evropský sociální fond v ČR
EVROPSKÁ UNIE
MINISTERSTVO ŠKOLSTVÍ, MLÁDEŽE A TĚLOVÝCHOVY
OP Vzdělávání pro konkurenceschopnost
INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Název: Using paramagnetic particles and PNA for isolation and electrochemical detection of DNA corresponding influenza virus

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Reg.č.projektu: CZ.1.07/2.4.00/31.0023

Název projektu: Partnerská síť centra excelentního bionanotechnologického výzkumu

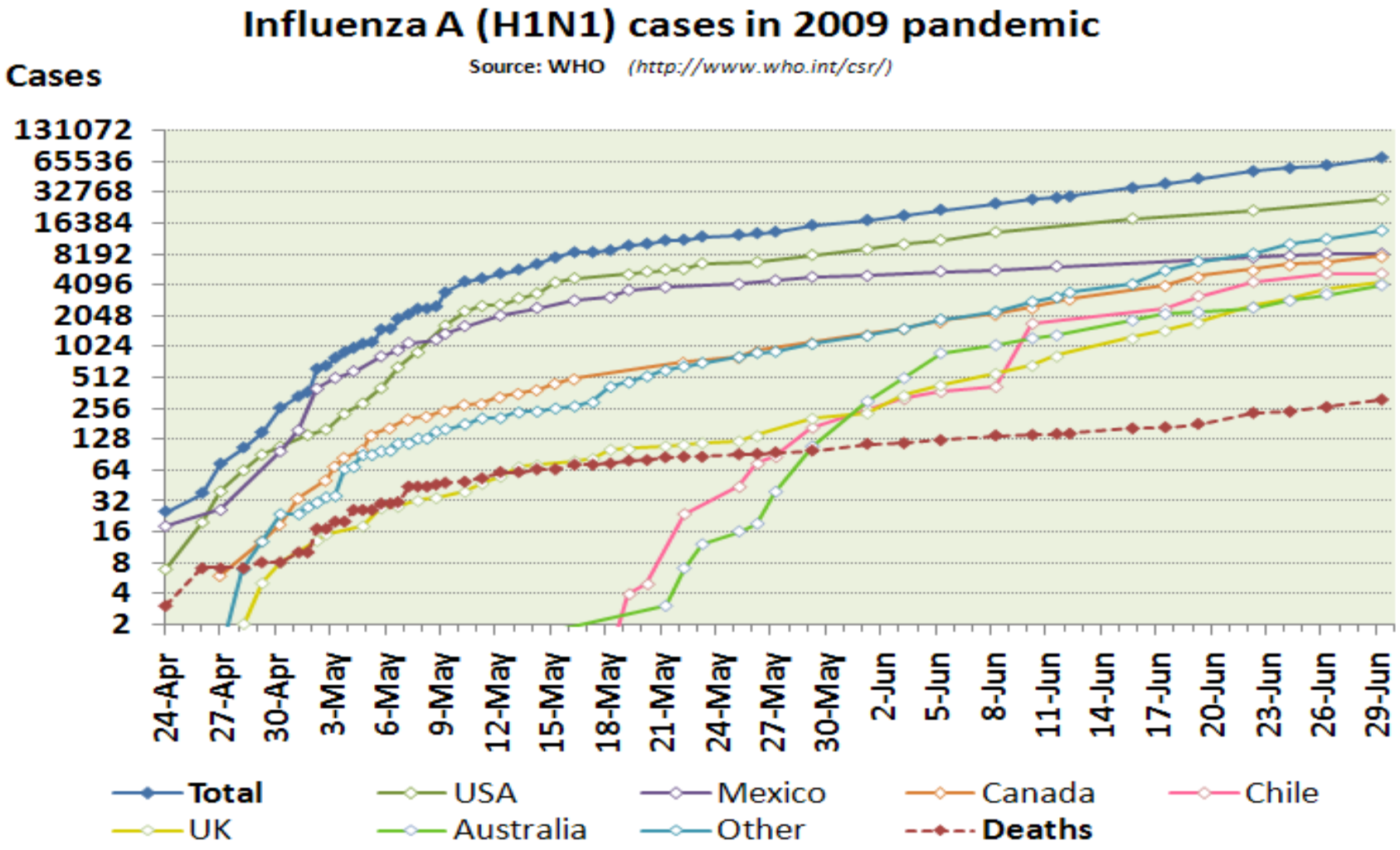
NanoBioMetalNet

- INTRODUCTION
- METHOD
- RESULT AND DISCUSSION
- CONCLUSION

- Influenza (flu) is a infectious disease from birds and mammals, which caused by RNA viruses (Influenza virus).

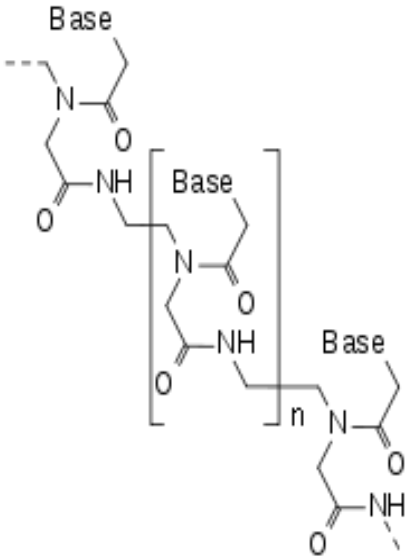
- 3-5 millions yearly cases of illness and 250 000-500 000 yearly deaths.

- Influenza virus A, B, and C


Influenza A (H1N1) cases in 2009 pandemic
Source: WHO (http://www.who.int/csr/)
Cases
131072
65536
32768
16384
8192
4096
2048
1024
512
256
128
64
32
16
8
4
2
24-Apr
27-Apr
30-Apr
3-May
6-May
9-May
12-May
15-May
18-May
21-May
24-May
27-May
30-May
2-Jun
5-Jun
8-Jun
11-Jun
14-Jun
17-Jun
20-Jun
23-Jun
26-Jun
29-Jun
Total
USA
Mexico
Canada
Chile
UK
Australia
Other
Deaths

Places suffered from H5N1 pandemic in Vietnam

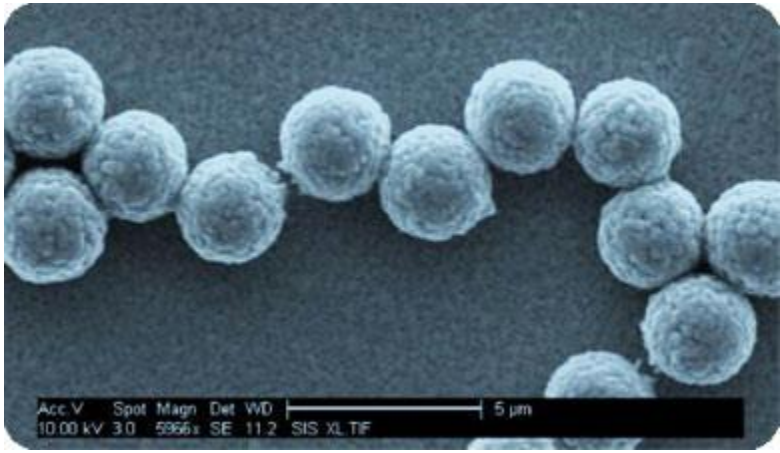
- PNA has a backbone made from repeating N-(2-aminoethyl)glycine units linked by peptide bonds. The different bases (purines and pyrimidines) are joined to the backbone by methylene or carbonyl linkages.



- PNA/DNA is better thermal stability than DNA/DNA

- PNA has various application such as: antigen and antisense therapy; PNA as molecular biology and functional genomics, PNA as a probe for diagnosis and detection, and PNA as biosensor.

❖ Small size but large surface (2 nm-10 μm), different variant of modification.



❖ Their ability to facilitate bioactive molecules binding

❖ Advantages of paramagnetic particles: easy using, short time.

METHOD

- Automatic pipetting station EP Motion 5075 (Eppendorf, Germany) was used for fully automated isolation process of target DNA sequence (5’–CCTCAAGGAG-3’) corresponding to influenza virus by using Oligo dt(25) and PNA (5’-AAAAACTCCTTGAGG-3’).

- Square wave voltammetry, square wave voltammetry coupled with adsorptive transfer technique, and differential pulse voltammetry method were used for electrochemical detection of nucleic acids.

RESULT AND DISCUSSION

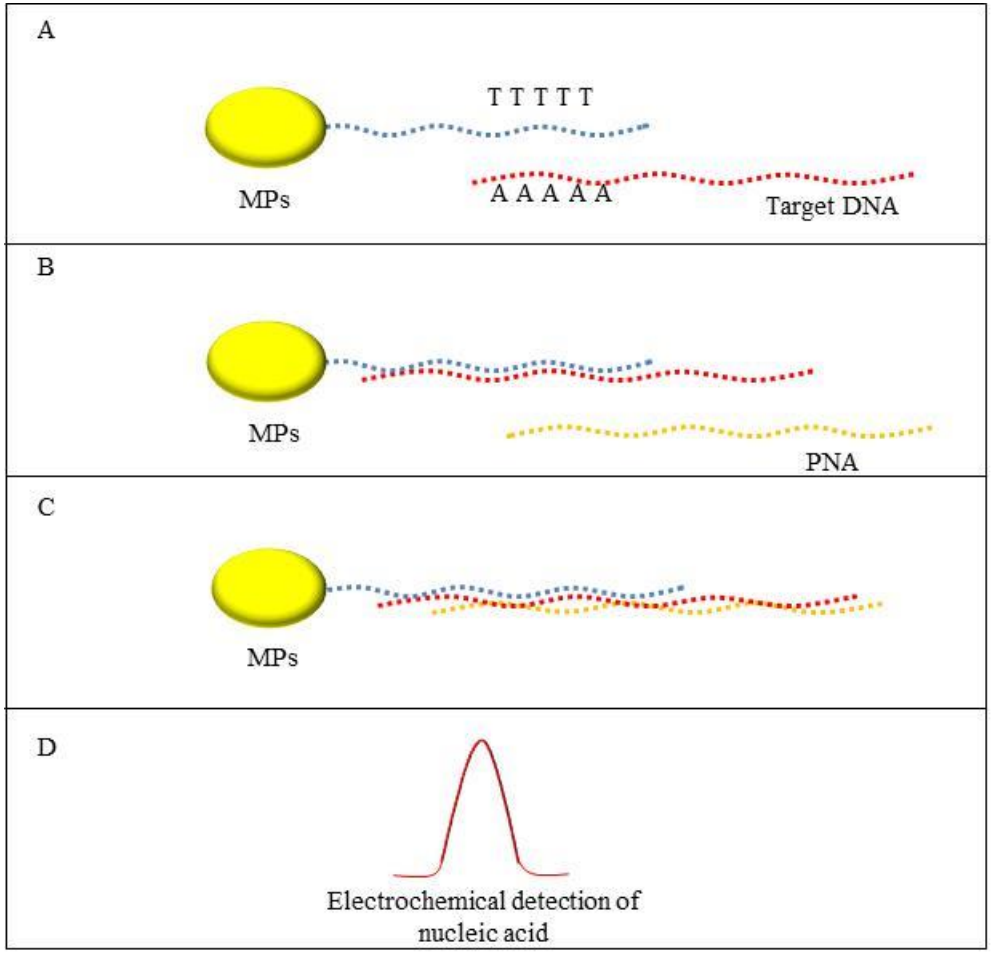
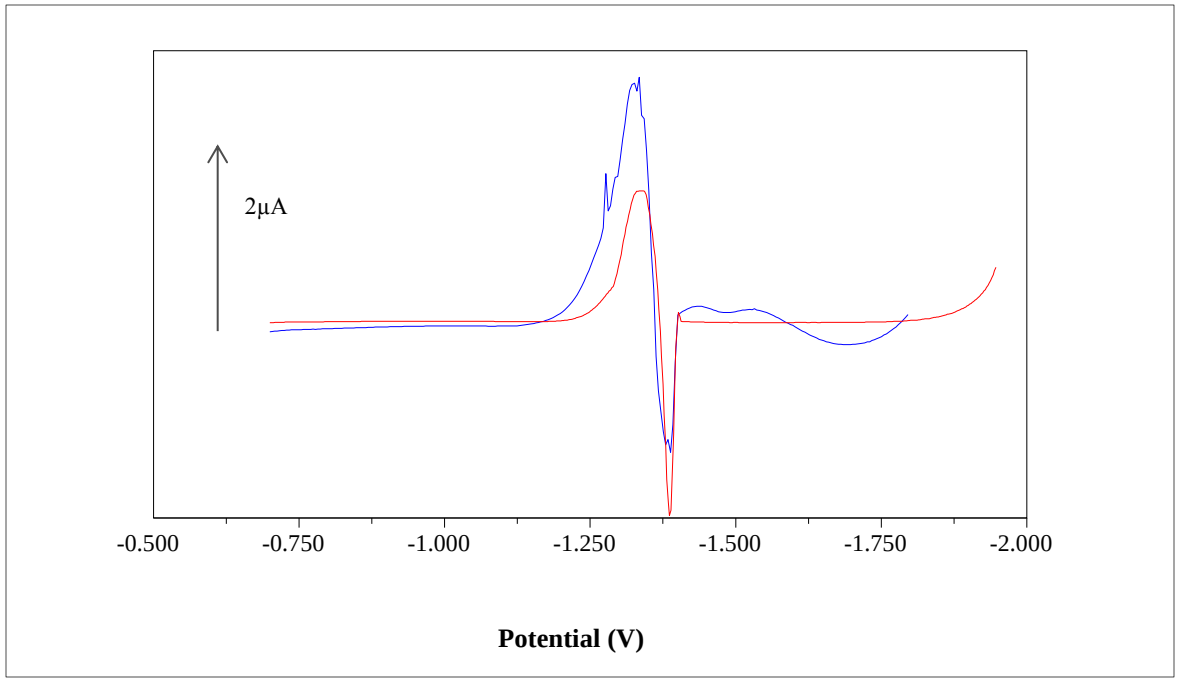


Figure.1**:** Scheme of isolation and detection of influenza derived oligonucleotide by MPs and PNA probe. A DNA biding MPs, B addition of PNA, C biding of PNA to MPs with DNA, D electrochemical detection of isolated product.



Blue: PNA

Red: DNA

Figure. 2: Characterization of PNA and DNA by differential pulse voltammetry, brdicka was used as an electrolyte. Parameters of DPV were as follows: ininitial potential -0.7V; end potential -1.8 V; time of accumulation 2 min; step potential 0.00495 V; amplitude 0.02505 V; interval time 0.2 s; modulation time 0.057 s.

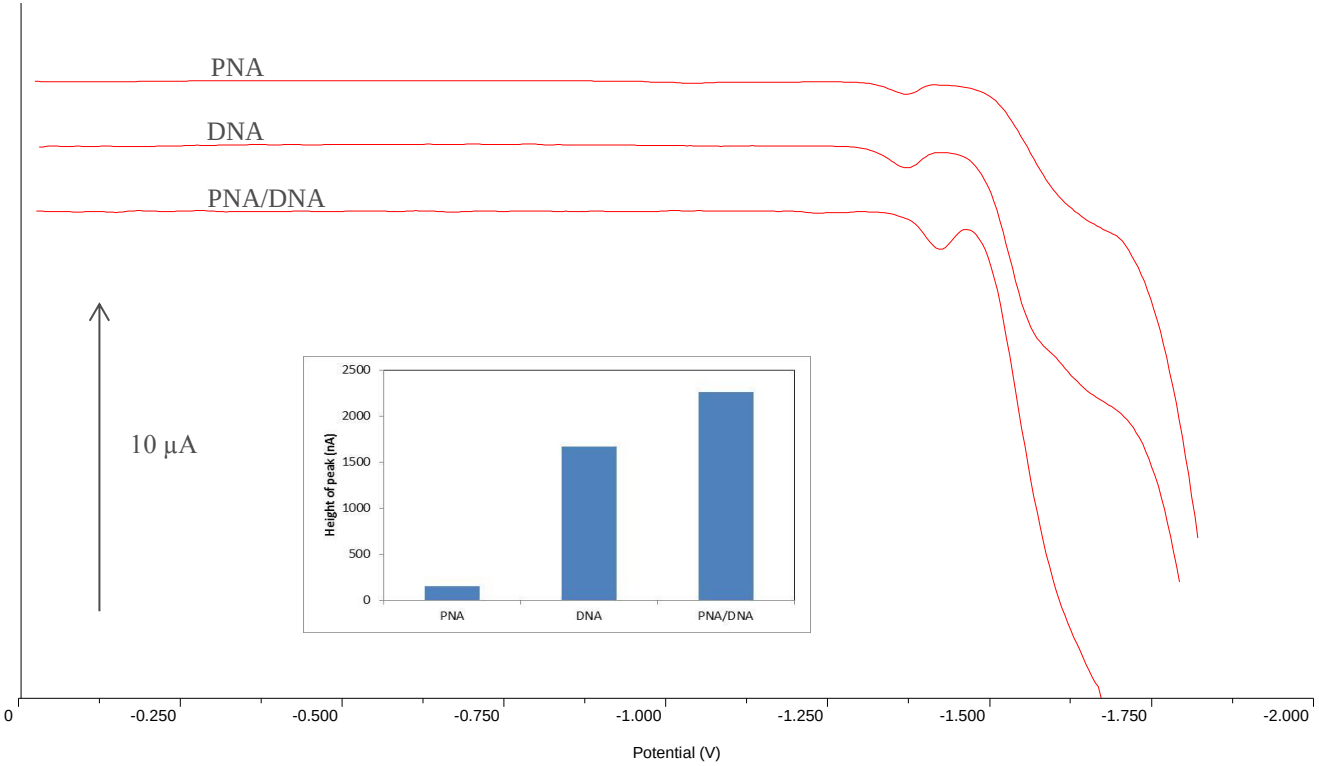


Figure 3: Voltammograms of PNA, DNA, and PNA/DNA. AdT SWV method was used. Parameters of AdT SWV was: time of accmulation 300s; purge time 60s; frequency 280 Hz; initial potential 0 V; end potential -1.8 V; step potential 0.00495 V; amplitude 0.02505 V.

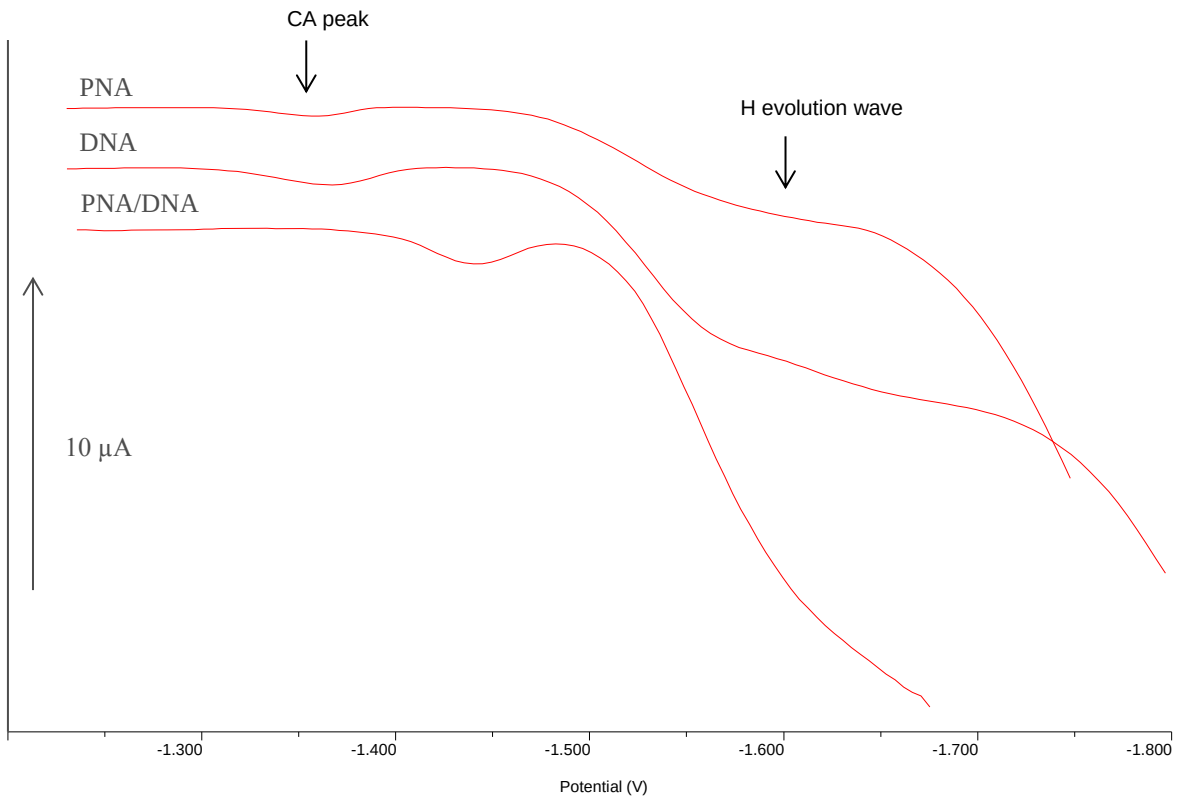


Figure 4: Voltammograms of PNA, DNA, and PNA/DNA. AdT SWV method was used. Parameters of AdT SWV was: time of accmulation 300s; purge time 60s; frequency 280 Hz; initial potential 0 V; end potential -1.8 V; step potential 0.00495 V; amplitude 0.02505 V.

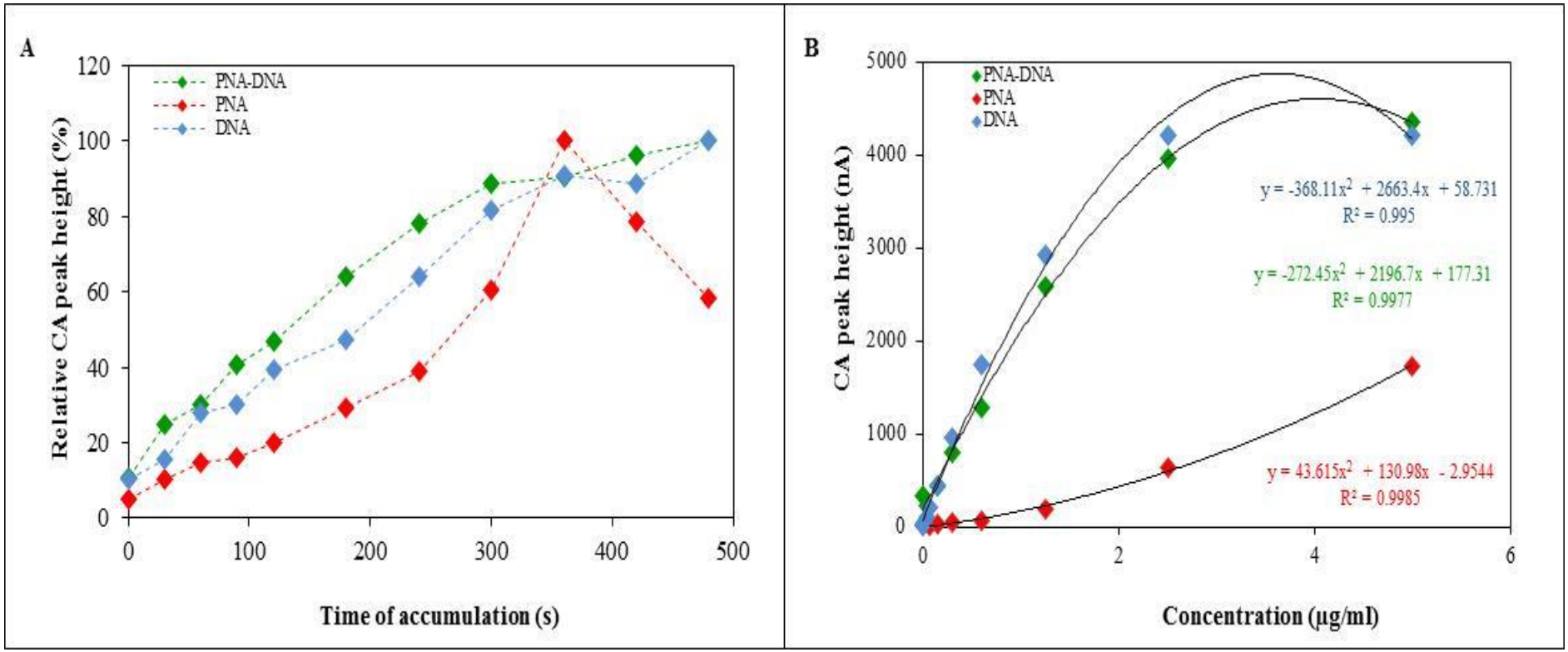


Figure 5: A Dependence of relative CA peak height (%) on time of accumulation of nucleic acid (s), AdT SWV method was used. B Dependence of CA peak height (nA) on concentration of nucleic acid (µg/ml), AdT SWV method was used. Parameters of AdT SWV was: time of accmulation 120s; purge time 60s; frequency 280 Hz; initial potential 0 V; end potential -1.8 V; step potential 0.00495 V; amplitude 0.02505 V.

- Electrochemical method is a powerful technique for nucleic acid determination.

- PNA can be used as biosensor for DNA target sequence because PNA shows ability to hybridize with DNA with high affinity and specify.

- Paramagnetic particles and PNA as a probe can be used for isolation of DNA target sequence because this established technique can facilitate DNA isolation process.

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Thank you for your attention

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