INFLUENZA VIRAL PROTEIN ISOLATION AND DETECTION USING QUANTUM DOTS

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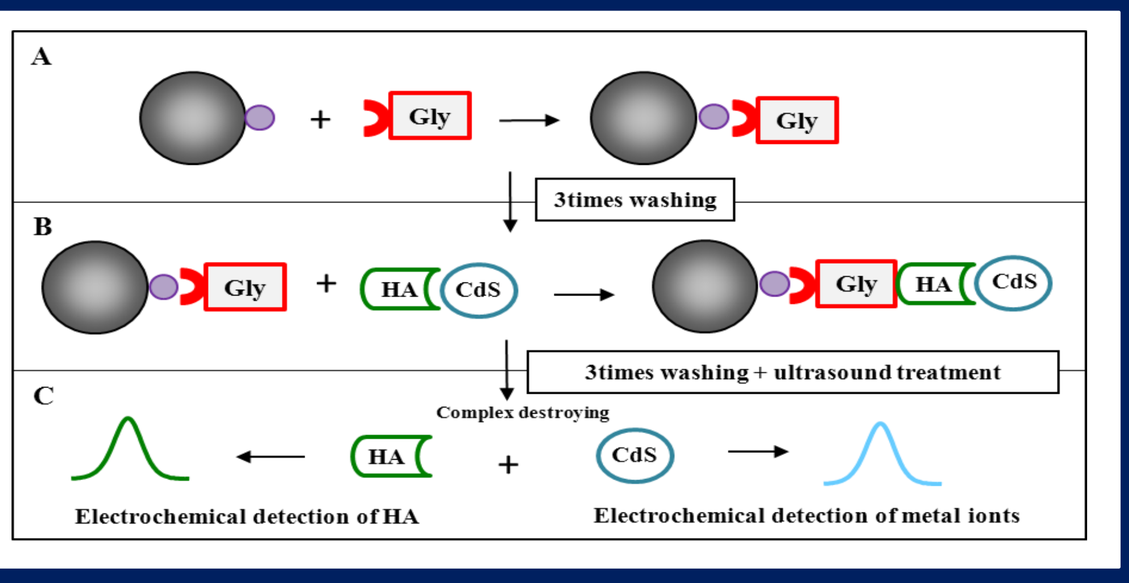


Fig. 1: Scheme of a fully automated method of detection H5N1 hemaglutinine (HA. (**A**) Biotinylated glycan binds to streptavidin modified MPs, (**B**) HA-CdS complex binding on glycan conjugated MPs (**C**) releasing of HA and cadmium(II) using ultrasound and their electrochemical detection.

1. INTRODUCTION

According to the World Health Organization (WHO), seasonal influenza require between 250 000 and 500 000 deaths each year [1] and is considered one of the most powerful potentionalagents.

There are three genera of the influenza viruses: Influenza virus A, B and C. Type A virus, the most virulent causes and can be subdivided into different serotypes. The influenza virus contains two surface antigens: hemaglutinin (HA) and neuraminidase (NA). HA mediates glycan receptor binding and initiate infection. NA is important for virus release[2]. Subtypes of influenza viruses are classified according to these antigens [3, 4]. More than 500 cases of avian H5N1 influenza infections in humans have been reported, of which nearly 60 % have resulted in death. A portable and standardized approach for large scale studies is examined. Electrochemical biosensors and bioassays have attracted considerable interest due to its high performance, miniaturized construction and low cost [5]. Quantum dots (QDs) have numerous possible applications in biosensing and can be applied as electrochemical targets.

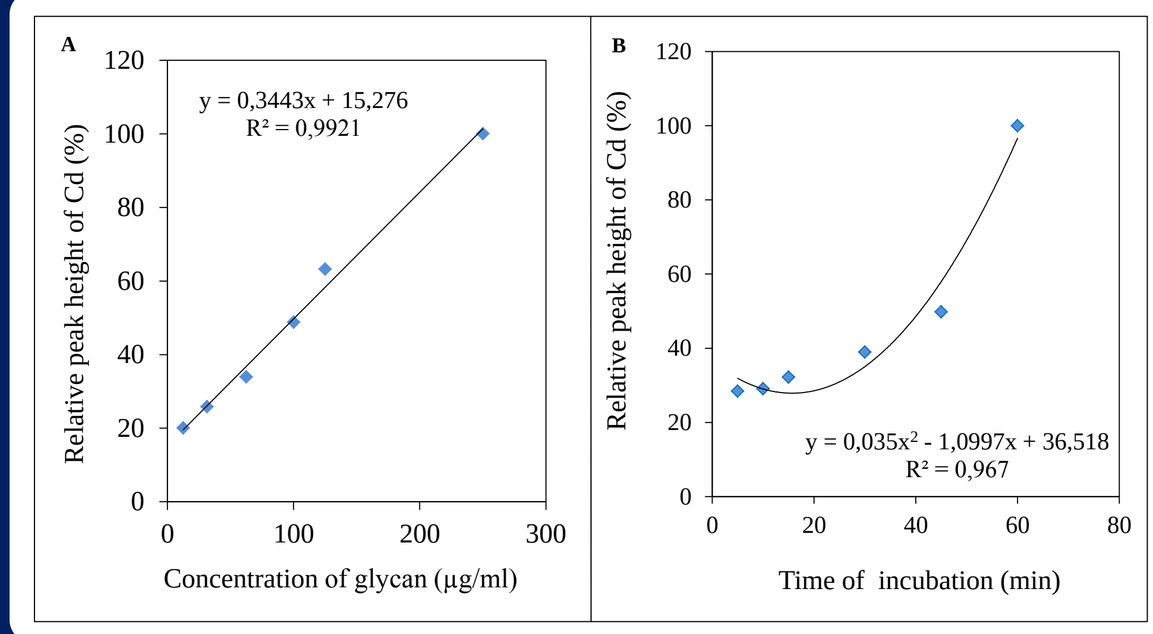
This study aimed to develop low cost and rapid MPs based isolation. Viral quantity is determined through CdS QDs using differential pulse voltammetry (DPV). The results are confirmed by Brdicka reaction. Particular attention was focused on the application of streptavidin coated paramagnetic particles (MPs) modified by biotinylated glycan and its selective binding properties to the influenza HA.

2. EXPERIMENTAL SECTION

CdS quantum dots (QDs) were prepared using a slightly modified version of a published method [6]. Prepared CdS were used for labeling of influenza HA: A/H5N1/Vietnam/1203/2004 (Prospec-Tany TechoGene Ltd., Tel Aviv, Israel). This step was folowed by automatic separation: Streptavidin Dynabeads M-270 (10 µl) was pipetted to microplates (PCR 96, Eppendorf), then subsequently transferred to a magnet. Modified MPs were washed three times with phosphate buffer (PBI). Biotinylated glycan were added to each of the wells and incubated (30 min, 25 ° C, 400 rpm). After incubation, samples were washed three times with PB1. Subsequently HA-CdS was added. It was further incubated (30 min, 25 °C, 400 rpm) and washed with PB1. Than Phosphate buffer 2 was added, followed by the treatment of ultrasound needle (2 min). The plate was transferred to the magnet and the supernatant was measured by DPV.

HA protein was measured by DPV method, using 747 VA Stand instrument connected to a 693 VA Processor and 695 Autosampler (Metrohm, Switzerland). It was equipped with a standard cell consisting of three electrodes, a cooled sample holder and cell (4 °C) (Julabo F25, JulaboDE, Seelbach, Germany). Brdicka supporting electrolyte containing 1 mM $Co(NH_3)_6Cl_3$ and 1M ammonia buffer (NH_3(aq) + NH_4Cl, pH = 9.6) was used. The parameters were: initial potential of -0.7 V, end potential of -1.75 V, modulation time 0.057 s, time interval 0.2 s, step potential 2 mV, modulation amplitude -250 mV, E_{ads} = 0 V.

Determination of Cd by DPV was performed using a 663 VA Stand (Metrohm) and a standard cell with three electrodes. GPES 4.9 software was employed for data processing. Acetate buffer (0.2 M CH_3COONa + CH_3COOH, pH 5) was used as a supporting electrolyte. The parameters were as follows: purging time 120 s, deposition potential -0.9 V, accumulation time 240 s, equilibration time 5 s, modulation time 0.057 s, interval time 0.2 s, initial potential -0.9 V, end potential -0.3 V, step potential 0.00195 V, modulation amplitude 0.02505 V, sample volume: 10 µl, cell volume: 1 ml (10 µl of sample; 990 ml acetate buffer).



3. RESULTS AND DISCUSSION

Transmission of influenza virus into the human population occurred due to changes in hemaglutinin (HA) and their glycan receptors. Glycan-HA interaction can be used as model for the virus isolation [7]. Current studies attempted to design a biosensor, which consists of glycan modified based isolation of HA labelled by CdS and subsequent electrochemical detection of target (complex: HA-QDs). Scheme of procedure shows Fig. 1. Isolation proces was followed by electrochemical analysis. DPV method was utilized for quantification of CdS from HA-QDs complex. Studies attempted to detect the complexe by Cd (II) ions using DPV. The following protein concentrations (from 0.5 to 45 µg/ml) were applied to CdS QDs. Typical dependence of Cd(II) concentration on protein concentration incubated with CdS QDs and measured at HMDE is shown in(Fig.2 A). In addition to detecting Cd(II), the presence of HA was investigated. DPV - Brdicka reaction, was utilized to detect Cd QDs-protein complex [8, 9]. The reaction mechanism is based on the catalytic evolution of hydrogen on mercury electrodes using Brdicka solution [10]. The signal increased with increasing HA concentrations, which is shown in (Fig.2 B). The detection limit of HA was 100 ng/ml.

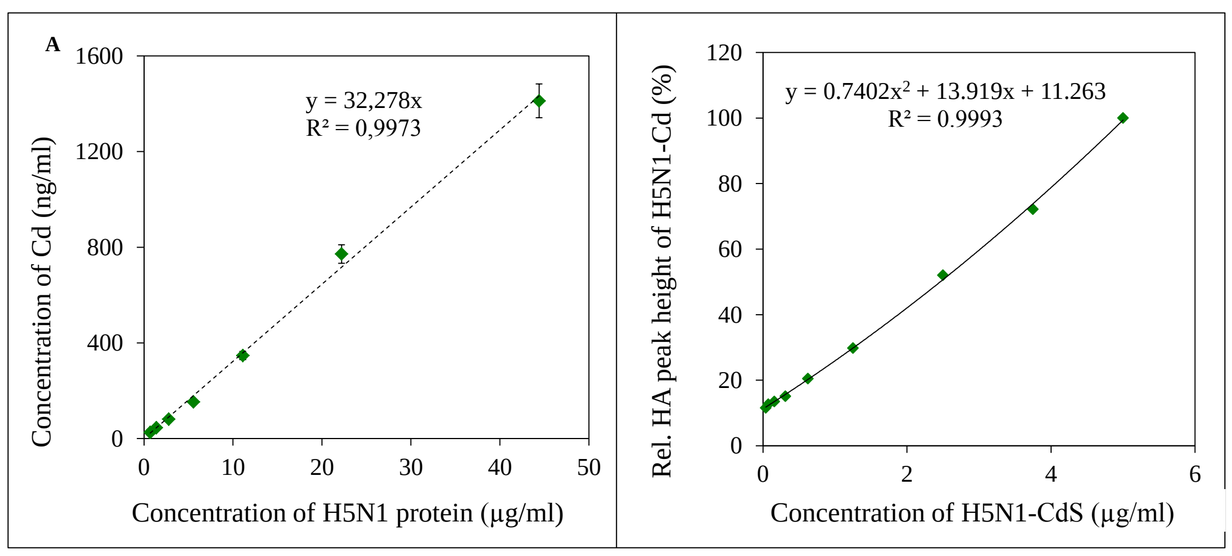


Fig. 2: (**A**) Dependence of cadmium concentration (related to protein) on various concentrations of complex H5N1-CdS. (**B**)Dependence of H5N1-CdS peak on applied concentration of HA; inset: voltammograms of protein peak measured at -1.55 V. Volume of measured sample was 5 µl. For other experimental part.

Fig. 3: Isolation of complex H5N1-CdS (5 µg/ml) on glycan conjugated MPs. Influence of (**A**) glycan concentration and (**B**) time of interaction on measured cadmium signal; inset: cadmium peaks. For other experimental conditions see Experimental section.

It was designed and implemented method for sensitive selection and determination of CdS labelled H5N1 viral protein. Streptavidin modified MPs were mixed with biotinylated glycan, on glycan conjugated MPs was bounded HA-CdS complex. described in Fig. 1. The isolation products were analysed using electrochemical methods (Fig. 1C). Selected components and its effect were tested to optimization of isolation proces. The influence of varying concentrations of glycan on the amount of isolated HA-CdS complex was investigated. Changes in Cd(II) ions peak height showed increasing dependency related on the concentration of biotinylated glycan. Maximum response gives 250 µg/ml of glycan (Fig. 3A). Maximum capacity of MPs was not achieved until concentrations reached 1 mg/ml, but this is economically disadvantageous. Increasing the surface coverage of coated MPs can also be achieved by imposing longer interaction time. From 30 to 60 minutes incubation (up to 60 %) show a dramatic increase of signal as shown in Fig. 3B.

4. CONCLUSIONS

Effective routine surveillance may be impossible in countries lacking basic public health resources. For a prevent of pandemic spread to be successful, low- cost, easy-to- use method is vital. We described a rapid quantum dots labelled HA detection method using automated isolation by glycan modified MPs. This method can be extended for detection of specific proteins of other pathogens or cancer markers.

Acknowledgement ***Financial support by NanoBioMetalNet CZ.1.07/2.4.00/31.0023 is highly acknowledged.***

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