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Detection of Newcastle disease virus with quantum dots-resonance light scattering system

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ABSTRACT

A sensitive QDs-based RLS assay method for the detection of Newcastle disease virus (NDV) antibody has been developed. CdTe quantum dots (QDs) were conjugated with Newcastle disease virus and used as RLS-based probes to detect NDV antibody. The electrostatic interaction between CdTe QDs and NDV resulted in enhanced resonance light scattering (RLS) signal characterized at 555 nm. Upon the addition of NDV antibody, QDs–NDV formed dispersive immunocomplex that can decrease the RLS signal. The decreased RLS intensity at 555 nm (ΔI_{RLS}) was linearly proportional to the concentration of NDV antibody ($C_{anti\text{-}NDV}$) in the range of 0.5–50 ng/mL, with correlation coefficient of 0.974 and detection limit of 0.1 ng/mL under the optimization conditions. The proposed method was applied to the determination of NDV antibody in spiked samples with satisfactory results.

1. Introduction

In recent years, semiconductor quantum dots (QDs) have attracted great interests because of their unique optical and surface properties, such as the size-tuned absorption, robust signal intensity, high photochemical stability, emission spectra achievable, large Stokes shifts and biocompatibility. As a new fluorescent label, QDs have made great progress in bioassay, including the detection of pharmaceuticals [1,2], biological imaging, immunoassays, virus detection [3–5], energy transfer and online monitoring. Because the high surface-to-volume ratios can be well-controlled and proper surface modified during the synthesis process, QDs surface nature plays an important role with the characteristics of nano-material. These characteristics mentioned above may endow QDs with more unique light scattering signals when QDs are conjugated with the biomolecules, e.g. DNA, peptides and proteins [6,7]. Since the resonance light scattering (RLS) technique first introduced into the quantitative determination of biomacromolecules in 1993 by Pastemack et al. [8], it has gradually gained regards from analytical chemists [9–11]. As a novel tool, the resonance light scattering technique is characterized by high sensitivity, convenience in performance and simplicity in apparatus. It detects light scattering signals with a common spectrofluorometer and has been widely employed in the designation of bio-assemblies and aggregation species [12–16]. Recent studies have shown resonance light scattering (RLS) is a valuable technique for the analysis of nucleic acid, proteins, and inorganic ions. Generally, after binding of biological dyes [17], artificial synthetic porphyrins and surfactants [18] to proteins, the strongly enhanced RLS signals of the bindings could be observed and measured by coupling and scanning simultaneously both the excitation and the emission monochromators of a common spectrofluorometer. Although the changed RLS signals can be easily measured for aggregated species or large particles in nanometer scale near UV absorption bands [8], RLS method has suffered from low selectivity and remains to be improved or combine with other detecting methods.

Newcastle disease virus (NDV) is a member of the Avulavirus genus in the Paramyxoviridae family, which has been found to infect a number of avian species. Naturally occurring NDV has been reported to be an effective oncolytic agent in a variety of animal tumor models. It has been used in vaccination with tumor cell oncolysates in people with tumors of digestive tract, colorectal carcinoma and other advanced cancers. Several of the naturally occurring strains of NDV have been used in multiple clinical trials against advanced human cancers [19]. In this paper, a QDs-RLS immunoassay method for the quantitative detection of NDV antibody has been proposed, coupling the advantages of the high sensitivity and easy operation of RLS technique and the specificity of immunoreaction. QDs were incubated with NDV in the phosphate-buffered saline, which will induce strong RLS signal with the maximum peak located at 555 nm. The added NDV antibody can recapture ND virus from the QDs–NDV bio-conjugate and simultaneously weakens the RLS signal of QDs–NDV. This can be explained by the specificity of immunoreaction between NDV and NDV antibody. Accordingly, a sensitive detection of NDV antibody using QDs-RLS probe can be constructed. The decreased RLS

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intensity was proportional to the concentration of antibody in a certain range. In this work, the spectral characteristics and the optimum reaction conditions were investigated. The proposed method exhibited high sensitivity and good selectivity.

2. Experimental

2.1. Chemicals

Mercaptosuccinic acid (MSA) (99%) was purchased from J&K Chemical Co. and tellurium powder (~200 mesh, 99.8%). $CdCl_2$ (99%) and $NaBH_4$ (99%) were purchased from Aldrich Chemical Co. Bovine serum albumin (BSA, 5 g) was obtained from Genview, and was dissolved by 2 mmol/L phosphate-buffered saline solutions (PBS, pH 7.4). NDV antigen and antibody were obtained from Harbin Veterinary Research Institute. Stock solutions were prepared at a final concentration of 1.0 mg/mL with occasionally gentle shaking, and stored at 0–4 °C. All chemicals used were of analytical reagent grade without further purification. The water used in all experiments had a resistivity higher than 18 MΩ cm^{-1}. Other chemicals used were of analytical reagent grade without further purification.

2.2. Apparatus

The RLS spectrum and intensity were recorded using a Shimadzu RF-5301PC spectrofluorometer (Shimadzu Co., Kyoto, Japan) equipped with a xenon lamp using right-angle geometry and a quartz cell. The excitation and emission monochromator wavelength were coupled and adjusted to scan simultaneously within the wavelength range from 250 to 800 nm. The slit widths of both excitation and emission light were kept at 10 nm. The response time was set as automatic. The pH value of the buffer solution used was measured with a PHS-3C pH meter (Tuopu Co., Hangzhou, China).

2.3. Preparation of CdTe QDs capped with MSA

Water-compatible CdTe QDs used in this study were synthesized by refluxing routes with mercaptosuccinic acid (MSA) as stabilizer [20]. Briefly, sodium hydrogen telluride (NaHTe) was produced in an aqueous solution by the reaction of sodium borohydride ($NaBH_4$) with tellurium powder at a molar ratio of 2:1 at first. Later, fresh NaHTe solution was added to 1.25 mM $CdCl_2$ solution at pH 11.2 in the presence of MSA in N_2 atmosphere, and the molar ratio of Cd^{2+}/MSA/HTe^- was fixed at 1:1.5:0.2. The CdTe precursor solution was subjected to a reflux at 100 °C under open-air conditions with condenser attached. Stable water-compatible MPA-capped CdTe QDs with PL emission wavelength at 640 nm were obtained and used in the present experiments.

2.4. Procedure for QDs-RLS immunoassay

150 μL of 1.25×10^{-4} mol/L CdTe QDs, phosphate-buffered saline and various amounts of NDV solution were added in a centrifugal tube. Then the solution was diluted and mixed thoroughly with gentle shake to form NDV-conjugated QDs. After incubation, the RLS spectrum of QDs–NDV was obtained by scanning excitation and emission monochromators synchronously through the wavelength range of 250–800 nm. The RLS intensity (I_{RLS}) was recorded. All RLS measurements were made against a parallel blank solution (I^0_{RLS}). The enhanced RLS intensity (ΔI_{RLS}) of QDs–NDV system is represented as $\Delta I_{RLS} = I_{RLS} - I^0_{RLS}$. After optimizing the conditions for the conjugation of CdTe QDs and NDV, NDV antibody was added into the system and treated with the same methods mentioned above.

3. Results and discussion

3.1. Spectra characteristics of CdTe QDs and QDs–NDV

The fluorescence spectrum of the CdTe QDs used in this work is shown in Fig. 1A. It can be seen that the CdTe QDs have a maximal PL emission wavelength at 640 nm, corresponding to the mean diameter of CdTe QDs of about 3.44 nm [21]. As CdTe QDs modified with mercaptosuccinic acid (MSA) have abundant carboxylic groups (–COOH), so CdTe QDs are water-soluble and carry negative charge in alkaline solution. When the pH value is lower than the isoelectric point of NDV, the ionization of amidogen takes the positive charge and the electrostatic interaction between QDs and NDV may occur. Fig. 1B is the RLS spectrum of QDs and NDV. From Fig. 1B, it can be seen that NDV exhibited no RLS signal in wavelength region of 250–800 nm. The QDs solution without NDV had a weak RLS peak at 630 nm. When QDs were mixed with NDV, the scattering intensity at 555 nm was enhanced obviously, and two shoulder peaks at 468 and 617 nm can also be observed. So a wavelength of 555 nm was chosen for further experiments.

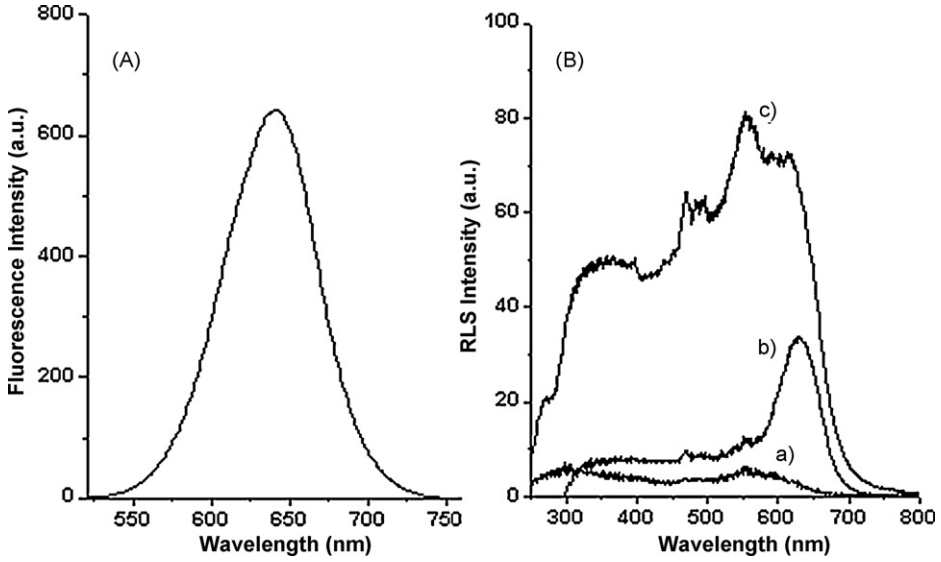

Fig. 1. (A) Fluorescence spectra of CdTe QDs. (B) The RLS spectra of (a) NDV, (b) QDs and (c) QDs–NDV.

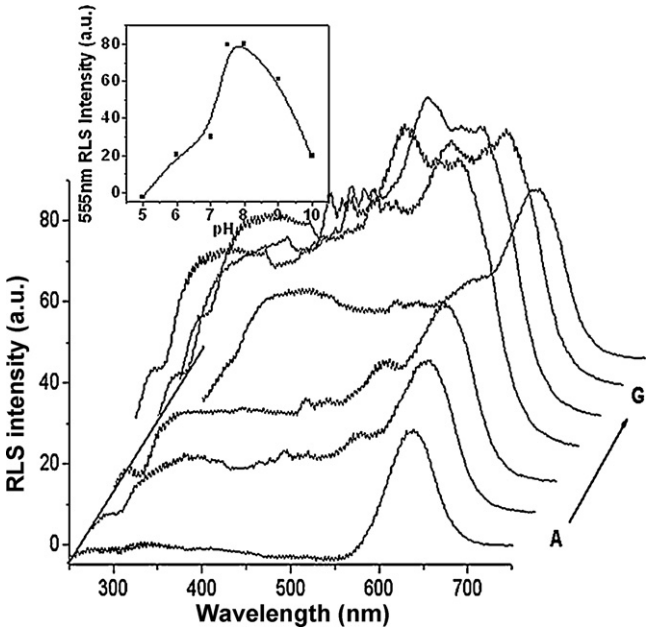

Fig. 2. The effect of pH on RLS spectra of QDs–NDV complex. [QDs] = 6.5×10^{-6} mol/L; [NDV] = 0.5 μg/mL; pH = 5.0 (A), 6.0 (B), 7.0 (C), 7.5 (D), 8.0 (E), 9.0 (F), 10.0 (G). The inset shows the relation between the RLS intensity at 555 nm and pH.

3.2. Optimization of the general procedure

3.2.1. Effect of pH

The effect of pH on the RLS intensity of QDs–NDV was investigated in the range of pH 5–10 (Fig. 2). Because CdTe QDs are stable in alkaline solution, too low pH value could induce the QDs denaturation. As QDs and protein take the same electric-charge at too high pH value, it can restrain the electrostatic interaction between CdTe QDs and NDV. On the other hand, protein consists of the clew containing a series of amino acid units, which make them intrinsically pH-selective conformations. In too low or too high pH condition, the biomolecules are in the same electric-charge (positive or negative). That results in the bilateral electrostatic repulsion between protein molecules, which can decrease the RLS intensity. The result in Fig. 2 indicated the maximum resonance light scattering appeared when pH was 7.5. It can also been noticed that RLS peak of the solution appeared at 630 nm at rigorous pH condition, which was the signal of single QDs. It indicated that less electrostatic interaction between CdTe QDs and NDV. So pH 7.5 was selected as the suitable pH value in this study.

3.2.2. Effect of ionic strength

The ionic strength of the medium had an effect on the interaction of CdTe QDs with NDV. Before the ionic strength reached 0.003 mmol/L, the RLS intensity of CdTe QDs in the presence of NDV increased with increasing ionic strength, as shown in Fig. 3. Then RLS intensity decreased when the ionic strength was above 0.003 mmol/L. When the ionic strength of the medium was lower than 0.5 mmol/L or higher than 0.02 mol/L, the RLS signals were scarcely detected. This phenomenon is closely associated with the suppression of the electric double-layer of protein in the presence of ionic strength. The appropriate ionic strength can decrease the bilateral electrostatic repulsion of protein and improve the interaction between CdTe QDs and NDV. Excessive salt makes an increase in the hydratability of protein and depresses the binding affinity. Therefore, PBS buffer solution with 3.0 mmol/L NaCl was chosen in this study.

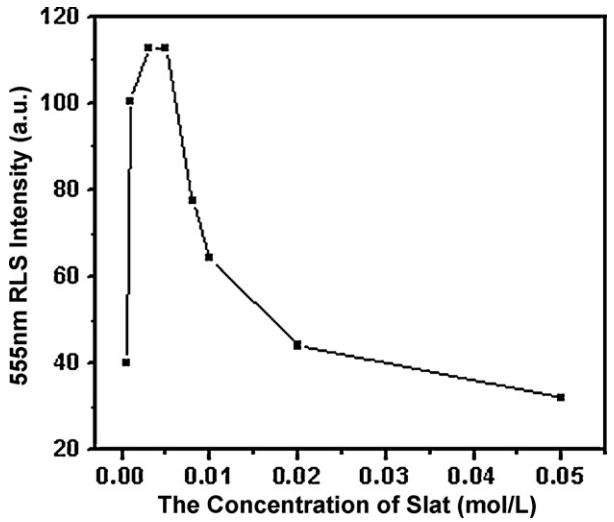

Fig. 3. The effect of ionic strength on RLS intensity of QDs–NDV complex. Ionic strength is: 0.0005, 0.001, 0.003, 0.005, 0.008, 0.010, 0.020, and 0.050 mol/L.

3.2.3. Effect of incubation temperature and time

The effect of incubation temperature on the resonance light scattering intensity was also investigated. The results are shown in Fig. 4. It can be seen that the resonance light scattering intensity increased with the increase of incubation temperature from 4 to 30 °C, and then it decreased. The reason for this phenomenon is that too high temperature could induce the protein denaturation, which can decrease the RLS intensity. While too low temperature is not favorable for the conjunct interaction of CdTe QDs and NDV. It indicated that the reaction can actually reach equilibrium at 30 °C.

In order to study the binding of electrostatic interaction between CdTe QDs and NDV, the effect of reaction time on the RLS intensity of QDs–NDV was investigated. For this purpose, QDs and NDV were mixed according to the procedure described above for different time intervals, and the results are shown in Fig. 5. It can be seen that the RLS intensity of QDs–NDV gradually increased with the increase in reaction time until 30 min. The results showed that the reaction between QDs and NDV occurred rapidly at 30 °C while incubated for 30 min.

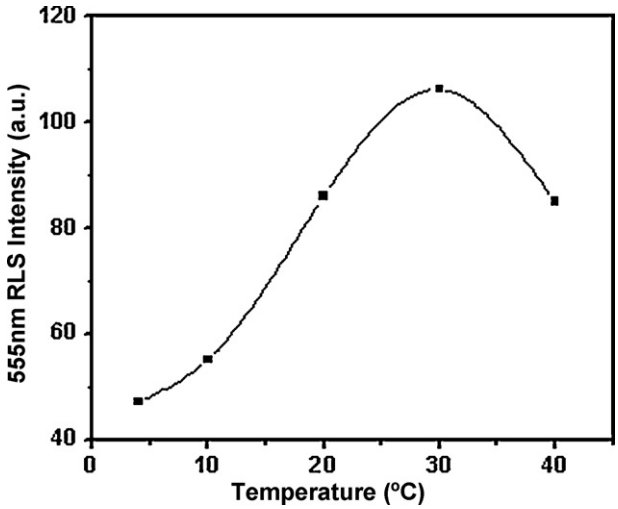

Fig. 4. The effect of incubation temperature on RLS intensity of QDs–NDV complex. The incubation temperature is: 4, 10, 20, 30, and 40 °C.

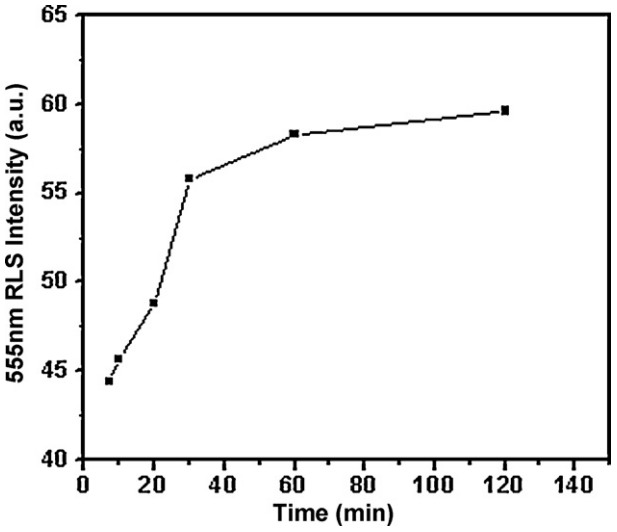

Fig. 5. The effect of reaction time on RLS intensity of QDs–NDV complex. The reaction time is: 7, 10, 20, 30, 60, and 120 min.

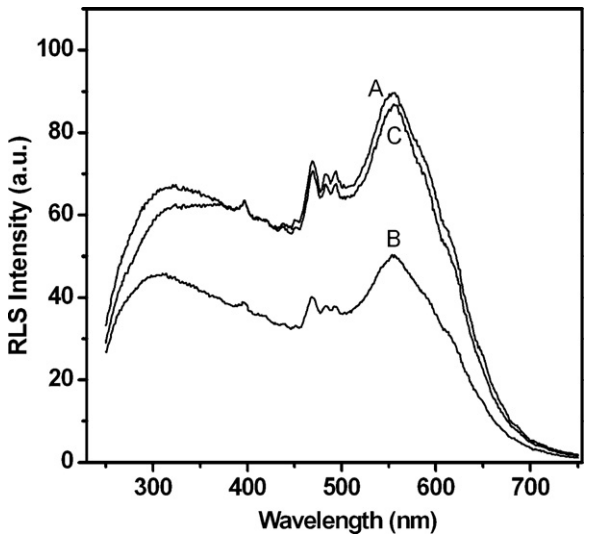

Fig. 7. The RLS spectra of (A) QDs–NDV, (B) QDs–NDV–NDV antibody, (C) QDs–NDV–goat anti-rabbit IgG. The concentrations of QDs, NDV, NDV antibody and goat anti-rabbit IgG are 6.5×10^{-6} mol/L, 0.1 μg/mL, 0.05 μg/mL and 0.1 μg/mL, respectively.

3.2.4. The effect of the concentration of NDV

NDV with different concentrations was added into CdTe QDs aqueous solution and the mixture underwent a RLS experiment. The results are displayed as Fig. 6. For a given concentration of CdTe QDs (6.5×10^{-6} mol/L), a significant enhancement of RLS intensity was observed with the increasing concentration of NDV. Then a turning point at 0.1 μg/mL and a slightly inclined platform emerged with increasing the amount of NDV. The saturated concentration of 0.1 μg/mL for NDV was adopted as the optimal conditions in the further experiment.

3.3. Immunoassay on the NDV-conjugated QDs

The specificity of immunoreactions is very high between NDV and NDV antibody. Due to the specificity of immunoreaction, NDV antibody competed with QDs for NDV in the solution. Upon the addition of NDV antibody, the occurrence of immunoreaction dispersed QDs–NDV complex aggregation, which will decrease the RLS signal intensity of QDs–NDV complex [22]. As shown in Fig. 7, the addition of NDV antibody in the QDs–NDV solution decreased RLS intensity obviously. In order to investigate the specificity of this method, goat anti-rabbit IgG was added into QDs–NDV solution as a blank test. The result showed that goat anti-rabbit IgG hardly affected the RLS signal of QDs–NDV (Fig. 7), indicating that the method has good specificity.

The relationship between the concentration of NDV antibody and the RLS intensity was further studied under the optimal conditions in this work. The results showed that a significant decrease of RLS intensity was observed with the increasing concentration of NDV antibody (Fig. 8). The RLS intensity decreased almost linearly with the concentration of NDV antibody in the range of 0.5–50 ng/mL. The linear regression equation was as follows: $I_{RLS}/I^0_{RLS} = 0.6465 - 0.0039\,C_{anti\text{-}NDV}$ (ng/mL), with the coefficient of correlation was 0.974. This approach can be used to detect as low as 0.1 ng/mL NDV antibodies. In order to evaluate the feasibil-

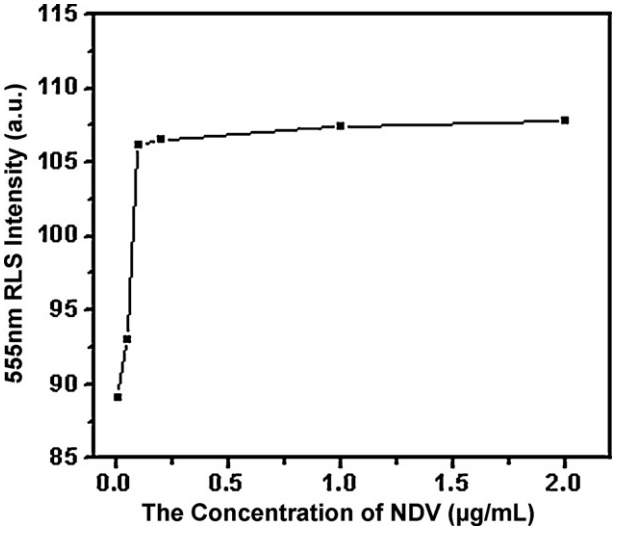

Fig. 6. The effect of the concentration of NDV on RLS intensity of QDs–NDV complex. Conditions: [QDs] = 6.5×10^{-6} mol/L; pH 7.5; the ionic strength is 0.003 mol/L (NaCl); incubation temperature is 30 °C; reaction time is 30 min; λ = 555 nm; [NDV] = 0.01, 0.05, 0.1, 0.2, 0.5, 1.0, and 2.0 μg/mL.

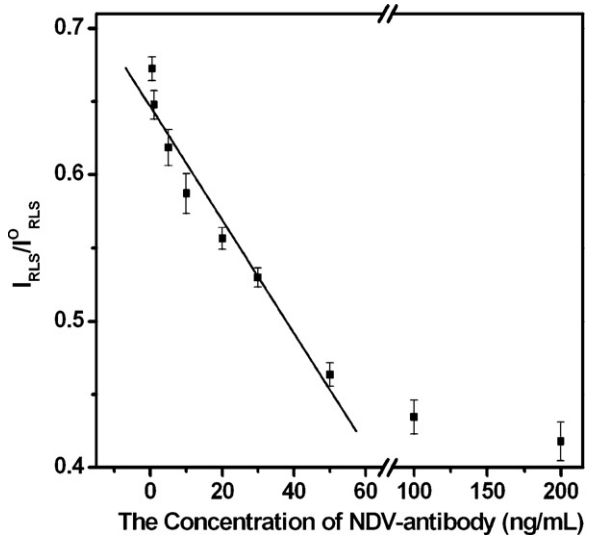

Fig. 8. The RLS spectra of QDs–NDV containing different NDV antibody concentrations: 0.5, 1, 5, 10, 20, 30, 50, 100, and 200 ng/mL. It shows the relationship between the RLS intensity at 555 nm and the concentrations of NDV antibody.

Table 1
Analytical results of NDV antibody in spiked samples ($n = 3$).

Sample	Added (ng/mL)	Found (ng/mL)[a]	Recovery (%)
1	2	2.30 ± 0.48	115.00
	10	9.35 ± 0.32	93.50
	20	19.81 ± 0.25	99.05
2	2	2.04 ± 0.39	102.00
	10	11.17 ± 0.64	111.70
	20	19.26 ± 0.46	96.30
3	2	2.17 ± 0.34	108.50
	10	9.90 ± 0.21	99.00
	20	18.91 ± 0.29	94.55

[a] Values are mean of three replicates (±R.S.D.).

ity of the method, three spiked samples were analyzed in this study. Spiked serum samples were prepared by adding appropriate volumes of NDV antibodies solution to blank chicken serum samples and the volume added was always less than 2% of the final sample volume to preserve the integrity of the samples. After stirring for 10 min and equilibrated at 37 °C for 60 min, the spiked samples were detected according to the described analytical procedure; the results were listed in Table 1. From Table 1, it can be seen that the recoveries of the three spiked samples were found to be in the range of 93–115%, validating the feasibility of immunoassay based on the QDs-RLS. Compared with virus neutralization tests, haemagglutination inhibition tests, enzyme-linked immunosorbent assays and agar gel precipitation tests [23–26], the proposed immunoassay technique based on QDs-RLS is simple, rapid with high sensitivity.

4. Conclusion

In summary, a sensitive QDs-based RLS immunoassay method for the detection of Newcastle disease virus (NDV) antibody is proposed in this paper, which took advantage of high sensitivity of RLS techniques and specific selectivity of immunoreaction. The effects of ionic strength, pH condition, incubation temperature and reaction time on QDs–NDV system were investigated. The QDs-RLS system can be used as a probe for the sensitive determination of protein with the advantages of simple, rapid and sensitive. With further studies in this field, the QDs-RLS system will be promising to be widely applied to aggregation-based immunoassays, DNA hybridization, enzyme–substrate interaction, etc.

Acknowledgements

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