Bio-Tek nanodrop principle

Spectrophotometric analysis is based on the principles that nucleic acids absorb ultraviolet light in a specific pattern. In the case of DNA and RNA, a sample is exposed to ultraviolet light at a wavelength of 260 nanometres (nm) and a photo-detector measures the light that passes through the sample. Some of the ultraviolet light will pass through and some will be absorbed by the DNA / RNA. The more light absorbed by the sample, the higher the nucleic acid concentration in the sample. The resulting effect is that less light will strike the photodetector and this will produce a higher optical density (OD)

Using the Beer-Lambert Law it is possible to relate the amount of light absorbed to the concentration of the absorbing molecule. At a wavelength of 260 nm, the average extinction coefficient for double-stranded DNA is 0.020 (µg/ml)−1 cm−1, for single-stranded DNA it is 0.027 (µg/ml)−1 cm−1, for single-stranded RNA it is 0.025 (µg/ml)−1 cm−1 and for short single-stranded oligonucleotides it is dependent on the length and base composition. Thus, an Absorbance (A) of 1 corresponds to a concentration of 50 µg/ml for double-stranded DNA. This method of calculation is valid for up to an A of at least 2. A more accurate extinction coefficient may be needed for oligonucleotides; these can be predicted using the nearest-neighbor model.

References

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