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RESEARCH PAPER

TITLE

USE AND ADVANCEMENT OF ANALYTICAL AND INSTRUMENTATION SYSTEMS: TWO-DIMENSIONAL GEL ELECTROPHORESIS, ELECTROSPRAY IONIZATION, MATRIX ASSISTED LASER

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Abstract

Gel electrophoresis is a technique which is used for the separation purpose and its applications are in pharmaceuticals, in health especially in cancer detection. Matrix assisted laser ionization is also a technique which is used for crude oil refinement and also used for protein separation. Electrospray ionization is a technique in which it works as a combination with the mass spectrometry. And it has more reproducibility. Electrospray also has both the acidic and basic ionization modes.

Introduction:

Gel electrophoresis is a flexible method which is mostly used in proteomics and is used for distillation of proteins. There are different methods for the separation of the proteins but the main technique which is used is two-dimensional gel electrophoresis.it not only separate the proteins but it can also quantify the number of proteins. This technique is mostly used as a combination with the mass spectrometry (Safmeh Magdeldin et al, 2014). Matrix assisted laser ionization is used for the petroleum base substances which cannot be separated by the mass spectrophotometer. It is a more suitable method for the crude oil which is processed by this method. Matrix assisted laser ionization is mostly used in the oil-based industry. It is also used for the recycling of biomass (Daniel *et a*l,2020). Electrospray is also a technique which gives us both the quality base readings and also gives us quantity base readings. In this technique the molecules are firstly added in the mass spectrometer and after this ionization process will be started. These techniques are mostly used in pharmaceuticals and in the protein separation as well (CS *et al.,* 2003).

Basic principle:

Two-dimensional gel electrophoresis has two main steps which is first dimension and second dimension (S Magdeldin *et al,* 2012).On the basis of their iso electric point, the protein molecules are sorted out and this action is performed in the first dimension. Proteins are separated as a pH gradient which permits the intense band recovering by the usage of tactics which are immobilized gradient electrophoresis and isoelectric focusing (S Magdeldin *et al,* 2012). By using the SDS Lemli or Tris-Tricine buffers proteins are separated on the basis of molecular weight. It's done in the second dimension.it is noted that the different proteins have same physiochemical properties but that are also separated by using the two-dimensional gel electrophoresis (Anderson *et al.*, 2001).

Differential gel electrophoresis (DIGE) was first conceived as a means of identifying mutated proteins. How do the biochemistry and physiology of cells adapt to new mutations? Maximum protein separation by 2-DE 1-3 was state-of-the-art when it was first developed. It was hypothesized that due to the heterogeneity of 2-DE and the complexity of cellular extracts, running side-by-side comparisons would be a futile

exercise, and that a more fruitful strategy would be to develop an internally controlled method in which the sample and its control are run on the same gel. A means of variably marking all proteins within a cellular extract would be necessary for this internally regulated strategy. Fluorescent labelling reagents were sought for to make the technique more accessible to the typical researcher. The fluorescent, DIGE dyes were developed using four design rules: I each matched set of dyes must react with the same amino acid residues, (ii) the dyes must have similar molecular masses, (iii) the dyes must have distinct fluorescent characteristics, and (iv) the charge of the target amino acid must be preserved. There were no appropriate fluorescent dyes available when the idea was first conceived. In order to fulfil these needs, the DIGE dyes were developed; they have a cyanine-dye backbone. The DIGE dyes were conceptualized at a lunch meeting between J. M. and Dr. Alan Waggoner, the "father" of using cyanine dyes in biological study. Carnegie Mellon undergraduate Mary Morgan and doctoral student Mustafa Ünlü were given the napkin with the dye structures on it and went on to synthesize the first batch of DIGE dyes. Using a liquid-cooled CCD camera and an IKEA kitchen cabinet, J. M. built the first 2-D-DIGE, gel-imaging system. In the future, Amersham Biosciences collaborated with the DIGE dyes' (CyDyesTM) commercial development (now GE Healthcare).

To identify proteins with fluorescent dyes

We set out to reduce the negative impact of most covalent modifications on protein migration on 2-DE gels. It is important to keep the tagged proteins at their natural isoelectric point, which is achieved by preserving the charge of the specific amino acid residue. Protein movement in the molecular mass dimension of 2-DE gels is always impacted by the use of dye labelling. Instead of actually changing the protein's mass, the dyes' influence on SDS binding is responsible for the observed mass change. There are two levels of protein labelling that have been proven to be suitable for reducing this apparent mass perturbation effect: minimal labeling, where each protein carries a maximum of one dye molecule, and saturation labeling, where 100% of all reactive residues are dye-coupled. Protein populations will exhibit size heterogeneity if labels are applied somewhere in between these two extremes. One set of DIGE dyes is reactive to lysine, and the other set is reactive to cysteine. Thirty lysine residues make up the average 50 kDa protein. Protein precipitation and a significant increase in apparent molecular mass resulted from attempts to mark all lysine residues. Therefore, we resorted to using lysine-reactive dyes for light protein labelling (referred to as minimal-labeling Cy2, Cy3 and Cy5). To restrict labelling to a single lysine residue per protein, conditions were created so that only around one in twenty proteins contained the label. It has been found that the addition of a single dye molecule has a minor effect on the apparent molecular mass of proteins larger than 30 kDa when compared to unlabeled proteins. Under 30 kDa proteins that have been minimally tagged typically show a shift in velocity of less than half the diameter of the protein spot compared to their unlabeled counterparts. Protein spots are often selectively removed for MS analysis 5 after being stained with a specific dye after electrophoresis.

A second group of DIGE dyes is reactive with cysteine. Despite there being fewer cysteines per protein, the very soluble zwitterions that make up cysteine-reactive dyes make them well suited for saturation labeling of all cysteine residues. Thus, low-protein content samples can be successfully analyzed by 2-

DE because of the increased sensitivity for protein identification. Saturation labelling has been used successfully in recent years to detect protein changes in small samples ranging from 1000 to 5000 cells, as shown by a number of publications (6-10). Both of these DIGE labelling approaches offer efficient strategies for generating differentially labelled samples for fluorescence-based proteome comparisons.

There was no available fluorescent gel imaging equipment for DIGE gels prior to the development of this method. The fluorescent signals from DIGE gels can be imaged using one of two methods. The first is a CCD-based setup with open-gel arrangement and wide-field illumination for easy access by a spot-cutting robot. Laser scanning of the gel between two low-fluorescence glass plates is used in the second imaging system. Both methods produce high-resolution, wide-dynamic-range photographs of DIGE gels. In order to remove protein spots from the gel for MS analysis, the laser scanning technique necessitates the use of a second instrument. The most important part of conducting insightful DIGE-based proteomics investigations is the experimental design and analysis, thanks to the development of acceptable DIGE dyes and sensitive imaging devices (Minden *et al.,*2009).

Recent Advances and technologies associated with two-dimensional gel electrophoresis:

Using images, a novel method called differential imaging gel electrophoresis has been developed. Two-dimensional gel electrophoresis is made more sensitive and repeatable by using multiplexed fluorescent dye-labeled protein samples (Falvo S, et al 2011). This method involves running multiple samples on a single gel to examine intra-gel and inter-gel variation. Proteins from various sources are labelled with fluorescent cyanine dyes. Proteins that are identical in each sample will transfer to the gel in the same way when the samples are run together, allowing their abundance to be calculated with relative ease using the corresponding dye and picture (Falvo et al., 2011).

Among the most effective methods for separating and fractionating complicated protein mixtures from tissues, cells, or other biological samples is two-dimensional polyacrylamide gel electrophoresis (2-DE). One gel can separate hundreds to thousands of proteins. When the fundamental improvements and high resolution 2D-E modification were made, the method quickly gained traction and became all-encompassing (Farrell *et al.,*1975). It was O'Farrell's improvement to the 2-DE method that made it possible to resolve up to 5000 protein-representing dots in a uniform 2-dimensional distribution and to reliably separate these spots (Magdeldin *et al.,*2012).

The 2-DE method is widely regarded as a driving force in the development of proteomics and protein research. It's the initial step toward learning more about the differentially regulated proteins found using mass spectrometry and western blotting. Two-dimensional (2-DE) analysis has been well defined in many applications, revealing physiological systems and proteins related with clinical diseases that can aid in the discovery of biomarkers.

Prefractionation, enrichment, and depletion prior to 2DE:

To reduce the sample complexity protein sample prefractionation has been implemented. The low abundance protein represented in 2DE.proteins have higher peptides which is perfectionated samples (Stasyk T, *et al* 2004). When the sample complexity reduces then low molecular proteins are easily identified. There are three

levels of sample prefractionation and these are cellular, subcellular and protein sub fractionation. For the whole proteome screening the cellular extraction method is used.NP-40 with buffer containing urea and thiourea used to recover proteins (Rabilloud *et al.*, 1997).

A density gradient centrifugation method (Biosvert FM, *et al* 2010) is used in subcellular prefractionation. On the base of physiochemical properties is another technique which is protein sub fractionation (Carsio S, *et al* 2011). In this technique, proteins which has strong cation exchange methods are fractionated. Protein fractionated also based on liquid phase on prefractionation (Zhu e*t al.,* 2004).

Blue native gel electrophoresis:

Schlager and von jagow was introduced the blue native gel electrophoresis. This technique is used to separate the enzymatically active membrane protein complexes. Protein sample is mixed with the anionic dye Coomassie Brilliant blue G-250 before the gel loading. Negative charges appear on the surface because of this dye. Protein complexes are bind with the dye and moves in the electrophoresis chamber (Kofoed EM, *et al* 2013). At the end the gel is stained with the dye and this technique is used in inflammasomes and integrin and in the placenta *(*Wang *et al., 2013)*.

Protein complexes in biological membranes and entire cell or tissue homogenates can b e isolated in a single step with blue native P AGE (BN-PAGE).

Physiological protein-

protein interactions can be identified, and n ative protein weights and oligomeric states c an be calculated.

Recovering native complexes from gels by electroelution or diffusion enables their usag e in in-

gel activity tests, native electroblotting and immunodetection, and 2D crystallisation and electron microscopy.

Methods for performing BN-

PAGE, followed by I native extraction or na tive electroblotting of separated proteins, (ii) a second dimension of tricine-SDS-

PAGE or modified BN-

PAGE, or (iii) a second dimension of isoele ctric focusing (IEF) followed by a third dim ension of tricine-SDS-

PAGE for the separation of subunits of com plexes, are detailed in this protocol (Witing et al., 2006).

2 DE for the post translational modifications:

For the cell biology and disease treatment post, translational modifications are used and in these two main tools are used and these are ProMoST (Halligan BD,*et al* 2004)and JvirGel (Hiller K *et al,* 2003).For the PTM monitoring specific dies are developed. Molecular probes, Pro-Q Diamond phosphoprotein gel stains are used to provide a precise method for results. Lissamine rhodamine B sulfonyl hydrazine is used for the staining of glycoproteins. Lectin binding strategy and enzymatic based techniques are used which have high resolution and high reproducibility (Farley *et al.,* 2009).

Post-translational modifications generate tremendous diversity, complexity and heterogeneity of gene products, and their determination is one of the main challenges in proteomics research. Recent developments in mass spectrometry-based approaches for systematic, qualitative and quantitative determination of modified proteins promise to bring new insights on the dynamics and spatio-temporal control of protein activities by post-translational modifications and reveal their roles in biological processes and pathogenic conditions. Combinations of affinity-based

enrichment and extraction methods, multidimensional separation technologies and mass spectrometry are particularly attractive for systematic investigation of post-translationally modified proteins in proteomics (Jensen., 2004)

Non-Equilibrium PH gel electrophoresis:

This technique is developed to sort out proteins with basic to high acidic pH and it is not possible to separate them by traditional method. The protein loss is less in this method. The non-equilibrium PH gel electrophoresis show good reproducibility in the basic gel zone and has also good reproducibility in acidic zone as well (Magdeldin *et al.,* 2012).

It is possible to separate proteins with very basic isoelectric points (pH 7.5-11.0) using a method called nonequilibrium pH gel electrophoresis (NEpHGE) (1,2). The addition of urea in IEF gels has a buffering effect, preventing the pH gradient from reaching the very basic values (with a pH above 7.3-7.6), making it difficult to resolve these proteins with regular IEF. Also, many extremely basic proteins are lost as they move away from the gel's edge due to cathodic drift. Instead of being concentrated at their isoelectric point, proteins undergo NEPHGE by moving at varying velocities throughout the gel as a result of their charges. Because of this, the pattern's distribution over the gel is established by the total number of volt hours. Therefore, reliable patterns can only be established if volt hours are consistently measured (Lopez *et al.,*1999).

2DE software analysis:

Spot detection and image alignment are the workflow for the software analysis. Sometimes some data is missing in the spot detection and thus leads to the mismatching error. Workflow is another method which is used to avoid the mismatching error. And in the workflow alignment of gel imaging is also used which increases the accuracy and provide more replications which in turn save time (Hoogland *et al.,* 1999).

The image analysis component of gel-based proteome research is critical to the experiment's overall effectiveness. The primary goal of software-assisted 2DE gel analysis is to recognize protein spots, match them across gels within an experiment, and discover any variations in protein expression between samples. Automated image processing techniques are required for effective protein expression analysis. There are various aspects to consider while selecting a software solution and implementing the analysis itself. The methods for spot matching, normalization, and background subtraction provided by the 2DE analysis program are critical to the successful quantification of protein expression levels. This chapter includes methodologies for quantitative and qualitative image analysis quality evaluation, in addition to generic protocols for image acquisition and subsequent 2DE image analysis (using Progenesis PG200) (Levanen at *el.,* 2009).

Applications and Advantages:

it is the most powerful technique which is used to separate the proteins at once.2DE justify the direct changes in the post translational modification which could not be detect by the genome sequence. This technique is used in whole proteome analysis (Magdeldin *et al.,* 2010), cell differentiation (Jungblut PR, 1990),detection of biomarkers and disease presence, drug research, cancer

biomarkers (Wu et *al.,* 2002), bacterial pathogenesis (Fanany *et al.,* 2013), purity checks, microscale protein purification and product characterization (Fanany *et al.,* 2013). There is robustness in the workflow of two-dimensional gel electrophoresis. There is a unique feature of the two-dimensional gel electrophoresis which is visualized mapping analysis. The main feature of the 2DE is that it can sort out the intact length of the proteins (Chevalier *et al.,*2008*)*. It also detects the physiochemical properties of the proteins with the possible quantification. Peptide mass finger could be used for the protein of interest.2DE also identify the PTMs and protein isoforms.it is also a compatible platform for further analysis (Chevalier *et al.,* 2008).

Electrospray ionization:

Principle;

Electrospray ionization techniques are widely used because it takes place at atmospheric pressure and thus it ionizes a wide range of acidic and basic functional groups which are polar hydrophilic molecules. The sample contains water, organic solvents and solvent generated ions. The mechanism of action is that there is a needle and capillary present and high voltage is passed from these which causes the sample to burst into the small droplets which are charged.

There are two categories of the electrospray ionization and these are direct and indirect modification. In the direct modification sample is directly mixed with the electrospray solvent for ionization (Anil Kumar *et al.,* 2017). The critical parameters for the formation of electrospray are flow rate, pressure, sheath gas, and voltage. In the indirect technique, the solution containing analytes and electrospray solvent did not produce ionization. Solvents and samples are mixed in this process via fusion and collisions (Kumar *et al.,*2017).

Electrospray ionisation is the most common ionization technique used in chemical and biological investigation today. It can investigate the molecular composition of liquid samples when connected to a mass spectrometer. A wide range of chemical compounds can be ionised using electrospray. Because there are no mass constraints, even massive noncovalent protein complexes can be studied. Because proteins can be identified and measured on trace levels in a high throughput fashion, its great ionization efficiency has radically transformed biomolecular sciences (Wilm.,2011). The tests were meant to establish the relative role of gas phase and solution phase mechanisms in the observed ionization suppression in biological sample extracts. According to the results, gas phase processes that result in the loss of net charge on the analyte are unlikely to be the most important activity involved in ionization suppression. The findings indicate that changes in droplet solution characteristics due by nonvolatile solute content are the primary source of ionization suppression in electrospray ionization of biological extracts (King *et al.,*2000).

Recent Advances

Flow rate has a tremendous effect on the efficiency of the electrospray ionization. Ionization efficiency is increased as the sample flow rate decreased (Kumar *et al.,* 2017). Due to this reason, it is used in nano electrospray for MS spray. The nano electrospray is dependent on the fluid surface tension, solvent composition, conductivity of the fluid and the applied voltage and pressure's ion source is pressurizing to a gas pressure which is high than the atmospheric pressure. Electrospray ionization has been demonstrated that it has

super atmospheric pressure. Without applying a voltage to generate spray, ionization can be achieved by gas flow with a sonic and supersonic speed. Cooks and his coworkers first describe the paper spray-MS. The paper substrate with the mixture of analytes van be achieved electrospray when a high voltage is applied (Kumar *et al.,* 2017). This technique is applied in different fields such as bio analysts, pharmacokinetics, forensics, microbiology and food science (Rachel *et al.,* 2007).

Electrospray ionization technique can be operated in both modes positive and negative ion modes. Each ionization spectra give different mass spectrum even if it has same sample because it depends upon the ionization mode. There is another technique which is used for employed to obtain structural information on the base of specific masses in the spectrum and this technique name is tandem mass spectrometry. Difference mass spectra is also a technique which is used to compare the two samples which are similar and it is prepared to highlight the changes in the chemical composition of the sample. Mostly it is used to visualize the variation in the spectrum. There is also a stand rise reference spectrum present which can be used to control and analyses the spectrum (Rachel *et al.,* 2007). This technique is mostly used to compare a jet fuel which is weathered and a jet fuel which is not weathered and thus it is also used to compare it with the jet fuel of the contaminated soil. Thus, it helps to determine the effect of weathering on the jet fuel and also identify the contaminants in the soil (Rachel *et al.,*2007).

Applications and Uses

Screening for inborn errors of metabolism, neonatal screening for galactosemic, neonatal screening for cholestatic hepatobiliary diseases, screening for peroxisomal disorders, screening for disorders in purine and pyrimidine metabolism, identifying and quantifying haemoglobin variants, and quantifying glycohemoglobin are all examples of clinical applications of this method (CS *et al*., 2003).

Understanding chemical processes through mechanistic studies allows scientists to create more environmentally friendly reactions by improving product yields, decreasing waste, and decreasing resource and energy usage. Kinetic investigations, isotope labelling, trapping of reactive intermediates, and sophisticated spectroscopic techniques are some of the conventional approaches for investigating reaction processes in solution (Schroder., 2012). Since scientists can directly examine liquid samples by mass spectrometry under mild conditions using electrospray ionization mass spectrometry (ESI-MS), it has become a useful technique for mechanistic studies within the past decade (Heravi *et al.,* 2021).

Matrix assisted laser ionization

Principle

Matrix-assisted laser ionization is a technique for detecting big, unbroken molecules. When combined with the growing number of available genome sequences, it is employed in proteomics to detect minute amounts of digesting proteins (Tyesha *et al.,* 2017).

carbohydrate analysis using matrix-assisted laser desorption/ionization (MALDI) mass spectrometry from 1991 to 1998. Carbohydrates benefit greatly from this method because both underivatized and derivatized molecules can be studied. Some examples of MALDI matrices used for carbohydrate analysis are given, and derivatization to increase mass spectrum detection limits is explained.

Highly sensitive mass spectrometric methods are examined with respect to sample preparation and the extraction of carbohydrates from biological medium prior to mass spectrometric analysis. The correlation between MALDI signal strength and mass and complex structure is discussed quantitatively. The potential of MALDI to generate fragmentation spectra and the importance of mass measurements to determine carbohydrate content are both emphasized. In this context, ions that reveal carbohydrate linking and branching patterns are of particular interest, thus we discuss their utilization in the context of in-source and post-source decay and collision-induced fragmentation. Methods for the examination of both N- and O-linked glycans are detailed, as is the use of MALDI mass spectrometry in conjunction with exoglycosidase sequencing to characterize glycans on glycoproteins. At the review's conclusion, we discuss how this method has been used to study carbohydrates in a wide range of contexts, including glycoproteins, bacterial glycolipids, sphingolipids, and glycolipids (Harvey, 1999).

Recent advances:

The matrix assisted laser ionization has different procedures and the quality of matrix deposition affect the detectability (Zavalin *et al.,*2013). Matrix solution spray coating used by the traditional method and also automatic method is used. In the matrix coating usually the particle size is about 10-15 micrometer which is adequate for the tissue sections. The high resolution of matrix laser assisted ionization is the advancement. There is another advancement in the MALDI which is targeted MALDI. There is one more technique which is advancement of the three-dimension imaging. In this technique the data compiles from multiple images that allow the researcher to visualize the 3D format. Within the whole organ 3D imaging can provide the insights.it is used for both on the two modes which are positive ion detection and negative ion detection mode. The low molecular weight compounds are imaged without targeted matrix assisted laser ionization (Franz *et al.,* 2000). The analytics obtained by the MS as to mass to charge ratios also shows 2 dimensions or 3 dimensions size maps. Matrix assisted laser ionization play a major role in image testing. Proper organic chemicals to act as matrices and the assumptions of different analytes can be selected based on in their acid base neutralization machine. High label testing is great sample set using mass spectrometry which is beneficial and increase the attention in latest years (Martin *et al.,* 2011). And thus reduced the numbers of specific side effects. The side effects advantage in contrast to non-matrix assisted laser ionization labeled tests methods such as fluorescence-based testing. And now recently requires a lot of samples preparations using the expensive label dyes. So far, the main focus of mass spectrometric HTS has been in electrospray ionization and solid-state matrix assisted laser desorption ionization. Electrospray ionization is a flexible and well-studied ionization platform which has a wide range of interesting pharmaceutical chemicals (Quan *et al.,* 2020). However, for a major setback for the electrospray ionization is the lack of speed in sample delivery which is required in HTS applications. One of the biggest advances in matrix assisted ionization over the past few years has been the expansion of space of MI decision. The local solution for the low molecular weight and width is that it often requires in many biological programs and their cellular fingers or under the cellular cells are required. This has resulted in refinement and development of high local adjustment and high MS sensitivity tools. Another way to expand the optics of

ionization source is to minimize laser beam size to 5mm (Wang *et al.,* 2013).

Applications:

Matrix assisted laser ionization is used in clinical settings, pharmaceutical and biological researches (Franz Hillenkamp*, Matrix-assisted laser desorption/ionisation, an experience, 2000). This technique is also used in the detection of metabolic biomarkers present in human diseases especially human cancer. Recently this technique is also used for the detection of chemotherapeutic pro-drug and to check its active metabolites. It is also used in the visualization of colon cancer and is also used as lipid biomarkers. In the standard matrix assisted test, expanded tissue type inserted into the thick pieces and these are inserted because the purpose is moving sample, for example the slides of glass are cover with indium titanium oxide Toaster this samples are joined with the matrix assisted laser ionization which are usually acidic or basic to extract analyses from the body tissues. Under the machine or in the matrix stress there are mainly two types of the energy draws which are ultraviolet and infrared rays which illumination of the laser beam to desorb and ionize the analytics in the plume of the matrix (Martin *et al.,* 2011).

References:

Anderson NG, M. A. (2001). Back to the future : the human protein index (HPI) and the agenda for post-proteomic biology. *proteomics*, 1;3-12.

Anil Kumar, M. a.-C. (2017). Electrospray Modifications for advancing Mass spectrometric Analysis. *mass spectrometry*, 6(2):S0057.

Biosvert FM, l. Y. (2010). A quantitative proteomics analysis of subcellular proteome localization and changes induced by DNA damage. *Mol cell proteomics*, 9:457-470.

Carsio S, Z. I. (2011). MUC1 protein expression in tumor cells regulates transcription of proinflammatory cytokines by forming a complex with nuclear factor-kappaB p65 and binding to cytokine promoters:importance of extracellular domain. *J Biol chern*, 286:42248-42256.

Chevalier M, L. S. (2008). Sweet silver:a formaaldehyde-free silver staining using aldoses as developing agents,with enhanced compatibility with mass spectrometery. *proteomics*, 8:4853-4861.

CS Ho, C. L. (2003). Electrospray Ionisation Mass Spectrometry: Principles and Clinical Applications. *CLIN biochem rev*, 1-10.

CS Ho, C. L. (2003). Electrospray ionization mass spectrometry:principles and clinical applications. *clin biochem Rev*, 1-10.

Daniel J ryan, q. q. (2020). laser based ionization:A review on the use of matrix assisted laser desorption/ionization mass spectrometry in petroleum research. *energy and fuels*, 11887-11896.

Falvo S, D. C. (2011). 2-D DIGE analysis of UV-C radiation-responsive proteins in globe artichoke leaves. *proteomics*, 12(3):448-60.

Fanany S, Y. Y. (2013). Two dimensional electrophoresis of the exo proteome produced from community acquired methicilin resistant staphylococcus

aureus belonging to colon complex 80. *MicrobiolRes*, 168:504-511.

Farley AR, L. A. (2009). Identification and quantification of protein posttranslational modifications. *Methods Enzymol*, 463:725-763.

Franz Hillenkamp*, M. K. (2000). Matrix-assisted laser desorption/ionisation, an experience. *international journey of mass spectrometery*, 71-77.

Franz Hillenkamp*, M. K. (2000). Matrix-assisted laser desorption/ionisation, an experience. *international journey of mass spectrophotometry*, 71-88.

Halligan BD, R. V. (2004). ProMoST (protein modification screening tool): a web based tool. for mapping protein modifications on two dimensional gels. *Nucleic Acids Res*, 32:W638-644.

Hiller K, S. M. (2003). JVirGel: calculation of two virtual two-dimensional protein gels. *Nucleic acids Res*, 31:3862-3865.

Hoogland C, S. J. (1999). The 1999 SWISS 2DPAGE database update. *Nucleic Acids Res*, 2000(28)286-288.

Jungblut PR, S. R. (1990). Analysis by high-resolution two-dimensional electrophoresis of differentiation-dependent alterations in cytosolic protein pattern of HL-60 leukmic cells. *j Biochem biophys methods*, 21:47-58.

Kofoed EM, V. R. (2013). Blue native polyacrylamind gel electrophoresis to monitor inflammasome assembly and composition. *Methods Mol Biol*, 1040:169-183.

Magdeldin S, L. H. (2010). Comparison of two dimensional electrophoresis mouse colon proteomes before and after knocking out aquaporin 8. *J Proteomics*, 73:2031-3040.

Martin Welker, E. R. (2011). systematic and applied microbiology. *journel of elsvier*, 2-11.

Quan, D. J. (2020). Laser-based ionization:A review on the use of matrix assisted laser desorption/ionization and laser desorption/ionization Mass spectrophotometry in petroleum research. *Energy and fuels*, 11887-11896.

Rabilloud T, A. C. (1997). Improvement of the solubilization of proteins in two-dimensions electrophoresis with immobilized pH gradients. *Electrophoresis*, 18:307-316.

Rachel L, S. a. (2007). The application of electrospray ionization coupled to ultrahigh resolution mass spectrometry for the molecular characterization of natural organic matter. *journel of mass spectrometery*, 42:559-574.

S Magdeldin, Z. Y. (2012). Biochemistry, Genetics and Molecular Biology *Gel Electrophoresis-Principles and Basics*. *Rijeka, Croatia: INTECH*.

Safmeh Magdeldin, S. e. (2014). basics and recent advances of two dimensional polyacrylamide gel electrophoresis. *clinical proteomics*, 11-16.

Stasyk T, H. L. (2004). zooming in: fractionation strategies in proteomics. *proteomics*, 4;3704-3716.

Tyesha C Baker, J. H. (2017). Recent Advancements in matrix assisted laser desorption/ionization mass spectrometry imaging. *current option in biotechnology*, 43.62-69.

Wang F, W. I. (2013). Identification and analysis of multi-protein complexes in placenta. *PloS one*, 8:62988.

Wu W, T. X. (2002). Identification and validation of metastasis-associated proteins in head and neck cancer lines by two dime signal electrophoresis and mass spectrometry. *Clin Exp Metastasis*, 19:319-326.

Zavalin A, Y. J. (2013). Laser beam filtration for high spatial resolution MALDI imaging mass spectrometry. *j am soc mass spectr*, 24:1153-1156.

Zhu K, Y. F. (2004). Proteomic analysis using 2-D liquid separations of intact proteins from the whole-cell lysates. *Cure protoc protein sci*, chapter 23:Unit 23.

Minden, J.S., Dowd, S.R., Meyer, H.E. and Stühler, K., 2009. Difference gel electrophoresis. *Electrophoresis*, *30*(S1), pp.S156-S161.

Magdeldin, S. ed., 2012. *Gel electrophoresis: Principles and basics*. BoD–Books on Demand.

Wittig, I., Braun, H.P. and Schägger, H., 2006. Blue native PAGE. Nature protocols, 1(1), pp.418-428.

Jensen, O.N., 2004. Modification-specific proteomics: characterization of post-translational modifications by mass spectrometry. Current opinion in chemical biology, 8(1), pp.33-41.

Lopez, M.F., 1999. Nonequilibrium pH gel electrophoresis (NEPHGE). 2-D Proteome Analysis Protocols, pp.129-131.

Levänen, B. and Wheelock, Å.M., 2009. Troubleshooting image analysis in 2DE. In Two-Dimensional Electrophoresis Protocols (pp. 112-129). Humana Press.

Harvey, D.J., 1999. Matrix-assisted laser desorption/ionization mass spectrometry of carbohydrates. *Mass spectrometry reviews*, *18*(6), pp.349-450.

Wilm, M., 2011. Principles of electrospray ionization. *Molecular & cellular proteomics*, *10*(7).

King, R., Bonfiglio, R., Fernandez-Metzler, C., Miller-Stein, C. and Olah, T., 2000. Mechanistic investigation of ionization suppression in electrospray ionization. *Journal of the American Society for Mass Spectrometry*, *11*(11), pp.942-950.

Tina Heravi, Jiewen Shen, Spencer Johnson, Matthew C. Asplund, David V. Dearden. Halide Size-Selective Binding by Cucurbit[5]uril–Alkali Cation Complexes in the Gas Phase. *The Journal of Physical Chemistry A* **2021,** *125* (36) , 7803-7812. https://doi.org/10.1021/acs.jpca.1c05060

Schröder, D., 2012. Applications of electrospray ionization mass spectrometry in mechanistic studies and catalysis research. *Accounts of chemical research*, *45*(9), pp.1521-1532.