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Graphitic carbon nitride embedded hydrogels for enhanced gel electrophoresis

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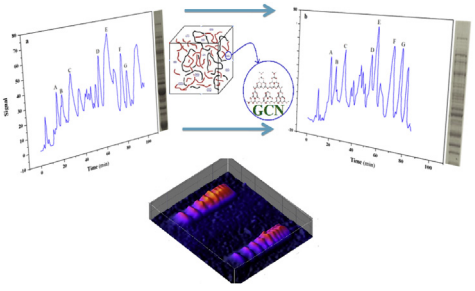
HIGHLIGHTS

- g-C_3N_4 nanosheets improved the resolution and efficiency in gel electrophoresis.
- g-C_3N_4 loading into polyacrylamide gel increases the thermal conductivity of gel.
- g-C_3N_4 nanosheets increase the resolution between bands.
- g-C_3N_4 nanosheets act a polymerization catalyst and as heat sink.

GRAPHICAL ABSTRACT



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ABSTRACT

Here, we show, for the first time, the use of graphitic carbon nitride (g-C_3N_4) nanosheets to improve the resolution and efficiency of protein separation in gel electrophoresis. By loading 0.04% (m/v) g-C_3N_4 nanosheets into the polyacrylamide gel at 25 °C, the thermal conductivity increased approximately 80% which resulted in 20% reduction in Joule heating and overall increase of separation efficiency. Also, polymerization of acrylamide occurred in the absence of tetramethylethylenediamine (TEMED) when the polyacrylamide gel contained g-C_3N_4 nanosheets. Hence, the g-C_3N_4 act simultaneously as a polymerization catalyst as well as heat sinks to lower Joule heating effect on band broadening.

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1. Introduction

The potential application of nanomaterials in various fields of science has been recognized and significant advances have been achieved [1–4]. The properties of nanomaterials including thermal, electrical, chemical, and mechanical are influenced by their size, shape, and composition [5]. High surface-to-volume ratio of nanoparticles (NPs) has led to their usage in applications such as detection and separation of biomolecules [4,6–9]. Various types of nanostructures such as carbon nanotubes [10], fullerenes [11], silica [12], latex [13], magnetic [14] and non-magnetic metal oxides [15], metal oxide semiconductor [15], silver [16], gold [17,18], ceria [4], and polymer-based NPs have been used successfully for the separation purposes and also for coating in electrophoresis [19,20]. Use of nanomaterials in separation science has been recognized in

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electrophoresis [4] and capillary electrophoresis [21], capillary electrochromatography [22,23], microchip electrophoresis [24], and chromatography separations [25,26]. Despite the widespread applications of nanomaterials in analytical chemistry, very little research has been devoted to their applications in gel electrophoresis.

With respect to the post-genome era and the importance of recombinant DNA technology, there has been revival in the use of gel electrophoresis to identify and characterize biological compounds [27]. Polyacrylamide gel electrophoresis (PAGE) is one of the most powerful techniques for separation of biological samples [28]. However, the efficiency and reproducibility of the separation are limited by some parameters such as band broadening due to the diffusion, Joule heating, adsorption, and other effects [29]. Temperature gradient in separation medium has a major influence on band broadening. For example, the electrophoretic mobility has a strong dependence on temperature, thus the parabolic profile of temperature leads to the parabolic velocity profile. The dispersion caused by a parabolic velocity profile due to flow is given by Ref. [30]:

$$\sigma_D^2 = \frac{R^2 \nu_{avg}^2 t}{24 D_y} \quad (1)$$

where ν_{avg} is the average linear velocity of the analyte across the medium, R is the internal radius of the medium, σ_D^2 is the peak variance due to dispersion, t is time, and D_y is the analyte diffusion coefficient. The expression relating the temperature induced electrophoretic velocity profile to band variance [31]; the peak variance due to the temperature gradient, $\sigma^2{}_{\Delta T}$ is given by:

$$\sigma_{\Delta T}^2 = \frac{R_i^6 E^6 \chi_e^2 \Omega_T^2 \mu^2}{1536 D_y k_b^2} t \quad (2)$$

where E and χ_e stand for the electric field strength and the electrical conductivity of the medium, respectively. The k_b is the thermal conductivity of the medium, Ω_T is the temperature coefficient of electrophoretic mobility, and μ is the solute electrophoretic mobility. The magnitude of $\sigma_{\Delta T}^2$ is inversely proportional to square of k_b. Therefore, any enhancement in thermal conductivity of medium leads to a dramatic reduction of the total peak variance generated by temperature gradient.

When voltage, U is applied on a separation medium, the heat produced, q can be calculated by Eq. (3):

$$q = UI\,t \quad (3)$$

where I and t stand for electric current and time, respectively. The temperature difference between the center line and the inside line of separation medium can be calculated by *Knox* equation [32]:

$$\Delta T = \frac{Qr^2}{4k_b} \quad (4)$$

where r is radius of separation medium and Q is the heat produced per unit volume and time:

$$Q = \frac{UI}{whl} \quad (5)$$

where w, h, and l are dimensions of gel. As Eq. (4) shows there is an inverse relationship between temperature difference and thermal conductivity of medium. It implies thermal conductivity plays a vital role for improvement of separation. Reduction in Joule heating in slab gel electrophoresis is important for improving the separation efficiency. One approach is using lower voltages at the expense of longer separation times.

Within the past decade, smart gels such as nanocomposite (NC) gels have been synthesized and used for a wide range of applications [33]. NCs have attracted much attention and are believed to be a revolutionary type of hydrogel [34]. They may improve the gel thermal and mechanical strength [35], interact with the analyte and force the transport of analytes through inter-particle channels [36] and change the gel cross-link density [37]. The NCs gels represent new opportunities for improving the balance between the thermal and mechanical properties [38,39].

Herein, we present a detailed study of graphitic carbon nitride nanosheets (g-C_3N_4) inclusion to the polyacrylamide (PA) gels. We chose g-C_3N_4 nanosheets because of their low electrical conductivity (band gap is in the range 3–4 eV) [40,41] as well as high thermal conductivity [42]. The aims of this study are to investigate the influence of g-C_3N_4 nanosheets in improvement of analytical figures of merit in gel electrophoresis and to study use of g-C_3N_4 nanosheets as catalyst for acrylamide polymerization in order to eliminate the use of TEMED.

2. Experimental

2.1. Chemicals

All reagents were of analytical grade. Tris-hydroxymethyla minomethane (Tris), glycine, silver nitrate, *N*,*N*-methylenebisa crylamide (Bis), acrylamide, sodium dodecyl sulfate (SDS), ammonium persulfate (APS), sodium carbonate, mercaptoethanol, formaldehyde, TEMED, and sodium thiosulfate were purchased from Merck (Germany). Melamine (99%) was prepared from Khorasan Petrochemical Complex (KPC, Iran) and used without further purification. *E. coli* protein samples were prepared from Biotechnology Research Center of Ferdowsi University of Mashhad.

2.2. SDS-PAGE of protein mixtures

One-dimensional gel electrophoresis of proteins was carried out in a 12% (m/v) vertical PA gel containing sodium dodecyl sulfate (SDS-PAGE) according to the Laemmli protocol [43]. The 30% (m/v) acrylamide/bis-acrylamide stock solution was prepared to produce the specific percentage of PA gel (12%). Acrylamide/bis-acrylamide stock solution 30% m/v (29:1) is based upon the total concentration (T) and cross-linker concentration (C) which are 30% T and 3.3% C, respectively. All samples were prepared in 5× concentrated Laemmli reducing buffer (1.0 mL Tris–HCl (0.125 M, pH 6.8), 1 mL glycerol (20%), 1.5 mL SDS (10%), 0.4 mL mercaptoethanol (2%), and 0.2 mL bromphenolblue (0.05%)) and boiled for 4 min before using. The protein concentration of the samples was adjusted so that about 7 μL of protein (0.04–0.09 mg/mL) was loaded per lane. Gels were stained by Coomassie Brilliant Blue and destained according to the standard protocol [44]. Gel images were analyzed using *ImageJ* [45] and *GelAnalyzer* [46] softwares. The SDS-PAGE was performed without cooling (thermostatization).

2.3. Synthesis of g-C_3N_4 nanosheets

The g-C_3N_4 nanosheets were synthesized by a two-step method in a tube furnace. In a typical procedure, a given amount (5.00 g) of melamine in a closed crucible was heated to 380 °C and 600 °C sequentially for 1 h and 2 h, respectively.

2.4. Characterization methods

The X-ray diffraction (XRD) analysis was carried out on Unisantis

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