Research Article

High-Efficiency DNA Extraction Using Poly(4,4′-Cyclohexylidene Bisphenol Oxalate)-Modified Microcrystalline Cellulose-Magnetite Composite

Aisha Nawaf Al balawi,1,2 Nor Azah Yusof,1,3 Sazlinda Kamaruzaman,1 Faruq Mohammad,4 Helmi Wasoh,5 and Hamad A. Al-Lohedan4

1Department of Chemistry, Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
2Haql College, University of Tabuk, 71491 Tabuk, Saudi Arabia
3Institute of Advanced Technology, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
4Surfactants Research Chair, Department of Chemistry, College of Science, King Saud University, 11451 Riyadh, Saudi Arabia
5Faculty of Biotechnology and Biomolecular Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Correspondence should be addressed to Nor Azah Yusof; azahy@upm.edu.my and Faruq Mohammad; fmohammad@ksu.edu.sa

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In this study, we studied the DNA extraction capability of poly(4,4′-cyclohexylidene bisphenol oxalate) following the surface modification and composite formation with that of microcrystalline cellulose (MCC) and magnetic iron oxide nanoparticles (NPs). The physical characterization techniques like scanning electron microscopy (SEM), Fourier-transform infrared (FTIR) spectroscopy, energy-dispersive X-ray analysis (EDX), and thermogravimetric analysis (TGA) were employed for the poly(bisphenol Z oxalate)-MCC-magnetite composite during different stages of its formation. The results confirmed the successful modification of the polymer surface. On testing in the presence of three types of binding buffers, a high value of 72.4% (out of 10,000 ng/μL) efficiency with a total yield of DNA at 2×10^6 ng and absorbance ratio of A260/A280 (1.980) was observed for the 2 M GuHCl/EtOH binding buffer. These results were compared against the other two buffers of phosphate-buffered saline (PBS) and NaCl. The lowest value of DNA extraction efficiency at 8125 ng/μL of 58.845% with absorbance ratios of A260/A280 (1.818) for PBS was also observed. The study has concluded an enhancement in the DNA extraction efficiency when the polymer is in the composite stage along with cellulose and magnetite particles as compared against the bare polymer.

1. Introduction

In recent years, nanocomposites have been extended to many different fields because of their greater potentials and ability to enhance the fundamental characteristics of any basic material. The nanocomposites in general are formed as a combination of two or more materials of different origins. For example, the polymer nanocomposites contain polymeric organics with metal/inorganic nanoparticles (NPs), while the magnetic nanocomposites are ferromagnetic or superparamagnetic materials such as iron, cobalt, and nickel with a diameter of 50-200 nm [1]. Several types of polymer nanocomposites have got interest in many studies such as polystyrene/nanoclay [2], silicates [3], graphene [4], carbon nanotubes (CNTs) [5], and magnetic polymer beads [6]. One important sector among many different applications of nanocomposites that employ polymeric matrices includes the DNA isolation and purification process.

The process to extract DNA is the first and essential step to do some activities that hold importance in the molecular biology such as hybridization, sequencing, ligation, amplification, biodetection, and cloning. The applications of the
DNA isolation technique are several and a few of them include the gene therapy, forensic science applications, diagnosis of diseases, and pathogen detection [7–9]. The chemistry and other related properties of DNA molecule structures help to separate and purify it through several methods [7, 10, 11]. The purification methods include the conventional methods that involve the use of fluid-phase to be phenol/chloroform mixture and cetyltrimethylammonium bromide (CTAB) and developmental methods such as solid-phase (e.g., glass fibers, silica, magnetic particles, and CNTs) [9]. Previous studies have discussed the methods to enhance the isolation efficiency of DNA by solid supports; for instance, Shakhmaeva et al. [10] studied the DNA adsorption by using CNTs as solid-phase extraction (SPE) sorbents. Other studies included the isolation of plant DNA using phenol-stacked CNTs [11], poly(hydroxethyl methacrylate)-magnetite particles to DNA purification from Escheria coli [12], magnetic nanoparticles (MNPs) with N-isopropylacrylamide (NIPAM) for DNA adsorption [13], and DNA isolation using hemoglobin-modified magnetic nanocomposite as solid-phase adsorbents [14].

Solid-phase support of nanocomposites involves the extraction and purification of DNA and has several advantages over the conventional methods like high accuracy, sensitivity, increased surface area for immobilization of DNA, and reduced time of incubation [15]. In addition, the isolation techniques involving the MNPs display benefit in limited use of chemicals, sustainable costs, minor physical or chemical damage, and ease of automation. The adsorption of DNA on polymer nanocomposite (solid-supported) takes place through driving force of hydrophobic, hydrogen bonding, and electrostatic interaction. These bondings are associated with functional groups (–COOH, –OH, and –NH2) of MNPs that have been surface-modified including coated with inorganic materials (e.g., iron oxide, silica, and gold) for investigation of high-efficiency loading (isolation) [16]. Polymer nanocomposites have been distinguished from other solid support materials because of the polymers with NPs new class and possess advanced properties with multi-applications. The overall characteristics and performances are determined based on the concentration and type of functional groups (e.g., carboxylic, iminodiacetate, amine, and sulfonic), structure of polymer, and methods to prepare polymer nanocomposites [17]. Moreover, the other surface influential physical properties of the polymer nanocomposites like surface area, micropore size, shape, and porosity play a crucial role towards the adsorption and isolation of DNA [18].

The present study has provided a novel process of producing the polymer nanocomposites that include magnetically responsive metal oxide and microcrystalline cellulose (MCC) for the extraction of high-efficiency DNA. Following the composite formation, the physicochemical techniques to fully understand the structure of poly(4,4′-cyclohexylidene bisphenol oxalate) having a benzene ring and oxygen bond to form covalent bonding with the iron oxide MNPs were studied by different instrumental techniques. Finally, the subsequent adsorption of DNA by the surface-modified polymer in the presence of three different buffers has also been studied.

2. Experiment

2.1. Materials. The DNA solution used as DNA specimen in this study was purchased from Sigma Company (D7290). Iron oxide (Fe3O4) with the particle size 50–100 nm, MCC with 20 μm particle size, urea, HNO3, and all other solvents were purchased from (Sigma-Aldrich). All the reagents used in this study were of the highest analytical grade (≥95%), which were received and utilized with no further purification. For the synthesis of poly(4,4′-cyclohexylidene bisphenol oxalate), we adopted the condensation-polymerization method where the product was obtained in form of white powder [19].

2.2. Synthesis of Poly(4,4′-Cyclohexylidene Bisphenol Oxalate)-MCC-Fe3O4 Composite. About 4.5 g of poly(4,4′-cyclohexylidene bisphenol oxalate) was added to 90 mL ultrapure water followed by the sonication for 15 min and cooled to 0°C by placing the reaction mixture in a refrigerator. In the next step, 6 g NaOH, 4 g urea, and 4 g MCC were added to the frozen suspension and stirred, and 4.5 g of Fe3O4 powder was added with stirring. The reaction mixture was kept in an ice bath for 10 min and then cooled to -20°C for 2 hours. After the period, the mixture was taken out from the cooler and 1 mL of 2M HNO3 was added at room temperature and stirred for 30 min. After this, 1 mL of 2M HNO3 was added to the composite and was left inside the acid overnight. This step was followed by washing the solution with distilled water for 15 min. Later, the synthesized composite was filtered and dried.

2.3. DNA Extraction Using the Polymer Nanocomposite. The study has used 2 M of guanidine hydrochloride (GuHCl) in 96% ethanol (EtOH), 2 M NaCl solution, and phosphate-buffered silane (PBS, 5 M GuHCl in 30% propanol) as three binding buffers for measuring the binding capacity of poly(4,4′-cyclohexylidene bisphenol oxalate)-MCC-Fe3O4 composite for the DNA analysis. A 200 μL of DNA solution (20 μL DNA and 180 μL of deionized water) was mixed with 300 μL binding buffer, whereas two different weights (0.2 g and 0.4 g) of poly(4,4′-cyclohexylidene bisphenol oxalate)-MCC-Fe3O4 composite were inserted into an Eppendorf tube including binding buffer and DNA solution with a total volume of 500 μL, followed by the incubation for 10 min. The solution was taken out using a pipette and washed with 70% EtOH to clean all the salts from the surface. The elution was separated from the modification of polymer, while the efficiency and purity of the assessing the DNA extraction was carried out in the elution buffer.

3. Purity and Yield Analysis of Extracted DNA

For the high-purity DNA, we measured the absorbance ratio between 260 nm and 280 nm using a nanophotometer device ranging from 1.8 to 2.0. In order to calculate the
total purity and extraction efficiency of DNA, we used the following equations:

\[
Total\ yield\ of\ DNA\ purification = \frac{Final\ elution\ of\ the\ solution's\ volume}{\times\ DNA\ concentration(\mu L)},
\]

\[
Extraction\ efficiency = \frac{Total\ DNA\ yield}{total\ DNA\ amount}\cdot(ng/input\ DNA\ volume,\mu L).
\]

4. Characterization of the Modified Polymer

For the Fourier-transform infrared (FTIR) spectroscopy analysis where it indicates the functional groups and bonding of the polymer at different stages of its formation, the PerkinElmer Spectrum 100 FTIR instrument was employed. For the analysis, the samples were recorded in the range of 4000-400 cm\(^{-1}\) using KBr pellets. The Mettler Toledo instrument was used to investigate the thermal stability of the polymer nanocomposite in the range of 60-700\(^\circ\)C by means of thermogravimetric analysis (TGA) and differential thermogravimetry (DTG). The scanning electron microscopy (SEM) technique was used to observe the changes in the surface morphology of the polymer composite using a Module NOVA NANOSEM 230 – FE 1™ instrument. The electron diffraction analysis by X-rays (EDAX) instrument with a Module NOVA NANOSEM 230 – FE 1™ was used for the elemental analysis. Lake Shore 7400 series was employed with vibrating sample magnetometer (VSM) to measure the magnetic properties of poly(4,4\(^{-}\)-cyclohexylidene bisphenol oxalate)-MCC-Fe\(_3\)O\(_4\) composite. The UV-Vis spectroscopic analysis was measured by the NanoDrop 2000 spectrophotometer.

5. Results and Discussion

Mixed polymer, NaOH, urea, MCC, and Fe\(_3\)O\(_4\) powder cooled at -20\(^\circ\)C condition were then dropped by HNO\(_3\) that resulted in the formation of a black powder having the magnetic properties. Such formed polymer composite along with the starting materials was analyzed by FTIR spectroscopy, and the results are shown in Figure 1. The surface-modified composite, poly(4,4\(^{-}\)-cyclohexylidene bisphenol oxalate)-MCC-Fe\(_3\)O\(_4\), resulted in the observation of bands for stretching vibration of the carbonyl group (C=O) in the polymer around 1725 cm\(^{-1}\)and the broad peak around 3270 cm\(^{-1}\)can be assigned to the C-H stretching vibrations of the cyclohexane of polymer. The C=C stretching vibrations from all the samples (except Fe\(_3\)O\(_4\)) were observed in the range of 1600-1638 cm\(^{-1}\), meaning that the functional groups related to C=C from the monomer, polymer, and cellulose are maintained in the polymer composite too. Also, the C-O-C group appeared in the finger print region of around 1012-1045 cm\(^{-1}\) is an indicative of the presence of carboxy-related groups from both polymer and cellulose in the final composite. The broadband in the composite around 1378 cm\(^{-1}\) is due to the bending vibrations of C-H groups, and the same band was observed as a sharp peak in the cellulose material around 1329 cm\(^{-1}\) due to the availability of a less number of C-H groups as compared to the final composite. Similarly, the observation of peak around 520 cm\(^{-1}\) in the samples of Fe\(_3\)O\(_4\) and the polymer composite can be assigned to the Fe-O stretching bands of magnetite material [20]. From the comparison and analysis of all other spectral bands, it can be confirmed that the poly(4,4\(^{-}\)-cyclohexylidene bisphenol oxalate)-MCC-Fe\(_3\)O\(_4\) sample maintains the functional groups of polymer, Fe\(_3\)O\(_4\), and MCC, which further confirms for the successful formation of the polymer composite.

The TGA and DTG curves for the final polymer composite were compared with those of the pure polymer and pure iron oxide, and the results have been shown in Figures 2(a) and 2(b). From Figure 2(a), it can be observed that the polymer after its composite formation due to the inclusion of Fe\(_3\)O\(_4\) seems to have slight variations in the multistep thermal degradation pattern. For the polymer composite, the first decomposition temperature of polymer was observed between 58\(^\circ\)C and 94\(^\circ\)C, with sample loss of 4%, and 52.41 to 1567\(^\circ\)C, with sample loss of 7% for the surface-modified polymer. The second thermal decomposition event started between 103\(^\circ\)C and 223\(^\circ\)C with sample loss of 12.5%, while surface modification of polymer observed sample loss of 14% between 157\(^\circ\)C and 338\(^\circ\)C. The last step to decomposition of polymer and surface modification of polymer was limited at 226-624\(^\circ\)C with the sample remained to be 81%, and in the temperature range of 351-672\(^\circ\)C, the sample amount remained to be around 71%. Similarly, the analysis of TGA results for the other samples indicated to have a weight loss of more than 95% and only 14% for the pure polymer and pure Fe\(_3\)O\(_4\), respectively. These results are anticipated when dealing with the pure organic and metal containing samples where there is no solid-supported content in the polymer and
organic content in the iron oxide material. In that way, the observation of the remained amount of 71% for the polymer composite sample is confirmed to have the remaining 29% as the polymer content, i.e., each 100 mg of polymer composite has about 71 mg of \( \text{Fe}_3\text{O}_4 \) and 29 mg of organic content. Further, the DTG curve (Figure 2(b)) for the polymer composite observed the integral peak around 86°C and loss of 0.26 mg as the beginning of the pyrolysis of polymer nanocomposite. The second integral peak was at 249°C with a mass loss of 1.98 mg, and the last pyrolysis reaction polymer nanocomposite was at 425°C, where the mass loss is 0.29 mg (Table 1).

5.1. Morphological Characterization. Figures 3(a)–3(c) have illustrated the surface modification of the polymer composite characterized by SEM and EDAX analysis. It is observed that the polymer before composite formation (Figure 3(a)) has a large gap and rough surface, which turned to be smoother and smaller beads shaped following its composite formation (Figure 3(b)). The formation of the composite in the beads shape following the surface modification can be attributed to the presence of MCC, while the magnetite presence was supporting the particle’s agglomeration. The average particle size of the beads was observed to be 136 nm, and the standard deviation for this particles was 45.74, while the average particle size of polymer before modification was 162 nm and they were uniform with slight agglomeration. The appearance of peaks for the respective elements in the sample confirmed the presence of Fe, O, and C from the EDAX analysis (Figure 3(c)). This provided enough evidence for the successful formation of the composite proposed in the present study.

The surface area of poly(4,4’-cyclohexylidene bisphenol oxalate)-MCC-\( \text{Fe}_3\text{O}_4 \) composite was measured by BET analysis, and the results have been shown in Figure 4 and Table 2. In this procedure, the \( \text{N}_2 \) gas was used for the physical adsorption, and for the analysis, the bath was maintained at 77.251 K. From Figures 4(a) and 4(b), the formation of IUPAC was classified through the appearance of isotherm linear plot type III adsorption model. Table 2 has shown three very important values to study the surface area of polymer composite like pore volume, total surface area, and pore size. It was observed that the \( \text{N}_2 \)-mediated BET surface area of the polymer composite was 2.6549 m\(^2\)/g. However, the surface area for the polymer before modification was measured to be 1.9294 m\(^2\)/g, which indicated increase in the surface area value. The results showed that the single-point adsorption and desorption total pore volume were 0.181943 cm\(^3\)/g and 0.198761 cm\(^3\)/g, respectively (Table 2). The pore sizes (adsorption and desorption average pore diameter) by (4 \( V/A \) by BET) were 2741.2863 Å and 2994.6726 Å, but for polymer before modification, it was 2426.3042 Å (242.63 nm) indicating an increase in the pore size (Table 2). Therefore, majority of the pores were classified into the range of macroporous with an average pore diameter of 242.63 nm. This helped in classifying IUPAC on pore dimensions, whereas three kinds of the pore dimensions of adsorbents were grouped as the micropore (\( d < 2 \) nm), the mesopore (\( d = 2-50 \) nm), and the macropore (\( d > 50 \) nm).

5.2. Magnetic Properties of Poly(4,4’-Cyclohexylidene Bisphenol Oxalate)-MCC-\( \text{Fe}_3\text{O}_4 \) Composite. Figures 5(a) and 5(b) have illustrated the magnetic hysteresis curves of pure \( \text{Fe}_3\text{O}_4 \) with those of poly(4,4’-cyclohexylidene bisphenol oxalate)-MCC-\( \text{Fe}_3\text{O}_4 \) composite measured by static hysteresis graph. From the comparison of both graphs, it is observed that the superparamagnetic property of the magnetite material (Figure 5(a)) is getting persisted in the polymer composite too (Figure 5(b)) as indicated by the generation of magnetic

<table>
<thead>
<tr>
<th>Pyrolysis temperature</th>
<th>Weight loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>86.53°C</td>
<td>0.26 mg</td>
</tr>
<tr>
<td>249.63°C</td>
<td>1.98 mg</td>
</tr>
<tr>
<td>424.96°C</td>
<td>0.29 mg</td>
</tr>
</tbody>
</table>
hysteresis loops. The highest magnetization value observed for the pure magnetite material has to be 83.55 emu/g while for the polymer composite to be 36.7 emu/g only. Such a decrease in the total saturation magnetization values for the polymer composite as compared against the pure magnetite material can be attributed to the presence of polymer in the composite. In general, the presence of polymer material onto the surface of magnetic core material usually decreases the total oscillation of the particles due to an increase in the polymer weight present at the surface.

Figure 3: SEM images of poly(4,4'-cyclohexylidene bisphenol oxalate) (a) and poly(4,4'-cyclohexylidene bisphenol oxalate)-MCC-Fe$_3$O$_4$ composite (b). The EDAX analysis of the final composite is shown in (c).

Figure 4: (a) BET isotherm linear plot for polymer composite. (b) Isotherm linear plot for polymer composite.
of the magnetic material [20, 21]. The observation of such property can be used to extrapolate the composite’s application to various other different fields like magnetic hyperthermia-based therapy and drug delivery. The various other magnetic properties like saturation magnetization (\(M_s\)), coercivity (\(H_c\)), and retentivity (\(M_r\)) for the poly(4,4′-cyclohexylidene bisphenol oxalate)-MCC-magnetite composite are presented in Table 3.

According to the obtained results, the value of \(H_c\) and the value of \(M_r\) were comparable with the values of \(M_s\) and \(H_c\) as depicted in the previous study [22]. The \(M_s\) of the Kenaf/magnetite40/polyester composite was 29.2 emu/g that was smaller than the \(M_s\) value obtained in the present study. However, the \(H_c\) was 112.2 Oe – (112.2 G) was higher than the results (99.88 G) proposed in the present study. Doulabi et al. [23] stated that \(M_s\) and \(H_c\) for Ni_{0.3}Zn_{0.7}Fe_{2}O_{4} polyvinyl acetate nanocomposite were 4.31 emu/g and 368.39 G, respectively, while the polymer composite in the present study was 36.70 emu/g and 99.88 G.

Typek et al. [24] measured the \(M_s\) and \(H_c\) values for the nanopowder sample, and the results of their study indicated that the \(M_s\) value was smaller than the present study results; however, the \(H_c\) values were higher. As compared to other studies, the observation of such decreased \(H_c\) values for composite in the present study can be attributed to the formation of composite structure with high

### Table 2: The values of surface area and pore volume for poly(4,4′-cyclohexylidene bisphenol oxalate).

<table>
<thead>
<tr>
<th>Relative pressure ((P/P_0))</th>
<th>Surface area</th>
<th>BET surface area</th>
<th>Single-point adsorption total pore volume of pores less than 842.856 Å radius</th>
<th>Single-point desorption total pore volume of pores less than 928.410 Å radius</th>
<th>Pore size (adsorption and desorption average pore diameter) by ((4V/A)) by BET</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.271</td>
<td>1.4475 m²/g</td>
<td>2.6549 m²/g</td>
<td>—</td>
<td>—</td>
<td>0.98761 cm³/g</td>
</tr>
<tr>
<td>0.988</td>
<td>—</td>
<td>—</td>
<td>0.181943 cm³/g</td>
<td>—</td>
<td>0.198761 cm³/g</td>
</tr>
<tr>
<td>0.989</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.181943 cm³/g</td>
<td>2,741.2863 Å and 2,994.6726 Å</td>
</tr>
</tbody>
</table>

### Table 3: The values of saturation magnetization \((M_s)\) and coercivity \((H_c)\) for the poly(4,4′-cyclohexylidene bisphenol oxalate)-MCC-magnetite composite.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Saturation magnetization ((M_s)) (emu/g)</th>
<th>Coercivity ((H_c)) (G)</th>
<th>Retentivity ((M_r)) (emu/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(4,4′-cyclohexylidene bisphenol oxalate)-MCC-magnetite composite</td>
<td>36.70</td>
<td>99.88</td>
<td>5.36</td>
</tr>
</tbody>
</table>
Table 4: Comparison of the extraction efficiencies of pure polymer, poly(4,4′-cyclohexylidene bisphenol oxalate) with that of poly(4,4′-cyclohexylidene bisphenol oxalate)-MCC-magnetite composite.

<table>
<thead>
<tr>
<th>Binding buffer</th>
<th>Sample weight</th>
<th>Pure polymer extraction efficiency (ng/μL)</th>
<th>Polymer composite extraction efficiency (ng/μL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 M GuHCl/EtOH</td>
<td>0.2 g</td>
<td>2247.5</td>
<td>7237.5</td>
</tr>
<tr>
<td>PBS</td>
<td>0.2 g</td>
<td>875</td>
<td>7177.5</td>
</tr>
<tr>
<td>2 M NaCl</td>
<td>0.2 g</td>
<td>1692.52</td>
<td>7222.5</td>
</tr>
</tbody>
</table>

The absorbance ratios indicated high purity of DNA with higher NaCl over, significant differences in the samples. The modifi-
cation of polymer NP showed high extraction efficiency for ssDNA (single-stranded DNA) at 10,000 ng/μL with total yield 2 × 10^6 ng. This indicated that the modification of polymer NPs had a sufficient affinity to capture the ssDNA. The differences of ssDNA purification and total yield amounts were dependent on the properties of adsorbent such as functional groups, structure, pore size of particles, surface area, and ligand density.

5.3. Extraction Efficiency and Purity of DNA. The comparison of the DNA extraction efficiency among the polymer before and after modification has been presented in Table 4, where the results confirmed improvement in the efficiency for all the tested conditions. The observation of such results can be attributed to an increase in the surface area due to the beads formed as part of the composite formation as against the pure polymer without the bead structure.

Table 5 has described the values of DNA extraction efficiency for the polymer composite, when tested under different weights and buffered conditions. It has been noted that there was no major difference in the percentage efficacy for all the three buffers tested and the values follows the order of GuHCl > NaCl > PBS of binding buffers at 0.4 g in the samples. Moreover, significant improvement was observed in the extraction efficiency with that of the polymer weighted. The values of absorbance ratios indicated high purity of DNA with higher extraction efficiency. A comparison of the maximum extraction efficiency and total yield of the modification of polymer NPs with those of some other affinity matrices has been provided in Table 6. The modification of polymer NPs showed high extraction efficiency for ssDNA (single-stranded DNA) at 10,000 ng/μL with total yield 2 × 10^6 ng. This indicated that the modification of polymer NPs had a sufficient affinity to capture the ssDNA. The differences of ssDNA purification and total yield amounts were dependent on the properties of adsorbent such as functional groups, structure, pore size of particles, surface area, and ligand density.

The objective of this study was to examine the effect of magnetic iron oxide-coated polymer composite towards the extraction of DNA, and for that, we have prepared the superparamagnetic Fe3O4-coated with MCC and poly(4,4′-cyclohexylidene bisphenol oxalate). The physical characterization of the prepared composite provided the information that the Fe3O4 particles are firmly surrounded by the MCC and poly(4,4′-cyclohexylidene bisphenol oxalate) polymer. This provides a primary evidence that there will not be any toxic effect for the direct interaction of the Fe3O4 particles with that of the bounded DNA, where the MCC and polymer serve as the protective shield from the oxidative behavior of iron, since the DNA possesses some protein groups having electron-rich surfaces and its direct interaction with the electron-deficient iron (Fe^{2+} or Fe^{3+}) has a tendency to absorb the electrons from those protein groups, and in that way, there is a chance for the destabilization of the adsorbed DNA [33]. In our case, the surface-coated groups like MCC and the polymer inhibit such oxidative action of Fe3O4 while simultaneously maintain the superparamagnetic behavior of magnetite iron oxide particles (Figure 5). Further, we observed a significant enhancement in the adsorption of DNA for the magnetic polymer composite as compared against the pure polymer (Table 4) and this increased extraction efficiency for the composite can be attributed to the density and associated stability offered by the solid support [34], since the polymer particles bound to the solid iron oxide surfaces allow for their increased and continuous interaction with that of the DNA molecules and thus contributed for the enhanced efficiency. As shown schematically in Figure 6, the polymer coating available at the surface of Fe3O4 particles can hold the DNA molecules by means of multiple forces like physical adsorption and chemical bonding. For example, the magnetic force means of interaction of DNA’s phosphate backbone structure with that of polymer sheets supported by the Fe3O4 particles increase the adsorption by π-π stacking which occurs between polymer and the nucleo-base ring structures [35]. Also, the MCC makes as a bridge between DNA and polymer that play a key role to increase the number of hydrogen bonding which also supports for the enhanced efficiency. In the present case, we have selected the magnetite particles having two different oxidation states of Fe^{2+} and Fe^{3+} and tested the efficiency linked to the magnetic properties. The iron oxide NPs can be available in other isomeric forms too having different oxidation states and changes of magnetic properties, and so our future studies have to deal with the DNA extraction studies of other iron oxide bound polymer composites like wüstite, maghemite, and hematite.

6. Conclusion

The present study has analyzed the structure of poly(4,4′-cyclohexylidene bisphenol oxalate) having a benzene ring and oxygen bond forming covalent bonding with the magnetic iron oxide NPs. It has used Fe3O4 NPs for the modification of poly(4,4′-cyclohexylidene bisphenol oxalate) polymer by means of MCC and magnetite particles. The physicochemical properties of the formed composite were thoroughly studied by means of SEM, thermal analysis, FTIR, EDAX, and magnetic hysteresis. The DNA extraction for the polymer poly(4,4′-cyclohexylidene bisphenol oxalate)-MCC-magnetite nanocomposite indicated high efficiency, i.e., 10,000 ng/μL for 2 M GuHCl/ethanol of binding buffer. The absorbance ratios of A260/A280 at 0.4 g of polymer composite weight were 1.980, 1.818, and 2.100 for 2 M GuHCl/EtOH, PBS, NaCl, respectively, indicating a highly purified DNA following the extraction process. The study results have confirmed that the composite prepared in the present study is superior to many different materials applied for the DNA extraction study. The composite exhibits superparamagnetic property due to its magnetic content, which can be explored in the future studies for other applications like
Table 5: Extraction efficiencies and total yields of DNA for the polymer composite under different weights and binding buffers.

<table>
<thead>
<tr>
<th>Binding buffer</th>
<th>Polymer weight</th>
<th>Concentration</th>
<th>260/280</th>
<th>Total yield (ng)</th>
<th>Extraction efficiency (ng/μL)</th>
<th>Extraction efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 M GuHCl/EtOH</td>
<td>0.2 g</td>
<td>2895</td>
<td>1.810</td>
<td>1,447,500</td>
<td>7237.5</td>
<td>52.417%</td>
</tr>
<tr>
<td>PBS</td>
<td>0.2 g</td>
<td>2871</td>
<td>1.763</td>
<td>1,435,500</td>
<td>7177.5</td>
<td>51.983%</td>
</tr>
<tr>
<td>2871</td>
<td>0.2 g</td>
<td>2889</td>
<td>2.254</td>
<td>1,444,500</td>
<td>7222.5</td>
<td>52.309%</td>
</tr>
<tr>
<td>2 M GuHCl/EtOH</td>
<td>0.4 g</td>
<td>4000</td>
<td>1.980</td>
<td>2,000,000</td>
<td>10,000</td>
<td>72.424%</td>
</tr>
<tr>
<td>PBS</td>
<td>0.4 g</td>
<td>3250</td>
<td>1.818</td>
<td>1,625,000</td>
<td>8125</td>
<td>58.845%</td>
</tr>
<tr>
<td>2 M NaCl</td>
<td>0.4 g</td>
<td>3592</td>
<td>2.100</td>
<td>1,796,000</td>
<td>8980</td>
<td>65.037%</td>
</tr>
</tbody>
</table>

Table 6: Comparison of the adsorption capacities for ssDNA from literature.

<table>
<thead>
<tr>
<th>Type of material</th>
<th>Adsorption capacity for ssDNA</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polystyrene beads</td>
<td>67 mg/g</td>
<td>[26]</td>
</tr>
<tr>
<td>Poly(glycidyl methacrylate) beads</td>
<td>90 μg/g</td>
<td>[27]</td>
</tr>
<tr>
<td>Fe₃O₄ NPs with polyethylene imine</td>
<td>69.2 mg/g</td>
<td>[28]</td>
</tr>
<tr>
<td>Polyethylene glycol dimethacrylate-glycidyl methacrylate</td>
<td>65.1 μg/g</td>
<td>[29]</td>
</tr>
<tr>
<td>Polyethersulfone membranes</td>
<td>10 mg/mL</td>
<td>[30]</td>
</tr>
<tr>
<td>Poly(hydroxyethyl methacrylate)-Fe₃O₄ MNPs</td>
<td>154 mg/g</td>
<td>[12]</td>
</tr>
<tr>
<td>Immobilized on MNPs as a SPE adsorbent</td>
<td>27.86 mg/g</td>
<td>[18]</td>
</tr>
<tr>
<td>N-Isopropylacrylamide-MNPs</td>
<td>83.40 mg/g</td>
<td>[13]</td>
</tr>
<tr>
<td>Diethylaminoethyl-Magarose as binding</td>
<td>8200 ng</td>
<td>[31]</td>
</tr>
<tr>
<td>Polyglycidyl methacrylate-ethylene glycol dimethacrylate beads</td>
<td>1.36 mg/mL</td>
<td>[32]</td>
</tr>
</tbody>
</table>

Figure 6: Schematic representation of the mechanism of adsorption and desorption of DNA onto the poly(4,4′-cyclohexyldiene bisphenol oxalate)-MCC-Fe₃O₄ composite.
gene separation, magnetic hyperthermia-based therapy, and magnetically controlled drug delivery.

**Data Availability**

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

**Conflicts of Interest**

The authors declare no conflict of interest.

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**References**


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