separations

Review

Magnetic Technologies and Green Solvents in Extraction and Separation of Bioactive Molecules Together with Biochemical Objects: Current Opportunities and Challenges

Xueting Feng [1,2], Hang Song [2], Tenghe Zhang [2], Shun Yao [2,*] and Yan Wang [3,*]

[1] College of Pharmacy, Southwest Minzu University, Chengdu 610041, China
[2] School of Chemical Engineering, Sichuan University, Chengdu 610065, China
[3] College of Life and Health Sciences, Anhui Science and Technology University, Chuzhou 233100, China
* Correspondence: cusack@scu.edu.cn (S.Y.); yanwang0129@126.com (Y.W.)

Abstract: Currently, magnetic technology and green solvents are widely used in chemical engineering, environmental engineering and other fields as they are environmentally friendly, easy to operate and highly efficient. Moreover, a magnetic field has positive effect on many physicochemical processes. However, related new methods, materials, strategies and applications in separation science still need to be developed. In this review, a series of meaningful explorations of magnetic technologies for the separation of natural products and biologic objects, including magnetic ionic liquids and other magnetic solvents and fluids, magnetic nanoparticles and magnetic fields, and the development of magnetic separators were reviewed. Furthermore, the difficulties in the application and development of magnetic separation technology were discussed on the basis of comparison and data analysis, especially for the selection of magnetic materials and magnetic field sources. Finally, the progress in the development of magnetic separators was also elaborated for researchers, mainly including that of the new high-efficiency magnetic separator through multi-technology integration and the optimization of traditional magnetic separators, which help current techniques break through their bottleneck as a powerful driving force.

Keywords: magnetic separation; magnetic ionic liquids; magnetic materials; magnetic field; natural products; biochemical objects

Citation: Feng, X.; Song, H.; Zhang, T.; Yao, S.; Wang, Y. Magnetic Technologies and Green Solvents in Extraction and Separation of Bioactive Molecules Together with Biochemical Objects: Current Opportunities and Challenges. *Separations* **2022**, *9*, 346. https://doi.org/10.3390/separations9110346

Academic Editor: Grzegorz Boczkaj

Received: 30 September 2022
Accepted: 1 November 2022
Published: 3 November 2022

1. Introduction

For chemical, pharmaceutical, food and other industries, natural products are an important resource of raw materials. The extraction, separation and purification of these active ingredients from various raw materials have been a hot research topic for scientists and engineers for many decades [1–6]. In this review, we refer to compounds with certain biological activities and unique functions in nature, mainly including alkaloids, polyphenols, flavonoids, saponins and volatile oils. There are obvious differences in the molecular structures, physico-chemical properties and state of these compounds in the cells of raw materials, so it is necessary to choose an appropriate method of extraction, separation and purification (e.g., crystallization, sublimation, partition, adsorption, and membrane separation) according to different situations and requirements. Further, small molecules including secondary metabolites, enzymes, nucleic acids, and proteins as well as cells, bacteria and virus are also very important research objects in the life sciences and bio-pharmaceuticals. Their efficient separation is necessary and meaningful for diagnoses, clinical treatments and fundamental studies in medicine and biology [7–11].

At present, there are an increasing number of problems associated with traditional separation methods, such as low work efficiency, long operation times, loss of target objects and their bioactivities, high solvent/energy consumption, and unsafety in addition to the lack of environmentally-friendly methods. So, these methods are gradually being replaced

by new cleaner or more efficient technologies in recent years. Magnetism is among the basic properties of substances, and all the common matters in the macrocosm have different degrees of magnetism. They can be divided into three types—ferromagnetic, paramagnetic and antimagnetic substances—according to their characteristics under an external magnetic field. To date, various magnetic materials have been used in medicine and the life sciences which have attracted wide attention. Some examples are ferric oxide, carbonyl iron, iron–nickel alloys, iron–aluminum alloys, zirconium dioxide, manganic oxide, $BaFe_{12}O_{19}$ and RCOMnP, and all of them have high magnetic permeability. Moreover, magnetic nanoparticles, magnetic nanofluids, magnetic ionic liquids, magnetosome/magnetotactic bacteria, magnetic carbon nanotubes, magnetic polymers, magnetic microcapsules and magnetic restricted access materials have shown great results when used for targeted delivery, controlled release, gene transfection, immunoassays, cell separation and transplantation, protein adsorption and immobilization, tracking of pathogenic microbes, tissue engineering, etc. [12–15].

At present, there is much innovation in and application of magnetic materials, and there are an increasing number of studies about the application of magnetic fields in the separation and analysis of drugs and active components. The special physico-chemical properties of objects in a magnetic field are attracting increasing attention from researchers. The advantages of using magnetic technologies, such as simplicity and rapidity, are being acknowledged and their potential is generally expected to be promising. Further, corresponding magnetic separators of different sizes and with features are also being developed and put into practice at diverse scales. Based on the collected data of references, the number of related articles per year indexed by the Web of Science™ (Thomson Reuters, London, UK) is summarized in Figure 1. There is an upward trend in the number of articles in the last nine years, and R&D of magnetic nanoparticles is predominant among the different subtopics in this field. Other technologies are temporarily lagging behind although still showing certain growth.

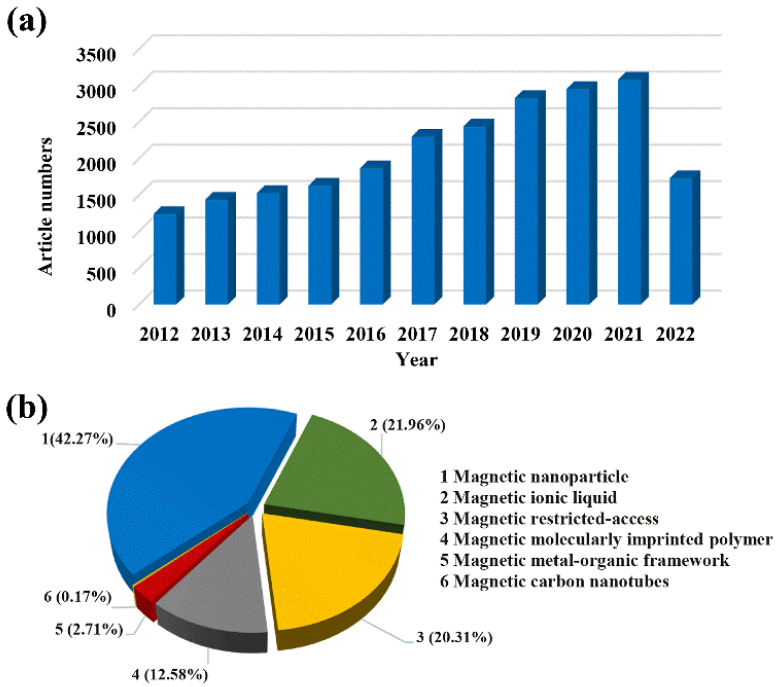

Figure 1. (**a**) The number of articles about magnetic technology in the separation of bioactive molecules and biochemical objects (search keywords: magnetic separate* OR magnetic extract* AND bioactive OR natural* OR medicine); (**b**) the percentage of research articles on different magnetic materials (January 2012~September 2022).

Based on the above research background, this paper comprehensively summarized related research on magnetic ionic liquids and other magnetic technologies in the separation of bioactive molecules and biochemical objects. In detail, the first part of this paper introduces the main role of magnetic fields and potential functions, and specifically magnetic separation; then common forms of magnetic technologies in related fields will be illustrated according to their popularity and research interest, followed by describing and summarizing the application of magnetic technology in the separation of bioactive molecules as well as biochemical objects, including systematic comparison and analysis of existing problems as well as prospects, which are expected to provide meaningful references for academic and industry scientists. At last, we provide a brief important summary on current magnetic separators for biological applications.

2. Roles of Magnetic Fields in Extraction and Separation

At present, extraction and separation processes under a gravity field, a centrifugal force field, an electrical field and microwave/ultrasound assistance have been studied in depth. A magnetic field is another vector force field with special energy and can exert its influence on electric charges in relative motion. Researchers have already found that measurable changes can be observed on optical properties, conductivity, dielectric constant, viscosity, absorption spectrum, chemical reactions, surface tension, adsorption/condensation characteristics and electrochemical effects of water or aqueous solution after they are treated by a magnetic field with certain intensity, which is mainly ascribed to the influence of the Lorentz force on intermolecular interactions in the solution system. Furthermore, when the magnetic field is removed, the above changes can last several hours or even days, and the presence of this phenomenon will make a special contribution to the separation process occurring in the solution environment [16,17].

The magnetic field can also change the physical and chemical properties of various solutes (including the dissolution, crystallization, precipitation and aggregation behavior of natural compounds or biological macromolecules), and further strengthen the phase separation, extraction, ion exchange, adsorption and flocculation process, etc. It has been found that a magnetic field with a suitable intensity can improve the separation performance of specific materials by increasing mass transfer speeds [18], and simpler objects and more easily popularized studies are usually found in the separation of two-phase emulsions [19,20] or ternary solvent systems [21]. According to related results, a magnetic field has a positive effect on the liquid–liquid extraction process, and liquid–liquid equilibrium data show that the distribution coefficients and resolution factors increase with the intensity of a magnetic field, which are measured under the magnetic field and can fit the non-random two-liquid (NRTL) model well [21]. These valuable findings can provide very meaningful references for the solvent extraction and multi-phase partition of natural products, and magnetic field-assisted extraction techniques began to be developed and popularized gradually—the target objects include flavonoids [22], alkaloids [23,24], vegetable oils [25], dye [26], polyphenols [22,24], proteins [27], polysaccharides [28], and mineral components [24]. All these explorations have achieved satisfactory results, and some enterprises have planned to apply related technological conditions in practice.

According to the current systematic study [29,30], the mechanism of magnetic fields on the separation process can be mainly elucidate as follows: (1) they can influence the charge distribution and network structure of water, and then the Lorentz force of a magnetic field on water molecules will be strengthened after the polarization of the latter, which can cause the secondary hydration layer in the structure of water being pushed off. As a result, water can be well dispersed in the single molecular state and the object particles (e.g., bioactive solutes and herbal materials) in the solution are easily separated from the water molecule binding to the precipitate; in addition, in terms of crystallization, magnetic treatment can reduce the critical radius of crystallization and form microcrystals easily. (2) A magnetic field can cause resonance in the liquid molecules and induce electric dipole moments; moreover, hydrocarbons in the magnetic field can easily transfer from the spin

simple state (S state) to the spin triplet state (T state); so, the number of molecules in the T state will increase in the solution, causing the breakage of H-bonds and the decomposition of associated groups to different degrees. Consequently, the solution properties can be changed with the spin state of electrons. (3) A magnetic field can result in additional magnetic moments in the liquid, producing additional magnetic fields and energy; their combined effect will weaken the cohesive force of an antimagnetic liquid and lower the molecular barrier, which also influences the physical properties of solutions; generally, the surface tension decreases, and the viscosity can be changed accordingly. Further, the diffusion coefficient, solubility and osmotic pressure will also increase, which can accelerate the extraction and ion-exchange process. (4) A magnetic field can promote the liquid-phase transition comprehensively (from the disordered state to the ordered state, from the symmetrical structure to the asymmetric structure, and from the mesh structure to the layered structure). (5) For molecules with anisotropic structures and magnetism, an external magnetic field can change them from disordered flow into ordered flow. (6) When a variable magnetic field is employed in extraction, it can produce a resonance effect, which will change the voltage of cell membranes in plant tissues. As a result, this can lead to more effective mass transfer of target substances via various microchannels (e.g., ion channels) by affecting the voltage of cell membranes.

As a representative, the effect of a magnetic field on crystallization will be illustrated to help readers further understand the above mechanisms, and other examples of extraction and separation technologies will be introduced in the latter sections of practical application. For instance, the crystallization process of the various solutes is very sensitive to their solution environment; the molecular barrier and cohesive force will change with the introduction of a magnetic field, which can further affect the nucleation and crystal growth rate significantly to promote crystallization (shown in Figure 2a,b) [31]. In some cases, the magnetic field can be used to boost the crystallization of the solution and increase the crystal size. Further, it is also applied to control the growth of the particle size and control crystal growth. Because there are many potential factors affecting the crystallization process in the solution environment, and the actual effect of a magnetic field is closely related to the treated object, if the operational parameters are not selected properly, they will not only be useless to the crystallization process and separation result, but also have the opposite effect. Therefore, these need to be carefully studied under the guidance of the relevant function models.

Currently, the effect of a magnetic field on the crystallization separation process of protein has been studied widely, and mainly includes the effect of the magnetic field on the nucleation rate [32,33], crystal growth [34], preferred orientation [35,36], crystal growth kinetics [37] and crystal quality [32,38]. Nucleation is the first step in crystal formation and is very important. Rareja-Rivera et al. [31] studied the effect of a magnetic field on lysozyme isomerase crystallization, where the crystallization system included 30 mg/mL lysozyme isomerase, 0.2 M ammonium acetate buffer (pH 7.0) and 30% (*w*/*v*) PEG 6000. After 48 hours of crystallization at 291~293 K (near r.t.), the number of lysozyme isomerase crystals was reduced under a magnetic field of 16.5 T, which was produced by a nuclear magnetic resonance (NMR) instrument. Furthermore, as shown in Figure 2a, the crystals were bigger than that in the controls (0 T). However, some studies also discovered that the number of lysozyme crystals was increased. For example, under the crystallization conditions for 30 mg/mL enol acyl reductase carrier protein developed by using 0.6 mol/L $NaNO_3$ and 0.5 mol/L adenosine deaminase (pH 5.6), the results showed that the number of crystal was increased under a magnetic field of 200 mT which was also produced by neodymium magnets for 48~72 h at 291, 293 and 295 K [32]. These contradictory results can be due to the different solution compositions and environments resulting from an external magnetic field. To date, there is no consensus on the effect of a magnetic field on protein crystal nucleation, but sufficient research has proved that a magnetic field can improve the quality of protein crystallization. In some cases, directional crystals can be obtained, and overall B-factor (temperature factor) values can be improved [31,34]. Further-

more, a magnetic field can affect the tertiary structure of proteins, and this is still a good research topic.

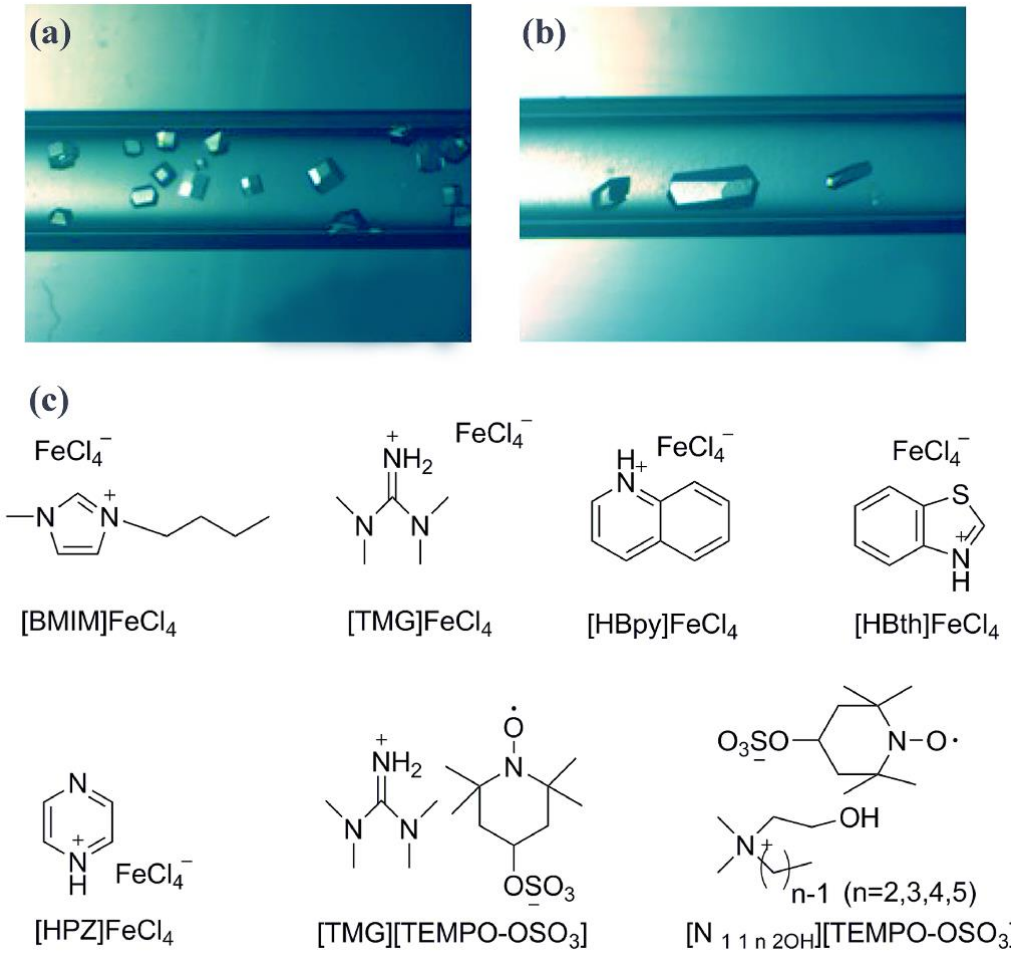

Figure 2. Crystals of lysozyme (**a**) without a magnetic field and (**b**) with a 16.5 T magnetic field [31]; (**c**) the structures of main MILs.

Diamagnetism is a universal property of substances. When the direction of the magnetizing force and that of gravity are the same or opposite, a supergravity, microgravity and even apparent zero gravity condition can be achieved; and the droplet can be levitated and used in containerless processing under an apparent zero gravity condition. Furthermore, it is known that high-quality crystallization can be obtained by containerless processing; now, superconducting magnets play important roles in containerless levitation, and strong magnetic fields can improve the quality of crystals. Cao et al. [39] used the JMTA-16T50MF superconducting magnet (maximum intensity: 16.12 T, magnetization level: −1500~1100 T^2/m, JASTEC Co., Ltd., Tokyo, Japan) to realize containerless levitation. It is worth mentioning that a strong and stable magnet is always very crucial for the development of magnetic separators in the future. In their study, it was found that the antimagnetic suspension technology could improve the crystal quality of Hen-egg white lysozyme, protein kinase, etc., including morphology and diffraction properties. The effect was even better than that of colloidal containerless, oil droplet containerless and conventional crystallization. Additionally, it was proved that through the action of a magnetic field, high-quality crystallization of biological macromolecules can be obtained, which has great significance for the subsequent structural analysis of biological macromolecules together with their preparation.

3. Magnetic Nanofluids and Ionic Liquids in Extraction and Separation

3.1. Magnetic Nanofluids

Magnetic nanofluids, also known as ferromagnetic nanofluids, describe a new type of functional medium, which has both the fluidity of liquid and the magnetism of solid magnetic materials. It is a stable colloidal liquid composed of magnetic solid particles with a nanometer diameter, base fluid (such as water, organic solvent, and oil) and a surface-active agent. The magnetism of magnetic fluids originates from magnetic solid nanoparticles, which generally show superparamagnetism; that is, the fluid shows magnetism only when an external magnetic field is applied; if the external magnetic field is removed, the magnetism disappears immediately. The roles of surface-active agents are (1) to prevent magnetic nanoparticles from aggregating and settling, (2) to make magnetic nanoparticles and carrier fluid have better compatibility, and (3) to maintain the stability of magnetic fluid. The carrier liquid should be a liquid with a low evaporation rate, low viscosity and high chemical stability. However, it is not easy to find an ideal carrier liquid which completely meets the above requirements. Therefore, current choices are based mainly on the literature, operator experience and adequate preliminary testing, and a water-based magnetic fluid is most widely used in the separation of natural products and in medicine; in addition, with the development of green solvents, an increasing number og magnetic nanofluids based on ionic liquids (ILs) and deep eutectic solvents (DESs) have been developed and applied.

Overall, magnetic fluid-based separation technology makes use of the dual properties of fluids and magnets, which is more environmentally friendly and energy efficient than the traditional magnetic separation technique; it is often more efficient for objects that are difficult to treat (e.g., stable/unstable suspensions or emulsions). When a magnetic fluid is applied for extraction and separation, the magnetic solid particles contained in magnetic fluids can be used as different adsorbents after appropriate modification; further, they can be easily recovered using an external magnetic field. The whole process is fast, efficient, mild and selective. However, the theory of magnetic fluids is difficult to fully validate similar to that of general hydrodynamics. Moreover, it is difficult to simulate magnetohydrodynamic phenomena because there are few alternative media at room temperature and a strong magnetic field is needed to observe magnetohydrodynamic phenomena. To some extent, this will restrict the application of magnetic fluids in extraction and separation.

3.2. Magnetic Ionic Liquids

Compared with above magnetic nanofluids, magnetic ionic liquids (MILs) have a simpler composition as a type of green molecular liquid. Magnetic ionic liquids belong to the family of functional ionic liquids and were first reported in 2004 [40]. Since then, MILs have attracted the attention of researchers and have been rapidly developed. Similar to conventional ionic liquids, MILs are composed of smaller anions together with greater cations and exist in the liquid state at room temperature. However, these anions and/or cations in MIL contain magnetic centers, which are often complexes of some metal ions or contain structures with single-electron organic free radicals (see Figure 2c); the atoms in the magnetic center of these structures have spin-parallel electrons or single electrons. These structural characteristics mean that MILs can be magnetized and attracted to an external magnetic field so that they can be separated from the whole system by the external magnetic field and reused. Moreover, MILs also have the the main advantages of ionic liquids, such as low vapor pressure, good thermal stability, and strong dissolving capability, so they are widely used, for example, in the chemical, pharmaceutical, and environmental protection fields. However, it has been reported that some ionic liquids have toxicity [41], and cations have a remarkable effect on the toxicity of ILs—for example, the toxicity of imidazolium ILs is more obvious than that of ammonium ILs and pyridinium ILs [42]. Moreover, a long alkyl substituent in the cation will increase the toxicity of ILs. As for magnetic ionic liquids, they are easier to recover than ordinary ionic liquids, which are less likely to have an impact on the environment. Even so, their potential toxicity should also be considered in the process of designing and screening green MILs.

At present, the preparation methods of MILs are basically the same as those of common ionic liquids, and two-step synthesis is frequently used. The first step is to synthesize halide intermediates, and the second step is to add anions containing magnetic centers using the ion-exchange process [43,44]. It is worth mentioning that magnetic liquids are generally mixtures, while MILs are easily prepared and relatively stable; so, the latter have higher application value.

According to the magnetic sources mentioned above, MILs can be divided into metal magnetic ionic liquids (MMILs) and organic magnetic ionic liquids (OMILs). The former mainly includes iron, dysprosium and other metal elements. For instance, 1,1,3,3-tetramethylguanidinium tetrachloroferrte ([TMG]$FeCl_4$) was synthesized by us and its magnetism originates from Fe(III) in the anion. Its magnetic susceptibility reaches 59.1×10^{-6} emu/g, which gives it strong paramagnetism at room temperature. When there is no magnetic field, it can be well dispersed in related solvents. Further, it has strong responsiveness to a small rubidium magnet and can be recovered from the solution after the extraction process. However, in the structure of organic magnetic ionic liquids that do not contain metal atoms, magnetism mainly originates from free radical groups, such as the 4-sulfonatooxy-2,2,6,6-tetramethylpiperidine-1-yloxyl (TEMPO-OSO_3)-based MIL ([TMG][TEMPO-OSO_3]) [45]. The MIL also shows ideal paramagnetism at room temperature for the existence of NO· free radicals in the anion and its droplets can be attracted and aggregated towards magnets with high speed. After first being reported in 2007 [46], the research and application of OMILs is falling behind that of metal-organic magnetic ionic liquids due to relatively weaker magnetism and lower stability. Further, their oxidability from the unique anion may affect the structure of unstable objects. In summary, the discovery of MILs solves the problems of conventional solvents in terms of extraction and separation as MILs do not result in recovery difficulty and low selectivity and are environmentally friendly. However, there are not enough types of MILs for selection, and the current application is still in the preliminary stage. Fortunately, their importance has been confirmed by existing applications in the separation and purification of tea polyphenols (TPs) [43], lysozyme [47], alkaloids [48], etc. It should be noted that, as another type of melting salt in liquid near room temperature, deep eutectic solvents (DES) and their polymers can also become magnetic when combined with various metallic structural fragments/supports to play their original role as green separation media for the aforementioned objects.

4. Magnetite Nanoparticles and Their Surface Modification

Although the topic of magnetic nanoparticles can be written as another special overview independently, this article mainly focuses on their key aspects for adsorption applications with industrial prospects. Generally, adsorption is a spontaneous thermodynamic process, which depends on the surface energy of the material to change the concentration of target compounds at the phase interface. Adsorption is a mass transfer phenomenon under various force fields. On the one hand, magnetic fields can change the separation efficiency of common adsorbents (e.g., active carbon and resins); on the other hand, it is beneficial for magnetic adsorbents to accelerate the adsorption speed and shorten the adsorption time in many applications. Magnetic adsorbents are a type of functional material with ideal magnetism, sufficient interaction sites and high surface energy, which can be composed by transition elements of iron, cobalt, nickel or their alloys. The relevant magnetic adsorption materials to this review are those that have a strong adsorption capacity for a variety of active molecules and can be used to separate them effectively from the mixture in the solid or the liquid phase (especially for medicinal active molecules hardly adsorbed or selectively enriched by other methods). The solid materials applied in the reported adsorption operation generally have porous particles or membranes with large specific surface areas, with suitable pore structures and surface arrangement structures.

What needs to be noted in particular is that superparamagnetism means the magnetic particles can show strong magnetism in the presence of an external magnetic field when their particle size is less than a critical value (e.g., Fe_3O_4 < 30 nm). Further, their magnetism will disappear and no remanence exists when the magnetic field is removed. So, superparamagnetic particles have been widely used for the capture of small molecules, biomolecules and cells. Bead composition directly impacts settling and magnetic separation profiles, which have implications for separation performance or diagnostic assay parameters such as incubation duration for binding and elution steps, and buffer change. At present, magnetic materials that are used widely are iron, cobalt and nickel, but these three metals are not stable enough and can be easily oxidized to their oxides with higher stability, and magnetic nanoparticles that are prepared by these metal oxides are used widely. In addition, gold and silver which are diamagnetic materials exhibit magnetism when the size of their nanoparticles is small enough; for example, gold nanoparticles exhibit magnetism when their size is less than 3 nm [49,50]. However, because their magnetism is weaker and their cost is higher than that of iron, cobalt and nickel oxides, they are not particularly widely used at a large scale. By further comparison, cobalt and nickel have weaker magnetic properties than iron, so iron oxides, especially Fe_3O_4 magnetic nanoparticles, are the most extensively employed because of their good superparamagnetism, versatile utilization and ideal biocompatibility. The most common method for preparing Fe_3O_4 magnetic nanoparticles is coprecipitation. In addition, hydrothermal, solvothermal, pyrolysis, sol-gel and microemulsion methods are also applied. Their comparison of these methods is shown in Table 1, which lists the advantages and disadvantages of each preparation method, as it is necessary to select the appropriate preparation method according to specific context and requirements. Among them, coprecipitation is the most popular method in biology because it is simple and efficient; and water is selected as the solvent to avoid the use of organic solvents. Moreover, the concentration of the solution, the temperature and the speed of adding precipitator can be utilized to control the morphology and size distribution of Fe_3O_4 particles.

Table 1. The comparison of the preparation methods of Fe_3O_4 magnetic nanoparticles.

Methods	Preparation Conditions	Solvent	Advantages	Disadvantages
Coprecipitation method	Nitrogen or argon atmosphere	Water	Short reaction time, simple operation and high yield	Size of particles cannot be easily controlled
Hydrothermal method	High temperature and high pressure	Water	Good magnetism, uniform particle size distribution and adjustable particle size	Severe requirements for temperature and pressure
Solvothermal method	High temperature and high pressure	Organic solvent	Mesoporous or hollow particles with larger specific surface area, controllable particle size	Harsh preparation conditions; more dangerous than hydrothermal methods for VOCs
Pyrolysis method	High temperature	Organic solvent	Monodisperse, high crystallinity, adjustable particle size and shape, hollow nanoparticles	High reaction temperature, complex responsive process; hydrophobic MNPs produced mainly
Microemulsion method	Complex system and conditions	Water and organic solvent	Narrow particle size distribution, regular morphology and good dispersibility	High preparation cost; difficulty to remove the surfactant
Sol-gel method	High temperature, drying	Organic solvent	Narrow particle size distribution, small particle size, good dispersibility	High cost of raw materials and long reaction duration

As shown in Table 1, although magnetic particles have many merits (such as a good surface effect, volume effect, magnetic effect, and biocompatibility), further modification is necessary for their long-term stability as well as for multiple functions; furthermore, naked magnetic nanoparticles (especially for Fe_3O_4 and γ-Fe_2O_3) usually have high chemical activity, so they can be easily oxidized and lose their magnetism. Therefore, appropriate technologies should be used to protect these nanoparticles in order to maintain their stability in subsequent applications. In addition, surface modification can make magnetic nanoparticles multi-functional, and different active groups with various characteristics can extend their available scope for various potential objects and affect the extraction and separation efficiency [51–54]. In summary, the main purposes of MNP modification include (1) improving separation efficiency and selectivity; (2) improving dispersion and stability; (3) endowing its surface with new physical, chemical and mechanical properties; (4) broadening its applied environment and compatibility with other substances.

On the whole, there are mainly four types of substances that are commonly used to modify the magnetic nanoparticles—inorganic substances, small organic molecules, biomolecules and polymers. The representative structures of several magnetic materials obtained after modification of nanoparticles are shown in Figure 3, and their comparison results are concluded in Table 2. Sometimes, making the structure increasingly complex may be just to make it out of the ordinary. After all, the more complex the structure, the more difficult it is to synthesize; there has been a considerable number of magnetic materials with satisfactory performance, and the rest is their further promotion. In practical applications, appropriate modification materials should be selected according to the specific context; the appropriate particle morphology should also be considered, in addition to the size of particles after modification and the uniformity of modification should be controlled to minimize the differences in the use process.

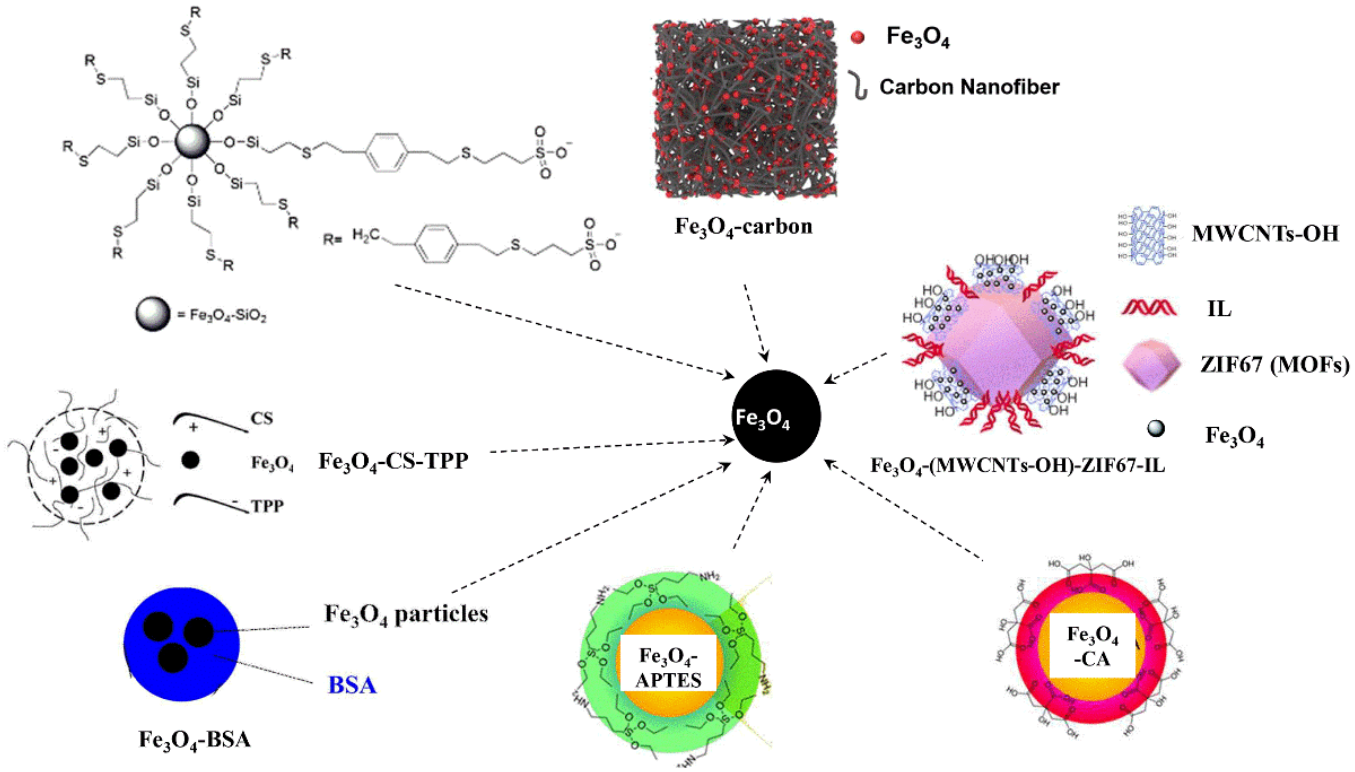

Figure 3. The structures of several representative magnetic materials [55–59].

Table 2. Magnetite nanoparticles and their surface modification.

Surface Modification	Composition	Methods	Purposes	Typical Applied Fields
Inorganic substance	Fe_3O_4-SiO_2	Stöber method, i.e., the hydrolyzed product of tetraethyl orthosilicate (TEOS) catalyzed by ammonia was coated on the surface of Fe_3O_4 nanoparticles and Fe_3O_4-SiO_2 MNPs were prepared	Improve the chemical stability of MNPs, cover the surface of MNPs with hydroxyl groups	Catalysts in synthetic reactions, or used for further surface modification
Inorganic substance	Fe_3O_4-carbon	(1) Fe_3O_4-carbon can be obtained by adding glucose in the process of the preparation of Fe_3O_4 nanoparticles by solvothermal method; (2) the polymer was coated on the surface of Fe_3O_4 nanoparticles firstly, and then annealed to prepare Fe_3O_4-carbon	Make MNPs more stable and more dispersible	Adsorbing organic substances, such as dyes; magnetic pigments
Organic molecules	Fe_3O_4-C_{18}, Fe_3O_4-APTES	Small molecules are added in the process of preparing Fe_3O_4 nanoparticles by pyrolysis method; ligand-exchange technology; (3) Fe_3O_4 nanoparticles are added to silane solution, and then react in autoclave after ultrasonic treatment	(1) Improve the water solubility and biocompatibility of MNPs; (2) provide sites for further grafting of other bioactive substances	Drug delivery system, or as magnetic solid-phase adsorbents to adsorb organic compounds
Polymers	Fe_3O_4-CS-TPP	Embedding method, i.e., Fe_3O_4 nanoparticles are dispersed in polymer solution, and cross-linking agents are added and then stirred; monomer polymerization method, i.e., Fe_3O_4 particles are dispersed in the solution of polymer monomers with functional groups, and then initiators and surfactants are added; (3) In situ synthesis, i.e., polymer microspheres are prepared and placed in the solution of Fe^{2+} and Fe^{3+}. After the iron ions are inserted into the microspheres, they will be converted in Fe_3O_4 by the effect of alkali	Improve the stability and biocompatibility of MNPs, provide more binding sites	Immobilized enzymes, cells, proteins, separation of nucleic acids and other bioactive substances or environmental pollutants (organic dyes, drug residues, etc.), and magnetic targeted drug carrier
Biological macro-molecules	Fe_3O_4-BSA	In general, a layer of organic small molecule or polymer is grafted onto the surface of Fe_3O_4 nanoparticles, and then the functional groups are used to graft biological molecules	Good biocompatibility	Biological fields such as separation, detection and biosensors
Metal-organic framework	Fe_3O_4-MOF	Fe_3O_4 nanoparticles are added in the preparation of MOF materials	Large specific surface area, magnetism	Biological fields such as separation, detection and biosensors

4.1. Surface Modification with Ionic Liquids and Other Inorganic Substances

The applied inorganic substances mainly include silicon dioxide, carbon and metal materials. Among them, the silica-coated Fe_3O_4 magnetic nanoparticle (Fe_3O_4-SiO_2) is a typical and important composite material in both basic research and application fields. The silica shell can not only shield magnetic dipole attraction among magnetic nanoparticles and then enhance their dispersity in sample solutions, but can also create the possibility of further modification with a variety of functional groups because of a great amount of Si-OH on the silicon-rich oxide layer of Fe_3O_4-SiO_2; and the complex can be easily activated with different conditions [60,61]. There are two main technical routes of the synthesis of Fe_3O_4-SiO_2 composite materials—the first method is based on the famous Stöber method [62] and the second approach is microemulsion synthesis [63]. To a certain extent,

the magnetic response of magnetic nanoparticles can be affected by the combination with SiO_2. As a result, the magnetic induction intensity (MII) of Fe_3O_4 magnetic nanoparticles after modification will become lower. As in previous reports, the magnetic induction intensity decreased with the increase in the silica content; when the silica contents were 50%, 30% and 10% (*w*/*w*), the experimental saturation magnetization values were 8.3, 18.2 and 34.5 emu/g, respectively, which were lower than that of bulk Fe_3O_4 nanoparticles (50.2 emu/g) together with the theoretical values of 35.1, 40.2 and 45.2 emu/g, respectively [64]. The difference between the experimental and the theoretical values was due to the reduction in non-magnetic SiO_2 to the magnitude of the saturation magnetization of Fe_3O_4, which was because of the quenching of surface moments [65]. However, they still have enough superparamagnetism in separation processes. The thickness of the SiO_2 shell will increase and the content of magnetic Fe_3O_4 will decrease with the increase in silica content. So in practical applications, silica content should be controlled to achieve effective magnetism and suitable stability. Moreover, the difference in the MII before and after modification will increase with an intensified magnetic field [66], and the magnetism of modified nanoparticles is still relatively stable. Nistler et al. [67] preparation iron oxide-shell silica-core nanocomposites and proved that their magnetism was basically unchanged after 77 days.

Fe_3O_4-carbon composites is another important magnetic functional material. It has become a hot topic in the surface modification field of Fe_3O_4 nanoparticles because carbon has good chemical and thermal stability together with high intrinsic conductivity. Fe_3O_4-carbon is often synthesized by solvothermal [68] or pyrolysis [58]. Wu et al. [69] synthesized magnetic microporous spheres of Fe_3O_4-carbon with an average diameter of 350 nm by this method. However, due to the shape change from cubical to spherical particles, the saturation magnetization determined by a vibration sample magnetometer (VSM) became 22.84 emu/g, which was lower than the saturation magnetization of 46.23 emu/g of the nanoparticles before modification. The saturated magnetic susceptibility of the modified magnetic nanoparticles is lower than that of the Fe_3O_4 nanoparticles because of the decrease in Fe_3O_4 content. It is a necessary loss during surface modification, and this influence does not hinder further application.

In addition, ionic liquids [56] have shown great potential in separation science for small molecules, proteins and nucleic acids in recent years, which are also very useful in MNP modification as a type of special green medium (see Figure 3).

4.2. Surface Modification with Small Organic Molecules

Organic small molecules are sometimes used in the modification process to obtain Fe_3O_4 magnetic nanoparticles with good properties. Currently, oleic acid (OA) or oleylamine have been considered to modify MNPs through solvothermal [70] or pyrolysis method [71]. These two compounds all have a C_{18} long alkyl chain, and this is good to maintain the stability of the magnetic fluid. Moreover, in the process of synthesizing MNPs, oleic acid can form a dense monolayer structure to ensure the high uniformity of the nanoparticles [72]. In general, the hydrophobic properties of magnetic nanoparticles will be enhanced after modification with a C_{18} chain, but the magnetic induction intensity will still be decreased. Commonly, the hydrophobicity and saturation magnetization of magnetic nanoparticles modified by noctadecyltrimethoxysilane (C_{18}, TMS) are higher than those modified by oleic acid [73]. Moreover, other surface groups including -NH_2, -COOH, -CN, C_8 and C_{12} have also been introduced on the surface of MNPs, which are extremely common in non-magnetic adsorbents and solid-phase extraction materials. These functional groups make them more suitable for enrichment of specific objects or as gene vectors.

Furthermore, natural compounds also are helpful to realize MNP functionalization, such as tannic acid, dopamine, 2,3-dimercaptosuccinic acid, citric acid, phosphatidyl vitamin B, and amino acids. They can be grafted on the surface of particles after magnetic nanoparticles are obtained or directly added in the preparation system of MNPs. After

their modification, MNPs show different solubility properties for water and oil together with amphiphilicity accordingly. Silane coupling agents are another type of modifier for the subsequent reactions with metal ions, polymers, biomolecules or other biomass. For instance, the magnetic iron oxide nanoparticles coated with (3-aminopropyl) triethoxysilane (APTES) are biocompatible and can be modified further because of the functional group of -NH_2 [59]. In particular, it was found that the saturation magnetization of modified magnetic particles first increased slightly and then decreased with a rise in the ratio of silane coupling agent KH-500 to Fe_3O_4. This is because the surface of magnetic particles was not completely wrapped when the silane coupling agent was added in the reaction system in a small amount, and the dispersion of magnetic particles was improved to a certain extent. However, the silane coupling agent in the particle surface was completely covered and the thickness increased on further increase in its dosage, which was not conducive to exert the effect of a magnetic field on particles, so the magnetic saturation intensity decreased [74].

The surface of the magnetic iron oxide nanoparticles prepared by pyrolysis is usually covered with non-polar groups, but most biological applications are carried out in polar solvents (such as water), so it is necessary to replace the non-polar groups by a functional ligand with hydrophilic to improve their water solubility. For example, an additional ligand, such as -COOH or BF_4^-, is often added into the magnetic fluid as a phase-transfer agent, which replaces the original ligand (such as C_{18}) on the nanoparticle and the hydrophobic layer interacts with the hydrophilic layer [75,76]. Dong et al. [77] prepared a magnetic fluid which remained stable for a long time in various polar or hydrophilic media by using $NOBF_4$ to replace the original coordination groups of C_{18} on the surface of the magnetic nanoparticles. They also found that the polar and non-polar conversion of nanoparticles was completely reversible.

4.3. Surface Modification with Polymers

Compared with small inorganic or organic molecules, polymers with a greater volume and a more complex structure used in the surface modification of Fe_3O_4 magnetic nanoparticles can not only provide better stability and more functional groups, but also have good biocompatibility and biodegradability. Therefore, this type of magnetic microsphere has more advantages than other magnetic materials used in biomedicine. Popular polymers usually include chitosan (CS), cyclodextrin (CD), polyethylene glycol (PEG), polystyrene (PS), polyetherimide (PEI), poly (ionic liquid) and poly (deep eutectic solvent) [78–82]. Generally, magnetic polymer microspheres can be classified into three categories according to their structure—(1) the core-shell structure, (2) the matrix-dispersed structure and (3) the shell-core-shell structure—and the core-shell structure is the most popular among them.

At present, the main methods of preparing magnetic polymer microspheres include entrapment, monomer polymerization and in situ synthesis [83–85]. Additionally, the second method is the most frequently used. According to the different polymerization conditions, it can be divided into three types—emulsion polymerization [86], dispersion polymerization [87] and suspension polymerization [69]. For instance, Chen et al. [88] synthesized magnetic chitosan microspheres (CS-MNPs) and magnetic chitosan-cyclodextrin microspheres (CS-CD-MNPs) by the suspension cross-linking technique, and their adsorption capability was compared to guide further structural design and optimization. In this frequently used method, water-insoluble monomers and an initiator are dispersed in a liquid medium with a dispersant in the form of small droplets by strong mechanical stirring to promote the polymerization reaction. The results showed that the saturation magnetization of Fe_3O_4, CS-MNPs and CS-CD-MNPs were 68.91, 41.03 and 37.59 emu/g, respectively.

4.4. Surface Modification with Biomolecules

In recent years, magnetic nanoparticles grafted with cells, enzymes, proteins, antibodies and nucleic acids have attracted increasing attention from chemists, biologists, engineers, etc. Additionally, nanomaterials are widely used in drug screening, antibodies and cell marker separation, together with detection, biosensors and other biological

fields because due to good biocompatibility. Compared with traditional cell separation methods, these modified magnetic nanoparticles can directly separate target cells from original samples such as blood, bone marrow, tissue homogenate, and culture media [89]. At the same time, the separation method is simple and fast; the conditions of the combination and elution processes are very mild and will not destroy cells; further, the magnetic separation technologies are more convenient to amplify and operate. By comparison, it is difficult to achieve these goals using traditional separation methods. Furthermore, it can be predicted that modification will become relatively more difficult for their great volume and high environmental requirements in both preparation and application. Generally, biomolecules can be not only directly or indirectly combined with magnetic nanoparticles through Van der Waals' forces, hydrogen bonds and coordination bond action, but also immobilized through reactions with the surface groups (-COOH, -CHO, -OH, and -NH) on MNPs [90]. The main method of preparing the magnetic nanoparticles is indirect, and sometimes there will also exist multiple layers of modification. First, a layer of organic small molecules or organic polymers was grafted on the surface of iron oxide nanoparticles and then the groups of the organic small molecules or organic polymers are functionalized by biomolecules (such as bovine serum albumin, human serum albumin, and adenosine triphosphate) [91–93]. Modification of magnetic nanoparticles with biological molecules (e.g., proteins and DNA) can not only improve their biocompatibility, but also identify specific target substances by bioactive substances attached to MNPs, which makes MNPs applicable to selective separation. So, for different target substances, surface modification may be different. Therefore, it is of great significance to develop more surface modification materials and methods for the development of magnetic separation in this field.

5. The Application of Magnetic Technology in Separation of Natural Products

5.1. Magnetic Solid-Phase Extraction

A typical process of magnetic solid-phase extraction is shown in Figure 4a. Compared with conventional sorbents in solid-phase extraction (SPE), MNPs have large specific surface areas and a short diffusion distance. Only a small amount of adsorbent and a short equilibrium time are needed to achieve effective microextraction for objects with low concentration, which has very high extraction capacity and efficiency. After use, they can be easily separated and collected from the system by an external magnetic field, avoiding tedious filtration or centrifugal processes [94]. So magnetic solid-phase extraction (MSPE) has the advantage of simpler operation, is less time consuming, and has a more ideal performance, which can effectively avoid column packing and blocking; it is also easier to amplify than preparative chromatography. At present, MSPE has been widely used in chemical analysis, ligand fishing, biological separation, food science, genomics, proteomics and other fields [95–97].

Magnetic solid-phase extraction materials usually have ferromagnetic and superparamagnetic properties, and it is reported that most of them contain Fe_3O_4 and γ-Fe_2O_3. Yamini et al. [98] prepared magnetic composite nanoparticles based on sodium dodecyl sulfate (SDS) and Fe_3O_4 successfully; then the magnetic nanoparticles (average diameter of 40 nm) were applied in the extraction of vitamin B12, which mainly exists in meat and soybean together with some herbs as an indispensable substance for the formation of red blood cells. Because MNPs have a high specific surface area, a short diffusion route and a magnetic response, this method has the following advantages when it is compared with other solid-phase extraction methods: (1) the amount of sorbent and the volume of eluent are smaller; (2) the time of extraction, magnetic separation and elution process is shorter. In this study, when 200 mL sample solution (pH 1.8) was treated, the amount of MNP needed was 40 mg and the extraction time was 7 min; the subsequent solid–liquid separation was completed in a few minutes by using the external magnetic field without centrifugation, filtration and other operations. After that, the solid was eluted with 500 μL of 0.1 mol/L NaOH-PrOH (30:70) for 2 min, and the enrichment factor reached 184.

Depending on the type of supports, reported magnetic solid-phase extraction materials can be divided into (1) magnetic silica gel, (2) magnetic carbon nanotubes, (3) magnetic graphene, (4) magnetic metal-organic frameworks (MMOFs), (5) magnetic covalent organic frameworks (MCOFs), (6) magnetic polymers, including magnetic molecular imprinting polymers (MMIPs), and (8) magnetic restricted access materials. There are enough studies on the synthesis of magnetic solid-phase extraction materials and their successful applications in the field of separation, and some representative ones are listed in Table 3. It can be concluded that although the properties of these magnetic solid-phase extraction materials were various and their magnetic response ability was different, they all accomplished their resolution tasks satisfactorily when faced with a variety of complex samples from natural products and organism. In particular the enrichment of target molecules from blood, urine, oil or milk is usually difficult. On the one hand, the intrinsic properties of samples make it difficult to separate them directly. Secondly, there are many coexisting components and a low target content.

Table 3. The application of magnetic solid-phase extraction in the separation of bioactive molecules.

Objects	Samples	Magnetic Materials	Separation Efficiency or Recovery	Ref.
Sesamol, sesamin, and sesamolin	Sesame oil	Graphene oxide-coated Fe_3O_4	84–86%, 82–92% and 83–94%	[99]
Cinnamic acid, ferulic acid and genistein	oilseeds	Graphene oxide-coated Fe_3O_4	84–98%, 94–95% and 83–109%	[100]
Aristolochic acid I and aristolochic acid II	*Aristolochiae* fructus and *Asari* radix et rhizome extrat	Adenine-coated magnetic multi-walled carbon nanotubes	92.7–97.5% and 92.6–99.4%	[101]
Morin, quercetin, and kaempferol	Dark tea, dark chocolate and tomato	Fe_3O_4-agarose	86–99%, 87–98% and 86–97%	[102]
Sudan dyes	Tomato sauce	Fe_3O_4-NH_2-MOF (MIL-101)	73–93%	[103]
Puerarin, daidzin, and daidzein	Pueraria lobata extrat	Fe_3O_4-SiO_2-MOF (ZIF-8)	98–101%, 94–102% and 97–104%	[104]
Rhodamine B	Chinese prickly ash extrat	Fe_3O_4-COF	92–98%	[105]
α-asarone and β-asarone	*Acorous Tatarinowii Rhizoma* and *Polygalae Radix* extrat	Fe_3O_4-COF	106–112% and 98–99%	[106]
Hydroxytyrosol	Chinese olive leaves extrat	MMIP	97–98%	[107]
Harmaline	*Peganum harmala* extrat	MMIP	96–105%	[108]
Hesperetin	The dried pericarp of *Citrus reticulata Blanco* extrat	MMIP	91–97%	[109]

Adsorbents with magnetic iron oxides can be easily recovered, so they are widely used in separation fields. However, some studies have shown that the adsorption capacity of adsorbents may be a little lower than that without magnetic iron oxides, and this type of loss is unavoidable for obtaining higher selectivity to the target. For example, Tang et al. [110] synthesized magnetic multi-walled carbon nanotubes (MMWCNTs) with the saturation magnetization of 8.06 emu/g based on multi-walled carbon nanotubes (MWCNTs) and iron oxides, and the SEM images of MWCNTs and MMWCNTs are shown in Figure 4c. The comparison showed that the saturated adsorption capacity of MWCNTs (19 mg/g) was slightly larger than that of MMWCNTs (18 mg/g), which may be due because the specific surface area of MWCNTs (162.99 m^2/g) was larger than that of MMWCNTs (138.66 m^2/g); magnetic iron oxides had the lowest adsorption capacity (13 mg/g) due to poor dispersion as another component part of MMWCNTs. However, MMWCNTs can be effectively separated from suspension by adding a magnet and this property is conducive to solve the problem that MWCNTs cannot be recovered from solution quickly and easily.

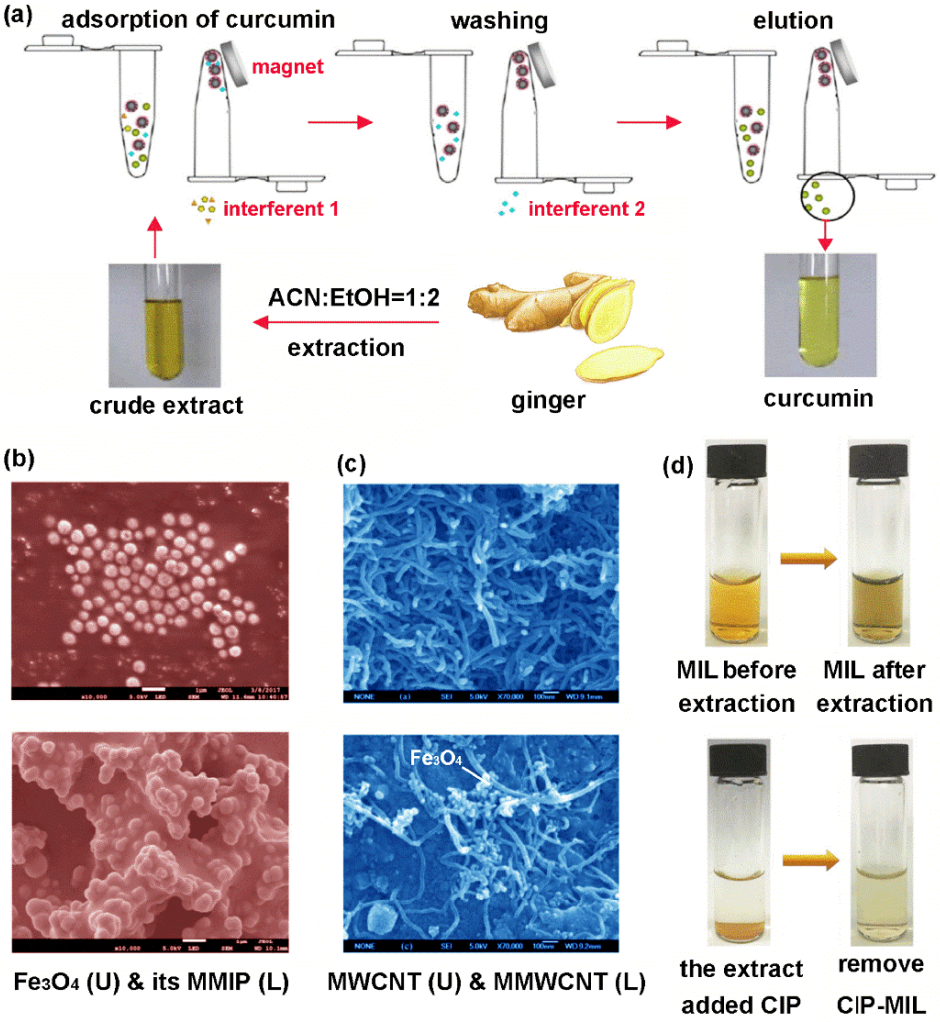

Figure 4. (**a**) The extraction process of curcumin from ginger by magnetic solid-phase extraction; (**b**) SEM images of Fe_3O_4 and its MMIP [109]; (**c**) MWCUT and MMWCNT [110]; (**d**) separation of TPs by MIL and CIP [43].

A variety of carbonaceous materials is an important driving force for the development of this field. At present, an increasing number of carbon-based magnetic adsorbents have been invented and applied. Rahimi et al. [111] successfully synthesized three-dimensional graphene-Fe_3O_4 (3D-G-Fe_3O_4) nanoparticles and measured the magnetic properties at room temperature. The results showed that the magnetic nanoparticles had high saturation magnetization (54.8 emu/g). Then, the adsorbent was successfully applied to extract caffeine according to an π–π reaction from tea, coffee chocolate and other food samples, and recovery reached 93.1~97.7%. This maybe due to its large specific surface area (216 m^2/g), resulting in a strong adsorption capacity. Furthermore, the adsorption and magnetic properties of the magnetic adsorbents remained basically unchanged after being reused 40 times, indicating that the adsorbent had good durability and a long service life.

In another study, Xiao et al. [112] developed a solid-phase extraction process to selectively extract the flavonoids (quercetin, luteolin and kaempferol) from urine samples based on magnetic carbon nanotubes (MCNTs) and an ionic liquid ([C_{16}mim]Br) for the first time. In particular, [C_{16}mim]Br has a very long alkyl chain which can form mixed hemimicelles on the surface of nanoparticles, and its selective separation performance is closely related to the intermolecular interaction with target flavonoids through hydrophobic, electrostatic and π–π interactions. Then, the recoveries comparison of flavonoids enriched with [C_{16}mim]Br-coated MCNTs-SiO_2 and [C_{16}mim]Br-coated Fe_3O_4-SiO_2 showed that

the two magnetite nanoparticles could result in more ideal results than many previous methods, and both of them can be separated from a suspension rapidly by a magnetic field. However, the recovery of flavonoids by [C_{16}mim]Br-coated MCNTs-SiO_2 was higher than that by [C_{16}mim]Br-coated Fe_3O_4-SiO_2, which was attributed to that MCNTs had a larger specific surface area. Moreover, the investigation on the effects of various separation conditions indicated that adding a small amount of [C_{16}mim]Br to form mixed hemimicelle solid-phase extraction adsorbents would be beneficial to obtain a higher recovery. When the excessive ionic liquid was used, recovery would decrease with the increase in the amount of [C_{16}mim]Br. This was because when the IL concentration in the solution (IL dosage) was higher than its critical micelle concentration, the IL micelles would also present in the solution, resulting in stronger retention in a part of flavonoid molecules, which could reduce their recovery accordingly.

In the process of extraction and separation, when there is a large amount of coexisting substances, the selectivity should be considered in addition to the extraction capacity, so a good adsorbent should not only have high adsorption capacity, but also have ideal selectivity. In order to strengthen the latter, one method is to use compounds with selectivity (such as IL and DES) to modify the adsorbent which was mentioned above, and another method is to use a magnetic molecular imprinted polymer (MMIP) which was developed and used to effectively enrich the target compounds with specific structural types. Hesperidin is a flavonoid compound, which is a main component in dried pericarp of *Citrus reticulata Blanco*. It has many pharmacological effects, such as antioxidant, anti-inflammatory and antitumor; so, it is widely used in medicines and functional products. At present, column chromatography and thin layer chromatography are two main methods for its separation and enrichment, but these methods are time consuming and have worse selectivity. Wang et al. [109] used hesperitin as a template to prepare a MMIP successfully and the SEM images of Fe_3O_4 and the MMIP are shown in Figure 4b. The type of MMIP was found with the better selective enrichment capacity of hesperetin from the dried pericarp of *Citrus reticulata Blanco* than that of MNIPs with luteolin, baicalein or ombuim (structural analogs of hesperetin) as a template. After 60 min of adsorption, solid–liquid separation can be achieved within 15 s by using the external magnetic field, and a recovery of 96.9% can be achieved at last, which proved that the method was very fast and efficient compare to traditional methods. Generally, the common mechanisms of the adsorption process can be divided into two types, which are specific adsorption and non-specific adsorption; but there was only non-specific adsorption in the above separation process. In addition, MMIPs were applied in the extraction of quercetagetin [105], rhodamine B [113], quercetagetin [114] and other natural active substances, and the results showed that the MMIPs had excellent adsorption capacity and significant selectivity. However, there are still some problems with this type of sorbent, such as the complicated preparation process and moderate adsorption capacity; further, there are a few functional monomers and most of them can only be polymerized and applied in the organic phase, so it is difficult to meet the separation requirements of many bioactive molecules, especially in their aqueous systems. All of these will become the focuses of further improvement.

In addition to the extraction separation which mentioned above, tracing and screening of lead compounds from natural products play important roles in drug development. Many researchers used magnetic nanoparticles which were modified with large molecules (such as protein, enzyme, receptor, and DNA) as bait to investigate and identify the ability of small bioactive molecules, and the so-called ligand-fishing method has been proved to be effective and convenient in searching and separating potential drug precursor molecules from the mixture of natural products. There are many examples of the successful application of magnetite nanoparticles in the targeted fishing for flavonoids, curcuminoids, saponins, anthraquinones or phenolic acids, and some of them are listed in Table 4. However, it is not easy to use these MNPs combined with macromolecules in large-scale preparative separation.

Table 4. The application of magnetite nanoparticles in the ligand fishing.

Samples	Magnetic Materials	Ligands	Ref.
Ginkgo biloba leaves extract	Human serum albumin-functionalized magnetic nanoparticles (HSA-MNPs)	Nine flavone glycosides	[115]
Dioscorea panthaica extrat	HSA-MNPs	Progenin II, progenin III, dioscin and gracillin	[116]
Dioscorea nipponica extract	HSA-MNPs	Three saponins (dioscin, gracillin, and pseudo-protodioscin)	[117,118]
Rhubarb extract	HSA-MNPs	Emodin and chrysophanol	
Epimedii Folium extract	α-glucosidase-functionalized magnetic nanoparticles	Eight flavonoid glycosides—baohuoside I, sagittatoside B, sagittatoside A, 2″-O-rhamnosyl icariside II, icariin, epimedin C, epimedin A, epimedin B	[119]
Selaginella doederleinii Hieron extract	Acetylcholinesterase-functionalized magnetic nanoparticles	Amentoflavone, robustaflavone, bilobetin and isoginkgetin	[120]
Pericarpium granati and *Cortex fraxini* extract	Monoamine oxidase B-functionalized magnetic nanoparticles	Calceolarioside B and ellagic acid	[121]
Bamboo leaf extract	Lipase-functionalized magnetic nanoparticles	Isoorientin, orientin, isovitexin	[122]

As shown in Table 4, many applied magnetic materials are protein (i.e., human serum albumin, HSA)-functionalized magnetic nanoparticles. HSA plays an important role in the transport and storage of endogenous and exogenous ligands, which is abundant in blood plasma, so it is of great significance to study the interactions between molecular ligands and HSA. The HAS-MNPs synthesized by Zhang and his coworker [123] have shown high magnetic responsiveness and good stability; performance remained basically unchanged (96.2%) after being reused 10 times. A total of 34 types of isoflavones and 13 types of astragalosides were screened from *Radix Astragali* by this sorbent. The method only needed three steps, including fishing, magnetic separation and elution; the whole process took less than 1.5 h, and the active substances can be screened without the need for the pre-purification. Bovine serum albumin (BSA) is also widely used because of its structural homology with HSA. It was found that the average size of the BSA-Fe_3O_4 MNPs prepared by Liu et al. [124] was 29 nm; when a magnet was placed next to the suspension, the NMPs could quickly gather together and then the magnet was removed and MNPs can be re-dispersed quickly and homogeneously by shaking. As a result, thirteen types of flavonoids were obtained by BSA-Fe_3O_4 MNPs from the methanol extract from *Pueraria* flower, and it was discovered that the affinity between flavonoids and BSA was closely related to the methylation and hydroxyl level of the B ring of the flavonoid structure.

In addition to the proteins mentioned above, enzymes are also frequently immobilized on MNPs in order to obtain the enzyme inhibitors from plant extracts which can not only enhance the activity, but can also be beneficial to their recycling use. According to the research of Chen and his coworkers [125], alcohol dehydrogenase (ADH), as a key enzyme in the conversion of ethanol to acetaldehyde, was immobilized on Fe_3O_4 nanoparticles modified by silica for the first time. It was found that the saturation magnetization of Fe_3O_4-SiO_2-ADH (32.4 emu/g) was less than that of Fe_3O_4-SiO_2 (40.0 emu/g) or Fe_3O_4 nanoparticles (69.9 emu/g). Similar to other immobilized enzymes, the thermal stability and pH stability of ADH on Fe_3O_4-SiO_2-ADH was better than that of free ADH, i.e., the activity of immobilized ADH at a different pH (5–7.5) or temperature (5~50 °C) was higher than that of free ADH. Then, the researchers used the Fe_3O_4-SiO_2-ADH nanoparticles to capture ADH inhibitors from *Glycyrrhiza uralensis* root, which can be used to treat alcoholism effectively. The optimum pH and temperature for the enzymatic activity of

immobilized ADH were 7.0 and 25 °C, respectively. Finally, nine active substances were focused that could bind to ADH and seven of them were found first, and the relative activity of immobilized ADH still remained 68.14% after being reused 10 times. In another study, Zhao et al. [126] immobilized neuraminidase (NA) onto magnetic beads (MBs) and applied it to fish NA inhibitor from *Flos Lonicerae*. NA is a glycoside hydrolase, which may be a target for the treatment of influenza; so, it is an important tool to find novel modulators of NA. As previously reported [127], the increasing amount of immobilized enzyme was not necessary for the increase in activity. When the mass ratio of NA to MBs was 1:8, the activity of the NA was highest for the higher surface density, reducing the contact of enzymes and other substances. In their research, four substances (luteolin-7-O-β-D-glucoside, luteolin, 3,5-di-O-caffeoylquinic acid and 3,4-di-O-caffeoylquinic acid) were fished out from the ethyl acetate extract of *Flos Lonicerae* and proved to be NA inhibitors, and the NA-MBs could also be reused for several separation process.

In addition to proteins and enzymes, DNA is also frequently immobilized on MNPs in order to obtain the DNA binders from plant extracts and study the interactions between them and they are helpful to repair impaired DNA. Yuan et al. [128] immobilized double-stranded DNA (ds-DNA) of different lengths (25, 200 and 1200 bp) onto Fe_3O_4 magnetic nanoparticles directly. Fe_3O_4-ds-DNA had a high saturation magnetization (42.97 emu/g) and was applied to fish DNA binders in *Rhizoma Coptidis* extract. After 10 min of adsorption at room temperature and 5 min desorption with deionized water at 80 °C, five compounds (columbamine, epiberberine, jateorhizine, palmatine and berberine) were found, and the order of the binding ability of MNP-ds-DNA was: MNP-ds-DNA (25 bp) > MNP-ds-DNA (200 bp) > MNP-ds-DNA (1200 bp). This difference might be caused by the steric hindrance between ds-DNA and nanoparticles, the ds-DNA length was longer, and the amount of ds-DNA that can be immobilized on Fe_3O_4 was less. In general, Fe_3O_4-ds-DNA can be used for screening ds-DNA binders from the complicated extract of traditional Chinese medicine. To date, magnetic technology has been widely used in the tracking and screening of lead compounds, but the magnetic materials are mostly based on Fe_3O_4, so in order to prevent oxidation and agglomeration, surface modification is necessary. This will reduce magnetic responsiveness and increase the cost of production. Additionally, the purpose of using magnetic extraction materials is mainly to make their recovery easier. Fixing the protein or enzyme on the magnetic carriers is not only relatively difficult, but also increases the cost of the whole process; and the special properties of these macromolecules will also limit the use of magnetic adsorbents to different extents. Therefore, the development of more feasible magnetic materials and applying them in this field for actual needs are necessary.

5.2. Magnetic Nanofluid Extraction

Magnetic nanofluids are composed of magnetic nanoparticles, base fluid (such as water, organic solvent, and oil) and surface-active agent, and they have been used to extract natural products due to their magnetic controllability and liquidity. They can be moved with the help of a magnetic field, so their recovery during the extraction process can be realized easily. In most extraction applications, magnetic nanofluids are quickly injected into the aqueous phase. The base fluid increases the dispersibility of the magnetic nanoparticles in the sample solution; thus, a large surface area of the magnetic nanoparticles is exposed, accelerating the extraction kinetics and decreasing the extraction time [129,130]. Given all of these findings, magnetic nanofluids has been accepted as a powerful material to extract natural products. For example, a magnetic nanofluid composed of hydrophilic DES (tetramethylammonium chloride/ethylene glycol, 1:2) and Fe_3O_4-SiO_2 was prepared and used to extract morin from apple and grape juices, diluted and acidic extracts of dried onion and green tea infusion samples—the hydrophilic DES enhanced the dispersion stability of the magnetic nanoparticles, resulting in the amelioration of the extraction kinetics, and recovery reached 97.7% with 1 min, which was higher than that of Fe_3O_4-SiO_2 in the absence of DES [131]. Due to high mass transfer efficiency, magnetic nanofluids are an ideal

option as extractants, but their application in the field of preparing natural products is still rare, and further application development is needed.

5.3. Magnetic Ionic Liquid Extraction

As motioned before, magnetic ionic liquids (MILs) have the advantages of conventional ionic liquids (such as good designability, environmental friendliness, low vapor pressure and extensive solubility), but also show strong magnetic responsiveness to the magnetic field. Because of the effect of hydrogen bonds, selective solubility and wide liquid ranges, MILs have been widely applied in various extraction and separation processes. Compared with volatile traditional extractants, the consumption of MILs is smaller under the same conditions, which satisfies the current needs of green chemistry to a large extent. In our previous research, [C_3mim]$FeCl_4$ aqueous solution was employed as a green solvent to extract and separate tea polyphenols (TPs) and caffeine, respectively [43], and the process is shown in Figure 4d. As the main active components in tea, TPs are often used in medicines and health care products because of their antioxidant, antiviral and antineoplastic activities. At present, TPs are usually extracted with water or alcohol as a solvent in heating or microwave-assisted modes. However, the low selectivity of water or alcohol usually leads to the inclusion of many other impurities in extracts, such as caffeine, which is a stimulant. Therefore, the content of coexisting caffeine has become an indicator of TP quality, which is often removed by resin adsorption from the mixture with TPs. The whole process of extraction together with separation is tedious, slow and reagent consuming. In our study, [C_3mim]$FeCl_4$ was found with selective interaction with TPs in the solution, and it can be well recovered after extraction by using carbonyl iron powders and magnetic field, and the recovery rate of this type of MIL reached 99.8%.

Similarly, Li et al. [132] used different MILs to extract sinomenine from *Sinomenium acutum*, which has analgesic, antitussive, hypotensive and anti-inflammatory activities. Comprehensively, the extraction performance of six imidazole-based MILs ([C_2mim]$FeCl_3Br$, [C_4mim]$Fecl_3Br$, [C_4mim]$FeCl_4$, [C_2OHmim]$FeCl_4$, [C_6mim]$FeCl_3Br$ and [C_8mim]$FeCl_3Br$) and three non-magnetic ILs ([C_4mim]Cl, [C_4mim]Br and [C_6mim]Br) was compared. The results indicated that the extraction rate of MILs was higher than that of non-magnetic ILs, which showed that the formation of metal complexes was beneficial to the extraction of compounds with hydroxyl groups in the structure. This mechanism is similar to that in the former study about the interaction between [$C_n$mim]$FeCl_4$ and TPs. Moreover, the extraction effect of $FeCl_3Br^-$ was better than that of $FeCl_4{}^-$; for lipophilic alkaloids such as sinomenine, it would also become more ideal with the increasing length of alkyl chains in MIL cations for their lowered polarity. After the extraction, the MIL was conveniently recovered by applying a magnetic field and the recovery rate reached 99.5%.

In addition, MILs have also been applied in the mode of aqueous two-phase separation (ATPS), and MIL-ATPS combines the advantages of MILs and traditional aqueous two-phase systems. Distribution coefficients of target molecules depend on their interactions with MIL and water in the presence of salts, including electrostatic force, hydrogen bond, hydrophobic effect and affinity. The studies about MIL-ATPS have attracted wide attention and become a hot research spot in separation science at present. In recent years, Nie et al. [44] synthesized a new type of cholinium-based organic magnetic ionic liquids [$N_{1\,1\,n\,2OH}$][TEMPO-OSO_3] (n = 1, 2, 3, 4 and 5). It was found that these five types of MILs were superparamagnetic, and the magnetism of MILs can be strengthened with the increase in the length of the cationic carbon chain. Among them, [$N_{1\,1\,5\,2OH}$][TEMPO-OSO_3] showed the strongest magnetism and the saturation magnetization was 23.39 emu/mol. Then, the ATPS was developed by using these types of MIL, inorganic salt and water. It was found that the MIL in the upper phase (MIL enrichment phase) can be attracted by magnets and moved to the magnets after the formation of ATPS. Finally, the system of 8 wt% [$N_{1\,1\,5\,2OH}$][TEMPO-OSO_3] (0.4 g) + 30 wt% K_3PO_4 (1.5 g) + water was successfully developed for the extraction and separation of berberine hydrochloride from the aqueous extract of *Coptis chinensis*. Berberine is the main active compound in many Ranunculaceae

plants, and it is usually used in clinical preparations as an antidiuretic, anti-inflammatory and antibacterial component. The researchers aimed to explore a different methodfrom the commonly used extraction methods (refluxing extraction, ultrasound-assisted extraction and supercritical fluid extraction) with some shortcomings. As a result, target alkaloids can be selectively enriched in the MIL phase through the electrostatic interaction between its electron-deficient pyridine ring and the anion [TEMPO-OSO_3] of the latter. As a result, an extraction duration of 10 min was sufficient, and the extraction rate and distribution coefficient reached 98.71% and 127.68%, respectively [48].

5.4. Magnetic Field-Assisted Extraction

Many studies on the application of a magnetic field in extraction and separation of bioactive substances have been carried out (see Table 5). In the extraction process assisted by magnetic fields with common solvents, the comparison in previous studies has shown that it can improve separation efficiency to a certain extent, and the effect of different magnetic field intensity will be different. For a clear understanding, related mechanisms have been summarized in Section 2, and here are some interesting examples. Orange peel contains many active substances, such as pigments, essential oil, and flavones, and many of them are important industrial raw materials. At present, the extraction method with conventional solvents is usually used to obtain active substances from all types of fruit peels. On this basis, Zhou and Gao et al. [133] used 95% ethanol as a solvent in heating extraction of active substances from *Citrus* peel in a magnetic field (90~450 mT) which was realized by magnets. The extraction efficiency was improved with the increase in the magnetic intensity in the lower level firstly; when the magnetic induction intensity reached 360 mT, the yield was 12.3% and then it began to decrease. Under optimum extraction conditions (temperature = 60 °C, material–liquid ratio = 1:7 g/mL, magnetic intensity = 140 mT and extraction time = 2 h), the highest yield of active substance reached 14.5%.

Moreover, in a study extracting the flavonoids from *Lycium barbarum* L. with magnetic field-assisted method, the magnetic field was produced by a NdFeB permanent magnet [134], and the ideal extraction efficiency of target constituents by this method reached 290.81 mg/100 g in 640 mT magnetic field after 60 min at 65 °C, higher than that achieved by traditional extraction at the same temperature (272 mg/100 g). It should be emphasized that a similar phenomenon appeared—the extraction rate showed a positive correlation with magnetic field intensity at the beginning ($\leq$640 mT), and the most suitable magnetic field intensity was closely related to raw materials, the target chemical profile and solvents. There are probably two main reasons—the first is that the physical and chemical properties of the solvent will be changed; for example, the hydrogen bond network of water molecules is deconstructed and the bond angle of H-O-H is changed to less than 105°. After the association is reduced, water molecules become smaller and easier to penetrate into the semi-permeable membrane of plant cells, and the solubility of flavonoids will be improved with the increase in magnetic field intensity; the second reason is that the distribution coefficient of this system decreases with the increase in magnetic field intensity. When the magnetic field intensity is low ($\leq$640 mT), the former plays a dominant role; but when the magnetic field intensity is higher than 640 mT, the latter plays a more important role. In addition to the above compounds with high or moderate polarity, magnetic field-assisted extraction is also suitable for strongly liposoluble components. Considering peony seed oil has blood circulation and blood fat regulating, anti-inflammatory sterilization and other functions, Zhang et al. [25] extracted the oil from peony seed with petroleum ether at 55 °C and magnetic field intensity ranging from 240~1200 mT that generated by self-made magnetic equipment. Additionally, it was found that when the magnetic induction intensity was 720 mT, the highest yield of peony seed oil (27.5%) was achieved.

In addition to the above plant raw materials, other natural resources with commercial value and development potential can also be considered. Astaxanthin is a carotenoid that has many physiological activities, such as antioxidant, anticancer, immunity enhancement, and improvement of eyesight. Zhao et al. [135] also used magnetic field-assisted extraction

technology to obtain astaxanthin from *Haematococcus pluvialis*. In their study, the experiment was carried out in an electromagnetic system (Shandong University, Jinan, China), and they found that when the magnetic field intensity was 15 mT, the extraction rate was 1.2 fold the rate without the effect of a magnetic field. Further, the improvement in the constant magnetic field on extraction for different substances is different, and has no impact on the extraction of some substances. Moreover, some studies have proved that the improvement effect of a variable magnetic field on extraction can be more obvious than that of a invariable magnetic field. This is because the solvent molecules and target substance will rotate continuously under the variable magnetic field, which can increase the temperature of the system and improve the extraction rate. Furthermore, the change of a high-frequency magnetic field can also make a liquid film (usually composed of strongly polar molecules) on the surface of the cells, which will increase the diffusion coefficient and accelerate the extraction rate [136]. As evidence, Zaguå A et al. [24] compared the effectiveness of the extraction of polyphenols together with caffeine from tea leaves using 100 °C water with a variable magnetic field (VMF) (a frequency of 50 Hz magnetic and induction of 100 mT) or permanent magnetic field (PMF) (magnetic induction of 100 mT). In this study, VMF was produced by an induction coil supplied by an alternating autotransformer, and PMF was generated between a pair of neodymium magnets 10 cm apart. The extraction was carried out under the conditions of a solid–liquid ratio of 1:50 and an extraction temperature of 100 °C for five minutes, which was proved effective for six different brands of tea (three types of green tea and three types of black tea). The results showed that under the constant magnetic field, the extraction efficiency did not improve significantly, but under the variable magnetic field, the extraction efficiency of the caffeine was significantly improved (green tea was 6~18%, blank tea was 4~12%)., Further, some improvement was seen in the extraction of polyphenols (green tea was 10%, blank tea was 15%). In addition, as another important ingredient in tea, amino acids, especially basic amino acids, have great nutritional value and can be used as the precursor of bioactive substances. Tarapatskyy et al. found that the extraction of the amino acids from tea was also improved by applying a VMF; in their study, the VMF with a frequency of 50 Hz and induction at 100 mT was produced by an induction coil supplied by an alternating autotransformer, and the extraction was conducted for 5 min with the solid–liquid ratio of 1:50. The results showed that the content of amino acids in green and black tea infusions increased by 8.5% and 4.7%, respectively, while the content of essential amino acids increased by 17.0% and 12.6%, respectively [137].

As mentioned above, the performance at the macro level is the improvement of extraction efficiency, and the mechanism at the micro level is that the magnetic field can change some key properties of solvent. Interestingly, these changes can be maintained for quite some time after removing the magnetic field, so some researchers directly use a magnetic field to treat/magnetize potential solvents before extraction. Chu et al. [138] used magnetized tap water, which was prepared by self-made magnetizing equipment, to extract stachyose; in this method, magnetization time was an important parameter under a certain magnetic field intensity in addition to extraction time and the solid–liquid ratio, and it was necessary to explore its effect on the product yield. Under the optimal conditions, i.e., water was magnetized under a magnetic field of 0.2878 T for 74.34 min, the solid–liquid ratio was 1:11 and the extraction time was 1.96 h, the extraction efficiency and content of stachyose increased by 40.02% and 20.27%, respectively, on the comparison of common tap water.

Table 5. Magnetic field in extraction and separation of bioactive molecules and together with biochemical objects.

Extract Objects	Solvent Systems	Magnetic Field Intensity	Time	Extraction Rate	Effect of Magnetic Field	Ref.
Scopolamine	Ethanol–water solution	400 mT	40 min (magnetization treatment), 60 min (ultrasonic extraction)	0.135%	The magnetic field destroyed the hydrogen bond network of solvents molecules, the molecules became smaller and easier to penetrate into the plant cells and dissolve the target substance, so the magnetic field is beneficial to improve the extraction rate of scopolamine, shorten the extraction time and improve the production efficiency	[23]
Polyphenols, caffeine and mineral component K	Demineralized water	100 mT, permanent magnetic field (PMF) and variable magnetic field (VMF)	5 min	Polyphenols: 0.990 mg/mL (PMF), 1.103 mg/mL (VMF); caffeine: 241.2 mg/L (PMF), 283.7 mg/L (VMF); K: 162 mg/L (PMF), 380 mg/L (VMF)	PMF had no significant effect on the extraction rate of the three target substances, the extraction rate of caffeine and K increased by VMF, but the effect of VMF on polyphenols was less obvious	[24]
Peony seed oil	Petroleum ether	720 mT	60 min	27.5%	Peony seed oil yield increased with the increase in magnetic induction intensity; when the magnetic induction intensity was 720 mT, the highest yield was achieved, and then the yield decreased with a further increase in magnetic induction intensity	[25]
Wolfberry flavonoids	Ethanol–water solution	640 mT	120 min (magnetization treatment), 60 min (reflux extraction)	2.9081 mg/g	The extraction rate increased with the increase in magnetic field intensity at the beginning; when the magnetic field intensity was higher than 640 mT, the extraction rate decreased with the increase in the magnetic field intensity	[134]
Astaxanthin	Acetic ether	18 mT	50 min	62.72%	A magnetic field was produced by a magnet, and the extraction yield of astaxanthin increased when the magnetic intensity increased from 0 to 15 mT, and then the extraction yield decreased	[135]
Stachyose	Magnetized water	287.8 mT	74.34 min (magnetization treatment), 1.96 h (extraction)	28.2%	The extraction rate was 1.4 fold the rate when the solvent was unmagnetized water	[138]

It should be noted that magnetized water also has certain antimicrobial activity, which is beneficial for its application in long-time extraction to prevent microorganisms from growing in solution, especially for solutions with proteins and saccharides as extracts. Zhang and her coworkers [139] compared the extraction of protein from oat bran by magnetized water, which was produced by a farm power magnetizing water machine (Safe Good Co., Ltd., Seoul, Korea) for 24 h at room temperature and distilled water. Under the conditions of a solid–liquid ratio of 15:1, a temperature of 40 °C, a pH of 10, and an extraction time of 2 h, the extraction rate and the purity of the protein obtained by magnetized water (the extraction rate was 63.88% and the purity was 72.10%) were higher than that of the distilled water (the extraction rate was 51.36% and the purity was 66.51%). So far, the mechanism is that the magnetized water has stronger permeability and solubility due to breaking the hydrogen bonds between the water molecules. Furthermore, a high extraction rate (71.06%) and high purity (75.28%) were obtained when the same magnetized water was used as the extracting solvent, the solid–liquid ratio was 16:1, the pH of the solution was adjusted to 10, and the higher extraction rate was obtained in one hour at 36 °C [28].

Oat polysaccharide which is another important bioactive substance in oat bran, is often extracted by water. Zhang and her coworkers [140] also investigated the effect of magnetized water, which was also produced by a farm power magnetizing water machine (Safe Good Co., Ltd., Seoul, Korea) for 24 h at room temperature and distilled water on the yield of oat polysaccharide. It was also found that under the conditions of a solid–liquid ratio of 18:1, a temperature of 68 °C, a pH of 8.7 and an extraction time of 1.25 h, the extraction rate of magnetized water was 13.9%, which was 37.55% higher than that of distilled water. It can be proved that the extraction efficiency can be effectively improved by either using magnetized water as an extraction solvent or applying a magnetic field directly during the extraction process.

6. Immunomagnetic Beads in Separation of Biological Objects

In recent years, magnetic technologies (especially immuomagnetic beads) have been used in numerous biological fields. Immunomagnetic beads are magnetic microspheres coated with specific groups (such as -NH_2 and -COOH), so they are special to bind DNA or other specific substance. The magnetic property of the particles has been utilized to achieve faster separation in the magnetic field. Similar to the aforementioned MSPE materials, the selected magnetic beads should be well dispersed in the solution without the presence of foreign magnetic field, but when there is an external magnetic field, these beads will respond to the magnetic field and then aggregate swiftly. This method is widely used in the separation and purification of nucleic acids, cells, proteins and enzymes because of its high specificity and simplicity of operation. In magnetic solid-phase extraction, magnetic metal-organic frameworks (MMOFs) which are composed of magnetic particles and MOFs are another commonly used sorbent. They have high selectivity, good dispersity and recyclability. Furthermore, some new magnetic materials are used in the field of biological engineering; one of them is famous magnetic graphene/mesoporous silica composites [66], including size-exclusive, "raisin-bread sandwich"-structured, quantum dots-capped, and phenyl-/sulfo-/targeting peptide-/Cu^{2+}-/polyethylenimine-modified ones.

6.1. Separation of Nucleic Acids

Nucleic acids are often separated from complex mixtures before starting some research and procedures, such as sequencing, amplification, hybridization, and detection. Traditional techniques of the separation of nucleic acids include chromatography, density gradient ultracentrifugation, or separation by adding chemical reagents. However, these methods are time consuming. At present, magnetic particles have been successfully applied in nucleic acid separation, which have achieved good results by the simple, quick and efficient method. Among the most commonly used method of separation and purification of DNA by magnetic particles, the mechanism of solid-phase reversible immobilization

(SPRI) is that DNA can selectively bind to functional surface of magnetic particles under a high concentration of PEG and salt. Furthermore, the process is reversible, i.e., DNA can be eluted and obtained [141].

In 2008, Saiyed et al. [142] prepared Fe_3O_4 magnetic nanoparticles with hydroxyl groups on the surface to isolate genomic DNA. By using co-precipitation, the size of these nanoparticles was approximately 40 nm, and they can be well dispersed in the TE buffer with pH 8.0 at room temperature. Then, the genomic DNA was isolated by using these magnetic nanoparticles as a solid-phase support, and the binding buffer was composed of 10% PEG 6000 and 1.25 M NaCl, while the eluent was 70% ethanol. Additionally, the whole process took place within 15 min, which was less than several hours taken by traditional phenol–chloroform extraction protocols. Moreover, the yield of DNA separated from samples by using magnetic nanoparticles was nearly 1.3 fold the yield using the traditional DNA extraction method or commercial kit (Qiagen method), and the agarose gel electrophoresis of DNA separated by magnetic nanoparticles is shown in Figure 5a. In another experiment, superparamagnetic magnetic nanoparticles were prepared by chemical co-precipitation. Because there was no use of polymers for surface modification of particles, they had a relatively small particle size (approximately 8 nm) and relatively better magnetic responsiveness; moreover, the magnetic nanoparticles could form stable colloidal suspensions in cell lysates. DNA can be directly bound to magnetic nanoparticles by electrostatic interaction, so MNPs were used to extract DNA from the *Escherichia coli* XL1 blue strain in binding buffer (10% PEG 6000 and 1.25 M NaCl), then eluted by 70% ethanol, The results showed that the method only took 25 min to obtain the DNA that did not contain protein and RNA basically, and the yield of DNA was 18~20 μg and the A_{260}/A_{280} was 1.8, which were similar to the yield of 20~22 μg and the A_{260}/A_{280} of 1.83, which were achieved by the purchased kit (Chromous Biotech Pvt Ltd., Bangalore, India), but the latter method was expensive and time consuming (approximately 2 h). Moreover, the yield and the purity of both methods were superior to the traditional phenol–chloroform method (yield: 18~20 μg, A_{260}/A_{280}: 1.5), which took more than 6 h. As a result, the agarose gel electrophoresis of DNA obtained by the three methods is shown in Figure 5b [143].

Magnetic technologies are considered a promising approach for high-efficiency nucleic acid separation; however, MNPs with different functional surfaces were reported to possess diverse capacities to bind nucleic acids. For example, Li et al. prepared three MNPs (MNP-NH_2, MNP-COOH and MNP-OH) and found that MNP-NH_2 had strongest affinity with nucleic acids, while MNP-COOH was the weakest. In addition, the size of MNPs also had a significant affect on their sedimentation and dispersion features, resulting in diverse separation results. MNPs of a too small size, such as a diameter of 50 nm, possessed a higher risk of incomplete recovery and residual risk, which would reduce the extraction yield and purity of nucleic acids; MNPs of an extra-large size, such as a diameter of 50 nm, could not effectively bind nucleic acids because of severe sedimentation and poor dispersibility [144].

6.2. Separation of Cells

With the development of cell therapy technology, systematically isolating cells in small-scale experiments or in high-throughput industrial settings has gradually become a hot research topic. Currently, various manual, semi-automated, automated, and robotic integration solutions can meet many specific demands, which will be selected by operators according to their budget and plan. Moreover, some isolation solutions can be coupled with downstream equipment conveniently, such as flow cytometry analyzers. At present, fluorescence-activated cell sorting (FACS) and magnetic-activated cell sorting (MACS) are the two commonly used cell separation methods for obtaining target cells with high purity. Furthermore, the latter is friendly to biological samples because of its simpler operation, so it is widely applied in different cell separation fields, such as sperm [145], epidermal stem cells [146], and lung multi-potent stem cells [147].

The MACS system is mainly composed of MACS microbeads, a MACS separation column and MACS separators. MACS microbeads are directly or indirectly bonded with the antibodies, and then can be bonded to particular cells to realize the separation of the particular cells in high-intensity magnetic fields; the diameter of most MACS microbeads is approximately 50 nm and their volume is much smaller than the cell volume. This is because MACS microbeads with a small size have a lower effect on cell activity and the cells can be determined by flow cytometry. Obviously, one disadvantage resulting from the small size is the weak magnetic responsiveness; this requires a much stronger magnetic field in the process of magnetic separation. There are some MACS microbeads which have a larger diameter (such as 200 nm or even 1200~4500 nm). These great particles have better magnetic responsiveness, and the involved magnetic separation process is simpler and more efficient, but the larger particles tend to produce mechanical pressure on cells, which may affect cell activity. Therefore, the appropriate size of magnetic beads should be chosen according to the specific context.

At present, there are many commercial products about the MACS system, such as Dynabeads Sterile Epoxy and the MIniMACS starting kit. Mesenchymal stem cells (MSCs) are the main source of cells for cell-based therapy, so it is very meaningful to isolate and purify MSCs and some methods have also been reported. For example, they were isolated from human synovial fluid by the MACS system, and CD90 microbeads (Miltenyi Biotec, Cologne, Germany) were chosen to immunolabel the cells in FcR Blocking Reagent (Miltenyi Biotec, Cologne, Germany) at 2~8 °C for 30 min, then MACS pro Separator (Miltenyi Biotec, Cologne, Germany) was used to realize magnetic separation, and the process is shown in Figure 5e [148]. MSCs obtained by the MACS system still had a high growth rate and differentiation potentials (chondrogenic, adipogenic, and osteogenic differentiation). Furthermore, the cost of the MACS was relatively low. At the same time, some studies have confirmed that rabbit mesenchymal stem cells (rbMSCs) are similar to human MSCs in many ways, such as cell biology and tissue physiology, so it is a good choice to use rabbit instead of human MSCs. Additionally, rbMSCs were isolated from a rabbit model by the same MACS system as follows: the mixture of CD90 microbeads (Miltenyi Biotec, Cologne, Germany) and cells was incubated in 4 °C for 15 min, and the magnetic separation process was carried out in a MiniMACS Separator (Miltenyi Biotec, Cologne, Germany) [149].

Although the equipment cost of the MACS system is lower than that of other methods (such as FACS), the current commercially available MACS system, especially MACS microbeads, is still expensive, and the MACS system is mainly used in small-scale isolation of a small number of cells; so, many researchers are working on the development of a new MACS system, especially new immune-magnetic beads. For example, Zhang et al. [150] successfully designed and synthesized four immuno-magnetic beads (IMNP-CD3, IMNP-CD4, IMNP-CD8 and IMNP-CD14) based on four different types of monoclonal antibodies for the separation of different T cells. These four immuno-magnetic beads can be suspended in ultrapure water without aggregate, and their diameters were approximately 710 nm. The magnetic field strength, which was provided by their designed separators, reached 550 mT, and the immuno-magnetic beads could successfully adsorb onto the separation column; the column was packed with steel balls and balls with a diameter of 250~550 μm were modified with parylene materials, and after magnetic separation three times, the separation efficiency of immune-magnetic beads reached 99.4%. Furthermore, the immuno-magnetic beads were used to isolate and enrich T cells from human blood. The effective selection rates of $CD3^+$, $CD4^+$, $CD8^+$ and $CD14^+$ T cells were 79%, 74%, 57% and 67.9%, respectively, similar to that of other methods reported previously, and the collected cells have good activity and can proliferate effectively in vitro.

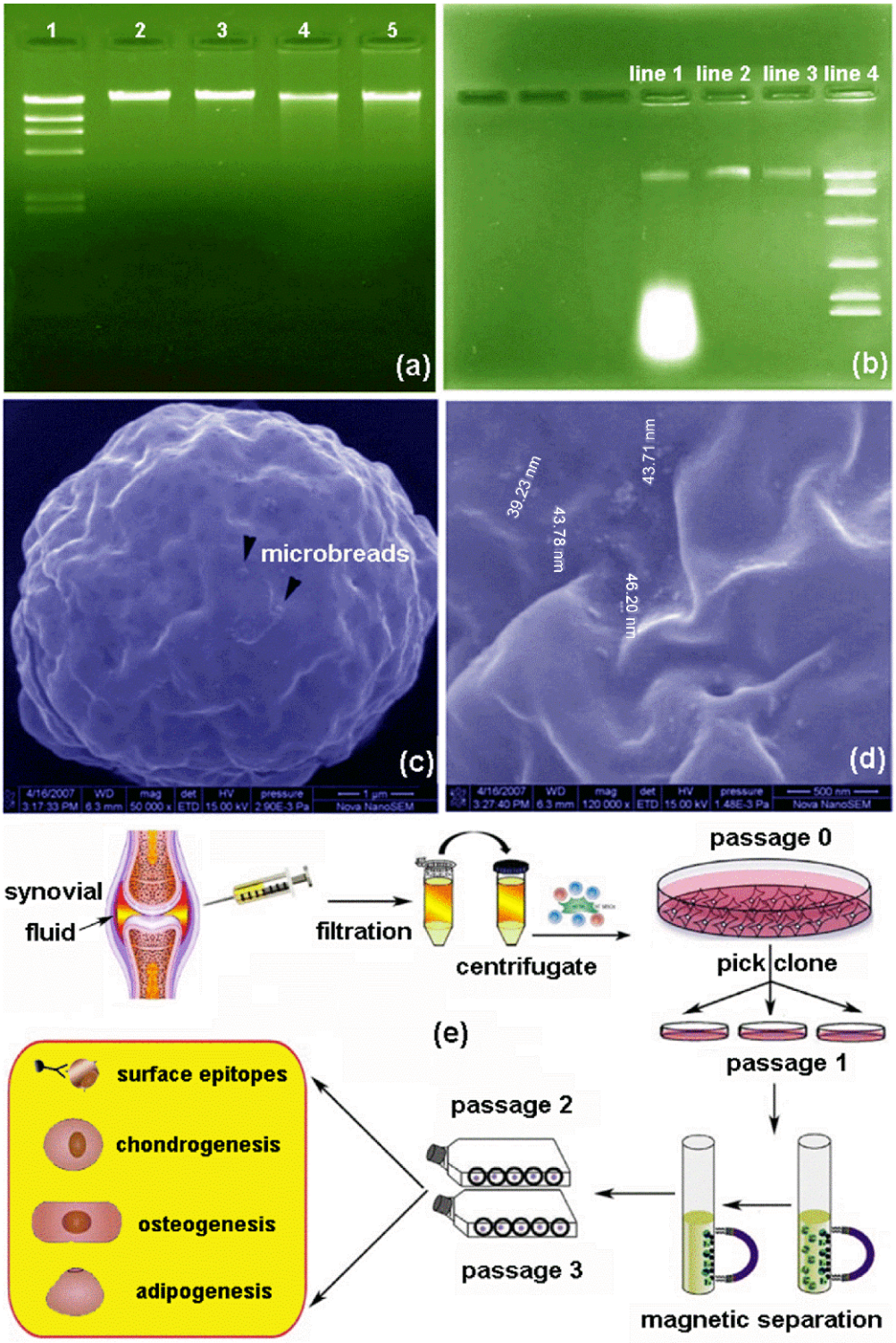

Figure 5. (**a**) The agarose gel electrophoresis of (1) a DNA molecular weight marker and the NDA obtained from (2) rat liver homogenate, (3) rat brain homogenate, (4) HCT116 and (5) human blood by magnetic nanoparticles [142]. (**b**) The agarose gel electrophoresis of (line 1) a DNA molecular weight marker and the DNA separated by (line 2) magnetic nanoparticles, (line 3) the commercial kit and (line 4) the traditional phenol–chloroform method [144]. SEM images of α6bri CD71dim cells marked by magnetic microbeads at (**c**) 50,000× and (**d**) 120,000× magnification, respectively [147]. (**e**) The separation of MSCs from human synovial fluid by the MACS system [149].

6.3. Adsorption and Immobilization of Enzyme or Protein

Isolation of pure proteins from biomaterials is a common task in biological engineering, and it is also conducive to the study of proteins and life activities. In addition, the separated proteins can also be used in medicine and the food industry, such as amylase which can be used in glucose production, or chymotrypsin which can be used for treatment of sprain. However, it is not easy to isolate proteins effectively from biological samples, and the highly efficient separation of these macromolecules from each other or a complex matrix is also worth exploring. The commonly used methods are precipitation, gel filtration chromatography, gradient density centrifugation and electrophoresis; but these methods usually require tedious treatment and often lower the activity of the target proteins to different extents. Compared with the conventional separation methods, magnetic separation technology has advantages of simplicity, less energy consumption, less activity loss and satisfactory recovery for proteins. In this field, Fe_3O_4 MNPs are the most commonly used because of the low toxic effects [151], and they bind proteins through bioconjugation, physical absorption, and covalent or non-covalent bonds [152–155]. Li et al. [156] used MNPs modified and joined with $–NH_2$ for isolating proteins from several traditional Chinese medicine (*Agaricus Blazei mushroom*, *Astragalus membranaceus*, *ginseng*, *Ganoderma lucidum*, and *Angelica sinensis*). In this study, MNP-NH_2 was added into the samples and placed at 4 °C for 15~20 min, the amino groups on the magnetic beads would bind with the carboxyl groups of the proteins, thus the binding between magnetic particles and protein was realized, then the protein could also be separated by a magnetic field at 4 °C within 20 min. The results showed that several proteins (*Agaricus blazei* protein, *Astragalus membranaceus* protein, *Panax ginseng* protein, *Ganoderma* protein, and *Angelicas* protein) were separated selectively.

Chitosan has good biocompatibility and contains amino groups, so the magnetic nanoparticles modified with chitosan (MNP-CS) are also used in protein adsorption and separation. In an attempt [157], the MNP-CS with a diameter of 8.7 nm was prepared to adsorb bovine serum albumin (BSA), which was widely used in biochemical experiments. This type of superparamagnetic particles can be well dispersed in water and the saturation magnetization was 50.05 emu/g. The isolation results showed that the adsorption of BSA in 0.05 mol/L Tris-HCl buffer with pH 6 reached equilibrium within 60 min and the saturated adsorption capacity was 300 mg/g. In addition to traditional MNPs, its combination with new materials/media is also considered by researchers, such as graphene oxides, CNTs, MOFs, or iLs. [158]. In recent experiment by Wei et al. [56], ionic liquid [C_6mim]Cl-modified magnetic metal-organic framework composites (Fe_3O_4-MWCNTs-OH-ZIF-67-IL) were prepared and used to extract α-chymotrypsin from the crude extract of *Porcine pancreas*, and the conditions were as follows: the solid–liquid ratio was 5 mg/mL, the solution pH was 6, and the incubation time was 2 hours at 35 °C. Then, α-chymotrypsin was eluted by phosphate buffer with pH 12. The results showed that the composite particles were superparamagnetic and the saturation magnetization was 7.1 emu/g. Moreover, Fe_3O_4-MWCNTs-OH-ZIF-67-IL had a high extraction capacity (635 mg/ g), and 93% of the initial activity of the extracted α-chymotrypsin remained. Furthermore, the extraction capacity of the magnetic extractant still reached 484.1 mg/g after being reused five times.

6.4. Enrichment and Trapping of Microorganism

Immunomagnetic separation (IMS) is an important application of magnetic technology in the detection of pathogenic microorganisms, and has a wide range of uses and broad prospects in the fields of diagnosis and treatment. It has been most widely used in capturing bacteria, e.g., *Escherichia coli* (*E. coli*), *Salmonella* and *Listeria monocytogenes*. In addition, the detection of protozoa (*Cryptosporidium*, *Giardia*, etc.) and viruses (*hepatitis A virus*, *Rona virus*, etc.) is achieved by the rapid, simple and highly specific method. For example, Wu et al. [159] established a method for tracking influenza A virus by integrating the methods of immunomagnetic beads enrichment and immunochromatography; the average particle size of spherical magnetic beads which were prepared by the EDC/NHS method was selected as 102 nm because of their good dispersion and little influence on chromatogra-

phy. The mixture of the sample and magnetic beads was incubated at room temperature for 15 min, and then magnetic separation was completed in 10 min. Furthermore, researchers compared the results of this method with commercial colloidal gold strips. This showed that the former method could detect samples with a cycle threshold (C_t) value of 28 in real-time PCR detection, while the colloidal gold strip could only detect samples with a C_t value of 22. This means that the method which combined immunomagnetic bead enrichment and immunochromatography can enrich the target samples to detect with lower concentrations. In another example, Gao and his coworkers [160] established an immunomagnetic enrichment real-time fluorescent PCR (IMS-PCR) technology for *Listeria monocytogenes*, *Staphylococcus aureus* and *Salmonella*, and applied it in the monitoring of food-borne pathogenic bacteria. The whole process only needed 2~3 h and the positive rate of IMS-q PCR detection was 8.12%, slightly higher than that of traditional culture method (7.57%).

When immunomagnetic beads are used to selectively isolate and purify microorganisms, the high specificity of immunological reactions and the magnetic correspondence of magnetic beads are crucial. Simply speaking, the types of antibodies as well as the size and properties of magnetic beads will affect the separation performance. Parham et al. [161] investigated the effect of magnetic beads of different sizes and properties on the detection of *Escherichia coli* O157:H7 from bovine feces, which was mainly reflected in the recovery of magnetic beads. The recovery of magnetic beads in the buffer with PBS + 0.01% Tween 20 was higher than that of PBS + 0.05% Tween 20. Moreover, different types of magnetic beads had different recovery rates. For example, the recovery rate of magnetic beads increased with the increase in iron content in magnetic beads; and if the size of magnetic beads was larger, the recovery rate of magnetic beads was higher. In this study, the recovery rate of greater magnetic beads (60~80% iron content) produced by Aureon was higher than that of smaller magnetic beads (12% iron content) produced by Dynal in the same external magnetic field. Then, the researchers compared the effect of different magnetic beads on *Escherichia coli* O157:H7 separated from bovine feces; the incubation process was performed at room temperature for 30 min, then the magnetic process was performed in a magnetic separator (MPC-S, Dynal) for 3 min. At last, the recovery of *E. coli* O157:H7 by Aureon magnetic beads (6.2 μm) reached 85% of spiked fecal suspensions (137 cfu/g), which was higher than the 35% of the Dynal beads (2.8 μm). Therefore, immunomagnetic beads should be screened according to the characteristics of the objects to be separated. However, it is not always the truth that larger magnetic beads are better—for example, smaller magnetic are suitable for cell sorting, which was mentioned earlier in this study. If the size of magnetic beads is too large, it will have a negative impact on the biological activity of some substances in the related system, and large magnetic beads are not suitable for continuous operation, e.g., a negative impact on the chromatographic separation, so it should be a choice according to the specific context.

In the last decade, the application of magnetic technology (especially immunomagnetic beads technology) in the field of biological science has become increasingly mature. There are many commercial immunomagnetic beads appearing in the market, but the same magnetic beads have different abilities in terms of recognition and binding for various substances, so it is necessary to develop new magnetic beads for more potential objects. As mentioned earlier, a series of internal and external factors can also affect their performance. For instance, when some magnetic beads are used to extract the substance, the mass ratio of them to the sample has an obvious influence on the product yield and purity. When the magnetic beads are not enough, the yield will be low; but when the magnetic beads are too much, the purity will be reduced and the cost will rise. For operators, it is necessary to weigh and consider balance; appropriate magnetic beads and separation conditions of the magnetic beads should be chosen for specific samples.

7. Magnetic Separators for Multi-Level Applications

The innovation of equipment and hardware is the fundamental motive to promote the development of different practical technologies. When the technical level of magnetic separation rises to a certain extent, powerful instruments are urgently needed to help them break through the technical bottleneck. The development of a new high-efficiency magnetic separator and the optimization and improvement of traditional magnetic separator are common methods. Currently, magnetic technologies have been widely used in the biological field because of mild operating conditions. Just as the beginning of this article introduced the separation of bioactive substances with magnetic beads, this part mainly introduces the application of magnetic separators which can generate various expected magnetic fields for separation in the scope of this review.

The magnetic-activated cell sorting (MACS) system which was mentioned earlier in Section 6.2 will be elaborated here in detail, and the basic principle of the immune magnetic beads sorting device is shown in Figure 6a. If it is a positive sorting process, the target compounds are labeled by the magnetic beads and become magnetic complexes. The magnetic complexes are adsorbed and remain in the separation column by placing the separation column in the separator and the unlabeled compounds (mainly non-target substances) flow away. After removing the magnetic field, the target compounds will be separated by eluting via the separation column. If it is a negative sorting process, the untargeted compounds are labeled by the magnetic beads and become magnetic complexes, so the target compounds can be separated directly and the untargeted compounds are adsorbed in the separation column by placing the separation column in the separator.

In the MACS system, the MACS separation column and MACS separators are the core components. The main function of MACS separators is to generate an ideal magnetic field. The MACS separation column is filled with iron beads, which are modified by hydrophilic substances. These iron beads can magnify the magnetic field, which is provided by MACS separators by 1000~10,000 fold. In this way, magnetic particles can be more effectively adsorbed on iron beads, thus the recovery rate and product purity will be improved. At present, there are many commercial products with the MACS system, and the most well-known companies are Miltenyi and Dynal. However, the magnetic beads of these companies differ in size, and the magnetic responsiveness is different, too. Therefore, in specific processes of separation, different magnetic field intensities are needed when the magnetic beads are different; in other words, different magnetic beads need different magnetic separation equipment. The cost will be very high, especially when multiple different magnetic beads are used. Therefore, it is meaningful to develop magnetic separation equipment with wider applicability. Sun et al. [162] proposed a separation method to adjust the magnetic field by adjusting the distance between the magnetic field and magnetic bead suspension, which provided a good idea for developing a separation system for magnetic beads with different properties.

At present, when MACS is widely used in separation and purification with a rapid development speed, there are still some problems that needed solving [163–167]. For example, if the filling of iron beads in the separation column is not uniform, the magnetic field will become uneven and the distribution of fluid flow can be affected; further, the price of the commercialized separation column is not low. As a result, a simple MACS tip was prepared by Oh et al. [168] as a substitute for the separation column in the magnetic cell sorting system, which was the magnetizable tip of a pipette. As shown in Figure 7a–c, the MACS tip was a 1 mL pipette tip with a nickel mesh layer which had regular rectangular microporous shape, and the cross-sectional area was 50.3 mm^2. The thickness of the nickel mesh was 200 μm and the porosity was 49.7%. It had a large surface area and the surface area of each mesh was 93.1 mm^2. The nickel mesh layer can be temporarily magnetized by a magnet which was placed by the pipette tip. After the magnet was removed, the nickel mesh lost its magnetism. The function of the nickel mesh layer in this MACS tip is similar to that of iron beads in the MACS separation column—it is designed to increase the magnetic field intensity in the sample flow channel. At the center of the flow channel,

the intensity of the magnetic field increased by approximately 10^5 fold compared with that when there was no nickel mesh. Subsequently, the researchers used the MACS tip with five nickel mesh layers to separate magnetic nanoparticles with a diameter of 200 nm which were labeled with *Escherichia coli*, and then the sample solution could flow through the MACS tip 20 times, and the recovery rate reached 94.1%. Furthermore, the recovery rate was almost 0 when there was no nickel mesh. This study showed that the magnetic field gradient can be increased by using nickel mesh layers, and that the uneven magnetic field gradient caused by the non-uniform filling of iron beads of a traditional MACS separation column would not happen.

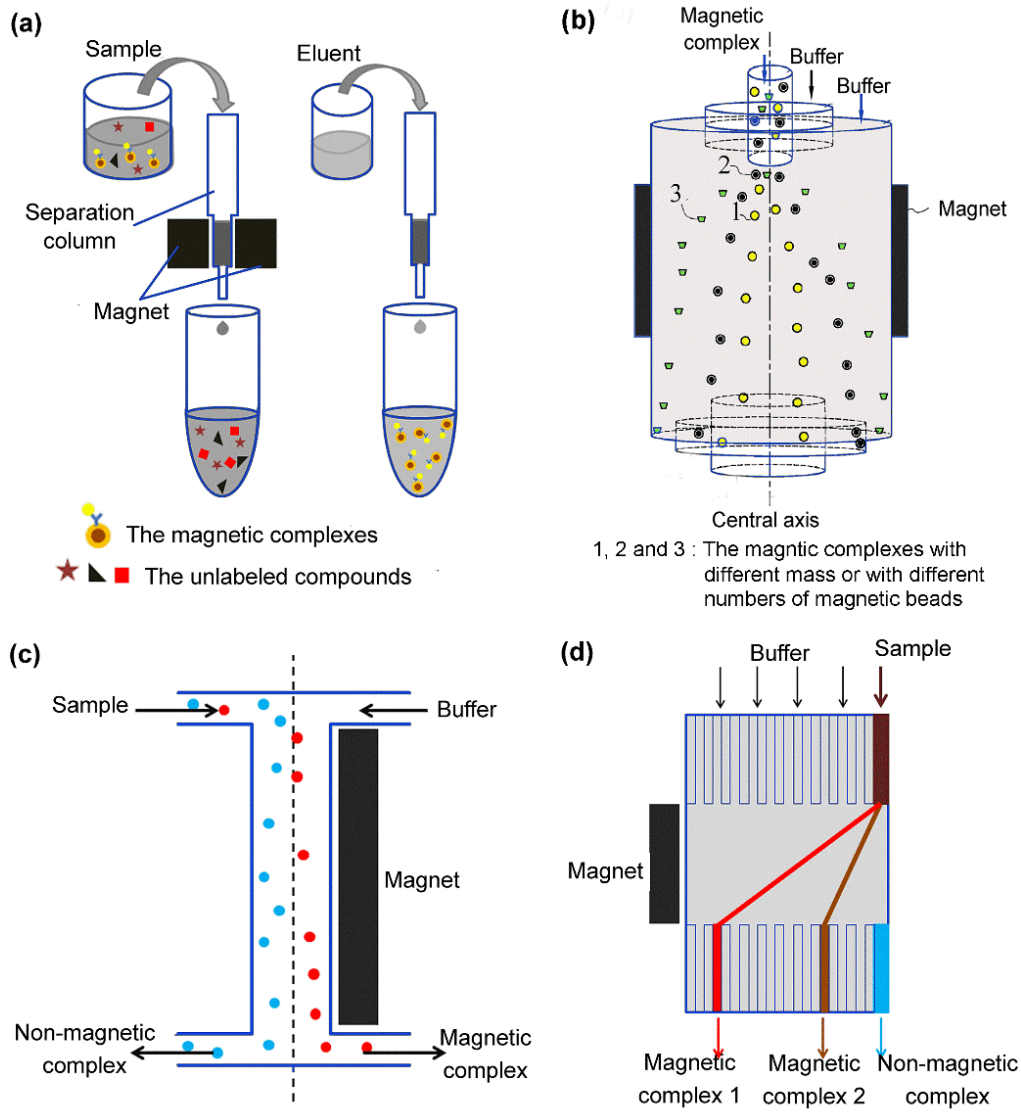

Figure 6. (**a**) The basic principle of the immune magnetic beads sorting device. (**b**) The cross-section view of the magnetic separator proposed by Murthy [162]. (**c**,**d**) The separation principle of the magnetophoresis [163].

Generally, all the devices mentioned above can only realize the separation of target objects that can be coupled with magnetic beads from objects that cannot. However, sometimes, more than one substance in the sample solution can be coupled with magnetic beads. If the separation device mentioned above is used, it is difficult to further separate these substances. Therefore, new instruments and equipment need to be studied and developed. In fact, as early as 2012, Murthy et al. [162] proposed a magnetic separator, and its cross-sectional area is shown in Figure 6b. When more than one substance in the

sample solution can coat magnetic beads, these substances might have a different relative molecular mass or coat different numbers of magnetic beads, and the result was that the magnetic complexes 1, 2 and 3 had different levels of magnetization. These sample mixtures could enter into the separation column, which was surrounded by the magnetic source from the inlet. Actually, the magnetic source was a number of magnets which were around the separation column. These magnets can be either permanent magnets or electromagnets. The rotating magnetic field was obtained by rotating the separating column around the central axis. The magnitude and gradient of the rotating magnetic field can be regulated by adjusting the magnetic field intensity, which was produced by the magnetic source and the rotating speed of the separating column. If the complex had a larger mass or more magnetic beads, it would be moved more away from the original trail and closer to the column wall, where the magnetic field was stronger. So, complexes 1, 2 and 3 with different mass or with different numbers of magnetic beads were separated under the action of a magnetic field and a centrifugal force, and the continuous separation of target objects with a different mass or with a different number of magnetic beads can be achieved by outflow from the different outlets, respectively. This separator can be used to separate biological samples; but in this type of equipment, the rotating magnetic field was obtained by rotating the separation column.

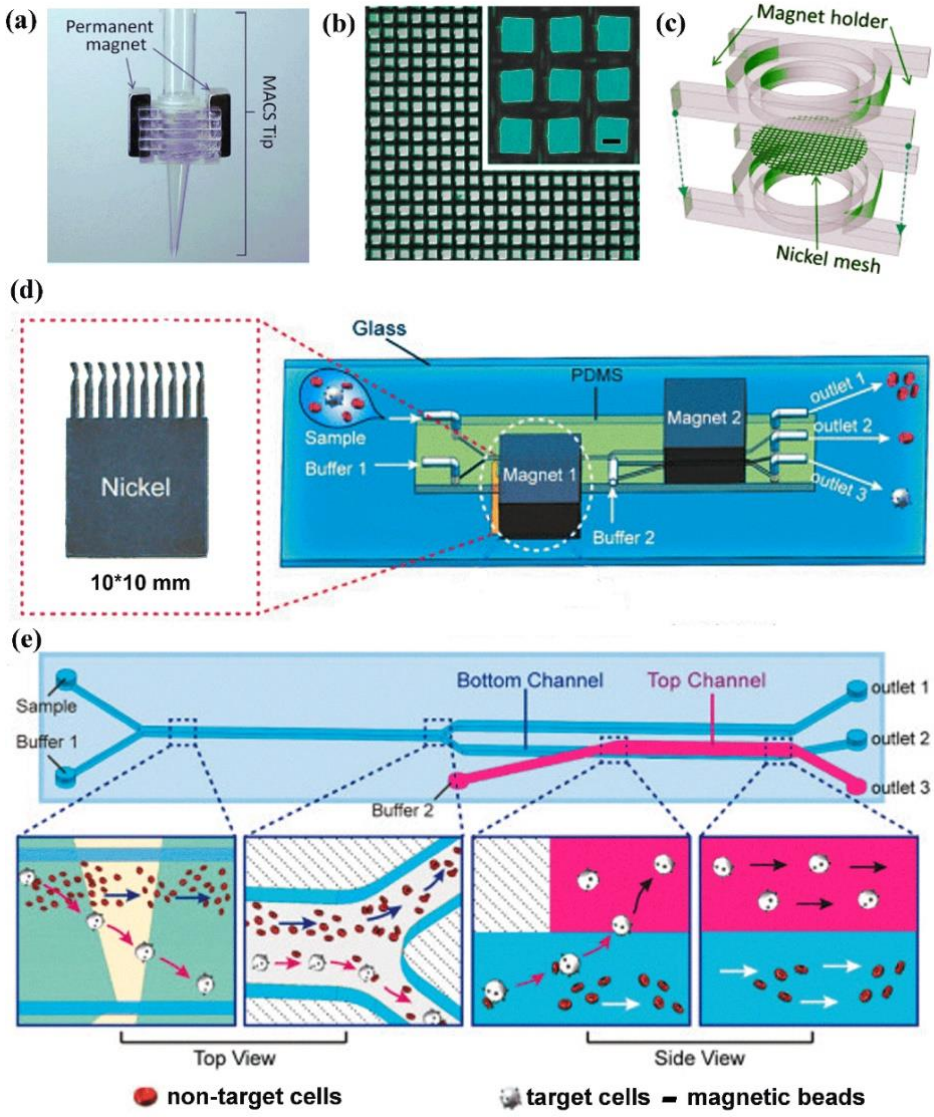

Figure 7. (**a**–**c**) The structure of the MACS tip [168]; (**d**) the device and (**e**) the separation principle of the magnetic separator which was proposed by Lin et al. [169].

Furthermore, in the separation process, it is not only the magnetic intensity that determines the outlet of the material, but also the centrifugal force. Therefore, it will affect the separation of some samples with a large centrifugal force and a small magnetic response force, or a small centrifugal force and a large magnetic response force. Therefore, this type of equipment has not been used widely at present; but if the separation column is not rotated and the external magnetic field is rotated, the interference of the centrifugal force will be avoided. This device is expected to achieve separation by changing the motion path of magnetic particles by the magnetic field, which can also be called magnetophoresis. In the field of bio-separation, magnetophoresis, magnetic separation on chips is also widely used. In Figure 6c, the magnetic substances will be shifted to the side of the magnet, thus the magnetic and non-magnetic substances are separated. In Figure 6d, materials with different magnetic susceptibility will be shifted to the magnet at the same time; but the angle of shifting is different, so that materials with different magnetic susceptibility can be separated.

Sometimes one-stage separation is not ideal because of the low purity or recovery, so Lin et al. [169] adopted two stages of lateral separation and vertical separation, and the device is shown in Figure 7d,e. In the lateral separation process, the magnet was a nickel microarray (NMA) with a special structure, which could significantly improve the magnetic field and its gradient; so, it can be used to separate as many target cells as possible from whole-blood samples. In the lateral separation stage, the target cell-magnetic beads and a few of the non-target cells that were non-magnetic could enter into the buffer stream from the sample stream. Subsequently, when the enriched cells in the buffer flow reached the vertical separation zone, which was an overpass structure, the target cells were elevated by the magnetic force and flowed into the top microchannel, while the unlabeled cells continued to flow through the vertical separation zone under the action of the bottom microchannel. Finally, high-purity target cells were collected at Exit 3. When the maximum magnetic field intensity on the surface of the magnet was set at 0.3 T, the 93% purity of the white blood cells (WBCs) can be achieved from the whole blood by two stages of lateral separation and vertical separation, which was higher than the 84% purity achieved by one-stage separation, and the cell survival rate was higher than 97.5%.

These devices, especially magnetic separation on chips, have been well developed in the field of biology. When they are used, the flow rate and the magnetic field intensity/gradient are needed for accurate control. A microfluidic chip has the characteristics of miniaturization and integration, and the application of magnetic materials used in a microfluidic chip has become a hot topic. According to current reports, there are a variety of ways to combine a magnetic field and a microfluidic chip. Magnetic microfluidic chips have been applied to the mixing and transportation of liquid in microfluidic chips, the switch and valve of chips, as well as the transportation, separation and capture of magnetic objects. At present, there are three ways to generate a magnetic field in microfluidic chips: an integrated electromagnet, an integrated soft magnet and an external magnet. (1) An integrated electromagnet integrates a tiny coil in the chip by micromachining and generates a magnetic field after applying a current. The magnetic field strength can be flexibly controlled by adjusting the current, but increasing the current will also cause the Joule heat problem. (2) An integrated soft magnet is also based on micromachining technology, and micro-soft magnets (such as nickel and ferronickel) that are easy to magnetize are integrated in microchannels of chips. Under the magnetization of an external magnetic field, the local magnetic field gradient increases significantly, so that the magnetic field force on the magnetic material is significantly increased; and the magnetic material can be controlled at a high flow rate, so as to achieve a fast and high-throughput separation of the target objects. (3) An external magnet is a conventional permanent magnet (e.g., NdFeB magnet, 500 mT) or electromagnet placed directly outside the microfluidic chip. Even if the external magnet is placed within a few millimeters from the channel, its magnetic field strength can also be used to control the magnetic particles. This method has the advantages of simple processing, a high success rate, low cost and unlimited design of microfluidic

channels. In addition, the magnet can be modified to improve the magnetic gradient, such as the unique structure shown in Figure 7d. It is believed that an increasing number of new magnetic separators will be developed and applied, and their performance breakthrough is worth awaiting.

8. Conclusions

This paper briefly introduces various magnetic technologies in the field of separation of bioactive molecules and biochemical objects in recent years. The simplicity, time-saving and high-efficiency properties have been elaborated. It is convenient and effective to apply them in the tracing and screening of lead compounds from natural products. The magnetite nanoparticles modified by all types of reagents have shown enough potential and ideal performance in the last decade, and numerous successful examples have provided sufficient guidance for the follow-up research. Further, related magnetic adsorbent materials are mostly based on Fe_3O_4, and most of them have complex structures, so their preparation process is long and their synthesis is relatively complex; some raw materials are a little expensive. For those with mature technology and the funds, more large-scale applications need to be carried out to fully verify their effectiveness in practice.

Overall, a magnetic field is an energy field, and can change some properties of a solvent. Whether magnetized water is used as a solvent or a magnetic field is applied directly in the extraction process, the extraction and separation efficiency can be further improved. The magnetic field intensity should be selected after careful investigation according to the specific context. In addition, an increasing number of new magnetic nanofluids and green solvents such as ionic liquids are being developed and applied in solid–liquid or liquid–liquid systems, and their performance is constantly being improved by optimization of structure and conditions. These innovative magnetic mediums are expected to solve the disadvantages of conventional solvents as they do not result in recovery difficulty and low selectivity and are environmentally friendly.

In the field of biological separation, magnetic separation technology has been widely used because as a mild technique—in particular, the immune magnetic bead sorting system, which not only shows immune specificity, but also has the rapid and mild characteristics of magnetic separation. It has been well applied in the biological field, and therefore numerous magnetic separators have been developed, such as MACS and various magnetic separation techniques on chips. In summary, the application of magnetic separation technology is becoming widely used in related fields, not only in the solid form and in a homogeneous system, but also in the liquid form and a heterogeneous system. Under the effort of researchers in different fields, magnetic separation technology is developing very fast. As a new green technology, magnetic separation is environmentally friendly and energy efficient, and it will be more attractive and powerful in the future.

Author Contributions: Conceptualization, X.F. and S.Y.; investigation, T.Z.; resources, H.S.; writing—original draft preparation, X.F.; writing—review and editing, S.Y. and Y.W.; visualization, T.Z.; supervision, H.S.; project administration, S.Y. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Natural Science Foundation of China (Grant No. 81673316) and the Fundamental Research Funds for the Central Universities, Southwest Minzu University (Grant No. ZYN2022093).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Bobinaitė, R.; Kraujalis, P.; Tamkutė, L.; Urbonavičienė, D.; Viškelis, P.; Venskutonis, P.R. Recovery of bioactive substances from rowanberry pomace by consecutive extraction with supercritical carbon dioxide and pressurized solvents. *J. Ind. Eng. Chem.* **2020**, *85*, 152–160. [CrossRef]
2. Li, P.F.; Qin, L.T.; Wang, T.; Dai, L.X.; Li, H.; Jiang, J.X.; Zhou, J.Y.; Li, H.; Cheng, X.Q.; Lei, F.H. Preparation and adsorption characteristics of rosin-based polymer microspheres for berberine hydrochloride and separation of total alkaloids from coptidis rhizome. *Chem. Eng. J.* **2020**, *392*, 123707. [CrossRef]
3. He, Q.; Guo, Z.X.; Cao, Y.; Yang, M.; Yao, S. Selective separation of main flavonoids by combinational use of ionic liquid-loaded microcapsules from crude extract of Tartary buckwheat. *Food Chem.* **2021**, *362*, 130225. [CrossRef] [PubMed]
4. Guo, Y.Y.; Li, Y.S.; Li, Z.C.; Yan, W.T.; Chen, P.; Yao, S. Extraction assisted by far infrared radiation and hot air circulation with deep eutectic solvent for bioactive polysaccharides from Poriacocos (Schw.) Wolf. *Green Chem.* **2021**, *23*, 7170–7192. [CrossRef]
5. Tang, J.Y.; Cao, Y.; Wang, X.; Yan, W.T.; Yao, S. A targeted extraction with solvent-free pressurized method based on analyte-like oxonium salts: An example for simultaneous determination of bamboo-leaf flavonoids by ultra-performance liquid chromatography/ultraviolet detection. *J Chromatogr. A* **2021**, *1658*, 462595. [CrossRef]
6. Andrade, T.A.; Hamerski, F.; Fetzer, D.E.L.; Roda-Serrat, M.C.; Corazza, M.L.; Norddahl, B.; Errico, M. Ultrasound-assisted pressurized liquid extraction of anthocyanins from Aronia melanocarpa pomace. *Sep. Purif. Technol.* **2021**, *276*, 119290. [CrossRef]
7. Phakoukaki, Y.V.; O'Shaughnessy, P.; Angeli, P. Intensified liquid-liquid extraction of biomolecules using ionic liquids in small channels. *Sep. Purif. Technol.* **2022**, *282*, 120063. [CrossRef]
8. Li, P.P.; Li, M.H.; Yuan, Z.J.; Jiang, X.B.; Yue, D.M.; Ye, B.; Zhao, Z.J.; Jiang, J.W.; Fan, Q.; Zhou, Z.C.; et al. 3D printed integrated separator with hybrid micro-structures for high throughput and magnetic-free nucleic acid separation from organism samples. *Sep. Purif. Technol.* **2021**, *271*, 118881. [CrossRef]
9. Ghosh, R.; Hale, G.; Durocher, Y.; Gatt, P. Dry-compression packing of hydroxyapatite nanoparticles within a flat cuboid chromatography device and its use for fast protein separation. *J Chromatogr. A* **2022**, *1667*, 462881. [CrossRef]
10. Song, Y.M.; Yang, Y.L.; Lin, X.; Zhao, Q.Z.; Li, Z.J.; Ma, G.H.; Su, Z.G.; Zhang, S.P. On-line separation and quantification of virus antigens of different serotypes in multivalent vaccines by capillary zone electrophoresis: A case study for quality control of foot-and-mouth disease virus vaccines. *J Chromatogr. A* **2021**, *1637*, 461834. [CrossRef]
11. Zhao, L.C.; Lv, X.R.; Cao, X.; Zhang, J.F.; Gu, X.K.; Zeng, H.Y.; Wang, L. Improved quantitative detection of VBNC Vibrio parahaemolyticus using immunomagnetic separation and PMAxx-qPCR. *Food Control* **2020**, *110*, 106962. [CrossRef]
12. Aslam, H.; Shukrullah, S.; Naz, M.Y.; Fatima, H.; Hussain, H.; Ullah, S.; Assiri, M.A. Current and future perspectives of multifunctional magnetic nanoparticles based controlled drug delivery systems. *J. Drug Deliv. Sci. Tec.* **2022**, *67*, 102946. [CrossRef]
13. Haghighi, A.H.; Khorasani, M.T.; Faghih, Z.; Farjadian, F. Effects of different quantities of antibody conjugated with magnetic nanoparticles on cell separation efficiency. *Heliyon* **2020**, *6*, 03677. [CrossRef]
14. Lee, G.; Song, J.; Han, H.; Kwon, D.; Park, J.; Jeon, S.; Jeong, S.; Kim, S. Zwitterion-coated colloidal magnetic nanoparticle clusters for reduced nonspecific adsorption of biomolecules. *Bioconjugate Chem.* **2021**, *32*, 1052–1057. [CrossRef]
15. Cai, Y.; Zhang, S.S.; Dong, C.; Yang, J.C.; Ma, T.; Zhang, H.; Cui, Y.L.; Hui, W.L. Lateral flow immunoassay based on gold magnetic nanoparticles for the protein quantitative detection: Prostate-specific antigen. *Anal. Biochem.* **2021**, *627*, 114265. [CrossRef]
16. Wang, Y.K.; Wei, H.N.; Li, Z.W. Effect of magnetic field on the physical properties of water. *Results Phys.* **2018**, *8*, 262–267. [CrossRef]
17. Zhang, L.; Yang, Z.; Deng, Q.J. Effects of pulsed magnetic field on freezing kinetics and physical properties of water and cucumber tissue fluid. *J. Food Eng.* **2021**, *288*, 110149. [CrossRef]
18. Feng, D.D.; Gao, J.M.; Zhang, Y.; Li, H.Y.; Du, Q.; Wu, S.H. Mass transfer in ammonia-based CO_2 absorption in bubbling reactor under static magnetic field. *Chem. Eng. J.* **2018**, *338*, 450–456. [CrossRef]
19. Guo, K.; Lv, Y.L.; He, L.M.; Luo, X.M.; Yang, D.H. Separation characteristics of water-in-oil emulsion under the coupling of electric field and magnetic field. *Energ. Fuel.* **2019**, *33*, 2565–2574. [CrossRef]
20. Guo, K.; Lv, Y.L.; He, L.M.; Luo, X.M.; Zhao, J. Experimental study on the effect of spatial distribution and action order of electric field and magnetic field on oil-water separation. *Chem. Eng. Process.* **2019**, *145*, 107658. [CrossRef]
21. Bahrpaima, K.; Bijanzadeh, A.R.; Behzadi, M. Effect of magnetic field on the liquid-liquid equilibria of (water + acetic acid + organic solvent) ternary systems. *Phys. Chem. Res.* **2017**, *5*, 125–134.
22. Cai, H.B.; Huang, L.H.; Qin, L.Q.; Jiang, L.X.; Huang, X.S. Extraction of active components from camellia oleifera tea by magnetized small molecules group water. *J. Guilin Norm. Coll* **2020**, *34*, 137–139.
23. Wu, Z.Y.; Chen, P.P.; Zhang, W.C.; Chen, T.Y.; Hui, A.L.; Xu, K. A Method for Ultrasonic Synergistic Magnetic Field Enhanced Solvent Extraction and Liquid-Liquid Extraction and Separation of Scopolamine. CN Patent 201810722377.6, 25 December 2018.
24. Zaguła, G.; Bajcar, M.; Saletnik, B.; Czernicka, M.; Puchalski, C.; Kapusta, I.; Oszmiański, J. Comparison of the effectiveness of water-based extraction of substances from dry tea leaves with the use of magnetic field assisted extraction techniques. *Molecules* **2017**, *22*, 1656. [CrossRef] [PubMed]
25. Zhang, W.L.; Fu, L.D.; Liu, D.; Zhu, H.T.; Wang, X.J.; Bi, Y.P. Extraction of peony seed oil by magnetization technology. *Coal Chem. Ind.* **2015**, *38*, 33–34.

26. Malek, F.H.; Alaridhee, T.; Hussein, A.A.; Al-Saeed, A.H.M. Effects of static magnetic field on dye extracted from Anchusa-ltalica through optimization the optoelectronic properties. *Int. J. Nonlinear Anal.* **2021**, *12*, 946–960.
27. Upadhyaya, L.; Semsarilar, M.; Quemener, D.; Fernandez-Pacheco, R.; Martinez, G.; Coelhoso, I.M.; Nunes, S.P.; Crespo, J.G.; Mallada, R.; Portugal, C.A.M. Block copolymer-based magnetic mixed matrix membranes-Effect of magnetic field on protein permeation and membrane fouling. *Membranes* **2021**, *11*, 105. [CrossRef]
28. Zhang, T.H.; Li, C.F.; Zhang, J.; Yu, Y.L. A process for enzymatic extraction of soybean oligosaccharide. CN Patent 202111019573.5, 26 November 2021.
29. Wang, Z.Q. The mechanism of action of magnetic fields on molecules of liquid. *J. China Univ. Pet. Nat. Sci. Ed.* **1998**, *04*, 119–122.
30. Du, J.; Feng, R.Y.; Zhao, J.; Yang, J.D. Research advance of the effect of magnetic field on physical and chemical properties of substances and their separation. *Hebei Chem. Eng. Ind.* **2006**, *11*, 21–24.
31. Pareja-Rivera, C.; Cuellar-Cruz, M.; Esturau-Escofet, N.; Demitri, N.; Polentarutti, M.; Stojanoff, V.; Moreno, A. Recent advances in the understanding of the influence of electric and magnetic fields on protein crystal growth. *Cryst. Growth Des.* **2017**, *17*, 135–145. [CrossRef]
32. Ryu, S.Y.; Oh, I.H.; Cho, S.J.; Kim, S.A.; Song, H.K. Enhancing protein crystallization under a magnetic field. *Crystals* **2020**, *10*, 821. [CrossRef]
33. Numoto, N.; Shimizu, K.I.; Matsumoto, K.; Miki, K.; Kita, A. Observation of the orientation of membrane protein crystals grown in high magnetic force fields. *J. Cryst. Growth* **2013**, *367*, 53–56. [CrossRef]
34. Nakamura, A.; Ohtsuka, J.; Miyazono, K.I.; Yamamura, A.; Kubota, K.; Hirose, R.; Hirota, N.; Ataka, M.; Sawano, Y.; Tanokura, M. Improvement in quality of protein crystals grown in a high magnetic field gradient. *Cryst. Growth Des.* **2012**, *12*, 1141–1150. [CrossRef]
35. Wu, Z.Q.; Liu, Y.M.; Liu, C.; Chen, J.J.; Chen, L.L.; Zhang, T.D.; Zhou, R.B.; Yang, C.Q.; Shang, P.; Yin, D.C. A first attempt investigation on crystallization screening and crystal quality of lysozyme under different simulated gravities in a large-gradient magnetic field. *CrystEngComm* **2019**, *26*, 4001–4010. [CrossRef]
36. Rodríguez-Romero, A.; Esturau-Escofet, N.; Pareja-Rivera, C.; Moreno, A. Crystal growth of high-quality protein crystals under the presence of an alternant electric field in pulse-wave mode, and a strong magnetic field with radio frequency pulses characterized by x-ray diffraction. *Crystals* **2017**, *7*, 179. [CrossRef]
37. Zhao, Y.H.; Hou, B.H.; Liu, C.H.; Ji, X.T.; Huang, Y.H.; Sui, J.C.; Liu, D.; Wang, N.; Hao, H.X. Mechanistic study on the effect of magnetic field on the crystallization of organic small molecules. *Ind. Eng. Chem. Res.* **2021**, *60*, 15741–15751. [CrossRef]
38. Yuan, C.X.; Xu, Y.; Jin, R.S. Effect of a magnetic field on the crystallization time and quality of phytosterols. *Sep. Eng.* **2019**, *52*, 401–405.
39. Cao, H.L.; Sun, L.H.; Li, J.; Tang, L.; Lu, H.M.; Guo, Y.Z.; He, J.; Liu, Y.M.; Xie, X.Z.; Shen, H.F.; et al. A quality comparison of protein crystals grown under containerless conditions generated by diamagnetic levitation. *Acta Crystallogr. D* **2013**, *69*, 1901–1910. [CrossRef]
40. Hayashi, S.; Hamaguchi, H.O. Discovery of a magnetic ionic liquid [Bmim]$FeCl_4$. *Chem. Lett.* **2004**, *33*, 1590–1591. [CrossRef]
41. Singh, K.P.; Gupta, S.; Basant, N. Predicting toxicities of ionic liquids in multiple test species—An aid in designing green chemicals. *RSC Adv.* **2014**, *4*, 64443. [CrossRef]
42. Peng, D.L.; Picchioni, F. Prediction of toxicity of Ionic Liquids based on GC-COSMO method. *J. Hazard. Mater.* **2020**, *398*, 122964. [CrossRef]
43. Feng, X.T.; Zhang, W.; Zhang, T.H.; Yao, S. Systematic investigation for extraction and separation of polyphenols in tea leaves by magnetic ionic liquids. *J. Sci. Food Agr.* **2018**, *98*, 4550–4560. [CrossRef]
44. Nie, L.R.; Yao, S.; Dong, B.; Li, X.L.; Song, H. Synthesis, characterization and physical properties of novel cholinium-based organic magnetic ionic liquids. *J. Mol. Liq.* **2017**, *240*, 152–161. [CrossRef]
45. Yao, T.; Zang, H.M.; Yao, S.; Dai, X.Z.; Song, H. Measurement and correlation of phase equilibria in aqueous two-phase systems containing functionalized magnetic ionic liquids and potassium phosphate at different temperatures. *J. Mol. Liq.* **2018**, *263*, 72–80. [CrossRef]
46. Yoshida, Y.; Tanaka, H.; Saito, G. Organic paramagnetic ionic liquids based on anion containing 2,2,6,6-tetramethyl-1-piperidinyloxyl radical moiety. *Chem. Lett.* **2007**, *36*, 1096–1097. [CrossRef]
47. Xu, W.; Dai, Q.Z.; Wang, Y.Z.; Hu, X.J.; Xu, P.L.; Ni, R.; Meng, J.J. Creating magnetic ionic liquid-molecularly imprinted polymers for selective extraction of lysozyme. *Rsc Adv.* **2018**, *8*, 21850–21856. [CrossRef]
48. Nie, L.R.; Song, H.; Yohannes, A.; Liang, S.W.; Yao, S. Extraction in cholinium-based magnetic ionic liquid aqueous two-phase system for the determination of berberine hydrochloride in Rhizoma coptidis. *RSC Adv.* **2018**, *8*, 25201–25209. [CrossRef]
49. Crespo, P.; Litran, R.; Rojas, T.C.; Multigner, M.; De La Fuente, J.M.; Sanchez-Lopez, J.C.; Garcia, M.A.; Hernando, A.; Penades, S.; Fernandez, A. Permanent magnetism, magnetic anisotropy, and hysteresis of thiol-capped gold nanoparticles. *Phys. Rev. Lett.* **2004**, *93*, 087204. [CrossRef]
50. Yamamoto, Y.; Miura, T.; Teranishi, T.; Suzuki, M.; Kawamura, N.; Miyagawa, H.; Nakamura, T.; Kobayashi, K.; Hori, H. X-ray magnetic circular dichroism study of gold nanoparticles protected by polymer. *J. Magn. Magn. Mater.* **2004**, *272*, 1183–1184. [CrossRef]
51. Li, H.Q.; Wang, Y.Z.; He, X.Y.; Chen, J.; Xu, F.T.; Liu, Z.W.; Zhou, Y.G. A green deep eutectic solvent modified magnetic titanium dioxide nanoparticles for the solid-phase extraction of chymotrypsin. *Talanta* **2021**, *230*, 122341. [CrossRef]

52. Zhang, B.W.; Wang, Y.F.; Zhang, J.; Qiao, S.Y.; Fan, Z.Z.; Wan, J.Q.; Chen, K.Z. Well-defined 3-Aminopropyltriethoxysilane functionalized magnetite nanoparticles and their adsorption performance for partially hydrolyzed polyacrylamide from aqueous solution. *Colloid. Surface. A* **2020**, *586*, 124288. [CrossRef]
53. Patil, S.S.; Rathod, V.K. Combined effect of enzyme co-immobilized magnetic nanoparticles (MNPs) and ultrasound for effective extraction and purification of curcuminoids from Curcuma longa. *Ind. Crop. Prod.* **2022**, *177*, 114385. [CrossRef]
54. Zheng, M.; Huang, Z.; Ji, H.D.; Qiu, F.G.; Zhao, D.Y.; Bredar, A.R.C.; Farnum, B.H. Simultaneous control of soil erosion and arsenic leaching at disturbed land using polyacrylamide modified magnetite nanoparticles. *Sci. Total Environ.* **2020**, *702*, 134977. [CrossRef] [PubMed]
55. Jiang, P.F.; Zhang, Y.X.; Zhu, C.N.; Zhang, W.J.; Mao, Z.W.; Gao, C.Y. Fe_3O_4/BSA particles induce osteogenic differentiation of mesenchymal stem cells under static magnetic field. *Acta Biomater.* **2016**, *46*, 141–150. [CrossRef]
56. Chen, Z.Q.; Wang, X.M.; Chen, Y.; Xue, Z.H.; Guo, Q.W.; Ma, Q.Q.; Chen, H.X. Preparation and characterization of a novel nanocomposite with double enzymes immobilized on magnetic Fe_3O_4-chitosan-sodium tripolyphosphate. *Colloids Surf. B* **2018**, *169*, 280–288. [CrossRef]
57. Wei, X.X.; Wang, Y.Z.; Chen, J.; Xu, P.L.; Zhou, Y.G. Preparation of ionic liquid modified magnetic metal-organic frameworks composites for the solid-phase extraction of [alpha]-chymotrypsin. *Talanta* **2018**, *182*, 484. [CrossRef]
58. Liu, Y.; Chen, J.S.; Liu, Z.K.; Xu, H.Y.; Zheng, Y.H.; Zhong, J.S.; Yang, Q.L.; Tian, H.F.; Shi, Z.Q.; Yao, J.L.; et al. Facile fabrication of Fe_3O_4 nanoparticle/carbon nanofiber aerogel from Fe-ion cross-linked cellulose nanofibrils as anode for lithium-ion battery with superhigh capacity. *J. Alloy. Compd.* **2020**, *829*, 154541. [CrossRef]
59. Ognjanović, M.; Stanković, D.M.; Jaćimović, Ž.K.; Kosović-Perutović, M.; Dojčinović, B.; Antić, B. The effect of surface-modifier of magnetite nanoparticles on electrochemical detection of dopamine and heating efficiency in magnetic hyperthermia. *J. Alloy. Compd.* **2021**, *884*, 161075. [CrossRef]
60. Nasrollahzadeh, M.; Issaabadi, Z. Reduction of Cr(VI) and 4-nitrophenol in aqueous media using N-heterocyclic palladium complex immobilized on the nano Fe_3O_4@SiO_2 as a magnetically recyclable catalyst. *Sep. Purif. Technol.* **2019**, *211*, 809–815. [CrossRef]
61. Ding, J.; Cheng, L.G. Core-shell Fe_3O_4@SiO_2@PANI composite: Preparation, characterization, and applications in microwave absorption. *J. Alloy. Compd.* **2021**, *881*, 160574. [CrossRef]
62. Stöber, W.; Fink, A.; Bohn, E. Controlled growth of monodisperse silica spheres in the micron size range. *J. Colloid Interf. Sci.* **1968**, *26*, 62–69. [CrossRef]
63. Da Silva, T.L.; Da Silva, A.M.H.; Assaf, J.M. Preparation of core-shell Pt@Fe_3O_4@SiO_2 nanostructures by oxidation of core-shell FePt@SiO_2 nanoflowers and their performance in preferential CO oxidation reaction. *Mater. Res. Express* **2019**, *6*, 015042. [CrossRef]
64. Lee, J.S.; Hong, S.K.; Hur, N.J.; Seo, W.S.; Hwang, H.J. Fabrication of spherical silica aerogel/magnetite nanocomposite particle. *Mater. Lett.* **2013**, *112*, 153–157.
65. Xu, H.; Tong, N.; Cui, L.; Liu, Y.; Gu, H. Preparation of hydrophilic magnetic nanospheres with high saturation magnetization. *J. Magn. Magn. Mater.* **2017**, *311*, 125–130. [CrossRef]
66. Liu, X.D.; Feng, J.N.; Li, Y. Preparation of carbon-functionalized magnetic graphene/mesoporous silica composites for selec-tive extraction of miglitol and voglibose in rat plasma. *Talanta* **2018**, *182*, 405–413. [CrossRef]
67. Nistler, A.; Hartmann, C.; Rümenapp, C.; Opel, M.; Gleich, B.; Ivleva, N.P.; Niessner, R.; Seidel, M. Production and characterization of long-term stable superparamagnetic iron oxide-shell silica-core nanocomposites. *J. Magn. Magn. Mater.* **2017**, *442*, 497–503. [CrossRef]
68. Gang, Q.; Akhtar, M.N.; Boudaghi, R. Development of high-efficient double layer microwave absorber based on Fe_3O_4/carbon fiber and Fe_3O_4/Rgo. *J. Magn. Magn. Mater.* **2021**, *537*, 168181. [CrossRef]
69. Wu, Q.J.; Mo, Z.L.; Guo, R.B.; Wang, B.; Li, L.; Liu, H.D.; Qi, L. Preparation and properties of C@Fe_3O_4 magnetic micro porous spheres. *J. Synth. Cryst.* **2016**, *45*, 2655–2660.
70. Nikolića, V.N.; Tadića, M.; Jovanovićbc, S.; Pasojevića, V.S. Tracking of the electronic re-ordering in Fe_3O_4/OA nanoparticles using magnetometry. *Ceram. Int.* **2019**, *45*, 17429–17437. [CrossRef]
71. Yue, T.T.; Huang, H.T.; Chang, Y.; Jia, J.C.; Jia, M.L. Controlled assembly of nitrogen-doped iron carbide nanoparticles on reduced graphene oxide for electrochemical reduction of carbon dioxide to syngas. *J. Colloid. Interf. Sci.* **2021**, *601*, 877–885. [CrossRef]
72. Lobato, N.C.C.; Ferreira, A.D.; Mansur, M.B. Evaluation of magnetic nanoparticles coated by oleic acid applied to solvent extraction processes. *Sep. Purif. Technol.* **2016**, *168*, 93–100. [CrossRef]
73. Yuan, Z.; Li, X.L.; Gao, Y.W.; Yan, F. Preparation and characterization of hydrophobic superparamagnetic magnetite nanoparticles. *Guangdong Chem. Ind.* **2015**, *42*, 144–145.
74. Zhang, L.J.; Liu, Y.J. Preparation and characterization of the epoxy silane modified magnetic nanoparticles. *J. Suzhou Univ. Sci. Tech. (Nat. Sci.)* **2013**, *30*, 45–49.
75. Klein, S.; Harreiβ, C.; Menter, C.; Hümmer, J.; Distel, L.V.R.; Meyer, K.; Hock, R.; Kryschi, C. $NOBF_4$-functionalized Au–Fe_3O_4 nanoheterodimers for radiation therapy: Synergy effect due to simultaneous reactive oxygen and nitrogen species formation. *ACS Appl. Mater. Interfaces* **2018**, *10*, 17071–17080. [CrossRef]

76. Yachi, T.; Matsubara, M.; Shen, C.; Asami, S.; Milbrandt, N.B.; Ju, M.; Wickramasinghe, S.; Samia, A.C.S.; Muramatsu, A.; Kanie, K. Water-dispersible Fe_3O_4 nanoparticles modified with controlled numbers of carboxyl moieties for magnetic induction heating. *ACS Appl. Nano Mater.* **2021**, *4*, 7395–7403. [CrossRef]
77. Dong, A.G.; Ye, X.C.; Chen, J.; Kang, Y.J.; Gordon, T.; Kikkawa, J.M.; Murray, C.B. A generalized ligand-exchange strategy enabling sequential surface functionalization of colloidal nanocrystals. *J. Am. Chem. Soc.* **2011**, *133*, 998–1006. [CrossRef]
78. Zhang, M.; Zhang, Z.; Peng, Y.Z.; Feng, L.; Li, X.H.; Zhao, C.L.; Sarfaraz, K. Novel cationic polymer modified magnetic chitosan beads for efficient adsorption of heavy metals and dyes over a wide pH range. *Int. J. Biol. Macromol.* **2020**, *156*, 289–301. [CrossRef]
79. Ning, J.H.; Chen, D.E.; Liu, Y.L.; Huang, S.E.; Wang, F.X.; Wei, R.; Hu, Q.C.; Wei, J.Q.; Sun, C. Efficient adsorption removal and adsorption mechanism of basic fuchsin by recyclable Fe_3O_4@CD magnetic microspheres. *J. Cent. South Univ.* **2021**, *28*, 3666–3680. [CrossRef]
80. Micurova, A.; Kluknavsky, M.; Liskova, S.; Balis, P.; Skratek, M.; Okruhlicova, L.; Manka, J.; Bernatova, I. Differences in distribution and biological effects of Fe_3O_4@PEG nanoparticles in normotensive and hypertensive rats-focus on vascular function and liver. *Biomedicines* **2021**, *9*, 1855. [CrossRef]
81. Ezzat, A.O.; Tawfeek, A.M.; Rajabathar, J.R.; Al-Lohedan, H.A. Synthesis of new hybrid structured magnetite crosslinked poly ionic liquid for efficient removal of coomassie brilliant blue R-250 dye in aqueous medium. *Molecules* **2022**, *27*, 441. [CrossRef]
82. Bide, Y.; Shokrollahzadeh, S. Toward tailoring of a new draw solute for forward osmosis process: Branched poly (deep eutectic solvent)-decorated magnetic nanoparticles. *J. Mol. Liq.* **2020**, *320*, 114409. [CrossRef]
83. Cryz, H.; Gabon, M.I.; Salehin, S.; Seviour, T.; Laycock, B.; Pikaar, I. Magnetic poly(acrylic acid)-based hydrogels for rapid ammonium sorption and efficient sorbent separation from sewage. *Environ. Sci. Ecotechnol.* **2021**, *6*, 100097. [CrossRef] [PubMed]
84. Shabzendedar, S.; Modarresi-Alam, A.R.; Noroozifar, M.; Kerman, K. Core-shell nanocomposite of superparamagnetic Fe_3O_4 nanoparticles with poly(m-aminobenzenesulfonic acid) for polymer solar cells. *Org. Electron.* **2020**, *77*, 105462. [CrossRef]
85. Yao, J.; Gao, F.; Liang, X.Q.; Li, Y.X.; Mi, Y.F.; Qi, Q.; Yao, J.; Cao, Z.H. Efficient preparation of carboxyl-functionalized magnetic polymer/Fe_3O_4 nanocomposite particles in one-pot miniemulsion systems. *Colloid. Surface. A* **2019**, *570*, 449–461. [CrossRef]
86. Resende, G.; Gutra, G.V.S.; Neta, M.S.B.; Araujo, O.A.; Chaves, S.B.; Machado, F. Well defined poly(methyl methacrylate)-Fe_3O_4/poly(vinyl pivalate) core-shell superparamagnetic nanoparticles: Design and evaluation of in vitro cytotoxicity activity against cancer cells. *Polymers* **2020**, *12*, 2868. [CrossRef]
87. Wu, J.M.; Cheng, X.; Li, Y.L.; Yang, G.S. Constructing biodegradable nanochitin-contained chitosan hydrogel beads for fast and efficient removal of Cu(II) from aqueous solution. *Carbohyd. Polym.* **2019**, *211*, 152–160. [CrossRef]
88. Chen, P.F.; Song, H.; Zhou, L.; Chen, J.; Liu, J.; Yao, S. Magnetic solid-phase extraction based on ferro ferric oxide nanoparticles doubly coated with chitosan and -cyclodextrin in layer-by-layer mode for the separation of ibuprofen. *RSC Adv.* **2016**, *6*, 56240–56248. [CrossRef]
89. Rashid, Z.; Shokri, F.; Abbasi, A.; Khoobi, M.; Zarnani, A.H. Surface modification and bioconjugation of anti-CD4 monoclonal antibody to magnetic nanoparticles as a highly efficient affinity adsorbent for positive selection of peripheral blood T $CD4^+$ lymphocytes. *Int. J. Biol. Macromol.* **2020**, *161*, 729–737. [CrossRef]
90. Fayazi, R.; Habibi-Rezaei, M.; Heiat, M.; Javadi-Zarnaghi, F.; Taheri, R.A. Glycated albumin precipitation using aptamer conjugated magnetic nanoparticles. *Sci. Rep.* **2020**, *10*, 10716. [CrossRef]
91. Ilyas, S.; Ullah, N.K.; Ilyas, M.; Wennhold, K.; Iqbal, M.; Schlößer, H.A.; Hussain, M.S.; Mathur, S. Mediating the fate of cancer cell uptake: Dual-targeted magnetic nanovectors with biotin and folate surface ligands. *ACS Biomater. Sci. Eng.* **2020**, *6*, 6138–6147. [CrossRef]
92. Sanli, S.; Ghorbani-Zamani, F.; Moulahoum, H.; Gumus, Z.P.; Coskunol, H.; Odaci Demirkol, D.; Timur, S. Application of biofunctionalized magnetic nanoparticles based-sensing in abused drugs diagnostics. *Anal. Chem.* **2020**, *92*, 1033–1040. [CrossRef]
93. Zhu, X.L.; Lu, N.; Zhou, Y.; Xuan, S.Y.; Zhang, J.J.; Giampieri, F.; Zhang, Y.P.; Yang, F.F.; Yu, R.S.; Battino, M.; et al. Targeting pancreatic cancer cells with peptide-functionalized polymeric magnetic nanoparticles. *Int. J. Mol. Sci.* **2019**, *20*, 2988. [CrossRef] [PubMed]
94. Kecili, R.; Ghorbani-Bidkorbeh, F.; Dolak, I.; Canpolat, G.; Karabork, M.; Hussain, C.M. Functionalized magnetic nanoparticles as powerful sorbents and stationary phases for the extraction and chromatographic applications. *TrAC-Trend. Anal. Chem.* **2021**, *143*, 116380. [CrossRef]
95. Yin, S.J.; Zhao, J.; Yang, F.Q. Recent applications of magnetic solid phase extraction in sample preparation for phytochemical analysis. *J. Pharmaceut. Biomed.* **2021**, *192*, 113675. [CrossRef]
96. Yuan, H.; Wan, H.; Hu, Y.K.; Ayeni, E.A.; Chang, Q.; Ma, C.; Liao, X. Fishing of alpha-glucosidase's ligands from aloe vera by alpha-glucosidase functionalized magnetic nanoparticles. *Molecules* **2021**, *26*, 5840. [CrossRef]
97. Cao, Y.H.; Zhao, C.X.; Tan, Q.T.; Gao, M.K.; Chen, G.; Zhai, R.Q.; Huang, X.D.; Xu, X.M.; Liu, G.Y.; Wang, J.; et al. Ternary magnetic Fe_3O_4@C_3N_4@covalent organic framework for facile extraction and determination of organophosphorus pesticides in fruit. *Microchem. J.* **2022**, *174*, 107103.
98. Yamini, Y.; Tahmasebi, E.; Ranjbar, L. Magnetic nanoparticle-based solid-phase extraction of vitamin B12 from pharmaceutical formulations. *Biol. Trace Elem. Res.* **2012**, *147*, 378–385. [CrossRef]
99. Wu, L.X.; Yu, L.; Ding, X.X.; Li, P.W.; Dai, X.H.; Chen, X.M.; Zhou, H.Y.; Bai, Y.Z.; Ding, J. Magnetic solid-phase extraction based on graphene oxide for the determination of lignans in sesame oil. *Food Chem.* **2017**, *217*, 320–325. [CrossRef]

100. Lang, H.H.; Yang, R.N.; Dou, X.J.; Wang, D.D.; Zhang, L.X.; Li, J.; Li, P.W. Simultaneous determination of 19 phenolic compounds in oilseeds using magnetic solid phase extraction and LC-MS/MS. *LWT* **2019**, *107*, 221–227. [CrossRef]
101. Shu, H.; Chen, G.N.; Wang, L.; Cui, X.; Wang, Q.; Li, W.; Chang, C.; Guo, Q.; Luo, Z.M.; Fu, Q. Adenine-coated magnetic multiwalled carbon nanotubes for the selective extraction of aristolochic acids based on multiple interactions. *J. Chromatogr. A* **2020**, *1627*, 461382. [CrossRef]
102. Majidi, S.M.; Hadjmohammadi, M.R. Development of magnetic dispersive micro-solid phase extraction based on magnetic agarose nanoparticles and deep eutectic solvents for the isolation and pre-concentration of three flavonoids in edible natural samples. *Talanta* **2021**, *222*, 121649. [CrossRef]
103. Shi, X.R.; Chen, X.L.; Hao, Y.L.; Li, L.; Xu, H.J.; Wang, M.M. Magnetic metal-organic frameworks for fast and efficient solid-phase extraction of six Sudan dyes in tomato sauce. *J. Chromatogr. B* **2018**, *1086*, 146–152. [CrossRef]
104. Zhu, J.Q.; Cheng, H.; Zhou, M.L.; Li, S.Y.; Tang, T.F.; Feng, J. Determining three isoflavones from Pueraria lobata using magnetic ZIF-8 nanoparticle-based solid-phase extraction and pressurized capillary electrochromatography. *Microchem. J.* **2022**, *212*, 114592. [CrossRef]
105. Liu, Q.L.; Xu, Q.; Sun, W. Facile preparation of core-shell magnetic organic covalent framework via self-polymerization of two-in-one strategy as a magnetic solid-phase extraction adsorbent for determination of Rhodamine B in food samples. *J. Chromatogr. A* **2021**, *1657*, 462566. [CrossRef]
106. Wei, W.; Han, Q.; Tian, S.; Wang, Y.P.; Zhang, H.Y.; Wang, H.B.; Liang, Q.L. Effective separation of α-asarone and β-asarone in TCM by covalent organic framework modified magnetic solid phase extraction. *Microchem. J.* **2022**, *175*, 107015. [CrossRef]
107. Yu, Q.Q.; Gan, H.X.; Feng, N.; Li, Y.F.; Han, Y.P. Hydroxytyrosol magnetic molecularly imprinted polymers as the sorbent for solid-phase extraction for selective recognition of hydroxytyrosol from Chinese olive leaves. Mater. *Today Commun.* **2021**, *29*, 102992. [CrossRef]
108. Sadegh, N.; Asfaram, A.; Javadian, H.; Haddadi, H.; Sharifpour, E. Ultrasound-assisted solid phase microextraction-HPLC method based on Fe_3O_4@SiO_2-NH_2-molecularly imprinted polymer magnetic nano-sorbent for rapid and efficient extraction of harmaline from Peganum harmala extract. *J. Chromatogr. B* **2021**, *1171*, 122640. [CrossRef]
109. Wang, D.D.; Gao, D.; Xu, W.J.; Li, F.; Yin, M.N.; Fu, Q.F.; Xia, Z.N. Magnetic molecularly imprinted polymer for the selective extraction of hesperetin from the dried pericarp of Citrus reticulata Blanco. *Talanta* **2018**, *184*, 307–315. [CrossRef]
110. Tang, W.W.; Zeng, G.M.; Gong, J.L.; Liu, Y.; Wang, X.Y.; Liu, Y.Y.; Liu, Z.F.; Chen, L.; Zhang, X.R.; Tu, D.Z. Simultaneous adsorption of atrazine and Cu(II) from wastewater by magnetic multi-walled carbon nanotube. *Chem. Eng. J.* **2012**, *211*, 470–478. [CrossRef]
111. Rahimi, A.; Zanjanchi, M.A.; Bakhtiari, S.; Dehsaraei, M. Selective determination of caffeine in foods with 3D-graphene based ultrasound-assisted magnetic solid phase extraction. *Food Chem.* **2018**, *262*, 206–214. [CrossRef]
112. Xiao, D.L.; Yuan, D.H.; He, H.; Pham-Huy, C.; Dai, H.; Wang, C.X.; Zhang, C. Mixed hemimicelle solid-phase extraction based on magnetic carbon nanotubes and ionic liquids for the determination of flavonoids. *Carbon* **2014**, *72*, 274–286. [CrossRef]
113. Su, X.; Li, X.; Li, J.; Liu, M.; Lei, F.; Tan, X.; Li, P.; Luo, W. Synthesis and characterization of core–shell magnetic molecularly imprinted polymers for solid-phase extraction and determination of Rhodamine B in food. *Food Chem.* **2015**, *171*, 292–297. [CrossRef]
114. Ma, R.T.; Shi, Y.P. Magnetic molecularly imprinted polymer for the selective extraction of quercetagetin from Calendula officinalis extract. *Talanta* **2015**, *134*, 650–656. [CrossRef]
115. Xiao, Y.R.; Ying, X.; Jian, L.; Li, S.D.; Xun, L. Selective extraction of flavonoids from Ginkgo biloba leaves using human serum albumin functionalized magnetic nanoparticles. *Chin. Chem. Lett.* **2013**, *24*, 1099–1102.
116. Qing, L.S.; Shan, X.Q.; Xu, X.M.; Xue, Y.; Deng, W.L.; Li, B.G.; Wang, X.L.; Liao, X. Rapid probe and isolation of bioactive compounds from Dioscorea panthaica using human serum albumin functionalized magnetic nano-particles (HAS-MNPs)-based ligand fishing coupled with electrospray ionization mass spectrometr. *Rapid Commun. Mass Sp.* **2010**, *24*, 3335–3339. [CrossRef] [PubMed]
117. Qinga, L.S.; Xue, Y.; Zheng, Y.; Xiong, J.; Liao, X.; Ding, L.S.; Li, B.G.; Liu, Y.M. Ligand fishing from Dioscorea nipponica extract using human serum albumin functionalized magnetic nanoparticles. *J. Chromatogr. A* **2010**, *1217*, 4663–4668. [CrossRef] [PubMed]
118. Xiong, J.; Ren, X.; Ding, L.; Xie, J.; Liao, X. Ligand fishing from rheum palmatum extract using human serum albumin functionalized magnetic nanoparticles. *Chin. J. Appli. Environ. Biol.* **2013**, *19*, 164–167. [CrossRef]
119. Shen, Y.P.; Wang, M.; Zhou, J.W.; Chen, Y.F.; Wu, M.R.; Yang, Z.Z.; Yang, C.Y.; Xia, G.H.; Tam, J.P.; Zhou, C.S.; et al. Construction of Fe_3O_4@α-glucosidase magnetic nanoparticles for ligand fishing of α-glucosidase inhibitors from a natural tonic Epimedii Folium. *Int. J. Biol. Macromol.* **2020**, *165*, 1361–1372. [CrossRef]
120. Zhang, F.; Li, S.H.; Liu, C.; Fang, K.; Jiang, Y.M.; Zhang, J.Y.; Lan, J.; Zhu, L.; Pang, H.Q.; Wang, G. Rapid screening for acetylcholinesterase inhibitors in Selaginella doederleinii Hieron by using functionalized magnetic Fe_3O_4 nanoparticles. *Talanta* **2022**, *243*, 123284. [CrossRef]
121. Jiang, X.L.; Yuan, Y.C.; Chen, L.B.; Liu, Y.M.; Xiao, M.W.; Hu, Y.D.; Chun, Z.; Liao, X. Monoamine oxidase B immobilized on magnetic nanoparticles for screening of the enzyme's inhibitors from herbal extracts. *Microchem. J.* **2019**, *146*, 1181–1189. [CrossRef]
122. Guo, H.; Chen, Y.F.; Song, N.; Yang, X.Y.; Yao, S.; Qian, J.Q. Screening of lipase inhibitors from bamboo leaves based on the magnetic ligand fishing combined with HPLC/MS. *Microchem. J.* **2020**, *153*, 104497. [CrossRef]

123. Zhang, Y.P.; Nie, M.K.; Shi, S.Y.; You, Q.P.; Guo, J.F.; Liu, L.L. Integration of magnetic solid phase fishing and off-line two-dimensional high-performance liquid chromatography–diode array detector–mass spectrometry for screening and identification of human serum albumin binders from Radix Astragali. *Food Chem.* **2014**, *146*, 56–64. [CrossRef]
124. Liu, L.L.; Ma, Y.J.; Chen, X.Q.; Xiong, X.; Shi, S.Y. Screening and identification of BSA bound ligands from Puerariae lobata flower by BSA functionalized Fe_3O_4 magnetic nanoparticles coupled with HPLC–MS/MS. *J. Chromatogr. B* **2012**, *887–888*, 55–60. [CrossRef]
125. Chen, M.; Liu, L.L.; Chen, X.Q. Rapid screening and analysis of alcohol dehydrogenase binders from Glycyrrhiza uralensis root extract using functionalized magnetic nanoparticles coupled with HPLC−MS/MS. *Can. J. Chem.* **2013**, *91*, 1147–1154. [CrossRef]
126. Zhao, Y.M.; Wang, L.H.; Luo, S.F.; Wang, Q.Q.; Moaddel, R.; Zhang, T.T.; Jiang, Z.J. Magnetic beads-based neuraminidase enzyme microreactor as a drug discovery tool for screening inhibitors from compound libraries and fishing ligands from natural products. *J. Chromatogr. A* **2018**, *1568*, 123–130. [CrossRef] [PubMed]
127. Megías, C.; Pedroche, J.; Del Mar Yust, M.; Alaiz, M.; Girón-Calle, J.; Millán, F.; Vioque, J. Immobilization of angiotensin-converting enzyme on glyoxyl-agarose. *J. Agr. Food Chem.* **2006**, *54*, 4641–4645. [CrossRef]
128. Yuan, L.; Xu, P.L.; Zeng, Q.; Liu, Y.M.; Ding, L.S.; Liao, X. Preparation of ds-DNA functionalized magnetic nanobaits for screening of bioactive compounds from medicinal plant. *Mater. Sci. Eng. C* **2015**, *56*, 401–408. [CrossRef]
129. Rastbood, S.; Hadjmohammadi, M.R.; Majidi, S.M. Development of a magnetic dispersive micro-solid-phase extraction method based on a deep eutectic solvent as a carrier for the rapid determination of meloxicam in biological samples. *Anal. Methods* **2020**, *12*, 2331–2337. [CrossRef]
130. Kabeer, M.; Hakami, Y.; Asif, M.; Alrefaei, T.; Sajid, M. Modern solutions in magnetic analytical extractions of metals: A review. *TrAC Trends Anal. Chem.* **2020**, *130*, 115987. [CrossRef]
131. Majidi, S.M.; Hadjmohammadi, M.R. Alcohol-based deep eutectic solvent as a carrier of SiO_2@Fe_3O_4 for the development of magnetic dispersive micro-solid-phase extraction method: Application for the preconcentration and determination of morin in apple and grape juices, diluted and acidic extract of dried onion and green tea infusion samples. *J. Sep. Sci.* **2019**, *42*, 2842–2850.
132. Li, Q.; Wu, S.G.; Wang, C.Y.; Yi, Y.J.; Zhou, W.L.; Wang, H.Y.; Li, F.F.; Tan, Z.J. Ultrasonic-assisted extraction of sinomenine from Sinomenium acutum using magnetic ionic liquids coupled with further purification by reversed micellar extraction. *Process Biochem.* **2017**, *58*, 282–288. [CrossRef]
133. Zhou, M.Q.; Gao, Y. Extraction of active substances from citrus peel under magnetic field. *Feed Husb.* **2009**, *10*, 47–49.
134. Zhou, Y.; Zhang, Z.; Zhang, S.G.; Bi, Y.; Zhang, P.; Pang, C.; Hu, W.; Liu, Z.B.; Zhou, L.; Xia, X. Using orthogonal array design to optimize the extraction of wolfberry flavonoids by magnetic field treatment. *Food Sci.* **2012**, *33*, 98–101.
135. Zhao, X.Y.; Fu, L.D.; Liu, D.; Zhu, H.T.; Wang, X.J.; Bi, Y.P. Assisted extraction of astaxanthin from Haematococcus pluvialis, journal of food processing and preservation. *J. Food Process. Pres.* **2016**, *40*, 463–472. [CrossRef]
136. Xiao, K.J.; Chen, J.; Li, Q.L.; Li, L.; Ruan, T.E. Intensified extraction of pectin from orange peel in an electromagnetis fiebl of super-high frequency. *Food Sci.* **2001**, *1*, 50–52.
137. Tarapatskyy, M.; Zaguła, G.; Bajcar, M.; Puchalski, C.; Saletnik, B. Field extraction techniques in preparing high-quality tea infusions. *Appl. Sci.* **2018**, *8*, 1876. [CrossRef]
138. Chu, L.N.; Lu, J.J.; Ma, S.; Gao, J.Y.; Du, L.F. Extraction of stachyose from radix rahmanniae by magnetization technology. *Mod. Food Sci. Technol.* **2009**, *10*, 1186–1189.
139. Jing, X.L.; Zhang, J.Q.; Zhang, L.P. Extraction process of oat bran protein by magnetized water. *Cereal Feed Ind.* **2014**, *12*, 32–35.
140. Zhao, H.X.; Liu, K.; Zhao, Z.L.; He, G.Y.; Zhang, L.P. Optimization of extraction for oat bran polysaccharides using magnetized water by response surface methodology. *Food Sci.* **2015**, *36*, 37–43.
141. Elkin, C.; Kapur, H.; Smith, T.; Humphries, D.; Pollard, M.; Hammon, N.; Hawkins, T. Magnetic bead purification of labeled DNA fragments for high-throughput capillary electrophoresis sequencing. *Biotechniques* **2002**, *32*, 1296, 1298–1300. [CrossRef]
142. Saiyed, Z.M.; Ramchand, C.N.; Telang, S.D. Isolation of genomic DNA using magnetic nanoparticles as a solid-phase support. *J. Phys. Condensed. Mat.* **2008**, *20*, 204153. [CrossRef] [PubMed]
143. Bandyopadhyay, A.; Chatterjee, S.; Sarkar, K. Rapid isolation of genomic DNA from *E. coli* XL1 Blue strain approaching bare magnetic nanoparticles. *Curr. Sci.* **2011**, *101*, 210–214.
144. Li, P.P.; Li, M.H.; Zhang, F.; Wu, M.Y.; Jiang, X.B.; Ye, B.; Zhao, Z.J.; Yue, D.M.; Fan, Q.; Chen, H.X. High-efficient nucleic acid separation from animal tissue samples via surface modified magnetic nanoparticles. *Sep. Purif. Technol.* **2021**, *262*, 118348. [CrossRef]
145. Aziz, N.; Novotny, J.; Brezinova, J.; Oborna, I. The effectiveness of different magnetic activated cell sorting columns and automated separation programs in depleting apoptotic sperm cells. *Fertil. Steril.* **2016**, *106*, 306. [CrossRef]
146. Chen, W.; Zhang, W.W.; Shi, C.; Lian, X.; Yi, S.; Yang, T. Enrichment of epidermal stem cells of rats by Vario magnetic activated cell sorting system. *Vitr. Cell. Dev. An.* **2013**, *49*, 583–588. [CrossRef] [PubMed]
147. Bie, Y.C.; Xu, Q.X.; Zhang, Z.Y. Isolation of dendritic cells from umbilical cord blood using magnetic activated cell sorting or adherence. *Oncol. Lett.* **2015**, *10*, 67–70. [CrossRef] [PubMed]
148. Jia, Z.; Liang, Y.; Xu, X.; Li, X.; Liu, Q.; Ou, Y.; Duan, L.; Zhu, W.; Lu, W.; Xiong, J.; et al. Isolation and characterization of human mesenchymal stem cells derived from synovial fluid by magnetic-activated cell sorting (MACS). *Cell Biol. Int.* **2018**, *42*, 262–271. [CrossRef]

149. Jia, Z.; Liang, Y.; Li, X.; Xu, X.; Xiong, J.; Wang, D.; Duan, L. Magnetic-activated cell sorting strategies to isolate and purify synovial fluid-derived mesenchymal stem cells from a rabbit model. *J. Vis. Exp.* **2018**, *138*, 57466. [CrossRef]
150. Zhang, Q.; Yin, T.; Xu, R.; Gao, W.; Zhao, H.; Shapter, J.G.; Wang, K.; Shen, Y.; Huang, P.; Gao, G.; et al. Large-scale immunomagnetic cell sorting of T cells based on a self-designed high-throughput system for potential clinical application. *Nanoscale* **2017**, *9*, 13592–13599. [CrossRef]
151. Naseri, M.G.; Saion, E.B.; Ahangar, H.A.; Hashim, M.; Shaari, A.H. Simple preparation and characterization of nickel ferrite nanocrystals by a thermal treatment method. *Powder Technol.* **2011**, *212*, 80–88. [CrossRef]
152. Bahrami, A.; Hejazi, P. Electrostatic immobilization of pectinase on negatively charged AOT-Fe_3O_4 nanoparticles. *J. Mol. Catal. B Enzym.* **2013**, *93*, 1–7. [CrossRef]
153. Saravanakumar, T.; Palvannan, T.; Kim, D.H.; Park, S.M. Optimized immobilization of peracetic acid producing recombinant acetyl xylan esterase on chitosan coated-Fe_3O_4 magnetic nanoparticles. *Process Biochem.* **2014**, *49*, 1920–1928. [CrossRef]
154. Sui, Y.; Cui, Y.; Nie, Y.; Xia, G.M.; Sun, G.X.; Han, J.T. Surface modification of magnetite nanoparticles using gluconic acid and their application in immobilized lipase. *Colloids Surf. B Biointerfaces* **2012**, *93*, 24–28. [CrossRef]
155. Cui, Y.; Li, Y.; Yang, Y.; Liu, X.; Lei, L.; Zhou, L.; Pan, F. Facile synthesis of amino-silane modified superparamagnetic Fe_3O_4 nanoparticles and application for lipase immobilization. *J. Biotechnol.* **2010**, *150*, 171–174. [CrossRef]
156. Li, J.; Qin, X.M.; Ding, H.P.; Chen, W.; Shen, H.B.; Li, X.Y. Magnetic nanometer particle separate the protein from Chinese herb medicine. *Biomagnetism* **2005**, *5*, 1–4.
157. Ma, Y.H.; Chen, G.; Zhao, J. Preparation and characterization of chitosan coated magnetic nanoparticles and their bsa adsorption properties. *Acta Polym. Sin.* **2013**, *13*, 1369–1375.
158. Reza, E.K.; Hossein, B.; Zeinab, A.; Hooman, A.M.A.; Milad, S.B.; Athar, N.; Farahnaz, D.; Behnam, T.; Farnoush, A.; Fateme, R.; et al. Functionalized magnetic nanoparticles for the separation and purification of proteins and peptides. *TrAC Trend. Anal. Chem.* **2021**, *141*, 116291.
159. Wu, X.M.; Zhang, L.P.; Ma, X.Z.; Zhao, Y.; Gao, J.M.; Hu, K.X. Integrating immunomagnetic bead enrichment with immunochromatography for the detection of in-fluenza A virus. *Chin. J. Microbiol. Immunol.* **2016**, *36*, 310–315.
160. Gao, T.; Zhang, L.P.; Wei, W.; Wu, Y.P.; Yang, H.F.; Zhang, K.J.; Chen, Z.X. Immunomagnetic enrichment real-time fluorescent PCR technology in the application of food borne pathogenic bacteria monitoring. *J. Med. Pest Contrl.* **2017**, *33*, 18–20.
161. Parham, N.; Spencer, J.; Taylor, D.; Ternent, H.; Innocent, G.; Mellor, D.; Roberts, M.; Williams, A. An adapted immunomagnetic cell separation method for use in quantification of Escherichia coli O157:H7 from bovine faeces. *J. Microbiol. Meth.* **2003**, *53*, 1–9. [CrossRef]
162. Srinivasa, M.S.; William, B.J.; Shankar, C.; Kumar, T.A.; Joseph, D.T.A.; Vishwas, I.M. Systems and Methods for Magnetic Separation of Biological Materials. U.S. Patent 2012/0135494 A1, 31 May 2012.
163. Kavanagh, D.M. *Microfluidic Based Magnetic Separator for Biological Applications*; Heriot-Watt University: Edinburgh, UK, 2013.
164. Sun, X.L.; Zhu, J.M.; Wei, H.Y.; Liu, J.; Zhang, D.H.; Zhou, H.R.; Tian, J.G.; Ye, Q.; Mei, J.C.; Huang, H.L.; et al. A magnetic cell sorting method with adjustable magnetic field. CN Patent 201410680428.5, 4 March 2015.
165. Wang, K.K.; Ding, R.R.; Ha, Y.P.; Jia, Y.N.; Liao, X.M.; Wang, S.S.; Li, R.J.; Shen, Z.H.; Xiong, H.; Guo, J.L.; et al. Hypoxia-stressed cardiomyocytes promote early cardiac differentiation of cardiac stem cells through HIF-1α/Jagged1/Notch1 signaling. *Acta Pharm. Sin. B* **2018**, *8*, 795–804. [CrossRef]
166. Ravelo, K.M.; Andersen, N.D.; Monje, P.V. Magnetic-activated cell sorting for the fast and efficient separation of human and rodent schwann cells from mixed cell populations. *Methods Mol. Biol.* **2018**, *1739*, 87–109. [PubMed]
167. Stimpfel, M.; Verdenik, I.; Zorn, B.; Virant-Klun, I. Magnetic-activated cell sorting of non-apoptotic spermatozoa improves the quality of embryos according to female age: A prospective sibling oocyte study. *J. Assist. Reprod. Gen.* **2018**, *35*, 1665–1674. [CrossRef] [PubMed]
168. Oh, S.; Jung, S.H.; Seo, H.; Min, M.K.; Kim, B.; Hahn, Y.K.; Kang, J.H.; Choi, S. Magnetic activated cell sorting (MACS) pipette tip for immunomagnetic bacteria separation. *Sensor. Actuat. B Chem.* **2018**, *272*, 324–330. [CrossRef]
169. Lin, S.J.; Zhi, X.; Chen, D.X.; Xia, F.F.; Shen, Y.H.; Niu, J.Q.; Huang, S.Y.; Song, J.; Miao, J.M.; Cui, D.X.; et al. A flyover style microfluidic chip for highly purified magnetic cell separation. *Biosens. Bioelectron.* **2019**, *129*, 175–181. [CrossRef]