Effects of NaCl and $MgCl_2$ Concentrations on the Adsorption of FAM-Labeled Single-Stranded DNA onto the Surface of Graphene Oxide Nanomaterial

Haniyeh Pourtaghi Khorsand[1], Farrokh Karimi[2]*
[1] Department of biotechnology, faculty of science, university of Maragheh, Maragheh, Iran
[2] Department of biotechnology, faculty of science, university of Maragheh, Maragheh, Iran
*karimifm@maragheh.ac.ir

ABSTRACT

The interaction of single-stranded DNA (ssDNA) with carbon-based nanomaterials has attracted considerable attention because of the distinctive surface properties of these materials and their broad applications in biosensing and nanobiotechnology. Among carbon-based nanomaterials, graphene oxide (GO), with its layered structure, high specific surface area, and abundance of oxygen-containing functional groups, provides a suitable platform for investigating nucleic acid–nanomaterial interactions. In this study, the interaction of FAM-labeled single-stranded DNA (ssDNA-FAM) with the GO surface was investigated using fluorescence spectroscopy. The fluorescence emission spectrum of ssDNA-FAM was recorded over the wavelength range of 500–600 nm, with a maximum emission at approximately 520 nm. Upon addition of GO, a substantial decrease in the fluorescence emission intensity of ssDNA-FAM was observed, indicating pronounced quenching of the FAM fluorescence. This reduction in fluorescence is consistent with an interaction between ssDNA and the GO surface that brings the fluorophore into close proximity to the nanomaterial surface. The effect of ionic conditions on the fluorescence response of the ssDNA-FAM/GO system was subsequently evaluated by examining different concentrations of NaCl and $MgCl_2$. Increasing the NaCl concentration from 0 to 100 mM resulted in a progressive decrease in fluorescence emission intensity, indicating enhanced quenching of the FAM signal at higher NaCl concentrations. A similar trend was observed with $MgCl_2$. Increasing the $MgCl_2$ concentration from 0 to 10 mM led to a reduction in fluorescence intensity, with a more pronounced decrease observed at higher concentrations, particularly over the range of 1–10 mM. The observed changes may be associated with alterations in electrostatic interactions within the ssDNA-FAM/GO system. Increased ionic strength can reduce electrostatic repulsion between negatively charged DNA phosphate groups and negatively charged regions of the GO surface, thereby facilitating closer association of ssDNA with the nanomaterial. In the presence of Mg^{2+}, charge screening may be further enhanced because of the higher charge density of divalent cations. These changes may promote closer proximity of the FAM fluorophore to the GO surface, thereby increasing fluorescence quenching. Overall, the results demonstrate that ionic composition is an important factor influencing the fluorescence response of the ssDNA-FAM/GO system. Increasing concentrations of both NaCl and $MgCl_2$ enhanced fluorescence quenching and reduced the emission intensity of FAM. Modulation of ionic conditions may therefore provide a useful approach for controlling ssDNA–GO interactions and optimizing the fluorescence response of GO-based nucleic acid sensing systems.

Keywords: single-stranded DNA (ssDNA), graphene oxide (GO), carbon-based nanomaterials, FAM, NaCl, $MgCl_2$, DNA nanosensor

1. *INTRODUCTION*

Single-stranded DNA (ssDNA), in addition to its essential roles in biological processes, has attracted considerable interest in the design and development of nanobiological systems, biosensors, and biosensing platforms because of its ability to interact with a broad range of nanomaterial surfaces. The structural and chemical characteristics of ssDNA, particularly its negatively charged phosphate backbone and exposed nitrogenous bases, facilitate interactions with solid surfaces and nanostructured materials. The nature and strength of these interactions are governed by the physicochemical properties of the nanomaterial surface

and the chemical composition of the surrounding environment. Among carbon-based nanomaterials, graphene oxide (GO) has emerged as a suitable platform for studying nucleic acid–nanomaterial interactions because of its distinctive physicochemical and surface properties [1]. GO exhibits a layered structure, a high specific surface area, and abundant oxygen-containing functional groups, including hydroxyl, epoxy, and carboxyl groups, which provide multiple sites for interactions with biomolecules. The adsorption of ssDNA onto the GO surface may induce changes in DNA conformation and molecular organization, thereby altering the local microenvironment of fluorophore-labeled molecules [2]. Characterizing these interactions can therefore provide valuable insights into the behavior of DNA at nanoscale interfaces. Fluorescent labeling of ssDNA with dyes such as 6-carboxyfluorescein (FAM) provides a sensitive approach for monitoring changes in the molecular microenvironment through fluorescence spectroscopy. The fluorescence emission intensity of FAM can be altered following interaction of ssDNA with the GO surface, particularly when the fluorophore is brought into close proximity to the nanomaterial surface [3]. Accordingly, comparison of the fluorescence response of FAM-labeled ssDNA in the free state and in the presence of GO provides a useful approach for assessing interaction-associated changes between DNA and the nanomaterial. In addition to the properties of the nanomaterial surface, the ionic composition of the surrounding environment plays an important role in determining DNA conformation and its interactions with nanoscale surfaces. Ions can modulate electrostatic interactions and influence the state of DNA near the GO surface by altering the ionic strength of the medium and interacting with charged groups within the DNA phosphate backbone [4]. NaCl, as a source of monovalent Na^+ ions, is commonly used to regulate the ionic strength of aqueous solutions. Changes in NaCl concentration can modify the electrostatic environment surrounding DNA and the GO surface and, consequently, influence the fluorescence response of the ssDNA–GO system [5]. Divalent ions such as Mg^{2+} can exert additional effects on electrostatic interactions because of their higher charge. Mg^{2+}can associate with the negatively charged phosphate groups of DNA, thereby modifying the local electrostatic environment and influencing the conformational state of DNA in aqueous solution. The use of $MgCl_2$ as a source of Mg^{2+} therefore provides an opportunity to investigate the contribution of divalent ions to the fluorescence response of the DNA–GO system [6]. Separate evaluation of NaCl and $MgCl_2$ effects can help distinguish the contributions of ionic strength and ion valence to the behavior of ssDNA in the vicinity of the GO surface. Given the importance of DNA interactions with carbon-based nanomaterials and the potential influence of ionic conditions on these interactions, this study investigated the fluorescence response of FAM-labeled ssDNA in the presence of GO. First, the fluorescence spectra of ssDNA–FAM in the free state and following incubation with GO were compared. Subsequently, the effects of different concentrations of NaCl and $MgCl_2$ on the fluorescence response of the system were evaluated. In addition, the morphological characteristics of GO and GO–ssDNA complexes were examined using microscopic techniques. The primary objective was to determine how the ionic conditions of the surrounding environment influence the fluorescence response of ssDNA in the presence of GO and to assess whether changes in FAM emission intensity can serve as an indicator of DNA–GO interactions. The findings may contribute to a better understanding of nucleic acid behavior at interfaces with carbon-based nanomaterials and provide a basis for optimizing DNA-based systems for biosensing and nanobiological applications.

MATERIALS AND METHODS

Fluorescence Emission Spectrum of ssDNA-FAM

To determine the fluorescence emission characteristics of FAM-labeled single-stranded DNA (ssDNA-FAM), the fluorescence emission spectrum was recorded over the wavelength range of 500–600 nm. The maximum emission wavelength and corresponding fluorescence intensity were determined from the resulting spectrum. The maximum fluorescence emission of ssDNA-FAM was observed at approximately 520 nm.

Preparation and Comparison of ssDNA-FAM and ssDNA-FAM/GO Samples

To evaluate the interaction between ssDNA-FAM and graphene oxide (GO), fluorescence emission spectra of ssDNA-FAM were recorded in the absence and presence of GO and subsequently compared. Spectra were acquired over the wavelength range of 500–600 nm. Changes in fluorescence intensity were evaluated to assess the fluorescence response of the ssDNA-FAM/GO system upon interaction with GO.

Sample Preparation and Scanning Electron Microscopy (SEM)

To evaluate the morphological characteristics of GO before and after interaction with ssDNA, scanning electron microscopy (SEM) images of GO and GO-ssDNA samples were acquired and compared. The images were examined for differences in surface morphology and structural features associated with the adsorption of ssDNA onto the GO surface.

Preparation of Samples with Different NaCl Concentrations

To investigate the effect of NaCl concentration on the fluorescence response of the ssDNA-FAM/GO system, samples containing 0, 10, 50, and 100 mM NaCl were prepared. The fluorescence intensity of each sample was measured under the corresponding NaCl concentration, and the obtained values were compared across the experimental conditions.

Preparation of Samples with Different $MgCl_2$ Concentrations

To investigate the effect of $MgCl_2$ concentration on the fluorescence response of the ssDNA-FAM/GO system, samples containing 0, 0.1, 1, 5, and 10 mM $MgCl_2$ were prepared. The fluorescence intensity of each sample was measured under the corresponding $MgCl_2$ concentration, and the obtained values were compared across the experimental conditions.

RESULTS AND DISCUSSION

Fluorescence Emission Spectrum of FAM-Labeled Single-Stranded DNA

The fluorescence emission spectrum of FAM-labeled single-stranded DNA (ssDNA-FAM) was recorded over the wavelength range of 500–600 nm (Figure 1). The fluorescence intensity increased progressively with increasing wavelength from 500 nm and reached a maximum of approximately 300 at around 520 nm.

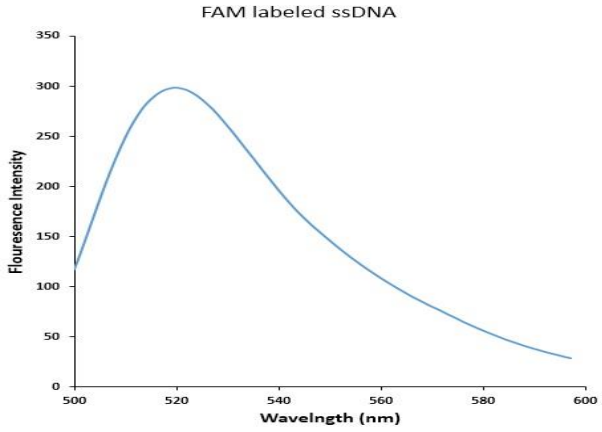


Fig. 1. *Fluorescence emission spectrum of FAM-labeled single-stranded DNA (ssDNA-FAM) recorded over the wavelength range of 500–600 nm.*

Effect of Graphene Oxide on the Fluorescence Intensity of ssDNA-FAM

To investigate the interaction between single-stranded DNA (ssDNA) and graphene oxide (GO), the fluorescence emission spectrum of free FAM-labeled ssDNA (ssDNA-FAM) was compared with that obtained in the presence of GO (Figure 2). Free ssDNA-FAM exhibited a distinct fluorescence emission profile with a well-defined maximum. In contrast, the addition of GO resulted in a pronounced reduction in fluorescence intensity throughout the examined wavelength range. The fluorescence signal of ssDNA-FAM in the presence of GO was substantially lower than that of the free sample, indicating efficient quenching of FAM fluorescence by GO. The observed fluorescence attenuation is consistent with the adsorption of ssDNA-FAM

onto the GO surface, which can bring the FAM fluorophore into close proximity to the nanomaterial. The GO surface contains extended sp^2-hybridized carbon domains that provide π-conjugated regions capable of interacting with the exposed nitrogenous bases of ssDNA. Adsorption of ssDNA onto GO has been attributed primarily to π–π interactions between the nucleobases and graphitic domains, together with other noncovalent interactions [7]. Close association of the fluorophore with the GO surface can facilitate nonradiative deactivation of the excited fluorophore, thereby resulting in substantial fluorescence attenuation. Depending on the molecular configuration and interfacial interactions, mechanisms such as fluorescence resonance energy transfer or electron transfer may also contribute to the observed quenching. Overall, the pronounced decrease in fluorescence emission observed in Figure 2 demonstrates the strong quenching effect of GO on ssDNA-FAM fluorescence. This response is consistent with the interaction and adsorption of ssDNA-FAM at the GO surface and confirms the suitability of the GO–ssDNA-FAM system for investigating DNA–nanomaterial interactions using fluorescence-based approaches.

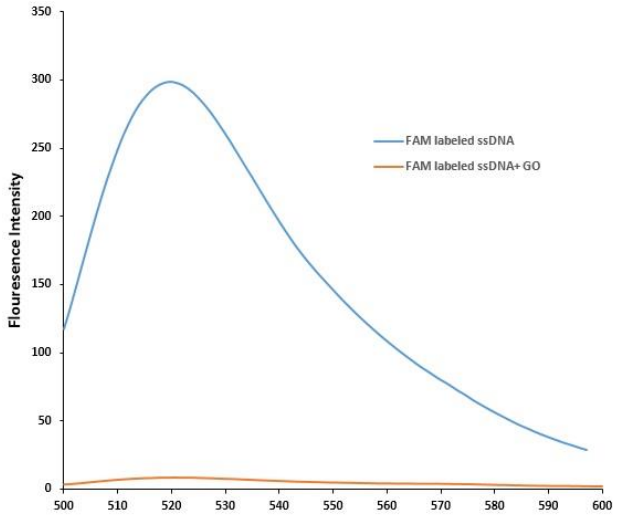


Fig. 2. *Comparison of the fluorescence emission spectra of FAM-labeled single-stranded DNA (ssDNA-FAM) in the free state and in the presence of graphene oxide (GO). The addition of GO markedly reduced FAM fluorescence intensity over the wavelength range of 500–600 nm, indicating substantial fluorescence quenching.*

Morphological Analysis of GO and GO-ssDNA Using Scanning Electron Microscopy

To investigate morphological changes associated with the interaction of single-stranded DNA (ssDNA) with graphene oxide (GO), scanning electron microscopy (SEM) was used to characterize and compare GO and GO-ssDNA samples (Figure 3). The GO sample exhibited the characteristic layered, sheet-like morphology of graphene oxide. Following incubation with ssDNA, the GO-ssDNA sample displayed a visibly altered surface morphology, characterized by a more compact and heterogeneous surface structure compared with that of pristine GO. The observed changes in surface morphology and structural organization suggest that the association of ssDNA with the GO surface may alter the surface characteristics of GO [8].

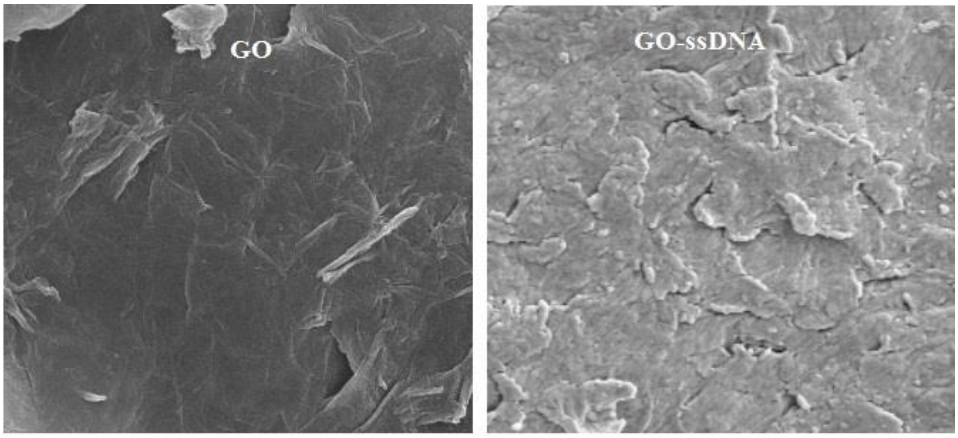


Fig. 3. *Scanning electron microscopy (SEM) images of GO and GO-ssDNA samples. The left image shows the characteristic layered and sheet-like morphology of GO, whereas the GO-ssDNA sample exhibits changes in surface morphology and structural organization following incubation with ssDNA.*

Effect of NaCl Concentration on the Fluorescence Intensity of the ssDNA-FAM/GO System

The effect of NaCl concentration on the fluorescence response of the ssDNA-FAM/GO system was investigated at NaCl concentrations of 0, 10, 50, and 100 mM. As shown in Figure 4, fluorescence intensity decreased progressively with increasing NaCl concentration. In the absence of NaCl, the fluorescence intensity was approximately 250. Increasing the NaCl concentration to 10 mM decreased the fluorescence intensity to approximately 160. A further increase to 50 mM resulted in a marked decrease to approximately 63, whereas the lowest fluorescence intensity, approximately 47, was observed at 100 mM NaCl. The concentration-dependent decrease in fluorescence intensity indicates enhanced quenching of the FAM fluorophore at higher ionic strengths. This effect may arise from changes in the electrostatic environment of the ssDNA-FAM/GO system. The phosphate backbone of ssDNA is negatively charged, and increasing ionic strength can screen electrostatic interactions between charged species within the system [9]. Such electrostatic screening may alter the conformation of ssDNA and its interaction with the GO surface, potentially affecting the distance between the FAM fluorophore and the GO surface. Because GO exhibits strong fluorescence-quenching capability toward fluorophores in close proximity to its surface, changes in ssDNA conformation or surface association may influence the efficiency of FAM quenching. Thus, the observed reduction in fluorescence intensity with increasing NaCl concentration is consistent with an ionic-strength-dependent modulation of the interaction between ssDNA-FAM and GO.

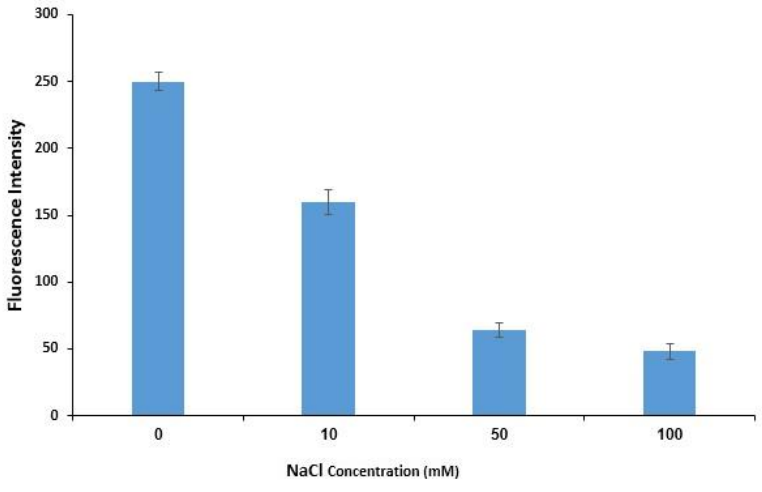


Fig. 4. *Effect of NaCl concentration on the fluorescence intensity of the ssDNA-FAM/GO system. Fluorescence intensity decreased progressively as the NaCl concentration increased.*

Effect of $MgCl_2$ Concentration on the Fluorescence Intensity of the ssDNA-FAM/GO System

The effect of $MgCl_2$ concentration on the fluorescence response of the ssDNA-FAM/GO system was evaluated at $MgCl_2$ concentrations of 0, 0.1, 1, 5, and 10 mM. As shown in Figure 5, fluorescence intensity decreased progressively with increasing $MgCl_2$ concentration. In the absence of $MgCl_2$, the fluorescence intensity was approximately 160. Increasing the $MgCl_2$ concentration to 0.1 mM resulted in a slight decrease to approximately 153, while a fluorescence intensity of approximately 149 was observed at 1 mM $MgCl_2$. A more pronounced decrease occurred at 5 mM, at which the fluorescence intensity declined to approximately 67. The lowest fluorescence intensity, approximately 27, was observed at 10 mM $MgCl_2$. The concentration-dependent decrease in fluorescence intensity suggests that increasing Mg^{2+} concentrations enhance the quenching of FAM fluorescence in the ssDNA-FAM/GO system. This effect may be related to changes in the electrostatic environment and conformation of ssDNA induced by Mg^{2+} ions. Because the DNA phosphate backbone is negatively charged, Mg^{2+} can interact electrostatically with phosphate groups and partially shield the negative charges along the DNA backbone. Such charge screening can reduce electrostatic repulsion within the DNA molecule and alter its conformation and spatial arrangement [10]. These conformational changes may, in turn, affect the interaction of ssDNA with the GO surface and modify the spatial proximity of the FAM fluorophore to GO. GO is an efficient fluorescence quencher when fluorophores are located in close proximity to its surface. Consequently, Mg^{2+}-induced changes in ssDNA conformation or orientation may alter the extent of FAM-GO interactions and thereby influence the fluorescence response. The relatively small changes observed between 0 and 1 mM $MgCl_2$, followed by a marked decrease at 5 and 10 mM, indicate that the effect of Mg^{2+} becomes substantially more pronounced at higher concentrations.

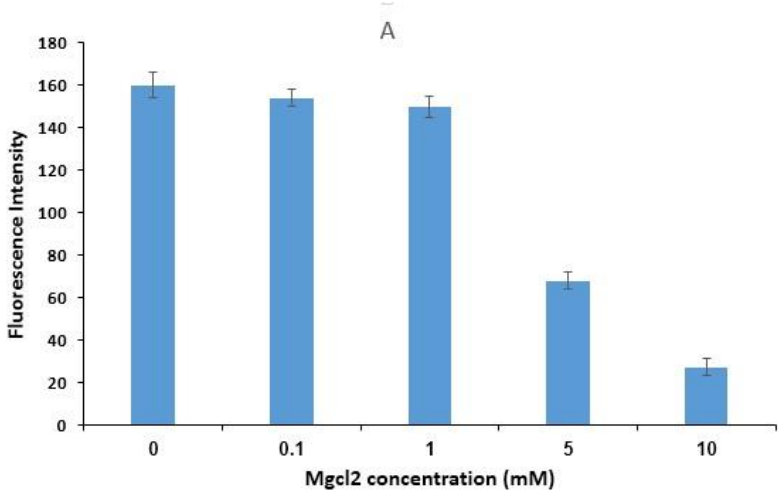


Fig. 5. *Effect of $MgCl_2$ concentration on the fluorescence intensity of the ssDNA-FAM/GO system. Increasing the $MgCl_2$ concentration from 0 to 10 mM resulted in a concentration-dependent decrease in fluorescence intensity. The change was relatively limited between 0 and 1 mM $MgCl_2$ but became substantially more pronounced at 5 and 10 mM.*

CONCLUSION

In this study, the interaction between FAM-labeled single-stranded DNA (ssDNA-FAM) and graphene oxide (GO) was investigated, with particular emphasis on the effects of ionic conditions on the fluorescence response of the ssDNA-FAM/GO system. Fluorescence spectroscopy showed that ssDNA-FAM exhibited a maximum emission at approximately 520 nm, whereas the presence of GO markedly reduced FAM fluorescence intensity. This pronounced quenching is consistent with close association of ssDNA-FAM with the GO surface, which may bring the FAM fluorophore into proximity with GO and facilitate fluorescence quenching. Scanning electron microscopy (SEM) also revealed morphological differences between GO and GO-ssDNA, providing complementary evidence for an interaction between ssDNA and the GO surface. The

fluorescence response of the ssDNA-FAM/GO system was strongly affected by ionic conditions. Increasing the NaCl concentration from 0 to 100 mM progressively decreased fluorescence intensity, indicating enhanced quenching at higher NaCl concentrations. This effect may be related to changes in the electrostatic environment of the system, which can influence ssDNA conformation and its interaction with the GO surface. Similarly, increasing the $MgCl_2$ concentration from 0 to 10 mM resulted in a concentration-dependent decrease in fluorescence intensity. The effect was relatively limited between 0 and 1 mM $MgCl_2$ but became more pronounced at 5 and 10 mM. The stronger effect observed at higher $MgCl_2$ concentrations may be associated with interactions between divalent Mg^{2+} ions and the negatively charged phosphate groups of DNA, which can modify DNA conformation and alter its association with GO. Overall, increasing concentrations of both NaCl and $MgCl_2$ enhanced the quenching of FAM fluorescence, demonstrating that both ionic strength and cation valency can influence the fluorescence behavior of the ssDNA-FAM/GO system. These findings emphasize the importance of controlling ionic conditions when studying DNA-GO interactions and optimizing GO-based fluorescence systems. In particular, changes in the concentrations of monovalent and divalent cations should be systematically considered because they can alter the physicochemical environment of the DNA-GO interface and consequently affect the measured fluorescence signal. The present findings provide an experimental basis for further optimization of ssDNA-GO-based fluorescence and biosensing platforms. Changes in FAM fluorescence may serve as a measurable readout of alterations in DNA-GO interactions; however, the contribution of ionic conditions should be carefully controlled to ensure reproducible and interpretable fluorescence measurements.

REFERENCES

[1] *Lopez, A., Liu, J. (2020). Covalent and Noncovalent Functionalization of Graphene Oxide with DNA for Smart Sensing. Advanced Intelligent Systems, vol. 2, no. 11, p. 202000123.*
[2] *Uten, S., Boonbanjong, P., Prueksathaporn, Y., Treerattrakoon, K., Sathirapongsasuti, N., Chanlek, N., Pinitsoontorn, S., Luksirikul, P., Japrung, D. (2024). Magnetic Graphene Oxide Nanocomposites for Selective miRNA Separation and Recovery. ACS Omega, vol. 9, no. 2, p. 2263-2271.*
[3] *Li, X., Li, T., Zhang, J., Zhang, Q., Deng, K., Ma, R., Wang, J., Kong, W. (2024). Establishment of a Dual-Signal Enhanced Fluorescent Aptasensor for Highly Sensitive Detection of Ochratoxin A. ACS Omega, vol. 9, no. 19, p. 21035-21041.*
[4] *Nitu, F. R., Burns, J. S., Ioniță, M. (2020). Oligonucleotide Detection and Optical Measurement with Graphene Oxide in the Presence of Bovine Serum Albumin Enabled by Use of Surfactants and Salts. Coatings, vol. 10, no. 4, p. 420.*
[5] *Lu, C., Liu, Y., Ying, Y., Liu, J. (2017). Comparison of MoS2, WS2 and Graphene Oxide for DNA Adsorption and Sensing. Langmuir, vol. 33, no. 2, p. 630-637.*
[6] *Muraru, S., Samoila, C. G., Slusanschi, E. I., Burns, J. S., Ionita, M. (2020). Molecular Dynamics Simulations of DNA Adsorption on Graphene Oxide and Reduced Graphene Oxide-PEG-NH2 in the Presence of Mg2+ and Cl− ions. Coatings, vol. 10, no. 3, p. 289.*
[7] *Gao, Y., Tian, J., Zhang, X., Qiao, B., Cao, Y., Wang, X., Wu, Q. (2020). A fluorescence assay for microRNA let-7a by a double-stranded DNA modified gold nanoparticle nanoprobe combined with graphene oxide. Analyst, DOI: 10.1039/c9an02274k.*
[8] *Ma, H., Xu, Z., Fang, H., Lei, X. (2020). Unexpected sequence adsorption features of polynucleotide ssDNA on graphene oxide. Physical Chemistry Chemical Physics, vol. 22, DOI: 10.1039/d0cp01066a.*
[9] *Hedley, J. G., Coshic, K., Aksimentiev, A., Kornyshev, A. A. (2024). Electric Field of DNA in Solution: Who Is in Charge? Physical Review X, vol. 14, no. 3, p. 031042.*
[10] *Serec, K., Dolanski Babić, S., Tomić, S. (2022). Magnesium ions reversibly bind to DNA double stranded helix in thin films. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, vol. 268, p. 120663.*