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REVIEW

Desorption Electrospray Ionization Imaging for Neurotransmitters evaluation: A Potential Approach to Parkinson's Disease Monitoring

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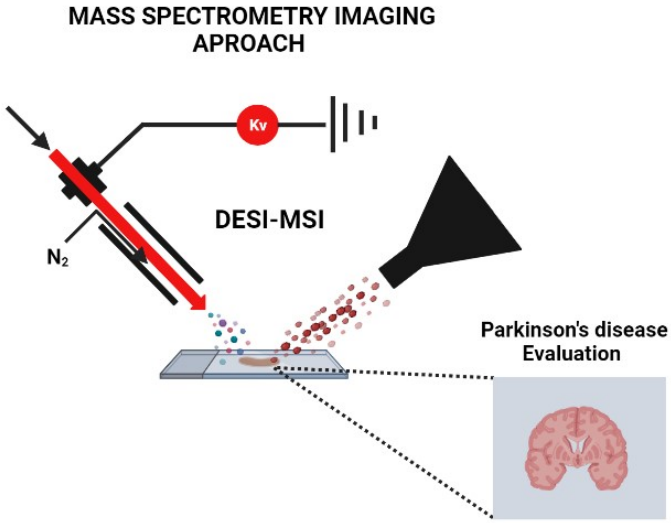

Parkinson's disease (PD) is globally known as the most common movement disorder and the second most common neurodegenerative disease. The disease includes the symptoms of involuntary limb tremors, stiffness, or inflexibility of limbs and joints, among others. Due to this, scientific reports on analytical methodologies to evaluate the progression of neurodegenerative diseases are extremely necessary. Traditional methods include histochemical, immunohistochemical, and ligand-based approaches, however, these approaches still suffer from selectivity limitations of association, leading to a wrong evaluation. In this context, mass spectrometry imaging methods, such as desorption electrospray ionization (DESI), are potential approaches to visualize the distribution of biomarkers that can lead to the information on the progress of PD. This review aims to bring a discussion of some DESI methodologies reported in the literature for the assessment of neurotransmitters associated with PD.

Keywords: Amino acids, DESI-imaging, Mass Spectrometry, Neurotransmitters, Parkinson's Disease

INTRODUCTION

The central nervous system is responsible for establishing a connection between the individual's body and the external environment.[1] In this context, small molecules known as neurotransmitters are a class of chemical messengers responsible to ensure the synaptic transmission between neural cells in the

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brain.[2] Knowing the central and peripheral nervous system, its chemical composition, and cytoarchitecture is extremally important to understanding the behavior of animals and the main neurological and neurodegenerative diseases.[3]

Among neurodegenerative diseases, Parkinson's Disease (PD) is the second most common disease in the elderly, affecting 1% of the world population over 65 years old, according to data from the World Health Organization (WHO). Nonetheless, about 10% of people with this condition have reported symptoms before age 40.[4] In Brazil, approximately 200,000 people suffer from this disease.[5]

Neuropathologically, PD can be defined by the loss process of substantial nigra (SN).[6] The most common synthons of the disease include involuntary limb tremors, stiffness or inflexibility of limbs and joints, bradykinesia or akinesia (slowness or lack of movement), and postural instability (impaired balance or coordination) (Figure 1). These symptoms directly interfere with the daily life of patients.[7] In addition to the perception of this disease as a movement disorder, there are other non-motor symptoms, such as a decline in cognitive activity, depression, sleep disorders, and hyposmia (low olfactory sensitivity).[8] Despite the great advances in the understanding of PD achieved in recent years,[9] the adopted criteria for diagnosis are still based on the mentioned symptoms above, as well as on neurological examinations.[7] Moreover, when new diagnostic criteria are correctly applied, about 20% of these diagnoses are still incorrect, due to the similarity with the symptoms of Parkinsonian disorders (PD symptoms caused by another clinical condition).[10] Because of this, the study of the fluctuations of some neurotransmitters and their concentration in the neural system has been an interesting approach to evaluating PD and its effect on human health.[2]

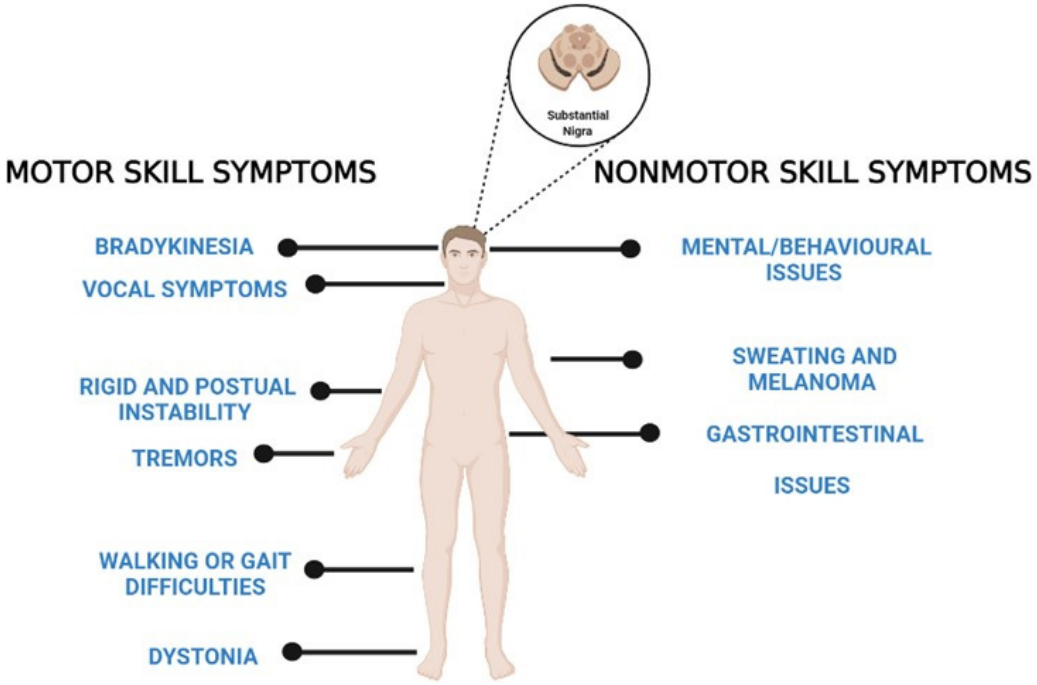

Figure 1. Schematic illustration of motor and nonmotor skill symptoms of Parkinson's disease.

As previously described, the loss of the SN is the main cause of PD, this loss of SN influences the neurotransmitters production.[11] According to some reports, nerve cells presented in SN are responsible for producing some important neurotransmitters that are linked to some neural messages related to body control movements, such as dopamine.[11–14] Consequently, the lower production of neurotransmitters causes the previously mentioned PD symptoms. Having this in mind, the monitoring and evaluation of some neurotransmitters have been extensively studied as favorably approaches to PD evaluation.[15,16]

According to Shariatgorji et al., the mapping of the distribution of neurotransmitters is mainly performed by histochemical, immunohistochemical, and ligand-based approaches.[2] In such approaches, the localization of specific enzymes or transporters acting as molecular markers is required. However, these approaches suffer especially from selectivity limitations of association, leading to a non-selectivity method. Due to this, the introduction of imaging techniques that are capable to performing a direct mapping and quantification of the total concentration of some neurotransmitters is extremally necessary. In this context, mass spectrometry imaging (MSI) methods, represents an alternative approach to performing the assessment and distribution of biomarkers that can lead to information about the progress of neurological disorders, such as PD.

Desorption electrospray ionization (DESI)

Traditionally, there are conventional ionization sources such as electrospray ionization (ESI), atmospheric pressure photoionization (APPI), and atmospheric pressure chemical ionization (APCI). These methods are capable of performing the analysis of many analytes in different matrices. Some of these methods also can be hyphenated with separation methods such as liquid chromatography (LC) and gas chromatography (GC). Although conventional methods have been well established in literature reports, these methods still require some specific conditions, such as the previous sample preparation of the sample, making the analysis exhausting and passive of analytical errors due to the improper sample preparation.[17]

Over the decades, advances in mass spectrometry (MS) have enabled the development of new ionization sources such as ambient ionization (AIMS) methods. These methods are capable of generating ions from different matrices without the need for previous sample preparation, such as the previously mentioned traditional ionization methods.[18,19] The great feature of these methods is the possibility of performing the analysis in environmental conditions, which contributes to the versatility of these analytical methods.[17,20,21] In addition to this, AIMS techniques are capable of promoting new advances in the development of new methods, which are expected to be faster and more sensitive methods for a greater variety of analytes in different matrices, such as biological samples.[22,23]

Among the main advantages of AIMS methods in comparison with the traditional methods, it can be mentioned a smaller sample volume, little or no use of organic solvent, and little or no sample preparation. Therefore, such methods promote the reduction of the need of separation methods such as the use in (LC) and (GC). Another advantage that has to be mentioned, is the possibility of associating this technique with fragmentation (MS/MS) and isotopic profile experiments, such as the traditional ionization methods.[24]

Among the AIMS methods, desorption electrospray ionization (DESI) was the first AIMS method to be introduced in 2004 by Cooks et al. and is considered the pioneer of the set of AIMS techniques.[25] The DESI technique is based on the desorption and ionization of analytes. First, a primary spray is formed with an electrically charged solvent and an inert gas under high pressure. The primary spray is directed at the sample on a given surface. The generated impact creates a thin layer of solvent on the surface, allowing a solid-liquid microextraction of these analytes.[26] Subsequently, with the molecules solubilized in this extraction process, the desorption of the analyte from the surface occurs as a result of the shock of the primary spray. A secondary spray is formed containing the desorbed analyte and sent to the mass spectrometer inlet (Figure 2).[25]

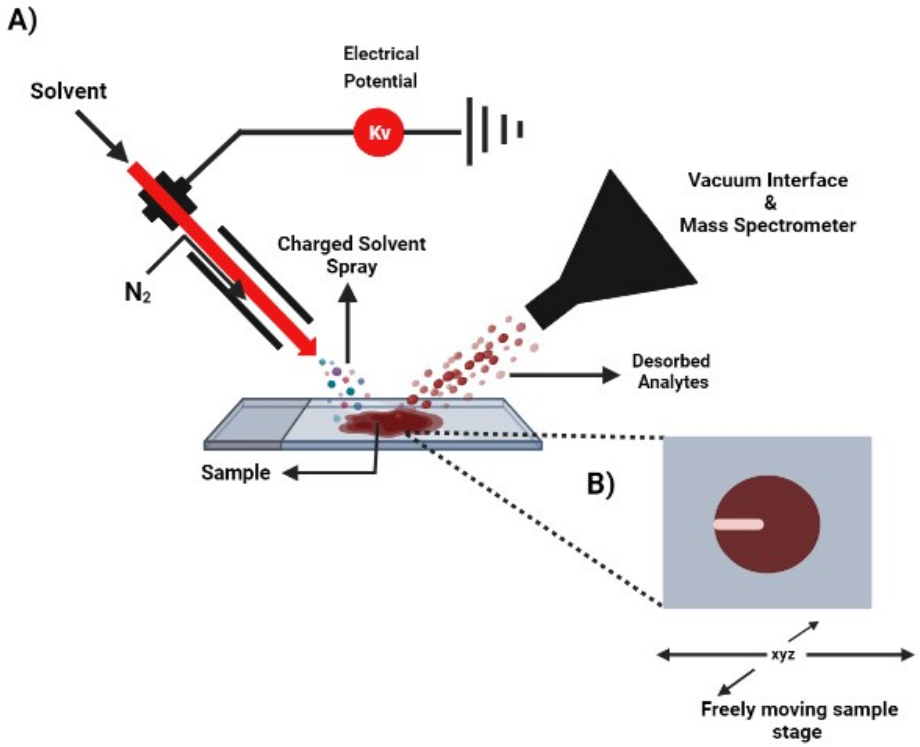

Figure 2. A) Schematic illustration of the DESI ionization process, and B) Zoom image of DESI spray movement into the sample surface.

DESI technique is part of the so-called imaging approaches, which are analytical methods capable of performing imaging chemical evaluation of a determined sample surface. Therefore, the technique has been being applied in many scientific fields, especially because of its potential to perform imaging analysis of many matrices, especially for biological tissues.[27]

Mass spectrometry imaging (MSI)

Mass spectrometry imaging (MSI) methods are commonly known as analytical approaches that allow the visualization and distribution of many molecules on a sample surface.[24,28,29] These methods are extremally applicable for numerous biomolecules analyses, such as lipids, peptides, and proteins from determinate human, animal, or plant tissue.[17,30,31] For biological tissue analysis, two main MSI methods are main applicated, these two methods comprehend the matrix-assisted laser desorption ionization (MALDI) and desorption electrospray ionization (DESI).[3,32] Although these two approaches are extremally used for simultaneous label-free visualization of surface compounds in biological samples, the MALDI approach still requires specific components for its performance.[14] For MALDI analysis, it is important to ensure the utilization of the proton donor matrix, which also influences the analyte desorption from the matrix, besides this, in MALDI technique, the matrix can hide the small-mass compounds from the spectra due to the presence of the matrix reducing the analytical performance of the method.[14,33,34] Due to this, when the evaluation of the sample surface is required, the DESI approach has been highlighted as a potential analytical method to perform such visualization.[21,35]

In DESI chemical imaging (DESI-MSI) a scanning *m/z* ratio peak on the “X” axis is performed in which the desorbed ions are then analysed, the generated data is then saved in a spectrum format.[36] In the same way, a scan of the same *m/z* ratio peaks is performed on the “Y” axis, from these scans a compilation of spectra is made, giving rise to a file, which allows the chemical images obtention.[37] The biggest advantage of chemical imaging is the possibility of visualizing the distribution of analytes in a sample surface, being

widely applied in the analysis of biological tissues.[30,38–41] Since the scanning is done continuously, the sample must be on a smooth and flat surface in order to obtain an image with the best possible resolution.[42]

For the obtention of the best image resolution via DESI-MSI some parameters need to be deeply evaluated, these parameters include the study of the geometry of the source (angle of the nebulization gas and the distances from the sprayer capillary to the sample surface and from the sprayer capillary to the inlet mass), desorption solvent, nebulizing gas pressure, solvent flow, and capillary voltage. In 2016, Tillner et al. demonstrated that applying different types of sprays and optimizing the capillary orientation towards the mass inlet can increase image resolution in DESI technique.[43] In 2014, Bodzon-Kulakowska et al. published a study with pork liver using DESI, which showed the possibility of improving from 1 to 224% the absolute signal intensity depending on the sprayer orientations adopted and the type of sprayer used.[44] The authors demonstrated that orientations closer to the capillary and solvent in relation to the mass spectrometer, a signal improvement was observed, while further orientations offered almost complete signal loss.

Recently, Wu et al. reported the development of a new method using DESI coupled with post-photoionization (PI) for the simultaneous imaging of polar and non-polar compounds in biological samples. In this study, dopants were used in a mouse homogenate model to increase the signal through ion molecules reactions.[45] The signal improvement for different compounds was explained by the ionization mechanism. In addition to this, the best dopant composition was applied to the MSI aided in the comprehensive imaging for different compounds of different polarities analysis. Finally, it was possible to quantify using toluene as a dopant by DESI-PI for any compounds in mice's brain tissue.

As the literature highlights (Table I), imaging approaches, such as DESI-MSI are interesting analytical methods to perform the evaluation of the chemical distribution of neurotransmitters on a sample surface. In addition to this, such methods, have also been widely applied for the chemical distribution visualization of some biomarkers, which could be used to evaluate the progress of a determined disease. Concerning this, this review aims to bring a discussion of the reported literature applications of DESI-MSI for the evaluation of Parkinson's disease.

Table I. Literature reports of neurotransmitters analysis by mass spectrometry imaging

Analyte(s)	Sample	Imaging technique	Derivatization agent	Ref.
Dop, DOPAC, 3-MT, 5-HT, Glu, Gln, Asp, GABA and Ado	Mice brain tissue	DESI-MSI and MALDI-MSI	DPP-TFB	2
GABA, Glu, Asp, Ser, ACh, Dop, and Chol	Mice brain tissue	DESI-MSI	-	3
Ach, GABA and Glu	Mice brain tissue	NANO-DESI-MSI	-	45
(R)-salbutamol	Mice brain tissue	DESI-MSI	-	46
Fluorobenzyl (DC20)	Mice brain tissue	DESI-MSI	-	47
DOP, Ado, xanthine and many kinds of important metabolites	Mice brain tissue	AFADESI-MSI	-	48
Cimbi-36	Mice brain tissue and kidney tissues	DESI-MSI, autoradiography and PET	-	49

(continues on the next page)

Table I. Literature reports of neurotransmitters analysis by mass spectrometry imaging (continuation)

Analyte(s)	Sample	Imaging technique	Derivatization agent	Ref.
5-HT, ACh, Dop, Tyr, 3-MT, GABA, Trp and Glu	Mice brain tissue	AFADESI-MSI	DPP-TFB	50
Cl, P, Fe, K, Ca and S	Mice brain tissue	DESI-MSI	-	51
GABA, Gln, Glu, Creatine and Ado	Mice brain tissue	DESI-MSI	-	52
Ala, Gly, Dop, GABA	Mice brain tissue	DESI-MSI	TPP-TFB	14
GABA, taurine, Dop	Mice brain tissue	DESI-MSI and MALDI-MSI	TPP-TFB and Br-TPP	53

Dop: Dopamine; DOPAC: Dihydroxyphenylacetic acid; GABA: ɤ-Aminobutyric acid; DPP-TFB: 2,4-Diphenyl-pyranylium tetrafluoroborate; Glu: Glutamate; Asp: Aspartate; 5-HT: serotonin; ACh: Acetylcholine; TA: tyramine; Ado: Adenosine; Tyr: Tyrosine; PEA: Phenethylamine; 3-MT: 3-methoxytyramine; α-GPC: L-alpha-glycerylphosphorylcholine; Tryp: Tryptamine; Gln: glutamine; DESI-MSI: Desorption Electrospray Ionization Mass Spectrometry Imaging; Nano-DESI-MS: Nano-Desorption Electrospray Ionization-imaging; MALDI-MSI: Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry Imaging; TMP-TFB: 2,4,6-trimethyl-pyranylium tetrafluoroborate; PEP-19: neuronal calmodulin-binding protein; Ser: Serine; Chol: Choline; AFADESI-MSI: air-flow-assisted Desorption electrospray ionization; PET: Positron Emission Tomography; Trp: Tryptophan; TPP-TFB: 2,4,6-trimethyl-pyranylium tetrafluoroborate; Br-TPP: 2-(4-bromophenyl)-4,6- diphenylpyranylium.

Applications of desi imaging for Parkinson's disease evaluation

The analysis of low abundance and difficulty to ionize compounds, such as neurotransmitters is the biggest challenge in tissue samples analysis due to the detection limit of these substances. To overcome this drawback, it is commonly applied some analytical protocols to improve the ionization of challenge compounds, which reflects in a better detection of such analytes. One of these approaches consists in the application of derivatization methods, which has been highlighted as one of the most applied methods to improve the analysis of neurotransmitters in tissue samples.[46–51]

To improve the detection limits, Shariatgorji et al. reported the use of a derivatization protocol for the identification of monoamine neurotransmitters in brain tissues by using 2,4,6-triphenylpyrylium (TPP) and 2-(4-bromophenyl)-4,6-diphenylpyranylium (Br-TPP) as derivatization agents followed by Mass Spectrometry Imaging employing Matrix-Assisted Laser Desorption/Ionization-mass spectrometry (MALDI-MSI) and Desorption Electrospray Ionization-mass spectrometry (DESI-MSI).[52] For the study, the capillary temperature was adjusted to 320 °C and with a spray voltage of 4.5 kV. The angle between the spray and the sample surface was adjusted to 75°. Methanol/water (95:5, v/v) at 1.5 μL min^{-1} was used for solvent spray, and nitrogen gas (N_2) at 7 bars as the nebulization gas. Through the derivatization reactions, primary amines N-alkyl- or N-arylpyridinium salts were formed. The produced salts with the TPP agent did not differentiate the target compounds from other non-derivatized compounds, however, the Br-TPP reaction provided a characteristic isotopic pattern for the derivatized compounds. MALDI-MSI was used to evaluate the feasibility of the derivatization protocol with TPP and Br-TPP. Such results corroborate with good detection of dopamine (TPP: *m/z* 444.19 and Br-TPP: *m/z* 522.10) GABA (TPP: *m/z* 394.18 and Br-TPP: *m/z* 472.09) and taurine (TPP: *m/z* 416.1 and Br-TPP: *m/z* 494.0) (Figure 3). DESI-MSI was used to evaluate the spatial distribution of molecules derivatized with TPP and Br-TPP. Through the analyses, the derivatization protocol employing Br-TPP as a derivatizing agent proved to be more advantageous than TPP, since Br-TPP acted as a marker for primary amines, by the bromine isotopic pattern. Furthermore, bromination through the pyrylium reaction solved the ionization problem

of other compounds by the desorption proton transfer mechanism, which makes it difficult to identify non-marked compounds by the isotopic profile. Therefore, this methodology proved to be effective in identifying monoamine neurotransmitters by the bromine isotopic pattern of bromine and improved the limit of detection in analyses by MALDI-MSI and DESI-MSI.

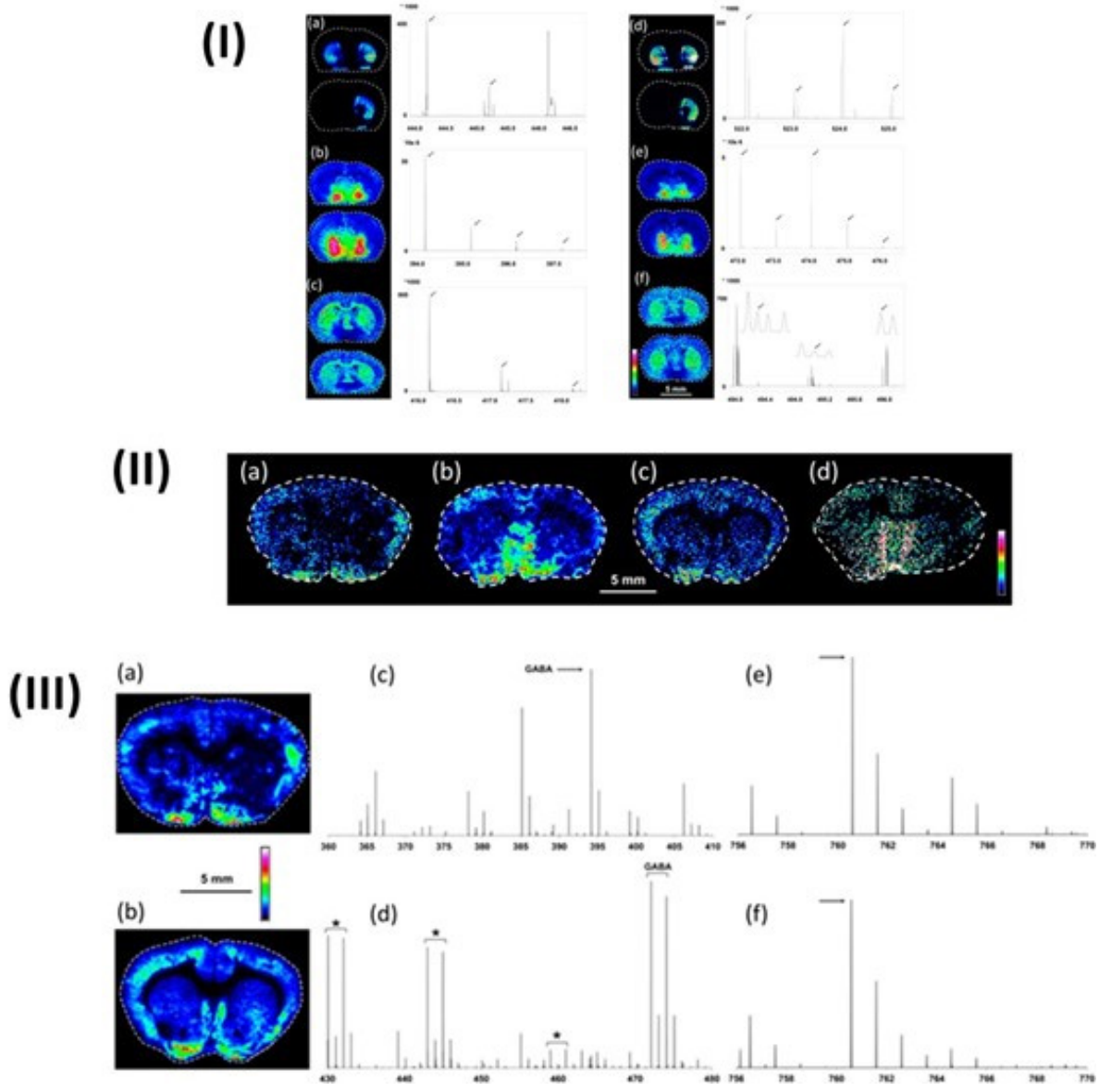

Figure 3. (I) MALDI-MSI analysis of neurotransmitters in rat brain tissue sections facilitated by TPP and Br-TPP on-tissue derivatization (a) TPP-derivatized dopamine (*m*/*z* 444.2), (b) TPP-derivatized GABA (*m*/*z* 394.2), (c) TPP-derivatized taurine (*m*/*z* 416.1), (d) Br-TPP-derivatized dopamine (*m*/*z* 522.1), (e) Br-TPP-derivatized GABA (*m*/*z* 472.1), (f) Br-TPP-derivatized taurine (*m*/*z* 494.0). (II) DESI-MSI analysis of TPP and Br-TPP derivatized rat brain tissue sections. Relative abundance and spatial distribution of TPP (a) *m*/*z* 380.1636 and (b) *m*/*z* 408.1951 and Br-TPP (c) *m*/*z* 458.07415 and (d) *m*/*z* 486.10535 derivatized compounds on control rat brain tissue sections. (III) DESI-MSI analysis of TPP- and Br-TPP-derivatized rat brain tissue sections. Relative abundance and lateral distribution of (a) TPP- and (b) Br-TPP-derivatized GABA (*m*/*z* 394.2, 472.1, respectively) on control rat brain tissue sections. Average spectra of (c) TPP- and (d) Br-TPP-treated brains showing different isotopic patterns for GABA. (e) Average spectra from TPP-derivatized rat brain showing an intense signal at *m*/*z* 760.5832. (f) The same signal was detected for a Br-TPP-derivatized rat brain tissue section without a bromine isotopic pattern, indicating that this compound was desorbed and ionized without derivatization. (Reprinted with permission from Reza Shariatgorji, Anna Nilsson, Nicole Strittmatter, et al. Bromopyrylium Derivatization Facilitates Identification by Mass Spectrometry Imaging of Monoamine Neurotransmitters and Small Molecule Neuroactive Compounds. *J. Am. Soc. Mass Spectrom.* **2020**, *31* (12), 2553–2557. https://doi.org/10.1021/JASMS.0C00166/SUPPL_FILE/JS0C00166_SI_001.PDF. Copyright 2020 American Chemical Society.[52])

Recently, Maciel et al. reported the use of DESI-MSI in brain tissues to investigate the distribution of neurotransmitters through derivatization protocols, based on the Katritzky reaction.[14] In this study, the capillary temperature was adjusted to 275 °C with a spray voltage of 4.5 kV. The angle between the spray and the sample surface was 55° and methanol at 1.5 μL min^{-1} was used for solvent spray and N_2 at 6.89 bar was used for nebulization gas. Mice brain tissues were submitted to derivatization protocols and compared with analyses performed on non-derivatized tissues, by DESI-MSI. Amino acids like Glycine (Gly) were not detected in non-derivatized tissues, however, with the application of derivatization protocols Gly could be observed at high MS intensity. In addition, alanine (Ala), gamma-aminobutyric acid (GABA), and dopamine (Dop) were detected at lower intensities than in derivatized tissues. The derivatization protocol based on the Katritzky reaction coupled to DESI-MSI allowed the monitoring of the derivatized Gly, Ala, GABA, and Dop and their spatial distribution on brain tissues since it is important to evaluate the dopaminergic integrity and function in the neurodegeneration process in PD. Through the methodology, the ability to be applied in models of mice with Parkinson's disease was proved, for the detection of the amino acids Gly, Ala, GABA, and Dop with high signal and quality images.

Elementary images are fundamental to understanding the pathogenesis of the disease, however, the use of biomarkers helps in the characterization and to observe the disease's evolution. De Jesus et al. reported the optimization of the method, for the correlation between the elementary markers (Fe, S, Zn) with lipids profiles presented in a single cut of fresh frozen tissue with a resolution of 50 μm.[53] In this context, it was used a pattern and uniform tissue, also known as a homogenized sample, which made it possible to test on different substrates regarding the compatibility of the elementary and molecular images. The study of the following ionization sources, Ion Beam Analysis (IBA), MALDI, and DESI, showed that the DESI-MSI achieved better results. According to the authors, the same substrates were investigated for other ionization approaches, however, using DESI approach with poly(ethylene terephthalate) (PET), higher contrast images were obtained compared to other substrates generated in standard glass. To generate images of tissue homogenates through a DESI source, a mass spectrometer was attached. The spray solvent was methanol/water 95:5 (% v/v) with the supply of a rate of 2 μL min^{-1} with the support of a pump and electrospray voltage of 4 kV, energy of collision of 35 V, and a capillary temperature being 100 °C. The entire database was generated by using the positive ion resolution mode and when used in particle-induced X-ray emission (PIXE), better results were obtained. Therefore, it was demonstrated methods to correlate the elements (Fe, S, Zn) with lipid profiles in fresh frozen tissue samples, with a resolution of 50 μm, it is important to report that the placement of Fe and lipids in the tissues was a new method, since, if there is an overload of iron, this can result in oxidative stress and subsequent damage to lipid membranes. Admittedly, when carrying out this search using DESI-MSI with PIXIE resolution, it allowed a greater horizon regarding the applicability of Fe in lipid profiles, thus generating a lot of studies in the area of inflammatory and chronic diseases, such as Parkinson's disease, cancer, and tuberculosis.

Hulme et al. observed the influence that microbes have on the microbiome-gut-brain axis from the production of neurotransmitters. In this study, DESI-MSI was used in order to locate molecular variations in the gut and brain in germ-free (GF) mice, which are cared for through antibiotics and control.[54] Therefore, a 1.5 mm spray tip was used for the analysis above the sample at an angle of 75°. The solvent of this process followed methanol/water (95:5 v/v) at 1.5 μL min^{-1}, with a voltage of 4.5 kV. Thus, the applied undirected MSI method allowed the discovery of new metabolites involved in microbiome gut brain (MGB) axis communication, being observed changes in the brain and intestine of GF when compared to control specific-pathogen-free (SPF). Therefore, the untargeted MSI identified four metabolites, vitamin B5 and 3-hydroxy-3-methylglutaric acid, while vitamin B5 decreased in free mice, 3-HMG was increased in SPF. This study also reported that there was no significant change in metabolism with the antibiotic's ingestion. In summary, the developed DESI-MSI method was capable to determine the levels of vitamin B5 that are controlled by the microbiota in the brain, which is responsible for several diseases, including Parkinson's disease, and, despite significant changes in the intestinal microbiota, neurotransmitters are not significantly altered in the brain.

Studies claim there's an association between the use of β2- Adrenoreceptor agonist (R)-salbutamol and reduced risk of developing PD, due to the regulate action of the α-synuclein gene which excess production may cause this disease.[55,56] Regarding this, in 2020, Zhang et al. analysed the spatial distribution of (R)-salbutamol in mice brains samples, using desorption electrospray ionization coupled with time-of-flight mass spectrometry (DESI-TOF-MS) with an imaging approach following two types of routes of administration: nasal and intravenous.[57] Concerning method optimization in this study, it should be mentioned that the flow rate was 2 μL min^{-1} and the nebulizing gas chosen was nitrogen. Moreover, the positive ionization mode was chosen in mass spectrometry (range of *m/z* 50-1000) and the electrospray solvent was methanol, water, and formic acid in the proportions of 95, 5, and 0.1 v/v respectively. In general, the authors reported that (R)-salbutamol delivery to the mouse brain was efficient and nasal route administration provided more exposure to the substance in question when compared with intravenous route administration. Thereby, it was verified through this study that investigating the spatial distribution, the delivery effect and choosing the best administration routine using DESI-MS as a tool is advantageous, efficient and helps studies involving PD.

The simultaneous imaging of neurotransmitters (dopamine, dihydroxyphenylacetic acid, 3-methoxytyramine, serotonin, glutamate, glutamine, aspartate, γ-aminobutyric acid, adenosine) and neuroactive substances (amphetamine, sibutramine, fluvoxamine) in brain tissues samples with PD model were performed by Shariatgorji et al. using DESI-MSI approach.[2] Through this study, the authors also demonstrated charge-tagging achieved through the chemical derivatization reaction using 2,4-Diphenyl-pyranylium tetrafluoroborate and how this approach could increase the sensitivity of the DESI-MSI analysis. For DESI-MSI analysis, a distance between the sprayer and the sample surface of 1.5 mm was used with an angle of 75°. A mix of methanol/water (95:5 v/v) as the electrospray solvent was used at a flow rate of 1.5 μL min^{-1} and spray voltage of ±4.5 kV with N_2 as the nebulizer gas. According to the obtained results, the application of DESI-MSI enabled the visualization of the target neurotransmitters in both negative and positive ion modes. However, the main focus of this analysis was to observe the difference in dopamine (one of the main biomarkers of PD) after the application of the PD model after the submission of a unilateral 6-hydroxydopamine (6-OHDA) lesioning of the nigrostriatal dopaminergic pathway. According to the authors, the developed imaging methodology was capable of confirming the decrease in the dopamine concentration in the striatal region of the 6-OHDA lesioned side of the brain. The drug imaging also showed the distribution of the neuroactive substances in different parts of the brain. For amphetamine, the substance was mainly localized in the cortex, cerebellum, hippocampus, and striatal structures of the brain. The sibutramine didn't undergo derivatized, however, its active metabolite N-di-desmethylsibutramine was successfully derivatized and was mainly localized in the cortical, striatal, and hippocampal structures of the brain. Finally, fluvoxamine was detected in the dosed brain tissue sections. According to the authors, the developed MSI method was successfully applied for the study of the PD model, especially after the application of a derivatization approach, which improved the detection limit of the studied compounds, in addition to the analysis of neuroactive substances which can lead to better information of the chemical distribution of such substances in the brain after consuming.

A direct visualization of neurotransmitters in mice brain tissues by DESI-MSI coupled to a hybrid quadrupole-Orbitrap mass spectrometer was reported by Fernandes et al.[3] The authors reported the spatial monitoring of the following neurotransmitters, γ-aminobutyric (GABA), glutamate, aspartate, serine, as well as acetylcholine, dopamine, and choline, and their distribution in coronal and sagittal slices of the brain. For the imaging analysis, the experimental condition of the method consisted of the use of 3.4 kV of spray voltage, 3.0 μL min^{-1} of solvent flow rate, with a surface scan of 600 μm s^{-1} with 75,000 image resolution. The comparison with three lines consisted of standards solutions, cerebellar, and cerebral extracts showed different accumulation of the studied neurotransmitters. For acetylcholine and choline, a larger abundance was found in cerebral extracts. On the other hand, glutamate was more abundant in cerebellar extracts, for other neurotransmitters, no difference in the extracts was observed (Figure 4A). According to the authors, the developed MSI method proved to be an efficient approach, especially for not

using derivatization protocols, which could cause ion suppression or interfering side reactions. Finally, the spatial distribution of sagittal and coronal sections proved the different distribution of the neurotransmitters (Figure 4B). The obtained results pointed out the potential application of the DESI-MSI method for the evaluation of neurotransmitters, which can be applied to the study of neurodegenerative diseases, such as PD.

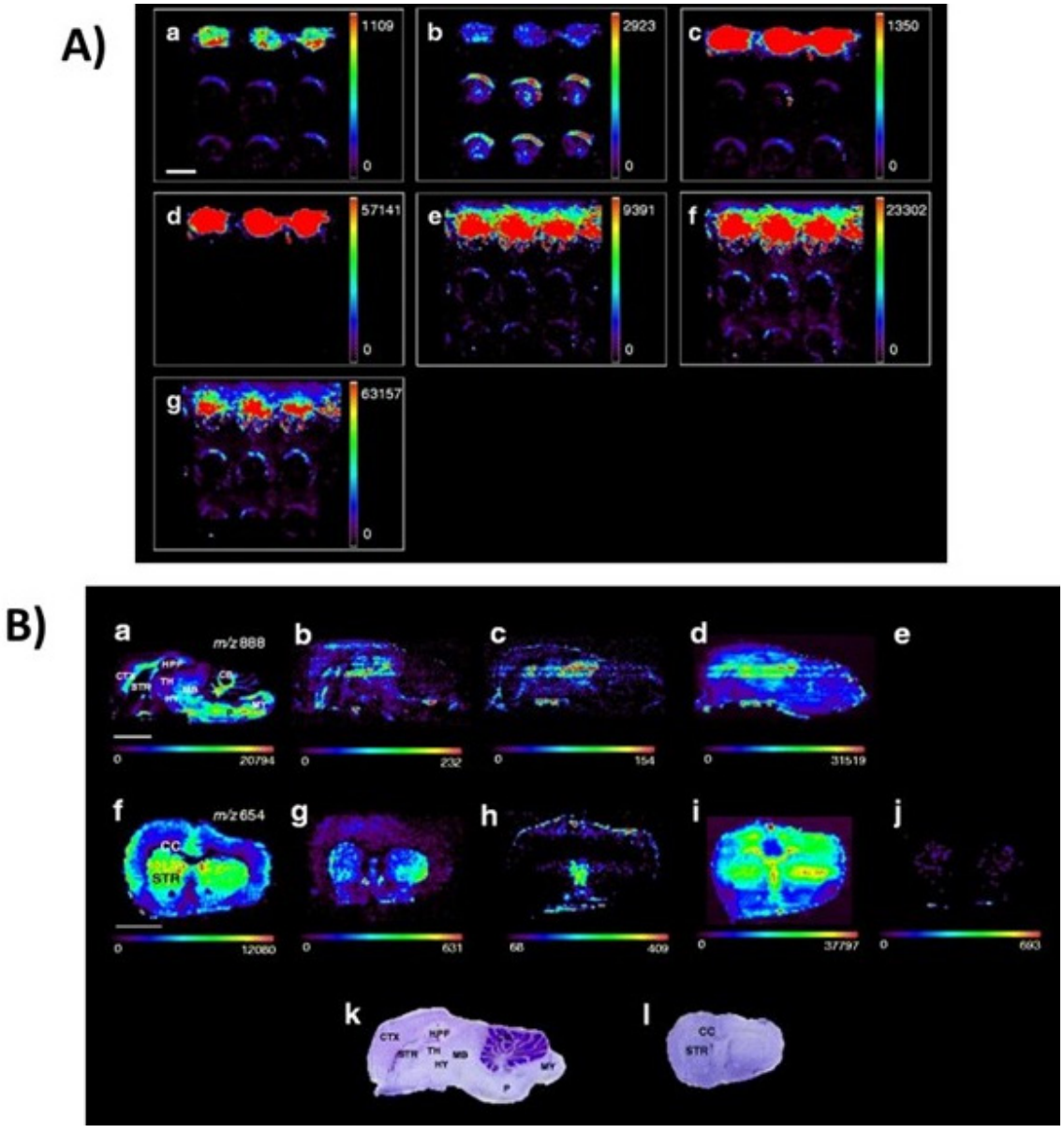

Figure 4. A) DESI-MSI of the spots of the standard solutions (first line in each Panel), cerebellar extract (second line in each Panel), and cerebral extract (third line in each Panel). (a–d) DESI (+)-MSI for (a) GABA, (b) choline, (c) acetylcholine, and (d) dopamine. (e – g) DESI (−)-MSI for (e) serine, (f) aspartic acid, and (g) glutamic acid. Scale bar: 2 mm, and B) (+)-DESI-MSI of neurotransmitters and metabolites in sagittal (b–e) and coronal (g–j) and (−)-DESI-MSI of lipids in sagittal (a) and coronal (f) rat brain sections. Relative abundance and spatial distribution of the ions of *m/z* 888.600 and 654.567 (a) and (f), respectively; STR = striatum, HPF = hippocampal formation, TH = thalamus, HY = hypothalamus, MB = midbrain, CB = cerebellum, P = pons, MY = medulla, CC = corpus callosum, CTX = cortex; acetylcholine (b) and (g); GABA (c) and (h); choline (d) and (i), and dopamine (e) and (j). Sagittal (k) and coronal (l) adjacent rat brain slices stained using the Nissl protocol. Scale bar: 5 mm. (Reprinted with permission from Anna Maria A. P. Fernandes, Pedro H. Vendramini, Renan Galaverna, et al. Direct Visualization of Neurotransmitters in Rat Brain Slices by Desorption Electrospray Ionization Mass Spectrometry Imaging (DESI - MS). *J. Am. Soc. Mass Spectrom*. **2016**, *27* (12), 1944–1951. https://doi.org/10.1007/s13361-016-1475-0. Copyright 2016 American Chemical Society.[3])

As could be seen in this section, DESI-MSI methods have been described in the literature for the neurotransmitter's evaluation, especially considering the chemical imaging of those which are related to neurodegenerative diseases. In addition to this, a variation technique of DESI has been recently highlighted as another powerful approach for the neurotransmitter's distribution evaluation, and it will be briefly discussed on the next topic of this paper.

Nano-Desorption electrospray ionization (Nano-DESI)

In addition to the DESI-MSI analysis, Nano-desorption electrospray ionization (Nano-DESI) has been highlighted as a potential approach for targeting neurotransmitters analysis in biological matrices. Nano-DESI is a variation of DESI that is also based on localized extraction on surfaces that allow sensitive quantitative and qualitative detection of molecules in complex samples and was first reported in 2010 by Roach et al.[58] This localized desorption is due to the presence of two fused silica capillaries directed analyzed surface with a continuous flow solvent between the capillaries (Figure 5).[32] This technique presents a high resolution and sensitivity, providing more details about the spatial distribution of analysed surfaces.[59] The main advantage of this technique is the obtentions of resolutions of 200 µm and 20 µm for biological matrices which promote the improvement of the generation of chemical imaging, having a potential application for the evaluation of the neurodegenerative disease, such as PD.[60]

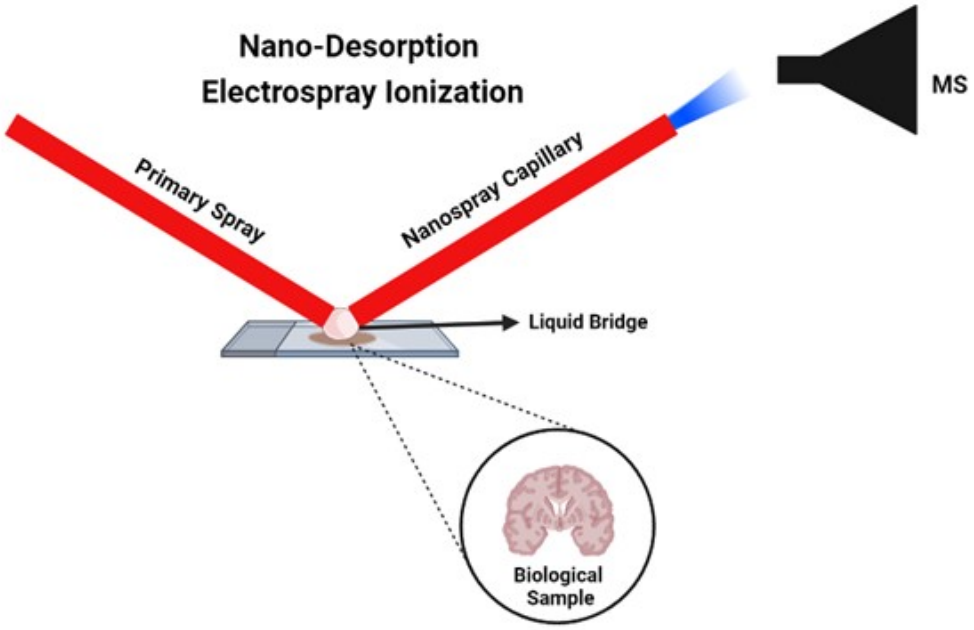

Figure 5. Schematic illustration of the Nano-DESI system.

In 2016, Bergman et al. reported the use of Nano-DESI incorporated for an online quantification of small molecules neurotransmitters, especially acetylcholine, aminobutyric acid (GABA), and glutamate in rat brain tissues sections.[61] In this study, was used the deuterated internal standards in the Nano-DESI solvent, which, demonstrated the identification, mapping, and quantification of these small molecules without a sample preparation step. The authors verified that GABA was more abundant in the medial septum band complex region (MSBC) than in cortex region. On the other hand, the glutamate was more abundant in the cortex region. Thus, the Nano-DESI was an adequate technique for the analyses and imaging evaluation of neurotransmitters, which according to the authors, could be successfully applied to the study of neurodegenerative diseases such as Parkinson's and Alzheimer's.

Mavroudakis et al. provided a method with Nano-DESI that combined host-guest chemistry targeting sodium and potassium ions and quantitative imaging of endogenous lipids and metabolites.[62] This method was applied to the ischemic stroke model which has a concentration by tissue of sodium and potassium highly dynamic. As the answer, it was verified the increase in these metals in the ischemic region compared with control (tissue without the ischemic stroke). Through this study, it was possible to observe the accumulation and depletion of lipids, neurotransmitters, and amino acids using the relative quantification with internal standards in a Nano-DESI solvent. Although the objective of this study wasn't just a neurotransmitters analysis, the developed method could be applied to these small molecules to enhance the diagnosis of other neurodegenerative diseases.

Nguyen et al. showed the depth of lipid coverage in Nano-DESI-MSI of mouse lung tissues compared to lipidomic analysis of liquid chromatography in tandem mass spectrometry (LC-MS/MS) of tissue extracts.[63] A combination of positive and negative Nano-DESI MSI ionization modes identified 265 unique lipids in 20 lipid subclasses and 19 metabolites (284 in total). Except for triacylglycerol (TG) species, its coverage was compared to LC-MS/MS experiments carried out using methanol/water tissue extracts and up to 50% with whole lung lipid analysis based on another extraction method. The results demonstrated the utility of Nano-DESI for spatial analysis of lipids in tissue sections. The combination between Nano-DESI and lipidomic LC-MS/MS is useful for the exploration of changes in lipids distributions during the development of lung, as well as resulting in diseases.

In summary, Nano-DESI allows application on different surfaces or with little preparation of the sample, with less volume of solvent, high sensitivity, a localized extraction, and high resolution by imaging. However, the technique is still low explored in studies of neurodegenerative diseases, however as previously presented in this paper, the technique has potential to be applied in distinct areas for studies of neurochemicals for comprehensions and progress evaluation of these diseases.

CONCLUSIONS

Since its introduction, imaging approaches have been used for many applications. The main advantage of such approaches is the possibility of the chemical imaging of the determined sample surface. Covering the MSI methods, this review pointed out the potential of the use of DESI-MSI as an imaging approach for the evaluation of biomarkers that can be associated with Parkinson's disease. Through a literature search, it was possible to note that this MSI method has been applied for the chemical evaluation and distribution of neurotransmitters in biological samples. Although some studies can be found in the literature, it is needed to highlight the need for more studies regarding the application of DESI-MSI for the analysis of neurotransmitters associated with PD. In addition to this, the variation of DESI known as Nano-DESI also needs a more critical view to demonstrate the application of such technique for the evaluation of PD, to amplify the application of the technique for chemical imaging of neurotransmitters associated with neurodegenerative diseases.

Conflicts of interest

The authors declare no conflicts of interest

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