Research and Reviews: Journal of Dairy Science and Technology
Volume 2, Issue 2, ISSN: 2319-3409

NALDI-TOF MS: Applications in Dairy and Related Sectors

Kamal Gandhi*, Anil Kumar, Prabin Sarkar, Ashok Aghav, Darshan Lal
Dairy Chemistry Division, National Dairy Research Institute, Karnal, Haryana, India

Abstract

NALDI-TOF (Nanotechnology-assisted laser desorption/ionisation time-of flight mass spectrometry) is a matrix free soft laser base MS technology used for low molecular weight hydrophobic biomolecule and organic molecule. NALDI-TOF MS is the new generation of Mass Spectrophotometers after MALDI-TOF MS. Its analyzing capacity is far better than MALDI-TOF MS in terms of speed, sensitivity and accuracy. This technique has found applications in a variety of disciplines including dairy, food, chemistry, biochemistry, microbiology, agriculture and physics. The purpose of this review is to summarize the contribution of the studies already performed with NALDI-TOF MS concerning dairy and related sectors.

Keywords: *NALDI-TOF MS, nanostructure, DIOS, biomolecule*

****Author for Correspondence:*** E-mail:kamalgandhi4444@gmail.com

INTRODUCTION

NALDI (Nanotechnology-assisted laser desorption/ionization) is among the first commercially available matrix-free methods for laser desorption ionization (LDI) of small organic compounds (< 3000 Da). Ionization takes place on a surface with densely packed alumina nanostructures (100/μm^2) coated with perfluorinated silane. The structures have diameters of approx. 20 nm and lengths of 100–500 nm [1, 2]. The perfluorinated coating adds ultra-hydrophobic properties to the nanostructured surface, which allows dense application of analytes and optimizes ionization, regardless of the type of solvent used. NALDI is somewhat similar to MALDI. For both methods, ionization occurs by illumination of a solid sample with a short laser pulse. In the case of MALDI, the analyte molecules are co-crystalized with a matrix, which absorbs in the wavelength of the laser, and this is spotted on a metal plate (target). The laser pulse results in rapid heating of the sample, leading to a micro explosion that ejects a plume of matrix and sample away from the target (Figure 1).

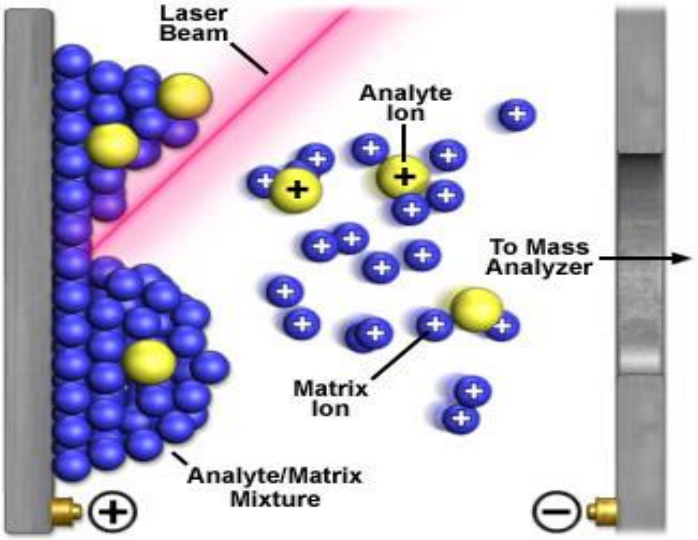

Fig. 1: *The MALDI Process.*

 © STM Journals 2013. All Rights Reserved

During this process the analytes are simultaneously desorbed and ionized within the plume by intermolecular interactions, usually proton transfer.

In NALDI, the laser pulse is absorbed by the nanostructured surface of the target. Some of the laser energy is transferred to analytes present on the nanostructures, causing them to be desorbed. Ionization is believed to occur just above the active layer of the NALDI surface by proton transfer reactions between analytes and protonated species originating from the active layer that are generated through a series of photochemical reactions [1]. In contrast to MALDI, NALDI and other matrix free techniques [2] do not suffer from matrix background in the mass range below m/z 700. In addition, non-uniform distribution of analytes within the matrix film, causing analyte "hot spots", can be minimized. Further, the process of selecting a suitable matrix that is miscible and may co-crystallize with the analytes is avoided. Thus, fast and sensitive analysis of polar and medium polar organic compounds is possible with little or no sample preparation using NALDI.

NALDI targets were previously shown to have excellent performance for small molecule analysis with 2–10 times lower detection limits in MS mode and up to 10 times lower detection limits in MS/MS mode in comparison to conventional MALDI preparations on polished steel targets. Their performance was also proven for capture and analysis of assays.

Principles of NALDI-TOF

A number of methods have been investigated for matrix-free analysis MS using nanostructured targets to avoid low mass (less than 1000 Da) interference from matrix ions. Researchers have investigated a number of nanostructured surfaces, including porous silicon, silicon nanowires, carbon nanotubes, and porous alumina. These matrix alternatives absorb the energy of the laser and aiding in ionization. Based on these architectures, 100 spot disposable nanostructure-assisted lasers desorption ionization (NALDI) targets have been commercially developed for the analysis of low mass molecules. The nanostructures are silicon nanowires grown from silane vapor with a diameter of 20 nm and range in length from 100 nm to 500 nm, with a density of 100 nanostructures/μm^2. The top layer of the inorganic nanostructures is coated with a hydrophobic organic layer that allows sample droplets to adhere to the surface of the NALDI target. Similar to the role of the MALDI matrix, the silicon nanowire structures are able to desorb the laser energy and aid ionization. A technique known as desorption ionization on silicon (DIOS) uses inert porous silicon wafers as substrates for the analysis of small molecules, such as peptides and peptide mixtures. DIOS has also been used for a number of other applications including the analysis of fatty acids, peptides, and proteins. Porous silicon targets are prepared by electrochemical etching, [3, 4] a process that affects the pore size and shape of the silicon wafers, which can influence the efficiency of LDI [5].

Efforts to improve spectral quality and the effective mass range for small molecules have led to the search for other inert substrates materials. Inert surfaces with nanostructures such as silicon nanowires, carbon nanotubes, and porous alumina have been synthesized and used for LDI MS [6]. LDI with nanostructured targets has also been used for molecules less than 1000 Da [7, 8]. Based on these Nanomaterials, 100 spot disposable nanostructure targets have been commercialized as Nano assisted laser desorption ionization (NALDI) targets for the analysis of small molecules less than 1000 Da and have been used for the analysis of polar and nonpolar compounds [9,7].

Characteristics of NALDI

1. NALDI is fast: Nano-Assisted Laser Desorption/Ionization omits time consuming ESI sample injection to the mass spectrometer. Thus it is the ideal approach for easy high-throughput analysis of loads of samples.
2. NALDI is matrix-Free: Disposable ready to use NALDI targets are nanostructured target plates for matrix free laser desorption ionization of low mass organic molecules. The nanostructures absorb the laser energy causing laser desorption of the samples placed on the target. As a result, mass spectra of small molecules are obtained at high sensitivity and with very low chemical background.

© STM Journals 2013. All Rights Reserved

The NALDI targets (Figure 2) are available in standard ¼ MTP format for use in Bruker. FLEX series instruments [10,11].

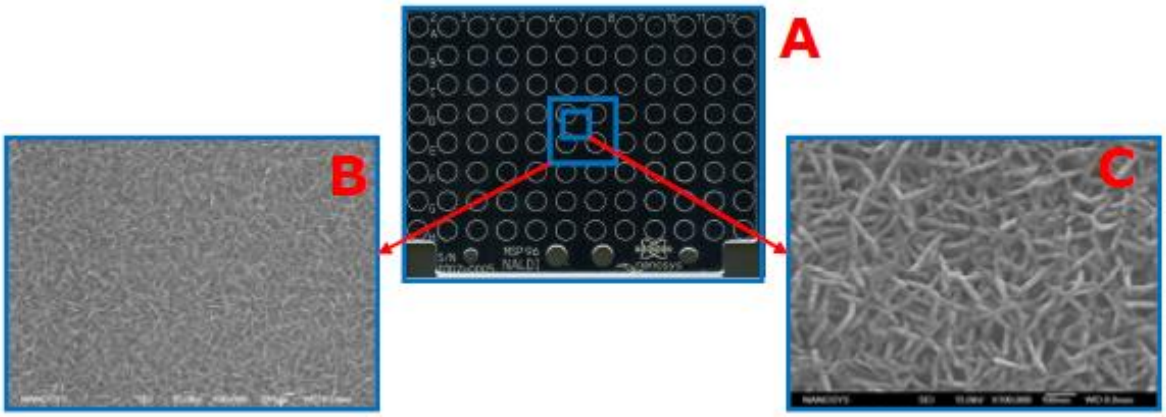

Fig. 2: *NALDI Target Plate (A) and Magnified Images of Nanostructured Surface with Magnification 30000X (B) and 100000X (C).*

3. NALDI is quantitative: Patented NALDI technology opens the door to routine analysis of small molecules such as pharmaceutical compounds by MALDI-TOF and TOF/TOF instrumentation, reaping the benefit of speed and sensitivity offered by these analytical systems. Now, a fast, quantitative and accurate mass spectral analysis of drug candidates after biological assays is possible and laborious sample processing is obsolete.
4. NALDI is sensitive: NALDI target plates can detect small drug compounds at low and even sub-femtomolar concentrations [10,11] as shown in Figure 3.

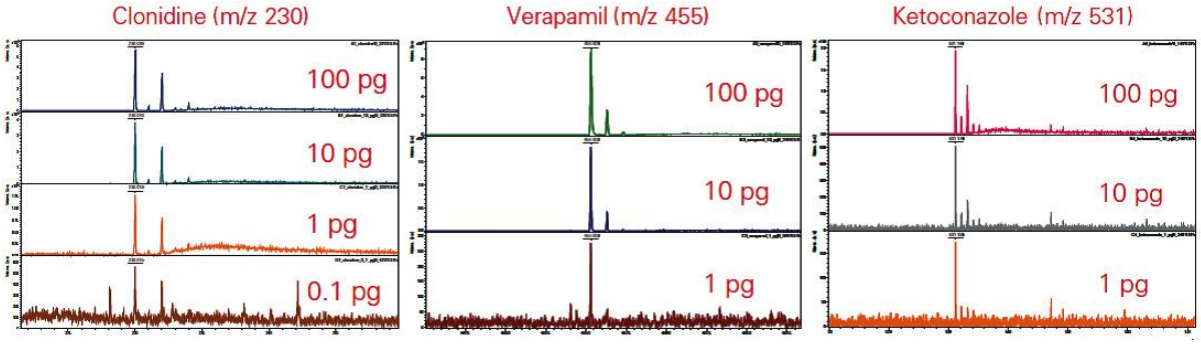

Fig. 3: *Naldi Spectra of Small Compounds.*

Applications of NADI-TOF

NALDI for Analysis of Peptides (Milk and Colostrum)

Several bioactive proteins have been identified in colostrum and milk, i.e., immunoglobulins, lactoferrin, cytokines, and antimicrobial proteins and peptides, such as defensins and cathelicidins [12, 13].

Some of the bioactive components may be applicable as food formulations or pharmaceuticals [14]. To increase knowledge about properties of individual bioactive components there are needs for technologies to identify these and to obtain the components in Purified form. One of the possibilities is application of sophisticated proteomics technologies. The most used techniques for analysis of proteins and peptides in different food matrices are electro spray ionization (ESI) and MALDI-TOF MS [15–18]. However, there are also reports available that analysis of low molecular weight compounds with MALDI can be complicated because of intense chemical noise from the matrix [19–21]. These problems can be reduced by using a matrix-free setup like NALDI which is

reported to show good performance for molecules up to 3000 Da [3, 10, 22] conducted a study to identify peptides from colostrum with molecular weight under 3000 Da.

Reports that even a million fold dilution gives enough signal to detect peptides with NALDI are available. In contrast, MALDI gives spectra with intensity less than 300, so they are not able to fragment any of these ions. The intensity of the signal from the β-casein fragment is at least 10 times higher with NALDI than with MALDI. Figure 4 shows that the spectra of NALDI and MALDI are not identical.

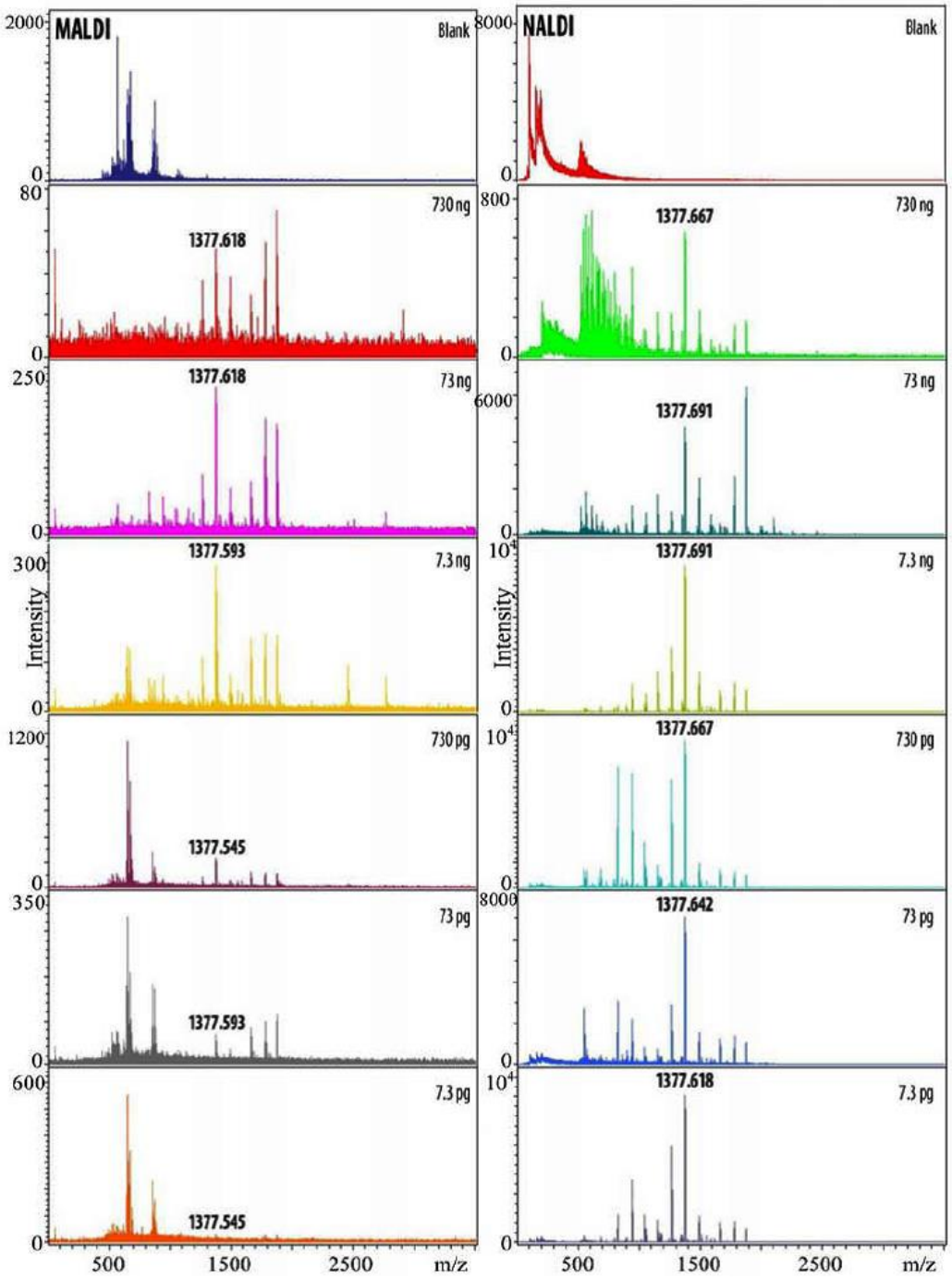

Fig. 4: *MS Spectra of Colostrum Sample Compared by MALDI and NALDI.*

In colostrum the most intense peak from the NALDI analysis was 1377.570 Da (Figure 5) and was fragmented with tandem mass spectrometry. [23]

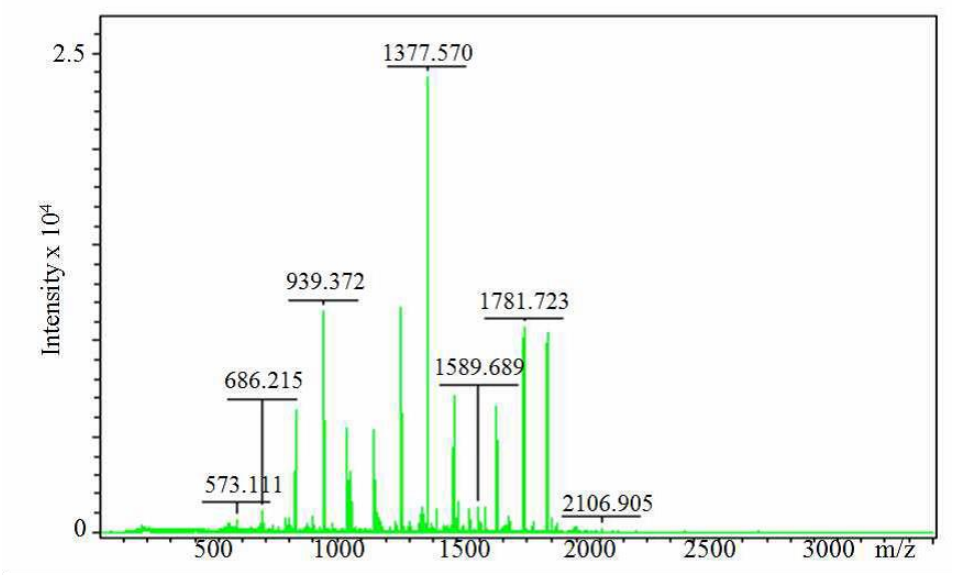

Fig. 5: *NALDI MS of Sample [23].*

The fragments were analyzed by *de novo* sequencing and Mascot search. The sequence was EPVLGPVRGPFPI (Figure 6), which is identical to β-casein [23].

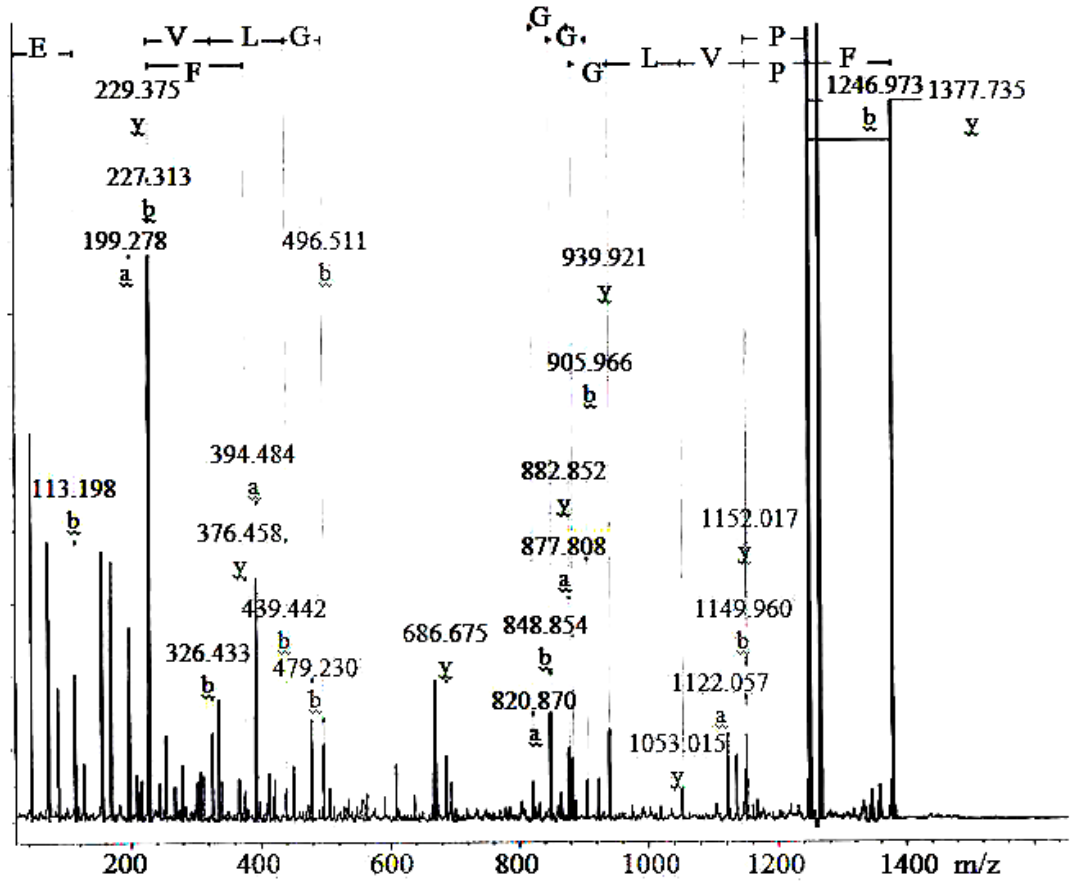

Fig. 6: *MS/MS Spectrum of ion 1377.570. The Figure shows Detection of a, b and y Ions after Fragmentation and the Fragment Sizes [23].*

From the results, it can be inferred that NALDI is useful for analysis of low molecular weight peptides. NALDI has high sensitivity and is easy to use. Moreover, comparison between NALDI and MALDI shows that peak intensities with NALDI were at least ten times

higher than those obtained with MALDI and they were able to detect a β-casein fragment from colostrum.

NALDI has been used for the analysis of a variety of lipids including PLs, TAGs, DAGs and FFAs derived from both standards and biological extracts [9, 24–26] in addition to PLs from tissue sections [27].

Dikler and Kowalski in 2009 [28] analyzed a mixture of tryptic digests of bovine transferrin, bovine serum albumin, yeast enolase and yeast phosphoglucose isomerase (also known as glucose-6-phosphate isomerase). The concentration of each of the four digests in the mixture is 250 fmol/µl. Their Findings are summarized below:-

1. The results from this mixture are characteristic of LC-NALDI experiments with large number of identified peptides and high Mascot peptide summary scores (Table 1).
2. Identified peptides spread across full mass range of tryptic peptides but NALDI targets show particularly high sensitivity to peptides under 1700 Da.
3. MS/MS spectra of peptides obtained from NALDI targets have the same fragmentation patterns and similar relative signal intensities as in case of conventional MALDI preparations.
4. There are additional advantages in analysis of peptides below 900 Da since there are fewer background signals in spectra acquired from NALDI targets.
5. The reproducibility between the LC-NALDI runs is good.

Table 1: *Results from 2 LC-NALDI Runs using Mixture of 4 Protein Digests each at 250 fmol/µL Concentration [28].*

Protein	Best run		Average from 2 runs	
	Mascot Score	**Number of peptides**	**Mascot score**	**Number of Peptides**
Transferrin	2541	53	2316	47
BSA	1886	35	2172	41
Enolase	1306	27	1361	26
Phosphoglucose isomerase	799	14	774	15

Pharmaceutical Industry

For novel drug discovery strategies in the pharmaceutical industry a new matrix-free LDI-MS method, named NALDI-MS for Nano-Assisted Laser Desorption/Ionization, was reported by Guénin et al. (2009) (19). In order to test the NALDI target, they studied 40 various small organic molecules including varieties of organic amine, peptides (organic acids, an organic amine, bisphosphonic acids, heteroaromatics, aromatics, ethers, polymers, peptides, amino acids, carbohydrates, lipids, and nucleoside) and evaluated different solvent preparations, concentration threshold, and ran MS/MS experiments. These (NALDI-TOF/MS) experiments were performed on a Biflex IV mass spectrometer (Bruker Daltonics) equipped with a nitrogen laser (337 nm).

Mass spectra were acquired in positive and negative reflector mode with a resolution generally greater than 6000. Several organic solvents could be used (acétonitrile, ethanol,

dichloromethane, THF) permitting the analysis of hydrophobic compounds, in contrast to classical MALDI-MS. Of these compounds 95% were successfully analyzed by NALDI/MS in positive or negative ion mode (90% in positive mode alone) in contrast to MALDI-MS. Moreover, this target could be re-used after simple washes. The use of NALDI for small organic molecule enables rapid MS analysis directly in organic solvents. Thus, the direct analysis of organic reaction media could be considered. This represents a new approach for the high-throughput analyses of organic synthesis and therefore NALDI could also be a method of choice for fast and automated analysis of combinatorial libraries.

Analytical Chemistry

Wyatt et al. (2010) [7] successfully analyze various chemicals, i.e., polymers, fluorinated

© STM Journals 2013. All Rights Reserved

porphyrin, hypervalent iodide compounds, Free-base porphyrins, Metal-porphyrin Complex, Organometallic/Coordination Compounds, Boron Compounds by NALDI-TOFMS. They observed-

1. High quality data can be acquired for non-polar compounds and metal complexes using NALDI technology.
2. NALDI has greater sensitivity than MALDI; at least 1000 times more for two porphyrin compounds.
3. Porphyrins can be observed as $[M + H]^+$, $M^{+\bullet}$ or $M^{-\bullet}$ species depending upon compound specific functionality.
4. NALDI may out-perform other mass spectrometry techniques, but more compounds must be characterized to fully evaluated capabilities of the technology.
5. More fragmentation may occur with NALDI compared to MALDI; extra structural information is provided.

Other Areas

1. In agricultural sector metabolic profiles of Maize can analyzed by (NALDI) – time of flight (ToF) mass spectrometry.
2. Use in analysis of microorganisms, i.e., E. coli and B. cereus species and analyzed number of phospholipids that can be used as identifiable biomarkers for both E. coli and B. cereus species [29].
3. Sahashi et al. (2010) [30] analyzed the bioactive compounds (triterpene glycosides called ginsenosides, main bioactive component) in Panax ginseng C.A. Meyer, a herbal products have been used in traditional Chinese medicine, by using nanoparticle-assisted laser desorption/ionisation mass spectrometry (MS).

Advantages of NALDI over MALDI

1. Matrix free technology.
2. Easy to handle.
3. One plate should use 30 times after simple wash.
4. Low cost compare to MALDI.
5. Sensitivity far higher for low molecular weight biomolecule.
6. No chemical noise interference.

Drawbacks

1. Nanoparticle and laser action and production of ion this phenomena is still not so clear for the scientist all hypothesis not totally meet the phenomena of laser base ion production in Nano surface.
2. Nano surface plate still is patent technology.

The use of exact Nano material for different biomaterial is not determined [9].

3. CONCLUSION

Although NALDI has found applications in a variety of disciplines including dairy, food, chemistry, biochemistry, microbiology, agriculture and physics, this phenomena is still not so clear for the scientist and all hypothesis is not meeting the phenomena of laser base ion production in Nano surface. It is still a patent technology. The use of exact Nano material for different biomaterial is not determined. Therefore there is a need for further research in this regard so that its applications could be diversified.

REFERENCES

1. C. Niu, R. H. Daniels, R. S. Dubrow, J. L. Goldman, US 7391018, 2008.
2. Peterson, D. S. Matrix Free Methods for Laser Desorption/Ionization Mass Spectrometry. *Mass Spectrometry Reviews* 2007; 26: 19–34p.
3. Thomas J. J., Shen, Z., Crowell J. E., *et al.* Desorption/Ionization on Silicon (DIOS): A Diverse Mass Spectrometry Platform for Protein Characterization. *Proceedings of the National Academy of Sciences of the United States of America.* 2001; 98(9): 4932–4937p.
4. Wei J., Buriak J. M., Siuzdak G. Desorption-Ionization Mass Spectrometry on Porous Silicon. *Nature*, 1999; 399 (6733): 243–246p.
5. Kumar P., Huber P. Effect of Etching Parameter on Pore Size and Porosity of Electrochemically Formed Nanoporous Silicon. *J. Nanomater*. 2007.
6. Shenar N., Cantel S., Martinez J., *et al.* Comparison of Inert Supports in Laser Desorption/Ionization Mass Spectrometry of Peptides: Pencil Lead, Porous Silica Gel, DiosChip and NALDI Target. *Rapid Commun. Mass Spectrom*. 2009; 23 (15): 2371–2379p.
7. Wyatt M., Ding S., Stein B., *et al.* Analysis of Various Organic and Organometallic Compounds Using

Nanostructure-Assisted Laser Desorption/Ionization Time of-Flight Mass Spectrometry (NALDI-TOF MS). *J. Am. Soc. Mass. Spectrom.,* 2010; 21 (7): 1256–1259p.

8. Go E.P., Apon J.V., Luo G., *et al.* Desorption/Ionization on Silicon Nanowires. *Anal. Chem*., 2005; 77 (6): 1641–1646p.
9. Guenin E., Lecouvey M., Hardouin, J. Could a Nano-Assisted Laser Desorption/ Ionization Target Improve the Study of Small Organic Molecules by Laser Desorption/Ionization Time-of-Flight Mass Spectrometry? *Rapid Commun Mass Spectrom*. 2009; 23:1395–400p.
10. Daniels R.H., Dikler S., Li E., *et al.* Break Free of the Matrix: Sensitive and Rapid Analysis of Small Molecules Using Nanostructured Surfaces and LDI-TOF Mass Spectrometry. *J. Assoc. Lab. Autom*., 2008; 13 (6): 314-321p.
11. Dikler S., Stacey C., Hardev V., *et al.* A Comparison of Matrix-free LDI-MS Using Nanostructured Targets and MALDI-MS for Small Molecule Analysis. Proceedings of the 55th ASMS Conference on Mass Spectrometry and Allied Topics, Indianapolis, Indiana, June 3-7, 2007.
12. Park Y. W. *Bioactive Components in Milk and Dairy Products*. Overview of Bioactive Components in Milk and Dairy Products. Wiley-Blackwell, 2009; 440p.
13. Stelwagen K., Carpenter E., Haigh B., *et al.* T. T. Immune Components of Bovine Colostrum and Milk. *Journal of Animal Science,* 2009; 87(13): 3–9p.
14. Wheeler T. T., Hodgkinson A. J., Prosser C. G., *et al.* Immune Components of Bovine Colostrum and Milk-a Historical Perspective. *Journal of Mammary Gland Biology and Neoplasia.* 2007; 12(4): 237–247p.
15. Shevchenko A., Sunyaev S., Loboda A., *et al.* Charting the Proteomes of Organisms with Unsequenced Genomes by MALDI-Quadrupole Time-Offlight Mass Spectrometry and BLAST Homology Searching. *Analytical Chemistry,* 2001; 73(9): 1917–1926p.
16. Trauger S. A., Webb W., Siuzdak G. Peptide and Protein Analysis with Mass Spectrometry. *Water,* 2002; 16: 15–28p.
17. Coon J. J., Syka J. E. P., Shabanowitz J., *et al.* Tandem Mass Spectrometry for Peptide and Protein Sequence Analysis. *Bio Techniques*. 2005; 38(4): 519–523p.
18. Jørgensen A. L. W., Juul-Madsen H. R., Stagsted J. Colostrum and Bioactive, Colostral Peptides Differentially Modulate the Innate Immune Response of Intestinal Epithelial Cells. *Journal of Peptide Science.* 2010; 16(1): 21–30p.
19. Cohen S. L., Chait B. T. Influence of Matrix Solution Conditions on the MALDI-MS Analysis of Peptides and Proteins. *Analytical Chemistry*. 1996; 68(1): 31–37p.
20. Knochenmuss R., Dubois F., Dale M. J., *et al.* The Matrix Suppression Effect and Ionization Mechanisms in Matrix-assisted Laser Desorption/Ionization. *Rapid Communications in Mass Spectrometry,* 1996; 10(8): 871–877p.
21. Go E. P., Shen Z., Harris K., *et al.* Quantitative Analysis with Desorption/Ionization on Silicon Mass Spectrometry using Electrospray Deposition. *Analytical Chemistry*. 2003; 75(20): 5475–5479p.
22. Lewis W. G., Shen Z. X., Finn M. G., *et al.* Desorption/Ionization on Silicon (Dios) Mass Spectrometry: Background and Applications. *Int. J. Mass Spectrom*. 2003; 226 (1): 107–116p.
23. Kütt M.L., Malbe M., Stagsted J. Nanostructure-Assisted Laser Desorption/Ionization (NALDI) for Analysis of Peptides in Milk and Colostrum. *Agronomy Research* 9 2011 (Special Issue II), 415–420p.
24. Muck A., Stelzner T., Hubner U., *et al.* Lithographically Patterned Silicon Nanowire Arrays for Matrix Free LDI-TOF/MS Analysis of Lipids. *Lab Chip*. 2010; 10:320–325p.
25. Pavlaskova K., Strnadova M., Strohalm M., *et al.* Time Dependent Oxidation during Nano-Assisted Laser Desorption Ionization Mass Spectrometry: a Useful Tool for Structure Determination or a Source of Possible Confusion? *Anal Chem*., 2011; 83:5661–5p.
26. Colantonio S., Simpson J., Fisher R, *et al.* Quantitative Analysis of Phospholipids using Nanostructured Laser Desorption Ionization Targets. *Lipids*, 2011; 46:469–77p.
27. Vidova V., Novak P., Strohalm M., *et al.* Laser Desorption Ionization of Lipid

Transfers: Tissue Mass Spectrometry Imaging without MALDI Matrix. *Anal Chem*., 2010; 82:4994–7p.
28. Dikler S., Kowalski P, Bruker Daltonics LC-NALDI: Using Matrix-Free
29. Nano structured Targets for Peptide Fractionation and Analysis MALDI-TOF/ http://www.bdal.com/uploads/media/ASMS09-MPZ572-LNALDI_SYD_01.pdf.
30. Juaneka Monet Hayes Detection of Microorganisms Using Maldi And Ion Mobility Mass Spectrometry. *PhD Dissertation*. Submitted to the Graduate Faculty of the Louisiana State University. 2013
31. Sahashi Y., Osaka I., Taira, S. Nutrition Analysis by Nanoparticle-Assisted Laser Desorption/Ionization Mass Spectrometry. *Food Chemistry*, 2010; 123: 865–871p.
32. Nørgaard A.W. Mass Spectrometric Study of Nanofilm Products Chemistry, Exposure and Health Effects. Ph.D. *thesis*. National Research Centre for the Working Environment, Denmark Department of Chemistry, University of Copenhagen, Denmark. 2010.

 © STM Journals 2013. All Rights Reserved