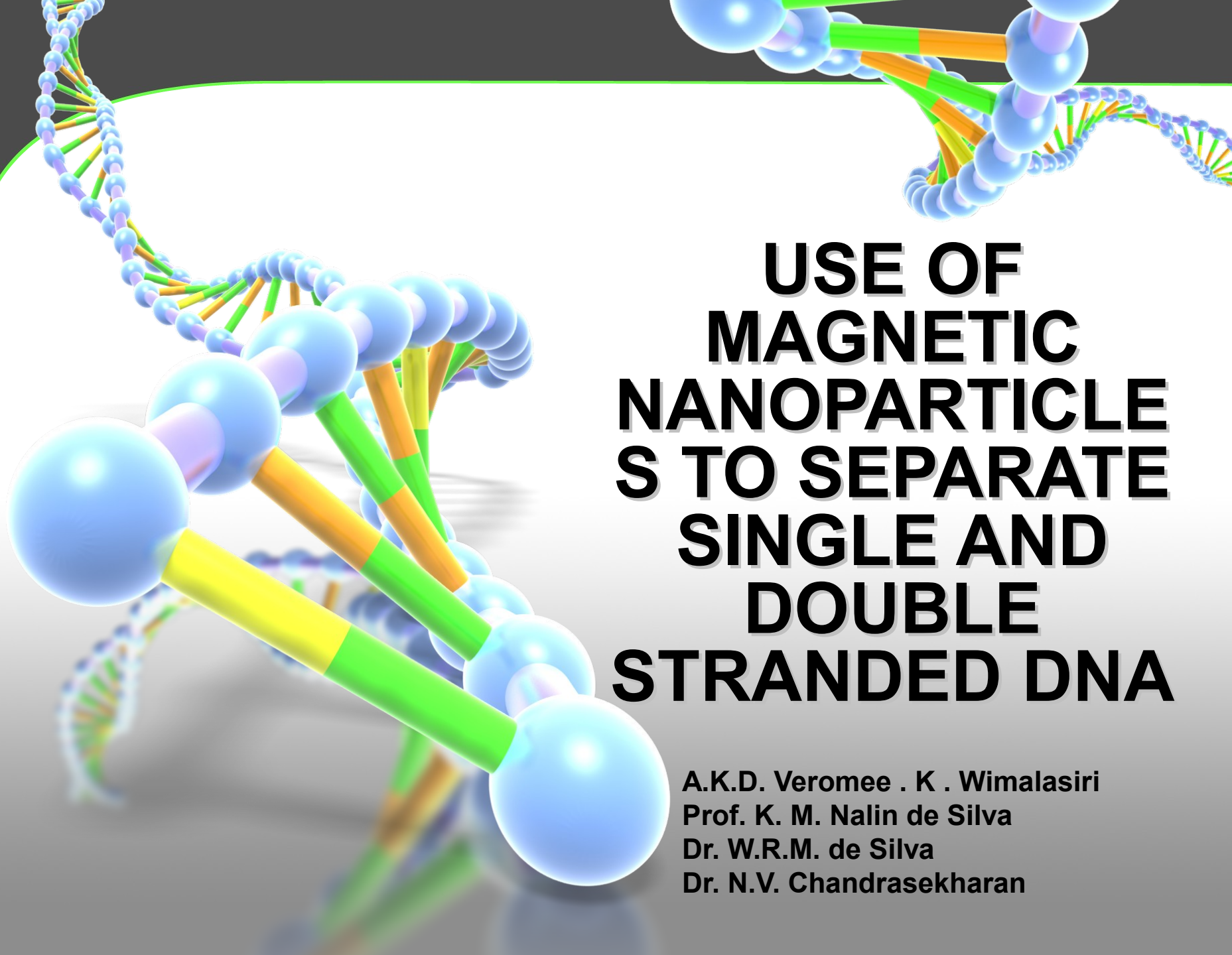

USE OF MAGNETIC NANOPARTICLES TO SEPARATE SINGLE AND DOUBLE STRANDED DNA

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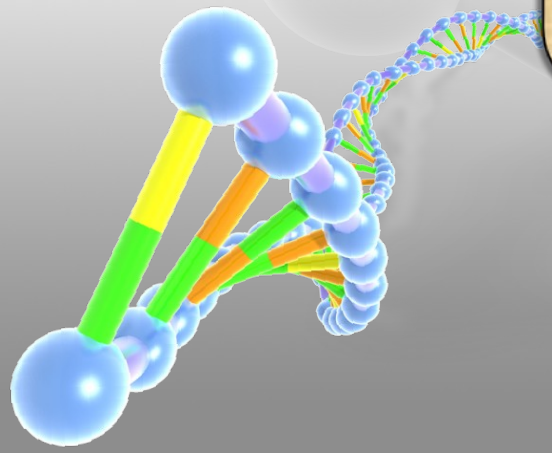
Overview

Synthesis of magnetically active iron oxide nanoparticles.

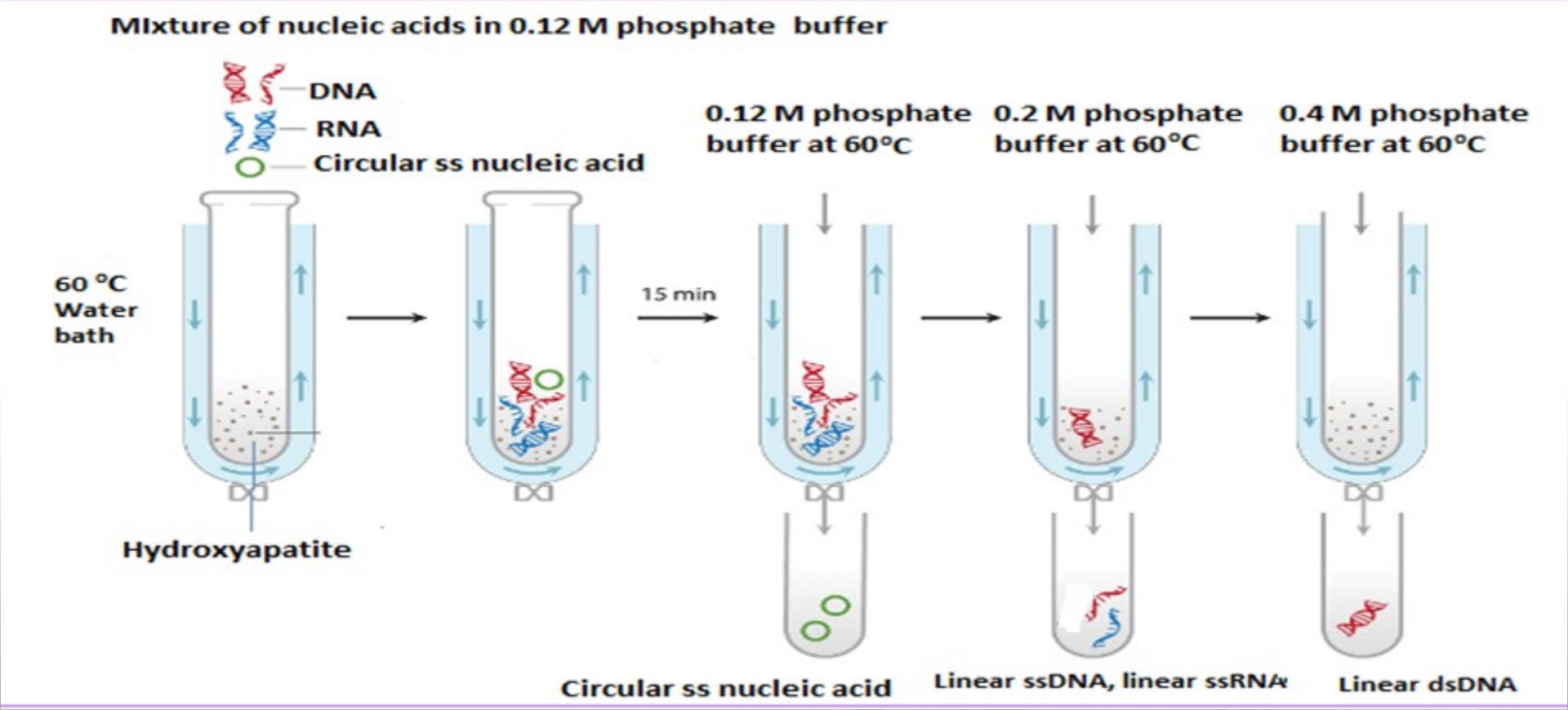
Modification of the surface of these particles using hydroxyapatite.

Separation of the single and double stranded DNA using modified nanoparticles.



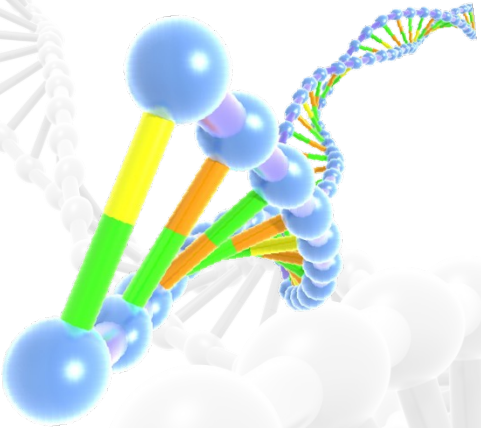
Available techniques for double and single stranded DNA separation

- Hydroxyapatite chromatography



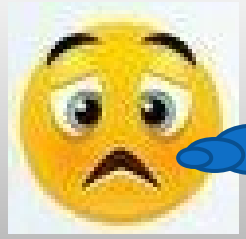
- Batch method
- Thermal elution in hydroxyapatite microcolumns

Why so many effort towards a new technique?



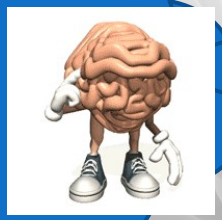
To produce

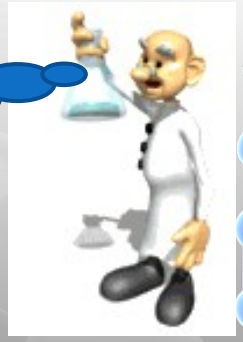
- Miniaturized
- Rapid
- High quality
- Cost effective DNA extraction method.

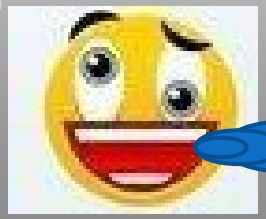


Oh ..how can I do this?

Just use your

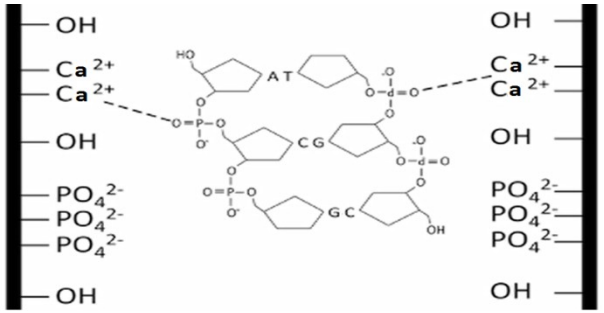






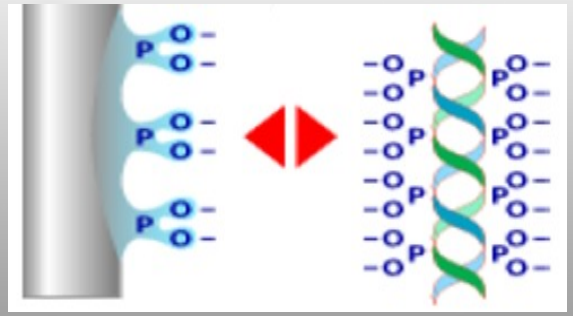
Yes ! I can coat hydroxyapatite on magnetic iron oxide nanoparticle .

DNA separation using hydroxyapatite (HAp)



Affinity interactions between DNA and 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.



Repulsion between DNA and HAp

- Shows electrostatic repulsion between negatively charged phosphate groups of HAp and DNA.

Synthesis of magnetically active oleic acid coated iron oxide (Fe_3O_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

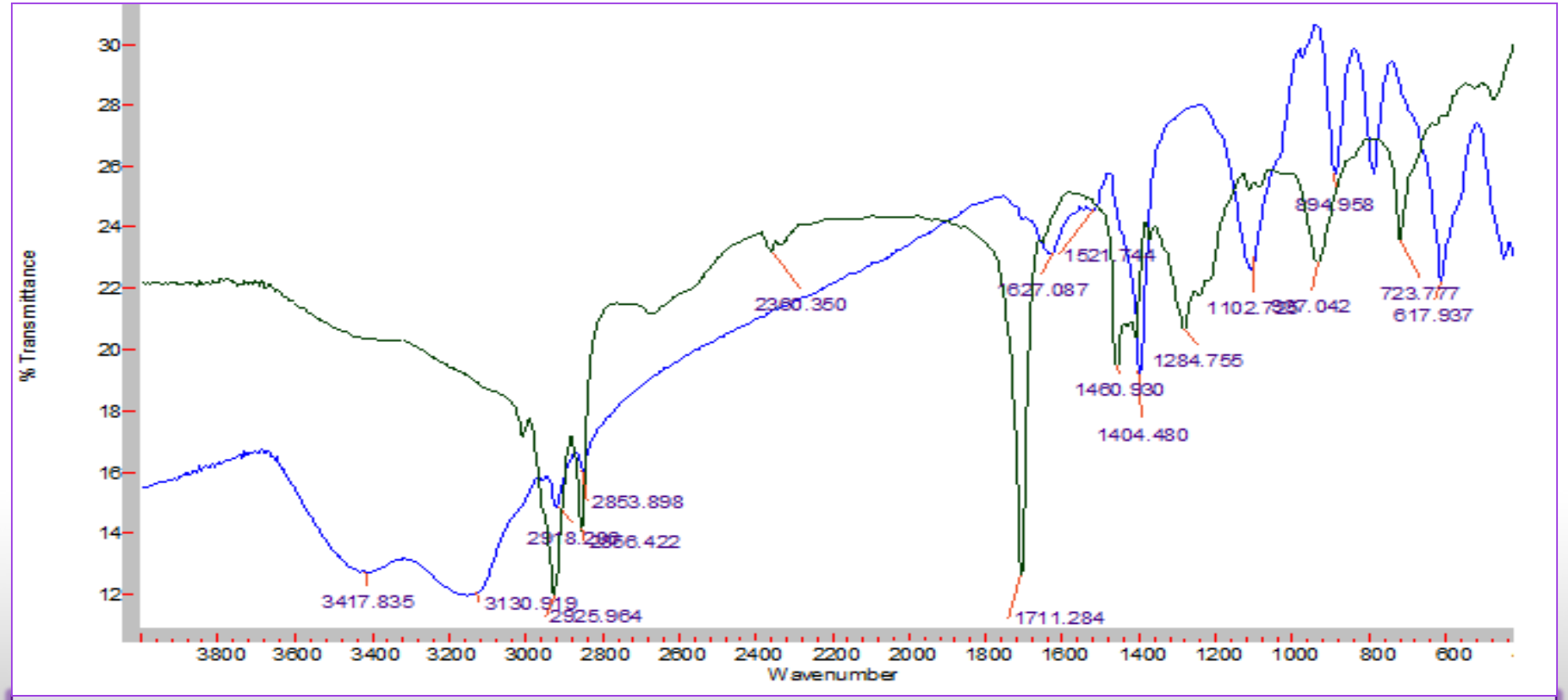


Figure 1: FT-IR spectrums of oleic acid coated iron oxide nanoparticles (blue) and neat oleic acid (green).

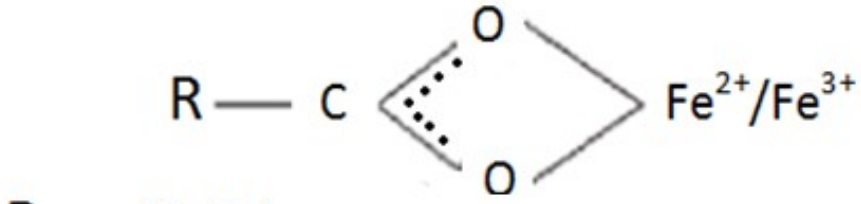


Figure 2: Iron carboxylate coordination.

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.

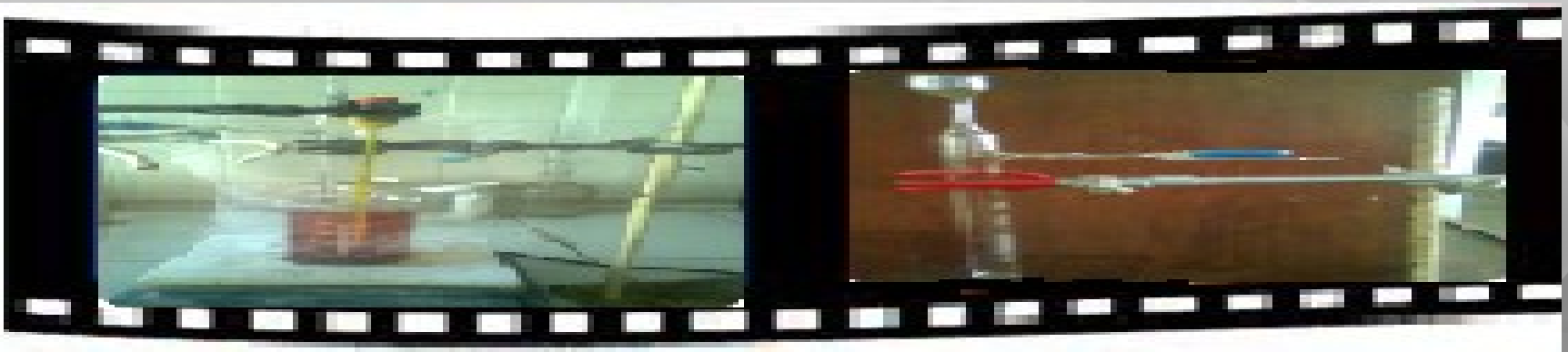
Homogeneous precipitation technique using a hydrothermal reaction

$$Ca(EDTA)^{2-}_{(aq)} \longrightarrow Ca^{2+}_{(aq)} + EDTA^{4-}_{(aq)}$$

$$10Ca^{2+}_{(aq)} + 6PO_4^{3-}_{(aq)} + 2OH^-_{(aq)} \xrightarrow{\Delta} Ca_{10}(PO_4)_6(OH)_{2\,(s)}$$

- HAp coating on IONPs were carried out using thermal dissociation of $Ca(EDTA)^{2-}$ chelate in NaH_2PO_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.

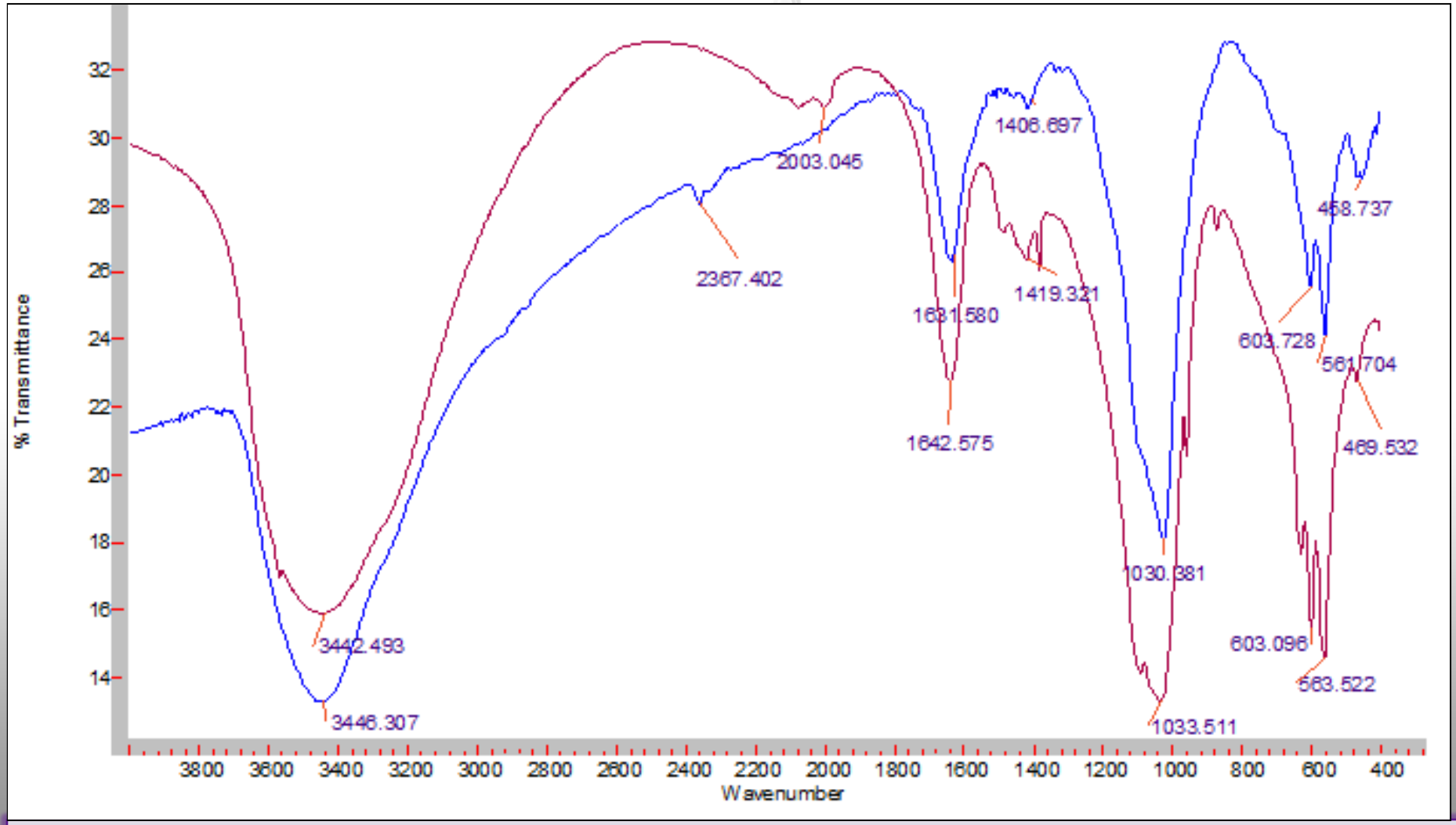
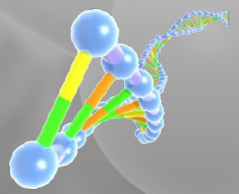


Figure 3: FT-IR spectrums of HAp coated IONPs at 180 °C (blue) and neat hydroxyapapite (red).

In-situ synthesis of hydroxyapatite coated IONPs

- HAp coating on IONPs were carried out by mixing the 0.15 M $(NH_4)_2HPO_4$ and 0.4 M $Ca(NO_3)_2$ solution with IONPs at 60 ^oC.

- Particles were characterized by using FT-IR and particle analyzer.



Characterization of HAp coated IONPs

- Size distribution is 18.17 - 28.21 nm.

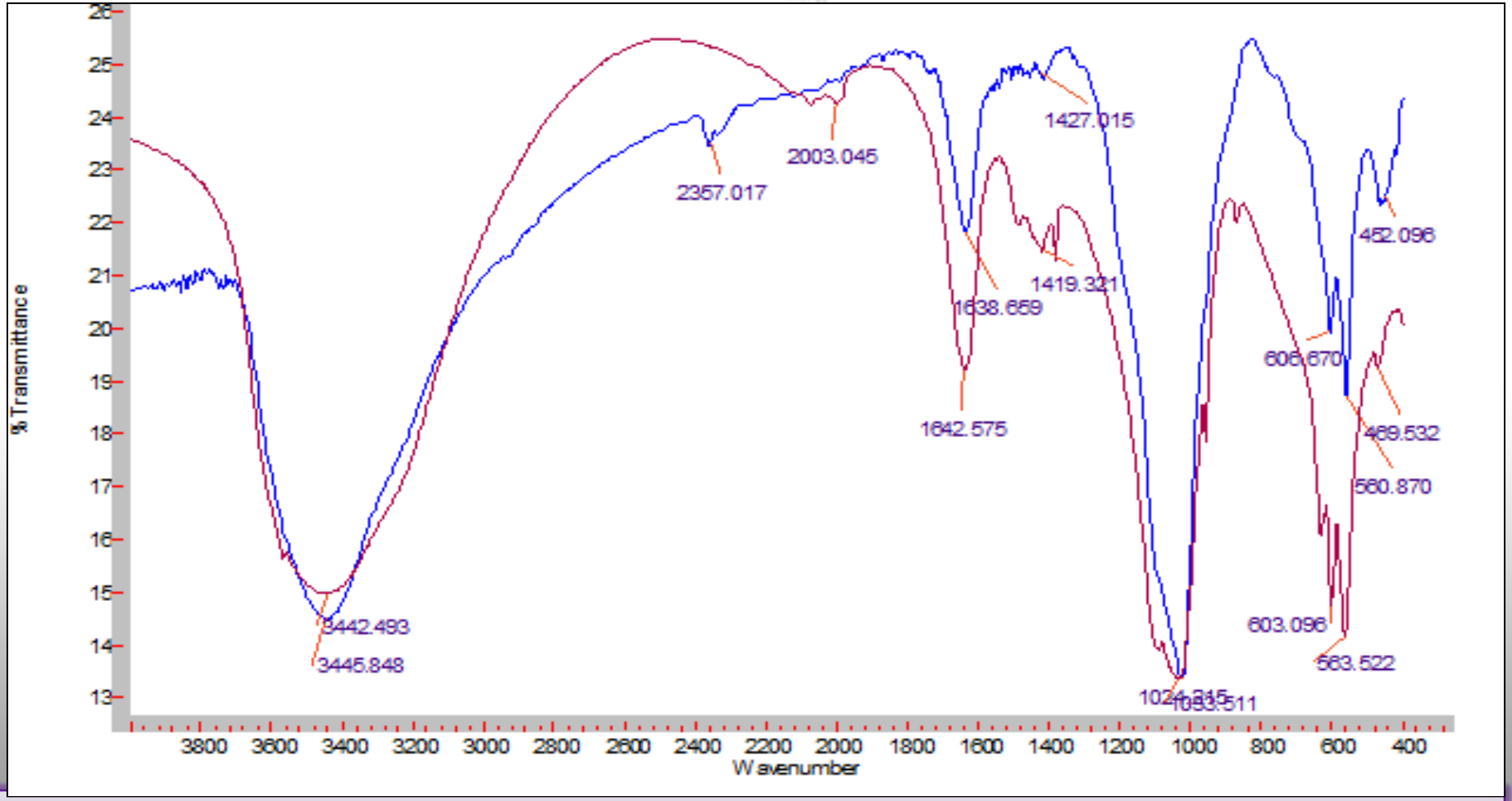


Figure 4: FT-IR spectrums of in situ synthesized HAp coated IONPs (blue) and neat hydroxyapatite (red) .

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.

Binding/washing buffer of DNA	Double stranded DNA elution buffer	Single stranded DNA elution buffer
• 0.10 M sodium phosphate buffer • pH 6.8	• 0.50 M sodium phosphate buffer • pH 6.8	• 0.20 M sodium phosphate buffer • pH 6.8

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 ^{o}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.

Figure 5: Gel image for double stranded DNA separation

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

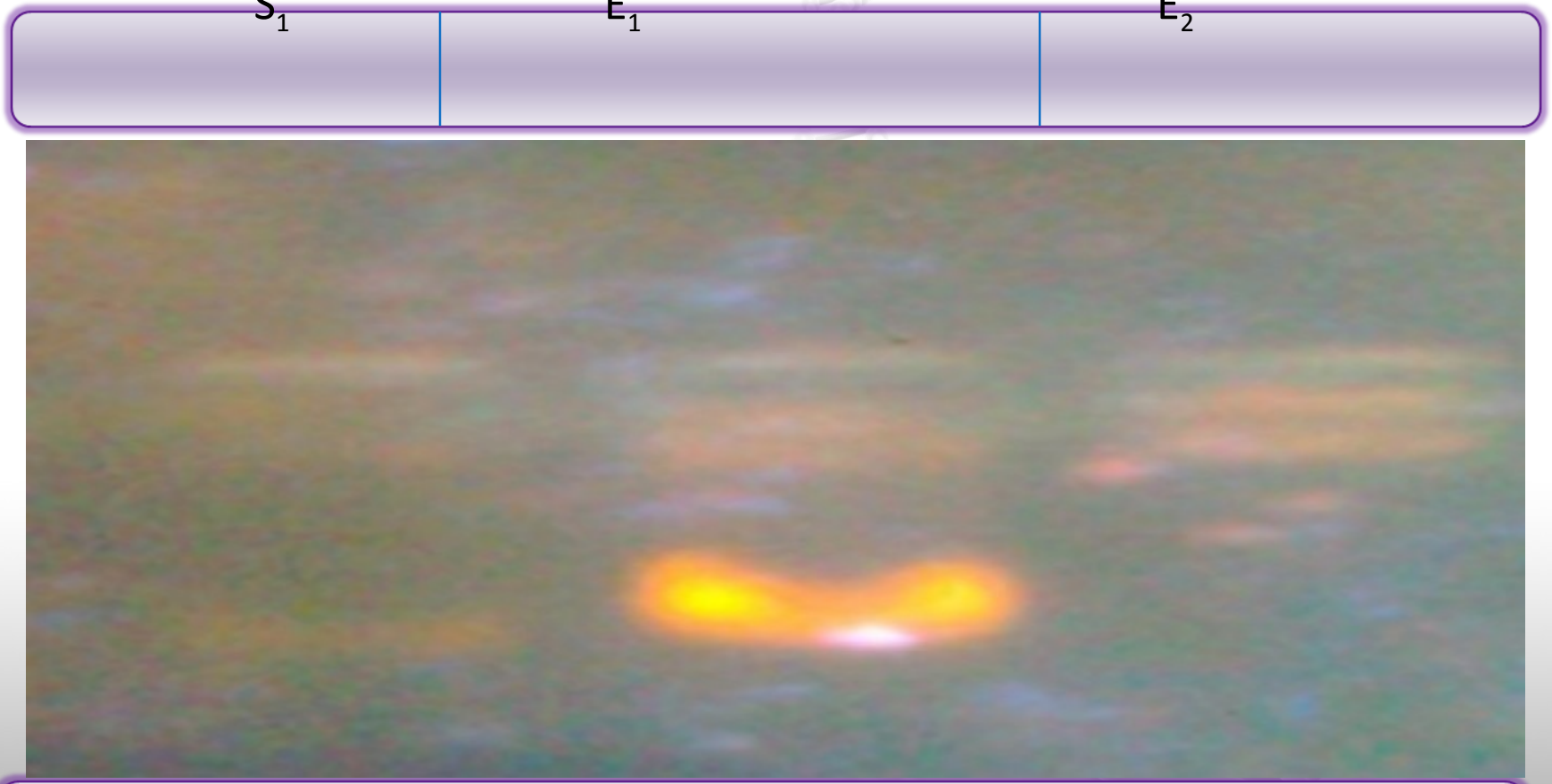


Figure 6: Gel image for double stranded DNA separation

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 ^{o}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.

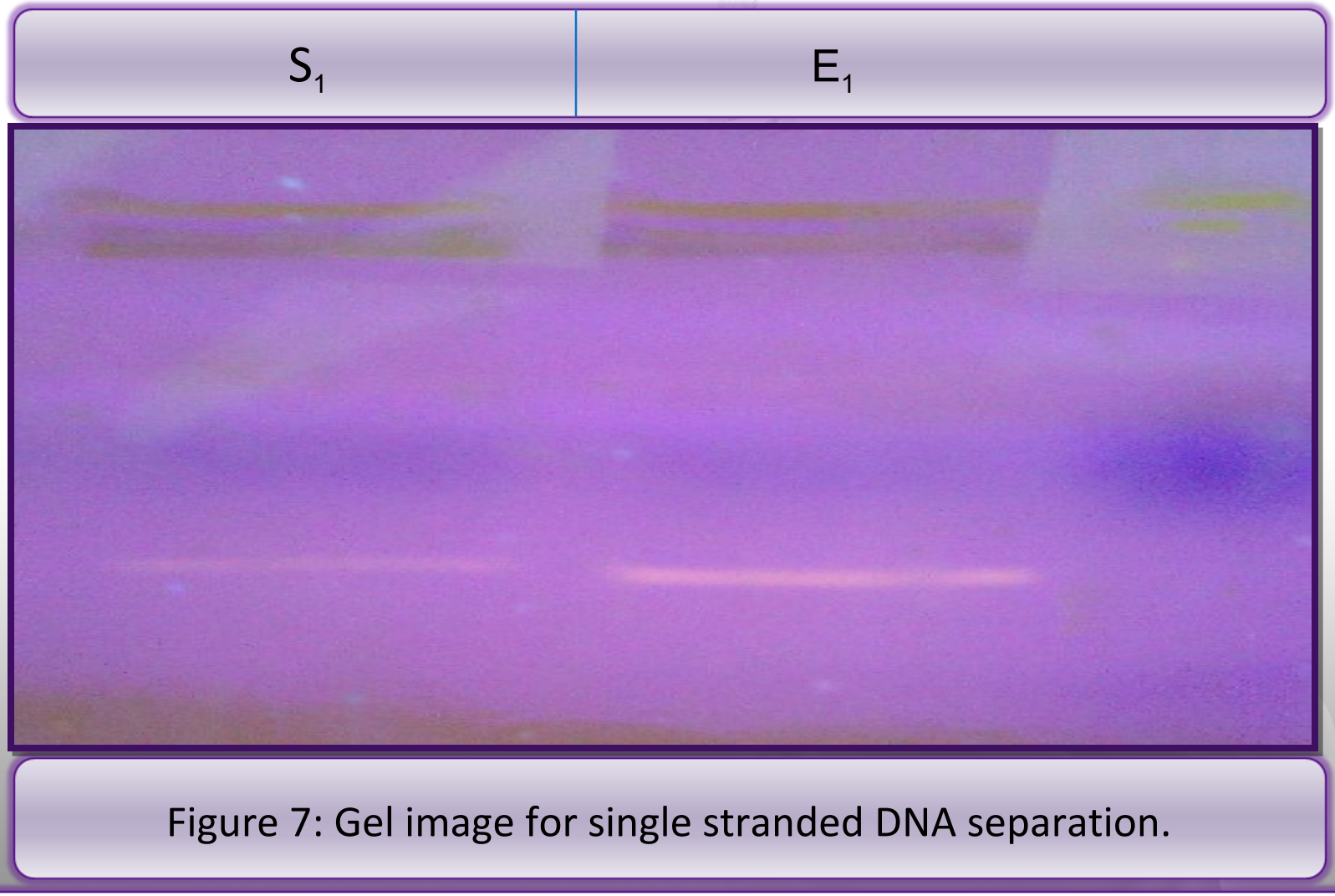


Figure 7: Gel image for single stranded DNA separation.

S_1: Unbound ssDNA remain in supernatant, E_1: Eluted DNA.

- HAp coated IONPs prepared by In-situ synthesis method

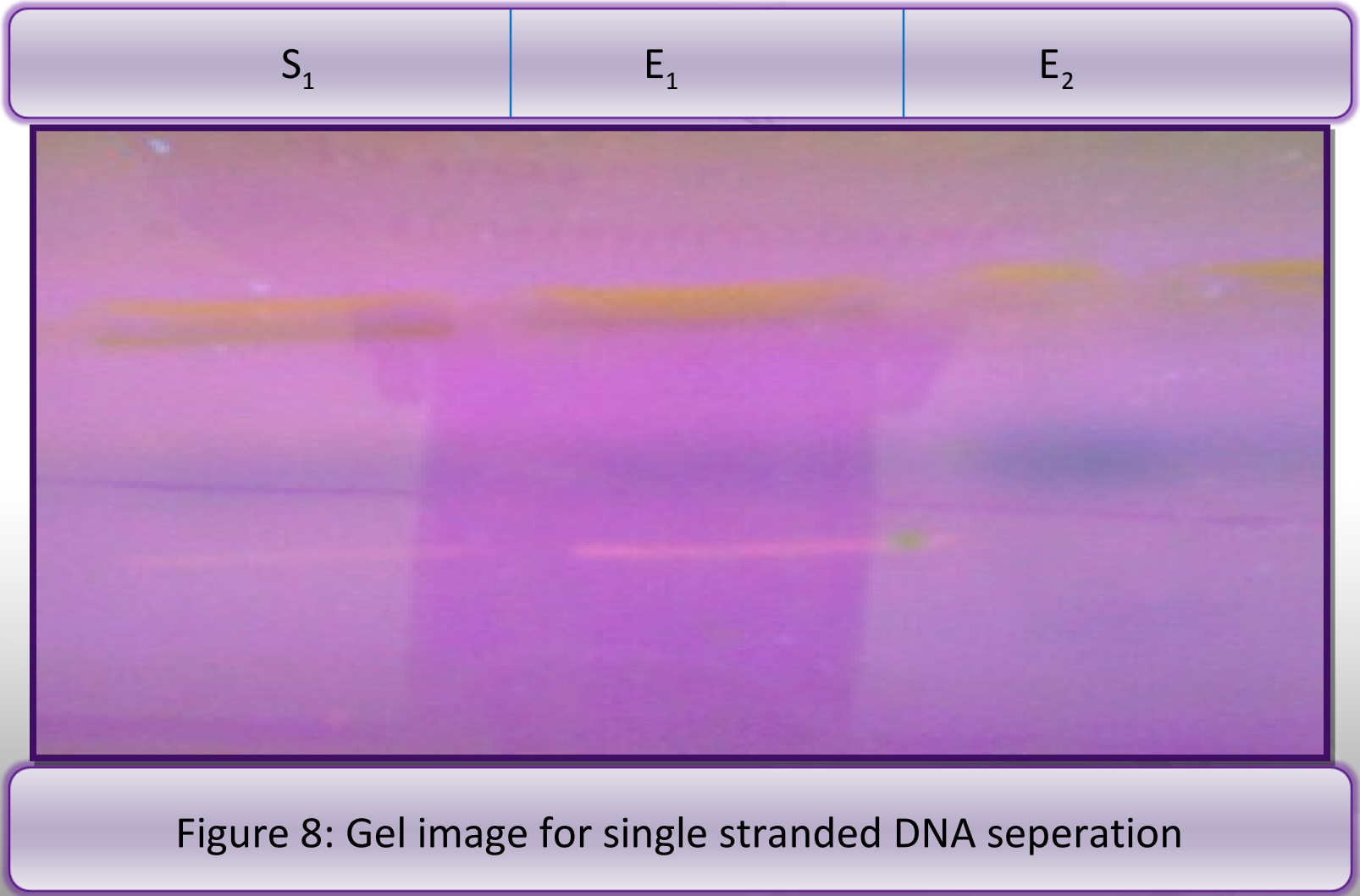


Figure 8: Gel image for single stranded DNA seperation

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

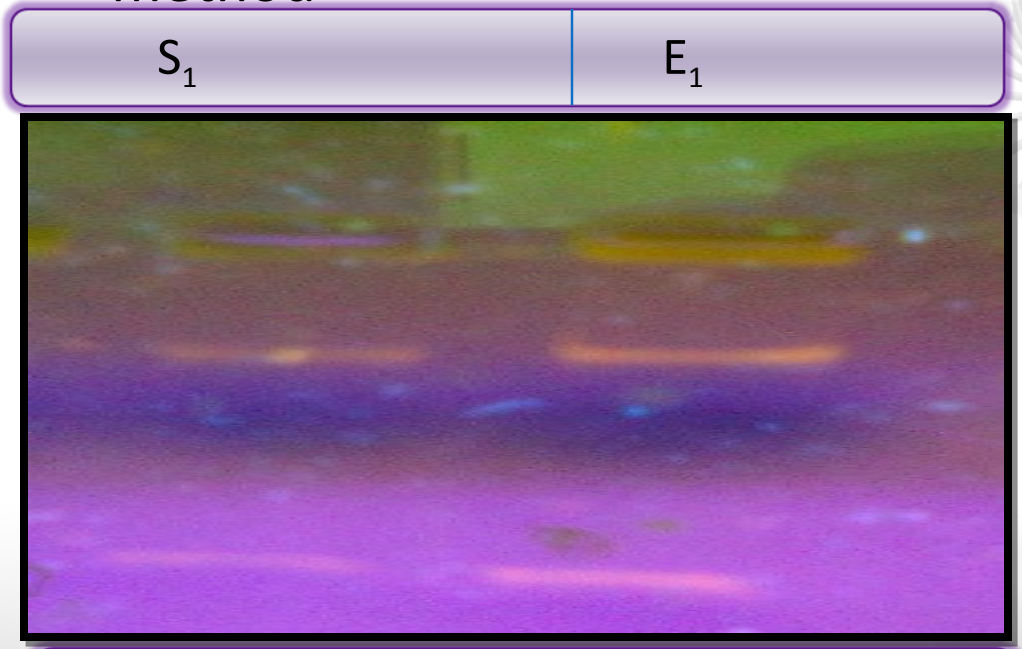


Figure 9: Separation of ssDNA using 0.20 M phosphate buffer.

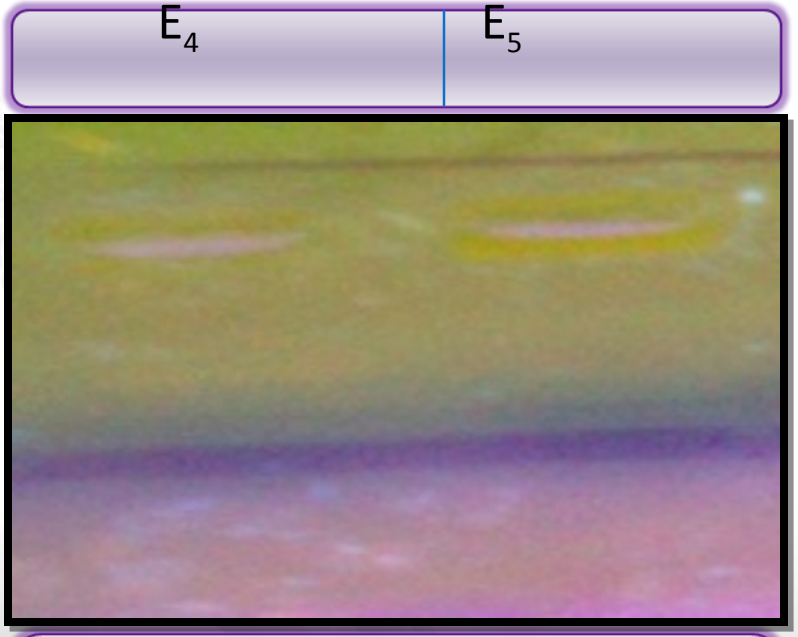


Figure 10: Separation of dsDNA using 0.50 M phosphate buffer.

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

- HAp coated IONPs prepared by In situ synthesis.

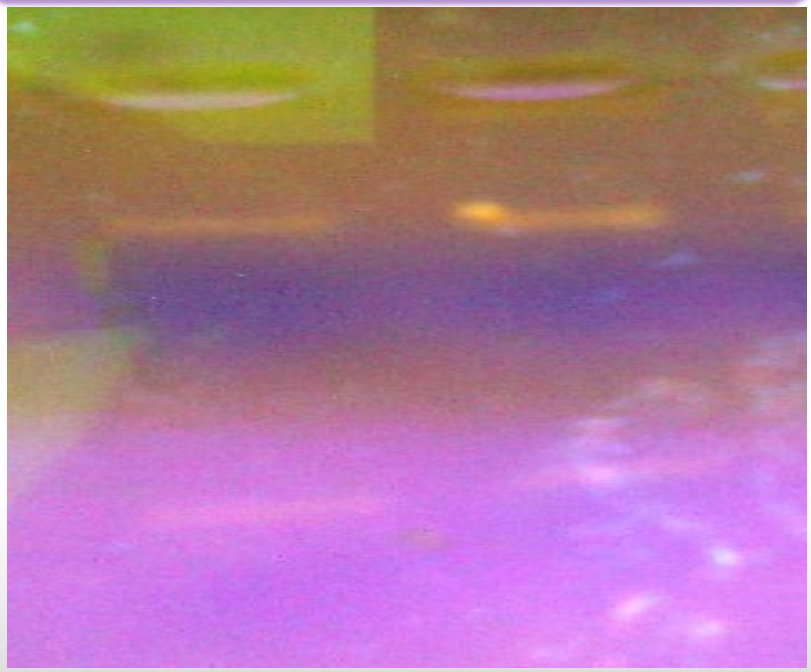


Figure 11: Separation of ssDNA using 0.20 M phosphate buffer.

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

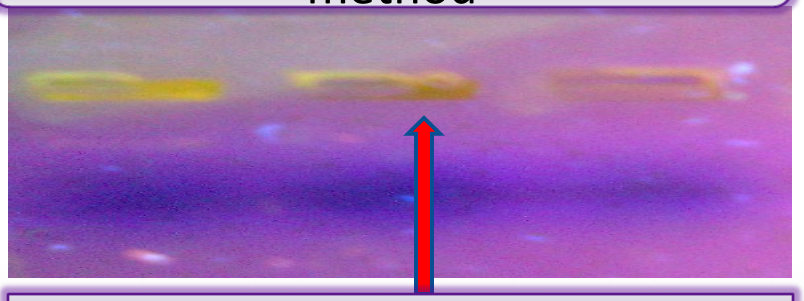
Which method is the best for coating the IONPs?

	HAp coated IONPs using Homogeneous precipitation method	HAp coated IONPs using In situ synthesis method
Colour	Yellow-brown	brown
FT-IR spectrum	√√√	√√
Ca level measured by AAS	√√√	√√
Seperation of DNA	√√√	√√
Tightness of the HAp binding to the surface of IONPs	weak	strong

Experimental evidences for loose-coupling of HAp on IONPs when it is prepared using homogeneous precipitation method

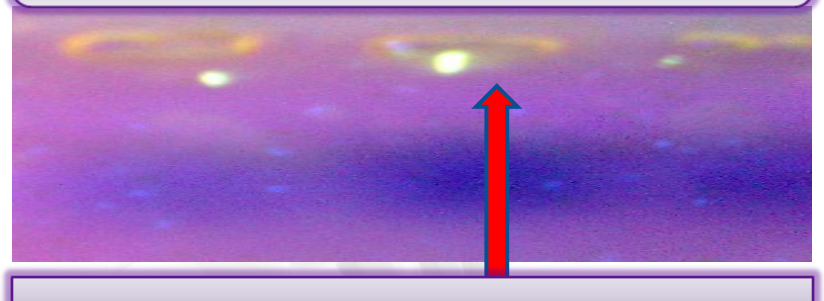
1

Homogeneous precipitation method



Yellow brown deposit in the wells

In-situ synthesis method



No such deposit

2

Yellow brown deposit appeared in the supernatant after the magnetic separation.

No such deposit

3

Yellow brown deposit appeared in 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.

↓

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 hydroxyapatte coated IONPs appear to be more promising.

Thank You !