Protein Quantification and Detection methods

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1. Protein quantification and detection methods

Many applications depend on the detection and quantification of proteins to follow purification and for further assays. Although in practice the biological and biochemical activity is more important (1), many systems are designed to measure protein concentration accurately, precisely, robustly and specifically. All these systems have their strengths and weaknesses (2, 3). A perfect protein determination assay reagent is yet to be developed. However, most researchers would agree on these characteristics:

- Non destructive
- Fast
- Easy to perform
- Sensitive
- Accurate
- Precise
- Tolerant on interfering substances

1.1. Assay characteristics

1.1.1. Interfering substances

Virtually every protein detection method known exhibits a particular sensitivity to the presence of biochemical reagents in the protein sample. Proteins are typically found in solution with detergents, buffer salts, denaturants, reducing agents, chaotropic agents or antimicrobial preservatives, depending on the preparation technique used to obtain the protein. When a component of a protein solution attenuates or artificially enhances the response of any assay, the component is considered to be an *interfering substance.* Substances that do not affect, or marginally affect, the results of an assay are known as *compatible substances.* Interfering substances can affect the assay of protein in one of the following ways:

1. They can suppress the response of an assay.
2. They can artificially enhance the response of an assay.
3. They can result in an elevated background reading.

Interference from many common substances can be compensated for with the *blank* designed for the specific assay. When only a “rough” estimate of protein is needed, a *blank-only* correction can be used. In this case, a blank is made up in the diluent of the unknown protein to correct for the raw diluent absorbance. The concentration is then calculated using the protein extinction coefficient.

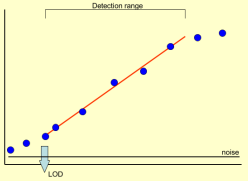
When a precise estimate is needed, a standard curve must be made in the same diluent that is used for the unknown protein. The concentration of the unknown protein is then determined from the standard curve.

Often, interfering substances can overwhelm the assay, making it difficult or impossible to perform. In these situations, the interfering substance can be removed by precipitation of the protein with trichloroacetic acid or acetone

before the assay is performed. Gel filtration chromatography or dialysis, performed prior to sample assay, can also be used to remove these interfering substances. When developing a protein assay, it is considered essential to identify those substances that are compatible with, or that may interfere with, the assay. Characterizing the assay in this manner helps to further define the utility of the test (8).

1.1.2. Detection limits

The minimum detection limit of a protein assay is the lowest concentration of the protein that can be accurately measured. This detection limit can be determined by measuring the response of a series of blanks made up in the same diluent in which the sample is prepared. The blank value, plus three times the standard deviation of the blank, represents the minimal sample value that can be detected above background. It should be noted that any interfering substance that increases the blank value also decreases the detectability of the assay by decreasing the signal-to-noise ratio test (8).



Detection limits

The maximum detection limit of a protein assay is the highest concentration that can be accurately measured. In general the maximum detection limit is at the end of the linear detection range.

1.1.3. Protein-to-Protein variability

All proteins are unique and can demonstrate their individuality in many ways. Each of the commonly used assay methods exhibit some degree of varying response toward different proteins. These differences relate to dissimilarity among proteins due to amino acid sequence, pI, structure and the presence of certain side chains or prosthetic groups that can dramatically alter the protein assay response. Most assays use bovine serum albumin (BSA) or immunoglobulin (IgG) as the standard protein against which to measure the concentration of a sample protein. Using these proteins as standards works well with most protein assay methods. However, if absolute accuracy is required, the standard curve must be prepared from a pure sample of the target protein. If a pure sample of the target protein is not available, select the standard protein that generates a colour response in the assay close to that of the target protein test (1, 8).

1.2. Detection methods

Most protein determination procedures can be grouped in four major techniques:

- Spectroscopic methods
- Colorimetric methods
- Dye binding methods
- Weighing

1.3. Spectroscopic methods

The absorbance technique is based on the absorption of light by liquid containing proteins. The ratio between the incident (P_0) and the transmitted (P) light is called the transmittance (T). The absorbance (A) is defined as 10 log of the reciprocal of the transmittance (7, 1).

$$T = \frac{P}{P_0} \qquad A = -\log T \tag{1.1}$$

In a spectrophotometer, mono-chromatic plane-parallel light enters the sample at right angles to the plane-surface of the sample. Under these conditions, the absorbance depends on the protein sample (ε), concentration (c) and the light path length (L) through the sample. The extinction coefficient (ε) is an intrinsic property unique for each protein. The absorbance A is defined via the Beer-Lambert law:

$$A_\lambda = \varepsilon\, c\, L \tag{1.2}$$

Because the concentration is normally in Molar and the path length is commonly 1 cm the ε is in $M^{-1}cm^{-1}$ (or L $mol^{-1}cm^{-1}$) and named the Molar extinction coefficient (ε^{1M}_{280nm}). The molar extinction coefficient is generally reported for the wave length of 280 nm.

A similar extinction coefficient is the absorbance of 1% solutions ($\varepsilon^{1\%}_{280nm}$). The relation between the molar and the 1% extinction coefficient is as follows:

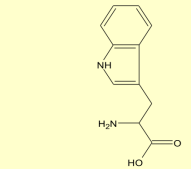
$$\varepsilon^{1M}_{280\ nm} = \left(\frac{\varepsilon^{1\%}_{280nm} \cdot Mr}{10} \right) (g^{-1}\ L\ cm^{-1}) \tag{1.3}$$

Most protein extinction coefficients ($\varepsilon^{1\%}_{280nm}$) fall in the range of 4.0-24.0 (7).

The absorbance method is rapid and non destructive. However, because the absorbance is not only limited to the proteins in solution, but also to the additives, an appropriate reference should be measured to correct for solvent effects.

Any absorbance calculation assumes that the peptide chromophores are exposed and contribute equally to the signal. It can be necessary to measure concentrations under denaturing conditions with for instance 6 M Guanidine HCl or 8 M urea.

A brief reading of the cited articles (2, 6) indicates that there is no single correct extinction coefficient value for a complex molecule like a peptide or protein. Even minor differences in buffer type, ionic strength and pH



Tryptophan

affects absorption values at least slightly. Most protein preparations, even those of equal purity, differ slightly in conformation and extent of modifications, such as oxidation, and these also affect absorption. Therefore, the best extinction coefficient value is one that is determined empirically using a solution of the study protein of known concentration dissolved in the same buffer as the sample.

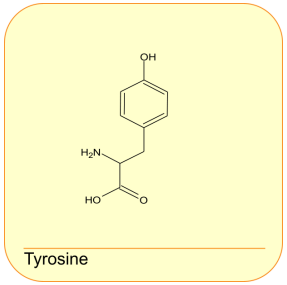
The extinction coefficient of a protein at 280 nm depends almost exclusively on the number of aromatic residues, particularly tryptophan and tyrosine and can be predicted from the sequence of amino acids. In addition cystine (cysteine does not absorb appreciably at wavelengths >260 nm) contributes a little to the absorbtion at 280 nm. Cystine is the amino acid formed when of a pair of cysteine molecules are joined by a disulfide bond.

Table: Molar extinction coefficients of chromophoric residues at 280 nm at neutral pH using a 1-cm cell.

Amino acid			**ε (cm^{-1} M^{-1})***			**Mr (Da)**
Tryptophan	Trp	W	5690	5500	5520	204
Tyrosine	Tyr	Y	1280	1490	1457	181
Cystine	Cys-Cys		120	125	173	121 (cysteine)

In 6.0 M guanidin hydrochloride, 0.02 M phosphate buffer, pH 6.5. (2)
* from ref (2), Pierce, (6).

The extinction coefficient of each chromophore in the peptide sequence is generally considered to be additive, that is, the overall molar extinction coefficient of the peptide depends on the types and number of these chromophoric residues in the sequence.



Tyrosine

Absorption coefficients (i.e., extinction coefficients) for many proteins have been compiled from the literature or reported in the Practical Handbook of Biochemistry and Molecular Biology 5. In addition, extinction coefficients can be calculated using the exact protein sequence on www.expasy.org. These values provide sufficient accuracy for most routine laboratory applications that require an assessment of protein concentration.

Most spectroscopes measure the absorbance between the values 0.000 and 3.999. However, measurements close to the extremes of the instrument are not desirable. In addition, depending on the wavelength used (type of absorbing components), there will be an optimal range to measure in. Concentrate or dilute the sample accordingly to obtain accurate results.

Table: Rough extinction coefficients

	Units	**Proteins**	**Immunoglobulins**
$\varepsilon^{1\,M}_{A280nm}$	$M^{-1}cm^{-1}$	Mr dependent	150000 Da
$\varepsilon^{1\%}_{A280nm}$	$(g/100\ ml)^{-1}cm^{-1}$	10	14
$\varepsilon^{1\,mg/ml}_{A280nm}$	$(mg/ml)^{-1}cm^{-1}$	1	1.4

1.3.1. BSA and immunoglobulin standards

Two proteins are commonly used to make a standard curve to compare to the unknown sample: Bovine serum albumin and mammalian immuno-globulin (7). The Pierce Company provides a calibrated BSA standard of 2 mg/ml in 0.9% NaCl. The $\varepsilon^{1\%}_{280nm}$ of this standard is equal to 6.67. The absorbance of the 2 mg/ml solution is thus:

$$A = \frac{\varepsilon \cdot C \cdot l}{10} = \frac{6.67 \cdot 2.000 \cdot 1}{10} = 1.334 \qquad (1.4)$$

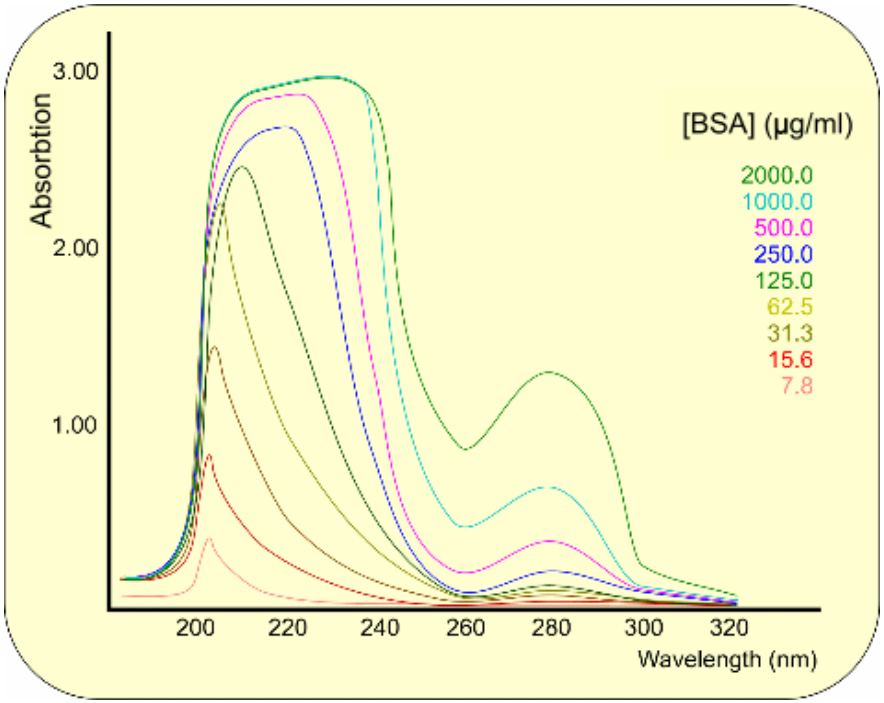


Figure: Spectroscopic wavelength scan (190 – 320 nm) of BSA.

1.3.2. Measurement at 280 nm

The absorption at 280 nm is mainly due to the phenolic group of tyrosine and the indolic group of tryptophan. It depends on the specific extinction coefficient of the protein. Detection limits are between 0.05 – 2 mg/ml protein. Interfering compounds are detergents, nucleic acids and particles. The advantage is that it is non destructive and rapid but relatively inaccurate. For measuring at 280 nm and below, quartz cuvettes are needed (1).

$$\text{protein (mg ml}^{-1}) = \frac{A_{280}}{\varepsilon_{280\,nm}^{1\,mg/ml}} \qquad (1.5)$$

1.3.3. Measurement at 280 – 260 nm

The absorption at 280 nm is mainly due to the phenolic group of tyrosine and the indolic group of tryptophan but the measurement at 260 nm can correct for nucleic acid contamination (1).

$$\text{protein (mg ml}^{-1}) = \frac{1.55\ A_{280\,nm} - 0.76\ A_{260\,nm}}{\varepsilon_{280\,nm}^{1\,mg/ml}} \qquad (1.6)$$

1.3.4. Measurement in the far UV

The absorption of light at 191 – 194 nm is mainly due to the peptide bonds. The sensitivity is 0.01 – 0.05 mg/ml protein. The quartz cuvettes and buffers must be clean. This methods is less dependent on amino acid composition but not feasible without special equipment due to UV absorption by oxygen (1).

1.3.5. Measurement at 205 nm

The absorption at 205 nm is mainly due to peptide bonds but at 205 nm the absorption by oxygen is minimal. Therefore the method is almost independent of the protein composition. The extinction coefficient of proteins at A_{205} nm appears to be between 30 - 35 ml.mg^{-1}.cm^{-1} (4, 9). In general a $\varepsilon_{205\,nm}^{1\,mg/ml}$ of 31 is used. The sensitivity is between 1 – 100 µg/ml protein.

The peptide bonds absorb very strongly but the linearity range of the absorption curve is less than with the $A_{280\,nm}$. Measurements with an $A_{205\,nm}$ absorbance above 2.00 are inaccurate.

$$\text{protein (mg ml}^{-1}) = \frac{A_{205\,nm}}{31} \qquad (1.7)$$

At the wavelength of 205 nm the amino acids tryptophan, phenylalanine, tyrosine and histidine can make a significant contribution to the total absorption. Arginine, methionine and cysteine have a moderate contribution to the absorption (9). With a correction for the absorption of tyrosine and tryptophan content, a good estimation of the protein extinction coefficient can be made (1, 4, 9):

$$\varepsilon_{205\,nm}^{1\,mg/ml} = 27.0 + 120 \times (A_{280}/A_{205}) \qquad (1.8)$$

For the measurement of the $A_{205\,nm}$ the solution has to be diluted at least 20 times in comparison with the $A_{280\,nm}$ measurement.

1.3.6. Calculation of peptide concentration

Lyophilized peptides may contain between 10% and 70% of bound water and salts by weight. More hydrophilic peptides generally contain more bound water and salts compared to hydrophobic peptides. Therefore, it is difficult to ascertain the actual peptide concentration based on the weight of the lyophilized peptide.

If the peptide has a chromophore in the sequence (Tryptophan; W or Tyrosine; Y), the peptide concentration can be conveniently determined based on the extinction coefficient of these residues.

Any absorbance calculation assumes that the peptide is unfolded and the chromophores are exposed, which is usually the case in short, soluble peptides. If there are doubts about the solubility or the folding of the peptide, it is advisable to make the measurement under denaturing conditions (e.g., 6 M Guanidine-HCl or 8 M urea). Obviously, these peptide solutions will be rendered useless for experimentation, unless the denaturants are removed. If the sequence does not have Tryptophan, Tyrosine or Cysteine, the only practical option is to perform amino acid analysis.

The concentration can be calculated form the absorbance and the molar extinction coefficient via the expression of Beer's law for concentration:

$$C = A \cdot DF / \varepsilon \cdot L \quad \text{(mol/L)} \qquad (1.9)$$

$$C = A \cdot DF \cdot Mr / \varepsilon \cdot L \,\text{(g/L)} \qquad (1.10)$$

Example: a 50x diluted solution of a peptide has an $A_{280\,nm}$ of 0.5 in a 1-cm cell. To calculate the original peptide concentration in the stock peptide solution (2):

C	= Concentration of peptide
A	= absorbance of peptide at 280 nm in 1 cm cell
DF	= dilution factor
ε	= molar extinction coefficient of each chromophore at 280 nm
L	= optical cell length (usual 1 cm)

Sequence: RRNWNQYKIQFGYSFSNSE

$\varepsilon_{peptide}$ = [(1 x 5590) + (2 x 1280)] + (3 x 120) = 8510 cm^{-1}M^{-1}

$Mr_{peptide}$ = 2746 Da

C = (0.5 x 50) / (8510 x 1) = 2.9. 10^{-3} M

C = (0.5 x 50 x 2746) / (8510 x 1) = 8.07 g/L

1.4. Colorimetric methods

The colorimetric methods make use of the property that certain ions change in absorption maximum on binding to other molecules, such as proteins.

1.4.1. Biuret method

Under alkaline conditions, copper (II) ion is bound to peptide nitrogen (peptide bonds) of proteins and peptides to produce a purple colour with an absorption maximum at 540 – 560 nm. The rather low sensitivity is 1 – 6 mg

protein/ml. Components like Tris and ammonia will interfere and the method has a relatively low sensitivity. The advantage is that the copper reagent reacts with peptide bonds and is therefore not dependent on the amino acid composition.

Protocol
Biuret reagent: 1.5 gram copper sulphate ($CuSO_4 \bullet 5H_2O$) and 6 gram sodium potassium tartrate in 500 ml of water. Add 300 ml of 10% (w/v) sodium hydroxide and make up to 1 litre. Store the solution in a plastic container in the dark. By adding 1 gram of potassium iodide reduction of the copper is inhibited and the solution can be kept indefinitely (1).

Protocol
- Add 0.5 ml protein solution (max 3 mg protein) to an eppendorf
- Add 2.5 ml of Biuret reagent and mix
- Let stand for 30 minutes
- Measure the absorbance at 540 nm against a blank containing 0.5 ml sample buffer and 2.5 ml Biuret solution

1.4.2. Lowry method (1951)

This is a modified Biuret reaction in which proteins react with copper (II) under alkaline conditions followed by the Folin–Ciocalteau phospho-molybdic-phosphotungstic acid reduction to heteropolymolybdenum blue by the copper-catalyzed oxidation of aromatic amino acids. The reagent reacts with the phenolic group of tyrosine, indole group of tryptophan and the –SH group of cysteine and give a maximum absorption at 750 nm. The sensitivity is 0.1 – 1.5 mg protein/ml for BSA and is more sensitive than Biuret. Among the interfering substances are amino acids, detergents, lipids, sugars, nucleic acids. In addition, this method is extremely pH dependent and pH should be between 10 and 10.5 (5, 1).

Protocol
- Stock reagent A: 1% (w/v) copper sulphate (CuSO4 • 5H2O)
- Stock reagent B: 2% (w/v) sodium potassium tartrate
- Stock reagent C: 0.2 M sodium hydroxide
- Stock reagent D: 4% (w/v) sodium carbonate

Prepare fresh reagent E by adding 49 ml reagent D to 49 ml reagent C, mix. Add 1 ml reagent A, mix and add 1 ml reagent B, mix.

Prepare fresh reagent F by adding 10 ml water to 10 ml of Folin-Ciocalteau reagent.

- Add 0.5 ml protein solution (max 0.5 mg protein) to a reagent tube
- Add 2.5 ml of reagent E.
- Mix and let stand for 10 minutes
- Add 0.25 ml of reagent F
- Mix and let stand for 30 minutes
- Measure the absorbance at 750 nm against a blank containing 0.5 ml sample buffer processed like the protein solution

1.4.3. Bicinchoninic acid method

The method is based on the alkaline reduction of the cupric ion to the cuprous ion by the aromatic amino acids in the protein, followed by

chelation and colour development by the BCA (Bicinchonininc acid) reagent. Maximum absorption of the colour is at 562 nm. The sensitivity is 0.1 – 1.2 mg protein/ml or 0.5 – 10 μg protein/ml depending on the modification used. The method tolerates amino acids, detergents, lipids, sugars, nucleic acids better than Lowry method but reducing sugars, copper and chelating agents still interfere (1).

Protocol standard BCA
Stock reagent A: 1% (w/v) BCA-Na_2, 2% (w/v) sodium carbonate, 0.15 (w/v) sodium tartrate, 0.4% (w/v) sodium hydroxide and 0.95% sodium bicarbonate. Adjust the pH to 11.25 with either 50% (w/v) sodium hydroxide or solid sodium bicarbonate. The solution is stable at room temperature.

Stock reagent B: 5% (w/v) copper sulphate ($CuSO_4 \bullet 5H_2O$). The solution is stable at room temperature.

Prepare fresh reagent C by mixing 4 volumes of reagent B to 100 volumes of reagent A. The colour will become apple green and the solution is stable for one week.

- Add 100 μl protein solution (0.1 – 1.2 mg protein/ml) to a reagent tube
- Add 2 ml of reagent C
- Mix and let stand for 30 minutes at 37°C
- Cool to room temperature
- Measure absorbance at 562 nm against a blank containing 0.5 ml sample buffer processed like the protein solution. The colour is stable for 1 hour.

Protocol micro BCA
Stock reagent A: 8% (w/v) sodium carbonate, 1.5 (w/v) sodium tartrate, 1.6% (w/v) sodium hydroxide. Adjust the pH to 11.25 with solid sodium bicarbonate. The solution is stable at room temperature.

Stock reagent B: 4% (w/v) BCA-Na_2. The solution is stable at room temperature.

Prepare fresh reagent C by mixing 4 volumes of 4% (w/v) copper sulphate ($CuSO_4 \bullet 5H_2O$) with 100 volumes of reagent B.

Prepare fresh reagent D by mixing 1 volume of reagent C with 1 volume of reagent A.

- Add 1 volume of protein solution (0.5 – 10 μg protein/ml) to a reagent tube
- Add 1 volume of reagent D
- Mix and let stand for 60 minutes at 60°C
- Cool to room temperature.
- Measure absorbance at 562 nm against a blank containing 0.5 ml sample buffer processed like the protein solution

1.5. Dye binding methods

Under appropriate conditions, the acidic and basic groups of proteins interact with the dissociated groups of organic dyes to from coloured precipitates. Dyes like Orange G, Amido Black and Coomassie Brilliant Blue G-250 are used.

1.5.1. Bradford CBB dye binding (1976)

The Bradford protein quantification assay is based on an absorbance shift in Coomassie brilliant blue G-250 (CBB). The dye binds in its anionic form to basic amino acids within the protein (particularly arginine residues), and when bound produces a complex that has an absorbance peak at 595 nm (3). Depending on the format, the sensitivity is 0.2 – 1.4 mg protein/ml or 5 - 100 μg protein/ml or with SDS page 40 – 50 ng per band. This assay is affected by a lot of commonly used chemicals. The method is rapid, simple, one step. However it is significant dependent on amino acid composition because CBB binds preferently to basic (arginine) and aromatic amino acid residues (1).

Dye reagent: dissolve 100 mg of Coomassie Brilliant Blue G-250 in 50 ml of 95% ethanol under vigorous stirring. Add 100 ml 85% (w/v) phosphoric acid and dilute with water to 1 litre. Filter the solution. The solution is stable for two weeks at room temperature.

Protocol standard assay

- Add 0.1 ml protein solution (0.1 – 1.4 mg/ml) to a reagent tube
- Add 5 ml of dye reagent
- Mix and let stand for 5 – 30 minutes
- Measure absorbance at 595 nm against a blank containing 0.5 ml sample buffer processed like the protein solution

Protocol micro assay

- Add 0.2 ml protein solution (5 – 100 μg mg/ml) to a reagent tube
- Add 0.2 ml of dye reagent
- Mix and let stand for 5 – 30 minutes
- Measure absorbance at 595 nm against a blank containing 0.5 ml sample buffer processed like the protein solution

1.5.2. Silver binding method

Upon silver binding the reaction is measured at 420 nm. It has an extremely high sensitivity of 150 ng – 20 μg protein/ml but is easily disturbed by EDTA, > 0.01% SDS and reducing agents like DTT and 2-ME. In addition, there is a high variation between different proteins and silver deposition on cuvette.

Protocol

Stock reagent A: 7.5% (w/v) tween 20, 100 mM Tris, 100 mM sodium carbonate.

Prepare fresh reagent B: 2.5% solution of gluteraldehyde from a 25% stock gluteraldehyde solution stored at 4°C.

Prepare fresh reagent C: Ammoniacal silver solution by adding to 18.2 ml of distilled water, 1.4 ml 20% (w/v) sodium hydroxide and 0.2 ml 29% ammonium hydroxide. Then add drop wise 0.2 ml of 20% (w/v) silver nitrate.

Prepare fresh reagent D: 3% (w/v) sodium thiosulphate.

- Add 0.1 ml protein solution (150 ng – 20 μg/ml) to a reagent tube
- Add 11 μl of reagent A

- Centrifuge at 450 g for 5 minutes through a 2 ml Bio-Gel P-2 column pre-equilibrated in 10x diluted reagent A and then drained of void volume
- Add 0.9 ml of distilled water to make the sample volume 1 ml.
- Add 20 μl of reagent B and vortex for 2 sec
- Add 200 μl of reagent C and vortex for 2 sec
- Let stand for precise 10 minutes at room temperature
- Ad 40 μl of reagent D
- Measure absorbance at 420 nm against a blank containing 0.5 ml sample buffer processed like the protein solution

1.5.3. Amino acid analysis

The amount of protein required for this detection method can be as low as 0.05 nmol pr 2.5 μg.

Boil the sample in 10 M HCl at 120°C over night. Label the released amino-acid either pre- or post column with a proper dye (e.g. fluorescent or equal). The disadvantage of this method is the sophisticated instrumentation needed.

1.6. Weighing

The protein is dissolved in iso-ionic water and dried at 95°C until all water is evaporated. Then the temperature is raised to 105-110°C to remove physical bound water. After cooling down in a vacuum exsiccator with dry P_2O_5 the amount of dry weight is determined. This value can be used to determine $\varepsilon_{A280}^{1\%}$ for the protein.

$$A_{280nm} = \varepsilon \cdot conc \cdot l \rightarrow while\ l\ is\ 1\,cm \rightarrow \varepsilon = \frac{A_{280nm}}{conc}$$

The concentration is the dry weight dissolved in 100 ml.

When for instance the A_{280} nm = 0.815 and the dry weight of 100 µl solution is 73 µg. 73 µg from 100 µl is 0.073 g / 100 ml = 0.073 % (10). The protein extinction coefficient is:

$$\varepsilon_{A280}^{1\%} = \frac{0.815}{0.073} = 11.16$$

The $\varepsilon_{A280}^{1\%}$ can be calculated on the web page of Expasy at

www.expasy.org/tools/protparam.html

Table: Overview of protein quantification methods.

Method		Range	Volume	Accuracy	Convenience	Interference
Absorbance	**280 nm**	20 μg – 3 mg	50 – 200 μl	Fair*	Excellent	Detergents, nucleic acids, particles
	205 nm	1 – 100 μg	50 – 200 μl	Fair	Very good	Detergents, nucleic acids, particles
	191–194 nm	0.01 – 0.05 mg		Fair	Poor	
Colorimetric assays	**Modified Lowry**	2- 100 μg	1 ml	Good	Fair	Strong acids, ammonium sulphate
	Biuret	1 – 10 mg	5 ml	Good	Good	Ammonium salts
	Bradford	1 – 200 μg	1 – 5.5 ml	Good	Excellent	-
	Amido Black	2 – 24 μg	2 ml	Good	Poor	-
	Colloidal gold	20 – 640 ng	1 ml	Fair	Poor	Strong bases
	Bicinchonic acid (Smith)	0.2 – 50 ug	1 ml	Good	Excellent	Strong acids, ammonium sulphate, lipids
Dye binding	**Bradford**	0.05-1.4 mg		Good	Excellent	
	Silver	150 ng – 20 ug		Fair	Fair	strong bases
Weighting				Excellent	Good	

from: Protein purification methods, a practical approach IRL Press Oxford.
*When the molar extinction coefficient is know, the accuracy is very good.

1.7. References

1. **Dunn, J. M.**; Protein Purification methods: Initial planning; 1-64; IRL Press, 1989.
2. **Gill, Stanley C. and von Hippel, Peter H.**; Calculation of protein extinction coefficients from amino acid sequence data. *Anal.Biochem.* **(182)**: 319-326; 1989.
3. **Knight, Matthew and Chambers, Paul**; Problems associated with determining protein concentration. *Molecular Biotechnology* **(23)**: 19-28; 2003.
4. **Kreusch, S. et al**; UV measurements in microplates suitable for high-throughput protein determination. *Anal.Biochem.* **(313)**: 208-215; 2003.
5. **Lowry, O. H. et al**; Protein measurement with the Folin phenol reagent. *J.Biol.Chem.* **(193)**: 265-275; 1951.
6. **Pace, C. N. et al**; How to measure and predict the molar absorption coefficient of a protein. *Protein Sci.* **(4)**: 2411-2423; 1995.
7. **Pierce Biotechnology**; Technical resource: Extinction coeffients. **(TR0006.0)**: 2004.
8. **Pierce Biotechnology**; Protein Assay - Technical Handbook; 2008.
9. **Scopes, R. K.**; Measurement of protein by spectrophotometry at 205 nm. *Anal.Biochem.* **(59)**: 277-282; 1974.
10. **Stocheck, C. M.**; Methods in enzymology: Guide to protein purification; 50-; Academic Press Inc, 1990.