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Spectrophotometry of DNA or RNA

For quantitating the amount of DNA or RNA, readings are taken at wavelengths of 260 nm and 280 nm. The reading at 260 nm allows calculation of the concentration of nucleic acid in the sample. An OD of 1 corresponds to ~50 µg/ml for double-stranded DNA, 40 µg/ml for single-stranded DNA and RNA, and ~33 µg/ml for single-stranded oligonucleotides. The ratio between the readings at 260 nm and 280 nm (OD_{260}:OD_{280}) provides an estimate of the purity of the nucleic acid. Pure preparations of DNA and RNA have OD_{260}:OD_{280} values of 1.8 and 2.0, respectively. If there is significant contamination with protein or phenol, the OD_{260}:OD_{280} will be less than the values given above, and accurate quantitation of the amount of nucleic acid will not be possible.

Because it is rapid, simple, and nondestructive, absorption spectroscopy has long been the method of choice to measure the amount of DNA and RNA in concentrated pure solutions. However, absorption spectroscopy is comparatively insensitive and, with most laboratory spectrophotometers, nucleic acid concentrations of at least 1 µg/ml are required to obtain reliable estimates of A_{260}. In addition, absorption spectroscopy cannot readily distinguish between DNA and RNA, and it cannot be used with crude preparations of nucleic acids. Because of these limitations, a number of alternative methods have been devised to measure the concentration of DNA and RNA (please see Table A8-4).

OD_{260}:OD_{280} Ratios

Although it is possible to estimate the concentration of solutions of nucleic acids and oligonucleotides by measuring their absorption at a single wavelength (260 nm), this is not good practice. The absorbance of the sample should be measured at several wavelengths since the ratio of absorbance at 260 nm to the absorbance at other wavelengths is a good indicator of the purity of the preparation. Significant absorption at 230 nm indicates contamination by phenolate ion, thiocyanates, and other organic compounds (Stulnig and Amberger 1994), whereas absorption at higher wavelengths (330 nm and higher) is usually caused by light scattering and indicates the presence of particulate matter. Absorption at 280 nm indicates the presence of protein, because aromatic amino acids absorb strongly at 280 nm.

For many years, the ratio of the absorbance at 260 nm and 280 nm (OD_{260}:OD_{280}) has been used as a measure of purity of isolated nucleic acids. This method dates from Warburg and Christian (1942) who showed that the ratio is a good indicator of contamination of protein preparations by nucleic acids. The reverse is not true! Because the extinction coefficients of nucleic acids at 260 nm and 280 nm are so much greater than that of proteins, significant contamination with protein will not greatly change the OD_{260}:OD_{280} ratio of a nucleic acid solution (please see Table A8-5). Nucleic acids absorb so strongly at 260 nm that only a significant level of protein contamination will cause a significant change in the ratio of absorbance at the two wavelengths (Warburg and Christian 1942; Glasel 1995; Manchester 1995, 1996; Wilfinger et al. 1997).

TABLE A8-5 Absorbance of Nucleic Acids and Proteins

% Protein	% Nucleic Acid	OD_{260}:OD_{280}	% Protein	% Nucleic Acid	OD_{260}:OD_{280}
100	0	0.57	45	55	1.89
95	5	1.06	40	60	1.91
90	10	1.32	35	65	1.93
85	15	1.48	30	70	1.94
80	20	1.59	25	75	1.95
75	25	1.67	20	80	1.97
70	30	1.73	15	85	1.98
65	35	1.78	10	90	1.98
60	40	1.81	5	95	1.99
55	45	1.84	0	100	2.00
50	50	1.87			

Using the predicted values in this table, Glasel (1995) derived an empirical equation to describe $\%N$ for a range of OD_{260}:OD_{280} ratios: $\%N = F([11.16R - 6.32],[2.16 - R])$, where $R = OD_{260}$:OD_{280}. Note that estimates of purity of nucleic acids based on OD_{260}:OD_{280} ratios are accurate only when the preparations are free of phenol. Water saturated with phenol absorbs with a characteristic peak at 270 nm and an OD_{260}:OD_{280} ratio of 2 (Stulnig and Amberger 1994). Nucleic acid preparations free of phenol should have OD_{260}:OD_{270} ratios of ~1.2.