Methods xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Methods

journal homepage: www.elsevier.com/locate/ymeth

Polymer-based metal nano-coated disposable target for matrix-assisted and matrix-free laser desorption/ionization mass spectrometry

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ARTICLE INFO

Article history:
Received 23 November 2015
Received in revised form 24 March 2016
Accepted 28 March 2016
Available online xxxx

Keywords:
MALDI
LDI
Disposable
Polymer-based
Nano-coated target
Mass spectrometry

ABSTRACT

The ideal MALDI/LDI mass spectrometry sample target for an axial TOF instrument possesses a variety of properties. Primarily, it should be chemically inert to the sample, *i.e.* analyte, matrix and solvents, highly planar across the whole target, without any previous chemical contact and provide a uniform surface to facilitate reproducible measurements without artifacts from previous sample or matrix compounds. This can be hard to achieve with a metal target, which has to be extensively cleaned every time after use. Any cleaning step may leave residues behind, may change the surface properties due to the type of cleaning method used or even cause microscopic scratches over time hence altering matrix crystallization behavior. Alternatively, use of disposable targets avoids these problems. As each possesses the same surface they therefore have the potential to replace the conventional full metal targets so commonly employed. Furthermore, low cost single-use targets with high planarity promise an easier compliance with GLP guidelines as they alleviate the problem of low reproducibility due to inconsistent sample/matrix crystallization and changes to the target surface properties. In our tests, polymeric metal nano-coated targets were compared to a stainless steel reference. The polymeric metal nano-coated targets exhibited all the performance characteristics for a MALDI MS sample support, and even surpassed the – in our lab commonly used – reference in some aspects like limit of detection. The target exhibits all necessary features such as electrical conductivity, vacuum, laser and solvent compatibility.

1. Introduction

Conventional MALDI MS targets consisting of plain metal (*e.g.* stainless steel) often require intensive cleaning involving copious amounts of solvents, additives and detergents, which adds to cost and creates an increased environmental footprint. The type of cleaning required depends on the analyte and target surface. For example, microorganisms need to be rendered inert after measurement via sterilization. Strongly adhering lipids, polymers or photo-polymerized UV MALDI MS matrices may need to be removed from the sample support's surface by force (abrasion). Furthermore the costs of microtiter plate-sized targets are considerable and make the use of a large number of targets expensive and longer storage of samples hard to justify. The surface properties and chemistry are also subject to change over time (*e.g.* rendering them more hydrophilic or hydrophobic) and even morphological damage of the surface can easily happen which was observed in our lab with nickel-coated aluminum targets.

In fields of work where a defined surface is not only required by the sample preparation method but also important for validation such as in the pharmaceutical industry or forensic sciences, it is necessary to ensure the exact same conditions for every measurement performed. For avoiding external influences disposable targets are an easy way of guaranteeing standardization, *e.g.* GLP requirements. Also in areas where high risk analytes such as human-pathogenic microorganisms or toxins are analyzed routinely, it is necessary to safely dispose the analyzed samples (because very often considerable parts of the samples are not completely consumed by the measurement). In such situations conventional full metal targets carrying the unused sample residues must be subjected to rigorous cleaning, such as sterilization. Obviously, in these instances inexpensive disposable sample holders are a great benefit.

In multi-user environments it is also convenient to have access to a repository of targets in contrast to having only a few expensive targets available that need to be communally shared. This is particularly true for the high-cost metal MTP (microtiter plate) format targets.

A number of polymer-based targets made it to the market and were evaluated [1–3]. Among these were the AnchorChip [4–6]

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http://dx.doi.org/10.1016/j.ymeth.2016.03.023
1046-2023/

(Bruker Daltonics) disposable targets with pre-spotted MALDI matrix with a varying number of spots for sample and calibrant, and the Mass·Spec·Focus Chip by Qiagen that features a focusing effect especially for phosphopeptides [7] and proteins [8]. This is achieved by creating an ultrahydrophobic boundary outside of the more hydrophilic sample well. Both offer specific functionality that comes with inherent limitations. The prespotted PAC AnchorChip is only available for Bruker mass spectrometers and is not suitable for samples which require different matrices and non-standard solvents. The Mass·Spec·Focus Chip is on the other hand limited by the low binding capacity and useable solvent systems [8]. Recently, Bruker brought the MALDI Biotarget 48 to market, a disposable MALDI target specifically designed for microorganism identification on their mass spectrometers.

We compare a disposable polymer target (DPT) consisting of a polypropylene support with carbon black (to facilitate optimal conductivity) coated with a stainless steel layer in the nanometer range with a standard stainless steel full metal target (FMT). The thin metal layer is a product of a sputtering process to emulate the surface usually encountered on current commercial available metal-based targets. The morphology [9] and surface composition [10,11] of a MALDI MS target greatly affects parameters such as sensitivity [1,12] and even selectivity [13]. The use of a patterned nano-structured gold film has even been demonstrated [11] to facilitate LDI measurements. Chemistry and morphological structure of the surface have a direct effect on the crystals (shape, crystal size/form/number) formed in the MALDI MS sample preparation [14–16], adding further complexity to the already difficult task of selecting the best matrix and solvent system for a given analyte. Even when using the same material composition, the way a target surface was crafted (*e.g.* polished by hand or machine, electro-polished, brushed) also has a direct influence on its microstructure. While most metal targets are polished to guarantee a certain area planarity and hydrophobicity, this creates microscopic polishing marks. The polymer-based target employed here is surface-treated with a sputter-coating process, resulting in a very smooth and uniform surface as will be shown later. The advantage here is that any small imperfections in the polymer surface are essentially leveled out due to the sputter deposition.

2. Experimental section

2.1. Targets

Disposable polymer targets (DPT; FlexiMass-DS targets, TO-430, Shimadzu Kratos Analytical) were designed and manufactured by Sony DADC Austria. The targets have the following dimensions, 76.2 mm × 26 mm × 1.6 mm (±0.1 mm, flatness and parallelism significantly less) and consist of carbon black-filled polypropylene with a stainless steel coating on the up-facing side of about 300 nm. The format used for the targets is based on the microscope slide-sized FlexiMass MALDI targets (Shimadzu Kratos Analytical). Four times 12 sample spot areas (2.8 mm ID) were marked allowing the preparation of 48 samples (four columns with 12 rows, designated A–L on the long side and 1–4 on the short; see Fig. 1(a)) and furthermore three calibration spot areas (2.3 mm ID) located in the top, center and bottom area of the target between the two central sample spot columns (see Fig. 1(a)). The targets were produced by an injection molding process followed by DC-pulsed magnetron sputtering of stainless steel onto the DPT's sample-carrying side.

As a reference target for performance comparison a full metal target (FMT; FlexiMass-targets TO-483R00, Shimadzu Kratos Analytical) which consists completely of stainless steel and exhibits a machine-polished surface was used.

An MTP-sized target holder (Precision adapter, TO-488, Shimadzu Kratos Analytical) was used for carrying the two different kinds of target (DPT and FMT) on the sample stage in the ion source. In this target holder up to 4 targets can be mounted and introduced simultaneously into the vacuum system of the ion source. The targets are pressed from the bottom into a frame (see Fig. 1(a), left target) by means of a spring plate to obtain optimal planarity across the whole target for optimal desorption/ionization.

2.2. Chemicals

Tyrocidine solution (kindly provided by the Viennese Chamber of Pharmacy; 1 μg/mL in ACN (acetonitrile):H_2O (1:1, v/v)), angiotensin II (Sigma-Aldrich, A8846; 10 pmol/μL in H_2O), α-cyano-4-hydroxycinnamic acid (CHCA; Agilent Technologies, G2037A) matrix solution (6.2 mg/mL in methanol (MeOH):ACN:H_2O (36:56:8, v/v/v)), sinapinic acid (SA, Sigma-Aldrich, S8313) matrix solution (10 mg/mL in ACN/0.1% aqueous trifluoroacetic acid (TFA) (1:1, v/v)), 2,5-dihydroxybenzoic acid (DHB; Fluka, 85707) matrix solution (20 mg/mL in ethanol:H_2O (1:1, v/v)), Pullulan 5600 Da (Polymer Standards Service; 2.02 mg/mL in H_2O with 0.05% NaN_3), IgM from human serum (Sigma-Aldrich, I8260, reagent grade; 0.8 mg/mL in 0.05 M Tris-HCl, 0.2 M NaCl, 15 mM NaN_3, pH 8.0), and γ-globulin (Sigma-Aldrich, 49030; 2 mg/mL in 0.01% aqueous TFA) were used without further purification. The peptide calibration mix consisted of angiotensin I (Sigma-Aldrich, A3178; $[MH]^+$ m/z = 1296.69, monoisotopic value), Glu1-fibrinopeptide B (Sigma-Aldrich, F3261; $[MH]^+$ m/z = 1570.68), N-acetyl renin substrate (Sigma-Aldrich, R5380; $[MH]^+$ m/z = 1800.94), ACTH 1-17 (Sigma-Aldrich, A2407; $[MH]^+$ m/z = 2093.09) and ACTH 18-39 (Sigma-Aldrich, A0673; $[MH]^+$ m/z = 2465.20) and contained these peptides at a concentration of 100 fmol/μL in ACN: 0.1% aqueous TFA (1:1, v/v) except for ACTH 7-38 (Sigma-Aldrich, A1527; $[MH]^+$ m/z = 3657.93) which was present in the mix at 150 fmol/μL. ACN (Merck, 100003), ethanol (Merck, 100983, absolute), TFA (Riedel-de-Haën, 61030) and methanol (Merck, 106007) were of analytical grade. Water was obtained from a reverse osmosis facility and further purified with Simplicity UV (Millipore). This water quality was used in all applications.

2.3. Mass spectrometer

Positive ion MALDI and LDI mass spectra were obtained by means of a time-of-flight instrument (Axima CFR^{plus}, Shimadzu Kratos Analytical) in the linear or reflectron mode. In case of the MALDI analysis of the glycoprotein IgM in the linear mode the mass spectrometer was employed in conjunction with an ultra-high mass detector (HM1 High-Mass System, CovalX) instead of the standard detector [17,18]. The instrument was used with a nitrogen laser (337 nm) at a pulse rate of 20 Hz and at an average analyzer pressure of 1.6×10^{-7} mbar.

2.4. Determination of mass spectrometric resolution, limit of detection (LOD), limit of quantification (LOQ) and linearity

Angiotensin II solutions (from 0.2 to 2 fmol/μL) were mixed in an Eppendorf vial with an equal amount of the described CHCA matrix solution. A volume of 1 μL of this mixture was pipetted on the DPT and the FMT and allowed to dry at room temperature (RT), resulting in absolute amounts of peptide from 0.1 to 1 fmol on target (preparation area of approx. 4.5 mm^2). The data obtained for these amounts were used to determine the LOD with an appropriate S/N ratio. Positive ion MALDI mass spectra of these spots were recorded in reflectron mode (484 unselected laser shots in a 700 μm × 700 μm raster) in the m/z range of 500–3000. All ions