# CHN microanalysis-a technique for the 21st century?

THE DETERMINATION OF PURITY IS OFTEN A KEY ASPECT OF SAMPLE CHARACTERISATION. MANY MODERN ANALYTICAL TECHNIQUES HAVE THEIR LIMITATIONS IN PURITY DETERMINATION WHICH MEANS THAT CLASSICAL TECHNIQUES, SUCH AS ELEMENTAL ANALYSIS, STILL PLAY AN IMPORTANT ROLE. CHN MICROANALYSIS IS A POWERFUL AND REALTIVELY STRAIGHTFORWARD METHOD FOR DETERMINING WHETHER OR NOT A SAMPLE IS PURE, BY PROVIDING A PRECISE AND ACCURATE ANALYSIS OF THE PERCENTAGE CARBON, HYDROGEN AND NITROGEN IN THE SAMPLE. CHN MICROANALYSIS CAN COMPLEMENT OTHER TECHNIQUES SUCH AS MS AND NMR, THUS ENHANCING THE RELIABILITY OF THE RESULTS OBTAINED.

BY KAREN J. ETHERINGTON, ALISON RODGER AND PAUL HEMMING

As analytical chemistry moves into the 21st century the demands on laboratory staff and equipment to become more productive with less resource and at lower cost are common place. As a consequence of these changes, the time of the analytical specialist is typically becoming increasingly focused on method development and problem solving tasks. This has provided a significant driving force to ensure that routine analyses can be undertaken by any scientist in an open access manner, at any time in a work programme.

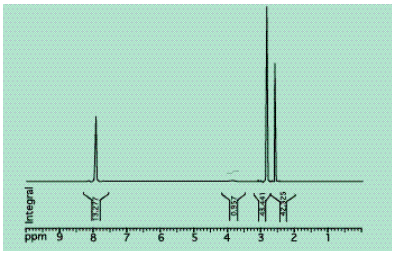

*Figure 1: NMR spectrum of LiCl dissolved in DMF. The peaks at 3 ppm correspond to the DMF methyl groups and the peak at 8ppm corresponds to the amide group of DMF.*

Among the techniques that have proven well-suited to this approach are gas chromatography (GC), GC-mass spectrometry (GC-MS), high performance liquid chromatography (HPLC), HPLC-mass spectrometry (LC-MS), mass spectrometry (MS), nuclear magnetic resonance (NMR) spectroscopy, LC-NMR and capilliary electrophoresis (CE).

A key aspect of sample characterisation is often determination of purity. The above mentioned techniques, some of which have revolutionised analytical chemistry, all have limitations when it comes to purity determination. Therefore, classical techniques such as elemental analysis still play an important role. Every technique has its advantages and limitations, so when fully characterising a sample, it is essential to use a range of methods to avoid biasing the results.

## CHN MICROANALYSIS

With a history stretching back to Lavoisier[1,2], the accurate elemental analysis of organic compounds has long been a key tool for characterising sample composition and purity in research and quality control environments. The use of CHN microanalysis as a routine technique to provide a precise and accurate elemental analysis of a sample is widely accepted across many industries worldwide. CHN microanalysis is a powerful and relatively straightforward method for determining whether or not a sample is pure by providing a precise and accurate analysis of a sample's percentage carbon, hydrogen and nitrogen content.

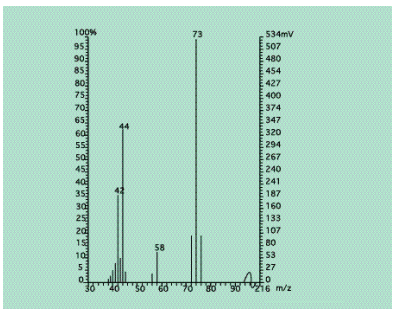

*Figure 2: EI Mass Spectrum of LiCl dissolved in DMF.*

With the advent of the horizontal furnace design, cross contamination and alteration of the gas flows have effectively been eliminated. This has led to enhanced accuracy and precision and the elimination of memory effects that occur in the vertical furnace configuration[3].

CHN microanalysis has now been developed so that samples can be run unattended in an automatic mode. The demands of accurate weighing of the microsamples has, to date, eluded attempts to replace the scientist with a robotic sample handler. Thus, the main drawbacks of CHN analysis are the requirements for accurate weighing of 1-2 mgs of sample (that is consumed in the analysis) and also the fact that samples are usually run as an automated batch rather than on an individual sample basis. Despite these perceived drawbacks, CHN microanalysis remains the most direct purity analysis and so is attractive in the context of increased regulatory pressure and global competition where high quality validatable purity data is essential.

As it has proven impossible to produce a fully automated, 'open access' CHN microanalyser, a body of opinion has grown suggesting that mass spectrometry, where a molecular ion is measured to a high degree of accuracy, or NMR, which enables an estimate of sample purity to be made, can be used to replace CHN analysis. This article uses example analyses from major industrial companies as well as from academia, to demonstrate the way CHN microanalysis complements other techniques such as MS and NMR and thus cannot be replaced.

All microanalysis data were obtained using a Model 440 CHN/O/S analyser (Exeter Analytical, North Chelmsford, MA, USA). NMR spectra were run on 250 MHz or 400 MHz spectrometers from (Bruker Analytische Messtechnik, Silberstreifen, Germany). E.I mass spectra were obtained using an AutoSpec (MicroMass, Manchester, UK) and MALDI mass spectra using a Kompact MALDI 4 (Kratos, Manchester, UK).

## PROBLEMS WITH COMMONLY USED TECHNIQUES

Many companies today benefit from the relative ease with which NMR can be used to determine target compound validity or elucidate the structure of an unknown in the absence of crystal data. However, whilst NMR is frequently also used as an easy method of assessing compound purity, any single species present at less than approximately 1% of the sample will remain invisible to this technique. The accuracy in determining total sample purity could therefore be seriously compromised if a number of low level impurities are present. As

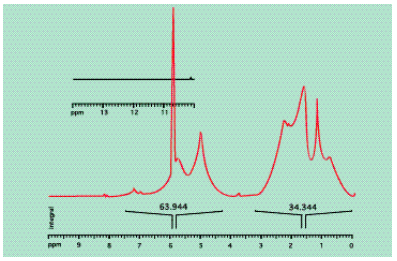

*Figure 3: NMR Spectrum of 4-bromo-2,6-bis(benzylthio) methylpyridine in CDCl3 ( + 3.5% methanol).*

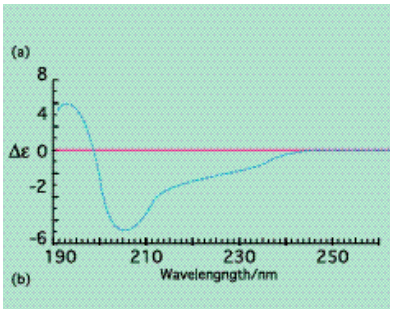


*Figure 4: Circular dichroism (0.1 mg/mL, 1 mm pathlength in water and 0.2% TFA) expressed as molar residue circular dichroism signal.*

NMR is a technique sensitive to nuclei with unpaired electrons, it will also fail to detect common inorganic contaminating species such as alkali metal chlorides. This is demonstrated in figure 1. In this example 5 % LiCl has been added to the common solvent dimethylformamide (DMF). The resultant spectrum clearly only shows the presence of DMF.

Similarly the presence of common inorganic contaminants can also elude MS analysis. Figure 2 illustrates how electron ionisation (EI) mass spectrometry also fails to show the 5 % LiCl in the DMF. The peak observed at m/z 42 suggests that only a small amount of LiCl is present, rather than the large excess that was actually added. However,

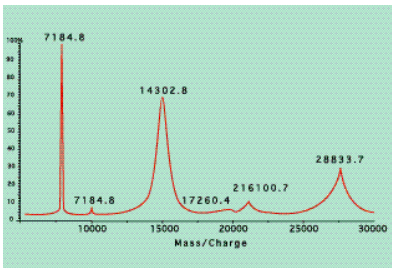


*Figure 5: MALDI MS (2 mg/mL protein in 50:50 MeOH:0.2% TFA in H2O; matrix: a-cyano-4-hydroxycinnamic acid in DMF) spectra of chicken egg lysozyme.*

CHN data clearly shows that it is not pure DMF in the sample. (DMF Theoretical. C:49.30%, H:9.65%, N:19.15%. Experimental. C:45.51%, H:9.01%, N:17.85%).
In a similar manner, the presence of low levels of solvents has little or no effect on the NMR and MS data collected. For example, analysis of a sample of 4-bromo-2,6-bis(benzylthio) methylpyridine (C21H20BrNS2) requires 0.5 moles of the solvent methanol per mole of analyte to make sense of the CHN data (C:57.51%, H:4.57%, N:3.24%). As shown in Figure 3, the NMR (in CDCl3) shows no evidence of the presence of methanol (at 3.5% level). As methanol has a very similar molecular weight to sulphur, the methanol present within this sample would also be effectively invisible to MS.

Mass spectrometric methods vary considerably in what they can detect. Mass spectrometry is usually used to identify the mass of the parent compound, and depending on the ionisation method used, may also be used to elucidate molecular structures from fragmentation patterns. LC- or GC-MS can only be used quantitatively if suitable standards are available. However, it is seldom possible to use MS alone as a accurate purity determination method since mass spectrometers measure ion currents which seldom relate directly to the concentration of analytes.

| | THEORETICAL | EXPERIMENTAL |
|---|---|---|
| CARBON | 51.44 | 44.39 |
| HYDROGEN | 6.75 | 6.04 |
| NITROGEN | 18.79 | 16.10 |

*Table 1: Elemental analysis of lysozyme. A comparison of the theoretical and experimental values can immediately indicate the degree of purity of the sample. Data expressed as percentage C, H and N.*

## Assessing the purity of supplied chemicals

Chemicals are usually received from suppliers with estimates of purity. However, particularly with biological samples such as proteins or DNA, water, salts and other materials from the production or isolation process may still be present and unaccounted for. The circular dichroism and matrix assisted laser desorption ionisation (MALDI) MS spectra for a commercial sample of chicken egg lysozyme are shown in figures 4 and 5 respectively. These results give no reason to expect that the lysozyme is in fact only about 60% protein. The MALDI shows the singly charged molecular ion at m/z = 14,300 as well as the +2 molecular ion and some aggregates and a protein fragment. The circular dichroism spectrum shows the presence of a protein with 32% a-helical content. However, the CHN data for this sample are very clear. They indicate that a significant part of the sample is not proteinaceous, as the experimental C and N percentages are significantly lower than would be expected for a protein (table 1). (Theoretical. C:51.44%, H:6.75%, N:18.79%. Experimental. C:44.39%, H:6.04%, N:16.10%).

Some sample types pose particular problems for NMR so that even the normal expectations of purity analysis by this techniques are unfounded. A particular case is the analysis of certain classes of organometallic compounds by NMR. If the metal is paramagnetic, coupling of other resonances to the paramagnetic atom broadens peaks and may even cause them to disappear into the baseline of the spectrum.
In this case the CHN microanalysis data is invaluable in confirming the identity and purity of the molecule (Theoretical. C:62.06%, H:6.13%, N:6.60%. Experimental. C:61.95%, H:6.08%, N:6.61%).

## Conclusion

No single analytical technique can be relied upon to provide the total picture when one desires to characterise fully a sample. CHN microanalysis still has a very important place alongside the other techniques in the modern analytical laboratory during characterisation and purity assesment of a wide range of samples. Due to recent advances in horizontal furnace CHN microanalysis instrumentation, precise and accurate results without constant system optimisation for sample type can be obtained for a wide range of samples from pure organic compounds, through organometallic species to proteins and peptides.

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