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Review

Advanced Materials for Immunosensing of Pharmaceutical and Drug Compounds

Jafar Soleymani*, Zahra Golsanamlou

Pharmaceutical Analysis Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

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Abstract

Real-time and accurate levels of pharmaceuticals undertake critical effects in the therapy process. Thus, reliable detection of pharmaceuticals is important for regulating the proper concentration of them to enhance the effectiveness and to decrease possible side effects. However, the development of new reliable sensory systems is the main prerequisite for the mentioned aims. Immunosensors can be regarded as an effective tool due to their sensitivity and unique specificity originating from the intrinsic nature of the antigen-antibody interaction. This review reports material tendencies in the development of immunosensors for pharmaceuticals (veterinary and human) which have been reported in the last few years. Carbon-based (graphene, graphene oxide, carbon nanotubes, etc.), gold, and magnetic materials are the main materials for the fabrication of pharmaceutical immunosensors. Also, this review reports benefits and limitations on the reported immunosensor and mechanism and analytical performance of the immunoplatforms to address future researches.

Introduction

Monitoring of the pharmaceutical levels in biological samples is crucial within the therapy process to guarantee efficient and safe therapy. Also, pharmaceutical residues should be monitored in the environment because of potential impact on the available environmental microorganisms like useful bacteria. In addition, veterinary pharmaceutical products which are employed to control various diseases on large scale must be monitored timely. It is obvious that any uncontrolled or unauthorized consumption of them may deposit residues in animal tissues and consequently may be unsafe for users. Thus, the concentration of both veterinary and human pharmaceuticals has to be determined in various matrixes of biological fluids, environmental samples, and food products. Therefore, the fabrication of reliable analytical methods with simple and fast detection procedures is the main request of the veterinarians and physicians to timely determined pharmaceuticals after medical or veterinary use.

To date, wide vast types of analytical techniques such as high performance liquid-chromatography (HPLC),[1] gas chromatography (GC),[2] electrochemical,[3–6] luminescence,[7–11] etc. have been used for the determination of pharmaceuticals in different biological and environmental media. Several detection strategies were used with the techniques to amplify the analytical signal or enhance the specificity of the probe towards the given analyte. Immunosensor is one of the most important strategies by which specific antibodies are utilized to detect analytes.

Basically, immunosensors are affinity-based sensing platforms with high potential for the fabrication of bioanalytical devices. A typical immunosensor is comprised of the bio-detection element, signal transducer, and the signal monitoring systems in which the bio-detection section (antibody-functionalized nanomaterial or substrates) perceive the analyte-related analytical signals, signal transducers translate recorded input to the measurable signals, and the monitoring systems display the produced signals.[12,13] Several signal transducers have been utilized in the fabrication of an immunosensor such as spectroscopic, electrochemical, and piezoelectric.[14,15]

Among various types of fabricated immunosensors, electrochemical-based immunosensors have been widely utilized because of their simplicity, sensitivity, and specificity in the determination of analytes.[16] Electrochemical immunosensors have high potential to be a point of care (POC) device for *in situ* detection and diagnostic aims.

This review summarizes the reported analytical methods that are fabricated to characterize the immunosensors for the detection of pharmaceuticals in various media, *i.e.*, biological and environmental with sufficient numbers of

***Corresponding Author:** Jafar Soleymani, Email: soleymanij@tbzmed.ac.ir

schemes. Also, this review aimed to provide a wide review of the advantages and disadvantages of applied advanced materials for the fabrication of various immunosensors to detect pharmaceuticals. In addition, the analytical performances of the reported methods are explained. Finally, future outlooks of pharmaceutical immunosensors to specific and reliable detection of pharmaceuticals were also explored.

Materials for immunosensing

Carbon-based materials

Carbon-based materials have gained great attention for biomedical applications. These materials present some exciting and promising features for immunosensing applications including biocompatibility, stability, high electron conductivity, and quantum yields.[17]

Özkütük et al[18] utilized graphene oxide to develop an immunosensor for the detection of clenbuterol in milk samples. They bound photosensitive amino acid to the GO particles and applied for modification of carbon paste electrode. The developed potentiometric electrode can detect clenbuterol as low as 0.87 pmol.L^{-1} where the sensor signal is linear in the range of 10 mmol.L^{-1} - 1.0 pmol.L^{-1}. Tablib et al[19] modified multi-walled carbon nanotube (MWCNTs) with poly(3,4-ethylenedioxythiophene) (PEDOT) molecules to provide more sensitive approaches. PEDOT is a conducting polymer that can enhance the electrochemical signal by facilitating and or increasing the electron transferring process through enhancing the surface area for anti-clenbuterol antibody (Ab)–CLB binding. MWCNTs/PEDOT was used to modify the screen printed electrode and then Ab was immobilized on the surface of the modified elected. This immunosensor provides a wide dynamic range of 0–250 ng.mL^{-1} with a LOD of 4.66 ng.mL^{-1}. Sensitivity of the clenbuterol immune-detection was more enhanced by modification of the surface of graphene with carboxyl-IgG groups. These materials were used to modify the surface of a gold substrate and then were applied for surface Plasmon resonance and impedance spectroscopy-based sensing. The developed assay detects clenbuterol from 0.01 ng.mL^{-1} to 10 ng.mL^{-1} with a lower detection limit of about 6.57 pg.mL^{-1}. As the authors declared this sensor could be applied for immunosensing of food additives.[20]

Graphene particles were also functionalized with AuNPs and metal-organic framework (MOFs) for immunosensing of monensin in milk samples. The AuNPs were anchored to the surface of Zn/Ni-ZIF-8-800@graphene using an electrochemical approach. Then, antibodies were attached on the AuNPs by physical adsorbed (Figure 1). Upon the optimized conditions, the linear range and LOD were 0.25–100 ng.mL^{-1} and 0.11 ng.mL^{-1}, respectively. The as-prepared immunosensor is able to detect monensin and may be applied as a promising sensor for recognition of other pharmaceutical drugs in food staffs.[21]

Graphene oxide (GO) is another allotropy of graphene riched with various active functional groups including carboxyl, hydroxyl, and epoxide which is highly dispersible in aqueous systems.[22,23] Tertis et al[24] used activated GO for sensitive sensing of acetaminophen in pharmaceutical formulations. The developed electrochemical sensor was constructed by layer-by-layer modification of a SPE with activated GO and anti-acetaminophen antibody (antiAPAP Ab) (Figure 2). The developed sensor can detect acetaminophen at 0.17 µmol.L^{-1} levels and could be used in serum environments. Chen et al[25] modified GO particles with palladium nanoparticles (Pd NPs) to develop an electrochemiluminescence (ECL) immunosensor for diclofenac detection. As shown in Figure 3, the Ab-Pd NPs/PEI-GO-QDs nanocomposite was used for the modification of the glassy carbon electrode (GCE). In the presence of the diclofenac, the current of the platform is drastically decreased which is a result of the blocking effect of diclofenac on the electron transfer ways between the

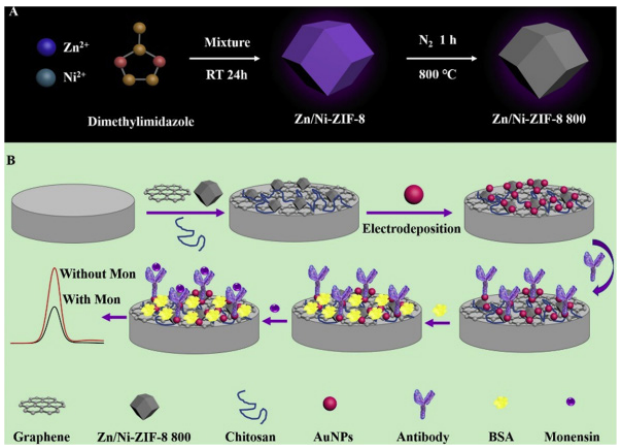

Figure 1. (A) Zn/Ni-8-800 synthesis steps and (B) monensin immunosensor fabrication steps. (Reprinted from [21] with permission license of 5045920970321)

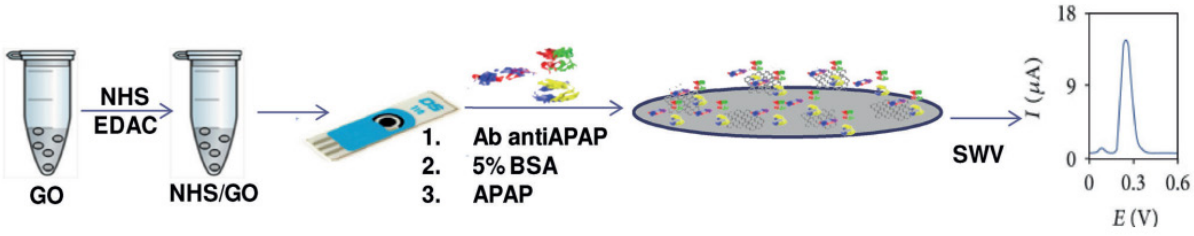

Figure 2. Schematic illustration of acetaminophen (APAP) immunosensor. (Reprinted from [24] with permission license of 5045921139387)

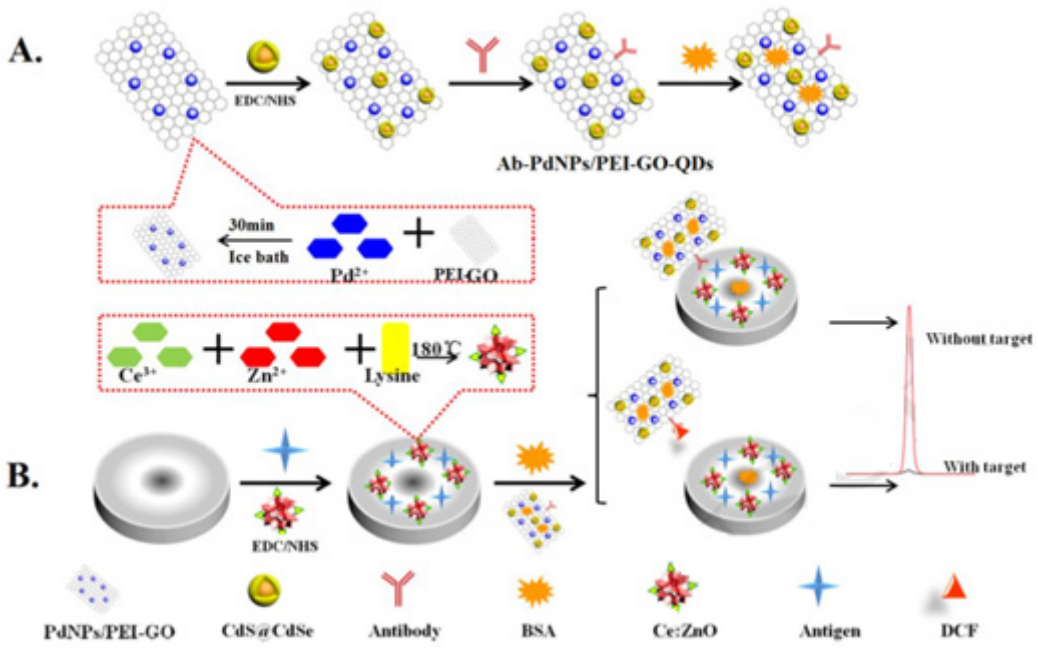

Figure 3. (A) Synthesis Process of Pd NPs/PEI-GO/QDs-functionalized antibody, and (B) Preparation steps of the GCE/ Pd NPs/PEI-GO/QDs-Ce:ZnO/Ag-OVA. (Reprinted from [25] with permission license of 5045921240388)

electrolyte and the modified electrode. It is worth noting that the presence of Ce and Zn ions increases the electron transfer rate. The proposed ECL-based immunoassay had shown high sensitivity for the detection diclofenac in the ranger of 0.001 to 1000 ng.mL^{-1} with LOD of about 0.3 pg.mL^{-1}. The sensitivity of the reported approach is as results of high technical sensitivity of ECL-based sensors, high surface area of GO and Pd NPs, and the effect of Ce and Zn ions on the final total current.

In another work, GO particles were firstly reduced and then modified with Cu/Cu_2O nanocrystals to produce Cu/Cu_2O/rGO nanocomposite for the determination of ractopamine in urine samples. To enhance the specificity of the nanoparticles towards ractopamine, its antibody was anchored on the surfaces of the nanocomposite and then used for developing an electrochemical immunosensor. In comparison with pristine rGO and Cu_2O, the antibody can easily attach onto the surface of Cu/Cu_2O@rGO nanoparticles due to its surface high-rich functional groups, which consequently resulted in enhanced sensitivity of ractopamine determination. This biosensor exhibited a low LOD of 7.5 pg.mL^{-1} with a dynamic range of 0.1 to 10 ng.mL^{-1}.[26]

He et al[27] utilized poly(L-lysine) to functionalize MWCNTs and then develop an enantioselective biosensor for the determination of ofloxacin. As presented in Figure 4, GCE electrodes were modified by MWCNTs at the first stage and then with Ag NPs, Ab_1 and Au nanoflowers (AuNFs). Multi-HRP–AuNFs–Ab_2 composites were used as the labeling agent to develop the enantioselective immunosensor. The reported immunosensor exhibited a selective response for ofloxacin, where its linear dynamic range and LOD are 0.26 - 25.6 ng.mL^{-1} and 0.15 ng.mL^{-1} for S- ofloxacin and 0.37 - 12.8 ng.mL^{-1} and 0.30 ng.mL^{-1} for R-ofloxacin, respectively.

Quantum dots (QDs) are a type of luminophores that are utilized in ECL-based methods for various bio-sensing aims. QDs showed tunable emission spectral features which have made them a favorable candidate for designing of ECL platform. QD-based ECL platforms have been applied widely for the ultra-sensitive immunosensing of different analytes. Li et al[28] polyamidoamine-coated carbon dots (CDs) as an ECL luminophore for detection of ketamine. Polyamidoamine (PAMAM) capped CDs

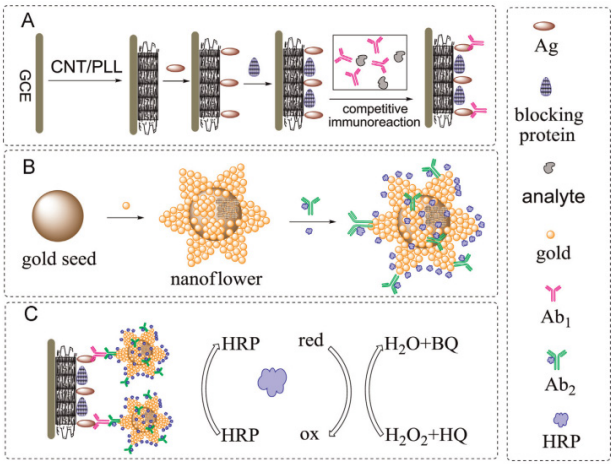

Figure 4. (A) Schematic illustration of ofloxacin electrochemical immunosensor using MWCNTs/PLL-multi-HRP–AuNFs–Ab2 bioconjugates, (B) syhtesis if antibody-funcationalized gold nanoflower, (C) mechanism of electrochemical sensing (Hydroquinone, HQ). (Reprinted from [27] with permission license of 5045930004926)

were attached to the surface of GCE through AuNPs and then ketamine antibody was anchored. The constructed immunosensor response is linear from 0.2 to 200 ng.mL^{-1} with a LOD of 67 pg.mL^{-1}. CDs-based ECL immunosensor can successfully detect ketamine in human blood plasma samples which paves new ways in label-free detection of drugs.

As a conclusion, the combination of ECL technique and carbon-based material presents various advantageous analytical features of high sensitivity and biocompatibility in biological media.

Nobel metals for immunosensing of pharmaceuticals

AuNPs

AuNPs have been utilized in the fabrication of different types of the sensory systems because of their superior electrical conductivity and biocompatibility. In addition, AuNPs possess a large surface-area-to-volume ratio, easy synthesis and modification, and favorable biocompatibility. AuNPs-based SERS approaches have been utilized for immunosensing of chloramphenicol after magnetic separation.[29] The use of AuNPs in immunosensing can enhance the surface availability to modify with different molecules, allowing to regulate the specificity of the developed method. In this work, AuNPs were functionalized with 4,4′-dipyridyl to provide a Raman reporter agent. To separate chloramphenicol from matrix, magnetic particles were functionalized with chloramphenicol antibodies. After magnetic separation, SERS reported nanocomposite were added to the analyte solution to recorded SERS signal (Figure 5). The developed SERS-based immunosensor is able to detect chloramphenicol as low as 1.0 pg.mL^{-1} over a wide dynamic range from 1 to 1×10^4 pg.mL^{-1}.[29] Li et al[30] reported a fiber-optic surface Plasmon resonance (FO-SPR) immunosensor for the determination of infliximab in inflammatory bowel disease (IBD) patients' serum samples. In order to obtain clinically applicable sensitivity of infliximab, the SPR signal was amplified using AuNPs functionalized with detection antibodies (Figure 6). LOD of the developed method is 2.2 ng.mL^{-1} which can cover biological concentration of infliximab. Obtained results confirmed that FO-SPR-based nanoprobe could be used as fast and real-time monitoring of infliximab, demonstrating its potential for designing a POC diagnostic device for determining other pharmaceutical drugs in serum samples. AuNPs can also be used in the developments of ECL-based sensors to intensify the emission of luminol. Pan et al[31] used the SPR technique for enrofloxacin detection in animal-derived foods. In this work, the gold nanofilms were functionalized with enrofloxacin-ovalbumin conjugate and enrofloxacin antibodies, where in the presence of the analyte, SPR signal is produced. The reported SPR immunosensor provided a favorable dynamic range sensitivity of 3.8 ng.mL^{-1} and low LOD of 1.2 ng.mL^{-1}.

Ya et al[32] improved indium–tin oxide (ITO) electrodes with glass gold nanoparticles to determine morphine by an immunosensing approach. Upon functionalization with AuNPs, a significant increase on the ECL signal has occurred where it is drastically diminished in the presence of morphine molecules. The dynamic range of this biosensor is 2–200 ng.mL with a LOD of 0.82 ng.mL^{-1}. Although favorable figure-of-merit, the application of the developed method was not checked in real samples where only spiked urine samples were determined by this immunosensor which cannot qualify it for successful determination of morphine in real samples. AuNPs are

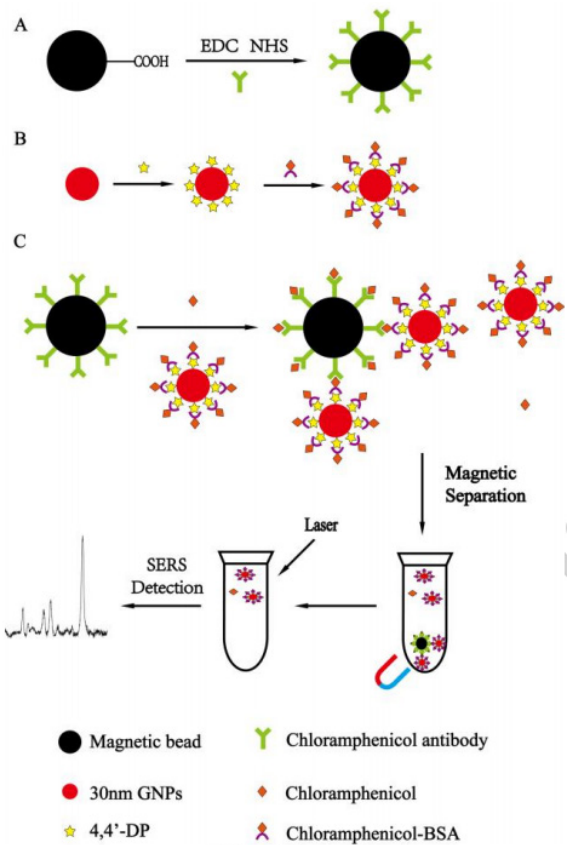

Figure 5. SERS-based immunosensor for detection of chloramphenicol. (Reprinted from[30] with permission license of 5045930299910)

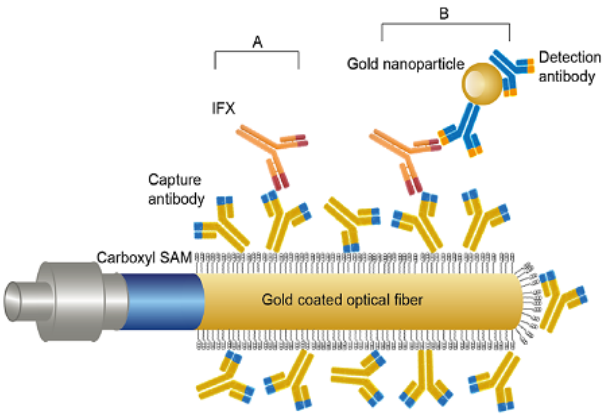

Figure 6. Plasmon resonance (FO-SPR) immunosensor for determination of infliximab. (Reprinted from[31] with permission license of 5045930477112)

one of the most frequently applied nanomaterials in developing of electrochemical approach by modification of the electrodes' surface especially electrochemical impedance spectroscopy (EIS)-based immunosensors. Lin et al[33] reported the application of AuNPs to modify screen-printed carbon electrode (SPCE) surface for immunosensing of salbutamol. As demonstrated in Figure 7, upon attachment of salbutamol antibody on the surface of the electrode, a specific probe was produced which the EIS-signal is linear from 0.1 pg.mL^{-1} to 1 µg.mL^{-1} with a LOD of 4 fg.mL^{-1}. The reported AuNPs/SPCE-based probe could detect salbutamol in diluted (1000-times) human serum samples and meets minimum requirements to be used in the determination of salbutamol in the real sample. This immunosensor showed potential for the designing of disposable label-free sensors for the quantifications of other molecules.

Qu et al[34] fabricated a capillary immunochromatographic assay (CICA) for the detection of clenbuterol in pig urine and other foodstuffs. They deposited antibody functionalized AuNPs on the inner surface of glass capillaries (d=1.1 mm). Clenbuterol- bovine serum albumin (BSA) and goat anti-mouse IgG are anchored on the inner surface of the test and control zones, respectively. Upon introduction of the mixture of AuNPs–mAb and sample, the clenbuterol of the sample compete with clenbuterol–BSA conjugates to react with AuNPs–mAb, resulting in to decrease in the AuNPs–mAb accumulation on the test zone and thus diminishes the color intensity. However, for blank samples, red-colored bands will be produced on both ends of test and control zones, while for clenbuterol positive samples, the red color of the test zone will reduce and even fade. The developed AuNPs–mAb-based CICA showed favorable sensitivity and specificity, for the detection of clenbuterol in real samples.

Ag based particles for immunosensing of pharmaceuticals

Ag NPs, as another member of noble metallic nanoparticles, exhibit some important features including high thermal stability and electrical conductivity and have been utilized at a wide range in biomedical sensing, labeling, imaging, etc. Bai et al[35] functionalized the GCE surface with MWCNTs and antibody of clenbuterol,

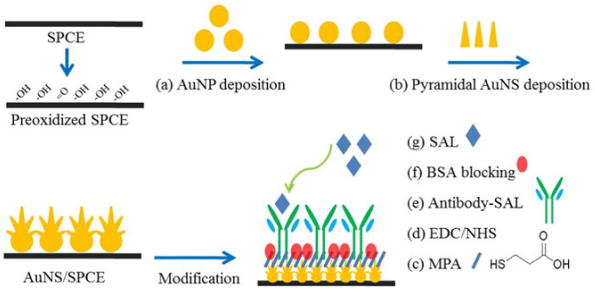

Figure 7. Schematic steps of the immunodetection of salbutamol detection using AuNPs. (Reprinted from [34] with permission license of 5045930662310)

however, AgNPs decorated GO particles were used as sensing nanocomposite to produce deferential pulse voltammetry current for immunosensing of clenbuterol. Obtained results showed a wide linear range from 0.01 to 10.0 ng.mL^{-1} with a LOD of 6.8 pg.mL^{-1}. The fabricated electrochemical immunosensor suggests fast and simple approaches for routine detection of clenbuterol. Wang et al[36] modified AgNPs with palladium to produce silver–palladium alloy nanoparticles (AgPd NPs) to enhance electrochemical response and label antibodies. rGO was used to further enhance the electrochemical current and therefore sensitivity of the immunosensor. SPCE was modified with the particles and antibody of the ractopamine, salbutamol, and clenbuterol for simultaneous quantifications. Results revealed that the fabricate biosensor can measure ractopamine, salbutamol, and clenbuterol concentrations from 0.01 to 100 ng.mL with LODs of 1.52 pg.mL^{-1}, 1.44 pg.mL^{-1} and 1.38 pg.mL^{-1}, respectively. While, Wu et al[37] fabricated silver triangular nanoprisms (AgTNPs)-functionalized with antibody to sense salbutamol by localized surface Plasmon resonance (LSPR). The reported dynamic range and LOD were 0.02–0.8 µg.mL^{-1} and 0.01 µg.mL^{-1}, respectively. As comped these two works, the AgPd NPs-based sensor can detect lower concertation of salbutamol which mainly results from the interstice feature of electrochemical methods and also applied nanoparticle for sensor designing.

SERS-based methods have gained tremendous interests due to their sensitive sensing ability. Most of the reported approaches have been utilized AuNPs as sensitizing particles to develop assay-based SERS probes. Although AuNPs exhibit a favorable SERS signal, however, the application of AgNPs is limited because of the low stability of their solutions. Thus, it is hypothesized that bimetallic particles of Au and Ag nanoparticles can exploit the physicochemical properties of both AuNPs and AgNPs to provide a more stable and sensitive sensing platform. In this regard, Zengin et al[38] functionalized Au-core@Ag-shell and magnetic nanoparticles (MNPs) nanoparticles with 2-mercaptobenzothiazole (SERS agent) and the kanamycin antibodies (as recognition agent, respectively, to fabricate SERS for kanamycin detection. The reported LOD was 2 pg.mL^{-1} in milk samples. The developed SERS-based sandwich-type assay exhibited favorable specificity to the determination of kanamycin with long-term structural stability for at least three months. Also, Yu et al[39] determined kanamycin using Ag@Fe_3O_4 NPs. As presented in Figure 8, the surface of GCEs was modified with thionine mixed graphene sheet (TH-GS) and antibody-functionalized Ag@Fe_3O_4 NPs. Upon addition of kanamycin, the current is produced which is linear with kanamycin concentrations from 0.050 to 16 ng.mL^{-1} with a LOD of 5 pg.mL^{-1}. Regarding the reported kanamycin detection approaches, the bimetallic method provides more sensitive manner for the immunosensing of kanamycin in pork meat samples.

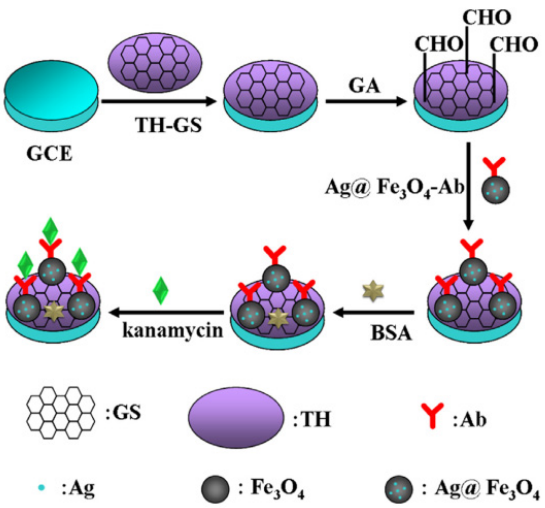

Figure 8. Immunosenig of kanamycin using Ag@Fe_3O_4-Ab-based electrochemical method. (Reprinted from [40] with permission license of 5045930972607)

Magnetic materials for immunodetection of pharmaceuticals

Recently, magnetic nanoparticles (MNPs) showed unique physicochemical properties including cheapness, biocompatibility, and high electron conductivity requiring for the fabrication of immunosensing platforms.

Liu et al[40] chitosan-magnetic nanoparticles for immunosensing of tetracycline. The anti-tetracycline monoclonal antibody was anchored on the surface of gold electrodes and the tetracycline was determined with the DPV technique. The developed immunosensor provided a wide dynamic range towards tetracycline concentrations from 0.08 to 1 ng.mL^{-1} with a LOD of about 0.0321 ng.mL^{-1}.

Magnetic beads (MBs) are wieldy used materials in the fabrication of the immunosensing platform which can automatically eliminate the most of the interfering agents. Hosu et al[41] designed a MBs-based approach for label-free immunosensing of acetaminophen. In this report, monoclonal antibody of acetaminophen was immobilized on the surface of MBs-functionalize-protein G molecules. Upon additions of acetaminophen, impedimetric signal (R_t) was changed which linear with acetaminophen concentrations. This sensor can detect acetaminophen as low as 1.76 µM. Despite the unfavorable sensitivity of the fabricated platform, it has simple construction steps which could be more developed for designing point-of-care devices.

Zhang et al[42] prepared Au@Ag_2S CS-NPs. magnetic chitosan/thionine matrix film (CSMCM) nanocomposite to modify gold electrode surface for immunosensing of ractopamine. The produced CSMCM enhances the loading capacity and stability of the developed immunosensor through bonds between S of Ag_2S shell and amine groups of biomolecules. The fabricate sensor detects ractopamine from 0.01 to 10 ng.mL^{-1} with a LOD of 2.5 pg.mL^{-1}. The fabricated assay provides a biocompatible and sensitive platform for the recognition of other biomolecules. Also, Chen et al[43] quartz crystal microbalance (QCM) approach for specific detection of ractopamine. To fabricate, PowerVision (PV) reagent was used as an enzyme-antibody functionalized polymer for signal labeling. However, magnetic β-CD NPs was implemented as ractopamine capture probe. The differences of the QCM frequency (as analytical signal) is linear from 0.03 to 25 ng.mL^{-1} with LOD as low as 0.01 ng.mL^{-1}. The developed sensor could be a portable device for fast detection of ractopamine in real fodder samples.

Abdelshafi et al[44] fabricated a microfluidic system for immunosensing of cocaine as an abused drug. In this work, antibodies were grafted on the MBs. Then, MBs were applied in ELISAs engaging horseradish peroxidase, catechol and TMB as the enzyme, electrochemical and colorimetric detection agents. In another step, the produced materials were embedded into a microfluidic sensing system. The developed approach is able to accomplish all the immunosensing steps in about 25 minutes. Cyclic voltammetry technique can detect cocaine within a wide dynamic range and LOD of 0.15 ng.L^{-1} in oral fluid and urine samples.

Other materials for immunosensing of pharmaceuticals

Photoelectrochemical (PEC)-based sensors are a new type of approach which has gained ever-rising interest because of their advantageous features and attractive potential in the biomedical sensing of various biomarkers. Combination of the spectroscopic and electrochemical properties aid ultra-sensitive detection ability to the PEC-based sensory systems as a result of decreased background noses. Wang et al[45] fabricated a PEC-based immunosensor based on a competitive approach for the selective recognition of dexamethasone (DXM). Bismuth sulfide modified graphitic carbon nitride was utilized as the biosensing agent to immobilize on the surface indium tin oxide (ITO) electrode. TiO_2@CdS was functionalized with antibodies to specific detection of dexamethasone. The competitive reaction between dexamethasone and BSA-dexamethasone diminishes the specific bonding, causing a decrease in the photocurrent intensity of the probe. This immunosensor exhibits an ultra-sensitive manner to the detection of dexamethasone with LOD of about 2 pg.mL^{-1}. Figure 9 demonstrates the fabrication steps of the immunosensor and the mechanism of action of the detection of dexamethasone.

Zhang et al[46] determined clenbuterol through an immunosensing approach using zinc sulfide QDs and polyaniline (ZnSQD@PANI). ZnSQD@PANI and antibody were utilized to modify the surface on a gold electrode, respectively. An amperometric immunosensor for clenbuterol. Collaboration of PANI enhances the sensitivity of the electrochemical probe by increasing the amount of the adsorbed clenbuterol antibody on its surface. The immunosensor displays a LOD and dynamic range of 5.5 pg.mL^{-1} and 0.01 to 10 ng.mL^{-1}, respectively. In addition, Yao et al[47] developed an ECL-based immunosensor for

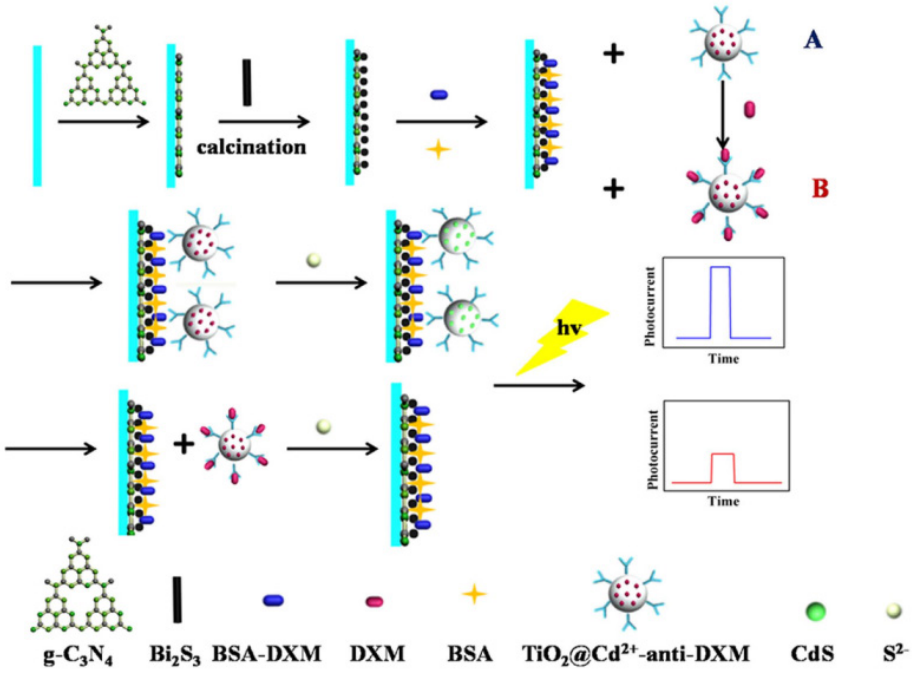

Figure 9. Schematic representation of the detection of DXM using Cd^{2+}@TiO_2-antibody probe. (Reprinted from [46] with permission license of 5045930972607)

clenbuterol detection. They modified the GCE with CdSe QDs, antibody functionalized chitosan and HRP labeled goat against rabbit IgG (HRP-GaRIgG). Using of the enzyme was significantly amplification the produced ECL signal, where in the presence of hydroquinone, a strong ECL signal is produced upon the cathodic reaction because of the product of H_2O_2 as an electrochemical reaction (Figure 10). The developed competitive immunosensor can detect clenbuterol from 0.05 to 1000 ng.mL^{-1} with a LOD of 0.02 ng.mL^{-1}. This immunosensor exhibited high signal and structural stability and reproducible fabrication steps.

Upconverting nanoparticles (UCNPs) are a new type of fluorescence materials with low-frequency excitations (near-infrared) that have been applied for the development of different sensing systems especially immunosensing platforms. In addition, UCNPs possess low leachability and large anti-Stokes shifts permitting to simultaneously detect more than one analyte at the same time. Hlaváček et al[48] fabricated an immunosensor using UCNPs to construct upconversion-linked immunosorbent assay (ULISA)-based diclofenac sensor for detection of diclofenac. In this work, carboxyl functionalized silica-coated UCNPs (signaling probe) were attached with

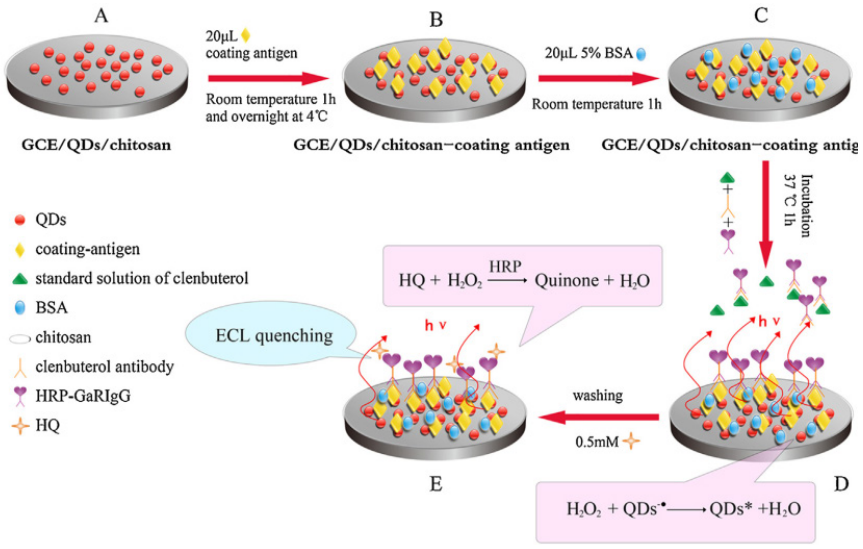

Figure 10. Fabrication steps of QDs/chitosan-based immunosensor for detection of clenbuterol. (Reprinted from [48] with permission license of 5045931210524)

secondary anti-diclofenac-IgG antibodies (Figure 11). The developed ULISA probe can detect diclofenac as low as 0.05 ng.mL^{-1} which is comparable with conventional ELISA results without needing to use enzyme for signal amplification. This sensor was utilized for the detection of diclofenac in water samples.

Conclusions

A comprehensive summary of the advances of nanomaterial in the fabrication of pharmaceutical immunosensors was presented in this article. Also, a brief of the synthesis and sensor designing steps were reported with summaries of their analytical performance. As reviewed, different sensing strategies with diverse signal amplification techniques were utilized in immunosensing of pharmaceuticals.

Material trends showed that various single and multi-component nanomaterials are synthesized with various shapes and sizes. Various categories of advanced materials were applied including carbon, gold, and other metal-based nanostructures. AuNPs have been mostly utilized as signal amplification and secondary functionalization particles. The second most used materials are carbon-based particles especially graphene originated ones. These materials found predominate applications in the immunosensing of pharmaceuticals because of some outstanding features such as biocompatible nature, cheapness, ease of access, high conductivity, and bright spectroscopic properties.

The majority of reported methods for immunosensing of pharmaceuticals are based on various types of electrochemical techniques due to their easy fabrication and ultrasensitive LODs. Among the optical sensors, luminescence-based materials have been repeatedly used for immunosensing platforms. While optical probes offer noninvasive and fast recognition, the electrochemical approaches provide us more sensitive recognition of the analytes.

Despite the advances in immunosensing of pharmaceuticals, future studies should be directed to develop more sensitive and specific approaches for the simultaneous detection of pharmaceuticals. Furthermore, *in situ* and real-time detection approaches such as lab-on-a-chip will more participate in the designing of the future probes.

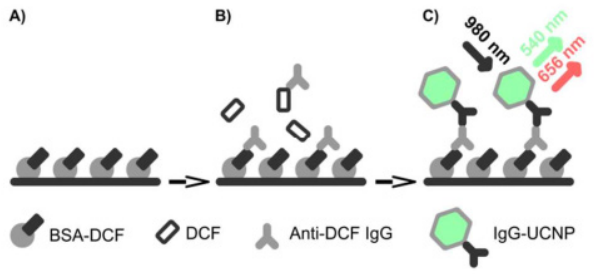

Figure 11. Schematic of indirect detection of diclofenac by ULSIA-based method. Reprinted (adapted) with permission from [49]. Copyright (2021) American Chemical Society

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Conflict of interests

There is no conflict of interests.

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