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Optimization of Binding Buffer Composition (Polyethylene Glycol, Sodium Chloride and pH) for Extraction of DNA from Biological Fluids Using Polyethyleneimine Functionalized Iron Oxide Nanoparticle-Based Method

Imran Khan[1], Gaurav Kaushik[1], Chaitenya Verma[2], Richa Vashishtha[3], Vinay Kumar[4]

[1]School of Allied Health Sciences, Sharda University, Greater Noida, Uttar Pradesh, 201310, India; [2]Department of Biotechnology, Sharda University, Greater Noida, Uttar Pradesh, 201310, India; [3]Department of Medicine, All India Institute of Medical Sciences, New Delhi, 110029, India; [4]Pennsylvania State University Hershey Medical Center, Hershey, PA, 17033, USA

Correspondence: Gaurav Kaushik; Vinay Kumar, Email gaurav.kaushik@sharda.ac.in; vinayktyagi07@gmail.com

Introduction: Efficient extraction of DNA from biological fluids is crucial for applications in molecular biology, forensic science, and clinical diagnostics. However, traditional DNA extraction methods often require costly reagents and lengthy procedures. This study aims to optimize the binding buffer composition for DNA extraction using polyethyleneimine-coated iron oxide nanoparticles (PEI-IONPs), which offer the dual benefits of magnetic separation and high DNA-binding efficiency.

Methods: The effects of three key binding buffer components—polyethylene glycol (PEG-6000), sodium chloride (NaCl), and pH—on DNA adsorption efficiency were systematically evaluated. Blood samples were treated with PEI-IONPs under various conditions, and DNA concentration, yield, and purity were quantified. Nanoparticle functionalization was confirmed through characterization, and DNA quality was validated via agarose gel electrophoresis.

Results: The optimized binding buffer composition consisted of a PEG-6000 concentration of 30%, NaCl concentration of 0M, and pH of 4, which yielded the highest DNA concentration (34 ± 1.2 ng/μL), yield (6.8 ± 0.2 μg), and purity (A260/A280 ratio of 1.81). These conditions significantly improved DNA recovery compared to suboptimal buffer compositions.

Conclusion: The findings highlighted the critical role of binding buffer composition in maximizing DNA recovery. The use of optimized PEI-IONPs provided a rapid and efficient method for DNA extraction, supporting its potential for applications in scientific and clinical research. Future studies should explore the robustness of these optimized conditions across diverse biological fluids and extraction settings.

Keywords: polyethyleneimine, nanoparticles, DNA, TEM, FTIR, blood

Introduction

Extraction of deoxyribonucleic acid (DNA) from biological samples is an important step in various scientific domains such as clinical diagnostics, forensic science, genetics and genomics. The efficiency and effectiveness of DNA extraction greatly affects downstream applications like Polymerase Chain Reaction (PCR), genotyping and sequencing. Therefore, optimization of DNA extraction protocols is important to obtain pure DNA from biological samples.

Over the years, a number of DNA extraction methods have been developed for the extraction of DNA from biological samples, each having their own advantages and limitations. Traditional DNA extraction methods, such as phenol-chloroform extraction and silica-based methods, have been widely used in various scientific domains due to their reliability. However, they have notable limitations that hinder their applicability in routine casework. Phenol-chloroform extraction, while being effective, is generally more time-consuming,[1–4] involves use of toxic reagents,[5,6]

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requires large sample volumes,[4] and can be inefficient in removing PCR inhibitors.[7,8] Similarly, silica-based methods, while effective in many cases, require sophisticated instruments like centrifuge and are often less efficient in removing PCR inhibitors, particularly in complex samples such as blood, saliva, or degraded tissues.[9] Although commercial kit-based methods are best known for their high efficiency, but due to their high cost they cannot be used in routine caseworks.[10,11] These challenges highlight the need for alternative approaches that are cost-effective, efficient, and compatible with a wide range of sample types.

In recent years, nanotechnology-based methods have emerged as promising alternatives for efficient DNA extraction from biological samples, offering potential advantages such as cost-effectiveness, ease of use, and minimal sample requirements.[12–16] In the past few years, a number of nanoparticles have been explored for biomedical applications like gold nanoparticles,[17,18] silica-coated magnetic nanoparticles, iron oxide nanoparticles,[19–22] etc. Gold nanoparticles exhibit good biocompatibility and surface functionalization potential[23,24] but have limitation in terms of higher production cost, genotoxic effects of the nanoparticles[25] and lack of magnetic properties, hindering their use in routine DNA isolation. Silica-coated magnetic nanoparticles combine the benefits of magnetic separation with effective DNA binding. However, it has limitations in terms of lower DNA adsorption capacity and higher particle size.[26] Out of various nanoparticles explored till date for DNA isolation, iron oxide nanoparticles stood out first offering several advantages like low production cost, ease of use, magnetic properties, etc.[12–14,27–29] To increase the efficiency and specificity of nanoparticles, a number of surface coatings of iron oxide nanoparticles have been explored till date like Polyethyleneimine, Graphene Oxide, Chitosan, Polyaniline, Polydopamine, silica, gold, etc.[4,26,30,31]

Among these, polyethyleneimine-coated iron oxide nanoparticles (PEI-IONPs) have received considerable attention due to their unique properties.[26,32] In comparison to others, PEI-IONPs were reported to offer a high free surface area for DNA binding, biocompatibility, a high DNA adsorption capacity (approximately 99%),[26] and the ability to specifically bind nucleic acids through electrostatic interactions. These nanoparticles have been successfully used in various DNA transfection studies and nucleic acid extraction from whole blood.[32,33]

The mechanism underlying the interaction between DNA and PEI-IONPs is primarily based on electrostatic attraction. DNA is a polyanionic molecule due to its phosphate backbone, which carries a negative charge under physiological conditions. PEI, a polycationic polymer, coats the surface of iron oxide nanoparticles, providing a dense array of positive charges. The positively charged amine groups ($-NH_2$) of polyethyleneimine (PEI) on the nanoparticle surface interacts with the negatively charged phosphate groups ($-PO_4$) of the DNA backbone through Coulombic forces.[26] This interaction is influenced by the composition of the binding buffer, which modulates the DNA–nanoparticle interaction to enhance specificity and efficiency. Given the widespread use of PEG-NaCl buffers in nanoparticle-based DNA isolation protocols,[19,31,34] it is imperative to determine the optimal concentrations of these components for this particular PEI-IONP-based DNA isolation protocol. PEG is a neutral, water-soluble polymer that creates a crowded macromolecular environment in the solution. This effect increases the effective concentration of DNA molecules, promoting aggregation and precipitation. PEG exerts an osmotic pressure that reduces the solubility of DNA, encouraging its adsorption onto the nanoparticle surface. This cooperative effect makes the DNA binding process more efficient.[31,35–37]

NaCl plays a pivotal role in stabilizing DNA–nanoparticle interactions through charge shielding, which modulates the electrostatic forces driving adsorption. At low ionic strengths, the minimal presence of Na+ ions allow for strong electrostatic interactions between the negatively charged DNA backbone and positively charged PEI-IONPs, facilitating efficient adsorption. Conversely, increasing NaCl concentration introduces a shielding effect, where Na+ ions partially neutralize the negative charges on the DNA backbone, reducing the strength of these interactions. This phenomenon can affect DNA adsorption efficiency by altering the balance of attractive and repulsive forces at the nanoparticle surface.

Additionally, at higher NaCl concentrations, competitive binding of Cl^- ions to the positively charged nanoparticle surface may occur. This competition can reduce the availability of binding sites for DNA molecules, further diminishing adsorption efficiency. Optimizing NaCl concentration is therefore critical to maximizing DNA yield and purity while minimizing non-specific interactions and maintaining the stability of the DNA-nanoparticle complex.[25,26,31,38]

Isolating DNA from biological samples using the PEI-IONP-based method involves three major steps: sample lysis, binding of DNA to the nanoparticle surface, and elution of DNA (see Figure 1).[32] Sample lysis is a common step in almost all DNA isolation protocols and has been optimized in many studies.[39] The elution buffer for the PEI-IONP-based

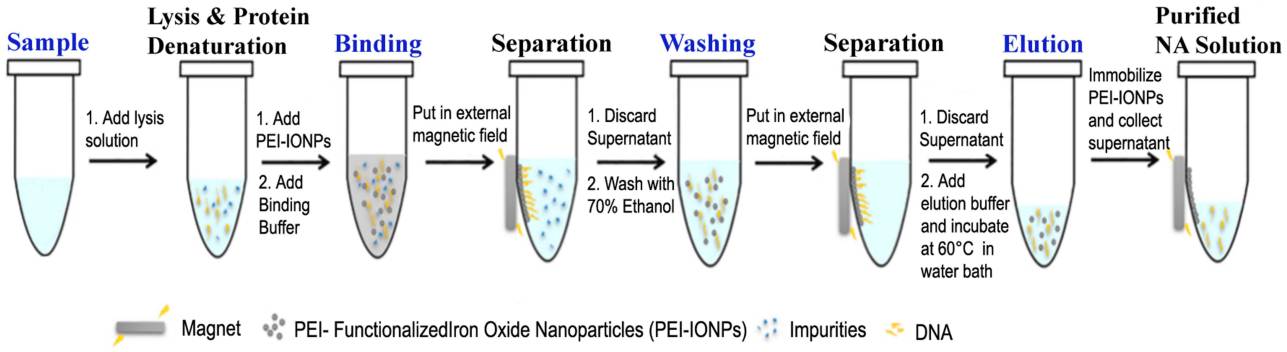

Figure 1 Basic Steps of DNA isolation from biological fluid using PEI-IONPs.

DNA isolation method has been optimized in a previous study by Danthanarayana AN et al.[32] However, an important factor in the efficiency of PEI-IONP-based DNA extraction is the nature and composition of the binding buffer. As discussed earlier, the binding buffer plays a crucial role in the selective and efficient binding of DNA to the surface of nanoparticle while minimizing non-specific interactions with other cellular components.[31]

Despite its importance, no study to date has systematically optimized the composition of binding buffer for the PEI-IONP-based method for isolating DNA from biological fluid sample. Optimizing the concentrations of its components is important because it ensures maximal DNA recovery and purity, which is essential for downstream applications.

The study aims to address the research gap by systematically investigating the effects of various concentrations of PEG and NaCl on the DNA adsorption efficiency of PEI-IONPs, as well as the quality and purity of isolated DNA. Small improvements in DNA yield and purity can have substantial implications for downstream applications such as PCR, sequencing, and forensic analysis. For example, in forensic science, as seen in most of the cases, degraded or trace quantities of samples are generally recovered from the scene of crime, so enhanced DNA recovery from degraded samples or small volumes can improve the success rate of genetic profiling. Similarly, in clinical settings, a high-throughput method with optimized conditions can streamline diagnostic workflows, reduce costs, and improve accuracy. By elucidating the optimal conditions for DNA binding, this study will contribute to the refinement of the PEI-IONP-based DNA isolation technique, which can be used in various scientific domains such as molecular biology, forensic science, genetics, and clinical research.

Materials and Methods

Materials

Ferric chloride hexahydrate, Ferrous chloride tetrahydrate, Cetyltrimethylammonium bromide (CTAB), Ammonia Solution (25%), Tris-HCl, Ethylenediaminetetraacetic acid (EDTA), Phosphate Buffered Saline, Proteinase K, RNase A Solution, Triton X-100, Polyethylene Glycol (PEG 6000), Sodium Chloride, Ethanol, Formamide, Hydrochloric acid, EDTA disodium salt, Acetic acid, Ethidium Bromide, 6X DNA Loading Dye, 1 kb DNA ladder, Agarose Powder, were purchased from Thermo Fisher Scientific. Polyethyleneimine branched (Mol. Wt. ~25 kDa) was purchased from Sigma Aldrich, Double-distilled water, Gel Electrophoresis Apparatus, UV-Vis Spectrophotometer.

Methods

Synthesis and Functionalization of Iron Oxide (Fe_3O_4) Nanoparticles

For the synthesis and functionalization of Iron oxide (Fe_3O_4) nanoparticles the method proposed by Danthanarayana AN et al (2018) was used.[32] Iron oxide (Fe_3O_4) nanoparticles were synthesized by co-precipitation method. First, 100 mL of a 0.1 M Fe(III) solution was mixed with 50 mL of a 0.1 M Fe(II) solution under an inert atmosphere for about 30 minutes. Then, 25 mL of a 0.1 M solution of cetyltrimethylammonium bromide (CTAB) was added to the mixture. Then, 5.0 M solution of NH_4OH was added drop-by-drop under nitrogen atmosphere until the pH of the solution reaches 12. The slurry thus formed will then be separated and washed with double distilled water and finally dried in a vacuum desiccator.[32]

Functionalization of Iron Oxide (Fe_3O_4) Nanoparticles with Polyethyleneimine

Twenty milliliters of 5M ammonium hydroxide solution and 8.5 mL of 20% polyethyleneimine solution in ethanol were mixed together in a flask followed by stirring at 50°C for 5 minutes. After that, 0.80 g of synthesized nanoparticles were added to the mixture with continuous stirring for 30 minutes. The slurry was then separated, washed and dried.[32]

Characterization of Synthesized Nanoparticles

The functionalized nanoparticles were characterized by Transmission Electron Microscope (TEM) for determination of size and Fourier-transform infrared spectroscopy (FTIR) to ensure proper functionalization.

Sample Collection

Blood samples were collected from 20 healthy individuals and stored in EDTA vials at 4°C till used.

Sample Lysis

Two hundred microliters aliquots of whole blood samples were transferred into microtubes and mixed with 20 µL of RNase A solution (10 mg mL^{-1}), 20 µL of Proteinase K solution (20 mg mL^{-1}) and 500 µL of lysis solution containing 400 mm Sodium Chloride, 10 mm Tris-HCl, 2.0 mm Disodium Ethylenediaminetetraacetic acid and 1% Triton X-100 in Distilled Water, adjusted to pH 8.2. The mixture was vortexed for 15 seconds to ensure homogeneity and then incubated for 10 minutes in a water bath at 65°C to ensure complete lysis of the cells.

Optimization of Binding Buffer Components

To optimize the binding buffer composition for extraction of DNA from biological fluid using PEI-IONPs, we evaluated the effects of different concentrations of PEG, NaCl, and varying pH levels. To validate the role of individual buffer components in DNA isolation efficiency, control samples were prepared and processed under the same experimental conditions but without specific components. For the effect of PEG concentration, a control experiment was conducted without the addition of PEG to the binding buffer. Similarly, for NaCl concentration, controls were performed without the inclusion of NaCl. To evaluate the effect of pH, a neutral pH (pH 7.0) binding buffer was used as a control. These controls allowed the comparison of DNA adsorption efficiency under optimized conditions against conditions lacking the respective component. All other parameters, including sample volume, incubation time, and PEI-IONP concentration, were kept constant. To assess the impact of PEG concentration on DNA capture efficiency, 300 µL of PEG at concentrations of 0%, 5%, 10%, 20%, 30%, and 40% were added to different aliquots of lysed blood samples, followed by the addition of 5 mg of nanoparticles. The samples were mixed by gently inverting the microtube 2–3 times. The microtube was then incubated for approximately 10 minutes at room temperature. Similarly, to determine the effect of NaCl concentration on DNA capture efficiency, 300 µL of NaCl solution at concentrations of 0M, 1M, 2M, 3M, 4M, 5M, and 6M were added to different aliquots of lysed blood samples, followed by the addition of 5 mg of nanoparticles. The samples were mixed by gently inverting the microtube 2–3 times. The microtube was then incubated for approximately 10 minutes at room temperature. Furthermore, to explore the effect of pH on DNA capture efficiency, 300 µL of binding buffer solution with pH levels ranging from 2 to 10 were added to different aliquots of lysed blood samples, followed by the addition of 5 mg of nanoparticles. The samples were mixed by gently inverting the microtube 2–3 times. The microtube was then incubated for approximately 10 minutes at room temperature. This systematic evaluation helped to determine the optimal conditions for the binding buffer components, enhancing the DNA adsorption efficiency of PEI-IONPs.

Elution of DNA from Nanoparticle Surface

After incubation, an external magnetic field was applied to immobilize the magnetic pellet, and the supernatant was removed. The pellet was then rinsed with 70% ethanol and left to dry. Once dried, the pellet was resuspended in 200 µL of elution buffer, consisting of 0.1 M Tris-HCl, 10% formamide and 1.5 M NaCl (pH 10.0), and incubated for 15 minutes in a water bath at 60°C.[16] The magnetic field was reapplied to immobilize the pellet, and the supernatant containing the eluted DNA was transferred to a new tube.

Qualitative and Quantitative Assessment of Extracted DNA

The concentration, yield and purity of the extracted DNA samples were assessed by UV-vis spectrophotometry by measuring the optical densities (OD) of the extracted DNA samples were measured at wavelengths- 260 nm and 280 nm using UV-vis spectrophotometer. The A_{260}/A_{280} ratios were calculated to determine the purity and yield and the resulting values were compared to find out the most effective binding buffer composition for optimal DNA recovery and purity. The data obtained from UV-vis spectrophotometry was cross-verified by agarose gel electrophoresis and the resulting DNA bands were compared for purity and integrity.

Statistical Analysis

The protocol was evaluated using blood samples collected from 20 individuals to ensure reproducibility. For each experimental condition (eg, PEG and NaCl concentrations, pH levels), the mean and standard deviation (SD) values of DNA concentration, yield, and purity (A260/A280 ratio) were calculated. Statistical differences between groups were evaluated using one-way ANOVA followed by Tukey's post hoc test, with a significance threshold of $p < 0.05$. All statistical analyses were performed using SPSS and MS Excel software.

Results

Characterization of Synthesized Nanoparticles

The size of the PEI functionalized iron oxide nanoparticles (PEI-IONPs) was determined using transmission electron microscopy (TEM). The TEM image obtained is given in Figure 2. The particles seem to have a diameter of 15 ± 2.0 nm. The particles appear embedded in a mesh of the PEI polymer with each iron oxide nanoparticle bound to multiple PEI strands, consistent with the branched structure of PEI (see Figure 2).

Proper functionalization of iron oxide nanoparticles with PEI was confirmed by Fourier-transform infrared (FTIR) spectroscopy. The resulting FTIR spectrum is given in Figure 3. The peak at 585 cm^{-1} corresponds to Fe-O bond vibration of iron oxide core. The peak around 1048 cm^{-1} is due to the weak CN stretching vibrations of Polyethyleneimine. The characteristic peak at 1329 cm^{-1} is due to scissoring vibrations of CH_2 and the peak around 1556 cm^{-1} correspond to -NH scissoring vibrations of PEI. The peaks between 2800 and 3000 cm^{-1} corresponds to CH_2 stretching vibrations of PEI. The

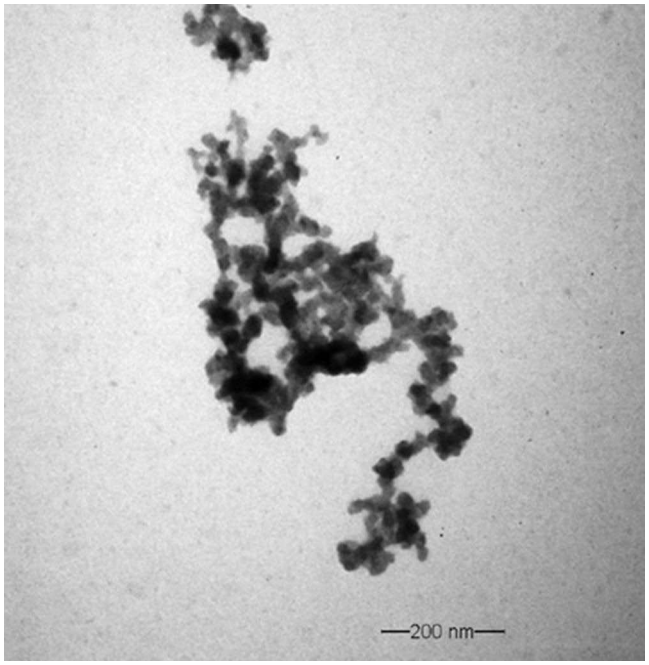

Figure 2 TEM Image of PEI functionalized Iron Oxide Nanoparticles.

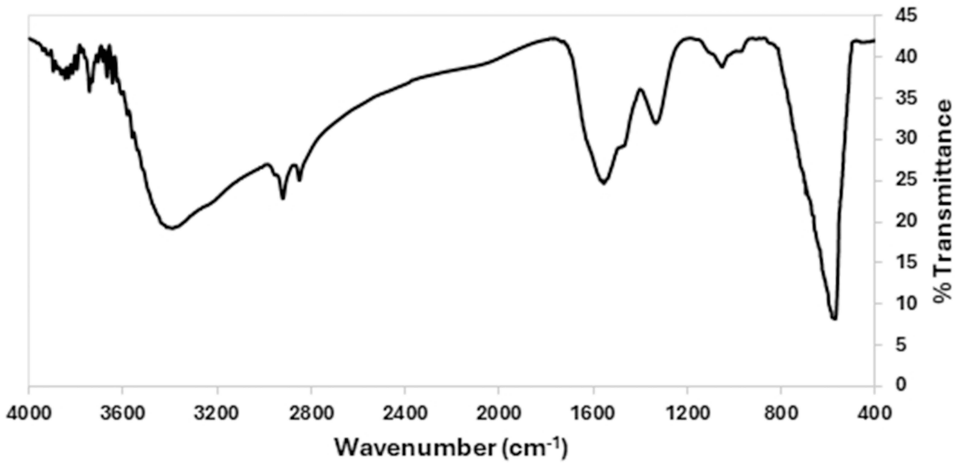

Figure 3 FTIR Spectrum of PEI functionalized Iron Oxide Nanoparticles.

peak at 3405 cm^{-1} corresponds to -NH stretching vibrations of Polyethyleneimine (see Figure 3). These peaks obtained were in good agreement to the previously reported results.[32]

Effect of Concentration of PEG on DNA Adsorption Efficiency of PEI-IONPs

Polyethylene glycol (PEG) significantly impacts DNA adsorption efficiency on PEI-IONPs nanoparticles. In this study, the effect of varying concentrations of polyethylene glycol (PEG) on the DNA adsorption efficiency of PEI-IONPs nanoparticles was investigated. The experimental data demonstrate a significant influence of PEG concentration on DNA yield and purity. The mean and standard deviation values of DNA concentration, DNA yield and A260/280 ratios at different concentrations of PEG are summarized in Table 1 and the data is illustrated in Figure 4.

As seen in Table 1, the DNA concentration and yield were highest at 30% PEG, with values of 34 ± 1.2 ng/µL and 6.8 ± 0.2 µg, respectively. The A260/280 ratios, ranging from 1.76 to 1.85, indicated high DNA purity across the different PEG concentrations. Notably, at 40% PEG, a lower A260/280 ratio was observed, suggesting co-precipitation of impurities at this higher concentration. Statistical analysis indicated significant differences in DNA yield at varying PEG concentrations ($p < 0.05$), with 30% PEG showing the highest efficiency.

Effect of Salt Concentration on DNA Adsorption Efficiency of PEI-IONPs

Salt concentration (NaCl concentration) influences DNA adsorption on PEI-IONPs nanoparticles by modulating the ionic strength of the solution, and this in turn affects the electrostatic interactions between the nanoparticle and DNA. In this study, the effect of varying salt concentration on the DNA adsorption efficiency of PEI-IONPs nanoparticles was also examined. Table 2 summarizes the mean and standard deviation values of DNA

Table 1 Impact of PEG Concentration on DNA Concentration, Yield, and Purity Isolated Using PEI-IONPs from 200 µL Blood Samples

	Concentration of Polyethylene Glycol (PEG- 6000)					
	0%*	5%	10%	20%	30%	40%
DNA Concentration (ng/µL)	16.5 ± 1.2	18.25 ± 1.3	19.0 ± 1.4	26.75 ± 1.8	34 ± 1.2	18 ± 2.0
DNA Yield (µg/200 µL of whole blood)	3.3 ± 0.2	3.65 ± 0.3	3.8 ± 0.3	5.35 ± 0.4	6.8 ± 0.2	3.6 ± 0.3
A260/280	1.84 ± 0.02	1.83 ± 0.02	1.85 ± 0.03	1.84 ± 0.02	1.81 ± 0.02	1.76 ± 0.03

Note: *Control Sample.

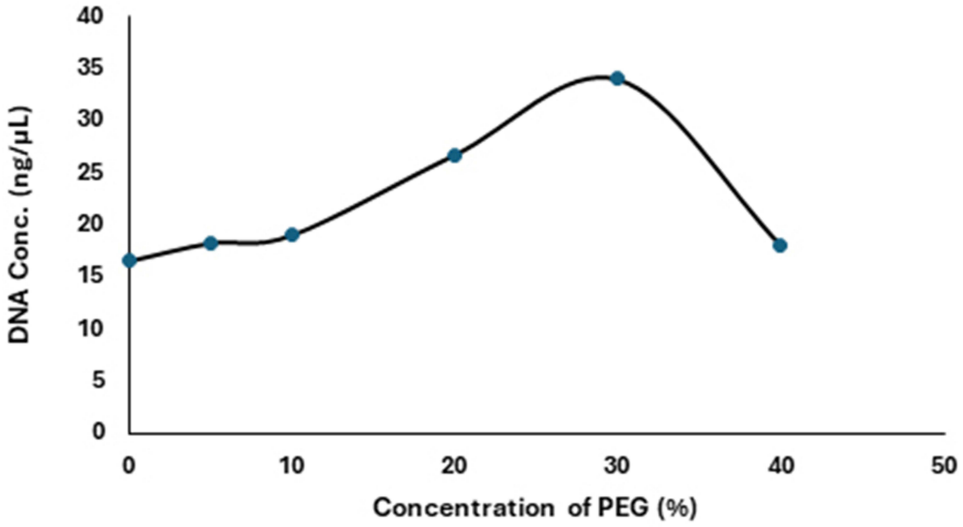

Figure 4 Effect of Concentration of PEG on DNA Adsorption Efficiency of PEI-IONPs.

concentration, DNA yield and purity ratio at different concentrations of NaCl. The results, shown in Table 2 and Figure 5, demonstrate that DNA concentration and yield were highest at 0M NaCl, with values decreasing significantly as NaCl concentration increased ($p < 0.05$).

The highest DNA concentration and yield were observed at 0M NaCl, with 33 ± 2.0 ng/µL and 6.6 ± 0.5 µg, respectively. The A260/280 ratios remained stable (1.81 to 1.84) across all NaCl concentrations, indicating consistent DNA purity. One-way ANOVA results confirmed statistically significant reductions in DNA yield as NaCl concentration increased ($p < 0.05$).

Effect of pH of Binding Buffer Solution on DNA Adsorption Efficiency of PEI-IONPs

The effect of pH on DNA adsorption efficiency of PEI-IONPs was also assessed. Table 3 summarizes the mean and standard deviation values of DNA concentration, yield, and purity across different pH values and the data is also illustrated in Figure 6.

The highest DNA concentration and yield were observed at pH 4, with values of 32.0 ± 2.0 ng/µL and 6.4 ± 0.4 µg, respectively. The A260/280 ratios at this pH indicated high DNA purity with minimal protein contamination. Statistical analysis demonstrated that DNA yield was significantly higher at pH 4 compared to other tested pH levels ($p < 0.05$).

Discussion

The Polyethyleneimine functionalized iron oxide nanoparticles (PEI-IONPs) were characterized by TEM and FTIR spectroscopy. TEM analysis revealed nanoparticles with a diameter of 15 ± 2.0 nm, embedded in a PEI polymer mesh.

Table 2 Impact of NaCl Concentration on DNA Concentration, Yield, and Purity Isolated Using PEI-IONPs from 200 µL Blood Samples

	Concentration of NaCl						
	0M*	**1M**	**2M**	**3M**	**4M**	**5M**	**6M**
DNA Concentration (ng/µL)	33 ± 2.0	22.75 ± 1.6	20.0 ± 1.5	17.5 ± 1.2	13.25 ± 1.0	11 ± 0.8	8.25 ± 0.6
DNA Yield (µg/200 µL of whole blood)	6.6 ± 0.5	4.55 ± 0.4	4.0 ± 0.4	3.5 ± 0.3	2.65 ± 0.2	2.2 ± 0.2	1.65 ± 0.1
A260/280	1.81± 0.02	1.82± 0.02	1.82± 0.03	1.84± 0.02	1.83± 0.02	1.83± 0.03	1.83 ± 0.03

Note: *Control Sample.

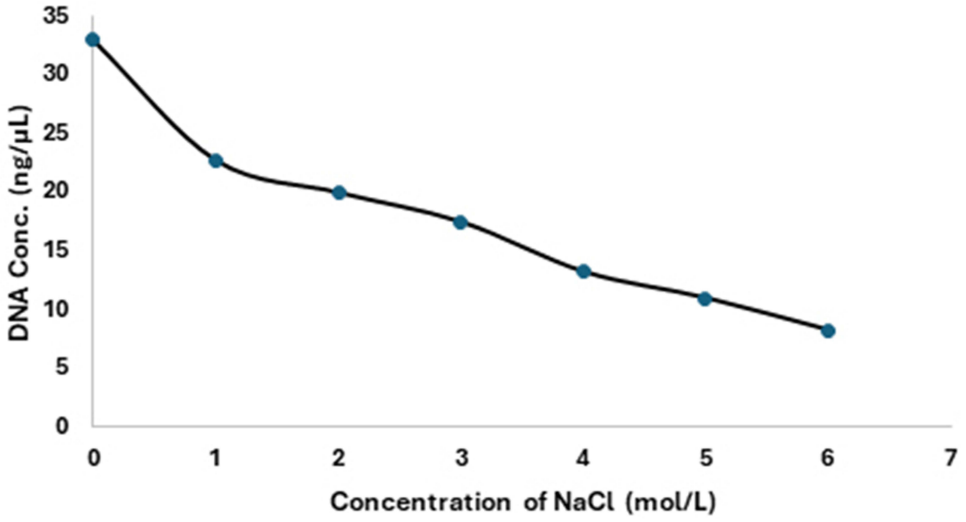

Figure 5 Effect of Salt Concentration on DNA Adsorption Efficiency of PEI-IONPs.

This morphology is consistent with the expected structure, where the branched PEI polymer binds multiple iron oxide nanoparticles (see Figure 2). FTIR spectroscopy further confirmed the successful functionalization of Fe_3O_4 nanoparticles with PEI. Characteristic peaks corresponding to Fe-O bond vibrations and various PEI functional groups were observed (see Figure 3). The results were in good agreement to the previously reported data.[32]

In addition to the structural characterization, the study explored the effects of polyethylene glycol (PEG-6000) concentration, NaCl concentration, and pH on the DNA adsorption efficiency of PEI-IONPs. The data clearly identified 30% PEG-6000, 0M NaCl, and pH 4 as the optimal conditions for DNA isolation, yielding the highest DNA concentration and purity. The optimized conditions were verified through agarose gel electrophoresis (results not shown), which confirmed the integrity and purity of the isolated DNA.

For PEG concentration, the DNA concentration and yield increased with rising PEG concentrations, reaching peak values of 34 ± 1.2 ng/µL and 6.8 ± 0.2 µg, respectively, at 30% PEG. Beyond this concentration, both DNA concentration and yield declined, suggesting that 30% PEG provides an optimal environment for DNA adsorption (see Figure 4). This is likely due to the enhanced precipitation and aggregation properties at this concentration.[31,37] The A260/

Table 3 Impact of pH on DNA Concentration, Yield, and Purity Isolated Using PEI-IONPs from 200 µL Blood Samples

pH	DNA Concentration (ng/µL)	DNA Yield (µg/200 µL of whole blood)	A260/280
2	31.75 ± 2.0	6.35 ± 0.4	1.81 ± 0.02
3	32.0 ± 2.0	6.4 ± 0.4	1.82 ± 0.02
4	32.0 ± 2.0	6.4 ± 0.4	1.82 ± 0.02
5	26.75 ± 1.5	5.35 ± 0.3	1.70 ± 0.03
6	22.0 ± 1.5	4.4 ± 0.3	1.73 ± 0.03
7*	17.5 ± 1.5	3.5 ± 0.3	1.75 ± 0.03
8	15.25 ± 1.0	3.05 ± 0.2	1.79 ± 0.03
9	14.0 ± 1.0	2.8 ± 0.2	1.81 ± 0.02
10	12.25 ± 1.0	2.45 ± 0.2	1.81 ± 0.02

Note: *Control Sample.

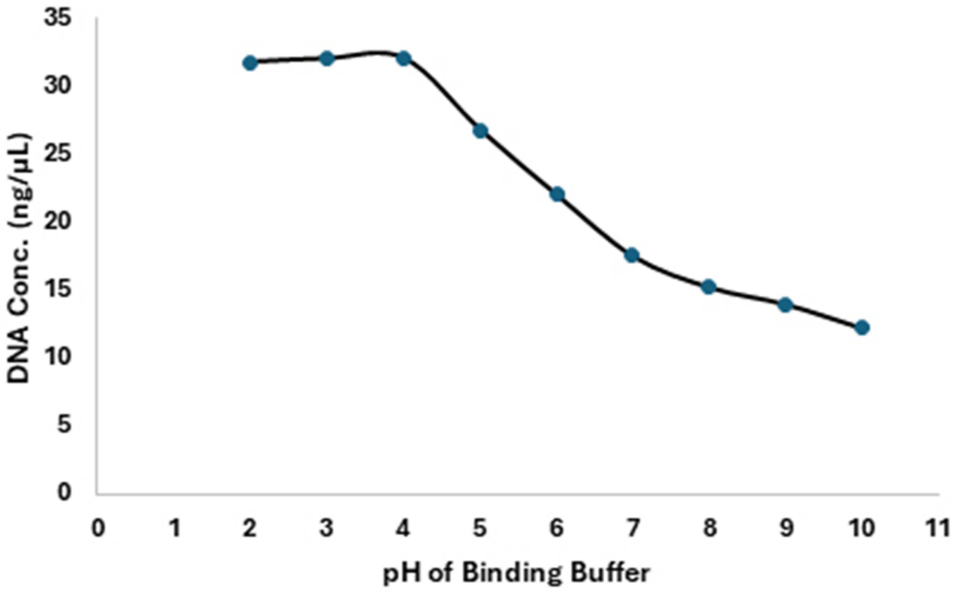

Figure 6 Effect of pH of Binding Buffer on DNA Adsorption Efficiency of PEI-IONPs.

280 ratios ranged from 1.76 to 1.85 for different concentrations of PEG, with a value of 1.81 ± 0.02 at 30% PEG, indicating high purity of the isolated DNA. At 40% PEG concentration, a comparatively lower A260/280 ratio was observed, which can be attributed to the co-precipitation of impurities along with DNA at this higher concentration (see Table 1). Control samples (0% PEG) exhibited the lowest DNA concentration (16.5 ± 1.2 ng/µL) and yield (3.3 ± 0.2 µg), reinforcing the importance of PEG in facilitating efficient DNA binding. Thus, 30% PEG was identified as the most effective concentration for DNA adsorption on PEI-IONPs, providing the highest yield while maintaining good purity.

In the case of NaCl concentration, the DNA concentration and yield were highest for control sample at 0M concentration of NaCl, with values of 33 ng/µL and 6.6 µg, respectively. As NaCl concentration increased, a decrease in DNA concentration and yield was observed, with the lowest values observed at 6M NaCl (8.25 ng/µL and 1.65 µg). This can be explained by the fact that the adsorption of DNA onto the surface of PEI-IONPs is mainly driven by electrostatic interactions.[26] The positively charged surface of the nanoparticles attracts the negatively charged molecules of DNA, facilitating effective adsorption on the surface of nanoparticles. At low ionic strength (0M NaCl), minimal interference from Na^+ ions allows for strong electrostatic interactions between DNA and PEI-IONPs, resulting in higher DNA adsorption efficiency. With higher NaCl concentrations, as the ionic strength of the medium increases, it leads to a weakening of the electrostatic interactions between the nanoparticles and DNA. This phenomenon, known as charge shielding, occurs when Na^+ ions in the solution shield the negative charges on the DNA, reducing the overall electrostatic attraction between the DNA and the positively charged nanoparticles.[26,40] The data obtained is in good agreement with the previously reported studies on nanoparticles with similar properties, such as PEI-$FePO_4$ nanocomposite.[40] The A260/280 ratios for all NaCl concentrations remained stable (ie 1.81 to 1.84), indicating that DNA purity was not significantly affected by ionic strength (see Table 2). Therefore, 0M NaCl is identified as the most effective concentration for maximizing DNA yield while maintaining purity.

Regarding pH, the data demonstrated that pH 4 is the optimal pH for DNA isolation using PEI-IONPs. At this pH, the DNA concentration and yield were highest (32.0 ng/µL and 6.4 µg/200 µL, respectively) with a high A260/280 ratio of 1.82, indicating pure DNA with minimal protein contamination (see Table 3). Control samples at neutral pH (pH 7) showed intermediate DNA recovery, with a concentration of 17.5 ± 1.5 ng/µL and yield of 3.5 ± 0.3 µg. The optimal performance at pH 4 can be attributed to the strong electrostatic interactions between the positively charged PEI-IONPs and negatively charged DNA backbone, which facilitates efficient DNA binding. At lower pH values (2–3), although DNA adsorption was efficient, slight degradation was observed on agarose gel, likely due to the harsh acidic conditions. The A260/280 ratios at

these pH levels remained around 1.81–1.82, indicating good DNA purity despite minor degradation. As the pH increased from 5 to 6, a significant decrease in DNA yield and purity was observed, with the A260/280 ratio dropping to 1.70–1.73. This was accompanied by increased protein adsorption, evidenced by the additional bands in the agarose gel electrophoresis results (pictures not shown). The increased protein contamination at these pH levels may be due to the proteins approaching their isoelectric points, resulting in reduced repulsion from the positively charged PEI-IONPs and increased co-precipitation with DNA. At pH levels above 6, the DNA yield continued to decrease, while the A260/280 ratio gradually improved, suggesting reduced protein contamination but also lower DNA adsorption efficiency. The reduced DNA binding at higher pH levels can be attributed to the decrease in positive charge on the PEI-IONPs, resulting in weaker electrostatic attraction with the negatively charged DNA. By pH 9–10, the DNA yield was lowest, but the purity ratios returned to around 1.81, indicating minimal protein contamination. The observed trends in DNA and protein adsorption with varying pH levels are in good agreement with findings reported for other nanoparticles with similar properties, such as PEI-$FePO_4$ nanocomposite.[40]

The findings from the agarose gel electrophoresis analysis, although not shown in the figures, corroborated the quantitative data. The gels indicated the highest DNA integrity and concentration at optimal PEG and NaCl concentrations, and at pH 4. The presence of faint or smeared bands at suboptimal conditions suggested lower DNA adsorption and potential co-precipitation of impurities.

Statistical analysis (one-way ANOVA with Tukey's post hoc test) further provided a robust framework for evaluating the significance of observed trends in DNA yield and purity. The results demonstrated that variations in PEG concentration, NaCl concentration, and pH produced statistically significant effects on DNA isolation efficiency ($p < 0.05$). These findings underscore the critical importance of optimizing each parameter for achieving high-yield and high-purity DNA.

Conclusion

The study systematically explored and identified the optimal conditions for DNA isolation using polyethyleneimine-coated iron oxide nanoparticles (PEI-IONPs), focusing on the effects of PEG-6000 concentration, NaCl concentration, and pH of binding buffer. The results conclusively demonstrated that a PEG-6000 concentration of 30%, an NaCl concentration of 0M, and a pH of 4 provide the highest DNA concentration and yield while maintaining high purity. These conditions maximize DNA adsorption efficiency by facilitating optimal electrostatic interactions and minimizing the co-precipitation of impurities. The findings of this study provide a robust framework for enhancing DNA isolation protocols, making this method valuable for various scientific and clinical applications. Future research should explore the scalability and applicability of these optimized conditions across different biological samples and settings to establish the robustness and versatility of this protocol as variability in the composition of fluids, such as differing protein, salt, or pH levels in different samples, may have an impact on DNA adsorption efficiency of these nanoparticles.

Abbreviations

PEI, Polyethyleneimine; PCR, Polymerase Chain Reaction; PEG, Polyethylene glycol; DNA, Deoxyribonucleic acid; PEI-IONPs, Polyethyleneimine functionalized iron oxide nanoparticles; FTIR, Fourier-transform infrared (FTIR) spectroscopy; TEM, Transmission Electron Microscopy; CTAB, Cetyltrimethylammonium bromide.

Data Sharing Statement

As the primary focus of this article was to optimize the binding buffer composition for Polyethyleneimine functionalized iron oxide nanoparticle-based DNA extraction method, it is important to note that the data and materials referred to in this paper were generated by the authors and not derived from publicly available sources. The data that support the findings of this study are available on request from the corresponding authors, Dr Gaurav Kaushik gaurav.kaushik@sharda.ac.in; Vinay Kumar vinayktyagi07@gmail.com.

Ethics Approval and Consent to Participate

Human related work (withdrawal of blood) was ethically approved by the Institutional Ethics Committee, Sharda University (Ref. No: SU/SMS&R/76-A/2024/01). In addition, we received the informed consent from the participants to participate in this study. All the authors agree that the study complies with the Declaration of Helsinki.

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Disclosure

The authors report no conflicts of interest in this work.

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