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Ceria nanocubic-ultrasonication assisted dispersive liquid–liquid microextraction coupled with matrix assisted laser desorption/ionization mass spectrometry for pathogenic bacteria analysis

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ABSTRACT

A new ceria (CeO_2) nanocubic modified surfactant is used as the basis of a novel nano-based microextraction technique for highly sensitive detection of pathogenic bacteria (*Pseudomonas aeruginosa* and *Staphylococcus aureus*). The technique uses ultrasound enhanced surfactant-assisted dispersive liquid–liquid microextraction (UESA-DLLME) with and without ceria (CeO_2) followed by matrix assisted laser desorption/ionization mass spectrometry (MALDI-MS). In order to achieve high separation efficiency, we investigated the influential parameters, including extraction time of ultrasonication, type and volume of the extraction solvent and surfactant. Among various surfactants, the cationic surfactants can selectively offer better extraction efficiency on bacteria analysis than that of the anionic surfactants due to the negative charges of bacteria cell membranes. Extractions of the bacteria lysate from aqueous samples via UESA-DLLME-MALDI-MS were successfully achieved by using cetyltrimethyl ammonium bromide (CTAB, 10.0 μL, 1.0×10^{-3} M) as surfactants in chlorobenzene (10.0 μL) and chloroform (10.0 μL) as the optimal extracting solvent for *P. aeruginosa* and *S. aureus*, respectively. Ceria nanocubic was synthesized, and functionalized with CTAB (CeO_2@CTAB) and then characterized using transmission electron microscopy (TEM) and optical spectroscopy (UV and FTIR). CeO_2@CTAB demonstrates high extraction efficiency, improve peaks ionization, and enhance resolution. The prime reasons for these improvements are due to the large surface area of nanoparticles, and its absorption that coincides with the wavelength of MALDI laser (337 nm, N_2 laser). CeO_2@CTAB-based microextraction offers lowest detectable concentrations tenfold lower than that of without nanoceria. The present approach has been successfully applied to detect pathogenic bacteria at low concentrations of 10^4–10^5 cfu/mL (without ceria) and at 10^3–10^4 cfu/mL (with ceria) from bacteria suspensions. Finally, the current approach was applied for analyzing the pathogenic bacteria in biological samples (blood and serum). Ceria assist surfactant (CeO_2@CTAB) liquid–liquid microextraction (LLME) offers better extraction efficiency than that of using the surfactant in LLME alone.

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1. Introduction

Matrix assisted laser desorption/ionization mass spectrometry (MALDI-MS) is a soft ionization technique which allows sensitive analysis of biomolecules [1,2]. Today, MALDI-MS has been considered as a routine technique as it can be applied to analyze bacteria [3–8]. MALDI-MS is a rapid technique for microorganism analysis [3–8] based on their biomarker peaks.

Analysis of bacteria in biological samples is usually a challenge task because of their diversity, complexity, and low concentration. Detection of bacterial infection at the initial stage is tremendously important for clinical treatment. In contrast, MALDI-MS analysis from biological analysis typically suffers from intense interferences or suppression effect due to the presence of complicated and huge amount of biological biomolecules. Therefore, it is necessary to preconcentrate the environmental/biological samples prior to MALDI-MS analysis. Preconcentration techniques such as liquid–liquid extraction (LLE) or solid phase extraction (SPE) are necessary to analyze trace amounts of analytes [9–11].

Recent trend in analytical techniques is to reduce the solvent amount or chemicals that were used in the preconcentration steps. It is also necessary to miniaturize the solvents in order to decrease the analysis time [11-13]. The first attempt of liquid phase microextraction (LPME) was the single drop microextraction (SDME). SDME is a rapid, simple, and inexpensive technique [14,15]. For high extraction

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efficiency, a method called dispersive liquid–liquid microextraction (DLLME) [16] was proposed based on a dispersive solvent such as methanol, propanol, or butanol. Further, the ultrasound enhanced surfactant assisted dispersive liquid–liquid microextraction method (UESA-DLLME) was proposed [17–21]. It has been developed to avoid the use of disperser solvent such as methanol, propanol [17–21]. The UESA-DLLME could accelerate the extraction efficiency based on the use of ultrasonication and it also offers many analytical merits, such as relatively low cost, eco-friendly, easy handling, no toxic effects, and it can provide satisfactory results with the use of the least volume of the solvent. Surfactants are amphiphilic organic compounds which contain both hydrophobic and hydrophilic moieties, so it is soluble in both organic and aqueous medium. Surfactants can reduce the interfacial tension between the organic and water layers resulting in increase the contact areas and to improve the extraction efficiency. Nanoparticles (NPs) prepared in organic layers have been extensively applied in order to increase the mass transfer of target analytes. NPs miniaturize the extraction solvents, improve detection sensitivity and increase the extraction efficiency [22–24]. Potential applications of nanoparticles in sample preparation are discussed in [25]. Comprehensive discussions for various microextraction methods have been reviewed in [26–30]. Cerium oxide nanoparticles or ceria (CeO_2) were applied in biomedical applications due to their nice biocompatibility [31]. Ceria (CeO_2) nanocubic exhibits high positive zeta potentials, thus it can assist protein adsorptions [32]. The electrostatic interactions are the driving forces for the protein adsorption and cellular uptake of the ceria nanoparticles [32].

The main aim of the present study is to develop a sensitive, and rapid microextraction technique based on UESA-DLLME with and without nanoceria (CeO_2) coupled with MALDI-TOF-MS for pathogenic bacteria analysis (*Pseudomonas aeruginosa* and *Staphylococcus aureus*). The CeO_2 nanocubic modified surfactant was prepared, characterized and applied for bacteria microextraction. For the first time, we applied CTAB modified CeO_2 (CeO_2@CTAB) nanoparticles dispersed into organic solvents as liquid microdroplets for the detection of target bacteria from their suspensions. Ceria was selected because it has excellent ability to adsorb bacteria biomolecules, such as proteins based on the high positive zeta potential [32]. UESA-DLLME with and without ceria were applied to extract both bacteria (*P. aeruginosa* and *S. aureus*) lysates from aqueous medium. Ceria (CeO_2) could increase the affinity toward protein adsorption; decrease the lowest detectable concentration for more than tenfolds, and improving the peaks resolution. Three different biological samples were used to check the applicability for the current approach. The results revealed that the ceria-surfactant ultrasonic assist liquid–liquid microextraction is a superior technique to extract the bacteria lysate from aqueous and blood suspensions over than the approach which only applies the surfactants for extraction.

2. Materials and methods

Tetraoctylammounium bromide (TOAB), sodium dodecyl sulfate (SDS) and cetyltrimethyl ammonium bromide (CTAB) were purchased from Sigma-Aldrich (USA). Dichloromethane was purchased from ECHO Co. (Taiwan). Chloroform was purchased from J.T. Baker (USA). Chlorobenzene and sinapinic acid were purchased from Alfa Aesar (Great British). Ultrapure water was obtained from a Milli-Q Plus water purification system (18.2 MΩ, Millipore, Bedford, MA, USA) was used for all experiments.

2.1. MALDI-TOF MS analysis

MALDI-TOF-MS spectra were obtained from Microflex (Bruker Daltonics, Bremen, Germany) equipped with a nitrogen laser (N_2, wavelength 337 nm). The spectra were recorded in positive and linear mode using an acceleration voltage of 20 kV and 10 ns extraction delay time. Sinapinic acid was used as a matrix for all experiments. Sample preparation procedures were discussed in the extraction procedures.

2.2. Ultrasonic instrument

The aqueous or real sample suspensions were ultrasonicated using an ultrasonication machine (LC30H, Sunway Scientific Corporation, Taiwan).

2.3. Characterization of ceria (CeO_2) nanocubic modified surfactant

The ultraviolet–visible (UV–vis) spectrum of CeO_2 nanoparticles was recorded using a double beam UV–vis spectrophotometer (Perkin Elmer 100, Germany) in the range of 200–700 nm. The size of the CeO_2 nanoparticles was further confirmed with the transmission electron microscopy (TEM, Philips CM 200, Netherlands) at an accelerating voltage of 200 kV. The samples were prepared for TEM by depositing 10.0 μL of an aqueous solution of the CeO_2 nanoparticles on a copper grid, and then it was dried under vacuum overnight. The sample was analyzed to confirm the particle size/morphology.

3. Experimental section

3.1. Preparation of ceria (CeO_2) nanocubic

The ceria CeO_2 were synthesized and then modified with the suitable surfactants as described below:

1. *Synthesis of ceria (CeO_2) nanocubic:* Ceria (CeO_2) nanoparticle was synthesized through a hydrothermal treatment. Ce $(NO_3)_3 \cdot 6H_2O$ (1.0 g) was dissolved in 10.0 mL of deionized water. A solution of NaOH (10%) was added rapidly with stirring to the previous solution. A light yellow precipitate of amorphous CeO_2 was observed. After about 1 h of stirring, all slurry was then transferred into a 50.0 mL of Teflon autoclave tube, and diluted with deionized water up to 80% of the total volume. The autoclave tube was heated at temperatures 200 °C for 24 h. After cooling, the precipitate was filtrated and washed several times with deionized water and then dried at 60 °C as shown in Fig. 1A. Some of the physical parameters such as temperature and time are cited from literature [33].
2. *Surface capping of ceria* (CeO_2) *with cetyltrimethyl ammonium bromide* (*CTAB*): About 0.5 g of ceria (CeO_2) was dispersed in organic solvent ($CHCl_3$, C_6H_5Cl, individually) that contain cetyltrimethyl ammonium bromide surfactant (CTAB, 0.5 g). The solution was subjected to strong stirring for 3 h. The synthesized nanoparticles were separated by ultracentrifugation (18 kg, 20 min), and then washed several time to remove all unassociated surfactant. The prepared CeO_2@CTAB was characterized using FTIR, TEM and UV spectroscopy.

3.2. Preparation of CeO_2@CTAB in organic solvent

About 0.5 g of CeO_2@CTAB was dissolved in 15 mL of organic solvents (chlorobenzene, CB and chloroform, $CHCl_3$), individually. The suspension solutions were used directly for the extraction procedures.

3.3. Bacteria cultivation

Both bacteria were cultivated using the conventional method as reported in Refs. [34,35]. *S. aureus* (BCRC 10451) and *P. aeruginosa*

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