MoS₂/Ag nanohybrid: a novel matrix with synergistic effect for small molecule drugs analysis by negative-ion matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

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Abstract:

This paper reports a facile synthesis of molybdenum disulfide nanosheets/silver nanoparticles (MoS_2/Ag) hybrid and its use as an effective matrix in negative ion matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). The nanohybrid exerts a strong synergistic effect, leading to high performance detection of small molecule analytes including amino acids, peptides, fatty acids and drugs. The enhancement of laser desorption/ionization (LDI) efficiency is largely attributed to the high surface roughness and large surface area for analyte adsorption, better dispersibility, increased thermal conductivity and enhanced UV energy absorption as compared to pure MoS_2 . Moreover, both Ag nanoparticles and the edge of the MoS_2 layers function as deprotonation sites for proton capture, facilitating the charging process in negative ion mode and promoting formation of negative ions. As a result, the MoS_2/Ag nanohybrid proves to be a highly attractive matrix in MALDI-TOF MS, with desired features such as high desorption/ionization efficiency, low fragmentation interference, high salt tolerance, and no sweet-spots for mass signal. These characteristic properties allowed for simultaneous analysis of eight different drugs and quantification of acetylsalicylic acid in the spiked human serum. This work demonstrates for the first time the fabrication and application of a novel MoS_2/Ag hybrid, and provides a new platform for use in the rapid and high throughput analysis of small molecules by mass spectrometry.

Keywords: MoS_2/Ag ; Matrix; Synergistic effect; Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; Small molecule drugs.

1. Introduction

Nowadays, matrix-assisted laser desorption/ionization (MALDI) time-of-flight mass spectrometry (MALDI-TOF MS) has been served as a powerful tool for the analysis of biomolecules such as peptides,[1] proteins,[2] and oligosaccharides.[3] However, while it is applied in the analysis of small molecules, it often meets challenges, largely due to interference of conventional organic matrix-related ions (e.g., α -cyano-4-hydroxycinnamic acid, CHCA) in low-mass regions (<500 Da)[4] and poor reproducibility originating from the heterogeneity of the matrix-analyte

crystals.[5] To overcome these drawbacks, great efforts have been made to develop nanomaterials as alternative matrices, especially for small molecule analysis. This technique is also known as surface-assisted laser desorption/ionization (SALDI).[6] Up to now, a variety of inorganic nanomaterials with different compositions and morphologies, including silicon,[7, 8] metal/metal oxide,[9-11] and carbon-based materials,[12-14] have been utilized as MALDI matrices. These nanomaterial-based matrices minimized the background interferences, increased sensitivity, and simplified the sample preparation. However, the need for elaborate nanofabrication process can limit their application. In addition, most of the metal oxide and carbon-based materials display poor dispersibility in solution that often yields inhomogeneous distribution of nanomaterials on the sample target. This usually results in poor shot-to-shot and sample-to-sample reproducibility and decreases sensitivity of MALDI-TOF MS.[15] Thus, exploration of better matrices with simple preparation approach, good dispersibility, and high laser desorption/ionization (LDI) efficiency is still very necessary and highly relevant for MS analysis.

To achieve sensitive and reproducible MALDI-TOF MS, great endeavors have been devoted to the combined functionalization of nanomaterials.[16-19] Shi *et al.* prepared highly water-dispersible polydopamine-modified carbon nanotubes to overcome the poor dispersibility of pristine multi-walled carbon nanotubes (MWCNTs) and demonstrated that the composites have improved the performance of the analysis of various water soluble small molecules.[16] Apart from the increased dispersibility, some hybrid nanocomposites can exhibit synergistic effect in the enhancement of the LDI efficiency of analytes. Xu *et al.* developed a hybrid nanocomposite with a large graphitized inner surface area, strong UV adsorption and excellent dispersibility, which have improved the LDI efficiency and prevented inhomogeneous distribution.[17] Kim *et al.* designed a graphene oxide/multi-walled carbon nanotube double layer and applied it as a MALDI substrate. The design showed enhanced surface roughness and surface area for analyte adsorption, and improved wettability in water because of the charged surface.[18] Although these methods have achieved some success, most of the nanomaterial-based matrices were based on the MS detection platform in positive ion mode. This often results in multiple alkali metal adducts in the mass spectrum, which is difficult to identify.[12, 17] In contrast, the negative ion spectrum is much clearer and easier to interpret with one deprotonated ion peak present[20, 21]. Furthermore, recent studies have showed that better sensitivity can be obtained in negative mode than positive mode

for analytes.[20, 22] Given the advantages of negative ion mode for small molecule analysis, exploring negative ion mode-compatible matrices with improved LDI efficiency is highly desirable.

As a two-dimensional (2D) single atomic layer of carbon atoms, graphene exhibits large surface area of its nanosheet structure, excellent optical and electrical properties, and has proved to be a useful matrix in the analysis of small molecules in negative ion mode.[22] Min *et al.* further prepared the N-doped graphene as a matrix and demonstrated it has higher LDI efficiency than other graphene-based matrices in negative ion mode.[20] Recently, considerable attention has been given to graphitic carbon nitride because of its 2D layer structure analogous to graphene.[21] The pyridinic nitrogen atoms in g-C₃N₄ nanosheets possess sp² electrons in the π -conjugated system, thus can promote the charging process in the negative ion mode.[21] Another graphene analogue, MoS₂ nanosheet, has similar features including 2D ultrathin atomic layer structure and high surface area, and has showed great potential in nanoelectronics, optoelectronics, and energy harvesting.[23] Especially, MoS₂ sheet edges have been identified as the active sites to dissociate H₂ and associate H for hydrogen evolution.[24, 25] MoS₂ nanosheets can be easily synthesized in large quantities and directly dispersed in solutions without surfactants or oxidation treatment comparing to graphene.[26] In addition, MoS₂ nanosheet has high absorption in the UV-visible range, and thus is suitable for MALDI experiments. Furthermore, MoS₂ is a promising supporting material to stabilize metal-nanoparticles such as gold and silver nanoparticles, forming hybrid composites. Berry's group has reported the increased thermal conductivity of MoS₂ after metal-nanoparticle incorporation,[27] which is propitious to the LDI process.[17] Additionally, AgNPs have been successfully utilized as an effective matrix for the determination of estrogens by negative ion MALDI-TOF MS where AgNPs have shown extremely high absorption coefficients in the near UV-vis region, a large heat capacity and high heat conductivity.[28, 29] Therefore, we anticipate that the combination of MoS₂ nanosheets with AgNPs would be very beneficial for enhancing the sample signal and reproducibility in negative ion MALDI-TOF MS.

Herein, we report the development of a facile method to prepare water-dispersible MoS₂/Ag hybrid and its application for the first time to negative ion MALDI-TOF MS analysis of small molecules (Scheme 1). The modification of Ag on the surface of MoS₂ nanosheet is expected to yield larger surface roughness and surface area for analyte adsorption, better dispersibility,

increased thermal conductivity and enhanced UV energy absorption. Compared with single component MoS₂ or Ag, the hybrid nanocomposites lead to the synergistic effect in the enhancement of the LDI efficiency for a range of analytes including amino acids, peptides, fatty acids, and drugs. In comparison with organic matrices in both positive and negative ion mode, the MoS₂/Ag hybrid matrix in negative ion mode exhibits significant advantages, such as high sensitivity, free matrix background, good salt tolerance, and excellent reproducibility. These characteristic properties allow it for simultaneous analysis of eight different drugs and further quantification of acetylsalicylic acid in the spiked human serum, making it a promising, generic platform for negative ion MALDI-TOF MS analysis of small molecules in complex, real world samples. The mechanism study of MoS₂/Ag with synergistic effect as matrix in negative ion mode is also discussed in detail.

< Insert Scheme 1 >

2. Experimental

2.1. Preparation of MoS₂ nanosheets and MoS₂/Ag hybrid.

The MoS₂ nanosheets were synthesized by chemical exfoliation according to the previously reported procedure with some modifications.[30] Briefly, pristine bulk MoS₂ was intercalated with lithium by reacting MoS₂ powder (3 g) with n-butyllithium in hexane (1.6 M, 30 mL) at 100 °C in a poly (tetrafluoroethylene) (Teflon)-lined autoclave (50 mL) for 4 h. Then the solution was transferred to a flask and stirred under N₂ atmosphere at 25°C for 48 h. The suspension was filtered over a filter paper and washed with 200 mL of hexane for 3 times, giving a black powder of intercalated MoS₂ compound. Exfoliation was then immediately suspended in 200 mL of Millipore water and sonicated for 4 h. The solution was centrifuged with 3000 rpm for several times to remove the unexfoliated materials. The concentration of the obtained MoS₂ solution was calculated as 0.1 mg mL⁻¹ by the drying and weighting method.

The MoS₂/Ag hybrid was prepared by sodium borohydride (NaBH₄) reduction of silver nitrate (AgNO₃) on MoS₂ sheets according to our previous report with some modifications.[31] Briefly, 16 mL of the obtained MoS₂ solution was mixed with 1.5 mL 50 mM trisodium citrate, 1.5 mL 6% polyvinylpyrrolidone, 600 µL 10 mM AgNO₃ and 4 mL H₂O for 5 min, then 48 µL of 500 mM NaBH₄ was added and stirred for 10 min. The obtained solution was then stood for 24 h and stored at room temperature for further use. AgNPs was synthesized as control by replacing the MoS₂ solution with 16 mL of ultrapure water. All the products were washed with Millipore water twice and dispersed in water to obtain a concentration of 0.5 mg mL⁻¹ before use.

2.2. Sample preparation for MALDI-TOF MS analysis.

CHCA (10 mg mL⁻¹) matrix was prepared as a solution in acetonitrile/water (2:1, v/v) containing 0.1% trifluoroacetic acid. 3-aminoquinoline (3-AQ) was dissolved in acetonitrile/water (1:1, v/v) at 10 mg mL⁻¹. For conventional analysis, an amount of 1 µL of analyte solution was deposited on the stainless steel sample plate, then 1 µL of CHCA or 3-AQ aqueous solution was deposited. For MoS₂, Ag, and MoS₂/Ag suspension solutions, 1 µL of each matrix solution was first pipetted onto the plate, and left in the air for about 30 min to form a thin matrix layer. Afterward, 1 µL of analyte solution was dropped onto the matrix layer and air-dried.

2.3. Mass spectrometry analysis.

MALDI-TOF MS experiments were performed on a 4800 *Plus* MALDI-TOF/TOF mass spectrometer (AB Sciex, U.S.A.) equipped with a pulsed Nd: YAG laser (355 nm wavelength) with both positive and negative reflection mode. A 384 spots ground-steel target was utilized for sample plate. For each spectrum, 50 shots from different positions of the target spot (automatic mode) were collected and analyzed. The Data Explorer Software from AB Sciex was used for data analysis.

3. Results and discussion

3.1. Characterization of materials.

The MoS₂ nanosheet was synthesized according to the procedure in the literature with some modifications (Scheme S1).[30] The water-dispersible, thin MoS₂ sheet was then modified with AgNPs by an *in situ* reduction reaction at room temperature (Scheme S1). The defects and edges on the MoS₂ sheet contain unbound sulfur, which can bind Ag nuclei and act as sites for Ag growth into bigger nanoparticles.[27] Fig. 1c shows the UV-vis absorption spectra of MoS₂ and MoS₂/Ag aqueous solutions. There is a strong absorption in the near UV region at about 355 nm, making MoS₂ a promising nanomaterial for absorbing and transferring laser energy used in MALDI to the analytes. After AgNPs modification, the absorption peaks at 405 nm and absorbance from 400 nm to 700 nm increased, which is resulted from the coupling of the surface plasmon resonance of AgNPs on MoS₂ surface.[32] The stronger UV absorption of MoS₂/Ag than MoS₂ indicates the synergistic effect of MoS₂/Ag as a matrix in MALDI-TOF MS analysis, since the LDI efficiency is related to the optical properties of nanomaterials.[17] The homogenous dispersion of nanomaterials in solution was also investigated since it determines if the material can form a homogeneous matrix layer on the sample target. Inset in Fig. 1c shows the suspensions of MoS₂ and MoS₂/Ag. Both can be dispersed well in water. The favorable dispersibility of the MoS₂

film is likely due to the matching surface free energies of the solvent with the exfoliated MoS₂ sheets.[33] After two weeks' storage at room temperature, however, aggregation was observed in the suspension of MoS₂. By contrast, MoS₂/Ag still exhibited excellent water dispersibility (Fig. S1). The improved water dispersibility of the MoS₂/Ag can be attributed to the citrate groups on the surface of AgNPs,[34, 35] making MoS₂/Ag a better matrix candidate in MALDI analysis.

The morphology and structure of MoS₂ and MoS₂/Ag were also investigated. From the transmission electron microscope (TEM) image of MoS₂ (Fig. 1a), the transparent and corrugated feature of the surface indicates that thin and isolated nanosheets are obtained. The lateral dimensions of the MoS₂ sheet are usually in the 400–1500 nm range. The nanosheet structure provides a larger surface area for analyte adsorption and allows the nanomaterial to attach to the sample target tightly, thus preventing the detachment issue in the vacuum system.[12] The high resolution TEM (HRTEM) image (Fig. S2a) shows the typical hexagonal single crystal structure with a lattice spacing of 0.271 nm for the (100) Mo atoms.[36] The six-fold selected area electron diffraction (SAED) pattern (Fig. S2b) also indicates that MoS₂ has a single crystal structure with hexagonal symmetry.[37] After the reduction of AgNO₃, AgNPs with diameters of about 9 nm formed and randomly distributed across the whole surface of the MoS₂ film (Fig. 1b), where some large-size AgNPs are observed along the edges. The latter may arise from the high density of defects on the MoS₂ edge sites; thus more silver nuclei load onto the edges to become larger AgNPs. The lattice fringe of about 0.205 nm can be assigned to the (200) plane of Ag (JCPDS no. 87-0720). The formation of Ag on the MoS₂ was also confirmed by energy dispersive X-ray spectrometer (EDX) with the presence of S, Mo, and Ag elemental peaks, as shown in Fig. S2d. It is worth noting that the strong interaction between MoS₂ and Ag cannot be disrupted after a long time sonication during the preparation of TEM specimen. The close contact produces a fast electronic transport and thus facilitates the charging process in negative ion mode.[38]

< Insert Fig. 1 >

3.2. MALDI-TOF MS analysis of amino acids.

To test the performance of the MoS₂/Ag nanosheet as the matrix for MALDI-TOF MS analysis, results for 0.5 mM Histidine (His, MW = 155.15) with three different sample preparation methods were compared (Fig. 1d). All deprotonated ions of His can be clearly observed, indicating the sample preparation procedures can promote efficient LDI process. The signal

intensity of His obtained by the matrix-first method was the highest ($S/N = 4053$), so this method was selected for further studies. A mixture of amino acids was analysed, with MoS_2 and Ag also employed as the matrices for comparison. Fig. 2a-e are the MALDI-TOF MS spectra of a standard solution containing four 0.5 mM amino acids of His, Phenylalanine (Phe, $\text{MW} = 165.19$), Arginine (Arg, $\text{MW} = 174.19$), and tryptophan (Try, $\text{MW} = 204.23$), analysed with CHCA matrix in positive ion mode as well as 3-AQ, MoS_2 , Ag and MoS_2/Ag matrices in negative ion mode. With CHCA matrix, the $[\text{M} + \text{H}]^+$ ions of all four amino acids were detectable with strong signals of matrix-related ions predominating the spectrum, which seriously suppresses analyte signals (Fig. 2a). 3-AQ, a commonly used organic matrix in negative ion mode, shows a low signal response of amino acids and abundant interfering matrix fragment peaks (Fig. 2b). By contrast, using MoS_2 , Ag and MoS_2/Ag matrices, all amino acids were successfully detected with characteristic $[\text{M} - \text{H}]^-$ ion signals and low background interference (Fig. 2c-e). Furthermore, the peak intensities of amino acids obtained from MoS_2/Ag were the highest as compared with the other two matrices, indicating that the modification of Ag on MoS_2 was highly effective for enhancing the LDI efficiency of small analytes.

< Insert Fig. 2 >

Given the higher intensities of MoS_2/Ag in MALDI-TOF MS, we further evaluated the limits of detection (LOD) of His and Phe at low concentrations on MoS_2/Ag matrix (Table 1). Each analyte was diluted with distilled water at different ratios to obtain samples with concentrations between 0.5 mM and 0.5 μM . As shown in Fig. S3, the signal intensity and S/N ratio of His and Phe decreased along with the decreasing concentrations. The LOD of 0.5 pmol was observed in the detection of His and Phe, with the S/N ratio of 19.96 and 36.65, respectively (Fig. S3a and S3c). This detection of limit was comparable to other matrices in the same ion mode [20, 21, 39, 40] and lower than those in positive mode (Table S1).[17, 18] To assess salt tolerance, His and Phe were further dissolved in 1×phosphate buffered saline (1×PBS), which has an ionic strength similar to the human body fluid, and dropped on MoS_2/Ag matrix layer for analysis (Fig. S3b and S3d). Although signal dropped and the LOD deteriorated by almost one order of magnitude, the MoS_2/Ag nanosheet was still suitable for direct analysis of samples with no need of desalting in MALDI-TOF MS. The solution of 0.5 mM of His was also employed to investigate the shot-to-shot signal reproducibility and precision of signal distribution, which were highly related

to the uniform distribution of matrix layer. From Fig. S4, we can see that MoS₂/Ag allows uniform spreading on the plate surface after solvent evaporation, generating a homogeneous film. For the same sample spot detection, continuous 10 mass spectra were obtained from 10 different regions in the MoS₂/Ag matrix spot. The MS signal intensity was stabilized at around 14000 and the relative standard deviation (RSD) was calculated to be 7.8 % (n = 10) (Fig. S5a). These results clearly indicate that the MoS₂/Ag nanofilm is featured with high inter-reproducibility without the need for “sweet spots” searching. The sample-to-sample reproducibility was also very good, with a low RSD of 8.7 % (n = 10) (Fig. S5b).

3.3. MALDI-TOF MS analysis of fatty acids and peptides.

Similarly, negative ionization of five peptides (Gly-Gly (MW = 132.12), Ala-Ala (MW = 160.17), Ala-Gln (MW = 217.22), Try-Phe (MW = 328.36), and Gla-Val-Phe (MW = 393.43)) and the fatty acids solution containing n-tetradecanoic acid (C14, MW = 228.37), n-hexadecanoic acid (C16, MW = 256.42), n-octadecanoic acid (C18, MW = 284.48), and n-eicosanoic acid (C20, MW = 312.53) were also examined to assess the performance of MoS₂, Ag and MoS₂/Ag matrices. Fig. S6a and S6b showed the positive-ion and negative-ion mass spectra of the five peptides with CHCA and 3-AQ matrices, respectively. Although high signals of the peptides were obtained, the matrix-related peaks still dominated the mass spectra, seriously interfering with the signals of the peptides. However, the deprotonated ions of all peptides and fatty acids were detected without any interference, as shown in Fig. 3. Moreover, peak intensities of these analyte molecules on MoS₂/Ag were more than twice enhanced in comparison to those on MoS₂ and Ag, which further demonstrated the outstanding performance of MoS₂/Ag. Encouraged by the above results, four peptides with higher mass range including Phe-Gly-Phe-Gly (MW = 426.46), Tyr-Gly-Gly-Phe-Leu (MW = 555.62), Arg-Ser-Gly-Phe-Tyr, (MW = 628.68), and Arg-Arg-Pro-Tyr-Ile-Leu (MW = 816.99) were also examined (Fig. S6c). As expected, clean background and sensitive deprotonated ions of the four peptides were achieved with the aid of MoS₂/Ag, displaying its good performance in higher mass range detection.

< Insert Fig. 3 >

3.4. LDI mechanism of MoS₂/Ag with synergistic effect.

To demonstrate if the enhanced MS intensity of MoS₂/Ag matrix is applicable to other analytes, some other small molecules including adenosine, uridine, and sulfadimidine were also

tested in negative-ion mode. The mass peak intensities are summarized as bar graphs in Fig. 4a for comparison. As expected, mass peak intensities of these analytes on the MoS₂/Ag matrix are more than twice higher than those on MoS₂ and Ag matrices. To investigate the relationship between the surface properties of the matrix and LDI efficiency, water contact angle measurement was carried out. Shi *et al.* have discussed in their work that the matrix material with better water dispersibility improved the performance in MALDI-TOF MS, since the surface hydrophilicity will affect the wettability of the matrix layer on sample plate for homogeneous sample spreading.[16] To measure the contact angle values, we deposited MoS₂ and MoS₂/Ag on a steel plate similar to the target plate. As shown in Fig. S8, bare MoS₂ surface showed a contact angle of 31°. AgNPs capped with citrate groups is beneficial for water dispersibility,[34, 35] so the presence of AgNPs on MoS₂ surface make the matrix more hydrophilic, with contact angle value decreasing to 17°. These results agree well with water dispersibility presented in Fig. S1. When small molecules were added onto the matrix layer, they can not only spread more homogeneously, but also induce stronger interaction between the analytes and the matrix for efficient electron transport. Meanwhile, previous study has demonstrated the thickness of the MoS₂/Ag was greater than that of the MoS₂ nanosheet.[38] Modification of AgNPs onto the MoS₂ surface apparently enhances surface roughness and surface area for analyte adsorption and, thus, increase the LDI efficiency.[18]

Both the improved surface wettability and surface roughness are important factors manipulating the interaction between analytes and matrix materials during the complicated LDI processes. The enhanced signal intensities could also be contributed by the synergistic effect originated from the interface of MoS₂ and Ag, where analytes can receive energies from both MoS₂ and Ag (as depicted in Fig. 4b).[18] AgNPs have extremely high absorption coefficient with $1.2 \times 10^8 \text{ M}^{-1}\text{cm}^{-1}$. [28] And MoS₂ has a direct band gap of about 1.8 eV, leading to a high absorption coefficient for incident light.[41] As a result, they both can absorb energies from the laser and co-transfer energies to the analytes for ionization. After nanoparticle deposition, the thermal conductivity of MoS₂ increases,[27] which benefits LDI processes since energy transfer from the matrix materials to the analytes likely via a thermally driven process[42].

< Insert Fig. 4 >

Unlike some nanomaterials that have the ability for giving protons or cations, the MoS₂ sheet obtained using chemical Li-intercalation and exfoliation method had no capability for providing free protons or metal ions thus it could make no contribution to positive ionization of analytes. However, MoS₂ edges and defects have been identified as the active sites for hydrogen evolution reaction.[25] The zeta potential of the MoS₂ was determined to be -30.15 mV, suggesting abundant Mo vacancy exists in MoS₂. This is well consistent with the relative atomic percent from the EDX spectrum, in which the ratio of Mo : S is calculated to be 0.83 : 2 (Fig. S2d). After MoS₂ modification with AgNPs, the zeta-potential became -54.93 mV, which can be attributed to the negative charges carried by AgNPs. These negatively charged sites can function as deprotonation sites for proton acceptance, thus facilitating the charging process in negative ion mode.[20, 21] Overall, the stronger interaction between analytes and matrices, enhanced laser absorption and thermal conductivity, and the negatively charged surfaces are all key factors enabling MoS₂/Ag for ionization in the negative ion MS.

3.5. MALDI-TOF MS analysis of small molecule drugs.

Therapeutic drug monitoring is the measurement of drug concentrations with narrow therapeutic ranges in blood and plasma, thereby improving patient care by individually adjusting the dose of drugs.[43] Though a useful tool for fast and high throughput analysis, MALDI-TOF MS has not been particularly effective for small drug molecules detection due to lack of suitable matrix. In our study, four common drugs of sulfadimidine (MW = 278.33), sulfamethoxazole (MW = 253.28), ampicillin (MW = 349.41), and amoxicillin (MW = 365.4) were first analysed using MoS₂/Ag as the matrix in negative ion mode. As presented in Fig. 5a, all the drug compounds at a concentration of 0.5 mM were detectable with high mass intensities. Antibacterial drugs, sulfadimidine and sulfamethoxazole, were detected as the only deprotonated $[M - H]^-$ ions at m/z 277.08 and 252.05, respectively. The other two antibiotics, ampicillin and amoxicillin, undergo possible rearrangement reactions and were detected with MS peaks assigned to the fragment ion $[M - C_6O_3NH_8]^-$ (m/z 207.07) for ampicillin, and $[M - C_6O_3NH_8]^-$ (m/z 223.07) for amoxicillin, respectively (the fragmentation pathways of them were showed in Fig. S9d and S9e). Three anticancer drugs with higher mass range were also analysed with the aid of MoS₂/Ag matrix. As presented in Figure 5b, the three molecules of nilotinib (MW = 529.52), imatinib (MW =

493.60), and dasatinib (MW = 488.01) at concentration of 0.5 mM were all detectable as the $[M - H]^-$ ions with significant intensity.

We also extended the application of MoS₂/Ag to the multiplex detection of eight target drugs to evaluate the use of MoS₂/Ag for simultaneous detection of small molecules. Drugs of acetylsalicylic acid, ketoprofen, mefenamic acid, and flufenamic acid are commonly used to reduce pain and fever for patients; drugs of sulfadimidine, sulfamethoxazole, ampicillin and amoxicillin are often used in the treatment and prevention of bacterial infection. Thus, the detection and identification of these drugs is of great importance for therapeutic drug monitoring. As shown in Fig. 6, all the target drugs with deprotonated ions or fragment ions were detected without any interference, indicating excellent ionization efficiency of MoS₂/Ag for complex small-molecule drug mixtures in a matrix-free background.

< *Insert Fig. 5* >

< *Insert Fig. 6* >

3.6. Determination and quantification of acetylsalicylic acid in spiked human serum.

To investigate the feasibility of rapid analysis in complex samples with MoS₂/Ag as a matrix, the profiling of drugs in biological media such as human serum should be carried out. As we have aforementioned, MoS₂/Ag showed homogeneous deposition on the target plate and had excellent sample-to-sample reproducibility. Therefore, we further investigated the use of MoS₂/Ag for quantitative measurement of acetylsalicylic acid in human serum by MALDI-TOF MS. A salicylate derivative, acetylsalicylic acid is routinely used for treatment of pain, fever, and inflammation. However, acetylsalicylic acid overdose has become a widespread issue and can lead to chronic poisoning.[44] The acetylsalicylic acid spiked serum was diluted with 4-fold acetonitrile to precipitate high abundant proteins, and the supernatant was collected for negative ion MALDI-TOF MS analysis using MoS₂/Ag matrix. Fig. 7a and 7b show the MS peaks of acetylsalicylic acid in human serum after the aforementioned simple sample preparation. The spiked acetylsalicylic acid can be readily detected at m/z 137.02 (the fragmentation pathway was showed in Fig. S9c), clearly indicating that this substrate is effective. At the concentration of 1 mM, the signal can be obtained with a relative clean background in the spectrum (Fig. 7a). When the concentration was decreased to 0.1 mM, the characteristic peak could still be detected with S/N ratio of 689.07 and a clear background (Fig. 7b). A quantitative standard curve of

acetylsalicylic acid was established. Without an internal standard, a linear correlation response was observed in the range from 0.1 to 1 mM ($18 \text{ mg L}^{-1} - 180 \text{ mg L}^{-1}$) with an excellent correlation coefficient, $r^2 = 0.9972$ (Fig. 7c). Typical acetylsalicylic acid serum level of a normally dosed patient in clinical monitoring is around $30 - 100 \text{ mg L}^{-1}$, while therapeutic doses yield a range from 0.1 to 1 mM, which is well covered by our analysis.[45] From these results, we believe that the MoS₂/Ag matrix-based MS can provide a simple, rapid, and reliable quantitative analysis method to monitor many other specific drugs and small molecules in biological media without the use of an internal standard.

< Insert Fig. 7 >

4. Conclusion

In summary, we have synthesized a novel MoS₂/Ag nanohybrid film and demonstrated its use as an efficient matrix for negative ion MALDI-TOF MS analysis of small molecules. The MoS₂/Ag nanohybrid integrates several favorable properties onto one substrate, including high surface roughness and large surface area for analyte adsorption, better dispersibility, increased thermal conductivity and enhanced UV energy absorption. The nanohybrid has exerted synergistic effect on the efficient detection of small-molecular-weight analytes including amino acids, peptides, fatty acids, and drugs. The MoS₂/Ag matrix offers several clear advantages for small molecule detection, in particular clear matrix background, good salt tolerance, good signal reproducibility and higher detection sensitivity. Furthermore, it allows for simultaneous analysis of eight different drugs and direct small molecule quantitative analysis in complex biological samples, as shown in the analysis of acetylsalicylic acid in the spiked real samples. Given its unparalleled performance for organic matrix-free mass spectrometric detection demonstrated in this work, it is strongly believed that the MoS₂/Ag nanohybrid is a highly promising nanomaterial for sensitive and reproducible MALDI-TOF MS detection of small-molecular-weight compounds.

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Scheme 1. Schematic illustration of MALDI-TOF MS analysis of small molecules using MoS₂/Ag as the matrix.

Fig. 1. TEM images of (a) MoS₂ and (b) MoS₂/Ag. (c) UV-vis spectra of MoS₂ and MoS₂/Ag aqueous solutions. Inset in (c) shows the photographs of MoS₂ (left) and MoS₂/Ag (right) aqueous solutions. (d) Mass spectra of His with different sample preparation methods using MoS₂/Ag as the matrix.

Fig. 2. MALDI-TOF MS spectra of 1 μ L amino acids mixture containing 0.5 mM His, Phe, Arg, and Try by using (a) the CHCA matrix in positive ion mode; (b) the 3-AQ matrix (c) the MoS₂ matrix (d) the Ag matrix, and (e) the MoS₂ /Ag matrix in negative ion mode. Laser intensities of 2800 were applied for all samples in positive and negative mode, respectively.

Fig. 3. MALDI-TOF MS spectra of 1 μ L peptides mixture containing 0.5 mM Gly-Gly, Ala-Ala, Ala-Gln, Try-Phe, and Gla-Val-Phe by using (a) the MoS₂ matrix, (b) the Ag matrix, and (c) the MoS₂/Ag matrix in negative ion mode; 0.5 μ L fatty acids mixture containing 0.5 mM C14, C16, C18, and C20 by using (d) the MoS₂ matrix, (e) the Ag matrix, and (f) the MoS₂/Ag matrix in negative ion mode. Laser intensity of 2800 was applied for all samples.

Fig. 4. (a) Comparison of mass peak intensities of various low-molecular-weight molecules with MoS₂, Ag, and MoS₂/Ag as matrices for MALDI-TOF MS. The concentration of all analytes was set as 0.5 mM. Laser intensity of 2800 was applied for all samples. (b) Scheme showing synergistic effect between MoS₂ and AgNPs as the matrix where analytes can receive energies from both MoS₂ and Ag.

Fig. 5. MALDI-TOF MS for antibacterial drugs and antibiotics (a) and anticancer drugs (b) at concentration of 0.5 mM using MoS₂/Ag matrix in negative ion mode. Laser intensity of 2800 was applied for all samples.

Fig. 6. MALDI-TOF MS spectra of the eight drugs mixture obtained using MoS₂/Ag matrix. The eight drugs shown are acetylsalicylic acid (a), ampicillin (b), ketoprofen (c), amoxicillin (d), mefenamic acid (e), sulfamethoxazole (f), sulfadimidine (g), and flufenamic acid (h). Laser intensity of 2800 was applied for all samples.

Fig. 7. MALDI-TOF MS spectra of (a) 1 mM and (b) 0.1 mM of acetylsalicylic acid in spiked human serum analysed using MoS₂/Ag matrix in negative ion mode. (c) Working curve for the

quantification of acetylsalicylic acid by MALDI-TOF MS with MoS₂/Ag matrix. Laser intensity of 2800 was applied for all samples.

Table 1. Table showing limits of detection and S/N ratio of His and Phe in distilled water and PBS buffer.

Scheme 1

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

(a)

(b)

Fig. 6

Fig. 7

Graphical Abstract

Table 1

Analytes	His in water	Phe in water	His in PBS	Phe in PBS
LOD (pmol)	0.5	0.5	5	5
S/N ratio	19.96	36.65	36.30	20.69