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Analysis of benzylpenicillin in milk using MALDI-TOF mass spectrometry with top-down synthesized TiO_2 nanowires as the solid matrix

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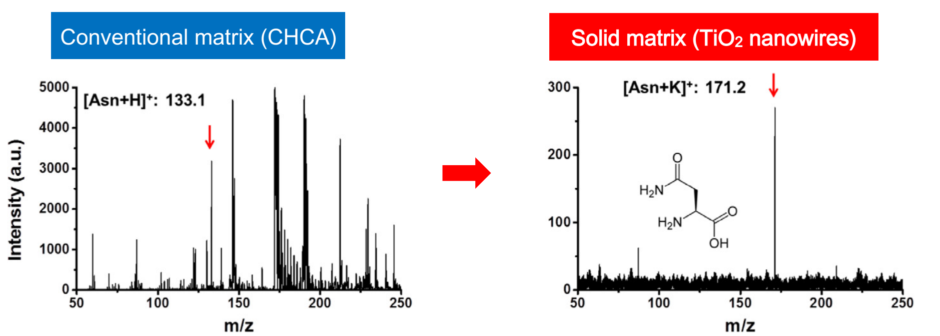
HIGHLIGHTS

- TiO_2 nanowires (NW) were synthesized as a solid matrix of MALDI-TOF mass spectrometry (MS).
- The synthesis of TiO_2 NW was optimized for the MALDI-TOF MS of small molecules ($m/z < 500$).
- The small molecule analysis was demonstrated using the TiO_2 NW and amino acids.
- The analysis of benzylpenicillin in daily milk were demonstrated according to EU directives.

GRAPHICAL ABSTRACT



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ABSTRACT

In this work, the wet-corrosion process for the synthesis of titanium oxide (TiO_2) nanowires in the anatase phase was optimized as the solid matrix in MALDI-TOF mass spectrometry, and the solid matrix of the TiO_2 nanowires was applied to the detection of antibiotics in a daily milk sample. The influence of the alkali concentration and the heat treatment temperature on the crystal structure of the TiO_2 nanowires was investigated. The ionization activity of the TiO_2 nanowires was estimated for each synthetic condition using amino acids as model analytes with low molecular weights. For the detection of antibiotics in milk, benzylpenicillin was spiked in daily milk samples, and MALDI-TOF mass spectrometry with the TiO_2 nanowires was demonstrated to detect the benzylpenicillin at the cut-off concentration of the EU directive.

1. Introduction

MALDI-TOF mass spectrometry has been widely used for the analysis of biomolecules with high molecular weights (Schrauzer and Guth, 1997; Glish and Vachet, 2003; Siuzdak, 2005). In this method, analytes were mixed with an excess of organic matrix molecules. When the dried mixture of analyte and matrix was exposed to laser radiation in the UV range (330, 350 nm), it was evaporated and the activated matrix molecules ionized the analyte. As the analytes could be ionized and analyzed without fragmentation, this ionization method (MALDI) has been widely used for the analysis of biopolymers with high molecular weights, such as proteins, nucleotides, polysaccharides, and so on. Additionally, MALDI-TOF has advantages of easy sample preparation, high sensitivity (fmol) and a wide detection range (600 kDa). However, because the organic matrix molecules were evaporated and ionized together with the analytes, the mass peaks of the fragmented matrix molecules were also produced, especially at the low range of the mass-to-charge ratio (m/z). Such mass peaks from

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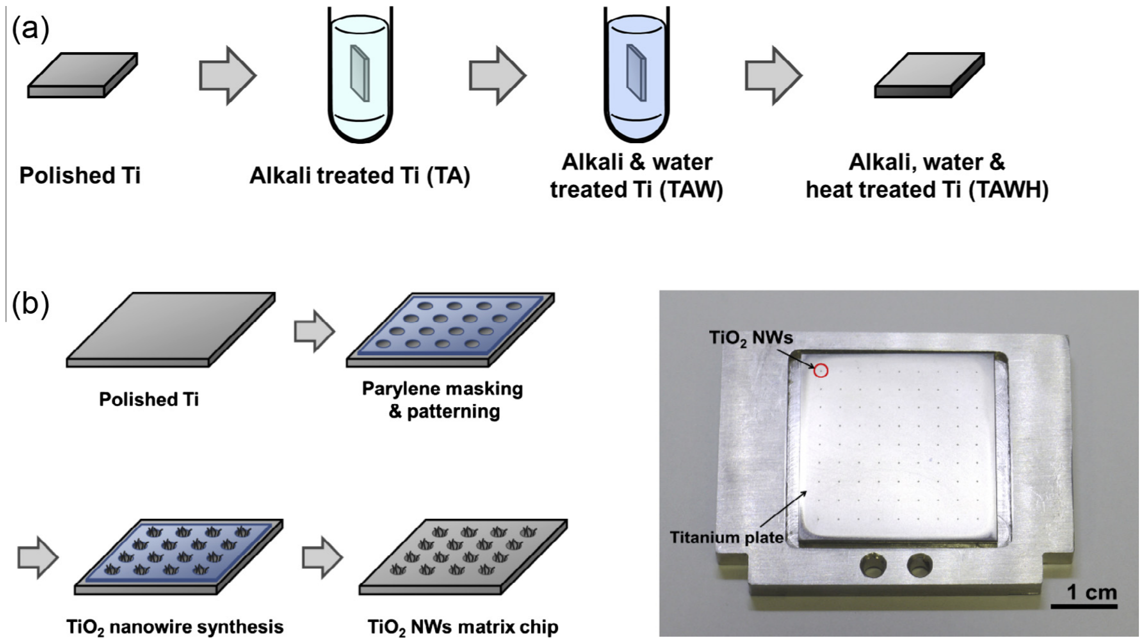

Fig. 1. Preparation of TiO_2 nanowires via the wet corrosion process. (a) Procedure of wet corrosion process consisting of (1) alkali treatment at 10 M KOH, (2) water treatment and (3) heat treatment at 600 °C. (b) Preparation of array-type target plate with TiO_2 nanowires using a parylene-N film as a mask for the wet corrosion process, and picture of array-type target plate.

fragmented matrix molecules were observed to be very irreproducible, resulting in a different pattern at each measurement. For this reason, it was very difficult to separate the mass peaks of the analytes at the low *m*/*z* range, and the analysis of small molecules was very restricted by using MALDI-TOF mass spectrometry (Wei et al., 1999; Kang et al., 2005).

For the analysis of small molecules with MALDI-TOF mass spectrometry, various types of solid matrices have been reported which could ionize analytes without producing the mass peaks of matrix molecules. Previously, metal nanoparticles (Kailasa and Wu, 2010; Nizioł et al., 2013; Khanam et al., 2013; Yang and Fujino, 2014), semiconductor nanowires (Kang et al., 2005; Kim et al., 2014a), two-dimensional nanostructures (Liu et al., 2012), and carbon nanotubes (Ren and Guo, 2006) have been reported to be used as solid matrices in MALDI-TOF mass spectrometry. As such solid matrices were known to ionize analytes without evaporation during the laser irradiation, they could be used for the analysis of small molecules without the unreproducible mass peaks from organic matrix molecules in the low *m*/*z* range. Recently, we reported that titanium oxide (TiO_2) nanowires, which were synthesized through bottom-up and top-down methods, could be used as a solid matrix (Kang et al., 2005; Kim et al., 2014a). Usually, the TiO_2 nanowires were known to have different crystal structures, such as rutile, anatase and layered structures. The photocatalytic effect of TiO_2 nanowires was reported to be observed with the anatase crystal structure, and the ionization of analyte was also reported to occur when using the anatase crystal structure. In particular, because of their strong absorption, TiO_2 nanomaterials have been considered the most promising materials; thin films (Torta et al., 2009; Sonderegger et al., 2011), nanoparticles (Castro et al., 2008), and nanowires (Kim et al., 2014a) have been reported as the solid matrix for MALDI-TOF mass spectrometry. The Ti-wire-based ionization method was also reported with probe electrospray ionization (PESI) mass spectrometry, which can be used to analyze small molecules (Mandal et al., 2010).

In the case of bottom-up synthesis, TiO_2 nanowires were synthesized on a silicon wafer as a substrate. The gaseous precursors were melted into the sputtered gold spot (catalysis) and the super-saturated precursors were grown into TiO_2 nanowires (Kang et al., 2005). Such a nanowire growth mechanism was well-known as a vapor–liquid–solid (VLS) mechanism, and the TiO_2 nanowires were tested to be applicable as a solid matrix for the analysis of small molecules and peptides. However, the bottom-up synthesis through a VLS-mechanism was restricted for the mass production of nanowires and the control of nanowire properties, such as the diameter, height, and density of nanowires, which could have an influence on the MALDI-TOF mass spectrometry results.

The TiO_2 nanowires were also reported to be synthesized through a top-down synthesis method (Kim et al., 2014a). In this method, the titanium plate was reacted with alkali solution at high concentrations with water and heat treatment, which was modified from a well-known hydrothermal wet-corrosion process (Kasuga et al., 1998). The TiO_2 nanowires could be used as a solid matrix for the simultaneous analysis of multiple analytes of amino acids and peptides. As the process could be carried out using aqueous solutions at ambient pressure and temperature without a gaseous reaction under vacuum, it was very compatible for the mass production of nanowires. The properties of the nanowires were estimated to be suitable for the MALDI-TOF mass spectrometry.

In this work, we investigated the optimal conditions for the wet corrosion process for the synthesis of TiO_2 nanowires for MALDI-TOF mass spectrometry. The influence of the alkali concentration and the temperature of heat treatment on the crystal structure of the TiO_2 nanowires were analyzed, and the ionization activity of the TiO_2 nanowires were estimated for each synthesis condition using model analytes with low molecular weights.

The solid matrix of the TiO_2 nanowires was applied to the detection of antibiotics in a dairy milk sample. Antibiotics are widely used in dairy cattle management for the treatment of disease and as dietary supplements. They may be administered orally as feed additives or directly by injection. The therapeutic use of antibiotics for the treatment of mastitis in dairy cattle can result in the contamination of milk with low amounts of the antibiotic if close attention is not paid to good therapeutic practice (Hamann et al., 1979; Bishop and White, 1984). Antibiotics in milk could cause bacterial resistance to the same antibiotics used to treat people for infections, which is considered to be a potential health hazard