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Surface-enhanced Raman spectroscopy (SERS) in food analytics: Detection of vitamins B_2 and B_{12} in cereals

Andreea Ioana Radu [a,b], Maria Kuellmer [a], Bernd Giese [c], Uwe Huebner [b], Karina Weber [a,b], Dana Cialla-May [a,b,*], Jürgen Popp [a,b]

[a] Institute of Physical Chemistry and Abbe Center of Photonics, Helmholtzweg 4, 07745 Jena, Germany
[b] Leibniz Institute of Photonic Technology Jena, Albert-Einstein-Str. 9, 07745 Jena, Germany
[c] Food GmbH Jena Analytik – Consulting, Orlaweg 2, 07743 Jena, Germany

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ABSTRACT

Food analysis has been gaining interest throughout recent decades for different reasons: the detection of hazardous substances in food and routine investigations of food composition and vitamin/nutrient contents. Regardless of the targeted component, food analysis raises a few challenges regarding the complexity of the matrix and detecting trace amounts of substances. We report herein the results obtained regarding the simultaneous detection of two B vitamins (riboflavin, vitamin B2 and cyanocobalamin, vitamin B12) by means of SERS. SERS provides molecular fingerprint identification and high analytical sensitivity together with a low processing time and cost. All these make SERS a promising tool for the development of food analytical methods.

1. Introduction

Throughout recent decades, people have become increasingly aware of the importance of having an equilibrated diet that would provide them with the needed amounts of nutrients, vitamins, antioxidants, and so on. This led to the establishment of organizations and implementation of norms regarding the permitted and recommended values of different food constituents (i.e., the European Food Safety Authority or U. S. Food and Drug Administration databases). At the same time, the food industry and research community have provided people with the opportunity to make an informed decision when choosing from a large variety of products. Even so, there are still problems with achieving the necessary consumption of different nutrients. For example, the recommended daily intake of vitamin B12 (cyanocobalamin) is estimated to be 1.5–3 μg [1–3]. While this value is easily consumed by most of the population, strict vegetarians, elderly, pregnant woman and teenagers encounter problems assimilating B12 and are exposed to an increased risk of developing vitamin B12 deficiency [2,3] because the main dietary sources of vitamin B12 are animal derived, such as meat (especially liver), fish and shellfish, poultry and milk products [2]. Furthermore, among the possible effects of a vitamin B12 deficiency, there is a form of anemia that results from the inhibition of DNA synthesis during red blood cell production, cardiovascular diseases, depression and even paranoia and dementia [1–5]. Vitamin B12 is not the only B vitamin that should be present in an organism. Another important B vitamin that is needed for the organism to function is vitamin B2 (riboflavin). As in the previous case, the needed daily intake of B2 (1–1.4 mg) [6] is easily consumed from a variety of foods, such as milk, cheese, dark green vegetables, liver, kidneys, yeast, mushrooms and almonds [6–8]. Nevertheless, deficiencies are encountered in the cases of the elderly population, pregnant woman and alcoholics, [8] and these deficiencies may result in eye fatigue, extreme unusual sensitivity to light, a swollen dark-colored tongue, digestive problems and a form of anemia characterized by normal cell size and normal hemoglobin content [9,10]. Thus, it is important that the required intake of these vitamins (and not only these vitamins) is ensured for every individual. Moreover, it is also important to certify that the different products available in the market contain these vitamins and that people are provided with information for self-ensuring a healthy lifestyle.

Different techniques are applied for the detection of food components. Among them, high performance liquid chromatography (HPLC) coupled with different detection methods (UV/Vis spectroscopy, mass spectrometry or diode array detection) [11,12]

* Corresponding author at: Institute of Physical Chemistry and Abbe Center of Photonics, Helmholtzweg 4, 07745 Jena, Germany.
E-mail address: dana.cialla-may@uni-jena.de (D. Cialla-May).

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has been developed towards being the gold standard in the analysis of many substances (i.e., vitamin B2, vitamin A, β-carotene, etc.) [13]. Nevertheless, for other analytes, microbiological assay analysis is still the gold standard analytical tool (i.e., vitamin B12 content is estimated by a microbiological assay using *Lactobacillus delbrueckii* as the test microorganism) [14]. In addition to being reliable and reproducible, these methods also have a major drawback regarding the total analysis time to obtain the results and the simultaneous detection of analytes. Accordingly, the development of a fast and specific technique for food analytics would be beneficial. One such technique is surface-enhanced Raman spectroscopy (SERS) [15–23]. SERS provides molecular fingerprint identification and high analytical sensitivity together with a low processing time and cost. To achieve these benefits, nanostructured surfaces are used in combination with monochromatic light [16,24,25]. Upon their interaction, a strong electromagnetic field is induced on the metal surface, and the normal Raman signal of the molecules in close vicinity of the nanostructured metal surface is enhanced by several orders of magnitude. Thus, SERS combines molecular specific Raman spectroscopy with an increased sensitivity due to field enhancement by metal nanostructures.

Within this contribution, we present the first steps towards the simultaneous detection of vitamins B2 and B12 in a simulated food matrix containing a relevant combination of sugars and starch followed by the identification of the two vitamins in an extract of a fortified cereal product by means of SERS. However, it is not the scope of this contribution to quantify these two analytes in the matrix using SERS. The fortified cereal matrix was the chosen food for analysis because cereals are a part of the diet of both omnivores and vegetarians and are an equilibrated source of various B vitamins [26]. In a first approach, this study analyzes the B2 and B12 vitamins from the total six B vitamins present in the cereal (thiamin, B2, niacin, vitamin B6, folic acid and B12). To do so, a food extraction protocol was applied in order to reduce the complexity of the final analyte solution and to insure for a good transfer of the two investigated B vitamins from the complex food matrix to the analyzed extract solution. To achieve this, we applied two different food extraction protocols optimized for the extraction of either B2 or B12 vitamins out of cereal matrices. The resulting extracts were measured by both SERS and HPLC, and based on the obtained data the following experiments were conducted towards the optimization of the protocol that yields less time-consuming results to develop a procedure for analyzing cereal samples by SERS.

2. Experimental

2.1. Chemicals and reagents

All reagents were of analytical or HPLC reagent grade. Riboflavin (vitamin B2, ≥98% pure), cyanocobalamin (vitamin B12, ≥98% pure), α-amylase (from *bacillus subtilis po*, 50 units/mg activity), starch (from potato, p.a., ISO, pH. Eur.), hydrochloric acid (≥98% pure) and Taka-Diastase (from *Aspergillus oryzae*) were purchased from Sigma Aldrich (Steinheim, Germany). D(+)-sucrose (≥99.5% pure), D(+)-water-free glucose (p.a., ACS), D(-)-fructose (≥99.5% pure), methanol (≥99.9% pure) and acetic acid (100% pure) were purchased from Carl Roth (Karlsruhe, Germany). Sodium acetate trihydrate (p.a., ACS, Reag. pH. Eur.) was purchased from Merck (Darmstadt, Germany). Distilled water was produced in-house. The fortified cereals used for the study were purchased from local stores in the area of Jena, Germany.

2.2. SERS active substrates

For the development of the SERS active substrates, e-beam lithography, vacuum evaporation and ion-beam etching were used based on the protocols by Huebner et al. [27–29] A 4⬠ fused silica wafer was cleaned using peroxymonosulfuric acid solution, and then, a thin undercoating (HMDS) and a 100 nm thick negative tone electron beam resist maN2401 (Micro Resist Technology GmbH) were spun on the wafer. The resist was baked for 3 min at 90 °C on a hotplate. A 10 nm gold layer was evaporated on top of the resist. The electron beam exposure, which was performed by using the unique character projection-based electron beam technique [28] of the shaped beam writer SB350OS (from Vistec Electron Beam GmbH), resulted in the formation of 140 chips/wafer (chip size: 5×10 mm^2). Each of the obtained chips contains 4 gratings with a size of 1×1 mm^2 for the SERS investigations. The exposure and the removal of the gold layer were performed afterwards. This was followed by the development of the resist in an AZ MIF 726 developer for 30 s and H_2O rinsing for 60 s. Next, the etching into the fused silica surface was performed with a CHF_3-SF_6-ICP etching process (inductively coupled plasma, ICP) by using an ICP power of 300 W. The etch depth of the 2D gratings with a period of 250 nm is approximately 100 nm. Lastly, the residual resist was removed using oxygen plasma, and the wafer was separated into single chips.

The silver films were freshly deposited (at the beginning of every measurement day) by means of thermal evaporation at an oil-free background pressure in the lower 10^{-7} mbar range. High-purity 99.999% silver granules were used as the raw material. The thickness as well as the deposition rate was controlled in situ using a quartz microbalance. The thickness of the silver layer was 40 nm.

The scanning electron microscopy (SEM) images of the measurement fields used through the experiments were obtained using a JEOL JSM-6700F system, and the as-prepared substrate's SEM image is presented in Fig. 1. Additionally, the substrates have already been spectroscopically characterized by Schneidewind et al. [29] and Hübner et al. [28] and they present multiple resonance plasmonic modes in the MIR to visible spectral domain. Freshly prepared SERS active substrates were applied for all SERS measurements.

3. Sample preparation

3.1. Sample preparation for the pH-dependent SERS measurements

For the pH-dependent measurements, stock solutions of 160 μM B2 and 98 μM B12 were prepared and then diluted with

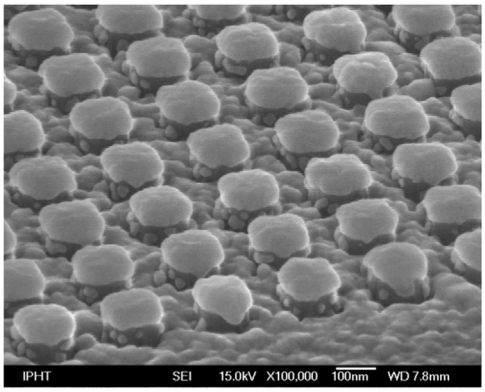

Fig. 1. SEM image of the SERS active substrate.