An efficient fiber fluorescence probe for the detection of trace elements*

HUA Li (华莉)[1]**，**GAO Hong-yun** (郜洪云)[1]，**LI Min** (黎敏)[1], **and SUN En-jie** (孙恩杰)[2]
1. Department of Physics, Wuhan University of Technology, Wuhan 430070, China
2. Department of Chemistry, Wuhan University of Technology, Wuhan 430070, China

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A high-efficiency capillary-fiber optic probe is developed for measuring the concentration of trace elements. The optimal probe consists of an excitation fiber (incident fiber) and a ring of collection fibers which is made up of 6 fibers. Both simulation and experiment results show that the structure gives the higher coupling efficiency with a reasonable capillary diameter. The coupling efficiency of the probe is determined by the number and arrangement of the fibers, internal diameter and length of the fiber optic sensing probe, and the end reflectivity of the capillary. The concentration of the carbonic anhydrase solution and dezincification reagents also affect the efficiency. A fluorescence efficiency of 2.4% is obtained in zinc detection experiment.
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Various optical fiber fluorescence probes for trace elements detection technology have been reported in recent years[1-4]. Fiber optic biosensors were also discussed with optical field and fluorescent effect to measure biological macromolecules species, like cells, proteins and DNA[5,6]. However, most optical fiber fluorescence probes for the detection of trace elements are intuitive, barely have complete theoretical quantitative analyses, and the coupling efficiency is about 0.8%. In this paper, a capillary-fiber probe with an efficiency of 2.4% is presented for trace elements detection. Because equimolar solution of carbonic anhydrase (CA) and 4,4-dimethyl-4-silapentance-1-ammonium trifluoroacetate (DSA) can generate specific binding with zinc, we present experiments with relative excess CA solution and fluorescent agent of DSA to detect the concentration of zinc. Our structure is also suitable for the detection of the concentration of other trace elements if we get the corresponding biological fluorescent probes for them. A novel fiber optic probe for trace elements detection is proposed in this paper based on the theory of axial resolution limit, and 5 probe parameters are discussed, which are number and arrangement of excitation fiber and collecting fibers, internal diameter and length of the fiber optic sensing probe and end reflectivity of the capillary. The fluorescence coupling efficiency is also affected by the concentration of detected CA solution and dezincification reagent.

The total efficiency of the fiber fluorescence probe (η_{total}) can be defined as the product of the efficiency of the fiber probe (η_{probe}) and the efficiency of the particular solution binding with the trace elements specifically (η_{solution})

$$\eta_{\text{total}} = \eta_{\text{probe}} * \eta_{\text{solution}}. \quad (1)$$

The fluorescence coupling efficiency is defined as the ratio of the emitted fluorescence intensity and the incident light intensity. If incident light intensity is fixed, the coupling efficiency is proportional to the emitted fluorescence intensity, which is affected by the absorption and scattering of the solution as well as the boundary yield of the far end surface and the side wall of the capillary according to axial resolution limit theory[7-9]. While the excitation light and the excited fluorescent light both propagate in the CA solution, the solution absorbs a certain amount of the light, so the light power is attenuated. The absorbance depends on both the composition and the concentration of the solution[10]. On the other hand, the particles in the solution can cause Rayleigh scattering, and the direction of scattered light rays is random[11].

Excited fluorescent bodies in fiber optic probe can be taken as a number of single illuminants in the CA solution, the probe can be divided into several cell areas, so we get the coupling efficiency of the total fluorescence from the capillary-fiber probe[12,13]. When the light from a point light source launches to a fiber, take the integral

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** E-mail: hualidehua@163.com

form of the coupling fluorescence power as[14]

$$P_0 = \int \exp(-\alpha L)\mathrm{d}L \int \mathrm{d}A \int I_0 \cos\theta \mathrm{d}\Omega\,, \tag{2}$$

$$\eta_{\text{probe}} = P_0 / P_{\text{e}} = (\int \exp(-\alpha L)\mathrm{d}L \times \int \mathrm{d}A \int I_0 \cos\theta \mathrm{d}\Omega) / P_{\text{e}}\,. \tag{3}$$

Attenuation coefficient α is dependent on the absorption and scattering of CA solution. The first integral is over the effective length of the capillary probe, the second integral covers the effective irradiate areas of fluorescent light source, and the third integral takes on all the possible directions of the fluorescent light emission. The fluorescence intensity has no unit since it stands for relative significance, and I_0 is a constant relative to the incident light intensity and CA solution concentration[15].

The structure of the proposed fiber probe is shown in Fig.1, where one excitation fiber (incident fiber) is in the center of 6 collecting fibers, and the bundle is in the center of a cylinder capillary. This design aims at a full excitation of the fluorescence in the solution and guarantees that a vast majority filling of effective light is incident in the receiving area of the probe.

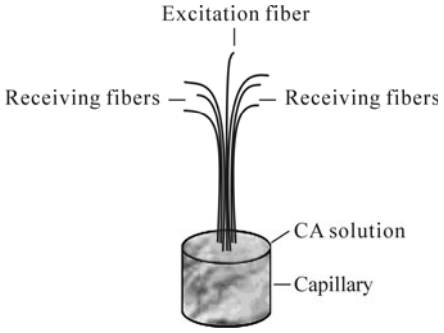

Fig.1 Sensor probe proposed with one excitation (incident) fiber in the center of collecting ring made up of 6 fibers

A large core UV silica fiber with diameter of 600 μm is chosen to be the excitation fiber to improve the coupling efficiency[16,17], the numerical aperture (*NA*) of the excitation fiber is 0.37, and the diameter of cladding is 660 μm. Similarly, the *NA* of the 6 receiving fibers is 0.39, diameter of the core is 600 μm, and the diameter of the cladding is 690 μm. To simplify the integral, we divide the probe region into several parts as shown in Fig.2. L, h and θ for each part are pre-determined according to the diameter of the capillary with the relation of $L=f(h, \theta)$. For example, L of part 1 is from 0 to 708.3 μm, h is from 0 to (708.3-L)tan(arcsin0.39), and θ is from 0 to arcsin0.39. After finding the upper and lower limits of the interval for each integral, we get the coupling power of every part.

With all the parameters in our model, we write the coupling optical power of part 1 as

$$P_1 = \int_0^L \exp(-7.3\times L\times 5.3\times 10^{-5} - L\times 5.3\times 10^{-5})\times \int_0^{(708.3-L)\tan(\arcsin 0.39)} 2\pi h \int_0^{\arcsin 0.39} 2\pi I_0 \cos\theta \sin\theta \mathrm{d}\theta \mathrm{d}h \mathrm{d}L. \tag{4}$$

Similar approach is used to get the coupling power of other parts.

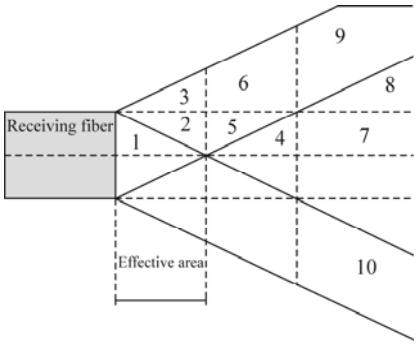

Fig.2 Axial-section view of the sensor probe with divided parts

To determine the fluorescence coupling efficiency of the proposed optical fiber probe, we carry out a set of experiments based on the system shown in Fig.3.

The excitation light source is a 365 nm-UV light (LS-325-1004, Ocean Optics). Excitation light launches into one of the 7 hard plastic clad optical fibers (HP 600/630-37/1040E, YOFC). Fluorescence is collected by the collecting fibers, and transmitted to the spectrometer (Advantest Q8381A) to get the fluorescence output power at 460 nm and 560 nm, respectively. In our measurements, the concentration of the CA solution is 2.29 mg/mL, the length of the optical path is 1 mm, and the attenuation coefficients are 5.3×10^{-5} L/(g·μm) at 580 nm and 0.387 L/(g·μm) at 468 nm (by SHIMADZU UV-1700UV spectrophotometer).

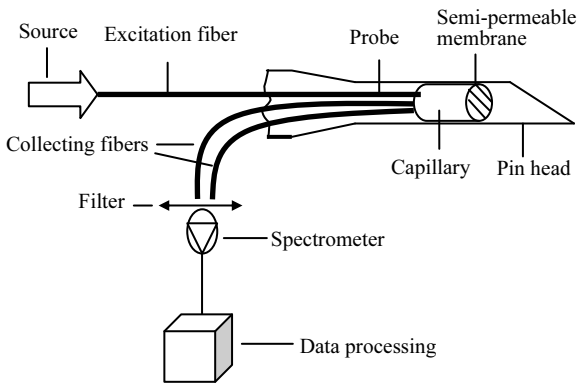

Fig.3 Schematic diagram of experiment system for Zn concentration detection with one excitation fiber and two collecting fibers

Because equimolar solution of CA and DSA can generate specific binding with zinc, the mixture of CA and DSA is a high selective biological molecule probe of Zn^{2+}. We prepare 4 groups of 5 mL CA solution (dissolved in absolute alcohol) with concentration of 2.29 mg/mL, and the diameters of four capillaries change from 3 mm to 4 mm (3 mm, 3.2 mm, 3.8 mm and 4 mm).

The total fluorescence coupling power versus capillary length with different diameters of capillary is shown in Fig.4. L is the distance from the end of the receiving fiber to the upper limit of its interval of integration. We find the receiving power increases with increasing L within 0.75 mm; once L is out of the range, the receiving power keeps constant. Thus we can get that the critical length of L is about 0.75 mm, and it is consistent with the simulation result. The diameter of the capillary has little influence on the receiving power when the diameter is large enough.

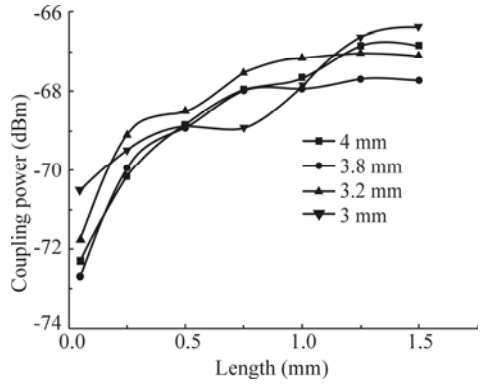

Fig.4 Relation between total fluorescence coupling power and capillary length with different diameters of capillary in solution of CA (2.29 mg/mL)

From the experimental results, we also find that the coupling fluorescence is too weak to be detected when the distance L is longer than $R/\tan\theta$, where R is the radius of the receiving fiber core, θ is the acceptance angle in the solution with a value of $\arctan(NA/n)$, and n is the refractive index of the CA solution of 1.3327. Choosing $R/\tan\theta$ as the probe length, and comparing the receiving power with the total fluorescence emission power, we can get the efficiency of 1.2% with the fiber structure in Fig.3 with 1 excitation fiber and 2 collecting fibers, which is consistent with the simulation result of 1.18% according to Eq.(3).

We get the coupling fluorescence power of the system with 1 excitation fiber and 6 collecting fibers as

$$P_0 = 3[P_1 + \int_{4519.2}^{L} \exp(-7.3\times L\times 5.3\times 10^{-5} - L\times 5.3\times 10^{-5})\times \int_{0}^{(4519.2-L)\tan(\arcsin 0.39)} 2\pi h\times \int_{0}^{\arcsin 0.39}\int_{0}^{2\pi} k(L,h) I_0 \cos\theta \sin\theta \mathrm{d}\theta \mathrm{d}h \mathrm{d}L \mathrm{d}\varphi],$$

$$k(L,h) = \frac{300^2}{(300+h)^2}, \quad (5)$$

where h is the vertical distance to the axis of the receiving fiber, and L is the distance between the chosen sections and the receiving fiber end face. P_0/P_e is 2.4% through the integral, which is the coupling efficiency η_{probe}.

The fluorescence spectra of apoCA, DSA, mixture of DSA and apoCA, mixture of DSA, apoCA and Zn^{2+} are measured respectively by 970CRT fluorescence spectrophotometer. Since CA itself contains zinc, we select the suitable dezincification reagent for CA in order to ensure the accuracy of experimental results. As shown in Figs.5 and 6, there is little florescence of apoCA. The fluorescence intensity has no unit for it only represents the relative significance. Emission spectrum of DSA mainly locates near 550 nm, and that of the mixture of DSA and apoCA mainly distributes in the range of 450−550 nm, but the fluorescence intensity at 464 nm in Fig.6 is smaller than that in Fig.5, which proves that the dezincification of CA with 2,6-pyridine dicarboxylic is more complete than that with EDTA. The coupling efficiency is increased with the concentration of CA solution increasing within a certain range (less than 2.45 mg/mL).

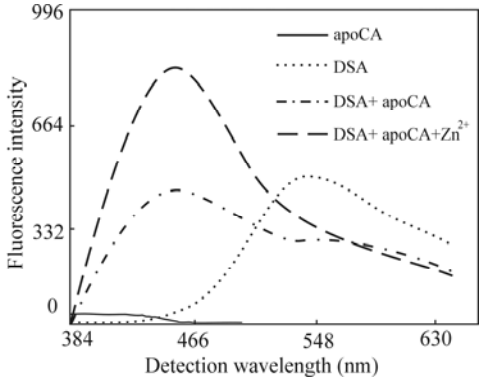

Fig.5 Fluorescence spectra of four solutions under dezincification of CA with EDTA

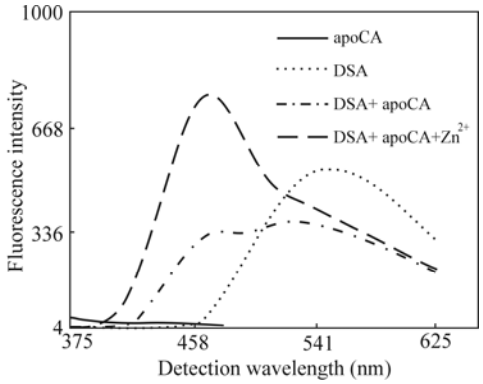

Fig.6 Fluorescence spectra of four solutions under dezincification of CA with 2,6-pyridine dicarboxylic

The efficiency of the particular solution combined with the trace elements ($\eta_{solution}$) also affects the total efficiency of the fiber fluorescence probe. For the detection of zinc, Tab.1 gives the parameters which affect $\eta_{solution}$ of CA and FITC solutions.

Tab.1 Parameters for CA and FITC solutions

Solution	Exciting light wavelength	Output light wavelength	Intensity of exciting light(I)	Fluorescence quantum efficiency(η)	Minimum concentration(C)
CA+DSA	326 nm	580 nm	22	0.84	0.58 mg/mL
FITC	490 nm	520 nm	35	0.30	0.10 mg/mL

The relationship between the sensitivity of the solution and the parameters of the solution is given by

$$\eta_{solution} = I\eta C . \quad (6)$$

We get the efficiencies of CA and FITC solutions as 10.7184 and 1.05, respectively. Therefore, the suitable binding reagents of the particular trace element can improve $\eta_{solution}$.

In conclusion, 5 probe parameters affecting fluorescence efficiency are analyzed, which are number and arrangement of excitation fiber and collecting fibers, internal diameter and length of the fiber optic sensing probe, the end reflectivity of the capillary, and the concentration of CA solution and dezincification reagent. The optimal structure is obtained as follows. The arrangement of one excitation fiber in the center of 6 collecting fibers gives the best fluorescence efficiency with a reasonable capillary diameter. Fluorescence efficiency increases only with increasing L within the range of 0−0.75 mm. The diameter of the capillary has little influence on the receiving power when the diameter is large enough. Both simulation and experiment results prove that fluorescence efficiency increases with the end reflectivity of the capillary. The fluorescence efficiency is also affected by the concentration of CA solution while it's below the saturation concentration. The result also shows that dezincification of CA with 2,6-pyridine dicarboxylic gives a higher detection sensitivity than that with EDTA.

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