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IMPLICATIONS OF ANALYTICAL NANOSCIENCE IN PHARMACEUTICAL AND BIOMEDICAL FIELDS: A CRITICAL VIEW

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ABSTRACT

Recent advancements have significantly transformed pharmaceutical analysis through the integration of nanomaterials, particularly in the evaluation and development of novel nanomedicines across preclinical to clinical stages. Over the past decade, a diverse range of nanomaterialssuch as carbon-based structures, metal nanoparticles, polymer-derived matrices, bio-based materials, and composite systemshave been increasingly adopted to enhance analytical capabilities in pharmaceutical research. These materials offer specialized physicochemical properties that facilitate improved analyte extraction from complex biological and environmental matrices, contribute to superior chromatographic resolution, and support the evolution of highly sensitive sensor technologies.The incorporation of nanomaterials has notably elevated the performance of detection platforms, including electrochemical sensors and biosensing techniques, enabling greater sensitivity, specificity, and efficiency in drug quantification across varied matrices. These innovations have led to substantial improvements in parameters such as detection limits, reproducibility, selectivity, and analytical turnaround times. However, challenges persist in terms of the standardized characterization and consistent quality control of these nanomaterials. Current analytical methods and instrumentation must evolve further to meet the growing demands of precision and reproducibility in nanomaterial-based pharmaceutical analysis.There remains a considerable scope for the deeper exploration of nanomaterial behavior under diverse

analytical conditions. Future efforts should focus on the refinement of measurement technologies and the development of advanced protocols for accurate characterization. Such initiatives will play a crucial role in ensuring the reliable integration of nanomaterials into routine pharmaceutical testing and the successful translation of nanomedicines into clinical practice.

KEYWORDS: Nanomaterials, Pharmaceutical analysis, Biosensing, Drug detection.

INTRODUCTION

The pharmaceutical landscape has experienced a transformative surge in recent years, largely attributed to the increasing prevalence of chronic illnesses, age-related disorders, and the evolution of clinical treatment strategies.This expanding demand for effective pharmaceutical interventions has simultaneously intensified the complexity of pharmaceutical applications and their subsequent impact on health, society, and the environment. In this evolving context, analytical chemistry has emerged as a pivotal discipline, providing essential tools for the development, monitoring, and quality assurance of pharmaceutical products across all stages of their life cyclefrom synthesis and manufacturing to post-marketing surveillance and environmental impact assessments. Contemporary pharmaceutical analysis must transcend conventional laboratory settings. There is an urgent need for robust analytical methodologies that not only ensure high accuracy, precision, sensitivity, and specificity, but also incorporate operational simplicity and sustainability. The demand extends to the capacity for spatial and temporal resolution in diverse matrices, including biological fluids, environmental samples, and complex drug formulations, whether in their native or metabolized forms. Furthermore, the shift toward minimal sample consumption and reduced use of reagents aligns with the global drive for environmentally conscious scientific practices, requiring analytical protocols that limit chemical waste and operational costs.

In response to these analytical challenges, nanotechnology has rapidly advanced as a transformative approach within pharmaceutical sciences. Since the initial exploration of nanomaterials (NMs) in the mid-20th century, their role in pharmaceutical research and analysis has expanded exponentially. These nanoscale materials characterized by unique thermal, electrical, mechanical, and optical properties have revolutionized drug delivery strategies, diagnostics, and analytical methodologies. Notably, nanotechnology-based drug delivery systems, such as liposomes, nanosuspensions, and polymeric nanoparticles, have

demonstrated significant improvements in drug solubility, bioavailability, and targeted delivery, thereby enhancing therapeutic efficacy while minimizing systemic toxicity.

A new subfield, Analytical Nanoscience and Nanotechnology (AN&N), has emerged at the intersection of analytical chemistry and nanotechnology. This discipline leverages the intrinsic properties of nanomaterials to develop sensitive, selective, and efficient analytical techniques. Within AN&N, nanomaterials can serve dual rolesas analytes in the characterization of nano-based pharmaceuticals, or as functional tools that improve various analytical stages, including sample preparation, separation, and detection. For example, metallic nanoparticles have been employed as sensor platforms, carbon-based nanostructures have been utilized in solid-phase extraction, and magnetic nanoparticles have facilitated rapid and efficient analyte isolation. However, the incorporation of nanomaterials into pharmaceutical analysis is not without its limitations. As novel nanostructures are continuously developed, the need for precise, reproducible, and universally accepted characterization protocols has intensified. This necessity has given rise to the field of nanometrology focused on the accurate measurement and standardization of nanomaterials. Nanometrology is critical in validating the size, surface charge, shape, and aggregation state of nanoparticles, parameters that directly influence their functionality and behaviour in biological or analytical systems. Despite its importance, industrial-scale nanometrology is still hampered by limitations in precision, scalability, and cost-effectiveness, highlighting a major bottleneck in the full integration of nanotechnology into mainstream pharmaceutical analysis. This review endeavours to explore the convergence of nanoscience, nanotechnology, and analytical chemistry, with a specific focus on pharmaceutical applications. It critically examines how various classes of nanomaterials have been employed over the past decade and a half to enhance analytical methodologies used in pharmaceutical research. Emphasis is placed on the practical roles of nanostructures within different stages of the analytical workflow, including extraction, separation, and detection processes. Moreover, the discussion extends to contemporary challenges in nanometrology and the necessity for standardized, high-throughput analytical techniques to ensure the quality, safety, and efficacy of nano-enabled pharmaceutical products.

I. Analytical Nanometrology in Modern Science

Over the past few decades, nanoscience and nanotechnology (N&N) have emerged as transformative disciplines, revolutionizing numerous sectors including medicine, agriculture, environmental science, energy, and consumer goods.The unparalleled physical, chemical, and

biological characteristics of nanomaterials (NMs)such as enhanced surface-to-volume ratio, quantum effects, and unique optical and catalytic propertieshave made them indispensable across these domains.This rapid expansion of nanotechnology into real-world applications has necessitated a parallel evolution in the methods used to analyze and regulate these novel materials.In the field of Analytical Chemistry, this convergence has given rise to a specialized sub-discipline often referred to as Analytical Nanoscience and Nanotechnology (AN&N). AN&N focuses on the integration of nanoscale materials and phenomena into analytical processes, both as tools and as objects of investigation. Originally, NMs were employed primarily to improve the performance of analytical methodsfor instance, enhancing sensitivity, selectivity, or miniaturization.However, as nanomaterials have become increasingly embedded in manufacturing, product development, and even healthcare systems, the need for rigorous analytical oversight has grown equally urgent.This demand has led to the development of Analytical Nanometrology (ANM), a relatively new but rapidly expanding field that deals with the measurement science of nanomaterials. ANM encompasses a wide range of objectivesfrom precise particle size and morphology characterization to understanding surface chemistry, aggregation behavior, and the fate of nanomaterials in complex matrices. The analytical challenges are numerous: heterogeneity of samples, limited availability of reference materials, lack of standardized protocols, difficulties in ensuring measurement traceability, and the requirement for high sensitivity and specificity in often trace-level quantifications.

A particularly pressing concern is the environmental and health impact of engineered nanomaterials. While these materials bring about significant advancements, they also raise questions about toxicity, bioaccumulation, and long-term ecological effects. Thus, analytical strategies must not only characterize and quantify nanomaterials but also monitor their transformation, degradation, and interactions in biological and environmental systems. This underscores the need for reliable, validated methods capable of operating across diverse sample types, including biological tissues, food products, wastewater, and atmospheric aerosols. To address such complexities, ANM employs a suite of advanced analytical techniques such as electron microscopy (SEM/TEM), atomic force microscopy (AFM), dynamic light scattering (DLS), nanoparticle tracking analysis (NTA), single-particle ICP-MS, and various spectroscopic methods including Raman and X-ray photoelectron spectroscopy (XPS). However, the diversity in nanomaterial types ranging from carbon-based nanostructures like graphene and fullerenes to metallic nanoparticles, quantum dots, and

polymeric nanocarriers demands tailored methodological approaches for each application. Recognizing the analytical challenges posed by nanomaterials, regulatory frameworks are also evolving. The European Commission (EC) has recently updated its criteria for defining and identifying nanomaterials, emphasizing the need for harmonized measurement approaches and standardized definitions based on parameters such as particle size distribution and surface characteristics. This regulatory clarity is pivotal for industries and laboratories alike to ensure compliance, safety, and quality control in nano-enabled products.

II. Role of Nanomaterials in Analytical Techniques

Due to their distinct physicochemical features such as an exceptionally large surface area relative to volume, nanometric scale, adjustable surface properties, and heightened reactivity nanomaterials have brought significant advancements to the field of pharmaceutical analysis. They have proven to be highly effective in key analytical stages, including sample preparation, compound separation, and molecular detection or sensing. These materials enhance analytical performance by offering greater sensitivity, increased selectivity, and the ability to handle complex pharmaceutical and biological samples with improved accuracy and efficiency.

a. Importance of Nanomaterials in Sample Preparation

In pharmaceutical analysis, the accuracy and reliability of drug detection largely depend on how effectively the sample is prepared beforehand. By integrating nanomaterials into these preparatory methods, researchers have achieved significant improvements in both the selectivity and efficiency of extracting pharmaceutical substances, especially when dealing with low-concentration or trace-level analytes.

b. Membrane-Mediated Extraction Techniques:

The convergence of nanotechnology and membrane science has resulted in a new generation of functional membranes, often referred to as nanocomposite or hybrid membranes. These systems incorporate nanomaterials into traditional membrane matrices, thereby enhancing their structural and functional properties well beyond the capabilities of conventional membranes. In pharmaceutical analysis, such nanomaterial-integrated membranes have proven instrumental in isolating and purifying drug molecules from complex media, such as blood plasma, urine, and pharmaceutical formulations. The inclusion of nano materials such as metal oxides, carbon-based structures, or functionalized polymers into membrane frameworks contributes to increased mechanical strength, improved chemical and thermal

resistance, and heightened selectivity. These properties are essential when dealing with intricate biological matrices, where interference from coexisting substances can significantly compromise analytical accuracy. Additionally, the incorporation of nanomaterials can help reduce membrane fouling, a common challenge that leads to reduced operational life and inconsistent separation performance.

One of the most promising innovations in membrane-based separation involves molecularly imprinted membranes (MIMs). These membranes are fabricated using a molecular imprinting technique where a target molecule, or "template", is embedded into a polymeric material during synthesis. Once the template is removed, it leaves behind a specific cavity that is structurally and chemically complementary to the target molecule. These "artificial recognition sites" allow the membrane to selectively rebind the target drug molecule with high affinity. In pharmaceutical contexts, MIMs have shown exceptional performance in selectively capturing specific drugs such as paclitaxel, a potent chemotherapeutic agent from mixtures containing numerous structurally similar compounds. However, despite their high specificity and reusability, certain limitations persist. The dense cross-linking needed to stabilize the imprinted structures often leads to reduced permeability and lower water flux, which in turn hampers process efficiency. Moreover, the rigid polymer matrix may lack the mechanical flexibility required for long-term operational durability.

To overcome some of the limitations of passive membrane-based separation, electro membrane extraction (EME) has emerged as a powerful alternative. This technique applies an external electric field across a membrane system to drive charged pharmaceutical compounds particularly ionizable drugs across a thin, immobilized liquid membrane. Incorporating nanomaterials into EME membranes further enhances their functionality. For instance, magnetic nanoparticles (e.g., Fe_3O_4) and carbon nanostructures can be embedded within the membrane matrix to significantly improve its porosity, electrical conductivity, and surface interaction potential. These enhancements facilitate a more efficient mass transfer process, enabling faster and more selective extraction of target drugs. Additionally, magnetic nanoparticles allow for easier handling and membrane retrieval using magnetic fields, which simplifies operation in automated or continuous-flow systems. The nanomaterial-enhanced EME setup is especially advantageous when dealing with trace-level drug detection, as it amplifies extraction efficiency while minimizing sample volume requirements. This makes it

highly suitable for bioanalytical applications, therapeutic drug monitoring, and pharmacokinetic studies.

c. Solvent-Based Extraction Approaches Enhanced by Nanomaterials

Traditional solvent-based extraction techniques, particularly liquid-liquid extraction (LLE), have undergone significant transformation with the advent of nanotechnology. Among the most impactful innovations is the integration of magnetic nanoparticles (MNPs) and metal oxide-based nanocomposites, which not only enhance the efficiency of analyte recovery but also simplify the separation process from complex biological matrices.One prominent example is the application of iron oxide nanoparticles (Fe_3O_4), which exhibit unique superparamagnetic properties. These nanoparticles can be easily manipulated using an external magnetic field, allowing for swift separation post-extraction. To enhance their surface characteristics and stability, Fe_3O_4 particles are often coated with mesoporous titanium dioxide (TiO_2), resulting in a composite material referred to as Fe_3O_4@mTiO_2. This hybrid structure combines the magnetic controllability of Fe_3O_4 with the high adsorption capacity and chemical stability of TiO_2.The mesoporous nature of TiO_2 contributes significantly to its performance. The pores offer a vast surface area and tailored surface chemistry that facilitate selective interaction with pharmaceutical compounds. The resulting Fe_3O_4@mTiO_2 nanocomposite demonstrates excellent dispersion in aqueous biological media and enables the efficient binding and removal of drug residues. This is particularly beneficial in sample pretreatment for chromatographic techniques such as high-performance liquid chromatography coupled with mass spectrometry (HPLC-MS), where reducing matrix interferences is essential for accurate quantification.

Another innovative solvent-extraction strategy utilizing nanotechnology is hollow fibre liquid-phase microextraction (HF-LPME). This technique employs a hollow fibre membrane, typically made of polypropylene or a similar polymer, which is used as a physical barrier between donor and acceptor phases. When functionalized with carbon nanofibers (CNFs) and impregnated with organic solvents such as dihexyl ether, the hollow fiber is transformed into an advanced extraction device. Carbon nanofibers are known for their exceptionally high surface area, robust adsorption capabilities, and chemical affinity toward a wide range of organic molecules. These characteristics enable rapid and selective partitioning of target analytes from the donor phase (usually a complex biological fluid) into the organic solvent phase within the fiber. The three-phase dynamic setup further improves extraction kinetics

and enhances recovery, especially for non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen, naproxen, diclofenac, and ketoprofen. The HF-LPME technique offers several compelling advantages, including minimal solvent usage, aligning with green analytical chemistry principles, and the ability to achieve high selectivity and preconcentration even at trace analyte levels. Its compatibility with automated systems facilitates seamless integration with modern analytical platforms, while its cost-effectiveness and operational simplicity make it highly suitable for routine pharmaceutical monitoring in both clinical and environmental applications.

d. Sorbent-Based Extraction Techniques Enhanced by Nanomaterials

These extraction strategies, essential for isolating trace-level pharmaceutical residues from complex matrices, have evolved considerably with the incorporation of nanotechnology. Among the most widely applied formats are solid-phase microextraction (SPME), dispersive solid-phase extraction (DSPE), magnetic solid-phase extraction (mSPE), microextraction by packed sorbent (MEPS), and stir-bar sorptive extraction (SBSE). The central advancement across these techniques lies in the engineering of novel sorbent materials using nanomaterials (NMs), which offer unique physicochemical properties not observed in conventional sorbents. One of the most impactful contributions comes from carbon-based nanostructures, particularly carbon nanotubes (CNTs). These cylindrical allotropes of carbon, characterized by their high aspect ratio and significant mechanical strength, exhibit exceptional adsorption capabilities. This efficiency is largely attributed to their expansive surface area and the presence of delocalized π-electron clouds along their tubular structure. These π-systems allow for strong π–π stacking interactions with aromatic drug molecules, facilitating efficient adsorption and retention. Additionally, CNTs are chemically inert and thermally stable, making them superior to traditional sorbents such as silica gel, C18 phases, or activated carbon.Their surface can be functionalized with various chemical groups, further enhancing selectivity towards specific pharmaceutical targets.

Another important class of nanomaterials employed in these extraction systems is carbon dots (CDs). These are quasi-spherical, zero-dimensional nanoparticles typically below 10 nanometers in diameter. CDs are prized for their luminescent behavior, which is strongly influenced by surface passivation and functionalization. There are two major subtypes: graphene quantum dots (GQDs) and carbon nanodots, both of which possess fluorescence properties that are excitation-wavelength dependent. These features enable their dual usenot

only in pre-concentration and purification during sample preparationbut also in detection, serving as fluorescent probes for trace pharmaceuticals. CDs are especially attractive because they offer a safer and more biocompatible alternative to traditional semiconductor quantum dots, which often suffer from heavy metal toxicity. A further enhancement in sorbent-based extraction involves the use of graphene and its oxidized derivative, graphene oxide (GO). Graphene is a single-atom-thick sheet of sp^2-hybridized carbon atoms arranged in a honeycomb lattice, exhibiting remarkable electrical conductivity, mechanical resilience, and surface area. GO, while structurally similar, contains oxygen-containing functional groups (e.g., hydroxyl, epoxide, and carboxyl) that provide active sites for chemical interactions. These groups enable GO to engage in hydrogen bonding, van der Waals forces, and electrostatic interactions with pharmaceutical molecules, thereby boosting extraction specificity. Moreover, the amphiphilic nature of GO facilitates dispersion in aqueous media, enhancing accessibility to target analytes.In analytical settings, both graphene and GO are employed as sorbent coatings in microextraction fibers or as dispersed sorbents in solution-phase techniques. Their surface modifiability allows for tailored interactions with specific drug molecules, further improving analytical performance metrics such as recovery, reproducibility, and sensitivity.

e. Composite and Functional Nanostructures:

In recent years, the focus of nanomaterial research in pharmaceutical analysis has extended beyond conventional carbon-based nanostructures to embrace more complex and highly engineered systems such as metal-organic frameworks (MOFs) and polymer-based composites. These advanced materials bring distinctive structural and functional properties that make them highly suitable for challenging analytical applications, especially in the extraction and selective identification of pharmaceutical compounds in diverse sample matrices. Metal-organic frameworks (MOFs) are crystalline hybrid structures composed of metal ions or clusters coordinated with organic ligands. One of their defining characteristics is an exceptionally high surface area combined with tenable porosity. This tunability allows precise customization of pore dimensions and surface functionalities, making MOFs highly effective for capturing specific drug molecules through size exclusion, hydrogen bonding, van der Waals forces, or electrostatic interactions. Furthermore, their modular architecture enables the selective interaction with analytes based on molecular shape and polarity, thereby enhancing both the sensitivity and specificity of the analytical process. MOFs have been employed in various sample preparation techniques especially in solid-phase extraction (SPE)

formats where they act as “smart sorbents” capable of isolating and enriching trace-level pharmaceuticals from complex biological fluids, wastewater, or environmental samples.On the other hand, polymeric nanocomposites, particularly those incorporating conductive polymers, have demonstrated remarkable potential in both extraction and detection workflows. When nanoparticles such as carbon nanotubes, metal oxides, or graphene derivatives are embedded within a polymer matrix, the resulting composite benefits from the synergistic properties of both components. The polymer provides mechanical flexibility and chemical stability, while the embedded nanomaterials contribute to enhanced surface area, electrical conductivity, and functional interaction with target molecules.

One promising area of application is in solid-phase microextraction (SPME), where such nanocomposites serve as high-efficiency sorbents. These materials not only offer excellent analyte retention capacities but can also be integrated with electrochemical sensors, thereby allowing for real-time, in-situ detection of pharmaceuticals following extraction**.** This dual-functionality enabling both isolation and quantification in a single platform not only streamlines the analytical workflow but also reduces the need for multiple processing steps. The versatility of these nanostructured composites extends to their adaptability across various analytical platforms, including chromatographic and spectrometric systems. Additionally, the ability to chemically tailor the surface functionalities of these materials further enhances their selectivity toward structurally diverse drugs. These composite systems are particularly valuable in detecting and quantifying compounds with low abundance or poor chromatographic behaviour, thus addressing longstanding challenges in pharmaceutical trace analysis.

A wide range of nanomaterial-based extraction techniques have emerged as integral components in modern pharmaceutical analysis due to their ability to enhance selectivity, sensitivity, and overall extraction efficiency. In membrane-based approaches, systems like molecularly imprinted polymer (MIP) coated graphene oxide–polyvinylidene fluoride–TiO_2 (GO@PVDF@TiO_2) and polysulfide (PSf) nanocavity-embedded membranes have shown promise in selectively extracting compounds such as norfloxacin and paclitaxel, though mechanical strength and water flux remain limiting factors. Liquid-liquid extraction (LLE) has advanced with magnetic core-shell materials such as Fe_3O_4@mTiO_2, which effectively recover a broad spectrum of psychoactive drugs from blood samples, with high recoveries (up to ~99.9%) and nanogram-level sensitivity. Likewise, hollow fiber liquid-phase

microextraction (HF-LPME) using carbon nanofibers (CNFs) has enabled precise quantification of NSAIDs like diclofenac and ketoprofen from urine. Solid-phase extraction (SPE) and its miniaturized versions have also benefited from functional nanomaterials. Sorbents such as multi-walled carbon nanotubes (MWCNTs), graphene oxide composites, imprinted polymer networks (IPNs), and dendrimer-modified MIPs have significantly improved the detection of various analytes across biological, pharmaceutical, and environmental matrices. These platforms have enabled the quantification of diverse drugs, including antidepressants, statins, anti-inflammatory agents, and antibiotics, often achieving recoveries above 90% and detection limits in the low ng/mL to pg/mL range. Integration with analytical methods such as HPLC-DAD, GC-MS, UHPLC-MS/MS, and chemiluminescence has further solidified the role of nanomaterial-enhanced extractions in high-precision pharmaceutical analysis.

f. Optical Detection Using Nanomaterials

The optical detection of pharmaceutical compounds has seen a paradigm shift with the integration of nanomaterials, owing to their unique optical characteristics and enhanced interaction with target analytes. These materials, by virtue of their quantum confinement effects, localized surface plasmon resonance (LSPR), and modifiable surface chemistry, have enabled the development of sensitive and selective optical sensors applicable across a variety of analytical platforms**.** Below is an in-depth discussion of key nanomaterials used for optical sensing in pharmaceutical analysis. Among the most widely utilized metallic nanomaterials in optical sensing, gold (AuNPs) and silver nanoparticles (AgNPs) are prominent due to their intense LSPR properties. These properties stem from the collective oscillation of conduction electrons at the nanoparticle surface when excited by light of specific wavelengths. This interaction leads to distinct absorption peaks in the visible region, making these nanoparticles ideal for optical transduction mechanisms. Gold nanoparticles are highly stable, exhibit excellent biocompatibility, and can be synthesized in various morphologies spheres, rods, starseach offering tunable LSPR peaks. Their optical properties are greatly influenced by interparticle distances; aggregation of AuNPs results in plasmon coupling and a visible color change (typically from red to blue), a feature effectively utilized in colorimetric assays for drug detection. Their ease of functionalization with biological ligands further enhances their application in biosensing, particularly in detecting antibiotics, anticancer agents, and hormones. Silver nanoparticles, on the other hand, display sharper and more intense plasmon bands compared to AuNPs, offering even higher sensitivity in certain assays. AgNPs are

frequently employed in combination with spectroscopic techniques such as SERS, chemiluminescence, and UV–visible absorption spectroscopy. Their plasmonic enhancement effect is particularly powerful in SERS, where AgNPs amplify Raman scattering signals by several orders of magnitude, allowing detection of drugs at ultra-low concentrations.

Zinc oxide nanoparticles represent a class of semiconductor nanomaterials with distinctive optical and electronic properties. Unlike traditional semiconductor quantum dots (e.g., CdTe, CdSe), ZnO nanoparticles are less toxic and more environmentally benign, making them a preferable choice for pharmaceutical and biological applications. Their bandgap (~3.37 eV) facilitates strong UV absorption, and their high exciton binding energy contributes to a high quantum efficiency. Chiral ZnO nanoparticles, in particular, have shown enhanced interaction with polarized light, allowing them to be used in optical sensors with improved enantioselectivity. These nanoparticles are also integrated into fluorescence and photoluminescence systems for the imaging and monitoring of drug distribution in biological specimens. Furthermore, their ability to act as energy donors or acceptors in fluorescence resonance energy transfer (FRET) systems further extends their applicability in multi-analyte sensing.

The emergence of carbonaceous nanomaterials such as carbon dots (CDs), graphene quantum dots (GQDs), and nanodiamonds (NDs) has added a new dimension to optical drug sensing due to their intrinsic photoluminescence and favorable biocompatibility profiles. Carbon dots are quasi-spherical, nanoscale carbon particles characterized by their small size (<10 nm), hydrophilicity, and stable fluorescence. The presence of oxygen-rich surface functional groups such as hydroxyl, carbonyl, and carboxylic acid allows for further conjugation with targeting ligands, making CDs excellent candidates for biosensing and imaging. They exhibit excitation-dependent emission and a high Stokes shift, reducing background interference during detection. Graphene quantum dots share similar fluorescence mechanisms with CDs but display superior electron mobility, photostability, and surface area due to their layered graphene structure. Their tunable emission properties, combined with low toxicity and ease of surface modification, make GQDs highly effective in fluorescence-based drug assays, especially for detecting small molecule pharmaceuticals and monitoring drug–protein interactions. Nanodiamonds offer a robust crystalline framework with modifiable surface chemistries. Their large surface area enables high drug-loading capacities, while their compatibility with host molecules like β-cyclodextrin and γ-cyclodextrin provides enhanced

selectivity and affinity toward specific pharmaceutical agents, such as anthracyclines. NDs also support resonance energy transfer mechanisms, making them suitable for sensing platforms that rely on fluorescence quenching or enhancement**.**

SWCNHs are a unique type of carbon nanostructure characterized by conical ends and the ability to aggregate into spherical clusters. These aggregates typically measure between 80 to 100 nm and offer both internal and external adsorption sites due to their hollow cores and porous surfaces. The inherent surface area and π-conjugated structure of SWCNHs enable the efficient adsorption of various guest molecules, including pharmaceutical analytes. This property is leveraged in constructing multifunctional sensing systems that combine electrochemical, fluorescent, and resistive signal outputs. Their structural uniformity and chemical tunability allow for precise control over analyte recognition and signal transduction. Nanozymes, or nanomaterial-based enzyme mimics, have gained attention as alternatives to natural enzymes in sensor applications due to their enhanced stability, long shelf life, and tolerance to environmental changes. Certain metal–organic frameworks (MOFs), such as MIL-53(Fe), display peroxidase-like activity and are employed in colorimetric assays.A notable example involves iron/nitrogen co-doped carbon nanozymes (Fe/NC NZs), which catalyze the oxidation of 3,3′,5,5′-tetramethylbenzidine (TMB) in the presence of hydrogen peroxide, producing a blue-colored oxidized product (oxTMB). When paired with orange-emitting carbon dots (O-CDs), a dual-mode detection system is achieved where oxTMB quenches the fluorescence of O-CDs via the inner filter effect. This synergistic system significantly enhances sensitivity and specificity, particularly in detecting thiol-containing drugs like captopril.

Room temperature phosphorescence (RTP) is an emerging phenomenon in nanomaterial-based sensors, offering delayed light emission and longer excited-state lifetimes that are advantageous for reducing background fluorescence. Traditional RTP materials often involve rare earth elements or heavy-metal complexes, which pose toxicity and environmental concerns. Carbon dots engineered to exhibit RTP behavior present a safer and more sustainable alternative. When doped with heteroatoms such as nitrogen and phosphorus and synthesized from biocompatible precursors like metformin or L-tryptophan, CDs can exhibit dual emission (fluorescence and phosphorescence). Embedding these RTP CDs in polymeric matrices like polyvinyl alcohol (PVA) enhances their solid-state emission due to restricted molecular motion and reduced non-radiative transitions. These RTP systems have been used

as fluorescent inks and dual-channel sensors capable of detecting specific amino acids and pharmaceutical molecules. Modifications such as L-tryptophan doping increase radiative recombination via hydroxyl groups and improve intersystem crossing to triplet states by narrowing the energy gap, thus boosting both fluorescence and phosphorescence intensities.

g. Surface-Enhanced Raman Scattering (SERS) and Plasmonic Sensing:

Surface-Enhanced Raman Scattering (SERS) is a powerful analytical technique that exploits the significant amplification of Raman signals through the interaction of light with metallic nanostructures. This enhancement is primarily due to the intense localized electromagnetic fields generated at specific sites, often referred to as "hotspots," on the surface of metal nanoparticles (NPs). Gold (Au) and silver (Ag) nanoparticles are the most commonly used substrates in SERS applications, owing to their strong surface plasmon resonance (SPR) properties and excellent chemical stability. The efficiency of plasmonic enhancement in SERS is influenced by several factors, including the size, shape, and spatial arrangement of the nanoparticles. Research has shown that optimal enhancement is typically observed in structures with dimensions ranging from 30 to 100 nanometres, particularly those with high aspect ratios and sharp edges. These geometric features facilitate the concentration of electromagnetic fields, thereby increasing the likelihood of Raman scattering events. Despite the advantages of SERS, challenges arise when analyzing analytes that either lack inherent chromophores or exhibit poor adsorption characteristics on metal surfaces. To address these limitations, researchers have developed indirect SERS strategies. These approaches often involve the use of SERS-active dyes to tag analytes or the application of bioconjugated nanoparticles that can selectively bind to target molecules. This enables the detection of a broader range of substances, including those that would otherwise be difficult to analyze using traditional SERS methods. A notable advancement in plasmonic sensing is the detection of ephedrine through the use of cerium (Ce^{3+})-modified gold nanoparticles (AuNPs). In this innovative approach, the presence of ephedrine leads to the aggregation of the Ce^{3+}-AuNPs complex, which significantly enhances Rayleigh scattering. This aggregation results in a pronounced increase in the Raman signal, allowing for sensitive detection of the analyte. Conversely, when pseudoephedrine is introduced, it induces dispersion of the nanoparticle complex, leading to a reduction in signal intensity. This dual-mode discrimination strategy is particularly advantageous for differentiating between these two closely related compounds in aqueous and biological samples.

Recent studies have further explored the potential of SERS in various applications, including environmental monitoring, food safety, and medical diagnostics. The integration of machine learning algorithms with SERS data analysis has also emerged as a promising avenue for improving the accuracy and speed of detection. By leveraging large datasets, these algorithms can enhance the interpretation of complex spectral information, facilitating the identification of target analytes with greater precision.

h. Electrochemical Detection Platforms

Electrochemical sensing has become a pivotal tool in pharmaceutical and biomedical analysis due to its intrinsic advantages: high sensitivity, rapid response, low-cost instrumentation, and compatibility with miniaturized systems. The performance of these platforms can be significantly enhanced through the integration of nanomaterials, which improve electron transfer kinetics, increase electroactive surface area, and enable tailored functionalization for specific analyte interactions.

Carbon nanomaterials, notably graphene, carbon nanotubes (CNTs), and carbon nanofibers (CNFs), continue to dominate sensor development owing to their outstanding electrical conductivity, large surface-to-volume ratio, and ease of surface modification. The incorporation of oxygen-containing functional groups in graphene oxide (GO) and the partially restored conductivity in reduced graphene oxide (rGO) provide dual benefits: enhanced dispersibility and electronic performance. Recent research has demonstrated the fabrication of dopamine sensors using composites like C_{60}-fullerene with GO, where π–π interactions facilitate uniform dispersion and strong interfacial contact. This synergistic assembly enhances redox peak separation and improves electron transfer efficiency, particularly useful in detecting neurotransmitters in complex biological matrices. Hybrid platforms combining GO with noble metals or conducting polymers such as GO-AuNP-polyaniline films have shown improved electrocatalytic behaviour and stability for simultaneous detection of catecholamines and uric acid.

Metal nanoparticles, especially those composed of silver (AgNPs), gold (AuNPs), platinum (PtNPs), and bimetallic systems like PtRu, have gained traction due to their high electrocatalytic activity and tunable surface chemistry. Silver nanoparticle-modified glassy carbon electrodes (AgNP-GCEs) have been utilized for the precise quantification of norepinephrine, employing cyclic voltammetry (CV) and differential pulse voltammetry (DPV). The enhancement in signal output is attributed to the increased electroactive surface

and favourable redox behaviour induced by AgNPs.Pt and PtRu alloy nanoparticles, particularly when immobilized on carbon-based matrices, have been used in electrochemical advanced oxidation processes (EAOPs) to degrade pharmaceutical residues like ibuprofen and diclofenac. These alloys exhibit superior tolerance to poisoning intermediates and offer robust performance in harsh electrochemical environments. Recent studies are also focusing on Au@Pd core–shell nanoparticles for multiplex drug detection, leveraging the high conductivity of gold with the electrocatalytic features of palladium.

Transition metal oxides, such as CuO, MnO_2, NiO, and their spinel or perovskite-type derivatives, exhibit multiple oxidation states, enabling diverse redox interactions essential for drug sensing. Copper oxide (CuO), in particular, stands out due to its affordability, environmental friendliness, and catalytic activity in redox reactions. It has been widely employed in detecting compounds like paracetamol, acetaminophen, and ascorbic acid. Advanced sensor designs utilize $CuFe_2O_4$ and delafossite $CuFeO_2$ anchored on rGO nanosheets. In ionic liquid-supported media, these composites show significant improvements in conductivity, surface area, and catalytic response. Such nanohybrids have demonstrated sub-micromolar sensitivity in detecting pharmaceuticals in serum and wastewater samples. Furthermore, zinc ferrite ($ZnFe_2O_4$) and cobalt ferrite ($CoFe_2O_4$) have emerged as promising candidates for electrochemical immunosensors, offering enhanced charge transfer and biocompatibility for diagnostic applications.

i. Miniaturized and Portable Sensing Systems

Recent advancements in lab-on-a-chip (LOC) and micro total analysis systems (μTAS) have transformed the landscape of drug detection, especially for point-of-care diagnostics. These platforms integrate highly responsive nano sensors that can detect pharmaceuticals with minimal sample volume and preparation. Among them, electrochemical mini-sensors have gained traction due to their ability to provide real-time outputs based on electrical parameters such as voltage, current, impedance, and capacitance. By leveraging the unique properties of nanomaterials (NMs)including high surface-to-volume ratios and tunable electrochemical behaviorthese systems achieve enhanced accuracy and faster response times. Notably, paper-based and flexible substrates are now being incorporated to enable truly portable diagnostics, extending applications to remote or resource-limited environments. Coupling these systems with wireless technologies (e.g., Bluetooth, NFC) has also facilitated real-time monitoring and data transmission, bridging the gap between laboratory-grade analysis and point-of-need

healthcare. Carbon nano-onions (CNOs) have emerged as promising functional nanostructures for drug sensing due to their excellent electron transfer kinetics and surface reactivity. When composited with nanoparticles such as gold (AuNPs) or titanium dioxide (TiO_2), these nanostructures demonstrate superior conductivity and signal amplification. Recent research indicates that modifying CNOs with nitrogen atoms (to create nitrogen-doped CNOs or NCNOs) significantly boosts their performance in electrochemical sensors.A notable application involves the use of aminated nanodiamonds (AM-NDs) to anchor NCNOs, creating a highly stable and conductive interface for analyte detection. For instance, this configuration has been employed for the sensitive and rapid quantification of acetaminophen, a commonly used analgesic, in biological fluids. This hybrid platform benefits from reduced background noise, increased electron mobility, and exceptional reproducibility, thereby aligning well with current demands for selective and high-throughput pharmaceutical analysis.

Molecularly imprinted polymers (MIPs) function by creating polymer matrices that exhibit selective binding sites, mimicking natural receptor-ligand interactions. The integration of MIPs with advanced nanomaterials such as graphene derivatives, carbon nanotubes (CNTs), and MXeneshas led to dramatic improvements in selectivity and analytical performance. One recent innovation includes a sensor platform for adrenaline detection, where the MIP layer is synthesized using hydroxymethyl-functionalized 3,4-ethylenedioxythiophene(hydroxymethyl-EDOT). This design enables the sensor to achieve a high degree of molecular recognition, stability over repeated use, and a low limit of detection. The synergy between the electroactive polymer base and nanoscale reinforcements results in heightened sensor sensitivity, faster rebinding kinetics, and long-term usability. Furthermore, researchers are exploring stimuli-responsive MIPs that adjust their binding properties in response to pH, temperature, or external fields opening new avenues for smart drug sensors that operate dynamically under physiological conditions.

j. Hybrid Nanocomposites Based on MOFs and COFs

Metalorganic frameworks (MOFs) and covalent organic frameworks (COFs) are emerging as front-runners in the development of next-generation sensor materials due to their exceptional structural tunability, intrinsic porosity, and customizable surface functionalities. These porous crystalline materials offer a large surface area and multiple functional sites that enable selective and sensitive detection of various analytes, particularly in electrochemical

sensing.Recent innovations involve the incorporation of conductive additives such as reduced graphene oxide (rGO) into MOFs, forming hybrid systems like MU-22@rGO. [214] These composites overcome the inherent poor conductivity of pristine MOFs, enhancing charge transfer kinetics and promoting stronger analyte-framework interactions. Such features are particularly advantageous in the electrochemical detection of neurotransmitters like L-DOPA, where sensitivity and selectivity are crucial. The MU-22–rGO composite facilitates efficient electron relay and improves detection limits through synergistic conductivity and active surface sites.On the other hand, COFs provide a purely organic scaffold with stable covalent linkages, enabling robust architecture under harsh conditions. Recent research demonstrates the functional integration of platinum nanoparticles (PtNPs) and carbon nanotubes (CNTs) into COF matrices, resulting in a highly electroactive ternary composite. These materials exhibit significant improvements in electron mobility, redox activity, and analyte adsorption. A notable application includes the sensitive detection of polyphenolic compounds such as tanshinol, a pharmacologically active compound, wherein the COF–PtNP–CNT composite provides a rapid and accurate sensing platform due to its large electroactive surface and catalytic enhancement from PtNPs.

k. Optical and Bio-Nano Sensing for Drug Detection

Innovations in the field of optical and bio-nano sensing have significantly enhanced the sensitivity, selectivity, and speed of drug detection.Various optical readout strategies such as fluorescence, colorimetric responses, surface-enhanced Raman scattering (SERS), and phosphorescence have been refined through the integration of advanced nanomaterials including graphene quantum dots (GQDs), molecularly imprinted polymers (MIPs), and lanthanide-doped metal-organic frameworks (MOFs).GQDs have emerged as highly promising fluorophores due to their excellent biocompatibility, tunable emission properties, and chemical stability. When embedded in MIPs, GQDs can selectively recognize and detect analytes like methamphetamine through mechanisms such as fluorescence quenching. This selective recognition arises from the specific binding cavities of the MIPs, which mimic the structural features of the target molecule. The fluorescence of the GQDs diminishes upon interaction with the analyte, enabling quantitative sensing. Similarly, lanthanide-based MOFs have gained attention for their sharp emission bands and long-lived luminescence. These frameworks exhibit analyte-responsive photoluminescence quenching primarily through the inner filter effect (IFE), where the incoming or emitted light is partially absorbed by the analyte, resulting in measurable luminescence suppression. Such systems have been

successfully employed in the detection of small molecules, including drugs of abuse and pharmaceutical residues, due to their high signal-to-noise ratios.

Another frontier in bio-nano sensing involves the development of DNA-based electrochemical platforms. For instance, pencil graphite electrodes (PGEs) functionalized with gold nanoparticles (AuNPs) and immobilized double-stranded DNA (ds-DNA) provide a robust interface for drug detection based on DNA intercalation. This design allows for the monitoring of interactions between genotoxic drugs such as temozolomide and nucleic acids, where the degree of intercalation alters the electrochemical signal in a concentration-dependent manner. Such platforms offer a low-cost, disposable, and portable approach for monitoring chemotherapeutic agents and assessing their genotoxic profiles. In addition to nucleic acid-based systems, enzyme and antibody-immobilized bio-nanocomposites are widely employed for drug assays. These nanostructured electrodes often incorporate carbon nanotubes, graphene, or conductive polymers to enhance surface area and electron transfer kinetics. Enzymatic biosensors utilize specific biocatalysts (e.g., acetylcholinesterase or tyrosinase) that undergo inhibition or catalysis upon exposure to drug molecules, translating the biochemical interaction into a quantifiable electrochemical or optical signal. Similarly, immunosensors exploit antigen-antibody interactions for high-specificity detection of therapeutic or illicit substances, benefiting from the increased sensitivity conferred by nanoparticle labelling and signal amplification techniques.

A 2023 study introduced a dual-sensing colorimetric platform combining silver nanoparticles (AgNPs) and graphene quantum dots (GQDs) for the visual detection of amphetamine and methamphetamine, leveraging aggregation-induced changes in optical properties. Additionally, lanthanide-based metal-organic frameworks (MOFs) doped with europium (Eu^{3+}) ions have been optimized for real-time monitoring of tetracycline residues in environmental water samples, offering sharp emission signatures and high sensitivity. Meanwhile, DNA-functionalized electrodes are gaining attention for early-stage screening of anticancer agents, with detection specificity governed by molecular docking interactions and nucleic acid hybridization efficiency, providing a promising approach for label-free genotoxicity assessment. Among these, Carbon Dot-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (CD-LDI-TOF-MS), Surface-Assisted Laser Desorption/Ionization Mass Spectrometry (SALDI-MS), and Surface-Enhanced Laser Desorption/Ionization Mass Spectrometry (SELDI-MS) have gained significant attention.CD-

LDI-TOF-MS leverages the exceptional optical and surface properties of carbon-based nanomaterials, such as carbon dots (CDs), which act as efficient energy-absorbing matrices. Unlike traditional organic matrices used in MALDI, carbon dots offer minimal background interference in the low-mass region, improving signal clarity for small drug molecules. The method provides high sensitivity for detecting trace levels of analytes, even at femtomolar concentrations, making it ideal for bioanalytical and forensic drug screening. Recent studies highlight its application in monitoring anticancer drugs and psychoactive substances directly from biofluids without extensive sample preparation. SALDI-MS utilizes nanostructured substrates often composed of metal nanoparticles (e.g., silver, gold), graphene, or silicon nanowiresto enhance laser energy absorption and analyte desorption. These substrates replace traditional matrices and allow for direct analysis of small molecules, including poorly ionizing pharmaceutical compounds. The nanostructures promote effective energy transfer and reduce chemical noise, enabling sensitive detection with minimal sample consumption. Modern SALDI applications include metabolomics, pharmacokinetics, and environmental drug residue monitoring.SELDI-MS, a variation of SALDI, incorporates biochemical affinity to enhance molecular recognition. It combines surface-enhancing materials with chemically modified chip surfaces that selectively bind target analytes. This method is particularly useful for proteomic profiling and drug–target interaction studies. It allows for the multiplex detection of biomarkers and therapeutic agents simultaneously, offering a platform for personalized medicine development and therapeutic drug monitoring.

In optical absorption methods, gold nanoparticles (AuNPs) have enabled the detection of Metformin and Sitagliptin in tablet form, with detection limits of 1.01 and 1.21 µg/L, respectively, within linear ranges of 100–1450 µg/mL and 50–1400 µg/mL. Graphene quantum dots coupled with AuNPs have been effective in sensing Phenytoin from plasma at a sensitivity of 5 ng/mL. Silver nanoparticles stabilized on polyethyleneimine (PEI-AgNPs) successfully detected Promethazine in diverse matrices such as urine, serum, and tap water at 0.01 µM, with a range extending to 200 µM. Folic acid-coated AgNPs (FA-AgNPs) showed dual-mode capability, achieving detection of 6-mercaptopurine at both 13.2 nM (absorption) and 2.3 nM (fluorescence) from biological fluids and pharmaceutical samples. In the domain of nanozymes, MIL-53(Fe)-based platforms enabled salicylic acid analysis from aspirin tablets with a detection limit of 0.26 µmol/L, while PEI-DHB nanozymes measured serum heparin at 0.02 µM. A manganese-calcium oxide nanozyme (NL-$MnCaO_2$) enabled metformin detection from serum samples with a lower limit of 0.17 µM.Fluorescence-based

platforms included ZnO nanoparticles functionalized with cysteine (ZnO NPs@Cys) for detecting dopamine in urine, reaching 0.15 µg/mL. Carbon nanohorns (CNHs) facilitated dopamine detection in serum down to 5 mM, while β-cyclodextrin-modified nanodiamonds were used to detect doxorubicin in urine at levels as low as 18 ng/mL. Iron/nitrogen-doped carbon nanozymes (Fe/NC) enabled captopril quantification with detection limits of 0.47 µM and 0.56 mM, depending on matrix complexity. Light scattering techniques have also shown promise. AuNPs detected procainamide in urine and water at femtomolar levels (10^{-11} M), and silver-gold nanocrystals (Ag@Au NCs) quantified fentanyl and its analogs in sewage at 0.1 mg/L. Ephedrine and pseudoephedrine were measured using Ce^{3+}-modified AuNPs in water and blood, reaching sensitivities of 1.9 and 3.8 ng/mL, respectively. Erlotinib, a cancer drug, was detected in plasma using AuNPs with a 40 nM sensitivity.In electrochemical detection, various modes such as amperometry, cyclic voltammetry (CV), and differential pulse voltammetry (DPV) have been utilized. CuO nanoparticle-coated graphene-β-cyclodextrin composites detected metronidazole down to 0.6 nM. A $CuFeO_2$ nanoparticle-reduced graphene oxide composite enabled rosuvastatin detection at 10 pM levels. GQDs detected doxorubicin hydrochloride from plasma at 16 nM. A silver nanoparticle-based GCE system (Delphinidin@AgNPs-GCE) identified neurochemicals like noradrenaline and uric acid in urine and pharmaceutical forms at micromolar levels. Similarly, mixed oxides ($CuO/CuFe_2O_4$) enabled dual drug detection (acetaminophen and codeine) in urine and plasma at 0.007 and 0.01 µM, respectively. Adrenaline was detected using MXene@CNH composites in urine samples at sub-nanomolar levels (0.3 nM), while Idarubicin was measured from serum using La_2O_3NP composites at a sensitivity of 1.3 nM.Square wave voltammetry (SWV) with nanodiamonds facilitated codeine detection at 54.5 nM, and a 3D hybrid of MoS_2, polyaniline, and rGO simultaneously measured dopamine, uric acid, and ascorbic acid with detection limits ranging from 0.36 to 22.2 µM. Other platforms like $ZnFe_2O_4$/ZnO-GO hybrids detected carbamazepine from biological matrices at 3 nM. Novel composites like rGO-TiO_2 or polydopamine@Fe_3O_4 modified electrodes demonstrated detection of rifampicin and diclofenac at low nanomolar ranges.

Sensing systems further demonstrated utility in complex environments. For example, calix-resorcinarene-derived AgNPs (KM20-AgNPs) allowed methylene blue detection at 0.16 nM, and PEDOT-integrated gold nanocomposites enabled acetaminophen sensing from urine at 10 µM. Graphene-based sensors detected multiple drugs including piroxicam and nimesulide with detection ranges spanning nano to micromolar levels. Complex formulations such as

TiO_2NPs-CNFs were used to detect idarubicin from both urine and serum at 3 nM. Vertically oriented reduced graphene oxide (VrGO) decorated with palladium nanoparticles (BFED&VrGO/PdNPs) enhanced sorafenib detection down to sub-nanomolar levels (9.9×10^{-10} M).Mass spectrometric techniques driven by nanomaterials show great promise. Carbon-dot assisted laser desorption ionization time-of-flight mass spectrometry (CALDI-TOF-MS) detected mefenamic acid in serum at concentrations as low as 0.46–0.51 ng/µL. Surface-assisted LDI (SALDI-TOF-MS) using magnetic UiO-66-based molecularly imprinted polymers (MUMIPs) detected luteolin in rat plasma and urine at 0.5 ng/mL. Surface-enhanced LDI (SELDI-MS) with magnetic carbon-black Fe_3O_4 nanocomposites enabled picogram-level detection of compounds like labetalol, metoprolol, doxepin, and desipramine in wine samples.

l. Nanomaterials in Instrumental Separation Techniques

Nanomaterials (NMs), owing to their large surface area-to-volume ratio and tunable adsorption properties, are emerging as promising tools in advancing separation science. Their integration into instrumental analytical techniques has significantly enhanced the resolution, selectivity, and sensitivity of various chromatographic methodologies. In particular, their roles as stationary phases, pseudostationary phases (PSPs), or as nanostructured supports have transformed conventional techniques into highly efficient platforms for complex sample analysis.In modern high-performance liquid chromatography (HPLC), capillary electrophoresis, and thin-layer chromatography, both carbon-based nanostructures and inorganic nanomaterials have found versatile roles. Among these, carbon nanotubes (CNTs) are well recognized for their mechanical strength, electrical conductivity, and sorption capacity. However, their limited protein exclusion capacity hinders their use in direct analysis of biofluids. To overcome this, restricted access carbon nanotubes (RACNTs) have been designed. These modified CNTs were recently employed in a two-dimensional liquid chromatography platform to detect tetracycline residues in dairy matrices, demonstrating their applicability in pharmaceutical quality control and food safety.Similarly, fullerene (C_{60}) has been investigated as a novel stationary phase due to its chemical inertness and resistance to hydrolytic degradation. While inherently hydrophobic, its surface can be functionalized to improve solubility and separation performance in both reversed-phase and hydrophilic interaction liquid chromatography (HILIC). A noteworthy innovation involves the synthesis of carbonaceous spheres, prepared via hydrothermal routes and subsequently functionalized with poly(amidoamine) (PAMAM) dendrimers. These nanospheres have shown excellent

performance as latex beads in agglomerated anion-exchange chromatography for pharmaceutical applications, offering enhanced analyte retention and stability. Precise enantioseparation is vital in the pharmaceutical industry for the isolation of single enantiomers in chiral drugs. Nanomaterials, when conjugated with chiral selectors such as metal-organic frameworks (MOFs), covalent organic frameworks (COFs), or ionic liquids, enable highly specific enantio-recognition. The immobilization techniques include covalent grafting, physical adsorption, or ionic interactions depending on the nanomaterial's chemical nature and the chiral selector's stability. Advances in metal nanoparticle-supported chiral selectors have contributed significantly to resolving enantiomers of therapeutic agents from complex biological matrices**.** Despite progress, continuous efforts are being made to develop novel NM-based columns with improved chiral recognition, sample throughput, and selectivity under low-volume, high-throughput conditions.

Various nanomaterials have been effectively integrated into advanced separation techniques to enhance the analysis of pharmaceutical compounds and biological matrices. For instance, in liquid chromatography (LC), a column-switching LC method employing restricted access carbon nanotubes (RACNTs) was developed for the rapid quantification of tetracycline-class antibiotics specifically tetracycline, chlortetracycline, oxytetracycline, and doxycycline in milk, achieving a total analysis time of just 12 minutes and detection limits ranging from 7.50 to 13.2 µg/L. Another LC-based technique utilizing magnetic nanoparticles functionalized with poly(N-isopropylacrylamide) and grafted with 10-ADMPhP was applied to detect rivaroxaban in plasma, urine, and pharmaceutical formulations, with a detection threshold of 3.06 mg/mL. Capillary electrochromatography (CEC) has also seen significant advancements. Graphene oxide-based CEC systems were used to separate naproxen, warfarin, and pranoprofen in tablet formulations, although specific analysis time and detection limits were not disclosed. A notable development involved a metal-organic framework (MOF) comprising pepsin and ZIF-8 integrated into a poly(GMA-co-EDMA) monolith, which enabled the profiling of multiple analytes including hydroxychloroquine, chloroquine, hydroxyzine, nefopam, clenbuterol, and amlodipine in pharmaceutical products over a 63-hour run time. Similarly, a covalent organic framework (TpPa-1) was applied in open-tubular CEC for analyzing tetracyclines in tablets, requiring 12 hours of analysis. Another monolithic CEC system employed β-cyclodextrin-capped gold nanoparticles (β-CD-AuNPs) for the determination of zopiclone, tropicamide, and chlorpheniramine in tablet samples, completing the analysis within 10 to 15 minutes and achieving detection limits as

low as 0.020 µg/mL. In microemulsion electrokinetic chromatography (MEEKC), fullerene-based materials were used to detect ciprofloxacin, norfloxacin, sulfamethoxazole, tetracycline, and oxytetracycline in animal feed within six minutes, with sensitivity levels between 0.700 and 1.50 µg/g. Furthermore, in capillary electrophoresis (CE), graphene quantum dots (GQDs) facilitated the determination of cinnamic acid and related derivatives in pharmaceutical tablets in under 18 minutes, with detection thresholds ranging from 11.7 to 41.8 mg/L. These innovations underscore the potential of nanostructured stationary phases to significantly improve separation efficiency, sensitivity, and selectivity in pharmaceutical analysis.

CONCLUSION

The emergence of nanomaterials has fundamentally reshaped the landscape of pharmaceutical and biomedical analysis, offering a sophisticated toolkit for enhancing sensitivity, selectivity, and functionality across analytical platforms. Their integration into sample preparation, separation, and detection stages has enabled more precise monitoring of therapeutic agents in complex matrices. Nanostructured materials such as metal-organic frameworks, carbon-based nanostructures, and engineered composites exhibit tunable properties that not only improve analytical throughput but also facilitate miniaturization and point-of-care applicability. Despite these advances, several critical issues remain unresolved. The complexity of nano formulations comprising multiple interactive components introduces challenges in stability, reproducibility, and regulatory classification. Standardized protocols for their analytical characterization are still lacking, impeding streamlined approval pathways and comprehensive safety evaluations. Moreover, the unique physicochemical behavior of nanomaterials at the nanoscale often limits the applicability of conventional techniques, necessitating either adaptation or the creation of novel hybrid methods. Moving forward, there is a pressing need to bridge methodological gaps by developing unified analytical frameworks and regulatory guidelines tailored specifically for nano-enabled pharmaceuticals. Interdisciplinary collaborations will be instrumental in driving innovations that align with both scientific rigor and regulatory compliance. Additionally, the long-term biocompatibility and environmental fate of these materials must be rigorously assessed to ensure sustainable integration into healthcare. As the field matures, embracing the full potential of nanomaterials will require not just technical advancement but also a holistic approach to risk management and ethical deployment. Ultimately, these efforts will pave the way for safer, smarter, and more effective pharmaceutical diagnostics and therapeutics.

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