Review Article

Electrochemical Biosensors Based on Nanostructured Carbon Black: A Review

Tiago Almeida Silva,[1] Fernando Cruz Moraes,[1] Bruno Campos Janegitz,[2] and Orlando Fatibello-Filho[1]

[1]*Department of Chemistry, Federal University of São Carlos, Rod. Washington Luís, Km 235, 13560-970 São Carlos, SP, Brazil*
[2]*Department of Nature Sciences, Mathematics and Education, Federal University of São Carlos, Rod. Anhanguera, Km 174, 13600-970 Araras, SP, Brazil*

Correspondence should be addressed to Bruno Campos Janegitz; brunocj@ymail.com

Carbon black (CB) is a nanostructured material widely used in several industrial processes. This nanomaterial features a set of remarkable properties including high surface area, high thermal and electrical conductivity, and very low cost. Several studies have explored the applicability of CB in electrochemical fields. Recent data showed that modified electrodes based on CB present fast charge transfer and high electroactive surface area, comparable to carbon nanotubes and graphene. These characteristics make CB a promising candidate for the design of electrochemical sensors and biosensors. In this review, we highlight recent advances in the use of CB as a template for biosensing. As will be seen, we discuss the main biosensing strategies adopted for enzymatic catalysis for several target analytes, such as glucose, hydrogen peroxide, and environmental contaminants. Recent applications of CB on DNA-based biosensors are also described. Finally, future challenges and trends of CB use in bioanalytical chemistry are discussed.

1. Introduction

Since the 1990s, after the carbon nanotubes properties reported by Iijima [1], the interest in conductive carbon nanostructured materials has been increasing in different areas, for example, medicine, food, and environment. Nanostructured carbonaceous materials should present some important characteristics, such as high surface area and electrocatalysis. Over the years, carbon nanotubes and graphene have been applied successfully in the development of electrochemical sensors in analytical chemistry [2, 3]. Carbon black (CB) is a conductive low cost nanomaterial made by petroleum products combustion, which presents defect sites. Today, CB represents an alternative material to be used for the development of electrochemical devices, specially sensors and biosensors, in order to detect several analytes. Usually, some research groups have used CB in the fabrication of electrodes based on thin films and composites. This nanomaterial can be easily modified with other materials, as

metallic nanoparticles [4] and polymers [5], as reported by Arduini and coauthors [6], which constructed an interesting screen-printed electrode modified with CB for electroanalysis proposes.

CB is comparable to other well-explored carbon nanomaterials, including graphene, carbon nanotubes (single-walled carbon nanotubes (SWCNTs) and multiwalled carbon nanotubes (MWCNTs)), fullerene (C_{60}), and single-walled carbon nanohorns (SWCNHs). Considering the electrochemical sensing of the neurotransmitter dopamine by using different carbon nanomaterials, CB has been highlighted an excellent modifier. In this context, by adopting similar electrode modification procedures, dopamine electrochemical sensors based on glassy carbon electrode (GCE) modified with graphene, carbon nanotubes, C_{60}, SWCNHs, and CB were reported by Sheng et al. [7], Habibi and Pournaghi-Azar [8], Zhang et al. [9], Zhu et al. [10], and Vicentini et al. [11]. In all mentioned works, by using voltammetric techniques, wide linear concentration ranges at submicromolar levels

TABLE 1: Physical and chemical properties of carbon black nanomaterial.

Property	Data
Shape	Spherical particles strongly bonded to form aggregates
Particle size (nm)	3.0 to 100
Electrical conductivity ($S m^{-1}$) [18]	1.0 to 2.0×10^3
Thermal conductivity ($W m^{-1} K^{-1}$) [19]	0.2 to 0.3
Tensile strength (MPa)[a] [20]	20 to 50
Surface area ($m^2 g^{-1}$) [21, 22]	15 to 1000
Young's modulus ($MN m^{-2}$)[a] [23]	1 to 50
Poisson modulus[a] [23]	0.494 to 0.500
Hydrodynamic diameters (nm)[b] [24]	794
Z-potential values, ζ (mV)[b] [24]	+29.4

[a] Values reported to a typical carbon black-filled rubber. [b] Aqueous dispersion of VALCAN@6 (ISAF, ASTM N220) CB from Cabot Co, USA, with primary particles diameter of ≈25–50 nm.

were obtained, as well as the following limits of detection: 2.5×10^{-7} mol L^{-1} (graphene sensor) [7], 3.1×10^{-7} mol L^{-1} (carbon nanotubes sensor) [8], 7.0×10^{-8} mol L^{-1} (C_{60} sensor) [9], 6.0×10^{-8} mol L^{-1} (SWCNHs sensor) [10], and 5.24×10^{-8} mol L^{-1} (CB sensor) [11]. In addition, it is interesting note that, using the CB sensor, the dopamine was simultaneously determined in the presence of ascorbic acid, paracetamol, and uric acid. Therefore, the simultaneous determination of this quaternary mixture of analytes in biological fluids via electrochemical tools was an analytical challenge solved by using a CB sensor.

In another exciting work from Compton group [12], the electrochemical sensing features of MWCNTs and CB-based sensors toward nicotine determination were compared. By applying adsorptive stripping voltammetry (AdSV) the MWCNTs and CB sensors provided limits of detection for nicotine of 5.0 μmol L^{-1} and 2.0 μmol L^{-1}, respectively. Therefore, an improvement in the voltammetric response was recorded using the CB modified electrode, which was observed significantly lower background signal and larger Faradaic currents achieved using this nanomaterial. In terms of electrochemical biosensing, the same comparative approach between carbon nanomaterials could be carried out considering an amperometric glucose biosensor. We will provide this detailed comparison in the following sections.

Electrochemical biosensors are selective devices using a biological recognition element immobilized on the transducer [14, 15]. Biosensors are able to provide quantitative or semiquantitative analytical information, which transforms the biorecognition reaction of the analyte to a measurable signal. These devices may be applied in different areas, such environmental, medical, biological, pharmaceutical, or industrial [14, 15]. In order to obtain a sensitive electrochemical biosensor based on CB, several architectures can be obtained in the preparation of the electrode surface such as monolayer, multilayers, and thin films. In the course of this article we will present a discussion about CB and its application in electrochemical biosensing, focusing on the surface architectures, materials, and preparation. Finally, we will present the prospects and future applications of CB in electroanalytical chemistry.
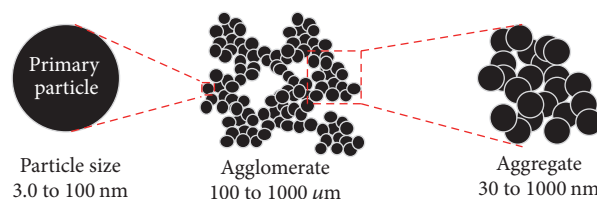

FIGURE 1: Schematic representation of nanometer-scale morphology of CB.

2. Considerations on Carbon Black Nanomaterial

CB is used in several areas of modern life, considering the range of relevant physical and chemical features, which are showed in Table 1. The most cited applications including its use in printer ink, reinforcements for rubber, active agent in electrically conductive plastic, and pigments in paints. Additionally, CB can be used as coatings, papers ink and cosmetics. CB can be produced by the following processes: "furnace," "channel," and "acetylene" [16, 17]. The most useful process is the "furnace," which produces more than 80% of the CB in the world. Depending on the process, CB can present different characteristics. In special, "acetylene" process produces small CB particles with high complexity of the agglomerates and higher graphitic content than those produced by furnace process.

CB primary particle is formed by elemental carbon arranged in fine particles, which has an amorphous and quasigraphitic structure. The average CB particle size ranges from 3.0 to 100 nm. However, one of the characteristics of CB is the formation of aggregates nanostructures, by presenting semispherical groupings. Moreover, these aggregates groups, with a distinctly long dimension, can form agglomerates. Figure 1 was adapted from the Kohjiya et al. [25] article and schematically displays the CB particle, CB aggregates, and agglomerates. Figure 2 shows typical scanning (SEM) and transmission (TEM) electron microscopy images recorded for CB particles. In this case, a VXC72R CB type was deposited on the GCE substrate [11]. From that, it is possible
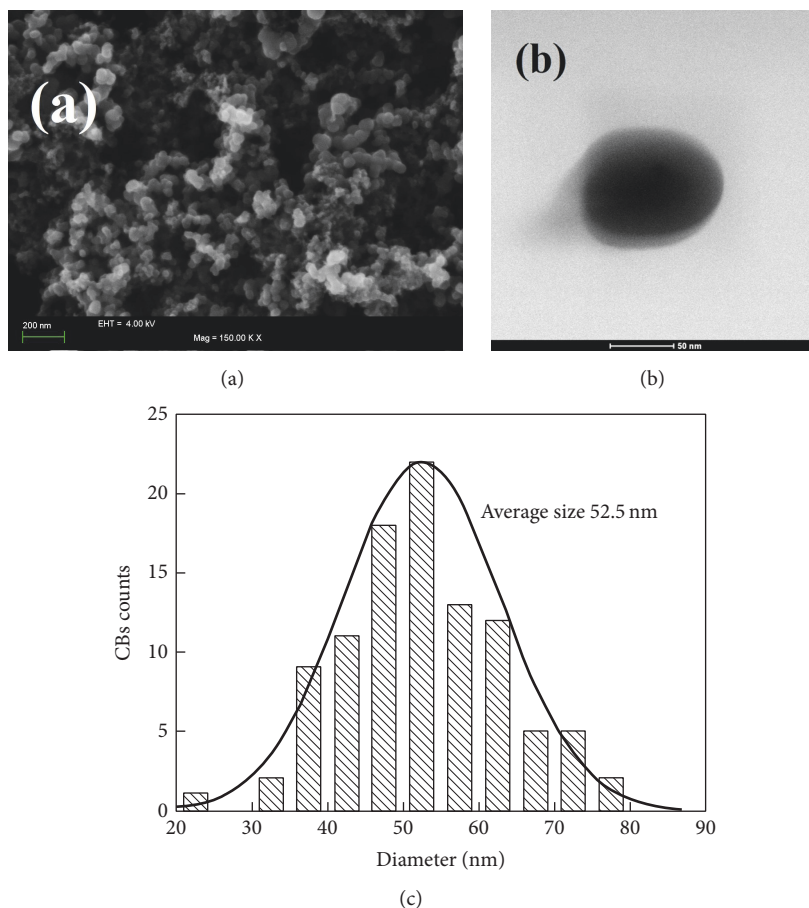
(a)

(b)

(c)

FIGURE 2: (a) SEM images of CB nanostructure, (b) HR-TEM micrographs of CB nanostructure, and (c) the corresponding histogram of the CBs diameters (the solid line corresponds to the Gaussian fit) (reproduced with permission of Elsevier [11]).

to verify the morphological aspects that confirm the scheme shown in Figure 1. CB formed porous structure based on beads (Figure 2(a)) and the spherical particles (Figure 2(c)) present an average diameter of 52.5 nm [11].

CB prepared from heat treatment up to 700°C [26] may increase the number of conjugated carbons in the sp^2 hybridization, leading to a progressive increase in the conductivity of this material. This factor can be associated with electrons with delocalized π-bonds that are available for charge storage [27]. In addition, oxygenates groups are preferentially formed at the edges of the graphite-like microcrystallites [28]. This behavior may be interesting to create an environment to immobilize organic molecules, such as enzymes and other biological material. The presence of a great number of sp^2 edge plans, the oxygenate species displayed over the CB particles, and the CB aggregation forms are intrinsic linked to the CB electrochemical behavior. These characteristics show that CB has the ability to intercalate ions on graphite layers [29], which affects electrical conduction between particles by tunneling [30] and the surface reaction. In particular, the surface area, the number of edges planned, and the structural defects depend on the synthesis or functionalization processes, which create several

functional groups that can be useful for the immobilization of various species, such as enzymes [31], genetic material [32], antibodies [33], and nanoparticles [4]. The sum of structural and electrical/electronic characteristics, ability to produce stable dispersion without the need for sophisticated procedures, and the fact that it is an extremely cheap material make the CB an interesting nanostructured material for the development of electrochemical devices for sensing and biosensing.

3. Biosensors Based on Nanostructured Carbon Black

CB is a relatively novel material in the field of electroanalytical chemistry. The first studies related to investigation of CB as modifier for sensors preparation were reported only in the last decade. One of the highlight pioneering works was the electrochemical sensors reported by Dang et al. [34] and Zhang [35, 36]. In these cases, CB was designated with acetylene black for the voltammetric sensing of tetracycline, colchicine, and rutin. The origin and/or type of CB can affect directly the electroanalytical performance of the designed electrochemical (bio)sensor. In this sense, our research group

TABLE 2: Electrochemical parameters of GCE and CB modified electrode.

Electrode	Electrochemical parameter							
	I_{pa} (μA)	I_{pc} (μA)	I_{pa}/I_{pc}	E_{pa} (mV)	E_{pc} (mV)	ΔE_p (mV)	$E_{1/2}$ (mV)	k^0 (cm s^{-1})
Bare GCE	26.0	−26.4	0.98	290	197	93	234	1.02×10^{-3} [72]
CB-GCE[a]	43.8	−43.6	1.00	272	206	66	211	9.26×10^{-2} [37]

[a]GCE modified with CB nanoparticles confined within a cross-linked chitosan film.
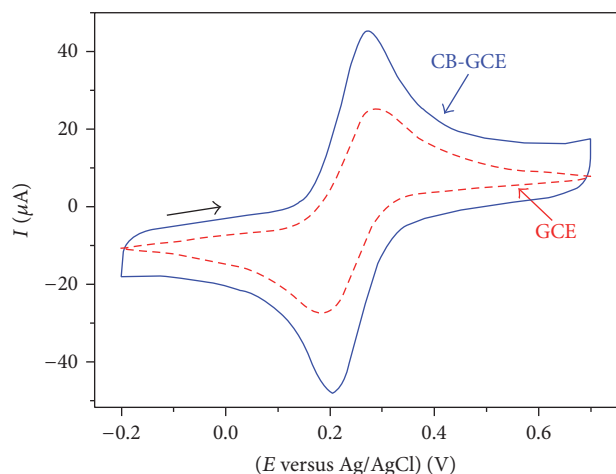
FIGURE 3: Cyclic voltammograms obtained for a 1.0×10^{-3} mol L^{-1} K$_3$Fe(CN)$_6$ in 0.1 mol L^{-1} KCl solution using a bare GCE and a CB-GCE. Scan rate = 100 mV s^{-1}. Black arrow is the scan direction.

reported recently a complete study about the electroanalytical performance of GCE modified with different CB structures [37]. The electrochemical features toward inorganic redox probes and biologically relevant molecules using as modifier the following kinds of CBs were evaluated: VXC72R, BP4750, and E2000. From these studies, VXC72R CB promoted remarkable properties in contrast with those recorded using BP4750 and E2000 CB. The properties of this low cost material include fast charge transfer kinetics and high analytical sensitivity. Therefore, the identification of the appropriated supplier and kind of CB is an important factor in the preparation of high analytical performance CB-based electrochemical (bio)sensing. CB powder could be purchased from different commercial sources. In the case of our works, VXC72R CB of excellent performance for electrochemical sensing was kindly supplied by Cabot® Corporation. The cyclic voltammograms recorded for a bare GCE and a GCE modified with CB in 0.1 mol L^{-1} KCl solution containing 1.0 $\times 10^{-3}$ mol L^{-1} redox probe [Fe(CN)$_6$$^{3-/4-}$] are presented in Figure 3. From these voltammograms, a set of electrochemical parameters were recorded and organized in Table 2. The anodic (I_{pa}) and cathodic (I_{pc}) peak currents increased close to 70% after the modification, demonstrating the increase of electroactive surface area from CB incorporation over GCE surface. The ratio between I_{pa} and I_{pc} components (I_{pa}/I_{pc}) was equal to 1.0 on the CB-GCE, which is a feature of the excellent reversibility of the studied redox probe. Considering the anodic (E_{pa}) and cathodic (E_{pc}) peak potentials, both anodic and cathodic peaks suffered a shift of potential in

the CB-GCE case. From that, the peak-to-peak potential (ΔE_p) separation decreased from 93 mV (GCE) to 66 mV (CB-GCE). The decrease of ΔE_p indicates the enhanced charge transfer kinetic on the modified GCE. To prove this statement, the heterogeneous charge transfer rate constant (k^0) for both electrodes was estimated and the obtained values are also shown in Table 2. As can be seen, the k^0 constant increased ≈90 times after the GCE modification with CB nanoparticles. All these data help to prove the excellent electrochemical behavior of CB nanoparticles, making it an exciting alternative carbon nanomaterial for construction of electrochemical sensors and biosensors. Currently, the use of CB for sensing purposes involves the electrochemical determination of pharmaceutical [4, 34–36, 38–43], environmental contaminants [44–57], food additives [58–61], biomolecules [46, 62–71], and nicotine [12].

The use of CB in the manufacture of sensors is a theme in full development, and design of biosensors based on CB is a recent research topic. Table 3 lists the biosensors based on CB reported up to date. Electrochemical biosensors can be constructed exploring different biological events in according to the target analyte and biological recognition element, ranging from biocatalytic assays (enzymatic biosensors) [73] to affinity assays (genosensors and immunosensors) [74, 75]. In analyzing Table 3, it is evident that CB has been mainly applied for enzymatic biosensors.

Glucose enzymatic biosensing is a research topic that has been explored widely in the literature, due to the relevance of continuous diabetes monitoring [88]. The use of CB as a carbon support for immobilization of glucose oxidase was investigated by Xiao-He et al. [82]. The better approach for preparation of the biosensor using poly(sodium 4-styrenesulfonate) (PSS) grafted on the CB surface (CB-g-PSS) was studied. The obtained results demonstrated that CB-g-PSS showed a good environment for glucose oxidase immobilization; however, selectivity and applicability tests were not provided. Carbon nanomaterials are widely explored to design biosensors based on glucose oxidase. Thus, it is an interesting approach to provide a comparison of analytical features recorded by using biosensors for glucose constructed with carbon nanomaterials, in order to verify the real advantages of low cost and widely available CB nanoparticles.

Table 4 shows a comparison of the analytical features of glucose oxidase (GOx) biosensors designed with SWCNTs, MWCNTs, C$_{60}$, pristine graphene, graphene oxide (GO), reduced graphene oxide (RGO), SWCNHs, and CB. The great part of works explored direct electron transfer (DET) between GOx enzyme and the nanostructured electrode surface. In these cases, a typical redox process is verified for

TABLE 3: Electrochemical biosensors based on nanostructured carbon black.

Analyte	Biological recognition element	Technique	Linear range (mol L⁻¹)	Limit of detection (mol L⁻¹)	Sensitivity (μA cm⁻² mmol⁻¹ L)	Repeatability (RSD, %)[a]	Long-term stability[b]	Ref.
Aldicarb	Acetylcholinesterase	Chronoamperometry	7.0×10^{-9}–1.0×10^{-5}	6.0×10^{-10}	19.2% mol⁻¹ L	2.3	80% (90 days)	[76]
Bisphenol A	Laccase	Amperometry	5.0×10^{-7}–5.0×10^{-5}	2.0×10^{-7}	71.4	3.0	Unchanged (14 days)	[77]
Carbofuran	Acetylcholinesterase	Chronoamperometry	1.0×10^{-10}–2.0×10^{-6}	2.0×10^{-11}	13.3% mol⁻¹ L	2.3	80% (90 days)	[76]
Catechol	Tyrosinase	Amperometry	1.3×10^{-8}–1.5×10^{-4}	6.0×10^{-9}	1720	3.3	100% (21 days)	[78]
Catechol	Tyrosinase	Amperometry	NR[c]	8.0×10^{-9}	625	3.0	NR	[79]
Catechol	Hemocyanin	Linear sweep voltammetry	3.7×10^{-7}–5.0×10^{-5}	8.7×10^{-7}	39.5 μA mmol⁻¹ L	NR	NR	[80]
Diethylstilbestrol	Cytochrome P450	Amperometry	2.0×10^{-7}–2.8×10^{-6}	5.9×10^{-8}	2.5×10^{4}	3.7	91% (10 days)	[81]
Glucose	Glucose oxidase	Chronoamperometry	1.1×10^{-3}–3.3×10^{-2}	NR	553.8 μA mmol⁻¹ L	<7.0	Unchanged (16 days)	[82]
Glucose	Glucose oxidase	Amperometry	2.5×10^{-4}–2.75×10^{-3}	7.0×10^{-5}	≈35	3.6	94% (10 days)	[83]
H₂O₂	Hemoglobin	Amperometry	1.0×10^{-3}–7.0×10^{-3}	NR	NR	NR	Unchanged (several days)	[84]
H₂O₂	Hemoglobin	Amperometry	8.66×10^{-7}–1.04×10^{-5}	3.26×10^{-7}	≈420	0.34	Unchanged (14 days)	[83]
Hydroquinone	Hemocyanin	Linear sweep voltammetry	4.5×10^{-7}–2.4×10^{-5}	8.0×10^{-9}	44.8 μA mmol⁻¹ L	NR	NR	[80]
Malaoxon	Acetylcholinesterase	Chronoamperometry	1.0×10^{-11}–1.0×10^{-6}	4.2×10^{-12}	18.6% mol⁻¹ L	2.3	80% (90 days)	[76]
Methyl-paraoxon	Acetylcholinesterase	Chronoamperometry	1.0×10^{-8}–7.0×10^{-6}	5.0×10^{-9}	23.3% mol⁻¹ L	2.3	80% (90 days)	[76]
Paraoxon	Organophosphorus hydrolase	Amperometry	2.0×10^{-7}–8.0×10^{-6}	1.2×10^{-7}	2.8×10^{3}	NR	NR	[85]
Sulfanilamide	Carbonic anhydrase	Amperometry	5.0×10^{-7}–5.0×10^{-6}	4.0×10^{-7}	7.55% μmol⁻¹ L	NR	76% (18 days)	[86]
Urea	Urease	Amperometry	2.0×10^{-4}–4.0×10^{-3}	NR	NR	3.7	50% (30 days)	[87]
ssDNA	ssDNA probe	Amperometry	1.0×10^{-13}–1.0×10^{-9}	2.0×10^{-14}	2.88 μA [log (mol L⁻¹)]⁻¹	4.4	NR	[32]
ssDNA	ssDNA probe	Differential pulse voltammetry	1.0×10^{-15}–1.0×10^{-10}	1.2×10^{-16}	5.08 μA [log (mol L⁻¹)]⁻¹	2.1	94.6% (15 days)	[13]

[a] RSD: relative standard deviation obtained for the analytical signal during successive measurements (6 to 10). [b] Long-term stability: percentage of initial signal that remained after a number of days stored in the refrigerator (typically at 4°C). [c] NR: not reported.

Table 4: Analytical features of glucose oxidase biosensors based on carbon nanomaterials.

Carbon nanomaterial	k_s (s^{-1})	Linear range (mol L^{-1})	Limit of detection (mol L^{-1})	Sensitivity (μA cm^{-2} mmol^{-1} L)	Repeatability (RSD, %)[a]	Long-term stability[b]	Reference
SWCNTs	NR[c]	5.0×10^{-4}–5.5×10^{-3}	8.3×10^{-5}	NR	4.3	78% (30 days)	[89]
	NR	2.0×10^{-5}–6.0×10^{-3}	1.0×10^{-5}	6	NR	88% (30 days)	[90]
	3.0	1.0×10^{-3}–1.0×10^{-2}	1.0×10^{-5}	NR	3.0	90% (7 days)	[91]
	7.73	0.0–7.8×10^{-3}	NR	7.42	NR	15 days	[92]
MWCNTs	1.69	2.0×10^{-5}–1.5×10^{-2}	9.0×10^{-6}	428.6	3.2	89% (30 days)	[93]
	1.56	5.0×10^{-5}–3.0×10^{-4}	1.0×10^{-5}	111.57	3.5	80% (10 days)	[94]
	4.6	2.0×10^{-5}–1.02×10^{-3}	1.0×10^{-5}	13	5.0	96% (20 days)	[95]
C$_{60}$	2.7	5.0×10^{-5}–1.0×10^{-3}	5.0×10^{-6}	NR	NR	NR	[96]
	2.92	1.0×10^{-4}–1.25×10^{-2}	3.5×10^{-5}	55.97	3.9	90.9% (6 days)	[97]
	NR	up to 8.0×10^{-3}	1.6×10^{-6}	$0.56^{[d]}$	4.26	80% (100 days)	[98]
Graphene	2.68	1.0×10^{-4}–1.0×10^{-2}	1.0×10^{-5}	110	NR	Unchanged (1-2 days)	[99]
	2.83	8.0×10^{-5}–1.2×10^{-2}	2.0×10^{-5}	37.93	NR	95% (7 days)	[100]
	NR	2.0×10^{-3}–1.4×10^{-2}	NR	NR	3.2	104.9 (7 days)	[101]
	5.9	2.0×10^{-3}–1.6×10^{-2}	7.0×10^{-4}	1.76	5.3	93% (30 days)	[102]
	NR	8.0×10^{-4}–2.0×10^{-2}	2.67×10^{-4}	0.767	3.4	92% (14 days)	[103]
GO	11.22	5.0×10^{-5}–2.32×10^{-2}	2.8×10^{-5}	1.1	2.87	82% (30 days)	[104]
	NR	2.0×10^{-5}–6.78×10^{-3}	7.6×10^{-6}	10	4.3	90% (30 days)	[105]
	NR	2.0×10^{-3}–1.6×10^{-2}	7.6×10^{-6}	NR	3.6	94.6% (14 days)	[106]
RGO	4.8	1.0×10^{-4}–2.7×10^{-2}	2.0×10^{-5}	1.85	2.72	93.4% (20 days)	[107]
	5.84	4.0×10^{-5}–6.0×10^{-3}	6.1×10^{-7}	278.4	NR	94% (21 days)	[108]
	3.02	1.0×10^{-5}–6.5×10^{-3}	4.7×10^{-6}	7.95	3.95	90% (30 days)	[109]
	NR	2.0×10^{-3}–1.8×10^{-2}	2.0×10^{-5}	7	4.0	95.4% (14 days)	[110]
	5.27	5.0×10^{-4}–1.25×10^{-2}	1.6×10^{-4}	3.84	6.2	87.3% (14 days)	[111]
SWCNHs	3.0	5.0×10^{-4}–6.0×10^{-3}	6.0×10^{-6}	15.1	2.6	Unchanged (14 days)	[112]
CB	6.26	2.5×10^{-4}–2.75×10^{-3}	7.0×10^{-5}	≈ 35	3.6	94% (10 days)	[83]

[a] RDS: relative standard deviation obtained for the analytical signal during successive measurements (6 to 10). [b] Long-term stability: percentage of initial signal that remained after a number of days stored in the refrigerator (typically at 4°C). [c] NR: not reported. [d] Value in μA mM^{-1} (electrode area not reported).

the biosensor response in buffer solution, which is attributed to the enzyme active centre. From cyclic voltammetric assays conducted at different scan rates, the authors stated that this process is controlled by the redox monolayer species adsorbed on the electrode surface. Using Laviron's equation the respective heterogeneous electron transfer rate constants (k_s) were predicted and the obtained values are showed in Table 4. Therefore, this constant provides information about the electron transfer kinetic between immobilized GOx and electrode surface. CB nanoparticles were able to ensure a fast DET, with k_s constant higher than those obtained using SWCNTs, MWCNTs, graphene, GO, RGO, and SWCNHs with only two exceptions among twenty-five revised works. From an analytical point of view, the CB-based GOx biosensor showed comparable linear range and limit of detection. In some cases, it is recorded limits of detection at micromolar levels. However, it should be observed that the glucose concentration in human blood is typically located in the millimolar range and, therefore, the linear range and limit of detection provided by the CB biosensor are adequate. A very important analytical parameter is the sensitivity. In this case, the most sensitive biosensors are those designed with MWCNTs, C_{60}, and RGO. On the other hand, the sensitivity of CB biosensor is higher than graphene biosensor and presented a high sensitivity than several biosensors constructed with SWCNTs, MWCNTs, pristine graphene, GO, RGO, and SWCNHs. Besides that, from repeatability studies, low relative standard deviations (RSD) were obtained for the biosensor response during consecutive measurements in all the cases. The long-term stability was evaluated as being the percentage of signal variation after some days of storing in a refrigerator at 4°C. There is not a standard in the works in terms of number of storing days and, in general, the biosensors maintained its initial response after many days, in special, for the CB biosensor case, which maintained 94% of its initial signal after ten days of storing.

The determination of the content of urea in milk samples, phenolic compounds in olive oil samples, and bisphenol A in tomato juice samples was interesting applications in the scenario of food analysis investigated by Laurinavicius et al. [87], Nadifiyine et al. [78], and Cammarota et al. [77], respectively. In the work proposed by Laurinavicius et al. [87], a CB paste electrode was modified with a flexible membrane based on terylene precoated with polyvinyl alcohol (PVA-T) containing the immobilized urease enzyme. The urea identification was achieved through the amperometric sensing of carbamic acid at a potential of +0.35 V, generated by the enzymatic conversion of urea. Exploring this strategy, the analytical curve presented a linear response in the range comprising 2.0×10^{-4} to 4.0×10^{-3} mol L^{-1}, and a good correlation was verified for the biosensor response in a 0.02 mol L^{-1} potassium phosphate buffer solution (pH = 6.5) in the absence and presence of milk sample. In another example, Nadifiyine et al. [78] developed a tyrosinase-based biosensor using a carbon black paste electrode matrix for the identification of phenolic species in olive oils. By using catechol as the enzymatic substrate, the amperometric response was linear of 1.3×10^{-8} to 1.5×10^{-4} mol L^{-1}, with a limit of detection of 6.0

$\times 10^{-9}$ mol L^{-1}. The applicability of the proposed biosensor was proved from the analysis of different olive oils with results statistically similar to those obtained by using a comparative Folin–Ciocalteu spectrophotometric method. In the work reported by Cammarota et al. [77], the bisphenol A was determined using a biosensor based on screen-printed electrodes (SPEs) modified with a bionanocomposite of laccase, thionine, and CB. In a previous work, the same research group reported a short communication paper demonstrating the remarkable analytical potentialities of SPEs modified with CB for the sensing of phenolic compounds (catechol, caffeic acid, gallic acid, and tyrosol) [58]. For the biosensor construction, in a first step, the SPE surface was modified with CB particles by dropping 6.0 μL of 0.1 mg mL^{-1} CB dispersion prepared in acetone solvent. Then, 5.0 μL of 0.4 mmol L^{-1} thionine aqueous solution was added on the CB-SPE surface, which was used as the electron mediator specie. Next, the laccase enzyme was supported on the previous modified surface using 5.0 μL of a laccase solution prepared with optimized enzyme units (1.19 U). Finally, the biosensor surface was mechanically protected using a Nafion® coating.

The construction of enzymatic biosensors based on CB for the detection of analytes of environmental interest is a well-explored topic. Cinti et al. [54] reported a work dedicated to investigate the electrochemical sensing and biosensing performance of CB paste electrodes prepared with CB type N220 from Cabot Corporation (Ravenna, Italy). Regarding the electrochemical sensing studies, the proposed CB paste electrode showed enhanced voltammetric response toward $[Fe(CN)_6]^{3-/4-}$ redox couple, ascorbic acid, acetaminophen, epinephrine, and 3,4-dihydroxyphenylacetic acid comparatively to a conventional carbon paste electrode prepared with graphite powder. A tyrosinase biosensor based on CB paste electrode showed remarkable analytical features toward catechol biosensing, including a sensitivity of 625 nA μmol L^{-1} and a limit of detection of 8.0×10^{-9} mol L^{-1}. It is interesting note that these values of sensitivity and limit of detection were enhanced comparing than those obtained using carbon paste electrodes prepared with graphite, SWCNTs, and MWCNTs. We advise the reader to analyze the results presented in Table 2 by Cinti et al. [54]. In another example of CB-based sensors, Deng et al. [59] reported the preparation of a CB-based biosensor for the identification of emerging contaminants as the organophosphorus pesticides [85]. The surface of a GCE was modified with a dispersion of mesoporous carbon (MC) and CB coated with Nafion with subsequent incorporation of the organophosphorus hydrolase. The obtained biosensor was applied on the identification of paraoxon, and the amperometric response was due to the p-nitrophenol produced in the enzymatic reaction. Interestingly, through comparative assays, the authors verified that MC and CB nanoparticles provided a higher analytical sensitivity toward p-nitrophenol comparatively to an architecture designed with carbon nanotubes.

In an analogous work, Radeke et al. [28] developed an electrochemical device to monitor paraoxon exploring the SPE technology and enzymatic inhibition mechanism. In this paper, a screen-printed carbon electrode was modified

with CB nanoparticles adopting a drop casting methodology and applied as a high performance platform for the enzyme immobilization. The paraoxon determination was performed through its inhibitory action on the butyrylcholinesterase (BChE) activity. This enzyme was immobilized on the CB-SPE surface using glutaraldehyde as a crosslinking agent. The BChE-CB-SPE biosensor was exposed to paraoxon solutions during an optimized 20 min incubation time, and the analytical response was defined as the decrease of current obtained for the oxidation of the dithiobischoline dimer formation [86]. From that, the paraoxon analyte was determined in the linear concentration range of 2.0×10^{-8} to 1.2×10^{-7} mol L^{-1}, with a limit of detection of 2.0×10^{-8} mol L^{-1}. Talarico et al. [53] proposed a biosensor based on inhibition of acetylcholinesterase (AChE) enzyme for the determination of different organophosphate and carbamate pesticides. A GCE surface was modified in a first step with a CB and pillar [5] arene as electron mediator, followed by the AChE enzyme using carbodiimide as linkage. Under the optimized experimental conditions, wide linear concentration ranges and very low detectability levels were obtained for the chronoamperometric identification of aldicarb, carbofuran, malaoxon, and methyl-paraoxon, as can be seen from data shown in Table 3. In addition, the analytes were quantified in spiked peanut and beetroot samples with satisfactory recoveries ranging from 86 to 121%.

DET between enzymes and electrodic surfaces is an important achievement to obtain data about protein structures and mechanisms of redox reactions related to some biological and metabolic processes [93, 113, 114]. A great number of technological innovations are derived, as the development of biofuel cells, bioelectronic devices, electrochemical biosensors, among others [115]. In this scenario, the analytical determination using DET method presented is interesting because this method can be performed directly without the use of secondary molecules, as mediator species, at solution, or immobilized biocatalyst on the electrode surface [116]. However, DET is not easily achieved, because the enzyme active centre is protected by a glycoprotein shell, hindering the efficient electron tunneling [117]. In this sense, DET has been reported using nanostructured electrode architectures based on carbon nanomaterials, especially graphene derivatives [100, 101, 118] and MWCNTs [93, 117]. DET between CB nanoparticles and hemoglobin (Hb) was diagnosed by the first time by Ma et al. [84], which was an important advance in the use of CB nanoparticles for biosensing purposes. In this interesting work, the biosensor architecture was constructed using a GCE as substrate, and this surface was modified with a casting of Hb-CB dispersion and the mixture of Hb and CB was fixed using Nafion film. Cyclic voltammograms were recorded using the proposed Hb-CB/GCE and comparative electrodes (CB/GCE and Hb/GCE). The electrode containing CB and Hb showed a well-define redox pair located at -0.295 and -0.361 V (versus SCE), which are attributed to the electrochemical reactions of Hb. Authors suggest that DET verified for Hb protein on the CB modified electrode is due to the presence of the oxygenated functional groups and active sites on the CB particles surface. The bioelectrocatalytic

activity of Hb immobilized on the CB/GCE was explored toward the hydrogen peroxide (H_2O_2) reduction reaction. From that, an amperometric curve was obtained for H_2O_2 concentration with a linear range between 1.0×10^{-3} and 7.0×10^{-3} mol L^{-1}. After the work published by Ma et al. other authors studied the DET between CB and enzymes. Suprun et al. [119] evaluated the DET of different Fe(III)-hemoproteins immobilized on electrodes modified with CB and didodecyldimethylammonium bromide (DDAB). The enzymatic biosensors were fabricated on a SPE surface and the DET for the following enzymes were evaluated: cytochrome c (cyt c), myoglobin (Mb), horseradish peroxidase (HRP), and cytochromes P450 (CYP 51A1, CYP 3A4, and CYP 2B4). All these hemoproteins presented a well-defined couple of redox peaks when immobilized on the proposed matrix, demonstrating the achievement of DET. Comparative assays were carried out for the disposable enzymatic biosensors constructed with SPEs modified with CB and SWCNTs. It was demonstrated that the CB/SPE provide a better environmental for the hemoprotein immobilization, because using the SPE/SWCNT an unsatisfactory signal-to-noise ratio was registered for the measurement conducted in a supporting electrolyte solution without deoxygenation. The SPE/CB/DDAB matrix was explored in the design of an immunosensor for cardiac Mb. The cMb-antibodies were immobilized on a CB/DDAB matrix. From square-wave voltammograms (SWVs) obtained for plasma samples from healthy donors and patients with acute heart attack, it was possible to verify that the proposed immunosensor was able to detect the variation of cMb concentration in different samples.

DET of cytochrome c and Hb biosensors designed with CB particles also were studied by Dai et al. [81] and Li et al. [83]. In the work reported by Dai et al. [81], the cytochrome P450 was entrapped on the surface of a GCE modified with a composite matrix of epoxy copolymers P and acetylene black. The cytochrome P450 incorporated on the proposed matrix showed electrocatalytic activity toward diethylstilbestrol. Diethylstilbestrol was amperometrically determined using the proposed biosensor in the linear concentration range from 2.0×10^{-7} to 2.8×10^{-6} mol L^{-1} with a limit of detection of 5.9×10^{-8} mol L^{-1}. This target analyte was successfully quantified in commercial tablet samples. In this scenario, the preparation of composite matrixes was carried out to promote DET using modified surfaces based on CB. Li et al. [83] described the synthesis of a quaternized cellulose nanoparticles (QCs)/acetylene black (AB)/enzymes. In this work, DET was verified for Hb and GOD enzymes. These biosensors were applied in the sensing of H_2O_2 and glucose. In a similar work, Yang et al. [80] investigated the preparation of a support matrix composed by gold nanoparticles, QCs and CB for immobilization of hemocyanin enzyme. The obtained enzymatic biosensor was explored in the simultaneous determination of hydroquinone and catechol in the linear ranges from 4.5×10^{-7} to 2.4×10^{-5} mol L^{-1} (hydroquinone) and from 3.7×10^{-7} to 5.0×10^{-5} mol L^{-1} (catechol), with limits of detection of 8.0×10^{-9} mol L^{-1} and 8.7×10^{-7} mol L^{-1} for hydroquinone and catechol, respectively.
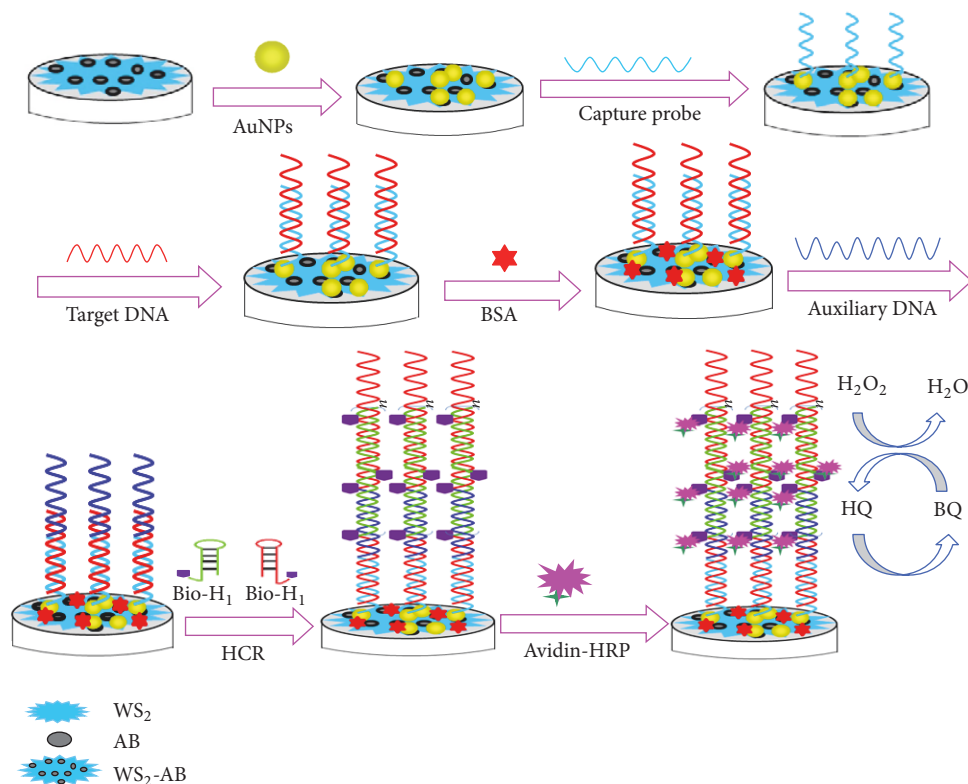
FIGURE 4: Schematic illustration of the working principle of DNA detection based on the hybridization chain reaction (reproduced with permission of The Royal Society of Chemistry [13]).

Recently, DNA biosensors based on CB were designed by Huang et al. [32] and Shuai et al. [13]. In another interesting work, Huang et al. [32] developed a new biosensor by using a combination of two-dimensional CuS nanosheets and acetylene black particles in a composite. The CuS-AB composite was synthesized by solvothermal method using ethylene glycol. For the biosensor preparation, the surface of a GCE was firstly modified with a CuS-AB film, followed by the electrodeposition of gold nanoparticles. CuS-AB and gold nanoparticles were explored as nanomaterials of high conductivity and large electroactive surface area for the biosensor signal amplification. The quantification was defined by the decrease of peak current for the $[\text{Fe(CN)}_6]^{3-/4-}$ redox couple after the hybridization event between the immobilized ssDNA probe and the target ssDNA. In this same research line, Shuai et al. [13] synthesized a two-dimensional tungsten disulfide-acetylene black (WS_2-AB) composite by a hydrothermal method for DNA biosensing purposes. The fabrication procedure and principle of detection are schematized in Figure 4. The WS_2-AB composite was deposited on a GCE surface and gold nanoparticles were electrodeposited in a following step. The authors used these two nanomaterials in the DNA biosensor preparation to ensure a large specific surface area and good electroconductivity. Exploring this strategy, the target ssDNA was determined in the range from 1.0×10^{-15} to 1.0×10^{-10} mol L^{-1} of the target ssDNA with a limit of detection of 1.2×10^{-16} mol L^{-1}.

4. Conclusions and Future Perspectives

CB is a very low cost nanomaterial, with relevant structural, morphological, and electronic properties for application in electrochemical sensing and biosensing. From its typical analytical sensitivity, selectivity, operational simplicity, and portability, the electrochemical biosensing routines have found a wide range of applications in different areas, including environmental, medical, biological, pharmaceutical, or industrial. The revision of literature showed that CB have been explored mainly in the construction of biocatalytic or enzymatic biosensors, dedicated to the determination of glucose, hydrogen peroxide, food additives, and environmental contaminants. The enhancement of the analytical performance of the proposed enzymatic biosensors is evident. A systematic comparison of the analytical features of glucose biosensors based on CB, SWCNTs, MWCNTs, graphene, GO, RGO, C_{60}, and SWCNHs demonstrated the efficacy of CB as an alternative carbon nanomaterial for preparation of biosensors. Therefore, the use of CB in electrochemical biosensing is a relatively young research topic and a wide range of applications can be carried out. CB can be explored as support to immobilization of more complex enzymes, incorporation of antibodies and specific DNA sequences for cancer diagnostic and predisposition, and development of point-of-care devices. Moreover, from the viewpoint of material chemistry, the CB chemistry is versatile, and hot

topics include the study of effect of chemical and physical treatments on the CB electrochemistry features, preparation of composites with different nanoparticles, and combination with ionic liquids. Researches devoted to fabrication of CB-based biosensors should be proposed in the near future, having into account topics about the use of new biosensitive interfaces, optimization of parameters, and tests in complex samples. Future applications of CB in electrochemical (bio)sensors are underway in our groups.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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