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Introduction

The ProfilerSGA (Figure 1) has been developed to address the need for high throughput measurements of ionisation (pKa) constants. A patented procedure² was devised by creating a linear pH gradient from a universal buffer in aqueous solution, injecting the sample, and measuring UV absorbance. In this way, large numbers of pKa measurements can be made very quickly, using small amounts of samples.

In this work, we have extended the technique to work in a mixed-solvent system. Our goal was to discover a mixture that dissolves the widest range of poorly soluble drugs in analytical quantities, and that also has good stability, low viscosity, low UV absorbance and minimal effects on the pH scale. We will present the results from our preferred solvent mixture.

Since pKa values are shifted in the presence of non-aqueous solvents it is instructive to look at simple rules for correction to aqueous values. This is examined for several functional group types.

Experimental



Figure 1. ProfilerSGA

A universal buffer in a mixed-solvent system was produced such that the final mixture contains 80% v/v H₂O : 6.67% v/v MeOH : 6.67% v/v 1,4-Dioxane : 6.67% v/v MeCN. We refer to this cosolvent system as 20% v/v MDM-mix. The universal buffer consists of a mixture of weak acids and bases that do not absorb significantly in the UV above 250nm, and provides an almost constant buffer capacity across a wide pH range.

A flowing pH gradient is created by blending acidified buffer (at pH2) with basic buffer (at pH12) using two computer controlled syringe dispensers that are inversely varied in speed whilst maintaining a constant total flow-rate (1.25ml/min). This enables a linear pH gradient to be established such that the pH can be changed from pH2 – pH12 (or vice versa) in a period of 2 minutes.

Samples for pKa measurement are presented as 10mM solutions prepared in DMSO in 96-well plates. 20µL of each sample is automatically diluted with 2mL of system solution via a liquid handling robot. Diluted sample is then injected directly into the flowing pH gradient at constant flow-rate (0.25mL/min) for the duration of the experiment. The system solution also consists of 20% v/v MDM-mix.

The pH gradient and sample pass through a diode array spectrophotometer where any changes in sample absorbance are measured in the UV wavelength range. The solution pH in the measurement flow cell is proportional to the time elapsed since the start of gradient generation, and the sample's pKa values are calculated from the change in UV absorbance at multiple wavelengths as a function of pH.

Results and Discussion

Figure 2 shows ProfilerSGA values for 55 compounds plotted against the corresponding values measured in 20% v/v MDM-mix by the industry standard pH-metric or DPAS/UV methods. A linear regression line has been fitted to the data with an R² value of 0.996, demonstrating that rapid measurement of pKas is possible in the MDM-mix.

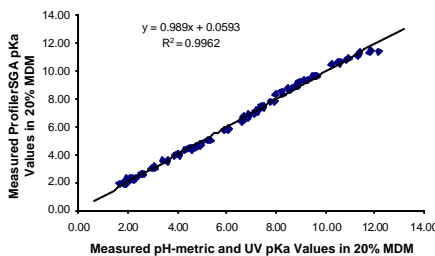


Figure 2. Validation of ProfilerSGA MDM pKa values

These compounds have been classified into different ionisable functional group types and the 20% v/v MDM-mix pKa results compared to aqueous pKa values. The results for acidic functional groups are presented in Figures 3-8. The results for basic functional groups are presented in Figures 9-14.

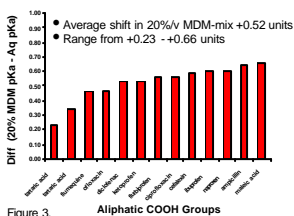


Figure 3. Aliphatic COOH Groups

Aliphatic and aromatic COOH

- Shifts largest for second ionisable group if compounds have two COOH groups
- Lowest shifts (<0.30) for nitrobenzoic acids and compounds with a salicylic acid group
- Substituting the phenol group for an acetyl group in acetylsalicylic acid results in a much larger shift.

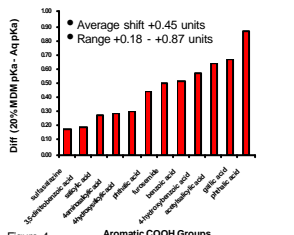


Figure 4. Aromatic COOH Groups

Maleic acid, tartaric acid and ibuprofen were measured pH-metrically on a GLpKa instrument since they had no chromophore or insufficient change in absorbance during ionisation to be measured by UV.

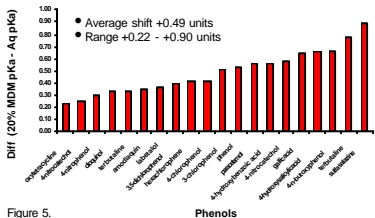


Figure 5. Phenols

Phenols

- Lowest shifts for nitro containing compounds
- Shifts largest for second ionisable group if compounds have two (or more) acidic groups
- Lower than average shifts for chlorophenols

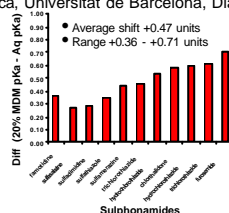


Figure 6. Sulphonamides

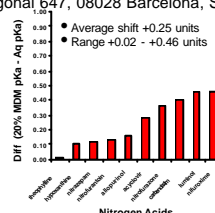


Figure 7. Nitrogen Acids

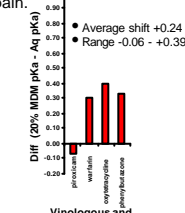


Figure 8. Vinologous and Carbon Acids

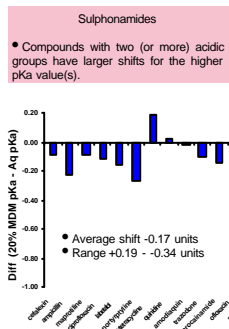


Figure 9. Primary, Secondary and Tertiary Amines

Nitrogen Acids

- Low shifts compared to many COOH groups and phenols, possibly caused by extensive delocalisation of negative charge in most nitrogen acids.

Vinologous and Carbon Acids

- Delocalisation of negative charge may cause small shifts.

Primary, Secondary and Tertiary Amines

- Most shifts are small and negative.
- The ring strain on the bicyclic tertiary amine in Quinidine may be relieved in MDM leading to a small positive shift.
- Unambiguous assignment of pKas may be difficult in oxytetracycline.

Cefalexin, ampicillin, maprotiline, nortriptyline, trazodone, imipramine, trimipramine and amiodarone were measured pH-metrically on a GLpKa instrument since they had no chromophore or insufficient change in absorbance during ionisation to be measured by UV.

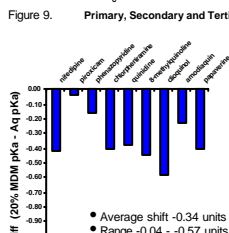


Figure 10. Pyridines and Quinolines

Primary, Secondary and Tertiary Amines

- Average shift -0.27 units
- Range -0.18 - -0.38 units

Anilines and Aminopyrimidines

- All shifts small and negative and covering a narrow range

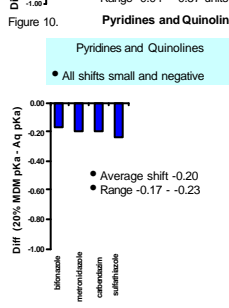


Figure 12. Imidazoles and Thiazoles

Pyridines and Quinolines

- All shifts small and negative

Anilines and Aminopyrimidines

- All shifts small and negative and covering a narrow range

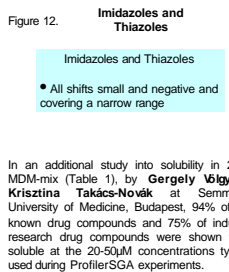


Figure 13. Imines

Imidazoles and Thiazoles

- All shifts small and negative and covering a narrow range

Imines

- All shifts negative and covering a narrow range

Amidines and Guanidines

- Small shifts
- Amiloride has positive shift indicating stabilisation of positive charge in MDM

In an additional study into solubility in 20% v/v MDM-mix (Table 1), by Gergely Völgyi and Krisztina Takács-Novák at Semmelweis University of Medicine, Budapest, 94% of well-known drug compounds and 75% of industrial research drug compounds were shown to be soluble at the 20-50µM concentrations typically used during ProfilerSGA experiments.

Concentration	Well-known compounds		Industrial samples		All samples	
	No.	%	No.	%	No.	%
1mM	19/32	59.4	48	50	23/40	57.5
50µM	30/32	93.8	68	75	36/40	90
20µM	30/32	93.8	68	75	36/40	90

Table 1. Number of compounds soluble in 20% v/v MDM-mix

Conclusions

The selection of 20% v/v MDM-mix cosolvent was done to help improve the solubility for difficult compounds during rapid pKa analysis. In order to maintain rapid throughput it is necessary to analyse at one cosolvent ratio only. Therefore, the cosolvent must not cause a large shift in pKa compared to the aqueous pKa. The cosolvents must be UV inactive above 225nm and must not be viscous. The cosolvents need to have a combination of polar and non-polar properties so that solubility is improved for hydrophobic compounds but is still good for polar compounds. We believe we have achieved these requirements with our 20% v/v MDM-mix. Such reagents are inexpensive, readily available, and have the added advantage of keeping the fluids clear.

The work in the 20% v/v MDM-mix has enabled us to come up with rule-of-thumb correction factors for many measured MDM pKas to true aqueous pKas.

Future work in collaboration with Semmelweis University will look to further optimise the cosolvent solubilising properties by adjusting relative proportions of each solvent in the mixture.

References

- Box, K., Bevan, C., Comer, J., Hill, A., Allen, R., Reynolds, D., High Throughput Measurement of pKa values in a mixed-buffer linear pH gradient system. Anal. Chem. 2003, 75(4), 883-892
- Hill, A., Bevan, C., Reynolds, D., Analytical method and apparatus therefor, 1999. UK Patent WO99/13328