Discrimination of Three Typical Amino Acids using PARAFAC

Yang Hui*[a], XIAO Xue[b], ZHAO Xuesong[b], WU Yan[a] , Zong Junjun[a] and Ye Jiesong[a]
a. New Star Application Technology Institute, Hefei, Anhui 230031, China; b. Key Lab. of Environmental Optics & Technology, AIOFM, CAS, Hefei, Anhui 230031, China

ABSTRACT

The intrinsic fluorescence characteristics of tryptophan, tyrosine and phenyloalanine were presented in this paper. The excitation/emission wavelength of tryptophan, tyrosine and phenyloalanine locate at λex/λem=280/350nm, 275nm/303 and 260/280nm respectively. The excitation and emission bands of these bio-fluorophores are quite overlapped within the EEM area whose excitation wavelength/emission wavelength scope is 230-270nm/260-340nm. Using the PARAFAC algorithm, 10 tryptophan, tyrosine and phenyloalanine mixed solutions and three compound amino acids samples were were successfully decomposed, the emission profiles, excitation profiles, central wavelengths and the concentration of the three components were retrieved with high precision, and finally, the tyrosine and taurine were detected from 1st and 2nd sample respectively.

Keywords: Fluorescence spectra, PARAFAC, tryptophan, tyrosine, phenyloalanine, compound amino acids

1. INTRODUCTION

Of the twenty naturally occurring amino acids that make all proteins, three are conjugated with aromatic ring side chains, and therefore intrinsically optically active: tryptophan (Trp), tyrosine (Tyr) and phenyloalanine (Phe)[1,2,3], and the excitation and emission bands of these bio-fluorophores are quite overlapped within the EEM area. So through the detection of intrinsic fluorescence characteristics of tryptophan, tyrosine, phenyloalanine, and other kinds of fluorophore, such as taurine, ginsenoside, FMN, FAD, and DPA, etc., the comparative contents of various kinds of organic components can be analyzed and resolved with high spectra resolution, and finally the classification of bio-aerosol/bio-agent can be reached.

PARAFAC, a three way-decomposition method, has been found to be very useful in identifying the independent spectra of different types of fluorophores [6]. Compared to its predecessor, principal component analysis (PCA) technique, and other methods, PARAFAC provides both a quantitative and qualitative model of the data and separates the complex signal measured into its individual underlying fluorescent phenomena with specific excitation and emission spectra. It can separate several independent groups of fluorophores from the overlapped components with a high resolution, so it is commonly used technique to monitor the mixed fluorescence EEMs. [7]

In this paper, the intrinsic 3D fluorescence spectra of tryptophan, tyrosine and phenyloalanine raw reagents with high purity, and the 3D fluorescence spectra of compound amino acids samples from two different factories were obtained with an F-7000 FL spectrophotometer, the excitation/emission spectra were decomposed successfully from the mixed solutions, and also from the simulated sample mixtures.

* sanpedroman@163.com

Optical Measurement Technology and Instrumentation, edited by Sen Han, Jiubin Tan, Proc. of SPIE
Vol. 10155, 1015509 · © 2016 SPIE · CCC code: 0277-786X/16/$18 · doi: 10.1117/12.2243750

2. EXPERIMENTAL SECTION

2.1 Instruments and Reagents

The Molecular ΣH_2O ultra pure water machine (Shanghai Molecular Co. Ltd) was used to generate the ultra purified water, UPW whose pH value is 5.4. L-Tryptophan mother liquid were compounded with L-Tryptophan from Sigma co.Ltd whose minimum purity equals to 99%, the L-Tyrosine mother liquid were compounded with L-Tyrosine from Sigma co.Ltd whose minimum purity equals to 98%, and the L-Phenylalanine mother liquid were compounded with L-Phenylalanine from USB co.Ltd whose minimum purity equals to 99%. All reagents and materials were weighed with Mettler Toledo precise electronic balance, and dissolved with KH_2PO_4 buffer, Na_2HPO_4 buffer or PBS with different pH values of 6.5, 6.6, 6.8, 7.0, 7.2, 7.3, 7.4, 7.6 and 8.0.

The L-Tryptophan, L-Tyrosine and L-Phenylalanine mother liquid concentration are 20, 21 and 40 mg/L respectively. The all mother reagent solutions were transferred through DragonLab whole disinfection manual single channel adjustable liquid shifter and diluted to working solutions of different concentrations. All reagents were of analytical grade, all solutions and put in amber glass bottles and stored in a refrigerator (4℃).

Two kinds of amino acids from the commercial market were measured and analyzed for the fluorescence characteristics. The 1st sample named amino acids nutrient solution is manufactured by Wuxi JianTe Medical Limited. China, its key components include chrysalis compound amino acids power, taurine, etc. The mother liquid of the sample 1st was compounded by put 100ul raw liquid into 250ml UPW. The 2nd sample named Bocheng american ginseng amino acids oral liquid comes from Jiangxi Bocheng Medical Limited China, the mother liquid of sample 2nd was compounded by put 50ul raw liquid into 250ml UPW.

3D fluorescence intensity measurements were carried out on an F-7000 FL spectrophotometer (Hitachi High-Technologies Corporation, Japan).

2.2 Instrument Settings and Experiment Procedure

Buffers with different pH values and mother liquids of different volumes were injected into the 10ml test tubes, and diluted with purified water to form the working liquids and background liquids.

For the fluorescence EEM measurements of L-Tryptophan, L-Tyrosine and L-Phenylalanine the spectrophotometer excitation wavelength ranged from 200.0nm, to 400.0nm, emission wavelength ranged from 250.0nm to 450.0nm. Scan speed was set at 12000nm/min with excitation and emission sampling interval of 2.0 nm, excitation and emission slit of 10.0nm, the PMT voltage was set at 600 V. All experiments were performed at room temperature at 25℃.

The 1st level and 2nd level Rayleigh scattering, Raman scattering and other background components within the fluorescence signals were corrected for the following analysis.

2.3 PARAFAC Method for Multi-components Discrimination

Based on the tri-linear decomposition theory, the parallel factor analysis method is a kind of mathematical model implemented by alternating least squares algorithm, which is widely applied to the analysis and application of three-dimensional and high-dimensional data, and used to decompose n-dimensional data to the N load matrixes.

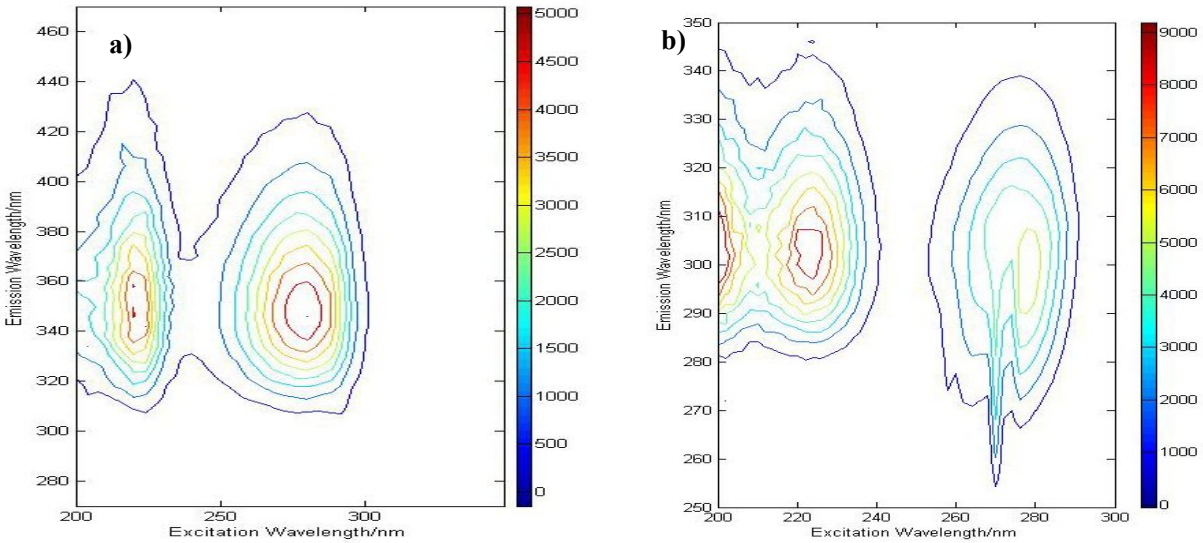
The measured fluorescence spectrum data matrix *EEM*s is a $I \times J \times K$ matrix, among it, *I* means the number of the samples, while *J* and *K* stand for the number of excitation wavelength and emission wavelength of samples respectively. With Parallel Factor decomposition model, score matrix *A*, load matrixes *B* and *C* can be decomposed and resolved from the fluorescence spectrum data matrix. The decomposition model can be represented as:

$$x_{ijk} = \sum_{f=1}^{F} \alpha_{if} b_{jf} c_{kf} + \varepsilon_{ijk} \quad , \text{i} = 1, 2, \ldots, \text{I}, \quad \text{j} = 1, 2, \ldots, \text{J}, \text{k} = 1, 2, \ldots, \text{K} \qquad (1)$$

where, x_{ijk} is the fluorescence intensity of sample i at excitation wavelength j and emission wavelength k, F is the column number of load matrix, representing the number of factors, ε_{ijk} is the residual element, indicating the vector's size which can't be expressed by the model, and α_{if}, b_{jf}, c_{kf} are the elements in load matrixes A, B and C respectively. The matrix A, B and C can be computed until the SSR is convergent, that means, the minimum loss function $f_{SSR} = \sum_{i=1}^{I}\sum_{j=1}^{J}\sum_{k=1}^{K} e^2{}_{ijk} < 10^{-6}$.

In this study, PARAFAC modeling was performed with MATLAB 7.0 code. The appropriate number of components was determined primarily based on the three diagnostic tools including residual analysis, core consistency and visual inspection of spectral shapes of each component, which are widely used by other similar studies. The components extracted by PARAFAC represent groups of the organic components that exhibit similar fluorescence properties. The component scores indicate the relative concentration of the groups, not the actual concentration of a particular material/fluorophore. However, it is typically assumed that the scores are proportional to the concentrations of the different components [8, 9].

3. FLUORESCENCE EEM CHARACTERISTICS OF THREE KINDS OF AMINO ACIDS



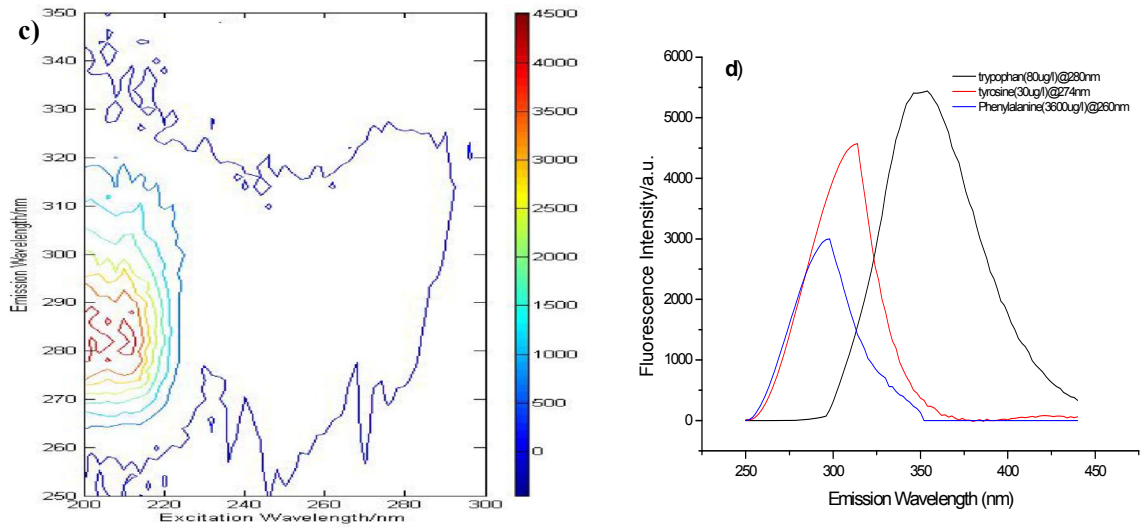


Figure1. Fluorescence intensity distribution of tryptophan@80ug/L, tyrosine@30ug/L, phenyloalanine @1350ug/L and their overlapped fluorescence emission spectra

From figure 1, it can be conclude that, the excitation/emission wavelength of tryptophan, tyrosine and phenyloalanine locate at λex/λem=280/350nm, 275nm/303 and 260/280nm respectively, the overlapping of these three 3D fluorescence spectra is obvious, as shown in figure 1.

4. DECOMPOSITION OF OVERLAPPED FLUORESCENCE SPECTRA OF MIXED STANDARD SOLUTIONS

Fluorescence EEM intensities of 10 solutions mixed with tryptophan, tyrosine and phenyloalanine of different concentration were retrieved by PARAFAC program, seen in figure 2.

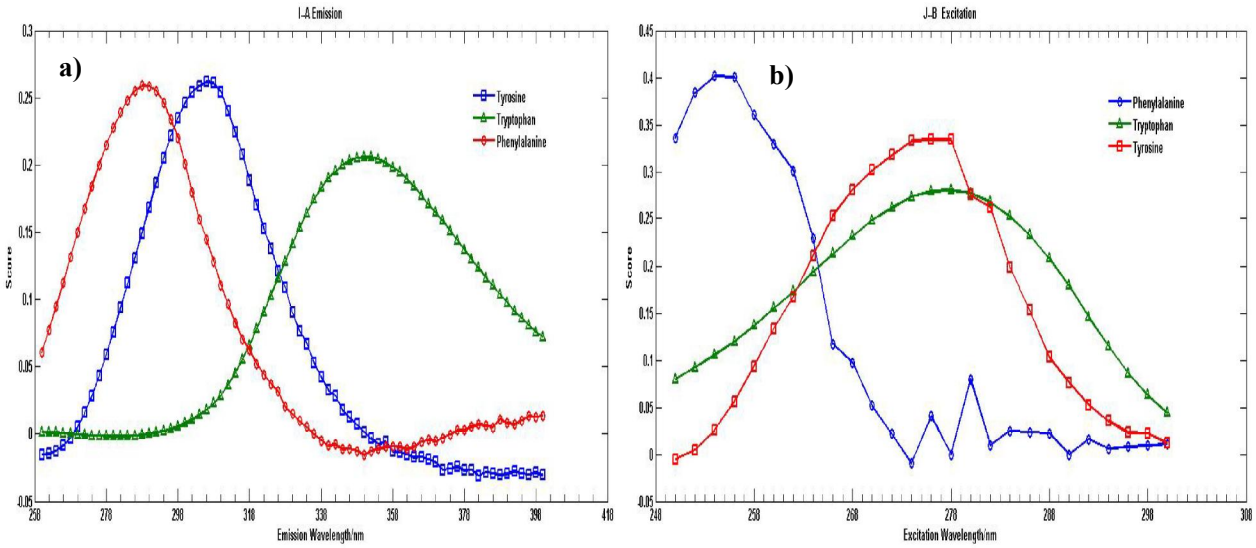


Figure2. Excitation and emission spectral profile retrieved by PARAFAC algorithm, (a) Emission spectra, (b) Excitation spectra circle

The emission spectra retrieved by PARAFAC are shown in Figure 2(a), the green curve with triangle symbol indicates the retrieved emission profile of tryptophan, the red curve with circle symbol indicates the retrieved emission profile of phenylalanine, and the blue curve with block symbol indicates the retrieved emission profile of tyrosine.

The excitation spectra retrieved by PARAFAC are show in Figure 2(b), the green curve with triangle symbol indicates the excitation profile of tryptophan, the red curve with block symbol indicates the excitation profile of tyrosine, and the blue curve with circle symbol indicates the excitation profile of phenylalanine. From figure 1 and 2, it can be seen that the retrieved emission and excitation profile and central wavelength of tryptophan, tyrosine and phenylalanine are coincident with their real excitation profiles. But as for the retrieved excitation profile of phenylalanine, there exists a extra zigzag fluctuating between 274nm and 284nm, and the central excitation wavelength locates at 256nm, a little bit ultraviolet shifted 4nm compared to the standard spectra.

5. ANALYSIS OF 3D FLUORESCENCE SPECTRA OF TWO SAMPLE SOLUTIONS

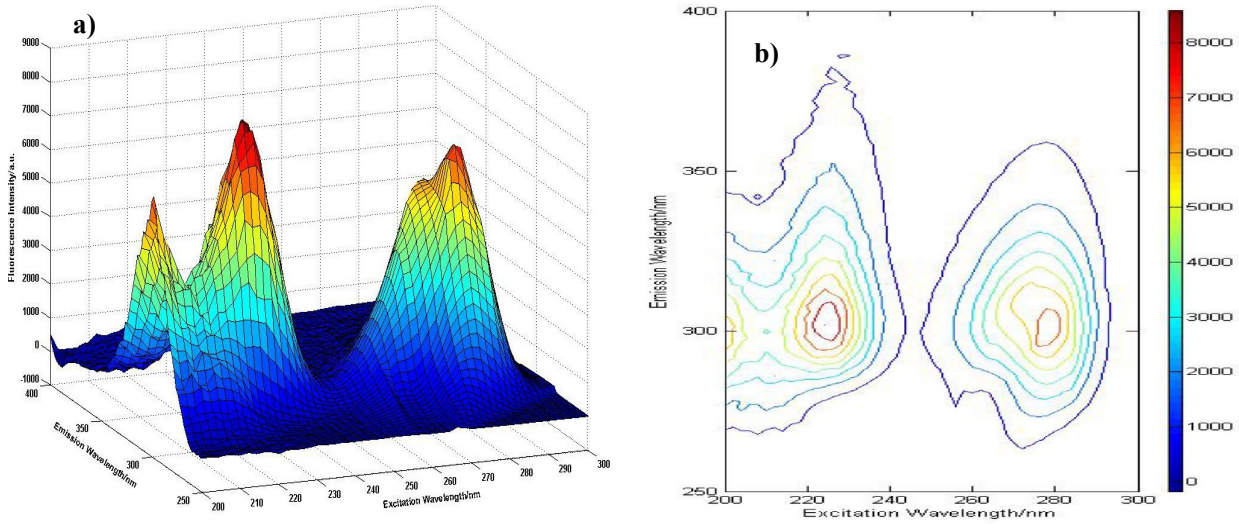


Figure3. Fluorescence spectra of the 1st sample

For the sample 1st, the fluorescence of tyrosine constituent was found obviously, and the fluorescence excited by tryptophan and phenylalanine were very weak, the concentration of tyrosine was about 40ug/L retrieved by PARAFAC method, as shown in figure 3.

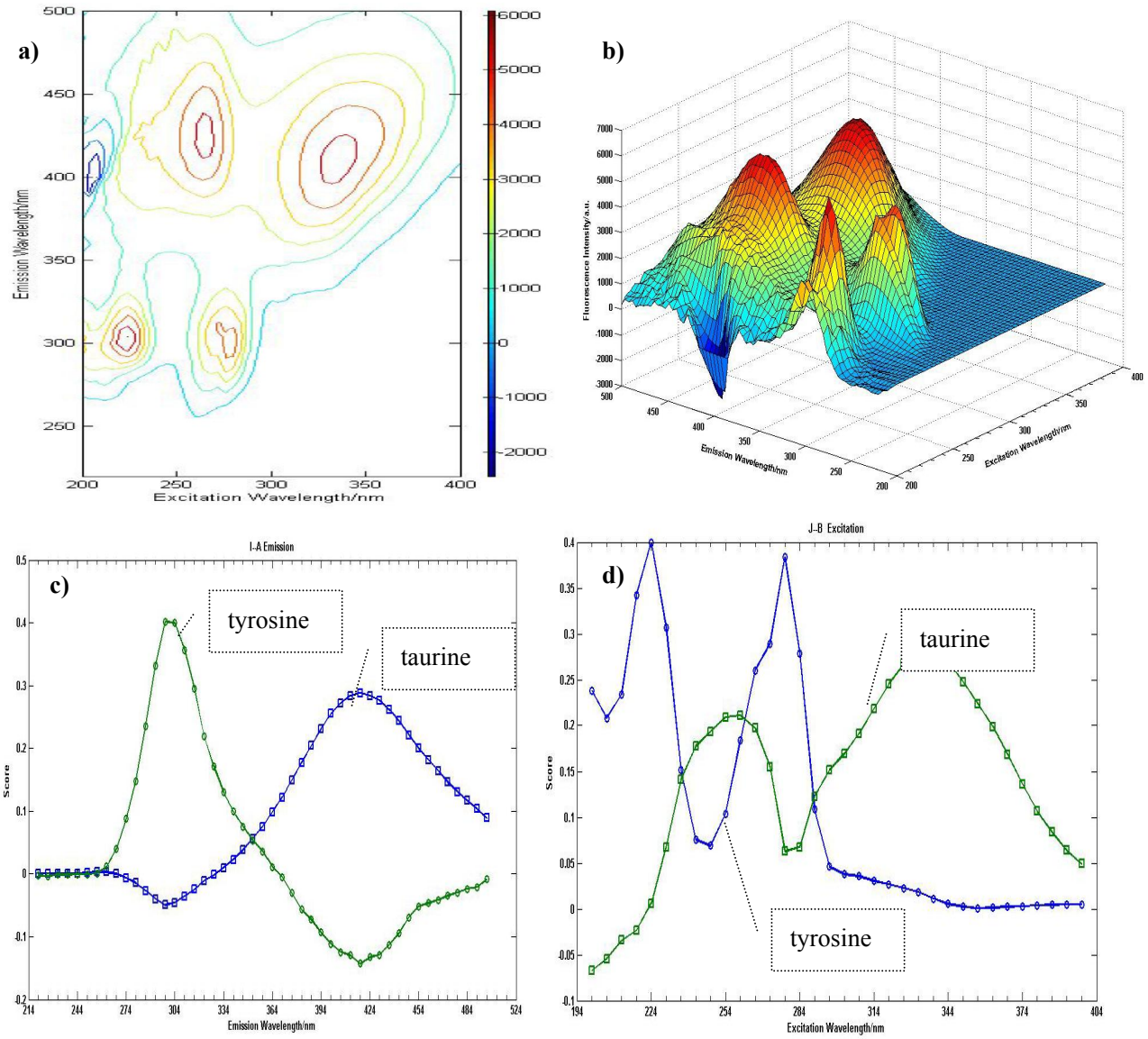


Figure4. Fluorescence spectra and retrieved fluorescence profiles of the 2nd sample (a) 2d Fluorescence EEM intensity distribution, (b) 3d Fluorescence EEM intensity distribution, (c) Emission profiles, (d) Excitation profiles

The 3D fluorescence spectra of sample 2nd and retrieved fluorescence excitation and emission profile ware shown in figure 4. The fluorescence peaks of tyrosine and taurine were clearly found. In figure4(c) and (d) the excitation and emission spectra of tyrosine and taurine were also deduced by PARAFAC method. The concentration of tyrosine and taurine are 27 ug/L and 18 ug/L respectively.

6. CONCLUSIONS

The fluorescence of a protein or bio-aerosol or bio-agent is a mixture of the fluorescence from individual aromatic residues and coenzyme. According to the law of Lambert-Beer, PARAFAC is only suitable to the low concentration solution because of the quenching effect by the high concentration of solution. On the other hand, PARAFAC is also

capable of analyzing the mixed trace elements of high fluorescence quantum efficiency.

Using fluorescence Spectrophotometer, the intrinsic fluorescent characteristics of tryptophan, tyrosine and phenylalanine were measured with solutions of different concentration. The overlapping of the fluorescence spectra of three components is obviously. The retrieved emission profiles, excitation profiles, central wavelengths and the concentration of three components are coincident precisely with real emission profiles, excitation profiles, and central wavelengths of each component, and finally, the tyrosine and taurine were detected from 1st and 2nd sample respectively.

ACKNOWLEDGMENTS

The work was supported by the National Natural Science Foundation of China named “Study on Technology of Ultraviolet Laser-induced Fluorescence LIDAR for Bioagent Remote Sensing” No. 41375026.

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