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Measurements of vitamin B12 in human blood serum using resonance Raman spectroscopy

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ABSTRACT

Vitamin B12 (cobalamin and its derivatives) deficiency has been identified as a potential modifiable risk factor for dementia and Alzheimer's disease. Chronic deficiency of vitamin B12 has been significantly associated with an increased risk of cognitive decline. An effective and efficient method for measuring vitamin B12 concentration in human blood would enable ongoing tracking and assessment of this potential modifiable risk factor. In this work we present an optical sensor based on resonance Raman spectroscopy for rapid measurements of vitamin B12 in human blood serum. The measurement takes less than a minute and requires minimum preparation (centrifuging) of the collected blood samples.

Keywords: Raman spectroscopy, vitamin B12, optical spectroscopy, dementia, Alzheimer's disease, cognitive decline

1. INTRODUCTION

Chronic vitamin B12 (cobalamin in its simplest form) deficiency has been identified as a potential modifiable risk factor for dementia and Alzheimer's disease[1]. It is important to have an effective, efficient and rapid method for measuring vitamin B12 concentration in human blood samples as established methods are very time-consuming and costly[2]. In recent years optical spectroscopy techniques have emerged as a potential alternative for rapid, sensitive and specific vitamin B12 measurements, with approaches such as fluorescence spectroscopy, plasmonic resonances and Raman spectroscopy[3].

Raman spectroscopy in particular is an attractive candidate technique as it uses the low-energy vibrations of chemical bonds to produce a unique optical fingerprint of a target molecule. The Raman spectrum of B12 has been shown to have distinct features[4] due to the chemical structure of cobalamin consisting of a corrin ring around a cobalt ion (shown in Fig. 1), a structure unique among biological molecules[5]. The main drawback of Raman spectroscopy is its low signal intensity, but a number of approaches have been developed to mitigate this issue, such as resonance Raman spectroscopy[6] and surface-enhanced Raman spectroscopy[7].

In this work we present an optical fiber sensor based on resonance Raman spectroscopy that can be used for rapid measurements of vitamin B12 added to human blood serum. The sensing system excites human blood serum samples at 532 nm, near the absorption electronic transition maximum of vitamin B12[8, 9], to detect the resonance-enhanced Raman signature of vitamin B12 against the background signature of diluted blood serum. Analysis of the recorded spectra allows us to quantify the amount of vitamin B12 added to human blood serum for concentrations as low as a few tens of micromolars. This work is a first step towards developing a measurement system based on Raman spectroscopy that can measure vitamin B12 in human blood serum at physiological concentration ranges (200-900 pg/ml)[10].

2. EXPERIMENTAL

2.1 Optical measurement system

A 532 nm continuous wave laser (Laser Quantum Gem) operating at a power of 80 mW is reflected off a sharp transition long-pass Raman filter (Semrock RazorEdge 532 nm Ultrasteep) and is focused onto the liquid sample placed inside a glass vial using a 4x microscope objective lens (Edmund Optics), as shown in Fig. 1.

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Raman light produced at in the sample is collected by the lens and is delivered through a long pass filter to a spectrometer (Horiba Jobin-Yvon iHR320) for spectral analysis.

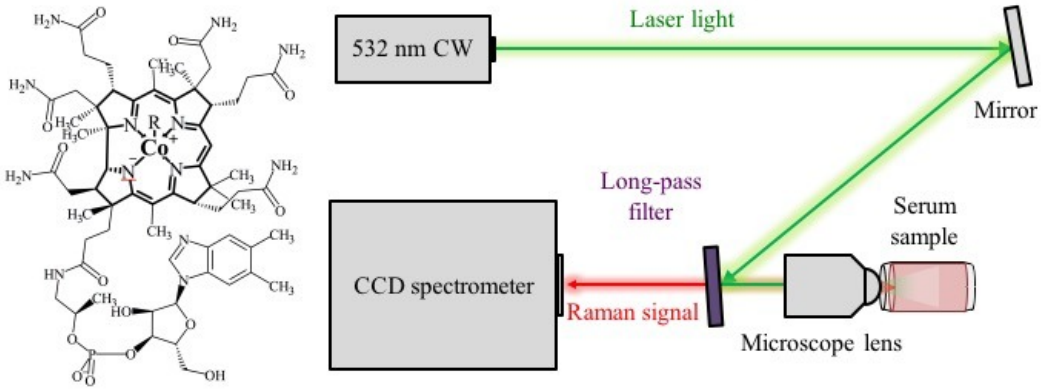

Figure 1. Chemical structure of vitamin B12 (left) and experimental setup used for resonance Raman measurements of vitamin B12 in human blood serum (right).

2.2 Sample preparation

Human blood samples were collected from a healthy young adult and were allowed to coagulate for 30 min before centrifuging to separate the serum (10,000 rpm, 15 min). The collected samples were then separated out into 200 µL plastic tubes and kept in a freezer at -23°C. At the beginning of the experimental measurements the serum was thawed and transferred to a glass vial and diluted 5 times by adding water (Millipore Milli-Q) to a total volume of 1 mL, followed by vortex mixing for 30s. Vitamin B12 in the form of cyanocobalamin (≥98%, Sigma Aldrich) was diluted in ultrapure water to a concentration of 1 mg/mL by vortex mixing for 60s. Controlled amounts of vitamin B12 solution were added to the diluted serum to produce a concentration series (20-110 µg/ml, 15-82 µM) for sensing experiments.

3. RESULTS AND DISCUSSION

The Raman spectrum of vitamin B12 in aqueous solution (100 µg/ml) is shown in Fig. 2, with the main Raman peak of vitamin B12 at 1507 cm^{-1} corresponding to the ground state vibration of the corrin ring in the cobalamin molecule[11]. This is the most prominent feature in the B12 Raman spectrum and therefore a good target for measurements in serum.

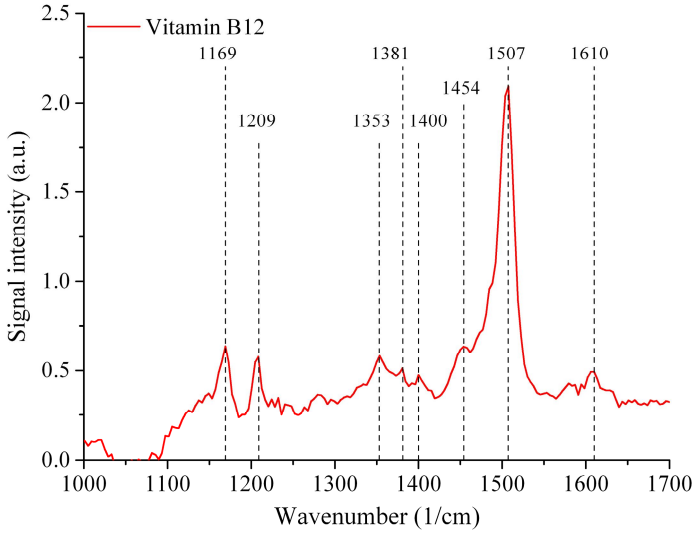

Figure 2. Background-subtracted Raman spectrum of vitamin B12 in the 1000 – 1700 cm^{-1} spectral region, showing the main vibrational peaks of vitamin B12.

The Raman spectra in the region of the main peak of vitamin B12 for diluted human serum samples with different B12 concentrations are shown in Fig. 3(a). Human blood serum in our measurements displays a peak at 1522 cm^{-1} that dominates the region of interest for vitamin B12. The spectral overlap of the two peaks poses a challenge as the signal from vitamin B12 is expected to be much weaker due to tits low concentration. A first approach towards spectral analysis is to fit a single Gaussian peak to the combined serum-B12 overlap and monitor its position as a function of vitamin B12 concentration; without B12 the peak should appear to peak at 1522 cm^{-1}, while a high concentration of vitamin B12 in the sample would show the peak shift towards 1507 cm^{-1}.

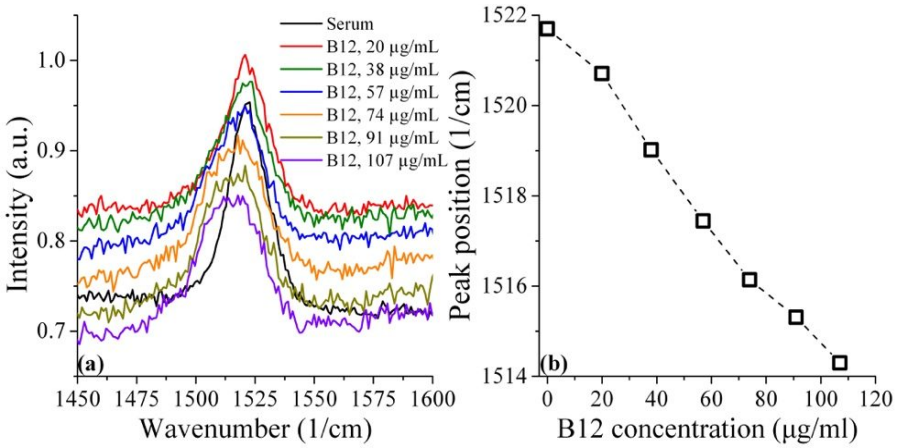

Figure 3. (a) Raman spectra from diluted human blood serum containing different concentrations of vitamin B12 in the spectral region of interest. (b) The position of the fitted Gaussian curve to the combined serum and vitamin B12 peak between 1500 and 1540 cm^{-1} as a function of vitamin B12 concentration.

Fig. 3(b) shows the position of the fitted Gaussian peak in diluted human blood serum from our measurements as a function of vitamin B12 concentration. The peak shifts monotonically to shorter wavenumbers as the vitamin B12 concentration increases, showing that it is possible to quantify the concentration of vitamin B12 in human blood serum in the region of 20-110 µg/mL (15-82 µM) based on its position.

4. CONCLUSIONS

In this work we have performed measurements of vitamin B12 in diluted human blood serum using resonance Raman spectroscopy. Our results show that it is possible to use this technique to both identify and quantify vitamin B12 in serum in the range between 20 and 110 µg/mL. Further work on enhancing the signal will enable us to investigate further lowering the limits of detection of this technique to measure physiological concentrations of vitamin B12 in human blood serum. We hope that this work will guide the development of a rapid diagnostic tool to aid diagnosis and monitoring of vitamin B12 deficiency as a powerful tool for establishing the effects chronic vitamin B12 deficiency has on the risk of dementia and Alzheimer's disease.

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