STATISTICAL OPTIMIZATION OF CARBON NANOTUBE PRODUCTION BY DS-CVD AND ITS APPLICATION IN PROTEIN PURIFICATION

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Introduction

Ever since Carbon Nanotubes (CNTs) discovery by Iijima [1] in 1991, they have been treated as the most promising nanostructured materials. The prospect of developing novel carbon-based nonmaterial has excited worldwide interest among researchers. CNTs have been of great interest; both from fundamental point of view and for potential applications because of their amazing mechanical, thermal, electric and magnetic properties [2, 3]. Various methods to grow CNTs have been developed, including laser ablation, arc discharge and Chemical Vapor Deposition (CVD). In this study, CNTs were produced using double stage CVD (DS-CVD) with acetylene (C_2H_2) and hydrogen (H_2) as the precursor gasses. Statistical optimization of the process parameters such as reaction temperature, reaction time, and gas flow rate of C_2H_2 and H_2 was carried out. Produced CNTs were submitted for purification to remove unwanted impurity such as catalyst via oxidation with nitric acid and sulfuric acid after which they were functionalized. These functionalized CNTs as well as the unfunctionalized batches were used as column chromatographic media to purify protein. It has been reported that the purification of CNTs by acid washing creates open end termini in the structure that are stabilized by –COOH and –OH groups left bonded to the nanotube at the end termini and/or the sidewall defect sites . Carboxylic group can also be introduced at the tube surface which can covalently bind proteins. This can be carried out via a two-step process of diimide-activated amidation between the carboxylic acid groups on the surface CNTs and amine groups on proteins. Thus, it makes perfect sense to employ carbon nanotubes as a support to purify proteins or enzymes

In this study we attempted to use CNTs to purify proteins sourced from skim latex serum. Skim latex is produced as a byproduct during the preparation of latex concentrate which is obtained upon centrifugation. It contains a dry rubber content of only 3 to 7 % with very low dirt content. Skim latex serum is the non rubber aqueous portion of latex which can be obtained via acid coagulation or membrane filtration. The serum contains a rich source of nitrogen, carbohydrates, proteins, lipids and trace metals. Some of these proteins are important enzymes which has great demand in pharmaceutical, food and detergent industries. Hence, there is need in art to improve the efficiency and yield of protein separation [4].

Experimental

Material

Three types of gases were used namely H_2 (99.99% purity) and C_2H_2 (99.9%) and Ar (99%). Ferrocene was used as the catalyst. Skim latex was supplied by Malaysian Rubber Board, Sungai Buloh, and Selangor.

Apparatus and Procedure

Experimental design and analysis of the optimization process of CNTs production was conducted at 4 levels Full Factorial Design using Design Expert® Version 6.08 software. The process parameters included in the experiments were reaction time, reaction temperature and flowrates of the two gasses used H_2 and C_2H_2. Production was carried out on hot-wall DS-CVD reaction chamber. A ceramic boat was placed 40 cm ahead of the furnace center and ferrocene catalyst was placed at the center of first furnace. The system was flushed with Ar in order to ensure oxygen free environment. In the mean time, the second furnace was heated to the desired reaction temperature. Heating was continued until the steady state was achieved. Ar flow was then stopped and heating of first furnace till 150°C was initiated. The flow for C_2H_2 along with H_2 was immediately opened. The reaction was carried on for the desired time period and on completion, the total amount of CNTs produced in the boats and walls of the second furnace were weighed. They were then acid purified, functionalized and characterized using SEM (JEOL JSM 6700F). As much as 2.0 g of functionalized and unfunctionalized CNTs were loaded into the column for chromatographic processes. Preparation of skim latex serum from skim latex, before being introduced into the column involved acetic acid coagulation, centrifugation and dialyzation against the running buffer.

Results and Discussion

For the statistical analysis, results from the analysis of variance (ANOVA) shows that the F-test is less than 0.05, showing the model is significant. In this design, the value of the correlation coefficient, $R^2 = 0.98$ and the value of the adjusted determination coefficient, $R^2_{(Adj.)}=0.97$, thus considered high indicating a high significance of the model.

Yield=+149.06+42.81*A+25.31*B-10.94*C-13.44*D +7.81*A*B-4.69*A*C-8.44*A*D-5.94*B*C+4.0*C*D

Equation (1)

where, the response is yield of CNTs, A is the coded value of reaction temperature, B is the coded value of reaction time, C is the coded value of H_2 flowrate, and D is the coded value of C_2H_2 flowrate. All terms are included in the model to give the optimum fit of the experimental data. **Equation 1** was used to predict the output of CNTs yield and compared with observed values as shown in **Figure 1**. **Figure 2** shows that the reaction time increases with respect to the increase in temperature. **Figure 3** shows a FSEM image of the produced CNTs which have diameters in a range of 30 to 35 nm and achived 95% purity. **Figures 4 and 5** show the protein separation profile on both unfunctionalized and functionalized CNTs.

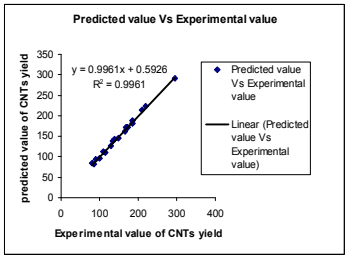

Figure 1. Relationship between predicted and experimental value of CNTs

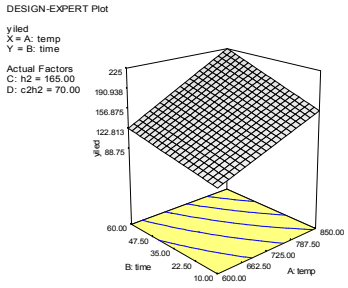

Figure 2. A 3-Dimensional structure of the interaction plot between process parameters

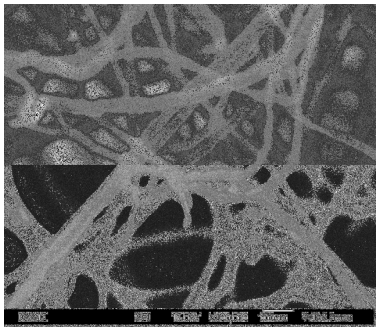

Figure 3. FSEM image of CNTs at 850°C

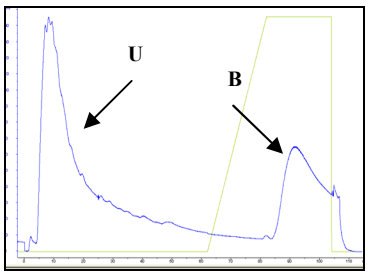

Figure4. Chromatogaraphic protein profile on unfunctionalized CNTs; U-unbound protein, B-bound protein

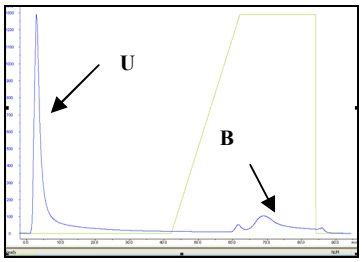

Figure 5. Chromatographic protein profile on functionalized CNTs; U-unbound protein, B-bound protein

Conclusion

The statistical analysis reveals that the optimized process parameters that give the best yield are at 60 mins reaction time, 850°C reaction temperature and the flowrated of C_2H_2 and H_2 at 150 and 40ml/min respectively. These produced CNTs were purified and functionalized. Both unfuctionalized and functionalized CNT were successfully used as column chromatographic media. Functionalized CNTs behaves like ion exchange chromatography (IEC) matrix, whereas unfunctionalized CNTs behaves like hydrophobic interaction chromatography (HIC) matrix. Purification of protein from skim latex serum mainly depends upon the pH and the ionic strength of the running buffer.

Reference

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