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Review

Electrochemical (Bio)Sensing of Maple Syrup Urine Disease Biomarkers Pointing to Early Diagnosis: A Review

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Abstract: Metabolic errors are inherited diseases, where genetic defects prevent a metabolic path, ending up in enzyme malfunction. In correspondence to its remaining or plenitude fall of enzymatic potency, there is an amassment of dangerous metabolites near the metabolic bar and/or a dearth of necessary products, inducing a certain disease. These metabolic errors may include deviations such as point mutations, expunctions or interferences, or further complicated genomic disorders. Based on these facts, maple syrup urine disease (MSUD) is a scarce metabolic disease, generated by huge concentrations of branched-chain amino acids (b AAs), i.e., leucine, isoleucine, and valine. In this situation, these large amounts of b AAs provoke abnormalities such as liver failure, neurocognitive dysfunctions, and probably death. To overpass those problems, it is crucial to implement a timely and agile diagnosis at the early stages of life in view of their immutable consequence on neonates. Thus, this review will describe MSUD and b AAs analysis based on electrochemical (bio)sensing.

Keywords: maple syrup urine disease; branched-chain amino acids; electrochemical (bio)sensing

1. Introduction

Amino acids are important in cellular metabolism, as they constitute the architectural parts for protein synthesis and metabolites, supplying the components for farther reactions occurring in living organisms. Amino acids such as aspartic acid (Asp), glutamic acid (Glu), γ-aminobutyric acid (GABA) and taurine (Tau) operate like neurotransmitters, regulating synaptic transference and recollection [1]. Branched-chain amino acids (b AAs) (leucine (Leu), isoleucine (Ile), and valine (Val)) partake in proteinic synthesis and protein catabolism. Phenylalanine (Phe) and tyrosine (Tyr) participate in the formation of trace amines and catecholamines [1].

Meanwhile, most AAs of nutritional significance lies on L-isomers. Natural proteins are entirely formed from L AAs [2,3]. The body cannot use vitamins or minerals in isolation. The enzymes, hormones, body tissues, even bones are constructed from AAs with vitamins and minerals hook-ups. The vitamins and minerals cannot perform this action without free AAs to create the required hook-ups. Therefore, AAs are necessary for vitamins and minerals to accomplish their task rightly. The free AAs are also needed to preserve neurotransmitters, as these are remarkably valuable for osmoregulation of cells and employed as an energy source.

For that matter, human body acquires 20-times more AAs than vitamins and about 4-times more AAs than minerals [4]. Both the D- and L AAs interconvert one to the other over a period, reaching the equilibrium by racemization. During food processing, the L AAs may be racemized to D-isomers. The concentration of L AA can likewise be used as a measure of the nutritive content of the food. Moreover, the chiral amino acids outline is a profitable mean for scanning countless

fermentation processes alongside with the detection of bacterial action. The D AAs have been thought as unnatural AAs.

When D AAs are replaced for L AAs in a protein, the protein goes through a switch in structure. This switch can shift the traits and functions of the protein. It is known that D AAs is a common element of bacterial cell wall [5]. The excretion of D AA in physiological fluids is aroused by age, diet, physiological state, and antibiotic therapies. The raised level of D AAs activates nephrotoxicity, growth inhibition, liver damage, fibrosis, and necrosis of kidney cell together with interference in the biosynthesis of certain fundamental neurotransmitters [6]. The white and gray matter of Alzheimer brain incorporates D AA, 1 to 4 times greater than the relevant sector of normal brains [7]. The human beings with renal breakdown have large levels of D AA in urine and serum [8–10].

On the other hand, rare metabolic diseases appear due to genetic abnormalities in the enzymes of the metabolic paths of dietary components. Generally, a scarce disorder in the broad populace is considered when a currency of at most 1 in 20,000 neonates occurs [11]. Presently, 400 million people all over the world are suffering of a scarce disease, about half of them are toddlers and 30% of those patents pass away in reach the antecedent of five years of living, as these diseases are exceptionally threatening in the cradle [12]. The symptoms, prediction, and precise decline of the catabolic paths are utterly distinctive in different metabolic diseases. Considering the aforementioned reasons, the assortment of the above-mentioned maladies is not rigorously prescribed and the most prevalent assortment takes into consideration the dominant molecule influenced (carbohydrates, fatty acids, AA and organic acids), provoking carbohydrate, fatty acid, amino, and organic acids-based diseases, respectively [13,14].

Amino acids maladies displace an autosomal recessive manner of inheritance which suggests that the mutation created a metabolic block is existing in the genetic element of both parents. Because of mutation, the inherited flaw is expressed downstream as a deficiency or a fragmentary biological activity of enzymes engaged in amino acids metabolism. Therefore, some substrates in these paths increase or are switched into different paths. Accordingly, amino acids complications are biochemically outlined by unusual levels of single or several amino acids and their downstream plasma and/or urine metabolites. Amino acid abnormalities are performed with variable and often nonspecific clinical symptoms. In alliance with medical assistance, these disorders are handled by nutritional constraints, supplements, and pharmaceutical food. In this sense, diseases due to amino acid disorders are one of the most dangerous metabolic diseases owed to their deadly repercussions on nurslings.

In particular, the most common diseases due to amino acid disorders are phenylketonuria (PKU), tyrosinemia, homocystenuria, arginase deficiency and maple syrup urine smelling disease (MSUD). All the particular strokes enjoy resemblances in clinical expression, generating mental obstruction in neonates, dementia, or are correlated with syndromes such as Parkinson's disease. They can also induce liver failure, rickets, hepatocarcinoma and death without medication in the early stage of essence [15,16]. Equivalently, a lack of these items can produce hypochondria, depression or albinism [16,17].

The case of MSUD is a scarce metabolic malady alongside a preponderance of 1:200,000 living childbirth [15]. It is provoked by tremendous amounts of the L-branched-chain amino acid (b AAs), such as leucine, isoleucine, and valine (Leu, Ile, and Val, biomarkers) [18]. In aforementioned malady, these excessive elevations of b AAs provoke complications liver illness, neurological impairments, and indeed death. To prevent these problems, it is particularly notable to obtain a timely and rapid diagnosis in the prime periods of life [14,15].

The most common methodologies that are used in the determination of branched-chain amino acids and therefore MSUD, are MS/MS, enzyme activity assays, HPLC, capillary electrophoresis, and genetic testing [19–27]. Notwithstanding, all the practices are time exhausting and, in a few incidents, crave a huge sample volume. A supplementary obstacle relevant to the diagnosis is the matter that for the newborn the monitoring criteria are different among various countries, and indeed vary in the same country, which makes it more troublesome to diagnose MSUD. In addition, there is no end treatment, but dietary constraints are pinpointed and accordingly, repeated monitoring of the target molecule should be performed during the patient's life to evade injurious influences.

Problems related to the diagnosis and monitoring of MSUD or the detection of L b AAs underscore the need for developing new diagnostic methods using easier-to-use, low-sample-volume approaches, which is particularly important in neonates [28–30]. These requirements are met by electrochemical methods and electrochemical (bio)sensors, which are a reliable and profitable appliance for the determination of metabolic biological markers, in order to facilitate their use in the diagnosis and monitoring of this scarce disease, due to their ease of use, simplicity, selectivity, sensibility and low cost [31,32]. In this manner, electrochemical method could be expanded in early diagnosis of other inborn errors of metabolism, including carbohydrate, fatty acid, and amino and organic acids-based diseases [33].

As it can be seen by the lack of literature, electrochemical sensors have been rarely used in the detection of b AAs related to rare diseases such as MSUD clinical diagnostics. Therefore, in this review, electrochemical (bio)sensors for the determination of branched-chain amino acids are summarized.

2. Maple Syrup Urine Disease

Amino acids (Figure 1) are necessary constitutional protein entities and precursors of neurotransmitters, porphyrins, and nitric oxide. Moreover, dietary proteins contain certain amino acids which are catabolized in human body and form organic acids, replenishing Krebs cycle and ammonia that expunge over the urea cycle and accordingly acts as an energy source [34].

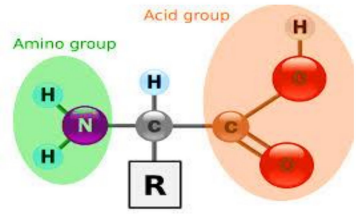

Figure 1. Structure of amino acid.

Aminoacidopathies (amino acids disorders) are a class of hereditary flaws of metabolism strokes, induced by the acquired deformities in paths engaged in amino acid metabolism. Primary amino acids disorders are caused due to mutations, resulting a metabolic block in both parents [34]. Consequently, the flaw is expressed downstream as a shortfall or a limited biological activity of enzymes engaged in amino acids metabolism [34]. Therefore, part of substrates in the particular paths acquire or are whirled toward surrogate paths. In other words, these maladies are biochemically outlined by irregular amounts of single or several amino acids and their metabolites. Aminoacidopathies bear a range of nonspecific clinical symptoms [34]. In alliance to therapeutic alimentation, these afflictions are handled by dietary constraints, supplements, and pharmaceutical foods, reducing the expenditure of a disturbing amino acid or in number incidents, protein expenditure [34,35].

MSUD is an autosomal relapsing disease which is the product of the lack of branched-chain α-keto acid dehydrogenase complex (bkAD) in the second step of catabolic path of b AAs [35]. MSUD is performed alongside five clinical phenotypes without a precise genotype-phenotype relationship. MSUD types can be classified according to the age at onset, the harshness of symptoms, the reply to thiamine supplementation and the biochemical outcomes [35]. In neonatal period classic and E3-deficient MSUD ordinarily appears, while the intermediate, intermittent, and thiamine-responsive types occur in any time of life [35]. It is presented by neurological and developmental delay, encephalopathy, feeding problems, and a maple syrup odor to the urine. It is as well biochemically described by risen plasma b AAs [34,35]. It is regulated by dietary leucine constraints; thus, all b AAs and allo-isoleucine are customarily monitored and alongside prompt medication, inmates generally enjoy satisfying clinical results [34,35].

Biallelic pathogenic variations in the catalytic segments of bkAD reduce its activity with reinforcing b AA amounts and inducing toxicity inward skeletal muscle and brain tissue [36,37]. b AA catabolism

is imperative for regular functions [38]. The prime step happens in the mitochondria and includes the alteration of leucine, isoleucine, and valine into their related α-ketoacids by branch-chain aminotransferase. b AAs can be identified in protein-rich nutrition and rest in the family of the nine amino acids indispensable for mankind, presenting influential provinces in protein synthesis and function, cellular signaling, and glucose metabolism [39,40].

On the next step in b AA catabolism, the bkAD complex commence oxidative decarboxylation of α-ketoacids [14], resulting in the alteration of α-ketoacids into acetoacetate, acetyl-CoA, and succinyl-CoA. The bkAD complex is composed of different segments, incorporating subunits E1α and E1β, E2, and E3. Reinforced b AA concentrations in the body renounce in these ingredients inducing MSUD [14,37,38]. Mouse models were used [41] as well as MSUD patients [42], where exaggerated loads of b AAs emerged and can generate serious tissue corruption if omitted without treatment. Abnormalities in b AA metabolism may induce implementations in glutamate synthesis, causing neurological implications [35]. The solution in preventing this symptomatology is the control of plasma concentrations of b AAs. In addition, the accretion of Leu is extremely neurotoxic [37] and excessive concentration of Leu may influence water homeostasis into the subcortical gray matter, resulting in bloating within the brain, convert nitrogen homeostasis farther draining glutamate amounts, reinforce oxidative stress, and wrestle along other amino acids, such as Tyr, in the central nervous system (CNS), which is engaged in protein signaling [35]. Moreover, α-ketoisocaproic acid, which is regarded to be an intervening in the metabolism of Leu, is a neurotoxin, advancing the encephalopathic disorder [35].

3. Conventional Detection Methods of Amino Acids

Different analytical techniques, such as high performance liquid chromatography (HPLC) and capillary electrophoresis (CE), have been adopted in the detection of AAs or b AAs. In recent past, ongoing types of columns, such as sub-2 µm-particle packed, monolithic silica, and core-shell columns, have been broadly employed in liquid chromatography (LC) testing of AA and the analysis time has been largely minimized. The on-chip LC methodology was also established for AA detection. Separation methods have been conjoined with varied determination processes, incorporating UV [23,24], FL [21,43,44], MS [45–47], and electrochemical [25,26,48] detection. Furthermore, MS has been broadly used and has turned out to be the most accepted determination method in AAs analysis. In summary, Song et al. [1] newly reviewed the recent trends in conventional analytical methods of amino acids in biological samples, where detailed information is given about their analytical features and advantages.

4. Electrochemical bAA Sensors and Biosensors

Electrochemical techniques rest on the fabrication of sensors or biosensors and are considered suitable for in situ determination of substances because they are extremely sensitive, simple, reproducible, cheep, relatively fast, and direct, without the use of extraction or preconcentration steps [49,50]. They can also be easily used in miniaturization [51]. Electrochemical detection is usually based on monitoring the signal of oxidation or reduction of the electroactive groups accumulated on the electrode surface [51] and measure features such as electrode potential, current intensity, the amount of electricity passing through the cell, resistance and the time [52].

Cyclic voltammetry (CV) is commonly used on electrochemical procedures, due to its qualitative and quantitative information. By applying CV, a wide range of sensors and biosensors could be developed, determining the analyte. Differential pulse voltammetry (DPV) and square-wave voltammetry (SWV) are pulsed methods [53,54]. The excitation potential in SWV inheres of a symmetrical square-wave pulse of a settled amplitude superimposed on a staircase waveform of step height. In this method the forward pulse of the square wave concurs with the staircase step [55]. DPV is a voltammetric method, comparable to SWV, where the potential excitation inheres of small pulses, which are superimposed upon a staircase waveform [56]. The major profit of the particular methods is the insignificant capacitive current which emerges on the enrichment of the sensitivity of the pulse voltammetric strategies. However, DPV is mostly administered on irreversible processes or

on systems posing slow-reaction kinetics, whereas SWV is usually administered on the examination of reversible processes (rapid reaction kinetics systems) [56,57]. Electrochemical sensors have been hardly practiced in the detection of b AAs such as Leu, Ile, and Val, associated with rare diseases clinical diagnostics. This is expounded by the evidence that these substances are electrochemically inert on bare electrodes [33]. Thus, in the subsequence chapters, electrochemical (bio)sensors for the determination of b AA are summarized.

4.1. Metal Nanoparticles and b AA Electrochemical (Bio)Sensing

The limited use of electrochemical sensors in the detection of b AA could be explained by the fact that these molecules are electrochemically inactive on bare electrodes. This limitation is can be solved by the modification of electrode's surface using metal nanoparticles. On this ground, iron oxides [58,59] CoNPs [60], strontium nanorods [61] as well as multiwall carbon nanotubes (MWCNTs) [62] have been used on the detection of some branched-chain amino acids.

Meanwhile, metal nanoparticles (NPs), such as silver and gold, have exceptional attributes, such as biocompatibility, high conductivity and high surface-to-volume ratio [63]. Thus, they are particularly alluring components for applications in electrochemical sensing and biosensing [64–67], as they are widely used in shaping the surface of electrodes in order to develop methodologies for detecting species of biological interest or to make diagnostic tools for various pathological conditions. Compared to stabilizers such as plant leaves [68], fruit extracts [69], plant roots [70], glucose [71] and carbonates materials [72], used in their composition, organic and natural dyes impart to the formed nanoparticles improved properties [73–75]. In addition, organic dyes have an advantage over the above factors because they have specific ionic, polar, non-bond functional groups (-azo dyes, -sulfites, -hydroxyl, and -nitro groups) and are usually systems that have π-conjugates [76] capable of being polymerized.

For example, Hasanzadeh et al. [59] used the magnetic (Fe_2O_3) mobile crystalline material-41 (MCM-41) to modify the surface of glassy carbon electrode (GCE). They found that the proposed electrode owned electrocatalytic activity against the electro-oxidation of the studied amino acids. In this work the amino acids at larger concentrations were determined by CV, hydrodynamic amperometry, and flow injection analysis. The linear range of the proposed method was in the range of 97–176 nM and the detection limit was found to be equal to 94 nM in the case of Val. The proposed sensor was shown to have rapid response, great catalytic activity, and ease of preparation.

Furthermore, Saghatforoush et al. [60] immobilized a Fe (III)-Schiff base on a modified GCE with multiwall carbon nanotubes (MWCNTs). They discovered that the proposed electrodes had great catalytic activity against the oxidation of amino acids at positive potential in acidic solution. The outcomes gave confirmation that these electrodes postured innate stability at a wide pH range, agile response, great sensitivity, low detection limit and a very positive oxidation potential of amino acids that declined the influence of interferences of the detection method. The linear concentration range of Val, the detection limits of Val (LOD), the limits quantization of Val (LOQ) and relative standard deviation of the above-mentioned sensor were found to be 25–1000 μM, 1.67 μM, 2.79–27.14 and 2.82%, respectively.

Meanwhile, cobalt hydroxide nanoparticles were practiced on the modification of a GCE (CHM-GC) and were employed on the investigation of the electrochemical behavior of some amino acids by Hasanzadeh et al. [61]. CV, chronoamperometry methods, and steady-state polarization measurements were used on the investigation of the oxidation and its kinetics. The results exposed that cobalt hydroxide sponsors the rate of oxidation by reinforcing the peak current, so the particular bimolecular reactions are oxidized at smaller potentials. CVs and chronoamperometry revealed a catalytic EC mechanism to be employable with the electrogeneration of Co(IV).

Strontium oxide nanorods (SrO NR) is another example of metal nanoparticle that can be used in the detection assays of b AAs. Thus, Hussain et al. [62] synthesized SrO NR in alkaline medium by a wet-chemical method. Their results showed that a thin-layer of the NR was accumulated on a GCE, fabricating an electrochemical sensor for L-Leu. The proposed sensor had good sensitivity,

a wide dynamic range, and good long-term stability. The response to Leu was studied by the current-voltage (I-V) technique. The calibration plot was linear between 0.1 nM to 0.1 mM. The sensitivity was equal to 2.53 nA·μM^{-1}·cm^{-2}, and the limit of detection was calculated and found equal to 37.5 ± 0.2 pM. The sensor was applied to real samples such as L-Leu spiked urine, milk, and serum, giving acceptable results.

Another useful tool on b AA analysis is multiwall carbon nanotubes. On this matter, Rezaei and Zare [63] developed a simple and sensitive leucine voltammetric detection assay in blood and urine samples. In their study a GCE was used and modified with MWNTs. The CV measurements revealed that MWNTs enhanced the oxidation of Leu GCE. They revealed that Leu was oxidized following multistep mechanism on the proposed electrode. A calibration curve was plotted under the optimum condition, and the sensor had a linear response in the range 9.0×10^{-6}– 1.5×10^{-3} mol L^{-1}. The LOD was found equal to 3.0×10^{-6} mol L^{-1} and a relative standard deviation (RSD%) was estimated below 3.0% (n = 5).

The use of metal nanowires (NWs) gives some advantages in clinical diagnosis such as simplicity, rapid sensor response and short total analysis time, and low sample consumption. Recently, García-Carmona et al. [77] demonstrated the fabrication of vertically aligned nickel nanowires-based electrochemical sensors (v-NiNWs) for fast determination of b AA, aiming to noninvasive screening of MSUD. v-NiNWs. The analytical features of the proposed methodology (for Leu as representative b AA in MSUD) such as LOD (8 mM) and linear range (25–700 mM) demonstrate that v-NiNWs are suitable disposable features for monitoring b AA in MSUD due to their ability to differentiate healthy and MSUD penitents. In addition, the results pointed an excellent intra and inter-electrodes repeatability. Total b AAs were also determined in positive samples with accuracy in just 5 min and using only 250 mL.

Furthermore, NiO NPs were electrochemically immobilized on a GCE and a platinum electrode [78]. In the particular study, CV in a flow cell was used, evaluating the sensors' capability to detect Val among other amino acid. The LOD was estimated to be 4 mM. In this work it was found that Val was electroactive sporadically.

4.2. Enzymatic Aproaches and b AA Electrochemical Biosensing

Over the last few decades enzyme biosensors have been widely developed, and it is evident that they are innovative assays in qualitative, as well in quantitative analysis of numerous analytes [79]. Electrochemical enzyme biosensors have distinguished advantages, because they are highly sensitive and specific, portable, cheap, and can be miniaturized and in this way can be used in the point-of-care diagnostic, which make them alluring for clinical analysis and routine measurements [79].

Generally, a biosensor is a device that operates to analyze a sample in the presence of a specific target analyze. Customarily, a biosensor is manufactured from a biological unit, which is roared as molecular recognition element, and a detector based on physicochemical process or transducer [79]. The biological unit (recognition element) is accumulated on the transducer's surface, interacts with target analyte [59]. The variations are then noticed by the transducer, and transformed to measurable signals, used to detect the concentration of the target molecule [79]. Biosensors are divided depending on either the recognition element, such as nucleic acids, antibodies, enzymes and cells, or by the class of the transducer (optical, mass-based, electrochemical, piezoelectric) [79].

On the other hand, electrochemical biosensors rely on the electrochemical properties of transducers and substances to be analyzed. This kind of biosensors were established as a development of the first glucose enzyme biosensor [80]. Intrinsically, variation in physicochemical features of elements such as current, voltage, resistance, or superficial charge, risen by oxidation-reduction processes are the output signals. The most popular class of transducers are amperometry, potentiometry, conductometry, and impedimetry.

Therefore, a recent example of an enzyme-based biosensor is that García-Carmona et al. [81] developed. The proposed biosensor is claimed to be fast, simple, selective, and sensible. The proposed

biosensor was fabricated with the on-line coupling of millimeter size motors (m-motors) and chronoamperometry for real-time analysis of b AAs in MSUD samples. Thus, an integrated cell device, including reservoirs for motors movement and electrochemical determination, was constructed. L-amino acid oxidase was introduced in m-motors by capillarity without coating chemistry, which is necessary for the enantioselective identification, and afterwards the released H_2O_2 was electrochemically screened with copper microwires. The results showed that the innate m-motor "*self-micro mixing*" characteristics and the extended delivery of new and free enzyme had the advantage on the one hand of avoiding the agitation or the physical adsorption of the enzyme, as well as its chemical bonding on transducer's surface and on any other way it reduced the investigation time.

Stefan-van Staden et al. [82] proposed four amperometric diamond paste-based biosensors and then used in the detection of are for the enantiopurity of Leu [82]. Biosensors' layout used physical accumulation of L- and D-oxidases on the proposed electrodes. In this paper, the characteristics of the different proposed sensors were examined in contrast. The results showed that the sensors were linear in pmol/L to nmol/L level. It was also found that the proposed sensor was reliable in detecting the enantiopurity using Leu as a raw material.

Moreover, Labroo and Cui [83] reported the fabrication of an amperometric bienzyme biosensor based on screen-printed electrodes and used it in the detection of Leu. The proposed biosensor was constructed by immobilizing p-hydroxybenzoate hydroxylase (HBH) and Leu dehydrogenase (LDH) on the proposed electrode, using $NADP^+$ and p-hydroxybenzoate as cofactors. The operating principle of this biosensor relied on the catalytic ability of LDH towards the specific dehydrogenation of Leu. The resulted NADPH prompts the hydroxylation of p-hydroxybenzoate by HBH in the existence of oxygen to generating 3,4-dihydroxybenzoate, developing an alteration in electron density on the proposed electrode. They claim that this sensor was linear in the range 10–600 μM and LOD was estimated to equal to 2 μM. Conclusively, they found that the sensor was rapid and reproducible with a total analysis time of 5–10 s.

Finally, the fabrication and analytical effectiveness of a bienzyme biosensors for the selective detection of AAs enantiomers using amperometric transduction is described in the literature [84]. The studied enzymes of the proposed method, as well as the mediator (L-Amino acid oxidase, horseradish peroxidase, ferrocene, respectively) was accumulated on a graphite-Teflon electrode with physical insertion in a graphite-Teflon solution. Experiments were made with and without the regeneration of electrode's surface on the useful lifetime of one single biosensor and on the reproducibility in the fabrication of different biosensors and gave evidence that the constructed biosensor, was robust and reproducible. The proposed modified electrode was employed in the successful detection of enantiomers of AAs in racemic mixtures which was indicative of the selectivity of the method, and to the evaluation of AAs in muscatel grapes.

4.3. Conducting Polymers and b AA Electrochemical (Bio)Sensing

Conducting polymers (CP) are of interest for their use as sensitive electrode surface coatings on electrochemical sensors and biosensors (electrode surface modifiers) [85]. They are outlined by large electrical conductivity and satisfying electrochemical reversibility and therefore upholding their application on sensor transducer signaling. Furthermore, CPs can be chemically acquired functional groups, which act as "tags" because of their qualification to identify biological or chemical items [86,87].

Nevertheless, the determination of small (bioactive) analytes such as b AAs remains an open continues to be an unclosed issue, as their mere correlation with detectable groups on the CP substrate is not adequate to generate the necessary electrochemical change for their detection. The key in this case is to build high-specific CP recognition points, which will strengthen selectivity and advance the sensitivity of the nidification procedure. In connection with the specificity of the above process, molecularly imprinted polymers (MIPs) can be administered in the synthesis of polymers with predetermined molecular recognition features and can be used in constructing sensors and

biosensors [88,89]. Molecular imprinting is the innovation of these designs, as they enjoy plentiful improved traits: they are sensible, fast, simple and can be portable [90,91].

Meanwhile, cellulose nanocrystals polymers, usually are used to upgrade sensitivity and selectivity of b AAs detection and may be an alternative solution [92]. This strategy counts on a rod-like network of eminently crystalline fibers and owns a huge specific surface area, contributing valuable electrical and optical attributes. Hence, Bi et al. [90] proposed an electrochemical sensor depended on 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO)-oxidized cellulose nanocrystals (TOCNCs). In this study, L-Cys modified Au electrode (TOCNC/L-Cys/Au) was formulated for determination and differentiation of the enantiomers of Phe, Leu, as well as Val. CV and DPV experiments showed that the constructed electrode had a peak current difference for the selected enantiomers. The proposed modifier was outlined by its various interactions with the different enantiomers, obtaining the identification of the enantiomers.

On the other hand, microfluidic chips (MCs) provide many advantages in diagnostics due to theirs fast response, the use of small quantities of sample with good reproducibility, opening new boundaries on point-of-care diagnostics (POC). In addition, electrochemical transducers are convenient for these devices because of their inherent miniaturization and sensitivity. In this sense, conjugated MCs with electrochemical methods which are simple, rapid, and cost-effective, is an attractive approach for a POC device that can be used in metabolic diseases such as MSUD [30,93].

Based on these, an electrochemical microfluidic assay was practiced in the partition, as well as the determination of AAs enantiomers of D-Met and D-Leu by Batalla et al. [94]. The proposed device admitted the adjusted microfluidic D AA partition, as well as the particular reaction among D-amino acid oxidase (DAAO) and one by one AA on a sole arrangement of a MC. The proposed system was claimed to be consistent with small sample expenditure, averting the need for supplements for the partition of enantiomers, and the covalent accumulation of the enzyme on the wall channels or on the electrode surface. Hybrid polymer/graphene-based electrodes were end-channel capped to the microfluidic apparatus, advancing the traits of the procedure. D-Leu were fortuitously determined, adopting the introduced system. D-Leu was detected indirectly by the detection of H_2O_2. The proposed method was claimed to have superlative precision in migration times and in peak heights, revealing the satisfying stability of the proposed sensor. It was found that the proposed sensor had also selectivity, due to the inactiveness of L AAs.

5. Conclusions

In this review, electrochemical (bio)sensors for the detection of b AAs were summarized. Electrochemical (bio)sensors as well as POC devices are relatively new avenues for reliable, accurate, sensitive, selective, green, cheap on the detection of b AA, involved in metabolic diseases such as MSUD, and are in line with current European and worldwide developments, concerning public health issues and representing the state of art on developing analytical methodologies. However, the use of electrochemical (bio)sensors in the detection of b AA have been poorly studied, as it can be seen from the lack of relevant studies in the literature. Although years of research have provided a lot of information on conventional analytical methods such as MS, limited research was made in the field of electrochemical (bio)sensors, which could improve the analytical features of b AA detection. Furthermore, investigating the prospects of increasing the accuracy, the sensitivity, the selectivity, the simplicity as well as minimizing the cost and toxicity of present b AA analytical methods is also an innovative approach to an old need for the world clinical diagnostics and electrochemical (bio)sensor are suitable tools towards this direction. In Table 1 selected studies in electrochemical (bio)sensing MSUD and b AA are summarized.

Table 1. Comparison of selected b AA electrochemical (bio)sensors.

Electrode	Analyte	Linear Range	LOD	Ref
MCM-41-Fe_2O_3/GCE	Valine	97–176 nM	94 nM	[59]
Fe (III)–Schiff base complex on GCE/MWCNTs	Valine	25–1000 μM	1.67 μM	[60]
SrO NR/GCE	L-Leukine	0.1–0.1 mM	37.5 pM	[62]
GCE/MWCNTs	Leukine	9.0×10^{-6}–1.5×10^{-3} M	3×10^{-6} M	[63]
v-NiNWs	Leukine	25–700 mM	8 mM	[77]
p-hydroxybenzoate hydroxylase and leucine dehydrogenase on a screen-printed electrode	Leukine	10 and 600 μM	2 μM	[83]

Abbreviation: GCE: glassy carbon electrode; MCM-41-Fe_2O_3/GCE: modified glassy carbon electrode with magnetic (Fe_2O_3) mobile crystalline material-41 (MCM-41); MWCNTs: multiwall carbon nanotubes; SrO NR/GCE: modified glassy carbon electrode with strontium oxide nanorods; v-NiNWs: vertically aligned nickel nanowires; LOD: limit of detection.

Meanwhile, greater analytical features are attained when electrochemical techniques are coupled with NPs. To that end, the great antifouling trait of NP electrodes is notably meaningful, considering that they are skilled to execute a considerable number of detections without the loss of their analytical features as has been disclosed by their valuable repeatability. This particular trait awards them respectable facilities to be practiced on the determination of biomarkers in real samples.

Notwithstanding, the dominant challenge continues existing, when real samples are to be analyzed, due to problems related to reproducibility, stability, as well as interferences. These elements can be worked out by evolving innovative sensors built on chiral nanostructured components, favoring the selectivity of the determination. Admirable analytical enforcement may be brought about through the coupling of enzymes to NPs and electroactive arbiters.

Conclusively, the captious prospects of the leading edge on the determination of b AAs, clearly launches innovative frontiers on electrochemical sensors for rapid monitoring of a disease, initiating modern concepts in diagnostics. A forthcoming advancement in electrochemical sensing may be the growth of implantable sensors for prolonged disease screening. Thus, novel (bio)materials must integrate into devices, achieving stability and limiting the infections with unwanted substances. Late determination methodologies, such as ultra-fast CV may be occupied for real-time b AA monitoring. The advancement of mercantile arrangements on b AA monitoring predicated on electrochemical (bio)sensors and chromatographic or electrophoretic techniques may be the next step in the field.

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