

A SIMPLE MEASUREMENT OF VITAMIN B₁₂ BY VISIBLE SPECTROSCOPY

Koichi Uminoto, Minae Matsuura, Yoshimasa Shimamoto, Katsunori Tachibana, Aki Kamada, Masahiro Miyata

Department of Biomedical Engineering Osaka Electro-Communication University

OBJECTIVES

Vitamin B₁₂ is well known to be a useful marker as a middle molecular substance as same as inulin for evaluating dialyzer clearance. Generally, method for measuring Vitamin B₁₂ is electrochemiluminescence immunoassay or chemiluminescence enzyme immunoassay, however, spectroscopy offers an alternative approach for clinical analysis. This approach is reagentless, thereby permitting real time analysis.

In this study, we investigated to measure vitamin B₁₂ directly by optical measurement.

METHODS

Vitamin B₁₂ (Wako chemicals, Ltd. Japan) was dissolved at a concentration of 0.78-12.5 mg/dl in pure water. The spectral measurement was performed by ultraviolet-visible spectrophotometer and those spectra were recorded in the 200-600 nm wavelength range. The concentration of Vitamin B₁₂ was extracted from spectrum based on the absorption band.

The compact diffusion equipment with two chambers and one membrane partition was made with polyacrylonitrile dialysis membrane (AN69 H12), and the permeability coefficient of vitamin B₁₂, glucose, creatinine and urea were measured for evaluating this vitamin B₁₂ optical measurement. The concentration of glucose, creatinine and urea were measured by auto-chemical analyzer.

RESULTS

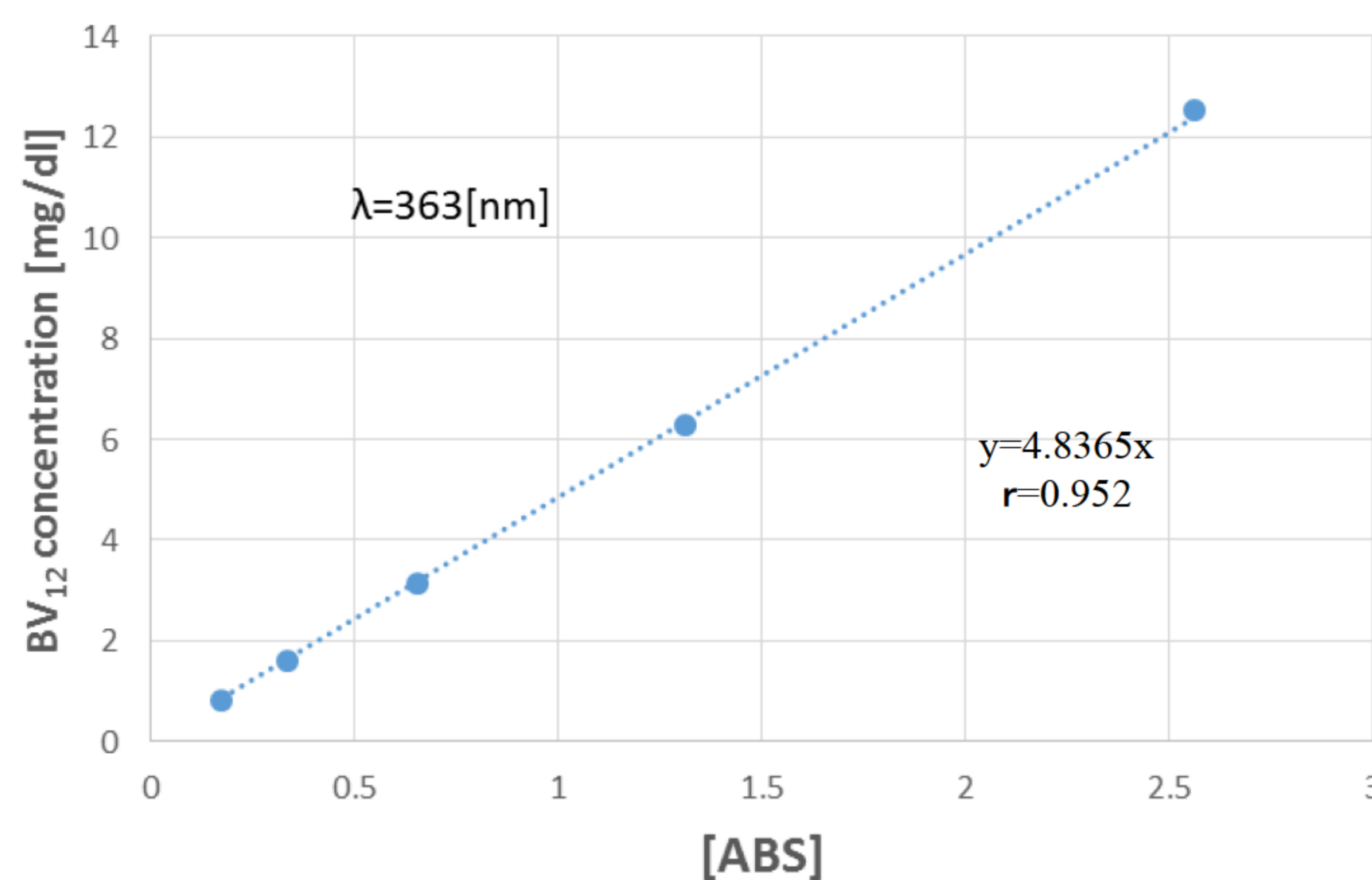
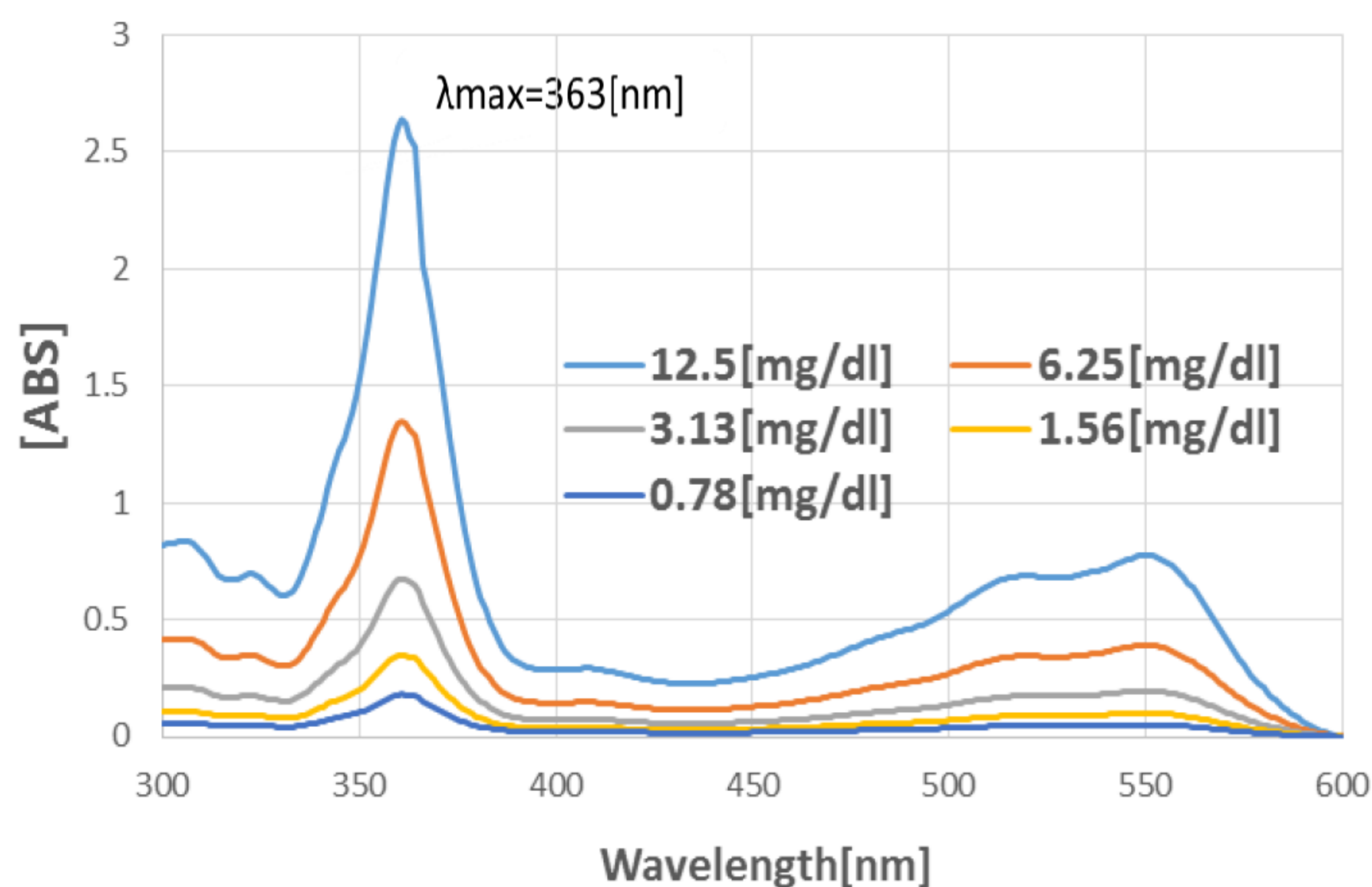


Fig.1 Absorbance spectrum of vitamin B₁₂ in the pure water

Fig.2 The peak values and predetermined concentration of vitamin B₁₂

The absorbance spectrum of vitamin B₁₂ in the pure water showed a large band at a peak of 363 nm and a small band at a peak of 550 nm (Fig.1). The absorption band of vitamin B₁₂ was determined at 363 nm based on the peak of its spectra. There was a significant correlation between the peak values and predetermined concentration of vitamin B₁₂ (r=0.952, P<0.001) (Fig.2).

Table 1 shows the permeability coefficient of four substances.

Table 1	Urea	Creatinine	Glucose	Vitamin B ₁₂
Molecular weight (dalton)	60	113	180	1355
Permeability coefficient (cm/s)	52.1 × 10 ⁻⁵	44.6 × 10 ⁻⁵	25.6 × 10 ⁻⁵	18.2 × 10 ⁻⁵

$$\text{Permeability coefficient} = \frac{V}{2At} \times \log_e \frac{C_1(0) - C_2(0)}{C_1(t) - C_2(t)}$$

V: Volume of Chamber, A: Membrane area, t: Diffusion time
 C₁, C₂: Concentration of substances in each chamber
 Urea: 100mg/dl, Creatinine: 10mg/dl, Glucose: 200mg/dl
 Vitamin B₁₂: 12.5mg/dl

CONCLUSIONS

The values of permeability coefficient of four different substances corresponded to their molecular weights. Therefore, it is possible to determine the concentration of vitamin B₁₂ based on the spectral values using spectrophotometer. The optical measurement technique is useful for evaluating dialyzer clearance of vitamin B₁₂.