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Ultrasonication-Assisted Cell Lysis of Chicken Liver Cells for DNA Isolation and Adsorption-Elution Studies on $CoFe_2O_4$@ZnO

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Abstract

Extracting nucleic acids for identification of suspects in the sphere of forensic examination is still an arduous assignment and requires less intensive and labour free protocols. Liver tissue is characterized by higher concentration of nuclei which leads to extraction of high amount of genetic material. Therefore, DNA has been extracted from chicken liver cells using newly developed lysis method with conventional method as well as the nanocomposites were brought in use to check the adsorption and elution capabilities. The developed magnetic nanocomposites CFO@ZnO were synthesized using solution combustion method and co-precipitation method and characterized using X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM) and Fourier Transform Infrared Spectroscopy (FTIR). The newly developed CFO@ZnO nanocomposites show exceptional quality of DNA extraction by the revamped approach of lysing the cells as compared to the traditional method of cell lysis. This cutting-edge method is cost efficient as well as less time consuming with superior performance and thus proves to be an updated strategy of cell lysis.

Keywords: *DNA adsorption, Magnetic separation, PCI, Sonication and Cobalt ferrite nanoparticles.*

1. Introduction

The integration of strong DNA evidence has strengthened criminal investigations and legal proceedings [1]. DNA isolation plays a vital role in linking suspects to the crime which has occurred [2]. The modern DNA isolation techniques have significantly enhanced the precision and reliability of forensic analysis in court of law [3]. DNA evidence is available in minute quantities and needs expertise to handle as it’s prone to contamination and loss during the processing of the sample [4]. Therefore, a thorough understanding is required to maximize DNA recovery while eradicating the contamination risks along with maintaining the integrity of the DNA sample. Traditional method of DNA extraction is labor

intensive as well as time consuming which often leads to degradation of the sample as it involves transferring the sample into multiple vials that in turn results in contamination [5]. Additionally, the traditional Phenol-Chloroform-Isoamyl alcohol (PCI) involves extensive use of hazardous chemicals, and it takes hrs to lyse the cells. With an objective of reducing the sample processing time, we have developed an efficient and accurate cell lysis protocol using bath sonicator.

The DNA was first isolated accidently by Friedrich Miescher in 1869 while he was working on proteins [6]. Over time, several DNA isolation techniques like silica-based method, magnetic bead method, chelax-100 method, alkaline extraction method and Phenol-Chloroform-Isoamyl alcohol (PCI) have emerged, each method offering its own unique approach [7]. A complex array of factors affect the efficiency of these methods such as the age of the sample, collection and storage method, chemicals used during isolation, technique used for isolation and the type of the exhibit provided. To approach this dilemma, DNA extraction is integrated with nanotechnology which provides transformative approaches to isolate and purify DNA. Nanotechnology is characterized by manipulation at nanoscale level which has provided remarkable outputs to revolutionize the landscape of molecular biology [8]. Nanomaterials leverage unique properties which can increase the efficiency, sensitivity and specificity of the isolation process. Recently, magnetic nanoparticles have secured a notable amount of popularity within the discipline of molecular science. These nanoparticles have an iron oxide core coated with a magnetic responsive material which will allow easy separation of DNA bound nanoparticles by using an external magnetic field [9]. Nanoparticles offer increased surface area per unit volume ratio thereby conferring sufficient binding sites for attachment of nucleic acids. Functionalization of the binding site with specific ligands, can enhance selectivity and thus make increasing the isolation of targeted DNA sequences more efficient [10]. These magnetic nanocomposites eradicate the employment of centrifugation processes in the extraction protocol and minimize the use of high-end equipment. Positively charged magnetic nanocomposites are brought in use which facilitates the adsorption process due to electrostatic interactions between the negatively charged DNA and positively charged synthesized nanocomposites, which ultimately prevents other debris being attached to the nanocomposite [11].

Herein, magnetic nanocomposites (MNC) are tested for its capacity to adsorb DNA isolated from chicken liver cells offering a high throughput DNA yield and purity. For this experiment, a fresh chicken liver was obtained from a local supplier. CFO@ZnO has been employed for isolating DNA, as $CoFe_2O_4$ (CFO) is an excellent ferrite endowed with good anisotropy and saturation magnetization. Zinc oxide (ZnO) has a positive zeta potential which favourably engages with DNA backbone that enables firm binding with the nanocomposite. According to our best knowledge, CFO@ZnO has not been used for similar experiments.

2. Experimental

$CoFe_2O_4$ (CFO) nanoparticles were developed using solution combustion method. 3 g of cobalt nitrate hexahydrate ($Co(NO_3)_2.6H_2O$) and 3 g of ferric (III) nitrate nonahydrate ($Fe(NO_3)_3.9H_2O$) were mixed uniformly in 50 ml ethanol. 6 g glycine was added to the mixture and was kept on magnetic stirrer for 4 hrs at 90 ºC. The mixture was allowed to dry in the oven at 180 ºC for overnight. The mixture was then exposed in the muffle furnace for 5 hrs at 500 ºC [12-13].

To synthesise the desired nanocomposite, we used 1 g of cobalt ferrite ($CoFe_2O_4$) nanoparticles and 1 g sodium hydroxide which were mixed in few drops of distilled water and later it was subjected to ultrasonication. The mixture was allowed to dry on hot plate at 40 ºC [14]. To that composition, a mixture of 0.15 g of zinc chloride ($ZnCl_2$) was added which was later subjected to hot air oven overnight at 250 ºC. The powder obtained was transferred to a ceramic vessel to cool down, it was then finely grinded with

the help of mortar and pestle to obtain fine granules of CFO@ZnO nanocomposite. The developed nanocomposite was then exposed to muffle furnace at 700 ºC for 6 hrs.

3. Results and Discussion

3.1 X-Ray Diffraction (XRD): Rigaku ultima-IV diffractometer was used for performing analysis, Fig. 1 shows that ZnO has hexagonal wurtzite structure with peaks at 2θ = 31.5º, 34.4º, 36.8º,47.5º, 56.4º, 63.1º, and 68.2º. The XRD pattern approximately attributed to (100), (002), (101), (102), (110), (103) and (112) respectively which is represented by "●". The XRD pattern of CFO nanoparticles exhibit peaks at 2θ=30º, 35.3º, 37.1º,43.2º, 53.2º, 56.8º, and 62.5º approximately attributed to (220), (311), (222), (400), (422), (511) and (440) respectively which is represented by "#". The mean size of CFO@ZnO nanocomposites was quantified using Eq. (1) which is as follow:

$$D = \frac{k\lambda}{\beta_{1/2} \cos\theta} \quad (1)$$

Where,

k = Scherrer constant

λ= X-ray wavelength

$\beta_{1/2}$ = Full width at half maxima of peak (FWHM)

θ = Angle of Bragg diffraction [14].

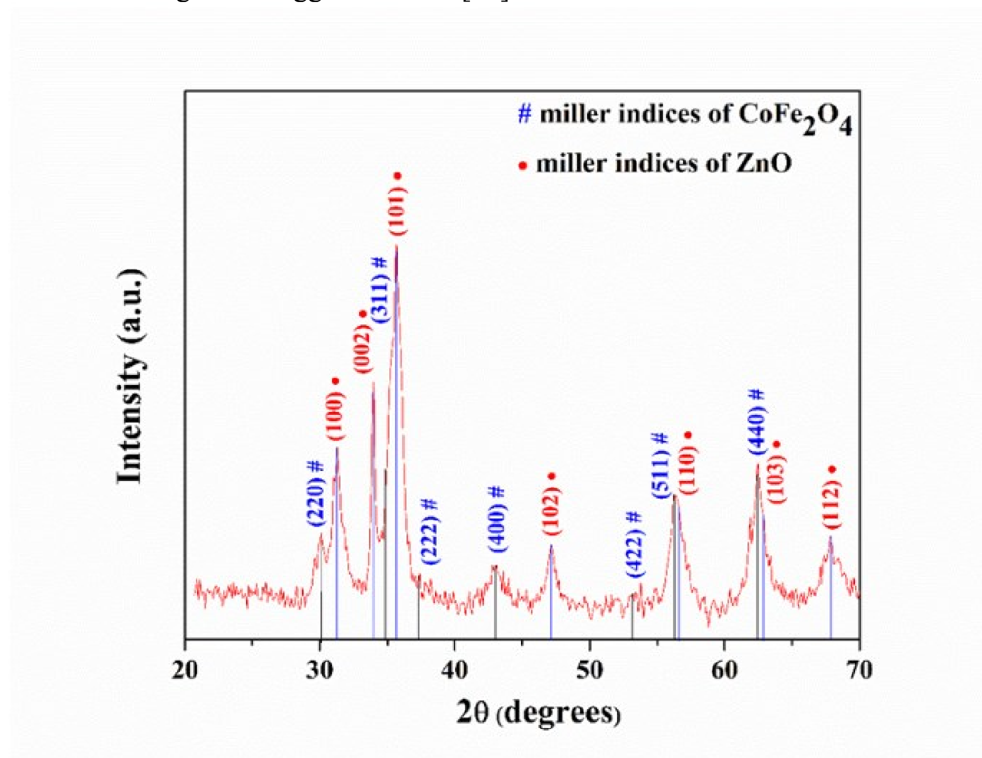

Figure 1. XRD pattern of CFO@ZnO.

3.2 Scanning Electron Microscopy (SEM): Hitachi TM3000 SEM was used for conducting analysis. In Fig. 2, morphology of nanoparticles can be described as pseudo-spherical like shape. Using Debye-Scherrer formula the crystalline size of developed nanoparticles was found approximately to be 9.78 nm.

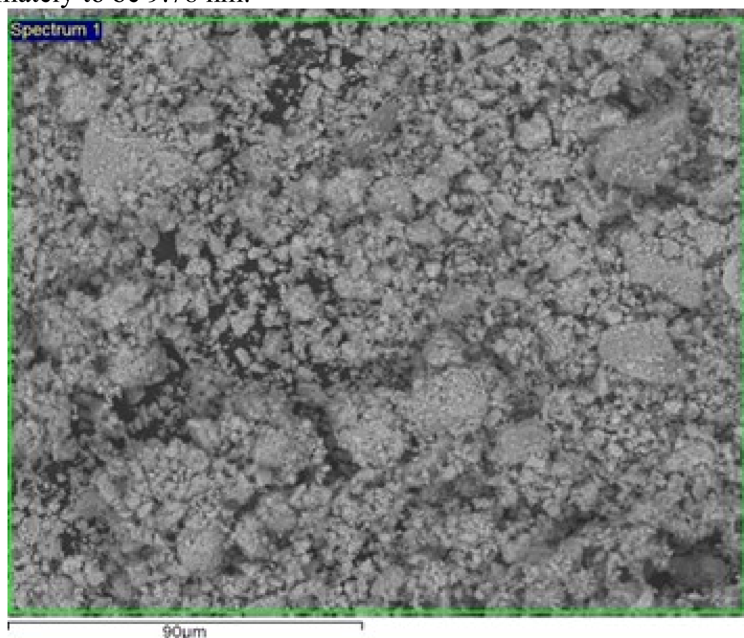

Figure 2. SEM image of CFO@ZnO.

3.3 Fourier Transform Infrared Spectroscopy (FTIR): The FTIR spectrum of CFO and CFO@ZnO nanocomposites are schematized in Fig. 3. The disparity resulting from both the spectra are due to the presence of the coatings. The peak seen at 598.03 cm^{-1} in Fig. 3 (b) is due to the vibrational stretching of the metal-oxygen bonds at the tetrahedral site. The adsorption depicted in Fig. (b) at 3426 cm^{-1} and 1548.39 cm^{-1} is due to H_2O stretching and the peak at 2917.95 cm^{-1} is because of the functional group C-H.

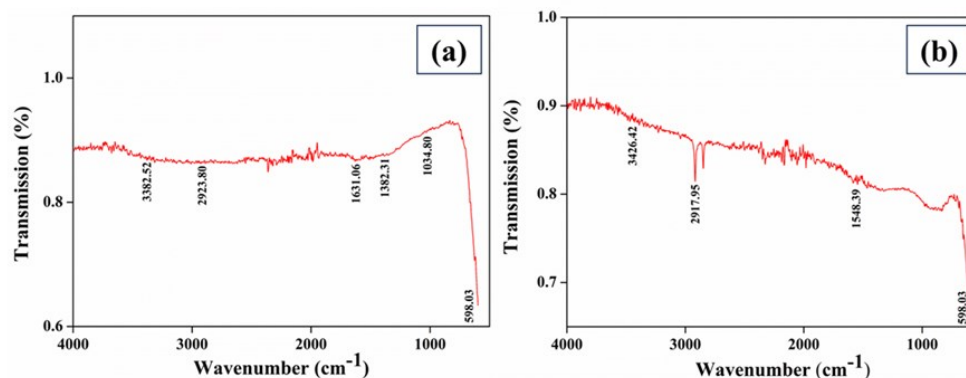

Figure 3. (a) FTIR analysis of CFO, **(b)** FTIR analysis of CFO@ZnO.

3.4 Extraction Using PCI Method with Integration of Bath Sonicator Lysis: Cell lysis is the crucial step in DNA isolation, a process that aims to disrupt the cell membrane to expose the internal cellular components like DNA, RNA, proteins, and organelles [15-16]. There are several techniques which are used to lyse the cell such as bead milling, sonication, chaotropic

agents, detergents, enzymatic digestion, and heat shock method. Sonication is a method which uses ultrasonic waves of high frequency to disrupt the cell membrane [17]. Sonicator produces a high frequency ultrasonic wave that is used for the disruption of cell. When the ultrasonicator waves are applied on cells, sound waves create an alternating high pressure and low-pressure cycles that induce mechanical stress on the cell membrane resulting in formation of transient pores or disruption in the membrane [18]. Sonication method is widely used as it offers the ease in handling large quantities, user friendly operations. There are several types of sonicator available but mostly probe sonicator is used for DNA isolation, these have drawbacks like heat generation, single-sample processing, contamination risks and potential DNA fragmentation. To address these issues, a bath sonicator was introduced which offers several advantages like the ability to process multiple samples at a time, reduced contamination as the tubes remains closed during the lysis process and reduced heat generation through vigorous ice addition in the bath sonicator.

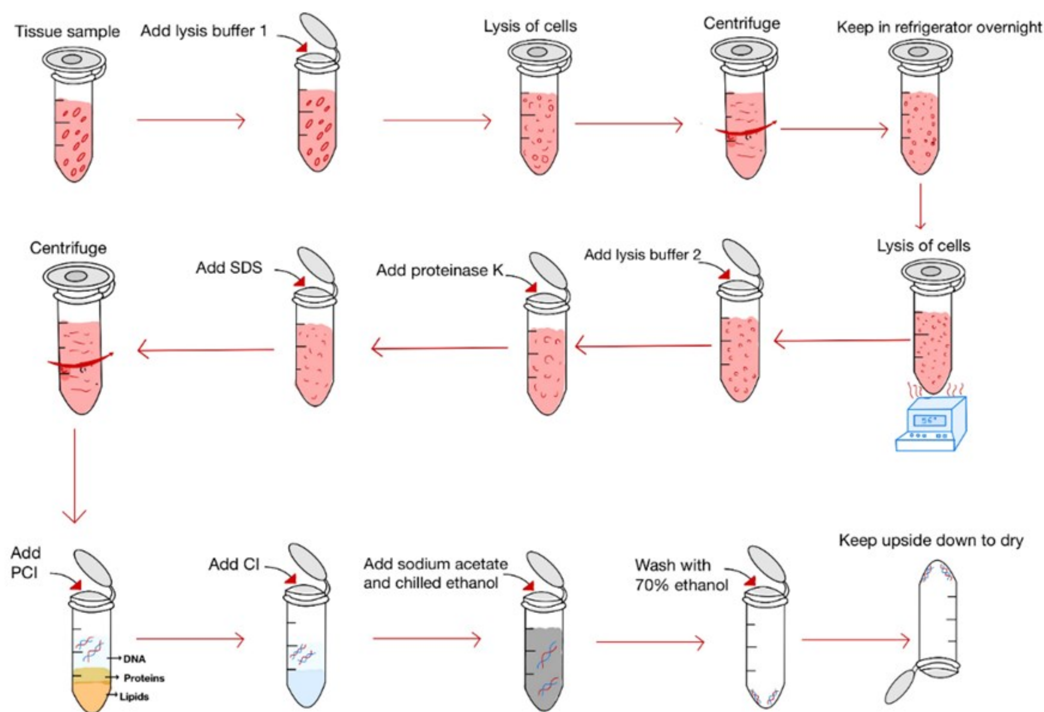

Figure 4. Procedure of DNA isolation using PCI method.

The protocol involves suspending the tissue sample in TE (Tris-EDTA) buffer and subjecting it to ultrasonication for 15 mins in cycles of 5 secs with ultrasonication and 5 secs without ultrasonication and similarly replacing TE (Tris-EDTA) buffer with the phosphate buffer. The efficacy of both the buffers was compared by extracting the genetic material using the PCI method as shown in Fig. 4 and the results were visualized through gel electrophoresis. The results obtained showed that DNA extracted using TE (Tris-EDTA) buffer exhibited two different bands, one with high-density containing high molecular weight DNA and low intensity band containing low molecular weight RNA and/or degraded DNA. While the DNA isolated using the phosphate buffer showed the presence of only a single band containing low molecular weight RNA and degraded DNA as shown in Fig. 5.

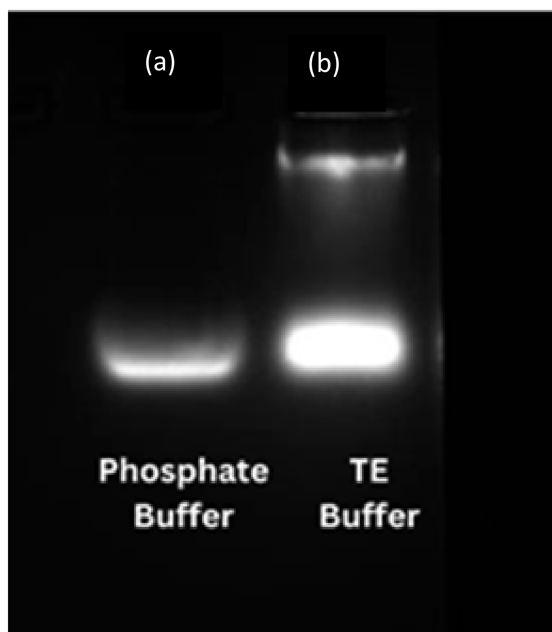

Figure 5. PCI extracted DNA using ultrasonicator for lysis using two different buffers: **(a)** Phosphate buffer **(b)** TE buffer.

The purity of isolated DNA was assessed by the absorbance ratio at 260 nm and 280 nm which revealed a ratio of 1.821 with TE (Tris-EDTA) buffer and 1.684 with phosphate buffer as indicated in Table 1. With these findings, it can be concluded that TE (Tris-EDTA) buffer is a superior buffer for cell lysis in juxtaposition to the phosphate buffer.

Table 1. Purity of DNA using two different suspension buffers during lysis.

Sample name	A260	A280	A260/A280
Ultrasonicator + Phosphate buffer	1.213	0.720	1.684
Ultrasonicator + TE buffer	1.555	0.854	1.821

3.5 Comparison Between Lysis Methods- Lysis Buffers and Bath Sonicator: When comparing two cell lysis methods, buffer-based lysis method comprises of two separate buffers which are used to disrupt the cell membranes. Buffer-based method is known for its simplicity, but it requires longer incubation time to reach its potential. On the flip side, ultrasonication method is quick and efficient which by inducing the mechanical stress on cell membranes facilitates the release of the genetic material.

Lysis buffer-based protocol was used wherein the liver tissue sample was minced and the sample was suspended in 500 µl lysis buffer I (Tris buffer 30 mM, EDTA 5 mM and NaCl 50 mM) later the sample was incubated at -20 ºC. The sample was then exposed to water bath at 80 ºC for cell lysis to take place via the heat and shock method. The samples were subjected to centrifugation at 10,000 rpm for 10 mins at 4 ºC. 500 µl lysis buffer II (EDTA 2 mM and NaCl 50 mM) was added to the sample and mixed uniformly using a vortex mixer. To the sample, proteinase K was added to make final concentrations as 10 µg/ml as total volume. Alongside, SDS was added to make the final concentration as 2% in the total volume. Samples were incubated at 56°C for 2 hrs. 500 µl of PCI (25:24:1) was added to the tube and it was gently mixed for 10 mins manually. The sample was subjected for centrifugation at

10,000 rpm for 10 mins at 4°C. After collecting supernatant, CI (Chloroform-Isoamyl alcohol 24:1) was added to the supernatant and was mixed gently for 10 mins. After mixing, it was again subjected for centrifugation at 10,000 rpm for 10 mins at 4°C. To the mixture, 1/30th volume of 3M sodium acetate and equal volume of chilled ethanol or isoamyl alcohol was added. After washing, the tubes were kept upside down overnight. The palette was dissolved in 50-100 µl of TAE (Tris-acetate-EDTA) buffer or ultrapure water and were incubated at 56°C [19]. 6 µl of dye was added to 4 µl of DNA sample and the samples were loaded and visualised [14], [20]. The bath sonicator process involves making fine paste and suspending them into TE (Tris-EDTA) buffer and later subjecting it to ultrasonication for 15 mins (5 secs exposure to ultrasonicator and 5 secs no exposure to ultrasonicator). After proper lysis, the extraction was carried out using PCI method.

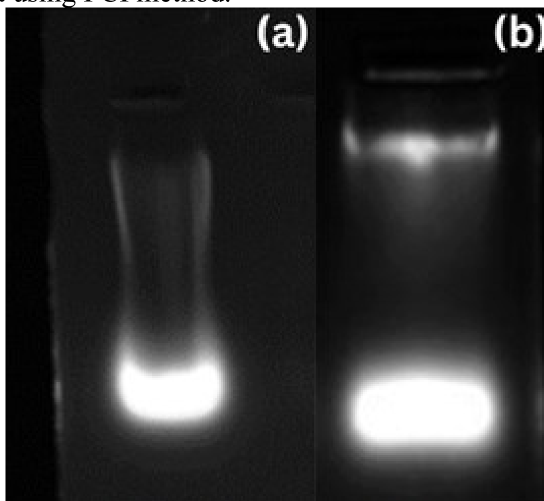

Figure 6. PCI extracted DNA using ultrasonicator for lysis using: **(a)** Lysis buffer **(b)** Bath ultrasonicator.

Table 2. Purity of DNA using two different lysis techniques.

Sample name	A260	A280	A260/A280
Using lysis buffer	1.234	0.772	1.597
Using bath sonicator	1.555	0.854	1.821

3.6 Adsorption and Elution of DNA Using CFO@ZnO: In this study, zinc oxide coated cobalt ferrite nanocomposites (CFO@ZnO) were synthesized for isolating the genetic material. Nanoparticle-based DNA isolation has gained attention over other conventional methods as it offers several advantages like reduced cross contamination, less mechanical stress, ease in operations, and cost-effectiveness. The adsorption of the genetic material onto the surface of the nanocomposites relies on the electrostatic interactions between the positively charged nanocomposite and negatively charged phosphate backbone of the DNA [21]. The protocol for assessing the adsorption and elution of DNA on the surface of nanocomposite is initiated by suspending the fine paste of the tissue sample into TE (Tris-EDTA) buffer and then subjecting it to the ultrasonication for 15 mins (5 secs exposure to ultrasonicator and 5 secs no exposure to ultrasonicator) at 10 ºC. After proper lysis, PCI method of extraction was carried out as mentioned in section 3.5 [22-25].

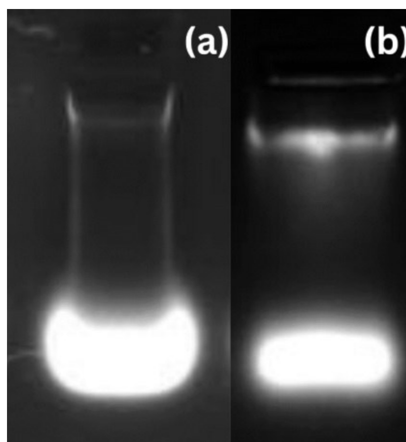

Figure 7. a) Adsorption elution of PCI extracted DNA through CFO@ZNO nanocomposites, **(b)** PCI extracted DNA.

This study underscores the efficacy of synthesized nanocomposites, which were able to adsorb genetic material and subsequently nanocomposites were subjected for elution at high temperatures. Fig. 7 (a) shows the DNA which was eluted from synthesized nanocomposites and Fig. 7 (b) shows the PCI extracted DNA. The purity of the isolated genetic material was assessed by calculating the absorbance ratio at 260 nm and 280 nm wavelengths. A ratio near 1.8 signifies that it is a pure DNA while the ratio below 1.5 indicates contamination of the sample by proteins or phenols [26-27]. Table 3 shows that absorbance ratio is 2.0 for DNA eluted from the surface of the nanocomposite whereas the absorbance ratio recorded for the DNA isolated using PCI method of extraction is 1.8. These findings suggest that the newly developed nanocomposite complements the adsorption and elution process making it a favourable candidate for DNA extraction in nanoparticle-based isolation techniques.

Table 3. Purity of PCI extracted DNA.

Sample name	A260	A280	A260/A280
Adsorption elution using nanocomposites	0.936	0.449	2.081
PCI extracted DNA	1.555	0.854	1.821

4. Conclusion

In this study, the primary goal was to investigate the binding capacity of the synthesised nanocomposite to absorb DNA on its surface and streamline the DNA isolation process which was achieved by employing bath sonicator for the lysis of the cell. The synthesised nanocomposites showed excelled binding efficiency, this paves the way for its employment in targeted DNA delivery and gene therapy. Among the buffers evaluated in this study, TE buffer proved to showcase superior performance during cell lysis. The breakthrough was achieved using bath sonicator for cell lysis which showed excellent results by completing the cell lysis within a timeframe of 15 mins. The bath sonicator lysis protocol was integrated with PCI method of extraction and the outcomes were compared with isolation using the lysis buffer. The results depicted that the lysis using bath sonicator showed exceptional output in accelerating the entire isolation process showcasing the potential in this method for enhancing the isolation processes in research and diagnostic research.

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Author Contributions

All authors listed on this manuscript have made substantial contributions to its creation and are accountable for all aspects of the work. Each author has participated in the following aspects of the research and manuscript preparation:

- **Conceptualization:** AV, SB
- **Methodology:** AV, SB
- **Formal Analysis:** AV, SB
- **Data Curation:** AV, SB
- **Writing - Original Draft:** AV, SN, SB
- **Writing - Review & Editing:** SN, SB
- **Visualization:** SN, SB
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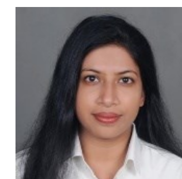

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