On-line coupling of gel electrophoresis and inductively coupled plasma-mass spectrometry

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The on-line coupling of gel electrophoresis with inductively coupled plasma-mass spectrometry (GE-ICP-MS) is a powerful tool for simultaneous separation, detection and quantification of bio-molecules, and has been applied to the determination of phosphorus in DNA, phosphoproteins, and phosphopeptides, gold in nano-particles, iron in metalloproteins, and iodine in aerosols, and cisplatin-oligonucleotide interactions. However, since the first report in 2005, relatively few papers have been published, perhaps reflecting the lack of familiarity with the benefits of this promising methodology. So, here for the first time, we critically review the applications of GE-ICP-MS, and explore the advantages and the limitations of the technique for various applications. Such scrutiny may be useful in not only the development of the technique but also highlighting its potential in proteomics, genomics and metallomics.

Keywords: Agarose gel; Bio-molecule; Buffer contamination; Capillary electrophoresis (CE); Elution buffer; Gel electrophoresis; High-performance liquid chromatography (HPLC); Inductively coupled plasma-mass spectrometry (ICP-MS); On-line coupling; Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

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1. Introduction

The rapid development of techniques based on inductively coupled plasma mass spectrometry (ICP-MS) has opened exciting new opportunities for the characterization of metal and non-metal associated bio-molecules [1]. However, there is still relatively little information about the role of the elements in biochemical processes which has prompted the development of the new field of metallomics. The combination of suitable separation techniques with ICP-MS detection has been a focus of research in recent years and several on-line and off-line techniques have been used for the separation and detection of bio-molecules {e.g., immobilized metal affinity chromatography (IMAC) [2], reversed-phase chromatography [3], size-exclusion chromatography (SEC) [4], and capillary zone electrophoresis (CZE) [5]}. However, for macromolecules, gel electrophoresis (GE) is still widely considered to be the definitive method of separation [6]. The combination of GE with detection methods {e.g., ICP-MS (GE-ICP-MS) [7] and laser ablation (LA) ICP-MS (GE-LA-ICP-MS) [8–12]} has enabled gel-based methodologies to obtain more detailed information about the elemental composition of bio-molecules.

The recently introduced on-line coupling of GE and ICP-MS (GE-ICP-MS) is a very effective way of obtaining such information. It was first described by Brüchert and Bettmer in 2005 [13] for the determination of double-stranded DNA (dsDNA) fragments. More recently, it has been reported for several applications {e.g., size characterization of gold nanoparticles (AuNPs) [14], detection of iron in metalloproteins [15], determination of the degree of phosphorylation in casein [16], iodide and iodate determination in aerosols [17], detection of phosphorus in phosphoproteins [18], cisplatin-oligonuleotide interaction [19] and detection and quantification of phosphorus in plasmid DNA [20]}. GE-ICP-MS could also be applied to other studies in metallomics, genomics and proteomics for separation, detection and quantification of metals and non-metals in biological fluids (e.g., serum, urine, saliva, cerebrospinal fluid, and drugs binding with DNA, RNA, and proteins).

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Coupled GE-ICP-MS enjoys a wide range of benefits over the conventional reversed-phase (RP)-LC-ICP-MS technique [18] (e.g.: cost of the gel is much cheaper than the cost of the column used in LC-ICP-MS and is readily replaceable in case of damage; the flow rate is flexible (μL/min to mL/min) and conventional nebulizers are usually satisfactory; the non-volatile buffers used are more tolerable than the volatile buffers and organic solvents used in most RP-HPLC-ICP-MS separations; and, a wider range of sizes of bio-molecules can be analyzed).

In micro (μ)-LC-ICP-MS and capillary electrophoresis (CE)-ICP-MS:

(1) low flow rates limit the choice of the nebulizer and spray chamber;
(2) miniaturization may reduce robustness of the system [21];
(3) μ-LC may not be very useful in analyzing large bio-molecules; and,
(4) limited sensitivity and poor migration time are considered the major disadvantages of CE-ICP-MS [22].

Similarly, in some cases, interaction of the analytes with the capillary wall may cause sample loss in CE [22]. However, in μ-LC and CE, smaller ID columns require low flow rates (μL/min or nL/min), which provide positive environmental and economic benefits.

The combination of CE with an ICP-MS detector potentially offers a tool of unique quality and enables both charged and neutral analytes to be separated in a single run [23,24]. Thus, a range of metalloproteins were determined using CE-ICP-MS [22], but this trend has been decreasing slowly for the past few years and the technique has recently been more popular for non-metalloprotein analysis [22].

GE-ICP-MS also has some advantages over SEC-ICP-MS. The commercially-available SEC columns are very expensive and the separating media, mostly made up of Sepharose or Superdex gels, cannot tolerate very high pressure. However, in GE-ICP-MS, the elution buffer is taken from a separate buffer reservoir or an embedded capillary inside the gel, so that a high flow rate can easily pass through and does not damage the gel. To prevent drying out, SEC columns also need some extra care. The most important disadvantage of SEC-ICP-MS is the common use of very high buffer concentration in the mobile phase, which may increase contaminant levels and thus increase the limit of detection (LOD) of the analytes. In the majority of SEC-based analyses (e.g., serum-protein analysis), the best separation is achieved by employing phosphate buffers that preclude the detection of P as an analyte by ICP-MS.

LA-ICP-MS is a powerful way of detecting analytes on solid substrates, but comparison of GE-ICP-MS with GE-LA-ICP-MS shows that the former has several benefits. The clear-cut benefit of GE-ICP-MS is that it is a single-step analysis [while GE-LA-ICP-MS requires two stages (i.e. separation followed by detection)] and is much less expensive. The two-step process may cause the loss of the analyte. Curling of the gel during LA analysis is also a difficult issue, usually requiring transfer of the analytes onto a membrane, and that may cause further sample loss. Moreover, poor LOD, loss of the weakly-bound metals and staining and de-staining of the analytes (in some cases) further reduce its efficacy compared with GE-ICP-MS.

However, GE-ICP-MS also has several limitations and requires further work to overcome the problems. These limitations include:

(1) broader peaks;
(2) the possibility of fragmentation of the proteins in the gel [18];
(3) contamination from the gel materials [18]; and,
(4) a time-consuming separation compared with CE [25] and μ-LC [26].

The separation of the analytes in GE, based on their isoelectric points (pI values), can be a very slow process and can take several hours, so it may not be well suited for GE-ICP-MS. For metalloproteins analysis by GE-ICP-MS, native-polyacrylamide GE (native-PAGE) is preferred to sodium dodecyl sulfate (SDS-PAGE) as significant loss of non-covalently bound metals has been reported in SDS-PAGE [10,27–29].

2. Gel-electrophoresis separation strategies

2.1. Polyacrylamide gel electrophoresis for separation of proteins

PAGE, either one-dimensional (1D) or two-dimensional (2D), is routinely used for the separation of proteins in proteomic studies. In this technique, polyacrylamide is used as a separating medium because it can withstand high voltages, and it is highly thermostable, chemically inert and can be prepared with different pore sizes. Protein separation can be performed using either SDS-PAGE or native-PAGE. Much has been written about PAGE-based separation methods and there is further information in the following references [17,30,31].

2.2. Agarose gel electrophoresis for separation of DNA and RNA

The basic reason for using agarose gels is the size of DNA and RNA molecules. The pore sizes of the polyacrylamide gel, even with very low percentage gels, are not suitable for separating the DNA molecules, because they are much larger in size, so an agarose gel with different percentages is used to achieve the separation of DNA. However, oligonucleotides can be separated using PAGE. More detailed information is available [32–34].