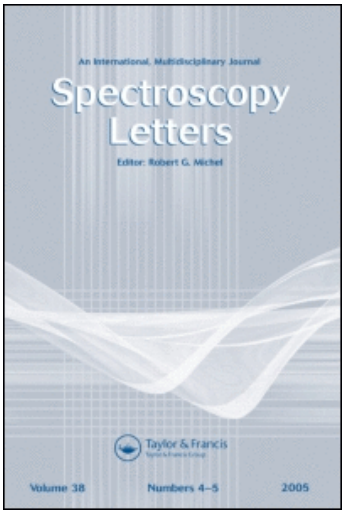

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Fluorometric Characteristics of a Wave-Guide Cell with Low Refractive Index

Kitao Fujiwara[a]; Tadahiro Kagoshima[a]; Tatsuya Uchida[a]; Takeshi Miyakawa[a]
[a] School of Life Science, Tokyo University of Pharmacy and Life Science, Hachouji, Japan

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Fluorometric Characteristics of a Wave-Guide Cell with Low Refractive Index

Kitao Fujiwara,* Tadahiro Kagoshima, Tatsuya Uchida, and Takeshi Miyakawa

School of Life Science, Tokyo University of Pharmacy and Life Science, Horinouchi, Hachouji, Japan

ABSTRACT

Recently, Teflon AF2400 (AF 1600, and AF 1601) was commercialized (DuPont Fluoroproducts, Wilmington, DE, U.S.A), that has a refractive index (1.29) lower than water (1.33), and which means that the wave-guiding of light is possible in water. In this study, we used Teflon AF 2400 as a waveguide capillary longpath cell for fluorometry. He-Cd and Ar^+ lasers were used as the excitation source, at 325 nm and 514.5 nm respectively. The length of the capillary wave-guide cell was 18.70 cm. The was wound twice on a flat surface (loop diameters: 2 cm and 3 cm). The excitation was executed through the wave-guide cell and the fluorescence from the wound capillary cell wall was collected in a perpendicular direction to the loop. With excitation at 325 nm, the fluorescence intensity at 450 nm emitted from the cell wall decreased

*Correspondence: Kitao Fujiwara, School of Life Science, Tokyo University of Pharmacy and Life Science 1432-1, Horinouchi, Hachouji, 192-0392, Japan; E-mail: kfujiwa@ls.toyaku.ac.

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along with the increase in the refractive index of the solvent. This can be caused by attenuation of the source light due to absorption by the solvent. In our experiment, the solvent of higher refractive index has the higher absorption at 325 nm. On the other hand, the fluorescence intensity at 590 nm, with excitation at 514.5 nm, increases with increased refractive index of the solvent. This result shows that an increase in the refractive index of the solvent is preferable for maintaining the wave-guiding of the source light. Here, the characteristics of fluorescence spectrometry are discussed in terms of the collection of fluorescence from the wave-guide capillary cell wall.

Key Words: Fluorometry; Water-core wave guide; Teflon capillary of low refractive index; Solvents.

Liquid core wave-guide long-path cells (LCW) are convenient for increasing the detection power for absorption or fluorescence spectrometry, although these systems have only limited application to solvents with a high refractive index,[1–3] because an aqueous solution can not be used as the core of a wave-guide. Recently, Waterbury et al. used a Teflon AF 2400 ($n_D = 1.29$) capillary in long path absorption spectrometry, in which the refractive index of the Teflon is lower than water ($n_D = 1.333$), and proved that total reflection is possible[4] in the wave-guide with a water core. They used Teflon AF 2400 as the absorption cell for the determination of several chemical species.[5,6] A LCW cell was also utilized as an absorption detector for HPLC.[7] Dasgupta et al. found its application is successful even for chemiluminescence.[8] They also utilized the high gas-permeability of this Teflon to detect several gases (H_2S, chlorine, NO_2) and acetone dissolved in water.[9] Absorption spectrometry of a water-core wave-guide was also applied to the determination of seawater color; colored dissolved organic matter.[10–12] The nano-molar detection of nitrate, nitrite[5] and phosphate[13] was also shown to be possible using a water-cored wave guide. Several papers were published on Raman spectrometry using the water-cored wave-guide.[14–19]

In the case of fluorescence spectrometry, Dasgupta et al. postulated the advantages of transverse excitation against the water-core, and detection of fluorescence through the water solution[9,20] in an LCW cell. Byrne et al. used this configuration for a compact spectrophotometer.[21] Although this configuration can exclude the attenuation of the excitation source, the self-absorption of a fluorescent analyte can not be excluded.[22,23] Here, we again tried the excitation of analyte through the liquid core (including the water) of

an LCW cell made from a capillary of Teflon AF 2400, and fluorescence was detected from the vertical side of the wave-guide cell. One of the merits of this configuration is that it can avoid the solvent absorption of the fluorescence. In our previous papers,[23,24] the solvent absorption was not considered in detail, therefore it was examined experimentally and theoretically in this paper.

EXPERIMENTAL

Reagents

The reagents 9-Anthracenecarboxylic acid (Wako Chem. Co.) and Rhodamine 640 (Exciton Inc.) were used as fluorescent dyes for 325 and 514.5 nm excitation, respectively. These dyes were dissolved in the solvents listed in Table 1. These are of analytical grade, and used without further purification.

Instruments

Figure 1 shows a schematic representation of the equipment used in this experiment. The light source used was a He-Cd laser (Kinmon Electric Co., Ltd., model, IK3251 R-F:325 nm) or an Ar^+ laser (Inn Laser Technology, model 5490ASL -00:514.5 nm). The analyte solution was sent to the LCW cell by a plunger pump (Sanuki Industry Co., Ltd., model AM3U-9069). Teflon AF 2400 is a product of DuPont Fluoroproducts, Wilmington, DE, U.S.A. The LCW cell length was 18.70 cm, in which the

Table 1. Solvents used in this experiment.

Solvent	Refractive index (n_D)
Water	1.333
Ethanol	1.361
1-butanol	1.399
Carbon tetrachloride	1.463
Toluene	1.496
Chlorobenzene	1.524
O-xylene	1.565

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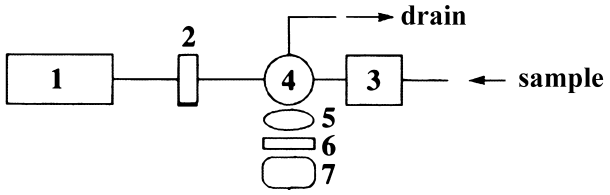

Figure 1. Schematic representation of the present experimental system. 1: light source (He-Cd or Ar^+ laser), 2: chopper, 3: pump, 4: LCW cell, 5: convex quartz lens, 6: filter, 7: photomultiplier.

Teflon AF 2400 capillary was wound twice on a flat surface. The loop diameters were 2 cm and 3 cm). The fluorescence from the entire view of the LCW coils was focused using a convex lens (f = 5 cm, diameter of lens = 5 cm) on a photomultiplier (Hamamatsu Photonics Co., Ltd., model R446, window:side-on type) through a combination of low pass and band-pass filters. Therefore, the face of the LCW coils was perpendicular to the window of the photomultiplier. Fluorescence from part of the LCW coil surface was collected. The design of the LCW cell, the inlet and outlet of the sample solution, and inlet of the light source are shown in Figure 2. The excitation light is chopped by a chopper (NF Electric Instruments Co., Ltd., model 5584), which was connected to a lock-in amplifier (NF Electric Instruments Co., Ltd., model 5516 B). The output signal of the photomultiplier was connected to the lock-in amplifier through a pre-amplifier (NF Electric Instruments Co., Ltd., model LI-76).

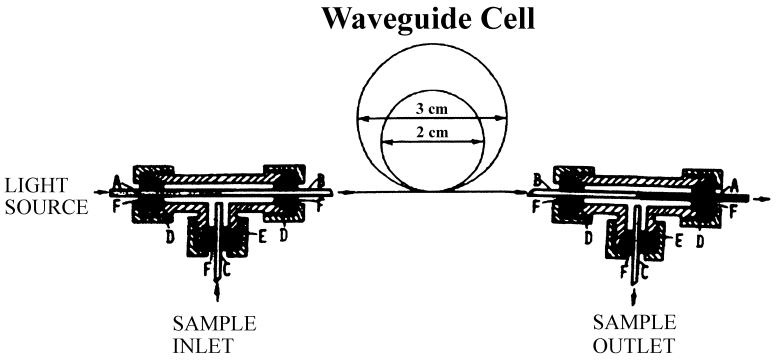

Figure 2. LCW cell design. A: quartz rod (1 mm diameter), B: LCW (Teflon AF 2400: i.d. = 0.6614 mm, o.d. = 0.894 mm), C: Teflon tube, D: graphite packing, E: stainless T tube, F: Teflon ring.

Procedure

For the 325 nm excitation, 9-anthracenecarboxylic acid was dissolved in ethanol (0.89 mM) and diluted in the solvents listed in Table 1. The fluorescence intensity of each solution was adjusted to equal that in a fluorometer (JASCO, model FP-777: excitation = 325 nm and emission = 450 nm) where 1 cm length cell was used. The concentration of the dye was as follows: water(17.8 .M), ethanol(7.12 .M), 1-butanol(7.57 .M), carbon tetrachloride(1.62 .M), toluene(1.78 .M), chlorobenzene (7.57 .M), and o-xylene (1.78 .M). In the case of the 514.5 nm excitation, Rhodanine 640 was dissolved in ethanol (21.9 .M), and diluted in each solvent as listed in Table 1 at 1% (0.219 nm). Therefore, the concentration of Rhodamine 640 in each solvent was 0.219 .M. The fluorescence intensity of each solution was adjusted to equal that by the fluorometer (excitation = 514.5 nm and emission = 590 nm). The flow rate of the dye solution was 0.5 mL/min.

RESULTS AND DISCUSSION

Figure 3 shows the dependence of the fluorescence intensity from the LCW cell on the refractive index of the solvent in the case of 325 nm excitation (He-Cd laser). Each dot is an average of five replicates. The standard deviation of the five measurements were less than 1%. As can be seen in the figure, the highest fluorescence intensity was obtained in the ethanol solution, and the fluorescence intensity decreased with an increase

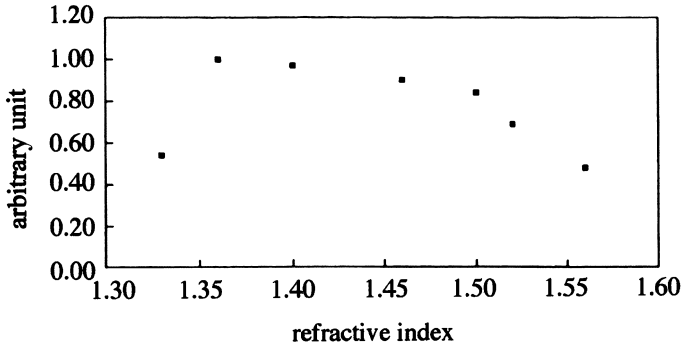

Figure 3. Dependence of the fluorescence intensity from the LCW on the refractive index of the solvent. (325 nm excitation). The decrease in the fluorescence intensity found in the solvents of higher refractive indexes is due to the absorption of solvent at 325 nm. (See the text.)

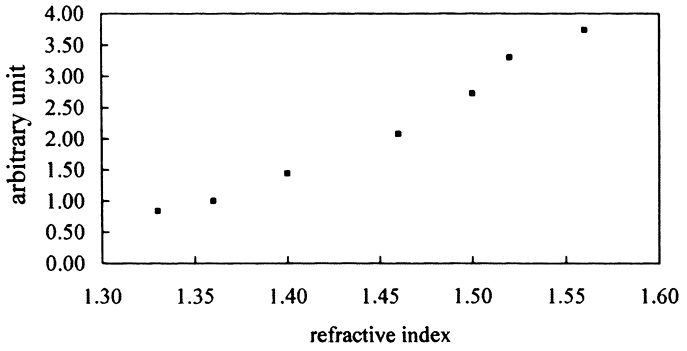

Figure 4. The dependence of the fluorescence intensity from the LCW on the refractive index of the solvent (514.5 nm excitation).

in the refractive index of solvent higher than ethanol. This result can be explained by the attenuation of the excitation light source through the LCW cell : 1 cm-absorbances of the excitation light at 325 nm are 0.01 (ethanol), 0.01 (1-butanol), 0.01 (carbon tetrachloride), 0.03 (toluene), 0.04 (chlorobenzene), and 0.05 (o-xylene). In this measurement, as ordinary double beam absorption spectrophotometer (JASCO, model V-570) was used, where water was taken as the reference.

On the other hand, the solvents listed in Table 1 show an absorbance (1 cm cell) of less than 0.01 at 514.5 nm. As can be seen in Figure 4, the fluorescence intensity from the LCW cell becomes stronger along with the increase in the refractive index of the solvent at 514.5 nm excitation The apparent difference between water and ethanol is smaller in Figure 4 than Figure 3. This is due to the relative scale of the ordinate. This fact shows that the fluorescence intensity from the LCW cell increases under the condition that the attenuation of the excitation source is negligible. Also, it is evident that the solvent effect on the fluorescence quantum yield of the dye is not dominant in the present case, because the fluorescence intensity in each solvent only depends on the absorption of solvent at the source light wavelength, but does not depend on either the polarity or the solubility of the solvent. The results obtained can be explained by the probability of the excitation light source passing through the LCW capillary. This situation can be expressed by the following expression:

$$I = kI_0\left[1 - \int_0^L e^{-1\{\varepsilon+q(n)\}}\varepsilon \mathrm{dl} - \int_0^L e^{-1\{\varepsilon+q(n)\}}q(n)\mathrm{dl}\right]$$
$$= kI_0e^{-L\{\varepsilon+q(n)\}}$$

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In this expression, I = fluorescence intensity observed from the LCW cell; k = the constant including the solid angle of luminescence collected by the photomultiplier, instrumental efficiency, and so on; I_0 = intensity of source light; XXX = absorbance of the solvent; q(n) = possibility of source light arrival at dl (a certain portion of the LCW cell); L = length of the LCW cell; and q(n) = probability of light dispersion inside the LCW cell. In the middle expression, the second integral term shows the absorption of solvent, and the third integral term shows the dispersion probability of the source light. These three terms in parentheses, [ ], can be calculated to the right side of the above expression. When the absorption of solvent is negligible (XXX = 0), the third terms can be described as:

$$P(n) = e^{-q(n)L} = \frac{2}{\pi}\tan^{-1}\left[\alpha\left(\frac{n-n_t}{n_t}\right)\right]$$

In this equation, the coefficient, $kI_{a>}$ is excluded. The above equation means the probability (P) of a light source photon reaching the end of the LCW capillary (length = L) under the condition that the absorption of the solvent is negligible. This probability is a function of the refractive indices of the solvent (n) and capillary (n_t), therefore, P can be written as P(n). As

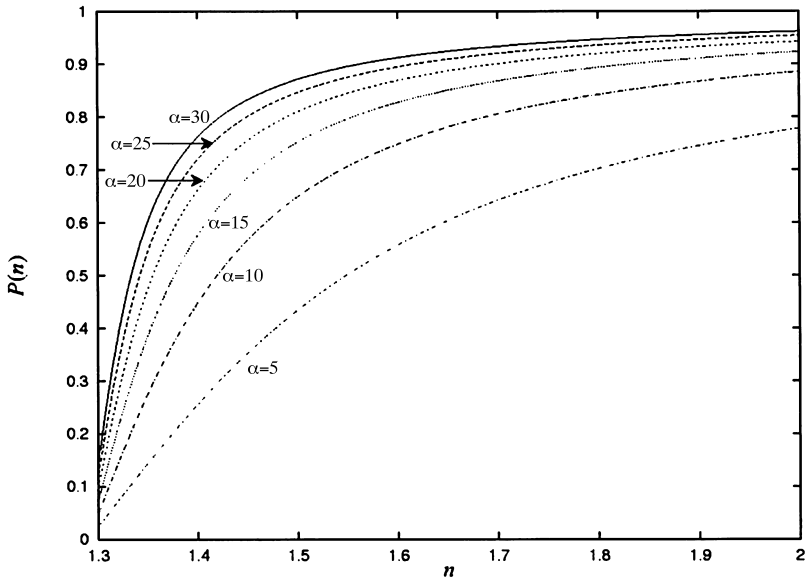

Figure 5. The probability P(n) of a source light photon reaching the end of the LCW capillary as a function of the refractive index of the applied solvent (n).

shown in Figure 4, this probability increases with the increasing difference between the refractive indices of the solvent and the capillary ($n - n_t$). It is noted that the above equation is valid when n is equal to or larger than n_t. In this case, P(n) is displayed in Figure 5, and n_t is 1.29 for the refractive index of Teflon AF 2400, and XXX in the equation includes the factor of L. The above expression is shown in the probability theory, but can be applicable for explaining the present results (Figures 3 and 4).

As a concluding remark, the fluorescence characteristics of the LCW cell using Teflon AF 2400 can be mathematically treated under the condition that the excitation is executed through the LCW capillary and fluorescence was taken from the perpendicular side of the LCW cell. For the 514.5 nm excitation, about a 10 times enhancement in the detection power compared with that of the commercial fluorometers can be obtained, even in the aqueous solution, by the present method using the optical configuration shown in Figures 1 and 2.

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