

High quality measurements from small sample amounts using the PionT3

A comprehensive validation study conducted using the PionT3 instrument has demonstrated that highly accurate and repeatable data are obtained for key physico-chemical parameters using sub milligram amounts for pK_a and logP measurements and from as little as 2 mg for solubility measurements. The study compared results obtained from Pion standard assays with literature values and excellent agreement was demonstrated.

This study was designed to assess performance of the PionT3 using a range of compounds possessing acidic, basic and ampholytic properties. This application note describes the study that was undertaken and provides some illustrative data sets. Full data from this study are available from Pion on request.



PionT3 Results

pK_a : Figure 1 shows a comparison of the pK_a results obtained in this study with the values reported in the literature¹⁻⁴. The correlation coefficient (R^2) of 0.998 illustrates that the data are in excellent agreement with the literature. Repeatability was excellent; the standard deviations were generally below 0.03 for aqueous assays and 0.11 for those in methanol/water cosolvent mixtures.

logP: Agreement between the measured logP values and those reported in the literature¹ was very good, with an R^2 value of 0.992. It was noted that the measured value of Maprotiline was 4.29, which is significantly lower than the literature value of 4.85. To investigate this, an additional measured value of 4.19 was independently obtained on a Pion GLpKa instrument. This suggests that the literature logP value is erroneous. The result obtained using the GLpKa instrument was therefore substituted as the comparison value for the purpose of calculating the correlation coefficient.

Experimental

The pK_a , logP and solubility of 12 compounds (chlorpheniramine, chlorzoxazone, fluoxetine, hydrochlorothiazide, imipramine, maprotiline, metoprolol, piroxicam, prochlorperazine, propranolol, tetracycline and warfarin) were investigated. 10 compounds (amiloride, furosemide, flumequin, benzocaine, quinine, papaverine, diazepam, labetalol, terbutaline and phenazopyridine) were analyzed for pK_a only. A minimum of 10 repeat measurements per compound was performed for each parameter investigated. Assay designs were selected from the templates provided in the PionT3 software. Minor modifications were made where necessary. Sample concentrations were in the range 0.4 – 1.5 mM for potentiometric pK_a and logP, and 20 – 30 μ M for UV-metric pK_a . Sample weights for solubility assays were within the range 2 – 140 mg.

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Repeatability was good. Typical standard deviations fell within the range 0.03 - 0.16. Results for hydrochlorothiazide and prochlorperazine showed poorer repeatability; the former having a very low logP and the latter being poorly soluble in octanol/water. No result could be obtained for tetracycline, whose logP was too low to measure reliably.

Solubility: Figure 2 compares the measured solubility values expressed as logS values with those reported in the literature, where S is the intrinsic solubility (equilibrium solubility of the fully neutral compound). Prochlorperazine was omitted from this section of the study, as its tendency to form an insoluble salt means that it would require special treatment. For the remaining 11 compounds, agreement between the measured values and those reported in the literature¹ was excellent ($R^2 = 0.994$). Typical standard deviations of less than 0.07 logS units were obtained.

Conclusion

The pK_a , logP and solubility results produced by the PionT3 are highly repeatable and agree well with results reported in the literature ($R^2 > 0.99$) over a wide measureable range. Sample requirements are low, needing just 0.5 mg for a potentiometric pK_a measurement or 5 μ L of 10 mM stock solution for a UV-metric determination.

References

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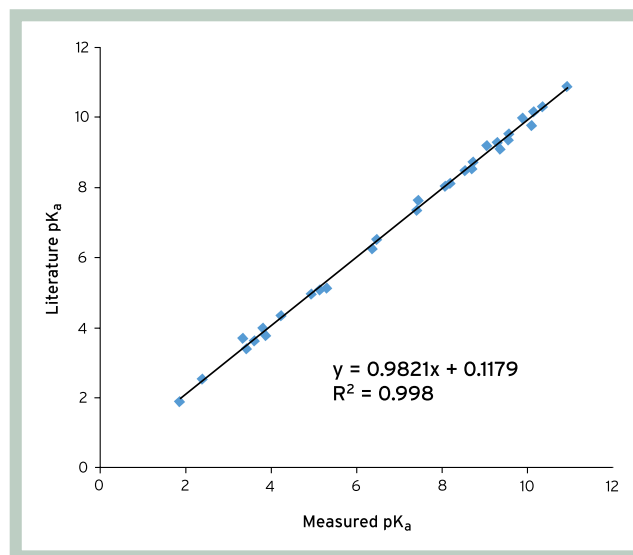


Figure 1. Literature vs Measured pK_a

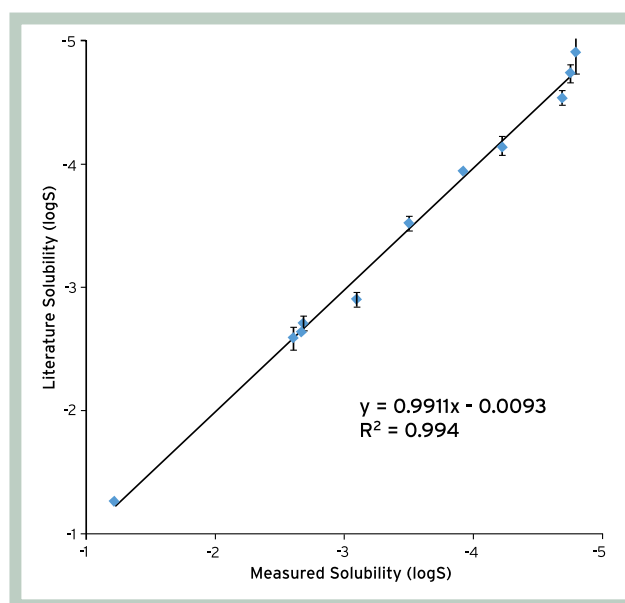


Figure 2. Literature vs Measured Solubility