$$[H^+]_{aer} * SOPR_{aer} = [NH_4OH]_s * k_H * Q_b$$

where $[H^+]_{aer}$ is the acid concentration in the aerosol droplets, $[NH_4OH]_s$ is the ammonia concentration in the bubbler, k_H is Henry's law constant expressed in appropriate units (moles/liter in air/molar concentration of ammonium), and Q_b is the air flow rate through the bubbler. Data from the literature of ammonia pressure as a function of weight percent concentration of $[NH_4OH]_s$ in solution were plotted and fit to a quadratic equation:

$$NH_3\ (\text{in mole of vapor/Liter of air}) = 2.0\times10^{-5}\,[NH_4OH]_s^{\,2} + 2.3\times10^{4}\,[NH_4OH]_s$$

With a solution pH of 2 and a liquid output rate of 150 μl/min, 3×10^{-6} mol/min of acid required neutralization. Using a concentrated ammonium hydroxide solution diluted with water at 0.003 g/g, the vapor pressure of ammonia was calculated to be 0.1 kPa. This is combined with the aerosol droplets at a flow rate of 0.3 l/min, yielding an ammonia production rate of 1.2×10^{-5} mol/min, which is adequate to neutralize the aerosol droplets. As the measured values of reconstituted aerosol particles were between pH 7 and 8, this suggests that liquid–vapor transfer of ammonia was rapid.

With the output rate and total air flow rate, the aerosol concentration was calculated to be 96.1 μg/L [36]. The respiratory minute volume (RMV) was calculated to be 0.15 l/min based on animal weight, which leads to a total inhaled dose of 115 μg for the 8 min exposure. From quantifying the deposited mass in the rat lung at the end of the exposure, an average of 6.9 μg was found, which corresponds to a 6% deposition of the inhaled dose. This is in reasonable agreement, albeit on the low side, of other inhalation studies in rats using a comparable aerosol particle size.

Along with determining the mass of compound deposited in the lung at the end of the exposure, a pharmacokinetic study was completed [36]. The lung concentration as a function of time was fit to a single exponential expression; $C = 2.50 * \exp(-0.03t)$, where the concentration was in μg/g and the rate constant is given in units of min^{-1}. The corresponding half-life is only 23 min, thus the estimated deposited mass in the lung has been underestimated as there will be a non-negligible amount of the compound eliminated from the lung during the 8 min exposure. Results from assessment of the safety and efficacy have been reported elsewhere [36].

4. Conclusion

The pH dependence of the amount of six structurally related compounds was found to be consistent with theoretical expectations of weak bases. The pH required to achieve the needed concentration for aerosol generation was low, but ammonia vapor, introduced into the air stream, effectively neutralized the aerosol droplets. With solvent removal, the resulting aerosol was suitable for evaluating the safety and efficacy of inhalation administration in rats.

Acknowledgements

The research was supported by the National Institutes of Health grant, 1R43HL102946-01.

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