Being originally designed as a tool in organic synthesis, click chemistry has emerged as one of the most highlighted techniques for engineering the architecture and functions of different types of surfaces. Therefore it can be employed in a wide range of applications in biotechnology and the development of nanomaterials. The covalent coupling on a specific and selective site, without altering the 3D configurations and orientation of active centers of biomolecules has the advantage to ensure the preservation of the biological activity and it can be applied for the design of biosensors. Thus, enzymes, antibodies, aptamers, DNA sequences can be easily coupled to polymers or other substrates such as carbon based nanomaterials, gold nanoparticles, and magnetic particles via the azide/alkyne reaction. Among the advantages of this technique there are two major points that makes it attractive for science: it can take place in an aqueous medium with applications in physiological matrix and being a chemoselective reaction can be used for the functionalization of biomolecules. The recent advances in the development of electrochemical sensors and biosensors based on click chemistry functionalization protocols are presented focused on a comparative and critical description of different configurations and their applications towards the detection of various target analytes.

Keywords: click chemistry, electrochemical (bio)sensors, immunosensors, surface functionalization

1. INTRODUCTION

The concept of "click chemistry" was introduced in 2001 by Sharpless and its coworkers [1] and was undoubtedly one of the main approaches in contemporary chemistry, being considered the success story of synthetic chemistry. The term "click" refers to a chemical process, which is efficient, selective and at the end it is obtained a single reaction product. It can be applied in physiological...
conditions and eco-friendly synthesis and due to the chemoselectivity it can be used for the tailoring of functional molecules such as peptides, nucleic acids and polysaccharides [1].

Click chemistry has been widely applied in synthetic organic chemistry, inorganic chemistry, polymer chemistry and biochemistry. Its use in almost all these domains is unique and demonstrates the high versatility of this technique. Being originally designed as a tool in organic chemistry, it emerged as one of the most employed methods for engineering the architecture and functions of different types of surfaces with important applications in biotechnology and in the development of nanomaterials [2, 3]. The electrochemical sensing applications require a stable, reproducible, highly sensitive and selective platform in order to ensure the controlled detection of the target analyte [4].

Adapting electrochemistry on multiplexed sensors systems represents one of the main approaches in biomedical research due to the wide range of envisaged applications [5]. The immobilization method for biocompounds such as enzymes [6], antibodies [7, 8], aptamers represents the critical step towards the achievement of fast electronic transfer in the fabrication of electrochemical (bio)sensors. Their direct adsorption onto the sensing platforms is a commonly used approach, but the stability of the obtained configurations in terms of reproducibility and sensitivity is a limited one [9]. The covalent coupling of biomolecules by the means of click chemistry on a specific and selective site makes it suitable for the construction of biosensors arrays, by maintaining their biological activity [10]. Moreover, this method can be applied in mild conditions, even in aqueous solvents, in the presence of a catalyst and of many functional groups without protection [11]. The process is fast, selective and resistant to side reactions [12].

Taking into account their mechanism, the click reactions were divided into four different categories: cycloadditions of unsaturated species (1,3-dipolar cycloaddition reactions and Diels-Alder transformations), nucleophilic substitution chemistry (ring-opening reactions of strained heterocyclic electrophiles), reactions which involved non-aldol carbonyl group and addition reactions to carbon-carbon multiple bonds [13].

Although there are several types of reactions as above-mentioned, the representative one of this technique is the cycloaddition, copper (I) catalyzed, between an azide group and an alkyne (CuAAC) [14]. In the absence of the catalyst, a transition metal, the cycloaddition is, in most cases, nonregioselective and quite slow. The CuAAC rate is $10^7$ higher than the noncatalyzed reaction and it can proceed at room temperature [15]. There are several approaches for the catalyst election. It can be added to the reaction environment or can be produced in situ by different strategies:

- Copper salts such as copper (I) iodide, chloride or acetate can be used, the limiting factor being the loss of its activity by oxidation to copper (II) or by dismutation to a mixture of copper (II) and (0). In this case the reaction must be performed under free-oxygen conditions.
- The catalyst’s synthesis in situ was achieved by combining copper (II) salts like copper (II) sulfate or acetate with a mild reducing agent such as sodium ascorbate, hydroquinone (HQ) and tris(carboxyethyl)phosphine [16, 17].
- Another way to produce the catalyst was the use of a copper piece. Even though the reaction time is longer at room temperature, this approach reduces the contaminations levels with copper [16].
Meldal and coworkers [18] have shown that, in the presence of Cu (I), which binds at the alkyne groups, the reactions are regioselective (position 1,4), rapid, at room temperature and in an organic medium.

Recent data have confirmed that this technique is important in biomedical applications such as: discovery of new substances with therapeutic potential, transport of molecules to target string, gene therapy and diagnostic. Drug discovery is one of the major applications due to the synthesis of complex molecules from simple molecular units. The Huisgen cycloaddition was able to control the kinetics of the synthesis of enzymes’ inhibitors [19]. Novel potent inhibitors for acetylcholine esterase, carbonic anhydrase, protein tyrosine phosphatase and HIV-1 protease were successfully constructed from large combinatorial libraries [20-22]. Another example of CuAAC application is the biochemical compounds’ synthesis such as biological oligomers, peptides, nucleic acids and carbohydrates. The oligopeptides’ functionalization was firstly presented in 2002 by Meldal et al [18]. The triazole linkage exhibited properties of aromaticity, rigidity and hydrogen bonds’ formation and was considered an interesting peptide mimetic [18]. The click reactions were also employed for materials’ synthesis such as hydrogels and microgels, which can be a base for tissue engineering, controlled drug-release matrix, and stationary phases for biological compounds [23]. One example is the synthesis of hyaluronan-based microgels networks, which exhibited an efficient controlled-release of doxorubicin [24]. The colloids tailored by CuAAC can be applied in the development of drug delivery carriers, gene carriers, contrast agents, bioseparation devices and diagnostics assays [25]. Magnetic particles were coupled to biological molecules such as fluorescent markers, steroid moieties (estrogen) or drugs (paclitaxel) [26].

The fluorogenic reaction based on this click method has emerged as an indispensable tool for in vivo labeling, cell imaging and metal sensing [27, 28]. In the recent years, designing of fluorescent chemical sensors with specificity and superior sensitivity for the detection of traces of toxic transition metal ions has gained popularity [29].

The recent research towards the development of electrochemical (bio)sensors based on click chemistry strategies will be discussed, the data being analyzed in a critical manner underlining the advantages and drawbacks of different configurations.

2. APPLICATION OF CLICK CHEMISTRY IN THE DEVELOPMENT OF SENSING PLATFORMS

The critical step in the development of a sensing device is represented by the engineering of the polymeric matrix properties in order to extend their functionalities. The enhancing of the surface is an effective and low cost alternative to the commercial available products. The most important strategy relies on the functionalization of self-assembled monolayers (SAM) [30]. Furthermore, their selective derivatization allows the coupling of nanoscale multicomponents with a controlled surface density.

The conventional immobilization techniques such as physical adsorption and layer by layer deposition do not meet the requirements of a reproducible detection platform. In this case the unreacted reagents and the possible side reactions are serious drawbacks, whereas click chemistry proved to be a suitable alternative for surface functionalization without the limitations stated above.
Pioneers in this field, Collman and Chidsey [31] reported numerous examples of SAMs functionalized via a triazole, ferrocene being the first model molecule used for surface characterization.

CuAAC was also used as a modern technique for hybrid materials’ synthesis known as polymer bioconjugates that combine the properties of synthetic and versatile polymers with those of biological compounds [32]. Proteins, enzymes, viruses, bacteria and cells could be also connected to polymers or low molecular weight functional groups via the azide/alkyne reaction. The experimental parameters need to be adapted to the physiological conditions in order to maintain their biological activity and to prevent denaturation or disassembly [1]. Initially, research was channeled on gold surfaces, but was later extended to other types of substrates such as silicon and glass. Due to the high levels of reagents, the reaction between molecules and substrate was spontaneous and rapid. A wide range of molecules was added to the planar surfaces using the CuAAC’s strategy: biotin, nucleosides, oligonucleotides, carbohydrates, porphyrins and proteins [2]. The essential condition is represented by the presence of the complementary functions involved in the CuAAC: the azide and alkyne groups on the immobilization platform and, respectively, on the modifier material or molecule. The functionalized compounds are commercially available or it can be easily synthesized depending on the previewed applications.

This technique was used for the functionalization of glassy carbon electrodes (GCE) modified with azide groups resulting from the diazonium grafting onto the surface. Gold nanoparticles (Au-NPs) with terminal alkyne groups were then coupled by click chemistry in the presence of electrochemically generated Cu (I). This electro-assisted method allowed the controlled coverage of the working surface [33]. The same functionalized electrode was employed for local click chemistry. By using a spectroelectrochemical microscopy Pt tip electrode that locally generated Cu (I), it was allowed the spatial covalent binding of alkyne groups. The results envisaged the possibility of spot immobilization of different molecules for the elaboration of small biomolecular device [34].

The attractive electronic, chemical and mechanical properties of carbon based nanomaterials such as graphene and carbon nanotubes (CNT) were combined with those of click chemistry for the enhancement of new materials’ features. Furthermore, the adsorption capacity, the possibility to be functionalized and their ability to promote the electron transfer reaction of a wide number of molecules made CNT a very attractive material for the development of electrochemical sensors and biosensors [35]. The interactions between the π-electrons on graphene and π-system donors such as pyrene can form π–π stacking structures without affecting the graphene’s sheets. The graphene modified with alkyne groups were employed for their immobilization by click chemistry on a gold surface modified with azide groups. It was then achieved a covalent binding, more stable and reproducible than the layer by layer deposition, which is based on low interactions like electrostatic adsorption, but in spite of this fact it is often used for the development of graphene platforms [36].

This protocol can be used for coupling different compounds on a gold surface in order to elaborate composite platforms with new properties [37]. A related example is represented by the covalent immobilization of amine multi-walled CNT (MWCNT) on carbon or gold screen printed electrodes (SPEs) by the means of diazonium salts’ click chemistry. The first approach used MWCNT, covalently attached to the gold electrode modified with the 4-aminothiophenol. After the generation of the corresponding diazonium salt, the MWCNT were grafted, assuring their covalent linking. An
alternative was represented by the direct electrografting of amine-MWCNT, after its diazotation, on gold or carbon substrate. The results were compared with the simple adsorption of the CNTs on the electrode’s surface, a technique that does not generate highly controlled detection platforms. Thus, there were proved their advantages towards the fabrication of a reproducible, ordered and completely covered electrode surface [35].

Other option of the click strategy is represented by the thiol-ene reaction, employed for the immobilization of pentenyl functionalized Ru(bpy')₃²⁺ on a (3-mercaptopropyl) trimethoxysilane pretreated indium tin oxide. This alternative proved a good reproducibility and effectiveness for the elaboration of a monolayer. The alkyne groups available on Ru(bpy')₃²⁺ can be further functionalized with compounds bearing thiol groups, an important approach for the fabrication of sensors [38].

2.1. Development of sensors

Click chemistry was used for many applications related to the chemical and electrochemical grafting on different materials and as a versatile method for electrode surface modification with direct applications in the development of sensors with customized superior characteristics. Therefore, it was used for the direct modification of the sidewalls of CNTs [39-42], gold [43] and GCE [44] for electrochemical applications. The immobilization of groups or other modifiers that present electrocatalytic effect at the electrode provides additional applications and is of great interest in the production of efficient, specific and sensitive electrochemical sensors.

Thereby, the new improved composite surfaces showed catalytic actions towards different analytes that cannot be electrochemically detected with unmodified substrates.

The hemoglobin (Hb) was immobilized onto an azide SAM on a gold surface. The electrode’s surface was functionalized with 4-pentynoic acid in the presence of Cu (I) generated from Cu (II) in the presence of ascorbic acid. Hb was attached by the carbodiimide reaction between the carboxylic group of 4-pentynoic acid and the amine group of the biomolecule. The covalent binding was proved by the electrochemical signal of Fe(III)/Fe(II) redox couple. This protocol offers a strategy for the covalent immobilization of functionalized biomolecules for the development of sensing device [45].

A different direction is represented by the coupling of metallophthalocyanines complexes that proved a proper catalytic activity for the detection of analytes such as hydrazine, amitrole and L-cysteine. In order to use them as click chemistry partners, they must be tailored with azide or alkyne functions, but the process is expensive, elaborated and can affect their structure in the absence of a proper protection. An alternative is represented by their immobilization by axial ligation of iron to ethynyl-pyridine, attached on a CNT surface functionalized with azide groups, being further employed for the coupling of iron phthalocyanine. The fabricated sensor was used for the detection of hydrazine with very good results [46]. A similar sensor was constructed by the immobilization of an alkyne modified cobalt phthalocyanine on a GCE after the electropolymerization of 4-azidoaniline. This configuration showed an electrochemical signal towards the detection of some pesticides, such as eserine, a cholinesterase inhibitor [47]. The detection of hydrazine and NADH was also achieved by
the immobilization of MWCNT on gold and carbon SPEs by diazonium chemistry as stated above [35].

The GCE surface can be also enhanced with alkyne Au-NPs in order to achieve the electrochemical separation of different analytes. After the modification of the carbon substrate with azide functions by diazonium chemistry, the Au-NPs were attached in the presence of Cu (II) and ascorbate. The Au-NPs determined the distortion of the hydrophobic 4-aminophenol monolayer, allowing the hydrophobic ions to penetrate it and to detect the electronic exchange with the surface. This configuration allowed the separation of the electrochemical signals for NO$_2^-$ and SO$_3^{2-}$ [48].

The click chemistry was used for the functionalization of gold electrodes previously modified with a tetrathiol-hexynyl derivative with an azide-ferrocene compound. The modification was studied by cyclic voltammetry and by the coupling of a fluorescent azide nucleotide that was detected by fluorescence microscopy [49]. The same strategy was also employed for the protein coupling on gold surfaces modified with polythiol-hexynyl compounds. The azide-PEG3-biotin was grafted in the presence of Cu (II), which was electrochemically reduced to Cu (I) at -0.3 V vs Ag/AgCl. The synthesized platform was used for the immobilization of streptavidin tagged fluorescent compounds by specific affinity interactions. The specific coupling of biotinylated human serum albumin was achieved by affinity interactions and it was detected by electrochemical impedance spectroscopy [50].

A novel tartrazine imprinted polymer-MWCNT-ionic liquid supported Pt nanoparticles composite film coated GCE was fabricated. Click chemistry was used for the MWCNTs functionalization with ionic liquid and Pt nanoparticles were then loaded on it using ethyleneglycol as a reducing agent. The MIP was prepared by typical free radical polymerization using 4-vinylpyridine as functional monomer and it was applied for the electrochemical determination of tartrazine in real samples with recoveries in between 88-108 % [51].

A three-step method was developed for the MIP thin films grafting onto gold surface. Propargyl acrylate was clicked onto an azidoundecanethiol/decanethiol mixed SAM, then polymerization was carried out directly on the electrode surface in the presence of $N,N'$-methylenebis(acrylamide) and azobisisobutyronitrile as the radical initiator and of HQ as electroactive template molecule, by applying UV light. The chronoamperometry was used for the detection of HQ and the results were compared to that of a sensor prepared by drop-coating MIPs onto gold substrate. It can be observed that in the case of the clicked-on MIP sensor for HQ the LOD was found to be about four times fold lower, while the sensitivity was found to be approximately three times higher than the coated-on MIP sensor. The improved performance is likely due to the favorable mass transfer characteristics of the clicked-on MIP sensing membrane [52].

2.2. Development of biosensors

Recent advances in the field of nanotechnology and new hybrid materials have also determined a tremendous development of new biosensors and smaller level systems able of delivering on site results of a wide variety of analytes without requiring sophisticated machinery and skilled operators. The applications of click chemistry in the design of biosensors are related to the controlled
immobilization of the catalytic compounds on a specific center onto the detection platform. Both ethynyl and azide groups, the partners involved in the CuAAC reaction, have a high energy level, but are inert to biomolecules, thereby assuring their immobilization on solid surfaces and on specific sites. These characteristics meet the critical aspects of enzymes’ immobilization and can contribute to the development of the third generation of biosensors where the electron transfer is directly performed in the absence of a redox probe.

The electrode’s surface modification with enzymatic compounds was mainly applied for the development of biosensors for the detection of hydrogen peroxide or paracetamol. The enzymatic compound is represented by the Horseradish peroxidase (HRP), which is a stable molecule and it can be easily functionalized with both of azide and alkyne groups. The immobilization of the tagged enzymes is regioselective, can be performed at room temperature on various conductive materials, preventing the proteins’ denaturation [53]. Its detection can be performed by registering the direct electronic transfer between its active center and the electrode’s surface and indirectly by the mean of a hydrogen peroxide and paracetamol, as redox probes.

By using this method, HRP functionalized with an azide group was covalently immobilized on the 1,4-dialkynylbenzene film deposited on a gold surface, successfully achieving the direct electron transfer. The immobilized enzyme showed a catalytic reduction activity towards hydrogen peroxide in a linear range between 5-700 μM, with a constant of heterogeneous electron transfer rate of 1.11 s⁻¹ and apparent Michaelis-Menten constant of 0.196 mM [54]. A narrow linear range of 5-50 μM for hydrogen peroxide’s detection was obtained after the immobilization of the same azide tagged HRP on a diazonium film previously grafted onto the working surface of planar graphite electrodes [55].

The coupling of neutravidin HRP was performed by specific affinity interaction with biotin-acylene after its CuAAC immobilization onto an azide functionalized layer on a carbon substrate. It was reported a concentration of 3.15 pmol cm⁻² enzyme and the catalytic response was found to be stable over time, proving the successful enzyme’s binding by click chemistry [56]. A different configuration for the immobilization of avidin tagged HRP was achieved by the nanopatterning of an azide polypyrrole layer by the means of nanosphere lithography. The immobilization of the enzyme was performed in two steps: the first one by click chemistry between the azide groups on the substrate and biotin-acylene. The final step determined the coupling of avidin-HRP by specific affinity interactions between avidin and biotin. The immobilization was indirectly proved by the detection of paracetamol. The analytical signal was ten times fold improved on the structured material, suggesting that the increase of the active surface area combined with the CuAAC immobilization strategy improved the properties of the biosensors for the detection of a pharmaceutical compound such as paracetamol [57].

This technique envisages the advantages of click chemistry towards the fabrication of enzymatic biosensors applied for the detection of pharmaceutical and biomedical compounds. The coupled biomolecules are commercially available, can be easily tailored with specific groups, without altering their catalytic activity and 3D configuration and can be attached on a specific recognition site with the complementary CuAAC function.
The immobilization can be performed directly onto the detection platform or by the means of specific affinity interactions biotin-avidin combined with click chemistry. The HRP was successfully detected by using target redox analytes such as paracetamol and hydrogen peroxide. The obtained results are promising and could be further exploited for the immobilization of other enzymes in order to develop third generation biosensors with applications in the biomedical field.

2.3. Development of immunosensors

Up to date the literature describes a wide range of examples for CuAAC applications in the development of immuno, apta and DNA sensors. The development of immunosensors is closely linked to the selective immobilization of the antibodies on the detection platform. The conventional methods such as direct or physical adsorption can affect the configuration of the molecules, reducing the stability and reproducibility of the sensing device [58]. When immunosensors arrays for the multiplexed detection of molecules are elaborated, the major drawback is represented by the contamination of the adjacent electrodes. This problem could be solved by click chemistry that ensures the target immobilization of the antibody onto an individual substrate. This approach was confirmed by the recognition of the target only on the specific electrode [59].

The detection of anti-human IgG was performed by using the sandwich model anti-human IgG and HRP-labeled anti-goat IgG, incubated on a previously electrochemical grafted phenylazide functionalized with alkyne human IgG by click chemistry. By this method it was achieved the specific on site immobilization of proteins, a promising route for electrochemical multianalyte detection. The elaborated configuration allowed the detection of the target with a LOD of 30 pg mL$^{-1}$ [59]. Another
configuration for anti-human IgG detection was elaborated after the coupling of alkyne-IgG on single-walled CNTs (SWCNTs) functionalized with azide groups by CuAAC. By using HRP as label, the target analyte was electrochemically quantified with the same LOD as mentioned above. Moreover, the proteins modified SWCNTs demonstrated an excellent dispersion in water and they preserved the biological activity of the immobilized molecules, proving that the proposed strategy for covalent immobilization of the protein is suitable for achieving a detection platform for immunoassays [60].

Due to the necessity of miniaturization, SPEs were used as a first hand substrate for the construction of different aptasensors. A label free DNA aptasensor for the detection of lysozyme was fabricated on carbon based SPEs after the deposition of Au-NPs on the surface followed by the self-assembly of 10-azidoundecan-1-thiol. This allowed the covalent coupling of alkyne anti-lysozyme binding aptamer. The detection of the target molecule was performed indirectly in the presence of hexaammineruthenium(III) as electrochemical redox probe also from egg white samples [61]. The carbon based working surface was functionalized with 4-((trimethylsilyl)ethynyl) benzene and p-nitrobenzen after the electrochemical reduction of the two corresponding aryl diazonium salts. An azide aptamer was then immobilized onto the surface by the click reaction in the presence of Cu (II) and ascorbate. It was achieved the impedimetric detection of ochratoxin A, a contaminant of a large range of foods, with a high affinity for this aptamer [62].

The click chemistry strategy was applied for the fabrication of a portable sensor for histidin based on the conventional portable glucose meters (PGM). The strategy was developed by immobilization of invertase-labeled alkynyl-DNA on the surface of streptavidin paramagnetic particles, modified with azide-DNA. When histidin was added into the system, in the presence of Cu(II), the reaction was blocked, the reduction of Cu(II) to Cu(I) being inhibited and a decrease in the PGM’s signal being observed [63]. The same sensor strategy was used for the detection of Cu (II). In this example, the reaction took place between the alkynyl-DNA immobilized on a carbon based SPEs and azide-DNA coupled to the invertase/magnetic bead conjugates. The catalyst was represented by Cu(I), generated in situ by the reduction of Cu (II) in the presence of ascorbate. The attached invertase promoted the conversion of sucrose to glucose, the signal being registered on a PGM, depending on the Cu (II) level [64]. The Cu (II) detection was also indirectly performed by monitoring the signal of a redox mediator coupled onto the working surface of a gold electrode by the click reaction. In this case the terminal azide group was represented by 1-azidoundecan-11-thiol and octylthiol previously assembled on the gold electrode surface. The coupling of propargyl-ferrocene was performed and the intensity of the ferrocene’s signal was linked to the Cu (II) concentration. The configuration was applied to the detection of Cu (II) in real samples with good recoveries and relative standard deviations [65].

Table 1 summarizes the data regarding the possible applications of click chemistry in the development of electrochemical sensors and biosensors. The substrates, alkyne and azide groups or the envisaged analytes are thus presented. The majority of electrode configurations imply the use of carbon or gold based substrates and the click reaction was used in order to bind biomolecules, carbon, gold or platinum based nanostructures or to modify the electrode with SAMs of tailored compounds improving the electrochemical performances in the presence of target molecules.
<table>
<thead>
<tr>
<th>Configuration</th>
<th>Substrate</th>
<th>Alkyne group</th>
<th>Azide group</th>
<th>Analyte</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSPE/MWCNT-NH$_2$</td>
<td>CSPE</td>
<td>-</td>
<td>-</td>
<td>NADH hydrazine</td>
<td>[35]</td>
</tr>
<tr>
<td>AuSPE/MWCNT-NH$_2$</td>
<td>AuSPE</td>
<td>-</td>
<td>-</td>
<td>NADH hydrazine</td>
<td>[35]</td>
</tr>
<tr>
<td>AuSPE/4-ATP/MWCNT</td>
<td>MPTMS - ITO</td>
<td>Ru(bpy$^3$)$_2$Cl$_2$</td>
<td>3-mercaptopropyl azido undecanethiol</td>
<td>Hb</td>
<td>[45]</td>
</tr>
<tr>
<td>Au/SAMs/Hb</td>
<td>Au</td>
<td>4-pentynoic acid</td>
<td>4-azidobenzene diazonium</td>
<td>hydrazine</td>
<td>[46]</td>
</tr>
<tr>
<td>GCE/SWCNT/Py/FePc</td>
<td>GCE/ SWCNT</td>
<td>ethynyl-Py</td>
<td>4-azidoaniline</td>
<td>eserine</td>
<td>[47]</td>
</tr>
<tr>
<td>GCE/PANI-N$_3$/alkyne-CoPc</td>
<td>GCE</td>
<td>alkyne-CoPc</td>
<td>4-azidoaniline</td>
<td>NO$_2^-$</td>
<td>[48]</td>
</tr>
<tr>
<td>GCE/Au-NPs</td>
<td>GCE-N$_3$</td>
<td>alkyne-AuNPs</td>
<td>4-azidoaniline</td>
<td>biotinylated human serum albumin</td>
<td>[50]</td>
</tr>
<tr>
<td>Au/bis(DTPA)-hexynyl/Azide-PEG3-Biotin</td>
<td>Au</td>
<td>polythiolhexynyl derivative</td>
<td>azide-PEG3-biotin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AuSAM decanethiol-N$_3$N$_2$-methylenesbis/N$_3$/(CH$_2$)$_3$SH</td>
<td>Au</td>
<td>propargyl acrylate</td>
<td>azido undecanethiol</td>
<td>HQ</td>
<td>[52]</td>
</tr>
<tr>
<td>GCE/MWCNTs-IL/PtNPs</td>
<td>GCE</td>
<td>propargyl IL</td>
<td>azide-MWNTs</td>
<td>tartrazine</td>
<td>[51]</td>
</tr>
<tr>
<td>Au/HRP/DEB</td>
<td>Au</td>
<td>1,4-dialkynyl benzene</td>
<td>azide-HRP</td>
<td>H$_2$O$_2$</td>
<td>[54]</td>
</tr>
<tr>
<td>CSPE/EthynylSAM/N$_3$-HRP</td>
<td>CSPE</td>
<td>ethynyl-monolayers</td>
<td>azide-HRP</td>
<td>H$_2$O$_2$</td>
<td>[55]</td>
</tr>
<tr>
<td>GCE/Nanostructured N-(10-azidodecyl)Ppy/ethynyl-biotin/HRP avidin</td>
<td>GCE</td>
<td>ethynyl-biotin/HRP-avidin</td>
<td>N-(10-azidodecyl) pyrrole</td>
<td>paracetamol</td>
<td>[57]</td>
</tr>
<tr>
<td>GCE/azideSWCNT/alkyne-IgG-HRP</td>
<td>GCE</td>
<td>alkyne-IgG</td>
<td>azide-SWNT</td>
<td>anti-IgG</td>
<td>[60]</td>
</tr>
<tr>
<td>GCE/phenylazide/alkyne IgG</td>
<td>GCE</td>
<td>alkyne-human IgG</td>
<td>phenylazide</td>
<td>anti-human IgG</td>
<td>[59]</td>
</tr>
<tr>
<td>SPCE/AuNPs/SAM 10-azidoundecan-1-thiol/antiLBA</td>
<td>SPCE with AuNPs</td>
<td>alkyne-antiLBA</td>
<td>10-azidoundecan 1-thiol</td>
<td>lysozyme</td>
<td>[61]</td>
</tr>
<tr>
<td>SPCE/ TMSi-Eth-Ar/p-NO$_2$-Ar/azide-Apt</td>
<td>SPCE</td>
<td>4-(TMSi-Eth-Ar) and p-NO$_2$-Ar</td>
<td>azide-aptamer</td>
<td>ochratoxine A</td>
<td>[62]</td>
</tr>
</tbody>
</table>
The click chemistry reaction involved in the development of immunosensors is mainly applied in the immobilization step of antibodies and aptamers on several substrates. The 1, 2, 3 triazole ring...
has a similar structure with the peptide bond maintaining the catalytic activity of the compounds and the site specific coupling. Additionally, nanomaterials such as SWCNT, Au-NPs, and magnetic beads were functionalized with azide and/or alkyne groups that participate to CuAAC, in order to enhance their properties and intermediate the covalent binding of biocompounds.

This new sensing approach provides a stable immobilization method and improves the sensitivity of the platform towards simultaneous and multiple target detection, reducing the nonspecific signal, a major drawback in the field of immunosensors. A disadvantage could be represented by the functionalization of antibodies or aptamers with the functional groups involved in the CuAAC, which is a time consuming and complicated procedure.

3. CONCLUSIONS

Being originally developed for organic synthesis, click chemistry has recently gained an increased attention due to its applications regarding the functionalization of biomolecules and surfaces. Features such as biocompatibility, high efficiency under mild conditions and no side reactions together with the specific on site binding, the preservation of the biomolecules’ 3D configuration and biological activity made it suitable for immobilization strategies. Several electrochemical (bio)sensors were already elaborated for the detection of a wide range of analytes with various applications in biomedical, pharmaceutical, environmental and food safety fields. Their configurations were studied mainly from the point of view of the fabrication protocol, highlighting the compounds with complementary groups involved in the click reaction. By combining this technique with the use of carbon based nanomaterials, metallic particles and nanostructuration of polymeric films a further enhancement of their properties could be achieved.

Based on the gathered data, the perspectives of this concept are promising especially in the development of electrochemical biosensors arrays for simultaneous multiplexed detection applied for point-of-care device fabrication.

ACKNOWLEDGMENTS

This paper was published under the frame of European Social Found, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/S/136893. This paper was published under the frame of European Social Found, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/S/138776. The authors are also thankful for the financial support to University of Medicine and Pharmacy “Iuliu Hațieganu” Cluj-Napoca for the research grant 1491/5/28.01.2014.

References