magnetochemistry

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Review

Bio-Catalysis and Biomedical Perspectives of Magnetic Nanoparticles as Versatile Carriers

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Received: 9 May 2019; Accepted: 27 June 2019; Published: 2 July 2019

Abstract: In recent years, magnetic nanoparticles (MNPs) have gained increasing attention as versatile carriers because of their unique magnetic properties, biocatalytic functionalities, and capabilities to work at the cellular and molecular level of biological interactions. Moreover, owing to their exceptional functional properties, such as large surface area, large surface-to-volume ratio, and mobility and high mass transference, MNPs have been employed in several applications in different sectors such as supporting matrices for enzymes immobilization and controlled release of drugs in biomedicine. Unlike non-magnetic carriers, MNPs can be easily separated and recovered using an external magnetic field. In addition to their biocompatible microenvironment, the application of MNPs represents a remarkable green chemistry approach. Herein, we focused on state-of-the-art two majorly studied perspectives of MNPs as versatile carriers for (1) matrices for enzymes immobilization, and (2) matrices for controlled drug delivery. Specifically, from the applied perspectives of magnetic nanoparticles, a series of different applications with suitable examples are discussed in detail. The second half is focused on different metal-based magnetic nanoparticles and their exploitation for biomedical purposes.

Keywords: green chemistry; magnetic nanoparticles; enzyme immobilization; controlled drug delivery; supporting materials

1. Introduction

Green or sustainable chemistry is the utilization of a set of principles that diminishes the use of toxic substances in the design, manufacture, and application of the chemical product. This fact encouraged the researchers and scientific community to discover simple and effective methods for the separation of homogenous catalysts from the reaction mixture and their subsequent recycling. The use of magnetic nanoparticles (MNPs) as efficient support materials for biocatalyst immobilization has become a theme of considerable interest. A range of attractive properties including high surface area, large surface-to-volume ratio, facile separation using external magnetic fields, and high mass transfer make MNPs ideal candidate for diverse biomedical applications [1,2]. MNPs exhibit their highest performance at sizes typical ranges from 10 to 20 nm due to the occurrence of the superparamagnetism property [3]. Recently, MNPs find potential use in catalysis including nanostructured material-assisted biocatalysts immobilization, biomedicine, target-oriented drug delivery, magnetic resonance imaging

(MRI), microfluidics, nanofluids, optical filters, data storage, and environmental remediation [4]. Herein, we focused on state-of-the-art two majorly studied applications of MNPs as versatile carriers for (1) matrices for enzymes immobilization, and (2) matrices for controlled drug delivery. Following the introduction, the formation and stabilization of magnetic nanoparticles are briefly discussed. From the applied perspectives of magnetic nanoparticles, a series of different applications with suitable examples are discussed in detail. The second half is focused on different metal-based magnetic nanoparticles and their exploitation for biomedical purposes.

2. Magnetic Nanoparticles: Formation and Stabilization

MNPs properties are firmly dependent on formation and construction method. For the synthesis of high-quality nanoparticles, particle-size distribution, particle size, symmetry, and crystallization have been governed with the help of advance colloidal system [5]. All these features of MNPs provide mono-dispersity and homogeneity in the target system. Sometimes, MNP required a stabilizing agent in a competitive environment to avoid from agglomeration, for example, in dipolar conditions, these particles face high surface-to-volume ratio [6]. Currently, there is a variety of nanomaterials available commercially [7]. Therefore, it reduces the effort of nanoparticle researchers and increases productivity. In spite of these facts, more work and experiments are needed to customize, develop, and synthesize purpose-built nanoparticles.

From a material chemistry perspective, the material used for the development of magnetic entity can be originated or constructed from iron (oxides), nickels, and cobalt. Sometimes elements such as strontium, zinc, barium, and zinc can also be conjugated with metals. Generally, MNPs belongs to the nanoalloy and metallic nanomaterials that are coated with specific kind of molecules [8,9]. These modifications make it target specific, enhance the stability, and improve the physiochemical properties (corrosion, oxidation, agglomeration, and toxicity) of nanoparticles [10,11]. Lastly, customized modification of MNPs both core and surface depend upon the system to be applied [12]. For example, agglomeration of MNPs with avidin–biotin (bifunctional linkers) increase the stability up to several months [13], oligos-based modification was used for DNA detection [14,15], modification with iron oxide or ferrites is the potential application for X-ray computed tomography or MRI [16]. Similarly, MNPs were coated with other conjugated molecules such as liposomes, micelles, polymeric coating, and core–shell structures (Figure 1) [17].

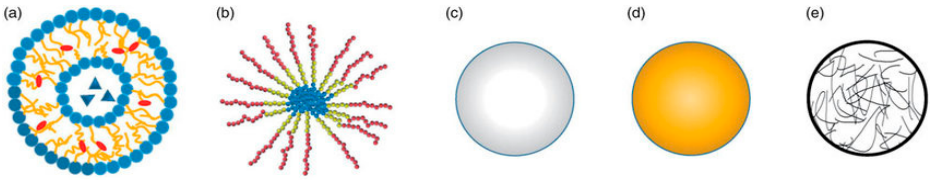

Figure 1. Representative structures of various NPs for drug delivery. (**a**) Liposomes, (**b**) polymeric micelles, (**c**) polymeric nanoparticle, (**d**) gold nanoparticle, and (**e**) nanogel. Reprinted from Wang et al. [17], an open-access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/). Copyright (2018) the author(s). Published by Informa UK Limited, London, UK, trading as Taylor & Francis Group.

3. Applications of Magnetic Nanoparticles

3.1. Bio-Catalysis Perspectives of Magnetic Nanoparticles

Over the past two decades, a wide variety of nano-carriers has been fabricated and applied as enzymes immobilization supports for diverse applications (Table 1). Among these support materials, MNPs have received substantial attention as versatile carriers, because of their unique physicochemical and magnetic properties, biodegradability, biocompatibility, low cost, and tailor-made surface chemistry. Also, MNPs allow facile, rapid, and efficient biocatalyst separation from the reaction media by using an external magnet [18].

Table 1. Summary of magnetic nanomaterials as versatile carriers for enzymes immobilization, and their applications

Magnetic Carrier	Name of Enzyme	Immobilization Technique	Improved Properties and Application of Immobilized Enzymes	References
Magnetic graphene oxide	Chloroperoxidase/glucose oxidase	Physical adsorption	Excellent catalytic efficiency, operational durability, and recyclability. Immobilized biocatalyst showed far greater thermal stability compared with the native enzyme. It presented an application in decolorization and degradation of many synthetic dyes from industrial wastewater.	[19]
hydrophobic virus-like organosilica nanoparticles	*Candida antarctica* lipase B	Covalent attachment	Improved pH and thermal resistance High tolerance to organic solvents and long-term storage stability. Efficient esterification reaction of levulinic acid retaining 75.7% of the levulinic acid transformation after 9 continuous biocatalytic cycles.	[20]
Chitosan-cross-linked magnetic nanoparticles	*Candida antarctica* lipase B	Covalent attachment	Superior separation and biocatalytic properties. Excellent storage stability and reusability. Production of bio-based photocurable oligo-esters by the ring opening esterification of polyols and itaconic anhydride.	[21]
Barium ferrite magnetic microparticles	Alcohol oxidase	Covalent attachment	Enhanced thermostability retaining > 65% of the original activity at 45 °C for 24 h. Good catalytic efficacy for oxidizing ethanol and methanol compared with the free enzyme. Recyclability for at three successive batches with 70% activity retention.	[22]
Glutathione-coated gold magnetic nanoparticles	Inulinase	Covalent binding	Enhanced storage and reusability stability. Immobilized biocatalyst preserved about 78% of its original activity after 10 repeated cycles. Improved enzyme performance at acidic pHs (3.0 and 4.0) and high temperature up to 80 °C. Complete hydrolysis of inulin to fructose and glucose.	[23]
Ionic liquid-modified magnetic chitosan composites	Lipase	Adsorption	Elevated catalytic activity (6.72-fold) as compared to the free enzyme Enhanced thermal stability and reusability retaining 92.1% of residual activity after 10 cycles of reuse.	[24]
Fe_3O_4 magnetic nanoparticles functionalized with wheat gluten hydrolysates	Inulinase	Covalent binding	High activity over a broader pH and temperature ranges, and also exhibited pronounced storage and thermal stability. The inulinase showed 12.3 folds rise in enzyme half-life value at 75 °C Potential recyclability retaining 70% of its preliminary catalytic activity after 12 continuous inulin hydrolysis cycles.	[25]
FunctionalizedAPTMS-magnetite nanoparticles	Cellulase and pectinase	Covalent immobilization	Improved characteristics such as high activities recovery, enhanced temperature stability (2.39-times greater than that to the free enzyme), and reusability for up to 8 continuous cycles in grape juice clarification.	[26]
Chitosan-montmorillonite nanocomposite beads	α-amylase	Cross-linking	High enzyme activity and stability at varying pH and temperature conditions than the free enzyme. Retention of about 53% enzyme relative activity after recycling 5 times	[27]
Chitosan magnetic nanoparticles	Pectinase	Cross-linking	Superior thermal stability than the soluble form of the enzyme. High stabilization retaining 87% of original activity after seven repeated cycles. Excellent durability. Potential apple juice clarification with up to 74% turbidity reduction after 2.5 h of treatment.	[28]
Amino-functionalized magnetic nanoparticle	α-amylase, cellulose, and pectinase	Cross-linking	Increased pH and thermal stability Encouraging enzyme reusability preserving up to 75% of activity after 8 reuse cycles. Clarification of fruit juices. Significant decrease in turbidity.	[29]
Magnetic cornstarch microspheres	Pectinase	Adsorption	Improved pH and thermal stability. Good reusability and operability of the immobilized biocatalyst preserving 60% of its initial activity after 8 reuses in apple juice processing.	[30]
Magnetic Fe_3O_4@chitosan nanoparticles	Lipase	Covalent immobilization	Immobilized biocatalyst presented more than 50% and 75% residual activity in the pH range 7.0–11.0, and 70 °C. Satisfactory reusability preserving 70% of its original activity after 10 repeated cycles. More than 50% conversion of ascorbic acid was achieved when used for ascorbyl palmitate synthesis in tert-butanol at 50 °C.	[31]

APTMS—3-aminopropyltrimethoxysilane.

3.2. Degradation of Dye Pollutants

Different dyes including acid blue 45, crystal violet, and orange G have shown wider applications in paper, textile, food, and many other industries. However, these dyes and other dyes containing pollutants pose severe threats to human health and aquatic organisms [32]. Due to their high photolytic and chemical stability, these dye pollutants are resilient to classical chemical, physical, and biological treatment methods. Enzymes as biocatalysts can be employed both in free as well as immobilized forms in the treatment of dyes or dyes-harboring industrial wastewater. However, immobilized enzymes present the advantages of durable catalytic stability, easy separation, and recovery, and multiple recycling, which improve the performance and trim-down the overall cost of industrial bioprocess [33–38]. Immobilized enzymes can be developed by different chemical and physical methods, which affect biocatalytic properties of the resulting immobilized system, and hence their applications in explicit processes [39,40].

Reports have shown the fascinating efficiency of chloroperoxidase (CPO) in degrading an array of synthetic dyes. Nevertheless, the lack of durable functioning stability and difficulty in recycling CPO hampered its large-scale application in wastewater bioremediation. To overcome this issue, Gao and coworkers, (2019) co-immobilized CPO and glucose oxidase (GOx) on the surface of magnetic graphene oxide (MGO). The catalytic performance of MGO-GOx-CPO considerably enhanced (96.6%) towards the degradation of orange G relative to MGO-GOx+MGO-CPO (86.2%), presumably because of reduced mass transfer limitation between CPO and H_2O_2 produced from GOx molecules (Figure 2) [19]. Remarkably, MGO-GOx-CPO exhibited its maximum activity at a temperature above 40 °C compared with the optimal temperature of 35 °C for the soluble biocatalyst. It also showed potential repetitive usability retaining ~38.5% of initial activity after six dye-decolorization cycles demonstrating the possibility of co-immobilized CPO and GOx in environmental applications. A peroxidase enzyme isolated and purified from the textile wastewater was immobilized on glutaraldehyde-functionalized Fe_3O_4 MNPs. The MNPs-insolubilized enzyme showed remarkable stability towards a range of pH and temperature perturbations than to the free form of the enzyme. It retained complete catalytic activity following storage at 4 °C and 25 °C for three months, and upon reusing for up to 100 repeated cycles. Moreover, the MNPs-assisted novel peroxidase was effectively used for the decolorization and degradation of industry wastewater containing direct green or reactive red azo dye pollutants in a prototype sequential lab-scale bioreactor [41]. In a recent study, Kashefi and coworkers [42] synthesized the magnetic graphene oxide (MGO) by integrating exclusive GO properties with the superparamagnetic characteristics of the $CuFe_2O_4$ nanoparticles. The amine group on MGO was functionalized chemically modified with 3-amino propyl trimethoxy silane and cross-linked activated with GLU. As-prepared functionalized MGO was utilized to covalently immobilize a laccase enzyme from genetically modified *Aspergillus* and exploited to degrade an azo dye Direct Red 23 using the response surface methodology. Results revealed that the immobilized nanobiocatalyst caused a maximum decolorization efficiency of 95.33% under the optimal conditions—i.e., pH, dye concentration, and enzyme dosage of 4.23, 19.60 mg/L, and 290.23 mg/L—respectively. In conclusion, it can be stated that the superparamagnetic nanomaterials-immobilized enzyme can potentially act as green and environmentally responsive nanobiocatalyst for efficient decolorization purposes [42].

3.3. Fruit Juice Clarification

In recent years, the development of new strategies for fruit juice clarification has gained a great interest in improving the quality of the juices. The turbid and cloudy appearance of the freshly prepared fruit juices resulting from the colloidal dispersion of pectin is one of the major issues in fruit juice processing [43]. Moreover, the presence of starch and other polysaccharides (i.e., cellulose and hemicellulose) tend to settle down during preservation resulting in haziness and poor-quality fruit juice [44]. Current employing microfiltration and ultrafiltration clarification technologies are restricted during the elimination of suspended pulp particles causing membrane fouling and reduce the membrane lifespan [45]. The use of immobilized enzymes has been substantially increased in

fruit juice industry to circumvent turbidity, cloudiness, and undesirable haziness accompanied by improving juice yield, quality, and shelf life [46]. Huang et al. [47] designed a biocompatible magnetic chitin nanofiber biocomposite using GLU cross-linker and used as a novel support material for chymotrypsin immobilization with excellent catalytic properties (Figure 3). The GLU-cross-linked insolubilized enzymes exhibited 70.7% of its original activity by incubating at 60 °C for 3 h, whereas the non-immobilized chymotrypsin showed only 29.6% of activity under identical conditions. After storage for 20 days, the immobilized nanobiocatalyst presented 84.9% of the initial activity, as compared to 18.8% for the free enzyme. After enzyme immobilization onto this magnetic nanobiocomposite, the loading capacity of the enzyme was improved up to 6.3-fold following GLU cross-linking. Moreover, the immobilized biocatalyst was easily recovered and recycled from the reaction mixture [47].

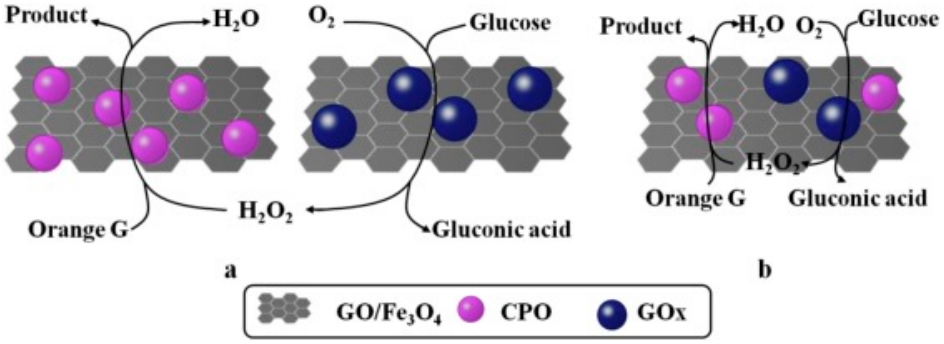

Figure 2. (**a**) Mass transfer resistance between chloroperoxidase (CPO) and glucose oxidase (GOx) for MGO-GOx+MGO-CPO; (**b**) no mass transfer resistance between CPO and GOx for MGO-GOx-CPO. Reprinted from Gao et al. [19], with permission from Elsevier. Copyright (2018) Elsevier B.V.

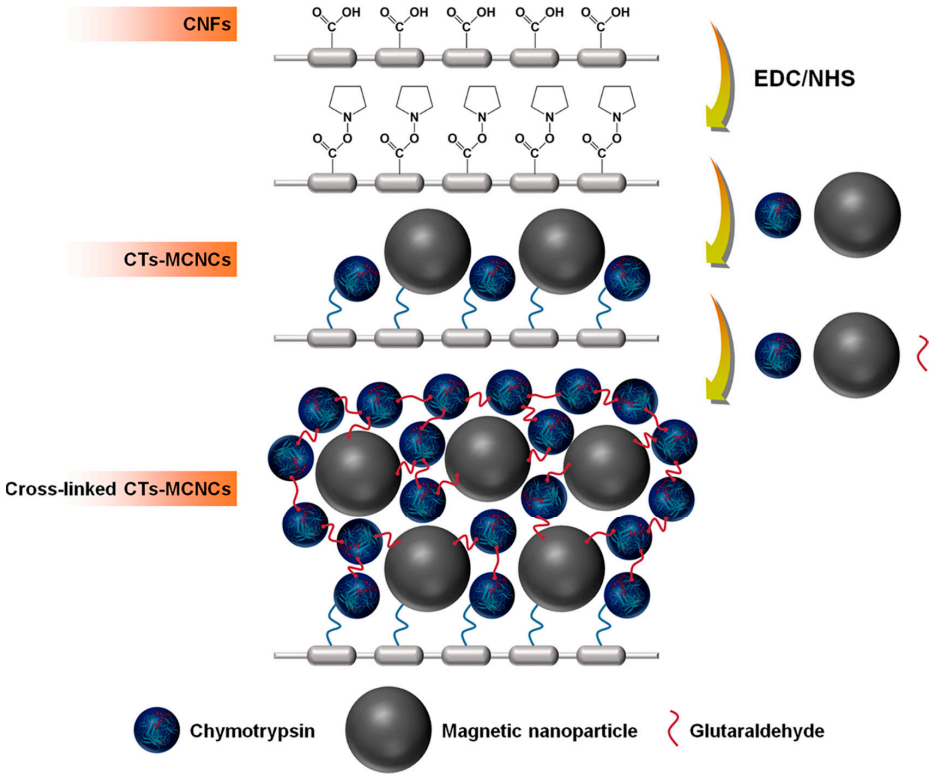

Figure 3. Schematic illustration of the immobilization of chymotrypsin onto the magnetic chitin nanofiber composite. Reprinted from Huang et al. [47], with permission from American Chemical Society. Copyright (2018) American Chemical Society.

Cross-linking agents play a critical role and have a direct effect on activity recovery and functional stability during cross-linking of the enzymes on nano-supports. Glutaraldehyde is considered the preferred cross-linking agent amongst a range of protein cross-linkers due to its cheapness, easy availability, and manipulation and the ability to form covalent bonding with the majority of the enzymes [48]. Nevertheless, some inherent drawbacks are associated with the use of glutaraldehyde as a cross-linker. The use of GLU cross-linker, in some instances, led to complete enzyme deactivation by penetrating into the catalytic site and cross-linking with biocatalytically important amino acids residues due to its small size [49]. Moreover, GLU-assisted cross-linking of enzyme molecules forms enzyme lumps that hinder the active sites resulting in mass transfer resistances and diminished catalytic efficiency [50]. Consequently, polysaccharides-based cross-linkers have gained great attention in the past years for cross-linking of proteins over the use of GLU. Sojitra and coworkers [29] synthesized magnetic tri-enzyme nanobiocatalyst by insolubilizing three enzymes, i.e., α-amylase, pectinase and cellulase onto chitosan MNPs using dextran polyaldehyde as a macromolecular cross-linker and utilized for fruit juice clarification. As compared to the soluble enzyme, the MNPs-immobilized biocatalysts presented more than 2-folds increment in half-life and enhanced tolerance to lower pH. Identical K_m and V_{max} values of the native and immobilized forms of pectinase revealed that conformational flexibility of enzyme was not altered after immobilization. In addition, the magnetic tri-enzyme presented 41%, 53%, and 46% reduction in turbidity for the clarification of apple, pineapple, and grapes juices after 2.5 h treatment. Results revealed that magnetic nanobiocatalysts might be a suitable technology for fruit juice clarification, owing to its possibility to separate enzymes from the reaction mixture and, subsequently, the reutilization of biocatalysts in multiple reaction cycles [29]. A significant improvement in pH and thermal stability of pectinase from *Penicillium oxalicum* F67 has also been achieved by immobilizing enzyme onto magnetic cornstarch microspheres. The resultant biocatalyst displayed about 60% of its preliminary activity after eight successive reuses for apple juice processing [30].

3.4. Biotransformation of Inulin to High Fructose Syrup

Inulin is a polysaccharide made up of fructose monomers and seems to be an abundant source for the production of fructooligosaccharides and fructose syrup in pharmaceutical and food industries. As a low caloric sweetener and prebiotic, fructooligosaccharides possess human health promoting effects such as a reduction in serum triglyceride and cholesterol levels, and significant improvement in the intestinal microbial flora [51,52]. Fructose is an inexpensive, low-calorie, and GRAS-approved sweetener and utilized as a safe alternative sweetener in diabetic patients [53]. Inulinase (EC:3.2.1.7) is well-known hydrolytic biocatalyst that can produce a high level of pure fructose syrup from inulin by a one-step enzymatic process in contrast to uneconomical and hazardous acid hydrolysis, as well as, multi-step enzymatic starch breakdown by glucoamylase, glucose isomerase, α-amylase, and pullulanase [54,55]. Due to lower solubility and high microbial contamination of inulin in the water at room temperature, industrial-scale inulin hydrolysis needs to be executed at elevated temperatures for higher inulin substrate utilization due to the increased solubility [56]. Therefore, thermostable inulinases are desirable biocatalysts for the chemical and food industry. In this context, Torabizadeh and Mahmoudi [25], covalently attached inulinase from *Aspergillus niger* onto wheat gluten hydrolysates (WGHs)-functionalized Fe_3O_4 MNPs in the presence of glutaraldehyde as a cross-linking agent. As-developed inulinase was found to be active over a broader pH and temperature ranges, and also exhibited pronounced storage and thermal stability. The inulinase showed 12.3 folds rise in enzyme half-life value following immobilization on MNPs at 75 °C and retained 70% of its preliminary catalytic activity after 12 continuous inulin hydrolysis cycles [25]. More recently, Mohammadi et al. [23] fabricated unique biocompatible support i.e., glutathione-coated gold MNPs (GSH-AuMNPs) and used for the covalent immobilization of the inulinase enzyme (Figure 4). The resulting magnetically recoverable and solid biocatalyst was applied for efficient biotransformation of inulin to high fructose syrup. After immobilization, the storage stability and reusability of inulinase were considerably

improved, and the immobilized biocatalyst preserved about 78% of its original activity after 10 repeated cycles. Immobilization on MNPs improved the enzyme performance at acidic pHs (3.0 and 4.0) and high temperature up to 80 °C. Chromatographic results revealed the complete hydrolysis of inulin and the end-products of both free and immobilized biocatalytic systems only consisted of 98% of fructose and up to 2% of glucose. The findings demonstrated that the magnetic nanomaterials-immobilized inulinase might display a high potential for larger scale synthesis of high fructose syrup and fructooligosaccharides useful for biotechnological, food, and biomedical industries [23].

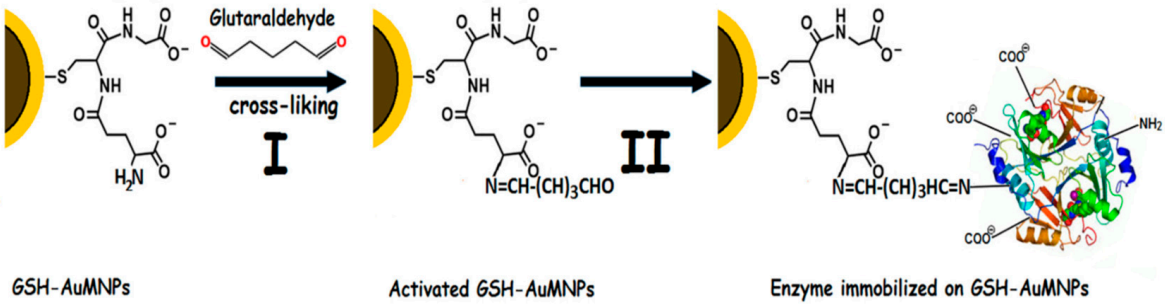

Figure 4. Enzyme immobilization steps including (**I**) cross-linkage of glutathione decorated Fe_3O_4-Au magnetic nanoparticles (GSH-AuMNPs) with GA, and (**II**) enzyme immobilization on GSH-AuMNPs surface. Reprinted from Mohammadi et al. [23], with permission from Elsevier. Copyright (2018) Elsevier B.V.

3.5. Other Applications

In recent years, fabrication of virus-like structured particles has appeared as one of the most creative and state-of-the-art support materials for enzymes immobilization. These novel materials offered a noteworthy biocatalytic platform enabling the durable stability and reusability of the enzymes [57]. For instance, P22 virus-like particles [58], Qβ virus-like particles [59], and cowpea chlorotic mottle virus particles [60,61] have been effectively created and employed for the enzyme immobilization. To date, a range of enzymes has been encapsulated onto these virus-like nano-carriers with the significant activity retention against extreme pH and temperature ranges [57]. For the first time, Jiang and coworkers [20] successfully synthesized hydrophobic virus-like organosilica nanoparticles (VOSNs) with a spherical core (Figure 5A) and employed for the covalent binding of the *Candida antarctica* lipase B (CALB). The synthesized hydrophobic VOSNs secured the active conformation of the CALB from the external hazardous environment because of stronger hydrophobic interfaces between the CALB molecules and VOSNs. As a result, the newly developed CALB@VSNs (Figure 5B) presented superior pH and thermal resistance, high tolerance to organic solvents and long-term storage stability. Under the optimized operating conditions, the CALB@VOSNs efficiently catalyzed the esterification reaction of levulinic acid and n-lauryl alcohol. Interestingly, it maintained 75.7% of the levulinic acid transformation even after nine continuous biocatalytic cycles [20].

Recently, subject to the requisite application, enzymes have been integrated with nanomaterials as immobilization carriers to engineer nano-biocatalysts [62]. Hosseini et al. [21] prepared new nano-magnetic biocatalyst particles following immobilization of CALB onto chitosan-cross-linked MNPs (Figure 6). The as-synthesized CALB-immobilized nanoparticles showed high storage stability and repeatability because of tightly cross-linked chitosan structure and covalent bonding. The newly developed magnetic biocatalyst efficiently catalyzed the ring opening esterification of itaconic anhydride as compared to the free enzyme [21]. In another study, *C. rugosa* lipase (CRL) coupled to new zwitterionic polymer-grafted silica nanoparticles (SNPs-pOD-CRL) displayed substantially improved enzyme–substrate affinity and catalytic performance than soluble enzyme due to the activation of lipase by the hydrophobic alkyl chains of the polymer (Figure 7) [63]. Moreover, a significant increase in thermal stability profile indicates that zwitterionic polymer with shorter alkyl side chains is advantageous to develop thermostable enzymes [63]. Of most recent, Suo et al. [24] developed a novel

immobilization strategy to increase the catalytic stability of lipase enzyme. In this method, lipase was adsorbed on ionic liquid (IL) modified magnetic chitosan (MCS) composites and graphene oxide (GO) nanosheets served as shell coating for anchoring the lipase structure (Figure 8) [24]. The GO-shielded novel biocatalytic system sustained 6.72-fold high activity as compared to the free enzyme. In addition, the thermal stability was also enhanced, and the immobilized enzyme retained 92.1% of residual activity after 10 cycles of reuse [24]. Wang et al. [31] synthesized stable magnetic Fe_3O_4@chitosan nanoparticles by an efficient and simple in-situ co-precipitation technique and used to chemically conjugate lipase from *Thermomyces lanuginosus* by covalent immobilization. Besides a broader pH and thermal tolerance, the immobilized lipase also exhibited good reusability maintaining 70% of original activity after 10 reaction batches. The nanobiocatalyst achieved higher than 50% conversion of ascorbic acid when used for ascorbyl palmitate synthesis in tert-butanol at 50 °C [31].

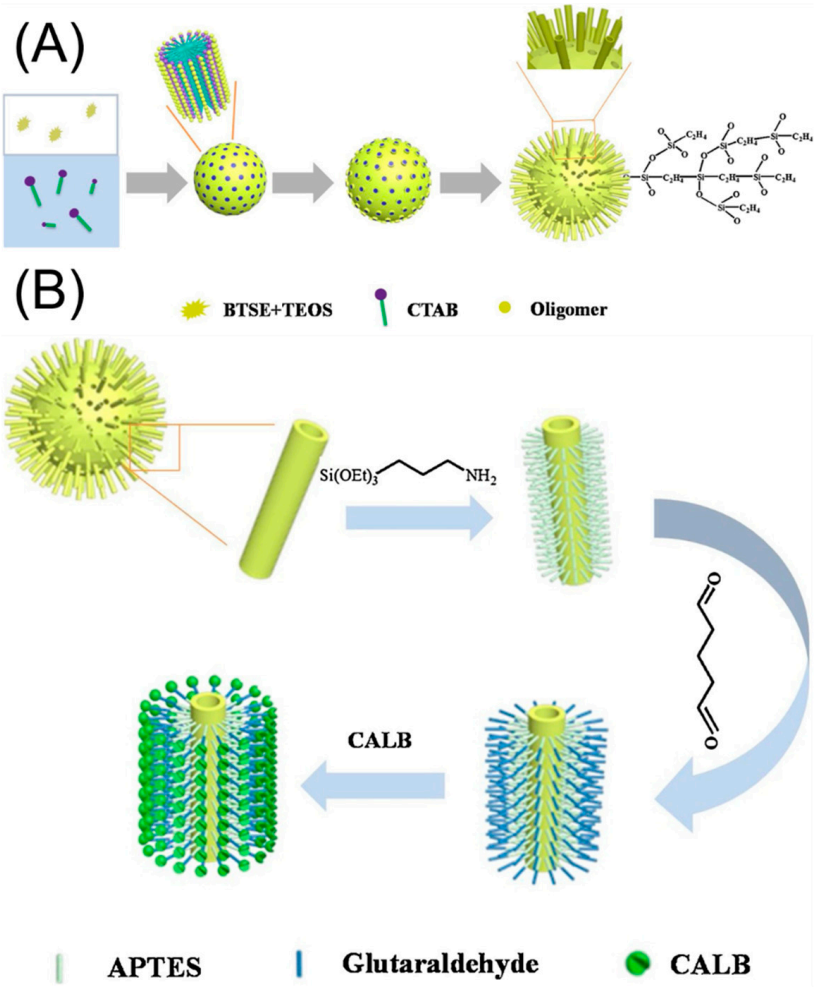

Figure 5. (**A**) Schematic illustration of the formation process of hydrophobic virus-like organosilica nanoparticles VOSNs and (**B**) preparation of the hydrophobic virus-like organosilica nanoparticles immobilized lipase B from *Candida antarctica*. Reprinted from Jiang et al. [20], with permission from Elsevier. Copyright (2019) Elsevier B.V.

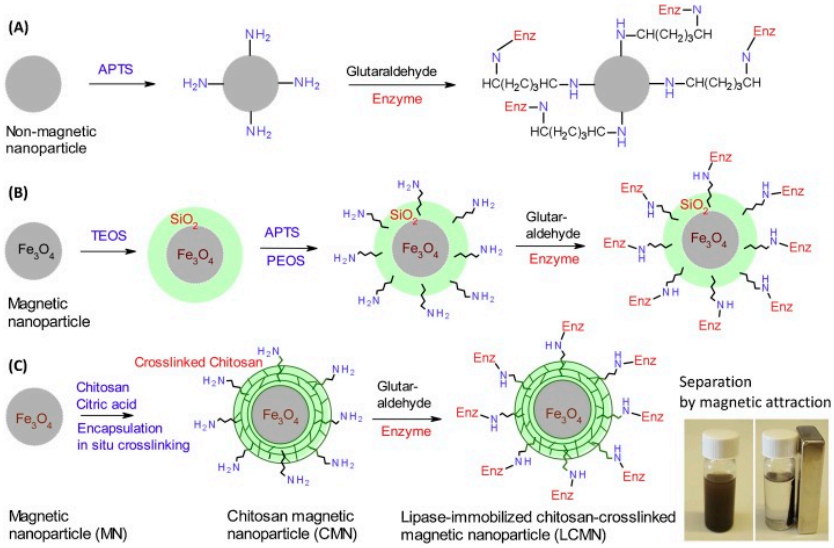

Figure 6. Representative illustrations of enzyme immobilization in the nanoparticle. (**A**) Conventional method using non-magnetic nanoparticle. (**B**) Conventional method using magnetic nanoparticle and organosilane compounds. (**C**) Present study using magnetic nanoparticle and chitosan crosslinked for immobilization of enzyme (lipase). APTS: 3-aminopropyltriethoxysilane, TEOS: tetra-ethoxy silane, PEOS: poly-ethoxysilane. Reprinted from Hosseini et al. [21], with permission from Elsevier. Copyright (2018) Elsevier B.V. (**A**) and (**B**) modified by Hosseini et al. [21] from Kim et al. [62], an open access article distributed under the Creative Commons Attribution License. Copyright (2018) the authors. Licensee MDPI, Basel, Switzerland.

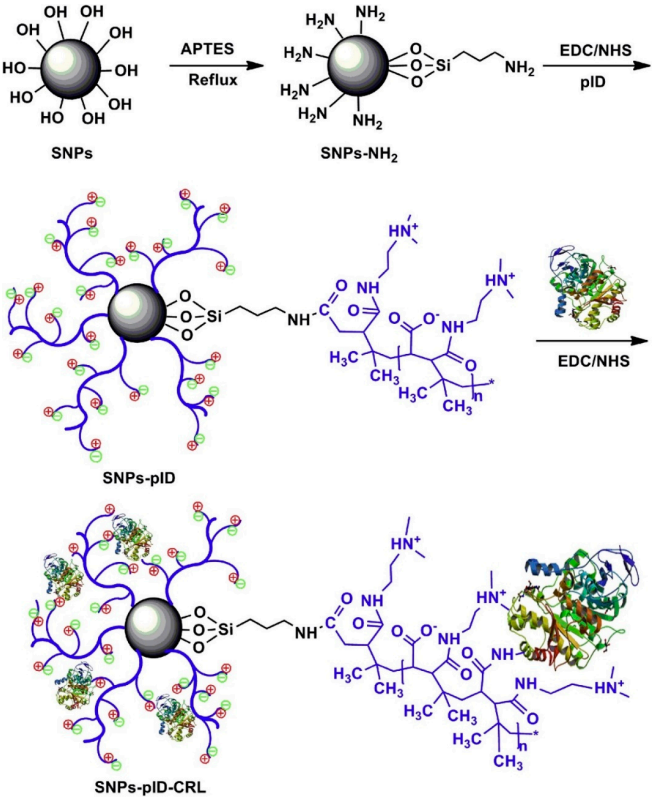

Figure 7. Schematic for the preparation of immobilized lipase on poly(4-((2-(dimethylamino)ethyl) amino)-4-oxobut-2-enoic acid-alt-isobutylene) (pID)-grafted silica nanoparticles. Reprinted from Zhang et al. [63], with permission from Elsevier. Copyright (2019) Elsevier B.V.

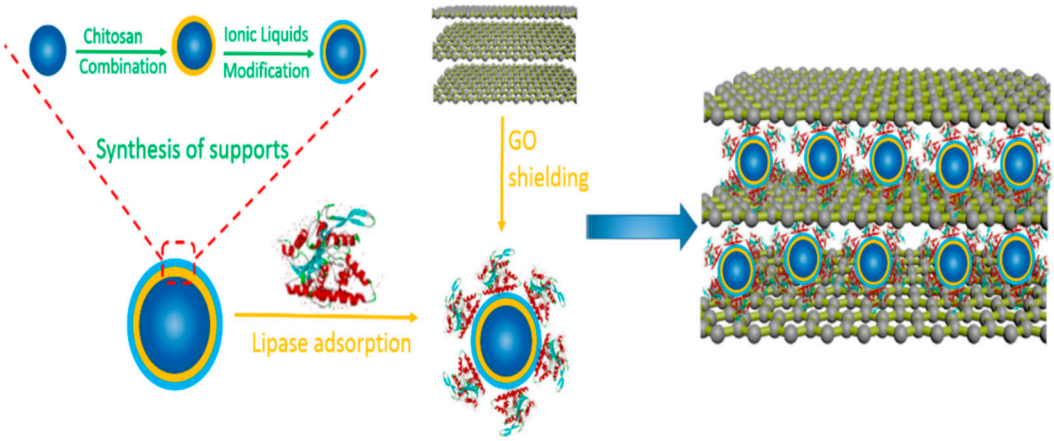

Figure 8. Immobilization of lipase on ionic liquid (IL) modified magnetic chitosan (MCS) composites using graphene oxide (GO) as shell coating. Reprinted from Suo et al. [24], with permission from American Chemical Society. Copyright (2019) American Chemical Society.

4. Biomedical Perspectives of Magnetic Nanomaterials

Magnetic nanoparticles are the most promising area and have a wide range in multi-disciplinary applications because of its unique features such as eco-remediation, biological purification, magnetic fluids, and a number of biomedical applications including hyperthermia mediated cancer treatment, biosensors for disease detection (MRI), and site-specific drug delivery. It varies in size from few to tens of nanometers (nm) [64]. It means MNPs are smaller than a cell (10–100 μm), protein (5–50 nm), gene (2 nm wide and 10–100 nm long), and virus (20–450 nm). Its small size makes enable to get closer to the desired targets. Although, NMPs coated with some biological interacting molecules that enable it to penetrate or tag the biological object [65]. MNPs obey the coulombs law that means it can be deployed by any outward magnetic gradient. These properties of MNPs open up the new site for immobilization or transportation of MNPs into the biological systems including human tissues. Therefore, these particles can act as a protective shield to deliver the variety of drugs especially anticancer, radionuclide entities, and target the hard sites of the human body like brain [66]. Another MNPs advantage is to response against the resonance applied by external system as a result transfer of energy from an excited medium to nanoparticles can be used for detection. For instance, hyperthermia is a nearly acceptable technique that is used for the treatment of cancer along with chemotherapy or radiotherapy, because it induces minimal damage to normal cells. In this technique, particles are used that act as hypothermic mediators [67]. These particles transfer the heat beyond the threshold level that are enough to kill the cells present in the cancer microenvironment. In various envisioned applications of MNPs, the size of the particles play a crucial role in the functionality, for example in various envisioned applications of particles are depends upon the size [68]. The particles have below critical size perform well. These particles are considered as a single functional magnetic domain which shows superparamagnetic properties when exceed the temperature threshold value. Sometime individual particles showed constant paramagnetic behavior and act like gigantic paramagnetic entity. These giant particles have a very quick response to the remnant magnetization or residual magnetization (magnetic force remains after external magnetization removed) and coercive force (the field required to bring the magnetization to zero). These distinctive features of particles make them very strong and potential candidate in biomedical applications [69–71].

Preparation of NPs are usually fall in the inductive method (bottom-up). In this method, NPs are fabricated from an atom level to the molecular level through self-organization pattern. In biomedical applications, core–shell of NPs is from the magnetic origin than make it more stable, compatible, and fast-penetrating through encapsulated with organic or inorganic polymers [72]. These supportive modifications enable them to use in various biomedical applications. Although morbidity rates globally comparatively decrease with the advancement in medical science, cancer is still one of the biggest

contributors to increase the mortality rate. Albeit according to the ACS (American Cancer Society) report mortality rate due to cancer decreased in the past few years. This is because of early diagnosis, target specific treatment, and decrease in smoking [73]. The conventional mode of treatment including chemotherapy, radiotherapy, sometimes surgery, and immunotherapy are unable to access and target the core micro tumor environment. Currently, combinatorial therapy also called multimodal treatment in which immunotherapy (relative advance), chemotherapy, and radiotherapy are used in combine fashion to get better results in cancer treatment [74].

Therefore, specific and targeted drug delivery provides a good opportunity to treat diseases. For the drug delivery, small particle (micro or nanoparticles) are being used to get momentous results including: (1) targeting the core area of a diseased body organ; (2) reducing the concentration of drug that is conquered by the surrounding cells of the target area; (3) reducing the maximum uptake of drugs by non-target cells that maximized the efficacy of drugs. Due to these reasons, the NP application graph exponentially increased in previously published articles (Figure 9) [75].

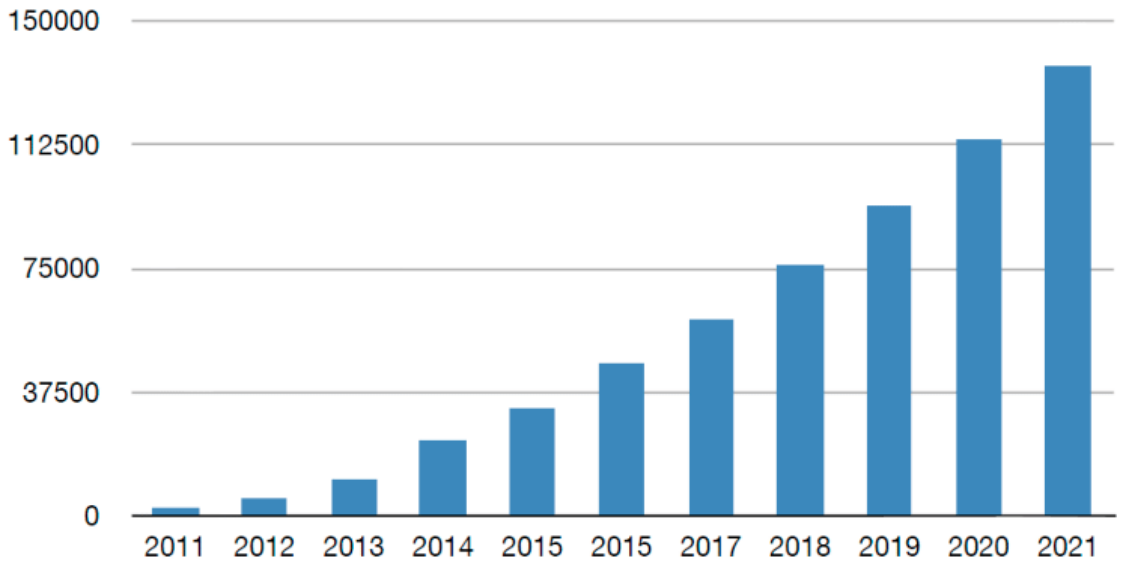

Figure 9. Global market trends for nanotechnology in drug delivery 2011–2021. Adopted from "Market Opportunities in Nanotechnology Drug Delivery". Available at: http://www.cientifica.com/market-opportunities-in-nanotechnology-drug-delivery/. Last accessed 9 May 2019.

NPs can easily internalization in tissue and cells because it can be phagocytosed due to its small size. Magnetic NPs based targeted delivery follows both the pattern of targeting active and passive target delivery. Previously reported that tumor microenvironment is highly permeable and drippy, so it called extravasation as a result of NPs target the tumor passively. These factors combine to enhance the permeability and retention of NPs so this phenomenon is known as enhanced permeation and retention (EPR) [76]. Along with all above-mentioned advantages, NPs have some limitations other than size. The main concern with NPs is the retention time in bloodstream. Therefore, the big problems with passive delivery of conventional NPs are that (i) they only target the reticuloendothelial system or mononuclear phagocyte system (MPS) and related organs including spleen, bone marrow, and liver; and (ii) they are unable to access the other tumor target sites or drug concentration is below the therapeutic level [68,77,78].

Previous studies have shown that in tumor cells were overexpressed different receptors such as G protein-coupled receptor GPR87 (pancreatic cancer) [79], GPR161 (Breast cancer) [80]. Therefore, active targeting vectors have been designed that are delicate to change in temperature, light, sound, magnetism, and pH and mounted it with drugs. Active targeting may be dependent on over-expression of low molecular weight species including Folic acid, sugars, thiamine, hyaluronic acid, transferrin, DNA, etc. [81,82]. There are different types of NPs such as Liposomes, polymeric micelles; polymeric

nanoparticle, emulsions, and nanogel are used for specific target delivery. These NPs are different in chemical nature, size, physical properties (light, temperature, pH, electric charge), hydrophobicity, hydrophilicity and pattern of conjugated with drugs (attached, adsorbed, encapsulated) [10]. Hereby, in this section, we mainly address the MNPs.

4.1. Efficacy of Nanoparticles-Based Drug Delivery

With the advancement of drug formulation, the designing of target-specific drugs is always the central and most discussed issue. Nanoparticle-based drug delivery is one of the most considerable and suitable options for target drug delivery. There are several reasons to considering the nanoparticle for a theranostic carrier and agent. Conventional drugs are administered through intravenous or oral dosing. Consequently, these drugs are not always be formulated at optimal dosage. Similarly, sometimes drug formulation is based on degradable biomolecules such as oligos, nucleotides, and proteins. Therefore, these kinds of drugs need an innovative and specifically targeted carrier system that thwarts them from annoying degradation [83,84]. It is reported that drug delivery system is directly linked to the size of particles because the large surface area with small size that enhances the physiochemical properties of particle including bioactivity, solubility, passes the blood–brain barrier (BBB), and crosses the skin endothelial cells [85]. Nanoparticles that are constructed from biodegradable (natural) and non-biodegradable (synthetic) polymers have envisaged the potential customized nanoparticles for drug delivery that help to escape the drugs from the endogenous invasion of enzymes [84]. Another advantage of introducing reliable drug delivery system that increase the sale growth rate of pharmaceutical companies. The state-of-the-art delivery system leads the companies to introduce new drug formulation and minimize the side effects of the drugs. Therefore, these innovative amendments will be valuable for patients [86]. Moreover, this not only increases the company's growth rate but also encourage the innovators to introduce new patents [87]. This nanotechnology will also offer new life to that drugs that are unmarketable due to the high toxicity, low bio absorptivity, etc. (Figure 10) [88,89].

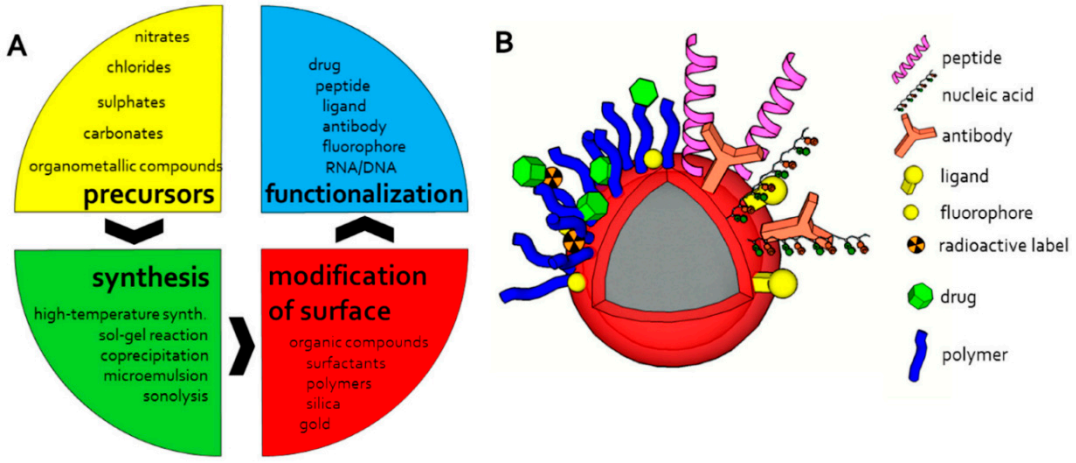

Figure 10. Scheme of magnetic particles design workflow (**A**) and possible modification and functionalization of magnetic particles (**B**). Reprinted from Kudr et al. [89], an open access article distributed under the Creative Commons Attribution License. Copyright (2017) the authors. Licensee MDPI, Basel, Switzerland.

4.2. Iron-Based MNPs for Biomedical Applications

Iron (transition element) is the most important and fourth copious element of earth's crust, as well as, considered as the backbone of modern infrastructure, around the globe. In past years, iron-based nanoparticles were abandoned due to its oxides and other renowned metals including nickel, cobalt, platinum, and gold. Nowadays, the development of iron-based MNPs is a trend in the field of nanoscience. Comparative analysis of ferromagnetic properties of iron with other magnetic material

has shown that iron is the leading element for nanoparticles applications. For example, gadolinium has low Curie temperature (Tc), that is below the room temperature and it shows high saturation magnetization (σs) at 0 K. Therefore, it is impractical in most of the experiments. Iron has high enough *Tc* and σs that are near to the optimum status in most of the experiments. Moreover, iron also shows via magnetocrystalline anisotropy that it was more feasible for work. Superparamagnetic behavior and maximum volume particles of iron at the required temperature is directly linked to magneto-crystalline anisotropy. This means that superparamagnetic behavior is much better and workable than any other metal nanoparticles.

Other than the magnetic feature, momentous features of iron nanoparticles that have been shown that these are less expensive, revealed the adequate biophysical and biochemical stability and compatibility as well as eco-friendly [76,90,91]. Surface modification of the MNPs is an integral part of defining the physiochemical properties and stability as well [92,93]. Surface modification elements should have a strong affinity with iron and also have functional compatibility [94]. Magnetite (Fe_3O_4) nanoparticles have been synthesized through Massart method and co-precipitation. These nanoparticles have varied between 7 to 13 nm in size. Later on, through the chemical alteration of Fe_3O_4 by aeration oxidation process were converted into maghemite (γ-Fe_2O_3) nanoparticles. These nanoparticles have greater biocompatibility, stability in the diverse field, and they demonstrate better heating capability [95]. Maghemite nanoparticles can also be used in ferrofluid that has an immense range of application in biomedicine, such as targeted drug delivery, hyperthermia in tumor treatment, and cell sorting and manipulation [96]. There is a number of methods to be implicated on maghemite nanoparticle synthesis including microemulsion [97], co-precipitation [98], organic decomposition at high temperature [99], and oxidation [100]. Similarly, there are many other ways to synthesize the iron MNPs, but innovative approaches are still needed for commercial production and environmental-friendly nanoparticles [101]. For iron oxide nanoparticles synthesis, sodium oleate, and iron chloride were mixed and add this mixture into cocktail solvent of hexane, ethanol, and water than heat it to get the waxy iron–oleate complex. Afterward, this complex mixed with oleic acid, dissolved into 1-octadecene and heated. Poly(D, L-lactide-co-glycolide) have a wide range of applications in biomedical sciences due to its non-toxic nature, biocompatibility, and bio-resorptive nature [102,103]. Therefore, MNPs-coated with this polymer is very fascinating and catch the attention of biomedical experts. Poly(D, L-lactide-co-glycolide) coated superparamagnetic iron oxide nanoparticles are widely used in MRI as a contrasting agent. For the preparation of this nanoparticle reaction of iron (III) acetylacetonate with 1,2-hexadecanediol, oleic acid, oleylamine, and phenyl at 260 °C in acidic pH [104]. polyethylene glycol (PEG) and polyethyleneimine (PEI) [105–107] coated superparamagnetic iron oxide nanoparticles are synthesized by co-precipitation method and cathodic electrochemical deposition (CED) [108] are a more effective method for this. Nanoparticles coated with PEG-PEI have shown high affinity to bind with DNA phosphate backbone [109].

There are the special amendments that have been occurred according to the area of applications such as improving the molecular images. A distinct group of MNPs having composite with the variety of different metal dopants (trace impurity elements) including M2+, Mn, Zn, Ni, or Co. Metal-doped iron oxides ($MnFe_2O_4$, $FeFe_2O_4$, $CoFe_2O_4$, and $NiFe_2O_4$) based nanoparticles were synthesized at high temperature and reaction occurred between the iron tris-2,4-pentadioate and divalent metal chloride [110,111]. Toxicological analysis of metal-doped nanoparticles has expressed that $MnFe_2O_4$ showed any toxicity in-vitro. While the other Co and Ni toxicity were limiting factors for their use [90]. Finally, the most imperative facet is to construct nanoparticles according to explicit biological applications, for example, magnetic fluid hyperthermia, thermoablation, targeted drug delivery, magnetic separation, and MRI [112].

4.3. Cobalt-Based MNPs for Biomedical Applications

Cobalt-based nanoparticles are rarely used in biomedical science due to their toxicity. However, in some cases, possible modification reduces the toxicity, for example, above-mentioned dopant

based MNPs ($CoFe_2O_4$) [112] and metal alloy MNPs ($Fe_{12}Co_{88}$, $Fe_{40}Co_{60}$, and $Fe_{60}Co_{40}$) [112,113]. There is the only way to make it applicable to biological systems. Commercially existing carbon-coated cobalt nanoparticles were functionalized with polyhydroxy-, polyamine-, or PEG2000-functionalized Dendron's or polymers and designed for theoretical biomedical applications as drug carriers [114]. Previous work reported that magnetic cobalt nanoparticle is auspicious material for retinal detachment cure if these nanoparticles were conjugated with poly (dimethylsiloxane), dicobalt octacarbonyl $Co_2(CO)_8$ [115]. Similarly, the above-mentioned application of cobalt that were only testified to repair the detach retina [116]. An altered polyol-process was pragmatic for crystalline cobalt nanoparticles with the possible implication in biomedicine [117].

4.4. Other MNPs for Biomedical Applications

In the traditional point of view refined form of Fe_3O_4 and γ-Fe_2O_3-based superparamagnetic nanoparticles are widely used. Some other materials that have desired features like $Y_3Fe_5O_{12}$, $SrFe_{12}O_{19}$, or $SmCo_5$ were also used [118]. However, the major problem with these materials is they are not prepared by conventional methods. These materials are separated and prepared by advanced methods like submicron particle preparation [119]. Another type of nanoparticle is based on Ni with different chemical modifications. Ni nanocrystals were encapsulated with carbon moieties Ni_3C phase in the core of the nanoparticles [120] and second modification was on the surface by NiO [121]. Similar, EDTA-caped NiO nanoparticles have been prepared by co-precipitation method, while nickel chloride hexahydrate, sodium hydroxide, and EDTA (optionally) served as reagents [89,122].

5. Concluding Remarks and Future Perspectives

Nanomaterials are being widely investigated as a powerful carrier for biomolecules immobilization, therapeutic, and other nanobiomedical applications. Immobilization of enzymes onto nanomaterial is highly encouraging in terms of catalytic activity, enhanced stability, and reusability. Amongst various immobilization techniques, the utilization of NPs is well perceived owing to the high-specific surface area, and thereby high biocatalyst loadings. Particularly, MNPs are becoming increasingly important in the immobilization arena because of their exceptional attributes including biocompatibility, uniform particle size, high surface area to volume ratio, and the recovery of the enzyme using an external magnetic field. Moreover, many MNPs possess noteworthy results in targeted-drug delivery MRI or theranostics. Therefore, MNPs can be anticipated to be the 'material of the future', which will considerably influence all areas of nano-biomedicine. However, despite the tremendous human health-related advantages of nanomaterials, concern has been raised regarding the adverse effects of these nanomaterials. Potential routes for drug delivery are also associated with the possible entry of toxic nanomaterials into the human body. For instance, inhalation-based drug delivery has been widely applied approach for direct delivery of drugs to the bloodstream, and the same route is particularly vulnerable to toxic nanomaterials. Similarly, drug delivery via the olfactory system has also arisen the toxicity issue of nanoparticle via the olfactory system. Therefore, in parallel to extensive research advances on the use of nanoparticles for biomedical purposes, their profound impact on human health also need to be deliberated by the same research. With particular reference to MNPs and their deployment in drug delivery, the following should at least be considered prior to designing MNP-based drug delivery systems. Aiming to uplift the unresolved problems associated to the MNPs based drug delivery, future studies should cover: (1) all types of toxicity issues, i.e., cytotoxicity, hemotoxicity, teratogenicity, and mutagenicity; (2) biocompatibility including cellular-compatibility and hematocompatibility; (3) immunogenicity and mutagenicity; and (4) biodegradation and/or effective release 'removal fate' from the body after targeted delivery. Encapsulation and/or coating of active MNPs using inert materials offer considerable potentialities for future research and could further limit the toxicity of free MNPs.

Author Contributions: Conceptualization, M.B. and H.M.N.I. Literature review, S.M., and T.R.; Writing—Original Draft Preparation, M.B., S.M., T.R., and H.M.N.I.; Figures, M.B. and S.M.; Tables, M.B.; Writing—Review & Editing, M.B., and H.M.N.I.; Revisions & Final editing, M.B. and H.M.N.I.; APC Funding Acquisition, H.M.N.I.

Funding: This research received no external funding. The APC (ID: magnetochemistry-513143) was funded by MDPI, St. Alban-Anlage 66, 4052 Basel, Switzerland.

Acknowledgments: All authors are thankful to their representative universities/institutes for literature services.

Conflicts of Interest: The authors report no conflict of interest in any capacity, i.e., competing or financial.

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