The importance of controlled concentration and drying in MALDI-TOF applications

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Matrix-assisted laser desorption/ionisation-time of flight mass spectrometry (MALDI-TOF MS) is a now an accepted and routine analysis for the elucidation and quantitation of biomolecules in life science research. In this technique a co-precipitate of a UV-light absorbing matrix and a biomolecule are irradiated by a nanosecond laser pulse. The technique involves spotting small concentrated aliquots of material on to a matrix-coated "target". The target is then positioned inside the Mass Spectrometer and the biomolecule of interest is desorped from the matrix surface and ionised by the laser. Most of the laser energy is absorbed by the matrix, which prevents unwanted fragmentation of the biomolecule, whilst some of the energy causes ionisation of the biomolecule. These ionized biomolecules are accelerated in an electric field and enter the flight tube of a time-of-flight mass spectrometer. During the flight in this tube, different molecules are separated according to their mass-to-charge ratio and reach the detector at different times. In this way each molecule yields a distinct signal. The method is used for detection and characterization of biomolecules, such as proteins, peptides, oligosaccharides and oligonucleotides, with molecular masses between 400 and 350,000 Da. It is a very sensitive method, which allows the detection of low (10^{-15} to 10^{-18} mole) quantities of sample with an accuracy of 0.1 - 0.01 %. Although the technique can be very sensitive, concentrated samples achieve the best results.

Protein identification by this technique has the advantage of short measuring times (a few minutes) and negligible sample consumption (less than 1 pmol) together with additional information on microheterogeneity (e.g. glycosylation) and the presence of by-products. Although molecular biology has provided powerful techniques for DNA analysis, this is not yet reflected in protein analysis. Genome sequencing has yielded a wealth of information on predicted gene products, but for the majority of the expressed proteins no function is known. Proteomics is an important new field of study of protein properties including expression levels, interactions and post-translational modifications and thus can be described as functional genomics at the protein level. The mass accuracy of MALDI-TOF MS is sufficient to characterise proteins (after tryptic digestion) from completely sequenced genomes such as methanogens and yeast. The use of MALDI-TOF MS for typing of single nucleotide polymorphisms using single nucleotide primer extension has also made important progress recently.

Oligonucleotides, proteins, antibodies and other larger biomolecules are all suited to MALDI-TOF analysis. However, these compounds can be difficult to concentrate without exposing them to thermal damage or cross-contamination. MALDI spotters use nano-scale liquid handling to pipette drops of sample on to the pre-coated target, but the sample is picked up from a well in a fairly standard microplate. The bulk sample is frequently formatted in a 96 well plate, with a number of plates contributing to each MALDI analysis run.

Concentrating large biomolecules in such microplates is not straightforward and it is here that Genevac's centrifugal evaporation technology can help. By protecting samples from over-exposure to heat and by controlling cross-contamination in the plates, Genevac evaporators can significantly improve the results generated from MALDI-TOF analysis. This article looks at how that protection is achieved in practice.

Centrifugal evaporation is, of course, not new. The technique has been used in life science research for 20 years or more, but it was rather crude until fairly recently. Samples were spun sufficiently fast (it was thought) to hold the sample in the bottom of the container as it boiled (evaporated) whilst atmospheric pressure was reduced to induce boiling close to, or below, room temperature. To speed drying, heat could be applied by warming the chamber walls. More recently manufacturers added powerful IR lamps to the system. These focus their IR energy onto the rotating sample, thus providing heat energy to the sample and speeding evaporation. The problems come with the behaviour of complex biological mixtures in such a system, starting with the problems of over-heating.

Heat energy is necessary to replace that lost as latent heat of evaporation in the boiling sample. As the solvent boils, it loses heat energy and cools itself and the container. This slows evaporation further and so energy must be directed into the drying sample to replace that which is lost if a continuous evaporation rate is to be maintained. Infra red heater lamps, which are a development of halogen lamp technology, are very good at providing the necessary heat flow. However, they can be too efficient, leading to over-heating of a sample that has already reached dryness. This is extremely undesirable where proteins and peptides are concerned, as they are thermally labile and easily damaged by temperatures above 40 °C. In order to prevent this situation, it is necessary to be able to measure the temperature of the sample as it spins around. Although that allows control of the heat energy flowing in, it in itself is quite difficult to accomplish. Many manufacturers gave up at this point and chose to control only the temperature of the chamber wall itself, but this is extremely unsatisfactory and provides no direct information on the physical status of the sample.

Genevac overcame this problem in the EZ-2 concentrator/drier by using a finely tuned IR pyrometer combined with sturdy solid aluminium sample holders. The non-contact sensor measures the surface temperature of the aluminium as it passes by and can be accurate to plus or minus 2.5 °C, quite adequate for this application. As heat flow through the aluminium sample block is uniform and because the instrument can control the heat flow to the samples by switching the IR lamps on or off, it is then possible to deduce the actual sample temperature from this data using a simple algorithm. In this way, the EZ-2 allows scientists to pre-select a sample protection temperature suitable for biology applications; normally 35 or 40 °C.

The second problem for highly sensitive samples such as DNA, protein isolates or peptides is one of contamination. While great care may be taken at the spot-picking, excision and loading stages to avoid cross-contamination, the sample micro plates present a unique problem at the concentration stage. In a conventional evaporator the plates may only be spinning at 250-300g. Independent trials by Glaxo Smith Kline have shown that this level of g-force is insufficient to entirely prevent cross-contamination within the plate. Contamination arises as samples begin to “bump” during the evaporation process. Bumping is a widely misunderstood phenomenon that is the major cause of spoilt or contaminated samples in such applications. It can be entirely eliminated by the use of Genevac’s DriPure™ bumping control system. With DriPure enabled, the vacuum is gently ramped down over a period of 30 minutes or so whilst at the same time, the applied g-force is increased to well over 450g to prevent bumping from occurring, by accentuating the boiling point/depth gradient and concentrating all the "hot enough to boil" solvent near the surface. This also creates active convection ensuring good mixing so that temperature gradients do not arise that could cause chaotic mixing of areas of liquid of dissimilar temperature. DriPure also ensures that any material that may eventually be ejected from the liquid surface is kept within the plate well.

GSK studies showed that with DriPure activated, bumping was eliminated even for difficult solvent/solute mixtures in micrtotitre plates, such as acetonitrile/water HPLC fractions and DCM / methanol mixtures.

Another advantage of using the Genevac EZ-2 when pre-concentrating samples in this way is the ability to achieve higher spotting densities, leading to greater sensitivity for low expression proteins, without complicated liquid handling procedures involving repeatable nano-spotting.

Combining these obvious benefits with the unprecedented ease of use that has made the EZ-2 so popular with researchers around the world since it was launched in 2002, it is not hard to understand why prestigious research groups are investigating this new addition to their MS armoury. Scott Dixon, Senior Researcher at UCSF Cancer Research Institute has had an EZ-2 working within his peptide laboratory and specifically with the MALDI-TOF facility for the last half year. Scott comments; *“It’s very easy to use. We spot when we perform MALDI applications as well as electrospray applications. Concentration is important in the spots so we use the EZ-2 concentrator to get the amount of material we spot to be consistent. In addition we use ICAT, which is a type of labelling, that allows us to get quantitative information from the mass spec. ICAT requires us to dry down our peptides completely and in this respect, the EZ-2 is very useful. It has helped us improve our results and by speedily concentrating or drying a number of plates simultaneously helps us get better utilization from the MALDI too.”* The EZ-2 performs the function which was previously done by lyophilisation according to Scott’s colleague Maria Pallavicini who purchased this EZ2 and is based at University of Merced in central California. *“Lyophilisation was very slow; using the EZ-2 to dry down our spots is much quicker,”* she said. Clearly there are significant speed and productivity advantages to be gained by integrating a centrifugal evaporator such as the EZ-2 into a mass spec laboratory.

To find out how the Genevac EZ-2 could fit into your mass spectroscopy programme, please visit our website at www.genevac.com.