Detection of Myeloperoxidase and Osteopontin in Human Serum Using Single Particle-ICP-MS with MoS$_2$ and ZnS Quantum Dots

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ABSTRACT: MoS$_2$ and ZnS quantum dots (QDs) were synthesized and applied as labels in magnetic immunoassays for the determination of myeloperoxidase (MPO) and osteopontin (OPN) via single-particle mode inductively coupled plasma mass spectrometry (ICP-MS). Primary MPO antibodies and primary OPN antibodies were captured by amino-modified magnetic nanoparticles, and the target biomarkers (MPO and OPN) were extracted and then specifically labeled with MoS$_2$ QD-secondary MPO antibody and ZnS QD-secondary OPN antibody conjugates, respectively. MoS$_2$ and ZnS QDs were employed as determination signal probes for ICP-MS measurements. Under the optimized conditions, the limits of detection obtained by magnetic immunoassay for MoS$_2$ and ZnS QD-labeled MPO and OPN were 0.004 and 0.005 ng mL$^{-1}$, respectively. The linear ranges for MPO and OPN were 0.01–50 and 0.02–50 ng mL$^{-1}$, respectively. From six replicates, the relative standard deviations of MPO and OPN were found to be 3.5% and 3.2%, respectively. The proposed strategy was applied to determine the MPO and OPN levels in real human serum samples.

INTRODUCTION

Acute coronary syndrome (ACS) is a common cardiovascular disease. It is a group of clinical syndromes based on complete or incomplete occlusions of coronary atherosclerotic plaques following rupture or invasion. For unpredictable cardiovascular events, reliable diagnostic information is essential to provide timely and life-saving therapeutic intervention. Myeloperoxidase (MPO), a type of heme-containing enzyme, is part of the peroxidase-cyclooxygenase superfamily, and is secreted by monocytes and neutrophiles. Systemic MPO levels are linked to coronary plaque erosion, and the number of MPO-expressing macrophages increases in eroded or ruptured plaques, resulting in ACS. MPO is recognized as a diagnostic and stratified marker of ACS. For patients with ACS, elevated blood MPO levels are known to indicate an increased risk for subsequent cardiovascular events. Osteopontin (OPN) is a highly phosphorylated glycoprotein with acidic characteristics. It is also a multifunctional protein that plays a significant role in cardiovascular diseases, cancer, kidney stones, diabetes, cell viability, biomineralization, and inflammation, as well as wound healing. As a latent biomarker for cancer, its targeted inhibition has also received widespread attention for its ability to prevent and alter tumor growth and metastasis. Therefore, the development of new MPO and OPN detection methods for biomedical research and cancer prognosis is important.

To date, several strategies for the detection of MPO and OPN...
include radioimmunoassay, electrochemical immunoassay, and enzyme-linked immunosorbent assays. These analytical methods exhibit excellent specificity; however, they are time-consuming and suffer from complicated preparation requirements and limited sensitivity.

Inductively coupled plasma mass spectrometry (ICP-MS) combined with elemental tag-based magnetic immunoassay has the advantages of wide dynamic ranges, multi-element detection, high sensitivity, rapid analysis capabilities, and low matrix effects, and has been widely employed for bioassays and clinical diagnoses that require the detection of proteins, cells, viruses, or bacteria. Huang et al. reported a new wash-free immunoassay for multiplexed biomarker (CA724, CA199, and CEA) monitoring by single particle mode (SP)-ICP-MS. Chen et al. used multiple lanthanide nanoparticle-tag labeling (NaEuF₄, NaTbF₄, and NaHoF₄) for the multiplex evaluation of breast cancer biomarkers CEA, CA153, and CA125 in human serum samples. Yang et al. reported CdSe/ZnS quantum-dot (QD) labeling for the counting and visualization of HepG2 cells in human whole blood. Wen et al. used nanosatellites (magnetic beads, @ NaLnF₄) and catalytic hairpin assembly amplification in combination with ICP-MS to diagnose cancer accurately. This method was used to detect ten types of miRNAs simultaneously with a limit of detection (LOD) of 0.01 fM.

Owing to their low background interference in biological samples, high sensitivity, low cytotoxicity, and good biocompatibility with ICP-MS, quantum dots (QDs) demonstrate the potential for application as elemental tags for ICP-MS-based assays. Reported QD-based elemental tags include CdSe QDs, CdTe QDs, ZnSe QDs, and Mn-ZnS QDs. Previously, we reported the use of ZnSe QDs, AuNPs, and AgNPs for magnetic immunoassays combined with SP-ICP-MS for the determination of carcinoembryonic antigen, cytokeratin fragment antigen 21-1, and carbohydrate antigen in real human serum samples. MoS₂ quantum dots (QDs) in the family of transition metal dichalcogenide nanomaterials have been used as fluorescent probes and are widely used in photodynamic therapy, bioimaging, and chemical and biosensors. ZnS QDs are one of the most popular semiconductor sulfides. They are environmentally friendly and have good chemical stability compared with other II–VI compound semiconductors. Magnetic nanoparticles (MNPs) possess unique superparamagnetism, which means they are endowed with an appropriate concentration of target molecules, which makes them suitable for performing simple and rapid immunoassays.

In this study, we propose a highly sensitive analytical method for detecting MPO and OPN based on a magnetic immunoassay and ICP-MS detection with MoS₂ and ZnS QDs serving as tags. First, we synthesized amino-modified magnetic nanoparticles (AMNPs), which have been conjugated with MPO Ab (primary MPO antibody, Ab1-MPO) and anti-OPN (primary OPN antibody, Ab1-OPN) to extract MPO and OPN, respectively. Second, the as-prepared MoS₂ and ZnS QD-tagged secondary MPO antibody (Ab2-MPO) and secondary OPN antibody (Ab2-OPN) were used to determine MPO and OPN in human serum samples via SP-ICP-MS. The MoS₂ and ZnS QDs were added to real human serum samples and the ⁹⁰Mo and ⁶⁴Zn content was successfully detected using SP-ICP-MS with a customized gas-pressure-assisted sample introduction system. The proposed method is accurate and rapid and only a small volume is required for the detection of MPO and OPN.

**EXPERIMENTAL**

**Instrumentation and operating conditions.** Table 1 lists the instrumental parameters for ICP-MS. ⁹⁰Mo and ⁶⁴Zn were detected using ICP-MS (NexIon 300X, Perkin Elmer, USA) with a customized gas-pressure-assisted sample introduction system, which we have reported previously. X-ray powder diffractometry (XRD, Rigaku D/max 2500/pc, Japan), Photoelectron spectrometry (ESCALAB 250, Thermo Fisher Scientific, USA), Fourier transform infrared (FTIR) spectroscopy (Perkin Elmer, USA), transmission electron microscopy (TEM, JEM-2100, JEOL, Japan), fluorescence spectrometry (RF-5301, Shimadzu, Japan), and UV-vis spectrophotometry (Cary 60, Agilent Technologies, USA) were used to characterize the prepared MoS₂ and ZnS QDs.

**Reagents.** NaCl, KCl, KH₂PO₄, K₂HPO₄·3H₂O, sodium sulfide nonahydrate, ethanol, citric acid, iron chloride hexahydrate, sodium hydroxide, iron (II) chloride tetrahydrate, formic acid, acetic acid, hydrochloric acid, and zinc acetate were purchased from Xilong Chemicals (Guangdong, China). Bovine serum albumin (BSA) was obtained from SolarBio. N-Hydroxysuccinimide (NHS, 98%), nitric acid, sodium molybdate (Na₂MoO₄·2H₂O, 99%), N-(3-dimethylaminopropyl)-N’-ethylcarbodiimide hydrochloride (EDC, 98%), glutathione (GSH), ammonium hydroxide (28%), and 3-(aminopropyl) triethoxysilane (99%) were obtained from Aladdin (China). Goat Table 1. Working Parameters for ICP-MS

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<td>Monitored isotope</td>
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</tr>
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</table>

**Table 1.** Working Parameters for ICP-MS

**Working Parameters for ICP-MS**
anti-mouse IgG antibody, MPO, MPO Ab, goat anti-rabbit IgG, OPN, and anti-OPN antibodies were obtained from Bioss Antibodies (Beijing, China). All other reagents were of analytical grade and were used without further purification. Ultrapure water (18.2 MW cm) was employed throughout this study.

**Data processing.** An iterative algorithm was applied to distinguish the particle pulse and background signal of the nanoparticles based on the standard deviation (3σ) for SP-ICP-MS. First, the mean and standard deviation of the entire dataset were calculated, followed by the collection of all the signal data that were 3σ combined with the mean for the entire dataset. For the remaining data, the standard deviation was recalculated, followed by the collection of all data that were greater than the new average plus 3σ. This process was repeated until there were no data points 3σ combined with the final mean. The collected data points represent the nanoparticle signals, and the remaining data represent the background.

**Synthesis of AMNPs.** A co-precipitation method was used to prepare Fe₃O₄ MNPs, as reported in our previous work. Iron (II) chloride tetrahydrate (0.9941 g) and iron chloride hexahydrate (2.3650 g) were dissolved in ultrapure water (100 mL) under sonication for 10 min and then reacted at 85 °C under N₂ with vigorous stirring. After 30 min, 10 mL of aqueous ammonia solution was added and the color of the solution changed to black. After separation using a magnet, the MNPs were alternately washed with ethanol and ultrapure water. The MNPs were dispersed in a mixture containing ethanol (75 mL) and ultrapure water (75 mL) under sonication for 30 min. The above solution was transferred into a three-neck flask under N₂. Subsequently, 3-(aminopropyl) triethoxysilane (3.7 mL) was added and stirred for 2 h at 60 °C. The AMNPs were alternately washed with ethanol and ultrapure water and separated using a magnet.

**Synthesis of MoS₂ and ZnS QDs.** MoS₂ QDs were prepared using a previously published hydrothermal method with slight modifications. Ultrapure water (12.5 mL) was added to Na₂MoO₄·2H₂O (0.1211 g) and sonicated for 5 min. The pH of the solution was adjusted to 6.5 by the addition of 0.1 mol L⁻¹ HCl. After mixing with GSH (0.6155 g) and ultrapure water, the mixture was sonicated for 10 min, transferred into a Teflon-lined stainless-steel autoclave, and heated at 200 °C for 24 h. MoS₂ QDs centrifuged for 5 min, and then stored in the dark until further use. ZnS QDs were prepared using zinc acetate and Na₂S·9H₂O as starting materials. Ultrapure water (30 mL) was added to 5.5044 g of zinc acetate and sonicated for 5 min. The pH of the above solution was adjusted to 9.0 by the addition of 0.2 mol L⁻¹ sodium hydroxide solution and saturated with N₂ for 30 min. Then, 0.1 mol L⁻¹ Na₂S·9H₂O was immediately injected into the mixture and refluxed for 20 h. ZnS QDs were centrifuged for 10 min and alternately washed with ultrapure water and ethanol.

**Immunoassay procedure.** Scheme 1 shows the principle of magnetic immunoassay for MPO and OPN. Briefly, the prepared AMNPs (15 mg) were added to phosphate buffer solution (PBS). Next, 100 mg of EDC and NHS (1:1) were mixed with the above solution and incubated for 1 h. Ab1-MPO and Ab1-OPN (1:1, 20 μL) were added and the solution was incubated for 2 h. Under an external magnetic field, AMNPs-Ab1 MPO/OPN were separated from the solution. The AMNPs-Ab1 MPO/OPN was washed with...
PBS. Further, 500 μL BSA was added to the AMNPs-Ab1 MPO/OPN to reduce non-specific adsorption. The preparation procedure for AMNPs-Ab1 MPO/OPN is shown in Scheme 1 (a).

To prepare MoS$_2$ QDs-Ab2-MPO conjugates, 500 μL of MoS$_2$ QDs were reacted with EDC (12.5 mg) and NHS (12.5 mg) for 1 h. The activated MoS$_2$ QDs were purified three times by centrifugation for 10 min. Subsequently, twenty microliters of the Ab2-MPO was mixed with the activated MoS$_2$ QDs and reacted for 2 h. The MoS$_2$ QDs-Ab2-MPO conjugates were collected by centrifugation and washed with PBS to remove the unreacted Ab2-MPO. Finally, the MoS$_2$ QDs-Ab2-MPO was blocked using BSA for 1 h. Scheme 1 (b) shows the preparation procedure for MoS$_2$ QDs-Ab2-MPO.

To prepare ZnS QDs-Ab2-OPN conjugates, 500 μL of ZnS QDs were mixed with EDC (12.5 mg) and NHS (12.5 mg) for 1 h. The activated ZnS QDs were gathered by centrifugation at 10000 rpm for 10 min. Ab2-OPN (20 μL) were added to the activated ZnS QDs and allowed to react for 2 h. The ZnS QDs-Ab2-OPN conjugates were purified three times by centrifugation and washed with PBS solution to remove unreacted Ab2-OPN. Finally, the ZnS QDs-Ab2-OPN was blocked by BSA for 1 h. Scheme 1 (b) shows the preparation procedure for ZnS QDs-Ab2-OPN.

MPO and OPN were added to 500 μL of AMNPs-Ab1 MPO/OPN and incubated with gentle shaking for 50 min. MPO and OPN were specifically captured by AMNPs-Ab1 MPO/OPN, and a magnet was employed to collect the complexes. Subsequently, 180 μL MoS$_2$ QDs-Ab2-MPO and 150 μL ZnS QDs-Ab2-OPN were mixed with the complexes. After incubation for 1 h, the AMNPs-Ab1-MPO/OPN-Ab2-MoS$_2$ QD/ZnS QD immunocomplex was separated using a magnet and washed with PBS solution. Formic acid (50 μL) was added to the immunocomplexes by sonication for 10 min to obtain free MoS$_2$ and ZnS QDs. The AMNPs-Ab1-MPO/OPN-Ab2-MoS$_2$ QD/ZnS QD solutions were dissociated using a magnet. The MoS$_2$ QDs/ZnS QDs were subsequently introduced to the ICP-MS instrument via the customized gas-pressure-assisted sample introduction system. The magnetic immunoassay procedure for MPO/OPN appears in Scheme 1 (c).

**RESULTS AND DISCUSSION**

**Characterization of AMNPs.** The prepared AMNPs were characterized using FTIR spectroscopy (Fig. 1(a)) and TEM. The FTIR spectra of the prepared MNPs exhibit bands at 1637 and 3438 cm$^{-1}$, corresponding to -OH bonds (Fig. 1(a)). The band at 607 cm$^{-1}$ was ascribed to the Fe-O bond stretching vibration. The FTIR spectrum for the AMNPs exhibits bands at 888, 1061, 1630, and 3435 cm$^{-1}$ which are related to the vibration of -NH$_2$ bonds, Si-O bonds, -OH bonds, and N-H bonds, respectively (Fig. 1(a)). These results demonstrate that the -NH$_2$ group was successfully added to the MNPs, as shown in the TEM image (Fig. 1(b)). The prepared AMNPs had an average diameter of 9.96 nm based on the statistical analysis of 100 random particles.

**Characterization of MoS$_2$ QDs.** Fluorescence spectroscopy, UV-vis spectroscopy, FTIR spectroscopy, TEM, and X-ray photoelectron spectroscopy (XPS) were employed to characterize the synthesized MoS$_2$ QDs. The fluorescence spectrum illustrates that the MoS$_2$ QDs have strong excitation and emission peaks (Fig. 2(a)) at 320 nm and 438 nm, respectively. The absorption peak appeared at 300 nm, which is consistent with the results of an existing study on MoS$_2$ QDs.$^{45}$ As shown in Fig. 2(b), the FT-IR spectra were used to identify the chemical groups of the MoS$_2$ QDs. The small peak at 615 cm$^{-1}$ corresponds to the vibration of the Mo-S bond. The bands at 1635, 1385, and 3433 cm$^{-1}$ correspond to the bending vibrations of N-H and C-N and the stretching vibrations of O-H/N-H, respectively. The above results demonstrate that the -COOH and amino groups were successfully added to the MoS$_2$ QDs. The TEM image (Fig. 2(c)) shows that the MoS$_2$ QDs were well dispersed without significant dispersion.
aggregation, and have a size distribution range of 1.73–4.22 nm and a mean diameter of 100 MoS$_2$ QDs per 2.77 nm (Fig. 2(d)).

The chemical states and compositions of the MoS$_2$ QDs were characterized using XPS. The spectrum shows that the MoS$_2$ QDs have six peaks at 161.9, 231.9, 284.1, 399.8, 495.1, and 530.9 eV (Fig. 3(a)), which were attributed to S2p, Mo3d, C1s, N1s, Mo3s, and O1s, respectively. The result in Fig. 3(b) indicates that the Mo3d spectrum has two peaks at 227.2 and 232.7 eV, which are attributed to Mo3d$_{5/2}$ and Mo3d$_{3/2}$, respectively. These results...
prove that Mo existed in the +4 oxidation state. In the S2p spectrum for the MoS2 QDs, there were characteristic peaks for S2p1/2 and S2p3/2 at 162.2 and 163.6 eV, respectively (Fig. 3(c)). This is due to the −2 oxidation state of S. The XPS results demonstrated the successful synthesis of MoS2 QDs.

**ZnS QD characterization.** UV-vis spectroscopy, TEM, XRD, EDS, and XPS were employed for characterization of the synthesized ZnS QDs. The absorption spectrum for ZnS QDs has a small absorption peak at 320 nm (Fig. 4(a)), which was consistent with the results of an existing study for ZnS QDs.41 The TEM image (Fig. 4(b)) shows that the prepared ZnS QDs exhibited good dispersion with an approximately spherical morphology. The size of the ZnS QDs is in the range of 1 to 3 nm and they have an average diameter of approximately 1.65 nm based on the statistical analysis of 100 random particles (Fig. 4(c)). The XRD pattern s of the ZnS QDs (Fig. 4(d)) exhibit characteristic diffraction peaks at 28.5° (111), 47.4° (220), and 56.5° (311). EDS was employed to study the elemental ratio of the ZnS QDs (Fig. 4(e)). The prepared ZnS QDs contain two elements, 75.41% Zn and 24.59% S.

The chemical composition and bonding states of the prepared ZnS QDs were characterized using XPS. Characteristic S2p and Zn2p peaks at 162.1 and 1021.1 eV, respectively, were observed in the spectra for the ZnS QDs (Fig. 5(a)). The high resolution XPS results for S2p show two peaks located at 162.6 and 161.6 eV, which are associated with S2p1/2 C-S and S2p3/2 Zn-S, respectively (Fig. 5(b)). The Zn2p XPS spectrum contains four peaks at 1045.8, 1044.9, 1022.5, and 1021.8 eV, which are associated with Zn2p1/2 Zn-O, Zn2p3/2 Zn-S, Zn2p1/2 Zn-O, and Zn2p3/2 Zn-S, respectively (Fig. 5(c)).

**Optimization of conditions.** Some experimental parameters, such as the BSA volume, the quantity and incubation time of the AMNPs-Ab1 MPO/OPN, MoS2 QDs-Ab2-MPO, and ZnS QDs-Ab2-OPN, and type of eluent, were optimized to achieve the best analytical performance.

The blocking reagent BSA solution was used to eliminate nonspecific adsorption. The influence of the BSA volume on the nonspecific sites was investigated via a process similar to the immunoassay procedure without the incubation of MPO and OPN.
Fig. 5 (a) XPS results; (b) high-resolution XPS results for S2p and (c) Zn2p of as-prepared ZnS QDs.

Fig. 6 Effect of BSA blocking volume.

Fig. 7 Effects of (a) volume and (b) incubation time of AMNPs-Ab1-MPO/OPN on the number of $^{98}$Mo/$^{64}$Zn detection events.

Fig. 8 Effects of (a) volume of MoS$_2$ QDs-Ab2-MPO, ZnS QDs-Ab2-OPN and (b) incubation time on the number of $^{98}$Mo/$^{64}$Zn detection events.

Fig. 9 Effect of a different type of eluent on the number of detection events.
500 μL, a complete block was achieved. The volume of BSA increased. When the volume of BSA reached 150 μL, the residue concentrations of MoS and ZnS QDs-Ab2-OPN also increased with increasing BSA volume. The intensity of the Mo/Zn increased as the incubation time of the AMNPs-Ab1 MPO/OPN were optimized. The incubation time was fixed at 1 h. The concentrations of MPO and OPN were fixed at 50 ng mL⁻¹. The concentration of AMNPs-Ab1 MPO/OPN was 5 μg mL⁻¹. The volume of the MPO and OPN standard solutions was 10 μL. The volumes of MoS QDs-Ab2-MPO and ZnS QDs-Ab2-OPN were 180 and 150 μL, respectively. To study the impact of AMNPs-Ab1 MPO/OPN volume on immunoreaction performance, the volume of AMNPs-Ab1 MPO/OPN in the range of 100 to 1000 μL was considered. As shown in Fig. 7(a), the number of detection events of ⁹⁸Mo⁶⁴Zn distinctly increased with the volume of AMNPs-Ab1 MPO/OPN from 100 to 500 μL, and then decreased slightly when the volume of AMNPs-Ab1 MPO/OPN was higher than 500 μL. Therefore, the optimal AMNPs-Ab1 MPO/OPN volume was determined to be 500 μL. The incubation times (20–120 min) of the AMNPs-Ab1 MPO/OPN and MPO/OPN were investigated. As shown in Fig. 7(b), the number of ⁹⁸Mo⁶⁴Zn determination events increased as the incubation time of AMNPs-Ab1 MPO/OPN and MPO/OPN increased between 20 and 50 min, and reached saturation at 50 min. Thus, 50 min was used as the incubation time for subsequent experiments.

Adding MoS QDs-Ab2-MPO and ZnS QDs-Ab2-OPN to the reaction system was essential to attain the desired performance for the immunoreactions. The concentrations of MoS QDs-Ab2-MPO and ZnS QDs-Ab2-OPN were fixed at 15 and 20 μg mL⁻¹, respectively. The impact of the volumes of MoS QDs-Ab2-MPO and ZnS QDs-Ab2-OPN was studied (Fig. 8(a)). The number of ⁹⁸Mo detection events increased with increasing volume of MoS QDs-Ab2-MPO from 60 to 180 μL. When the volume of MoS QDs-Ab2-MPO was increased from 180 to 300 μL, the number of ⁹⁸Mo detection events remained constant. Thus, 180 μL of MoS QDs-Ab2-MPO was selected for subsequent studies. Similarly, the optimal volume of ZnS QDs-Ab2-OPN was 150 μL (Fig. 8(a)). The effect of various incubation times on the number of ⁹⁸Mo⁶⁴Zn detection events was revealed in Fig. 8(b). The number of detection events for ⁹⁸Mo⁶⁴Zn increased as incubation time increased from 20 to 60 min and then plateaued with longer incubation times. Therefore, the incubation time was set to 60 min for subsequent experiments.

An appropriate eluent was selected to elute MoS and ZnS QDs from the solution of AMNPs-Ab1-MPO/OPN-Ab2-MoS QDs/ZnS QDs for ICP-MS determination. Different types of acids (1 mol L⁻¹) were applied as eluents to release the MoS and ZnS QDs from the AMNPs. As shown in Fig. 9, formic acid exhibited optimal elution capability for MoS and ZnS QDs compared to nitric, citric, and acetic acids. Hence, we selected formic acid to use as an eluent in subsequent experiments.

**Analytical performance.** The results of the quantitative analysis by the MPO and OPN methods under the optimal parameters are presented in Fig. 10. The calibration plots for the number of

![Fig. 10](https://example.com/figure10.png)

**Fig. 10** (a) MPO and (b) OPN calibration plots obtained using SP-ICP-MS.

### Table 2. Comparison with Other Methods for MPO and OPN Determination

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**Abbreviations:** ICP-MS, inductively coupled plasma mass spectrometry; LOD, limit of detection; MPO, myeloperoxidase; OPN, osteopontin.

(Fig. 6). When the volume of BSA was insufficient to eliminate the non-specific sites, the residue concentrations of MoS QDs-Ab2-MPO and ZnS QDs-Ab2-OPN also increased with increasing BSA volume. The intensity of the Mo/Zn increased as the volume of BSA increased. When the volume of BSA reached 500 μL, a complete block was achieved.

To obtain a high capture efficiency, the volume and incubation time of the AMNPs-Ab1 MPO/OPN were optimized. The incubation time was fixed at 1 h. The concentrations of MPO and OPN were fixed at 50 ng mL⁻¹. The concentration of AMNPs-Ab1 MPO/OPN was 5 μg mL⁻¹. The volume of the MPO and OPN standard solutions was 10 μL. The volumes of MoS QDs-Ab2-MPO and ZnS QDs-Ab2-OPN were 180 and 150 μL, respectively. To study the impact of AMNPs-Ab1 MPO/OPN volume on immunoreaction performance, the volume of AMNPs-Ab1 MPO/OPN in the range of 100 to 1000 μL was considered. As shown in Fig. 7(a), the number of detection events of ⁹⁸Mo⁶⁴Zn distinctly increased with the volume of AMNPs-Ab1 MPO/OPN from 100 to 500 μL, and then decreased slightly when the volume of AMNPs-Ab1 MPO/OPN was higher than 500 μL. Therefore, the optimal AMNPs-Ab1 MPO/OPN volume was determined to be 500 μL. The incubation times (20–120 min) of the AMNPs-Ab1 MPO/OPN and MPO/OPN were investigated. As shown in Fig. 7(b), the number of ⁹⁸Mo⁶⁴Zn determination events increased as the incubation time of AMNPs-Ab1 MPO/OPN and MPO/OPN increased between 20 and 50 min, and reached saturation at 50 min. Thus, 50 min was used as the incubation time for subsequent experiments.

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An appropriate eluent was selected to elute MoS and ZnS QDs from the solution of AMNPs-Ab1-MPO/OPN-Ab2-MoS QDs/ZnS QDs for ICP-MS determination. Different types of acids (1 mol L⁻¹) were applied as eluents to release the MoS and ZnS QDs from the AMNPs. As shown in Fig. 9, formic acid exhibited optimal elution capability for MoS and ZnS QDs compared to nitric, citric, and acetic acids. Hence, we selected formic acid to use as an eluent in subsequent experiments.

**Analytical performance.** The results of the quantitative analysis by the MPO and OPN methods under the optimal parameters are presented in Fig. 10. The calibration plots for the number of

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Table 3. Analytical Results (mean ± standard deviation, n=3) for MPO and OPN in Human Serum Samples

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Target</th>
<th>Determined (ng mL⁻¹)</th>
<th>CLIA (ng mL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MPO</td>
<td>0</td>
<td>0.1±0.03</td>
</tr>
<tr>
<td></td>
<td>OPN</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>MPO</td>
<td>10.1±0.08</td>
<td>10.0±0.05</td>
</tr>
<tr>
<td></td>
<td>OPN</td>
<td>1.70±0.12</td>
<td>1.73±0.06</td>
</tr>
<tr>
<td>3</td>
<td>MPO</td>
<td>15.4±0.15</td>
<td>14.9±0.81</td>
</tr>
<tr>
<td></td>
<td>OPN</td>
<td>4.85±0.61</td>
<td>4.68±0.45</td>
</tr>
</tbody>
</table>

Abbreviations: CLIA, chemiluminescent immunoassay; MPO, myeloperoxidase; OPN, osteopontin.

98Mo⁹⁴Zn detection events and the concentrations are linear in the ranges of 0.01–50 ng mL⁻¹ for MPO (y = 17.70 x + 8.65, R² = 0.9991) and 0.02–50 ng mL⁻¹ for OPN (y = 13.19 x + 15.44, R² = 0.9993). The LODs obtained by magnetic immunoassay with MoS₂ and ZnS QD-labeled MPO and OPN were 0.004 and 0.005 ng mL⁻¹, respectively. Calculated from six replicates, the relative standard deviations of MPO and OPN were 3.5% and 3.2%, respectively. Comparisons with reported methods for the determination of MPO and OPN (Table 2) indicate that the LOD obtained in this study is relatively low.¹,⁵,⁸,⁹,⁴⁸ however, it is higher than those of electrochemical immunosensor methods.⁶

Human serum sample analysis. To study the practicability of the method, we measured the concentrations of MPO and OPN in human serum samples from Guilin Fifth People’s Hospital (Guilin, China). AMNPs were mixed with the samples, and the mixture was then separated via magnetic separation and not required for sample pretreatments. Human serum samples were also analyzed by chemiluminescent immunoassay for validation (Table 3). The concentrations of MPO and OPN obtained by the proposed method were in agreement with those obtained by the chemiluminescent immunoassay.

CONCLUSION

In this study, a novel strategy based on ICP-MS multiple immunoassays was proposed to determine the MPO and OPN content in human serum samples. The MPO and OPN were determined using Ab1-MPO and Ab1-OPN conjugated with AMNPs, while the MoS₂ and ZnS QDs were used as element tags for the detection of MPO and OPN using SP-ICP-MS. The proposed method was validated by the determination of MPO and OPN using a chemiluminescent immunoassay. Compared to conventional methods, our simultaneous immunoassay of MPO and OPN reduces sample consumption and improves the assay efficiency.

ASSOCIATED CONTENT

The supporting information (Figs. S1–S12, Tables S1–S3, and Texts S1–S3) is available at www.at-spectrosc.com/as/home.

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