Polymer coat improves the detection ability of sensing enzymes

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Coating carbon nanotubes with a polymer membrane facilitates the homogenous immobilization of enzymes on their surface to form compounds used as biosensors.

Carbon nanotubes (CNTs) have become very popular due to their many applications as sensors, biomedical devices, and in electronics.\textsuperscript{1–4} Recent research efforts in the field have focused either on developing completely new nanoscale sensors\textsuperscript{4} or on improving macroscale devices using functionalized CNTs, for example, by immobilizing enzymes on their surface. The role of each enzyme determines its ability to detect substances of interest. For example, carbonic anhydrase recognizes oxygen (O\textsubscript{2}) and carbon dioxide (CO\textsubscript{2}). At the molecular level, the functionalization process involves the formation of one or more covalent—i.e., strong—bonds between CNT surface amino groups and those of enzymes using the compound cyanuric chloride as linker. Commercially available CNTs come pretreated with surface amino groups. However, depending on the method used, enzymes are not always distributed in a homogenous manner. This is problematic as it affects the quality of biosensors.

Currently, functionalization of CNTs is carried out in solution or in the solid state,\textsuperscript{5} where plasma treatment is used to deposit carboxylic acid groups on the nanotube surface. A disadvantage of this method is that it prohibits addition of any other functional groups, such as enzymes, which require different reaction conditions. Moreover, when working in solution, one of the biggest challenges is to prevent nanotubes from aggregating to avoid nonhomogenous surface modification, which affects the reliability of measurements. We have developed a new method of functionalizing CNTs in solution using a membrane reactor made of polysulfone, a polymer known for its toughness and stability at high temperatures.\textsuperscript{6}

![Fourier-transform IR (FT-IR) spectra](image)

Figure 1. Fourier-transform IR (FT-IR) spectra of (a) a polysulfone (PS)-carbon nanotube (CNT) composite membrane, (b) a PS-CNT composite membrane with a cyanuric chloride linker and functionalized with three enzymes, including (c) a diastase, (d) an invertase, and (e) a carbonic anhydrase.

To ensure homogenous immobilization of enzymes, we first coated nanotubes with a porous membrane made by dispersing them in a polysulfone solution using a precipitation technique known as phase inversion-immersion.\textsuperscript{7} The CNTs were then functionalized with different enzymes. These included O\textsubscript{2} and CO\textsubscript{2} to detect carbonic anhydrases, and enzymes that recognize specific sugars such as invertases and diastases. In this process, cyanuric chloride played the role of a linker, reacting with amino groups on both the nanotubes and the enzymes. Finally, the functionalized CNTs were isolated by dissolving the membrane at the conclusion of the reaction.

Using the composite made of the polysulfone membrane attached to CNTs as a reactor facilitated the dispersion and access of enzymes to the amino groups on the CNT surface. The

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membrane pores aided transport of the enzymes by diffusion thanks to their conical shape, which created a small pressure difference between the two sides of the polysulfone coating. In addition, phase inversion-immersion enabled us to adjust the size of the pores to values that promoted diffusion.

To test the effectiveness of our method, we first analyzed the membrane-CNT composite using Fourier-transform IR (FT-IR) spectroscopy: see Figure 1(a). Except for the specific bands attributed to the polysulfone and the amino groups from the nanotubes, no other absorption band was observed (i.e., there was no chemical interaction between the membrane and the nanotubes). We then established the FT-IR spectrum of CNTs with cyanuric chloride linkers—see Figure 1(b)—and used that as a baseline for comparison with the spectra of functionalized CNTs: see Figure 1(c, d, and e). We analyzed the modifications of the FT-IR spectrum before and after enzymes were attached to the nanotubes.

The most important absorption bands at 1150, 2870, and 3210cm\(^{-1}\) were attributed to the sulfur dioxide (SO\(_2\)) groups, the methyl (CH\(_3\)) groups, and the aromatic rings from the polysulfone, respectively. The band at 1620cm\(^{-1}\) was attributed to the amine (NH\(_2\)) of the CNT amino group. After functionalization, the absorption band at 1620cm\(^{-1}\) disappeared, and a large band with a maximum at 1493cm\(^{-1}\) appeared. We concluded that it resulted from the formation of an NH group, a byproduct of the reaction binding amino groups of the enzymes to those of the CNTs mediated by the cyanuric chloride linker: see Figure 2 (top). A wide band at 1697cm\(^{-1}\) also appeared, which we assumed was nitrogen (N) from the aromatic ring of cyanuric chloride.

To confirm that the polysulfone coating used as a reactor was indeed responsible for homogeneous enzyme distribution on nanotubes, we examined the membrane using scanning electron microscope (SEM) imaging. We first covered the sample with a thick layer of gold to protect it from destruction during the imaging process. The SEM image showed a uniform membrane surface structure: see Figure 2(a). A closer look revealed its asymmetry, with the average diameter of pores—see Figure 2(b)—ranging from 10nm to between 1.2 and 2\(\mu\)m. This means that the pores were wide enough for the cyanuric chloride linker (measuring 5–6 Å) and the enzymes (150–200 Å) to access the reaction centers.

These results suggest that our method can be used to produce biosensors based on functionalized CNTs in two ways, i.e., with or without the polysulfone coating. If retained, the conical shape of the membrane’s pores protects the enzymes in applications where they are exposed to adverse conditions, such as oxidative media or those with extreme values (acid or alkaline) of pH. Next, we plan to develop methods of manufacturing functionalized CNTs on a large scale to produce commercial biosensors.

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**References**