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Fast DNA Diagnostic Using Fe_3O_4 Magnetic Nanoparticles and Light Emitting ZnS/Mn Nanoparticles

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Abstract: In this study, the fast DNA diagnostics was successfully developed using high fluorescent Mn doped ZnS nanoparticles and NH_2-modified SiO_2 coated Fe_3O_4 nanoparticles in a *sandwich structure*. In one end of the sandwich structure, we employed NH_2-modified SiO_2 coated Fe_3O_4 nanoparticles as a docking matrix. A so-called *capture probe* oligonucleotide chain, that specifically identifies the target DNA, linked to the docking magnetic particles. In the other end, other oligonucleotide chain – named *detector probe* – contacted with the signaling semiconductor particles. The complementary hybridization of the *detector probe – target – capture probe* formed the *sandwich* configuration that attached the fluorescent particles to the docking matrix. This configuration was used to detect DNA of Epstein-Bar virus (EBV) and as the result, a fine affinity between the luminescent intensity at 586 nm of Mn doped ZnS colloids showed with the initially added DNA target concentration, which could detect the presence of target DNA within 2×10^6 copies/ml (~0.3 fM). The mobility of the as-prepared solutions used in this methodology promised an attractive applicability for designing a DNA detecting fast KIT.

Keywords: DNA fast kit, ZnS nanoparticles, Fe3O4 nanoparticles, Fluorescent.

1. Introduction

Semmiconductor nanoparticles have been mostly attracted in recent years for the application in biomedicine due to their fluorescent feature. The small size of semiconductor nanoparticles make them become suitable to conjugate with various biology molecules with size scale from bigger size of DNA, RNA molecules to medium size of enzymes, antibodies, as well as small size of organic ligands [1,2]. Besides, their relatively fixed emission spectrum with limited Stokes shift (that is separation from emission band and excitation band) attracts very specific labeling applications in biology therapeutic such as visualization of cell-imaging [3-6], pH probes [7], detection of organic compounds [8] and

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different biomolecules [9,10] … Among of those biomedicial applications, DNA detection plays a very important role; not only for gene studying investigation, but also ready used for criminal detecting, disease examination and early cancer caution. In recent years, the very small size semiconductor nanoparticles, which usually called quantum dots – QDs, brought an enormous evolution in DNA labeling according to special classic biological methods. For instance, emission quenching via the presence of gold nanoparticles was applied for detection the concentration of target DNA in solutions [8,11,12]. In addition, QDs-organic compound FRET (Fluorescence Resonance Energy Transfer) acceptor-donor couples were appropriate to enhance the classic FRET approach for DNA concentration counting [8,9].

Mn^{2+} doped semiconductor nanoparticles can be a very good candidate of labeling agents in DNA detection. They are easily synthesized in normal conditions by co-precipitation [13,14], hydrothermal [15] and sol-gel methods [16]. Despite that, the usual obtained peak of these materials are normally broadened, the lightened orange color photo luminescent intensities are relatively elevated, thus could be visualized by naked eye under normal conditions [13]. These properties make Mn^{2+} doped semiconductor nanopartilces attractive in order to replace classic organic fluorescent compound for cell-imaging [17] and/or DNA detection.

In most applications, however, the QDs still stayed in biology and/or reaction environment. In some early publications, the emission wavelength of the QDs strongly depends on the present of organic compound occur in solution [18-20], hence might create peak-shift during the investigation. Magnetic particles were moderated to solve this problem. Simple SiO_2 coated Fe_3O_4 particles have been successfully applied for DNA selection [21,22], which gave us a very good idea to use them as donor-system that could stock up the QDs hybridized DNA target by outside magnetic field. From that, they could announce information about DNA concentration due to photo luminescence measurement.

In this publication, two types of DNA detector oligonucleotides and two kind of the above mentioned nanoparticles were used to create a sandwich-structural multifunctional colloids, that hybridized by specific gene section of Epstein-Bar virus (EBV). The whole structure was collected by outside permanent magnet and was investigated by photoluminescence spectrometer. The observed peak intensities at specified 590 nm were elaborated with the initial added target DNA concentration, hence appraised the possible applicability of the multifunctional structure for DNA detecting.

2. Materials and Methods

2.1. Design of the multifunctional detecting structure

Typical sandwich structure of DNA detecting probe contains 3 main parts: magnetic donor (or catcher), high photo luminescent detector and target DNA (Figure 2.1). Here, the target DNA was selectively cloned from the plasmid that contains 434 nucleic base-pairs specific Epstein-Bar viral gene (which is normally double chain, but could releases into 2 single complementary chains under 98^{o}C). The donor part contains magnetic nanoparticles that are conjugated with the so called catcher

probes; and the detector part contains detector probes-conjugated high intensity light emitting semiconductor nanoparticles. The catcher probe and detector probe are two single oligonucleotide chains, that are could specifically hybridize with the target DNA, were purchased from Key Laboratory of Protein and Enzymes, Hanoi University of Science.

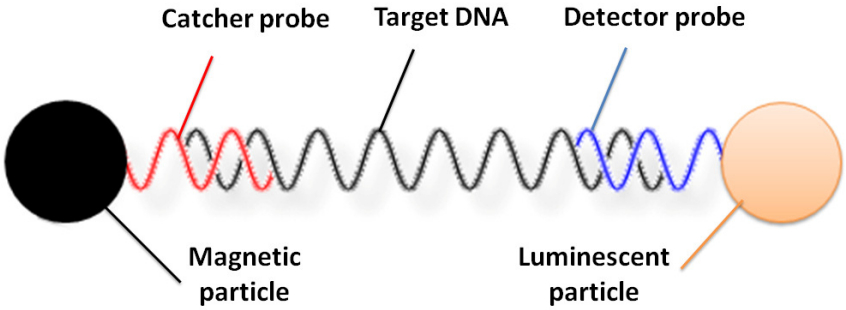


Figure 2.1. Schematic structure of multifunctional magnetic-QDs sandwich applied for DNA detection.

During experiment, the kit were separated to 3 solutions containing magnetic donor particles, QDs detector particles and target DNA molecules, respectively. After mixing, the magnetic donor – target DNA – QDs detector complexes assemble via the specific hybridization of catcher probe and detector probe with the target DNA, which are easily collected by outside magnetic field. The higher concentration of target DNA molecules in initial solution, the more complexes occur, hence, increases the luminescent intensity of the after-selected solution. By this, the named multifunctional magnetic-QDs sandwich structure would be applied for fast KIT DNA detection during scene-investigation and viral DNA detection…

2.2. Materials

DNA catcher, detector probes and target DNA were purchased from Key Laboratory of Protein and Enzyme, Hanoi University of Science. Amino (-NH_2) modified SiO_2 coated Fe_3O_4 magnetic nanoparticles were obtained from Magnetic Group, Nano Center of Energy, Hanoi University of Science. Zinc acetate, sodium sulfide, polyvinyl pyrrolidone (PVP - MW 40000 Dalton), manganese acetate methyl imidazole (MIA), N-(3-Dimethylaminopropyl)-N'-ethyl carbodiimide hydrochloride (EDC) and 4-aminthiophenol (4-ATP) were purchased from MERCK, Germany. All inorganic materials were purity checked by energy dispersive X-ray spectroscope (EDS), Center for Materials Science, Faculty of Physics, Hanoi University of Science, VNU Hanoi .

Conjugation of amino modified SiO_2 coated Fe_3O_4 (Fe_3O_4/SiO_2-NH_2) nanoparticles

Magnetic donor nanoparticles were created by conjugating the Fe_3O_4/SiO_2-NH_2 particles with catcher probes under catalytic presence of MIA and EDC [23,24]. 3 ml containing 1 mg/ml Fe_3O_4/SiO_2-NH_2 particles was added into 10 ml test tube before mixing with 1 ml of 25 µmol/ml catcher probe solution. The reaction then was initiated by adding 1 ml of MIA and 1 mg EDC, and then was stored in 4°C through 12 hours. After 12 hours, when the reaction fully completed, the *magnetic donor* particles containing solution was collected by washing with phosphate buffer saline

solutions 1X pH = 7 (PBS 1X). After collection the magnetic donor particles was divided into 2 parts: one part was diluted in PBS 1X pH=7 up to 10 ml and stored under 4°C condition – later called *magnetic donor solution*, while the other part was dried for magnetic property investigation.

Synthesis, surface modified and conjugation of Mn doped ZnS nanoparticles

The QDs detectors containing solutions (later called *Detector solution*) was synthesized by co-precipitation of Mn doped ZnS nanoparticles, functionalizing with 4-ATP and conjugating with detector probes. First 20 mL of droplet Na_2S 0.075 M was added into 100 ml containing mixture of zinc acetate 0.01M, Manganese acetate 0.1mM and 10 g/L PVP. The individual white color precipitation of Mn doped ZnS (ZnS/Mn) accumulated and stabilized after 10 minutes stirring. 3 mL of 7×10^{-4} 4-ATP in alcohol was added. The reaction between 4-ATP and ZnS/Mn formed $ZnS/Mn_{crystal}$-S-C_4H_4-NH_2 stable covalent linkage between the organic molecules with the crystalline colloids [25], which has free $–NH_2$ group (ZnS/Mn-NH_2). After washing at least 6 time with two-times distilled water, the ZnS/Mn-NH_2 particles containing solution was washed with phosphate buffer saline solution 1X pH=5 3 times.

The free $–NH_2$ groups then reacted with the PO_4^{-3} groups from the 3'-end of detector probes under the catalyst of MIA and EDC. 50 mL of ZnS/Mn-NH_2 particles containing solution was mixed with 1 ml Detector probe 25 μL/mL, before adding 1mL mixture of MIA and 1mg EDC. After 12 hours reaction under 4°C condition, the solution was purified by centrifugation with PBS buffer 1X pH=7. The end solution that contains only Detector probes conjugated ZnS/Mn particles (*Detector solution*) was diluted in 20 mL PBS 1X pH=7 then stored in 4°C.

Before use, photo luminescence property of Detector solution was investigated by FL3-22TAU fluorescence spectroscope, Center for Materials Science, Hanoi University of Science. The solution was diluted respectively 2 times, 5 times, 10 times, 100 times, 1000 times, 10000 times, 50000 times and 100000 times. Emission spectrum of each diluted solution was investigated under 326 nm light excitation in order to check the detection limit of the measurement. Besides, one part of the Detector solution was dried for magnetic property investigation by VMS mode of PPMS (Physical Properties Measurement System, Quantum Design) at Nano and Energy Center, Hanoi University of Science, VNU Hanoi.

Target DNA

Target DNA purchased from Key Laboratory of Protein and Enzyme was a specific gene section of EBV cloned from plasmid with very high concentration of 10^{11} copies/ml (~ 1.66 pM). After receiving, we diluted the sample in PBS 1X pH=7 down to 10^7 copies/ml concentration then dispensed in 1mL Ependorf tubes and stored in refreeze under -4°C condition. Each measurement, one different Ependorf was acquired and diluted to investigation concentration.

2.3. Measuring method

We considered the measurement as a suitable fast test in order to check the present of the EBV DNA in solution. In this fast KIT, the ready solutions were *Detector solution*, *Magnetic donor solution* and the target DNA solution that was needed to be investigated.

The target DNA solutions were prepared from the stock solution of 10^7 copies/ml concentration. After acquiring from refreeze, we dilute the target DNA containing solution in to different concentrations, respectively 5×10^5, 10^6, 2×10^6, 2.5×10^6 and 5×10^6 copies/ml in PBS 1X pH=7. Negative control sample was simple PBS 1X solution without any organic molecules.

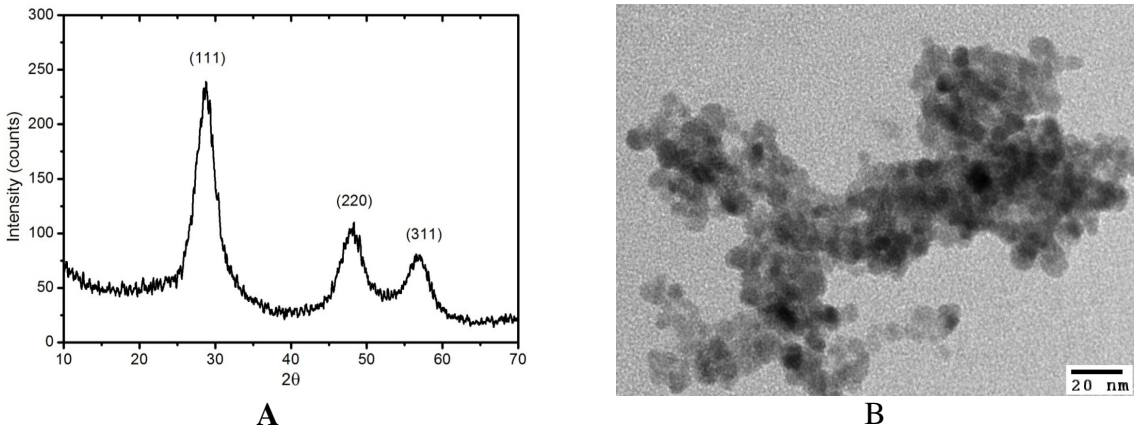
First , to begin the fast test, each target DNA containing solution (later called target DNA solution) with different concentrations was bath in boiled water for 5 minutes. The double helix structure released into 2 single chains and was fixed by immediately emerged in ice. Then, we mixed 1ml of *Detector solution* with 1 ml of *Magnetic donor solution* and later dispensed 1 ml of target DNA solution after fixed. The sandwich structural complex was hybridized in 30 minutes. In third step, we used permanent magnet to collect the occurred complex structures. Forth step, the solution was distributed 3mL PBS 1X pH=7 and was investigated by Fluorescence spectroscope (FL3-22TAU, Center for Materials Science, Hanoi University of Science). Finally, fifth step, data manipulation; characteristic peak height at 588 nm of fluorescence spectra was considered as function of initial target DNA concentration.

We repeated the measurements at least twice with each DNA target concentration to check the reproductivity. The repeating times were shown in Table 2.1.

Table 2.1. Table of repeating times for each target DNA concentration in order to check the reproductivity of the measurement.

Concentration of target DNA	Repeating times
5×10^5	2
10^6	2
2×10^6	3
2.5×10^6	3
5×10^6	3

3. Result and Discussion



A B

Figure 3.1. Typical XRD (A) and TEM image (B) of ZnS/Mn-NH_2 particles prepared by co-precipitation, 4-ATP surface-modification.

Typical X-ray diffraction (XRD) of ZnS/Mn-NH_2 particles is shown in Figure 3.1. The diffraction peaks corresponding to (111), (220), (311) planes are separated explicitly at 29.12˚, 47.56˚and 54.42˚, which mostly agree with standard close-packed face central cubic (FCC) structure of sphalerite mineral (JCPDS number 05-0566). The data was fitted to the three peaks. We used Debye-Scherer formula applied for these peaks to calculate the average size of crystalline of the particles, which is shown in table 3.1. From the table, we assumed that the particles have spherical shape and average size ~ 3nm, which agrees with the transferred electron microscopic (TEM) result (Panel B, Figure 3.1).

Table 3.1. Gaussian multi-peak fitting results of ZnS/Mn-NH_2 particles' XRD spectrum.

Miller index	FHMW	θ	Calculated average size (nm)
(111)	2.805	14.280˚	2.92±0.02
(220)	3.274	23.783˚	2.66±0.04
(311)	3.114	28.214˚	2.87±0.07

We did the same measurements (XRD and TEM investigations) with the Fe_3O_4/SiO_2-NH_2 nanoparticles. The results are shown in Figure 3.2. In panel A, there is a broadened peak occurred at about 24^o, which responses to the amorphous phase of SiO_2 coat. The peaks obtained at 2θ of 30.07^o, 35.55^o, 43.05^o, 53.59^o, 57.20^o and respectively at 62.68^o agree with the standard close-packed FCC thiospinel structure of magnetite chalcogenide (JCPDS No. 79-0417).

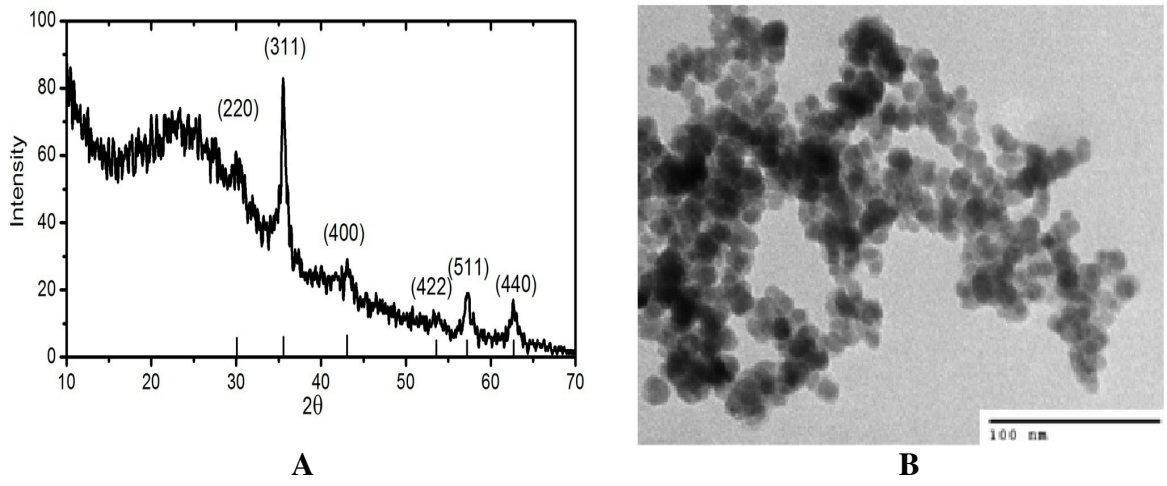


Figure 3.2. XRD (A) and TEM image (B) of Fe_3O_4/SiO_2-NH_2 particles.

Magnetic properties of the nanoparticles

At small size of 15-20 nm (Figure 3.2B) the ferrite oxide particles showed typical superparamagnetic property with comparatively high saturated magnetization M_s of about 61.2 emu/g, while the semiconductor particles illustrated very low saturated magnetization (~0.5 emu/g).

High external permanent magnet treatment (not shown) demonstrates that the Fe_3O_4/SiO_2-NH_2 particles could be totally collected under 15 min, while it was not experienced in the case of ZnS/Mn-NH_2 particles. Figure 3.3 shows the magnetization of Fe_3O_4/SiO_2-NH_2 and ZnS/Mn-NH_2 particles

depending on the applied field. The saturate-magnetization of *magnetic donor colloids* reaches relatively high quantity of 61 emu/g at 15 kOe applied field, while that quantity of *Detector particles* obtained at 0.5 emu/g. The reversibility of both lines demonstrates the superparamagnetic and paramagnetic property of the Magnetic donor particles and the Detectors, respectively.

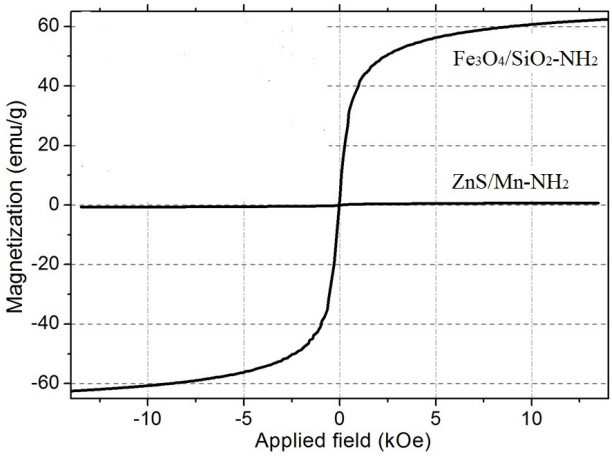
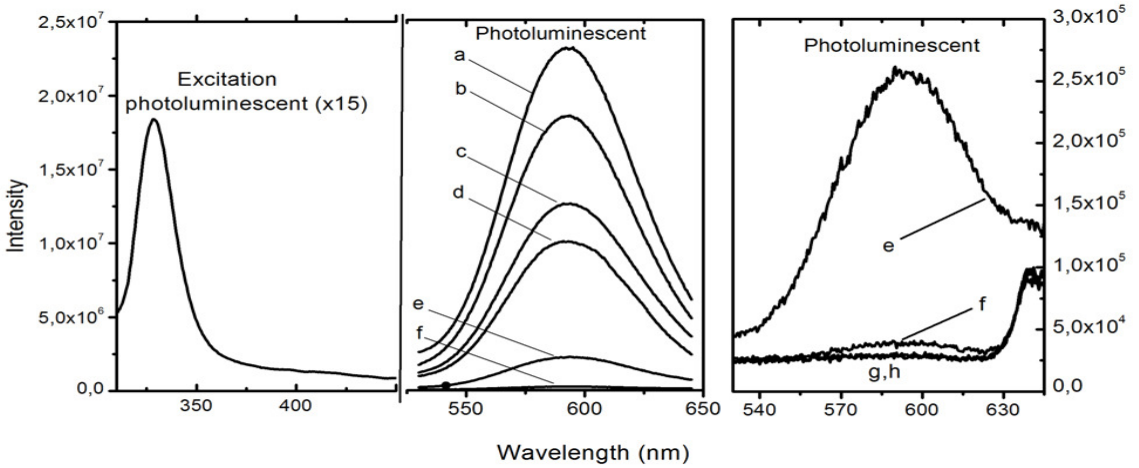


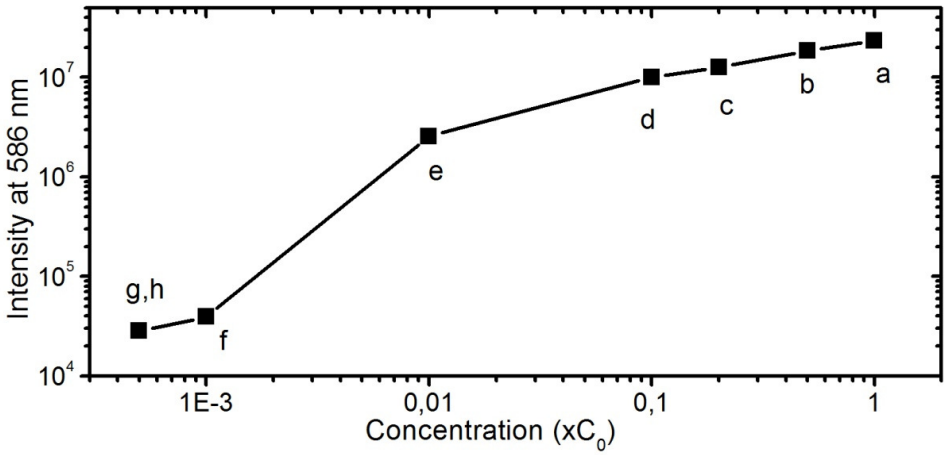
Figure 3.3. Magnetization curves of Fe_3O_4/SiO_2-NH_2 and ZnS/Mn-NH_2 nanoparticles.

Photo luminescent property

The emission intensity of Mn^{2+} from ZnS:Mn clusters was obtained at 588 nm that agrees with early published results [13-15]. The observed intensity decreases depending on decreasing of the solution concentration (Figure 3.4). As visualized in g and h line (A panel), the observed signals could not be distinguished when the solution was diluted to 5×10^3 times and 10^4 times. In log scale, the photoluminescence intensity was proportion with the solution concentration when the dilute rate was smaller than 10^3 times, corresponding to a, b, c, d and e points in Panel B of the figure.



A



B

Figure 3.4. Photo-Luminescence property of ZnS/Mn-NH_2 Detector particles with different dilution time. A panel: a. 2 times, b. 5 times, c. 10 times, d. 100 times, e. 1000 times, f. 10000 times, g. 50000 times and h. 100000 time dilution. B panel: illustration of the peak intensity of the ZnS/Mn-NH_2 Detector solution at 588 nm depending on the dilution times in log-scale.

Diagnostic measurement

Figure 3.4 demonstrates the schematic progress of the DNA test experiment. The A, B, C, D panel correspond to first 4 steps of respectively sandwich hybridization, magnetic collection, washing and redistribution of the collected sandwich complex to PBS 1X pH=7.

In the first step, the sandwich formula of *magnetic donor – DNA – Detector* accumulates in solution. During this step, the complementary hybridization was macroscopic stochastic progress. The concentrations of accumulated sandwich structures are normally proposal with the initial target DNA concentrations (Panel A). After applying outside permanent magnet, all magnetic colloids orientate to the magnetic side. The remained uncoupled *Detector particles* distribute in solution (panel B). By washing, the uncouple *Detector particles* sweeps out (panel C) and the coupled *Detector particles* (in the complex) proportional with the number of the target DNA are distributed in the ending solution (Panel D). The photo-luminescence (PL) intensity of the ending solution is the PL intensity of *Detector particles* formulated in the complexes.

The observed photoluminescence spectra of the sandwich complex (data not showed) indicated that when the concentration of initially added concentration increased the characteristic peak of Mn^{2+} at 588 nm increased. The detection limit was about 2×10^6 copies/ml.

In Figure 3.5, the emitting intensity at 588 nm gave better concentration distinguishing when the initial DNA concentration higher than 2×10^6 copies/ml, and would obtain misinterpretation with typical error at lower concentrations. Under same measuring condition with the upward measurements of *Detector solution*, the obtained limited distinguishable intensity was nearly 10^6 counts at 588 nm. Below this magnification, the equipment could not detect accurately the presence of the *Detector particles* (here the negative control shown relatively low intensity at 588 nm – data not shown). Above

the intensity of 10^6 counts, the equipment can detect the presence of the *Detector particles* quite well (see Figure 3.4B) and is able to recognize the present of the DNA in the solution, which corresponds to the 2×10^6 copies/ml initial concentration of target DNA. We can assume that this diagnostic method can help detect the presence of the EBV DNA higher than 2×10^6 copies/ml (it is about 0.3 fM).

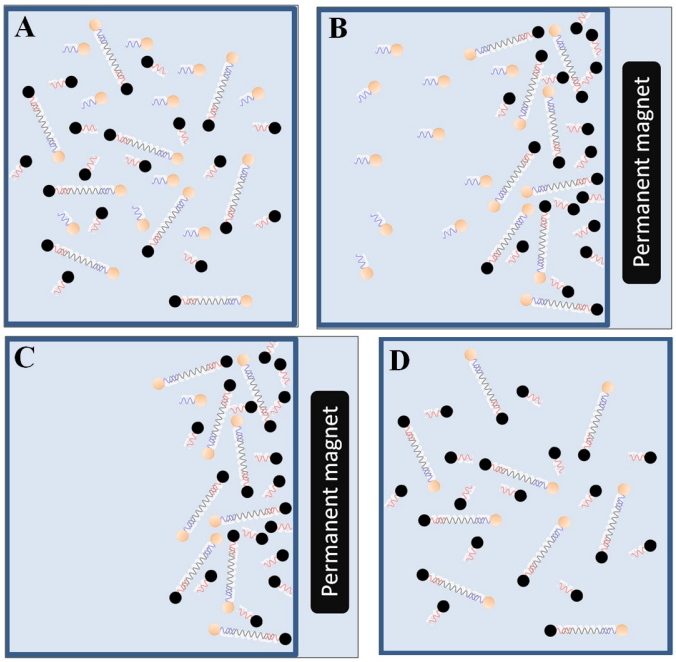


Figure 3.4. Schematic progress of the diagnostic experiment.

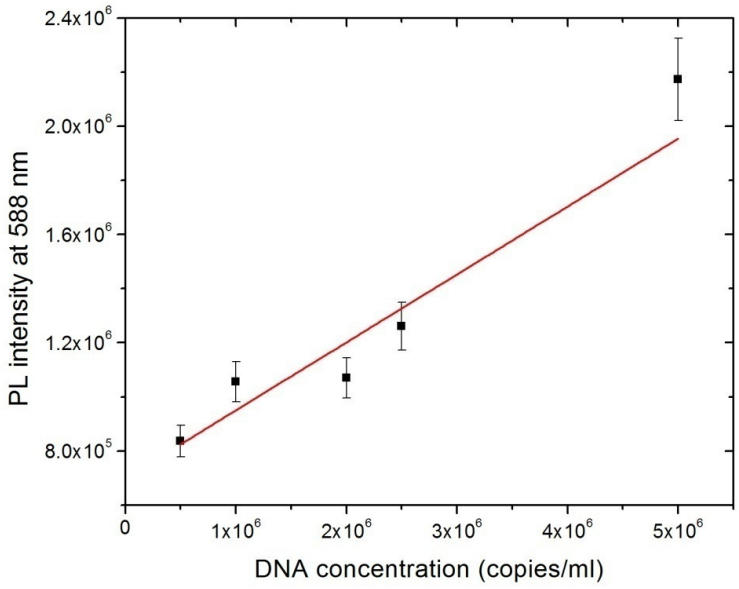


Figure 3.5. Dependence of obtained PL intensity at 588 nm on initial target DNA.

4. Conclusion

The fast KIT of DNA detection was successfully developed using multifunctional nanoparticles in a sandwich structure. The measurement, however, need to be developed to have the suitable form for scene detection of present DNA. The solutions – *Detector solution, Magnetic donor solution* – are comfortably mobile, but we still have to use the laboratorial equipment, that is the PL3-22TAU, which has relatively big size. The light source, as inventing, could be replaced by 335 nm emitting Deep UV LED and the emitted light could be collected by visible light detector, which are commercially purchasable. Hence, the method promises a fine applicability for fast viral DNA detection, early disease warning and scene-criminal detection.

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