DETECTION OF INFLUENZA VIRUS OF H5N1 SUBTYPE

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ABSTRACT

In spite of the fact that cadmium is considered as a toxic metal can be used in numerous biotechnological applications. The aim of this study was isolate and detect of cadmium sulphide (CdS) quantum dots labelled influenza oligonucleotide-SH (ODN-SH) H5N1. We described and designed method based on paramagnetic particles (MPs) for isolation of viral nucleic acid labelled quantum dots (QDs) with further electrochemical quantification of cadmium in the label.

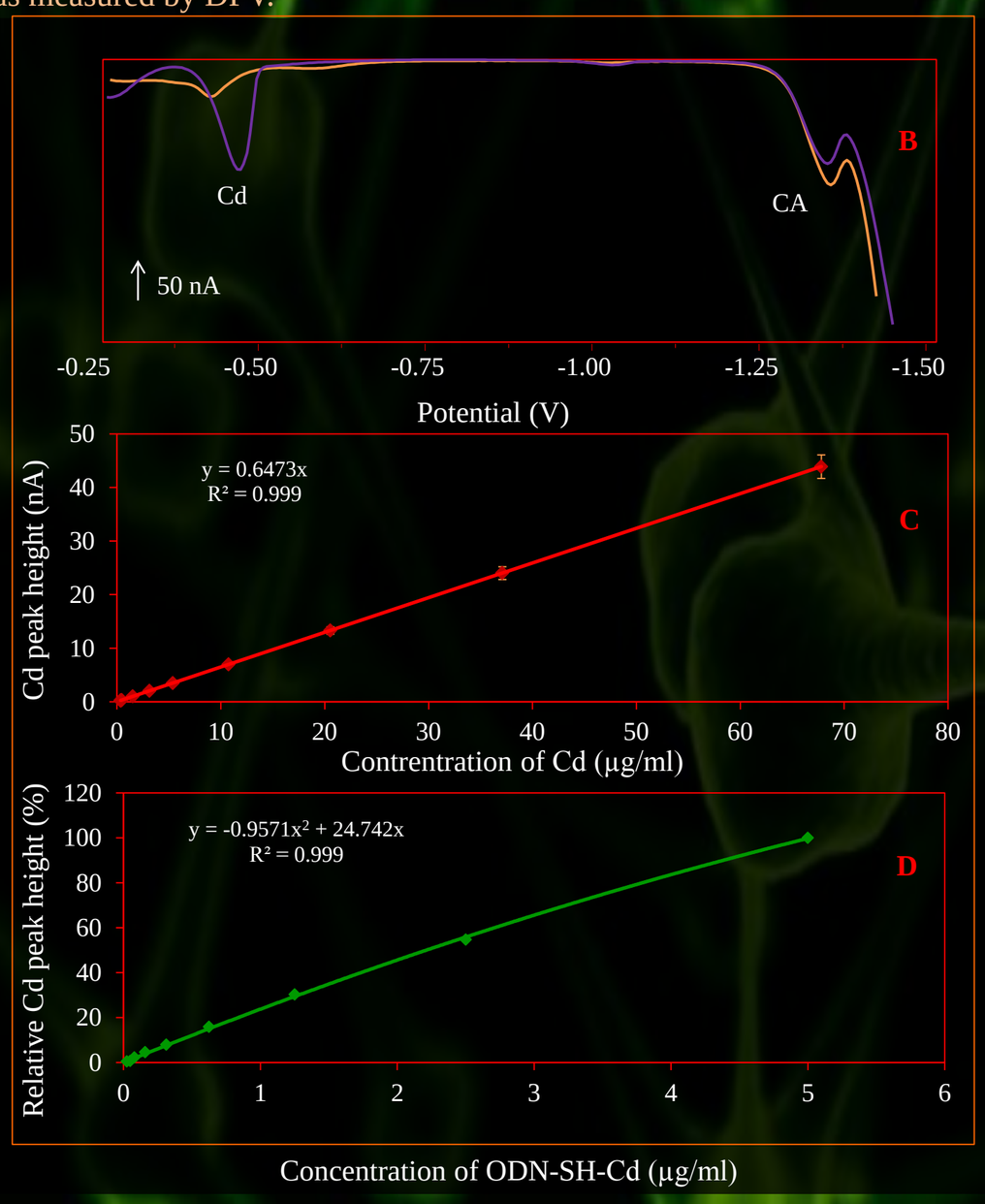
METHODS

Detection of Cd labelling influenza oligonucleotide (ODN-SH-Cd) was carried out by differential pulse voltammetry. The optimized parameters were as follows initial potential -0.9 V; end potential -0.45 V; deposition potential -0.9 V; duration 240 s; modulation amplitude 0.025).

RESULTS AND DISCUSSION

We designed and described MPs assay based on electrochemical detection of QDs labelled influenza oligonucleotide. The method for CdS QDs detection (Cd peak) was optimized and part of oligonucleotide isolation was fully automated. The hybridization process was influenced by wide range of hybridization conditions such as temperature, time, pH and composition of hybridization buffer. The effect of hybridization temperature was observed. The increasing temperature enhanced amount of hybridized target (ODN-SH-Cd) and thus increase Cd peak height, but only to Tm of isolated DNA (Tm influenza derived ODN was 28 °C). Optimal temperature was 25 °C.

Fig.B: Comparison of real SWV voltammograms of ODN-SH (-) and ODN-SH-Cd (-).
Fig.C: Calibration curve of Cd. For electrochemical determination DPV method was applied.
Fig.D: Limit of detection for Cd peak of detected ODN-SH-Cd complex. Cd peak was measured by DPV.



ACKNOWLEDGEMENT

The financial support from the following project CEITEC CZ.1.05/1.1.00/02.0068 is acknowledged.

Fig. A: Optimization of time of acumulation (s) for Cd peak of ODN-SH-Cd; concentrtion of both ODNs was 2µg/ml. For determination of CA peaks SWV as an electrochemical method were applied, Cd peaks were determined by DPV.

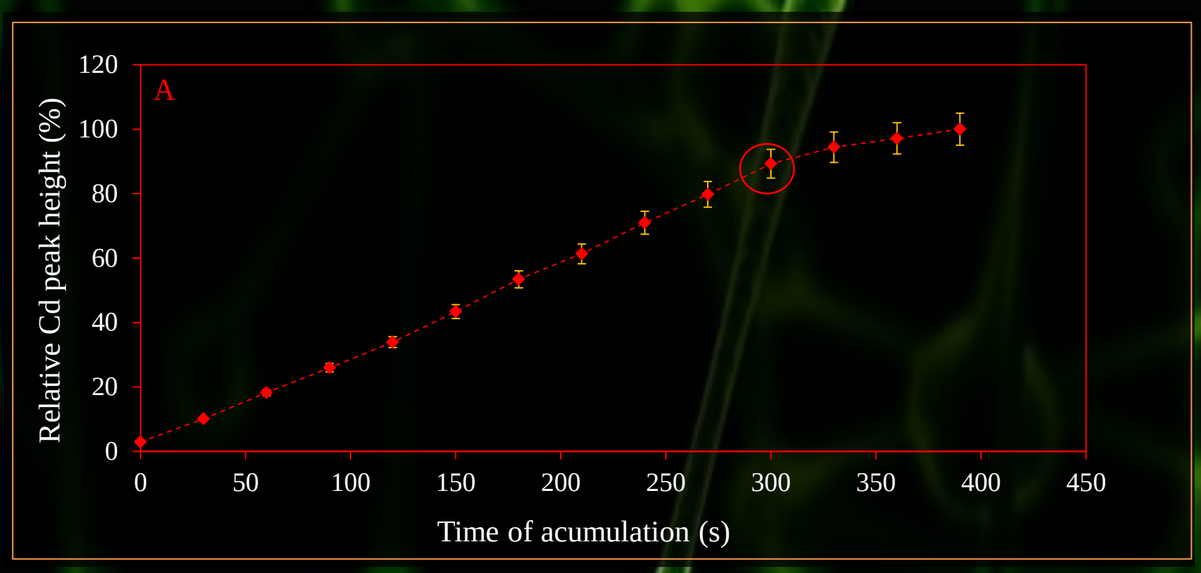
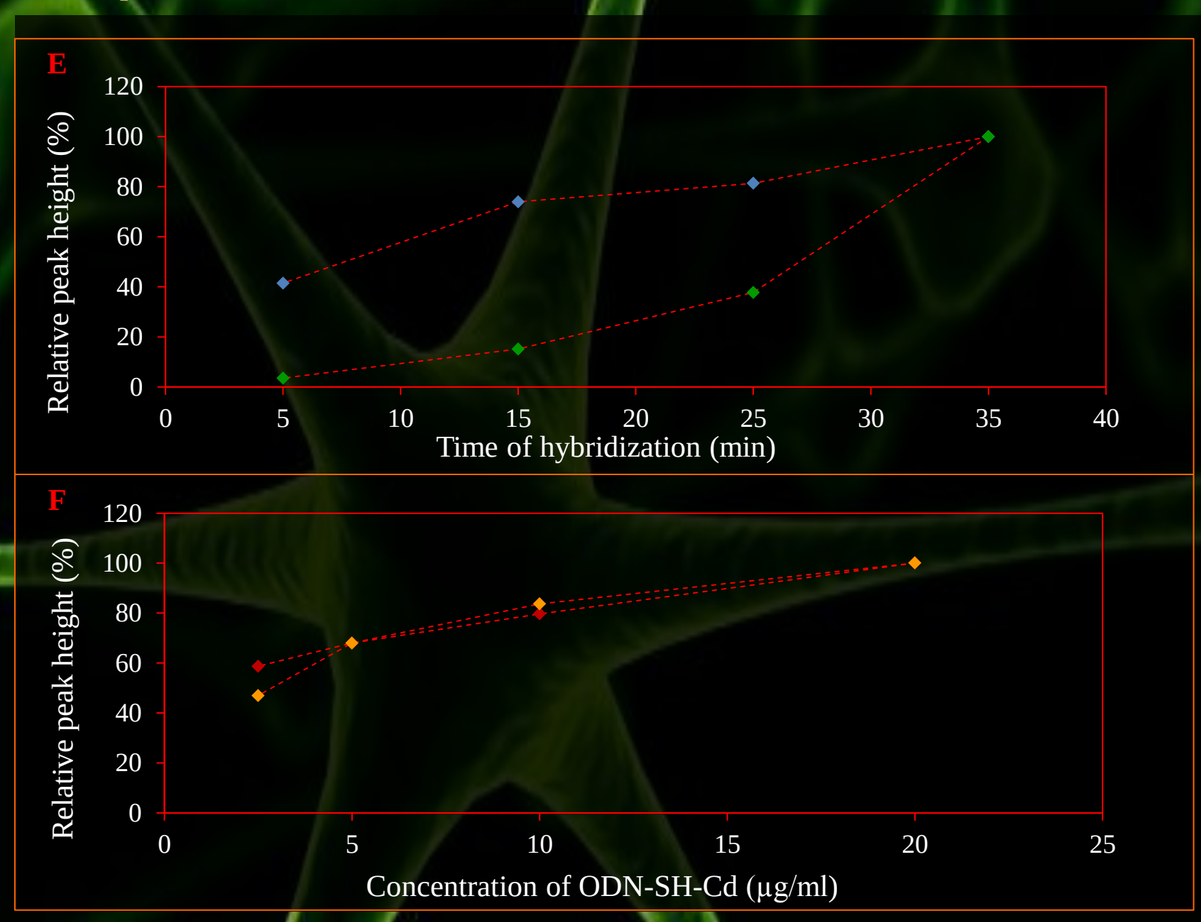


Fig.E: Change of relative peak height (relate to maximum value) with changing time of hybridization (5, 15, 25 and 35 min). CA peak and Cd peak were used.
Fig.F: Dependence of relative peak height (relate to maximum value) on concentration of ODN-SH-Cd (2.5, 5, 10, 20 µg/ml) under hybridization temperature 25 °C. CA peak and Cd peak were used.



CONCLUSION

It was proposed and optimized method for automated isolation of Cd labeled influenza oligonucleotide. It was observed the effect of hybridization temperature (second hybridization) on height of CA and Cd peak. And it was demonstrate the supposed effect. With increasing hybridization temperature amount of hybridized ODN-SH-Cd increased. Our system will be used as a miniaturized electroanalytical tool for rapid detection of target oligonucleotide based on isolation by probe conjugated MPs.