Review Article
Volume 11:4, 2021

Metabolomics: Open Access

ISSN: 2153-0769 Open Access

Ce-Ms-Based Metabolomics: Recent Advances

Thomas Geisberger*

Technical University Munich (TUM), Germany

Abstract

An overview of the application of CE-MS in the field of metabolomics is provided. The study of endogenous low-molecular-weight compounds in biological samples is known as metabolomics. CE-MS has proven to be an effective technique for profiling polar metabolites in biological samples. This review discusses the application of various CE separation modes, capillary coatings, MS analyzers, sample preparation techniques, and data analysis methods . The use of CE-MS in metabolomics research is demonstrated by analysing bacterial extracts, plant extracts, urine, plasma, and cerebrospinal fluid samples. The relevant CE-MS metabolomics studies published between 2000 and 2008 are tabulated, including information on sample type and pretreatment and MS detection mode. Future developments are discussed, including the use of alternative ionization techniques, coupled separation systems, and the potential of microchip CE systems for metabolomics.

Keywords: Systemic Metabolome • Electrophoresis-Mass Spectrometry • CE-MS

Introduction

Over the last decade, metabolomics has grown in importance in clinical applications. Metabolomics research is important because disease conditions have a direct impact on the systemic metabolome. Metabolome-based biomarkers are being actively developed for early detection and indicating the stage of specific diseases. Understanding the effect of a molecular intervention on a living organism is an important strategy for comprehending novel or unexpected biological processes. The simultaneous advancements in advanced analytical techniques, sample preparation techniques, computer technology, and databank content have enabled metabolomics to yield more valuable scientific information than ever before. There is no single analytical technique capable of analyzing the entire metabolome, with over 15,000 known endogenous metabolites. However, capillary electrophoresis-mass spectrometry (CE-MS) is a novel technique for analyzing metabolites that are not accessible via liquid or gas chromatography techniques. CE's analytical capability combined with a recent sample preparation techniques focused on extracting polar-ionic compounds, make CE-MS a perfect technique for metabolomics studies.

Literature review

This review discusses the various analytical aspects of non-targeted and targeted metabolomics, as well as the new technological developments used in CE-MS for metabolomics. The use of CE-MS in metabolomics research is demonstrated by analyses of biomedical and clinical samples, as well as bacterial and plant extracts. A table summarises the relevant papers on CE-MS for metabolomics, including information on sample type and pretreatment, as well as MS detection mode. Future considerations include the challenges of large-scale and (quantitative) clinical metabolomics studies, as well as the use of sheathless interfacing and various ionisation techniques.

***Address for Correspondence:** Thomas Geisberger, Technical University Munich (TUM), Germany, E-mail: sdattfbt@iitr.ac.in*

Received 11 July 2021; **Accepted** 26 July 2021; **Published** 2 August 2021

Technological Developments

Coupling CE to MS is a lively research field, getting to help obtain stable, reproducible, and sensitive analysis with interfaces compatible with CE instrumentation [1]. Recently, Zhang et al. reported the planning and performance of a sheath less CE-MS interface. This design utilized a 70-cm long fused-silica capillary with 3 mm long bare fused silica at one end exposed by burning and subsequently etched in acid to make a symmetrical tapered tip. The tip was then washed and dried before being evenly smeared with quick-drying epoxy and quickly twined with a bit of foil (Fig. 1A and B). The sheath less interface is prepared to use once the epoxy dries. The constructed emitter is smooth and flat, thus, easy to make a stable electrospray plume (Fig. 1C). The authors flushed the capillary alternatively with hexadimethrine bromide (HDB) and dextran sulfate (DS) solutions to make three-layered coatings for the analysis of organic acids, and four-layered coatings for the analysis of cationic analyses. The evaluation of the interface performance was investigated and a stable MS signal was obtained when the flow rate was within the range of 80 to 510 nL/min and therefore the ESI voltage in range of 1.9 to 2.2 kV. The applicability was demonstrated with the analysis of 4 alkaloids, using 20 mm ammonium formate in 50% v/v acetonitrile (ACN) (pH 3.0). For the tested compounds, absolute LOD values below 1 fmol might be acquired with satisfactory migration-time repeatability [2]. Additionally, this work also included the appliance of this setup for the analysis of organic anions. The convenience in manufacturing of this proposed interface and its capability to separate cations and anions render it a really promising tool for metabolomics studies.

Applications

The applicability of CE-MS for metabolomics in various fields was demonstrated in 58 publications within the period from July 2018 to June 2020. The search terms “metabolomics,” “metabolic profiling,” “metabolic fingerprinting,” [3] “capillary electrophoresis and mass spectrometry” were used for choosing these studies from ISI Web of Science and PubMed databases. an summary of those studies, which provides information about the sort of sample and compounds analyzed, the BGE, sample pretreatment procedure, the MS analyzer employed, LOD (when provided by the authors), and remarks on the sort of approach, the sort of capillary coating, and whether CE was used as a complementary method. Within the following sections, some representative CE-MS-based metabolomics studies are discussed in additional detail. Could be acquired with satisfactory migration-time repeatability. Additionally, this work also included the appliance of this setup for the analysis of organic anions. The

convenience in manufacturing of this proposed interface and its capability to separate cations and anions render it a really promising tool for metabolomics studies.

Biomedical and Clinical Applications

Wells et al. developed a MS-compatible electrokinetic supercharging (EKS) strategy for the sensitive and robust analysis of biogenic amines in biological samples. This work utilized a coffee pH BGE that consists of fifty mM ammonium formate (pH 2.5) and 40% methanol, and a number one electrolyte (LE) that contained 250 mM ammonium formate (pH 2.5). a standard sheath-liquid interface was used for coupling CE to MS. To perform EKS, hydrodynamic injection of LE at 50 mbar for 30 s and water at 50 mbar for 1 s was first conducted, followed by electrokinetic injection of sample at 30 kV for 150 s. LOD values right down to 10 pM were obtained for the investigated neurotransmitters in EKS, leading to a 5000-fold sensitivity enhancement compared to hydrodynamic injection. The utility of this method was demonstrated within the determination of several neurotransmitters in rat brain homogenate and whole Drosophila homogenate, emphasizing its capability of simultaneously measuring pM and μM concentrations. Furthermore, the authors compared quantified concentrations of the amines in rat brain homogenate by the EKS method with those by an existing LC-MS/MS method [3]. Greater differences (20–46%) between the 2 analytical approaches were discovered in concentration for compounds containing moieties susceptible to oxidation. it had been speculated that more rapid analysis and efforts in oxidation prevention may help to further increase the accuracy of the proposed CE-MS method.

The field of nanomaterial (NM) corona is predominately focused on the adsorption of proteins to the NM surface, and has only seen a couple of studies investigating a subset of the metabolome. Insights into the interactions between metabolites and NMs can help form a comprehensive understanding of the role of the corona in determining the biological consequences of NMs. to review metabolite recruitment characteristics on the surface of NMs employed CE-MS with both sheath less and sheath-liquid interfaces. Low-pH separation conditions were used throughout the entire study, with normal CE polarity for cationic detection and reverse CE polarity for anionic detection. Targeted compounds were incubated along side six biologically relevant NMs in water, and therefore the effect of proteins on the adsorption characteristics was also investigated by introducing either intact or protein-free plasma within the incubation mixture. Since it's difficult to directly analyze the depleted amount of metabolites, the authors analyzed the rest fraction within the supernatant after incubation. Unique adsorption characteristics were demonstrated for various NMs while the mechanism underlying such interactions was still unclear. Metabolite recovery studies were conducted by using three rounds of washing steps, however, although the recovery data clearly indicated the quantity of metabolites not detected within the supernatant was adsorbed to the NMs, the adsorbed amount couldn't be fully recovered. By completing incubation experiments with either intact or protein-free plasma, the authors illustrated that protein portion of the corona is important to the formation of the metabolite corona to make an entire bimolecular corona. Despite the discoveries of the many intriguing phenomena, this pilot study generated many unanswered questions that need more follow-up research.

The pathogenesis of Alzheimer's disease (AD) has been shown to involve dysregulation in multiple biochemical pathways. to check whether deregulation of choline-related biochemical pathways within the brain are associated with AD pathogenesis, Mahajan et al. performed targeted and quantitative CE-MS metabolomics analysis on human brain tissue samples and transcriptomics study. CE-MS analyses were conducted consistent with the protocol from Human Metabolome Technologies (HMT) for both cations and anions. This work focused on 26 quantified metabolites that represented biochemical reactions related to transmethylation and polyamine synthesis/catabolism mainly in two brain regions, inferior gyrus (ITG) and middle gyrus (MFG). Significant alterations in metabolites (mainly within the inferior gyrus [ITG]), like choline, S-adenosyl methionine, cysteine, reduced glutathione (GSH), spermidine, N-acetyl glutamate, N-acetyl aspartate, and gamma-Aminobutyric acid (GABA) distinguished AD from control groups, and were also indicative of severity of AD pathology. However, the transcriptomic analyses focused on other brain regions, which hindered its integration with metabolomics data. Additionally, the findings during this work got to be further validated in studies with larger sample sizes.

Recently, Piestansky et al. developed a CE-MS/MS method for the analysis of AA in urine samples of inflammatory bowel disease (IBD) patients. A sheath-liquid interface was used for coupling CE to a Triple Quadrupole tandem MS instrument. A 500 mM acid solution was selected as BGE for the separation of 20 proteinogenic AA. the tactic evaluation revealed satisfactory performance parameters, and yielded LOD values of 0.22 to 8.73 μM in urine. The validated CE-MS/MS method was then applied for the targeted analysis of AA in urine from regional enteritis patients who were treated with azathioprine, and representative extracted profiles are shown in Fig. 5. The obtained concentrations of AA in urine were normalized thereto of creatinine, and showed high consistency with the info produced by a UHPLC-MS system. This emphasized the potential of CE-MS as an economic and reliable alternative to UHPLC-MS for clinical routine AA monitoring. The statistical analysis of the info revealed a moderate decrease in seven AA in IBD patients in comparison to the healthy control subjects.

Plant and Microbial Applications

To comprehensively characterize water-soluble metabolites in three major seaweed groups and explore the influence of various extraction protocols on metabolic profiles, Hamid et al. utilized a cross-platform metabolomics approach with both LC-MS/MS and CE-MS [37]. This study included three groups, 11 algae species before instrumental analyses, authors conducted two different metabolite extraction protocols using methanol-water, with and without chloroform. CE-MS analysis was employed for both cationic and anionic profiling, that specializes in free AA, organic acid, and charged metabolites, while LC-MS/MS approach was adopted for the analysis of free sugars. it had been revealed that the majority AA might be found altogether the tested species, with their concentration differences mainly attributed to their algae groups. Among the AA, alanine was found to be the foremost abundant altogether algae species, which may be a reflection of the metabolism change caused by the oxygen flux as a consequence of high and low water within the sea [3]. The obtained metabolite profiles from LC-MS and CE-MS were subjected to statistical method, revealing clear inter- and intragroup distinctions within the principal component analysis (PCA) plots. However, clustering analysis showed that no seaweed group characteristics with AA were uncovered. Instead, the sugar profiles demonstrated a characteristic alignment with the taxonomy tree representing three seaweed groups. Although some effect of the extraction method was observed on the compound concentrations, the extent of such effect was very limited on the result and therefore the differences within the metabolite profiles obtained were mainly the results of interspecies dissimilarities.

It was reported that Scheffersomyces stipitis uses N-acetyl-d-glucosamine (GlcNAc) as its sole carbon source, but the GlcNAc metabolic pathway in S. stipitis remains poorly understood. So as to research the metabolic responses to GlcNAc in S. stipitis, Inokuma et al. conducted both cationic and anionic analyses on a CE-MS system with sheath-liquid interface . The harvested yeast cells were first subjected to anaerobic fermentation for twenty-four h before metabolite extraction employing a boiling ethanol method at 95°C for five min. This study verified over 130 metabolites in total, among which were 106 compounds related to carbon and nitrogen metabolism. The PCA demonstrated clear separation between the metabolic data of yeast cells cultivated with GlcNAc and people with xylose and glucose. The GSSG/GSH ratio, an indicator of intracellular oxidative stress, was found to be approximately twofold higher in GlcNAc-grown cells than in glucose-grown cells, indicating the previous are exposed to high levels of oxidative stress. a good range of nitrogen-containing compounds, like AA, purines, and pyrimidines, showed increased accumulation during GlcNAc assimilation in S. stipitis. The RT-PCR showed that the rise of AA in GlcNAc-grown cells was thanks to the induction of expression of 5 genes liable for encoding certain AA synthases, while the elevated concentrations of purine and pyrimidine might be attributed to the use of ammonia because the amino donor for glutamine-

dependent amidotransferases that help the biosynthesis of varied purines and pyrimidine intermediates. However, future work is required to characterize these amidotransferases in S. stipitis. Interestingly, many of those nitrogen-containing compounds are valuable thanks to their pharmaceutical properties, which potentially could render S. stipitis a tool for the direct production of those compounds from GlcNAc.

The different composition of gastrointestinal microbiota across the intestinal tract reportedly contributed to the difference between the tiny and enormous intestinal metabolome profiles, however, little is understood about the metabolome profiling throughout the alimentary canal and its correlation with gastrointestinal microbiota. to realize more insight into this, Yamamoto et al. conducted metabolomics assays using both CE-MS and LC-MS/MS for the measurement of gastrointestinal luminal metabolite concentrations across different sections of the intestinal tract in specific pathogen-free (SPF) and germ-free (GF) mice. CE-MS-based metabolic profiling included both cationic and anionic metabolites. Cationic metabolites were separated on fuse-silica capillaries in low-pH condition while the separation of anionic metabolites was done on a COSMO(+) capillary at high-pH separation conditions. Metabolome analysis by CE-MS and LC-MS/MS identified a complete of 382 metabolites in gastrointestinal luminal contents from SPF and GF mice. The results revealed a significantly higher number of gastrointestinal luminal metabolites in SPF mice than in GF mice. Significantly more metabolites from the upper and lower colon might be detected in SPF mice than in GF mice, suggesting that colonic microbiota may produce unique sort of metabolites only within the former. The examination of the metabolome data obtained for various parts of the alimentary canal in SPF and GF mice indicated that gut microbiota is liable for the assembly of specific metabolites, and therefore the signature of various metabolites between these two sorts of mice. Further exploration remains needed to raised comprehend the contribution of gut microbiota to the gastrointestinal luminal metabolome.

Food Applications

In meat industry, a comprehensive understanding of the alterations that happen in postmortem muscle metabolites could offer essential information on the way to manipulate the assembly of key compounds to enhance the standard of meat. Recently, Muroya et al. employed a sheath-liquid CE-MS approach in an effort to seek out metabolites and pathways relevant to postmortem aging and beef quality in Japanese Black (JB) cattle. Lean muscle pieces at an equivalent position were taken from three steers at 0, 1 day, and 14 days postmortem and stored at −80°C until use. The frozen muscle pieces were homogenized in 50% 0°C ACN, followed by centrifugation and ultrafiltration through a 5 kDa cutoff membrane. The filtrate was subsequently lyophilized and reconstituted for both cationic and anionic profiling with HMT protocol. The CE-MS-based metabolomics study detected 197 compounds, of which 171 were annotated and 70 quantitated. Among the annotated metabolites, a complete of 89 metabolites showed significant changes during postmortem aging of beef (adjusted $P<0.05$, false discovery rate<0.10). These metabolites were assigned to functional pathways and 6 of them were highlighted because the characteristic biochemical events associated with meat quality including glycolysis, the acid cycle, the pentose phosphate pathway, protein digestion, aminoalkanoic acid generation, and purine metabolism. These progresses are expected to contribute to the standard improvement of aged beef, in aspects of tenderness, flavor, and functional value. Although significant changes in metabolite contents illustrated during this study agreed with previous studies of postmortem meat aging, the authors did not consider the impact on postmortem metabolite generation by the variation of weight among the tested animals.

To determine d-AA in vinegars, Lee et al. developed a CE-MS method for the enantiomeric separation of underivatized free AAs. This work made use of a sheath-liquid interface for the coupling of CE to MS and a separation buffer that consisted of 30 mM (18-crown-6)-2,3,11,12-tretracarboxylic acid ($18C_6H_4$), used as a chiral selector (CS). to attenuate ESI contamination by the nonvolatile CS and improve the ionization efficiency, a partial filling approach was employed by filling approximately 70% of the capillary with the separation buffer after flushing with 1 M FA before hydrodynamic injection of the sample solution. In practice, AA enantiomers interacted with the CS and got separated before entering the FA segment and arriving to the ESI source. The separated AA peaks observed during this work were free AA ions, $[AA+H]+$. This approach provided resolution values (Rs) between 0.6 and 32.4 and LOD values from 0.07 to 1.03 µg/mL, suitable for detecting traces of D-AAs in food. This method was then applied within the analysis of AA enantiomers in three sorts of vinegars, where little matrix effect was observed. D-AAs might be detected altogether the vinegars, taking over 0.4 to 2.0% of the entire AAs determined. The varying abundance of D-AAs in vinegars can indicate the extent of fermentation. Moreover, the tactic can potentially be utilized for assessing the taste profile of fermented foods supported the content of D-AAs.

Conclusion

Previous reviews of CE-MS-based metabolomics are evaluated in this paper to highlight recent advancements in this technique. We specifically look at papers published in the last two years (2018 and 2019) on CE-MS based metabolomics. The current state of affairs and the challenges confronting metabolomic studies are discussed in order to demonstrate the high potential of CE-MS for future research, particularly in biomarker development studies.

References

1. Rawi Ramautar , Govert W Somsen, Gerhardus J de Jong. "CE-MS in metabolomics: National Center for Biotechnology Informatiom". 30(2009).
2. Ramautar R." CE-MS for metabolomics: Developments and applications in the period 2008-2010". 32(2011).
3. Wei Zhang, Rawi Ramautar."CE-MS for metabolomics: Developments and applications in the period 2018–2020". *Wiley Analytical Science* (2020).

How to cite this article: Thomas Geisberger. "Ce-Ms-Based Metabolomics: Recent Advances." *Metabolomics (Los Angel)* 11 (2021):293.