Accepted Manuscript

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Author: Celine I.L. Justino, Armando C. Duarte, Teresa A.P. Rocha-Santos

PII: S0165-9936(16)30008-5
DOI: http://dx.doi.org/doi: 10.1016/j.trac.2016.04.004
Reference: TRAC 14722

To appear in: *Trends in Analytical Chemistry*

Please cite this article as: Celine I.L. Justino, Armando C. Duarte, Teresa A.P. Rocha-Santos, Critical overview on the application of sensors and biosensors for clinical analysis, *Trends in Analytical Chemistry* (2016), http://dx.doi.org/doi: 10.1016/j.trac.2016.04.004.

Critical overview on the application of sensors and biosensors for clinical analysis

Celine I.L. Justino[a,b,*], Armando C. Duarte[a], Teresa A.P. Rocha-Santos[a]

[a]*Department of Chemistry & CESAM, University of Aveiro, Campus de Santiago, 3810-193 Aveiro, Portugal*

[b]*ISEIT/Viseu, Instituto Piaget, Estrada do Alto do Gaio, Galifonge, 3515-776 Lordosa, Viseu, Portugal*

*Corresponding author: celinejustino@ua.pt (Celine I.L. Justino)

Tel.: +351 232 910 100; Fax: +351 232 910 183.

Highlights

- We present the state of the art of recent sensors and biosensors employed in clinical analysis.
- Their analytical performance was compared identifying their advantages and limitations.
- Approaches to improve the analytical performance of sensors and biosensors are reported.

Abstract

The application of sensors and biosensors for clinical analysis has aroused the attention of scientific community due to the possibility of their miniaturization and portability, allowing the construction of diagnostic devices for point-of-care testing. This paper presents an up-to-date overview and comparison of the analytical performance of recent sensors and biosensors used in clinical analysis, namely for cancer and cardiac biomarkers, hormones, biomolecules, neurotransmitters, bacteria, virus, and cancer cells, along with their significant advances since 2011. Some approaches to improve the analytical performance of sensors and biosensors through their figures of merit are also discussed.

Keywords: analytical performance; biosensor; clinical analysis; figure of merit; sensor.

1. Introduction

The advances in technologies for improvement of global health services are nowadays highly demanded involving the diagnosis, treatment, and prevention of any disease in humans [1]. Although effective, the laboratory techniques based mainly on optical analytical principle,

such as immunoassays, are limited by sample enrichment and purification before analysis, and they are expensive and slow. Thus, strategies for signal amplification have been recently proposed to increase immunoassay sensitivity in order to use such analytical techniques for further clinical diagnostics [2]. Sensors and biosensors have been widely discussed in current literature due to their importance in detection and monitoring of different types of analytes in the field of food safety, environmental monitoring, clinical analysis, and medical diagnosis [3]. The application of immunosensors in clinical diagnostics have also been recently reviewed considering the detection and identification of cardiac and cancer biomarkers as the central point in the development of point-of-care technologies, having potential to analyze real biological samples (for continuous monitoring of physiologically important analytes) in routine clinical use [4].

There are three main types of sensors and biosensors commonly used in clinical analysis considering their transduction principle, that is, electrochemical, optical, and piezoelectric-based sensors and biosensors. Electrochemical biosensors are self-contained integrated device capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element that is retained in direct contact with an electrochemical transduction element (e.g., a pair of electrodes or field-effect transistors) [5]. Electrochemical biosensors are thus based on monitoring of electro-active species that are produced or consumed by the action of the biological elements, and following potentiometric, amperometric, or impedimetric transduction principles [6]. The analytical output in optical sensors is due to the interaction of analyte with transducer based in optical properties such as absorbance, reflectance, luminescence, fluorescence, refractive index, and surface plasmon resonance (SPR) [6]. Concerning piezoelectric sensors, they employ materials that resonate under the application of an external alternating electrical field, and quartz crystals are typically used producing an oscillating electric field in which the resonant frequency of the crystal depends on its chemical nature, size, shape, and mass [6]. According to the recent literature, the electrochemical principle is mainly applied in clinical sensors and biosensors [4,7-10]. For example, the electrochemical immunosensors have been used in clinical diagnostics as point-of-care devices, since they are portable, simple, easy to use, cost-effective, and disposable in most cases and studies of electrochemical immunosensors for cardiac and cancer biomarkers have demonstrated the potential usefulness of these approaches in public health applications [4]. On the other hand, the incorporation of various nanomaterials in electrochemical biosensors has been pointed as advantageous in the technological progress of health monitoring [1]. In the same direction of analysis, implantable

electrochemical biosensors for *in vivo* continuous monitoring of individual subjects has been identified as an advance in point-of-care technology in order to perform personalized diagnosis, since several functions are combined in individual microchips [1,11].

Another classification is based in the recognition principle of the analyte of interest, for example, immunosensors (antibody-antigen interaction) and enzymatic biosensors (enzyme-target analyte interaction). The recognition elements, both classical, such as enzymes and antibodies, and recent, such as aptamers and molecularly imprinted polymers are important parts of chemical sensors and biosensors and they are responsible for the recognition of target analytes of interest [12].

The nanotechnology applied in the construction of clinical sensors and biosensors is also an important accomplishment in latest years with the incorporation of new materials taking advantage of their conductive properties, high surface-to-volume ratio, and good biocompatibility, thus contributing to the improvement of sensor performance [13-15]. For example, for the detection of clinically significant biomarkers, nanomaterials such as nanowires synthesized from metals (e.g., Ni, Cu, Au, Pt), metal oxides (ZnO, SnO_2, Fe_2O_3), and silicon/indium/gallium semiconductors (Si, InP, GaN), quantum dots based on CdSe, CdTe or CdSeTe, carbon nanotubes (CNT), and metal nanoparticles (based on Au, Cu, Pd, Co, Ag, or Pt) have been incorporated in biosensors [1,9,13]. Proteins, neurotransmitters and cancer biomarkers have been also detected and quantified with biosensors where CNT were used as transduction elements [1]. In addition, the nanotechnology in biosensing platforms is also associated to the miniaturization of systems providing lower consumption of power, lower sample volumes, shorter assay times, and low operating costs, and several nanomaterial properties such as the mechanical stiffness, high carrier mobility, thermal conductivity, high surface-to-volume ratio, and improved electron transfer of CNT, as well as the high surface-to-volume ratio and electrical current capacity of nanowires are useful when applied in sensors and biosensors [13].

The analytical performance is crucial in the construction of final prototypes in order to lead to the commercialization of clinical sensors and biosensors, which is actually mainly verified for glucose biosensors [16]. Several directions for the improvement of the analytical performance through the enhancement of figures of merit have been considered in the development of new sensors and biosensors, particularly to construct more sensitive and reproducible sensing platforms. For example, the incorporation of nanomaterials in the transduction substrate or their association with metal nanoparticles or polymers has been

tested to improve the sensitivity of sensors and biosensors as well as the stability of their analytical response.

Due to the continuous interest in the application of sensors and biosensors for clinical analysis, it would be useful to update a previous literature review published in 2010 by Justino et al. [3] in the same field of research. Thus, the present paper has the objective of reviewing the state of the art of recent sensors and biosensors employed in clinical analysis covering the period of 2011-2015 for comparison of their analytical performance, and also considering their advantages and limitations according to the transduction principle. Some approaches are also reported in order to improve the analytical performance of sensors and biosensors.

2. Analytical figures of merit

The validation of a method undergoes the assessment of their figures of merit, which are those quantifiable terms that may indicate the extent of the quality of the process, and their assessment is required for ensuring the quality of results [17]. Similarly, to the validation of sensors and biosensors, the main figures of merit that should be studied are the sensitivity, selectivity, LOD, repeatability, and reproducibility [3]. Such figures of merit are also important to characterize and to compare the analytical performance of sensors and biosensors. Table 1 shows the definitions of the main figures of merit used for the validation of an analytical method such as sensors and biosensors.

< Table 1 >

The figures of merit should be assessed during the development of a technique or device and verified periodically during the routine analysis, in order to estimate their analytical performance characteristics such as reliability, capacity, and variability.

3. Improvement of the analytical performance of sensors and biosensors

The improvement of the analytical performance of sensors and biosensors is an important phase along their development since improved sensing systems can further be constructed as prototypes and commercialized for practical application in environmental monitoring, food safety, or clinical analysis. Thus, the study of various approaches for enhancing the analytical performance of sensors and biosensors through the improvement of the associated figures of merit is one of the main aims in the recent research. The sensitivity can be considered the

central figure of merit and, when classical (univariate) calibration is followed, its definition is based in the change of the analytical response of the instrument divided by the corresponding change in the stimulus (concentration of the analyte of interest), that is the slope of the analytical calibration curve [17]. The nanotechnology has been applied to improve the performance of biosensors due to the large surface area-to-volume ratio available on nanostructures for the immobilization of labels and biological recognition elements leading to the amplification of the analytical signal and consequent improvement of sensitivity [18].

3.1. Nanomaterials type and characteristics

The incorporation of nanomaterials in sensors and biosensors offer improved biocompatibility, additional binding sites, and higher signal intensities (through enhanced electrical properties) when compared with the use of traditional materials, thus improving the sensitivity and specificity of the detection [14,19]. In the works of Zhang et al. [20], Zhang et al. [21], Baek et al. [22], Feng et al. [23], Wang et al. [24], Wang and Zheng [25], Lozano et al. [26], Guo et al. [27], and Sun et al. [28], the influence of the presence of nanomaterials (i.e., gold nanoparticles, ZnO nanoparticles, ZnO-gold nanocomposites, and CNT) was studied on the analytical performance of the constructed sensors and biosensors [i.e., in sensitivity, LOD, concentration range, and stability], as following reported. In the work of Zhang et al. [20], where an electrochemical DNA sensor is reported, the presence of gold nanoparticles significantly enhanced the sensitivity and the LOD. In the absence of gold nanoparticle amplification, only a slight increase in the peak current was observed even after hybridization with 1 μM DNA target, and with gold nanoparticle amplification, a significant enhancement of peak current only with 10 pM DNA target was observed, demonstrating that the presence of gold nanoparticle allows the DNA detection with high sensitivity [20]. In addition, the LOD (10 fM) is lower with gold amplification, since the sensor only detect 0.5 nM target DNA in the absence of gold nanoparticle amplification. Also in the work of Zhang et al. [21], the LOD is improved with the incorporation of gold nanoparticles. Zhang et al. [21] developed a microfluidic beads-based immunosensor for detection of α-fetoprotein using horseradish peroxidase coupled with gold nanoparticles for amplification of signal. A greatly enhanced sensitivity for the target analyte is also verified and mainly due to the large surface area of gold nanoparticles, which is responsible to the several binding events of the enzyme on each nanosphere [21]. The microfluidic beads-based immunosensor showed a 50-fold increase in LOD when compared to microfluidic beads-based immunosensor without gold nanoparticles. Baek et al. [22] developed an optical sensor for thrombin based on SPR

principle where a dual nanoparticle amplification strategy was used, that is, two different gold nanoparticle shapes (nanorods and quasi-spherical nanoparticles) were incorporated in the sensor. The authors verified that the use of real-time SPR measurements to detect target concentrations as low as 0.1 aM is a 10-fold improvement compared to single nanoparticle-enhanced SPR detection methods [22]. Feng et al. [23] verified a great enhancement of LOD for the electrochemical sensor used in DNA hybridization recognition due to the synergistic effect of two nanomaterials (gold nanoparticle and polyaniline nanotube membranes), which combined the very large surface areas of nanomaterials with their excellent conductivities. The sensor displays a much lower LOD ($3.1x10^{-13}$ mol L^{-1}) for the DNA detection when compared with other DNA sensors with LOD between $1.4x10^{-12}$ and $2.4x10^{-11}$ mol L^{-1} [23]. The incorporation of metal semiconductor compounds such as ZnO in conjugation with gold nanoparticles can improve the detection sensitivity of SPR sensors, as reported in the work of Wang et al. [24], where an SPR biosensor based on ZnO-gold nanocomposites was constructed for the detection of human immunoglobulin M (hIgM). The biosensor exhibits its analytical response for hIgM in the concentration range of 0.30-20.00 μg mL^{-1}, but without ZnO-gold nanocomposites, the biosensor shows a response for hIgM in the concentration range of 1.25-20.00 μg mL^{-1}. Wang et al. [24] also verified that when the biosensor based on the ZnO-gold nanocomposites was applied, the sensitivity for determination of hIgM is enhanced with a maximum shift of resonant wavelength (analytical response at 20.00 μg mL^{-1}) of 7.53 nm in comparison to the analytical response of 4.10 nm in sensors only with gold film. In another work, Wang and Zheng [25] reported the improvement of the sensitivity by 3-fold in an electrochemical sensor based on glass carbon electrode for hydrogen peroxide with the electrodeposition of silver nanoparticles on a ZnO film when compared to similar sensors without ZnO. The improvement of stability of sensors with ZnO was also verified with the analytical response dropping by 2% in comparison to the drop of response by 30% in sensors without ZnO film. According to Wang and Zheng [25], such improvement is due to the capacity of ZnO in immobilizing inorganic nanoparticles, facilitating the formation and more intensive distribution of small silver nanoparticles. In the work of Lozano et al. [26], an electrochemical sensor based on a CNT paste electrode (CNTPE) with polymer electrogenerated from Fe(III)-5-amino-1,10-phenantroline solution was tested to detect glucose in comparison with a similar sensor based on graphite paste electrode (CPE). According to the authors, the CNT display an important role on the generation of poly(Fe(III)-5-amino-1,10-phenantroline) since with the CNTPE/poly(Fe(III)-5-amino-1,10-phenantroline), an enhancement of 200-fold in the sensitivity at -0.100 V was observed

(sensitivity of 130 μA M^{-1}) when compared with that obtained with the CPE/poly(Fe(III)-5-amino-1,10-phenantroline) where a sensitivity of 0.65 μA M^{-1} was verified. Recently, Guo et al. [27] reported the impact of gold nanoparticle aggregation for DNA colorimetric sensors. The gold dimers (selectively formed upon target binding) significantly improved the long-term stability (approximately 10-fold) and the dynamic range of detection (approximately more than 2 orders of magnitude) when compared with conventional colorimetric sensors, where larger nanoparticle aggregates are formed. Sun et al. [28] proposed a multilayer film based on gold nanoparticles and polyaniline/carboxylated multi-walled CNT (MWCNT)-chitosan nanocomposite in order to fabricate an electrochemical immunosensor for detecting chlorpyrifos. The analytical response of the immunosensor, that is, the change of the reduction peak current before and after immunoreaction, increased after the adsorption of gold nanoparticles onto the surface of the polyaniline/carboxylated MWCNT-chitosan nanocomposite film due to the fast direct electron transfer, which was facilitated by the gold nanoparticles [28]. Thus, the authors concluded that gold nanoparticles on the nanocomposite film improve the electrochemical signal and adsorption capacity of antibody, enhancing the detection sensitivity. According to Wei et al. [29], electrochemical sensors that commonly use multilayer electrodes, that is, thin layers of polymers, nanoparticles, or nanoparticle-polymer composites stacked on top of the electrode, have enhanced sensitivity due to their action as 3D matrix in order to capture nucleotide probes and reducing the interference from nonspecific molecules, which can contribute to background noise.

Other works also reported the improvement of the analytical signal after the incorporation of gold nanoparticles, quantum dots, and graphene, enhancing the sensitivity of respective sensors [30-33]. For example, Kavosi et al. [30] constructed an electrochemical immunosensor for the detection of α-fetoprotein, where polyamidoamine dendrimer-encapsulated gold nanoparticles were immobilized as sensing interface on a gold electrode. It was verified that the gold nanocomposite was responsible to the high sensitivity of the immunosensor since it was used as an amplification factor due to its high conductivity and remarkably large surface area [30]. According to the authors, it was verified that when α-fetoprotein was incubated with its antibody directly exposed to the gold electrode, only a small response could be observed and a slightly larger response (10-fold increase) was shown upon the interaction of α-fetoprotein with electrode modified with the gold nanocomposite. The large specific surface area of the gold nanocomposite with abundant surface functional groups available to capture much more antibodies at the sensing interface and the accelerated electron transfer caused by the presence of gold nanocomposite are the causes of such signal

amplification [30]. The quantum dots are nanoparticles containing over ten thousands metal ions and they also could be used for signal amplification of sensors. For example, Zhou et al. [31] proposed an electrochemiluminescence immunosensor for α-fetoprotein using CdS quantum dots and gold nanoparticles labelled with antibodies for α-fetoprotein for signal amplification. According to the authors, the signal intensity from the CdS/gold composite film is about 2.5-fold higher than that observed from the pure CdS film, which may be due to the great catalytic activity and enhanced electrical conductivity of the gold nanoparticles, also enhancing the detection sensitivity [31]. Xie et al. [32] fabricated an electrogenerated chemiluminescence assay for the determination of thrombin using CdSe/ZnS quantum dots as sensing probe on a graphene modified glassy carbon electrode covered with gold nanoparticles. As results, Xie et al. [32] verified that the analytical signal in the absence of the quantum dots was very low and after addition of quantum dots (coupled with avidin and DNA), the electrogenerated chemiluminescence intensity increased greatly. Concerning the incorporation of graphene, Du et al. [33] proposed an electrochemical immunosensor for detection of α-fetoprotein where dual signal amplification is verified using graphene sheets in the sensor platform and functionalized carbon nanospheres labelled with horseradish peroxidase. Thus, a 7-fold increase in detection signal was verified in immunosensor when compared to the immunosensor without graphene modification and carbon nanospheres labelling, also improving its sensitivity [33]. According to the authors, the amplification strategy employed is mainly due to the presence of graphene, which increases the surface area to capture more antibodies on the electrode surface and also accelerates the electron transfer.

3.1.1. Density of nanomaterials and width of nanomaterial channel

The influence of the nanomaterials characteristics, such as CNT density [34-36] and CNT width channel [37] has been reported on the analytical performance of sensors and biosensors because they can affect figures of merit such as signal-to-noise ratio, reproducibility, sensitivity, and LOD. Ishikawa et al. [35] have studied how the control of network single-walled CNT (SWCNT) density affects the analytical performance of field effect transistor (FET) devices. They have classified the density of nanotubes as low, medium, and high according to the time of incubation in ferritin solution, which is associated to the density of catalyst. Ishikawa et al. [35] have concluded that the lower the density of CNT, the higher the sensitivity in relation to LOD and magnitude of response, as well as, the reproducibility of such biosensors. According to Ishikawa et al. [35], the enhanced sensitivity associated to low density of SWCNT is partially explained by the elimination of direct metallic nanotube

pathways, enhancing the semiconductor behaviour of nanotubes and providing lower capacitance and high ON/OFF ratios, while the high density of SWCNT reflects their quasi-metallic behaviour. The sensing performance of those devices was investigated using streptavidin as a model analyte, but the final use of optimized FET devices was for the detection of nucleocaspid protein, a biomarker associated to SARS coronavirus. Taking into account the plot of the analytical signal (normalized conductance *vs.* log of the streptavidin concentration) for each device with different nanotube density, the devices with low density of nanotubes clearly offers the highest magnitude of response, while the device with the high density nanotube offers the lowest magnitude of response. The LOD was estimated in ranges of 100 pM to 1 nM, 10 to 100 pM, and 1 pM to 10 pM for high, medium and low density devices, respectively. Fu et al. [34] have also verified the impact of nanotube density in the sensitivity of FET devices; they reported the detection of DNA molecules in FET biosensors with networks of SWCNT, where the density of nanotubes has improved the ON/OFF ratios of devices from 5 to 2000 with high and low SWCNT density, respectively, as well as improved the LOD from 10 pM to 0.1 fM with high and low SWCNT density, respectively. This can be due to the impurity behaviour of DNA molecules for carrier charge scattering, which increased drastically the ON/OFF ratio. The increase of ON/OFF ratio was more significant when lower SWCNT densities were used due to the decrease in number of metallic SWCNT percolative paths. Recently, Okuda et al. [36] reported the fabrication of electrolyte-gated sensors based on a FET consisting of horizontally aligned multiple SWCNT synthesized on single-crystal quartz for the label-free immunosensing of human immunoglobulin E. The developed sensors with aligned channels exhibited a drain current (analytical response) of 400-fold that of single-channel devices and they exhibit much lower drain current fluctuations, since the calculated residual standard deviation (RSD) was 2.4% and 0.05% for the single- and aligned-channel FET, respectively, demonstrating their usefulness for highly sensitive and practicable solution sensing [36]. Concerning the influence of CNT width channel on the analytical performance of sensors, Lee et al. [37] reported the construction of highly-sensitive nanoscale sensors for Hg^{2+} by controlling the structure of aligned SWCNT networks. Lee et al. [37] have concluded that a better signal-to-noise ratio and a higher sensitivity was observed with narrower channels rather than with wider channels, which may be due to a decrease in effective length of the current paths (i.e., damaged current paths leads to poor conductivity of SWCNT channels); LOD was improved from 10 nM using 2 μm-wide SWCNT network sensors to 1 pM using 100 nm-wide SWCNT network sensors. Lee et al. [37] also obtained mathematical relations between SWCNT channel width and sensitivity

(sensitivity ~ width$^{-1.6}$) and between SWCNT channel width and signal-to-noise ratio (signal-to-noise ratio ~ width$^{-1.1}$).

3.1.2. Number, diameter, and doping level of nanomaterials

Other nanomaterials characteristics such as number, diameter, and doping level of nanomaterials also affect figures of merit of sensors, enhancing the sensitivity in sensors with low nanowires (NW), higher NW diameters, and lower doping concentration and also improving the LOD in sensors with lower doping concentration. In their work, Li et al. [38] tested sensors based on NW for the detection of human immunoglobulin G (hIgG) as a model analyte. When NW-based sensors with different number of NW were tested, Li et al. [38] verified that the sensitivity of sensors decreases with increasing number of NW, that is, a larger number of NW (4 and 7 NW-based devices) exhibited poor sensitivity (decrease of ~38 and ~82%, respectively) when compared to device with just one nanowire. According to the authors, this decrease in sensitivity is attributed to the depletion of hIgG molecules from surrounding solution, reducing the binding events between NW and analyte which results in smaller current changes of sensing devices. When NW-based sensor with different NW diameters were tested, that is, with diameters in ranges of 60-80 nm, 81-100 nm, and 101-120 nm, Li et al. [38] verified that sensors with larger NW diameter (81-100 nm and 101-120 nm) exhibited higher decrease in sensitivity (~37%) when compared to devices with thinner NW of 60-80 nm (decrease in sensitivity of ~16 %), essentially due to the decrease of surface-to-volume ratio in thick wires at μm order. On the other hand, Li et al. [38] observed that a 2 orders of magnitude change in doping concentration resulted in an increase of approximately 3.2-fold in biosensor sensitivity, that is, increase of 69% in sensitivity with the decreasing of NW doping from 10^{19} to 10^{17} atoms cm^{-3}. In addition, Li et al. [38] also observed that a lower LOD for hIgG (~10 fg mL^{-1}) was verified for NW-based sensors with doping concentration of 10^{17} in comparison with sensors with 10^{19} atoms cm^{-3} (LOD of ~10 pg mL^{-1}).

3.2. Presence of label molecules

In immunoassays and other analytical techniques, as well as sensors and biosensors, the presence of labels helps in the fast detection of target analyte, amplifying the analytical signal; for example, for fluorescence-based labelled methods, a superior sensitivity and specificity is verified when compared to label-free methods [39]. Recently, Granqvist et al. [39] proposed an optical biosensor based on SPR principle where the presence of labelled molecules (strongly absorbing dye molecules) significantly improved the sensitivity and the

specificity of the SPR sensor. Two simple model assays were used to demonstrate the performance of such SPR method: the small molecule assay was used to demonstrate the sensitivity enhancement of label SPR and it consists in sensor with bovine serum albumin (BSA) and avidin, and the DNA assay was used to demonstrate the selectivity enhancement of the assay and consists in sensor with single-stranded DNA bound through biotin to the surface-bound avidin [39]. According to the authors, the sensitivity is significantly enhanced (100-fold) when compared to conventional label-free SPR and the influence of noise factors like temperature, pressure, and bulk liquid composition variations is also significantly reduced by using label-enhanced SPR sensing [39]. On the other hand, the specificity, with respect to the label, is very high and stable, which reduces the problem of non-specific binding [39].

Nanoparticles have been also used as labels in sensors and biosensors, improving the analytical signal of sensing systems and enhancing their sensitivity to target analytes. For example, Shen et al. [40] developed an electrochemiluminescence immunosensor to detect human cardiac troponin I (cTnI) by using N-(aminobutyl)-N-(ethylisoluminol)-functionalized gold nanoparticles (ABEI-functionalized gold nanoparticles) as labels for attachment of antibodies. ABEI is a derivative of isoluminol, used as electrochemiluminescence reagent, but when compared with luminol, ABEI is of relatively high efficiency if chemically attached to specific analytes and thus acts as a favourable label for bioassays [40]. In the work of Shen et al. [40], three kinds of probes including ABEI, luminol-functionalized gold nanoparticles, and ABEI-functionalized gold nanoparticles were tested. According to the authors, the immunoassay system using ABEI-functionalized gold nanoparticles as labels exhibited higher analytical signal than that with other labels, that is, the electrochemilumiscence intensity of ABEI-functionalized gold nanoparticles is 12.5-fold higher than the intensity of ABEI and 1.5 fold higher than luminol-functionalized gold nanoparticles, which demonstrated the enhanced sensitivity of the assay with ABEI-functionalized gold nanoparticles labelling [40]. In another work, Su et al. [41] constructed an electrochemical immunosensor for detection of α-fetoprotein using nanogold-enclosed titania nanoparticles as labels to antibodies. Su et al. [41] verified that the electrochemical signal with nanogold-enclosed titania nanoparticles was greatly amplified in comparison with pure nanogold or titania-based labels, enhancing the sensitivity of the immunosensor to α-fetoprotein. Zhang et al. [42] reported an electrochemiluminescence immunosensor for cancer biomarkers detection using Ru-silica capped onto nanoporous gold composite as label, and verified that such electrochemiluminescence label is responsible to the signal amplification and greatly

increased the sensitivity and extended the detectable concentration range by 2 orders of magnitude of the immunosensor.

Magnetic nanoparticles used as labels can also enhance the sensitivity and stability of sensors and biosensors [43]. For example, Wang et al. [44] reported the construction of a SPR biosensor employing magnetic nanoparticles (with iron oxide core) as labels for detection of human chorionic gonadotropin. The sensitivity of the biosensor was investigated as a function of mass transport of the analyte to the sensor surface driven by diffusion (free analyte) or by the magnetic field gradient (analyte bound to magnetic nanoparticles) [44]. According to the authors, the sensor response of SPR biosensor with the magnetic nanoparticles is ~17 times higher than that for direct detection format (without magnetic nanoparticles), which is due to the larger mass and higher refractive index of the magnetic nanoparticles [44]. In addition, the sensitivity of human chorionic gonadotropin detection was improved by four orders of magnitude compared with the regular SPR sensor with direct detection format, and mainly due to the larger mass and higher refractive index of magnetic nanoparticles [44]. In another work, Liu et al. [45] reported the construction of an electrochemiluminescence immunosensor for the detection of cancer biomarkers using CdTe quantum dots coated silica nanospheres as signal amplification labels. With CdTe quantum dots coated silica nanospheres, the immunosensor exhibited an higher electrochemiluminescence signal of about 4.1-fold when compared to immunosensor with pure CdTe quantum dots, thus improving the sensitivity of the immunosensor [45].

3.3. Nature of recognition element

The recognition elements are important parts of sensors and biosensors since they are responsible for the recognition of target analytes of interest and new recognition elements such as aptamers, molecularly imprinted polymers, DNAzyme, and affibodies have been studied for the recognition improvement [12].

The use of aptamers as recent recognition elements in sensors and biosensors has many advantages such as they are thermally stable and reusable, and they can be easily modified for their immobilization by incorporating reporter molecules (e.g., fluorophores or enzymes) and functional groups [12]. On the other hand, their versatility and avoidance of the use of animals in their production provide major advantages over antibodies, and they also have a higher surface density of receptors [12]. Their incorporation in sensors and biosensors lead to an improved analytical performance, mainly through enhanced LOD. Yao et al. [46] compared the performance of a quartz crystal microbalance (QCM) biosensor using two types

of recognition elements: aptamer and antibody, for the detection of immunoglobulin E (IgE) in human serum. The aptamers or antibodies specific to IgE were immobilized on the gold surface of a quartz crystal and the frequency shifts of the QCM were measured. Although the linear range with the antibody-based QCM (10-240 μg L^{-1}) was comparable to that of the aptamer-based QCM (2.5-200 μg L^{-1}), a lower LOD was reported in the aptamer-based biosensor (2.5 μg L^{-1}) in comparison with that of an antibody-based biosensor (10 μg L^{-1}). According to the authors, the aptamers could tolerate the repeated affine layer regeneration after ligand binding, recycling the biosensor with little loss of sensitivity. When stored for three weeks, the frequency shifts of the aptamer-coated crystals were all greater than 90% of those on the response at the first day [46].

Enzymes and DNAzyme were also used to improve the sensor performance in terms of sensitivity and LOD. According to Du et al. [47], one of the most popular strategies to enhance the detection sensitivity is using enzyme-functionalized nanoparticles as labels by loading a large amount of enzyme toward an individual sandwich immunological reaction event. Du et al. [47] developed an electrochemical immunosensor for detection of phosphorylated p53 by using graphene oxide as a nanocarrier of enzymes (horseradish peroxidase). In order to enhance detection sensitivity, a multienzyme labelling strategy was used instead of a single-enzyme label during the immunoassay [47]. In addition, the LOD is 10-fold lower than that of a conventional sensor with single horseradish peroxidase label. Concerning DNAzyme, it is a catalytic nucleic acid containing deoxyribozyme, which is also used as recognition element, and it is synthesized by SELEX (systemic evolution of ligands by exponential enrichment) technique in order to generate well-ordered, three-dimensional structures that catalyze specific chemical reactions [48]. Huang et al. [49] developed a label-free colorimetric aptasensor based on DNAzyme amplification for detection of thrombin. The sensor can detect thrombin specifically with a LOD of 1.5 pM, which is at least four orders of magnitude lower over the unamplified colorimetric assay [49]. Zhao et al. [50] developed a versatile DNAzyme-based amplified biosensing platforms for detection of nucleic acid, protein, and enzyme activity. A fluorescence enhancement was verified together with the multiple turnover capability of the activated DNAzyme, which could improve the sensitivity of the sensing system [50].

The molecularly imprinted polymers are also recent recognition elements with various advantages such as high selectivity, stability, short time of synthesis, high thermostability, and cost effectiveness, and presenting a great potential for replacing biological antibodies; they are also known as artificial antibodies or plastic antibodies [12]. Their use in sensors and

biosensors lead to an improvement of the analytical signal, sensitivity, and LOD, as following reported. In the work of Gholivand et al. [51], a selective and sensitive electrochemical sensor was fabricated for propylparaben, and based on a nanosized molecularly imprinted polymer-carbon paste electrode, verifying its high recognition ability in comparison to a non-imprinted polymer-carbon paste electrode. The analytical signal obtained with cyclic voltammograms were recorded and the signal of the molecularly imprinted polymer-based sensor was higher than that of the non-imprinted polymer-based sensor, indicating that the molecularly imprinted polymer intensively absorbs propylparaben from the aqueous solution in comparison to non-imprinted polymer-based sensor [51]. In another work, Kong et al. [52] fabricated an electrochemical sensor for ascorbic acid using as recognition element a molecularly imprinted copolymer [the poly(*o*-phenylenediamine-*co*-*o*-aminophenol)], observing a high sensitivity and selectivity. When compared to a polypyrrole-based sensor for ascorbic acid (concentrations range between 250 and 7000 µM and LOD of 74 µM), the analytical performance of the imprinted copolymer sensor was improved (concentrations range between 100 and 10000 µM and LOD of 50 µM), which is explained by the broadened usable pH range of the poly(*o*-phenylenediamine-*co*-*o*-aminophenol) (from pH 1.0 to pH 8.0) [52]. According to the authors, the sensor also exhibited a good reproducibility and stability, and it has been successfully applied in the determination of ascorbic acid in real samples. It should be referred that the lack of simple and robust techniques to the synthesis of molecularly imprinted polymers can affect their further performance in practical applications. This is due to the requirement of different combinations between monomers and cross-linkers in order to obtain selective molecularly imprinted polymers. Concerning the application in sensors and biosensors, the main limitation is the difficulty in the integration of molecularly imprinted polymers in transducers, which then need more research to obtain an improved analytical signal. The detection of proteins is also limited with sensors based on molecularly imprinted polymers since a three-dimensional element is commonly lacking in the polymer surface.

3.4. Modes of power delivering

The modes of delivering electric power to sensors and biosensors, that is, the delivering of alternating current (AC) or direct current (DC) [53] and the use of a specific frequency [54] also can be tested in order to improve the sensor output (i.e., analytical signal), thus enhancing figures of merit of respective sensing systems, such as sensitivity and LOD. For example, Yamamoto et al. [53] fabricated FET biosensors based on CNT for BSA and

verified the improvement of the sensitivity of biosensors (by increasing the signal-to-noise ratio) using AC measurement instead of using DC measurement. According to the authors, the AC measurement with a lock-in amplifier suppresses the fluctuations in drain current in the biosensors without decreasing the signal level, thus the noise level of FET biosensors used in buffer solutions was greatly decreased by AC measurement. The signal-to-noise ratio of sensors measured using AC was six times higher than that obtained by DC measurement. The sensors were tested to BSA but they constitute promising technique for further sensitive applications by using the AC measurement. Recently, March et al. [54] proposed a highly sensitive and versatile high fundamental frequency (HFF) QCM immunosensor tested for pesticide (carbaryl and thiabendazole) analysis, and the sensitivity of conventional QCM sensors was estimated with the help of a new electronic characterization approach based on phase change measurements at a constant fixed frequency. The already established and commercially available systems usually employ frequencies ranging from 5 to 20 MHz but their operation at suitable HFF has been imperative for liquid applications in which the sensitive detection of small changes is required [54]. March et al. [54] verified that the highest sensitivity was exhibited by the 100 MHz HFF-QCM immunosensor, and when the results were compared with those reported for 9 MHz QCM, an improvement of one order of magnitude for sensitivity and two orders of magnitude for the LOD was verified (sensitivity of 30 μg L^{-1} and 0.66 μg L^{-1}, and LOD of 11 μg L^{-1} and 0.14 μg L^{-1}, for 9 and 100 MHz, respectively). A 50 MHz QCM was also tested and both the sensitivity (1.95 μg L^{-1}) and LOD (0.23 μg L^{-1}) was verified as intermediate values in relation to the previous two tested QCM [54].

4. Analytical performance of sensors and biosensors for clinical analysis

The study of the analytical performance of sensors and biosensors is reported in the following subsections through the estimation of associated figures of merit and taking into account the configuration of the various sensors and biosensors applied to the detection of physiologically important analytes considered in clinical analysis.

4.1. Sensors and biosensors for cancer and cardiac biomarkers

A wide number of publications have been found reporting the fabrication of sensors and biosensors for detection of cancer and cardiac biomarkers in comparison to other groups of analytes determined in the field of clinical diagnostics. That may be related to the clinical

relevance of such biomarkers or the urgent need of employment of point-of-care diagnostic device for their early detection.

Table 2 includes the figures of merit of current sensors and biosensors (from literature published between 2011 and 2015) for comparison of their analytical performance when used to the detection of: 1) cancer biomarkers [e.g., prostate specific antigen (PSA), interleukins (IL-6 and IL-8), matrix metalloproteinases (MMP-2 and MMP-3), α-fetoprotein, and carcinoembryonic antigen (CEA)] and 2) cardiac biomarkers [e.g., C-reactive protein (CRP), amino-terminal pro-brain natriuretic peptide (NT-proBNP), cardiac troponins (cTnT and cTnI), and myoglobin], and Table 3 shows the normal values of such physiologically important analytes.

< Table 2 >

< Table 3 >

4.1.1. Cancer biomarkers

The determination of the levels of cancer biomarkers in blood or tissue provided crucial information for the screening of cancer, and the development of sensing devices or other analytical methodologies for their sensitive and reliable point-of-care measurement has been a significant challenge for the early cancer detection and treatment monitoring, since high sensitivity, accuracy, minimal technical expertise, rapid and facile system maintenance should be obtained [105]. Sensors and biosensors for PSA, IL-6, IL-8, MMP, α-fetoprotein, CEA, and CA-125 have been fabricated in latest years, as shown in Table 2.

PSA is the most typically tumour marker for diagnosis of prostate cancer, providing direct clinical information through its concentration in blood, when levels are higher than 4.0 ng mL^{-1} (Table 3). Various biosensors are proposed for the detection of PSA in serum samples, as shown in Table 2. For example, Yang et al. [55] and Li et al. [56] employed electrochemical immunosensors for the detection of PSA in human serum samples, using the functionalization of graphene sheets with respectively CdS quantum dots or cobalt hexacyanoferrate nanocomposites for the immobilization of antibodies. In the work of Yang et al. [55], a better LOD (0.003 ng mL^{-1}) and wider linear range (0.005-10 ng mL^{-1}) were obtained when compared to such figures of merit reported with the immunosensor fabricated by Li et al. [56], where 0.01 ng mL^{-1} and 0.01-2 ng mL^{-1} were found for the LOD and linear range, respectively. Fig. 1 shows an image from transmission electron microscopy (TEM) of

graphene sheets (Fig. 1i), where a transparent and paper-like structure is observed, and a TEM image of graphene sheets functionalized with quantum dots (Fig. 1ii), where a large number of quantum dots were observed on the surface of graphene sheets [55]. In Fig. 1iii, the UV-Vis spectra of graphene sheets (curve a), quantum dots (curve b), and quantum dots-graphene sheets (curve c) are depicted and no absorption peaks were observed for graphene sheets, two absorption peaks were observed around 250 and 440 nm when pure quantum dots are analyzed, also verifying that the shift of such two absorption peaks is ascribed to 240 and 400 nm when quantum dots was anchored onto graphene sheets [55]. In addition, Fig. 1iv compares the analytical response of the immunosensor by using square wave voltammograms of the quantum dots on bare and graphene modified electrodes; the peak current (at -0.8 V) is enhanced about three times with the graphene modified electrode, when compared to the bare electrode (only with quantum dots), exhibiting an acceptable response for the further biosensing of PSA.

< Fig. 1 >

According to Yang et al. [55], the graphene sheets have very large surface area, allowing a large number of quantum dots to be immobilized onto their surface, thus increasing the sensitivity of the immunosensor. In addition, the authors referred that the low LOD may also be attributed to the large amount of antibodies immobilized to the graphene sheet modified electrode and the good conductivity of graphene sheets, which enhanced the detection sensitivity of Cd^{2+} (from the CdS quantum dots). The immunosensor was successfully applied for the detection of PSA in serum samples, since the PSA contents agreed well with the ELISA measurement (a correlation coefficient of 0.898 was found between the PSA contents obtained by these two methods), which means that the immunosensor can be used for routine clinical testing. Yang et al. [55] and Li et al. [56] also investigated the selectivity of the immunosensors by studying their responses towards human IgG, BSA, lysozyme, vitamin C, and glucose. Both works verified that the current variation due to the interfering substances was less than 8% of the current measured in the absence of the interfering compounds, when 1 ng mL^{-1} of PSA solution containing 100 ng mL^{-1} of interfering substances were tested, which means that both immunosensors exhibited good selectivity. When the analytical performance of the biosensors reported in Table 2 is compared for the detection of PSA, the LOD obtained in the work of Zani et al. [57] is the highest (1.4 ng mL^{-1}) observed. Zani et al. [57] proposed an electrochemical immunosensor based on protein G-coated paramagnetic

microparticles modified with antibodies specific to PSA, which were immobilized on screen-printed graphite 8-sensor arrays as working electrodes and functioning as multiplexed electrochemical platforms. Recently, Li et al. [60] fabricated an electrochemical immunosensor based on graphene (functionalized with ferrocene-carboxaldehyde composites) and silver hybridized mesoporous silica nanoparticles immobilized on glass carbon electrodes for the detection of PSA in serum samples. A low LOD was obtained (0.002 ng mL^{-1}) possibly due to the use of mesoporous silica materials which have large specific surface area, uniform structure, and controlled pore size [60]. Good stability (RSD lower than 10% after three weeks of stand-by) and reproducibility (RSD of 6.4%) were also observed with the immunosensor, concluding that the structure of the nanocomposites can be advantageous for the immobilization of biomolecules other than PSA useful in clinical diagnostics [60]. According to the authors, the use of silver nanoparticles to amplify the signal can improve the sensitivity of electrochemical immunosensors due to their biocompatibility and high electrical conductivity. Li et al. [60] demonstrate the applicability of the immunosensor for clinical routine, testing five different concentrations of PSA (1.0, 2.0, 4.0, 6.0, and 8.0 ng mL^{-1}) in human serum samples (without any treatment) using the standard addition method, and recoveries between 97.0 and 102.5% with RSD lower than 5.4% were obtained. In addition, the analytical results from the immunosensor was compared with those obtained with a commercial enzyme-linked immunosorbent assay (ELISA) and RSD from -4.61 and 4.10% were obtained between the two methodologies, which also indicates the feasibility of the immunosensors for clinical application [60]. Concerning selectivity, the immunosensor was incubated in 2 ng mL^{-1} PSA solution containing 200 ng mL^{-1} of interfering substances (hIgG, lysozyme, and α-fetoprotein) and the current variation due to the interfering substances was less than 5.2% of that without interferences, which means that the selectivity of the immunosensor was good [60].

Interleukins belong to the family of cytokines and they have an important function in the inflammatory response from illness such as psoriasis, rheumatoid arthritis, cardiovascular disease, and inflammatory bowel disease, as well as relevant in other diseases such as diabetes and Alzheimer's illness [64,66]. For example, IL-6 is a useful biomarker over-expressed by several types of cancer such as prostate cancer or head and neck squamous cell carcinoma, when concentrations in serum increase up to nanogram per millilitre range; the IL-6 level in serum of healthy individuals is about 6 pg mL^{-1} (Table 3). The traditional analytical techniques for the detection of IL-6 are, for example, ELISA, fluorescent microarray, chemiluminescence immunoassay, biosensors based on fluorescence, or conductimetry [65].

Other biosensors are currently used based on electrochemistry, as shown in Table 2. For example, Liu et al. [63] reported an immunosensor based on electrochemical and optical principles with graphene oxide nanosheets, polyaniline nanowires, and CdSe quantum dots nanocomposites immobilized on glass carbon electrodes for the detection of IL-6 in serum samples. The immunosensor has a long-term stability since the analytical response has not significantly changed when the sensor was stored for 14 days at 4ºC with RSD of 7.4% and it also has a good reproducibility since when tested in five independent sensors, the detection of 10 pg mL^{-1} of IL-6 provides a RSD of 7.6%. In addition, the analytical results obtained with the immunosensors were compared with those of ELISA methodology and relative deviations from -5.84 and 6.21% were found between the two methodologies, which means that the sensor could be satisfactorily applied to the clinical determination of IL-6 levels in human samples [63]. According to the authors, the nanocomposite shows excellent biocompatibility, dispersability, and solubility, which can improve the electrochemiluminescence biosensing method applied for the detection of IL-6 in serum samples or other proteins in clinical samples [63]. Liu et al. [63] also verified that the proposed immunosensor has sufficient selectivity for IL-6 detection and it is capable of differentiating IL-6 from its analogs in complex samples, since an acceptable RSD (6.3%) was obtained when the response for IL-6 was compared to that of BSA, CEA, and hIgG. Recently, Wang et al. [66], Ojeda et al. [64], and Lou et al. [65] proposed electrochemical biosensors for the detection of IL-6 in serum samples (Table 2), and based on gold nanoparticles/graphene/silica sol-gel, carboxyl-functionalized magnetic microparticles, and gold/palladium/silver nanoparticles, respectively. In the first two works, similar LOD were obtained (0.3 and 0.39 pg mL^{-1}) but with a wider linear range (1.75-500 pg mL^{-1}) and better sensitivity (336 nA pg^{-1} mL^{-1}, respectively) for the work of Ojeda et al. [64] when compared to such figures of merit obtained by Wang et al. [66] which were about 1-40 pg mL^{-1} and 20.4 nA pg^{-1} mL^{-1}, respectively. Wang et al. [66] have used an indium tin oxide (ITO) electrode, where the gold nanoparticles/graphene/silica sol-gel film was *in situ* synthesized providing a stable network for the labelling of the composite constituted by gold nanoparticles/polydopamine/CNT used for immobilization of antibodies specific to IL-6. The polydopamine is a novel, simple, inexpensive, “green”, and stable material, and its association with gold nanoparticles and CNT is promising in clinical diagnosis [66]. In addition, Wang et al. [66] found that the results of serum samples with the sensor have acceptable agreement with the reference ELISA methodology, since a correlation coefficient of 0.990 was found between the two methodologies, which means that the fabricated immunosensor can be incorporated in bioelectronic systems. An acceptable

selectivity of the immunosensor was also verified since a variation of 2.2-8.8% was obtained concerning the response of the immunosensor to interfering analytes (α-fetoprotein, human chorionic gonadotropin, and PSA) [66]. From the work of Ojeda et al. [64], the advantage of the biosensor fabricated is related to the excellent storage stability provided by the anti-IL-6-magnetic beads conjugates, which provides amperometric responses with no significant loss during at least 36 days. In addition, the analytical results obtained with the biosensor were statistically in agreement with those provided by a commercial ELISA kit, since no significant differences were found between both methodologies (at α=0.05). In comparison to such two works, the LOD obtained through the electrochemical immunosensor reported by Lou et al. [65] was lower (0.059 pg mL^{-1}) in a wider linear range (0.1-100000 pg mL^{-1}). Lou et al. [65] combined the electrically heated carbon electrode technique with gold nanoparticles constituting a competitive dual signal amplification strategy. According to the authors, the electrically heated carbon electrode technique is a way to accelerate the reaction kinetic without changing the bulk solution temperature, thus improving the mass transport by changing temperature of electrode and leading to an enhanced electrochemical signal together with a higher signal-to-background ratio, which can be responsible to the high analytical performance. Concerning its stability, more than 90% of initial responses for IL-6 was obtained with the biosensor when it is stored for 14 days at 4ºC, which can be attributed to the good biocompatibility of retaining the bioactivity of proteins. In addition, the immunosensor was tested to detect IL-6 in serum samples and the results were compared with those obtained with ELISA methodology; RSD less than 6.3% were reported for eight individual biosensors tested to 100 pg mL^{-1} of IL-6, suggesting its potential application towards the early evaluation of tumour diseases [65]. The selectivity of the immunosensor was also tested and no significant differences (RSD from 6.5% to 10.9%) were observed when 10 pg mL^{-1} IL-6 was mixed with 100 pg mL^{-1} BSA, cTnI, CEA, and MMP-2, indicating that such analytes could not cause observable interference in the immunosensor [65]. Another electrochemical immunosensor is proposed by Tang et al. [62] for the detection of IL-6 in serum samples and it is based on the microfluidic principle, providing a LOD in the femtogram per millilitre range (10 fg mL^{-1}, that is, 0.01 pg mL^{-1}) and a linear range from 10 to 1300 fg mL^{-1} (0.01-1.3 pg mL^{-1}), which is very promising for the detection of other biomarkers for cancer diagnostics. According to Tang et al. [62], microfluidics provided excellent control of mass transport that improved the signal-to-noise ratio while making the detection system more portable. In addition, the low cost of the sensors is the major advantage associated to such fabrication method, also leading to high quality microchips with minimized sample volume of

sample required of 1 μL [62]. The schematic representation of the microfluidic chips proposed by Tang et al. [62] is shown in Fig. 2.

< Fig. 2 >

According to Tang et al. [62], the microfluidic array has an excellent potential as a cheap, disposable chip-based diagnostic tool for early cancer detection and monitoring, and the very low LOD allows extensive dilution of samples to help the minimization of non-specific binding of potentially interfering biomolecules in serum, and may also be useful in tests for cancer recurrence.

The MMP family of zinc-dependent endopeptidases represents a class of proteins that are crucial for the regulated degradation and processing of extracellular matrices, also facilitating the host and tumour communication [67]. For example, MMP-2 is an important MMP in tumour growth, invasion, and metastasis, playing an important role in physiological and pathological states including morphogenesis, reproduction, and tissue remodelling [67]. Another MMP is the MMP-3, which elevated expression is associated to head and neck squamous cell carcinoma and adrenal tumours, constituting a potential clinical tool for diagnosing and monitoring of these diseases [61]. In Table 2, three works are reported for the detection of MMP-2 and MMP-3 with different biosensors. For MMP-2, Yang et al. [67] reported the construction of an electrochemical immunosensor based on gold nanoparticles and nitrogen-doped graphene composite immobilized on a glass carbon electrode and Song et al. [68] constructed an optical biosensor based on graphene oxide. According to Song et al. [68], the graphene oxide has been used in order to develop biosensors based on fluorescence resonance energy transfer due to its superior fluorescence quenching capacity and unique adsorption characteristics for biomolecules. With the electrochemical immunosensor proposed by Yang et al. [67], a better LOD was obtained (0.11 pg mL^{-1}) when compared to the optical biosensor (2500 pg mL^{-1}) proposed by Song et al. [68]. On the other hand, the immunosensor showed a wide linear range (0.5-50000 pg mL^{-1}) with high selectivity and long-term stability (90% of the initial response was remained after storage of biosensors at 4ºC for one week), and could be satisfactorily applied for the detection of proteins in clinical laboratories [67]. In addition, analytical results from the immunosensors were compared with those of an ELISA methodology and acceptable agreement was verified since relative deviations between -5.97 and 2.94% were obtained, suggesting that the immunosensor could be applied to clinical determination of MMP-2 levels in human plasma [67]. Also according

to the authors, the composite constituted by gold nanoparticles and nitrogen-doped graphene sheet has many advantages suitable for biosensing, that is, facilitating robust immobilization of antibodies, promoting electron transfer, and exhibiting excellent electrochemical activity, thus enhancing the analytical performance of the immunosensor due to the homogeneous dispersion of gold nanoparticles on nitrogen graphene sheet.

The α-fetoprotein, an oncofetal glycoprotein, is the most common and important liver cancer tumour marker, mainly produced by the liver, yolk sac, and gastrointestinal tract of a human foetus and its increased level in adult serum is an indicator of hepatocellular carcinoma or endodermal sinus tumour [71]. The conventional techniques for the detection of α-fetoprotein are ELISA, electrochemistry, electrochemiluminescence, mass spectrometry, QCM, and SPR immunoassays but their limitations such as relatively sophisticated instrumentation, significant sample volume, limited sensitivity, and clinically unrealistic expense and time, have been lead to the development of new, simple, and inexpensive methods to detect α-fetoprotein or other biomarkers in both normal and cancer patient serum [71]. For the detection of α-fetoprotein in serum samples, various electrochemical sensors are proposed (Table 2), where nanomaterials were used for signal transduction. CdTe quantum dots, MWCNT/gold nanoparticles, gold/silver nanoparticles, and Fe_3O_4/ZrO_2/nanogold composite were employed by Li et al. [71], Ge et al. [72], Lai et al. [73], and Gan et al. [70], obtaining LOD of 0.00013, 0.00015, 0.0039, and 0.01 ng mL^{-1}, respectively. Such different levels can be due to the different transduction principles employed; all sensors are based on electrochemical principle but, for example, optical mechanism (exciting detection technique using quantum dots as photoactive materials) and magnetic mechanism (preparation of magnetic particles: Fe_3O_4-ZrO_2-Au-polylysine) were combined with electrochemistry as reported in the works of Li et al. [71] and Gan et al. [70], respectively. In the work of Li et al. [71], good long-term stability of two weeks at 4ºC was reported since no apparent change in photocurrent response was found when detecting 10 ng mL^{-1} of α-fetoprotein, and the feasibility for clinical application was demonstrated since relative deviations between -3.6 and 3.0% were obtained when analytical results of immunosensor were compared with those obtained with ELISA. In the work of Lai et al. [73], the same sensor stability is reported (90% of initial response remained after two weeks at 4ºC) and when human serum samples were tested for α-fetoprotein detection with the immunosensor and with a commercial electrochemiluminescence test, relative errors between -10.5% and 8.5% were observed, indicating good accuracy of the reported biosensor for clinical sample detection. Concerning

the stability of the biosensor reported in the work of Gan et al. [70], it is higher, since no apparent change in response was observed with measurement of α-fetoprotein every 3-5 days, with RSD of 3.0% during 30 days (at 4ºC). The authors referred that the good stability may be due to the consistency of Fe_3O_4-ZrO_2-Au-polylysine membrane and the fact of protein molecules were attached firmly onto the surface of glass carbon electrode. The immunosensor can be applied to clinical determination of α-fetoprotein levels in human serum since average concentration of α-fetoprotein with the biosensor (4.56 ng mL^{-1}) was similar with that obtained with ELISA (4.89 ng mL^{-1}), indicating no significant difference between such results. In addition, Gan et al. [70] verified that a good selectivity of the immunosensor for the determination of α-fetoprotein, since the sensor response for various analytes (100 ng mL^{-1} CEA, 1 μg mL^{-1} hIgG, 20 ng mL^{-1} carbohydrate antigen 19-9, 20 ng mL^{-1} human chorionic gonadotropin antigen, 2 μg mL^{-1} BSA, 2 μg mL^{-1} dopamine, 2 μg mL^{-1} L-lysine, 2.5 μg mL^{-1} uric acid, 2.5 μg mL^{-1} glucose, 5 μg mL^{-1} ascorbic acid, and 5 μg mL^{-1} Na^+) did not interfere with the determination of 5.0 ng mL^{-1} α-fetoprotein (signal changed below 5%). Recently, Wang et al. [74] proposed an electrochemical immunosensor based on mesoporous silica nanoparticles, which were used as carrier for the immobilization of secondary antibody as well as Fe_3O_4 nanoparticles and the enzyme horseradish peroxidase, both employed as signal amplification labels. The mesoporous silica nanoparticles are known to have a large specific surface area and good biocompatibility and constitute promising sensing materials for electrochemical sensors [74], and they are also cheaper materials in comparison to noble metal nanoparticles (Au, Al or Pt) often used in electrochemical sensors, as shown in the previous examples. A good linear relationship between current change and concentrations of α-fetoprotein was obtained with the electrochemical immunosensor (0.01-25 ng mL^{-1}) with a low LOD (0.004 ng mL^{-1}), suggesting a wide potential application in clinical analysis or detection of other tumour markers [74]. In addition, when compared to the analytical results obtained with ELISA methodology, for detection of α-fetoprotein in serum samples, the immunosensor has relative deviations between -6.1 and 1.3%, indicating suitability in real sample detection.

Concerning CEA, a glycoprotein, it is the most extensively used tumour marker for clinical diagnosis of lung cancer, ovarian carcinoma, breast cancer, and cystadenocarcinoma and its cutoff value in clinical diagnosis is 5 ng mL^{-1} [78,80]. When inflammation or tumours arise in any endodermal tissue including gastrointestinal tract, respiratory tract, pancreas, and breast, the levels of CEA can become elevated [106]. Several analytical techniques based on

immunoassays have been reported for the determination of CEA, including radioimmunoassays, enzyme immunoassays, and fluoroimmunoassays, but drawbacks such as radiation hazards, long analysis times, or the need for qualified personnel and/or sophisticated instrumentation have been reported [107]. As an early diagnosis and therapeutics of cancer are necessary, the development of sensitive, precise, and accurate analytical techniques are required for the determination of CEA with low concentration levels in complex biological samples [79]. For example, electrochemical biosensors have several advantages in comparison with the molecular detection approaches previously identified, including the ability to analyze complicated body fluids, high sensitivity, and compatibility with microfabrication technologies, low manpower requirements, and compact instrumentation that is compatible with portable devices [107]. In Table 2, various electrochemical biosensors are proposed for the detection of CEA in serum samples with different levels in their figures of merit such as linearity and sensitivity. For example, Han et al. [76], Kong et al. [77], and Lai et al. [73] fabricated electrochemical immunosensors for CEA based on different transduction materials. Han et al. [76] fabricated an electrochemical immunosensor based on chitosan-ferrocene, nano-TiO_2 film, and gold nanoparticles-graphene nanohybrid, Kong et al. [77] proposed an electrochemical immunosensor based on gold nanoparticles-thionine-reduced graphene oxide nanocomposites film, and Lai et al. [76] fabricated an electrochemical immunosensor based on gold and silver nanoparticles. In the works of Han et al. [76] and Kong et al. [77], similar LOD (0.0034-0.004 ng mL^{-1}) were obtained but different sensitivities (4.2 and 0.53 µA ng^{-1} mL^{-2}, respectively) and linearity (0.01-80 ng mL^{-1} and 0.01-0.5 ng mL^{-1}, respectively) were reported, as shown in Table 2. In the work of Han et al. [76], the testing of real serum samples was reported with the biosensor and ELISA as reference method, indicating good agreement between both analytical methodologies, since relative deviations of biosensor results were between -8.31 and 7.58%. In the work of Kong et al. [77], recovery experiments were performed by standard addition method (CEA concentrations from 40 and 200 pg mL^{-1}) in human serum and analyzed with the proposed immunosensor; an acceptable recovery in the range of 92.5 and 113.0% was reported, thus indicating the feasibility of immunosensor to determined CEA in human serum for routine clinical diagnosis. Wang et al. [79] have constructed a simple label-free electrochemical immunosensor based on nafion membrane containing SiO_2 nanoparticles immobilized on glass carbon electrodes for the detection of CEA in serum samples. Wang et al. [79] obtained an excellent LOD in the femtomolar range (1 fg mL^{-1}, that is, 0.000001 ng mL^{-1}) with the immunosensor, which response was linear from 0.000001 to 0.1 ng mL^{-1}. In addition, the analytical results obtained

with the immunosensor for the detection of CEA in human serum samples are consistent with the data determined by a microparticle enzyme immunoassay with relative errors between -8 and 5% [79]. The specificity of the immunosensor was also determined by mixing 1 ng mL^{-1} of CEA with 1 ng mL^{-1} of glucose, uric acid, α-fetoprotein, BSA, hIgG, and ascorbic acid [79]. Wang et al. [79] verified that the variation of the analytical response obtained from interference substances was less than 7.6% when compared to that of pure CEA, which means that the selectivity of the immunosensor was acceptable. Recently, Lin et al. [80] and Sun et al. [81] constructed electrochemical immunosensors for the detection of CEA in serum samples where transduction principle is based on nano-gold functionalized mesoporous carbon foam (immobilized on glass carbon electrodes) and gold-silver bimetallic nanoparticles (immobilized on paper working electrodes), respectively, which were used to immobilize the antibodies specific to CEA. As shown in Table 2, the LOD obtained in such two works, together with the work of Wang et al. [79], were the lowest in comparison to the other works reporting the detection of CEA with electrochemical immunosensors. Lin et al. [80] proposed a method to amplify the immunosensor signal by the incorporation of a commercial silver enhancer solution, which is a mixture of silver and colloidal gold. According to the authors, such new designed amplification strategy holds great potential for ultrasensitive electrochemical biosensing of other analytes, which can be reflected in the LOD; the LOD found in the work of Lin et al. [80] was better (0.000024 ng mL^{-1}) than that found in the work of Sun et al. [81] (0.0003 ng mL^{-1}), probably due to the signal transduction, which was amplified in the work of Lin et al. [80], thus enhancing the stability of metal particles. In both works, the results obtained with fabricated biosensors for detection of CEA in real human serum samples were compared to reference methodologies, that is, commercial electrochemiluminescence tests, and acceptable agreements with relative errors less than 11.9% and less than 3.2% were respectively obtained by Lin et al. [80] and Sun et al. [81].

Cancer antigen 125 (CA 125) is a serum cancer biomarker for monitoring and follow-up of ovarian, breast, and uterine cancer patients, also used for prognosis of response to various cancer therapies [9] and its cut-off value in clinical diagnosis is 35 U mL^{-1} (Table 3). In Table 2, Wang et al. [78] and Ge et al. [72] reported multiplexed electrochemical immunosensors based on MWCNT, functionalized on a microfluidic paper-based analytical device (μPAD) denominated by wax-patterned microfluidic paper-based three-dimensional electrochemical device (3D-μPED) and on MWCNT/gold nanoparticles (functionalized on a electrode array-based paper), respectively, with ability to detect simultaneously more than one cancer biomarker in serum samples. In the work of Wang et al. [78], a simple, sensitive, low-cost,

disposable, and portable biosensor for the detection of CEA and CA 125 is reported and Ge et al. [72] proposed a multi-analyte biosensor for simultaneous detection of four cancer biomarkers (α-fetoprotein, CEA, CA 125, and CA 153) based on electrode arrays with high throughput, low cost, small consumption, simple operation, and high sensitivity. The schematic configurations and calibration curves obtained in the two works are showed in Fig. 3.

< Fig. 3 >

The multiplexed configuration can enhance the sample throughput, shortening the assay time, and decreasing the sample consumption and costs [72]. For both works, the calibration plots obtained for CA 125 and CEA showed good linear relationship between the analytical response (peak currents) and the logarithm values of CA 125 and CEA concentration, with correlation coefficients of 0.9968 and 0.9964, respectively, as reported in the work of Wang et al. [78] and correlation coefficients of 0.9965 and 0.9954, respectively for CA 125 and CEA, as shown in the work of Ge et al. [72] (Table 2). Both for CEA and CA 125, the LOD obtained in the work of Ge et al. [72] are better (0.00002 ng mL^{-1} and 0.000037 U mL^{-1}, respectively) than those obtained by Wang et al. [78] (0.01 ng mL^{-1} and 0.2 U mL^{-1}, respectively), but with linear ranges that covered most of the levels in human serum with both biosensors (Table 2). MWCNT have high chemical stability, good electrical conductivity, and strong adsorptive ability, and their high surface area provides a large loading capacity for the nanoparticles, thus improving catalytic activity when metal nanoparticles are supported on MWCNT [108], as shown with the biosensor fabricated by Ge et al. [72], where MWCNT function as heterogeneous catalyst supports for gold nanoparticles. The sensor developed by Wang et al. [78] and Ge et al. [72] were applied for detection of CA 125 and CEA in human serum samples and the analytical results were compared with those obtained with a reference methodology (commercial electrochemiluminescence method). In the work of Wang et al. [78], relative errors between the sensor and the reference method were less than 5.8% for CA 125 and less than 6.5% for CEA, and in the work of Ge et al. [72], RSD are between 2.5 and 5.9% for CA 125 and between 1.4 and 6.3% for CEA, showing an acceptable agreement between the two methodologies and for both works.

4.1.2. Cardiac biomarkers

Cardiovascular diseases (CVD) such as cerebral vascular accident and coronary heart disease are among the major causes of ill health, invalidity, and death worldwide [109], and the determination of specific blood compounds (biomarkers) has been suggested as a tool for the identification of high-risk patients in terms of the CVD risk. Thus, the early detection of cardiac biomarkers with high sensitivity is required in clinical diagnostics. Various analytical techniques have been used such as turbidimetry, SPR and immunoassays, which need of pre-treatment and labelling, and they are time consuming to be considered in practical clinical routine; nowadays the construction of biosensors for the rapid detection of cardiac biomarkers is very promising due to their miniaturization, portability, flexibility, and sensitivity [86,87].

In Table 2, some recent works are reported with the fabrication of biosensors for the detection of CRP, cardiac troponins, NT-proBNP, and myoglobin in serum samples. The employment of nanomaterials or combination of nanomaterials with composites for that construction was observed for the majority of works, mainly due to their large surface area, good biocompatibility, enhancing their analytical performance [87]. For example, ZnO nanotubes, Fe_3O_4-SiO_2 magnetic nanoprobes, and SWCNT were used in the works of Ibupoto et al. [84], Zhou et al. [87], and Justino et al. [88] as labels for the immobilization of antibodies in immunosensors for CRP detection. CRP is an acute-phase protein and levels higher than 3 mg L^{-1} are used to evaluate the risk assessment for CVD development such as atherosclerosis, angina, coronary heart disease, peripheral artery disease, myocardial infarction, and stroke, and it can be used as reliable marker for tissue injury, infection, and inflammation [82]. Low LOD were found in the works of Ibupoto et al. [84], Zhou et al. [87], and Justino et al. [88], that is, 0.001, 0.0003, and 0.1 ng mL^{-1}, respectively, which were lower than those obtained with traditional immunoassays (30-200 ng mL^{-1}) [84,110]. All works reported enhanced analytical performance with the immunosensors. The time response of the electrochemical immunosensor fabricated by Ibupoto et al. [84] was less than 10 seconds, detecting CRP with good reproducibility (RSD less than 5%), and stability (3 days), but the immunosensor was not tested in real samples, which constitutes a limitation for further clinical application. Ibupoto et al. [84] tested the proposed sensor into a solution with common interferents (glucose, urea, uric acid, as well as sodium, potassium, and iron ions) but, as referred by the authors, a negligible response was obtained. Both the piezoelectric immunosensor and electrochemical immunosensor reported by Zhou et al. [87] and Justino et al. [88], respectively, were validated by detection of CRP in serum and saliva samples. Zhou et al. [87] obtained good recoveries (87-107%) when the detection of CRP was tested in various serum samples by the immunosensors and classical ELISA methodology, as well as

good reproducibility (CV of 2.4%) and repeatability (CV of 3.4%). The major advantage of such piezoelectric immunosensor is the possibility of surface regeneration and repeated use (stability of analytical response of two weeks), constituting a versatile analytical technology for clinical analysis of CRP or other cardiac biomarkers. In the work of Justino et al. [88], the electrochemical immunosensors are considered as disposable, label-free, low-cost, and of easy analysis, which are advantageous to the development of a point-of-care testing methodology for CVD risk assessment. The immunosensors were validated by analyzing serum and saliva samples with ELISA methodology, showing comparable analytical performance; linear correlations were found between the immunosensor and ELISA when applied to serum (r^2=0.9904) and saliva (r^2=0.9991) samples. Thus, non-invasive sampling methodology reported in the works of Zhou et al. [87] and Justino et al. [88] for the detection of CRP can be useful for diagnosis and monitoring of CVD. Recently, the group of Esteban-Fernández de Ávila [85,89] proposed two amperometric magnetoimmunosensors for the detection of CRP both using carboxylic acid-modified magnetic beads as labels for the immobilization of antibodies; the first biosensor [85] was constructed in disposable gold screen-printed electrodes for the detection of CRP in serum samples and the second one [89] was constructed in screen-printed carbon electrodes for the multiplexed detection of CRP and NT-proBNP, which is another cardiac biomarker (Table 2). According to Esteban-Fernández de Ávila et al. [85], magnetic beads have been useful in the development of electrochemical immunosensors contributing to the improvement of their sensitivity, reduction of the time of analysis, and minimization of matrix effects. Comparable sensitivity (0.203-0.386 μA ng^{-1} mL^{-2}) and reproducibility (RSD of 6.3-6.5%) were found with the CRP immunosensors but with LOD of different orders of magnitude: 0.021 ng mL^{-1} in the work of Esteban-Fernández de Ávila et al. [85] and 0.47 ng mL^{-1} for the multiplexed immunosensor proposed by Esteban-Fernández de Ávila et al. [89]. According to Esteban-Fernández de Ávila et al. [85], the low LOD observed have a practical advantage in the case of existing strong matrix effects for real clinical samples, which makes a sample dilution necessary before the analysis. Both immunosensors were successfully applied to spiked serum samples, which demonstrate the applicability of such sensing platforms for clinical diagnosis of CRP. With the same multiplexed immunosensor, but for the detection of NT-proBNP, Esteban-Fernández de Ávila et al. [89] obtained a similar LOD than that obtained when analyzing CRP, but with lower reproducibility (9.4%). According to Esteban-Fernández de Ávila et al. [89], the low cost, easy automation, and miniaturization of multiplexed immunosensor, together with the use of disposable mass-produced electrodes make such approach as a promising and attractive

alternative diagnosis tool for the development of point-of-care devices for *in situ* clinical diagnosis. Another biomarker for cardiac risk prediction is the NT-proBNP with an important role in the natriuretic, diuretic, and vasodilatory system as well as in the inhibition of the rennin-angiotensin-aldosterone system and the sympathetic nervous system [91]. Zhuo et al. [90] and Yi et al. [91] also reported electrochemical immunosensors for the detection of NT-proBNP based on gold-MWCNT composite and avidin functionalized magnetic nanoparticles, respectively (Table 2). The biosensor reported by Zhuo et al. [90] is responsible to a better LOD (0.006 ng mL^{-1}) and wide linear range (0.02-100 ng mL^{-1}) in comparison to that reported by Yi et al. [91], where a LOD of 30 pg mL^{-1} and a linear range between 0.04-2.5 ng mL^{-1} are observed. The main advantage of the immunosensor proposed by Yi et al. [91] is the simple regeneration procedure which consists in washing the magnet with buffer in order to remove the avidin functionalized magnetic nanoparticles, antigen and antibodies.

Troponins are the most specific and sensitive biomarkers of myocardial cell injury and they have been highlighted for the testing of acute myocardial infarction [96]. Troponins such as cTnT and cTnI can control the calcium-mediated interactions between actin and myosin in cardiac and skeletal muscles; the cTnT is expressed in skeletal muscle to a smaller extent, whereas cTnI has not been recognized outside of myocardium [99]. Increased levels of cTnT (higher than 0.3 ng mL^{-1}) are highly specific to cardiac injury and they are strongly associated to an increased risk of re-infarction and death, and an early diagnosis is crucial to plan therapeutics [93]. As shown in Table 2, Fonseca et al. [93], Gomes-Filho et al. [94], Silva et al. [95], and Mattos et al. [96] proposed immunosensors respectively based on gold nanoparticles, carboxylated CNT, amine-functionalized CNT, and *o*-aminobenzoic film for immobilization of antibodies specific to cTnT, obtaining LOD between 0.0015 and 0.033 ng mL^{-1}. All immunosensors were applied to serum samples with high sensitivity and suitable for clinical applications as potential tools for point-of-care acute myocardial infarction diagnostics. For example, Gomes-Filho et al. [94] and Silva et al. [95] compared the cTnT concentrations obtained with both immunosensors in human serum samples with the levels found with electrochemiluminescence immunoassays and correlation coefficients of 0.987 and 0.990 were obtained between the two methods ($p<0.0001$) for the works of Gomes-Filho et al. [94] and Silva et al. [95], respectively. Recently, Singal et al. [99] and Cho et al. [100] reported electrochemical and optical immunosensors, respectively, for the detection of cTnI. The electrochemical immunosensor (based on impedance) was prepared with functionalized platinum nanoparticles immobilized on a graphene monolayer deposited on a glass carbon electrode, obtaining a LOD of 0.0042 ng mL^{-1} with reproducible results (RSD of 4-11%) and

a linear range between 0.01 and 10 ng mL^{-1} [99]. According to Singal et al. [99], the combination of graphene monolayer composite with functionalized platinum nanoparticles may result into an increased biomolecular immobilization for immunosensor fabrication, since graphene is an ideal transducer material due to its large surface area, high heterogeneous electron transfer rates, high intrinsic mobility, and good electrical and thermal conductivity, and the functionalized platinum nanoparticles have a large specific surface area, excellent biocompatibility, strong adsorption ability and good conductivity. In addition, good shelf-life stability was reported with the immunosensor fabricated by Singal et al. [99] since 96% of its initial sensitivity is retained (for 1.0 ng mL^{-1} of cTnI) when the analytical performance was monitored after 30 days storage at 4ºC. The optical immunosensor (based on fluorescence) is constituted by a membrane strip installed within a cartridge to simultaneously detect cTnI and myoglobin, which are two acute myocardial infarction biomarkers [100]. For cTnI, a LOD of 0.05 ng mL^{-1} was obtained with reproducible results (RSD of 7.5%) and linear range between 0 and 50 ng mL^{-1}. Both cTnI biosensors can be used in clinical diagnostics taking into account that the cut-off concentration of cTnI in the blood between 0.01 and 0.1 ng mL^{-1}; a level between 0.1 and 2 ng mL^{-1} indicates a diagnosis of unstable angina and other heart disorder and a level greater than 2 ng mL^{-1} indicates a person with a significant myocardial injury and increased risk for future serious heart events [99].

Concerning myoglobin, it is used for early diagnostics of acute myocardial infarction and it occurs at a level in blood of 50-100 ng mL^{-1} increasing about 10-500 times in acute myocardial infarctions [100]. With its optical immunosensor, Cho et al. [100] obtained reproducible results (RSD of 2.3%) and a linear range between 2.0 and 100 ng mL^{-1}. Four different potentially interfering substances (40 mg dL^{-1} bilirubin, 2000 mg dL^{-1} triglycerides, 2000 mg dL^{-1} haemoglobin, and 100 mg dL^{-1} ascorbic acid) were also tested in samples to determine whether they influenced the immunoassay and it was showed that their presence did not cause a significant change (±5% variation) in the result compared to that of the negative control, except for the presence of bilirubin [100]. Zapp et al. [102] and Kim et al. [103] also recently reported immunosensors for myoglobin detection, as shown in Table 2, and based on electrochemical and optical transduction principles, respectively. The electrochemical immunosensor provides a lower LOD (6.29 ng mL^{-1}) but narrower linear range (9.96-72.8 ng mL^{-1}) than those obtained with the optical immunosensor (31.0 ng mL^{-1} and 100-1000 ng mL^{-1}, respectively). The limitation of the immunosensor fabricated by Zapp et al. [102] is the lack of validity in real samples; only simulated serum samples with myoglobin were used. Kim et al. [103] proposed an optical immunosensor based on SPR

which functions continuously in real-time, in a reproducible way, verifying the myoglobin levels over 8 hours with periodic one-point calibration every 3 hours with acceptable variations (average CV of 4.91%), when tested in real serum samples. Thus, according to Kim et al. [103], the immunosensor could be used as a companion diagnostic technique with real-time electrocardiographic measurement, significantly enhancing the sensitivity of acute myocardial infarction diagnosis and thereby enabling treatment at an early stage [103].

4.2. Sensors and biosensors for hormones, biomolecules, and neurotransmitters

Table 4 includes the figures of merit of current sensors and biosensors (literature published between 2011 and 2015) for comparison of their analytical performance when used to the detection of: 1) hormones such as cortisol; 2) biomolecules such as glucose and cholesterol; and 3) neurotransmitters such as acetylcholine, epinephrine, norepinephrine, and dopamine, and Table 5 shows the normal values of such physiologically important analytes.

< Table 4 >

< Table 5 >

4.2.1. Hormones

Cortisol belongs to the family of glucocorticoid hormones and it is known as a potential biomarker for psychological stress estimation. The abnormality in cortisol levels is a good indicator of chronic conditions such as Cushing's syndrome (symptoms of obesity, fatigue, and bone fragility) when levels are in excess, or Addison's disease (manifested by weight loss, fatigue, and darkening of skin folds and scars) when levels are in deficit [112,136]. The detection of cortisol is mostly limited to laboratory techniques such as chromatography, ELISA, SPR, and QCM, which limitations are long time from sampling to results (from days to a few weeks), complex sample preparation, and expensive diagnosis [111,112]. Thus, point-of-care technologies have been required in order to get rapid and selective cortisol detection in relevant biological fluids such as saliva. A good correlation in the amount of free cortisol present in the saliva and the total cortisol present in the blood is known ($r=0.60$; $p<0.001$) with normal levels of cortisol in serum in the range of 100-500 nM [131]. Recently, Pasha et al. [111] and Vabbina et al. [112] proposed electrochemical immunosensors based on cyclic voltammetry for the detection of cortisol in saliva samples (Table 4) and the obtained

analytical results were validated using ELISA methodology. Pasha et al. [111] constructed a simple, low-cost, and label-free immunosensor based on interdigitated microelectrodes (IDE) and Vabbina et al. [112] proposed a label-free, sensitive, and selective immunosensor with nanomaterials (zinc oxide 1D nanorods and 2D naoflakes) for the immobilization of cortisol antibodies. Both schematic representations of immunosensing principle are showed in Fig. 4.

< Fig. 4 >

In the work of Pasha et al. [111], the interdigitated microelectrodes were modified with a self-assembled monolayer (SAM) of dithiobis(succinimidylpropionate) used for covalent immobilization of cortisol antibodies (anti-C_{ab}), as shown in Fig. 4i. In the work of Vabbina et al. [112], nanomaterials were synthesized through sonochemical technique and they are used to immobilize cortisol antibodies. Both immunosensors were able to detect cortisol in saliva samples and they can be further used to monitor physiological stress for health monitoring and to understand various physical, behavioural and psychological variables which may affect the central nervous system.

Estradiol or 17β-estradiol is a natural steroid estrogen, also considered as an endocrine disrupting chemical, with impact on the reproductive and sexual functioning and the quantification of their levels in serum (normal levels are about 200-600 pg mL^{-1}) has been crucial in various clinical evaluations such as investigations of fertility treatments, post-menopausal status, hyperandrogenism, and breast cancer [113,114]. Analytical techniques such as gas chromatography and high performance liquid chromatography (HPLC) or electrochemical methods involving voltammetry and chronoamperometry have been reported to the determination of estradiol in biological, pharmaceutical, and water samples but involving labour intensive, time consuming, and expensive equipments [113-115]. In the clinical field, the fabrication of biosensors has been also observed due to their high selectivity and sensitivity. For example, Hao et al. [113] fabricated a sensor for estradiol detection based on MWCNT and gold nanoparticles immobilized on graphite electrode through layer-by-layer assembling technique. The sensor was tested in serum samples obtained from pregnant women using the standard addition method to determine the estradiol content, and good recoveries (98.5-103.5%) were reported, which means that the sensor can be applied for the determination of estradiol in real samples. Ojeda et al. [114] proposed a disposable electrochemical immunosensor based on graphed *p*-aminobenzoic acid immobilized on screen-printed carbon electrodes for the detection of estradiol and a low LOD (0.77 pg mL^{-1})

and a wide linear range (1.0-250 pg mL^{-1}) were obtained. The immunosensor was also applied to the analysis of certified serum and spiked urine samples obtaining mean recoveries between 96 and 102% for serum samples and between 95 and 100% for urine samples. Recently, Wang et al. [115] proposed an electrochemical sensor for estradiol based on nanoporous polymeric film on glass carbon electrodes, and good recoveries (99-103%) were reported when the sensor was applied to human urine samples, which demonstrates the applicability of the sensor to real samples. Wang et al. [115] also studied the selectivity of the sensor by measuring the current responses of some potential interfering substances such as L-cysteine, L-lysine, and ascorbic acid ($1.0x10^{-4}$ mol L^{-1}), as well as uric acid and tryptophan ($5.0x10^{-5}$ mol L^{-1}) and no interference (standard deviation lower than 5.0%) was found in the determination of $1.0x10^{-6}$ mol L^{-1} of estradiol, which indicates good selectivity on the estradiol determination.

4.2.2. Biomolecules

The monitoring of glucose and cholesterol is crucial in clinical analysis for the diagnosis and treatment of diabetes mellitus and coronary heart diseases, respectively. Recent sensors are proposed for their detection, as shown in Table 4, where nanoparticles (CuO, Fe_2O_3, or ZnO) are mainly employed for the enhancement of their analytical response. For example, Dung et al. [118], Yang et al. [119], and Zhang et al. [121] have employed CuO-SWCNT nanocomposites, carboxyl-modified graphene oxide/magnetic nanoparticles, and CuO nanoparticles/carbon spheres, respectively, for the detection of glucose with electrochemical sensors. Dung et al. [118] and Zhang et al. [121] reported the best sensitivities (2981 µA mM^{-1} cm^{-2} and 1610 µA mM^{-1} cm^{-2}, respectively) and wider linear ranges (0.05-1800 µM and 0.5-2300 µM, respectively) using non-enzymatic sensors in comparison to the enzymatic-based sensor proposed Yang et al. [119], where sensitivity of 1074.6 µA mM^{-1} cm^{-2} and linear range of 100-1400 µM were reported (Table 4). The non-enzymatic sensors have been highly recommended in comparison to the sensors fabricated with enzymes which have drawbacks such as high cost associated to enzyme isolation and purification, chemical and thermal deformation of enzyme, limiting the analytical applications; the non-enzymatic glucose sensors are based on direct oxidation of glucose at the electrode surface and the use of nanostructured metals (for example, Pt, Au, Ni or Cu) and metal oxides (for example, CuO or

NiO) have been explored due to their enhanced catalytic performances [116,121]. In addition, the synergic interaction between various nanostructures employed in the transduction mechanism of sensors also enhanced the analytical performance of such systems. For example, in the work of Zhang et al. [121], where the best sensitivity and wider linear range were verified, CuO nanoparticles attached on carbon spheres were immobilized in the glass carbon electrode without needing of pre-surface modification. CuO nanoparticles have high specific surface area, good electrochemical activity, and the possibility of promoting electron transfer reactions at lower over-potential, and carbon spheres have an increasing electrochemical potential due to their physical properties such as homogeneity of particle size, high specific surface area, and conductivities [121]. The sensor was also highly selective to glucose in the presence of commonly interfering species such as ascorbic acid, dopamine, uric acid and chloride ion, and successfully applied to monitor the concentration of glucose in human serum samples [121].

Hong et al. [122], Umar et al. [123], and Ahmad et al. [124] employed CuO nanoparticles, Fe_2O_3 nanoparticles, and ZnO nanotubes, respectively, in the transduction mechanism of sensors for the detection of cholesterol in serum samples. The lower LOD (0.0005 μM) was obtained in the work of Ahmad et al. [124], where a high performance cholesterol biosensor based on ZnO grown on Si/Ag electrodes was proposed. The response of the electrode was obtained in 2 seconds when different concentrations of cholesterol (from 1.0 to 13000 μM) were tested, as shown in Fig. 5i, which indicates the good catalytic property of the electrode [124]. In addition, the biosensor has a high stability for cholesterol detection retaining about 93% of its original response to cholesterol after 60 days of storage, which can be due to the electron transfer between ZnO nanotubes and the electrode, the higher specific surface area of ZnO nanotubes, and their ability to maintain the bioactivity of cholesterol oxidase, as explained by Ahmad et al. [124]. From the interference test, Ahmad et al. [124] verified that interfering species (glucose, ascorbic acid, L-cysteine, and uric acid) do not have any obvious effect on the biosensor response, which means that the fabricated biosensor is selective to cholesterol in the presence of various interfering species.

< Fig. 5 >

Umar et al. [123] proposed another electrochemical biosensor for cholesterol detection, where the cholesterol oxidase was immobilized on Fe_2O_3 particles as the sensing active area that

have a particular morphology similar to pine, as shown in Fig. 5iii. Such Fe_2O_3 micro-pine shaped hierarchical structures were synthesized by facile hydrothermal process in large quantity, and characterized with field emission scanning electron microscopy (FESEM). Fig. 5iii shows a FESEM image where the regular pine-shape morphology and very high density of Fe_2O_3 structures is observed, Fig. 5iv and Fig. 5v demonstrate the well-crystallinity and rhombohedral α-Fe_2O_3 crystal structures with a clear central trunk (~7±2 μm) where highly ordered parallel branches (~3±1 μm) are distributed in both sides of the trunk [109]. According to the authors, the high sensitivity (78.56 μA mM^{-1} cm^{-2}) is attributed to the large specific surface area of micro-pine shaped α-Fe_2O_3 hierarchical structures, enhanced cholesterol immobilization, and direct and fast electron communication between the active sites and the electrode [123].

4.2.3. Neurotransmitters

The acetylcholine is a neurotransmitter found in both peripheral nervous system and central nervous system in humans. Two sensors with different transduction principles are reported in Table 4; Rahman [125] fabricated an electrochemical sensor as a smart-chip based on enzymatic principle using the acetylcholine oxidase, which was immobilized in the electrode surface via peptide conjugation by the amide bond formation and Zhang et al. [126] constructed an electrochemical sensor based on MWCNT and ZnO nanoparticles for the detection of acetylcholine and choline, which is an enzymatic product obtained with the oxidation of acetylcholine. The monitoring the levels of acetylcholine and choline is very important to detect neurodegenerative diseases such as Alzheimer's and neuromuscular diseases, myasthenia gravis, and impaired cholinergic neurotransmission [127]. As shown in Table 4, the lower LOD and higher sensitivity were obtained with the smart-chip, which employed a simple and efficient approach to the immobilization of enzymes onto active sensitive surface, thus enhancing the sensor performance to a large group of biomolecules for wide range of biomedical applications in health care fields, as referred by Rahman [125]. In addition, the high sensitivity, small sample volume (70 μL), stability (1 month), and reproducibility (RSD of 1.8%) are other advantages of such smart-chip, which was fabricated by conventional photolithographic techniques. The main advantage of the electrochemical sensor proposed by Zhang et al. [126] is its long-term storage of 90 days, which can be due to the unique multi-layer structure employed (MWCNT-ZnO nanoparticles) providing a favourable microenvironment to maintain the bioactivity of choline oxidase and acetylcholinesterase. Zhang et al. [126] tested the sensor and a HPLC-MS/MS technique for

the detection of choline in human plasma samples, obtaining consistency in the analytical results since mean concentration of total choline of 2.67±0.21 mM in human plasma was obtained with the sensor and mean concentration of 2.54±0.12 mM in human plasma was obtained with the HPLC-MS/MS technique.

Epinephrine, also known as adrenaline, is one of the most important neurotransmitters in mammalian central nervous systems and it exists in the nervous tissue and body fluid in the form of large organic cations [128]. The determination of the levels of neurotransmitters such as epinephrine, dopamine and norepinephrine in biological fluids has an important role in clinical analysis since they are diagnostic marker molecules for a variety of metabolic and neurological disorders; for example, increased levels in urine have been used as a tumour marker for neuroblastoma and phaeochromocytoma, and stress level [129]. Akyilmaz et al. [128] constructed an interesting electrochemical biosensor based on fungal cells (from *Phanerochaete chrysosporium*) for the voltammetric determination of epinephrine (Table 4). The protocol for the sensing of epinephrine with the electrochemical biosensor begins with the lyophilisation of fungal biomass and its immobilization in gelatine on a platinum working electrode; then, the immobilized cells were used as a source of laccase to develop the epinephrine biosensor, where the current increases due to the redox activity resulting of the catalytic action of the laccase in the biosensor, which released an epinephrinequinone [128]. According to the authors, the proposed biosensor has many advantages such as low cost and simplicity of construction when compared to biosensors based on isolated enzymes. Other sensors for the detection of epinephrine as well as other neurotransmitters such as dopamine and norepinephrine in urine samples were reported in Table 4, using optical fibre with alginate/laccase matrix [129] or fluorescence [130]. The three neurotransmitters (dopamine, epinephrine, and norepinephrine) were tested in both sensors constructed by Silva et al. [129] and Silva et al. [130], with better LOD (0.00059-0.00088 ng mL^{-1}) for the fluorescence sensor in comparison to the optical fibre sensor based on alginate/laccase matrix, where LOD in the range of 4.1 and 5.5 ng mL^{-1} were obtained. Figures of merit such as LOD (0.00059-0.00088 ng mL^{-1}) and linear range (between 0.001 and 0.3 ng mL^{-1}) of the fluorescence optical fibre sensor were found as better than those obtained with a HPLC-electrochemical detector (HPLC-ED) (0.0045-0.0051 ng mL^{-1} for the LOD and linear range between 0.005 and 0.125 ng mL^{-1}) when dopamine, norepinephrine, and epinephrine were tested. Thus, Silva et al. [130] suggested that, in combination with the compact design, low-scale instrumentation, and effective cost of analysis, the fluorescence sensor is an interesting alternative to the existent methodologies for the determination of catecholamines in clinical samples.

4.3. Sensors and biosensors for bacteria, virus, and cancer cells

Table 6 includes the figures of merit of current sensors and biosensors (literature published between 2011 and 2015) for comparison of their analytical performance when used to the detection of: 1) pathogenic bacteria such as *Escherichia coli*; 2) virus such as hepatitis C virus core antigen and avian influenza virus; and 3) cancer cells.

< Table 6 >

4.3.1. Bacteria

Traditional detection methods for pathogenic bacteria such as *E. coli* and *Streptococcus pyogenes* involve cell culture and polymerase chain reaction (PCR) but limitations such as long processing times (24-48 hours of cultivation) or need for high cost equipment avoid the rapid and *in situ* monitoring of such pathogens, thus they are not practical for point-of-care diagnostics. For example, Yang et al. [137] and Safavieh et al. [138] proposed two electrochemical immunosensors based on microfluidics for the detection of *E. coli* in urine samples (Table 6). The human urine is effectively used to detect urinary tract infections commonly related with kidney, and it is known that *E. coli* is responsible for up to 80% of such infections, when concentrations are equal or higher than 10^5 CFU mL^{-1} [137]. Concerning microfluidics, it reduces the consumption of the costly reagents since the manipulation of small amount of liquid occurred in closed microscale channels, and thus, increasing the surface-to-volume ratio, it enhances the undergoing reactions, which are benefit for the development and application of miniaturized biosensors [138]. The biosensor fabricated by Yang et al. [137] is considered as a lab-on-a-chip that consists of two chambers (for concentration and sensing) connected in series and an integrated impedance detector. The role of such two chambers is the reduction of the nonspecific absorption of proteins such as albumin that can coexists with *E. coli* in urine, in order to improve the sensitivity. In the concentration chamber, the bacteria are separated from the urine sample due to the existence of microsized magnetic beads conjugated with *E. coli* antibody, and then, the immobilized *E. coli* is transferred to the sensing chamber for impedance measurements in order to estimate the *E. coli* concentration [137]. With such portable biosensor, a LOD of $3.4x10^4$ CFU mL^{-1} was obtained, which is lower that the threshold of urinary tract infections (10^5 CFU mL^{-1}). With the microfluidic biosensor proposed by Safavieh et al. [138], the LOD is better (24 CFU mL^{-1}) than that obtained by Yang et al. [137]. Safavieh et al. [138] performed a loop-mediated

isothermal amplification in the biosensor principle which can explained such result since it has a major role in the quantification of the bacteria. The isothermal amplification techniques can amplify the target nucleic acids in a constant temperature; for example, the loop-mediated isothermal amplification is accurate, fast, cost-effective with high sensitivity and specificity and it can amplify a few copies of DNA to 10^9 copies in less than an hour at 60-66 ºC [138]. Recently, Barreiros dos Santos et al. [139] reported the construction of a label-free immunosensor based on epoxysilane obtaining a LOD of 1 CFU mL^{-1}, which is lower than those reported previously. The biosensor was constructed onto ITO electrodes where the antibodies specific to *E. coli* were immobilized through epoxysilane covalent attachment. According to the authors, the low LOD obtained can be due to the robust surface functionalization used, which was based on the immobilization of biomolecules through silane monolayers using trifunctional silanes. The immunosensor also shows good reproducibility (RSD of 3%) and good stability (48 hours at 4ºC). Barreiros dos Santos et al. [139] also tested a traditional technique (ELISA) to the detection of *E. coli* in urine samples in order to compare the analytical results but a higher LOD (10^4 CFU mL^{-1}) was obtained. The same research group [151] has previously developed another immunosensor for the detection of *E. coli* but using gold substrates rather than ITO electrodes and referred that although the LOD was similar (2 CFU mL^{-1}), the ITO allows lower experimental time; the immobilization of antibodies onto ITO required 4 hours and in gold, such procedure is overnight required for functionalization.

4.3.2. Virus

The most sensitive and specific assays for diagnosis of infectious diseases are PCR or ELISA methodologies, but their high time consuming and cost are responsible for their no suitability for clinical screening or point-of-care molecular diagnosis; as alternative, immunosensors have the potential of simple, rapid, label-free, and low-cost molecular diagnosis [152].

Hepatitis C virus is the major causative agent of chronic viral hepatitis which can develop into cirrhosis and hepatocellular carcinoma and its early detection is crucial to prevent chronic infection [141]. Concerning avian influenza virus, it spread easily by air transmission, and infection through the respiratory system is quickly acquired, resulting in an urgent need to detect the influenza virus rapidly and reliably, since conventional virus detection methods such as diagnostic test kits, ELISA, and PCR are either poor in specificity, low in sensitivity, time-consuming, expensive, or require a laboratory and a trained technician [143]. Recent

immunosensors are reported in Table 6 for the detection of hepatitis C virus core antigen, avian influenza virus, and dengue virus, and nanomaterials were used in the construction of all biosensors reported. For example, Ma et al. [141] employed gold nanoparticles and zirconia nanoparticles (inorganic oxide with thermal stability and chemical inertness without toxicity) in a chitosan nanocomposite to construct an immunosensor based on glass carbon electrode for the detection of hepatitis C virus core antigen, and Singh et al. [143] employed SWCNT to the construction of an immunosensor for the detection of avian influenza virus. Both schematic representations of the construction of immunosensors and SEM images of nanomaterials immobilized in sensing platforms are shown in Fig. 6.

< Fig. 6 >

The label-free amperometric immunosensor reported by Ma et al. [141] displays a high sensitivity to the hepatitis C virus core antigen (13.7 μA ng^{-1} mL^{-2}) in the concentration range between 2 and 512 ng mL^{-1}, with a LOD of 0.17 ng mL^{-1}. The SEM image observed in Fig. 6ii(a) displays zirconia-chitosan nanoparticles with smooth morphology and diameter of about 100 nm, as indicated by solid arrow, and the SEM image with the morphologies of nanocomposites with immobilized hepatitis C virus core antibodies are shown in Fig. 6ii(b), where it is observed the higher sizes of the antibody immobilized nanocomposites in comparison with zirconia nanoparticles alone (Fig. 6ii(a)). In addition, the immunosensor has a high reproducibility (RSD of 4.2%) and high stability since the immunosensor could remain about 98.5% of the initial current response after a storage period of 30 days at 4°C, which can be due to the good biocompatibility of gold-zirconia-chitosan nanocomposites with the immobilized antibodies [141]. The immunosensor was tested in six serum samples and the results were compared to those of a traditional ELISA technique; the concentrations of hepatitis C virus core antigen found were similar, with relative deviations of the proposed immunosensor in the range of -4.35 to 6.65%, which suggests that the immunosensor can constitute a feasible alternative tool for the clinical detection of hepatitis C virus core antigen in human serum samples. For avian influenza virus detection, the SWCNT immunosensor reported by Singh et al. [143] displays a LOD of 1 PFU mL^{-1} in a linear concentration range between 1 and 10^4 PFU mL^{-1} with a detection time of 30 minutes. According to Fig. 6iv(a) and 6iv(b), uniformly distributed, aligned, and aggregation-free SWCNT are observed after the PDDA assembly, demonstrating that the majority of the individual SWCNT were reasonably aligned in parallel to the electrodes, there was reproducibility in the deposition

SWCNT due to the PDDA monolayer (Fig. 6iv(c)) as well as successful immobilization of avidin and biotinylated antibodies onto the SWCNT surfaces is observed since uniform morphology appeared in Fig. 6iv(d), and Fig. 6iv(e) shows a single influenza virus captured by the antibodies distributed on the SWCNT with several virus aggregates [143]. According to the authors, the immunosensors have the potential to become an important component of a point-of-care test kit for rapid and simple clinical diagnosis or a component of a portable lab-on-a-chip system.

Dengue is a self-limiting, non-specific illness characterized by fever, head-ache, myalgia, and constitutional symptoms, and its severe forms (hemorrhagic fever and shock syndrome) may lead to multi-system involvement and death, mostly amongst children [145]. As referred by Kumbhat et al. [153], the ELISA and immune-fluorescence assay are the gold standard serological tests for detection of specific antibody and viral antigen, respectively, but a timely diagnosis of dengue virus in vectors and human population employing real-time biosensors is the current demand to prevent the spread of the disease. Dias et al. [145] proposed an amperometric immunosensor for the detection of the non-structural protein 1 (NS1) of the dengue virus employing carboxylated MWCNT on screen-printed electrodes with antibodies covalently linked to such transducing platform (Table 6). In primary infections, NS1 levels range from 0.04 to 2 μg mL^{-1} in serum samples of patients in the disease acute phase (up to 7 days) [145]. According to the authors, the proposed imunosensor is a point-of-care approach and it allows the diagnosis of early clinical phase of dengue infection since successful analytical results were obtained when spiked blood serum samples were evaluated, obtaining excellent recovery values (98-116%), and also considering the excellent LOD (12 ng mL^{-1}) and sensitivity of 85.59 μA mM^{-1} cm^{-2} [145].

4.3.3. Cancer cells

Cytosensors have been recently developed for the detection of cancer cells due to the use of simple instrumentation and the reduction of cost and time required for analysis [147]. For example, Zheng et al. [146] developed a robust electrochemical cytosensing approach for both selective detection of leukaemia cells with LOD as low as ~40 cells and quantitative evaluation of DR4/DR5 expression status on leukaemia cell surfaces. DR4 and DR5 are death receptors and their quantification of their expression status on leukaemia cell surfaces is of vital importance to the development of diagnostic tools to guide death receptor-based leukaemia treatment [146]. The electrochemical cytosensor has: a) multifunctional hybrid nanoprobes used for the cytosensing and they are based on horseradish peroxidase and on Au

nanoparticle-decorated magnetic Fe_3O_4 beads for the specific recognition of DR4/DR5 on leukaemia cell surfaces, and b) nano-architectured electrode interfaces which specifically target the analytes and significantly facilitate the electron transfers between the analytes and electrodes [146]. According to the authors, the multifunctional hybrid nanoprobes and the nano-architectured electrode interface provide a signal amplification which is responsible to the low LOD. In addition, the electrochemical cytosensing platform is believed to be of great clinical value for the early diagnosis of human leukaemia and the evaluation of therapeutic effects on leukaemia patients after radiation therapy or drug treatment [146]. Liu et al. [147] constructed a label-free electrochemical cytosensor with surface-confined ferrocene as signal indicator and SWCNT for the selective detection of human cervical carcinoma cells. The cytosensor has a wide detection range from 10 to 10^6 cells mL^{-1} with a LOD as low as 10 cells mL^{-1}, which was reached even in the presence of a large amount of non-cancerous cells. The poly(ethylyimine) functionalized with ferrocene was used as the signal indicator and assembled on the electrode surface, thus providing an amplified signal to improve the detection sensitivity [147]. Recently, Mir et al. [148] constructed a nanobiosensor for the selective detection of human non-small-cell lung cancer cells using hydrazine and aptamers attached on gold nanoparticles for signal amplification. The sensitive and accurate early diagnosis of lung cancer is of extreme significance for its prevention, therapeutic efficacy assessment, and minimal residual disease determination since if the associated cancerous cells are recognized in the early stages of lung cancer, the treatment and five year survival rate increases to over 60% [148]. In the cytosensor proposed by Mir et al. [148], the lung cancer cells were recognized by the strong interaction between their glycoproteins on the cell membrane surface and specific aptamer, obtaining an excellent dynamic range from 15 to $1x10^6$ cells mL^{-1} with a LOD of 8 cells mL^{-1}. According to the authors, the proposed cytosensor provides a simple, low cost and biocompatible strategy for the sensitive analysis of non-small-cell lung cancer, which could be highly applicable for observing the differentiation between cancerous and non-cancerous cells and for improving the early clinical diagnosis and treatment of non-small-cell lung cancer and related diseases. Human liver hepatocellular carcinoma cells were also detected through cytosensor by the same research group but using two types of sensing principles. Sun et al. [149] proposed an electrochemical cytosensor based on aptamers covalently attached to a gold electrode to capture the cancerous cells using horseradish peroxidase and gold nanoparticles in the sensing probe and Sun et al. [150] also proposed an electrochemical cytosensor based on aptamers but attached on a glassy carbon electrode with gold nanoparticles. Both electrochemical cytosensors delivered a wide

detection range from 10^2 to 10^7 cells mL^{-1} but with different LOD which is of 30 cells mL^{-1} in the work of Sun et al. [149] and 15 cells mL^{-1} in the work of Sun et al. [150]. The advantage of such cytosensors is the regeneration of the sensing system through an electrochemical reductive desorption method performed to break gold-thiol bond and remove all components on the gold nanoparticles/glassy carbon electrode surface. For example, in the work of Sun et al. [150], for cancer cell detection, the regenerating cytosensor still retains 90% of its original sensitivity for two times.

5. Conclusions and future prospects

This review covered the recent applications of sensors and biosensors in clinical area for the detection and monitoring of different types of physiologically important analytes such as cancer and cardiac biomarkers, hormones, biomolecules, neurotransmitters, bacteria, virus, and cancer cells. The analytical performance of such sensors and biosensors has been compared and electrochemical biosensors continue to be the most proposed for clinical applications, mainly due to the possibility of miniaturization, easy handling, and portability, advantageous in the point-of-care testing, as well as due to their high sensitivity and selectivity, rapid response, and low cost. We would highlight that the detection of physiologically important analytes with fabricated sensors and biosensors is now highly performed in real biological samples (blood serum and plasma, urine, and saliva), as shown in Tables 2, 4, and 6 of the present paper, when compared to the literature and reported in our previous paper [3]. Concerning saliva, it has been considered as an alternative sample type in recent works reporting the construction of biosensors to the determination of various analytes such as IL-6, CRP, and cortisol. It is mainly due to the associated non-invasive collection, cost-effectiveness, pain-free, stress-free and no need of special laboratory equipment [154], and new directions in this field is the improvement of nanoscale and microscale biosensors for point-of-care testing of salivary analytes. Prototypes of sensors and biosensors in clinical field are mainly constructed for glucose and cholesterol detection and used as point-of-care technologies in the routine diagnosis. Further concerns in application of current sensors and biosensors in clinical analysis are the improvement of their stability for other physiologically important analytes such as cancer and cardiac biomarkers as well as hormones or neurotransmitters, which is associated to the problem of interferences with undesired molecules during their determination in real biological samples. This field is of extreme importance in the characterization of analytical performance of an analytical technique, as shown in the guideline developed by the Clinical and Laboratory Standards Institute [155],

where background information, guidance, and experimental procedures for investigating, identifying, and characterizing the effects of interfering substances in clinical chemistry test results were discussed. Concerning sensors and biosensors, the recent literature has reported the use of specific recognition elements (such as synthesized elements as aptamers) to determined physiologically important analytes and the chemical surface modification as solutions to reduce the non-specific binding of interfering molecules in order to improve the sensor selectivity [12]. In addition, the improvement of the analytical performance of sensors and biosensors through the enhancement of their figures of merit is an important accomplishment in analytical chemistry and it should be considered in the development of sensing systems in order to lead to prototype commercialization. It must also be highlight that the study of all figures of merit should be considered in the validation of sensors and biosensors, both in clinical area as well as in other applications such as food safety and environmental monitoring. In the literature concerning the development of clinical sensors and biosensors, a lack in the study of selectivity of sensors and biosensors was verified. When the selectivity is tested, it is possible to determine if the method is completely able to quantify accurately an analyte in the presence of interferences, and thus, such figure of merit is crucial to be studied [3].

Acknowledgments

This work was funded by Portuguese Science Foundation (FCT) through scholarships (ref. SFRH/BPD/95961/2013) under POCH funds, co-financed by the European Social Fund and Portuguese National Funds from MEC. This work was also funded by national funds through FCT/MEC (PIDDAC) under project IF/00407/2013/CP1162/CT0023. Thanks are also due, for the financial support to CESAM (UID/AMB/50017), to FCT/MEC through national funds, and the co-funding by the FEDER, within the PT2020 Partnership Agreement and Compete 2020.

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Figure Captions

Fig. 1 i) TEM image of graphene sheets; **ii)** TEM image of graphene sheets functionalized with quantum dots; **iii)** UV-Vis spectra of a) graphene sheets, b) quantum dots, and c) quantum dots-graphene sheets; and, **iv)** Square wave voltammograms response of electrodes with a) only quantum dots and b) electrodes with quantum dots in graphene sheets (Reprinted from Yang et al. [55], with permission from Elsevier).

Fig. 2 i) Fitting the 8-electrode immunoarray into the microfluidic device. The array is sandwiched between two layers of poly(methylmethacrylate) (PMMA) and one layer of poly(dimethyl)siloxane (PDMS) acting as microfluidic channel above the sensor electrodes. The red arrows indicate the flow of buffer; **ii)** Computer generated design of the gold array showing microwells around electrodes; and, **iii)** Completed array of 8 electrodes with individual microwells containing 1 µL aqueous droplets (Reproduced from Tang et al. [63] with permission of The Royal Society of Chemistry).

Fig. 3 i) Schematic representation of 3D-µPED. (A): wax-patterned paper sheet; (B): 3D-µPED [a) paper working zones and b) paper auxiliary zone]; (C): screen-printed electrodes [c) carbon working electrodes, d) Ag/AgCl reference electrode, e) carbon counter electrode, f) silver conductive channel and pad, and g) transparent polyethylene terephthalate substrate]; (D): after stacking, the paper working zones and the paper auxiliary zone will be aligned to the screen-printed working electrodes, counter and reference electrode (Reprinted from Wang et al. [78], with permission from Elsevier); **ii)** Calibration curves for CA 125 and CEA (eleven measurements for each point) (Reprinted from Wang et al. [78], with permission from Elsevier); **iii)** (A) Layered structure of the device; (B) Schematic diagram of the addressable electrode array detection system [a) and d) polyethylene terephthalate substrates with column electrodes (C*n*) and row electrodes (R*n*); b) reference electrode (RE), c) sensing sites with columns electrodes (W*n*) and counter electrode (CE)] (Reproduced from Ge et al. [72] with permission of The Royal Society of Chemistry); **iv)** Calibration curves for immunoassay of tumour markers (Reproduced from Ge et al. [72] with permission of The Royal Society of Chemistry).

Fig. 4 i) Schematic representation of IDE, where the binding of cortisol with antibody (Anti-C_{ab}) block the electron transport generated from medium to IDE and fabrication of immunosensor proposed by Pasha et al. [111] (Reproduced from Pasha et al. [111] by permission of The Electrochemical Society). CE: counter electrode; WE: working electrode; SAM: self-assembled monolayer; **ii)** Illustration of ZnO nanorods and ZnO nanoflakes along with immobilization of monoclonal anti-cortisol antibody to fabricate electrochemical cortisol immunosensor proposed by Vabbina et al. [112] (Reprinted from Vabbina et al. [112], with permission from Elsevier).

Fig. 5 i) Amperometric response to different cholesterol concentrations in 0.10 PBS at +0.38 V and **ii)** Calibration curve (Reprinted from Ahmad et al. [124], with permission from Elsevier). FESEM images of as-synthesized α-Fe_2O_3 micro-pine shaped hierarchical structures at: **iii)** low-magnification, **iv)** and **v)** high-resolution (Reprinted from Umar et al. [123], with permission from Elsevier).

Fig. 6 i) Fabrication and measurement procedures for the immunosensor proposed by Ma et al. [141] and **ii)** Sizes and morphologies of nanocomposites [(a) SEM images of ZrO_2 nanoparticles-chitosan and (b) antibodies-Au nanoparticles-ZrO_2 nanoparticles-chitosan] (With kind permission from Springer Science+Business Media: Microchimica Acta, Ma et al. [141]); **iii)** Schematic illustration of the SWCNT immunosensor for H1N1 virus detection proposed by Singh et al. [143] and **iv)** SEM images of (a) PDDA-SWCNT, (b) PDDA-SWCNT at higher magnification, (c) SWCNT deposited by sedimentation, (d) H1N1 antibody immobilized on PDDA-SWCNT, and (e) H1N1 antibody immobilized on PDDA-SWCNT after capturing a single influenza virus (Reproduced from Singh et al. [143] by permission of The Royal Society of Chemistry).

Table 1 Main figures of merit used in the validation of sensors and biosensors [3,17].

Figure of merit	Definition
Sensitivity	Slope of the analytical calibration curve. An analytical method is sensitive when a small change in analyte concentration causes a large change in the response.
Selectivity	Ratio of the slopes of the calibration lines of the analyte of interest and a particular interference. A method is selective when the response of the analyte can be differentiated from every other response.
LOD	Concentration or the quantity derived from the smallest signal that can be detected with acceptable degree of certainty for a given analytical procedure.
Repeatability	Closeness of the agreement between the results of successive measurements of the same measure and carried out in the same conditions related to operators, apparatus, laboratories and/or intervals of time analysis.
Reproducibility	Closeness of the agreement between the results of successive measurements of the same measure and carried out in different conditions related to operators, apparatus, laboratories and/or intervals of time analysis.
Signal-to-noise ratio	Ratio of the useful analytical signal to the background noise, which is identified as a measure of the statistical fluctuations in a blank signal.

Table 2 Analytical parameters of recent clinical sensors and biosensors for cancer and cardiac biomarkers.

Analyte detected (matrix)	Sensor type	Linear range	LOD[a]	Sensitivity (slope)	Additional information	References
CANCER BIOMARKERS						
PSA (serum samples)	Electrochemical immunosensor based on CdS quantum dots functionalized graphene sheets	0.005-10 ng mL^{-1}	0.003 ng mL^{-1}		RSD=7.9%; n=5	[55]
PSA (serum samples)	Electrochemical immunosensor based on graphene-Co hexacyanoferrate nanocomposites (GCE)	0.02-2 ng mL^{-1}	0.01 ng mL^{-1} (S/N=3)		RSD=6.7%; n=5	[56]
PSA (serum samples)	Electrochemical immunosensor based on antibody modified paramagnetic microparticles (screen-printed 8-sensor arrays based on 8 graphite working electrodes)	0-20 ng mL^{-1}	1.4 ng mL^{-1} ($3y_0$+s)	0.05 µA ng^{-1} mL^{-2}	RSD=8%; n=5 r^2=0.995	[57]
PSA (serum samples)	Electrochemical immunosensor based on MCWNT (SPCE)	0.005-4 ng mL^{-1}	0.005 ng mL^{-1}	0.077 µA pg^{-1} mL^{-2}	r^2=0.97	[58]
PSA (serum samples)	Electrochemical immunosensor based on Au nanoparticles-MWCNT-cross-linked starch nanocomposites (GCE)	0.01-0.5 ng mL^{-1}	0.007 ng mL^{-1} (S/N=3)	63.94 µA ng^{-1} mL^{-2}	RSD=4.1%; n=6 r^2=0.9936 Recovery=91.0-106.3%	[59]
PSA (serum samples)	Electrochemical immunosensor based on graphene and Ag hybridized mesoporous silica nanoparticles (GCE)	0.01-10 ng mL^{-1}	0.002 ng mL^{-1}	3.6807 µA ng^{-1} mL^{-2}	RSD=6.4%; n=5 r^2=0.9963 Recovery=97.0-102.5%	[60]
IL-8 (serum samples)	Electrochemical immunosensor based on MCWNT (SPCE)	8-1000 pg mL^{-1}	8 pg mL^{-1}			[58]
IL-8 (serum samples)	Electrochemical immunosensor based on superparamagnetic particles and Au nanoparticles	0-2000 pg mL^{-1}	0.001 pg mL^{-1}	0.0543 nA pg^{-1} mL^{-2}	r=0.9782	[61]
IL-6 (serum samples)	Electrochemical immunosensor	0.01-1.3 pg mL^{-1}	0.01 pg mL^{-1}		r=0.9914	[62]
IL-6 (serum samples)	Electrochemiluminescence immunosensor based on graphene oxide nanosheets/polyaniline nanowires/CdSe quantum dots nanocomposites (GCE)	0.5-10000 pg mL^{-1}	0.17 pg mL^{-1}		RSD=5.9%; n=3	[63]
IL-6 (serum, urine, and saliva samples)	Electrochemical magnetoimmunosensor based on carboxyl-functionalized magnetic microparticles (SPCE)	1.75-500 pg mL^{-1}	0.39 pg mL^{-1} ($3y_0$+s)	336 nA pg^{-1} mL^{-2}	RSD=6.9-8.1%; n=8 r=0.999 Recovery=98-103%	[64]
IL-6 (serum samples)	Electrochemical immunosensor based on Au-Pd-Ag nanoparticles (electrically heated carbon electrode)	0.1-100000 pg mL^{-1}	0.059 pg mL^{-1} (S/N=3)		RSD=6.3%	[65]
IL-6 (serum samples)	Electrochemical immunosensor based on Au nanoparticles-graphene-silica sol-gel (ITO)	1-40 pg mL^{-1}	0.3 pg mL^{-1} (S/N=3)	20.4 nA pg^{-1} mL^{-2}	RSD=7.2%; n=3 r=0.9919	[66]
MMP-2 (serum samples)	Electrochemical immunosensor based on Au nanoparticles and nitrogen-doped graphene composites (GCE)	0.5-50000 pg mL^{-1}	0.11 pg mL^{-1} (S/N=3)	4.20 µA ng^{-1} mL^{-2}	RSD=5.7% r=0.997	[67]
MMP-2 (serum samples)	Optical biosensor based on graphene oxide	10000-150000 pg mL^{-1}	2500 pg mL^{-1}		RSD=0.53-4.56% Recovery=93.6-98.8%	[68]

MMP-3	Electrochemical immunosensor based on graphene oxide/polypyrrole ionic liquid nanocomposite (ITO)	1-1000 pg mL^{-1}	1 pg mL^{-1}			[69]
α-fetoprotein (serum samples)	Electrochemical magnetoimmunosensor based on Fe_3O_4/ZrO_2/nano-Au composite (GCE)	0.05-10 ng mL^{-1}	0.01 ng mL^{-1} (S/N=3)	0.2906 μA ng^{-1} mL^{-2}	RSD=2.5% r=0.9905	[70]
α-fetoprotein (serum samples)	Photoelectrochemical immunosensor based on CdTe quantum dots-glucose oxidase bioconjugate (ITO)	0.0005-10000 ng mL^{-1}	0.00013 ng mL^{-1}	4.60 μA ng^{-1} mL^{-2}	RSD=0.8-1.5%; n=5 r=0.9997	[71]
α-fetoprotein (serum samples)	Electrochemical sensor based on MWCNT and Au nanoparticles	0.0005-100 ng mL^{-1}	0.00015 ng mL^{-1} (S/N=3)		RSD=2.7-5.1% r=0.9969	[72]
α-fetoprotein (serum samples)	Electrochemical immunosensor based on Au and Ag nanoparticles (SPCE)	0.005-5.0 ng mL^{-1}	0.0039 ng mL^{-1} (S/N=3)		CV=5.3-7.9%; n=5 r=0.9954	[73]
α-fetoprotein (serum samples)	Electrochemical immunosensor based on mesoporous silica nanoparticles with Fe_3O_4 nanoparticles (GCE)	0.01-25 ng mL^{-1}	0.004 ng mL^{-1} (S/N=3)		RSD=1.8-3.5%; n=5	[74]
CEA (serum samples)	Electrochemical immunosensor based on CNT/Au nanoclusters (GCE)	0.1-2.0 ng mL^{-1}	0.06 ng mL^{-1} (S/N=3)	4.69 μA ng^{-1} mL^{-2}	CV=5.6%; n=5 r=0.992	[75]
CEA (serum samples)	Electrochemical immunosensor based on chitosan-ferrocene and nano-TiO_2 film and Au nanoparticles-graphene nanohybrid	0.01-80 ng mL^{-1}	0.0034 ng mL^{-1} (S/N=3)	0.5289 μA ng^{-1} mL^{-2}	RSD=2.5%; n=5 r=0.9991	[76]
CEA (serum samples)	Electrochemical immunosensor based on Au nanoparticles-thionine-reduced graphene oxide nanocomposites film (GCE)	0.01-0.5 ng mL^{-1}	0.004 ng mL^{-1} (S/N=3)	4.2 μA ng^{-1} mL^{-2}	RSD=4.6%; n=5 r=0.9978 Recovery=92.5-113.0%	[77]
CEA (serum samples)	Electrochemical sensor based on MWCNT and Au nanoparticles	0.0005-100 ng mL^{-1}	0.00002 ng mL^{-1} (S/N=3)		RSD=1.9-5.8% r=0.9954	[72]
CEA (serum samples)	Electrochemical immunosensor based on MWCNT (μPAD)	0.05-50 ng mL^{-1}	0.01 ng mL^{-1}	2.587 μA ng^{-1} mL^{-2}	RSD=4.2%; n=10 r=0.9964	[78]
CEA (serum samples)	Electrochemical immunosensor based on Au and Ag nanoparticles (SPCE)	0.005-5.0 ng mL^{-1}	0.0035 ng mL^{-1} (S/N=3)		CV=4.7-5.9%; n=5 r=0.9975	[73]
CEA (serum samples)	Electrochemical immunosensor based on nafion membrane-SiO_2 nanoparticles (GCE)	0.000001-0.1 ng mL^{-1}	0.000001 ng mL^{-1} (S/N=3)	0.10016 μA fg^{-1} mL^{-2}	RSD=3.5%; n=3 r^2=0.9944	[79]
CEA (serum samples)	Electrochemical immunosensor based on nano-Au functionalized mesoporous carbon foam (GCE)	0.00005-1 ng mL^{-1}	0.000024 ng mL^{-1} (S/N=3)		RSD=7.6%; n=3 r=0.9997	[80]
CEA (serum samples)	Electrochemical immunosensor based on Au-Ag bimetallic nanoparticles (paper working electrode)	0.001-50 ng mL^{-1}	0.0003 ng mL^{-1} (S/N=3)	15.58 μA ng^{-1} mL^{-2}	RSD=5.1%; n=11 r=0.9965	[81]
CA 125 (serum samples)	Electrochemical immunosensor based on MWCNT (μPAD)	0.001-75.0 U mL^{-1}	0.2 U mL^{-1}	1.285 μA U^{-1} mL^{-2}	r=0.9968	[78]
CA 125 (serum samples)	Electrochemical sensor based on MWCNT and Au nanoparticles	0.0001-100 U mL^{-1}	0.000037 U mL^{-1} (S/N=3)		RSD=3.6-5.9% r=0.9965	[72]
CARDIAC BIOMARKERS						

CRP (serum samples)	Electrochemical immunosensor based on aldehyde-terminated nanocrystalline diamond	1-1000 nM	10 nM			[82]
CRP (serum samples)	Electrochemical immunosensor based Fe_3O_4-Au magnetic nanoparticles (SPCE)	1.2-200 ng mL^{-1}	0.5 ng mL^{-1}			[83]
CRP	Electrochemical immunosensor based on ZnO nanotubes	10-1000000 ng L^{-1}	0.0010 ng mL^{-1}	13.17 mV mg^{-1} L^{-1}	RSD<5%; n=6 r^2=0.99	[84]
CRP (serum samples)	Electrochemical magnetoimmunosensor based on carboxylic acid-modified magnetic beads (gold SPE)	0.07-1000 ng mL^{-1}	0.021 ng mL^{-1} (3s/m)	0.203 µA ng^{-1} mL^{-2}	RSD=6.5%; n=9 r=0.997	[85]
CRP (serum samples)	Electrochemical immunosensor (polycrystalline Au electrodes)	60000-6000000 ng L^{-1}	19000 ng mL^{-1} (S/N=3)			[86]
CRP (serum samples)	Piezoelectric immunosensor based on Fe_3O_4-SiO_2 magnetic nanoprobes	0.001-100 ng mL^{-1}	0.0003 ng mL^{-1}	30.1 Hz pg^{-1} mL^{-2}	CV=2.4%; n=5 r=0.9943 Recovery=86.7-107.0%	[87]
CRP (serum and saliva samples)	Electrochemical immunosensor based on SWCNT	0.1-100000 ng mL^{-1}	0.1 ng mL^{-1}		CV=1.0-9.4%; n=3 r^2=0.9924	[88]
CRP (serum samples)	Electrochemical magnetoimmunosensor based on carboxylic acid-modified magnetic beads (SPCE)	2-100 ng mL^{-1}	0.47 ng mL^{-1} (3s/m)	0.386 µA ng^{-1} mL^{-2}	RSD=6.3%; n=10 r=0.996	[89]
NT-proBNP	Electrochemical immunosensor with Au and MWCNT composite	0.02-100 ng mL^{-1}	0.006 ng mL^{-1} (S/N=3)	2.023 µA ng^{-1} mL^{-2}	RSD=3.4%; n=10 r=0.997	[90]
NT-proBNP (serum samples)	Electrochemical magnetoimmunosensor based on avidin functionalized magnetic nanoparticles	0.04-2.5 ng mL^{-1}	0.03 ng mL^{-1} (S/N=3)		r=0.9827	[91]
NT-proBNP (serum samples)	Electrochemical magnetoimmunosensor based on carboxylic acid-modified magnetic beads (SPE)	0.12-42.9 ng mL^{-1}	0.02 ng mL^{-1}		RSD=9.7%; n=9	[92]
NT-proBNP (serum samples)	Electrochemical magnetoimmunosensor based on carboxylic acid-modified magnetic beads (SPCE)	2-100 ng mL^{-1}	0.47 ng mL^{-1} (3s/m)		RSD=9.4%; n=10 Recovery=105-111%	[89]
cTnT (serum samples)	Piezoelectric immunosensor based on Au nanoparticles	0.003-0.5 ng mL^{-1}	0.0015 ng mL^{-1}	63.82 Hz ng^{-1} mL^{-2}	CV=7%; n=5 r=0.989	[93]
cTnT (serum samples)	Electrochemical immunosensor based on carboxylated CNT	0.1-10 ng mL^{-1}	0.033 ng mL^{-1}		CV=3.7%; n=10 r=0.9996	[94]
cTnT (serum samples)	Electrochemical immunosensor based on amine-functionalized CNT (SPE)	0.0025-0.5 ng mL^{-1}	0.0035 ng mL^{-1} (3s/m)	3.25 µA ng^{-1} mL^{-2}	RSD=3.8%; n=7 r=0.995	[95]
cTnT (serum samples)	Electrochemical immunosensor based on *o*-aminobenzoic film	0.05-5.0 ng mL^{-1}	0.016 ng mL^{-1} (3s/m)		RSD=6.2%; n=6 r=0.992	[96]
cTnI	Electrochemical immunosensor based on Au nanoparticles (ITO)	1-100 ng mL^{-1}				[97]
cTnI	Electrochemical immunosensor (SPE)	1-100 ng mL^{-1}		5.5 µA ng^{-1} cm^{-2}	r=0.987	[98]
cTnI	Electrochemical immunosensor based on Pt nanoparticles and graphene composite (GCE)	0.01-10 ng mL^{-1}	0.0042 ng mL^{-1} (S/N=3)		RSD=4-11%; n=3 r=0.991	[99]

cTnI (serum samples)	Optical immunosensor (fluorescence)	0-50 ng mL^{-1}	0.05 ng mL^{-1}	RSD=7.5%; n=6 r^2=0.9984	[100]
Myoglobin (plasma samples)	Electrochemical immunosensor based on Au nanoparticles	10-400 ng mL^{-1}	5 ng mL^{-1} (S/N=3)	RSD=15%; n=3 r=0.981	[101]
Myoglobin	Electrochemical immunosensor based on liquid crystal and Au nanoparticles (GCE)	9.96-72.8 ng mL^{-1}	6.29 ng mL^{-1} (3s/m)	RSD=4.3%; n=5 Recovery=99.7-109.1%	[102]
Myoglobin (serum samples)	Optical immunosensor (SPR)	100-1000 ng mL^{-1}	31.0 ng mL^{-1} (S/N=3)	CV=4.9% r^2=0.99	[103]
Myoglobin (serum samples)	Optical immunosensor (fluorescence)	0-340 ng mL^{-1}		RSD=2.3%; n=6 r^2=0.979	[100]

CA 125: cancer antigen 125; CEA: carcinoembryonic antigen; CNT: carbon nanotubes; CRP: C-reactive protein; cTnI: cardiac troponin I; cTnT: cardiac troponin T; CV: coefficient of variation; GCE: glassy carbon electrode; IL-6: interleukin-6; IL-8: interleukin-8; ITO: indium tin oxide electrode; LOD: limit of detection; MMP-2: matrix metalloproteinase-2; MMP-3: matrix mettaloproteinase-3; MWCNT: multi-walled carbon nanotubes; NT-proBNP: amino-terminal pro-brain natriuretic peptide; PSA: prostate specific antigen; RSD: residual standard deviation; SPCE: screen-printed carbon electrode; SPE: screen-printed electrode; SPR: Surface Plasmon Resonance; SWCNT: single-walled carbon nanotubes; µPAD: microfluidic paper-based analytical device. [a]Determination of LOD: "S/N=3": LOD is three times the signal-to-noise ratio; "3s/m": LOD is 3 times the standard deviation (s)/slope of calibration plot (m); "$3y_0+s$": LOD is 3 times the blank response (y_0) plus the standard deviation (s).

Table 3 Normal values of cancer and cardiac biomarkers in human blood.

Analyte	Normal value	Reference
CANCER BIOMARKERS		
PSA	4.0 ng mL^{-1}	[60]
IL-6	6 pg mL^{-1}	[64]
IL-8	13-20 pg mL^{-1}	[61]
MMP-2	367–770 ng/mL	[104]
MMP-3	15–72 ng/ml	[104]
α-fetoprotein	< 20 ng mL^{-1}	[70]
CEA	5 ng mL^{-1}	[78]
CA-125	35 U mL^{-1}	[78]
CARDIAC BIOMARKERS		
CRP	3 mg L^{-1}	[82]
NT-proBNP	1 ng mL^{-1}	[89]
cTnT	0.3 ng mL^{-1}	[93]
cTnI	0.01-0.1 ng mL^{-1}	[99]
Myoglobin	50-100 ng mL^{-1}	[100]

Table 4 Analytical parameters of recent clinical sensors and biosensors for hormones, biomolecules, and neurotransmitters.

Analyte detected (matrix)	Sensor type	Linear range	LOD[a]	Sensitivity (slope)	Additional information	References
HORMONES						
Cortisol (saliva samples)	Electrochemical immunosensor (interdigitated microelectrodes)	10-100000 pg mL^{-1}	10 pg mL^{-1}	6 µA pg^{-1} mL^{-2}	r=0.99	[111]
Cortisol (saliva samples)	Electrochemical immunosensor based on ZnO 1D nanorods and 2D nanoflakes	0.001-100 nM	0.001 nM	7.74-11.86 µA M^{-1}		[112]
Estradiol (serum samples)	Electrochemical sensor based on MWCNT and Au nanoparticles (graphite electrodes)	70-42000 nM	10 nM (S/N=3)	7.57 µA $µM^{-1}$	RSD=3.9%; n=8 r=0.994 Recovery=98.5-103.5%	[113]
Estradiol (serum and urine samples)	Electrochemical immunosensor based on graphed *p*-aminobenzoic acid (SPCE)	1.0-250 pg mL^{-1}	0.77 pg mL^{-1}		RSD=5.9%; n=8 r=0.990 Recovery=95-102%	[114]
Estradiol (urine samples)	Electrochemical sensor based on nanoporous polymeric film (GCE)	100-10000 nM	50 nM (S/N=3)	0.1866 µA $µM^{-1}$	RSD=4.3%; n=5 r=0.996 Recovery=98.7-103.0%	[115]
BIOMOLECULES						
Glucose (serum samples)	Electrochemical immunosensor based on Ni nanoparticles and MWCNT	1-1000 µM	0.5 µM (S/N=3)	1438 µA nM^{-1} cm^{-2}	r=0.995 Recovery=90.7-106.2%	[116]
Glucose (serum samples)	Electrochemical sensor based on graphene oxide and NiO nanofibers (GCE)		0.77 µM (S/N=3)	1100 µA mM^{-1} cm^{-2}		[117]
Glucose (serum	Electrochemical sensor based on CuO-SWCNT nanocomposites	0.05-1800 µM	0.050 µM	1610 µA mM^{-1} cm^{-2}		[118]

samples)						
Glucose	Electrochemical enzymatic sensor based on carboxyl-modified graphene oxide and magnetic nanoparticles	100-1400 µM		1074.6 µA mM^{-1} cm^{-2}	RSD=5.8% r^2=0.988	[119]
Glucose (serum samples)	Electrochemical sensor	2-15000 µM	0.5 µM (S/N=3)			[120]
Glucose	Electrochemical sensor based on CuO nanoparticles attached in carbon spheres (GCE)	0.5-2300 µM	0.1 µM (S/N=3)	2981 µA mM^{-1} cm^{-2}	RSD=1.6%; n=6 r=0.999 Recovery=97.1-98.5%	[121]
Cholesterol (serum samples)	Optical enzymatic sensor based on CuO nanoparticles	0.625-12.5 µM	0.17 µM		RSD=5.9%; n=6 r=0.9994 Recovery=96.5-104.9%	[122]
Cholesterol (serum samples)	Electrochemical enzymatic biosensor based on Fe_2O_3 nanoparticles	100-8.0 mM	18 µM (S/N=3)	78.56 µA mM^{-1} cm^{-2}	r^2=0.9951 Recovery=95.0-98.0%	[123]
Cholesterol (serum samples)	Electrochemical enzymatic biosensor based on ZnO nanotubes	1.0-13000 µM	0.0005 µM (S/N=3)	79.40 µA mM^{-1} cm^{-2}	r=0.9997	[124]
NEUROTRANSMITTERS						
Acetylcholine	Electrochemical enzymatic biosensor	0.001-1000 µM	0.00014 µM (S/N=3)	7354 µA mM^{-1} cm^{-2}	RSD=1.8% r=0.9951	[125]
Acetylcholine	Electrochemical sensor based on MWCNT and ZnO nanoparticles	1.0-1000 µM	0.3 µM	180 µA mM^{-1} cm^{-2}		[126]
Choline (plasma samples)	Electrochemical sensor based on MWCNT and ZnO nanoparticles	1.0-800 µM	0.3 µM	178 µA mM^{-1} cm^{-2}	Recovery=95-106%	[126]
Choline (serum samples)	Electrochemical enzymatic biosensor based on MWCNT and ZnO nanoparticles (GCE)	0.005-200 µM	0.01 µM	0.1929 mA μM^{-1}	CV=2.97%; n=6 r^2=0.9799	[127]
Epinephrine	Electrochemical biosensor based on fungal cells	5-100 µM	1.04 µM (3s/m)		CV=3.83%; n=5 r^2=0.9948	[128]
Epinephrine (urine samples)	Optical fibre sensor based on alginate/laccase matrix	8.2-205 ng L^{-1}	5.5 ng L^{-1} ($3y_0$+s)		r^2=0.9996	[129]
Epinephrine (urine samples)	Optical fibre sensor based on fluorescence	0.001-0.3 ng mL^{-1}	0.00088 ng mL^{-1} ($3y_0$+s)		r^2=0.9998 Recovery=96-101%	[130]
Norepinephrine (urine samples)	Optical fibre sensor based on alginate/laccase matrix	8.9-222 ng L^{-1}	5.3 ng L^{-1} ($3y_0$+s)		r^2=0.9997	[129]
Norepinephrine (urine samples)	Optical fibre sensor based on fluorescence	0.001-0.3 ng mL^{-1}	0.00065 ng mL^{-1} ($3y_0$+s)		r^2=0.9998 Recovery=96-101%	[130]
Dopamine (urine	Optical fibre sensor based on alginate/laccase matrix	9.8-245 ng L^{-1}	4.1 ng L^{-1}		r^2=0.9996	[129]

samples)			($3y_0$+s)		
Dopamine (urine samples)	Optical fibre sensor based on fluorescence	0.001-0.3 ng mL^{-1}	0.00059 ng mL^{-1} ($3y_0$+s)	r^2=0.9999 Recovery=96-101%	[130]

CV: coefficient of variation; GCE: glassy carbon electrode; LOD: limit of detection; MWCNT: multi-walled carbon nanotubes; RSD: residual standard deviation; SPCE: screen-printed carbon electrode; SWCNT: single-walled carbon nanotubes. [a]Determination of LOD: "S/N=3": LOD is three times the signal-to-noise ratio; "3s/m": LOD is 3 times the standard deviation (s)/slope of calibration plot (m); "$3y_0$+s": LOD is 3 times the blank response (y_0) plus the standard deviation (s).

Table 5 Normal values of hormones, biomolecules, and neurotransmitters in human blood.

Analyte	Normal value	Reference
HORMONES		
Cortisol	100-500 nM	[131]
Estradiol	200-600 pg mL^{-1}	[113]
BIOMOLECULES		
Glucose	4-8 mM	[132]
Cholesterol	5.2-6.2 mM	[133]
NEUROTRANSMITTERS		
Acetylcholine	1115 -1413 pg mL^{-1}	[134]
Epinephrine	0.02 - 0.46 nM	[135]
Norepinephrine	0.45 - 2.49 nM	[135]
Dopamine	0.01 - 0.48 nM	[135]

Table 6 Analytical parameters of recent clinical sensors and biosensors for bacteria, virus, and cancer cells.

Analyte detected (matrix)	Sensor type	Linear range	LOD[a]	Sensitivity (slope)	Additional information	References
BACTERIA						
Escherichia coli (urine samples)	Electrochemical microfluidic immunosensor based on magnetic beads	$6.4x10^4$-$6.4x10^8$ CFU mL^{-1}	$3.4x10^4$ CFU mL^{-1} ($3y_0+s$)			[137]
E. coli (urine samples)	Electrochemical microfluidic immunosensor	4.8 -$4.8x10^8$ CFU mL^{-1}	24 CFU mL^{-1}			[138]
E. coli (serum samples)	Immunosensor based on epoxysilane (ITO)	10-10^6 CFU mL^{-1}	1 CFU mL^{-1}		RSD=3%; n=3 r^2=0.999	[139]
Streptococcus pyogens (saliva samples)	Electrochemical immunosensor based on polytyramine (SPE)	10^4-10^7 cells mL^{-1}				[140]
VIRUS						
Hepatitis C virus core antigen	Electrochemical immunosensor based on Au nanoparticles- ZrO_2 nanoparticles-chitosan nanocomposite (GCE)	2-512 ng mL^{-1}	0.17 ng mL^{-1} (S/N=3)	13.68 µA ng^{-1} mL^{-2}	RSD=4.2%; n=5 r=0.9968	[141]
Hepatitis C virus core antigen (serum samples)	Electrochemical immunosensor based on mesoporous carbon-methylene blue nanocomposites	0.00025-0.3 ng mL^{-1}	0.00001 ng mL^{-1} (S/N=3)	355 µA pg^{-1} mL^{-2}	RSD=5.2%; n=5 r=0.997 Recovery=94.8-105.6%	[142]
Avian influenza virus H1N1	Electrochemical immunosensor based on SWCNT	1-10^4 PFU mL^{-1}	1 PFU mL^{-1}		r^2=0.99	[143]
Avian influenza virus H9N2	Electrochemical immunosensor based on magnetic beads	50-2000 ng mL^{-1}	1 ng mL^{-1} (S/N=3)		RSD=4.8%; n=3 r=0.997	[144]
Dengue virus NS1 protein (serum samples)	Electrochemical immunosensor based on carboxylated MWCNT (SPE)	40 ng mL^{-1}-2 µg mL^{-1}	12 ng mL^{-1}	85.59 µA mM^{-1} cm^{-2}	CV=3.4%; n=6 r=0.996 Recovery=98-116%	[145]
CANCER CELLS						
Leukemia cells	Electrochemical cytosensor based on HRP and gold nanoparticles-decorated magnetic Fe_3O_4 beads	10^3-10^6 cells mL^{-1}	660 cells mL^{-1}		r=0.995	[146]
Human cervical carcinoma cells	Electrochemical cytosensor based on ferrocene and SWCNT	10-10^6 cells mL^{-1}	10 cells mL^{-1}		RSD=2.8%; n=5	[147]
Human non-small-cell lung cancer cells	Electrochemical cytosensor based on hydrazine and aptamers attached on gold nanoparticles	15-10^6 cells mL^{-1}	8 cells mL^{-1}		RSD=2.8%; n=10 r=0.9987	[148]
Human liver cancer cells	Electrochemical cytosensor based on aptamers, horseradish peroxidase and gold nanoparticles	10^2-10^7 cells mL^{-1}	30 cells mL^{-1}		RSD=3.7%; n=3 r=0.9952	[149]
Human liver cancer cells	Electrochemical cytosensor based on aptamers and gold nanoparticles	10^2-10^7 cells mL^{-1}	15 cells mL^{-1}		RSD=5.6%; n=3 r=0.9917	[150]

CFU: colony-forming unit; CV: coefficient of variation; GCE: glassy carbon electrode; HRP: horseradish peroxidase; ITO: indium tin oxide electrode; LOD: limit of detection; MWCNT: multi-walled carbon nanotubes; PFU: plaque-forming unit; RSD: residual standard deviation; SPE: screen-printed electrode; SWCNT: single-walled carbon nanotubes. [a]Determination of LOD: "S/N=3": LOD is three times the signal-to-noise ratio; "$3y_0+s$": LOD is 3 times the blank response (y_0) plus the standard deviation (s).