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Abstract:
Many diseases can be identified by the appearance of the markers in body fluids like blood, urine, saliva, tears, etc. This article aimed to summarize the newest studies about electrochemical sensors with molecularly imprinted polymers as sensitive and selective layers on the electrode to detect protein-based biomarkers of neurodegenerative diseases. The article describes the detection of the biomarkers of Alzheimer's disease (Amyloid-β oligomers and p-Tau), Parkinson's Disease (α-Synuclein), and stress biomarker (*α*-amylase) using molecular imprinting technology, and also, analyses the development of the sensor design, and the influence of used materials. The research methods, the application of different electrodes, the influence of the polymers, and the established detection limits are reviewed and compared.

Keywords: molecularly imprinted polymer (MIP); conducting polymer (CP); biosensor; electrochemical sensor; disease biomarkers; Alzheimer's disease biomarker; Parkinson's disease biomarker; stress biomarker.

1. Introduction

The selectivity and sensitivity of some sensors can be improved by the application of various structures that increase the selectivity of these sensors [1-3]. The semiconducting properties of some conducting polymers (CPs) are very suitable for sensing purposes, therefore, structures based on conducting polymers can be applied in various sensing devices [4-6]. Some conducting polymer formation technologies enable to increase in the selectivity of sensors toward selected analytes [4, 7-11]. CPs possess remarkable semiconducting properties [12-15], and can be deposited over the signal transducer [16, 17]. Polymer-based structures can be exploited for charge transfer from the redox enzymes [18].
Different polymer formation approaches are applied for the formation of CPs-based sensing structures, these methods include chemical [19], electrochemical [13], enzymatic [20], and/or

microorganism-assisted [21-23] formation methods. During the formation of CPs some biomaterials (e.g.: enzymes [24-26], antigens [27] antibodies [4, 20, 28] and various receptors), [29] which are able to bind selected targets and provide advanced selectivity for biosensor. However, the stability and the price of biomolecules are mostly not sufficient, therefore, some reasonable replacements are demanded to reduce the price and increase the stability of designed bioanalytical systems. A very reasonable alternative is the employment of molecularly imprinted polymers (MIPs) [30, 31] here among many other polymers CPs can be very efficiently applied [30-36]. MIPs can be used in the development of analytical systems for the diagnosis of infectious [37] and some other diseases.

Recently, the diagnosis of some diseases is very often based on the determination of particular biomarkers. Moreover, biomarkers are essential for the rational development of drugs and medical technologies. Common and recurrent disorders such as cancer, neurological, and cardiovascular diseases are considered to be the leading causes of human death. Using biosensors, these disorders could be detected by biomarkers at an early stage of pathogenesis. To increase patient survival, rapid and targeted treatment is necessary. According to the National Cancer Institute, a biomarker is a biological molecule found in blood or other body fluids, or tissues that is a sign of a normal or abnormal process, or of a condition or disease. Such compounds also may be used for following the body's responses to a treatment of a disease or condition [38].

This study aims to overview MIPs for the determination of protein-based biomarkers for the diagnosis of some diseases.

2. Major principles and methodologies used for MIP formation

MIPs are formed by polymerizing functional monomers in the presence of an analyte to form cavities for target molecules and deposited on different types of electrode surfaces (pencil graphite electrode [39], graphite electrode [40], quartz crystal microbalance sensor [8, 41, 42], surface plasmon resonance sensor [43], boron-doped nanocrystalline Diamond [31, 44, 45], platinum electrode [46, 47], etc.). After analytes are removed, they leave complementary imprinted cavities to recognize target molecules such as low molecular weight molecules (e.g. theophylline [31, 44], caffeine [8], histamine [45], uric acid [42, 48], tryptophan [40], etc.), or large molecular weight objects (e.g. proteins [46, 47, 49], DNA [39], viruses [50], or bacteria [51]). Numerous traditional immunoanalytical methods provide precise measurements of different analytes. However, most of these methods, including the enzyme-linked immunosorbent assay (ELISA), depend on the use of expensive immune chemicals and/or lengthy analyte determination methodologies, as well as high-end tools, and are required to be operated by a specialist. MIPs provide a wide range of possibilities for the development of biosensors including low-cost, easy preparation, advanced storage stability, quick read-out time, good specificity, and the possibility of clinical or point-of-care testing. MIP-based sensors in comparison with antibodies or receptors-based sensors offer superior chemical and thermal stability. Thus, MIP-based electrochemical biosensors have become very attractive for the detection of disease biomarkers. The extraction of proteins from polymer films, the reusability of these imprinted protein-based sensors, conformational changes of the proteins during the imprinting phase, and the proper orientation of the proteins during the imprinting phase are the difficulties associated with molecular imprinting of proteins [30]. The imprinting of small molecules is less complicated compared to macromolecules, for instance, proteins. The diffusion of proteins into the cavities imprinted in the polymer matrix is greatly hindered by their vast size. Functional monomers, highly reactive free radicals, cation radicals, or organic solvents can affect the proteins or amino acids during the polymerization process and it can alter the morphology of the imprinted cavities. The tertiary and quaternary structure of protein templates may be affected by the sum of all these processes, which would reduce the sensitivity

and selectivity of the developed biosensor, thus, there are relatively few selective biosensors for diseases' proteins based on MIPs [52, 53]. Also, some drawbacks of MIPs previously were emphasized [54]. As drawbacks of MIPs, studies noted difficulties with electropolymerisation, especially the mass production, and bulk polymerization due to heterogeneous binding sites and other methods that provide a poor synergy with the electrochemical detection [54].

Different combinations of monomers, initiators, cross-linkers, and solvents are used to create MIPs. In aqueous solutions or organic solvents, electrically conducting polymeric structures are conducted at room temperature. Flexible MIP synthesis offers a significant benefit for imprinting biomolecules, it provides an opportunity to elude conformational changes and denaturation. Although, MIP-based assays have demonstrated competitiveness with assays like ELISA in terms of specificity, sensitivity and compatibility with technologies like lateral flow lab-on-a-chip, the commercial potential of these assays has not yet been completely realized [55].

In pursuance to create a suitable template for MIP, many different conducting polymers are used. Due to their various qualities and biocompatibility, conducting polymers have been extensively used in the fields of bioanalytical and biomedical research, drug delivery, tissue engineering, and cell culture. Conducting polymers' most favoured characteristics include electrochemical activity and conductivity, mechanical elasticity, biocompatibility, and environmental stability. These qualities are necessary to improve the sensing capabilities of the analytical and bioanalytical systems [4]. Because of their electrical properties, which enable the conversion of biochemical data into electrical signals, conducting polymers provide the most promising materials for the biosensors [6]. Conducting polymers can be prepared by polymerization from the chemical [19, 56], electrochemical oxidative techniques [8, 42], or enzymatic [57-60], etc. Cyclic voltammetry is the most used technique for electropolymerized conducting polymers. It is also feasible to control the polymer thickness by adjusting the voltage range [61].

3. The application of MIPs for the determination of Alzheimer's disease

As the population of many developed countries ages, the World Health Organization predicts that neurodegenerative diseases such as Alzheimer's disease (AD) and other forms of dementia, as well as conditions like Parkinson's disease and amyotrophic lateral sclerosis, will overtake cancer to become the second leading cause of death after cardiovascular disease by 2040 [62].

AD is a multifactorial neurodegenerative disease. Its pathogenesis has been attributed to the extracellular deposition of amyloid β plaques, the intracellular neurofibrillary tangles that are made of hyperphosphorylated-Tau protein, and synaptic, and neuronal loss. These factors mostly damage cortical and limbic areas of the human brain [63]. The accurate diagnosis of AD is complicated and mostly performed by invasive, time-consuming, and expensive analysis like cerebrospinal fluid analysis or neuroimaging techniques such as positron emission tomography and magnetic resonance imaging. Since no inexpensive test to diagnose AD using biomarkers at all stages of the disease has been developed, new detection methods such as MIP-based electrochemical biosensors are being developed to address this limitation. The biomarkers that are the most indicative of AD are amyloid-β peptides (Aβ40 of 40 amino acids and Aβ42 of 42 amino acids), total-Tau, and phosphorylated-Tau (p-Tau). Because of their hydrophobicity, amyloid-β peptides can bind to plasma proteins, resulting in incorrect results of the analysis. Usually, proteins and other substances made by brain cells are detected in cerebrospinal fluid but also it could be measured by sensitive blood tests [64]. Although these biomarkers are promising molecules for the detection of AD, further investigation is still needed [65]. Besides, the asymptomatic AD phase can last decades [66]. The physiological levels of Aβ42 in the cerebrospinal fluid of AD patients are reported to be

less than 500 pg/mL [67]. AD is characterized by the production and deposition of amyloid-β oligomers in the brain, therefore, this peptide is a promising biomarker in the diagnosis and monitoring of AD progression.

3.1. MIPs of amyloid-β oligomers

In recent years, it has been suggested that cerebrospinal fluid measurement of β-amyloid peptides, especially Aβ42 and Tau proteins, including total and phosphorylated forms of Tau, may alter the positron emission tomography image in determining the pathophysiology of AD. In cerebrospinal fluid, the changes observed include a 50% reduction of Aβ42 as a consequence of amyloid deposition in the brain. The changes also include an increase in p-Tau and total-Tau. In clinical practice, the use of Aβ42 as a biomarker for AD is largely limited by the absence of reference values. Measurable values of Aβ42 in plasma of cognitively normal individuals ranged from 8.12 to 29.00 pg/mL [68]. The levels of the cut-off of 680 pg/mL for abnormal cerebrospinal fluid Aβ42 were also determined [69].

R. Dehdari Vais *et al.* [70] approached the MIP-based biosensor for Aβ42 detection. Pyrrole monomer was electropolymerized on a screen-printed carbon electrode in the presence of an Aβ42 template. Polypyrrole was chosen as a polymer film because of its easy electropolymerization and good stability in biological samples with neutral pH values. To evaluate the effectiveness of the biosensor, it was tested for the detection of Aβ42 in artificial cerebrospinal fluid and the results confirm the applicability of the biosensor for the diagnosis of AD. Based on the calibration curve, Aβ42 could be determined in cerebrospinal fluid by a LOD of 2.3 pg/mL. A low LOD of 1.2 pg/mL was achieved, indicating the suitability of the biosensor for the detection of Aβ42 at clinically relevant levels. Graphite and some other carbon-based electrodes can be modified by roughing, increasing surface area or oxygenated functional groups on the surface, M.V. Pereira *et al.* [71] designed for the first time a MIP-based electrochemical biosensor for Aβ42 on a flexible paper platform. The carbon ink electrode was doped on the paper support, the poly(3,4-ethylenedioxythiophene) and amino thiophenol were used as a pretreatment and finally, the poly(*o*-phenylenediamine) MIP film was polymerized to trap Aβ42 molecules. In comparison to metal electrodes, carbon provides many advantages due to its low cost, wide range of possibilities, and high chemical stability. Commercial serum was used to test this designed biosensor. A Biosensor showed good sensitivity (LOD is 0.067 ng/mL with a linear range from 0.1 ng/mL to 1 μg/mL) in a healthy individual's blood sample. This biosensor could be improved in the future to determine the reference level of amyloid in the blood of patients with AD.

MIP techniques for Aβ42 detection were first applied by F.T.C. Moreira *et al.* [72]. *α*-cyclodextrin was implemented to trap target molecules on a polymer matrix layered on top of conducting polymer polyaniline layer. The LOD value of such MIP was 0.20 ng/mL. Later the same group improved the biosensor [73] by using polyaniline as a polymer matrix to entrap target molecules and used nanoparticles modified with copper oxide as conducting substrate for enriched electrolytic activity and electron transferability. Copper and copper oxide show notable electrocatalytic activity among other electroactive materials. The copper oxide nanowires, with their increased surface-to-volume ratio, provided an improvement in detection sensitivity. Significant improvement was obtained reaching a LOD of 0.4 pg/mL in a range from 0.1 ng/mL to 66.0 ng/mL in diluted blood samples without the need to incubate an active redox element on the working electrode under optimal conditions. This biosensor showed satisfactory results in Aβ42 detection for AD patients. To make the results more sensitive, several modifications can be made at once. N. Ozcan et al. [74] formed a highly sensitive MIP/target/aptamer sandwich of delaminated titanium carbide MXene and multi-walled carbon nanotubes (MWCNTs) composite including a MIP assay for β-amyloid protein recognition. Delaminated titanium carbamide MXenes are easy to make and possess a high electrical

conductivity, while MWCNTs prevent MXenes from aggregation. The biosensor showed a very low 0.3 fg/mL threshold of detection. The recovery in blood plasma was 99.99% - 100.04%. These results seem to bring out the relevant role of highly sensitive electropolymerized pyrrole as a template entrapment matrix, MWCNTs as electrode modifiers, and the benefit of MXene as electrode construction material. Based on the low LOD of this biosensor, it may be a suitable sensing platform for the detection of β-amyloid in the blood of patients with AD.

MIP technology with conducting polymers has been used in AD sensors also to detect chemicals found even in the breath of AD patients. S. Emam *et al.* [75] developed an innovative butylated hydroxytoluene air sensor and chose pyrrole as a conducting functional monomer due to its well-studied properties. It is important to note that non-invasive and less stressful interventions than blood or cerebrospinal fluid tests are important in AD diagnostic sensors research, and MIP is a suitable technology for this purpose.

The most sensitive platform for β-amyloid was created by N. Ozcan *et al.* [74], combining polypyrrole with d-$Ti_3C_2T_X$ MXene and MWCNTs. F.T.C Moreira *et al.* [73] used polyaniline modified with copper oxide resulting in low LOD. R. Dehdari Vais *et al.* [70] study also showed satisfactory results by entrapping molecules just in polypyrrole. It can be concluded that polypyrrole is a suitable and commonly used polymer for the detection of AD markers in the blood, cerebrospinal fluid, and even breath.

3.2. MIPs of p-Tau

p-Tau is usually examined in cerebrospinal fluid but can also be identified in blood, values vary due to different isoforms, age, stages of AD disease, and other factors. A. Ben Hassine et al. [76] reported an electrochemical biosensor based on a MIP for p-Tau-441 protein detection. *3*-aminophenol as a monomer was chosen because poly(3-aminophenol) is beneficial for high permselectivity, and simple control of polymer density that self-limits its growth. The stability of the target protein is favoured by its interaction with the amine and hydroxyl groups during the rebinding step. The LOD of this biosensor was 0.024 pM in buffer and 0.067 pM in spiked and diluted human serum samples. This suggests that other molecules present in the serum may interact with vacancy recognition and affect sensitivity. The results have shown a good overall performance of precision and LOD, and in the future, it may be suitable for the detection of p-Tau protein.

Analysing a mixture of currently examined markers such as β-amyloids and Tau proteins in combination with neurotransmitters and genetic markers from blood samples could improve the usual laboratory methods, allowing the AD diagnostic test to be made available to the general population. Given the global incidence of AD, there is still a need for a low-cost, environmentally friendly biosensor with high stability and selectivity in many circumstances.

4. The application of MIPs for the determination of Parkinson's Disease

Protein misfolding disorders are the term used to describe neurodegenerative diseases such as AD and Parkinson's disease. α-Synuclein is a protein containing 140–amino acids (14.3 kDa). α-Synuclein protein accumulation in the neuronal cell body ('Lewy bodies') and neurites ('Lewy neurites') is related to all cases of Parkinson’s disease [67, 77]. α-Synuclein has been detected in cerebrospinal fluid, blood, saliva, skin (cutaneous nerves), enteric nervous system, and retina of Parkinson’s disease patients [78]. Thus, it could be a marker of PD, and its level may be an important factor in the severity of the disease.

Y. Ma *et al.* [77] synthesized a biosensor for *α*-Synuclein detection. The sensor was made of a GCE electrode modified with graphene nanosheets and nanospherical conjugated

microporous polymer with the imprinted template *α*-Synuclein. Porous organic polymers in particular stand out due to their distinctive morphologies, customizable design, and various preparation techniques. Conjugated microporous polymers, a subset of porous organic polymers, are a type of material with a 3D network, a durable nanoporous structure, and a framework with a π-electron conjugated structure resulting in higher thermal and chemical stability, bigger surface areas, and precisely calibrated porosity. The LOD of 3.5×10^{-5} ng/mL was achieved on this sensor and the results in serum showed the potential for diagnosing Parkinson's disease in the early stages.

α-Synuclein can be accurately detected in cerebrospinal fluid by various methods of immunoassays (ELISA and electrochemiluminescence); serum and plasma levels can be carried out using standard immunoassays, single-molecule sandwich immunoassays, and ELISA. However, these kinds of methods are expensive and require laboratory preparation, it makes sense to develop a simple, inexpensive, and portable α-Synuclein detection sensor.

5. MIP application for detection of stress biomarker *α*-amylase

Saliva collection is non-invasive, relatively simple, less likely to cause stress, and can reflect real-time levels of biomarkers. The most valuable potential markers of stress in saliva are cortisol, lysozyme, *α*-amylase, and Chromogranin A [79]. Acute stress is associated with activation of the sympatho–adreno–medullary system, which is reflected by salivary *α*-amylase and chromogranin A. Salivary amylase levels rise considerably faster than cortisol in response to psychological stress, showing that it is a superior stress indicator. The enzyme is also thought to be an indicator of a state of calm or relaxation [80]. Normal *α*-amylase ranges in blood serum 0.05–0.125 U/mL and saliva 19–308 U/mL [81]. As a result of increased physiological and psychological stress, the concentration of *α*-amylase in saliva (above 0.5 mg/mL) rises.

T.S.C.R. Rebelo *et al.* [82] developed a disposable point of care stress biomarker *α*-amylase using MIP technology with a LOD of 3.0×10^{-2} mg/mL in human saliva. First, a cysteamine self-assembled monolayer was formed on the AuSPE, then the pyrrole was electropolymerized. In the rebinding process, the obtained sensor demonstrated high sensitivity and selectivity on *α*-amylase in human saliva and a buffer solution containing other biomarkers. With a portable system and disposable chips, this MIP biosensor demonstrated analytical capabilities as a suitable candidate for diagnostic point-of-care testing devices for the detection of *α*-amylase in saliva.

From the reviewed studies, it can be concluded that electrochemical biosensors based on MIP technology can be applied to detect Aβ42 p-Tau 441, *α*-synuclein, and *α*-amylase. This suggests that MIPs can be used to detect neurodegenerative diseases such as Alzheimer's and Parkinson's disease as well as to identify patients dealing with stress. Conducting polymers are an excellent choice for precise and simple MIP biosensing devices.

Conclusions

To conclude, this review article discusses the newest studies about electrochemical sensors for the detection of protein-based biomarkers for different diseases. Bearing in mind that more accurate and better detection methods are needed today, molecular imprint technology and biosensors based on MIPs are considered an alternative, due to their attractiveness, easy synthesis, price, and short laboratory testing time. This article reviews the application of MIP to the detection of biomarkers of Alzheimer's, Parkinson's diseases, and stress. After reviewing various MIP variants, it was assumed that conducting polymers are an excellent choice for precise and simple MIP biosensing devices. Moreover, polypyrrole is a suitable and commonly used polymer for the detection of AD markers in the blood, cerebrospinal fluid, and even

breath. Electrochemical biosensors based on MIP technology can be applied to detect Aβ42 p-Tau 441, α-synuclein, and α-amylase.

MIP-based biosensors have a lot of potential for diagnostics. This review shows MIP's achievable detection limits. The ability to create such sensors quickly and efficiently contributes to a better quality of human life through more targeted diagnosis and detection of some neurodegenerative diseases.

CRediT authorship contribution statement:
Conceptualization: Ar. R. and V. R.; Writing - Original Draft: G. P., R. B., and V. R.; Writing - Review & Editing: Ar. R., D. P., M.B. and V. R.; Supervision: Ar. R.; Project administration: U. S.-B.; Funding acquisition: Al. R. and Ar. R.

Declaration of Competing Interest
The authors declare no conflict of interest.

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