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ZnO nanoparticle modified polymethyl methacrylate assisted dispersive liquid-liquid micro extraction coupled MALDI-MS for rapid pathogenic bacteria analysis

Gangaraju Gedda[2], Hani Nasser Abdelhamid[1, 3], M Shahnawaz Khan[2], and Hui-Fen Wu[1, 2, 4, 5, 6*]

[1]Department of Chemistry, National Sun Yat-Sen University, Kaohsiung, 70, Lien-Hai Road, Kaohsiung, 80424, Taiwan
[2]Doctoral Degree Program in Marine Biotechnology, National Sun Yat-Sen University and Academia Sinica, Kaohsiung, 80424, Taiwan
[3]Department of Chemistry, Assuit University, Assuit, 71515, Egypt
[4]School of Pharmacy, College of Pharmacy, Kaohsiung Medical University, Kaohsiung, 807, Taiwan
[5]Center for Nanoscience and Nanotechnology, National Sun Yat-Sen University, 70, Lien-Hai Road, Kaohsiung, 80424, Taiwan
[6]Institute of Medical Science and Technology, National Sun Yat-Sen University, Kaohsiung, 804, Taiwan
*Corresponding author, Phone: +886-7-5252000-3955; Fax: +886-7-5253908
E-mail: hwu@faculty.nsysu.edu.tw (Prof. HF Wu)

Abstract

A new, fast and nano-based approach to extract the pathogenic bacteria lysates from aqueous samples was reported. The zinc oxide nanoparticles modified with polymethyl methacrylate (ZnO@PMMA) were synthesized and applied for the dispersive liquid-liquid microextraction (DLLME) in order to detect and extract the bacteria. The extracted lysates were further identified by matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS). The results indicate that the present approach is a simple, rapid and efficient micro extraction technique for the analysis of pathogenic bacteria lysate (*Staphylococcus aureus* and *Pseudomonas aeruginosa*). Under the optimal conditions, the minimum detectable concentration are 9.7×10^3 and 1.7×10^4 cfu/mL for *S. aureus* and *P. aeruginosa*, respectively. The ZnO@PMMA nanoparticles provide multifunctional forces to strengthen the interactions with the cell

lysate. The large surface area of ZnO@PMMA enhance the separation efficiency, improves the sensitivity and quality of MALDI-MS spectra. The present method was validated by the real sample analysis such as tap and drinking water. Data reveal that the ZnO@PMMA-DLLME is a promising microextraction technique for pathogenic bacteria analysis and also enables for clinical investigation in the near future.

Keywords: Dispersive liquid-liquid microextraction, ZnO nanoparticle, polymethyl methacrylate, pathogenic bacteria, matrix-assisted laser desorption/ionization mass spectrometry

1. Introduction

Rapid identification of pathogenic bacteria is a crucial demand nowadays for food, water safety, clinical diagnosis and treatment[1-3]. Conventional methods, such as cell culture, polymerase chain reaction (PCR), enzyme-linked immunosorbent assays (ELISA), microscopic diagnosis, and flow cytometry, [1-3] are sensitive and even could be selective, the difficulty of the analysis procedures, including culture selection, isolation, and morphologic and biochemical characterization are limited by their efficiency. Recently, Matrix assisted laser desorption/ionization mass spectrometry (MALDI-MS) have been intensively used as an alternative tool for the bacterial identification and characterization in various bio-applications.

MALDI-TOF MS can be used to get fingerprint marker protein peaks from microbial cells[4]. These marker peaks can be compared with known library of intact microbial cells of MALDI-TOF spectral fingerprints or proteomics database. Therefore, direct analysis of microorganism in real world sample is simple and straightforward but ion suppression effect may occur due to the interferences or the presence of highly ionizable matrices. These drawbacks could be magnified for real sample analysis or clinical samples because the

presence of interfacial species such as salts or other biomolecules that may produce more unwanted extraneous signals that resulting in failure in identification. Secondly, those microorganisms are present in extremely low concentration in real samples. Therefore, to improve MALDI-MS analysis, pre-concentration or pretreatment steps are necessary for the analysis of trace amount of bacteria from real world samples[5].

Conventional separation techniques, such as liquid-liquid microextraction (LLME) or solid phase micro extraction (SPME) are laborious; consume long time for optimization, and require large amount of toxic and eco-unfriendly solvents [6]. A popular liquid phase micro extraction (LPME) method, called dispersive liquid–liquid micro extraction (DLLME), is an important microextraction technique because it is rapid, simple operation, inexpensive and require short training. The main advantage of this approach is that it is feasible to couple with other analytical techniques, such as gas chromatography (GC-MS), high performance liquid chromatography (HPLC), UV–Vis spectrophotometer and atomic absorption spectroscopy (AAS)[7]. To improve the extraction efficiency, nanoparticles (NPs) assisted microextraction has been reported[8]. NPs can minimize the extraction solvent, improve detection sensitivity and enhance the extraction efficiency. NPs could interact with various biomolecules through different forces such as non-covalent interactions (hydrophobic interaction, electrostatic attraction, hydrogen bond, vander Waals) or covalent bond via the capping or stabilizing agent the coat the NPs surface. [9]. Among the different nanoparticles, surface modified ZnO nanoparticle was applied in different biological application including drug delivery, sensors, biological images [10], and as stationary phase for solid-phase micro extraction of proteomics [11].

Herein, we introduce a novel nanoparticle assisted liquid-liquid microextraction that based on ZnO nanoparticles modified with polymethyl methylacrylate (ZnO@PMMA) for the extraction of pathogenic bacteria (*Staphylococcus aureus* and *Pseudomonas aeruginosa*). The extracted bacteria were then analyzed by MALDI-TOF-MS for further identification. The ZnO@PMMA–DLLME approach is based on a multiple phase-solvent system involving an aqueous media containing bacteria, water immiscible solvent (extracting solvent) containing ZnO@PMMA. The present method requires tiny volume of both sample and solvent (µL) and present a novel analytical platform for sensitive and rapid analysis of pathogenic bacteria. Additionally, we also successfully applied this method for the detection of pathogenic bacteria from two different real water sources; tap water and drinking water.

2. Experimental

2.1. Chemicals and methods

Polymethyl methacrylate (PMMA) polymer was purchased from ACROS organics (New Jersey, USA). Zinc nitrate hexahydrate and trifluoroacetic acid (TFA) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Sinapinic acid (SA) was purchased from Alfa Aesar (Ward Hill, MA 01835, USA). Methanol, acetonitrile (ACN), ethanol and chloroform were obtained from J.T.Baker (Phillipsburg, NJ, USA). Dichloromethane was purchased from ECHO chemicals (Miaoli, Taiwan). Chlorobenzene was obtained from TEDIA (Fairfield, USA). All aqueous solutions were prepared using ultrapure deionized water from a Milli-Q purification system (Millipore, Milford, MA, USA).

2.2. Instrumentation

Transmission electron microscopy (TEM) images of ZnO@PMMA were obtained by Philips CM200 (Switzerland, operated at 300 keV). Fourier transform infrared (FTIR) measurements were carried out at room temperature on a Perkin-Elmer Spectrum 100FT-IR. UV-visible spectrum was recorded by using double beam UV–visible spectrophotometer (Hitachi U-3501, Tokyo, Japan). Mass spectra were collected by MALDI–TOF mass spectrometer (Microflex, Bruker Daltonics, Bremen, Germany) equipped with a 337 nm nitrogen laser 1.25 m flight tube, sinapinic acid as matrix, positive and linear mode (with average shots 200). The laser power was adjusted to ~10% above the threshold energy in order to obtain good resolution as well as high signal to noise ratio (S/N). Flex Control and Flex Analysis 3.0 software used for the collection of all MALDI–TOF mass spectra.

2.3. Synthesis of ZnO@PMMA polymer

ZnO@PMMA polymer nanocomposites were synthesized by hydrolysis of zinc precursor in basic medium [12]. Typically, 2 g of PMMA polymer was dissolved in 50 mL chloroform at room temperature. The solution was stirred to dissolve the PMMA. Ethanolic solution of zinc nitrate was prepared by a dropwise addition of equal volumes of zinc nitrate hexahydrate (0.5 M) and ethanolic solution to NaOH ethanolic solution (0.5 M, 25 mL) within 30 min. Then, the resultant mixture was kept at 25 °C for 2 hours under vigorous stirring. The solution mixture was then refluxed at 60 °C for one hour followed by casting onto a Petri dish for solvent evaporation. The resultant dry polymer nanocomposite film was heated at 120 °C under vacuum for 2 h. The ZnO@PMMA composite was crushed and dispersed in organic solvent (chlorobenzene, dichloromethane and chloroform).

2.4. Bacterial cultivation

A biosafety level 1 cabinet (Nuaire, Plymouth, MN, USA) was used for all bacterial experiments. The standard bacterial strains used in this study, *Staphylococcus aureus* (BCRC 10451) and *Pseudomonas aeruginosa* (BCRC 10303), were purchased from the culture collection at Bioresource Collection and Research Center (BCRC), Hsin-Chu, Taiwan. Both pure strains were cultured individually on Luria-Bertani (LB) agar plates (15 g/L agar) for 24 h incubation at 37 °C. The bacterial cells were transferred to an Eppendorf tube with 1 mL of sterile distilled water. The bacterial concentration in the resulting suspension was estimated using the traditional plate counting method. All glassware and media used in this experiment were autoclaved at 15 lbs pressure for 15 min. All experiments were performed three times to check reproducibility. **2.5. ZnO@PMMA-DLLME extraction and MALDI-MS analysis procedure**

Initially ZnO@PMMA (2 mg/mL) dispersed in organic (extraction) solvents such as chlorobenzene, dichloromethane and chloroform. Fig. 1 shows the approach used for pathogenic bacteria extraction from the aqueous samples and analysis by MALDI-MS. Briefly, 900 µL of aqueous bacterial suspension was taken into a 1.5 mL sterile Eppendorf tubes (Fig.1a). Subsequently, the PMMA modified ZnO NPs (2 mg/mL) containing microextraction solvent and disperser solvent were rapidly injected with a 1 mL syringe (gastight, Hamilton, USA) into the sample solution (Fig. 1b). The Eppendorf tubes were vortexed for 15 mins at room temperature and then incubated for 10 mins to separate the organic layers containing NPs from aqueous layers (Fig. 1c). During this process, the bacterial cells were shifted from the aqueous layer to the organic layer by adhesion to ZnO@PMMA surface. The NPs attached bacterial cells were collected at the bottom of the Eppendorf tubes (Fig.1d). This organic layer containing bacterial cells bound to nanoparticles by hydrophobic adhesion was transferred to Eppendorf tubes with a 10 µL syringe (Fig. 1d). Then, 1 µL of the separated

mixture was mixed with 1 μL of sinapinic acid (SA, 50 mM). Finally, 1 μL of above solution was placed onto the MALDI target plate (Fig. 1e), dried at room temperature, and then analyzed by MALDI–MS (Fig. 1f).

2.6. Real water sample collection

Tap water and drinking water samples used in this study were collected in sterilized glass bottles. The water samples were collected from our laboratory (Chemistry building, C2007), National Sen Yat-Sen University, Kaohsiung, Taiwan). *S. aureus* (4.8×10^8 cfu/mL) and *P. aeruginosa* (6.2×10^8 cfu/mL) were separately spiked in both water samples. Bacteria were extracted from the water samples according to the ZnO@PMMA-DLLME extraction procedures mentioned above (Fig. 1).

3. Results and Discussion

3.1 Synthesis and Characterization of the PMMA polymer grafted ZnO NPs

ZnO modified PMMA was prepared by hydrolysis of zinc precursor in presence of PMMA that capped ZnO surface via ester group ($R\text{-}COOCH_3$) as shown in Fig.S1.[12]. The prepared material was characterized by TEM, UV-Vis and FTIR as showed in Fig. 2. The morphology of ZnO@PMMA is analysis using TEM image (Fig.2A). TEM image indicate that ZnO@PMMA has spherical shape (Fig. 2A) and the average diameter is 6 nm (Fig.1B). The PMMA polymer grafted ZnO@PMMA display an absorption maximum at 330 nm that are very closed with the laser wavelength of our MALDI-MS (N_2 laser (337 nm). Fig.2C indicate that the ZnO@PMMA could assist the desorption/ionization process during MALDI analysis. The surface modification of ZnO by PMMA was confirmed by FTIR (Fig. 2D). The PMMA grafting to ZnO NPs shows a broad peak at 3350 cm^{-1} corresponding to the stretching vibrations of the –O–H group on the surface of ZnO NPs. The sharp peaks at 1700-1750 and 1147 cm^{-1} were due to stretching vibrations of the ester

groups of PMMA. Additionally, the characteristic peaks of stretching vibrations of C–O–C were observed at 1150-1250 cm^{-1}. The FTIR spectrum confirms the surface modification of ZnO by PMMA.

3.2. ZnO@PMMA -DLLME method coupled with MALDI-MS for bacteria analysis

MALDI-MS is one the most useful technique for the identification of pathogenic bacteria due to the following reasons: 1) it can provide an universal bacterial identification; 2) it can identify bacteria strains from samples quantitatively/qualitatively; 3) it can be useful for detect uncharacterized bacteria/biomolecules that exist in the real sample; 4) high-throughput analysis (96-365 samples per run) is achieved; 5) it is sensitive, simple, and fast. In MALDI-MS The bacteria identification can be characterized by two different methods: (1) mass spectra compared with a fingerprint database and (2) matching of biomarker masses to a proteome database. Here we report a standard bacteria spectrum for each strain in order to evaluate the separation/extraction efficiency for pathogenic bacteria. This is highly recommended than the use of the proteome database because the MALDI MS detection on the intact cell (whole cell) methods of bacteria has various variables such as the use of growth medium, culture time and incubation time. Thus, the obtained mass spectra may show some mass shifts if we searched from proteomics database. Next, the identification of low-abundant cell biomarkers in the real world samples is still a main challenge due to the presence of low concentration analytes and the interferences from matrices Thus, the ZnO@PMMA was used in DLLME for extraction and the extract lysate were identified by MALDI-MS. Shen et.al reported a fast solid-phase microextraction method using core-shell ZnO@polymethyl methacrylate nanobeads (ZnO@PMMA) as an adsorbent for proteomics analysis of different proteins[11]. They found that the proposed approach showed high enriching efficiency and salt tolerance capability for MALDI-MS.

3.2.1. Selection of suitable extracting solvent

In general, the extraction solvent must meet the following criteria in order to achieve high performance; 1) the density of the solvent must be different from that of water to enable phase separation, 2) it should possess low toxicity and eco-friendly; 3) it should have high affinity to trap the target analyte and extremely low solubility in water, 4) it solvent should be able to disperse the hydrophobic NPs, and 5) it should not cause any changes in the protein signals. Based on the above conditions, we select dichloromethane, chloroform, and chlorobenzene as possible extraction solvents. These solvents are able to solubilize ZnO@PMMA over than other solvents.

In order to select the best extracting solvent from various extraction solvents, the ZnO@PMMA was dispersed in each halogenated solvents and performed the experiments individually.. Here the volume of bacteria aqueous sample (1000 µL), extracting solvent (50 µL) and disperser solvent (50 µL) were used. Fig. 3 shows the MALDI-MS spectra of two bacteria, *S. aureus* and *P. aeruginosa,* obtained using the ZnO@PMMA-DLLME extraction method using three different extracting solvents. Fig. 3 shows the control (a) MALDI-MS spectra of *S. aureus* and *P. aeruginosa* in the absence of ZnO@PMMA-DLLME. The MALDI-MS spectra of *S. aureus* and *P. aeruginosa* obtained by employing ZnO@PMMA-DLLME with dichloromethane, chlorobenzene and chloroform as microextraction solvents are shown in Fig. 3A (b-d) and Fig. 3B (b-d). The MALDI-MS results reveal that compared to the other two solvents, dichloromethane serves as the best DLLME solvent in terms of extraction efficiency for both pathogenic bacteria.

3.2.2. Optimization of volume of extracting solvent

Optimization of volume of the extracting solvent is not only reducing the toxic solvents but it is also important to obtain high resolution. If the extraction solvent volume is increased, the volume of the microdroplets obtained by vortex and incubation would increase resulting in a decrease in the enrichment factor. Hence, to achieve a high separation performance, the extraction solvent volume should be minimized, while still sufficient to serve as the sediment drop after vortex/incubation.

In order to estimate the effect of extracting volume on the extraction efficiency, an experiment was conducted with the extracting solvent (dichloromethane) volume was varied in range 20 to 80 µL (increments of 10 µL). The results are presented in Fig. 4. The extracting solvent successfully extracted maximum number of proteins from each bacterium with 20 µL that was considered for all the further experiments. The extracting solvent (dichloromethane, 20 µL) was used for both bacteria i.e *S. aureus* and *P. aeruginosa.*

3.2.3. Selection of suitable dispersive solvent and volume

The dispersive solvent causes the extraction solvent to form fine droplets in aqueous samples. A selected disperser solvent must be highly miscible in both aqueous and organic phase. Here, methanol was used as a disperser solvent due to low cost and commonly used in microextraction methods. Moreover, in order to facilitate efficient extraction of bacteria, the disperser solvent volume must be optimized. If it is too small, a homogenous dispersion may not form. In contrast, at too large volumes, the solubility of other molecules in water could increase resulting in the reduction of extraction efficiency.

To investigate the effect of the dispersive solvent volume on exaction efficiency, an array of experiments were conducted with the disperser solvent volume was varied from 20 to 80 µL by increments of 10 µL while maintaining the extraction solvent according to

the above optimization. The results reveal that 20 µL and 60 µL of disperser solvent were optimized to perform further analysis for *S. aureus* and for analysis of *P. aeruginosa,* respectively (Fig.5).

3.3 The potentiality of ZnO@PMMA in microextraction of bacteria

The influence of ZnO@PMMA concentrations on bacteria adhesion and extraction efficiency were investigated with different amount (0.05, 0.1, 0.2, 0.5,1.0mg/mL) as shown in Fig.S2. The MALDI-MS mass spectra of protein profile results indicate that by increasing concentration from 0.05 to 0.1 mg/mL, the number of bacteria protein signals and their signal intensities are highly improved. In the presence of 0.2 to 0.5 mg/mL, showed similar pattern but in 1 mg/mL condition, the signals were reduced. Thus, we selected 0.2mg/mL as the optimized concentration. The bacteria extraction efficiency was enhanced with the increased amount of ZnO@PMMA. However, in higher concentration of the nanoparticles, the MALDI signals show slightly decreasing.

3.4 Method for reaching lowest detectable concentration of pathogenic bacteria

The infectious pathogen bacteria in contaminated environmental and clinical samples are typically in ultra low concentrations. It is difficult to detect low concentration pathogens by using the conventional MALDI-MS. Our developed perconcentration method has been applied to test the lowest detectable concentrations of pathogenic bacteria. For this, we prepared different concentrations of bacteria from stock bacteria solution as below. For *S. aureus:* 4.8×10^{8} , 9.7×10^{6} , 3.2×10^{5} , 1.8×10^{4}, 9.7×10^{3} and 8.9×10^{2} cfu/mL and for *P. aeruginosa:* 6.2×10^{8} , 4.5×10^{7}, 1.2×10^{6},3.2×10^{5},1.7×10^{4} and 3.8×10^{3} cfu/mL are prepared to find the lowest detectable concentration. The bacterial concentration was calculated by standard plate count method. Fig.S4 & Fig.S5 provide the MALDI mass spectra for lowest detectable concentration of bacteria. These results suggest the lowest detectable concentration for *S. aureus* (Fig.S3)

and *P. aeruginosa* were 9.7×10^3 and 1.7×10^4 cfu/mL for *P. aeruginosa* (Fig.S3), respectively. Due to the ZnO@PMMA with high affinity of adhesion to bacteria, this method allowed to preconcentrate and separate bacteria even at very low concentration. The lowest detectable concentration is much lower than those previous reported MALDI-MS methods for bacterial analysis (Table.1).

3. 3. Mechanistic interaction of ZnO@PMMA with bacteria

Bacterial adhesion to nanomaterials depends on physicochemical interactions among them. Nanomaterial functionalized with hydrophobic capping agent show highest affinity towards bacterial adhesion through hydrophobic interactions. In this regards, several researcher have focused on developing hydrophobic adhere interaction between materials and bacteria[14]. Since hydrophobicity was induced on ZnO NPs by a surface modification with hydrophobic PMMA polymer (Fig.S1& Fig.2A). PMMA enhance the mechanical properties of ZnO NPs[12, 15], thus it may improve the interaction with bacteria during agitation. The small size (6nm) of the ZnO@PMMA (Fig.2A) has greatly influenced bacteria adhesion due to the availability of large surface area-to-volume ratios. The ZnO@PMMA is hydrophobic in nature, thus the bacteria cells were likely to be extracted by ZnO@PMMA-DLLME via the hydrophobic interactions. Several researchers studied physical and chemical interactions between the PMMA polymers with the bacteria[16]. They investigated that the physical and chemical interaction between the PMMA polymers with bacteria are hydrophobic interactions. Both gram positive and gram negative bacteria contain several hydrophobic proteins[17]. These hydrophobic proteins interact with hydrophobic NPs through hydrophobic interactions[18]. Additionally, the bacteria cell membranes contain several hydrophobic components such as extracellular molecules, fimbriae, and flagella[19]. Furthermore *S. aureus* (Gram-positive) contains

long alkyl chain LPS (thickness around 8-10 nm) and *P. aeruginosa* (Gram-negative) long alkyl chain teichoic acid[20]. These hydrophobic components may be responsible for the hydrophobic interactions between bacteria and ZnO@PMMA[21].

The MALDI-MS results suggest that under the optimal conditions, the ZnO@PMMA combined DLLME effectively transfer the bacteria from the aqueous phase to organic phase (Fig.S3). These results suggested that in the absence of bacteria, we did not find any proteins peaks. In contrast, in the presence of bacteria, it generated significant protein profiles. It was conformed that the NPs adhesion bacteria transferred from the aqueous phase to the organic phase. Increasing the amount of NPs also increase the number of protein peaks, the signal intensity and the peak resolution (Fig.S2). The TEM microscopy was used to confirm the surface morphology of bacteria with and without ZnO@PMMA(Fig.6). These results reveled that the ZnO@PMMA coated on the surface of bacteria without disturb the morphology. It is due to the ZnO@PMMA affinity towards bacteria. These observations clearly indicate that the ZnO@PMMA have high affinity toward the adhesion on bacteria.

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5. Real sample analysis

The PMMA polymer shell with high enriching efficiency to proteins and week interactions with the inorganic salts, detergents and other matrices components[11,22]. Thus, bacteria can easily extract from the contaminated matrix sample using ZnO@PMMA-DLLME. In MALDI-MS analysis, bacterial identification is based on the protein fingerprint given by the mass spectra. To demonstrate the applicability of the current method to real sample analysis. We employed the ZnO@PMMA DLLME method for the extraction of the two pathogenic bacteria from tap water and drinking water. First, *S.aureus* and *P.aeruginosa* were spiked into both tap water and

drinking water and the extraction was followed by the above optimized method. The extract bacteria lysate were analyzed by MALDI-MS and the results are displayed in Fig.7. The results indicate that the bacterial protein signals obtained from both the water samples closely matched the standard bacteria protein signals for (A) *S. aureus* (Fig.7A) and (B) *P. aeruginosa* (Fig.7B). These results suggest that our developed method was successfully applied for bacteria lysate extracted from the matrices contain water samples. Hence, the ZnO@PMMA -DLLME method present a rapid and sensitive microextraction approach for pathogenic bacteria i.e *S. aureus* (Fig.7A) and *P. aeruginosa* (Fig.7B). The present approach is a promising technique for the clinical medicine and biomedical applications in the near future.

7. Conclusion

We have successfully introduced a simple, rapid, and sensitive microextraction approach for pathogenic bacteria based on ZnO@PMMA - DLLME coupled with MALDI-MS. Effective extraction was achieved by exploiting the hydrophobic interactions between the pathogenic bacteria and the ZnO modified PMMA nanoparticles. This approach has potential application for bacteria separation at very low colonies as 9.7×10^3 and 1.7×10^4 cfu/mL for *S. aureus* and *P. aeruginosa*, respectively. It is simple, easy and rapid for very short analysis time. We believe that the present method is a novel platform for the fields of clinical microbiology and clinical medicine as well as any fields to investigate the presence of pathogenic bacteria in the environmental samples.

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Figure Captions

Figure 1. Schematic illustration of ZnO@PMMA-DLLME coupled with MALDI-MS for pathogenic bacteria extraction and analysis; (a) bacterial sample solution, (b) rapid injection of micro extraction solvent and disperser solvent into sample solution, (c) separation of micro extraction solvent phase from sample solution, (d) micro extraction solvent transfer to new test tube, (e) sample spotted on MALDI target plate and analysis by MALDI-MS, (f) MALDI-MS spectra, (g) ZnO@PMMA interaction with bacteria.

Figure 2. Characterization of ZnO@PMMA using (A) TEM image, (B) histogram for particle size distribution (C) FTIR of ZnO@PMMA and (D) UV-Vis absorption.

Figure 3. Effect of type of extracting (organic) solvent on the efficiency of (A) *S. aureus* and *(*B) *P. aeroginosa* with (a) control (b) dichloromethane, (c) dichlorobenzene and (d) chloroform.

Figure 4. Extracting solvent volume effect on extraction efficiency of (A) *S. aureus. (*B) *P. aeruginosa* with (a) 20 (b) 30 (c) 40 (d) 50 (e) 60 (f) 70 (g) 80 µL of dichloromethane.

Figure 5. Dispersive solvent volume effect on extraction efficiency of (A) *S. aureus. (*B) *P. aeruginosa* with (a) 20 (b) 30 (c) 40 (d) 50 (e) 60 (f) 70 (g) 80 µL of methanol.

Figure 6. TEM analysis of (A) *S. aureus* and (B) *P. aeruginosa* before (a) and after (b) interaction with ZnO@PMMA

Figure 7. Extraction of (A) *S. aureus* (B) *P. aeruginosa* from (a) Sterilized de-ionized water (control) (b) tap water and (c) drinking water.

Table 1: Comparison between different Nanomaterials-assisted micro extraction methods coupled with MALDI-MS

S.No	Nano particle	Bacteria	Extraction Method	Real sample	LOD(cfu/mL)	Referance
1	ZnO@PMMA	*P.aeruginosa&S. aureus*	DLLME	Tap& Drinking water	9.7×10^3 &1.7×10	Here
2	IL@ Pt	*E.coli&Serratia marcescens*	SDME	Water	10^6	8(a)
3	CeO2@CTAB	*P.aeruginosa &S.aureus*	LLME	blood &serum	10^3-10^4	8(a)
4	Amine modified Fe3O4	*Aeromonas, Salmonella, Pseudomonas,Enterococcus ,Bacillus,Staphylococcus*	Anion-exchange Magnetic	Tap& reservoir water	1×10^3	23(a)

		and E.coli				
5	Vancomycin-Immobilized Fe3O4	*S.saprophyticus &S. aureus*	Magnetic	Urine	7×10^4	23(b)
6	Fe3O4@Al2O	*E. coli*	Magnetic	Urine	9.6×10^4	23(c)

Note: PMMApolymethyl methacrylate, DLLME-dispersive liquid-liquid microextraction, SDME-single drop microextraction, LLME-liquid-liquid microextraction,