論 文 の 内 容 の 要 旨

論文題目　Electrophoretic migration behavior of double stranded DNA and small molecules in graphene oxide-doped polymer gels

（酸化グラフェンをドープしたハイドロゲル中における、2 本鎖 DNA 及び低分子物質の電気泳動挙動）

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Introduction

Polymer nanocomposites based on nanoparticles have had tremendous impact in science and technology. As opposed to traditional composites, the fillers in polymer nanocomposites have dimensions below 100 nm and therefore exhibit high surface to volume ratios. Addition of a small volume of nanofillers produces a great amount of interfacial area between polymer matrix and nanofillers, resulting in either improved or new properties without compromising attractive properties of neat materials. One of the important challenges in this field is to control the nanofiller dispersion state so that the desired property can be achieved. In addition to achieving the desired properties, incorporation of nanofillers into polymers can impart superior and unique properties that traditional fillers are unable to achieve, such as mechanical strength, electrical conductive etc. Furthermore, nanofillers also change polymer viscosity, a critical parameter that influences molecular transport and flow behavior. Hydrogels, 3D networks of cross-linked hydrophilic polymers, also provide an excellent platform for molecule separation and membrane technology. Because they are biocompatible, and can be tailored to be sensitive to changes in pH, temperature, and ionic strength, hydrogels have broad applicability, such as biosensors, separation science and drug delivery. Transport of molecules through the confined regions is extremely important in drug delivery. For example, drug transport through hydrogels directly determines the rate of release to target cells. Current challenges in drug delivery and separation science require better understanding of solute interactions with local environment as well as their mobility and diffusion.

Capillary gel electrophoresis is one of the most important techniques for DNA analysis. Combining with an organic dye (ethidium bromide (EB)), DNA fragments could be well separated according to the size and expediently detected using photomultiplier tube. On the other hand, the limitations in the separation of low molecular weight compounds in gel matrix pose a great disadvantage over a free solution electrophoresis. In particular, separation of oligomers of less than 20 base pairs attracted a lot of attention over the past several years. Practically speaking, it is nearly impossible to decrease the mesh size of the network to decrease diffusion of such small molecules, which opened up field for new material design.

Herein, we propose incorporation of 2D planar graphene oxide (GO) sheets into polymer gel network in

order to control diffusion and mobility of DNA fragments and small molecular weight compounds through specific interactions between GO sheets and migrating solutes. GO has a layered structure and contains hydroxyl, epoxide and carboxyl groups on its surface, and it has been used to improve the mechanical and physical properties of polymers. In this study we used GO-doped polymer gel for the separation of both DNA and small molecules. Our results show a promising potential for graphene oxide and derivative materials to be utilized for separation and detection of biomolecules and small charged compounds.

Results and discussion

Understanding the chemistry behind the aqueous dispersibility of GO sheets is of fundamental importance for understanding size fractionation of GO sheets. Here, we used zeta potential measurements and pH titrations to establish the chemistry underlying the aqueous dispersibility of GO sheets at different values of pH. The zeta potentials of GO aqueous dispersions at different values of pH are shown in Figure 1. Generally, particles with absolute value of zeta potentials more than 30 mV is considered to form stable dispersions due to interparticle electrostatic repulsion.

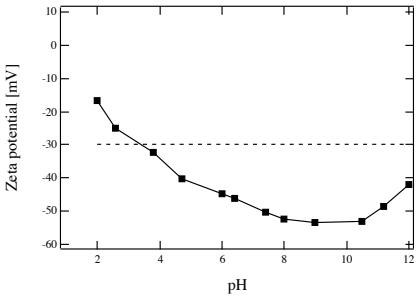

Fig.1. Zeta potential of GO in buffer solutions of wide pH range

GO samples formed stable suspensions in pH range of 4-12, with lowest value of -52.4 mV at pH 9, which suggests that the stability of the GO dispersions is a consequence of the negative charge on the graphene sheets.

One the challenges in preparation of composite materials is to achieve stable dispersion of GO in polymer matrix. GO was added to the hydrogel precursor solutions and then pre-gel solution was left to gel for up to 30 min. During the gelation time there was a possibility for GO settling due to gravity because of higher GO density (~2.1 g cm^{-3}) as compared to PEG aqueous solution (~1.2 g cm^{-3}). Therefore, we examined the role of surfactant on dispersion of GO in PEG polymer matrix. Surfactants are commonly used to aid carbon based materials dispersion in aqueous solutions. We specifically focused on hyaluronic acid (HA), which has been shown to disperse GO due to its electrostatic charge: at buffer pH of 7-8, HA is anionic and provides an electrostatic hindrance which causes a repulsion between GO aggregates, effectively breaking it into smaller ones. Additionally, as a major component of extracellular matrix, HA is biocompatible and promoting tissue repair. The presence of HA did not hinder crosslinking process, which was confirmed by mechanical testing. To confirm homogenous GO dispersion in PEG matrix, we examined GO density as a function of hydrogel depth. Figure 2 shows a phase contrast image of GO-PEG composite divided into 5 equal sections. We estimated that there was a similar number of GO aggregates within each section (around

300) with an average area of around 70 μm^2. Therefore, we conclude that we successfully distributed GO throughout the hydrogel. Prepared composite materials were first tested for mechanical properties and swelling behavior. All composite samples retained GO in the network after reaching equilibrium for prolonged immersion times (5-7 days).

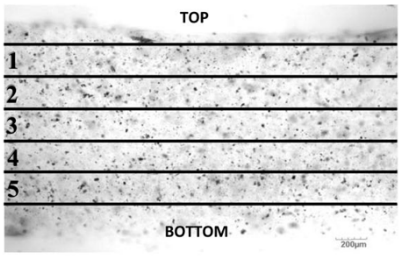

Fig. 2. GO distribution within GO/PEG composite as a function of hydrogel depth

Although GO content had negligible impact on the time required to reach swelling equilibrium (~12 hours), equilibrium swelling ratio decreased gradually with increasing GO content. This can be attributed to the covalent attachment of GO sheets to tetra-PEG amine pre-polymers that increases the crosslink density of the network and possibly the formation of network defects. This result is supported by the increase in elastic modulus of GO/PEG composites with increase of GO content.

Prepared GO/PEG hydrogels were first used to investigate the influence of GO inclusion on the migration charged molecules under electric field. First we prepared GO/PEG hydrogels using two combination of mutually reactive tetra-PEG pre polymers: amine and activated NHS ester (gelation time ~10 min). Electrophoretic mobility of dsDNA up to 3.5 kbp was independent of GO concentration in system with 1:1 molar ratio of tetra-PEG pre-polymers (Fig. 3(A)), indicating that dsDNA does not feel additional constraints from GO sheets incorporated into network. Therefore, we deemed to decrease the effect of the main network by employing incorporating of GO sheets into critical gel, which is formed from 2 types of mutually reactive clusters prepared near critical gelation point. In the case of critical gel, the concentration of tetra-PEG network can be decreased to as low as 6 gL^{-1} (Fig. 3(B)). Decrease in main network concentration shows clear difference in mobility of both short and long dsDNA in our experimental conditions, comparing to control sample. In addition, separation resolution was considerably improved in GO/PEG hydrogels.

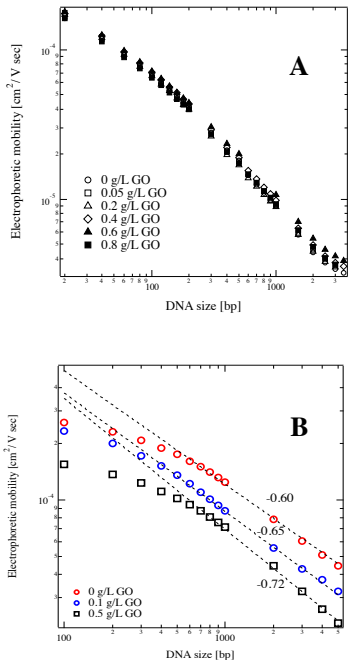

Fig. 3. Electrophoretic mobility of dsDNA in tetra-PEG gel doped with GO, prepared from equal stoichiometric ratios of pre polymers (A) and using critical clusters (B)

The doping of GO in tetra-PEG gel resulted in the significant increase of the shift distances of both the single DNA fragment and the adjacent DNA fragments. The increased shift distance of DNA fragments could be attributed to excellent conductivity of GO, promoting the electrophoresis rate of DNA fragments. The improved separation resolution for DNA fragments could be attributed to the successive adsorption-desorption processes between the surfaces of GO sheets dispersed in the gel network and DNA fragments

with different length.

Considering profound decrease in mobility of small dsDNA, mobility of charged small molecular weight compounds was investigated. Figure 4 shows electrophoretic mobility of 2 fluorescent dyes in GO doped tetra-PEG gels with different GO content. Mobility of dyes discontinuously decreased with increase in GO content. Clear crossover in slope values from -0.25 to -0.75 was observed for both dyes on GO content of around 0.1 g/L. Our viscosity measurements indicated that this crossover concentration is attributed to the overlapping concentration of GO sheets in buffer solution at pH 7.4. Electrophoretic mobility of both dyes decreased 10 times with adding of 1 g/L GO, which can at first be attributed to strong electrostatic interactions between GO sheets and dye molecules. On the other hand, incorporation of negatively charged polystyrene (PS) particles in the concentrations equal to that of GO sheets showed that electrophoretic mobility of charged dye was independent of negatively charged PS beads, suggesting that planar shape of GO sheets plays prominent role in retarding migration of dyes (Fig.5). Therefore, we checked adsorption of dyes on GO to understand the mechanism of retardation.

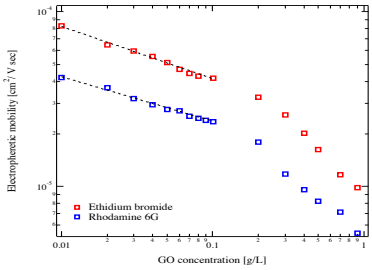

Fig. 4. Mobility of ethidium bromide and rhodamine 6G as a function of GO content

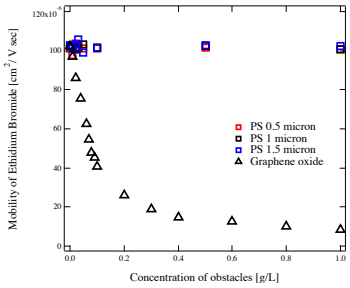

Fig. 5. Effect of incorporation of charged PS beads of different size and GO on mobility of small dye

First, we observed that upon addition of GO to the R6G solution, the fluorescence intensity reduced significantly due the adsorption of R6G molecules on the GO surface and the quenching of R6G fluorescence. Langmuir isotherm yielded a straight line that indicated the Langmuir behavior. From this data, we estimated the free energy of adsorption of R6G−GO complex (ΔG) to be −2.3 kJ/mol. Since ΔG is negative and low, we can conclude that the R6G adsorption process is spontaneous and the adsorption is caused by weak molecular interactions.

Conclusions

Novel electrophoresis strategy has been developed for separation of DNA fragments using GO-doped tetra-PEG gel. The doping of GO resulted in the significant increase of resolution of both the single DNA fragment and the adjacent DNA fragments. This increase in DNA resolution could be attributed to the successive adsorption-desorption processes between the surfaces of GO sheets dispersed in the gel network and DNA fragments. In addition, electrophoretic mobility of small molecules was reduced 10 times by adding a small amount of GO in the gel network. This indicates that inclusion of GO into the network can decrease diffusion rate of charged compounds and lead to new strategies in drug release systems design. This work is first study that investigate the effect of GO-doping on electrophoresis of biomolecules and small molecules which shows potential for separation and detection of DNA fragments and other charged molecules.