

Fluorescence detection of carbon quantum dots assessed by stratospheric platform

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Using of 3D printing technology (acrylonitrile butadiene styrene as material) stratospheric probe (SP) was developed for the purpose of this experiment. Fluorescent behavior of carbon quantum dots (CQDs) in concentration range 0-32 mg.ml⁻¹ was monitored by stratospheric probe and classical fluorescence spectroscopy. Balloon flight lasted 120 minutes. During this time it traveled 90 km. At a height of approximately 40 km there was a rupture of balloon. Thanks to a parachute SP landed in a controlled manner in the wood in the cadastral area of municipality Brusnica (Slovak Republic). It was found that the fluorescence intensity of CQDs changed slightly before and after the flight, however this was caused by the CQDs instability and not by the detector design.

Keywords: 3D printing; Carbon quantum dots (CQDs), Fluorescence; Probe; Stratosphere

1. Introduction

Measurement of molecular fluorescence is one of the most sensitive methods to detect the signal of interest. It can be realized in numerous arrangements including flow-through geometry (i. e. chromatography, capillary electrophoresis) [1,2], imaging and microscopy [3,4], however the most commonly used is the stationary fluorescence spectrometry [5]. The diversity of the detectors is based on several factors such as light source and/or geometry of the detection cell. Currently, the utilization of the light emitting diodes as a light source is a trend enabling miniaturization of the instru-

mentation [6-8]. The portable devices moreover allow the in situ analyses, which have number of advantages such as obtaining the data in real time, lowering the time of the analysis as well as its costs. Especially in cases where the detection site is difficult to reach, the remote-controlled analyzers are beneficial. Stratosphere is one of these hard-to reach places and therefore stratospheric or space research is a new challenge for these devices [9]. Nowadays, balloons are a main tool for stratospheric in situ research mostly focused on the stratosphere composition. In case that balloon is above the ozone layer, nearly the same radiation as in free space affects

the balloon. This fact is used to test durability of space materials in cheap way [10]. Ghysels et al. used optical sensor carried by balloon to quantify amount of carbonic dioxide in upper parts of troposphere and stratosphere [11]. Photometric quantification was also used to quantify vertical distribution of oxides of bromine [12]. However, to our knowledge, no fluorimetric device has been used in stratospheric conditions so far.

To monitor the behavior of the detector above the ground, carbon quantum dots (CQDs) can be employed. CQDs are new type of nanomaterial. They retain portion of bulk material properties and gain new, which arise from their nanometer diameter. They are biocompatible and possess chemical inertness and low toxicity [13]. The importance of CQDs is reflected in their electronic, mechanical, chemical and optical properties. All of these properties allow using CQDs in different fields of research such as catalysis, sensing, bioimaging, tissue engineering, optoelectronic and electronic devices [14-16]. The fluorescent labeling of DNA using nanoparticles enables DNA to be observable in vivo or in vitro experiments [17].

The aim of this work was to test the fluorescence analyzer made by 3D printer in stratospheric conditions. For testing of this stratospheric equipment 0-32 mg.ml⁻¹ CQDs were used. Testing was performed prior and after the return of stratospheric probe from the stratosphere

2. Results and Discussion

2.1 Miniaturized fluorescence analyzer

The analyzer was composed mostly from parts, fabricated using 3D printing technology, Fig. 1 A. These parts are good heat insulators due to use of fused deposition modeling technology of 3D printing. The other advantages of 3D printing are speed of fabrication, which also enables fast testing of prototypes. The final shape of the probe was cylindrical with diameter 26 cm, high 28 cm and weight 2200 g. Servomotors were used to eject the samples out from the probe. The detection part of the probe includes a light emitting diode (LED) source of radiation of wavelength 245 nm (Fig. 1 B-a), photomultiplier suitable for these measurements with optical filter, Fig. 1 B-c,d. Temperature of this part of the probe is stabilized. Heaters and temperature sensors maintain the proper temperature. The detection is based on irradiation of the sample (Fig. 1 B-e) using luminescent diode and detection of emission intensity ($\lambda = 450$ nm). The stratospheric probe contained two UV cuvettes with solution (1 ml) containing CQDs/DNA conjugate. One cuvette was ejected to outer environment regularly, where it was exposed to UV irradiation. The second reference cuvette stayed within probe. Experimental data were transferred on-line to computer in control center.

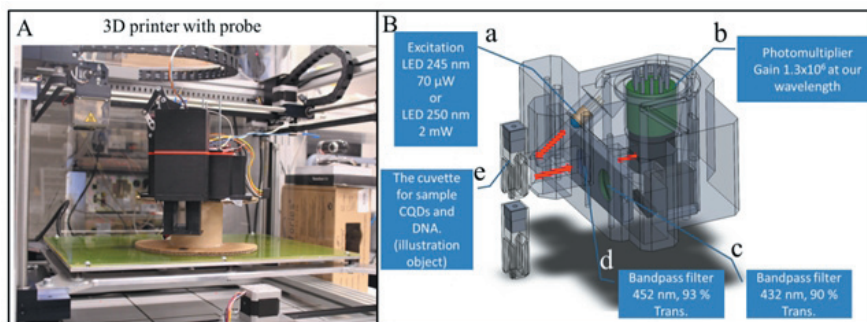


Figure 1: (A) 3D printer with detection part of probe. (B) 3D model of stratospheric probe detection part.

2.2 Flight computer - Julo-X

The board, which is connected with Julo-X (flight computer was developed by SOSA, Fig. 1 A and 1 Aa) included 7 power outputs for heating elements, 10 temperature sensors, high voltage source for powering the photomultiplier tube (PMT), LED current driver for the operating point stabilization, SD card storage for all of data and three current measurements on servo1, servo2 and PMT output. ATmega128 was employed as the micro control unit. For the temperature measurement was chosen because of easy connection to MCU. The power on servos was detected and from this data, the load of devices was derived. Julo-X recorded outside temperature, humidity, pressure, GPS position and altitude and sent them into ground PC. Before stratospheric launch was Julo-X (Fig. 1 A and 1 Aa) connected with miniaturized fluorescence analyzer, Fig. 1B. Finally, the stratospheric probe was wrapped in a thermal protection layer and attached to parachute and balloon.

2.3 Launch of stratospheric probe

The proposed detector was implemented to the platform Julo-X (flight computer) and released to stratosphere using balloon filled with helium. Prior to the launch of stratospheric probe, the last tests of communication between the probe and the control center were done, Fig. 3 A. Subsequently, the stratospheric balloon was filled with helium (Fig. 3 B) from the storage bomb. SP was launched 12. 9. 2014 at 7:30 a.m. from the airport situated in the Spišská Nová Ves (Slovak republic). Balloon flight lasted 120 minutes. During this time, it traveled 90 km. At a height of approximately 40 km there was a rupture of the balloon. Thanks to a parachute SP landed in a controlled manner in the wood in the cadastral area of municipality Brusnica (Slovak Republic). Thanks to radio and GPS module spacecraft was found, Fig. 3 C.

2.4 Verification of fluorescence functionality the stratospheric probes

Water soluble CQDs were prepared according to the general method reported by Wang et al. [18]. Fluorescent activity of 0-32 mg.ml⁻¹ CQDs was determined by stratospheric probe. The intensity of fluorescence was recorded before the flight into the stratosphere in field conditions (Fig. 4 A-a) and subsequently after completion of the flight, Fig.4 A-b. It was found that the fluorescent signal of CQDs after completion of the flight was decreased compared to that before the flight. The same analysis was also performed using fluorescence analyzer Infinite M 200 pro, Fig 4 B-a (before the flight in the laboratory conditions) and Fig. 4 B-b (after flight in laboratory conditions). The fluorescent signals of CQDs were also lower. From these results, it can be concluded that the decrease in the fluorescence signals of CQDs was not caused by the construction faults of the fluorescence analyzer, but probably by the instability of CQDs in time. In this way, it was verified that the fluorescent analyzer manufactured by 3D printer could be used in stratospheric environment.

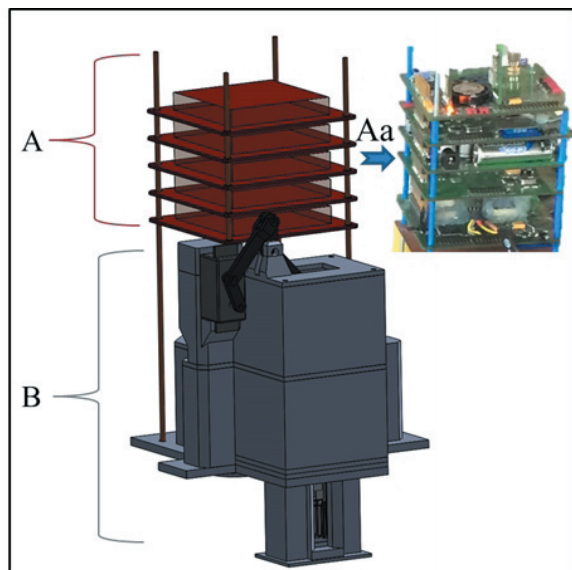


Figure 2: (A) 3D model of flight computer Julo-X and its real photo (Aa). B) Schematic illustration of miniaturized fluorescence analyzer connected with Julo-X.

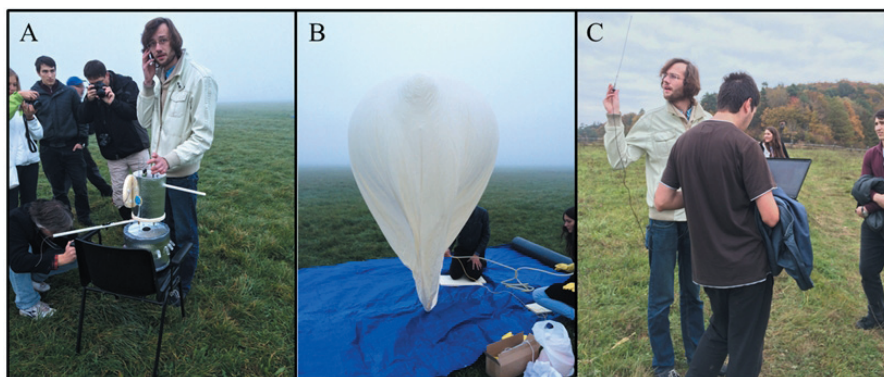


Figure 3: (A) Test of communication between the stratospheric probe and the control center. (B) Filling of latex balloon with helium. (C) Localization of the stratospheric probe by radio signal.

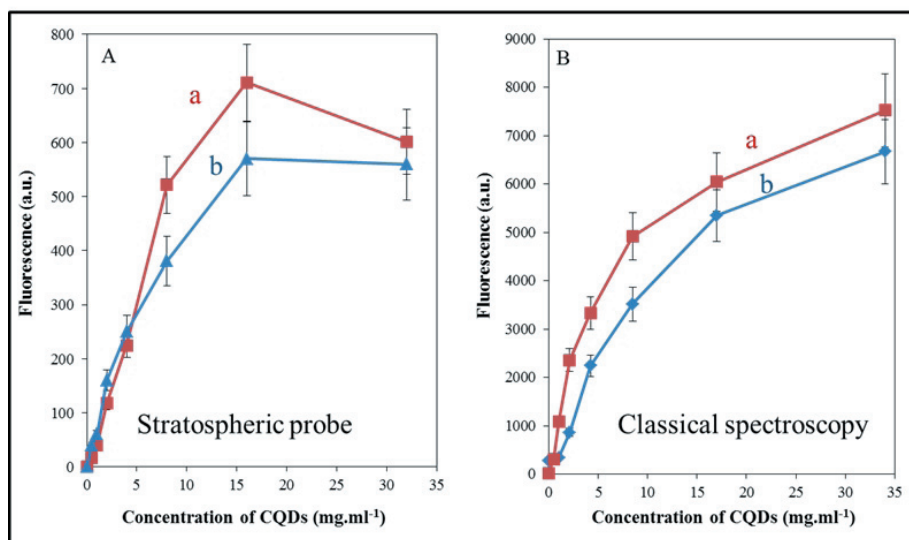


Figure 4: (A) Fluorescent activity of 0-32 mg.ml⁻¹ CQDs determined by stratospheric probe a) before launch, b) after landing probe. (B) Fluorescent activity of 0-32 mg.ml⁻¹ CQDs determined by fluorescence analyzer a) before launch in the laboratory, b) after landing probe in the laboratory.

3. Experimental Section

3.1 Chemicals and materials

All the reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA) in ACS purity, unless noted otherwise. Deionized water underwent demineralization by reverse osmosis using Aqua Osmotic O2 (Aqua Osmotic, Tisnov, Czech Republic) and was subsequently purified using Millipore RG (MiliQ water, 18 M Ω , Millipore Corp., Billerica, Massachusetts, USA).

3.2 Synthesis and characterization of CQDs

The preparation of water soluble CQDs was carried out following the protocol of Wang et al [19]. The fluorescence of resulting CQDs was measured using multifunctional microplate reader Tecan Infinite 200 PRO (Tecan group Ltd. Männedorf, Switzerland) [20]. The average particle size and size distribution were determined by quasielastic laser light scattering with a Malvern Zetasizer (NANO-ZS, Malvern Instruments Ltd., Worcestershire, U.K.). Nanoparticles/distilled water solution (1 mg.mL⁻¹) was put into a polystyrene latex cell and measured at a detector angle of 173°, a wavelength of 633 nm, a refractive index of 0.30, a real refractive index of 1.59, and a temperature of 25 °C.

3.3 Construction of miniaturized fluorescence analyzer by 3D printer

Rest of the probe was crafted on Profi 3D maker (3DFactories, Straznice, Czech Republic) by using acrylonitrile butadiene styrene. All parts of the device were changeable. The advantage in 3D printing technology is the short time of production and designing the model in CAD software Solidworks (Dassault Systèmes SolidWorks Corp., a subsidiary of Dassault Systèmes, S. A. (Vélizy, France) where the components were tested for noncollision in final assembly.

3.4 fluorescence spectroscopy

Fluorescence spectra were acquired by multifunctional microplate reader Tecan Infinite 200 PRO (TECAN, Männedorf, Switzerland). Briefly, 50 μ L of sample CQDs was placed in a transparent 96 well microplate with flat bottom

by Nunc (Thermo Scientific, Waltham, USA). 245 nm was used as an excitation wavelength and the fluorescence scan was measured with the range from 585 to 800 nm per 2-nm steps. Each intensity value was an average of three measurements. The detector gain was set to 100.

4. Conclusions

The developed concept was used to construct of fluorescence detector, which was carried to stratosphere by balloon. The detector was also tested after return to ground. The successful functioning of the SP under stratospheric conditions was verified and the applicability of 3D printing technology for the stratospheric detection devices was confirmed

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Conflicts of Interest

The authors have declared no conflict of interest.

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