Food Control 65 (2016) 99–105

Contents lists available at ScienceDirect

Food Control

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

Analysis of trace methylene blue in fish muscles using ultra-sensitive surface-enhanced Raman spectroscopy

Chunying Li [a, b], Yiqun Huang [a, c], Keqiang Lai [a], Barbara A. Rasco [c], Yuxia Fan [a, *]

[a] College of Food Science and Technology, Shanghai Ocean University, No. 999 Hucheng Huan Road, LinGang New City, Shanghai, 201306, China
[b] Chifeng Institute for Drug Control, Tianyi Road, New City, Chifeng, Inner Mongolia, 024000, China
[c] School of Food Science, Washington State University, Pullman, WA, 99165, USA

ARTICLE INFO

Article history:
Received 31 July 2015
Received in revised form
4 January 2016
Accepted 12 January 2016
Available online 13 January 2016

Keywords:
Surface-enhanced Raman spectroscopy
Methylene blue
Gold nanoparticle
Rapid analysis
Quantitative detection

ABSTRACT

Surface-enhanced Raman spectroscopy (SERS) is an emerging technology with great potential for sensitive and rapid analysis of trace methylene blue (MB) in fish muscle. Five different SERS substrates including two commercial nanostructured surface substrates and three types of Au nanoparticles (NPs) with different diameters were compared to optimize enhancement effects. The highest sensitivity with the lowest detection limit of 5 ng/mL for MB standard solutions and 10 ng/g for MB in fish muscles was achieved using the 18 ± 2 nm Au NPs. Partial least squares regression models developed for quantitative analysis showed high linear correlation between the actual and predicted values for MB (*R2 cv* = 0.958, RPD = 4.84, RMSE = 6.97 ng/g) in fish muscle. Since Au NPs are easy to prepare, stable and affordable, the high sensitivity and predictability of this method has the potential to be widely used for trace analysis of prohibited drug residues in fish and potentially other foods or biological samples.

1. Introduction

Methylene blue (MB) is a heterocyclic aromatic compound containing a thiazine ring (Fig. 1), and has many applications in microbiology, medicine and diagnostic fields (Oz, Lorke, Hasan, & Petroianu, 2011). MB is an effective antifungal dyes, as effective as malachite green and crystal violet for preventing and curing saprolegniasis, red mouth disease, ichthyophthiriasis and other fish diseases (Xu et al., 2012). However, exposure to MB may cause adverse health effects in human such as vomiting, shock, and tissue necrosis (Muthuraman & Teng, 2009; Razmara, Daneshfar, & Sahrai, 2011). The use of MB in aquaculture is banned in the USA and Japan, but there is no clear regulation about the use of MB in aquaculture in many other countries such as China. Development of rapid MB detection methods for seafood products helps address consumer concern about the product safety and ensure that the products meet the regulatory requirement for global markets.

Surface-enhanced Raman spectroscopy (SERS) is an emerging technology that enables detection of trace amounts of target molecules with high sensitivity and excellent selectivity; it is based on the vibrational signatures of functional groups of the target molecule. The Raman scattering signals of target molecules can be enhanced by as much as 10^{14}–10^{15}, reaching the level of single molecule detection (Nie & Emory, 1997). Recent studies show that SERS is a powerful analytical tool in the field of food safety (Sharma, Frontiera, Henry, Ringe, & Van Duyne, 2012), particularly for the detection of chemical hazards in food. Typical studies included rapid detection of banned or restricted residual pesticides (Fan, Lai, Rasco, & Huang, 2014; Fan, Lai, Rasco, & Huang, 2015; Wang et al., 2014; Zhang, Yu, Li, Mustapha, & Lin, 2015), antibiotics such as nitrofuran (Li et al., 2015; Yu et al., 2014) and tetracycline (Filgueiras, Paschoal, Dos Santos, & Sant'Ana, 2015), illegal industrial dyes in fish (Harraz et al., 2015; Li et al., 2014; Zhang, Yu, Li et al., 2015; Zhang, Yu, Pei, et al., 2015), legal or illegal food additives (He et al., 2015; Pan et al., 2014) and other prohibited chemicals in foods (Di Anibal, Marsal, Callao, & Ruisánchez, 2012; Rajapandiyan, Tang, & Yang, 2015). The use of appropriate SERS substrates is one of the most important factors needed to achieve effective and reproducible enhancement effect so that high selectivity and sensitivity can be obtained. To date, there is no such thing as universal substrate and the selection of a SERS substrate depends upon the physical and chemical properties of the target compound and the sample matrix in which the target compound is present;

* Corresponding author.
E-mail addresses: chunyingli1984@163.com (C. Li), yqhuang@shou.edu.cn, yhuang1@wsu.edu (Y. Huang), kqlai@shou.edu.cn (K. Lai), rasco@wsu.edu (B.A. Rasco), yxfan@shou.edu.cn (Y. Fan).

http://dx.doi.org/10.1016/j.foodcont.2016.01.017
0956-7135/

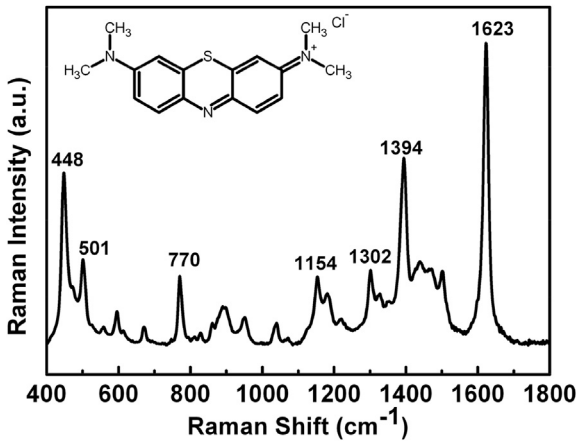

Fig. 1. Chemical structure and Raman spectrum of methylene blue.

the selection is often empirical.

The objective of this study was to investigate the potential of applying SERS to detect MB in fish muscle. Five different substrates were compared and chemometric models were established based upon SERS spectral data for quantitative analyses. The study provides important information for further development of a rapid and highly sensitive SERS method to quantitatively analyze trace levels of drug residues in complex food matrices such as seafood products.

2. Material and methods

2.1. Reagents and preparation of MB standards

Methylene blue (ACS reagent grade, ≧95%) was purchased from MP Biomedicals (Solon, OH, USA). Acetonitrile (HPLC grade) and methanol (HPLC grade) were acquired from Sigma–Aldrich (St. Louis, MO, USA). Na_3-citrate ($Na_3C_6H_5O_7 \cdot 3H_2O$, 99%) and chloroauric acid ($HAuCl_4 \cdot 3H_2O$, ≧49%) were supplied by J&K Scientific (Logan, UT, USA) for synthesizing gold nanoparticles. All other chemicals were obtained from Sinopharm Chemical Reagent Ltd. (Shanghai, China).

A series of MB acetonitrile solutions (0–200 ng/mL) was prepared and stored in dark at 4 °C for no more than one month before use.

2.2. Synthesis of gold nanoparticles and substrates

Gold nanoparticles (Au NPs) were synthesized by the reduction of $HAuCl_4$ with sodium citrate (Frens, 1973). Three different particle sizes of Au NPs were synthesized by adjusting the ratio of $HAuCl_4$ and sodium citrate used. In brief, 100 mL of 0.01% $HAuCl_4$ solution was first heated to boil with continuously stirring. Then, 1 mL of sodium citrate (0.5, 1.0 or 1.5% w/w) was added into the boiling solution, and stirred for 15 min until the solution color turned to dark wine red. Finally, the pH of the gold colloidal suspension was adjusted to 7 by adding 0.2 mol/L potassium carbonate solutions. The characterizations of Au NPs were analyzed with a transmission electron microscopy (TEM; JEM-2100F, JEOL Ltd., Tokyo, Japan). The average particle sizes of Au NPs were calculated based upon TEM images of 50 particles.

2.3. Fish sample preparation

Tilapia fillets (Zhenye Aquatic and Cool Storage Ltd., Guangdong, China) were homogenized (8010s, Waring Commercial, Torrington, CT, USA) for 5 min at high speed and stored at −18 °C. Tilapia fillets were analyzed for MB using a conventional LC-MS method (FDA, 2007) to ensure that the fillets used contained no measurable MB. Tilapia homogenates were spiked with various concentrations of MB to obtain homogenates with 10.0, 20.0, 30.0, 50.0, 100.0 or 200.0 ng/g of MB.

A preparation method based upon Andersen et al. (2009) for triphenylmethane dyes was used to extract MB from fish muscle with details described in our group's previous study for analysis of malachite green (Zhang, Yu, Li et al., 2015; Zhang, Yu, Pei, et al., 2015) and crystal violet (Li et al., 2014). A further modification was applied for MB extraction, which involved deletion of the oxidation step used for the analysis of triphenylmethane dyes.

Most of the malachite green and crystal violet were converted to their reduced forms in fish body, and the oxidation step during sample preparation was to change the reduced forms of malachite green and crystal violet back to their original oxidized forms. Although MB in fish body may be reduced to leuco-methylene blue (LMB), LMB was easily oxidized to MB once exposed in air during sample preparation, and therefore no additional oxidation step was required for MB analysis (Turnipseed et al., 1997; Borwitzky, Haefeli, & Burhenne, 2005).

2.4. SERS substrates and spectral collection

The SERS spectra were acquired using a Nicolet DXR microscopy Raman spectrometer (Thermo Fisher Scientific Inc., Waltham, MA) equipped with a CCD detector with spectral resolution of 5 cm^{-1}. A 633 nm He–Ne laser with 2 mW laser power and 20× objective lens with a slit width of 50 cm^{-1} were used. Each spectrum (300–2000 cm^{-1}) was the average of 5 scans with total acquisition time of 10 s. The substrates used included two commercial gold substrates, Q-SERS (Nanova Inc., Columbia, MO, USA) and Klarite substrates (Renishaw Diagnostics Ltd., Glasgow, U.K.), and Au NPs with three different particle sizes synthesized in our lab. The structure of two commercial substrates was different.

To use Au NPs, 10 μL of Au colloidal solution was dropped onto a clean glass slide and dried in air for about 5 min. Then, 5 μL of an MB standard solution or fish muscle extract was dispersed onto the substrate surface. For the commercial substrates, 1 μL MB standard or fish muscle extract was dropped onto the substrate surface. SERS spectra were acquired after the evaporation of solvent.

Three spectra from different locations on the surface of the substrate were collected and the average was used for data analyses. Experiments were repeated four times.

2.5. Statistical analysis

Partial least squares regression (PLSR) was used to establish quantitative relationship between spectral data and the amounts of MB in standard solutions or fish extract (Delight 3.2 Dsquared Development Inc., LaGrande, OR, USA). Leave-one-out cross-validation was applied to validate PLSR models (Martens & Martens, 1986; Martens & Naes, 1989). The predictability of the models was evaluated using coefficients of determination (R^2) between the actual values and predicted values of MB, the root mean square errors (RMSE) and the ratio of performance to deviation (RPD = Sample standard deviation/RMSE). An ideal model should have an R^2 value close to 1, and the RMSE of the model should be as small as possible (Saeys, Mouazen, & Ramon, 2005; Williams, 2004). An RPD value of 2–5 indicates that the model could be used for screening purpose, while a greater than 5 RPD indicates good predictability of the model (Huang et al., 2001; Smyth et al., 2008; Williams, 2001).