A MICRO-FLUIDIC ULTRA-VIOLET EMISSION SOURCE FOR DIRECT FLUORESCENCE OF TRYPTOPHAN

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Abstract— **This paper describes a fluidic microchip for observing the direct fluorescence of tryptophan and other amino acids, which are used in studying protein structure and dynamics. Since the excitation wavelengths of these are in the 250-290 nm ultra-violet range, it is a major challenge to find appropriate light sources that can be integrated onto micro-total analysis systems. This effort demonstrates that illumination from a micro-discharge can be used to observe the direct fluorescence of tryptophan. The discharge is ignited across an air gap between an on-chip metal anode and a liquid cathode made of a saturated solution of lead nitrate. Atomic transitions in Pb atoms that are consequently sputtered into the discharge provide the wavelengths necessary to excite amino acids. Unwanted wavelengths are rejected by an optical filter that separates the micro-discharge from the tryptophan sample. Measured results include the unfiltered and filtered spectral output of the micro-discharge, as well as that of the resulting tryptophan emission, which has the characteristic broad peak from 300-450 nm.**

Keywords— **Tryptophan, fluorescence, microfluidics, micro-total analysis systems, micro-discharge**

I. INTRODUCTION

Fluorescence detection is a widely used technique for medical diagnostics and biochemical analysis. The molecules of interest fluoresce at characteristic emission wavelengths when they are illuminated at characteristic excitation wavelengths, which are shorter (and hence more energetic).

In one diagnostic approach, a fluorescent dye is used to chemically label the quantity of interest. For DNA detection, dyes which intercalate into the double-helix provide very high sensitivity and make it possible to detect attomoles of DNA base-pairs [1]. A contributing factor to high sensitivity is quantum efficiency, which is the ratio of the number of photons emitted to those absorbed in the excitation wavelengths. For example, SYBR Green I gel stain is a cyanine dye that has a quantum efficiency of 0.8 [2]. When bound to dsDNA it is most efficiently excited by radiation over 491-503 nm, and has a broad emission spectrum over 510-600 nm, with a peak at 522 nm.

While dyes offer many attractive features, their use is not always favored or even possible. For example, proteins can be fluorescent even without the presence of a dye, and changes in this intrinsic or direct fluorescence can be indicative of structural transformations [3,4]. The intrinsic fluorescence of proteins and peptides is due to the presence of tryptophan, tyrosine or phenylalanine, which are amino acids. In contrast to the excitation and emission wavelengths for the SYBR green dye, which are in the visible portion of the spectrum, these three have absorption peaks over 250-290 nm and emission peaks over 280-350 nm, all in the deep ultra-violet (UV) region. The characteristics for tryptophan are shown in Fig. 1 [5,6]. (Note that the wavelength at which this fluorescence peaks is highly sensitive to the microenvironment, and hence it is widely used for studying protein structure and dynamics.) It is noteworthy that the quantum efficiencies of these amino acids are relatively low. For example, tryptophan, which tends to dominate in fluorescence over the other two, has a quantum efficiency of only 0.19 when dissolved in water as a free amino acid [3]. These characteristics can make it relatively challenging to observe direct fluorescence.

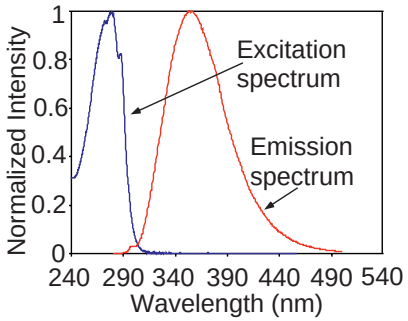

Fig. 1: Typical excitation and emission spectra of tryptophan show that the UV source must operate over 250-290 nm wavelengths, whereas the fluorescence peaks over 330-390 nm [5,6].

In a typical fluorescence imaging system, the radiation source is often broad-band, so a low-pass filter is located between the source and the sample to reduce its illumination by the longer wavelengths. In addition, a high-pass filter located between the sample and the detector so as to restrict the measured signal to the fluorescence wavelengths and minimize the radiation from the source that might inadvertently leak through.

In recent years significant research has been devoted to miniaturization of biochemical instrumentation, leading to micro-total analysis systems (also referred to as "lab-on-a-chip"). With respect to fluorescence detectors, the efforts have focused on solid-state sources such as light-emitting diodes (LEDs) and lasers (VCSELs) [7-10]. However, making these sources for deep UV wavelengths and integrating them with microfluidic systems is a major challenge.

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This effort demonstrates an alternative approach which not only provides a source of illumination in the 250-290 nm wavelengths, but can be easily integrated with micro-total analysis systems because it is a microfluidic device.

II. DEVICE STRUCTURE AND CALIBRATION

The device structure is illustrated in Fig. 2. An electrical micro-discharge is ignited across an air gap between a metal anode and a liquid reservoir containing a saturated solution of $Pb(NO_3)_2$ which serves as the cathode. The micro-discharge sputters the solution from the cathode into its glow region. Atomic transitions in Pb atoms provide the wavelengths necessary to excite amino acids. Unwanted wavelengths are rejected by an optical filter that separates the micro-discharge from the tryptophan sample. The tryptophan is located in a reservoir or channel as part of a micro-total analysis system. The overall footprint of the device is approximately 1 cm x 1 cm, with the reservoirs covering half the area. The assembly is held horizontally during operation. The filtered emission from the discharge pulse follows optical path I (Fig. 2), and stimulates fluorescence in the sample reservoir, which is detected along optical path II. These paths are orthogonal to minimize the signal feed-through from the arc to the detector, which is an Ocean Optics USB 2000™ spectrometer that connects to the USB port of a computer.

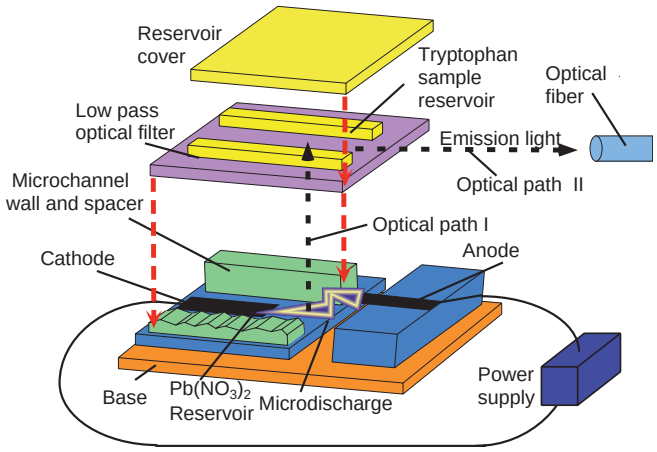

Fig. 2: An exploded schematic of the device showing various components. The tryptophan sample reservoir can be replaced by a micro-total analysis chip.

The overall architecture of this device is based on a fluidic microchip that we have reported for detecting inorganic contamination in water samples [11], and on another device (using $BaCl_2$) for optical fluorescence of bio-chemicals in the visible regime [12]. However, the necessity of working with UV wavelengths adds many unique challenges. It is necessary not only to find an appropriate liquid source that is easily available for widespread usage, but also develop means to accommodate the relatively weak emission strengths in the 250-290 nm range, and the relatively poor quantum efficiencies of the amino acids. The emission reservoir must be sealed from the amino acid because Pb^{2+} and a number of other metal ions are known to quench fluorescence in tryptophan [4]. Additional constraints are placed on the structural materials with respect to the transmission of these wavelengths.

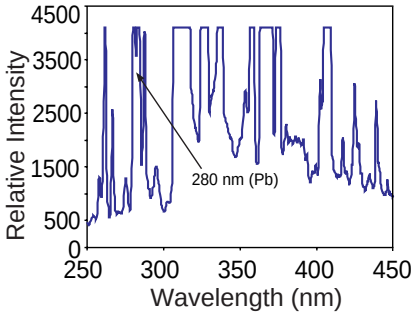

Fig. 3: Measured spectrum from lead nitrate with concentration of 5g/10ml water (saturated solution), showing substantial emission intensity in the 250-290 nm wavelengths.

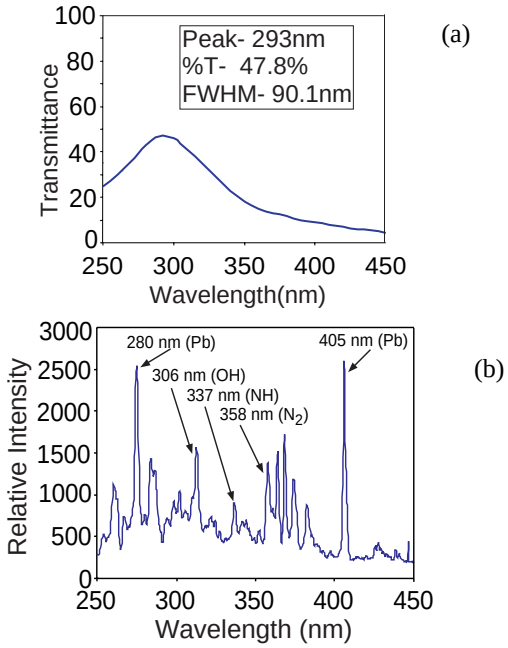

Fig. 4: (a) Transmission curve of the filter separating the UV source from the tryptophan sample. (b) Measured spectrum of the filtered emission, showing increased prominence of 250-290 nm wavelengths as compared to the unfiltered emission in Fig. 3.

The spectral emission obtained by a micro-discharge to the saturated $Pb(NO_3)_2$ solution is shown in Fig. 3. There is a substantial peak near 280 nm, which is characteristic of Pb, and closely matches the excitation peak for tryptophan as shown in Fig. 1. However, there are also a number of other peaks with high relative strength, which should be suppressed to reduce the likelihood of signal feed-through to the detector. As shown in Fig. 2, this is done by using a filter to separate the discharge source from the amino acid. In the present version of the device a UV-grade filter with a fused silica substrate (#300-W-1D from Acton Research

Corp, Acton, MA) is used. It has peak transmission wavelength of 293.0 nm, and its FWHM is 90.1 nm, with a peak transmittance of 47.8%. The transmission characteristics of the filter and the resulting spectrum that is used to excite the tryptophan are shown in Fig. 4. The undesired wavelengths from the lead nitrate are substantially attenuated. The remaining large peaks (such as those near 368 nm and 405 nm) can be cancelled by a simple calibration in which the spectrum of a control sample of water is subtracted from the spectrum of the tryptophan dissolved in water, as will be demonstrated in the next section.

III. EXPERIMENTAL RESULTS

A number of measurements were performed using commercially available tryptophan, which was dissolved in DI water at room temperature to prepare a saturated solution [13]. In all the measurements presented, the background radiation in the room was measured immediately before each experiment and subtracted out of the results.

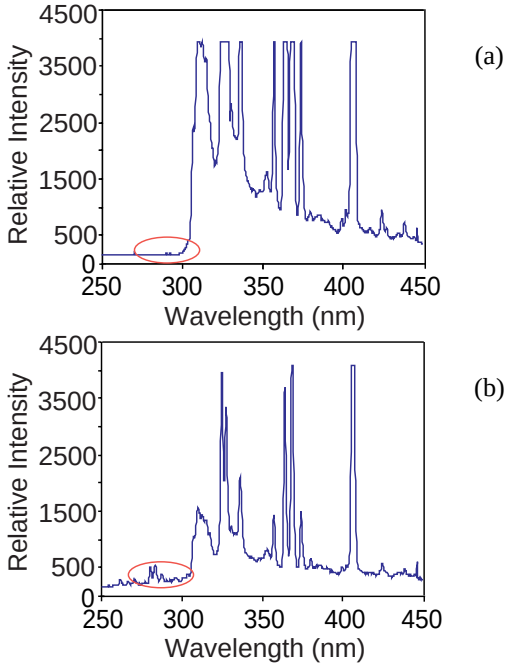

Fig. 5: (a) Light transmitted through tryptophan shows that the peaks near 280 nm have been absorbed; (b) These wavelengths are transmitted through the control sample of water.

The absorption of the UV light was studied by placing the optical fiber on the same axis as the incident light source (i.e. along optical path I). Figure 5(a) shows the light spectrum after it passes through a 2.5 mm deep sample of tryptophan solution. Comparing with the excitation spectrum, we can see that tryptophan absorbs the lines in the 280nm range. To verify this result, a control experiment was done by placing water instead of tryptophan in the sample chamber. Figure 5(b) shows that the 280 nm lines pass through the water and can be clearly seen in the transmitted spectrum. This confirms that the tryptophan sample absorbs them. Also, the similarity of the remainder of these two spectra suggests that other attenuation is due to water.

The fluorescence spectrum was observed by placing the fiber along optical path II, transverse to the excitation path to remove the interfering background radiation. Figure 6 shows the observed output from a tryptophan sample, with evidence of the characteristic broad peak between 300 nm and 450 nm. A control experiment with water in place of tryptophan provides the spectrum shown in Fig. 7, with no evidence of the characteristic broad peak of tryptophan. The peaks near 368 and 405 nm are from spikes of high relative intensity in the lead nitrate spectrum which are not sufficiently attenuated by the filter and are coupled from optical path I to II. The curves in Figures 6 and 7 were both obtained by integrating the spectrum for 10 sec. and further averaging along the wavelength axis over ±15 points from each data point, using a triangular weighting.

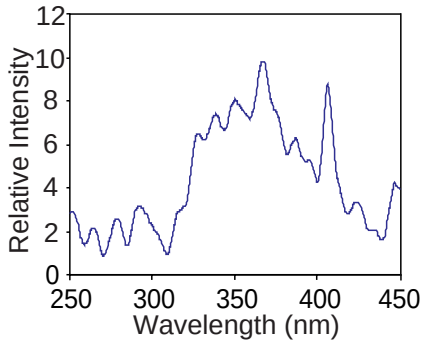

Fig. 6: Optical emission measured along optical path II (as shown in Fig. 2) indicating the existence of the direct fluorescence of tryptophan by the broad peak between 325 nm and 425 nm.

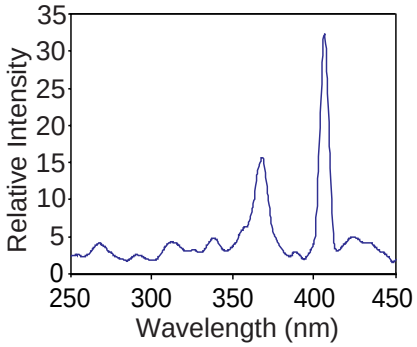

Fig. 7: Spectrum measured with a DI water control sample in a manner analogous to Fig. 6, shows the absence of the broad peak between 325 nm and 425 nm.

A simple calibration method in which the spectrum of the water sample is subtracted from that obtained with the tryptophan sample can be used to make the fluorescence emission clearer. However, before doing this, the data in Figs. 6 and 7 are scaled so that the base-to-peak amplitude at 405 nm are of equal magnitude. It is noteworthy that this

calibration at 405 nm also removes the spurious peak at 368 nm.

Figure 8 shows the observed tryptophan spectrum after the signal processing, superimposed with the reference tryptophan spectrum from [6]. The characteristic broad peak of tryptophan fluorescence is clear. This establishes that the microdischarge UV source can be used to excite the UV fluorescence.

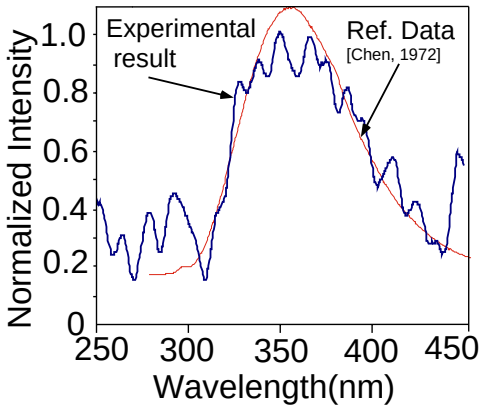

Fig. 8: The net output from the tryptophan sample, obtained by subtracting the curve of Fig. 7 from that of Fig. 6. The reference emission curve of tryptophan [6] has been superimposed.

IV. CONCLUSION

It has been experimentally demonstrated that spectral emission from electrical micro-discharges can be used to fluoresce amino acids with excitation wavelengths in the deep UV range. The discharges are generated in air, between a metal anode and a liquid cathode, both of which are on-chip. The characteristic fluorescence of tryptophan in a solution of water can be clearly observed using a lead nitrate solution as the source of 280 nm radiation used for the optical excitation of the sample. Since the quantum efficiency of tryptophan fluorescence is about three-fold to four-fold higher in dimethyl sulfoxide (either as free amino acid or when incorporated into a polypeptide chain), it is expected that this method can be used in a variety of tests. Additionally, since the emission stems from characteristic spectra of ions in the liquid cathode, it can be tuned with various species and replenished as needed using a microfluidic system that is disparate from micro-total analysis system that it is serving. Deep UV radiation which is difficult to generate from any other miniature source, can be easily generated using simple chemicals in this manner. The microfluidic nature of the emission source also makes it easier to integrate with the micro-total analysis system, and tightly couple the optical emission.

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