RESEARCH ARTICLE

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Design and Development of Fe_3O_4@Prussian Blue Nanocomposite: Potential Application in the Detoxification of Bilirubin

Zainab Ali Hadi[1], Atheer Hameid Odda[1], Ammar Fadhil Jawad[2], Fadhil Jawad Al-Tu'ma[1]*

Abstract

Background: Prussian blue nanoparticles (PBNPs) due to their high solubility, stability, flexible molecular structure, tunable size, easy synthesis, and surface modification have attracted the attention of researchers as high-efficiency therapeutic agents. Recently, it has been reported that magnetic nanoparticles can be to bind pathogenic substances on their surface, followed by a recollection by magnetic separation. Considering the potential application of PB and magnetic nanoparticles, in the current study we aimed to strategically design and synthesize a highly efficient nano-magnetic bilirubin scavenger system based on iron oxides@prussian blue nanocomposites (Fe_3O_4@PB) NCs. **Materials and Methods:** The Fe_3O_4@PB NCs were synthesized by an improved shell-growing procedure and identified using advanced characteristic techniques TEM, SEM, XRD, DLS, and Zeta potential. Synthesized Fe_3O_4@ PB NCs showed good magneton properties and also demonstrated dramatic absorbent properties that empower use as an eco-friendly adsorbent nano agent for the detoxification of toxins. In addition, Fe_3O_4@PB nanoparticles showed high performance of bilirubin absorption in the serum and blood of sickle cell anemia patients. (Temp. 37.7ºC, the dose of adsorbent: 1 mg/mL, incubation time 30 min, and initial concentration: 0.25 mg/mL). **Results:** The results demonstrated an ideal adsorption capacity (86%) of Fe_3O_4@PB NCs which is significant compared to the reported adsorbents agents. These results pave the way for the application of Fe_3O_4@PB NCs for the effective purification of toxins from patients' body fluids.

Keywords: Sickle cell anemia- magnetic nanoparticles- bilirubin- blood purification- prussian blue nanoparticles

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Introduction

Sickle cell disease (SCD) is a general term that defines a group of inherited diseases, including HbSβ-thalassemia, HbSC, and sickle cell anemia (SCA) that are characterized by mutations in the gene encoding the hemoglobin β subunit (HBB). Sickle-cell hemoglobin (HbS) is caused by a mutation in the β-globin gene that results in the substitution of valine for glutamic acid at position 6 of the β-globin (βS) subunit of the hemoglobin and reduce the flexibility of red cells (Odame, 2023). Less flexible red blood cells cause blockages in the microcirculation of the smallest vessels of tissues, which leads to reduced oxygenation of tissues and chronic damage to almost every organ in the body (Eaton, 2022). The most common symptoms of SCD include mild to severe anemia, painful crises, hand-foot syndrome, frequent infections, stroke, growth retardation, bone defects, and liver disorders (Azmet et al., 2020).

Most liver complications of SCD include hepatic sequestration, acute intrahepatic vaso-occlusion, and intrahepatic cholestasis. Moreover, SCD treatment can cause liver damage from iron overload and transfusion-associated viral hepatitis (Darbari et al., 2006; Distelmaier et al., 2020). In addition, recent studies have shown that elevated ferritin, hyperbilirubinemia, and alkaline phosphatemia are factors independently associated with mortality in SCD patients (M Frankool and Jawad Al-Tu, 2010; Feld et al., 2015). Acute liver failure is the main cause of death in 8-10.7% of patients with SCD (Karacaoglu et al., 2016). Liver markers are frequently abnormal in SCD patients but are often limited to a mild increase in total bilirubin without an increase in alanine aspartate aminotransferase (AST) or aminotransferase (ALT) (Al-Tu'ma et al., 2017; Haydek et al., 2019). In patients with liver diseases, due to leakage of bilirubin glucuronides from hepatocytes, reverse transfer from hepatocyte to plasma, or return from bile, the produced bilirubin is not destroyed in time and accumulates in the serum (Hansen, 2002; Blau et al., 2014). As a result,

*[1]Department of Chemistry and Biochemistry, College of Medicine, University of Kerbala, Kerbala, Iraq. [2]Department of pharmacognesy, College of pharmacy, University of Kerbala, Kerbala, Iraq. *For Correspondence: fadhil.jawad.altuma@gmail.com*

the body's toxic substances such as bilirubin and bile acids increase sharply in a short period of time, which seriously threatens the patient's life (Ju et al., 2019). Hence, detoxification through blood purification therapy is needed, which helps the recovery of organs (Rochon et al., 2011; Das, 2020).

Direct elimination of disease-causing compounds is an inherently attractive therapeutic approach for a wide variety of pathological conditions, including blood stream infections and intoxications (Kang et al., 2014), Compounds with low molecular weight such as urea, potassium, etc. are easily removed from the circulation by membrane-based processes (hemodialysis and hemofiltration). Whereas, compounds with high molecular weight are removed through sorption-based processes of hemoperfusion and hemoadsorption (Vanholder et al., 2008). Despite hopeful findings, the practical usage of hemoperfusion is still polemical and concerns have been raised owing to side effects for instance loss of blood cells, non-specific protein uptake, and activation of inflammatory and coagulation pathways (Ma et al., 2021). Studies showed that the use of free-floating nanoparticles showed significant potential in treatment of various diseases and removing pathogenic particles compared to porous membranes (Kang et al., 2014; Nejati et al., 2022; Dadashpour et al., 2023).

Lately, it has been reported that magnetic nanoparticles can be used to attach pathogens to their surface, followed by magnetic separation. In blood detoxification by magnetic separation-based, detoxification agents that are attached to magnetic nanoparticles injected into extracorporeal blood circulation, then form a complex consisting of the toxifications bound to the magnetic particle that can be swiftly cleared from the blood by magnetic separation. The efficiency of suchlike blood detoxification methods is basically determined through the target-ligand binding (specificity and binding site accessibility), and the efficiency of the magnetic separation-based procedure (Figure 1) (Herrmann et al., 2015; Alagheband et al., 2022).

Magnetic iron-oxide nanoparticles (Fe_3O_4) are one of the most widely operated magnetic materials owing to their biocompatibility, chemical stability, high magnetic susceptibility, safety, and low cost (Fathi Karkan et al., 2017; Sadeghzadeh et al., 2017; Khiavi et al., 2019; Ganapathe et al., 2020; Salmani Javan et al., 2022). For the preparation of Fe_3O_4, various experimental techniques are available, including thermal decomposition, microemulsion, co-precipitation, hydrothermal electrochemical process, sonochemical synthesis, sol-gel synthesis, and laser pyrolysis (Davaran et al., 2014; Maiyong et al., 2017).

Prussian Blue (PB), also known as "Berlin blue," was the first pigment to be obtained synthetically and was discovered by Berlin artist Dietsch in the early 18th century. Prussian blue nano-particles (PBNPs) have interesting properties, such as stability, flexible molecule structure, porosity, and adjustable physical and chemical properties (Guari and Larionova, 2019). Prussian blue nanoparticles have been broadly operated in biomedical fields, such as targeted drug delivery and tissue engineering. By adjusting Prussian blue morphology, it becomes an ideal molecular carrier for diagnosis and therapy (Qin et al., 2018). Recently, it has been found Fe nanoparticles could be surface modified with PB which is a potential candidate for detoxification applications, also shown that Fe_3O_4 nanoparticles could be attached to PB based on surface charge mechanism, and the fraction of PB depends on the particle size of Fe_3O_4 (Arun et al., 2013).

Overall, considering the qualities that Prussian blue possesses, in the current study we used it to absorb the bilirubin without affecting the components of the blood. Furthermore, magnetizing Prussian blue will make it easier to remove toxins. To the best of our knowledge, no previous studies have been conducted on bilirubin removal using iron oxides@ PB NCs. Therefore, in the current study, we aimed to synthesize and characterization of nano-magnetic bilirubin scavenger system based on iron oxides@prussian blue nanocomposites (Fe_3O_4@PB) NCs.

Materials and Methods

Patient characteristics

A case-control study was conducted between May, 2022 and Feb. 2023 in Kerbala Teaching Hospital for pediatrics and the advanced postgraduate laboratories of the chemistry and biochemistry department in the college of medicine, University of Kerbala University, Kerbala - Iraq. In this research, 50 participants with sickle cell disease with an age range of 14 to 60 years were included in the study. The samples were collected after the diagnosis of sickle cell disease by the laboratory from the hereditary hematology center of Karbala Children's Teaching Hospital. The following information is taken from the patients and tagged with detailed information such as (name, age, height, weight, body mass index, smoking, family history, and exercise). Five ml of venous blood was obtained by venipuncture using 5 ml disposable syringes from each subject (patient and healthy control). Blood was settled in gel tubes and left to clot for 20 minutes and then centrifuged for 10 minutes at 290°C to obtain serum.

Synthesis and characterization of Magnatic Iron Oxides@ prussian bluenanocomposites (Fe_3O_4-PB NCs)

In this work, a new nanocomposites based on PB-conjugated magnetic nanoparticles were synthesized and operated to eliminate bilirubin from blood. This nanocomposites possesse both the properties of PB as a good adsorbent as well as iron oxide as a good magnetic material.

Synthesis of magnetic iron oxide nanoparticles (Fe_3O_4)

The Fe_3O_4 nanoparicles are synthesized by co-precipitation method (Saraçoğlu et al., 2023), which is considered the best traditional way, and because of the uses of non-toxic solvent has a high yield, and is easy to reproducibility. The co-precipitation process involves ferric (Fe3+) precipitation and ferrous (Fe2+) salts aqueous solutions by adding a base.

Preparation of Fe_3O_4-PB nanocomposite .

The Fe_3O_4@PB NCs were fabricated through a

modified shell-growing method according to the literature (Fu et al., 2014). Briefly, 10 mL Fe_3O_4 NPs aqueous dispersion was added dropwise into 20 mL aqueous K4[Fe- (CN)6] solution (2.0 mM, pH = 3.0) under vigorous mechanical stirring at room temperature. The color of the Fe_3O_4 NPs aqueous dispersion slowly altered to light green during the addition procedure. Next, 20 mL aqueous FeCl3 solution (2.0 mM, pH = 3.0) was added dropwise into theabove mixed solution. The gained Fe_3O_4@PB NCs were separated from the aqueous solution and washed three times with deionized water using an external magnetic field. The obtained nano-composite were finally re-dispersed in deionized water to produce the aqueous dispersion of the Fe_3O_4@PB NCs.

Characterizations of PB-coated iron oxide nanoparticles

The UV- visible absorbance spectral analysis was done by using UV-VIS spectrophotometer (Shimadzu, 1900, Japan) after diluting a small amount of the sample into distilled water at wave length 540 nm for the assessing the formation of Fe_3O_4@PB NCs. Additionally, the surface zeta potentials of the magnetic nanoparticles and nanocomposite were measured using a (Zetasizer Nano-ZS DLS particle size analyzer, Malvern). The phase and crystallinity were analyzed using powder X-ray diffraction (XRD) analysis obtained by an X-ray diffractometer (pw1730, hilips, Holland) in the range of 2θ=10–80°. The mean crystallite size of α- Fe_3O_4NPs was quantitatively determined from XRD data by employing Debye Scherer's equation: Where D is the average crystallite size, K=0.9 is the shape factor, λ is the X-ray wavelength (in nm), θ is the Bragg diffraction angle, and β is the full width at half maximum (FWHM) of the intense peak (in radians).

Removing bilirubin from blood

For this purpose, patient serum samples were mixed in pool and tacked (1 ml) from it and was mixed with (1 ml) of NCs. Under optimum conditions (Temp. 37.7 oC, conc. of NCs. 0.25 mg /ml , time 30 min.) was measured the absorbance to calculate concentration of T.B. All serum was compared with serum pool of controls under same conditions.

Statistical Analysis

For all statistical steps, SPSS version 28.0 (IBM: SPSS and Chicago, Illinois, USA) was used and descriptive statistics were performed on the data of the participants in each group. Also, the mean ± standard deviation is used to show variable values. The Shapiro test was used to check the data distribution. Analytical statistical tests showed that the parameters had categorical variables that were different in important ways. Statistically significant was assumed for all tests of hypothesis with a p-value of less than 0.05.

Results

The morphology and structure of the as-prepared nanoparticles were characterized by using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The SEM images showed that the synthesized Fe_3O_4 NPs exhibited a smoothly spherical shape (Figure 1a). Also, after conjugating PB on the surface of Fe_3O_4 NPs, a sphere-like structure is maintained well with an enlarged diameter, revealing the existence of the coating layer. The synthesized Fe_3O_4@PB nanocomposites displayed a spherical external layer and the cube-like nanoparticles around the sphere, which is the common morphology of PB, Figure 2b.

Furthermore, the TEM images well demonstrated the spherical-shaped structure of the as-prepared Fe_3O_4 NPs with an irregular average particles size of 70 nm, meanwhile after the in situ formulation PB around the Fe_3O_4NPs, fused Fe_3O_4@PB nanocomposites resulted in an increase of particle size to be around 160 compering to the pristine Fe_3O_4 NPs, Figure 3. The particle sizes

Table 1. The Effectiveness of PB@Fe_3O_4 NCs for Remove of Bilirubin from Aqueous Solution, Blood and Patients Serum Pool

Fluid type	Precipitate material	C_0	C_e	RE %
Aqueous solution	Bilirubin	1.5371	0.3771	76%
Blood	Bilirubin	1.559	0.2701	83%
Serum pool	Bilirubin	1.4252	0.2622	81%

RE (%), removal efficiency; C_0, is the initial concentration; C_e, is the concentration at equilibrium after 30 min.

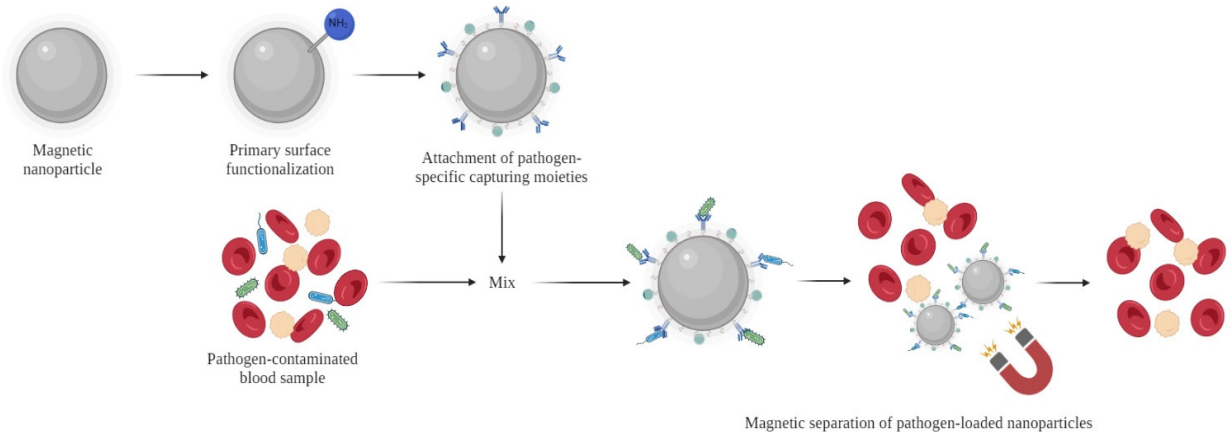

Figure 1. The Principle of Magnetic Separation-Based Blood Purification: Elimination of Pathogen.

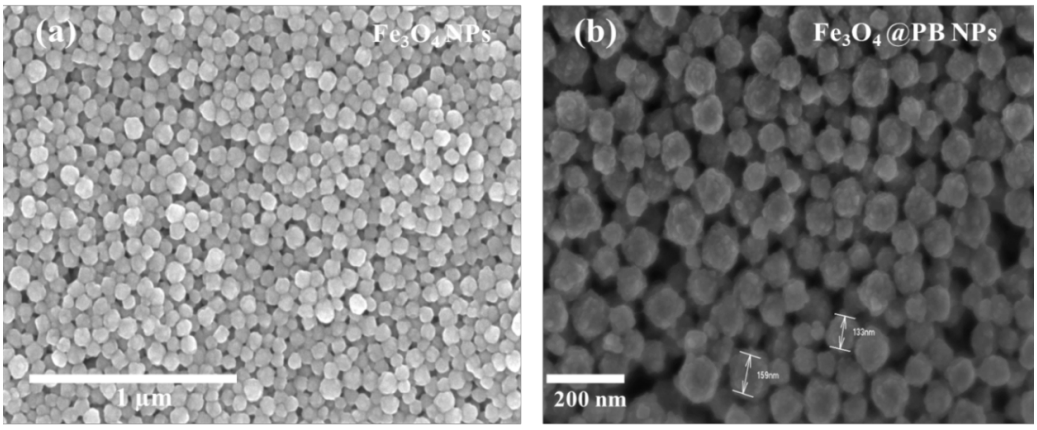

Figure 2. SEM Images of (a) Fe_3O_4NPs, (b)Fe_3O_4@PB nanocomposites.

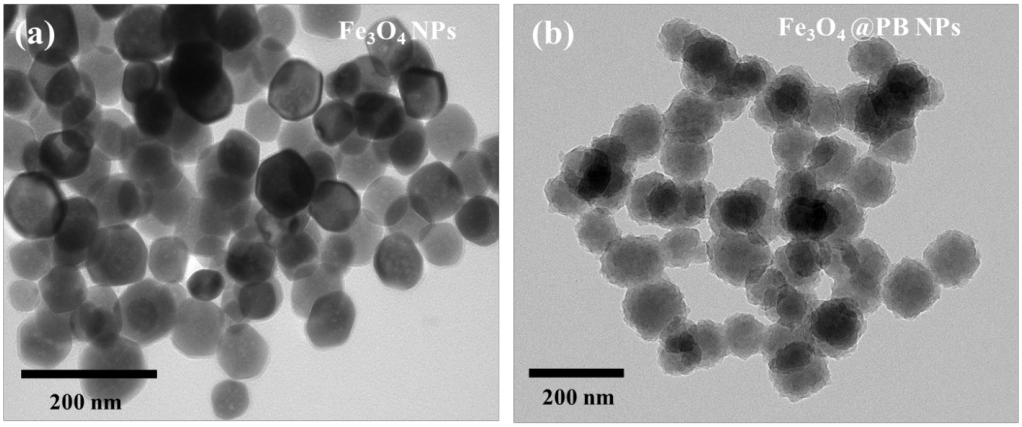

Figure 3. TEM Images of (a) Fe_3O_4NPs, (b) Fe_3O_4@PB Nanocomposites.

of the nanomaterials were also further assessed by the dynamic laser scattering (DLS) technique, and it appears to be larger than those determined by TEM (because of the dehydration of NPs in TEM method). The diameter of Fe_3O_4NP was ~100 nm while, after incorporation with Prussian blue, an increase in average size from ~100 nm to ~250 nm was observed. As shown in Figure 4, the results of the DLS method demonstrated the successful incorporation of Fe_3O_4@PB nanocomposites.

Furthermore, the zeta potentials of the as-prepared nanoparticles were evaluated; as shown in Figure 5, after incorporating with PB, the Fe_3O_4@PB NCs surface

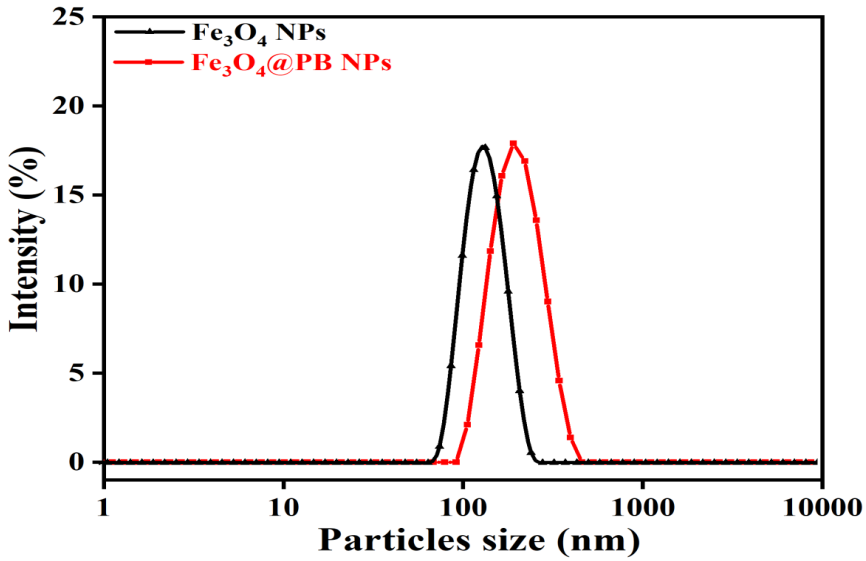

Figure 4. DLS Results of Fe_3O_4NPs (black line), Fe_3O_4@PB Nanocomposites (red line). The results demonstrated Fe3O4NP is successfully incorporated with Prussian blue, the diameter of Fe3O4NP was ~100 nm while, after incorporation with Prussian blue, an increase in average size from ~100 nm to ~250 nm was observed.

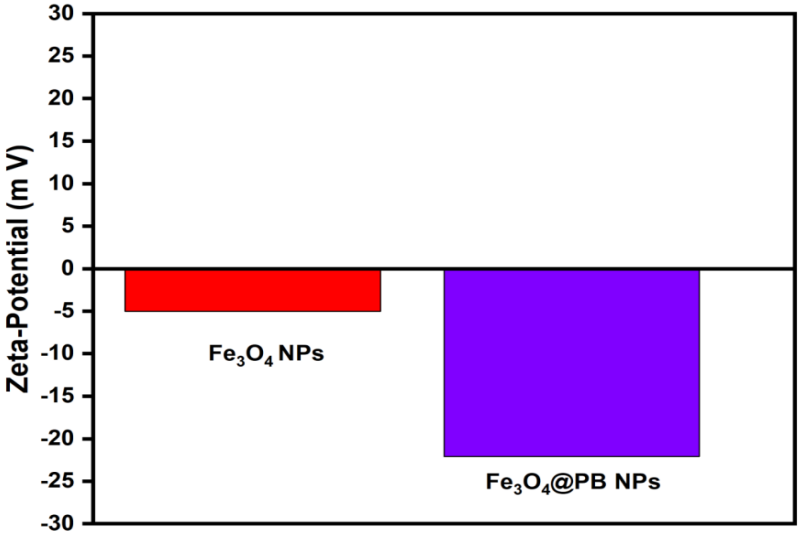

Figure 5. Zeta-Potentials of Fe_3O_4NPs, Fe_3O_4@PB NCs, The incorporation of Fe3O4@PB with PB increased the surface charge from -5.2 to -20.1 mV, which is due to the presence of cyanide ions (CN^-) in the PB structure.

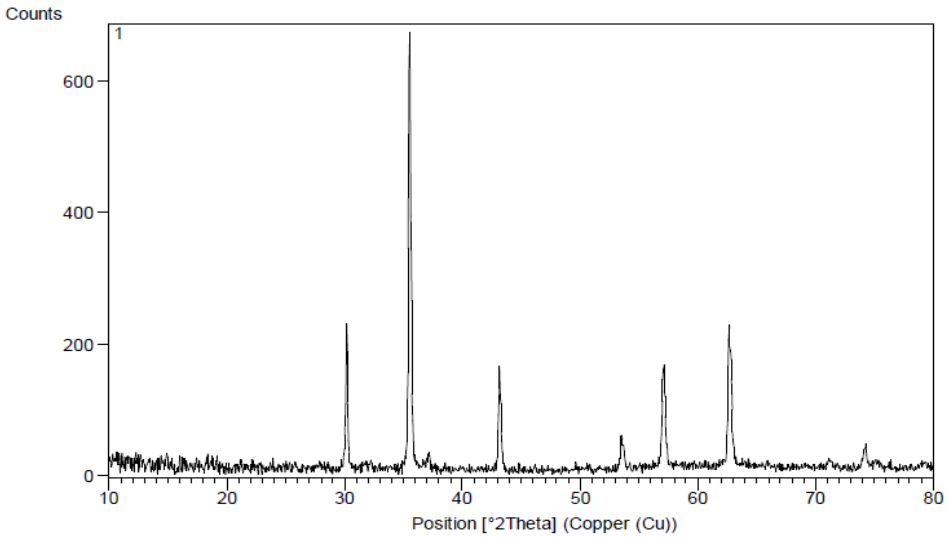

Figure 6. XRD Results of (a) Fe_3O_4NPs, Fe_3O_4@PB NCs

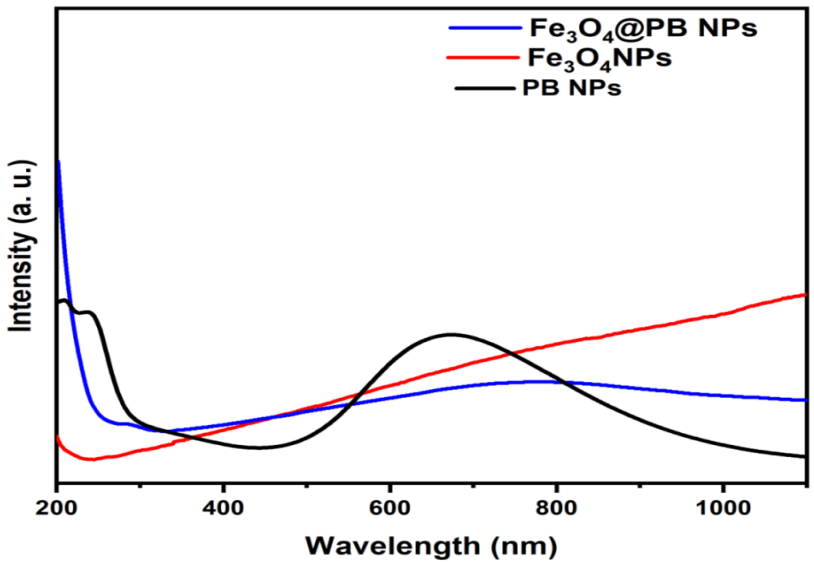

Figure 7. UV−Vis-NIR Spectra of Fe_3O_4NPs (red line), PBNPs (black line), and Fe_3O_4@PB NCs (blue line).

charges, and the zeta potentials changed from -5.2 to -20.1 mV. The negative change indicates the presence of negatively charged cyanide ions (CN^-) in the PB structure.

The crystalline structure of Fe_3O_4@PB NCs was further identified by the X-ray diffraction (XRD) method (Figure 6). X-ray diffraction (XRD) patterns were obtained using a Rigaku Ultima III X-ray diffractometer equipped with a CuKα radiation source. Results demonstrated larger particle size did not result in better attachment of PB With Fe_3O_4. Besides,the optical absorption results of the as-prepared samples were investigated by UV−Vis-NIR spectra (Figure 7). Fe_3O_4@PB NCs have the characteristic peak of both Fe_3O_4NPs and PB NPs which is clear evidence for the formation of Fe_3O_4@PB NCs. To investigate the toxic compound performance of PB@Fe_3O_4 NCs, aqueous solutions are firstly employed to investigate their ability to adsorb the bilirubin under optimal conditions and then applied on patiant blood and on serum pool for each cases and controls.

Discussion

The results of the present work showed PB@Fe_3O_4 NCs have great potential to employ as a nonhazardous and bio-adsorbent for effective adsorption of bilirubin in aqueous solution, blood, and patient serum pool. As can be seen in Table (1) the removal efficiency (RE %) of PB@Fe_3O_4 NCs for bilirubin from aqueous solution was 76% while from blood was 83% and for patient serum, the pool was 81%, respectively. This result in a line with previous studies has indicated magnetic separation exhibits greater potential than classical resin-based blood purification in the clinical therapy of toxic compounds (Guo et al., 2019; Luo et al., 2020).

In conclusion, In the current study, new nanocomposites based on PB-conjugated magnetic nanoparticles were synthesized and operated to eliminate bilirubin from the blood of sickle cell anemia patients. Synthesized NCs were characterized by TEM, SEM, XRD, and DLS techniques. Synthesized Fe_3O_4@PB NCs showed good magneton properties and also demonstrated dramatic absorbent properties that empower use as an eco-friendly adsorbent nano agent for the detoxification of toxins. Furthermore, PB@Fe_3O_4 NCs showed great potential to employ as detoxification of bilirubin from SCD patients. In summary, the easy preparation, high catalytic capacity, and excellent reusability of PB@Fe_3O_4 nanoparticles make it a promising approach for bilirubin detoxification from the plasma and serum of SCD patients.

Author Contribution Statement

Writing - original draft preparation: Zainab Ali Hadi; critical review of study: Atheer Hameid Odda and Ammar Fadhil Jawad Conceptualization, Supervision: Fadhil Jawad Al-Tu'ma

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Ethical Approval

All procedures involving human participants in research projects were conducted in compliance with the ethical standard of the research committee of Kerbala University, as well as the 1964 Helsinki declaration and any revisions or other ethical standards deemed equivalent.

Informed Consent

Consent to participate in the study was received from each individual person who took part in the research.

Availability of data and materials

The data and materials that support the findings of this study are available from the corresponding author, upon reasonable request.

Competing Interests

No potential competing interest was reported by the authors.

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