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The effect of ether treatment of influenza virus on the efficiency of chitosan nanoparticles encapsulation

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Introduction: The researches have shown that the potential of virus antigen encapsulation in Chitosan Nanoparticles is higher and more efficient than the whole virus. Hence in this study, the effect of Ether treatment of A/H9N2 sub type of Influenza virus on Efficiency of Chitosan nanoparticles encapsulation was investigated.

Methods: A/H9N2 Virus was purified from allantoic fluid by Sucrose gradient (20-60%). Then, the part of purified virus was mixed by Tween- 80- 10% for 5 min. This mixture was combined with equal volume of ether and incubated in 4°C. After 15 min, the upper layer that contain ether and virus envelops were eliminate and the virus capsid and spikes were collected from lower. This solution was evaluated by SDS-PAGE and Hemagglutination assay (HA). Ion gelation method with Na-Tripolyphosphate (Tpp) anions as cross linker was used to prepare the antigen-loaded nanoparticles. Influenza A/H9N2 ether treated and un-treated was incorporated separately in the Na-Tpp solution. After antigen encapsulation, physicochemical properties of nanoparticles such as: Morphology, particle size and zeta potential were investigatedwith photon correlation spectroscopy (PCS) and Laser Doppler Anemometry (LDA) using a Zetasizer 3000-HS (MalvernIns., UK) respectively. Finally, the loading efficacy, loading capacity and Ag- releasing efficacy of them were evaluated by HA and Nano drop.

Results: The SDS-PAGE results show that Albumin was eliminated from virus solution. These results also reveal that treatment of Influenza virus by ether were take place with proper quality. So, the efficiency of virus purification and ether treated was confirmed by SDS-PAGE. The particle size and zeta potential of Cs nanoparticles were similar for treated and untreated viruses. The loading efficacy of treated and un-treated virus loaded nanoparticles was 15 and 10HAU respectively and the Nano drop results of *in-vitro* releasing for treated virus loaded nanoparticles was more than the untreated.

Conclusions: In this study it was found that ether treatment is a successful method for influenza spikes purification. The ether-treated antigen was far superior to the untreated antigen in loading efficacy and antigen releasing yield; although there was no significant difference in morphological and physicochemical properties of both. Therefore, ether treatment method could be proposed as a suitable and affordable manner to encapsulation improvement of enveloped virus in chitosan nano particles, such as influenza virus.

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