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**Abstract:** Bovine insulin solutions (pH=9.3) have maximum absorption at 278 nm and an intrinsic emission at 305 nm when excited at 282 nm. The relative fluorescence intensities show linear dependence on its concentration:  $10 \mu\text{g/mL} < [\text{Ins}] < 200 \mu\text{g/mL}$ . When  $\text{Eu}(\text{PDA})_3^{3-}$  tris complex is added to these solutions, it has a hyperchromic effect at 278 nm absorption band of insulin, sensitizing the emission intensities of central  $\text{Eu}^{3+}$  metal ion of the complex at 590 and 615 nm, and simultaneously quenching the emission intensity of hormone at 305 nm. Stern-Volmer plots show that a mechanism of bimolecular quenching at 305 nm and sensitization at 615 nm are valid up to a mole ratio,  $R = [\text{Eu}(\text{PDA})_3^{3-}]/[\text{Ins}] < 2.0$ . An intramolecular rather than an intermolecular energy transfer is proposed. An apparent binding constant,  $\log K_{\text{app}} = 4.70 \pm 0.13$ , is calculated for  $\text{Ins}-[\text{Eu}(\text{PDA})_3^{3-}]_2$  type product, the presence of which may offer a new luminescence technique as a diagnostic tool and an alternative to radio-iodinated ( $^{131}\text{I}$  -) insulin. A simple, rapid and accurate quantitation of insulin is proposed by using a fixed concentration of  $\text{Eu}(\text{PDA})_3^{3-}$ , and measuring its initial  $F_0$  at  $\lambda_{\text{exc}}/\lambda_{\text{em}} = 282/615 \text{ nm}$  and the difference,  $\Delta F$  after sensitization when  $\sim 100$  microliters of insulin sample is added. The coefficient of variation (CV), the relative error and minimum detectable amount of bovine insulin hormone are found to be 3.0%, 1.2% and  $7.3 \pm 0.2 \mu\text{g/mL}$  respectively.

**Key Words:** Insulin, Fluorescence Spectroscopy, Eu(III) - { Pyridine - 2,6 - Dicarboxylate } Tris Complex Stern-Volmer Plots, Apparent Binding Constant

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