USE OF MAGNETIC NANOPARTICLES TO SEPARATE SINGLE AND DOUBLE STRANDED DNA

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Overview

Synthesis of magnetically active iron oxide nanoparticles.

Modification of the surface of these particles using hydroxyapatite.

Separation of the single and double stranded DNA using modified nanoparticles.
Available techniques for double and single stranded DNA separation

- Hydroxyapatite chromatography

- Batch method

- Thermal elution in hydroxyapatite microcolumns
Why so many effort towards a new technique?

To produce
- Miniaturized
- Rapid
- High quality
- Cost effective DNA extraction method.

Oh ..how can I do this?

Just use your...

Yes ! I can coat hydroxyapatite on magnetic iron oxide nanoparticle .
DNA separation using hydroxyapatite (HAp)

- Shows electrostatic interaction between positively charged Ca$^{2+}$ ions on the surface of the HAp and negatively charged phosphate moieties in nucleic acid backbone.

- Shows electrostatic repulsion between negatively charged phosphate groups of HAp and DNA.
Synthesis of magnetically active oleic acid coated iron oxide (Fe$_3$O$_4$) nanoparticles

- Oleic acid coated IONPs were prepared by mixing 0.1 M Fe$^{2+}$ and Fe$^{3+}$ (1:2) solution with 20% oleic acid under basic conditions.

- Particles were characterized by using FT-IR.
Characterization of oleic acid coated IONPs

Figure 1: FT-IR spectrums of oleic acid coated iron oxide nanoparticles (blue) and neat oleic acid (green).

Figure 2: Iron carboxylate coordination.
Synthesis of hydroxyapatite functionalized IONPs

- In our work, mainly two synthetic routes were used for this purpose.

  I. Homogeneous precipitation method under a hydrothermal reaction.

  II. In-situ synthesis method of hydroxyapatite functionalized Iron oxide nanoparticles.
Homogeneous precipitation technique using a hydrothermal reaction

\[
\text{Ca(EDTA)}^{2-}_{(aq)} \rightarrow \text{Ca}^{2+}_{(aq)} + \text{EDTA}^{4-}_{(aq)}
\]

\[
10\text{Ca}^{2+}_{(aq)} + 6\text{PO}_4^{3-}_{(aq)} + 2\text{OH}^-_{(aq)} \rightarrow \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2_{(s)}
\]

- HAp coating on IONPs were carried out using thermal dissociation of Ca(EDTA)$^{2-}$ chelate in NaH$_2$PO$_4$ solution at 180 °C in a sealed pyrex glass tube.

- Particles were characterized by using FT-IR and particle analyzer.
Characterization of HAp coated IONPs

- Size distribution is 78.82 - 164.2 nm.

Figure 3: FT-IR spectrums of HAp coated IONPs at 180 °C (blue) and neat hydroxyapatite (red).
In-situ synthesis of hydroxyapatite coated IONPs

- HAp coating on IONPs were carried out by mixing the 0.15 M (NH₄)₂HPO₄ and 0.4 M Ca(NO₃)₂ solution with IONPs at 60 °C.

- Particles were characterized by using FT-IR and particle analyzer.
Characterization of HAp coated IONPs

- Size distribution is 18.17 - 28.21 nm.

Figure 4: FT-IR spectrums of in situ synthesized HAp coated IONPs (blue) and neat hydroxyapatite (red).
Determination of amount of hydroxyapatite coated on IONPs

- It was determined by measuring atomic absorbance of Ca at 422.7 nm after dissolving 40 mg of HAp coated IONPs from each method in a HCl solution.
Results

- Atomic absorbance measurement for HAp coated IONPs prepared by homogeneous precipitation method is 0.0919.

- According to calibration plot and stoichiometry ratio, amount of HAp in 40 mg of HAp coated IONPs is 6.3155 mg (~16% (w/w)).

- Atomic absorbance measurement for in situ coated IONPs is 0.0845.

- According to calibration plot and stoichiometry ratio, amount of HAp in 40 mg of HAp coated IONPs is 5.8310 mg (~14% (w/w)).
Application of HAp coated IONPs in DNA separation

- Separation mainly based on phosphate ion concentration of the buffer.

<table>
<thead>
<tr>
<th>Binding/washing buffer of DNA</th>
<th>Double stranded DNA elution buffer</th>
<th>Single stranded DNA elution buffer</th>
</tr>
</thead>
<tbody>
<tr>
<td>• 0.10 M sodium phosphate buffer</td>
<td>• 0.50 M sodium phosphate buffer</td>
<td>• 0.20 M sodium phosphate buffer</td>
</tr>
<tr>
<td>• pH 6.8</td>
<td>• pH 6.8</td>
<td>• pH 6.8</td>
</tr>
</tbody>
</table>
Extraction of double stranded DNA by using HAp coated IONPs

- A mixture of dsDNA in binding buffer was incubated with HAp coated IONPs at 60 °C for 10 minutes for DNA binding.
- Absorbed DNA was eluted by using 0.50 M phosphate buffer.
- Finally, unbound DNA in supernatant and eluted DNA were loaded onto a 0.8% agarose gel.
Results

- HAp coated IONPs prepared by homogeneous precipitation method.

$S_1$: Unbound DNA remain in supernatant, $E_1$: 1st eluted DNA, $E_2$: 2nd eluted DNA
HAp coated IONPs prepared by In-situ synthesis method

Figure 6: Gel image for double stranded DNA separation

$S_1$: Unbound DNA remain in supernatant, $E_1$: 1st Eluted DNA, $E_2$: 2nd eluted DNA
Preparation of single stranded DNA (ssDNA)

- Asymmetric polymerase chain reaction was used for synthesis of single stranded DNA.
- Amplification of the ssDNA was carried out by using unequal primer ratios.
Extraction of single stranded DNA by using HAp coated IONPs

- A mixture of ssDNA in binding buffer was incubated with HAp coated IONPs at 60 °C for 10 minutes for DNA binding.

- Absorbed DNA was eluted by using 0.20 M phosphate buffer.

- Finally, unbound DNA in supernatant and eluted DNA were loaded onto a 0.8% agarose gel.
Results

- HAp coated IONPs prepared by homogeneous precipitation method.

Figure 7: Gel image for single stranded DNA separation.

$S_1$: Unbound ssDNA remain in supernatant, $E_1$: Eluted DNA.
HAp coated IONPs prepared by In-situ synthesis method

Figure 8: Gel image for single stranded DNA separation

$S_1$: Unbound ssDNA remain in supernatant, $E_1$ & $E_2$: Eluted DNA
Separation of Single stranded and double stranded DNA from a mixture of ssDNA and ds DNA HAp coated IONPs.

- Separation was carried out by combining the ssDNA and dsDNA procedures.
  - Firstly, ssDNA was separated.
  - Secondly, dsDNA was separated.
Results

- HAp coated IONPs prepared by homogeneous precipitation method

<table>
<thead>
<tr>
<th>S₁</th>
<th>E₁</th>
<th>E₄</th>
<th>E₅</th>
</tr>
</thead>
</table>

S₁: Unbound DNA remain in supernatant
E₁: Eluted DNA after adding 0.2 M phosphate buffer
E₄ and E₅: Eluted DNA after adding 0.5 M phosphate buffer

Figure 9: Separation of ssDNA using 0.20 M phosphate buffer.

Figure 10: Separation of dsDNA using 0.50 M phosphate buffer.
HAp coated IONPs prepared by In situ synthesis.

Figure 11: Separation of ssDNA using 0.20 M phosphate buffer.

Figure 12: Separation of dsDNA using 0.50 M phosphate buffer.

$S_1$: Unbound DNA remain in supernatant

$E_1$: Eluted DNA after adding 0.2 M phosphate buffer

$E_4$ and $E_5$: Eluted DNA after adding 0.5 M phosphate buffer
Which method is the best for coating the IONPs?

<table>
<thead>
<tr>
<th></th>
<th>HAp coated IONPs using Homogeneous precipitation method</th>
<th>HAp coated IONPs using In situ synthesis method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour</td>
<td>Yellow-brown</td>
<td>brown</td>
</tr>
<tr>
<td>FT-IR spectrum</td>
<td>✔ ✔ ✔ ✔</td>
<td>✔ ✔</td>
</tr>
<tr>
<td>Ca level measured by AAS</td>
<td>✔ ✔ ✔ ✔</td>
<td>✔ ✔</td>
</tr>
<tr>
<td>Seperation of DNA</td>
<td>✔ ✔ ✔ ✔</td>
<td>✔ ✔</td>
</tr>
<tr>
<td>Tightness of the HAp binding to the surface of IONPs</td>
<td>weak</td>
<td>strong</td>
</tr>
</tbody>
</table>
Experimental evidences for loose-coupling of HAp on IONPs when it is prepared using homogeneous precipitation method

1. Homogeneous precipitation method
   - Yellow brown deposit in the wells

2. In-situ synthesis method
   - No such deposit

Yellow brown deposit appeared in the supernatant after the magnetic separation.
A 2 ml aliquot of 2 M HCl was added.

Yellow brown solid dissolved

Ca\(^{2+}\) level was measured using AAS and it was 5.929 ppm.

No such deposit

A 2 ml aliquot of 2 M HCl was added.

Yellow brown deposit appeared in supernatant after the magnetic separation.

No such change

Ca\(^{2+}\) level was measured using AAS and it was 0.567 ppm.
Conclusion

- Hydroxyapatite coated IONPs prepared by the homogeneous precipitation method is suitable for separation/isolation of either single stranded or double stranded DNA than HAp coated IONPs prepared by the in situ synthesis method. However, it is not suitable for separating a single or double stranded DNA from a mixture of ssDNA and dsDNA unless the binding of HAp to IONPs are enhanced. In this respect, in-situ synthesized hydroxyapatite coated IONPs appear to be more promising.
Thank You!