Study on the Detection of the Content of the Phenylalanine by Rare Earth Ions Fluorescence Probe Method

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Abstract. Phenylketonuria (PKU) is a kind of genetic and metabolic disease which caused by mutations. High concent of phenylalanine and its metabolites in the blood which result in impairment of brain development, even mental retardation. In recent years, due to the impact factors of diet and environment, PKU disease was significantly increased. Studies have shown that early detection of abnormal levels of phenylalanine and treatment will greatly reduce the rate of mental retardation. Therefore, monitoring of blood levels of phenylalanine is particularly important for phenylketonuria patients. This study discussed a new method of terbium rare earth ions fluorescence probe to detect the content of phenylalanine. At the same time, the experimental conditions were optimized. The results showed that the optimal pH of the system was 8, the optimum surfactant was SDBS(1.0×10⁻³ mol/L) and the optimum concentration of Tb³⁺ was 3×10⁻² mol/L. Under optimal test conditions, the fluorescence intensity had a good linear relationship with the concentration of phenylalanine in the range of 6×10⁻⁷~3×10⁻⁵ mol/L. And the linear equation as follows: F= 1.0979C_Phe +23.50268, r = 0.998. It provided a new theoretical approach for the clinical test of Phenylketonuria.

1. Introduction
Phenylketonuria (PKU) is a genetic and metabolic disease [1], which is the main reason of intellectual disability. In 1934, Follinh first described the disease. In 1937, Penrose and Quastel named the disease as “phenylketonuria”. In 1939, Jervis proposed that PKU is an autosomal dominant inheritance disease and confirmed that the disease was caused by liver phenylalanine hydroxylase deficiency in 1953[2]. Pediatric PKU patients if not treated early, will lead to the discoloration of skin and hair, and the exacerbations of mental disorders. Approximately 25% of children develop epilepsy, and the bone, skin and white matter abnormalities of patients were appeared among other clinical symptoms.

The Chinese government had already embarked on developing of neonatal disease screening for 20 years. As one way of Three-grade-defence, it can improve the quality of the population and prevent born defect [3]. Now, it is get remarkable achievement beneath high concerned by government. But for technical reasons, the current detection and treatment of PKU disease is still restricted, and the family with PKU children still suffered serious physical and psychological burden. In 2008, in Shanghai Municipal Health Bureau, Chen Tian, et al. together with a number of hospitals in Shanghai screened the evaluation of parents of neonatal. Only 3.7% of parents were satisfied with the treatment method, while 96.3% of parents were unsatisfied and worried about their children's rehabilitation effect and future [4]. Feng Hui, et al. [5] also found that parents of children with PKU SCL-90 compared to the normal value showed more psychological problems, such as depression and psychotic performance. Higher affect of depression of children may be related to mood of parent who has pessimism, disappointment and even thinking about death after learned more knowledge about PKU.
This showed that parents of children with PKU disease will face harsh reality, they will suffer from more psychological problems compared to the normal parents, and should be given more attention [6, 7].

In recent years, healthcare workers had taken great concern in testing and treating PKU. It was found through in low-phenylalanine diet that we can keep levels of phenylalanine in blood in the ideal range of 60~120mol/L (1~2 mg/dl) to prevent serious mental disorders and secondary epilepsy of children. In a word, PKU neonatal screening is vital important [8-14]. In this research, the method of rare earth fluorescence probe was used to detect the content of phenylalanine. Differ from traditional fluorescence kit, this method simplified experimental procedures, reduced the cost of the experiment, and also had positive meaning for PKU screening and treatment [15-20].

In this paper, phenylalanine was complexed with rare earth terbium ions, then optimized the experimental conditions and detected the content of phenylalanine. The results showed that phenylalanine had a linear relationship with the fluorescence intensity of the system in the concentration range of $6 \times 10^{-7}$~$3 \times 10^{-5}$ mol/L.

2. Experimental method

Tb$^{3+}$ solution, phenylalanine solution, Tris-HCl (0.1mol/L, pH=9.0), SDBS solution, and redistilled water were mixed and added to a 10ml colorimetric tube. Then shaked it for 10 min, and the fluorescence intensity of the system was tested under certain conditions (Ex=371nm, Em=544nm, 1cm fluorescence cuvette, band pass of excitation and emission both were 10nm).

3. Results and discussion

The characteristic fluorescence spectra of the Tb$^{3+}$ solution were not shown, but the characteristic fluorescence spectra of the phenylalanine-Tb$^{3+}$ complex solution showed the characteristic peaks of Tb$^{3+}$. The fluorescence intensity of phenylalanine was significantly lower than that of the complex at 452 nm, because the energy was transferred from phenylalanine to Tb$^{3+}$ and the fluorescence properties were revealed by the phenylalanine-Tb$^{3+}$ complex.

3.1. Effect of surfactant

Phenylalanine-Tb$^{3+}$ complex solution showed characteristic fluorescence of Tb$^{3+}$, but the intensity was weak. Then added an appropriate amount of surfactant to complex solution can greatly enhance the fluorescence intensity, and it can be used for quantitative detection, the results were shown in Figure 1.

This experiment investigated the effect of different surfactants (including non-ionic surfactants solid (GA, β-CD), a cationic surfactant (CTMAB) and anionic surfactants (SDS, SDBS)) on the fluorescence intensity of the system. The results showed that the sensitizing strength of GA, β-CD and CTMAB was limited, while the anionic surfactants SDS and SDBS were better sensitized, and the SDBS is the most effective surfactant. So in this study, SDBS was used as a sensitizing surfactant.
Figure 1. The fluorescence emission spectrum curve of phenylalanine rare Earth fluorescence Probe 1. Phenylalanine; 2. Tb\(^{3+}\) phenylalanine; 3. Tb\(^{3+}\) phenylalanine-SDBS \(C_{Tb}^{3+}=3\times10^{-3}\) mol/L, \(C_{Phe}=1\times10^{-5}\) mol/L, \(C_{SDBS}=1\times10^{-3}\) mol/L, pH=8.0

Figure 2. Effect of the concentration of the surfactant on the fluorescence intensity \(C_{Tb}^{3+}=3\times10^{-3}\) mol/L, \(C_{Phe}=1\times10^{-5}\) mol/L, pH=8.0

3.2. Effect of the concentration of the surfactant
The concentration of SDBS had a significant effect on the fluorescence intensity of the system. The results were showed in Figure 2.

Figure 2 indicated that the fluorescence intensity of the system was strong when the concentration of SDBS was 1.0×10\(^{-3}\) mol/L. So in this study, 1.0×10\(^{-3}\) mol/L was used as the optimum concentration of SDBS.

3.3. Effect of the pH
In Figure 3, it was showed that the effect of different pH (at the range of 2.0–10.0) uorescence intensity of phenylalanine-Tb\(^{3+}\)-SDBS. The fluorescence intensity continued to increase when pH was at the range of 5.0–8.5, when the pH was 8.0, the fluorescence intensity reached the maximum value. So, pH=8 was chosen as the optimum pH.

In the experiment, pH = 8 can be set by using a variety of different buffers. The buffers were as follows: Tris-HCl, NH\(_4\)Ac-HAc, NaAc-HA and NH\(_4\)Cl-HCl. The experimental results showed that 0.1mol/L Tris-HCl buffer solution had the best effect. So, 0.1mol/L Tris-HCl buffer solution should be chose in this experiment to adjust the pH.
3.4. Effect of Tb$^{3+}$ concentration

The fluorescence intensity of the system was studied under different concentrations of Tb$^{3+}$ solution when the concentration of SDBS was 1.0×10$^{-3}$ mol/L, the concentration of Tris-HCl buffer solution was 0.1mol/L, and the pH was 8 and the fixed phenylalanine concentration.

It was found that when the concentration of Tb$^{3+}$ was in the range of 2.5×10$^{-3}$ mol/L~3.5×10$^{-3}$ mol/L, the fluorescence intensity was very strong. And when the concentration was 3×10$^{-2}$ mol/L, the fluorescence intensity value was the largest. So the concentration of Tb$^{3+}$ was choosed for 3×10$^{-2}$ mol/L for this experiment. The results were showed in Figure 4.

3.5. Accuracy and stability test

Under the best conditions above test: the concentration of Tris-HCl was 0.1mol/L, the concentration of Tb$^{3+}$ was 3.0×10$^{-2}$ mol/L, the concentration of SDBS was 1.0×10$^{-3}$mol/L and pH was 8. Then performed parallel detection of phenylalanine (1×10$^{-6}$mol/L) for ten times, the result was that RSD% was 1.4.

The reaction time which related to stability of system was studied; the result indicated that the system had maximum fluorescence intensity in the stability range of 5 min~10 min. In this experiment, the assay was carried out after the system was fully reacted for 5 min and completed in 10 min.
3.6. Linear range and detection limit
Under optimal test conditions, the fluorescence intensity had a good linear relationship with the concentration of phenylalanine in the range of $6 \times 10^{-7}$ to $3 \times 10^{-5}$ mol/L. And the linear equation as follows: $F = 1.0979C_{\text{Phe}} + 23.50268$, $r = 0.998$.

3.7. Sample analysis
Different batches of Puritan's Pride tablets (the labeled amount of phenylalanine was 0.5 g/tablet) were finely grinded and weighed, then dissolved in dilute hydrochloric acid solution and sondered in a volumetric flask (100 mL) with re-distilled water for ultrasound. After nature sedimentation, 1mL of mixed solution was taken to another volumetric flask (100 mL) and quantitatively measured using the standard curve method. Simultaneously, according to Ch.P (2010 ed), using titration method to measure the content as controlled experiment. The results were showed in Table 1, and RSD %= 1.016 (n = 5).

| Samples | method(g/p) | Ch.p.(g/p) | Recovery rate(%) |
|---------|-------------|------------|------------------|
| 1       | 0.502       | 0.499      | 100.6            |
| 2       | 0.498       | 0.496      | 100.4            |
| 3       | 0.496       | 0.495      | 100.2            |
| 4       | 0.506       | 0.502      | 100.8            |
| 5       | 0.508       | 0.505      | 100.6            |

4. Conclusions
A new convenient method of rare earth fluorescent probe technique for the detection of phenylalanine was presented in this study, which can be used to detect low concentrations of phenylalanine and improve the sensitivity of the detection test. Compared with the old fluoroscopy method for detecting the PKU, the new method is more convenient to use and reduces the use of copper. Copper is a kind of metal material, in addition to polluting the environment, it may also cause a long-term damage to infants and researchers. This new method for determining the PKU will have a better effect.

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