
Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Analytica Chimica Acta 1186 (2021) 339134

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

# A homogeneous nucleic acid assay for simultaneous detection of SARS-CoV-2 and influenza A (H3N2) by single-particle inductively coupled plasma mass spectrometry

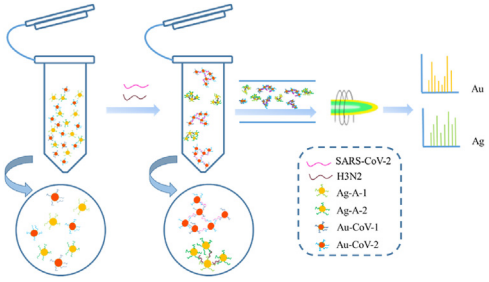
Yan Xu , Beibei Chen , Man He , Bin Hu *

*Department of Chemistry, Wuhan University, Wuhan, 430072, China*

## HIGHLIGHTS

- Homogeneous assay for simultaneous detection of SARS-CoV-2 and H3N2 were developed by SP-ICPMS with nanoparticle probes.
- The method doesn't require separation and washing procedure, which will be beneficial for highly infectious virus detection.
- The developed method is simple, sensitive and fast.
- The developed method is easy to extend to detect other biotargets by changing modification sequences on the NPs probe.

## GRAPHICAL ABSTRACT




## ARTICLE INFO

*Article history:*
Received 22 July 2021
Received in revised form
14 August 2021
Accepted 28 August 2021
Available online 2 October 2021

*Keywords:*
SP-ICP-MS
Simultaneously homogeneous detection
COVID-19
SARS-CoV-2
H3N2 influenza
Nanoparticles probe

## ABSTRACT

In recent years, single particle inductively coupled plasma mass spectrometry (SP-ICP-MS) has become a powerful tool for biological quantitative analysis. Homogeneous analysis method requires no separation and washing steps, which is suited for the analysis of highly infectious pathogens, so as to reduce the risk of infection during the operation. SARS-CoV-2 spreads all over the world, and its early infection symptoms are similar to influenza, which brings inconvenience to triage. Therefore, developing novel analytical method for simultaneous detection of multiple viral nucleic acids is essential. Taking the advantages of SP-ICP-MS and homogeneous analysis strategy, a SP-ICP-MS homogeneous nucleic acid assay by using gold nanoparticles (Au NPs) and silver nanoparticles (Ag NPs) probes was established for simultaneous sensitive analysis of SARS-CoV-2 and influenza A (H3N2). In the present of target SARS-CoV-2 or H3N2 nucleic acids, corresponding Au NPs or Ag NPs probes form larger aggregates, resulting in increased pulse signal intensity and reduced pulse signal frequency of the corresponding NPs in SP-ICP-MS measurement. In this assay, the reaction system of Au NPs and Ag NPs probes does not interfere with each other, and there was no separation and washing procedure, which facilitates operation, saves the analysis time, and improves the analysis efficiency. The linear range of this method is 5 −1000 pmol $L^{-1}$, with low-level limits of quantification of target nucleic acid. The developed SP-ICP-MS simultaneous homogeneous detection method has a good potential for detecting nucleic acid, protein, cell and other biological samples by changing different modification sequences on the NPs probes.



* Corresponding author.
*E-mail address:* binhu@whu.edu.cn (B. Hu).

https://doi.org/10.1016/j.aca.2021.339134
0003-2670/

## 1. Introduction

Since 2019, the Corona Virus Disease 2019 (COVID-19) which was caused by SARS-CoV-2 virus infection has spread worldwide. As of August 9, 2021, there are over 202 million confirmed cases and more than 4.2 million deaths worldwide [1], and they are still on the rise. Common symptoms of COVID-19 patients include high fever, cough, sore throat, headache, and difficulty in breathing, which are similar to flu-like illnesses. However, the infection of COVID-19 may cause acute respiratory distress syndrome in some severe cases within a short time. The pattern of changes in the viral load between COVID-19 patients and influenza patients are similar. The viral load in the early respiratory tract is very high, and there is a high risk of transmission before the onset of symptoms. Therefore, it is very important to diagnose infected individuals early and accurately to prevent the widespread spread of this deadly disease. Up to now, quantitative reverse transcription-polymerase chain reaction (qRT-PCR) assays with different designs are the main method for the diagnosis of SARS-CoV-2 [2]. The simultaneous detection of SARS-CoV-2 and influenza A/B by qRT-PCR has been studied recently [3,4]. But qRT-PCR was limited by sophisticated operation steps, highly specialized equipment, personnel, and reagents. These limitations will extend the turnaround times. Many researchers are trying to find alternative ways to solve these problems. For instance, some researchers use isothermal amplification techniques such as reverse transcription loop-mediated isothermal amplification (RT-LAMP), clustered regularly interspaced short palindromic repeats (CRISPER) [5] and recombinase polymerase amplification (RPA) [6] to eliminate thermal cycling and reduce the experimental time. Simultaneous detection of multiple pathogens is increasingly needed under the current epidemic situation, but most of them are fluorescence methods, which is not conducive for simultaneous detection of multiple targets due to spectral overlap. Simultaneous, rapid, sensitive and accurate detection of SARS-CoV-2 and influenza A/B virus is beneficial to provide more effective assistance in the early stage of triage.

Inductively coupled plasma mass spectrometry (ICP-MS) as an element-specific detector has high sensitivity, wide linear range, strong anti-matrix interference ability, high sample throughput and multi-element analysis ability. ICP-MS based on element labeling for bioanalysis has become a powerful biological quantitative analysis tool in recent years, and has been widely used in the analysis of proteins [7–9], nucleic acids [10,11], viruses [12], cells [13,14], and so on. For instance, Tanner's group had simultaneously detected multiple proteins in complex samples by using the element-tagged antibodies [15]. Peng et al. used polymer-based elemental tags to simultaneously determine multiple glycoproteins (Haptoglobin, hemopexin and ovalbumin) [16]. Wang's group had proposed a sensitive and multiplex method for viral DNAs detection [17]. With multicomponent nucleic acid enzymes (MNAzymes) amplification strategy, lanthanide labeling ICP-MS has also been used for simultaneous detection of three miRNAs recently [18]. Simultaneous detection of circulating tumor cell of HepG2 and MCF-7 cells in human peripheral blood was achieved by ICP-MS using Au NPs and CdSe quantum dots labels combined with magnetic separation [14]. All these facts demonstrate that ICP-MS multi-element detection has great application potential in simultaneous detection of multi-target substances.

Single particle (SP)-ICP-MS [19] is a special ICP-MS detection strategy. By using time - resolved acquisition mode, the pulse signal intensity and frequency of single particles in solution can be obtained, which are related to the size and concentration of particles, respectively. Zhang et al. [20] developed a SP-ICP-MS method with NPs labeling for quantitatively detection of multiplex DNA. Three DNA targets associated with three diseases (HIV, HAV and HBV) were captured on a 96-well plate and labeled to DNA probes with three noble metal NPs (Au, Ag, Pt NPs) via DNA sandwich assay. Then the DNA probes were released for SP-ICP-MS detection. The simultaneous detection of multiple target analytes was realized using the correlation between the pulse signal frequency of different NPs and the corresponding concentration of target analytes. However, it is a heterogeneous assay which includes multiple separation and washing steps.

Homogeneous analysis has attracted more attention in recent years. Homogeneous assay principles rely on signal generation in solution without immobilization, separation or washing steps [21,22], and has the advantages of simple operation, fast analysis speed, and easy to automation. Han et al. [23] used single-NP labeling to realize a one-step homogeneous DNA assay by SP-ICP-MS. They hybridized DNA target with DNA probes immobilized on Au NPs, resulting in decreased concentrations of Au NPs as well as increased sizes of Au NPs aggregation. Quantification of DNA can be obtained by SP-ICP-MS measurement of the degree of the aggregation of Au NPs. Huang et al. [24] applied the SP-ICP-MS homogeneous detection method to detect antigens by using Au NPs labeled antibody as elemental tag. Later on, they [25] extended the SP-ICP-MS homogeneous immunoassay to simultaneous analysis of multiple cancer related biomarkers. Yin et al. [26] established a universal detection method for different kinds of nucleic acids (such as DNA, RNA, and microRNA) by combining SP-ICP-MS homogeneous detection with MNAzyme amplification. Li et al. [27] used target-induced hybridization chain reaction (HCR) to generate a corresponding number of Au NP aggregates for SP-ICP-MS detection, realizing analysis of DNA at fM level.

When dealing with infectious agents, the risk of contamination or infection during testing cannot be ignored, and a homogeneous method that does not require separate operations will be beneficial for facilitating operation and safety. Therefore, in this work, SP-ICP-MS homogeneous assay with noble metal NPs probes was developed to simultaneously analyze two viral sequences of SARS-CoV-2 and influenza A (H3N2) with similar symptoms in the early stage of infection. The specific scheme is shown in Fig. 1. Firstly, two 15-nt segments of SH-DNA (SH—CoV-1 and SH-CoV-2 complementary to the characteristic sequence of SARS-CoV-2) were modified on Au NPs to prepare two kinds of Au NP probes, and two kinds of Ag NP probes were prepared by modifying 15-nt sequence of SH-DNA (SH-A-1 and SH-A-2 complementary to H3N2 characteristic sequence) on Ag NPs. When the target nucleic acids were present, larger aggregates were formed by corresponding probes hybridizing with target nucleic acids, which increased the pulse signal intensity and reduced the pulse signal frequency of the

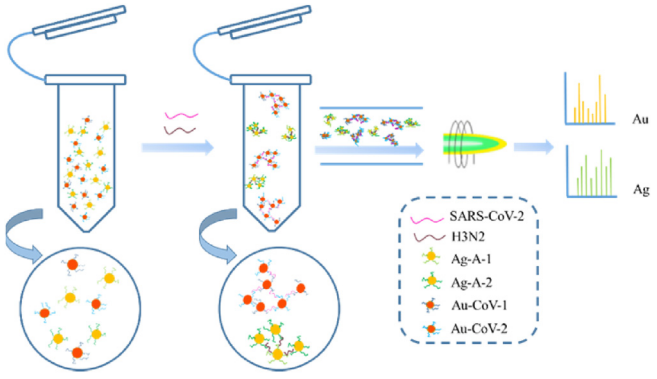



**Fig. 1.** Schematic illustration of a simultaneously homogeneous detection system based on Au NPs, Ag NPs and SP-ICP-MS.

corresponding NPs in SP-ICP-MS measurement. According to the relationship between the pulse signal intensity and frequency of NPs and the concentration of target analytes, the dual mode quantification of two different target analytes can be achieved.

## 2. Experimental section

### 2.1. Reagents and instruments

The nucleic acid sequences used are shown in Table S1 and Table S2, and the information of other reagents and buffers used in this work is listed in Text S1.

Thermo Fisher Scientific X Series II ICPMS (USA) was used for SP-ICP-MS detection. Table S3 showed the specific instrument operating parameters. Other instruments used in this work are shown in Text S2.

### 2.2. Synthesis of Au NPs and Ag NPs probes

The preparation of Au NPs and Ag NPs probes was based on our previous work [26] with slight modifications. Briefly, 10 μL of ammonium acetate buffer solution (500 mmol $L^{-1}$ with 10 mg $mL^{-1}$ Tris(2-carboxyethyl)phosphine (TCEP), pH 6.8) was added into 10 μL of SH-CoV-1 and SH-CoV-2 solutions (100 μmol $L^{-1}$), respectively. The mixtures were mixed and incubated at 37 °C for 1 h. After that, 1 mL of Au NPs solution (preparation of Au NPs with different particle sizes was detailed in Text S3) was added and incubated at room temperature overnight for 12 h. Then 20 μL of 0.01% Tween-20 solution was added to the mixture, and 3 mol $L^{-1}$ NaCl solution was added every 40 min for 6 times, and the final concentration of NaCl in the solution was 0.3 mol $L^{-1}$. The mixture was incubated at room temperature overnight, centrifuged at 8500 rpm for 15min, and washed by PBST buffer for 4 times. Finally, the Au NPs probes were stored at 4 °C for following experiments. The preparation of Ag NPs probes (SH-A-1 and SH-A-2 labeled) were similar to the preparation steps of Au NPs probes.

### 2.3. Simultaneous homogeneous detection by SP-ICP-MS based on Au NPs and Ag NPs probes

The four synthesized probes (2.5 μL Au NPs-CoV-1, Au NPs-CoV-2, Ag NPs-A-1, and Ag NPs-A-2 probes) were mixed with 10 μL 30 mmol $L^{-1}$ $MgCl_2$, 20 μL Target-CoV and 20 μL Target-H3N2 at different concentrations (the final concentration in 60 μL reaction solution was 0, 5, 10, 20, 50, 100, 200, 500, 800, 1000 pmol $L^{-1}$, respectively), and incubated for 10 min at 60 °C, and then cooled slowly to room temperature. After that, the sample was diluted 3000 times with PBST for SP-ICP-MS determination.

## 3. Results and discussion

### 3.1. SP-ICP-MS detection

Four kinds of Au NPs with different sizes were synthesized for SP-ICP-MS detection. The prepared Au NPs were characterized by transmission electron microscope (TEM). As shown in the TEM images (Fig. S1), four kinds of Au NPs were dispersed well with regular shape and uniform particle sizes. According to the size distributions (Fig. S2), the particle sizes of four kinds of Au NPs were 20.8 ± 2.4, 29.9 ± 4.2, 34.9 ± 3.9 and 42.8 ± 5.4 nm, respectively.

The intensity and frequency of pulse signals of the above prepared Au NPs were detected by SP-ICP-MS, and the two quantitative models of SP-ICP-MS pulse intensity and frequency variation were validated. As shown in Fig. 2A, SP-ICP-MS was used to measure four kinds of Au NPs whose particle concentration was about 3 × $10^6$ particles/mL. It can be seen that the pulse signal intensity increased with the increase of the diameter of Au NPs. A fine linear fitting was made between the average pulse signal intensity and the diameter of Au NPs (Fig. 2C). It also demonstrates that SP-ICP-MS is capable to distinguish aggregates containing different number of gold atoms. Then, the 29.9 nm Au NPs with different particle concentrations were determined by SP-ICP-MS, and the mass spectra are shown in Fig. 2B. As the concentration of Au NPs increased, the pulse signal frequency gradually increased, and there was a good linear correlation between the pulse signal frequency and particle concentration (Fig. 2D). These results indicate that SP-ICP-MS has a good ability to distinguish the particle concentration of NPs in solution as well. Therefore, the degree of hybridization of NPs in solution can be identified by SP-ICP-MS in both pulse intensity and frequency variation modes.

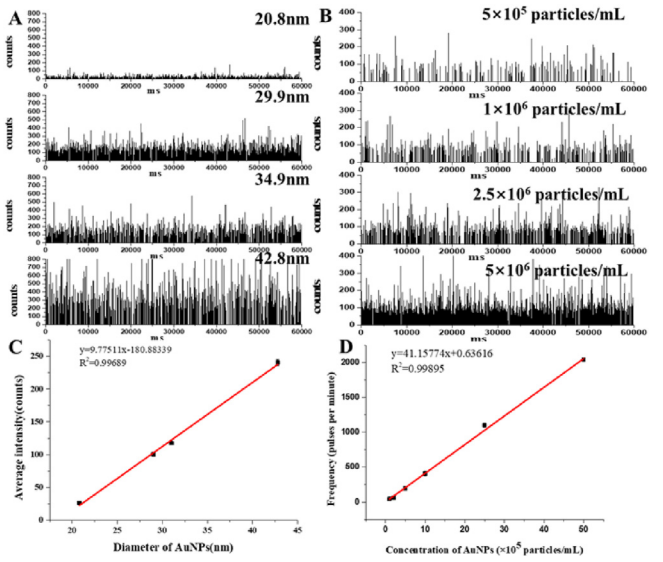



**Fig. 2.** SP-ICP-MS spectra of Au NPs with various diameters (concentration: 3 × $10^6$ particles/mL) (A). SP-ICP-MS spectra of Au NPs with various concentrations (particle size: 29.9 nm) (B). Correlational relationship between the diameter of Au NPs and average intensity (C). Correlational relationship between the concentration of Au NPs and pulse signal frequency (D).

### 3.2. The feasibility of the designed homogeneous nucleic acid assay

In the designed homogeneous nucleic acid assay (shown in Fig. 1), Au NPs and Ag NPs probes are used to hybridize with target nucleic acids (SARS-CoV-2 and H3N2, respectively) to aggregate larger NPs, and the Au NPs and Ag NPs under different hybrid conditions are simultaneously detected by SP-ICP-MS. To validate the feasibility of the designed strategy, a series of target nucleic acids with different concentrations were determined by the developed SP-ICP-MS homogenous assay. The aggregation of Au NPs and Ag NPs was determined by SP-ICP-MS and characterized by TEM images. As can be seen from SP-ICP-MS mass spectra (Fig. 3), with the increase of the concentration of the target nucleic acid, the intensity of pulse signals gradually increased while the frequency decreased correspondingly. It indicates the specific aggregation between NP probes and target nucleic acids occurred, and the more target nucleic acids, the higher degree of NPs aggregation. In the TEM images (Fig. 4), no NPs aggregation was found without the addition of targets, while the degree of hybridization of NPs increased with the increase of concentration of target nucleic acids. The TEM results further verified that SP-ICP-MS detection was able

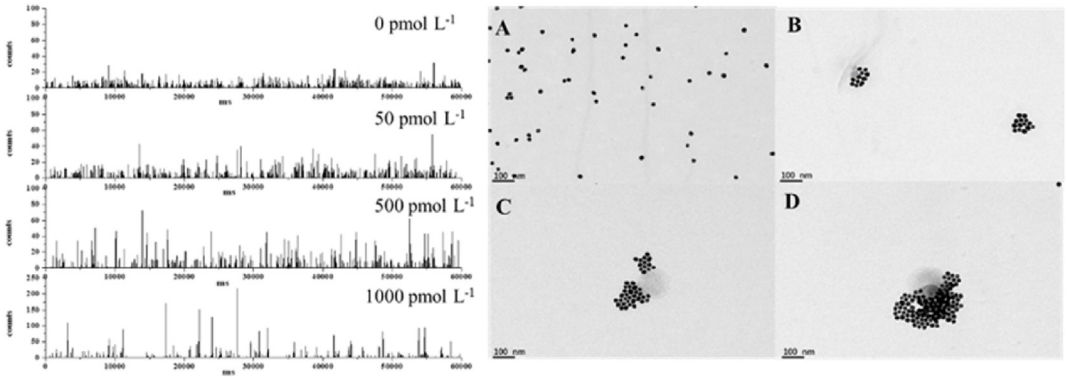



**Fig. 3.** SP-ICP-MS results (left) and TEM images (right) of Au NPs probes with 0 (A), 50 (B), 500 (C) and 1000 (D) pmol $L^{-1}$ target-CoV.

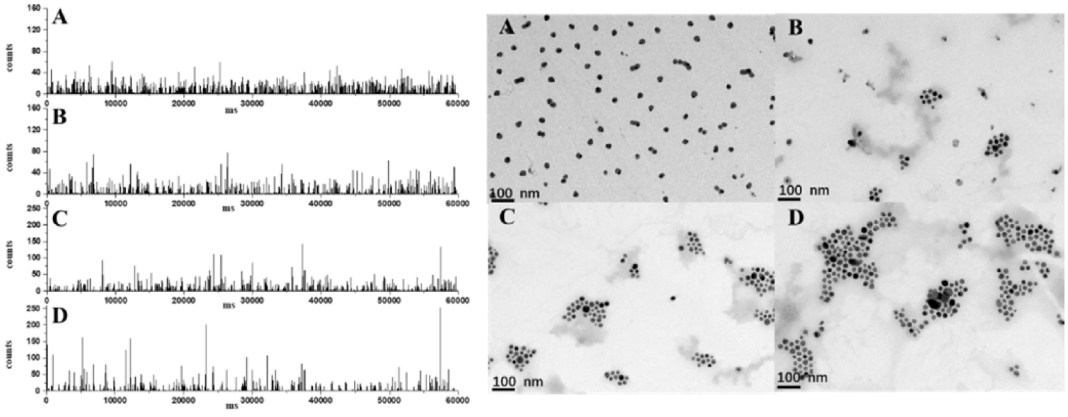



**Fig. 4.** SP-ICP-MS results (left) and TEM images (right) of Ag NPs probes with 0 (A), 50 (B), 500 (C) and 1000 (D) pmol $L^{-1}$ target-H3N2.

to distinguish the concentration of targets by characterizing the degree of aggregation. By fitting the concentration of target nucleic acid with the pulse signal intensity and frequency of NPs aggregation, a dual mode quantification of SARS-CoV-2 and H3N2 viral sequences can be achieved.

The average number of labeled DNA molecules modified on the surface of Au NPs and Ag NPs was determined by fluorescence method (details were illustrated in Text S4 and Fig. S3). The results indicate that the average number of the labeled DNA molecules of each Au NP was 308, and the number of the labeled DNA molecules per Ag NP was 212.

### 3.3. Optimization of experimental conditions

To achieve best analytical performance, important parameters related to the experimental conditions were optimized, including dwell time for ICP-MS detection; concentration of Au NPs/Ag NPs probes; the concentration of $Mg^{2+}$ as a stabilizer for DNA complementary hybridization. Detailed steps and the results for experimental conditions optimization were provided in supporting information (Text S5 and Fig. S4-S6). Finally, the optimal experimental conditions were chosen as follows: the dwell time of ICP-MS was 2 ms; the concentration of Au NPs/Ag NPs probes was 100 pmol $L^{-1}$; and the concentration of $Mg^{2+}$ was 5 mmol $L^{-1}$.

### 3.4. Specificity of probes

In order to investigate the specificity of the probes, three sequences with single, double and triple mismatched bases were designed, respectively, and analyzed by the developed SP-ICP-MS homogenous assay. The results are shown in Fig. S7. The intensity of all the mismatched sequences was similar with the blank group, indicating that the method had excellent specificity for target nucleic acids with high sequence similarity.

### 3.5. Cross-reactivity between Au NP probes and Ag NP probes

Since in the developed homogeneous assay, the targets of SARS-CoV-2 and H3N2 were detected simultaneously with the addition of both Au NPs/Ag NPs probes, it was necessary to confirm that there was no cross reaction between the two reaction systems. The selectivity and cross-reactivity of the developed assay was studied. As can be seen from Fig. 5, when only the target-CoV was added, the signal of Au increased, but the signal of Ag did not change significantly compared with the corresponding blank signals (Ag NPs + Au NPs). Similarly, when only the target-H3N2 was added, the Ag signal increased, while the Au signal did not change significantly compared with the corresponding blank signals. Meanwhile, when the target-CoV and target-H3N2 existed alone or coexisted with each other, the generated signals were almost the same. These results indicated that there was no obvious interference between the synthesized Au NP probes and Ag NP probes and their target nucleic acids, and there was no cross-reaction during simultaneous detection of multiple elements.

### 3.6. Analytical performance

The analytical performance of the developed method was

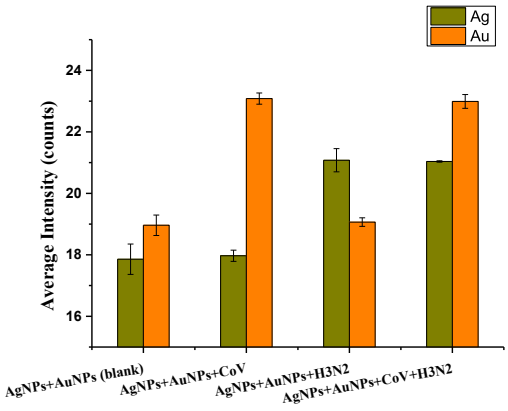



**Fig. 5.** Selectivity and cross-reactivity of the mixed probes.

investigated under optimal experimental conditions. The final fitting results between the average intensity of NPs and the concentrations of target nucleic acids were shown in Fig. 6. Wherein, for determination of target-CoV with Au NPs probe, the linear range was 10–1000 pmol $L^{-1}$ (Y = 0.01322X +10.64070, $R^2$ = 0.9985), the relative standard deviation (RSD) was 4.9% (c = 100 pmol $L^{-1}$, n = 7). For determination of target-H3N2 with Ag NPs probe, the linear range was 10–1000 pmol $L^{-1}$ (Y = 0.01539x+18.11898, $R^2$ = 0.9996), and the RSD was 2.1% (c = 100 pmol $L^{-1}$, n = 7).

The correlation between the frequency obtained and the target nucleic acids concentrations was also explored, and it was found that with the increase of the concentration of the target nucleic acid, the frequency gradually decreased, showing an exponential correlation with the concentration of the target nucleic acid (Fig. S8). The frequency and log nucleic acid concentration have a good linear relationship as shown in Fig. 7. The linear equation between the frequency of Au NP probes and concentration of the target-CoV was Y = −101.77927logx+364.129, $R^2$ = 0.9941, in a fitting range of 5–1000 pmol $L^{-1}$, the RSD was 4.0% (c = 100 pmol $L^{-1}$, n = 7). And the linear equation between the frequency of Ag NP probes and concentration of the target-H3N2 was Y = −99.61924logx+325.82568, $R^2$ = 0.9887, in a fitting range of 5–1000 pmol $L^{-1}$, the RSD was 3.1% (c = 100 pmol $L^{-1}$, n = 7).

The analytical performance of the proposed homogenous SP-ICP-MS method was compared with other reported nucleic acid detection methods, and the results were listed in Table S4. The limits of quantification (LOQs) of this method were lower than the fluorescence methods [28,29] and scattering spectroscopic method [30]. The LOQs and linear range of this method were comparable with those SP-ICP-MS methods. Specifically, since no nucleic acid amplification method was introduced in this method, the LOQs of this method were at the same level as DNA detection assay based on SP-ICP-MS methods [20,23], but higher than that of other SP-ICP-MS methods [26,27] which introduced nucleic acid amplification. Compared with electrochemical methods, the LOQs of the developed method was lower than the method reported in Refs. [31,32], but slightly higher than the work with nucleic acid amplification involved [33]. In addition, this method was a homogeneous detection method, which was simple to operate. Meanwhile, this method can detect two different target nucleic acids simultaneously by using two different modes of pulse signals frequency and average intensity.

### 3.7. Real-sample analysis

Saliva and urine are useful non-invasive specimen types. In healthy human saliva and urine samples, both of two target nucleic acids were not detected. To validate the applicability of the developed method, spiking experiments were carried out. Target nucleic acids were spiked into saliva and urine with 50, 100, 500 pmol $L^{-1}$ respectively, and the determined results are summarized in Table S5 and Table S6. For each spiking level, the measured concentrations matched well with spiking concentrations with satisfactory recovery, demonstrating that the method had good anti-interference ability to biological matrix and was expected to be useful in actual sample analysis. In addition, the concentrations obtained from intensity mode and frequency mode were tested by *t*-test. As can be seen from the calculated P value, there was no significant difference between these two modes, indicating that the two modes can be used for accurate quantitative analysis of target analytes in actual samples, and what is more, they can verify each other.

## 4. Conclusion

In this work, a homogeneous detection method of SP-ICP-MS was established by using Au NPs and Ag NPs as probes for simultaneous sensitive analysis of nucleic acid sequences of two different viruses. Homogeneous analysis method requires no separation and washing steps, and is easy to operate, which is very suitable for the treatment and detection of highly infectious pathogens to reduce the risk of infection during the operation. SP-ICP-MS can be used for simultaneous and highly sensitive detection of multiple elements. The reaction systems of Au NPs and Ag NPs did not interfere with each other, which made it possible to simultaneously detect SARS-CoV-2 and H3N2 with high analytical efficiency. Besides, the design and synthesis of probes in this method was very simple. This method can be further extended to SP-ICP-MS simultaneous

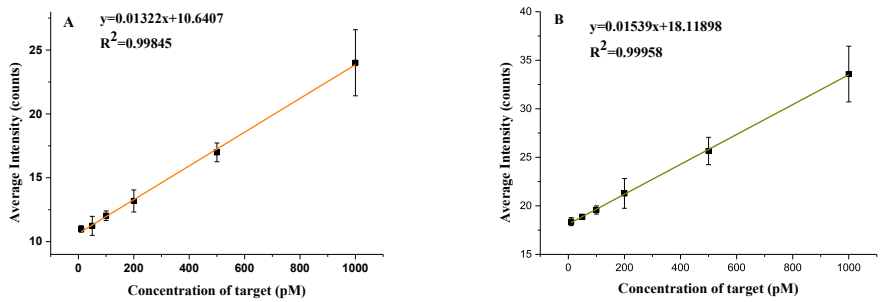



**Fig. 6.** Linear relationship between average intensity of Au NPs (A)/Ag NPs(B) and concentration of target nucleic acids.

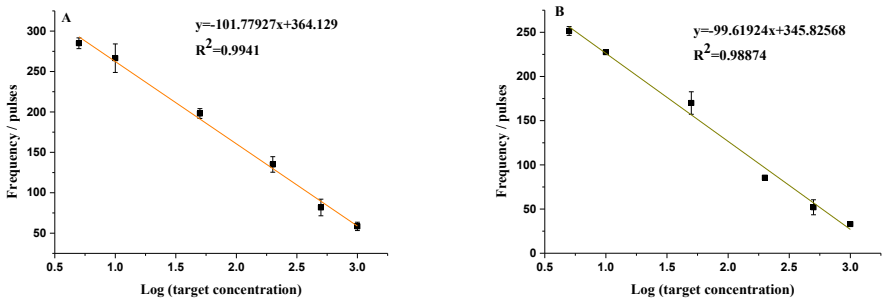



**Fig. 7.** Linear relationship between frequency of Au NPs (A)/Ag NPs(B) and concentration of target nucleic acids.

homogeneous detection of multiple nucleic acids, proteins, cells and other biological molecules by changing different modification sequences and using different NPs probes.

## CRediT authorship contribution statement

**Yan Xu:** Investigation, Methodology, Writing – original draft. **Beibei Chen:** Methodology, Writing – review & editing. **Man He:** Methodology. **Bin Hu:** Supervision, Conceptualization, Writing – review & editing, Funding acquisition.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

This work is financially supported by the National Nature Science Foundation of China (Grant No. 21974100, 21775113).

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aca.2021.339134.

## References

[1] World Health Organization. Coronavirus disease (COVID-19) Situation Report. https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports (Accessed 9 August 2021).
[2] V.M. Corman, O. Landt, M. Kaiser, R. Molenkamp, A. Meijer, D.K. Chu, T. Bleicker, S. Brünink, J. Schneider, M.L. Schmidt, D.G. Mulders, B.L. Haagmans, B. van der Veer, S. van den Brink, L. Wijsman, G. Goderski, J.-L. Romette, J. Ellis, M. Zambon, M. Peiris, H. Goossens, C. Reusken, M.P. Koopmans, C. Drosten, Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR, Euro Surveill. 25 (2020) 2000045.
[3] D. Nörz, A. Hoffmann, M. Aepfelbacher, S. Pfefferle, M. Lütgehetmann, Clinical evaluation of a fully automated, laboratory-developed multiplex RT-PCR assay integrating dual-target SARS-CoV-2 and influenza A/B detection on a high-throughput platform, J. Med. Microbiol. 70 (2021), 001295.
[4] M.H. Ji, Y. Xia, J.F.-C. Loo, L. Li, H.-P. Ho, J.N. He, D.Y. Gu, Automated multiplex nucleic acid tests for rapid detection of SARS-CoV-2, influenza A and B infection with direct reverse-transcription quantitative PCR (dirRT-qPCR) assay in a centrifugal microfluidic platform, RSC Adv. 10 (2020) 34088–34098.
[5] B. Pang, J.Y. Xu, Y.M. Liu, H.Y. Peng, W. Feng, Y.R. Cao, J.J. Wu, H.Y. Xiao, K. Pabbaraju, G. Tipples, M.A. Joyce, H.A. Saffran, D.L. Tyrrell, H.Q. Zhang, X.C. Le, Isothermal amplification and ambient visualization in a single tube for the detection of SARS-CoV-2 using loop-mediated amplification and CRISPR technology, Anal. Chem. 92 (2020) 16204–16212.
[6] P. Wang, C. Ma, X. Zhang, L.Z. Chen, L.Y. Yi, X. Liu, Q.W. Lu, Y. Cao, S. Gao, A ligation/recombinase polymerase amplification assay for rapid detection of SARS-CoV-2, Front. Cell. Infect. Microbiol. 11 (2021) 680728.
[7] X. Yin, B.B. Chen, M. He, B. Hu, Simultaneous determination of two phosphorylated p53 proteins in SCC-7 cells by an ICP-MS immunoassay using apoferritin-templated europium(III) and lutetium(III) phosphate nanoparticles as labels, Microchim. Acta 186 (2019) 8.
[8] G.W. Sun, B. Huang, Y. Zhang, Y.Q. Zhang, Z. Xing, S.C. Zhang, X.R. Zhang, A combinatorial immunoassay for multiple biomarkers via a stable isotope tagging strategy, Chem. Commun. 53 (2017) 13075–13078.
[9] J.M. Liu, X.P. Yan, Ultrasensitive, selective and simultaneous detection of cytochrome c and insulin based on immunoassay and aptamer-based bioassay in combination with Au/Ag nanoparticle tagging and ICP-MS detection, J. Anal. Atom. Spectrom. 26 (2011) 1191–1197.
[10] G.J. Han, S.C. Zhang, Z. Xing, X.R. Zhang, Absolute and relative quantification of multiplex DNA assays based on an elemental labeling strategy, Angew. Chem. Int. Ed. 52 (2013) 1466–1471.
[11] N. Wu, K. Wang, Y.T. Wang, M.L. Chen, X.W. Chen, T. Yang, J.H. Wang, Three-dimensional DNA nanomachine biosensor by integrating DNA walker and rolling machine cascade amplification for ultrasensitive detection of cancer-related gene, Anal. Chem. 92 (2020) 11111–11118.
[12] G.Y. Xiao, B.B. Chen, M. He, K.M. Shi, X. Zhang, X.T. Li, Q.M. Wu, D.W. Pang, B. Hu, Determination of avian influenza A (H9N2) virions by inductively coupled plasma mass spectrometry based magnetic immunoassay with gold nanoparticles labeling, Spectrochim. Acta B 138 (2017) 90–96.
[13] Y. He, S.L. Chen, L. Huang, Z.W. Wang, Y.N. Wu, F.F. Fu, Combination of magnetic-beads-based multiple metal nanoparticles labeling with hybridization chain reaction amplification for simultaneous detection of multiple cancer cells with inductively coupled plasma mass spectrometry, Anal. Chem. 91 (2019) 1171–1177.
[14] X.T. Li, B.B. Chen, M. He, H. Wang, G.Y. Xiao, B. Yang, B. Hu, Simultaneous detection of MCF-7 and HepG2 cells in blood by ICP-MS with gold nanoparticles and quantum dots as elemental tags, Biosens. Bioelectron. 90 (2017) 343–348.
[15] Z.A. Quinn, V.I. Baranov, S.D. Tanner, J.L. Wrana, Simultaneous determination of proteins using an element-tagged immunoassay coupled with ICP-MS detection, J. Anal. Atom. Spectrom. 17 (2002) 892–896.
[16] H.Y. Peng, Y. Jiao, X. Xiao, B.B. Chen, M. He, Z.R. Liu, X. Zhang, B. Hu, Magnetic quantitative analysis for multiplex glycoprotein with polymer-based elemental tags, J. Anal. Atom. Spectrom. 29 (2014) 1112–1119.
[17] Y.C. Luo, X.W. Yan, Y.S. Huang, R.B. Wen, Z.X. Li, L.M. Yang, C.J. Yang, Q.Q. Wang, ICP-MS-based multiplex and ultrasensitive assay of viruses with lanthanide-coded biospecific tagging and amplification strategies, Anal. Chem. 85 (2013) 9428–9432.
[18] Q. Kang, M. He, B.B. Chen, G.Y. Xiao, B. Hu, MNAzyme-catalyzed amplification assay with lanthanide tags for the simultaneous detection of multiple microRNAs by inductively coupled plasma-mass spectrometry, Anal. Chem. 93 (2021) 737–744.
[19] F. Laborda, E. Bolea, J. Jimenez-Lamana, Single particle inductively coupled plasma mass spectrometry: a powerful tool for nanoanalysis, Anal. Chem. 86 (2014) 2270–2278.
[20] S.X. Zhang, G.J. Han, Z. Xing, S.C. Zhang, X.R. Zhang, Multiplex DNA assay based on nanoparticle probes by single particle inductively coupled plasma mass spectrometry, Anal. Chem. 86 (2014) 3541–3547.
[21] H.Q. Zhang, F. Li, B. Dever, X.-F. Li, X.C. Le, DNA-mediated homogeneous binding assays for nucleic acids and proteins, Chem. Rev. 113 (2013) 2812–2841.
[22] A. Sassolas, L.J. Blum, B.D. Leca-Bouvier, Homogeneous assays using aptamers, Analyst 136 (2011) 257–274.
[23] G.J. Han, Z. Xing, Y.H. Dong, S.C. Zhang, X.R. Zhang, One-step homogeneous DNA assay with single-nanoparticle detection, Angew. Chem. Int. Ed. 50 (2011) 3462–3465.
[24] Z.L. Huang, C.Q. Wang, R. Liu, Y.Y. Su, Y. Lv, Self-validated homogeneous immunoassay by single nanoparticle in-depth scrutinization, Anal. Chem. 92 (2020) 2876–2881.
[25] Z.L. Huang, Z.Y. Li, M. Jiang, R. Liu, Y. Lv, Homogeneous multiplex immunoassay for one-step pancreatic cancer biomarker evaluation, Anal. Chem. 92 (2020) 16105–16112.

[26] X. Yin, B.B. Chen, M. He, B. Hu, A homogeneous multicomponent nucleic acid enzyme assay for universal nucleic acid detection by single-particle inductively coupled plasma mass spectrometry, Anal. Chem. 93 (2021) 4952–4959.
[27] B.R. Li, H. Tang, R.Q. Yu, J.H. Jiang, Single-nanoparticle ICPMS DNA assay based on hybridization-chain-reaction-mediated spherical nucleic acid assembly, Anal. Chem. 92 (2020) 2379–2382.
[28] X.W. He, N. Ma, A general strategy for label-free sensitive DNA detection based on quantum dot doping, Anal. Chem. 86 (2014) 3676–3681.
[29] M. Zhang, B.C. Yin, W.H. Tan, B.C. Ye, A versatile graphene-based fluorescence "on/off" switch for multiplex detection of various targets, Biosens. Bioelectron. 26 (2011) 3260–3265.
[30] S.F. Lv, F. Chen, C.Y. Chen, X.M. Chen, H. Gong, C.Q. Cai, A novel CdTe quantum dots probe amplified resonance light scattering signals to detect microRNA-122, Talanta 165 (2017) 659–663.
[31] S. Yang, M. You, L.Z. Yang, F. Zhang, Q.J. Wang, P.G. He, A recyclable electrochemical sensing platform for breast cancer diagnosis based on homogeneous DNA hybridization and host-guest interaction between cucurbit [7]uril and ferrocene-nanosphere with signal amplification, J. Electroanal. Chem. 783 (2016) 161–166.
[32] J.H. Wu, W.X. Lv, Q.T. Yang, H.Y. Li, F. Li, Label-free homogeneous electrochemical detection of MicroRNA based on target-induced anti-shielding against the catalytic activity of two-dimension nanozyme, Biosens. Bioelectron. 171 (2021) 112707.
[33] J.J. Jiang, X.Y. Lin, D. Ding, G.W. Diao, Enzyme-free homogeneous electrochemical biosensor for DNA assay using toehold-triggered strand displacement reaction coupled with host-guest recognition of Fe3O4@SiO2@β-CD nanocomposites, Biosens. Bioelectron. 114 (2018) 37–43.