

## **CHAPTER 5 CONCLUSIONS AND RECOMMENDATIONS**

### **5.1 Conclusions**

The aggregation of the carbon nanoparticles was studied. The storage time has been shown to affect the aggregation of the carbon nanoparticles, leading to larger size particles, but only up to 30 days after sonication. The average size of the carbon nanoparticles was quite similar after a month of storage. Nonetheless, the sizes of the carbon particles were still smaller than the pore size of nitrocellulose membrane. Therefore, the aggregation of carbon should not affect the movement and the intensity.

In this study, all the experiments were conducted on a nitrocellulose membrane. The nitrocellulose membrane was firstly spotted by NGAL sample and then blocked with BSA solution. Carbon nanoparticles were used as a label for this assay. Three hundred and fifty  $\mu\text{g/ml}$  of monoclonal anti-NGAL antibody was conjugated to the 0.2% carbon nanoparticles with 90% conjugation efficiency. After that, in the first method, antibody-carbon conjugate solution was spotted on the membrane at 2  $\mu\text{l/spot}$ , while the alternative was to dip some part of the membrane into 20  $\mu\text{l}$  of antibody-carbon nanoparticle conjugate and incubated at room temperature. The first method, which the antibody-carbon conjugate solution was spotted directly, gave clearer and better results than the second method, which the antibody-carbon conjugate solution was allowed to flow through the membrane. The results of monoclonal anti-NGAL antibody testing could not indicate the presence of NGAL protein. In the same way, the results of human lipocalin2 NGAL biotinylated antibody testing could not indicate the presence of different concentrations of NGAL protein also which was shown in term of the color intensity of each spot.

### **5.2 Recommendations**

Although the new antibody which was more sensitive and specific was replaced, the results were still unsatisfactory. Maybe the carbon conjugate is not suitable for this membrane system because of its stain. Therefore, a gold label would be more appropriate to be used in the lateral flow system.