Ans: In these calculations,
terms like Optical Density (O.D) and Absorbance (A) are used Interchangeably.

So, as given in Question,

Absorbance of NADH at 340nm, $A_{NADH\,(340)} = 0.233$

∵ Only NADH absorbs at 340nm, we can use the following formula to calculate the concentration of NADH in Solution.

$$\boxed{\text{Absorbance } A = \underset{\text{co-efficient}}{\varepsilon\,(\text{molar extinction})} \times \underset{\text{of substance}}{\text{concentration}} \times \underset{\text{of Cuvette}}{\text{length}}}$$

∴ $A_{NADH\,(340nm)} = \underset{\text{(Given in Question)}}{6220} \times C_{NADH} \times 1$ (Given in question).

$$0.233 = 6220 \times C_{NADH} \times 1$$

$$C_{NADH} = \frac{0.233}{6220} = 3.74 \times 10^{-5}\,M$$

$$\boxed{C_{NADH} = 3.74 \times 10^{-5}\,M} \quad (1)$$

At 260 nm,

$$\underset{(\text{at } 260\text{ nm})}{A_{NADH}} = 3.74 \times 10^{-5} \times \underbrace{15000}_{\varepsilon \text{ of NADH at } 260\text{ nm}} \times 1$$

$$\therefore \boxed{A_{NADH} = 0.56} \text{ — } (2)$$

As we know Total Absorbance = Sum of Individual Absorbances of substances present in a solution.

At 260 nm,

$$A_{total} = A_{NADH} + A_{NAD^+}$$

$$\underset{(\text{Given in Question})}{1.000} = \underset{(\text{from } (2))}{0.56} + A_{NAD^+}$$

$$A_{NAD^+} = 1.000 - 0.56 \Rightarrow 0.44$$

$$\boxed{A_{NAD^+} = 0.44} \text{ — } (3)$$

from 3 ;

given in Question

$A_{NAD^+} = C_{NAD^+} \times \varepsilon_{NAD^+ \text{ at } 260\,nm} \times \text{length}$ (Given in question)

$0.44 = C_{NAD^+} \times 18000 \times 1$

from 3

$C_{NAD^+} = \dfrac{0.44}{18000} = 2.44 \times 10^{-5}\ M$

$\therefore$ C_{NAD^+} at 260 nm

$\therefore$ $\boxed{C_{NAD^+} \text{ in solution} = 2.44 \times 10^{-5}\ M}$