Studying the Effect of Ionic Composition concentration and Immobilization Buffer pH on Single-Stranded DNA Adsorption onto Carbon Nanoparticles

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ABSTRACT

Carbon nanoparticles have emerged as versatile platforms in nucleic acid–based biosensing owing to their tunable surface chemistry and strong affinity toward single-stranded DNA (ssDNA). The efficiency and stability of ssDNA adsorption onto carbon nanomaterials critically determine the performance of such systems and are strongly governed by buffer conditions, particularly ionic composition, ionic strength, and pH. In this work, the effects of stabilizing buffer ionic composition and pH on ssDNA adsorption onto carbon nanoparticles were systematically investigated. Buffers containing controlled concentrations of mono- and divalent cations were examined across a defined pH range to elucidate their influence on DNA–nanoparticle interactions. The results indicate that increasing ionic strength enhances ssDNA adsorption primarily through ionic screening, leading to attenuation of electrostatic repulsion between the negatively charged DNA backbone and carbon surface. Furthermore, divalent cations induce additional enhancement via ionic bridging effects, promoting stronger and more stable adsorption. Buffer pH was found to modulate adsorption behavior by altering surface charge density and protonation states, with mildly acidic to neutral conditions favoring increased adsorption efficiency compared to alkaline environments. Notably, the simultaneous optimization of buffer pH and ionic composition produced a synergistic enhancement in adsorption kinetics, surface coverage, and hybrid stability. These findings provide mechanistic insight into the electrostatic and ionic contributions governing ssDNA adsorption and offer rational guidelines for buffer design in DNA–carbon nanoparticle interfaces for biosensing and bioanalytical applications.

Keywords: *Carbon nanoparticles; DNA concentration; Nanoparticle solubility; Dispersion; Colloidal stability; Biosensing*

1. *INTRODUCTION*

Carbon-based nanosensors have emerged as a versatile class of biosensing platforms, enabling highly sensitive detection of biomolecules through controlled interactions with nucleic acids [1,2]. Incorporation of single-stranded DNA (ssDNA) into these nanosensors allows selective biomolecule detection, nucleotide sequence recognition, and biomarker identification in diagnostic and bioanalytical applications [3,1]. The performance of these systems critically depends on the stability of the DNA–nanomaterial interface, which is strongly influenced not only by solution pH but also by the ionic composition and strength of the buffer [4,3]. Interactions between ssDNA and carbon-based nanomaterials—including graphene oxide (GO), carbon nanotubes (CNTs), and other functionalized carbon nanostructures—have attracted significant attention due to their ability to form stable DNA–carbon hybrids with broad applications in nanobiotechnology and biosensing [5,2,2]. Adsorption of DNA onto carbon surfaces is primarily governed by π–π stacking interactions between nucleotide bases and the sp^2 carbon lattice, hydrophobic interactions, and electrostatic forces between the negatively charged DNA phosphate backbone and surface functional groups of the nanomaterials [6,1]. Since both DNA and oxidized carbon surfaces typically carry negative charges, electrostatic repulsion can substantially limit adsorption. In this context, ions in solution, particularly cations, accumulate near DNA and

carbon surfaces, partially neutralizing their charges. This ionic screening reduces electrostatic repulsion and facilitates DNA adsorption [4,3]. The nature and concentration of these ions, including monovalent (Na^+, K^+) and divalent cations (Mg^{2+}, Ca^{2+}), play a critical role in modulating electrostatic interactions and can enhance adsorption stability through charge compensation mechanisms [4,3]. A key parameter regulating these interactions is the stabilizing buffer. The buffer not only sets the solution pH but, through its ionic strength and composition, directly influences electrostatic forces between DNA and carbon nanomaterials. At low ionic strengths, electrostatic repulsion dominates, limiting DNA adsorption. Increasing ionic strength enhances screening, reduces the effective Debye length, and promotes physisorption of DNA onto the carbon surface. Buffer pH also plays a decisive role in controlling adsorption. At lower pH, partial protonation of surface functional groups reduces net negative charge, decreasing repulsion and enhancing adsorption [3,4]. In contrast, at higher pH, increased negative charge density on both DNA and carbon surfaces intensifies repulsion, reducing adsorption efficiency [4,3]. The combined effects of buffer pH and ionic strength often exhibit synergistic behavior, where optimized pH and ionic composition together enhance DNA adsorption and hybrid stability beyond the sum of their individual contributions [4,7]. Given the critical influence of both pH and ionic composition, this study systematically investigates the effects of stabilizing buffer pH and ionic strength on ssDNA adsorption onto carbon nanoparticles. By analyzing electrostatic, π–π, hydrophobic, and ion-mediated interactions, this work provides mechanistic insight and practical guidance for the rational design of DNA–carbon nanomaterial interfaces in biochemical and biosensing applications [4,3].

2. *Materials and Methods*

2.1 Characterization

Scanning Electron Microscopy (SEM): Morphology, DNA surface coverage, and aggregation state were examined to study the quality of dispersion and surface interactions. High-purity carbon nanoparticles with stable aqueous dispersibility were used as the adsorption substrate. Single-stranded DNA (ssDNA, 20-mer, random sequence) was purchased in lyophilized form and dissolved in nuclease-free water to prepare a 100 µM stock solution. Working ssDNA solutions were freshly prepared by dilution in the corresponding buffers. Sodium chloride (NaCl), potassium chloride (KCl), magnesium chloride (MgCl2), and calcium chloride (CaCl2) were of analytical grade and used as received. Phosphate, Tris–HCl, and acetate buffers were used to cover a wide pH range. The pH of each buffer was adjusted using HCl or NaOH and verified using a calibrated pH meter.

2.2 Preparation of Buffers with Defined Ionic Composition

To investigate the effect of ionic composition and ionic strength, stabilizing buffers were prepared with systematically varied concentrations of monovalent and divalent ions. Monovalent salt concentrations (NaCl or KCl) were adjusted to 5, 25, 50, 100, and 200 mM. Divalent cation concentrations (MgCl2 or CaCl2) were set to 0.5, 1, 2, 5, and 10 mM. These concentration ranges are commonly used to study DNA–carbon nanomaterial interactions and ionic screening effects [1,2,8]. All buffers were filtered through 0.22 µm membrane filters and equilibrated at room temperature before use.

2.3 Effect of Buffer pH on ssDNA Adsorption

Adsorption experiments were performed at pH values of 4.0, 5.0, 6.0, 7.0, 8.0, and 9.0. This pH range was selected to capture changes in protonation and deprotonation of DNA phosphate groups and surface functional groups of carbon nanoparticles. Buffer capacity and ionic strength were kept constant during pH variation experiments to isolate the effect of pH.

2.4 ssDNA Adsorption Experiments

Carbon nanoparticles were dispersed in a stabilizing buffer at a concentration of 100 µg/mL and sonicated for 10 min to ensure homogeneous dispersion. ssDNA was then added to achieve a final concentration of 2 µM, a value commonly employed in adsorption studies involving carbon nanomaterials [1,3]. The mixtures were incubated at room temperature for 60 min to allow the adsorption equilibrium to be reached. Following incubation, samples were centrifuged at 12,000 rpm for 10 min to separate nanoparticle-bound DNA from unadsorbed DNA. The concentration of ssDNA remaining in the supernatant was quantified using UV–Vis

spectroscopy at 260 nm, a standard approach for monitoring nucleic acid concentration [4,6]. The amount of adsorbed DNA was calculated by subtracting the unadsorbed DNA concentration from the initial DNA concentration.

2.5 Data Analysis

All experiments were conducted in triplicate, and results were reported as mean ± standard deviation. Adsorption efficiency was analyzed as a function of ionic strength, ionic composition, and pH. The synergistic effects of buffer pH and ionic composition were evaluated by comparing adsorption efficiencies under combined optimal conditions with those obtained by varying each parameter independently [4,3].

3. *Results and Discussion*

3.1 Microscopic Analysis of Carbon Nanotubes

The purchased carbon nanotubes were characterized for morphology and structure using a scanning electron microscope (SEM, MIRA3 TESCAN). SEM analysis confirmed that DNA was bound and hybridized onto the nanotubes [Fig.1].

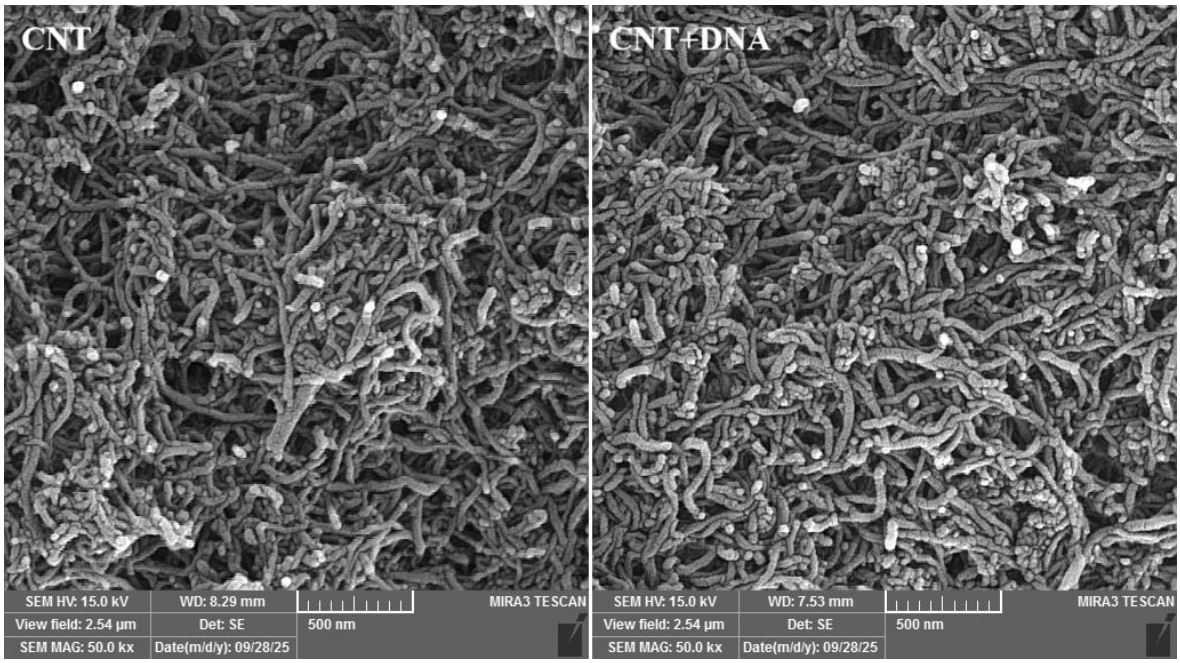

Fig. 1. Scanning Electron Microscopy (SEM) images of (A) carbon nanotubes and (B) immobilization of the DNA probe on the CNT surface.

3.2 Investigation of the solubility and dispersion of CNTs in water in the absence of DNA and CNTs in water in the presence of DNA

Figure 2 shows a photograph of CNTs in water in the absence of DNA (left) and the solution/dispersion of CNTs in water in the presence of DNA (right). Without DNA, CNTs were insoluble in water, aggregated, and sedimented. The dispersion/solution of CNTs was obtained in black and optically transparent when DNA was used as the solvent.

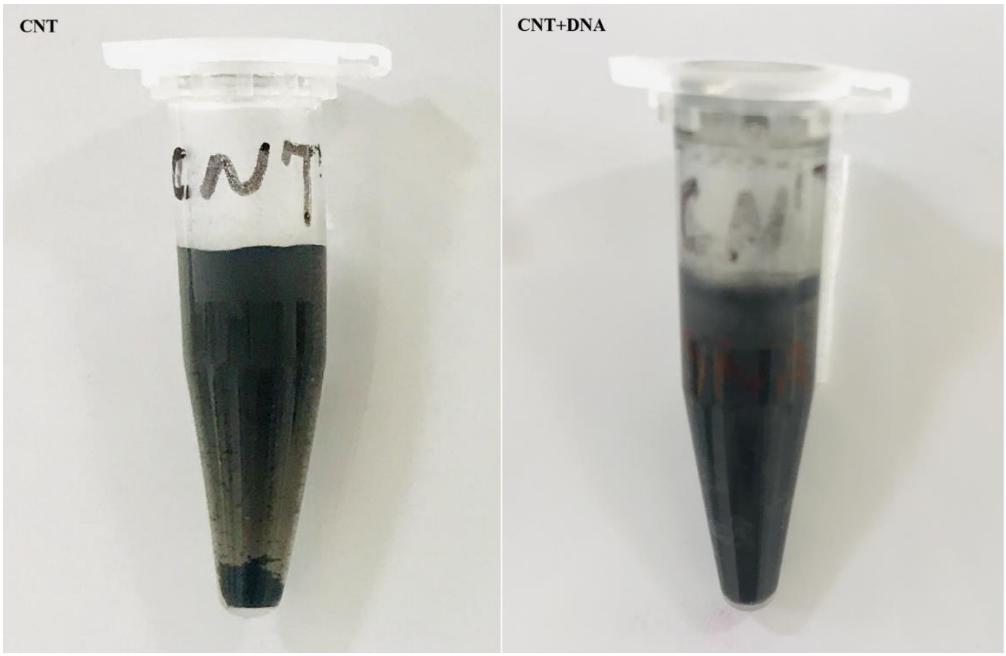

Fig. 2. A photograph of the aggregation and sedimentation of carbon nanotubes (CNTs) in water in the absence of DNA (left) and the dissolution/dispersion of carbon nanotubes in water in the presence of DNA (right).

3.3 Effect of Ionic Composition and Ionic Strength on ssDNA Adsorption

The ionic composition and overall ionic strength of the buffer significantly influence the adsorption behavior of ssDNA on carbon nanomaterials. Increasing ionic strength enhances DNA adsorption by reducing electrostatic repulsion between the negatively charged DNA backbone and the negatively charged carbon surface through improved ionic screening [4,3]. Experimental studies have demonstrated that the rate and extent of ssDNA adsorption on graphene oxide increase with higher ionic strength, as a greater concentration of cations screens phosphate negative charges and allows a closer approach of DNA to the carbon surface [4,3]. Higher ionic strength promotes tighter packing of adsorbed ssDNA due to more effective shielding of electrostatic repulsion. Structural and kinetic studies have shown that increasing ionic strength alters ssDNA conformation and surface coverage on carbon nanomaterials. Scattering and structural studies on ssDNA adsorbed to single-walled carbon nanotubes have shown that increasing solution ionic strength alters ssDNA conformation and surface coverage, consistent with enhanced screening of phosphate charges and more compact adsorption layers [9,8]. The specific ionic composition also plays a critical role. Divalent cations such as Mg^{2+} can facilitate stronger adsorption compared to monovalent ions by enhancing electrostatic stabilization and surface interaction [4,6]. Molecular dynamics simulations further indicate that divalent cations can mediate interactions between DNA and graphene oxide surfaces, reinforcing electrostatic contributions to adsorption beyond simple charge screening [6,9]. These findings are consistent with electrostatic models of DNA adsorption, in which increased counterion concentration reduces the effective Debye length and enables ssDNA to overcome repulsive forces more readily [4,3]. Collectively, both the magnitude and valency of ions in solution significantly modulate adsorption equilibria, DNA conformation, and surface coverage [Fig. 3].

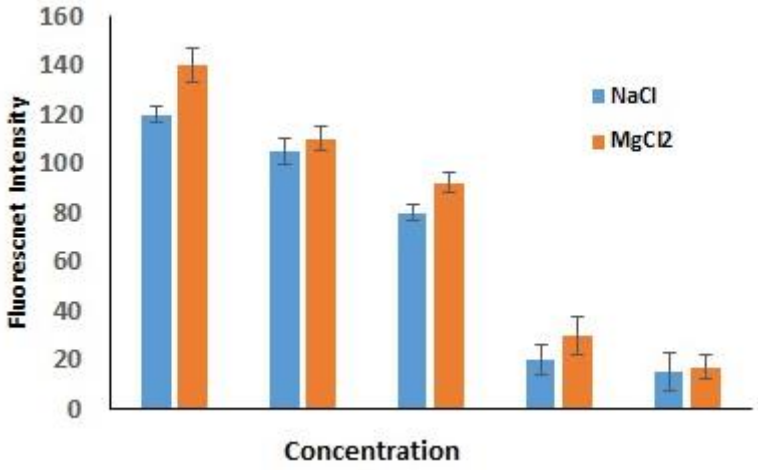

Fig. 3. Fluorescence spectra of FAM-labelled ssDNA probe in the presence of different concentrations of NaCl and MgCl2 in the Immobilization buffer (Excited at 485 nm, Emission at 520 nm).

3.4 Effect of Buffer pH on ssDNA Adsorption

In addition to ionic composition, buffer pH plays a decisive role in the adsorption of ssDNA onto carbon nanoparticles. At lower pH values, partial protonation of surface functional groups on graphene oxide and related carbon nanomaterials reduces net negative charge, thereby lowering electrostatic repulsion and facilitating adsorption [4,7]. Experimental investigations have demonstrated that DNA adsorption on graphene oxide is enhanced under mildly acidic conditions, where increased protonation of surface oxygen-containing groups promotes stronger interfacial interactions [4,6]. This behavior can be attributed to changes in the surface charge density of both DNA and carbon materials. As pH decreases, partial protonation of nucleotide bases (e.g., adenine and cytosine) and surface functional groups reduces effective electrostatic repulsion, thereby improving ssDNA affinity for carbon surfaces [4,6]. Conversely, at higher pH values, increased deprotonation leads to greater negative charge density on both DNA and the nanoparticle surface, intensifying electrostatic repulsion and reducing adsorption efficiency [1]. Systematic studies on DNA adsorption to graphene oxide and related carbon substrates have consistently reported optimal adsorption under moderately acidic to near-neutral pH conditions [4,3]. For instance, adsorption efficiency improves significantly when pH is lowered from alkaline or neutral values toward ranges where protonation of surface functional groups increases interfacial attraction and promotes closer DNA–surface contact [4]. Collectively, these findings highlight the central role of buffer pH in modulating surface charge density, protonation equilibria, and electrostatic interactions, ultimately governing ssDNA adsorption behavior on carbon nanomaterials [Fig. 4].

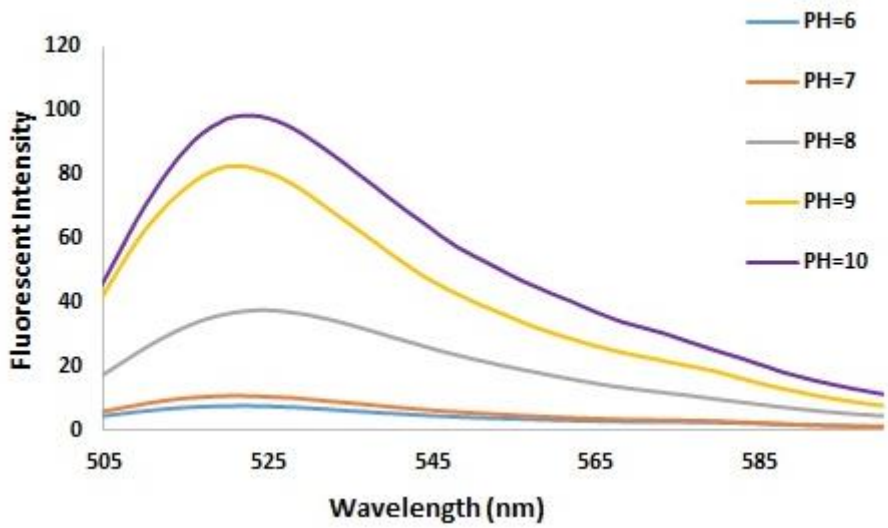

Fig. 4. Fluorescence emission spectrum of DNA probe in the presence of carbon nanotubes (CNTs) at different pH values of the immobilization buffer.

3.5 Synergistic Influence of Ionic Composition and pH

The combined effects of ionic composition and buffer pH exhibit a clear synergistic influence on ssDNA adsorption onto carbon nanomaterials [4,3]. Low pH alone may not sufficiently promote adsorption if ionic strength remains too low to effectively screen electrostatic repulsion between negatively charged DNA backbones and carbon surfaces. Conversely, elevated ionic strength under non-optimal pH conditions may only partially enhance adsorption due to persistent surface charge effects [4]. When both parameters are optimized simultaneously, adsorption capacity and interfacial stability increase significantly compared with the adjustment of either parameter alone. At moderately acidic pH, increasing ionic strength accelerates adsorption kinetics and enhances equilibrium surface coverage, demonstrating that ionic screening and protonation states cooperatively govern adsorption thermodynamics [4,5].This synergistic behavior can be rationalized through a combination of mechanisms: enhanced ionic screening (reduction of Debye length), moderated surface charge density via protonation of functional groups, and strengthened electrostatic or cation-bridging interactions, particularly in the presence of divalent ions such as Mg^{2+} [4,6]. Molecular-level simulations further support the role of multivalent cations in reinforcing DNA–graphene oxide interactions beyond simple charge screening [3]. Collectively, these cooperative effects shift adsorption equilibria toward stronger binding and higher surface coverage, which is critical for optimizing the performance of DNA–nanocarbon biosensors and nanobiotechnological platforms.

4. Conclusion

In this study, we systematically investigated the influence of buffer ionic composition and pH on the adsorption of single-stranded DNA onto carbon nanoparticles. Our findings demonstrate that both ionic strength and cation identity play central roles in modulating electrostatic interactions, ionic screening efficiency, and cation-mediated bridging, thereby strongly affecting adsorption efficiency and surface coverage of ssDNA [6,3,2]. Buffer pH was shown to critically determine the effective surface charge density of both DNA and carbon nanomaterials. Lower pH conditions facilitate adsorption by reducing electrostatic repulsion through partial protonation of surface functional groups and nucleobases [4,7]. Importantly, a synergistic interaction between ionic composition and pH was observed, highlighting that simultaneous optimization of these parameters significantly enhances adsorption capacity and interfacial stability beyond the effect of each factor independently [4,3]. These findings provide mechanistic insight into DNA–carbon nanoparticle interactions and offer practical guidance for the rational design of DNA-functionalized carbon nanomaterials for applications in biosensing, molecular diagnostics, and nanobiotechnology.

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