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Design, characterization and applications of new ionic liquid matrices for multifunctional analysis of biomolecules: A novel strategy for pathogenic bacteria biosensing

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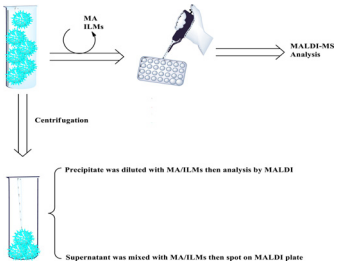
HIGHLIGHTS

- Design and characterize novel UV absorbed-ionic liquid matrices series.
- Apply the new series for different analytes.
- Introduce a novel methodology for pathogenic bacteria biosensing.
- Tabulate the physical parameters of the new series.

GRAPHICAL ABSTRACT



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ABSTRACT

The design, preparation and performance for novel UV-light absorbing (room-temperature) ionic liquid matrices (UV-RTILMs) for matrix assisted laser desorption/ionization mass spectrometry (MALDI-MS) were reported. A series of UV-RTILMs was prepared by ultrasonication of equimolar of acid (mefenamic acid) and bases (aniline (ANI), pyridine (Pyr), dimethyl aniline (DMANI) and 2-methyl picoline (2-P)). The UV-RTILMs have not only significant absorbance at the desired wavelength (337 nm of the N_2 Laser), but also have available protons that can easily undergo proton transfer reactions to ionize the target molecules. The novel UV-RTILMs have the ability to ionize different and wide classes of compounds such as drugs, carbohydrate, and amino acids. The new UV-RTILMs series have been successfully and selectively applied for biosensing the lysates of pathogenic bacteria in the presence of the cell macromolecules. A new strategy for biosensing pathogens was presented via sensing the pathogens lysate in the cell suspension. The new materials can effectively detect the bacterial toxins without separation or any pretreatment. They offered excellent ionization of labile oligosaccharides with protonated peaks. They could significantly enhance the analyte signals, produce homogeneous spotting, reducing spot-to-spot variation, excellent vacuum stability, higher ion peak intensity, and wide application possibility. The physical parameters such as molar refractivity, molar volume, parachor, surface tension, density and polarizability were calculated and tabulated. The new UV-RTILMs could offer excellent reproducibility and great repeatability and they are promising matrices for wide applications on MALDI-MS.

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1. Introduction

The soft ionization technique such as matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) has become an indispensable tool for analytical chemistry, biology, biotechnology, biomedicine and organic chemistry [1,2]. The wide success of MALDI-MS is mainly related to the great ability and wide application of the matrix that co-crystallizes and transfers the laser radiation energy to the thermal labile and non-volatile analytes such as high molecular weight (MWt) molecules i.e., polymers, sugars, and proteins. Simply, the analyte is co-crystallized into a solid ultraviolet or infrared-absorbing organic acid matrix (depending on the laser types) which can vaporize the molecules after absorbing the laser radiation and after carrying the analytes and it can undergo ionization via ion-molecular reaction (IMR), charge transfer, gas-phase photoionization, disproportionation, excited-state proton transfer, energy pooling, thermal ionization, desorption of preformed ions or others [3]. The key point of MALDI MS success is due to the wide number of effective matrices and increased understanding of their roles and applications [3]. A wide range of different substances have been used for various applications in MALDI-MS. It may be organic [4–8], inorganic and nanoparticles [9–18]. This bank of different compounds enables MALDI MS circumventing their drawbacks such as fragmentation, interferences (<500 Da), destruction of non-covalent interaction, and increase their selectivity. However, the organic matrix still the crux for MALDI-MS analysis due to high sensitivity, can be used for MS-images, stable, cheap and able for further modifications.

In 2001, Armstrong et al. introduced the potential use of a new type of matrix called ionic liquid matrices (ILMs) [19]. Ionic liquids (IL), also called room-temperature ionic liquids (RTIL), are salts that have melting point below 100 °C. Since that time, ILMs attracted a lot of research attention of many groups [17,19–53]. ILMs were reported for effective analysis of several classes of biological molecules including peptides and proteins, [53] phospholipids [54], oligonucleotides [24], oligosaccharides [25], polymer [43] and low molecular weight compounds, such as amino acids and sugars [21,25,54,55]. A potential number of review articles that discuss merits and application of ILs in mass spectrometry and other analytical techniques have been reported [56–63]. ILMs offer many advantages over conventional organic and nanoparticles matrices. They can dissolve a broad range of different analytes, possess negligible vapor pressure, test countless substances; their properties can be tuned by variation of the cations and anions moieties, can form homogenous solution; they can be used for quantitative analysis and application for softer ionization of labile analytes. Due to the last two advantages, they enable relative quantification of biomolecules such as amino acids [21], sugars [25], and peptides [27], short proteins, and oligonucleotides [57] when applying appropriate internal standards.

Here, we introduce novel UV light absorbing room-temperature ionic liquids (UV-RTILMs) for MALDI-MS for the first time. The ILMs were prepared by ultrasonication of equimolar of mefenamic acid and bases such as pyridine (Pyr), 2-methyl pyridine (2-P), aniline (ANI), and dimethyl aniline (ADANI). All the prepared ILMs have UV absorption coincide with the wavelength of N_2 laser (337 nm), thus they can serve for the energy transfer and ionization process of wide range of different analytes such as drugs, carbohydrates, amino acids. For the first time, we successfully applied them for biosensing pathogenic bacteria based on their endotoxin moieties. Data reveal a great promise for these new materials in analytical chemistry, biosensing and organic chemistry.

2. Materials and methods

Mefenamic acid (MA), aniline (ANI), *N,N*-dimethylaniline (DMANI), pyridine (Pyr), 2-picoline (2-P), sulfathiazole, maltoheptaose hydrate, D-panose, palatinose and methanol (CH_3OH) were purchased from Sigma-Aldrich (USA). All chemicals were used directly without any purification. *Staphylococcus aureus* (BCRC 10451) and *Pseudomonas aeruginosa* (BCRC 10303) standard cultures were purchased from Bioresource Collection and Research Center (Hsin-Chu, Taiwan).

2.1. Instruments

2.1.1. MALDI-TOF analysis

The MALDI-TOF-MS analysis was performed by employing positive ion mode on a time-of-flight mass spectrometer (Microflex, Bruker Daltonics, Bremen, Germany) with a 1.25 m flight tube. Desorption/ionization was obtained by using a 337 nm nitrogen laser with a 3 ns pulse width. The accelerating potential is +20 kV. Laser power was adjusted to slightly 10% above the threshold to obtain good resolution and signal-to-noise ratios. The data were repeated more than three times to confirm repeatability. Data were collected using Microflex-Control software (Bruker Daltonics, Bremen, Germany) and processed with Flex Analysis software (Bruker Daltonics, Bremen, Germany). Data were drawn using Origin V 6.0 program and the physical parameters were calculated using ACS/ChemSketch V 12.

2.2. Matrices preparation

2.2.1. Preparation of conventional matrices mefenamic acid (MA)

Mefenamic acid was prepared in 50 mM concentration by dissolving 120.5 mg mL^{-1} in CH_3OH (10 mL). The solutions were stored in the refrigerator.

2.2.2. Preparation of ionic liquid matrices (ILMs)

Ionic liquid matrices of MA series (MA/ANI, MA/DMANI, MA/Pyr, MA/2-P) were prepared just prior to use by adding a 1.2 equivalent of organic base to a solution containing 120.5 mg mL^{-1} of MA (1.0 equivalent) in CH_3OH. Solutions were then sonicated for 10 min. The matrix solutions were investigated interday and intraday in order to check stability during storage. The structures of the series is shown in Fig. 1A.

2.2.3. Application of ionic liquid matrices (ILMs)

The prepared ionic liquid matrices were tested by sulfathiazole, maltoheptaose hydrate; D-panose, palatinose pathogenic bacteria (*S. aureus* and *P. aeruginosa*). The compounds were prepared in aqueous solution and labeled as stock solution (1 mM). From the stock solution, different solutions were prepared to determine the limit of detection (LOD). For pathogenic bacteria, it were cultivated at 37 °C and maintained on Difco™ Nutrient broth (Becton, Dickinson, France, 8.0 g per 1.0 L) and Agar plates (Gen Chain Scientific, GCS, New York, USA, with 1.5% agar). The two bacteria were grown individually overnight at 37 °C using agar medium. The bacteria were dispersed in de-ionized water (1 mL) using a sterilized needle. The cell numbers (cfu mL^{-1}) were evaluated using plate counting protocol. To probe the small molecules of the pathogenic bacteria, we test it in three different batches (Fig.1B). Briefly, after 24 h, the cultured bacteria were removed from the agar plate and then dispersed in de-ionized and sterilized water. The suspension (10 μL) was directly mixed with the matrices (10 μL) and then spots 2 μL in MALDI plate for analysis (Fig.1B). The same suspension was precipitated using centrifugation (10 kg) and then the precipitate and supernatant were investigated. The precipitate was diluted with matrices solution and then 2 μL was