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HPLC–NMR–MS: past, present and future

The development of technology to enable a practical and useful direct coupling of HPLC with NMR spectroscopy to get real-time detection and identification of the eluting compounds is an interesting story [1]. It is a case of waiting for technology to catch up with demand. Early studies were conducted in the late 1970s and early 1980s; these included the concept of placing the HPLC column inside the NMR superconducting magnet [2–4]. However, these failed, largely as a result of technical limitations. It was the advent of higher-field NMR magnets and digital electronic technology that improved NMR sensitivity and, coupled with improvements in methods for suppressing the large NMR peaks from solvents, the methodology became viable and commercial instruments appeared on the market.

The desire for real-time analysis led to initial studies of HPLC–NMR using the continuous flow approach. This led to poor detection limits and it soon became clear that stopped-flow approaches, where the entire armoury of NMR tools for molecular identification could be used, was the way forward. Later, in 1995, the first attempt to combine HPLC with both NMR and MS was published [5]. Then, the debate ensued as to whether the NMR and MS should be in series or in parallel; the parallel arrangement soon became the norm because it enabled either the NMR or the MS to be used as the intelligent detector in finding eluting peaks of interest. For example, if the eluting compound contains fluorine, then ^{19}F NMR spectroscopy would be a highly selective approach for detecting fluorinated molecules with no background. By contrast, if the molecule of interest contains a well-defined molecular fragment, for example, a sulfate conjugate of a drug (a typical drug metabolite), then searching for a mass loss of 80 amu is, again, a selective and sensitive MS approach. Thus, more recently, the stopped-flow method with NMR and MS detection in parallel has become standard.

More recently, the idea of trapping the eluting peaks into capillary loops for later off-line analysis has gained much support and is increasing in usage; commercial instruments for performing this process are now available. This process can select up to 36 peaks from a running chromatogram and later conduct all necessary NMR and MS analyses under computer automation. This is of great benefit to industrial laboratories, such as those performing drug metabolite identification in the pharmaceutical industry.

Several further developments should not be forgotten. For example, it is possible to hyphenate even more techniques into a total combined analysis system (hypernation) [6]. One way in which this has good practical use is to combine more than one type of MS into the analytical system. Methods that are beginning to be used include inductively-coupled plasma (ICP) MS, which causes all molecules to be reduced to their atomic content, enabling the assay of a wide variety of heavy atoms. For a drug molecule that contains chlorine and is extensively metabolised in the body, a single HPLC–ICPMS run will detect all of the chlorinated molecules in a sample. Such a 'chloratogram' can be a useful tool for identifying both the number and quantity of such metabolites. If the HPLC analysis is then combined with a high resolution MS system, such as a time-of-flight or a Fourier transform MS, then masses can be determined highly accurately, leading directly to the elemental formula [7].

Further developments are occurring in the NMR component of HPLC–NMR–MS, including the development of NMR detectors that are cooled to cryogenic temperature. This causes a thermal noise reduction of ~500% and, hence, results in substantially improved signal:noise ratios, with a consequent impact on detection limits. Combining such developments with on-going attempts at miniaturization [8] will see the old adage of NMR being an insensitive technique stamped out forever.

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Ion channels in drug discovery and development

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Approximately 100 attendees and speakers enjoyed the atmosphere and information exchange at the second annual *Ion Channels* conference at the historic Bellevue Hotel (14–15 July 2003, Philadelphia, USA), hosted by the Strategic Research Institute. A good mix of pharmaco scientists, academics and technology reps made up the attendees and presenters.

The conference was co-chaired by Wilhelm Lachnit (Molecular Devices; http://www.moleculardevices.com) and Chris Mathes, who opened with a brief historical overview of the patch-clamp technique. The patch-clamp method has been an enabling technology in the study of ion channels since it was first reported in 1976 [1], being improved, thereafter, by the discovery of gigaseal recordings for low-noise, single-channel recordings [2]. In fact, the patch-clamp is one of the only methods for viewing the biological activity of proteins working in action in living cells. In 1991, Neher and Sakmann received the Nobel Prize in Medicine for inventing the patch-clamp technique. This technique uses glass micropipettes, with tips of 1–2 microns, that form tight seals (>1 gigaohm) against cell membranes. With this technique, single channel or whole cell currents through ion channels can be recorded with specialized amplifiers that enable recordings of miniscule currents (i.e. 10^{-12} A; see, for example, http://www.axon.com).

Conventional patch-clamp is slow and tedious, but well suited for PhD-level scientists, working out the detailed biophysical properties of ion channels. Until recently, however, there was no hope that this method could be used for screening ion channels in the drug discovery process. Seven of the talks at the conference highlighted new technologies in automated patch-clamp, several of which use planar patch-clamp electrodes. These planar electrodes are simply flat substrates (usually glass) with a 1–2 micron aperture separating intracellular and extracellular solution. 'On the surface', planar patch-clamp appears to be a modern technology, but it was actually first reported by Russian scientists in 1975 [3].

The new frontier: automated and chip-based patch-clamp systems

Seven talks presented recent advances in automated patch-clamp. Appropriately, the first technology reported was the AutoPatch® from Xention Discovery (http://www.mvfund.com/xention.htm), presented by John Ford, Director of Biology. The AutoPatch-1 (AP-1) was one of the first automated patch-clamp systems to hit the scene in 1998; at the heart of it is the Interface Patch technology. This clever method involves

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