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Carbon nanotube-modified sodium dodecyl sulfate–polyacrylamide gel electrophoresis for molecular weight determination of proteins

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ABSTRACT

The effect of incorporating carbon nanotubes (CNTs) in the gel matrix on the electrophoretic mobility of proteins based on their molecular weight differences was investigated using sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). More specifically, a reduction in standard deviation in the molecular weight calibration plots by 55% in the case of multiwalled carbon nanotubes (MWCNTs) and by 34% in the case of single-walled carbon nanotubes (SWCNTs) compared with that of pristine polyacrylamide gels was achieved after incorporating an insignificant amount of functionalized CNTs into the gel matrix. A mechanism based on a more uniform pore size distribution in CNT modified polyacrylamide gel matrix is proposed. Furthermore, the impact of SWCNTs and MWCNTs on the mobility of proteins in different molecular weight regimes at a given acrylamide concentration offers a tunable gel matrix in terms of the selection of molecular weight ranges of proteins. The robustness and excellent reproducibility of the CNT–PAGE protocol are expected to have a significant impact on the molecular weight determination of newly isolated proteins.

Carbon nanotubes (CNTs)[2] have found tremendous applications in a number of domains ranging from electronics to space technology. In particular, they are among the most investigated nanomaterials for the separation of DNAs and proteins due mainly to their uniquely selective adsorptive power toward these biomolecules [1,2]. More significantly, the adsorptive power of CNTs has been deployed at different levels of proteomics. For example, CNT–peptide conjugates have been employed to selectively destroy the anthrax toxin [3]. On the other hand, CNTs have been used as the stationary phase for protein separation using capillary electrophoresis [4]. Tunable diameter and flexibility for controlling hydrophobic–hydrophilic character and ease of functionalization (by covalent, electrostatic, and other interactions) to create desired biocompatible functional groups in a selective manner, both at the tips and along the sidewalls, are additional advantages of CNTs for these applications.

In view of their compliance with many polymers and specific interactions with biomolecules such as DNA and proteins, CNTs can be combined with selected polymers as matrices for enhancing the efficiency of the electrophoretic separation of proteins. Polyacrylamide (PAM) is one of the most widely studied polymers for protein separation in a technique, commonly called polyacrylamide gel electrophoresis (PAGE) [5]. It briefly involves the separation of proteins and peptides in a porous gel matrix, which offers a sieving action in the presence of an external electric field. Protein separation by PAGE is normally based on the differences in charge density, size, and shape of the protein molecules, which is commonly referred to as native PAGE. However, the effect of charge density and shape could be virtually eliminated by denaturing the proteins by a suitable surfactant, namely sodium dodecyl sulfate (SDS), so that the separation is based exclusively on their molecular weight (referred to as SDS–PAGE). In this technique, prior to electrophoresis, the protein mixture is treated with SDS, which coats all proteins in proportion to their mass (1.4 g SDS/1.0 g protein) and ensures that they all have a net negative charge on the surface. Because the SDS-coated proteins assume linear structures such as rods and random coils, a linear relationship between their molecular weight and the logarithm of their relative electrophoretic mobility (R_f) is achieved at a given gel

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[†] Dedicated to the memory of our beloved colleague, Dr. Islam Khan, who passed away on November 12, 2010.
[2] *Abbreviations used:* CNT, carbon nanotube; PAM, polyacrylamide; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate; MWCNT, multiwalled CNT; SWCNT, single-walled CNT; CVD, chemical vapor deposition; sccm, standard cubic centimeter per minute; TEMED, *N,N,N′,N′*-tetramethylethylenediamine.

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concentration, so that the proteins are separated based on their sizes [5,6]. More significant, this procedure is found to be more precise and reproducible than the contemporary techniques such as gel filtration for the molecular weight determination of proteins [5,7].

A number of attempts have been made to improve the efficiency of protein separation by SDS–PAGE and also to achieve unanimous applicability in the whole range of protein size from a few to several hundred kilodaltons. It is particularly challenging to separate low-molecular-weight proteins (1–100 kDa) [5]. This limitation arises from the "pores" (defined as the separation between two successive crosslinks) in a PAM gel not being uniform in size (ranging from 0.5 to 3 nm) and shape [5,8]. The pore size is found to follow a slightly skewed non-Gaussian distribution, as explained by the Ogston theory of gel electrophoresis, which considers the gel as a random meshwork of fibers [9,10]. Recently, gel electrophoresis has been used successfully to separate gold nanoparticles (15 ± 2.7 nm) loaded in an agarose gel based on the differences in their size and shape [11]. In this context, composites of PAM with high-aspect-ratio nanomaterials could be essentially effective for protein separations owing to the unique features of the latter such as flexibility due to self-organization and dynamics of assembly, adsorption, and percolation behavior in the polymer matrix [12]. For example, multiwalled CNT (MWCNT) modified PAM gels have been used for the separation of apolipoprotein and complement C3 of human serum using native PAGE [13]. However, the above study employed a very high loading of the nanotubes (up to 0.1 wt.%) in the gel matrix and used the differential adsorption of the proteins on CNTs. Nonetheless, CNT matrices with very low loading could work well for SDS–PAGE because the SDS-denatured proteins might not interact with CNTs as strongly and specifically as do the native proteins, although the SDS added to the buffer solutions (anode as well as cathode buffers) may have subtle adsorptive interactions with the nanotubes present in the gel matrix by virtue of its long alkyl (C_{12}) chain [14]. In addition, SDS is capable of displacing proteins adsorbed on CNT surfaces to ensure that the nanotubes affect protein separation mainly by altering gel morphology instead of by interacting with the proteins directly through their surface functional groups [15].

Here we report a systematic approach to separate proteins of sizes ranging from 14 to 100 kDa with unprecedented efficiency based purely on the differences in molecular weight using CNT/PAM composite gels in SDS–PAGE. Furthermore, we compare the protein separation efficiency of single-walled CNT (SWCNT) and

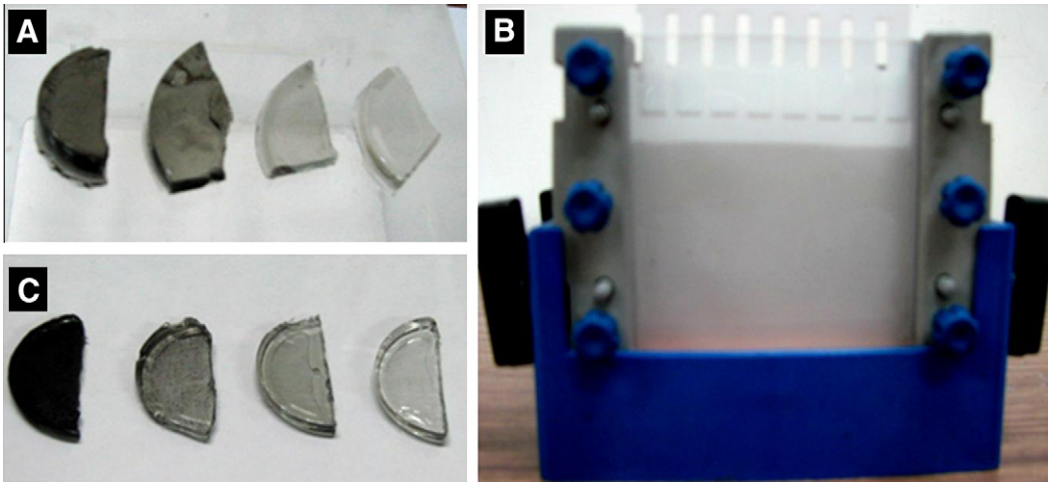

Fig.1. (A) SWCNT/PAM gels (composition [wt.%] from right to left): 0.0015, 0.0025, 0.005, and 0.01%. (B) Homogeneous SWCNT/PAM gel in the casting unit before electrophoresis. (C) MWCNT/PAM gels (composition [wt.%] from right to left): 0.0015, 0.0025, 0.005, and 0.01%.

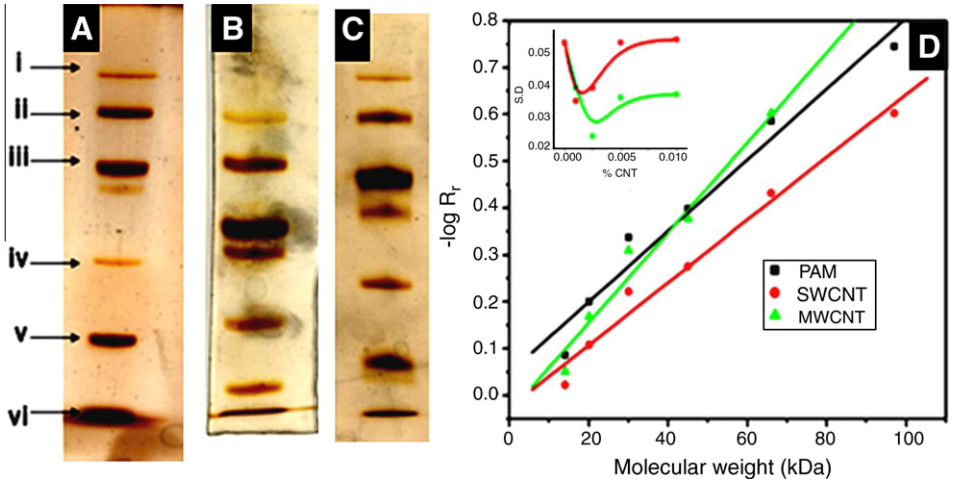

Fig.2. Separation of 14- to 97-kDa marker proteins by electrophoresis in PAM gel (12% T) (A), SWCNT/PAM composite gel (B), and MWCNT/PAM composite gel (C), each with 0.001 wt.% CNT content, after silver staining. The protein mixture consists of the following: (i) phosphorylase *b* (97 kDa); (ii) albumin (66 kDa); (iii) ovalbumin (45 kDa); (iv) carbonic anhydrase (30 kDa); (v) trypsin inhibitor (20.1 kDa); (vi) lactalbumin (14.4 kDa). (D) Variation of R_f values (logarithm) of the individual proteins with their molecular weights. The inset shows the dependence of standard deviations from linearity in these plots on CNT content for both SWCNTs and MWCNTs.