Enhancement of Polymerase Chain Reaction using Graphene Nano-flakes

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Abstract-**The excellent heat transfer properties of nanoparticles have potential applications in various fields including biology during the last two decades. Recently, the use of various nanoparticles in polymerase chain reaction (PCR) resulted in significant enhancement of its efficiency and specificity. In this research we have demonstrated the effects of a novel material, graphene nano-flakes on PCR. The rationale behind the use of graphene flakes is its unique physical and heat transfer properties. A number of experimental results including the effect of graphene flakes on denaturation of DNA and annealing step will also be discussed. The preliminary results clearly show that enhanced heat transfer effect of nano-flakes augment PCR yield and ultimately overall enhancement in reaction efficiency.**

Keywords-component; Graphene flakes, Nano-PCR, nanoscale heat transfer

I. INTRODUCTION

Nano-PCR is relatively a near area of research in the field of biotechnology [1, 2]. Recently, the idea of adding nanoparticles into polymerase chain reaction (PCR) [3-5] for enhancing its efficiency and specificity has attracted several researchers [3-5]. A number of nanoparticles including metallic [6, 7], oxide [8, 9], carbon nanotubes (CNT) and nanoparticles [10-12], have been used in several investigations. The reports have witnessed that the addition of an optimum concentration of nanoparticles results with enhanced yield i.e., efficiency [6], enhanced specificity [12-14], reduction in overall reaction time [8]. It is also apparent that the nanoparticle concentration plays a dramatic role in the viability of PCR, and higher concentrations could lead to PCR inhibition [7, 8]. Although a few mechanisms are proposed for the enhancements [9, 15], the basis is still unclear and needs further study. However, the enhanced heat transfer effect of nanoparticles, is a common perception of various existing reports [6, 8]. Therefore further study in this area will explore the key factors for enhancements and thus enable more reliability.

In this present study, we have examined PCR with the recently discovered graphene flakes [16]. It is one layer of atomic carbon and its theoretical specific surface area is up to 2600 m^2/g [17]. It has excellent in-plane thermal conductivity up to 5200 W/mK [18]. Furthermore, outstanding properties of graphene includes, quantum Hall effect, high values of Young's modulus, mobility of charge carriers and fracture strength [19].

II. MATERIALS, METHODS AND CHARACTERIZATION

A. Preparation of Graphene Nanofluids

Graphene nano-flakes of 8 nm thickness (Graphene Labs Inc. USA) were suspended in molecular biology-grade distilled water at 1 mg/mL concentration. To ensure proper mixing of the nanoparticles in water, sonication was performed for 2 hours using bath-sonicator. This well dispersed nanofluid was used as a stock solution and was appropriately diluted to various concentrations. The UV-Vis spectroscopy (Perkin-Elmer Lambda 35 spectrophotometer) was performed to confirm the characteristic peak of the samples.

B. Scanning Electron Microscopy (SEM)

The graphene samples were mounted on stubs with conductive carbon tape and coated with platinum. All samples were analyzed for their physical-structure and elemental compositions, Energy-dispersive X-ray spectroscopy (EDX) using SEM (JOEL JSM 6400 LV, Japan).

C. PCR Methodologies

PCR was carried out to amplify segments of DNA using gene-specific primers (table 1). Optimization and amplification of these DNA segments were performed using PCR master mix (2X) from the Fermentas Inc. Canada. The typical 25µL PCR reaction mixture contained the following final concentrations: 12.5 µL of master mix that includes 0.05 units/µL Taq DNA Polymerase in reaction buffer, 4 mM MgCl2, 0.4 mM dATP, 0.4 mM dCTP, 0.4 mM dGTP and 0.4 mMdTTP, 1 µM of each forward/reverse primer and 10–20 ng of template DNA per 25 µL reaction with or without different concentrations (0.1–3.2 nM) of nanoparticles. The PCR protocol began with a 94 ◦C denaturation step for 5 min, followed by a Touchdown program (94 ◦C denaturing step for 30 s followed by initial annealing temperature of 70 ◦C, subsequently run down to 55 ◦C at 1 ◦C/cycle, and a 72 ◦C extension step for 1 min), followed by a uniform three-step amplification profile (94 ◦C denaturing step for 30 s, 54 ◦C annealing step for 30 s, 72 ◦C extension step for 1 min) for another 23 cycles, then 72 ◦C for 10 min, and finally held at 4 ◦C.

The PCR reactions were carried out using a thermocycler with maximum ramping rate of 2.5 ◦Cs−1 (Eppendorf Mastercycler gradient). After PCR, the products were run in

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agarose gels (1.1% wt/vol) and visualized by ethidium bromide staining.

TABLE 2: PRIMERS USED FOR PCR AMPLIFICATIONS

Endoglucanave I from Fuvarium oxyoporum sp	
Forward primer: EGlclnF	5`-TTCGAAATGCAGACCCCCGAC-3` T_m: 51.2 GC content: 57.14%
Reverse primer: EGlclnR	5`-CACGCTCAGCCCTTACAAGCG-3` T_m: 53.1 GC content: 61.90%

III. RESULTS AND DISCUSSION

A. UV-VIS Spectrophotometry

The characteristic spectra of graphene nanofluids (8 nm) were observed for concentrations such as 0.1, 0.06, 0.08 w/w%. The absorbance was increasing with the increase in concentration and the peak was observed approximately at 225 nm wavelength Fig.1. This measurement is in good agreement with the existing reports [20].

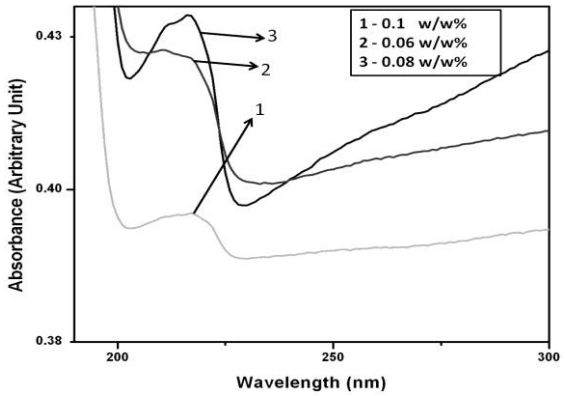

Figure 1. UV-Vis spectra of graphene nanofluids. 1 - 0.1; 2 - 0.06; 3 – 0.08 w/w%;

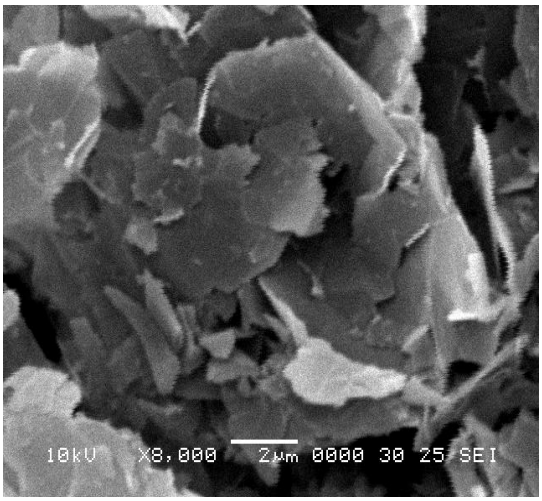

Figure 2. Left: Right: Scanning electron microscope (SEM) image of 8 nm graphene nano-flakes.

The SEM images with 2 µm magnification confirmed the expected physical nature of graphene flakes Fig.2. The appearance of graphene shows that the flakes are single layered and stable without agglomeration. After EDX analysis, using quantitative method the graphene flakes were analyzed and normalized results are presented in the Table 2.

TABLE 3. ELEMENTAL COMPOSITION OF GRAPHENE FLAKES

Element	**Elemental composition %**
	Graphene 8 nm
C	98.30
Zr	0
P	1.70
Si	0

B. Effect of Graphene on PCR

Initially graphene nano-flakes suspended in distilled H_2O was used to test the viability of PCR. The preliminary results showed unambiguous enhancement in the PCR yield (image not shown) of the order of ~10 folds. In order to find the optimum concentration, a series of experiments with increasing graphene concentrations from 0.01 – 0.2 w/w% were performed. Similar to the existing reports on other nanoparticles [6, 21, 22], graphene too exhibits strong concentration dependent PCR enhancement Fig.3. The increasing nanoparticles concentration from 0.01 w/w% shows a gradual increase in the PCR yield till lane-6 (0.1 w/w%) and gradually decreases with increasing concentrations. However concentrations with >0.1 w/w% results in gradual decrease in PCR yield and finally inhibition (image not shown).

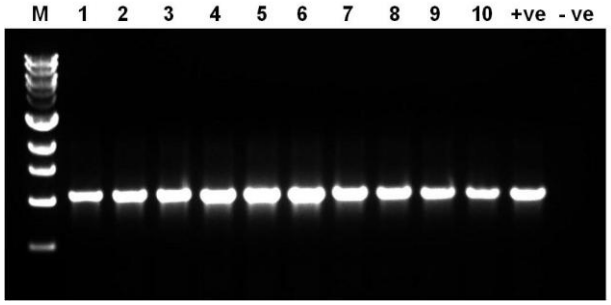

Figure 3. Agarose gel electrophoresis of PCR products showing concentration-dependent effect of. PCR amplification of a region of EG-1 was carried out in the presence of 0.001–0.1 w/w% graphene nano-flakes. The lane labeled as '+ve' indicate the PCR products obtained under identical conditions without addition of the nanoparticles and the lane labeled as '−ve' indicates the negative control reaction in which water was added instead of template DNA. The lane labeled as 'M' shows positions of the DNA molecular weight markers.

CONCLUSION

The preliminary results obtained in this research works clearly shows that, graphene nano-flakes has a great potential to be used as PCR enhancers. In agreement with the previous studies, the current results explore that the enhancement is strongly particle concentration dependent. And the higher concentrations may inhibit the PCR process owing to enhanced thermal conductivity effect. However further studies are highly essential to understand the mechanism completely.

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