CHAPTER 1

Spectroscopic Methods for Matrix Characterization

1.1. INTRODUCTION

The objective of many bioassay methods is to selectively quantitate a single biomolecule, such as a particular enzyme or antibody, or to determine the presence or absence of a known DNA sequence in an unknown sample. Methods for these very selective assays will be considered in later chapters.

When faced with a true unknown, for example, during the isolation or purification of a biomolecule, it can be important to characterize the unknown matrix, or the components in the unknown solution that are present along with the species of interest. This involves the estimation of the total quantity of the different types of biomolecules.

Biochemists often estimate the total quantity of protein and nucleic acid in an unknown by the nomograph method.[1] In this method, the absorbance of the unknown solution in a 1-cm cuvette is measured at 260 and 280 nm. The nomograph (Fig. 1.1) is then used to estimate concentrations.

The nomograph method is rapid and involves no chemical derivatization, but suffers from several disadvantages. Interferences result from any species present in the unknown, other than protein or nucleic acid, that absorb at these wavelengths. Phenol, for example, which is used at high concentrations during the purification of nucleic acid, absorbs at both 260 and 280 nm and leads to overestimation of total nucleic acid. Furthermore, the nomograph method does not distinguish between deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), and is useful only for samples containing relatively high concentrations of protein and nucleic acid.

In this chapter, we will consider simple colorimetric and fluorometric methods for the quantitation of total protein, DNA, RNA, carbohydrate, and free fatty acid. All of the methods are based on the generation of a chromophore or fluorophore by a selective chemical reaction.

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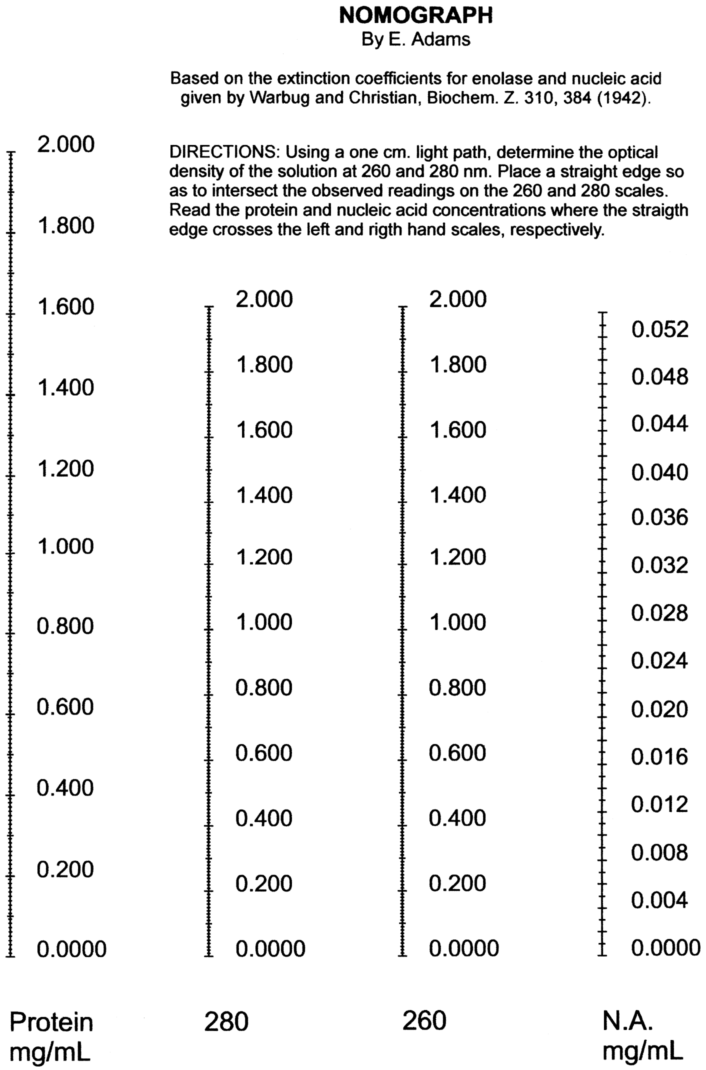


Figure 1.1. Nomograph used to estimate total protein and nucleic acid concentrations. [Reprinted, with permission, from Calbiochem, San Diego, CA.]

1.2. TOTAL PROTEIN

The three most common assays for total protein[2] are the Lowry (enhanced copper), Smith (bicinchoninic acid, BCA), and Bradford (Coomassie Blue) methods. All are colorimetric methods, and are based on the generation of absorbing species in proportion to the quantity of protein present in the sample. The ninhydrin assay is a recently reported promising method.

1.2.1. Lowry Method

The Lowry, or enhanced alkaline copper method, begins with the addition of an alkaline solution of Cu^{2+} to the sample. The copper forms a complex with nitrogen atoms in the peptide bonds of proteins under these conditions, and is reduced to Cu^{+}. The Cu^{+}, along with the R groups of tyrosine, tryptophan, and cysteine residues of the protein then react with the added Folin–Ciocalteau reagent, which contains sodium tungstate, sodium molybdate, phosphoric acid, and HCl (W^{6+}/Mo^{6+}). During this reaction, the Cu^{+} is oxidized to Cu^{3+}, which then reacts with the peptide backbone to produce imino peptide (R_1—CO—N=C(R_2)—Co—R_3) plus Cu^{2+}, and the Folin reagent is reduced to become molybdenum–tungsten blue. Absorbance is measured in glass or polystyrene cuvettes at 720 nm, or if this value is too high (>2), at 500 nm.

A calibration curve obtained with standard protein solutions [e.g., bovine serum albumin (BSA)] is used to obtain total protein in the unknown. Under optimum conditions, and in the absence of reactive side chains, it has been shown that two electrons are transferred per tetrapeptide unit; however, proteins, with significant proline or hydroxyproline content, or with side chains that can complex copper (such as glutamate) yield less color. The side chains of cysteine, tyrosine and tryptophan contribute one, four and four electrons, respectively.[3] Note that different proteins will produce different color intensities, primarily as a result of different tyrosine and tryptophan contents.

With reagents prepared in advance, the Lowry assay requires ~ 1 h. A 400-μL sample is required, containing 2–100-μg protein (5–250 μg/mL). Nonlinear calibration curves are obtained, due to decomposition of the Folin reagent at alkaline pH following addition to the sample that results in incomplete reaction. Interferences include agents that acidify the solution, chelate copper, or cause reduction of copper(II).

1.2.2. Smith (BCA) Method

The Smith total protein assay is also based on the initial complexation of copper (II) with peptides under alkaline conditions, with reduction to copper(I). The ligand BCA is then added in excess, and the purple color (562-nm peak absorbance) develops upon 2:1 binding of BCA with Cu^{+} (Fig. 1.2).

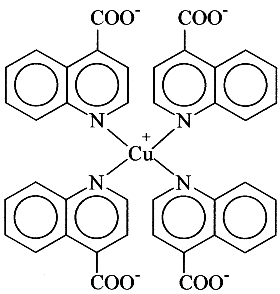


Figure 1.2. Complex formed upon reaction of bicinchoninic acid with Cu^{+}.

The Smith assay takes ~ 1 h, and requires a minimum volume of 500 μL with 0.2–50-μg protein (0.4–100 μg/mL). As with the Lowry assay, calibration curves are nonlinear, with negative deviation at high protein concentration. Interferences include copper reducing agents (such as reducing sugars) and complexing agents as well as acidifying agents in the unknown sample. Common membrane lipids and phospholipids have also been shown to interfere, and an apparent protein concentration of 17 μg of protein (as BSA) was found for 100 μg of 1,3-dilinoleoyglycerol.[4] Since BCA is a stable reagent at alkaline pH, it can be added to the alkaline copper reagent, so that only one reagent addition is required. The Smith assay has been adapted for 96-well microtiter plates,[5] and a linear dependence of absorption (570 nm) on protein concentration was observed over the 1–10-μg/mL concentration range, with 10-μL sample volumes.

1.2.3. Bradford Method

The Bradford method is based on the noncovalent binding of the anionic form of the dye Coomassie Blue G-250 with protein.[6] The dye reacts chiefly with arginine residues, which have a positively charged side chain, and slight interactions have also been observed with basic residues (histidine and lysine) and aromatic residues (tyrosine, tryptophan, and phenylalanine). In the absence of protein, the dye reagent is a pale red, and upon binding to protein, a blue color is generated with an absorbance maximum at 590 nm. The structure of the dye is shown in Figure 1.3.

The Bradford assay is very popular because it is rapid (5 min) and involves a single addition of the dye reagent to the sample. It provides a nonlinear calibration curve of A_{590} against concentration over the 0.2–20-μg range of total protein contained in a 20-μL sample volume (10–1000 μg/mL). Negative deviation from linearity occurs with this method as with the Lowry and Smith methods. In the Bradford assay, curvature is due to depletion of free dye at high protein concentration, and a better approximation of linearity can be achieved by plotting A_{595}–A_{465} against concentration, to take dye depletion into account.

The Lowry and Bradford methods have been compared for protein quantitation in samples containing membrane fractions.[7] While the Lowry method yielded reproducible results over 2 weeks of sample storage at −20 °C, the Bradford method was shown to significantly underestimate membrane proteins, even after

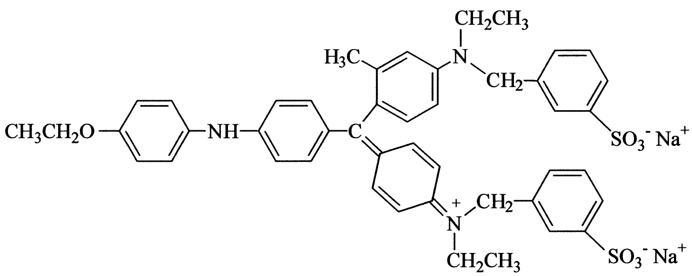


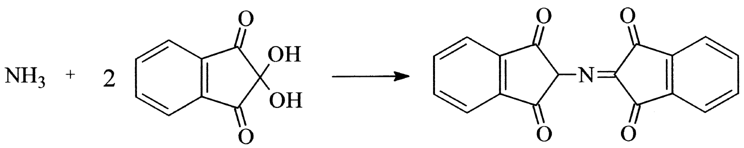
Figure 1.3. Coomassie Blue G-250, used in the Bradford total protein assay.

treatment with base or surfactants, with lower estimates occurring after longer periods of sample storage. Tissue homogenates from rat brain assayed by Bradford method initially showed 52% of the protein estimated by the Lowry method; after 14-days storage, this value had decreased to ~ 30%. Since membrane lipids and phospholipids interfere with the Smith total protein assay, membrane proteins should be quantitated by the Lowry method, or by the new ninhydrin method described below.

1.2.4. Ninhydrin-Based Assay[8]

This method is based on the quantitation of total amino acids following acid hydrolysis of proteins present in tissue samples. Microtiter plates are used in this assay. Tissue samples (10 μg) are first hydrolyzed in 500 μL of 6 *M* HCl at 100 °C for 24 h to liberate ammonium. The samples are then lyophilized (chilled and evaporated), and the residue, containing ammonium chloride, is dissolved in a known volume of water.

Ninhydrin reagent, containing ninhydrin, ethylene glycol, acetate buffer, and stannous chloride suspension, initially a pale red color, is added to 1–10 μg of protein hydrolysate in a flat-bottom microtiter plate. During the 10-min incubation at 100 °C, ammonia reacts with the ninhydrin reagent to produce diketohydrindylidene-diketohydrindamine (Eq. 1.1).



(1.1)

Under these conditions, the product exhibits a broad absorption band between 560 and 580 nm, and a microtiter plate reader set at 575 nm may be used for measurement. Correlation of absorbance with protein concentration has been performed using several protein standards, as shown in Figure 1.4, which also shows results obtained with the same protein standards by the Bradford method. These data demonstrate much better sensitivity with the ninhydrin method, and suggest an ~ 10-fold improvement in detection limit. An important advantage of this method is that the differences in calibration curves obtained using different protein standards are relatively small. Interferences include free amino acids as well as other compounds containing amine groups.

1.2.5. Other Protein Quantitation Methods

The method of nitration has been reported for protein quantitation. In this method, aromatic amino acid residues are treated with nitric acid, and the products of nitration absorb at 358 nm. In the case of tyrosine, nitration occurs at the 3-position of the aromatic ring, producing 3-nitrotyrosine. This method has been compared to the Smith and Bradford assays using BSA standards, and practically identical results were obtained, as shown in Figure 1.5. The detection limit of this assay is 5 μg, and known interferences included Triton X-100 and phenolic compounds.[9]

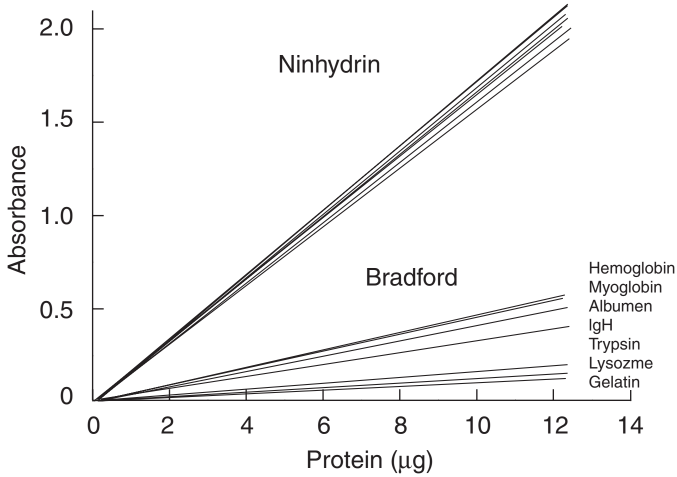


Figure 1.4. Comparison of results of ninhydrin and Bradford methods for total protein concentration in tissue samples. Proteins in both assays are the same.[8] [Reprinted, with permission, from B. Starcher, *Anal. Biochem.* **292**, 2001, 125–129. "A Nirhydrin-Based Assay to Quantitate the Total Protein Content of Tissue Samples." Copyright © 2001 by Academic Press.]

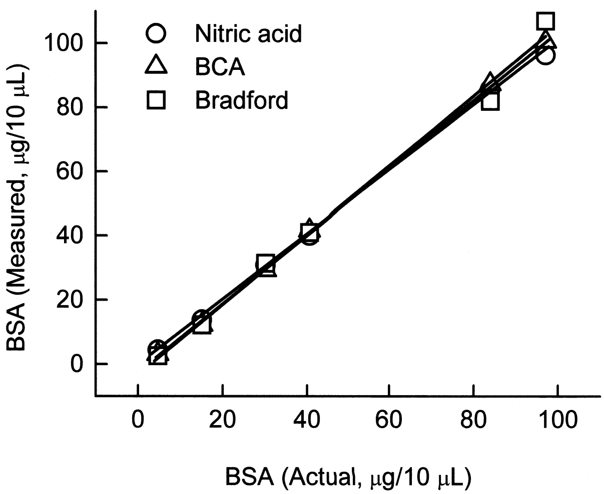


Figure 1.5. Relationship between actual and measured BSA concentration in six samples, showing that this method produces comparable results to the Bradford and BCA total protein assays. [Reprinted, with permission, from K. C. Bible, S. A. Boerner, and S. H. Kaufmann, *Anal. Biochem.* **267**, 1999, 217–221. "A One-Step Method for Protein Estimation in Biological Samples: Nitration of Tyrosine in Nitric Acid." Copyright © 1999 by Academic Press.]

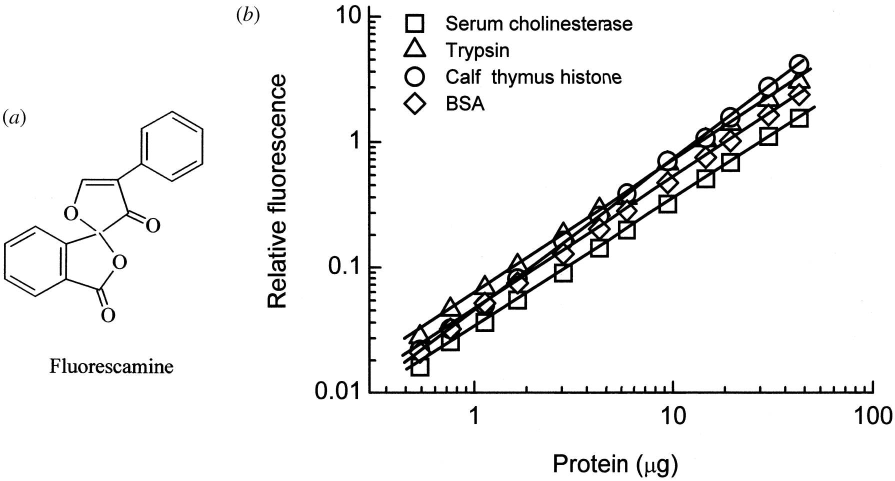


Figure 1.6. (*a*) Chemical structure of fluorescamine, and (*b*) calibration curves obtained for different proteins. [Reprinted, with permission, from P. Böhlen, S. Stein, W. Dairman, and S. Undenfriend, *Archives of Biochemistry and Biophysics* **155**, 1973, 213–220. "Fluorometric Assay of Proteins in the Nanogram range." Copyright © 1973 by Academic Press, Inc.]

An older method employing fluorescamine may be used if improved detection limits are required. In this method, fluorescamine (Fig. 1.6) reacts with the primary amine groups of the protein, generating flourophores that can be excited at 390 nm to generate emission at 475 nm. Figure 1.6 also shows calibration curves for a variety of proteins; this method is capable of detecting as little as 10-ng total protein.[10]

Table 1.1 summarizes detection limits and interferences for the five protein assay methods described here.

1.3. TOTAL DNA

Several methods are available for the colorimetric or flourometric determination of total DNA. The first two that will be considered involve depurination of DNA under acidic conditions, and are followed by chemical reactions with 3,5-diaminobenzoic acid (DABA) or diphenylamine (DPA). Two fluorescence methods based on selective, noncovalent interactions of a probe molecule with DNA will then be considered.

1.3.1. Diaminobenzoic Acid Method

Samples containing DNA are incubated in 1 *N* perchloric acid for 10 min. Under these conditions, DNA is depurinated, deoxyribose is released, and converted to ω-hydroxylevulinylaldehyde. An equal volume of 1.3 *M* DABA dihydrochloride is

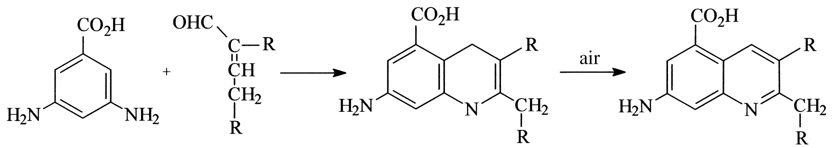
TABLE 1.1. Detection Limits and Interferences for Protein Assay Methods

Assay	Detection Limit	Interferences[a]
Lowry	2 μg	Acidifiers, Cu chelating agents (e.g., high phosphate concentration), Cu^{2+} reducing agents (e.g., reducing sugars, thiols), Tris, HEPES, EDTA, neutral detergents
Smith	0.2 μg	Acidifiers, Cu chelating, reducing agents, Tris, ammonium sulfate, EDTA, lipids + phospholipids. (Neutral detergents and SDS do not interfere)
Bradford	0.2 μg	Lipids + phospholipids, neutral detergents, SDS
Ninhydrin	0.02 μg	Amino acids, amine-containing compounds
Nitration	5 μg	Triton X-100, phenolic compounds. (Tris, SDS, urea, thiols, reducing agents, glycerol, ammonium sulfate, neutral detergents do not interfere)
Fluorescamine	10 ng	Primary amine groups, secondary amines at high concentration, TRIS, ammonium sulfate (Phosphate does not interfere)
Ninhydrin	0.02 μg	Free amino acids and other compounds containing amine groups

[a]Tris = tris(hydroxymethyl)aminomethane; HEPES = *N*-(2-hydroxyethyl)piperazine-*N*′-ethanesulfonic acid; SDS = sodium dodecyl sulfate; EDTA = ethylenediaminetetraacetic acid.

added and the solution is heated at 60 °C for 30 min. Under these conditions, the following reactions (Eqs. 1.2 and 1.3) occur:[11]

$$R-CH_2-CHO \longrightarrow R-CH_2-CH{=}\overset{\displaystyle R}{\overset{|}{C}}-CHO \qquad (1.2)$$

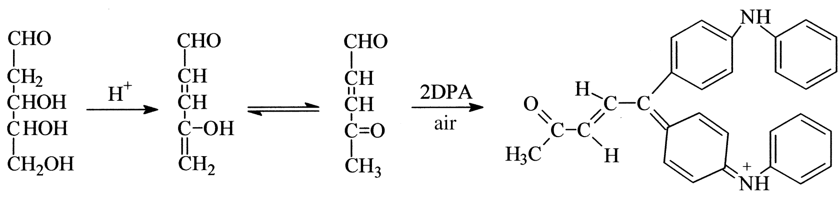


(1.3)

Following a 10-fold dilution of the sample with 0.6 *M* perchloric acid, the product of this reaction absorbs maximally at 420 nm. The original assay, developed by Kissane and Robins,[12] uses DABA dihydrochloride for depurination (no perchloric acid was used) and employs fluorescence measurements at 520 nm after excitation at 420 nm. More recent work shows that the assay with perchloric acid can be conducted with 10-μL DNA sample volumes and provides a linear fluorescence calibration curve over the 10–500-ng total DNA range (1–50 μg/mL).[13] The DABA method has since been modified for colorimetric measurements at 420 nm, and has a detection limit of 25 μg.[14] The DABA reaction is specific for DNA, but measurements are affected by the presence of lipids, saccharides, salts, and the surfactant Triton X-100, these species can be removed form DNA samples by ethanol precipitation.[15]

1.3.2. Diphenylamine Method

The DPA method is based on the colorimetric measurement of products formed by reaction of ω-hydroxylevulinylaldehyde (from the deoxyribose released after depurination of DNA) with diphenylamine in 1 *M* perchloric acid.[14–16] The reaction yields a mixture of products, such as that shown in Eq. 1.4,[17] which together have an absorbance maximum at 595 nm. This method has a similar dynamic range to the DABA assay with colorimetric measurement. Interferences include proteins, lipids, saccharides, and RNA.



(1.4)

1.3.3. Other Fluorometric Methods

Sensitive fluorometric assays for double-stranded (native) DNA in tissue extracts are based on the noncovalent interaction of intercalating dyes (Fig. 1.7 such as ethidium bromide, 4′,6′-diamidino-2-phenylindole (DAPI), or 2-(2-[4-hydroxyphenyl]-6-benzimidazolyl)-6- (1-methyl-4-piperazyl)benzimidazol trihydrochloride (Hoechst 33258), with double-stranded DNA in neutral, aqueous solutions.

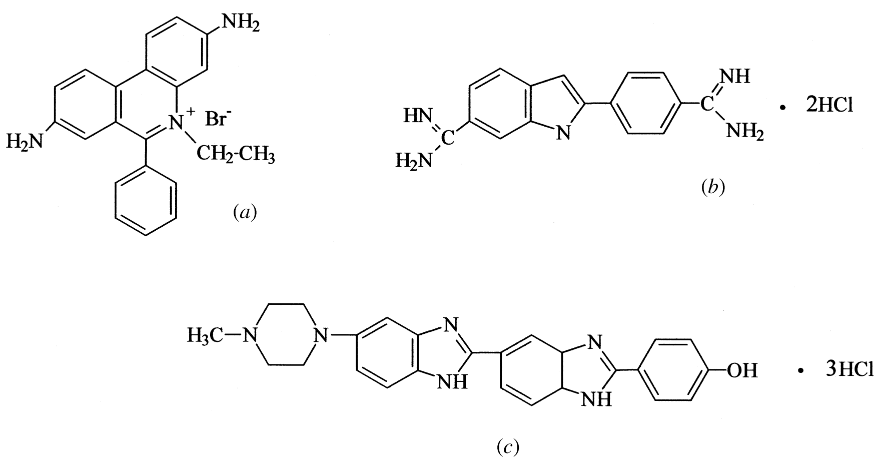


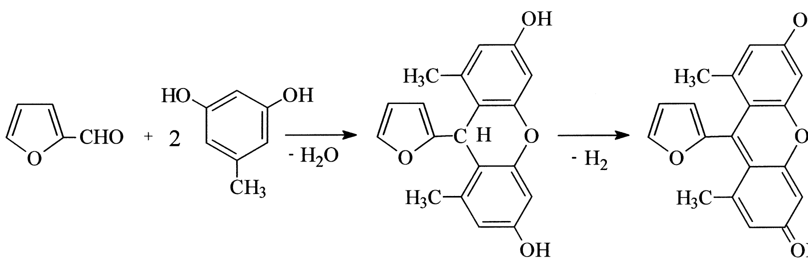
Figure 1.7. DNA-intercalating dyes: (*a*) ethidium, (*b*) DAPI, and (*c*) Hoechst 33258.

When free in aqueous solution, these dyes exhibit limited fluorescence, but upon binding DNA, their fluorescence increases markedly. Ethidium binds to RNA and DNA while DAPI and Hoechst 33258 selectively interact with DNA. Hoechst 33258 at a final concentration of 1 µg/mL in 0.05 *M* phosphate, pH 7.4, with 2 *M* NaCl has been used to quantitate as little as 10-ng double-stranded DNA.[18] Because these dye-binding methods involve intercalation between the bases of double-stranded DNA they are not useful for the quantitation of single-stranded DNA.

Single-stranded DNA containing deoxyguanosine residues can be quantitated in the presence of terbium(III). Ten micromolar Tb^{3+} in a pH 6 cacodylate buffer shows no detectable fluorescence, with excitation at 290 nm and emission measured at 488 nm. In the presence of single-stranded DNA, Tb^{3+} coordinates with deoxyguanosine-5-phosphate nucleotides, and a linear dependence of fluorescence on concentration has been observed over the 1–10-µg/mL range of thermally denatured rat liver DNA.[19]

1.4. TOTAL RNA

The only method used to quantitate ribonucleic acid is the orcinol assay.[20] With this method, RNA is depurinated in concentrated HCl and the resulting ribosephosphates are dephosphorylated and dehydrated to produce furfural (Eq. 1.5). Furfural then reacts with orcinol in the presence of Fe^{3+} to yield colored condensation products, as shown in Eq. 1.4, which together possess an absorption maximum at 660 nm.



(1.5)

The DNA also undergoes a limited reaction under these conditions, and yields ~ 10% of the color of a similar concentration of RNA; the dehydration step to form furfural is not readily accomplished by 2-deoxyribose.

The original orcinol assay has been modified to improve its selectivity toward RNA over DNA and sugars. The improved method[21] uses a 1.0-mL RNA sample incubated 24 h at 40 °C with 4.0 mL of 85% sulfuric acid prior to addition of the orcinol reagent (containing no Fe^{3+}). Under these conditions, colorimetric measurements are made at 500 nm, where products formed by the reaction of levulinic acid (from DNA) with orcinol do not absorb. Proteins (e.g., BSA) do not interfere, and the method is sixfold more selective to RNA over DNA than the original orcinol assay.

1.5. TOTAL CARBOHYDRATE

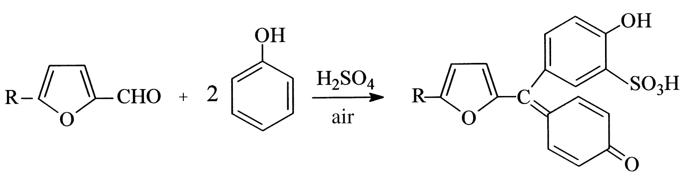
Methods reported for the quantitation of reducing sugars, that is, those that possess a terminal aldehyde group, involve redox reactions with oxidizing agents such as Cu^{2+} (in the presence of BCA) or $Fe(CN)_6{}^{3-}$. Nelson reagents (alkaline copper sulfate and solutions of molybdate and arsenate in dilute sulfuric acid that reduce to molybdenum blue),[22] or 2-cyanoacetamide.[23] The Cu^{2+}-BCA assay has been described earlier for protein quantitation, and the method used for quantitation of reducing carbohydrates is almost identical to the total protein method.[24] Methods for total carbohydrate determination employ strong acids to affect hydrolysis of oligosaccharides and polysaccharides, and dehydration to reactive species such as furfural. These reactive intermediates form chromophores in the presence of phenol, 2-aminotoluene[25] or anthrone[26] and fluorophores with 2-aminothiophenol. We will consider the ferricyanide method for reducing sugars, the phenol-sulfuric acid and 2-aminothiophenol methods for total carbohydrate, and the purpald assay for bacterial polysaccharides.

1.5.1. Ferricyanide Method

This assay employs an alkaline solution of potassium ferricyanide (2.5 mg/mL in phosphate buffer) as the only reagent. A reducing sugar solution of 80 μL is combined with 20 μL of this reagent. The mixture is heated in a sealed tube to 100 °C for 10 min. Following the addition of 300 μL of cold water, absorbance is measured at 237 nm, which is the wavelength of maximum absorbance for ferrocyanide. This method allows the quantitation of mannose over the 1–25-nmol (~ 1.3–30-μg/mL) range of reducing monosaccharides. Tryptophan and tyrosine residues of proteins were found to interfere significantly with the assay, but the extent of interference can be quantitated by measuring A_{237} following an initial incubation at 40 °C, where these residues reduce ferricyanide, but reducing sugars remain inert. This method was developed to determine the carbohydrate content of glycoproteins.[27]

1.5.2. Phenol–Sulfuric Acid Method

In concentrated sulfuric acid, polysaccharides are hydrolyzed to their constituent monosaccharides, which are dehydrated to reactive intermediates. In the presence of phenol, these intermediates form yellow products, such as that shown in Eq. 1.6,[28] with a combined maximal absorbance at 492 nm.

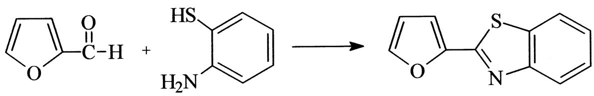


(1.6)

The phenol–sulfuric acid method has been adapted for use with 96-well microtiter plates. Carbohydrate samples are combined with an equal volume of 5% (w/v) phenol in the wells of a microtiter plate, the plate is placed on a bed of ice and 0.125 mL of concentrated sulfuric acid is added to each well. Following incubation of the covered plate at 80 °C for 30 min and cooling to room temperature, the absorbance of each well measured at 492 nm. This method shows good linearity for maltose over the 0.2–25-μg range, using an initial sample volume of 25 μL (10–200 μg/mL).[24]

1.5.3. 2-Aminothiophenol Method

In this assay, furfural and related monosaccharide dehydration products formed in strong acid are reacted with 2-aminothiophenol to form highly fluorescent 2-(2-furyl)benzothiazole and related species (Eq. 1.7).



(1.7)

The reagent solution contains 0.4% (w/v) 2-aminothiophenol, and is prepared by combining equal volumes (0.34 mL) of aminothiophenol and ethanol, and diluting to 100 mL with 120 m*M* HCl. Samples (0.5 mL) are combined with reagent (0.2 mL) and 30% (w/v) H_2SO_4 (0.5 mL) in sealed vials, heated to 150 °C for 15 min, cooled, and diluted by the addition of 0.8-mL water.

Fluorescence is measured at 411 nm with excitation at 361 nm. The assay can easily detect 50 ng of carbohydrates such as galactose, glucose and xylose, and pentoses such as arabinose are detectable at the 10-ng level. Calibration curves show slight curvature, with negative deviations from linearity. Most of the carbohydrates studied yielded useful curves over the 50–600-ng range (0.5–6 μg/mL).[29] Interferences from surfactants (0.5% w/v) such as SDS and Triton X-100 are significant and are thought to alter the polarity of the environment of the fluorophores. Tris buffers and high protein concentration (e.g., 0.1-mg/mL BSA) should also be avoided, unless blanks containing similar concentrations of these species can be prepared.

1.5.4. Purpald Assay for Bacterial Polysaccharides

Polysaccharides containing substituted or unsubstituted glycols in residues such as glycerol, ribitol, arabinitol, furanosyl galactose, and sialic acid may be quantitated by reaction with sodium periodate, follow by reaction with the purpald reagent, as shown in Figure 1.8. The formaldehyde produced in the first step reacts with the purpald reagent, and the product of this reaction is further oxidized by periodate to form a purple product with an absorbance maximum at 550 nm. The limit of detection has been reported as 15 μg/mL using the native polysaccharide from *Salmonella pneumoniae*.[30]

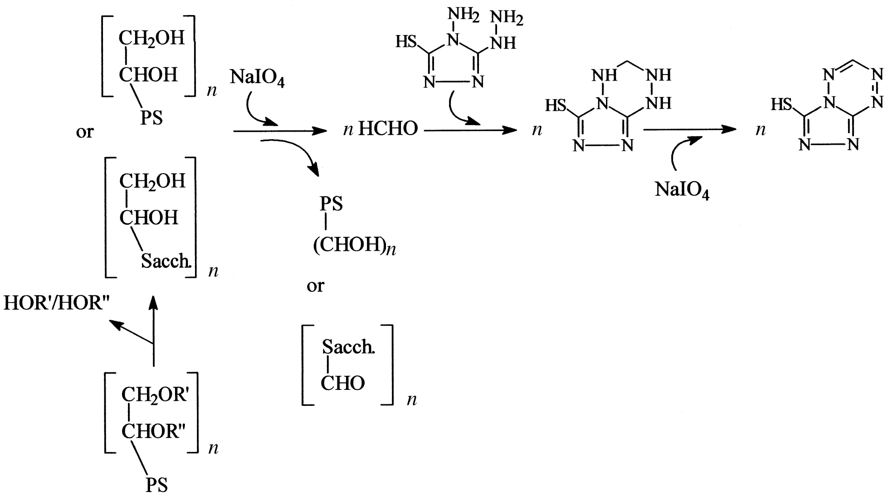


Figure 1.8. Chemistry of the purpald assay for bacterial polysaccharides (PS). The substituent groups R′ and R″ must be released during treatment with NaOH and H_2SO_4, before the periodate reaction.

1.6. FREE FATTY ACIDS

Most methods for the quantitation of free fatty acids involve chemical derivatization followed by gas chromatography–mass spectrometry. However, a colorimetric method is available for the quantitation of long-chain ($C > 10$) free fatty acids in plasma, and is based on the color developed by cobalt soaps of free fatty acids dissolved in chloroform.[31]

The assay involves four solutions: (1) chloroform:heptane:methanol (4:3:2 by volume); (2) 0.035 *M* HCl; (3) the salt reagent, consisting of 8 mL of triethanolamine added to 100 mL of an aqueous salt solutions containing 20 g Na_2SO_4, 10 g Li_2SO_4, and 4 g $Co(NO_3)_2.6H_2O$; and (4) the indicator solution consisting of 20-mg 1-nitroso-2-naphthol in 100 mL of 95% ethanol.

The procedure uses 100-μL samples (heptane blank, standard, or plasma sample) combined with 4.0 mL of chloroform/heptane/methanol and 1.0 mL 0.035 *M* HCl in sealed vials. After mixing and centrifugation steps, the upper aqueous methanol phase is discarded, and 2.0 mL of the salt reagent is added and mixed. A second centrifugation step is followed by combining 2.0 mL of the upper phase with 1.0 mL of the indicator solution. After 20-min absorbance is measured at 435 nm.

Linear calibration curves were obtained for palmitic acid over the 0.10–1.2-μ*M* concentration range, with few interferences. Protein (2% BSA) and many major metabolites such as lactic acid or β-hydroxybutyrate do not interfere, but some color development is observed for high concentrations of phospholipids such as lecithin.

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PROBLEMS

1. Total protein in an unknown sample was estimated using the Lowry and Bradford assays. Results were 33 ± 2 μg/mL from the Lowry assay and 21 ± 1 μg/mL from the Bradford assay, using stock solutions of BSA as standards in each assay. The unknown sample was then thoroughly oxygenated, and the assays were repeated. The Lowry method yielded 22 ± 1 μg/mL, while the Bradford results did not change. Why did the results of the Lowry assay decrease by 33%?

2. The Lowry, Smith, and Bradford assays provide an estimate, rather than an exact measure of total protein. Why? What is the importance of the standard protein chosen for the construction of calibration curves?

3. An analyst suspects that an unknown sample containing double-stranded DNA also contains a significant quantity of carbohydrate. The DABA assay for total DNA yields a concentration of 44 ± 3 μg/mL, while the ferricyanide assay for reducing carbohydrate, conducted on an 10-fold dilution of the unknown sample, yielded a result of 1.8 ± 0.3 μg/mL. The formula weight of one deoxynucleotide monophosphate residue of DNA is ~ 330 g/mol, while monosaccharides have a molecular weight of ~ 180 g/mol. Assuming that interference in the DABA assay is due solely to reducing monosaccharides, and that one monosaccharide molecule yields the same absorbance as one deoxynucleotide monophosphate in the DABA assay, calculate the actual quantity of DNA in the sample. Suggest a more selective assay for double-stranded DNA that could be used to confirm this value.