# Ανάλυση πρωτεϊνών

Protein analysis is important for:

1. **Nutrition labeling**
2. **Pricing:** The cost of certain commodities is based on the protein content as measured by nitrogen content (e.g., cereal grains; milk for making certain dairy products, e.g., cheese).
3. **Functional property investigation:** Proteins in various types of food have unique food functional properties: for example, gliadin and glutenins in wheat flour for breadmaking, casein in milk for coagulation into cheese products, and egg albumen for foaming (see Chap. 15).
4. **Biological activity determination:** Some proteins, including enzymes or enzyme inhibitors, are relevant to food science and nutrition: for instance, the proteolytic enzymes in the tenderization of meats, pectinases in the ripening of fruits, and trypsin inhibitors in legume seeds are proteins. To compare between samples, enzymes activity often is expressed in terms of specific activity, meaning units of enzyme activity per mg of protein.

Protein analysis is required when you want to know:

1. Total protein content
2. Content of a particular protein in a mixture
3. Protein content during isolation and purification of a protein
4. Nonprotein nitrogen
5. Amino acid composition (see Chap. 15)
6. Nutritive value of a protein (see Chap. 15)

# Φασματομετρικός προσδιορισμός συνολικής πρωτεΐνης

- Αντίδραση διουρίας (Biuret)
- Μέθοδος Lowry
- Μέθοδος BCA
- Επιλογή πρότυπης πρωτεΐνης

# Μέθοδος διουρίας

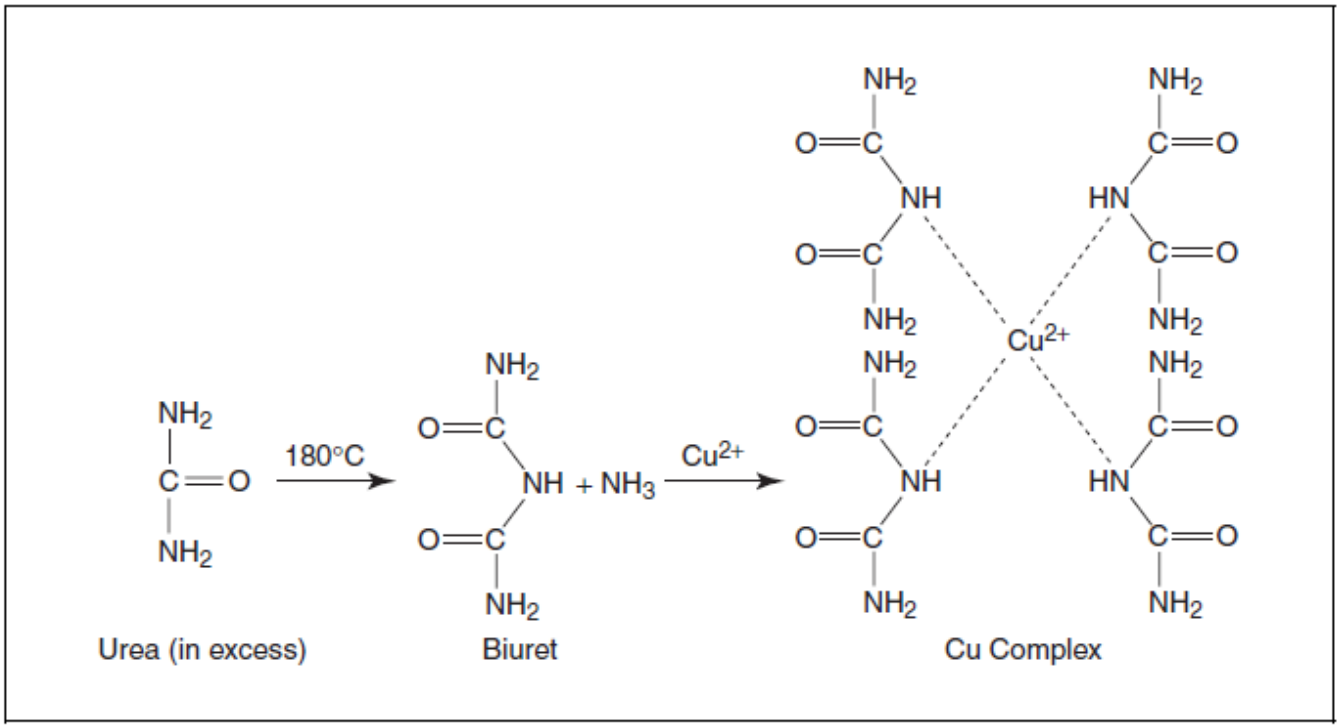
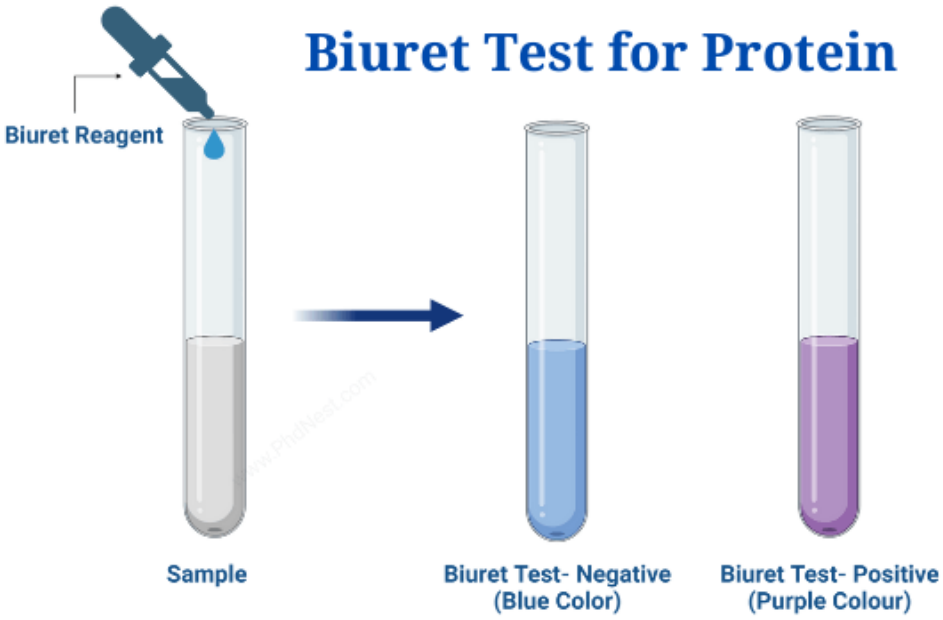



**Figure B1.1.5** The schematic of the Biuret reaction.

- 540 nm
- 4-6 πεπτιδικοί δεσμοί / $Cu^{2+}$




Advantages:

1. Less expensive than the Kjeldahl method; rapid (can be completed in less than 30 min); simplest method for analysis of proteins.
2. Color deviations are encountered less frequently than with Lowry, ultraviolet (UV) absorption, or turbidimetric methods (described below).
3. Very few substances other than proteins in foods interfere with the biuret reaction.
4. Does not detect nitrogen from nonpeptide or nonprotein sources.

Disadvantages:

1. Not very sensitive as compared to the Lowry method; requires at least 2–4 mg protein for assay.
2. Absorbance could be contributed from bile pigments if present.
3. High concentration of ammonium salts interfere with the reaction.
4. Color varies with different proteins; gelatin gives a pinkish-purple color.
5. Opalescence could occur in the final solution if high levels of lipid or carbohydrate are present.
6. Not an absolute method: color must be standardized against known protein (e.g., BSA) or against the Kjeldahl nitrogen method.

# Μέθοδος Lowry

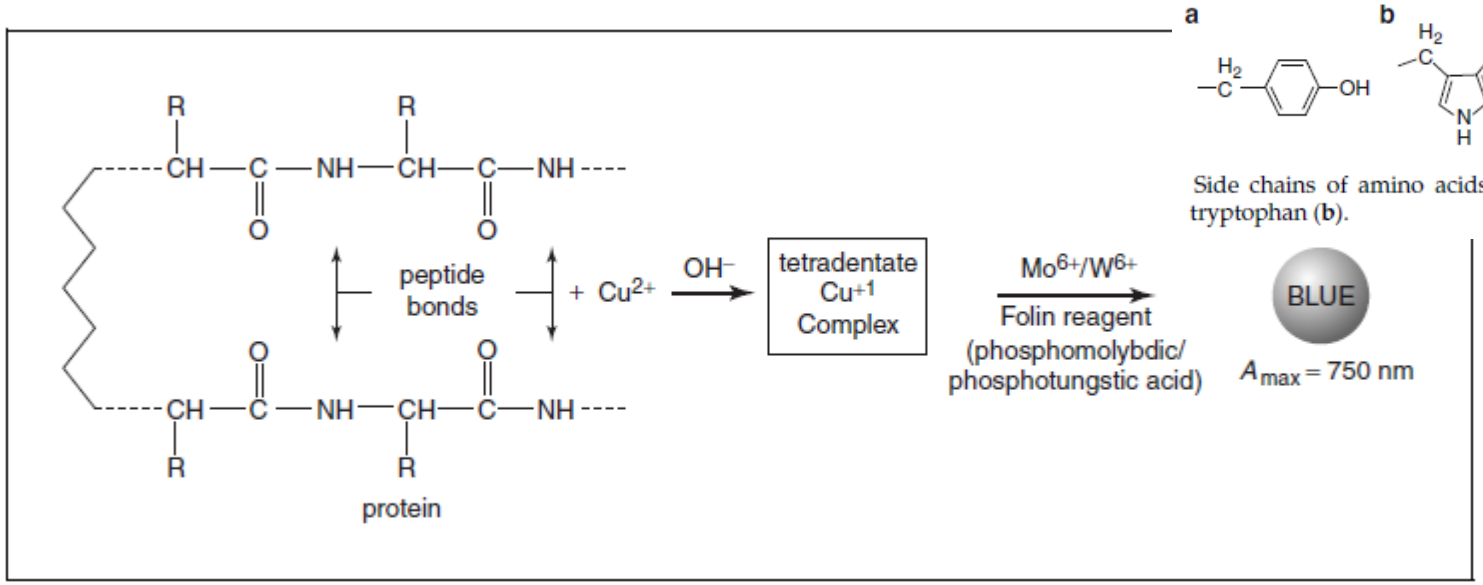






**Figure B1.1.1** The reaction schematic for the Lowry Protein Assay.

Advantages:

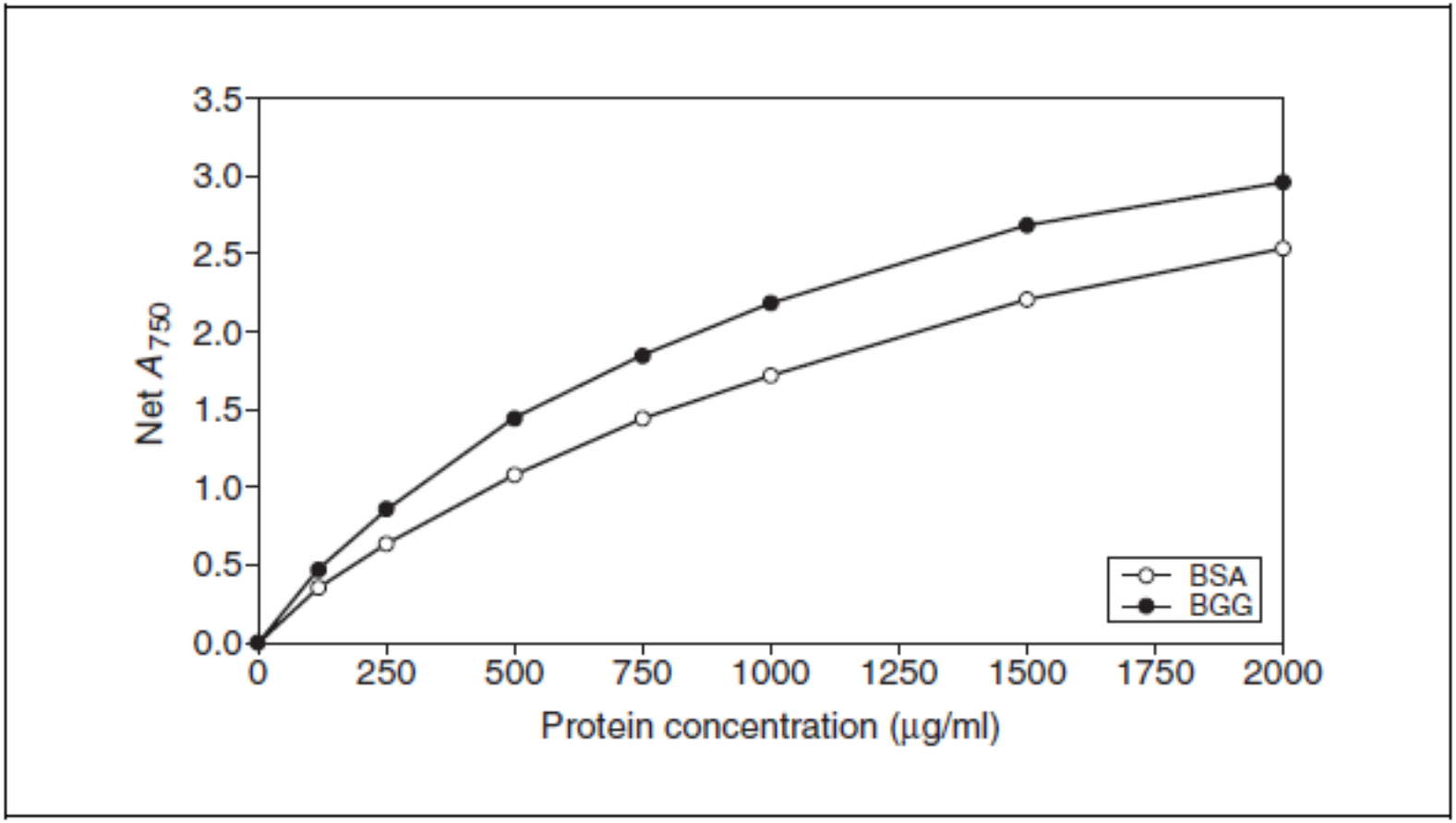
1. Very sensitive
   (a) 50–100 times more sensitive than biuret method
   (b) 10–20 times more sensitive than 280-nm UV absorption method (described below)
   (c) Similar sensitivity as Nesslerization; however, more convenient than Nesslerization
2. Less affected by turbidity of the sample.
3. More specific than most other methods.
4. Relatively simple; can be done in 1–1.5 h.

Disadvantages:

For the following reasons, the Lowry procedure requires careful standardization for particular applications:

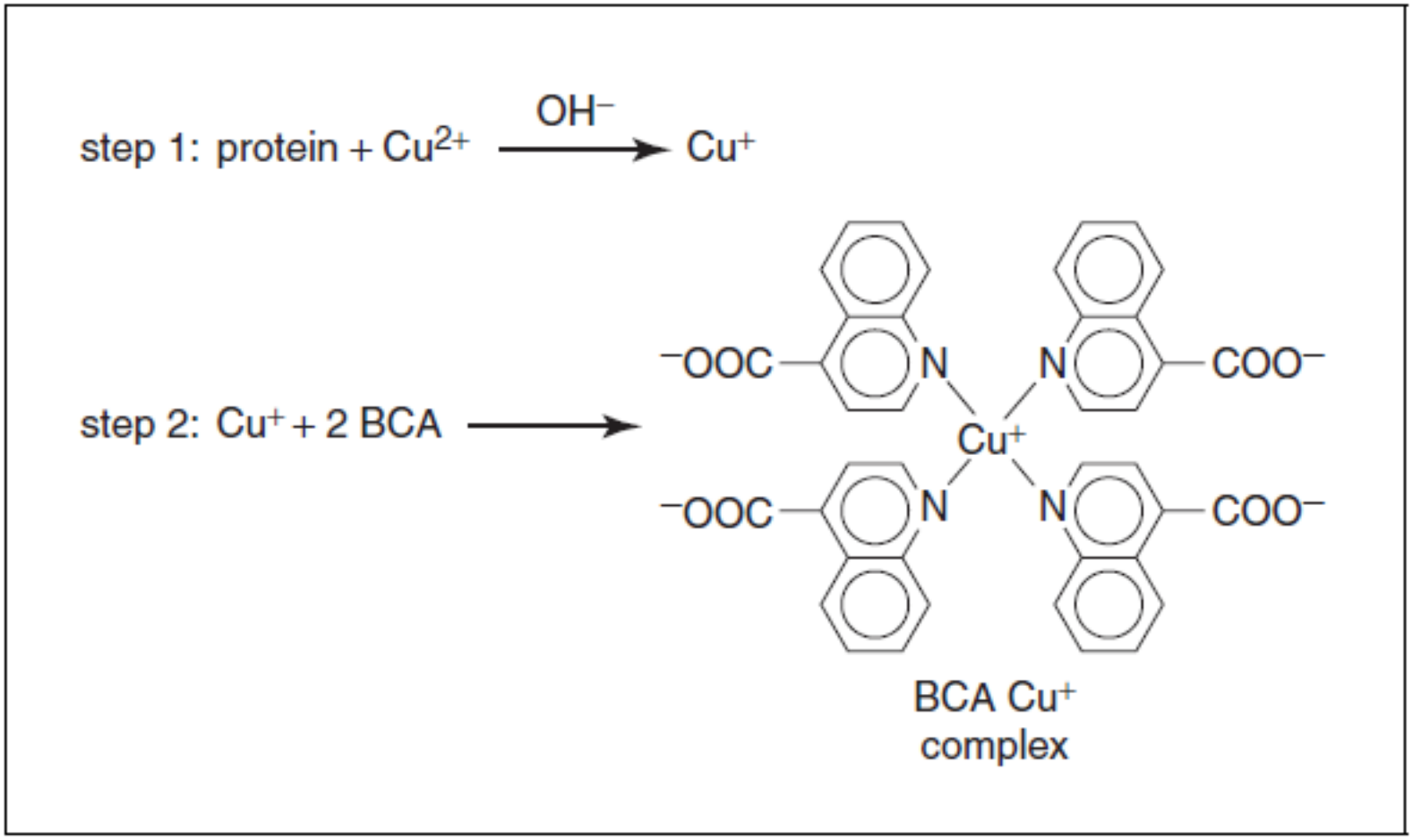
1. Color varies with different proteins to a greater extent than the biuret method.
2. Color is not strictly proportional to protein concentration.
3. The reaction is interfered with to varying degrees by sucrose, lipids, phosphate buffers, monosaccharides, and hexoamines.
4. High concentrations of reducing sugars, ammonium sulfate, and sulfhydryl compounds interfere with the reaction.

# Πρότυπη πρωτεϊνη




- BSA, bovine serum albumin
- BGG, bovine gamma globulin

# Μέθοδος BCA


step 1: protein + $Cu^{2+}$ $\xrightarrow{OH^-}$ $Cu^+$
step 2: $Cu^+$ + 2 BCA
$^-OOC$
N
N
$COO^-$
$Cu^+$
$^-OOC$
N
N
$COO^-$
BCA $Cu^+$ complex

# Προσδιορισμός συνολικής πρωτεϊνης μέσω μέτρησης συνολικού αζώτου

- Kjeldahl
- Dumas
- Μέθοδος καύσης

Υπόθεση : Οι πρωτεϊνες περιέχουν 16% άζωτο.

- Συντελεστής 100/16=6.25
- Μη πρωτεϊνικό άζωτο ?

**Table B1.2.1** Conversion Factors from Percent Nitrogen to Percent Total Protein for Various Commodities and Their Products

| Products | Conversion factors |
|---|---|
| Animal | 6.25 |
| Cottonseeds | 5.30 |
| Peanuts | 5.46 |
| Soybeans | 5.71 |
| Sunflower seeds | 5.30 |
| Safflower seeds | 5.30 |
| Coconut meat | 5.30 |
| Sesame seeds | 5.30 |
| Corn | 6.25 |
| Millet | 5.83 |
| Rice | 5.95 |
| Wheat | 5.83 |

# Μέθοδος Kjeldahl

Το δείγμα διαλύεται με $H_2SO_4$ και καταλύτη $CuSO_4/TiO_2$ , το N μετατρέπεται σε $NH_3$, η οποία αποστάζεται και τιτλοδοτείται.

$$\text{Protein} \xrightarrow[\text{Heat, catalyst}]{\text{Sulfuric acid}} (NH_4)_2SO_4$$

$$(NH_4)_2SO_4 + 2NaOH \rightarrow 2NH_3 + Na_2SO_4 + 2H_2O \quad [2]$$

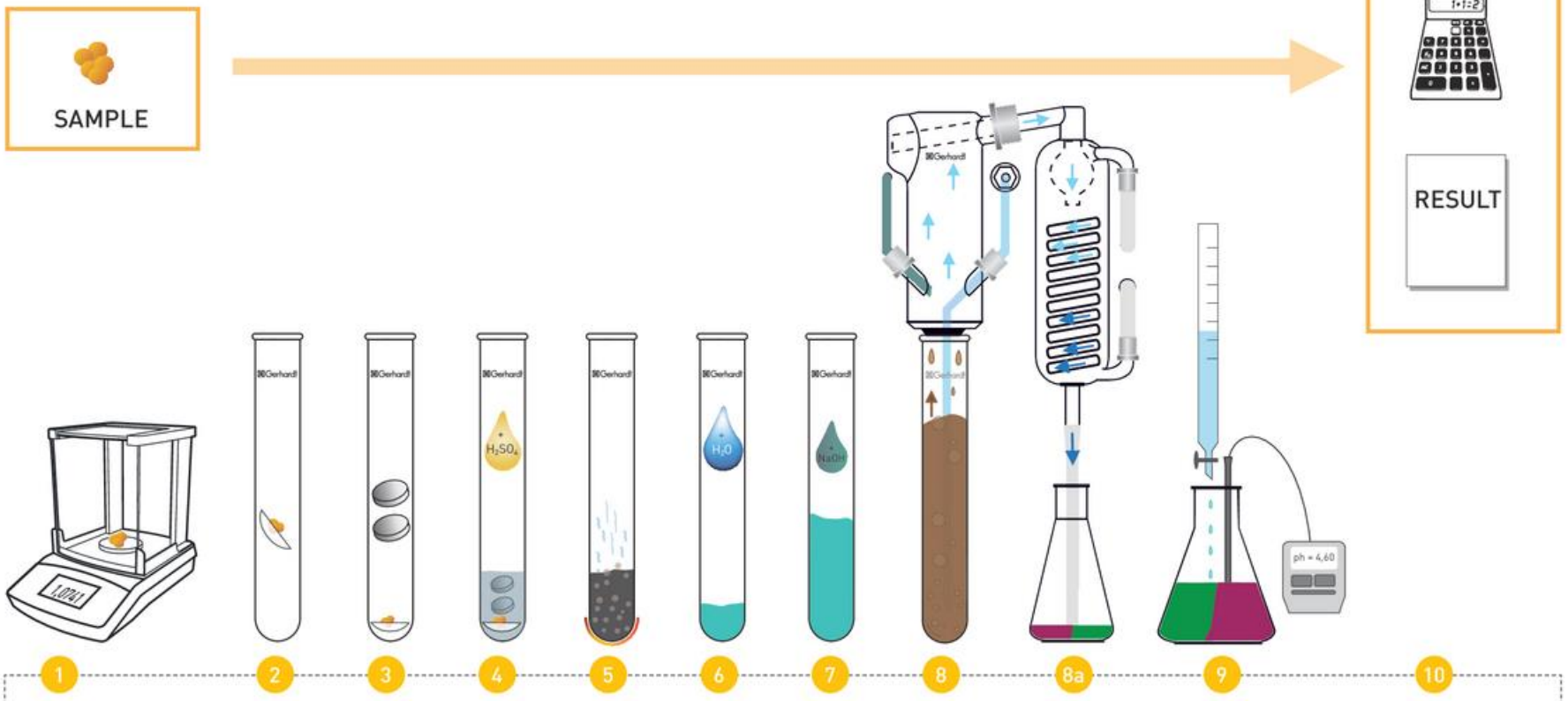
$$NH_3 + H_3BO_3\,(\text{boric acid}) \rightarrow \underset{(\text{borate ion})}{NH_4^+ + H_2BO_3^-} \quad [3]$$

$$H_2BO_3^- + H^+ \rightarrow H_3BO_3 \quad [4]$$

tube.com/watch?v=TWaPfYqAt4c

# Kjeldahl Analysis for Nitrogen and Protein

Gerhardt Analytical Systems




1. Weighing in sample on N free paper.
2. Transfer of sample with weighing boat into digestion tube
3. Addition of salt to raise boiling point and as catalyst, e.g. KJELCAT Cu
4. Addition of sulphuric acid
5. Digestion at boiling point, for about 60 to 180 minutes
   $C_nH_mN_x + H_2SO_4 \longrightarrow n\,CO_2 + \frac{1}{2}\,m\,H_2O + \frac{1}{2}\,x\,(NH_4)_2SO_4$ (solv)
6. Dilution of sample solution with water to prevent strong reactions by following addition of caustic soda. This can be done automatically in some VAPODEST steam distillation units.
7. Addition of caustic soda to release ammonia, today this is done automatically in modern steam distillation units like VAPODEST $NH_4^+ + OH^- \longrightarrow NH_3\uparrow + H_2O$
8. Seperation of ammonia by steam distillation and trapping the condensated ammonia-water solution in boric acid (8a).
   $NH_3 + H_3BO_3 \longrightarrow NH_4^+ + H_2BO_3^-$
9. Quantitative determination of nitrogen by titration with sulfuric or hydrochloric acid. With direct pH measurement with pH electrode or with pH indicator.
   $NH_4^+ + H_2BO_3^- + HCl \longrightarrow NH_4CL + H_3BO_3$
10. Calculation

**Nitrogen content:**

$$\%\,N = \frac{1{,}4007 * c * (V - V_b)}{E}$$

c - $H^+$ Ion concentration of standard volumetric solution: hydrochloric acid c (H+) = 0,1 mol/l alternatively: sulfuric acid c ($H^+$) = 0,1 mol/l (c($H_2SO_4$) = 0,05 mol/l)
V - Consumption volumetric standard solution sample [ml]
$V_b$ - Consumption volumetric standard solution blank [ml]
E - Weight [g]

**Calculation protein content:**

% Crude protein = % N * PF

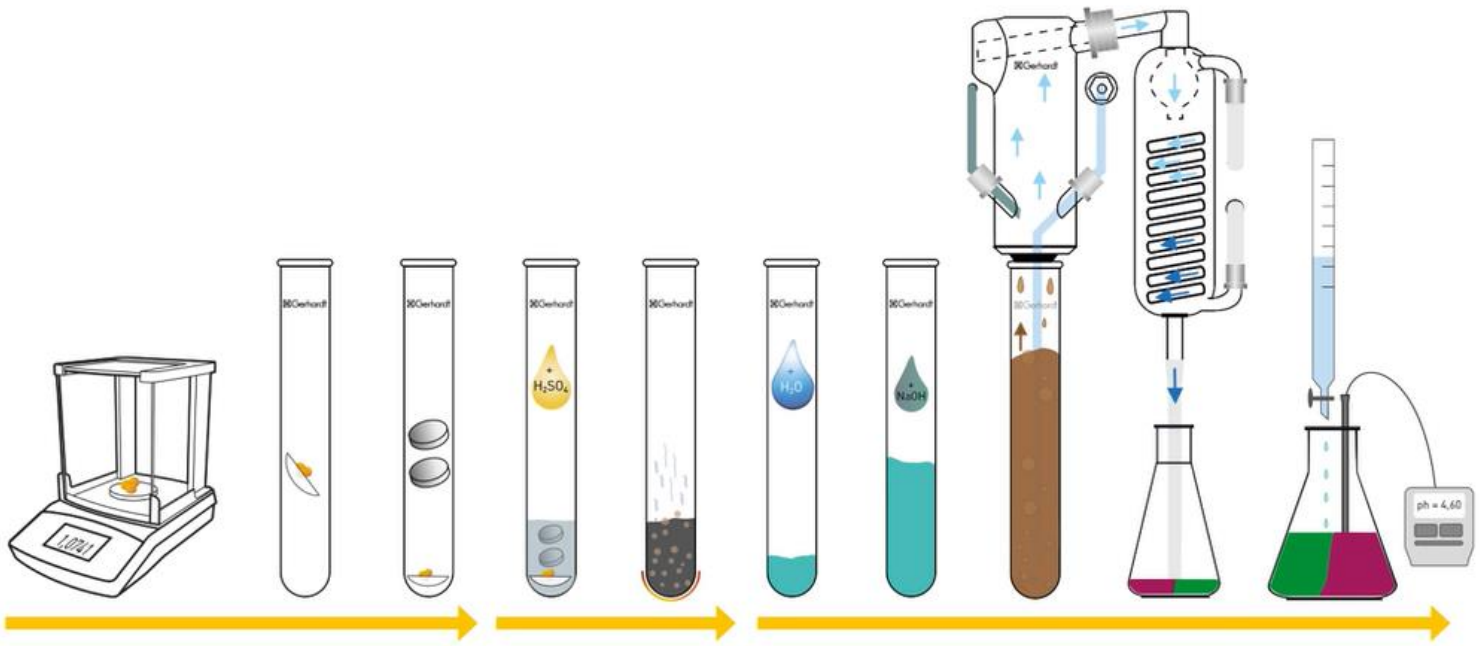
Protein content is calculated by a factor (PF)

Examples of protein factors:

| | |
|---|---|
| 6.38 | Milk, cheese, milk powder and milk powder products (including milk-based infant formulae, milk protein concentrate, whey protein concentrate, casein and caseinates) Not applicable for samples containing ammonium caseinate |
| 6.25 | Meat, fish, poultry, egg, vegetables, fruits, different types of grain, corn, legumes, feed |
| 5.95 | Rice |
| 5.71 | Soy beans |
| 5.7 | Wheat and wheat flour |
| 5.55 | Gelatin |
| 5.4 | Oilseeds, Nuts |

Note: The listed protein factors are often applied. Depending on regulation the analysis is based on, different protein factors must get applied. Especially in grain, nuts and oilseeds or single components of the above described sample types.

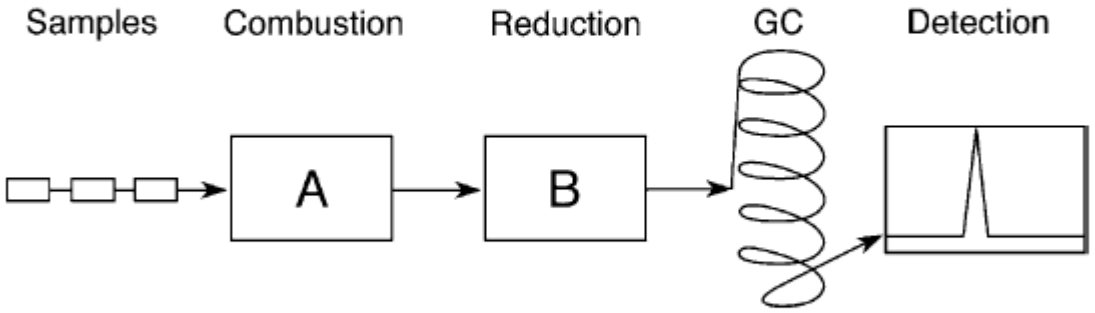
# Kjeldahl-Analyis



# Μέθοδος Dumas και καύσεως

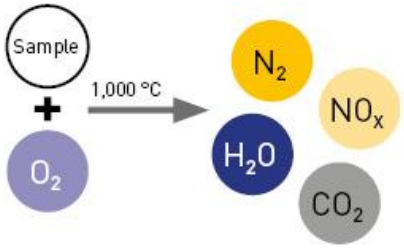
Το δείγμα καίγεται πλήρως σε $CO_2$ και νερό, τα οποία κατακρατούνται, ενώ ο όγκος του $N_2$ που παράγεται μετρείται με κατάλληλη συσκευή.

Samples (approximately 100–500 mg) are weighed into a tin capsule and introduced to a combustion reactor in automated equipment. The nitrogen released is measured by a built-in gas chromatograph. Figure 9-1

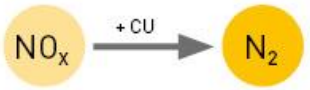



**9-1 figure**

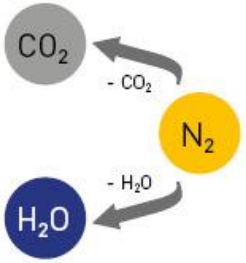
General components of a Dumas nitrogen analyzer. *A*, the incinerator; *B*, copper reduction unit for converting nitrogen oxides to nitrogen; and *GC*, gas chromatography column.



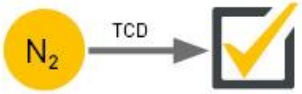

Step 1:
Combustion of the sample and addition of oxygen ($O_2$) at approx. 1,000 °C



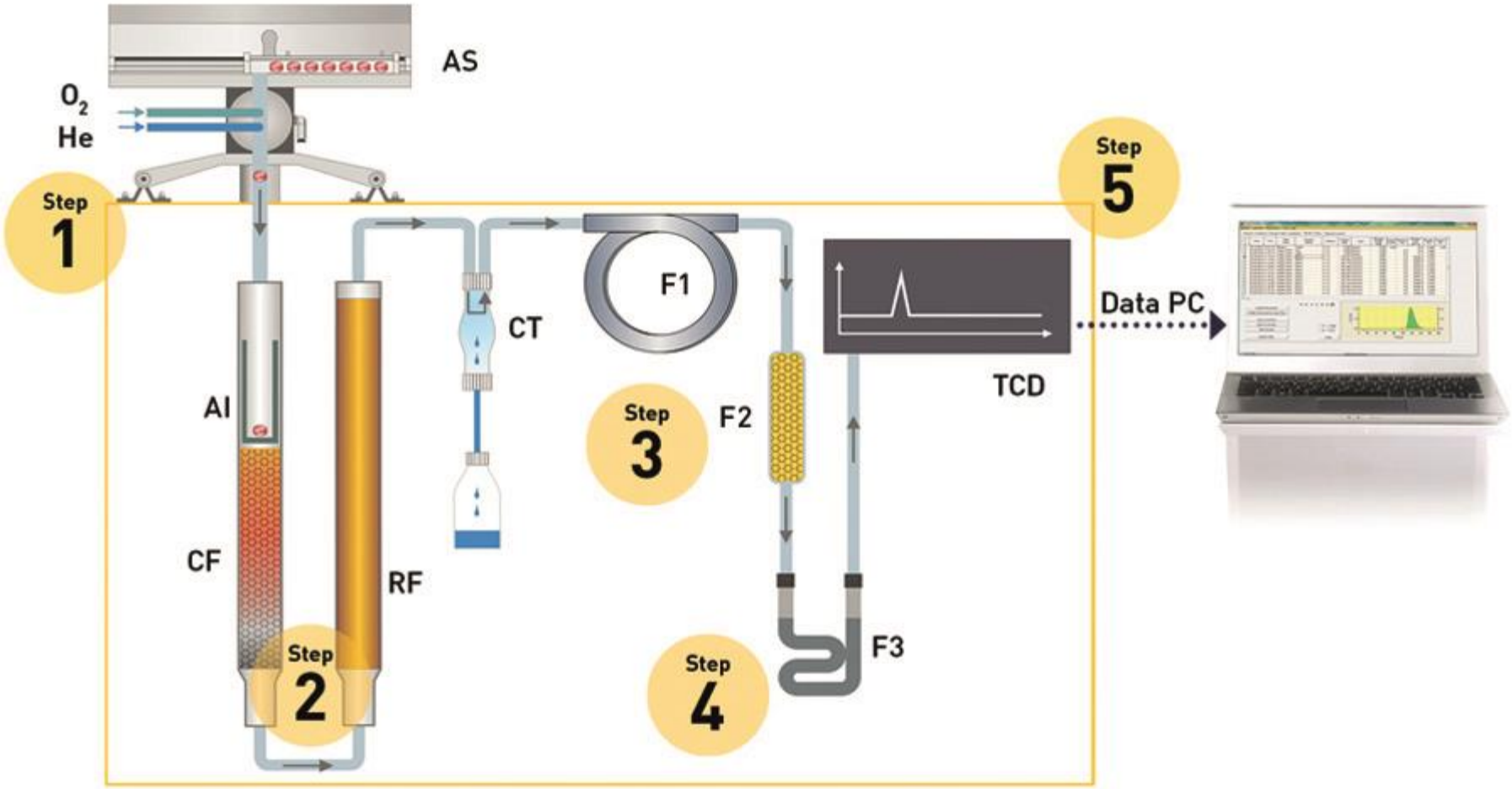

Step 2:
Reduction of nitrogen oxides ($NO_x$) to elemental nitrogen ($N_2$) through copper (CU)

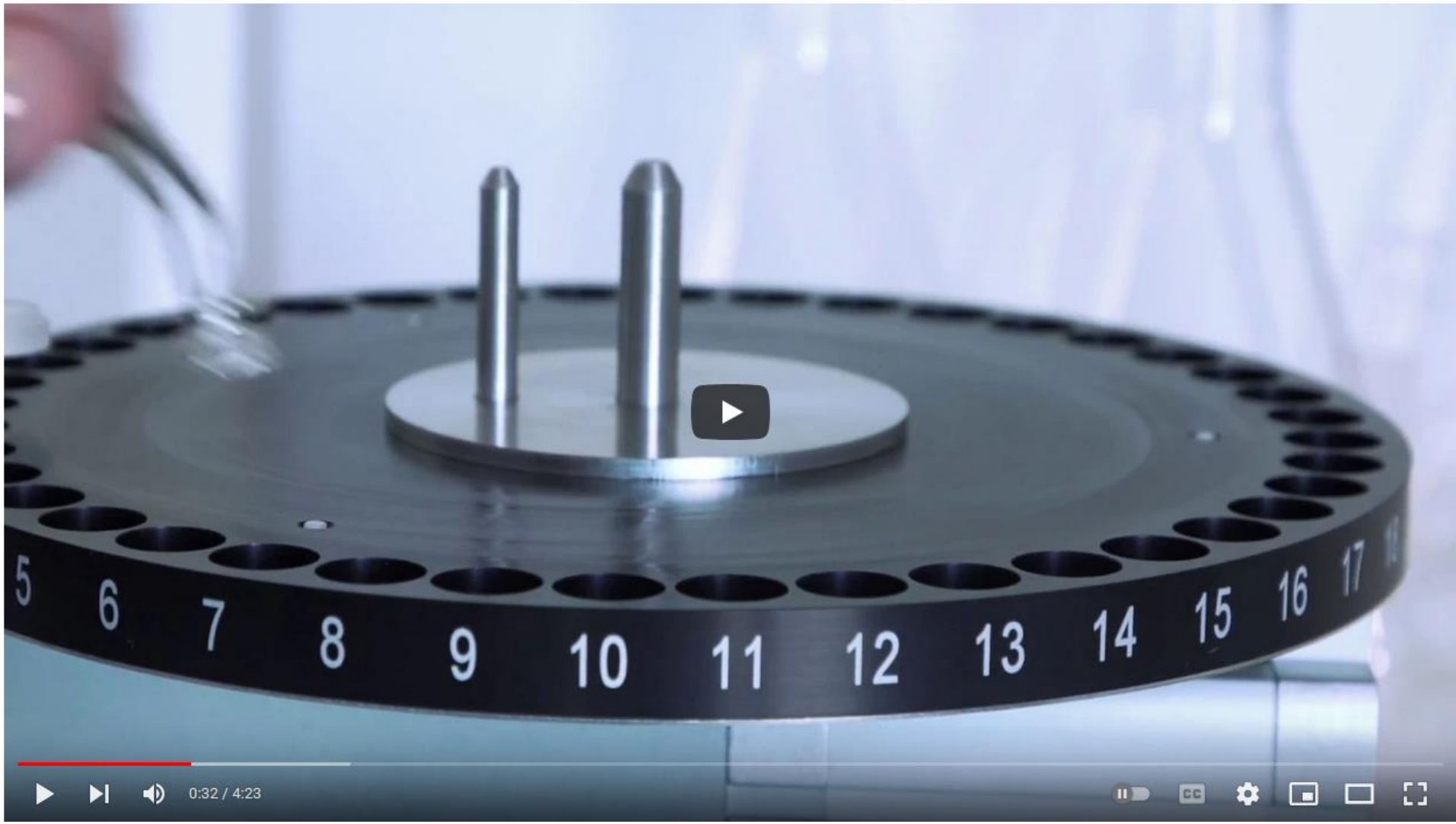



Step 3 and 4:
Separation of water ($H_2O$) and carbon dioxide ($CO_2$)




Step 5:
Determination of the nitrogen content with the help of a thermal conductivity detector and calculation by software.




5 6 7 8 9 10 11 12 13 14 15 16 17
0:32 / 4:23
CC

# UV Φασματομετρικός προσδιορισμός πρωτεϊνών

1. Απορρόφηση στα 280 nm (τρυπτοφάνη, τυροσίνη, κυστίνη)
- Νόμος του Beer
- 20-3000 μg/mL

$$\text{concentration (mg/ml)} = \frac{A_{280}}{a_{280} \times b}$$

Advantages:

1. Rapid and relatively sensitive; At 280 nm, 100 μg or more protein is required; several times more sensitive than the biuret method.
2. No interference from ammonium sulfate and other buffer salts.
3. Nondestructive; samples can be used for other analyses after protein determination; used very widely in postcolumn detection of proteins.

Disadvantages:

1. Nucleic acids also absorb at 280 nm. The absorption 280 nm/260 nm ratios for pure protein and nucleic acids are 1.75 and 0.5, respectively. One can correct the absorption of nucleic acids at 280 nm if the ratio of the absorption of 280 nm/260 nm is known. Nucleic acids also can be corrected using a method based on the absorption difference between 235 and 280 nm (37).
2. Aromatic amino acid contents in the proteins from various food sources differ considerably.
3. The solution must be clear and colorless. Turbidity due to particulates in the solution will increase absorbance falsely.
4. A relatively pure system is required to use this method.

# UV Φασματομετρικός προσδιορισμός πρωτεϊνών

2. Απορρόφηση στα 205 nm (πεπτιδικός δεσμός)
•1-100 μg/mL

3. Φθορισμός
•5-50 μg/mL

**Table B1.3.3** Fluorescence Properties of Aromatic Amino Acids[a]

| Amino acid | Excitation wavelength | Emission wavelength | Quantum yield |
|---|---|---|---|
| Phenylalanine | 260 nm | 283 nm | 0.04 |
| Tryptophan | 285 nm | 360 nm | 0.20 |
| Tyrosine | 275 nm | 310 nm | 0.21 |

[a]Values are for aqueous solutions at pH 7 and 25°C (Hawkins and Honigs, 1987; Fasman, 1989).

# Μέτρηση βαθμού υδρόλυσης, DH, πρωτεϊνών

- Αντίδραση νινυδρίνης

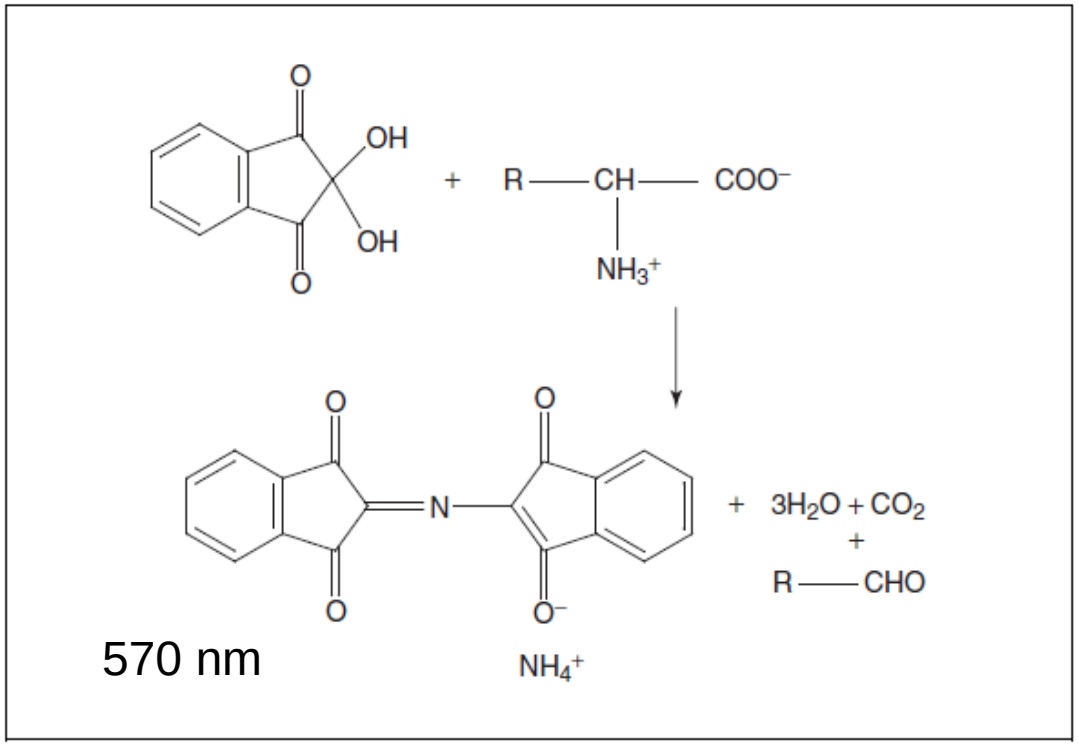
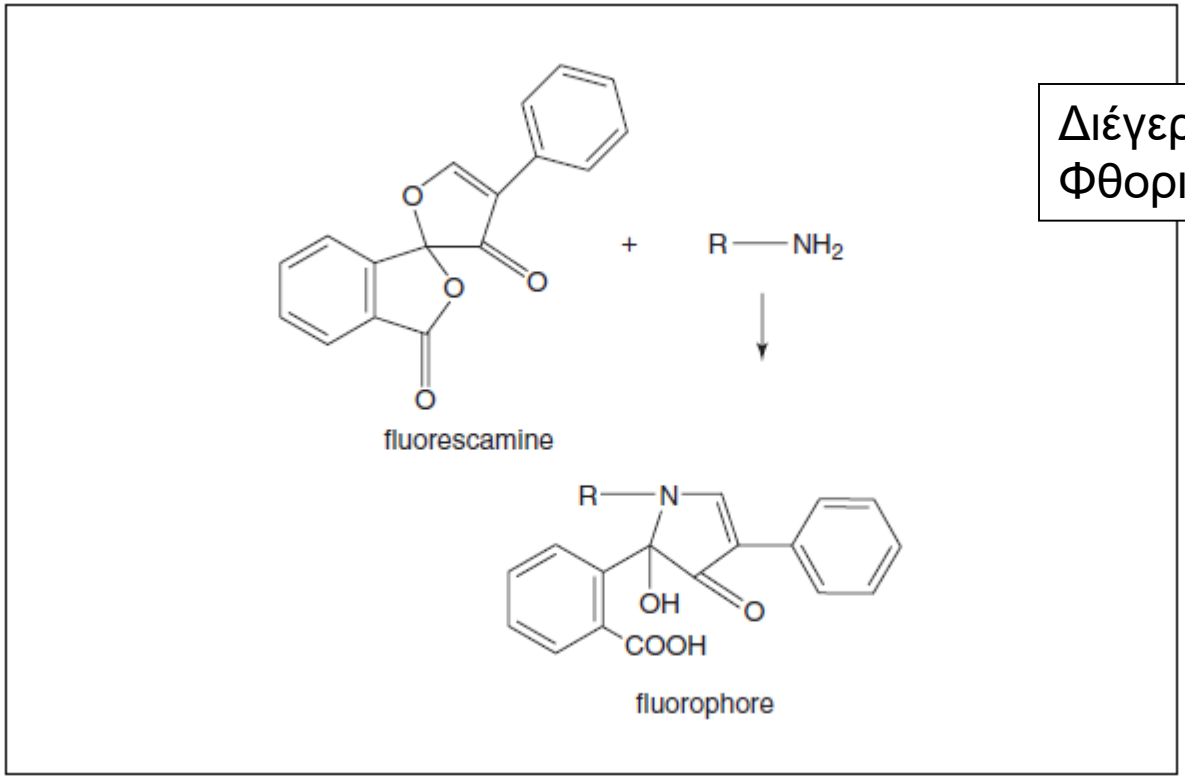



**Figure B2.2.1** Reaction of ninhydrin with an amino acid, producing the purple-blue product.

- Αντίδραση fluorescamine




Διέγερση στα 390 nm
Φθορισμός στα 475 nm

**Figure B2.2.4** Fluorescamine reaction.

# Ηλεκτροφόρηση πρωτεϊνών

•Παρακολούθηση αλλαγών στις πρωτεϊνες κατά την επεξεργασία τροφίμων.
•Οι πρωτεϊνες διαχωρίζονται με βάση το μέγεθος και το φορτίο τους, υπό την επίδραση εξωτερικού ηλεκτρικού πεδίου.

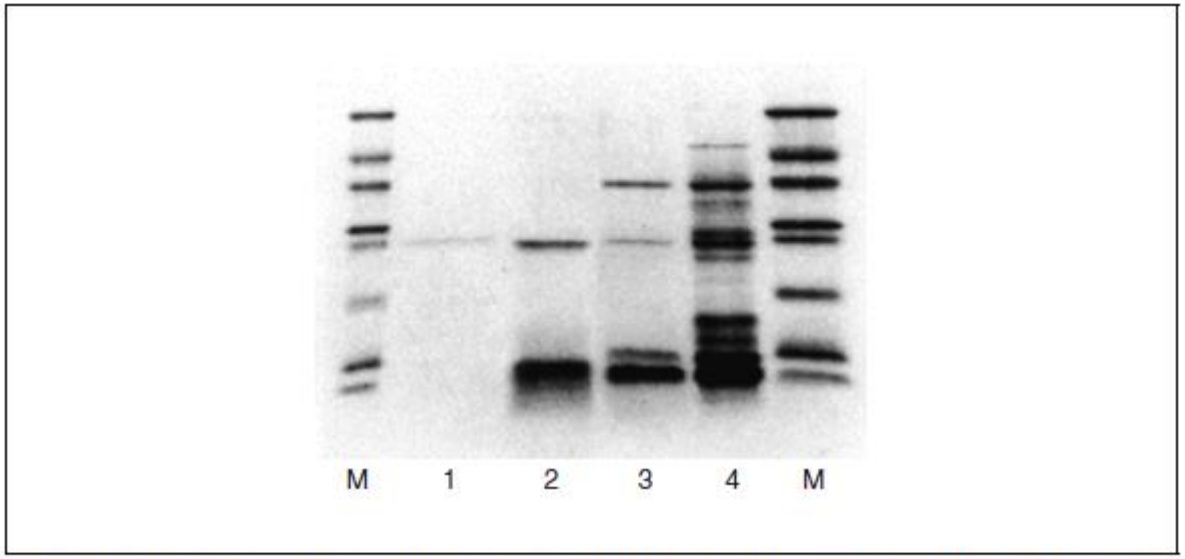



**Figure B3.1.1** A 15% SDS-polyacrylamide gel stained with Coomassie brilliant blue. Protein samples were assayed for the purification of a proteinase, cathepsin L, from fish muscle according to the method of Seymour et al. (1994). Lane 1, purified cathepsin L after butyl-Sepharose chromatography. Lane 2, cathepsin L complex with a cystatin-like proteinase inhibitor after butyl-Sepharose chromatography. Lane 3, sarcoplasmic fish muscle extract after heat treatment and ammonium sulfate precipitation. Lane 4, sarcoplasmic fish muscle extract. Lanes M, low-molecular-weight standards: aprotinin ($M_r$ 6,500), α-lactalbumin ($M_r$ 14,200), trypsin inhibitor ($M_r$ 20,000), trypsinogen ($M_r$ 24,000), carbonic anhydrase ($M_r$ 29,000), gylceraldehyde-3-phosphate dehydrogenase ($M_r$ 36,000), ovalbumin ($M_r$ 45,000), and albumin ($M_r$ 66,000) in order shown from bottom of gel. Lane 1 contains 4 μg protein; lanes 2 to 4 each contain ~7 μg protein.

# Προσδιορισμός αλλαγών στη διαμόρφωση πρωτεϊνών με φθορισμό

•Ο φθορισμός των αμινοξέων τρυπτοφάνη και τυροσίνη επηρεάζεται από τη διαμόρφωση της πρωτεϊνης (folding).
• Μελέτη μετουσίωσης πρωτεϊνών (θέρμανση, pH, άλατα κλπ.)

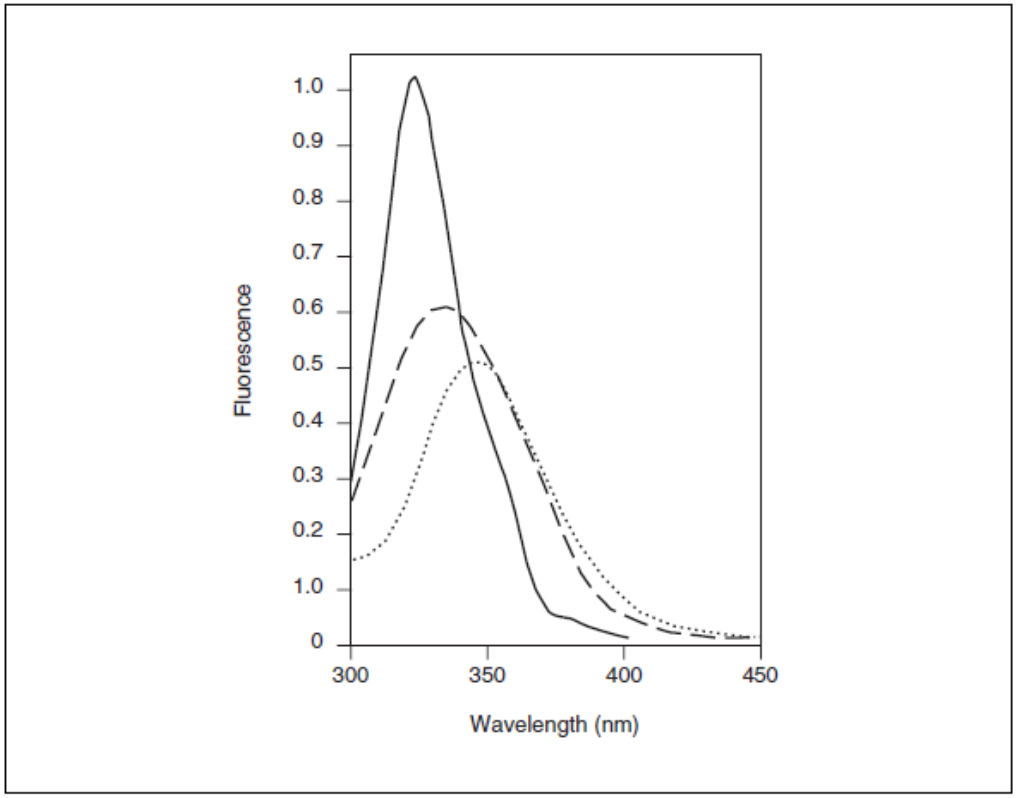
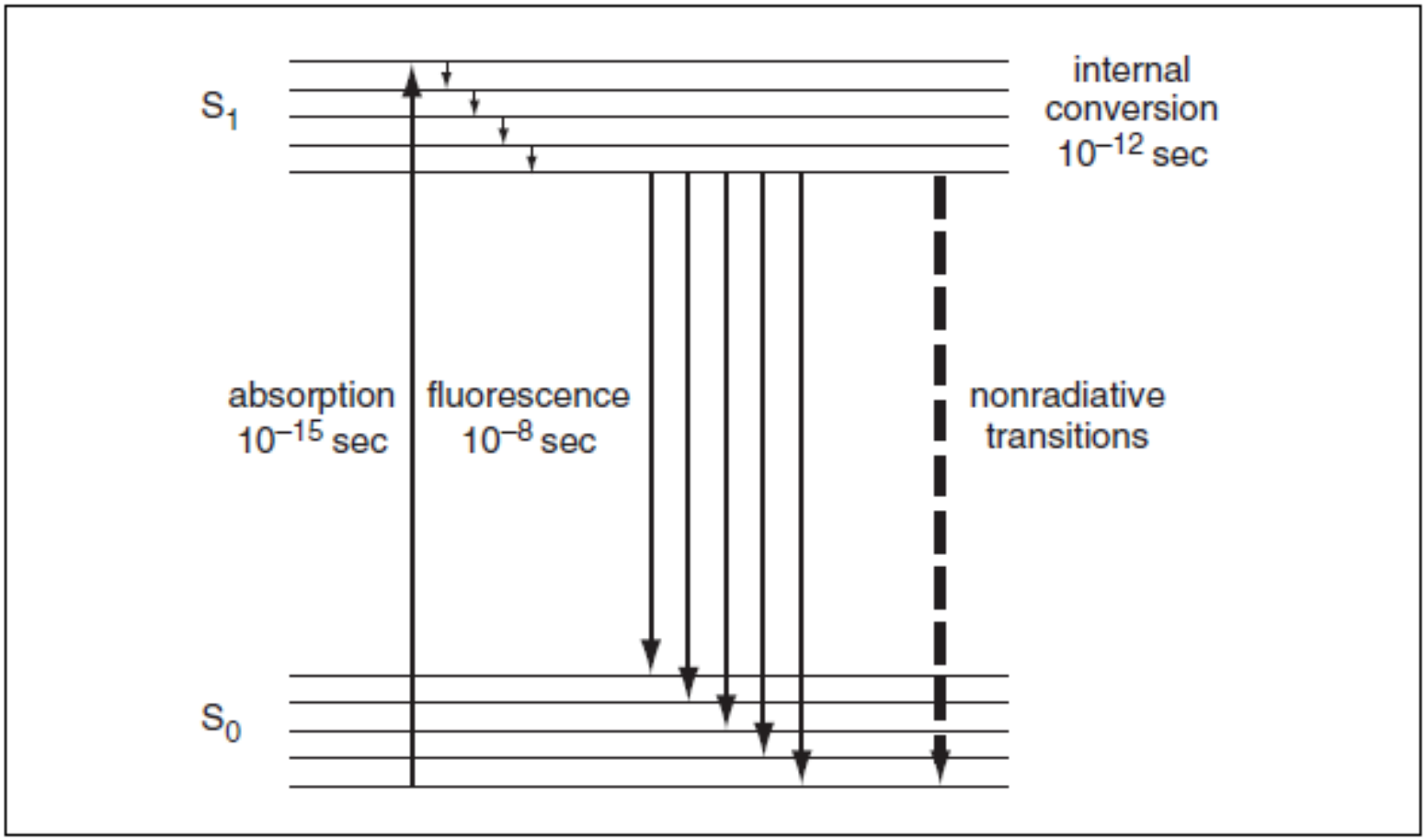



**Figure B3.6.9** Quenching of fluorescence of tumor necrosis factor (TNF) on denaturation. Fluorescence emission spectra are shown for native TNF (solid line), guanidine-unfolded TNF (dotted line), and acid-denatured TNF (dashed line). Parameters: TNF concentration, 30 μg/ml; $\lambda_{ex}$ = 280 nm; bandwidths ranging from 16 to 24 nm. Measurements made using Perkin-Elmer MPF3 spectrofluorimeter. Reprinted from Hlodan and Pain (1994) with permission of Elsevier Science.

# Φθορισμός


$S_1$
internal
conversion
$10^{-12}$ sec
absorption
$10^{-15}$ sec
fluorescence
$10^{-8}$ sec
nonradiative
transitions
$S_0$

# Μέτρηση ενζυμικής ενεργότητας

Πολυγαλακτονουράση
- Μέτρηση αναγωγικης ικανότητας
- Μέτρηση ιξώδους

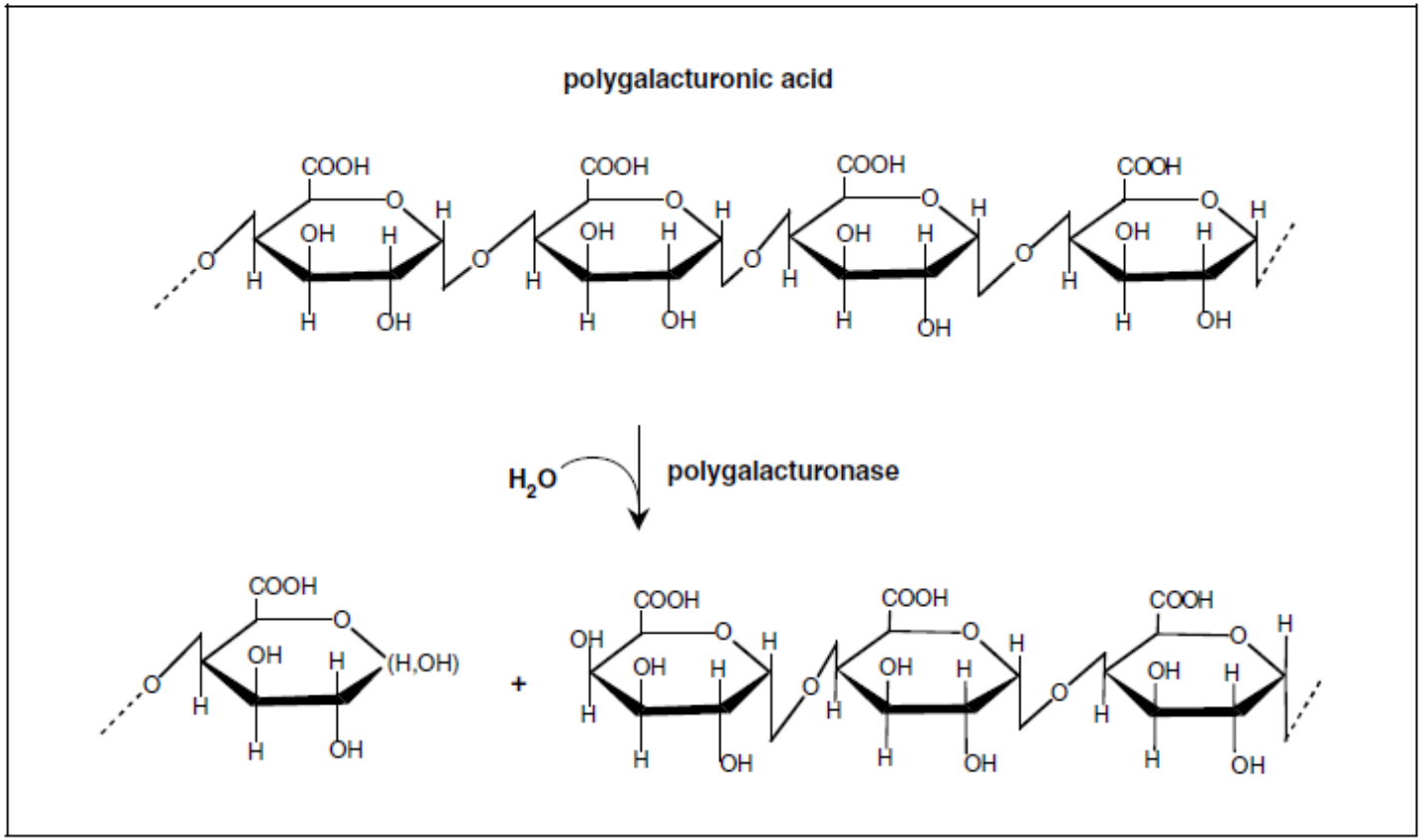



**Figure C1.2.1** Reaction catalyzed by polygalacturonase.

# Ενεργότητα ενζύμου σαν συνάρτηση της συγκέντρωσης

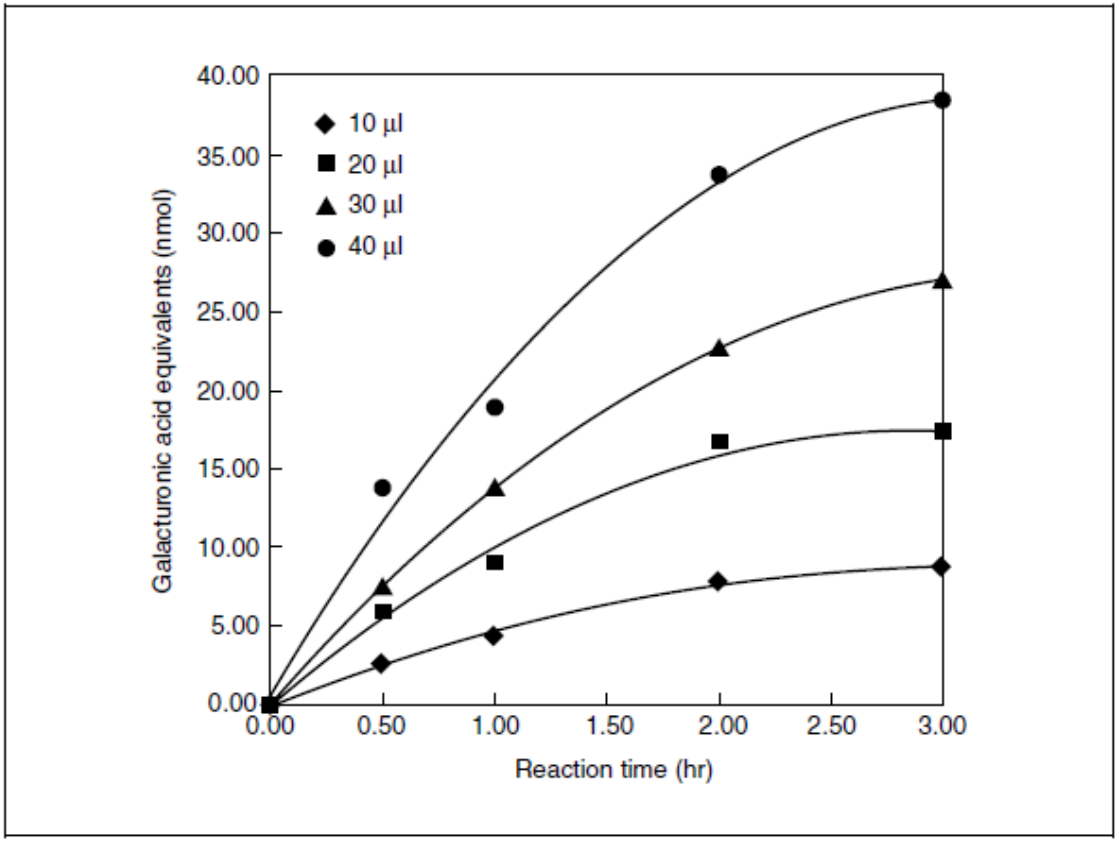



**Figure C1.2.3** Reaction progress curves for the production of new reducing ends (measured as galacturonic acid equivalents) at different enzyme loads.