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Evaluation of solvent effects on protonation using NMR spectroscopy: Implication in salt formation

Hyungchul Kim[a,1], Jinhai Gao[b], Diane J. Burgess[a,*]

[a] Department of Pharmaceutical Sciences, School of Pharmacy, University of Connecticut, Storrs, CT 06269, USA
[b] Analytical Science, Novartis Institute for BioMedical Research, Inc., Cambridge, MA 02139, USA

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ABSTRACT

Investigation of the use of solution NMR spectroscopy to determine the effect of organic solvents on chemical shift changes in bases on addition of acids is reported. This information can be useful in the evaluation of solvents and counterion selection for salt formation. ^{1}H and ^{15}N chemical shift changes in three bases (pyrazine, phthalazine, and pyridine) on the addition of acids (1:1 ratio) were determined in various solvents. The effect of acid strength on chemical shift changes was examined. ^{1}H and ^{15}N chemical shift changes indicated protonation (salt formation). The media used affected the observed chemical shift changes. In D_2O the data followed the ΔpK_a (base–acid) general rule, that the pK_a value of the acids should be 2 units lower than the pK_a of the base to ensure proton transfer. Protonation, as measured by chemical shift changes using solution NMR spectroscopy, provided novel insight on potential salt formation in different media. Solution NMR spectroscopy appears to be a useful tool to evaluate counter ion and solvent selection for salt formation reaction.

1. Introduction

The physicochemical properties of drug candidates often dictate their successful development into pharmaceutical products (Wenlock et al., 2003). For example, many drug candidates fail due to low solubility and/or low stability. Salt formation is routinely employed in modern drug discovery to overcome such failure by changing the physicochemical properties of drug candidates without modifying their chemical structures. There have been numerous literature reports concerning the selection process to achieve an optimal salt form for new drug candidates (Gould, 1986; Morris et al., 1994; Bastin et al., 2000) and impact on solubility (Serajuddin and Pudipeddi, 2002), dissolution rate and bioavailability (Berge et al., 1977; Engel et al., 2000). A common problem during salt screening is that glassy material may form following solvent evaporation. This may result from any of the following: high solubility of the salt form in the solvent system; insufficient time for nucleation and crystal growth; and/or insufficient proton transfer from acid to base in the solvent system.

A general rule for appropriate counter ion selection for salt formation for a weak base is that the pK_a value of the acid should be 2 units lower than that of the base to ensure proton transfer (Bastin et al., 2000; Serajuddin and Pudipeddi, 2002; Black et al., 2007). The difference between the pK_a of the base and the pK_a of the acid is known as ΔpK_a. Although the ΔpK_a is a useful guideline for initial counter ion selection, it is important to remember that pK_a values can shift with the solvent system due to differences in dielectric constant and proton donor or acceptor properties. Most pK_a values reported in the literature are based on the aqueous phase, whereas organic solvents are usually employed in salt formation. Bordwell reported differences in the pK_a values of various compounds in water and DMSO (Bordwell, 1988). Even though changes in the pK_a values of acids and bases in organic solvents are well known, no guidelines have been reported regarding selection of solvent systems for pharmaceutical salt formation based on protonation between acids and bases. The concept of pK_a change in different media has been explained using speciation diagrams for ephedrine and acetic acid (Black et al., 2007). The pK_a of ephedrine and acetic acid in water are 9.74 and 4.76, respectively, whereas, in methanol they are 8.74 and 9.71, respectively. Thus the ΔpK_a values of ephedrine and acetic acid are 4.98 and −0.97 in water and methanol, respectively. Accordingly, there is no pH range to ensure sufficient proton transfer from acid to base in methanol, suggesting that salt formation between ephedrine and acetic acid is unlikely to occur in this solvent.

The pK_a in non-aqueous solutions can be explained by autoprotolysis. Autoprotolysis can be defined as the proton transfer reaction between two identical molecules, one acting as a Brønsted acid and

* Corresponding author. Tel.: +1 860 486 3760; fax: +1 860 486 0538.
E-mail address: d.burgess@uconn.edu (D.J. Burgess).
[1] Current address: Early Development, AstraZeneca Pharmaceuticals LP., 1800 Concord Pike, Wilmington, DE 19850, USA.

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Table 1
Autoprotolysis constants of some amphiprotic solvents at 25 °C (Reichardt, 2003).

Sovents	Lyonium ion	Lyate ion	pK_{auto}
Water	H_3O^+	HO^-	14.00
Acetic acid	$CH_3C(OH)_2^+$	$CH_3CO_2^-$	14.45
Methanol	$H_3C—OH_2^+$	$H_3C—O^-$	17.20
Ethanol	$H_5C_2—OH_2^+$	$H_5C_2—O^-$	18.88
1-Propanol	$H_7C_3—OH_2^+$	$H_7C_3—O^-$	19.43
2-Propanol	$(H_3C)_2C—OH_2^+$	$(H_3C)_2C—O^-$	20.80
Ethyl acetate	$H_3C—C(OH)=O^+C_2H_5$	$H_2C=C(O^-)—OC_2H_5$	22.83
N,N-dimethylacetamide	$H_3C—C(OH)=N(CH_3)_2$	$H_2C=C(O^-)—N(CH_3)_2$	23.95
2-Methyl-2-propanol	$(H_3C)_3C—OH_2^+$	$(H_3C)_3C—O^-$	26.8
Acetone	$H_3C—C(OH^+)—CH_3$	$H_2C=C(O^-)—CH_3$	32.5
Dimethyl sulfoxide	$H_3C—S^+(OH)—CH_3$	$H_2C=S(O^-)—CH_3$	33.3
Acetonitrile	$H_3C—C\equiv NH^+$	$H_2C=C=N^-$	≥33.3

the other as a Brønsted base. The autoprotolysis constant of water (=autoionization constant = ion product constant of water) can be described as below and the pH is defined as $pH = -\log a(H_3O^+)$.

$$H_2O + H_2O \leftrightarrow H_3O^+ + OH^- \rightarrow K_w = a(H_3O^+)a(OH^-) \approx [H_3O^+][OH^-]$$

Even though the value of $-\log K_w$ (pK_w) changes with temperature, it is reasonable to assume that the pH window of water is approximately between 0 and 14 and the width is ~14. In an amphiprotic solvent SH, autoprotolysis occurs as shown below and the autoprotolysis constant is constant at a given temperature (Izutsu, 2002; Reichardt, 2003).

$$SH + SH \leftrightarrow SH_2^+ + S^- \rightarrow K_{auto} = [SH_2^+][S^-]$$

For example, the autoprotolysis of methanol can be expressed as below.

$$H_3COH + H_3COH \leftrightarrow H_3C-OH_2^+ + H_3C-O^- \rightarrow K_{auto} = [H_3C-OH_2^+][H_3C-O^-]$$

The pH concept in water can be applied to define the pH in non-aqueous solutions as $pH = -\log a(SH_2^+)$. The width of pH of non-aqueous solutions corresponds to pK_{auto} which is shown in Table 1.

Since pH scales differ depending on the medium, (autoprotolysis) ion activity is different in different media, pK_a of electrolytes in non-aqueous media will be different compared to that in water. Aqueous pK_a is important to understand the pH-solubility behavior in water and can be used in initial counter-ion selection for salt screening. However, salt formation (acid–base) reaction usually is performed in non-aqueous solutions, and so the pK_a in non-aqueous solutions will play an important role in the reaction. The pK_a in non-aqueous solutions can be defined as below.

$$HA + SH \overset{K_a}{\longleftrightarrow} SH_2^+ + A^-,\ K_a = \frac{[SH_2^+][A^-]}{[HA]},\ pK_a = -\log K_a$$

As can be seen, the only difference is the lyonium ion rather than the hydronium ion in the equation. Currently, hundreds of pK_a values of electrolytes are available for non-aqueous solutions in the literature, but not many for pharmaceutically used acids and bases (Chantooni and Kolthoff, 1979; Barrette et al., 1984; Barbosa et al., 1988; Bosch and Rosés, 1989; Izutsu, 1990; Maran et al., 1991; Kim et al., 2001; Izutsu, 2003).

To effectively use the ΔpK_a rule, knowledge of the pK_a values in different solvent systems is necessary. Interestingly, not many pK_a values in organic solvents for frequently used pharmaceutical salts have been reported in the literature even though there have been many reports regarding pK_a values in different organic solvents. The lack of knowledge of pK_a values of drug candidates and counterions

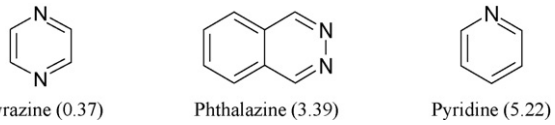

Fig. 1. The chemical structure of the bases and their aqueous pK_a values.

in different solvent systems results in considerable time and effort being spent in a trial and error fashion in the salt selection process. Therefore, it would be very useful to have a simple experimental method to predict salt formation (protonation) in different solvent systems.

The objective of this study is to evaluate the effect of the solvent on proton transfer from acid to base using solution NMR spectroscopy. The effect of counter ions on protonation of bases was investigated using three model compounds, pyrazine, phthalazine and pyridine, which have a broad range of pK_a values, 0.37, 3.39, and 5.14, respectively. These model compounds were selected since they cover a range of basicity. D_2O, DMSO-d6, MeOD-d4 and acetone-d6 were used as solvent systems to compare the proton (^1H) and nitrogen (^{15}N) chemical shift changes.

2. Materials and methods

2.1. Materials

Pyrazine, phthalazine and pyridine were purchased from ACROS ORGANICS (New Jersey, USA). Fumaric acid was purchased from Fisher Scientific (Fair Lawn, NJ, USA). Orotic acid was purchased from Alfa Aesar (Ward Hill, MA, USA). The other organic acids were purchased from ACROS ORGANICS. Deuterated NMR solvents (DMSO-d6, MeOD-d4, D_2O and Aceton-d6) were purchased from Aldrich (Milwaukee, WI, USA). All the materials were used as supplied.

2.2. NMR spectroscopy

NMR data were acquired using a Bruker AVANCE 600 NMR spectrometer equipped with a 5 mm TCI CryoProbe probe with z-gradient operating at a frequency of 600.13 MHz for ^1H. Chemical shifts for ^1H spectra were referenced by setting the internal TMS (tetramethylsilane) to 0 ppm. The ^{15}N chemical shifts were extracted via a heteronuclear multiple bond correlation (HMBC) experiment and the shifts reported were indirectly referenced to liquid NH_3 using the resonance of pyridine in DMSO-d6 at 317 ppm.

2D ^1H-^{15}N HMBC spectra were acquired in the non-phase-sensitive mode without low-J filter and decoupling of ^{15}N during acquisition (Bax and Summers, 1986). The HMBC experiments were optimized for long-range coupling constants of 7 Hz. The spectra were recorded with 32 increments in F1 and 32 scans per increment. The sweep widths in F2 and F1 were 7246 Hz and 12164 Hz, respectively. The data were processed using linear prediction and zero filling in the F1 domain and sine-bell apodization of factor 0 in both dimensions with a final matrix of 2 k × 2 k.

2.3. Preparation of samples for ^1H and ^{15}N NMR spectroscopy

The chemical structure and aqueous pK_a values of the three bases are shown in Fig. 1. Physical mixtures of pyrazine, phthalazine and pyridine with various acids are summarized in Table 2. All the physical mixtures were prepared by mixing 0.2 M of base and acid (1:1 ratio) in the different media.

Table 2
Physical mixtures of free bases and acids for protonation studies using NMR spectroscopy.

Pyrazine, phthalazine, and pyridine		Pyridine	
D_2O	DMSO-d6	Acetone D6	Methanol D4
HCl	HCl	Sulfuric acid	Sulfuric acid
Methanesulfonic acid	Methanesulfonic acid	Methanesulfonic acid	Methanesulfonic acid
Oxalic acid	Benzenesulfonic acid	Oxalic acid	Oxalic acid
Maleic acid	Oxalic acid	Maleic acid	Maleic acid
Ethanesulfonic acid	Maleic acid	Ethanesulfonic acid	Ethanesulfonic acid
Malonic acid	Ethanesulfonic acid	Malonic acid	Malonic acid
Glycolic acid	Malonic acid	Glycolic acid	Glycolic acid
Lactic acid	Glycolic acid	Malic acid	Malic acid
Succinic acid	Lactic acid	Benzoic acid	Benzoic acid
Acetic acid	Benzoic acid	Acetic acid	Acetic acid
	Succinic acid		
	Acetic acid		
	Orotic acid		

Note: Pyridine + sulfuric acid and pyridine + oxalic acid in acetone showed immediate precipitation following mixing, probably due to salt formation. Pyridine + methanesulfonic acid and pyridine + ethanesulfonic acid in acetone showed immediate color change to brown following mixing. Therefore, these four samples were not run on the NMR.

3. Results and discussion

3.1. pK_a values of acids and bases

Table 3 shows the available literature pK_a values for various acids and bases in different media. All the pK_a values in water are cited in the Handbook of Pharmaceutical Salts (2002). The pK_a values in non-aqueous solvents have been collected from multiple articles (Bos and van der Linden, 1996; Rived et al., 1998; Barbosa et al., 1999; Sarmini and Kenndler, 1999; Serajuddin and Pudipeddi, 2002). As can be seen, the pK_a values of both the acids and bases change with the different media and are very solvent specific. For example, the pK_a value of pyridine did not change considerably between water and methanol (5.22 and 5.44, respectively). However, the pyridine pK_a value was considerably different in acetonitrile (12.3) and dimethyl sulfoxide (3.4). On the other hand, the pK_a values of the acids cited were considerably different in methanol compared to water. For example, the pK_a values of malonic acid in water are 2.83 and 5.7 and in methanol they are 7.66 and 10.64. Table 4 lists various physicochemical properties of the solvents listed in Table 3. On comparing Tables 3 and 4 it is apparent that changes in the pK_a values of these acids and bases in different media do not correlate with their solvent properties (*i.e.* dielectric constant, relative polarity, hydrogen bond donor or acceptor propensity). For example, it is known that the dielectric constant of organic solvents may suppress the ionization of acids and bases (Kolthoff, 1974). However, for the acids and bases compared in Table 3, there is no correlation between their dielectric constants and the changes in their pK_a values in the various organic solvents. For example, acetonitrile and dimethyl sulfoxide have higher dielec-

Table 3
Acids and their available literature pK_a values in water and various organic solvents.

	pK_{a1}[a] H_2O	pK_{a2}[a] H_2O	pK_{a1}[b,c] MeOH	pK_{a2}[b,c] MeOH	pK_{a1}[d] ACN	pK_{a2}[d] ACN	pK_{a1}[d] DMSO	pK_{a2}[d] DMSO
Acids								
HCl	−6.1				8.9		2.1	
Sulfuric acid	−3	1.92			7.8	25.9	1.4	14.7
Ethanesulfonic acid	−2.05							
Toluenesulfonic acid	−1.34				8.7		0.9	
Methanesulfonic acid	−1.2				10.0		1.6	
Benzenesulfonic acid	0.7							
Oxalic acid	1.27	4.27	6.1[e]	10.7[e]	14.5	27.7	6.2	14.9
Maleic acid	1.92	6.23						
Malonic acid	2.83	5.7	7.66	10.64	15.3	30.5	7.2	18.6
Tartaric acid	3.02	4.36	8.12					
Fumaric acid	3.03	4.38		9.78				
Music acid	3.08	3.63						
Citric acid	3.128	4.761						
Glycolic acid	3.28							
Malic acid	3.459	5.097						
Lactic acid	3.86							
Benzoic acid	4.19		9.3		20.7		11.0	
Succinic acid	4.21	5.64	9.14	11.3				
Acetic acid	4.76		9.63		22.3		12.6	
Orotic acid	5.85	8.95						
Bases								
Pyrazine	0.37							
Phthalazine	3.39							
Pyridine	5.22		5.44		12.3		3.4	

Note abbreviations: Methanol (MeOH), acetonitrile (ACN), dimethyl sulfoxide (DMSO).
[a] Handbook of Pharmaceutical Salts (2002).
[b] Rived et al. (1998).
[c] Sarmini and Kenndler (1999).
[d] Izutsu (2002).

Table 4
Comparison of physicochemical properties of various solvents.

Name	Molecular Weight	Dielectric constant[a]	Relative polarity[b]	$\sum\alpha$[a]	$\sum\beta$[a]
Water	18.02	78.36	1	1.17	0.47
Acetonitrile	41.05	35.69	0.75	0.07	0.32
1,2-Dichloroethane	98.96	10.13	0.81	0.1	0.11
Dimethyl sulfoxide	78.13	46.83	1.00	0	0.88
Methyl alcohol	32.04	32.61	0.6	0.43	0.47
Isopropyl alcohol	60.10	19.26	0.48	0.33	0.56
t-butyl alcohol	74.12	12.5[c]			
Pyridine	79.10	12.98	0.87	0	0.52

$\sum\boldsymbol{\alpha}$: Summation of the hydrogen bond donor propensities of the solvent.
$\sum\boldsymbol{\beta}$: Summation of the hydrogen bond acceptor propensities of the solvent.
[a] Gu et al. (2004).
[b] Marcus (1993).
[c] Myers.

tric constants compared to methanol, but the ionic suppression of acetic acid and benzoic acid in these two solvents was higher than that in methanol. Although the pK_a values do not correlate with specific solvent properties, there is a general trend that relative pK_a values are maintained within a specific solvent (*i.e.* stronger acids have lower pK_a values and weaker acids have higher pK_a values).

3.2. NMR spectroscopy

Fig. 2 shows the ^{1}H–^{15}N HMBC spectra of pyrazine, phthalazine and pyridine. For each base, the ^{1}H–^{15}N HMBC cross peaks between the protonated nitrogen and the neighboring proton of the free base and the mixtures of base and various acids were overlaid to demonstrate the chemical shift changes. In the ^{1}H–^{15}N HMBC spectra, the ^{1}H and ^{15}N chemical shifts can be extracted from the x and y axes, respectively. The ^{1}H and ^{15}N chemical shift of the three bases were used to calculate chemical shift changes on the addition of acids. The delta ^{1}H and ^{15}N chemical shift is defined as the ^{1}H and ^{15}N chemical shift of base on the addition of acid, subtracted by ^{1}H and ^{15}N chemical shift of the base. The delta ^{1}H and ^{15}N chemical shifts are summarized in Table 5.

The addition of acids with different pK_a values caused ^{1}H chemical shift changes in the NMR spectra of the bases in the vicinity of the nitrogen, as well as ^{15}N chemical shifts of the nitrogen. These chemical shift changes indicate protonation, *i.e.* salt formation reaction has occurred. Since significant changes in proton NMR occurred only in the vicinity of the nitrogen, this was used to monitor and

Table 5
Delta ^{1}H and ^{15}N chemical shifts in ppm of bases on addition of acids (ratio 1:1).

Acids	NMR Solvent	Pyrazine		Pthalazine		Pyridine	
		^{15}N	^{1}H	^{15}N	^{1}H	^{15}N	^{1}H
HCl	D_2O	11	0.19	67.9	0.93	96.3	0.3
Methanesulfonic acid	D_2O	11.2	0.19	64.6	0.91	95.6	0.3
Oxalic acid	D_2O	7.5	0.13	64.5	0.89	95.3	0.29
Maleic acid	D_2O	2	0.04	50.5	0.77	94.6	0.28
Malonic acid	D_2O	2	0.03	45.8	0.7	93.2	0.28
Glycolic acid	D_2O	0.7	0.02	27.1	0.5	83.8	0.26
Lactic acid	D_2O	0.3	0.02	25.1	0.5	75.9	0.23
Succinic acid	D_2O	0	0.02	19.9	0.43	83.1	0.26
Acetic acid	D_2O	0	0.01	9.1	0.3	66	0.2
HCl	DMSO-d6	1	0.01	72	0.49	99.4	0.35
Methanesulfonic acid	DMSO-d6	0.9	0.01	73	0.5	98.3	0.35
Benzenesulfonic acid	DMSO-d6	1.2	0.01	78	0.52	105.7	0.37
Oxalic acid	DMSO-d6	0.1	0.01	2.8	0.02	24.3	0.02
Maleic acid	DMSO-d6	0.1	0.01	6.9	0.04	24.4	0.06
Malonic acid	DMSO-d6	0	0.01	0.9	0.01	7.1	0
Glycolic acid	DMSO-d6	0.1	0.01	0.4	0.03	1.7	0
Lactic acid	DMSO-d6	0	0.01	0.4	0.03	2.5	0
Benzoic acid	DMSO-d6	0.2	0.01	0.2	0	0.7	0
Succinic acid	DMSO-d6	0.1	0.01	0.4	0	1.4	0
Acetic acid	DMSO-d6	0.1	0.01	0.2	0	1.3	0
Orotic acid	DMSO-d6	0.2	0	2.6	0.01	6.7	0.02
Maleic acid	Acetone-d6					16.5	0.04
Malonic acid	Acetone-d6					10.2	0.01
Glycolic acid	Acetone-d6					3.3	−0.01
Malic acid	Acetone-d6					7.6	0
Benzoic acid	Acetone-d6					3.2	0
Acetic acid	Acetone-d6					1.8	−0.02
Sulfuric acid	MeOD-d4					93	0.34
Methanesulfonic acid	MeOD-d4					82.2	0.3
Oxalic acid	MeOD-d4					24	0.09
Maleic acid	MeOD-d4					6.2	0.01
Malonic acid	MeOD-d4					8.2	0.02
Glycolic acid	MeOD-d4					2.9	−0.01
Malic acid	MeOD-d4					4.6	0.01
Benzoic acid	MeOD-d4					1.5	−0.01
Acetic acid	MeOD-d4					0.9	−0.01

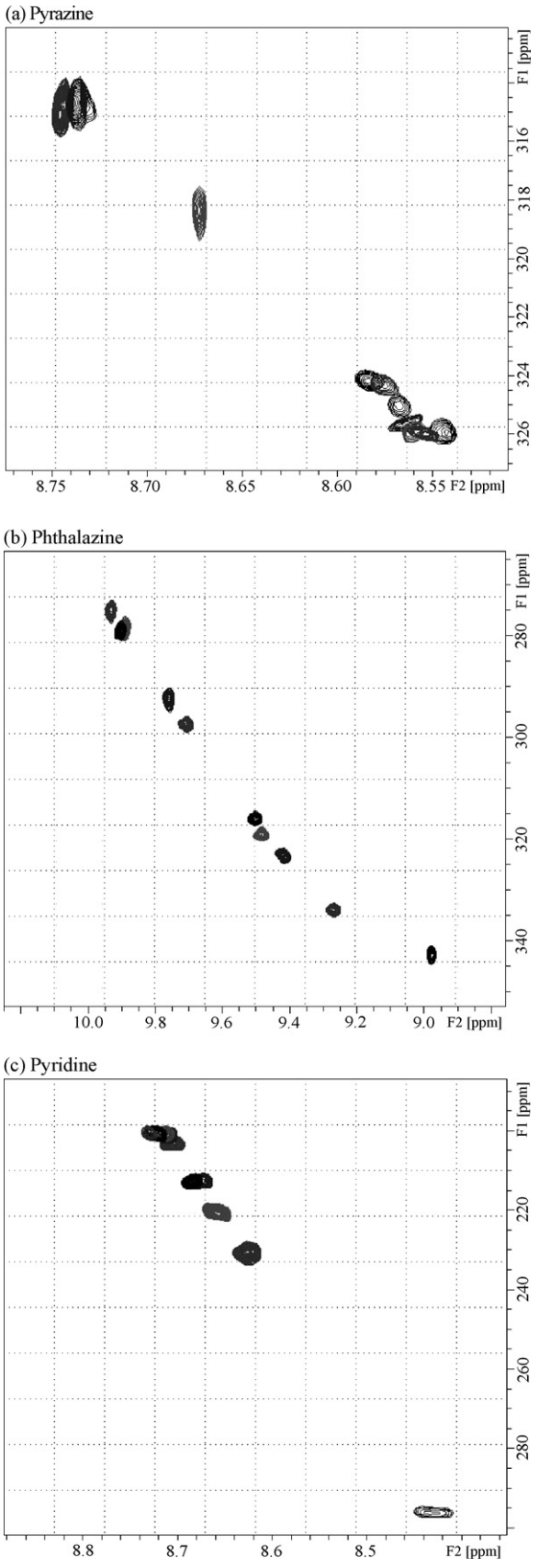

Fig. 2. ^{1}H–^{15}N HMBC spectra of the three bases (pyrazine, phthalazine, and pyridine) on addition of acids in D_2O.

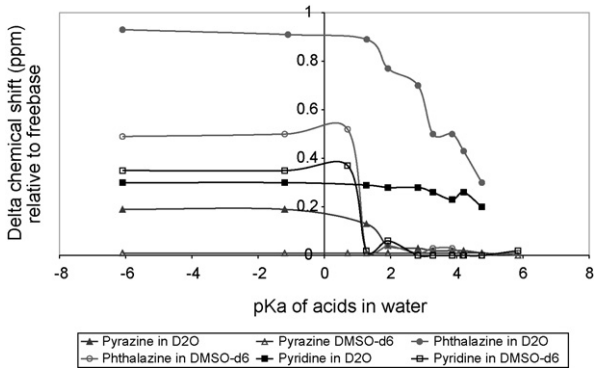

Fig. 3. ^{1}H chemical shift changes of the three bases (pyrazine, phthalazine, and pyridine) on addition of acids in D_2O and DMSO-D6 plotted against the aqueous pK_a values of the acids.

calculate the chemical shift difference between each base and the mixture of base and acid.

The ^{1}H and ^{15}N chemical shift changes observed for the bases in D_2O and DMSO-d6 solution, on addition of the various acids, are compared in Figs. 3 and 4 by plotting the chemical shift changes against the aqueous pK_a values of the acids. The aqueous pK_a values are used as a measure of the relative strength of the acids since these values are readily available. Although the absolute pK_a values vary with the solvent used, the relative acid strength does not (as discussed above). The chemical shift changes observed in D_2O and DMSO-d6 were significantly different. Such differences may be expected since the physicochemical properties of different solvents (*e.g.* dielectric constant, relative polarity and hydrogen bonding ability) are known to affect acid–base reactions. However, plots of ^{1}H and ^{15}N NMR chemical shift changes against the acid pK_a values showed a similar trend even though the extents of the chemical shift changes were different. The larger chemical shift changes obtained for ^{15}N NMR compared to ^{1}H NMR are due to the direct protonation of the nitrogen atom and the higher shielding effect for the nitrogen compared to the proton nuclei. The extent of the chemical shift changes were related to the pK_a values of the different acids with greater chemical shift changes occurring with the stronger acids.

The three bases investigated, pyrazine, phthalaazine and pyridine, have aqueous pK_a values of 0.37, 3.39, and 5.22, respectively. The higher the pK_a of the base the greater chemical shift changes observed for ^{15}N chemical changes. In general, this can be related to the strength of the base since the nitrogen experiences direct protonation. The ^{1}H chemical shift change was lowest for the lowest pK_a value of the base, however this trend did not follow for pyrazine and phthalazine. This may be due to proton shift changes being affected

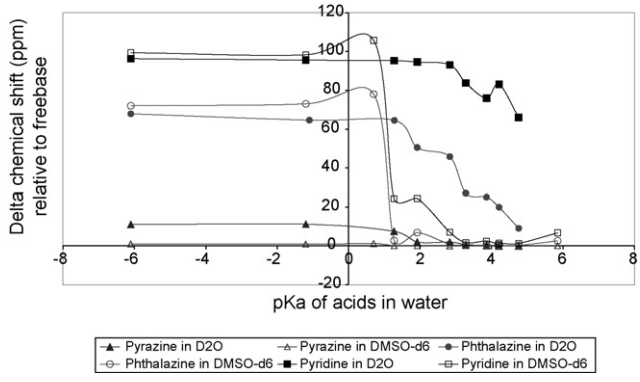

Fig. 4. ^{15}N chemical shift changes of the three bases (pyrazine, phthalazine, and pyridine) on addition of acids in D_2O and DMSO-D6 plotted against the aqueous pK_a values of the acids.

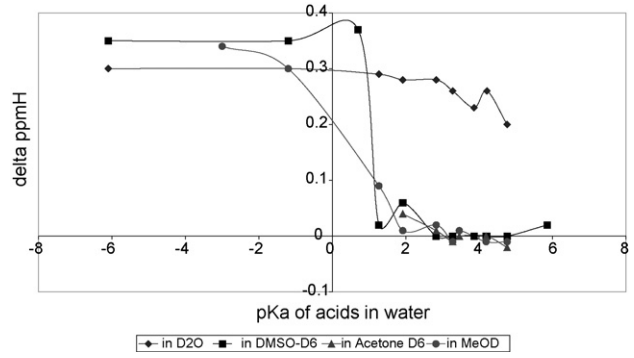

Fig. 5. ^{1}H chemical shift changes of pyridine on addition of acids in D_2O, DMSO-D6, acetone-d6 and MeOD-d4 plotted against the aqueous pK_a values of the acids.

by neighboring atoms. The magnitude of ^{1}H and ^{15}N chemical shift changes can be affected by the molecular structure (*i.e.* how easily protonation affects the electron density distribution) as well as by experimental conditions.

Figs. 5 and 6 show the ^{1}H and ^{15}N chemical shift changes of pyridine on addition of various acids in four different solvent systems. The chemical shift changes are plotted against the aqueous pK_a values of the acids. There is a gradual change in the chemical shift of pyridine in D_2O on addition of different acids as the acid pK_a values increase. Whereas, in the various organic solvents a sharp change in the chemical shift of pyridine is noted on addition of weak acids at the point of transitioning from strong to weak acids. For example, in DMSO-d6 the ^{1}H chemical shift changed from 0.37 (benzenesulfonic acid) to 0.02 (oxalic acid). Chemical shift data could not be obtained for strong acids (sulfuric acid, methanesulfonic acid, ethanesulfonic acid, and oxalic acid) in acetone. Precipitation occurred in the case of sulfuric acid and oxalic acid and color change occurred in the case of methanesulfonic acid and ethanesulfonic acid. Precipitation is an indicator of immediate salt formation and is likely to be due to lack of solubility of the salt in the solvent system. Color change is an indicator of chemical reaction which is most probably salt formation in this case.

Considering the available pK_a values in methanol (Table 3) pyridine is not expected to form salts with malonic acid, tartaric acid, benzoic acid, succinic acid, and acetic acid since the pK_a values of these acids are higher than that of pyridine. This is in agreement with the data presented Figs. 5 and 6. Similarly, pyridine is not expected to form salts with benzoic acid and acetic acid in DMSO-d6, but is expected to form salts with hydrochloric acid and methanesulfonic acid in DMSO-d6. These predictions are confirmed by the data presented in Figs. 5 and 6. When complete pK_a values of pharmaceutically useful counter ions in different organic solvents are available such solution NMR studies will help to prioritize the counter ions for initial salt screening. This can also assist in the selection of a second organic solvent when a solvent mixture is needed to increase the acid base reaction. Since the complete pK_a values in many different organic solvents are not available, solution NMR experiments can provide an experimental method to probe the extent of the acid–base reaction in the organic solvent of interest. Although this study provides very useful information on salt formation reaction in organic solvents, it should be pointed out that this technique would not replace traditional salt selection methodologies at this time. This technique could be used as a secondary troubleshooting method.

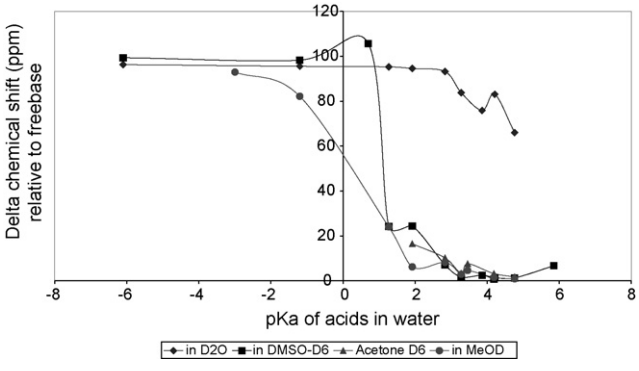

Fig. 6. ^{15}N chemical shifts changes of pyridine on addition of acids in D_2O, DMSO-D6, acetone-d6 and MeOD-d4 plotted against the aqueous pK_a values of the acids.

For the pyridine + sulfuric acid and pyridine + oxalic acid mixtures, precipitation in the crystalline form took place in methanol but not in D_2O, DMSO-d6, and actone-d6. Precipitation during preparation of NMR samples could be used as a first screening step for salt formation. Generally, precipitation is the first step to obtain solid material during salt screening and therefore, precipitation during preparation of mixtures of base and acid is a favorable result. Most of the instrumental characterization methods such as XRPD, TGA, and DSC are performed on solid material to identify suitable crystalline salt forms. The pK_a value is a thermodynamic parameter that is generated in the equilibrium state. However, crystallization is a kinetic process. Solvent dependent precipitation of a crystalline salt form may be due to: the solubility of the salt; a difference in the nucleation/crystal growth rate; and/or the extent of proton transfer from acid to base in the solvent system. Solubility and rate of nucleation/crystal growth can be controlled to some extent by processes such as slow evaporation. However, the lack of proton transfer from acid to base in the solvent system needs to be precluded in the experimental design during salt screening. The key to the crystallization step during salt screening is the inter-molecular attractive force needs to be sufficient to overcome ion/molecule-solvation in the medium and the arrangement of molecular units in the three dimensional space. Higher chemical shift changes imply stronger acid–base interaction and may have a higher chance of crystallization due to higher inter-molecular attractive force.

Even though deuterated solvents are used in this study, it is possible to use non-deuterated solvents in the NMR study by applying a solvent peak suppression technique while collecting NMR data. Non-deuterated organic solvents have been successfully used in our laboratory. The chemical complexity of pharmaceutical drug candidates from proton NMR can be reduced using ^{15}N NMR since number of nitrogen atoms in a molecule is much less than the number of protons.

4. Conclusion

The ^{1}H and ^{15}N NMR chemical shift changes of three bases on addition of acids with different acidity determined using solution NMR spectroscopy showed different trends in different media. These results indicate that the extent of acid–base reaction in different media will be different and so the solvent system should be taken into account to ensure sufficient salt formation reaction. The maximum chemical shift changes were achieved at around ΔpK_a values of 2 in D_2O which confirms the ΔpK_a (base–acid) general rule that the pK_a value of the acids should be 2 units lower than the pK_a of the base to ensure proton transfer. The solution NMR technique can be used to confirm the ΔpK_a general rule in different organic solvents when the complete pK_a values in many different organic solvents are available. Since the pK_a values of drug candidates may not be available for all solvent systems solution this NMR technique may provide useful information and rationale to select suitable solvent system for salt formation reaction in organic solvents.

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References

2002. Handbook of Pharmaceutical Salts: Properties, Selection and Use. Wiley-VCH, Zurich.
Barbosa, J., Barron, D., et al., 1988. Dissociation constants and assay of weak bases in non-aqueous solvents. J. Pharm. Biomed. Anal. 6, 709–716.
Barbosa, J., Barrón, D., et al., 1999. Chromatographic behaviour of ionizable compounds in liquid chromatography. Part 1. pH scale, pK_a and pHS values for standard buffers in tetrahydrofuran-water. Anal. Chim. Acta 389, 31–42.
Barrette, W.C., Johnson, H.W., et al., 1984. Voltammetric evaluation of the effective acidities (pK_a') for Broensted acids in aprotic solvents. Anal. Chem. 56, 1890–1898.
Bastin, R.J., Bowker, M.J., et al., 2000. Salt selection and optimisation procedures for pharmaceutical new chemical entities. Org. Process Res. Dev. 4, 427–435.
Bax, A., Summers, M.F., 1986. Proton and carbon-13 assignments from sensitivity-enhanced detection of heteronuclear multiple-bond connectivity by 2D multiple quantum NMR. J. Am. Chem. Soc. 108, 2093–2094.
Berge, S.M., Bighley, L.D., et al., 1977. Pharmaceutical salts. J. Pharm. Sci. 66, 1–19.
Black, S.N., Collier, E.A., et al., 2007. Structure, solubility, screening, and synthesis of molecular salts. J.Pharm. Sci. 96, 1053–1068.
Bordwell, F.G., 1988. Equilibrium acidities in dimethyl sulfoxide solution. Acc. Chem. Res. 21, 456–463.
Bos, M., van der Linden, W.E., 1996. A knowledge based advisory system for acid/base titrations in non-aqueous solvents. Anal. Chim. Acta 332, 201–211.
Bosch, E., Rosés, M., 1989. Ionic equilibria in neutral amphiprotic solvents; resolution of acid strength in tert-butyl alcohol. Talanta 36, 627–632.
Chantooni, M.K., Kolthoff, I.M., 1979. Resolution of acid strength in tert-butyl alcohol and isopropyl alcohol of substituted benzoic acids, phenols, and aliphatic carboxylic acids. Anal. Chem. 51, 133–140.
Engel, G.L., Farid, N.A., et al., 2000. Salt form selection and characterization of LY333531 mesylate monohydrate. Int. J. Pharm. 198, 239–247.
Gould, P.L., 1986. Salt selection for basic drugs. Int. J. Pharm. 33, 201–217.
Gu, C.-H., Li, H., et al., 2004. Grouping solvents by statistical analysis of solvent property parameters: implication to polymorph screening. Int. J. Pharm. 283, 117–125.
Izutsu, K., 1990. Acid–Base Dissociation Constants in Dipolar Aprotic Solvetns, IUPAC Chemical Data Series No. 35. Blackwell, Oxford.
Izutsu, K., 2002. Electrochemistry in Nonaqueous Solutions. WILEY-VCH Verlag GmbH.
Izutsu, K., 2003. Acid-base reactions in non-aqueous solvents. In: Electrochemistry in Nonaqueous Solutions, pp. 59–83.
Kim, H.s., Chung, T.D., et al., 2001. Voltammetric determination of the pK_a of various acids in polar aprotic solvents using 1,4-benzoquinone. J. Electroanal. Chem. 498, 209–215.
Kolthoff, I.M., 1974. Acid–base equilibriums in dipolar aprotic solvents. Anal. Chem. 46, 1992–2003.
Maran, F., Celadon, D., et al., 1991. Electrochemical determination of the pK_a of weak acids in N,N-dimethylformamide. J. Am. Chem. Soc. 113, 9320–9329.
Marcus, Y., 1993. The properties of organic liquids that are relevant to their use as solvating solvents. Chem. Soc. Rev. 22, 409–416.
Morris, K.R., Fakes, M.G., et al., 1994. An integrated approach to the selection of optimal salt form for a new drug candidate. Int. J. Pharm. 105, 209–217.
Myers, B.J., Common organic solvents Table of properties, http://organicdivision.org/organic_solvents.html.
Reichardt, C., 2003. Solvents and Solvent Effects in Organic Chemistry. WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.
Rived, F., Rosés, M., et al., 1998. Dissociation constants of neutral and charged acids in methyl alcohol. The acid strength resolution. Anal. Chim. Acta 374, 309–324.
Sarmini, K., Kenndler, E., 1999. Ionization constants of weak acids and bases in organic solvents. J. Biochem. Biophys. Method. 38 (2), 123–137.
Serajuddin, A.T.M., Pudipeddi, M., 2002. In: Stahl, P.H., Wermuth, C.G. (Eds.), Salt selection strategies. Handbook of Pharmaceutical Salts: Properties, Selection, and Use. Wiley-VCH, Zurich, pp. 135–160.
Wenlock, M.C., Austin, R.P., et al., 2003. A Comparison of physiochemical property profiles of development and marketed oral drugs. J. Med. Chem. 46, 1250–1256.