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Magneto-separation of Genomic Deoxyribose Nucleic Acid using pH Responsive Fe_3O_4 @silica@chitosan Nanoparticles in Biological Samples

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Abstract

Magnetic adsorption technology of polymer functionalized superparamagnetic nanoparticles is showing great potential in biomedical applications because of their facile "on and off" state of magnetization.Highly magnetized superparamagnetic nanoparticles based magnetic adsorption technology was employed for the purification of DNA. The core-shell Fe_3O_4 nanoparticles were synthesized by alkaline precipitation and subsequently coated with silica by sol gel method. Further, silica coated Fe_3O_4 nanoparticles functionalized with chitosan was used as an adsorbent for DNA. The surface adsorption of DNA onto the modified nanoparticles was optimized as a function of pH, time and temperature. The adsorption efficiency of Fe_3O_4 @silica@chitosan nanoparticles was examined by U.V visible spectroscopy**.** Fe_3O_4 @silica@chitosan nanoparticles with the high adsorption efficiency of~ 88% and a high elution efficiency of ~ 98% were employed for DNA isolation from human saliva. Agarose gel electrophoresis was used to validate the quality and quantity of DNA isolated from human saliva. Square wave voltammetry was carried out for monitoring the quantitative information about DNA fragments concentration. This method has potential application in the field of bioelectrochemical analysis. The isolation results of genomic DNA from saliva indicated that the Fe_3O_4@silica@chitosan nanoparticles have outstanding advantages in operation, selectivity, and capacity over the present existing isolation protocols (Phenol–chloroform extraction). Furthermore, it opens up new opportunities in detection and isolation of nucleic acid.

Keywords: Fe_3O_4 nanoparticles, Chitosan, DNA adsorption, Genomic DNA isolation, Square Wave Voltammetry.

1. Introduction:

Magnetic adsorption technology has become a promising tool for the separation of biological molecules. Robinson.et.al. used magnetic separation for the first time in biotechnological context [1]. Materials employed for this are defined as magnetisable, which can become magnetic under the influence of an external magnetic field and can facilitate the isolation or extraction of a target molecule. The nonmagnetic target binds to the surface of magnetisable solid phase support, either through a specific affinity interaction or other mechanism,for example ion exchange or hydrophobic interaction so that it can then be isolated or extracted by application of an external magnetic field [2].Nano-size magnetic adsorbents have attracted wide attention owing to their high surface to volume ratio, which result in a higher binding rate, and magnetically controllable aggregation, making the separation easier [3].

In order to improve the colloidal stability, biocompatibility and reduce toxicity,the nanoparticles are often encapsulated with suitable biocompatible polymers [4].Coating of polymers over magnetic nanoparticles induces interparticle repulsion and thereby lowers the magnetic attraction between particles which prevents them from agglomeration in presence of an external magnetic field [5]. Surface functionalized magnetic nanomaterials have been widely used in biotechnological and clinical applications, such as bioseparation[6,7,8], drug and gene deliverysystem [9], enzyme and protein immobilization [10], and hyperthermia treatment [11-13] along with DNA separation and adsorption studies [14 ,15] and biosensing [16]. The nucleic acids can be isolated directly from crudesample materials such as blood, tissue homogenates,cultivation media and water.

The magnetic properties of the solid materials provide an additional advantage due to their possibility of adjusting magnetization and demagnetization that, they can be removed relatively easily and selectively evenfrom viscous sample suspensions. In fact, magnetic separationis the method of choice for the recovery of smallparticles in the presence of biological debris and other fouling material of similar size.Furthermore, the efficiency of magnetic separation isalso suited for large-scale purifications [17].

Generally, interactions of biological macromolecules with surfaces are of great significance in nature and biotechnology. DNA immobilization and adsorption on surfaces have direct applications in biosensing,DNA purification, and nanofabrication [18]. Many methods of DNA immobilization were developed, including cross-linking, electrostatic adsorption, self-assembled monolayer, and entrapment in porous magnetic silica microspheres surface [15]. DNA is a negatively charged biomolecule due to the deprotonation of thephosphate groups above pH 1.0 [8]. Exploiting the property of DNA, the use of electrostatic capture of DNA has been shownby decreasing the solution pH below the pKaof theimmobilized residue. Further, in order to release the DNA from these devices,it is necessary to switch the charge on support by increasing the pH above pKa of immobilized residue [19]. Wanget al. [20] have reported extraction of calf thymus DNA using ionic liquid 1-butyl-3-methylimidazolium hexafluorophosphate. A high binding efficiency (~90%) was achieved, which mainly resulted from the strong electrostatic attractionbetween the cationic groups and the phosphate groups of DNA [21]. However, low back extraction efficiency has been facedalso due to the strong electrostatic forces.

In the present work, a study on the adsorption of DNA on surface of Fe_3O_4 @silica@chitosan nanoparticles and its elution in the medium was conducted. The silica coating on iron oxide nanoparticles is employed in order to screen themagnetic dipolar interactions between the MNPs. Moreover, silica shells present other advantages arising from their chemical stability, easy surface modification (reactive surface silanol groups) and biocompatibility, thus offering the possibilityof introducing new functionalities onto the nanoparticle surface [22].The core shell nanoparticles were further functionalized with chitosan to exploit the positive amino group of chitosan for DNA adsorption**.** The amine activated surface can be used to covalently link specific biomolecules[23]

The work reports the utility of Square-Wave Voltammetry, for measurements of nucleic acids at glassy carbon electrodes. The adsorption efficiency of ~ 87% is obtained with high elution efficiency of ~ 98% which is a desirable parameter. The high adsorption efficiency as well as high elution efficiency of the proposed nanoparticles makes it an appropriate candidate to be used in bioseparation. The high adsorption efficiency of the nanoparticles wastested for DNA isolation from human saliva. The proposed method is suitable for both miniscale as well as large scale DNA purification.

2. Materials and Methods

2.1 Chemicals

Calf thymus DNA and Low molecular weight Chitosan (>90% degree of deacetylation) were procured from Sigma Aldrich. Acetate buffer for electrochemical analysis was used(0.2 M CH_3COOH + 0.2 M CH_3COONa, pH 5.0). Ferrous chloride ($FeCl_2$), polyethylene glycol (PEG, M_W = 6000), sodium chloride (NaCl), Tris-HCl, ethylene diaminetetraacetic acid (EDTA), sodium dodecyl sulphate (SDS), sodium hydroxide (NaOH), hydrochloric acid (HCl), were purchased from Hi media, India. All solutions were prepared using double distilled water.

2.2 Fabrication of Fe_3O_4 @silica@chitosanNanoparticles.

Fe_3O_4@silica@chitosan nanoparticles were synthesized by alkaline precipitation method as per previously reported method [24]. 2 g of $FeCl_2.4H_2O$ was dissolved in 50 mL of 1 M HCl by heating up to 70 º C. 50 mL of 3 M NaOH was added to it at 60º C drop by drop with constant stirring. The appearance of black precipitate indicated the formation of iron oxide nanoparticles. The precipitate was magnetically separated and washed thoroughly with water. The synthesized Fe_3O_4 nanoparticles were silica coated in an acid catalyzed sol-gel reaction. Magnetic suspension (0.1 gm/10 mL Fe_3O_4) was ultrasonically dispersed in a round bottomed flask. Acid catalysis was done by adding 0.1 mL of HCl vigorously. After one hour 3 mL of water, 5 mL of ammonium hydroxide and 3 mL of TEOS was added to the flask in 5 mL of methanol. The hydrolysis of TEOS was carried out for 7 h at 80 º C under continuous stirring at 550 rpm. The resultant product was separated by an external magnet and washed thoroughly with double distilled water thrice. Silica coated Fe_3O_4 nanoparticles (0.2 gm) were dispersed in 7.5 mL of chitosan solution (4 mg/mL chitosan dissolved in 2 % acetic acid). Suspension was mixed by sonication for 30 min. Finally, the chitosan functionalized silica coated Fe_3O_4 nanoparticles were dried at room temperature.

2.3 Size distribution and colloidal stability

Size distribution and colloidal stability of the sample was performed on PSS/NICOMP 380 ZLS particle sizing system (Santa Barbara, CA, USA) with a red He–Ne laser diode at

632.8 Å in affixed angle 90° plastic cell was used for the measurement of surface charge and colloidal stability.

2.4Procedure for adsorption and desorption of DNA from Fe_3O_4@silica@chitosan Nanoparticles:

Study on the adsorption of DNA, from aqueous solutions, on the surfaces of nanoparticles was investigated batch-wise. The Aliquot of known volume of DNA solution, with selected initial concentration and pH, was transferred into a beaker. A known dosage of the magnetic nanoparticles was added to the beaker. After mixing time elapsed, the nanoparticles were separated magnetically.The concentration of the DNA residue in the supernatant was determined by measuring absorbance before and after adsorption of DNA by UV–vis spectrometer at 260 nm. The percent adsorption of DNA on nanoparticles was calculated using the following equation:

$$\% \text{ Adsorption} = (C_0 - C_f)/C_0 * 100\% \quad (1)$$

where C_0 and C_f, respectively, represent the initial and final(after adsorption) DNAconcentration (in mg L−1) in the aqueous phase.For potential applications, regeneration and reusability of anadsorbent are important factors to be reported. The desorptionratio was calculated using the following equation:

$$D = Ci*V/Q*100 \quad (2)$$

Where Ci is the concentration of DNA in the desorption media, V is the volume of this media, and Q is the amount of DNA in the solid phase.

2.5 Time, temperature dependent relative DNA isolation studies:

The effect of adsorption time was studied by keeping the DNA with magnetic nanoparticles at optimized pH for different time. The role of temperature on adsorption of DNA to the surface of magnetic nanoparticles was studied at selected experimental temperatures ranging from 25ºC to 90ºC.All the experiments were performed batch wise.

2.6 Reusability study

In order to test the reusability of magnetic nanoparticles, DNA adsorption and elution procedure was repeated 5 times by using same group of magnetic nanoparticles. Each experiment was repeated in triplicate to validate the reproducibility.

2.7 XRD analysis

X-ray diffraction (XRD) patterns of Fe_3O_4@silica@chitosan and DNA adsorbed Fe_3O_4@silica@chitosan nanoparticles were recorded on a Rigaku D/MAXIIA diffractometer using Cu Kα radiation (λ = 1.5406ºA), operating at 40 kV and 40 mA in the 2θ range from 20º to 80º.

2.8 DNA isolation using Fe_3O_4 @silica@chitosan magnetic nanoparticles from Human saliva.

The saliva (3 mL) was collected and mixed with 500 µL of cell lysis buffer (1 % SDS and 0.2 M NaOH solution) for 2 min at 50ºC.The tubes were vortexed and centrifuged. The saliva lysis buffer mixtures were transferred to fresh Eppendorf tubes, and 200 µL of magnetic nanoparticles were added. Magnetic solid phase adsorption of genomic DNA was done by adding nanoparticles to the lysed solution. Then the Binding buffer (25 % PEG- 6000 and 2.0 M NaCl, pH 8.5) was added. After that the mixtures were placed at room temperature for 10 minutes to ensure target DNA was completely bonded onto the surface of the magnetic nanoparticles. After placing the tube beside a magnet for 20 seconds to attract the nanoparticles, the supernatant was discarded with a pipette and DNA pellet was retained. The DNA pellet was resuspended in 100 µL of 70% ethanol to purify it, and was then centrifuged at room temperature for 20 seconds at 600r/min and then returned to the magnetic to attract the beads for 20 seconds. The supernatant was discarded again. The purification procedure was repeated once to remove any residual contaminants. The purified DNA was eluted by the addition of 50 µL of elution buffer (10 mM Tris HCl, 1.0 mM EDTA , pH 8.5) dissociate the bonded DNA from the magnetic nanoparticles, then vortexed until the pellet was (visually) completely resuspended. With this approach, the DNA could be isolated and purified from saliva samples and stored at 4ºC until use. The DNA concentration was measured by spectrophotometer (DN-1000 spectrophotometer, NanoDrop, Wilmington, DE, USA) and verified by agarose gel electrophoresis.

2.9Agarose gel Electrophoresis and Electrochemical analysis

The isolated DNA was analyzed by 1.2% agarose gel electrophoresis. The running buffer was TAE (40mM Tris, 40mM acetic acid, 1mM EDTA, pH8.0. DNA ladder (New England BioLabs) within the size range from 0.5 to 1.5 kb was used to monitor the size of analyzed fragment The electrophoresis (Bio-Rad) was running at 100 V and 6 ºC for 45 min. The bands of interest were cut out with a sterile blade and transferred to a microcentrifuge tube was divided into three aliquots and TE buffer (10 mM Tris–HCl pH 8, 1 mMEDTA, ACS purity, Sigma-Aldrich) was added (dilution gel:TE buffer 1:0.5, 1:1 and 1:2)

. Electrochemical measurements were performed using a CHI660C electrochemical workstation (CH Instruments, Shanghai, China). A three-electrode system was employed, including a working GCE electrode, an Ag/AgCl reference electrode and a platinum wire counter electrode using a standard cell with three electrodes. The adsorptive transfer technique is based on the sample accumulation (120 s) onto the working electrode surface and consequently on the electrode washing and square wave voltammetric (SWV) measurement. All experiments were carried out at room temperature (21°C). SWV measurements were carried out in the presence of acetate buffer pH 5.0. SWV parameters: start potential -0.65V, end potential 0.15, potential step 4 mV, frequency 150 Hz, and amplitude 25.05 mV. In this study, we demonstrate that the electrochemical analysis is a sensitive and powerful tool for this type of investigations.

3. Results and Discussion

3.1 Size distribution and colloidal stability

The morphological characteristics of bare Fe_3O_4 and Fe_3O_4 @silica@chitosan Nanoparticles are shown in Fig 1a and b respectively. Spherical morphology was observed in SEM images of bare Fe_3O_4 and Fe_3O_4 @silica@chitosan Nanoparticles .It is evident that the morphology appearing in SEM images is the surface morphology of the clusters of nanoparticles. Dynamic light scattering analysis of the sample revealed that the nanoparticles were monodispersed with average hydrodynamic diameter of 155.3 nm and a variance of 0.036 [22, 24]. However, the average size of nanoparticles as determined by TEM was approximately 17 nm as reported in our previous work. The reason for the difference in size in DLS and TEM is attributed to sample preparation which is different in each of them.In TEM, the chitosan-coated MNPs were spread out in a thin film and dried prior to

measurement, while DLS measured particle size in solution. Thus, TEM was capable of distinguishing individually coated MNPs, while DLS measured the aggregation of MNPs in solution.

The cationic character of the elaborated particles was pointed out via electrokinetic study by measuring the zeta potential as a function of pH.It is noted that the particles are positively charged at acidic pH with a surface potential greater than +40 mV. This confirms the presence of amino groups on the particle surface in their protonated form, which is attributed to the presence of chitosan on the particle surface.Because chitosan has multiple amino groups, it is sensitive to the pH of the solution. The pKa of chitosan is 6.2, indicating that at or near biological pH half of the amino groups are deprotonated, promoting intramolecular hydrogen bonding and leading to precipitation. Chitosan modified particles were found to produce a net positive charge at lower pH. The reason for positive charge is because of the protonation of free amino group present on surface of chitosan modified nanoparticles. At higher pH, that is towards basic the value of zeta potential changes from positive to negative. The data implied that the nanoparticles could electrostatically bind negatively charged DNA [25].

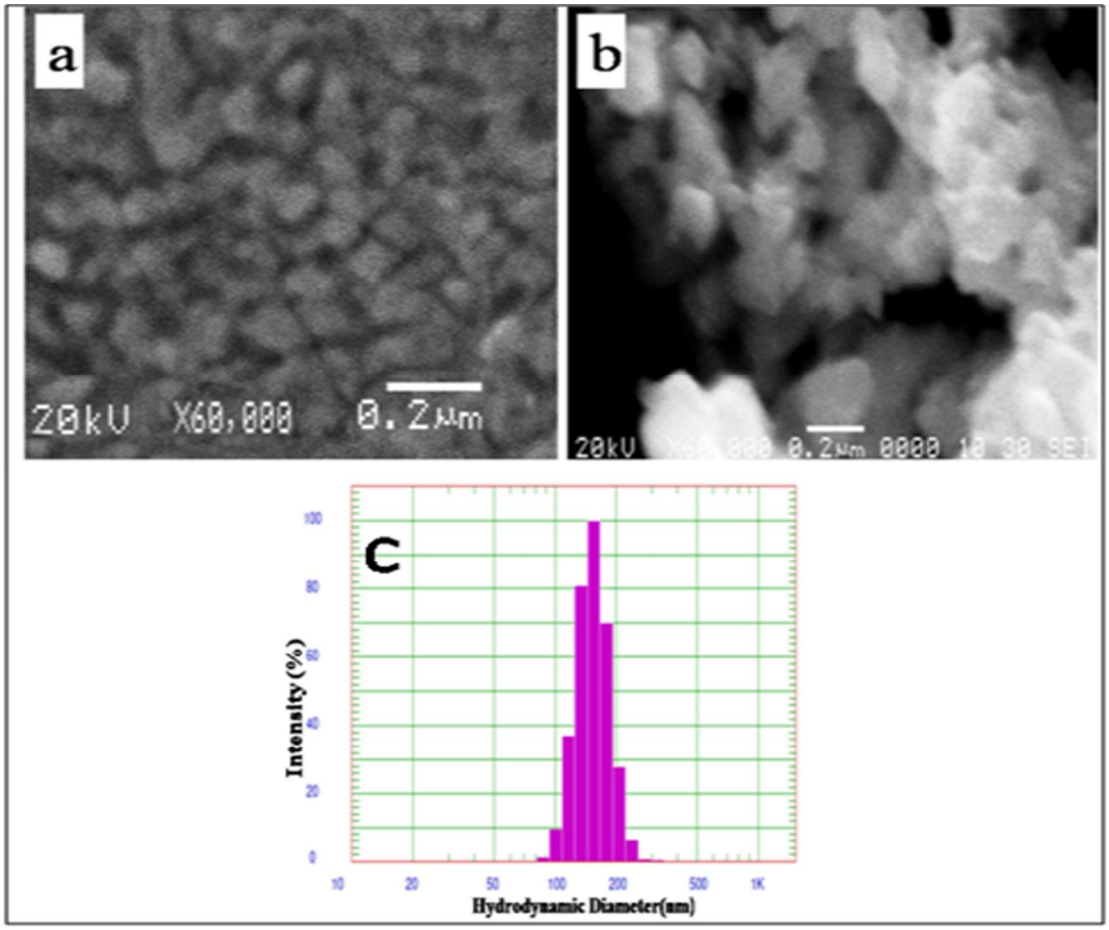

Fig 1a. SEM of bare Fe_3O_4 nanoparticles, b. SEM of Fe_3O_4 @silica@chitosan Nanoparticles and c. Size distribution of chitosan functionalized Fe_3O_4.silica superparamagnetic nanoparticles

3.2 DNA adsorption on Fe_3O_4 @silica@chitosan Nanoparticles.

The effect of the pH of buffer on the adsorption of DNA onto the surface of Fe_3O_4 @silica@chitosan nanoparticles was studied at different pHvalues ranging from 3.0 to 7.0 .The initial concentrations of DNA and nanoparticles were set at 20.0 mg/Land 20 mg, respectively. Fig. 2 reveals that the maximum adsorption of DNA occurs at pH 5.0. As shown in Fig. 4, the maximum amount of captured DNA (52μg) occurred at pH 5. The DNA binding efficiency at pH 5 was calculated to be 88%, as per Equation 1.The binding mechanism of DNA on the surface of Fe_3O_4 @silica@chitosan nanoparticles is most likely to be electrostatic

interactions between positively charged surface of the nanoparticle and negatively charged phosphate backbone of DNA. DNA is the molecule with a backbone of phosphodiester linkages. Phosphate groups are present in phosphodiester bond, and these are responsible for imparting negative charge to DNA. At neutral pH DNA molecule is negatively charged. It is a strong acid with pK_a value less than 1.0 Chitosan; a polycationic molecule has free amino groups at low pH which gives positive charge to molecule. The pK_a of protonated amino groups of chitosan is 6.2. Hence chitosan can hold positive charge below 6.2 and provide a DNA capture state below 6.2. According to the data shown in figure maximal binding of DNA occurs at pH 5.0. Above this pH the concentration of adsorbed DNA remains almost constant to about pH 6.0. At pH above 6.0 there is a drastic decrease in amount of DNA adsorbed owing to the pK_a of chitosan at which chitosan is deprotonated [26].

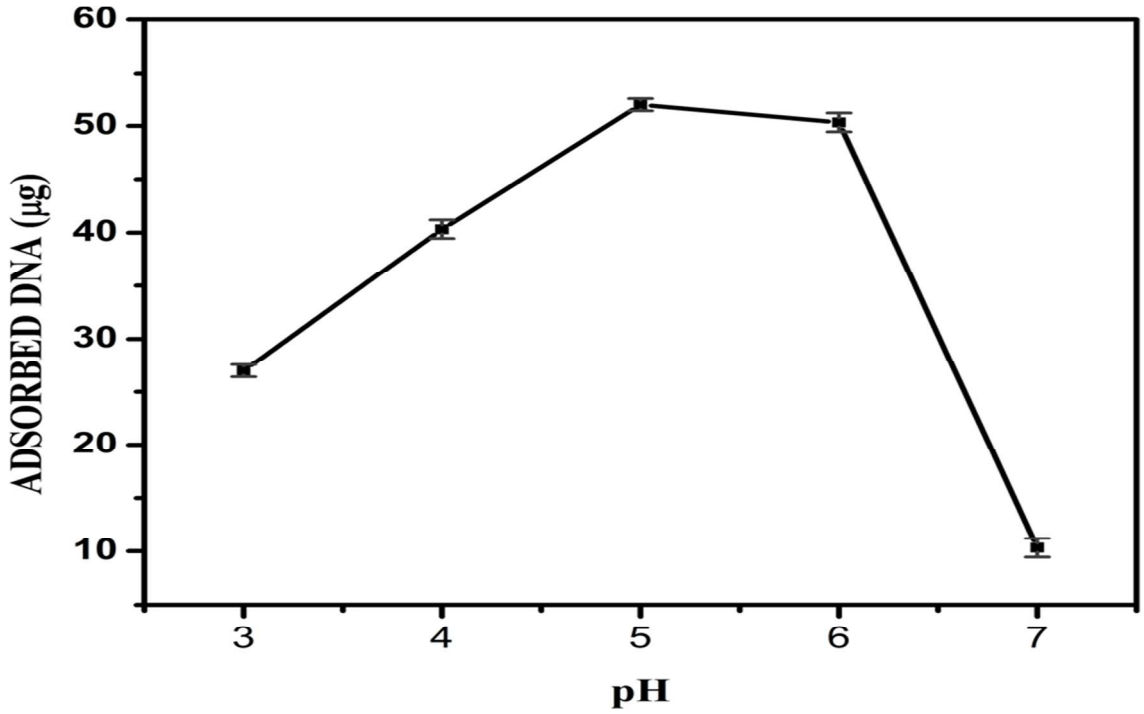

Fig.2. Effect of pH on adsorption of DNA on Fe_3O_4 @silica@chitosan nanoparticles.

3.3 DNA desorption from Fe_3O_4 @silica@chitosan Nanoparticles.

Fig. 3 represents the amount of DNA desorbed from the Fe3O4 @silica@chitosan nanoparticles at basic pH. The amount of eluted DNA is maximumat pH 8.5. The desorbed amount increases with the increase in pH of Tris buffer from 7.5 to 8.5. At pH8.5 the desorbed amount was found to be ~ 51.0 µg. The elution efficiency was calculated to be nearly98% whichwas expressed as per equation 2. Above pH 8.5, the desorbed DNAamount reached a plateau. At pH 9.0, the amino groups in thechitosan modified MNPs are expected to be deprotonated and thecaptured DNA can be eluted from the electrically neutral surfaceof the MNPs with gentle agitation at room temperature.The elution of DNA occurs due to separation of negatively charged DNA from positively charge nanoparticles. The positive charge of particles is due to the amino group which deprotonates and become neutral above pH 6.2. Hence negative charge separates from the surface of neutral nanoparticles [18].

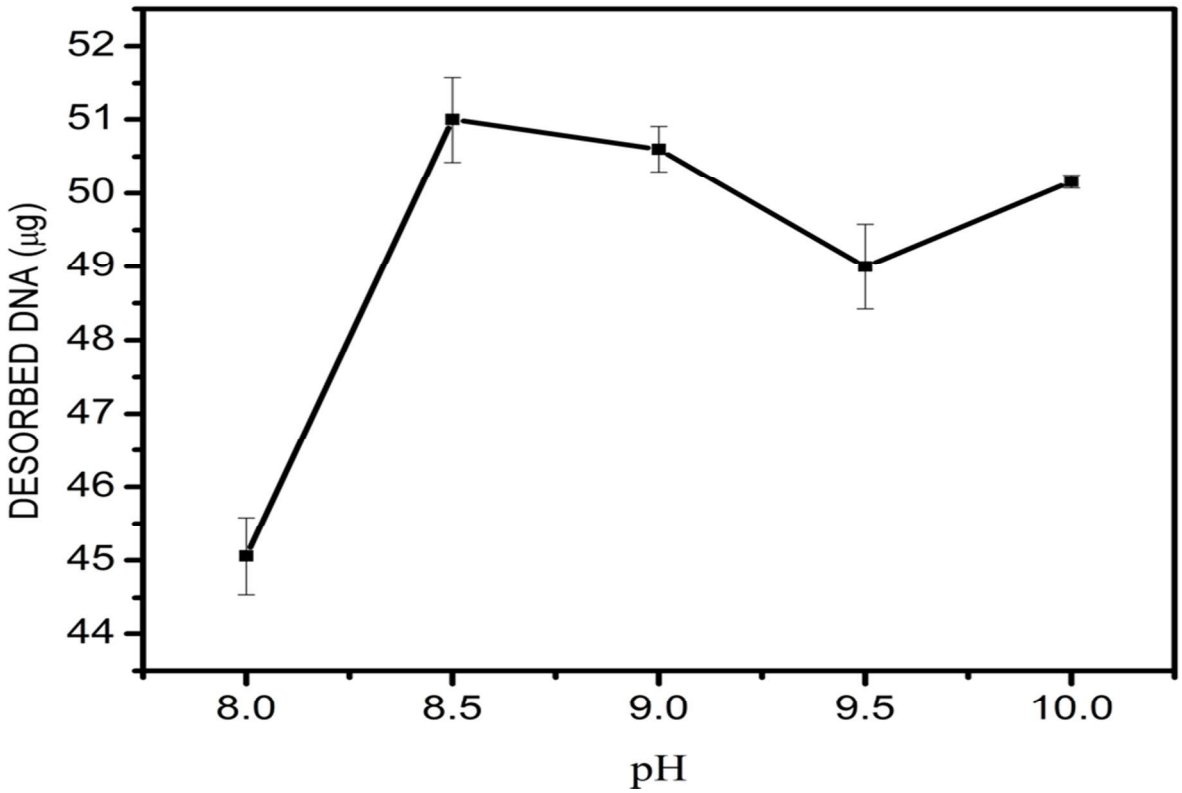

Fig.3. Effect of pH on desorption of DNA from Fe_3O_4 @silica@chitosan nanoparticles.

3.4 Effect of contact time

The effect of contact time on the amount of DNA adsorbed on Fe_3O_4 @silica@chitosan nanoparticles surface was assessed.The amount of nanoparticles was kept

20.0 mg and optimum pH of 5.0 was selected for this study. The result indicated maximum adsorption at 20 minutes as shown in Fig. 4. However about 93% of DNA was adsorbed in less than 10 min. The results point out the ability of nanoparticles to quickly adsorb DNA from solutions in less time and hence can be utilized for fast isolation protocol.

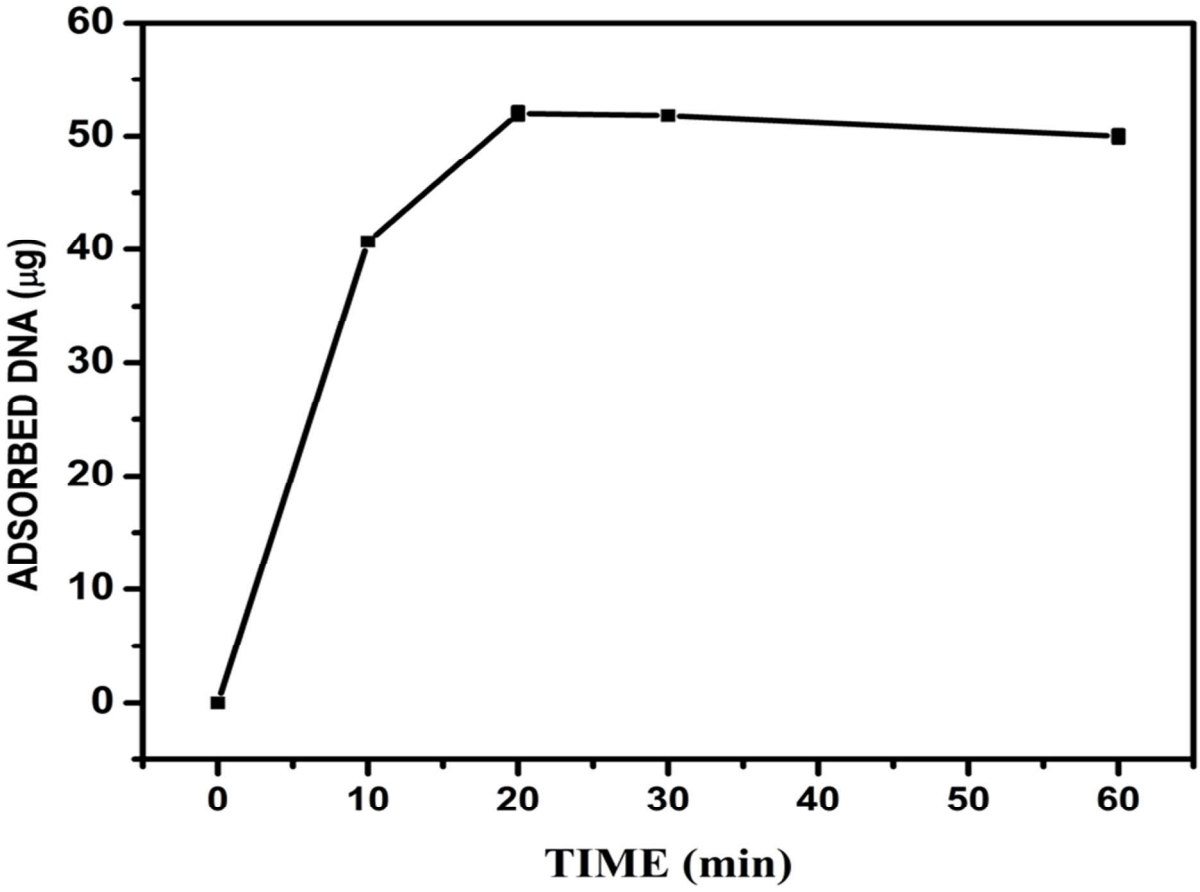

Fig.4. Effect of contact time on the amount of DNA adsorbed on Fe_3O_4 @silica@chitosan nanoparticles .

3.5 Effect of Temperature on binding of DNA to Fe_3O_4 @silica@chitosan nanoparticles.

The effect of temperature on adsorption of DNA onFe3O4 @silica@chitosan nanoparticles was studied at optimal pH and stirring time of 10 min. Table.1 represents the purity of DNA when subjected to different adsorption temperatures. The temperature has a great role to play in DNA Adsorption. The double stranded DNA structure is maintained by hydrogen bonds between the two strands. At temperatures above 90 ° C double stranded form

is separated into single strands and hence no adsorption of DNA should be expected. At low temperatures (≤ 50 ºC) both DNA and RNA are adsorbed on the particle. However when the temperature reached 70 ºC pure DNA was isolated and no RNA contamination was found and the purity remained constant to up to 90 ºC.The A_{260}/A_{280} ratio indicates the presence of protein contamination at 30 ºC to 50ºC adsorption temperatures and RNA contamination at temperatures above 80 ºC [27].

Table.1 Purity of DNA when subjected to different adsorption temperatures.

Temperature	30 °C	50 °C	70 °C	80 °C	90 °C
Purity (A260/A280)	1.24	1.30	1.85	1.87	2.0

3.6 Reusability studies

Reusability studies were performed by assayingthe concentration of DNA obtained from same amount of human saliva by using same set of Fe_3O_4 @silica@chitosan Nanoparticles for four subsequent experiments. The first experiment provided 52 µg DNA from 3mL of saliva. When the same set of nanoparticles was used for second time the concentration of DNA obtained was 51 µg. The obtained concentration was only 3% lower than that of the first experiment. Interestingly, even after fourth experiment high concentration of DNA was obtained. The yield after fourth experiment was 50.5 µg which are just 4% lower than the first experiment.It means even after repeated use the number of free positive groups are not decreasing significantly and hence taking part in adsorbing and eluting DNA with change in pH. This also indicates that the chitosan modified nanoparticles are highly stable. Excellent reusability of the particle makes it a suitable candidate for repeated DNA isolation. In the procedure reported in this paper, more than 96 % of DNA was desorbed in 30 min.

3.7 XRD pattern

The XRD pattern of Fe_3O_4@silica@chitosan nanoparticles and DNA adsorbed Fe_3O_4 @silica@chitosan nanoparticles are shown in Fig.5. From the absence of (210) and (300) peaks in the XRD pattern, it can be concluded that separate maghemite (g-Fe2O3) is not

present in the samples [28]. The appearance of hump at 2θ ≈ 13°, which represents the presence of a DNA molecule [29].The XRD pattern ofChitosan@Silica@Fe_3O_4nanoparticles and DNA adsorbed Chitosan@Silica@Fe_3O_4nanoparticles.

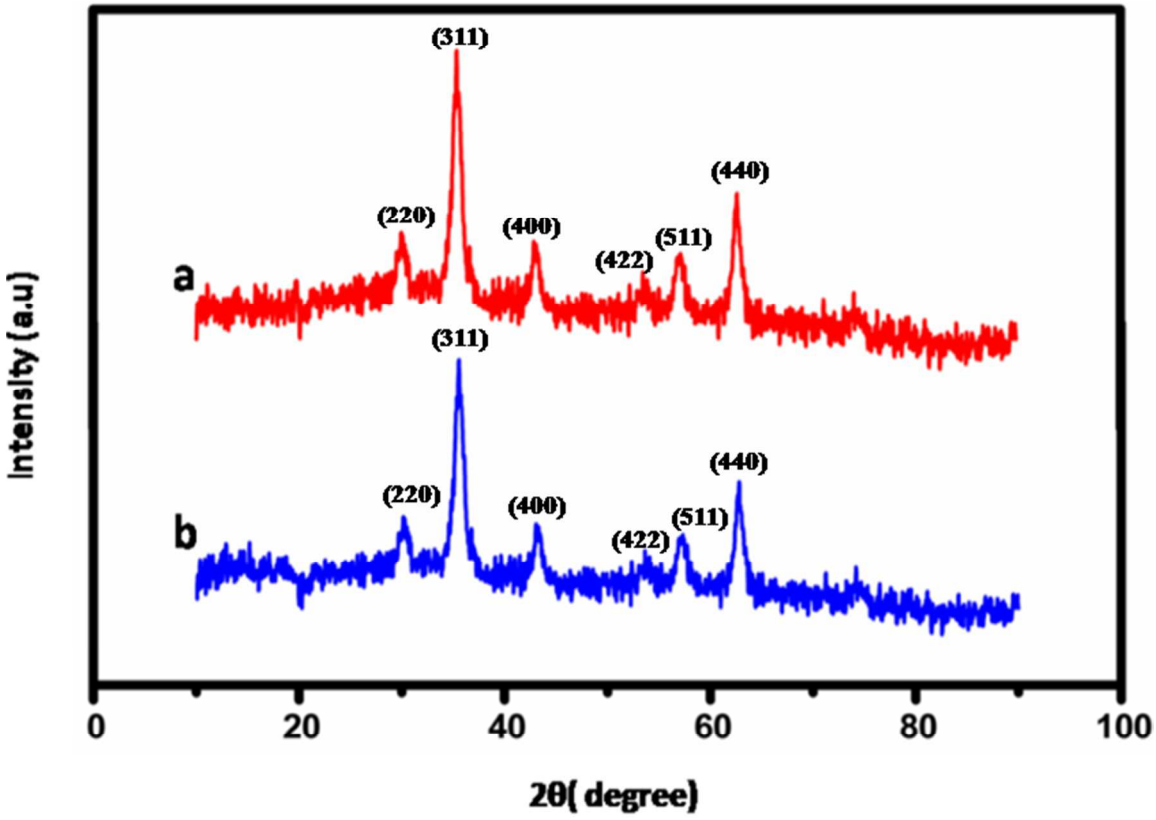

Fig.5 The XRD pattern of Fe_3O_4 @silica@chitosan nanoparticles and DNA adsorbed Fe_3O_4 @silica@chitosan nanoparticles

3.8 Agarose gel electrophoresis and electrochemical analysis of the isolated DNA

DNA isolated by different concentration of MNP's is displayedin Agarose gel electrophoresis; the isolated DNA samples provided sharp bands with size of 496 bp (Fig. 6 inset). To obtain the quantitative information about DNA fragment concentration, square wave voltammetry was used. The voltammetry is very sensitive method for DNA detection, suitable for miniaturization as well as monitoring of DNA interactions [30]. The electrochemical signals of extracted DNA are shown in Fig.6 illustrating that the extraction process is dependent on DNA concentration. The linearity of this dependence is expressed by correlation coefficient $R^2 = 0.9945$ as depicted in **Supplementary No. 1**.Fig.6.Square Wave

Voltammetry and inset: Agarose gel electrophoresis of isolated DNA with (a) 20mg, (b) 16mg (c) 12mg, (d) 8mg, (e) 4mg of Chitosan@Silica@Fe_3O_4nanoparticles and (f) electrolyte.

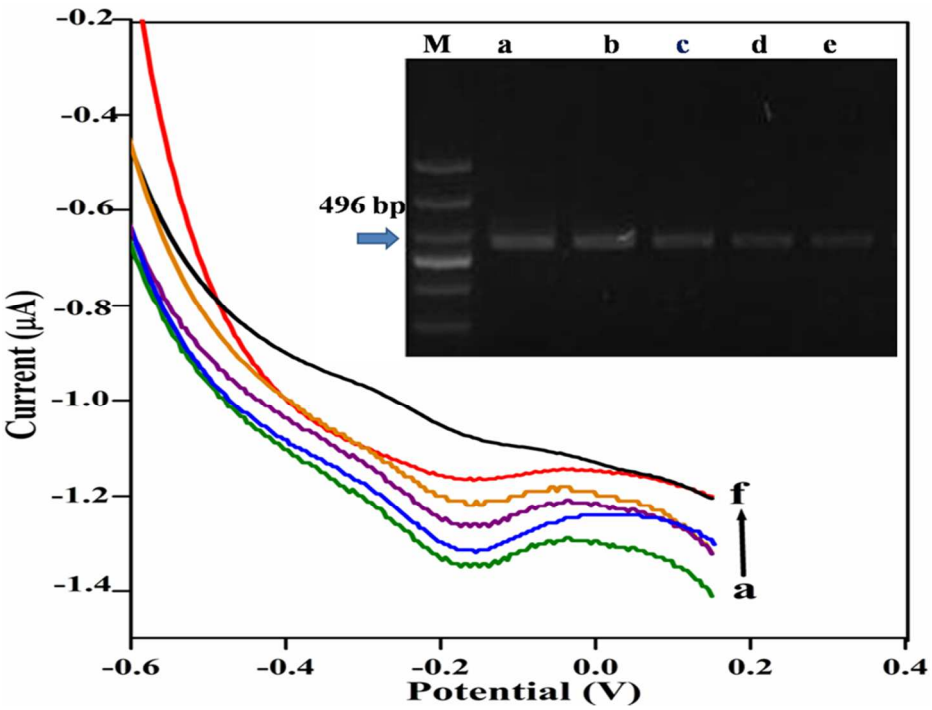

Fig.6.Square wave voltammetry and Inset: Agarose gel electrophoresis of isolated DNA with (a) 20mg, (b) 16mg (c) 12mg, (d) 8mg, (e) 4mg of chitosan functionalized Fe_3O_4-silica superparamagnetic nanoparticles and (f) electrolyte

3.9 Comparing DNA adsorption efficiency of Fe_3O_4 @silica@chitosan nanoparticles with Fe_3O_4 @silica

The study completed in this work highlights the characteristics of Fe_3O_4 @silica@chitosan nanoparticles that make it advantageous for DNA adsorption applications. The work compares DNA adsorption by chitosan coated Fe_3O_4 @silica under totally aqueous conditions with Fe_3O_4 @silica under chaotropic conditions. As evident from the Fig.7, the adsorption efficiencies of Fe_3O_4 @silica@chitosan and Fe_3O_4 @silica nanoparticles was ~ 88% and ~80% respectively. Interestingly, the elution efficiency of Fe_3O_4 @silica@chitosan nanoparticles (~ 98%) was found much higher than elution efficiency of Fe_3O_4 @silica nanoparticles (60%).

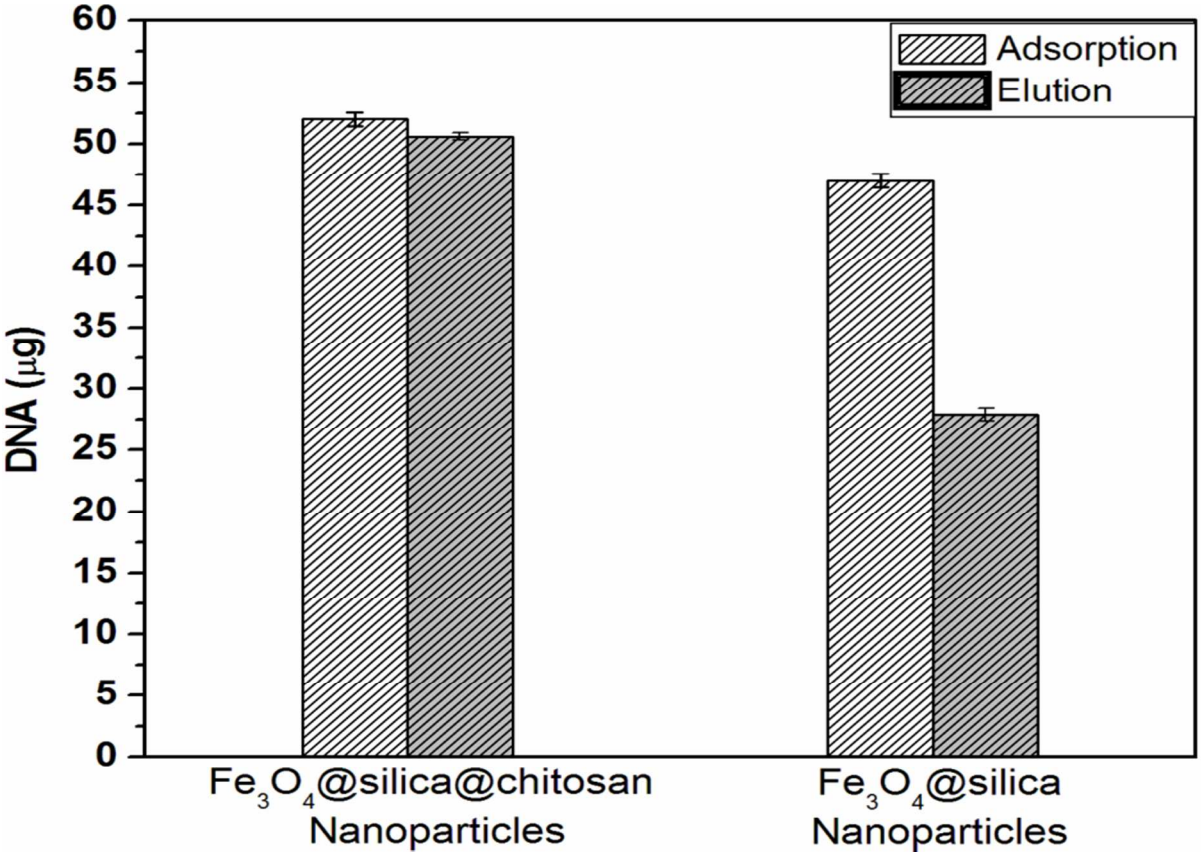

Fig.7.Comparison of Adsorption and Elution efficiency of Fe_3O_4 @silica@chitosan nanoparticles with Fe_3O_4 @silica nanoparticles.

The properties of Fe_3O_4 @silica@chitosan nanoparticles are compared with some recently reported materials used for DNA isolation and adsorption in Table 2.

Table. 2 Comparative study of present work with some recently reported materials used for DNA isolation and adsorption

Materials	Properties favorable for DNA purification	Reference
1.Fe_3O_4/SiO_2-GPTMSDEAE	a) Paramagnetic, pH tunable surface charge, highly stable. b) Size:200 nm, Magnetization: 30.081emu/g A_{260}/A_{280}: 1.79,yield:4.75 µg from 0.2 mL Blood.	[2]
2.Haemoglobin modified	a) Superparamagnetic,	

magnetic nanocomposites	pH tunable surface charge, highly stable . b) Average crystallite size: 15.3nm A_{260}/A_{280}:1.84,yield:10.5 µgm from 1ml bacterial culture.	[31]
3.Iron oxide nanoparticles	a) Superparamagnetic, stable, large surface area. b) A_{260}/A_{280}:1.7, yield :18-20 µg	[32]
4.Fe_3O_4@PANI	a) High magnetization, well dispersed, b) Average crystallite size: 30 nm, A_{260}/A_{280}:1.79 yield: 20.8 µg with 10mg of Fe_3O_4@PANI from 20µl of crude extract.	[33]
5.Salicylic acid coated magnetic nanoparticles.	a) Higher surface area to volume ratio, stable monodispersable. A_{260}/A_{280}:1.83, yield: 160.6 µg/µL from mammalian cells.	[34]
6.Chitosan functionalized Fe_3O_4-silica superparamagnetic nanoparticles	a) Superparamagnetic, highly stable, small size, pH tunable surface charge, can be reused. b) Average crystallite size: 17nm A_{260}/A_{280}: 1.85, yield: 52 µg from 3mL Human saliva.	Present work

It is evident that the proposed material not only has comparable properties similar to recently reported materials but is better in terms of superparamagnetic nature, pH tunable surface charge, and in isolation of high yields pure DNA.

4. Conclusion

Isolation and extraction of DNA from biological samples has always been of great interest and challenging to scientific fraternity. Higher percentage of isolation with maintaining the good quality of DNA has been concern for various known DNA isolating systems. We have developed pH responsive magnetic nanoparticles for successful isolation of genomic DNA

from human saliva. This has potential to extend the usability in other biological samples too. An investigation on the adsorption and desorption of DNA to the surface of magnetic nanoparticles was performed. The optimized parameters of pH, temperature and concentration were utilized for isolation of genomic DNA from human saliva. We could determine ~95% DNA isolation ability, faster isolation with in 10 min of time frame and significant reusability, with ~55% DNA isolation even after 5 cycles of use, make these particles highly potent in this process. It also can be concluded that the proposed magnetic nanoparticles can very efficiently bind to DNA and rapidly isolate DNA from real samples. The pH sensitive magnetic nanoparticles are well suited not only for routine laboratory use, but also the simplicity of this approach specifies their potential for automated DNA purification.The prepared Fe_3O_4 @silica@chitosan nanoparticle present promising prospects to be used as magnetic carrier in medical diagnosis for non specific capture, detection and isolation of nucleic acid.

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