LAB-EXPERIENCE INTRODUCTION

Important knowledges/ requirment

1- Basical knowledge(s)

2- Lab equipment

3- How to use lab equipment

4- Stock solution preparation

- [ ] 1) Solution preparation from a solid substrate
- [ ] 2) Solution reparation by dilution of a concentrated stock solution
- [ ] 3) Buffer solution preparation
- [ ] 4) Dilution v/v, serial dilution

5- Analytes' extraction principle

6- Analysis' building block(s)

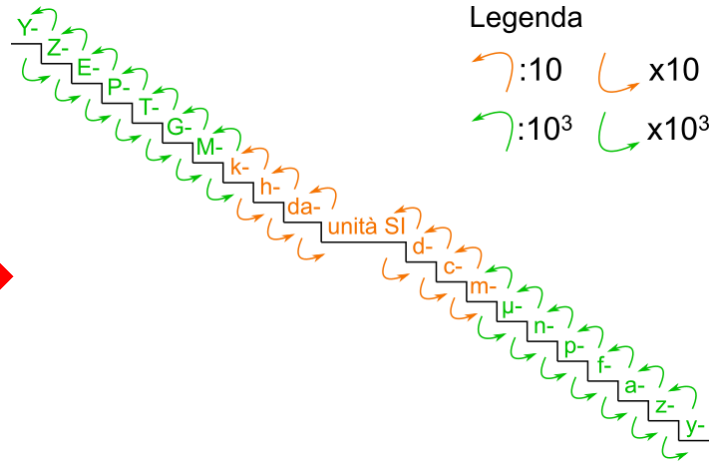
7- Analytical strategies

Basical knowledge(s)

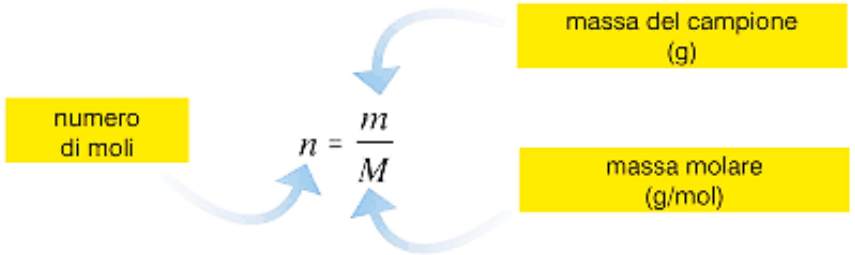
UNIVERSITÀ DEGLI STUDI DI TERAMO

Formulary

SI Base Units		
Physical Quantity	**Name of Unit**	**Abbreviation**
Mass	kilogram	kg
Length	meter	m
Time	second	s
Temperature	kelvin	K
Amount of substance	mole	mol
Electric current	ampere	A
Luminous intensity	candela	cd



Moles and [C]



$$\text{molarità} = \text{M} = \frac{n_{\text{soluto}}\,(\text{mol})}{V_{\text{soluzione}}\,(\text{L})}$$

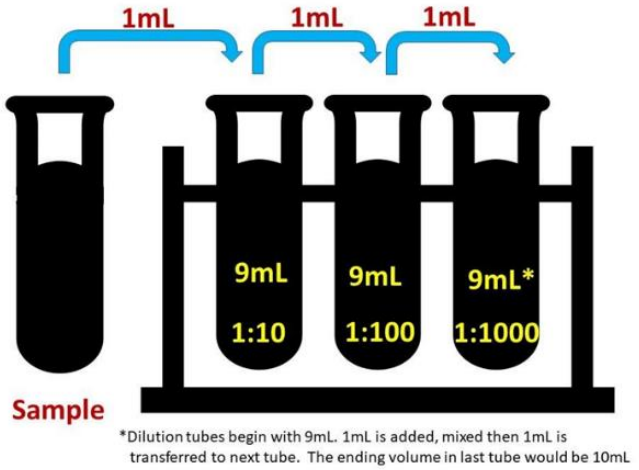
The Dilution Equation

$$M_1V_1 = M_2V_2$$

M_1 = initial molarity ("stock solution")
V_1 = initial volume (Liters)
M_2 = final (desired) molarity
V_2 = final volume (Liters)

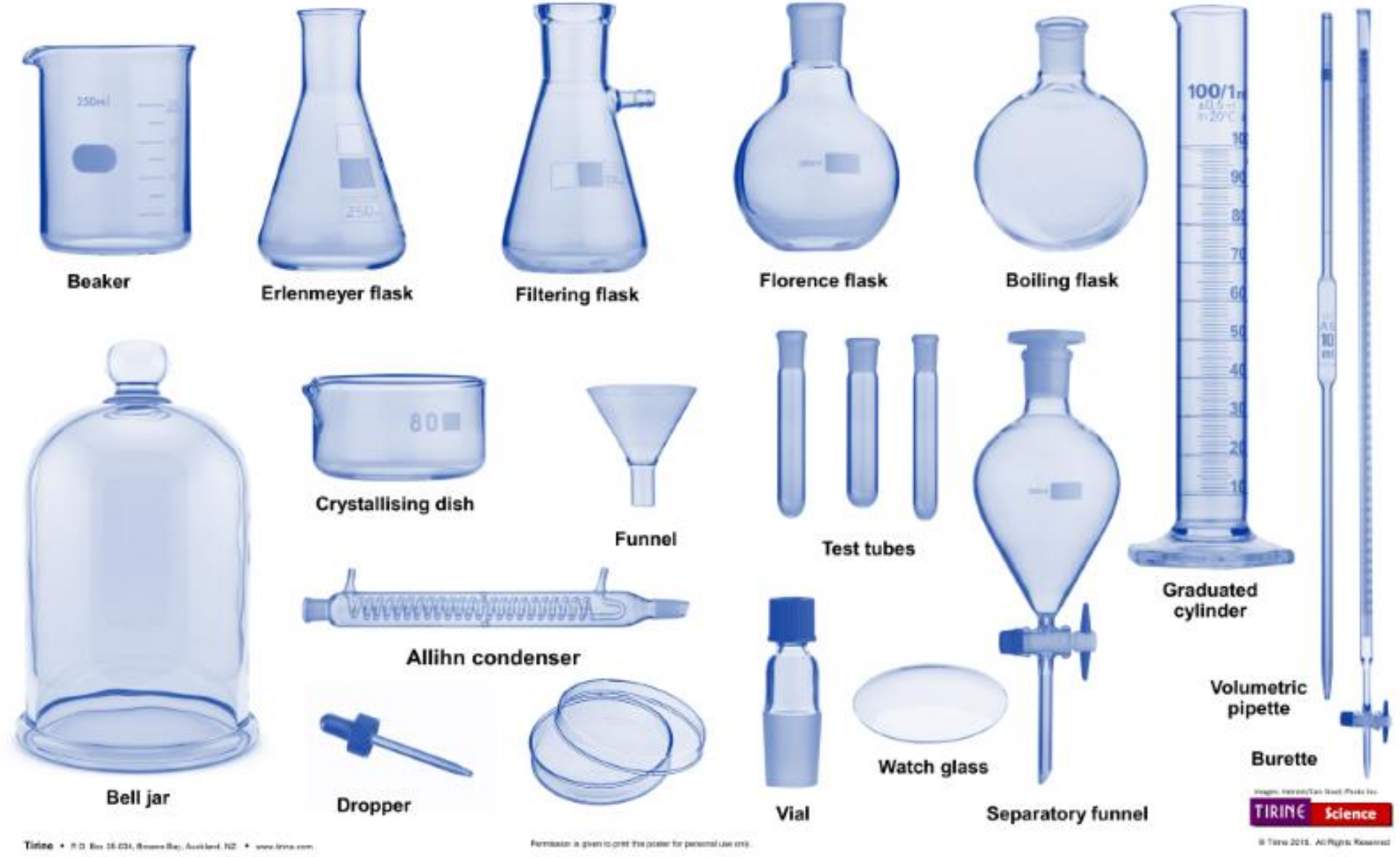
This equation is used when you have a "stock solution" of higher molarity than you need and you need to dilute it to a lower molarity by adding additional solvent.

Serial dilution



*Dilution tubes begin with 9mL. 1mL is added, mixed then 1mL is transferred to next tube. The ending volume in last tube would be 10mL

Lab glassware



Lab glassware

UNIVERSITÀ DEGLI STUDI DI TERAMO

- Spray bottle

- Eppendorf

- Rak

- Flacon

Lab glassware

- Desiccator/ Deumidificator

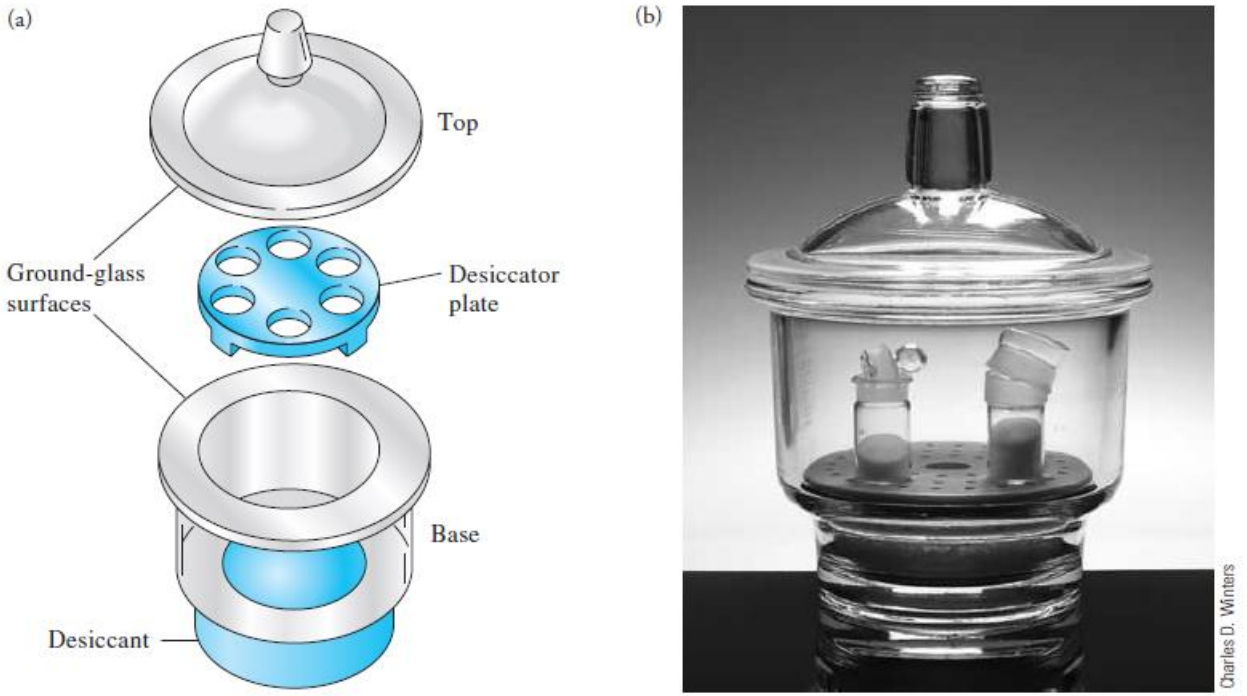
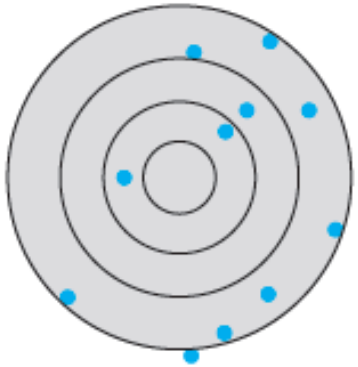
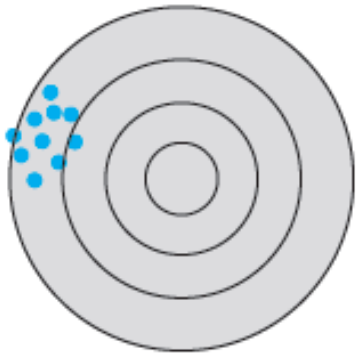


Figure 2-8 (a) Components of a typical desiccator. The base contains a chemical drying agent, which is usually covered with a wire screen and a porcelain plate with holes to accommodate weighing bottles or crucibles. (b) Photo of desiccator containing weighing bottles with dry solids.

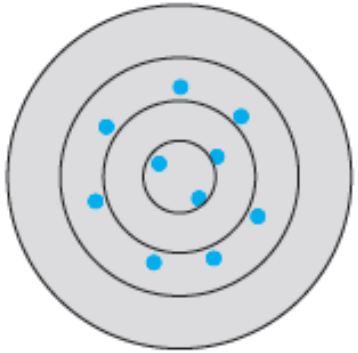
Precise or accurate?



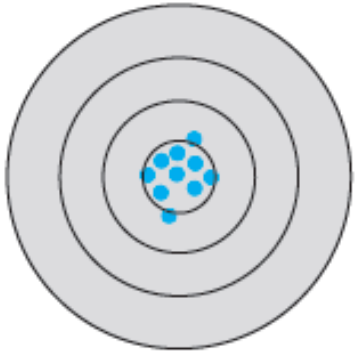
Low accuracy, low precision



Low accuracy, high precision



High accuracy, low precision



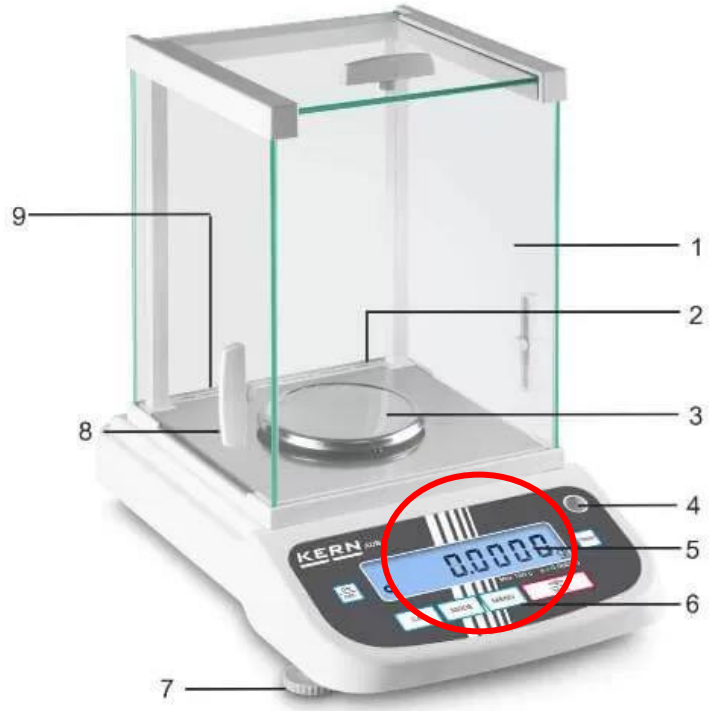
High accuracy, high precision

What precision means?

What accuracy means?

Measuring solids

- Technical and Analytical balance

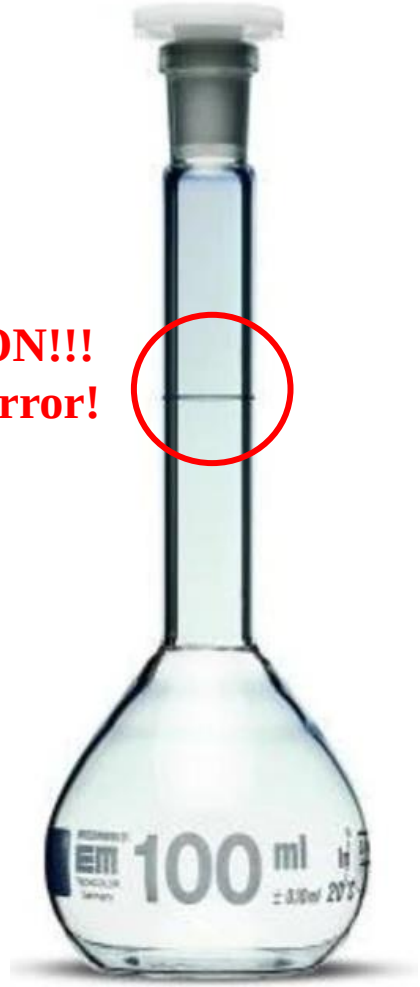


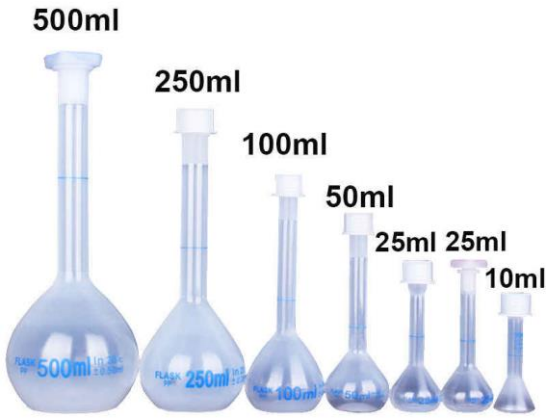
Measuring volume

UNIVERSITÀ DEGLI STUDI DI TERAMO

- Flask

PAY ATTENTION!!!
Parallasse error!





- Buret

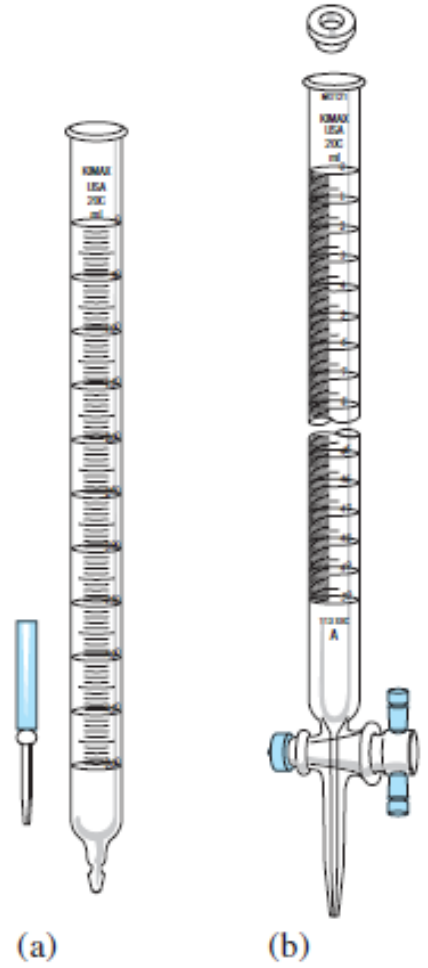
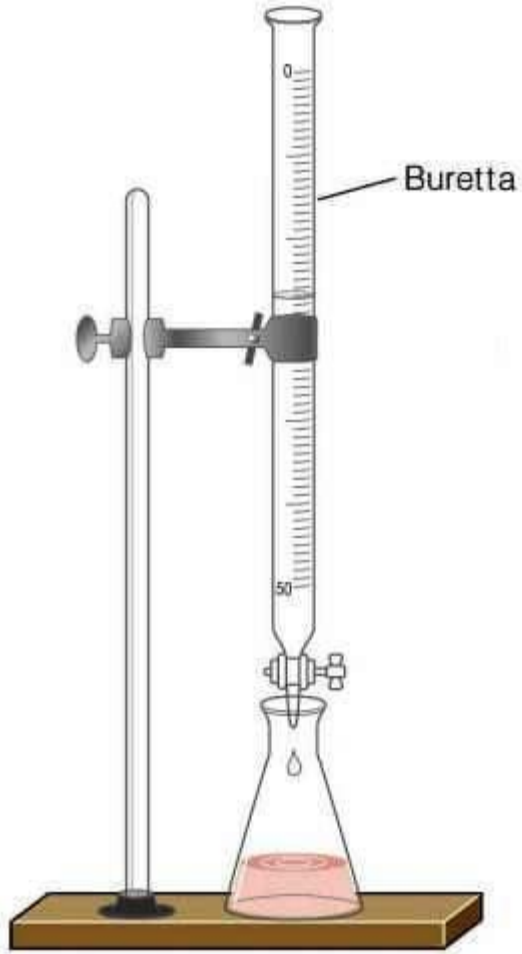


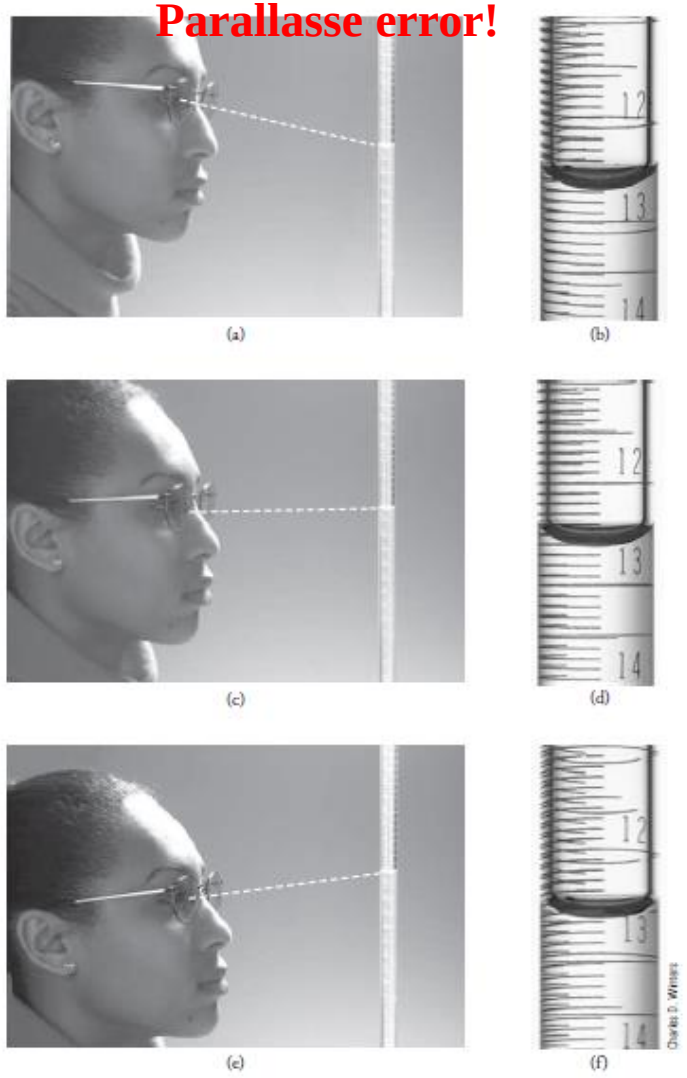
Figure 2-19 Burets:
(a) glass-bead valve,
(b) Teflon valve.

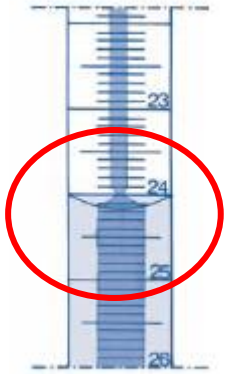
Measuring volume

- Buret



PAY ATTENTION!!!
Parallasse error!





Measuring volume

- Pipette

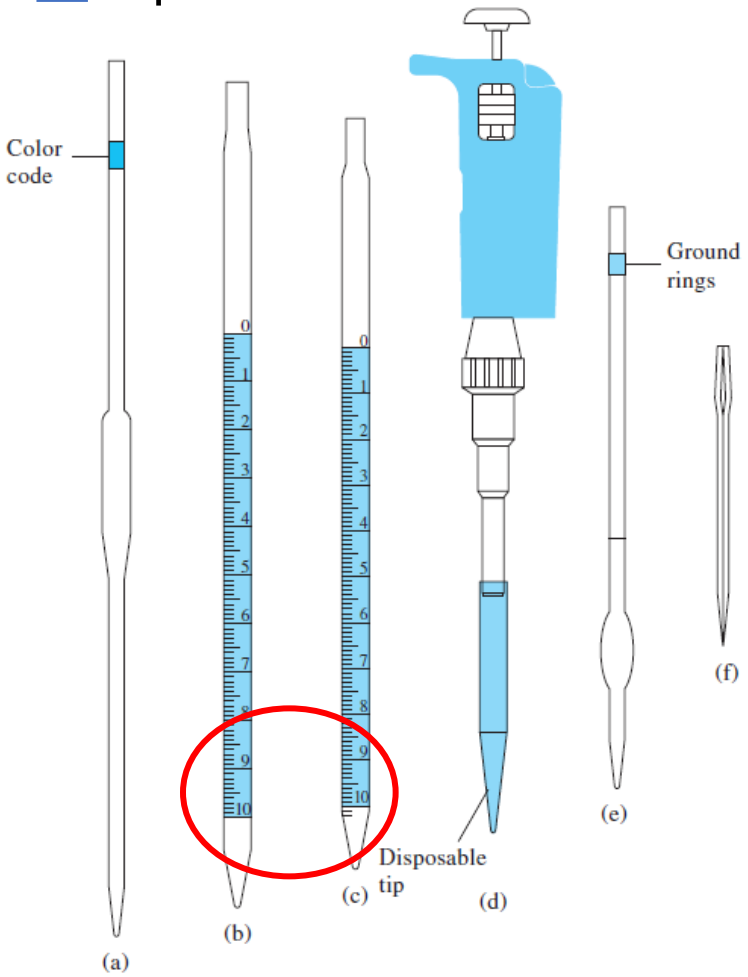


Figure 2-17 Typical pipets: (a) volumetric pipet, (b) Mohr pipet, (c) serological pipet, (d) Eppendorf micropipet, (e) Ostwald–Folin pipet, (f) lambda pipet.

Tolerances, Class A Transfer Pipets

Capacity, mL	Tolerances, mL
0.5	±0.006
1	±0.006
2	±0.006
5	±0.01
10	±0.02
20	±0.03
25	±0.03
50	±0.05
100	±0.08

TABLE 2-2
Characteristics of Pipets

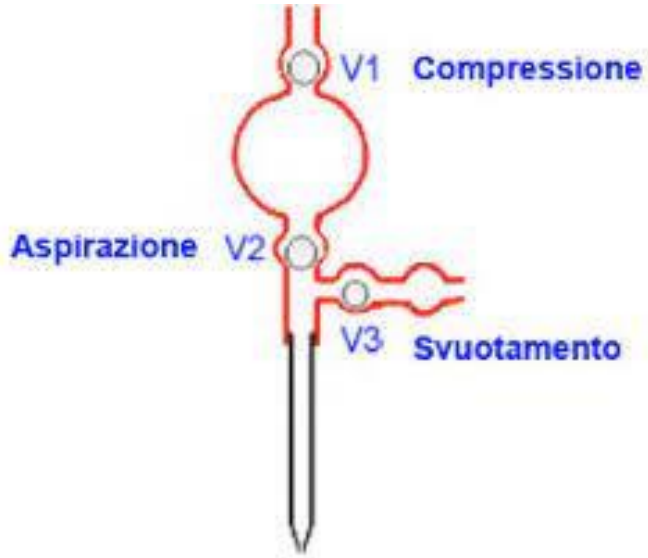
Name	Type of Calibration*	Function	Available Capacity, mL	Type of Drainage
Volumetric	TD	Delivery of fixed volume	1–200	Free
Mohr	TD	Delivery of variable volume	1–25	To lower calibration line
Serological	TD	Delivery of variable volume	0.1–10	Blow out last drop**
Serological	TD	Delivery of variable volume	0.1–10	To lower calibration line
Ostwald-Folin	TD	Delivery of fixed volume	0.5–10	Blow out last drop**
Lambda	TC	Containment of fixed volume	0.001–2	Wash out with suitable solvent
Lambda	TD	Delivery of fixed volume	0.001–2	Blow out last drop**
Eppendorf	TD	Delivery of variable or fixed volume	0.001–1	Tip emptied by air displacement

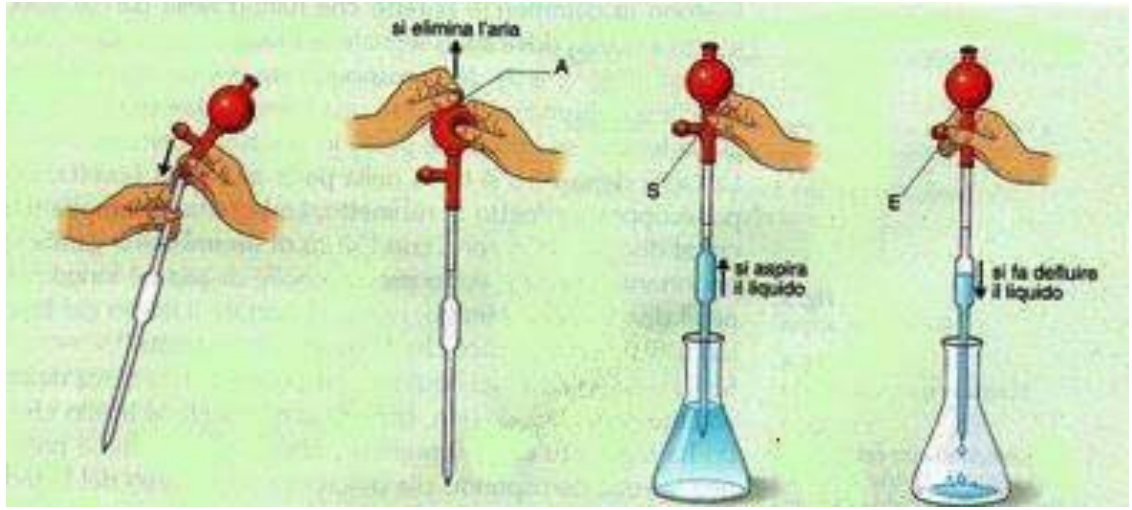
*TD, to deliver; TC, to contain.
**A frosted ring near the top of pipets indicates that the last drop is to be blown out.

Measuring volume

- Glassware pipette

PELEUS BALL

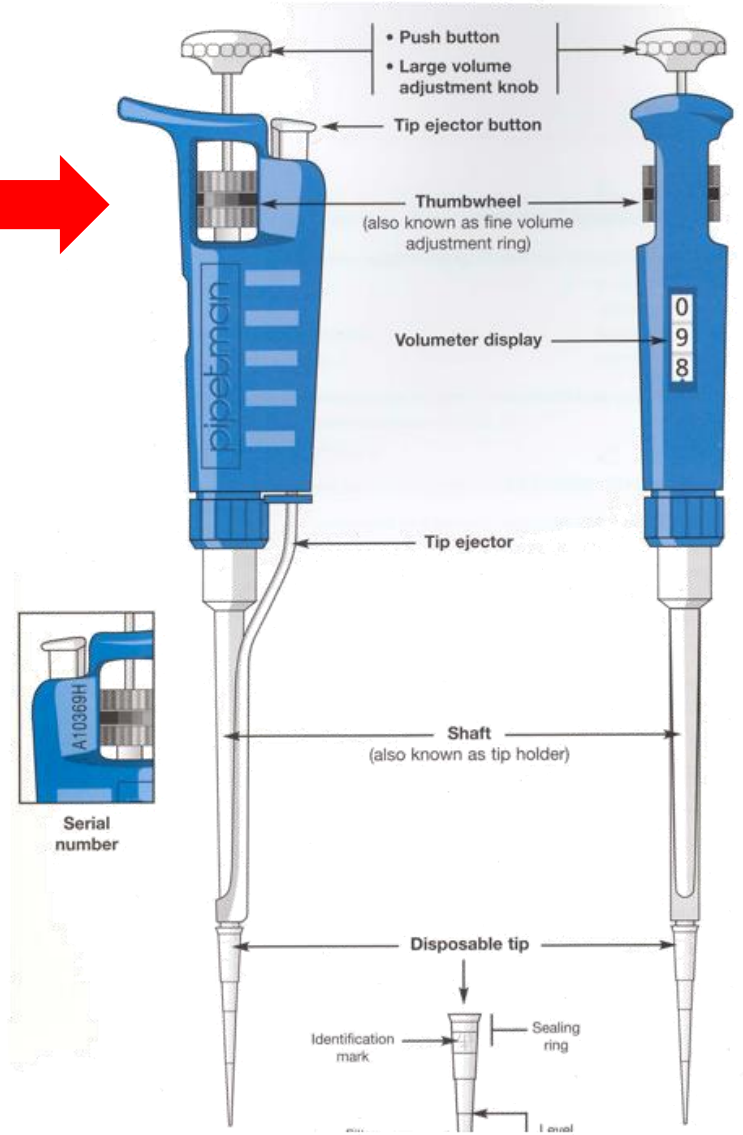




Measuring volume

- Gilson pipette

PAY ATTENTION!!!

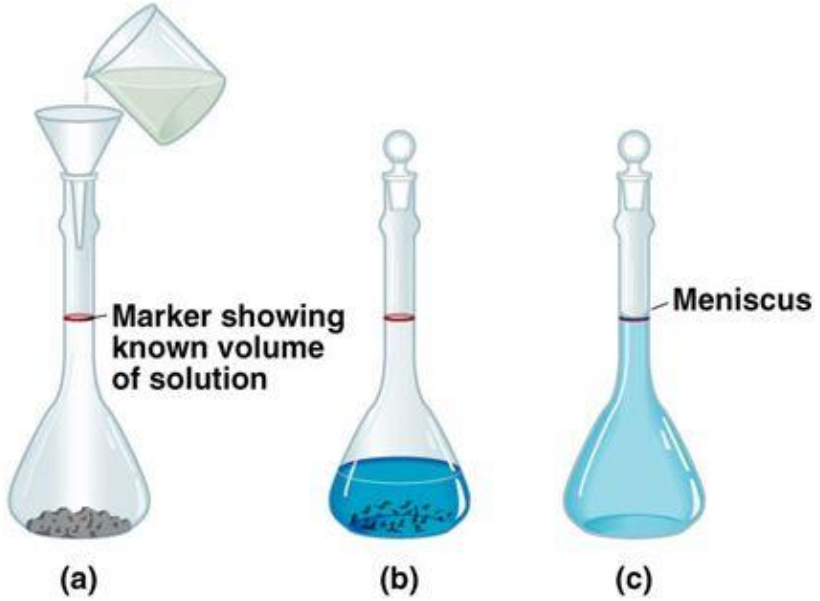


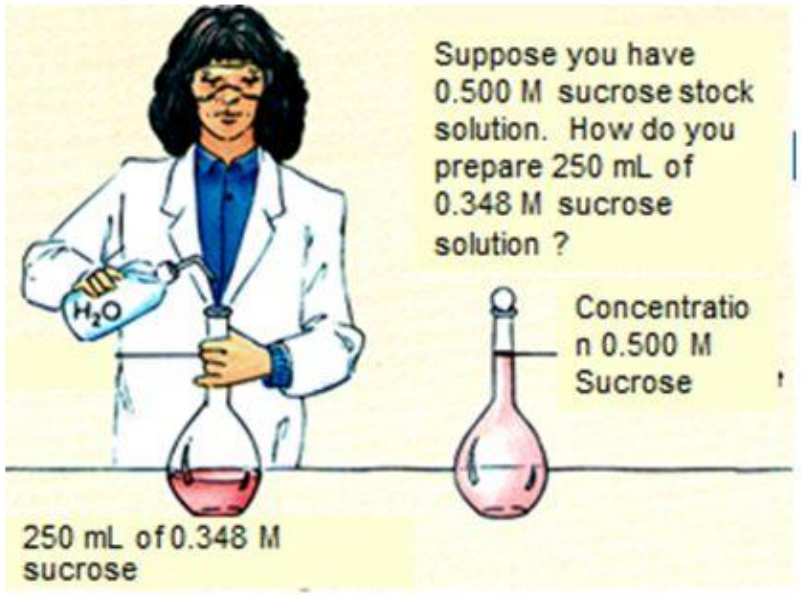
Range and Precision of Typical Eppendorf Micropipets

Volume Range, µL	Standard Deviation, µL
1–20	<0.04 @ 2 µL
	<0.06 @ 20 µL
10–100	<0.10 @ 15 µL
	<0.15 @ 100 µL
20–200	<0.15 @ 25 µL
	<0.30 @ 200 µL
100–1000	<0.6 @ 250 µL
	<1.3 @ 1000 µL
500–5000	<3 @ 1.0 mL
	<8 @ 5.0 mL

Two methods for Preparation of a desired volume of a Molar Solution

- 1) Preparation from a solid solute.
- 2) Preparation by Dilution of a Concentrated Stock Solution.

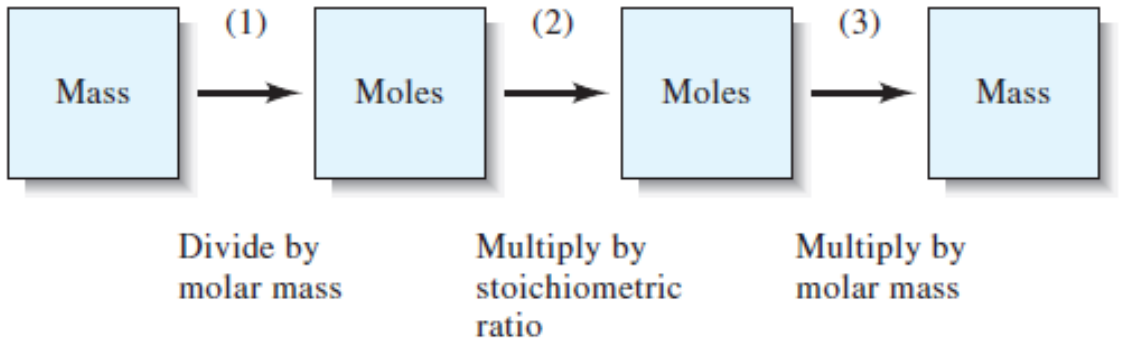


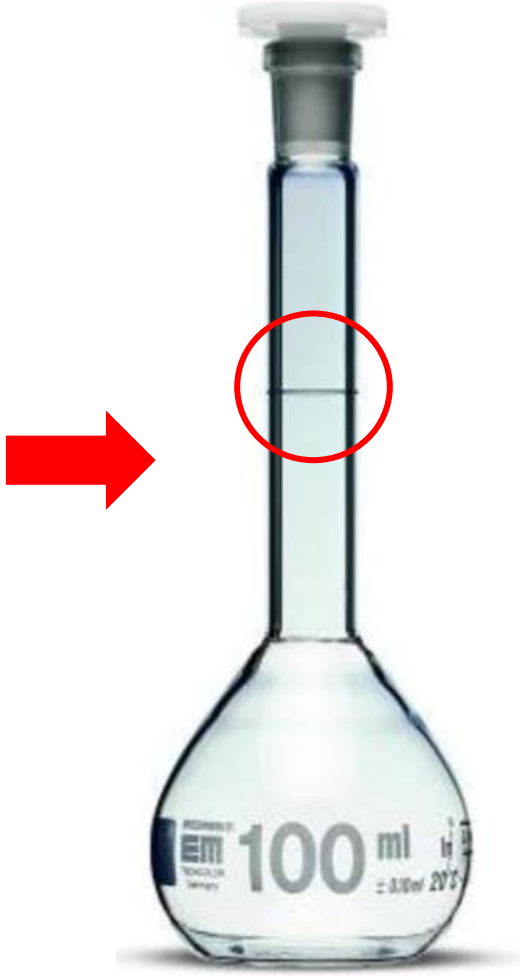


How to solve?

Stock solution preparation

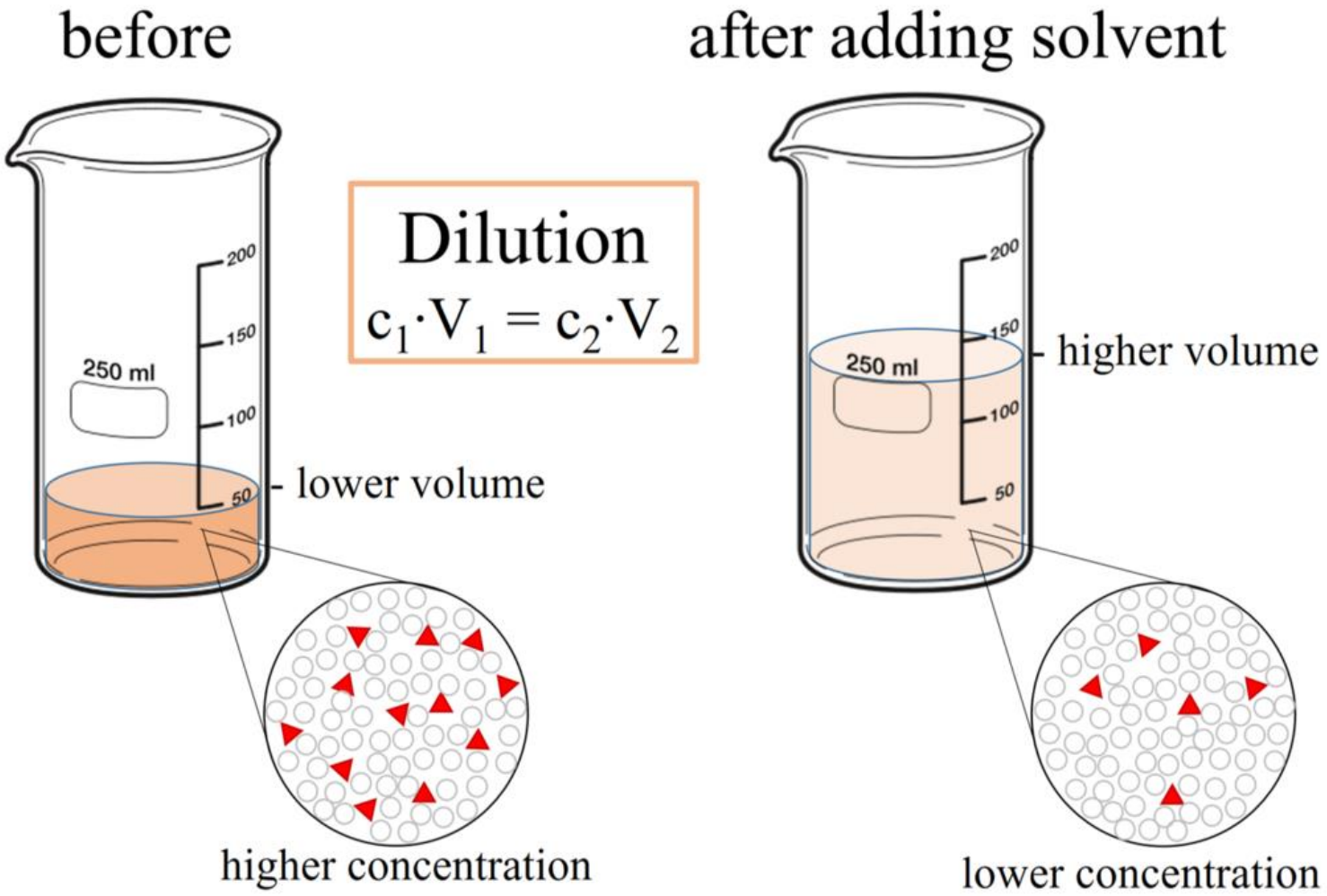
- 1) Preparation from a solid substrate





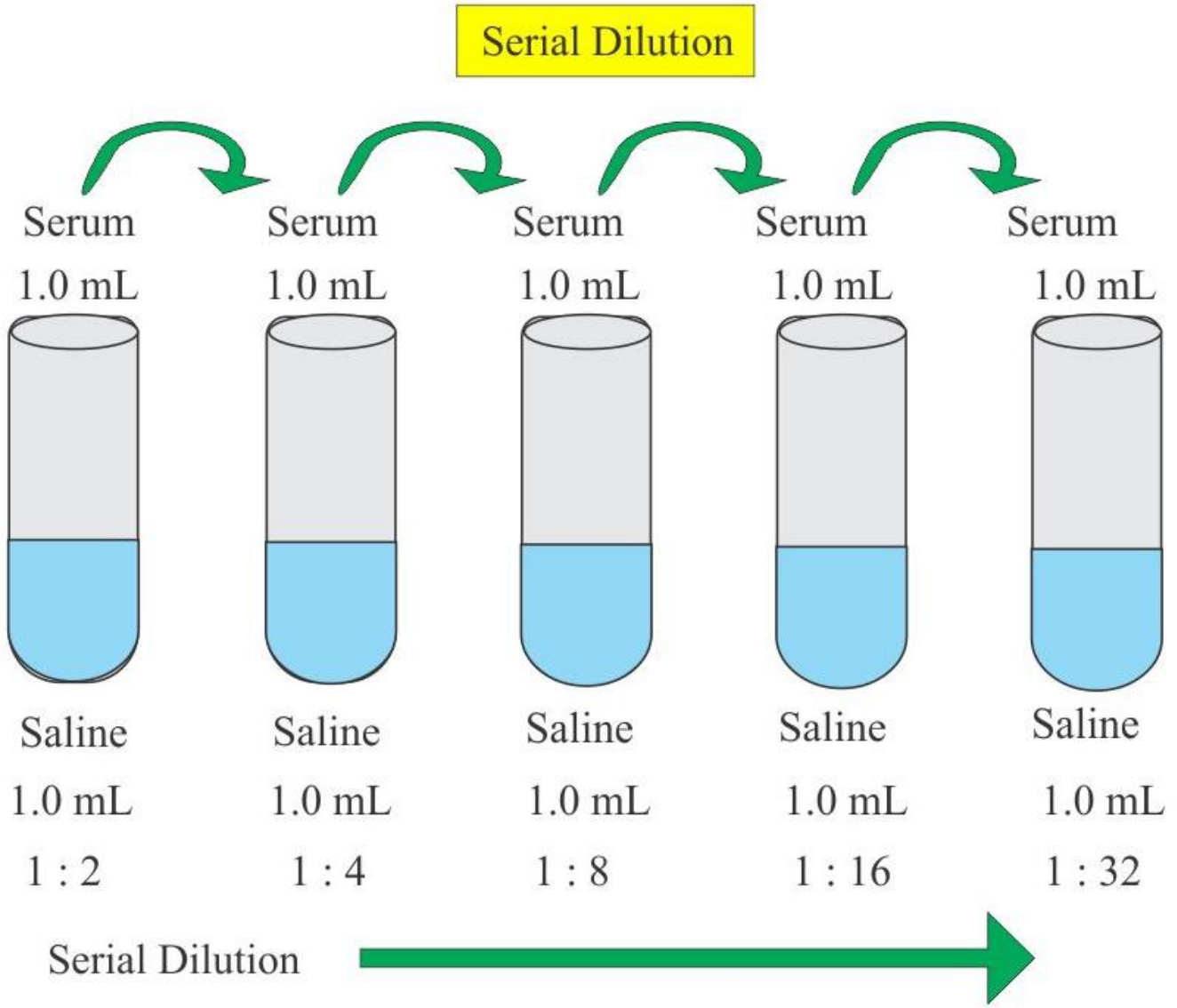
Stock solution preparation

- 2) Preparation by dilution of a concentrated stock solution



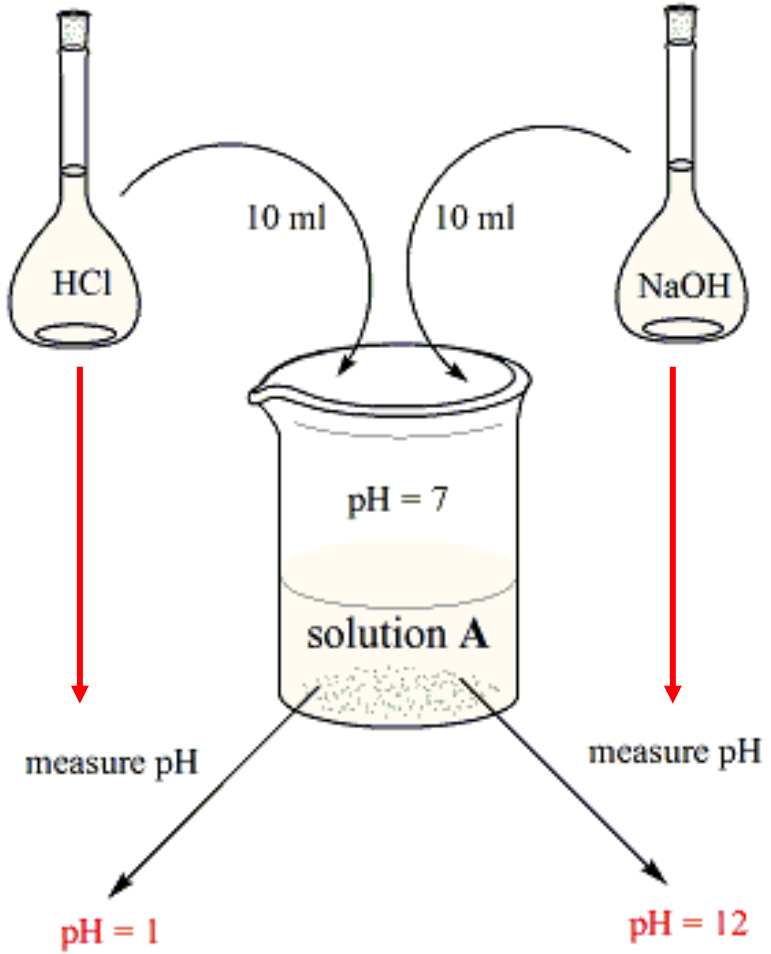
Stock solution preparation

- Serial dilution

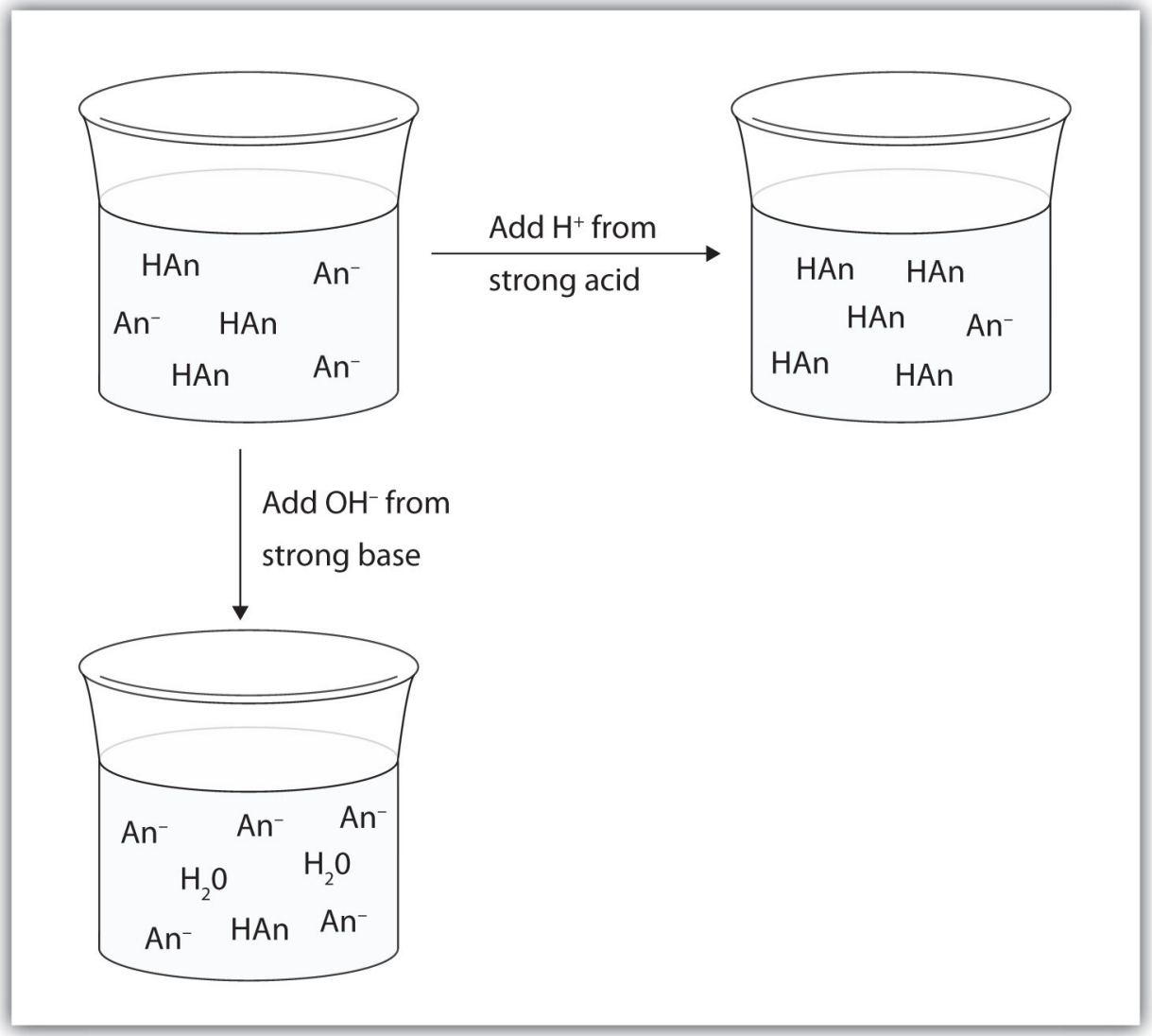


Stock solution preparation

- 3) Buffer solution preparation



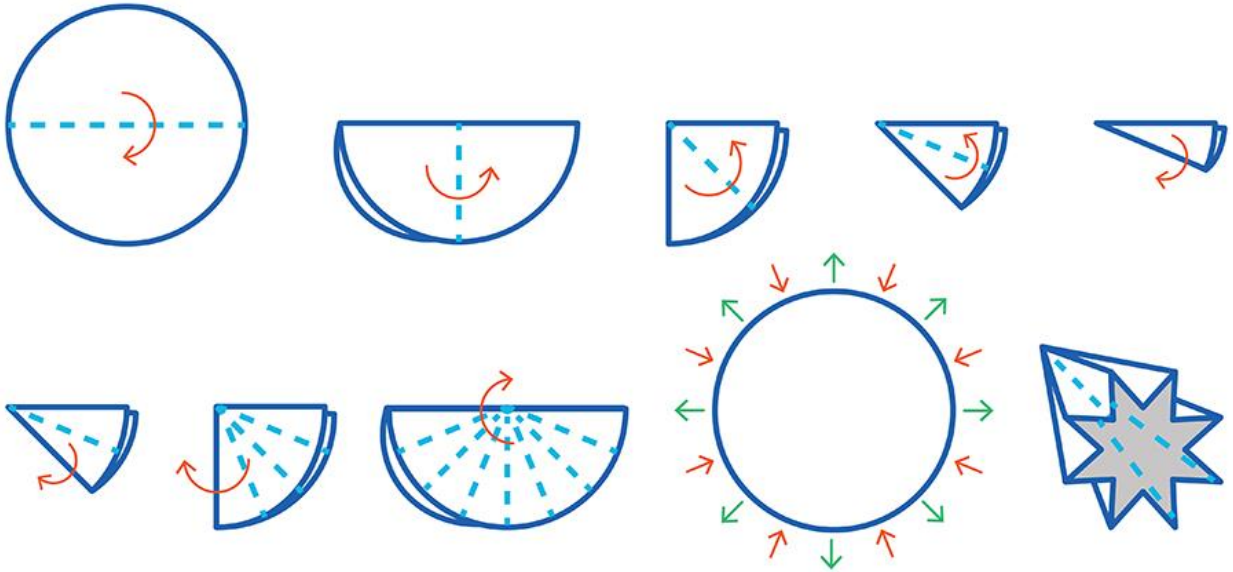
?



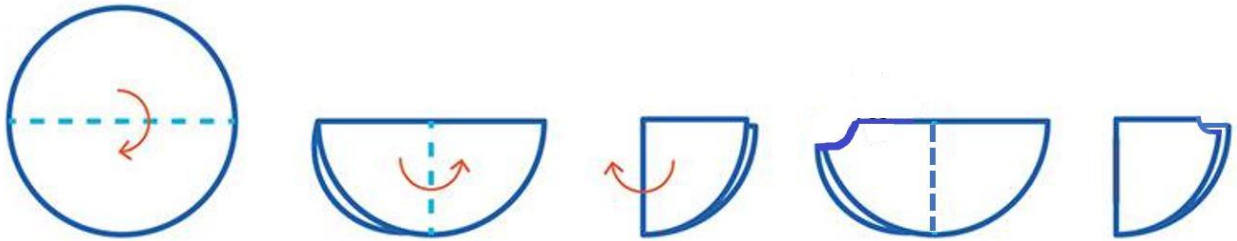
Separation system

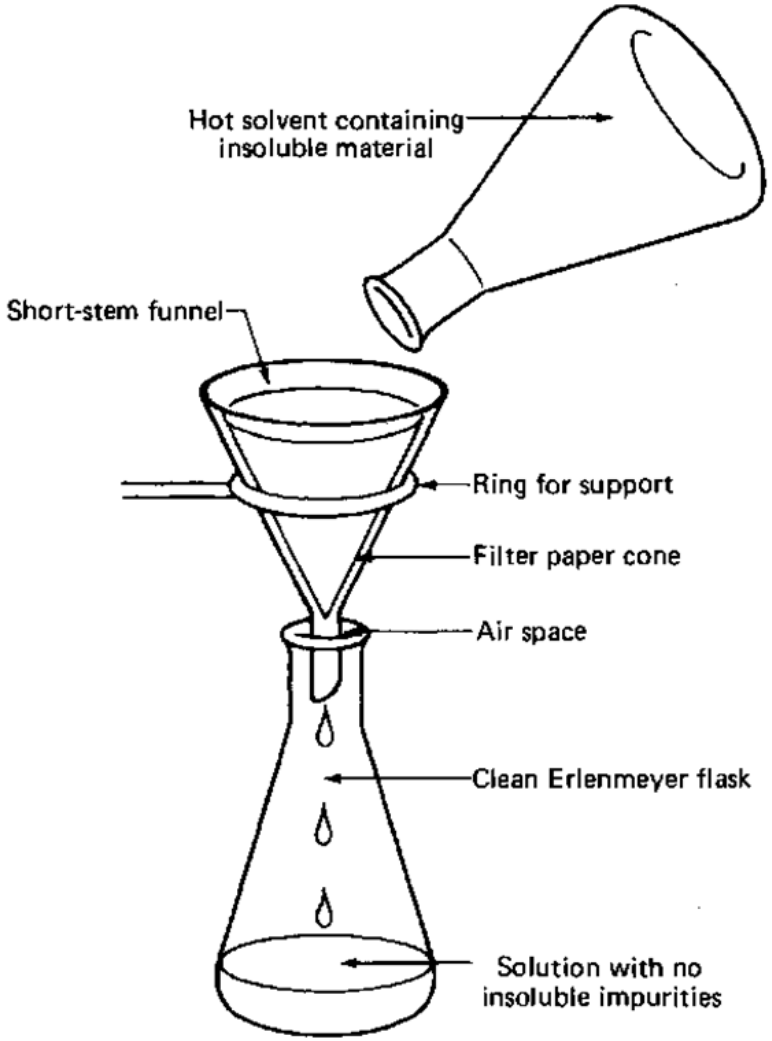
- How to build up a paper filter

A)



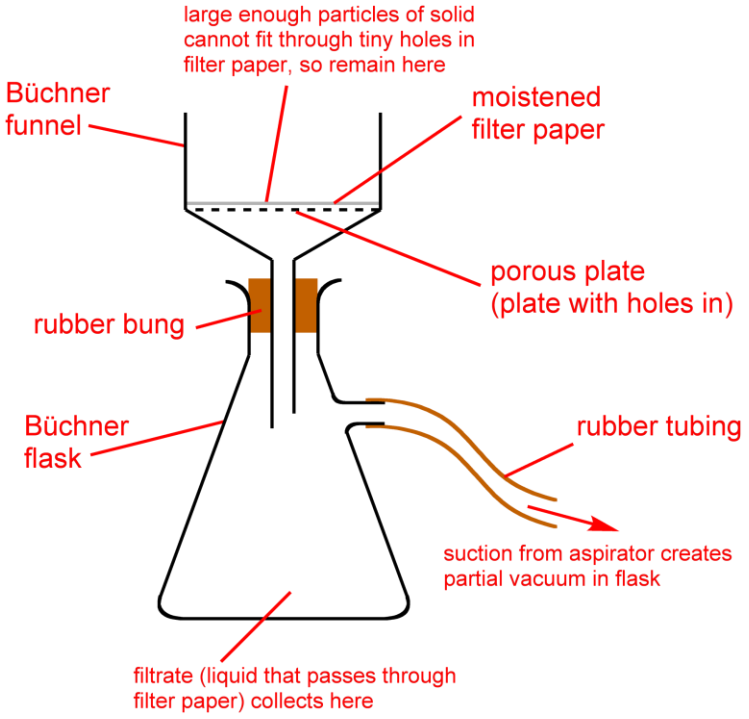
B)



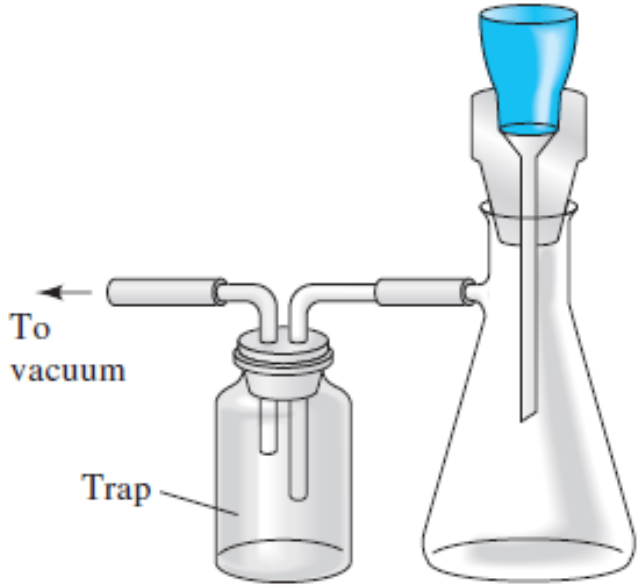


Separation system

- Vacuum filtration



Vacuum system

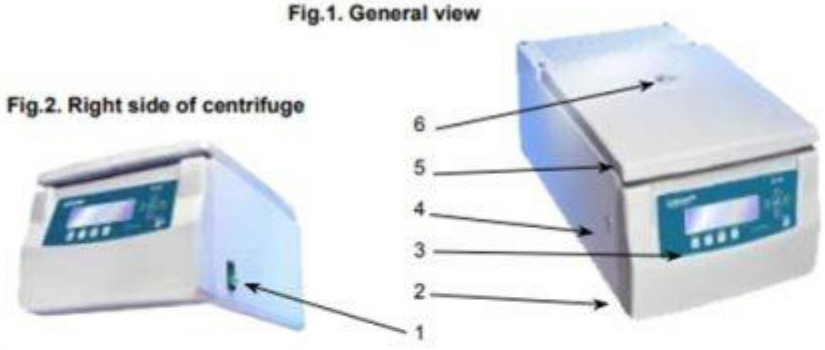


Separation system

- Centrifuge

1

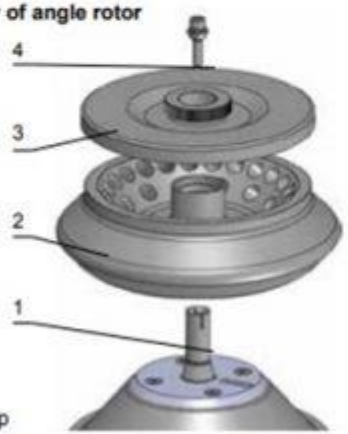
Fig.1. General view



1. Power switch
2. USB
3. Control panel
4. Point of emergency lid opening
5. Lid
6. Inspection glass

Fig.2. Right side of centrifuge

Fig.3. Assembly of angle rotor

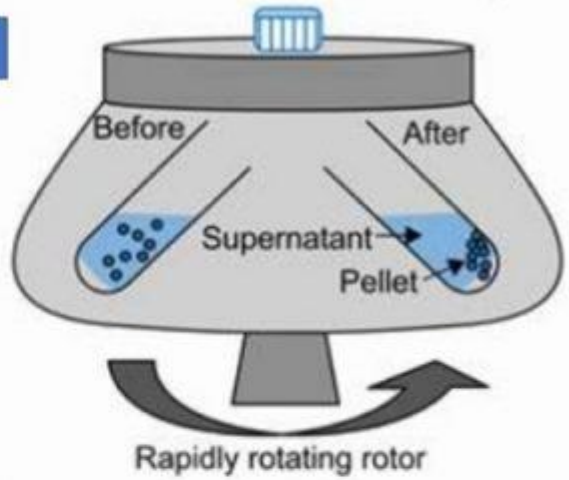


1. Motor axle
2. Rotor
3. Rotor lid
4. Complete clamp

Fig.4. Mains socket back of the centrifuge

1. Main socket
2. Fuse socket

2



3

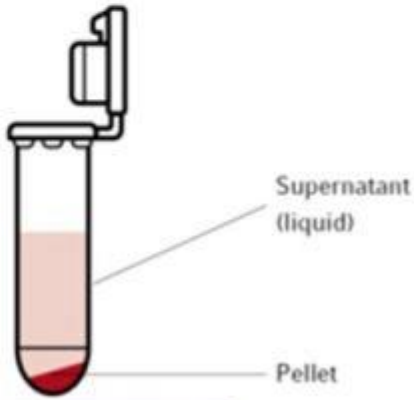


Fig. Centrifuge: General View, Centrifugation procedure and components separation

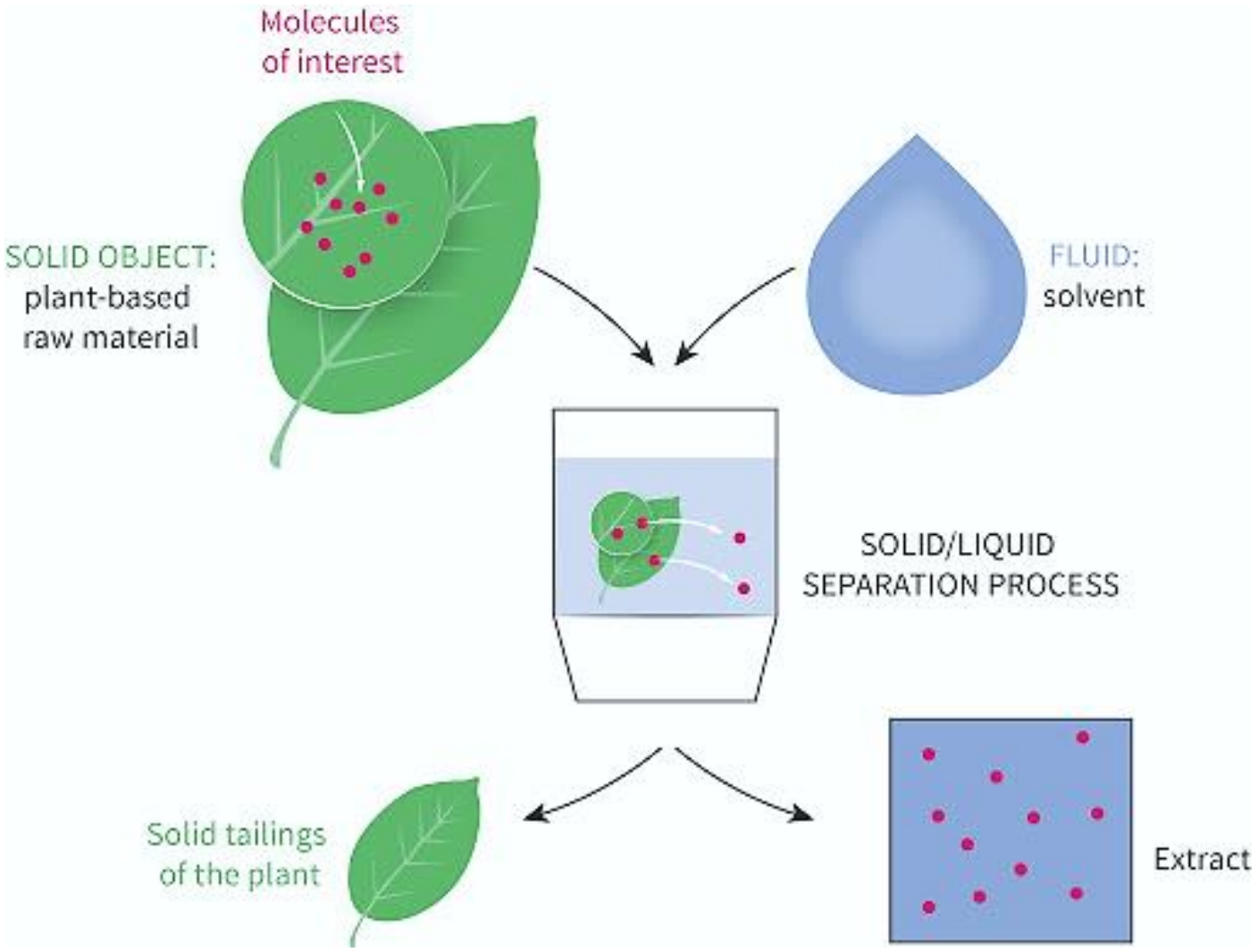
Image sources: handling-solutions.eppendorf.com & labnetinternational.com

Analytes' extraction principle

Solid-liquid extraction. Extraction form solid sample.

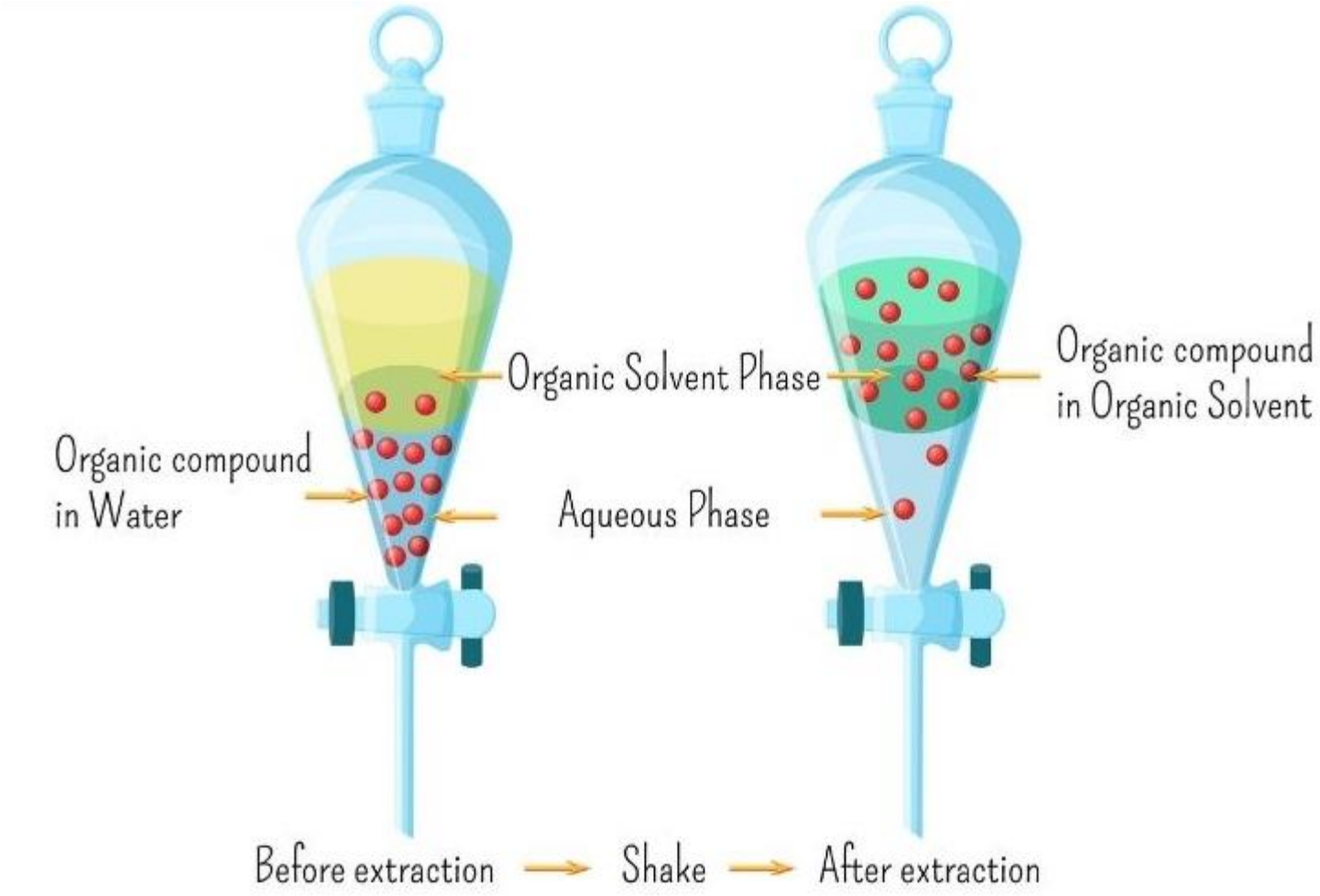
A) **Sample Weight**

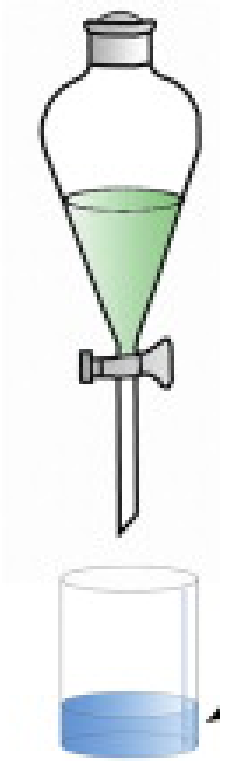
B) **Sample Extraction**



Analytes' extraction principle

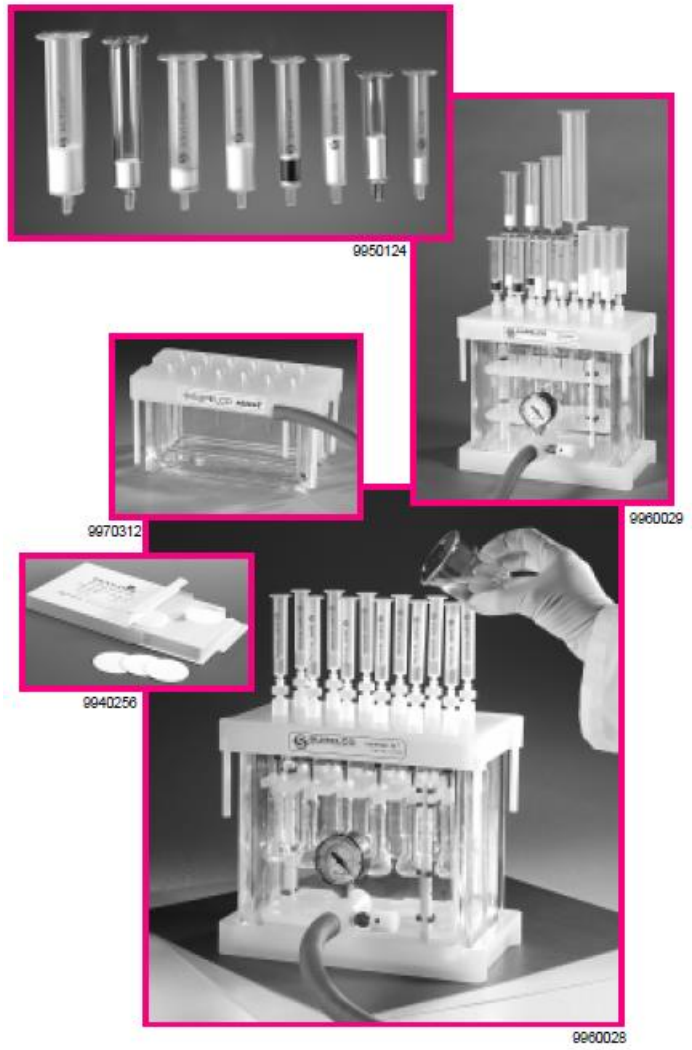
Liquid-liquid extraction. Extraction form liquid sample.

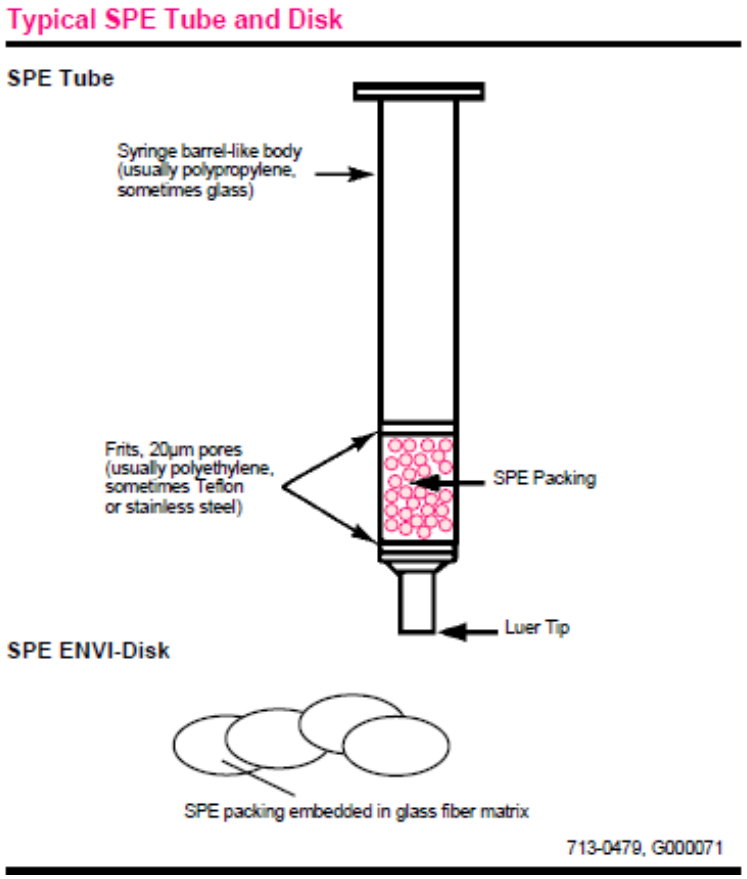




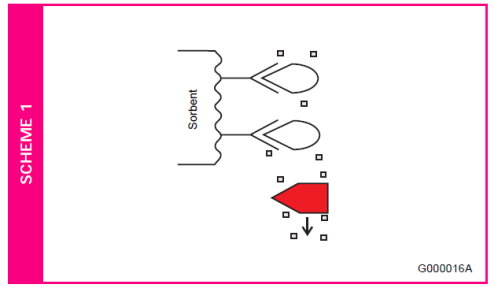
Analytes' extraction principle

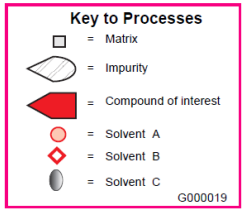
Solid phase extraction (SPE). Extraction form liquid sample.

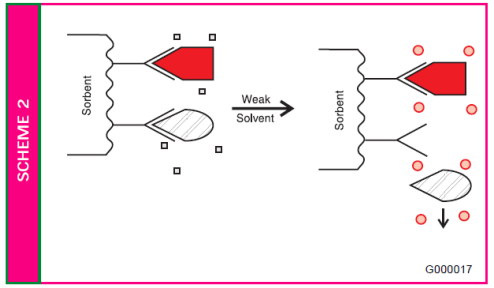


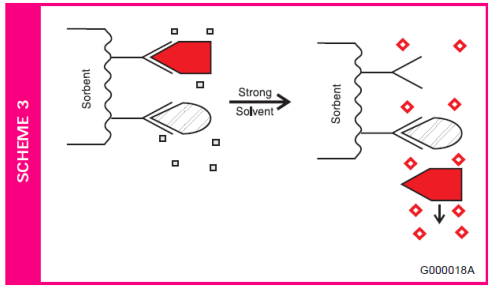


How to use SPE









SOLID PHASE EXTRACTION OF PHOLYPHENOLS FROM OLIVE OIL

Antioxidants

Antioxidants could be defined as sacrificial molecules.
Antioxidants are natural or synthetic molecules able to scavenge reactive species, such as reactive oxygen and nitrogen species (ROS and RNS), contributing to oxidative homeostasis.

Phenolic compounds

Beneficial effect on human health

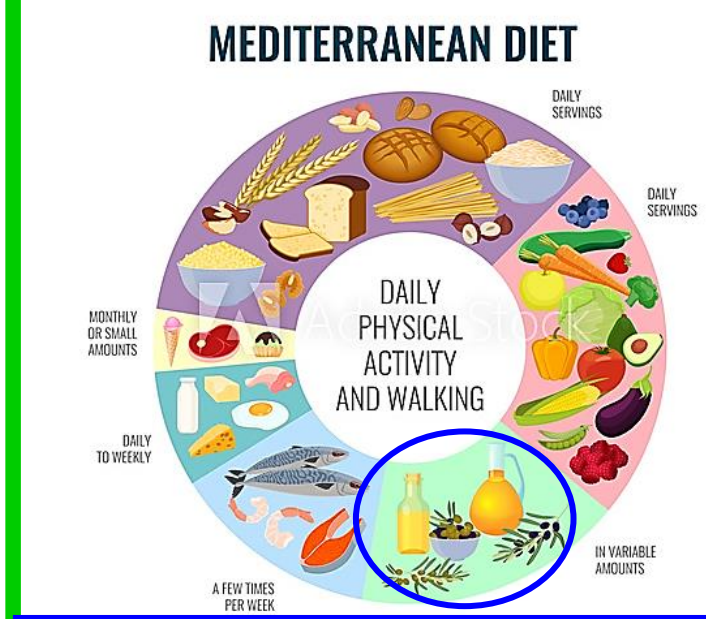
Anti-microbial property

Additives in biomedicine practices

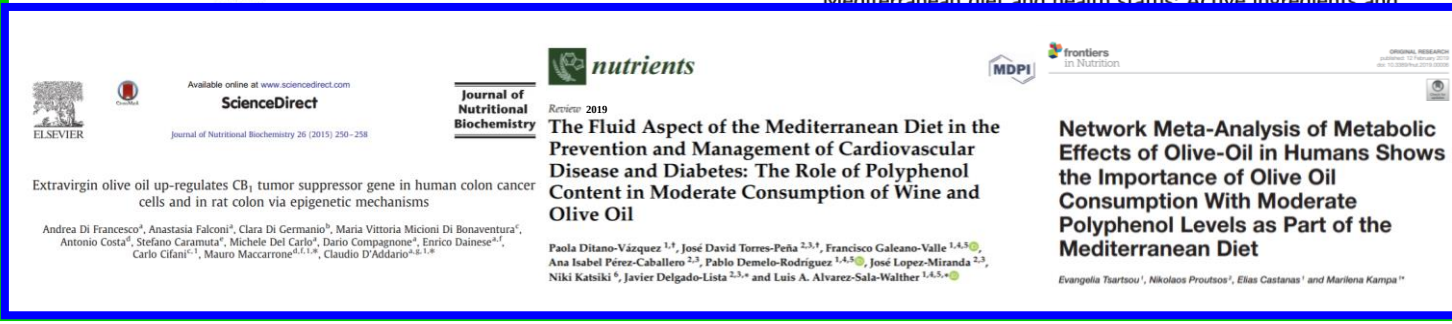
Food supplements (sensory and nutritional properties, shelf-life)

Quality and process indicators

Potential tools for functionalization of materials



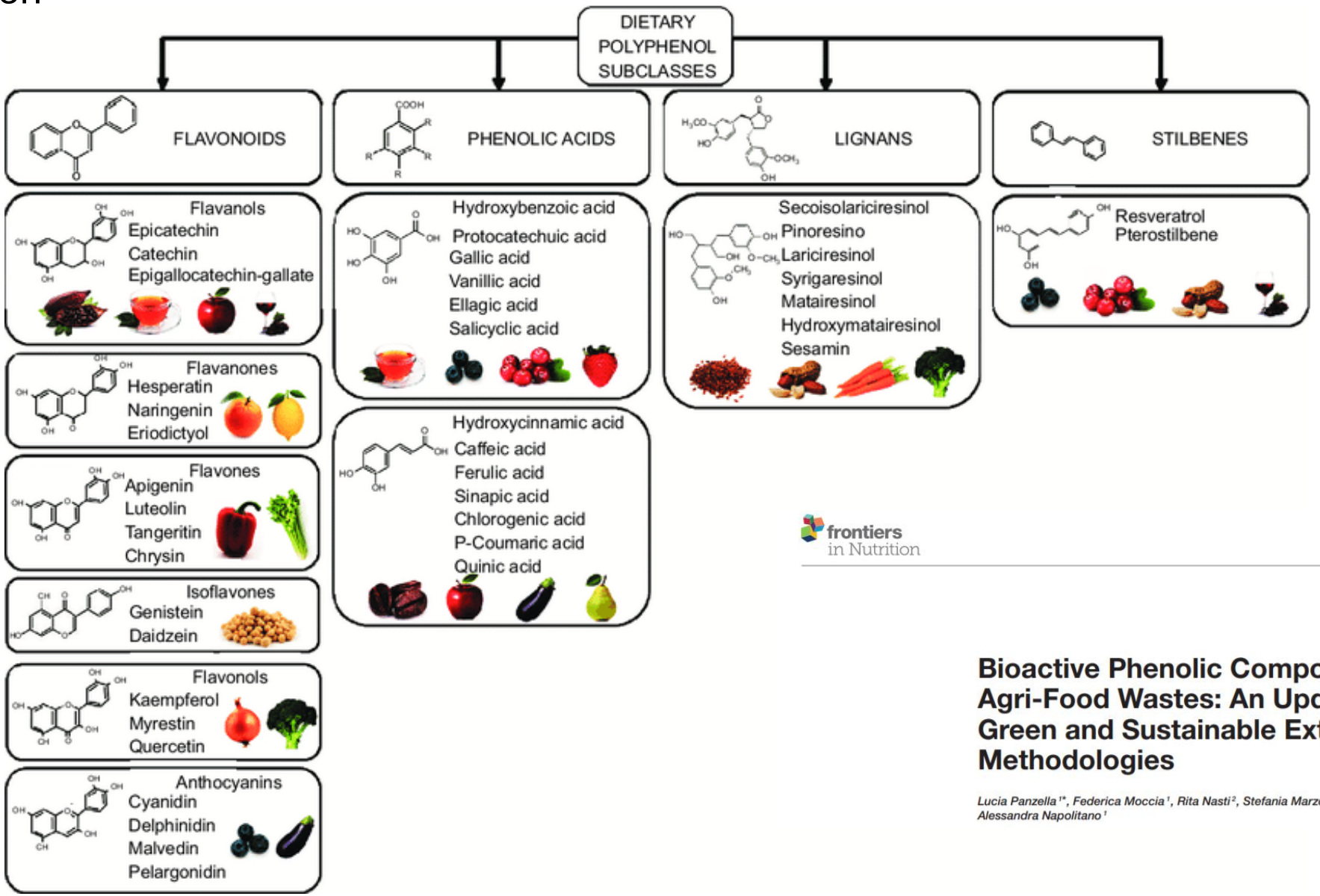

Maturitas 132 (2020) 65–69
Contents lists available at ScienceDirect
Maturitas
journal homepage: www.elsevier.com/locate/maturitas
The Mediterranean diet: A historical perspective on food for health
Juan José Hidalgo-Mora, Alicia García-Vigara, María Luz Sánchez-Sánchez, Miguel-Ángel García-Pérez, Juan Tarín, Antonio Cano
Molecular Aspects of Medicine 67 (2019) 1–55
Contents lists available at ScienceDirect
Molecular Aspects of Medicine
journal homepage: www.elsevier.com/locate/mam
Benefits of the Mediterranean diet: Epidemiological and molecular aspects
Lluis Serra-Majem, Blanca Román-Viñas, Almudena Sanchez-Villegas, Marta Guasch-Ferré, Dolores Corella, Carlo La Vecchia
Received: 10 April 2019 | Revised: 8 June 2019 | Accepted: 17 June 2019
DOI: 10.1111/bph.14778
Themed Section: The Pharmacology of Nutraceuticals
REVIEW ARTICLE
Mediterranean diet and health status: Active ingredients and


Available online at www.sciencedirect.com
ScienceDirect
Journal of Nutritional Biochemistry 26 (2015) 250–258
Journal of Nutritional Biochemistry
Extravirgin olive oil up-regulates CB_1 tumor suppressor gene in human colon cancer cells and in rat colon via epigenetic mechanisms
Andrea Di Francesco, Anastasia Falconi, Clara Di Germanio, Maria Vittoria Micioni Di Bonaventura, Antonio Costa, Stefano Caramuta, Michele Del Carlo, Dario Compagnone, Enrico Dainese, Carlo Cifani, Mauro Maccarrone, Claudio D'Addario
nutrients
Review 2019
The Fluid Aspect of the Mediterranean Diet in the Prevention and Management of Cardiovascular Disease and Diabetes: The Role of Polyphenol Content in Moderate Consumption of Wine and Olive Oil
Paola Ditano-Vázquez, José David Torres-Peña, Francisco Galeano-Valle, Ana Isabel Pérez-Caballero, Pablo Demelo-Rodríguez, José Lopez-Miranda, Niki Katsiki, Javier Delgado-Lista and Luis A. Alvarez-Sala-Walther
MDPI
frontiers in Nutrition
Network Meta-Analysis of Metabolic Effects of Olive-Oil in Humans Shows the Importance of Olive Oil Consumption With Moderate Polyphenol Levels as Part of the Mediterranean Diet
Evangelia Tsartsou, Nikolaos Proutsos, Elias Castanas and Marilena Kampa

Antioxidants

UNIVERSITÀ DEGLI STUDI DI TERAMO

Classification



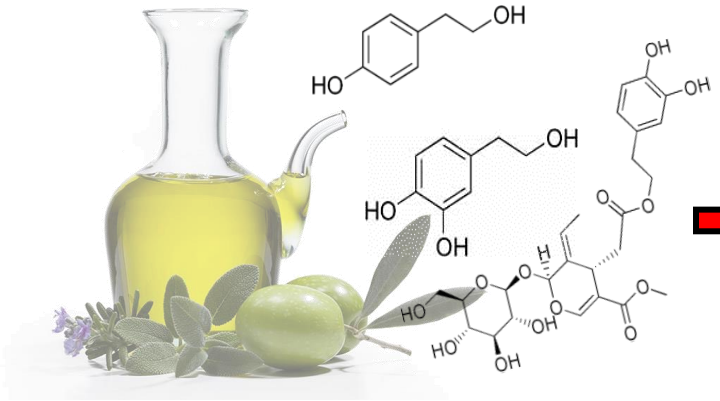
frontiers in Nutrition

REVIEW
published: 07 May 2020
doi: 10.3389/fnut.2020.00060

Bioactive Phenolic Compounds From Agri-Food Wastes: An Update on Green and Sustainable Extraction Methodologies

Lucia Panzella[1], Federica Moccia[1], Rita Nasti[2], Stefania Marzorati[2], Luisella Verotta[2] and Alessandra Napolitano[1]*

Phenolic content evaluation in Extra Virgin Olive Oil. Main strategies



Extraction (LLE / SPE)

Solvent evaporation and phenols concentration

Separative / Chromatographic strategies

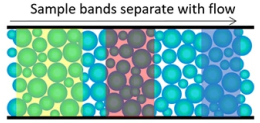
Plant Foods Hum Nutr (2012) 67:326–336
DOI 10.1007/s11130-012-0315-z

ORIGINAL PAPER

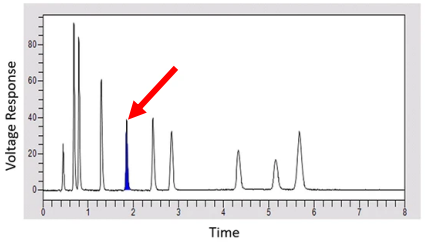
Comprehensive Analysis of Polyphenols in 55 Extra Virgin Olive Oils by HPLC-ECD and Their Correlation with Antioxidant Activities

Banu Bayram · Tuba Esatbeyoglu · Nicole Schulze · Beraat Ozcelik · Jan Frank · Gerald Rimbach

Sample bands separate with flow



Detection



Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Olive oil polyphenols: A quantitative method by high-performance liquid-chromatography-diode-array detection for their determination and the assessment of the related health claim

Massimo Ricciutelli[a], Shara Marconi[b], Maria Chiara Boarelli[b], Giovanni Caprioli[c], Gianni Sagratini[c], Roberto Ballini[b], Dennis Fiorini[b,*]

Electrochemical-based strategies

Microchimica Acta (2019) 186: 363
https://doi.org/10.1007/s00604-019-3418-5

ORIGINAL PAPER

Nanohybrid carbon black-molybdenum disulfide transducers for preconcentration-free voltammetric detection of the olive oil o-diphenols hydroxytyrosol and oleuropein

Daniel Rojas[1,2] · Flavio Della Pelle[1] · Michele Del Carlo[1] · Emiliano Fratini[3] · Alberto Escarpa[2,4] · Dario Compagnone[1]

Food Research International 54 (2013) 2075–2082

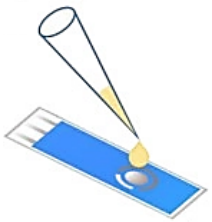
Contents lists available at ScienceDirect

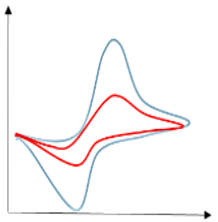
Food Research International

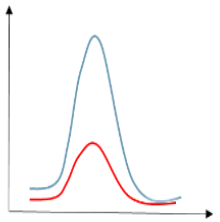
journal homepage: www.elsevier.com/locate/foodres

Voltammetric e-tongue for the quantification of total polyphenol content in olive oils

Irina Mirela Apetrei[a], Constantin Apetrei[b,*]







Optical-based strategies

Research Article

Evaluation of total phenolic content in virgin olive oil using fluorescence excitation–emission spectroscopy coupled with chemometrics

Giacomo Squeo,[a] Francesco Caponio,[a] Vito M Paradiso,[a] Carmine Summo,[a] Antonella Pasqualone,[a] Igor Khmelinskii[b] and Ewa Sikorska[c]*

Computers and Electronics in Agriculture 173 (2020) 105445

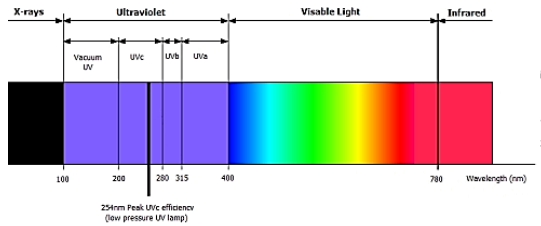
Contents lists available at ScienceDirect

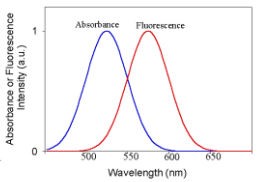
Computers and Electronics in Agriculture

journal homepage: www.elsevier.com/locate/compag

Visible/Near Infrared (VIS/NIR) spectroscopy as an optical sensor for evaluating olive oil quality

Nawaf Abu-Khalaf[b,*], Mohammed Hmidat[b]





Phenolic content evaluation in Extra Virgin Olive Oil

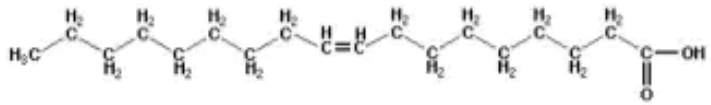
Why the extraction is required?

COMPOSIZIONE CHIMICA DELL'OLIO EXTRAVERGINE DI OLIVA

L'olio extravergine di oliva è costituito da:

- **98% gliceridi e acidi grassi monoinsaturi**

  (oleico, linoleico, linolenico)

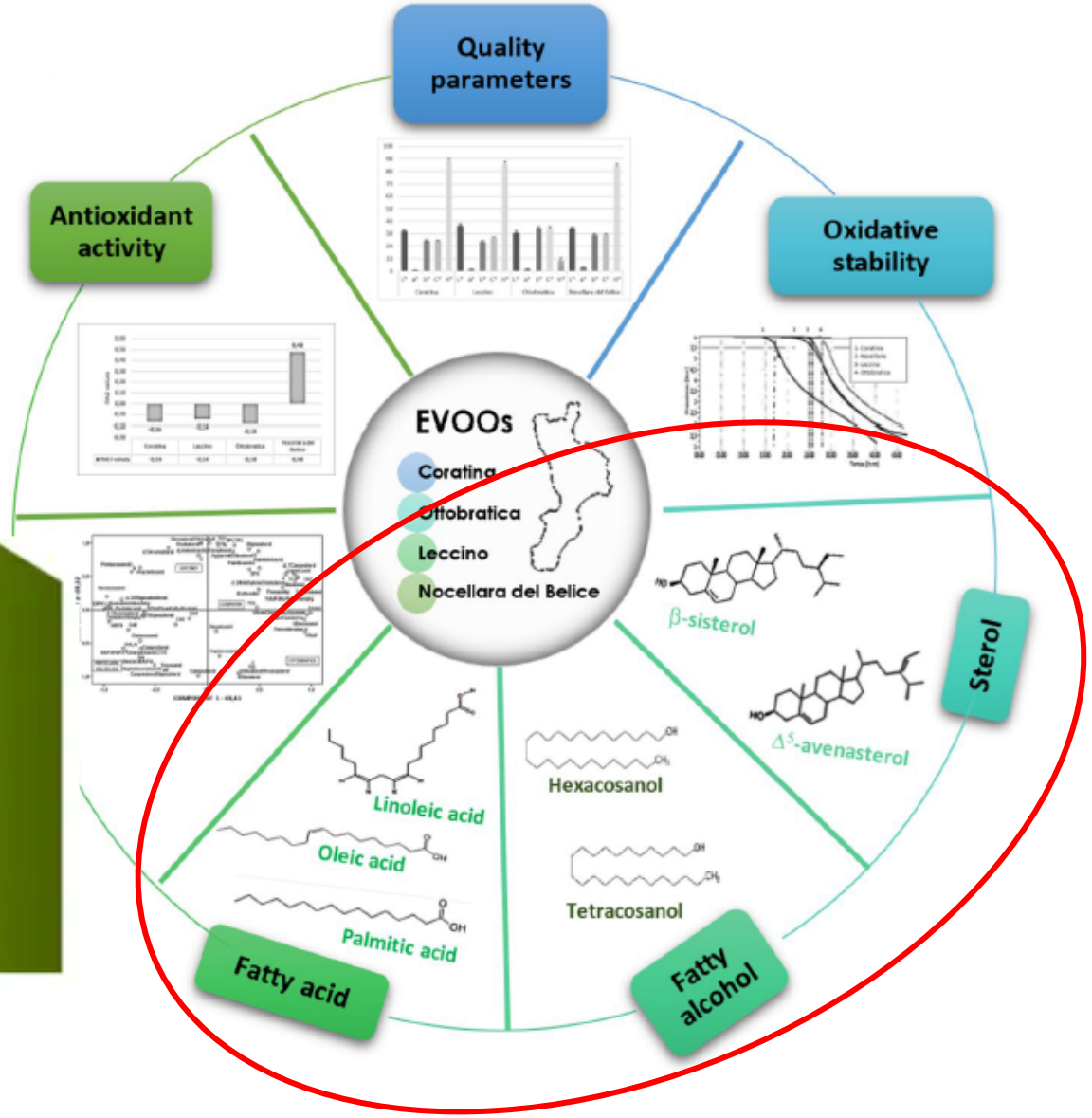


- **2% componenti minori**

  (polifenoli, vitamine e sostanze minerali)

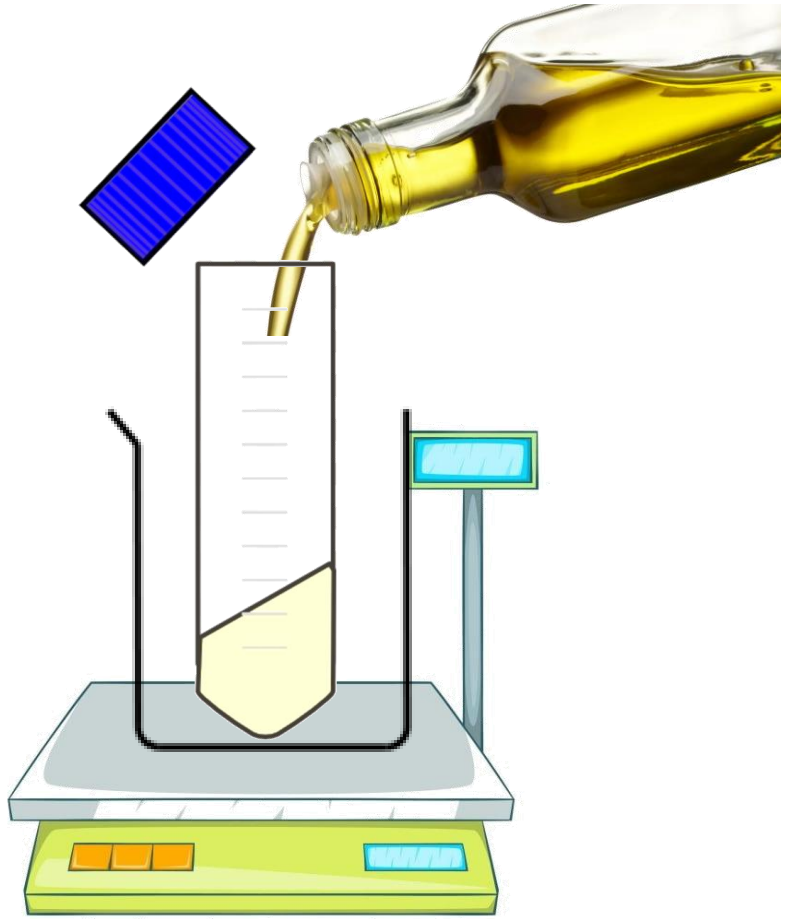
05/03/13

29

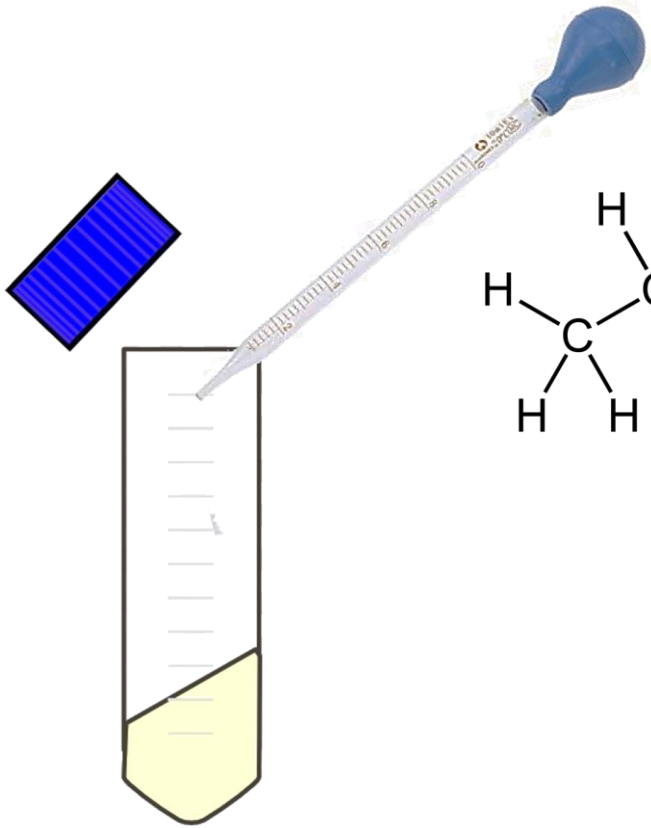


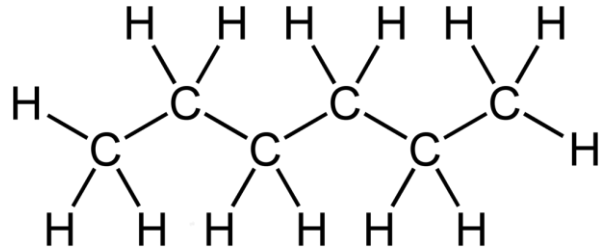
Preparazione del campione

- Pesare 1.0 g di campione



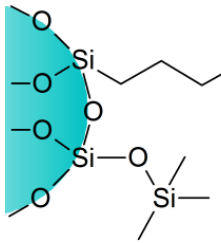
- Scioglierlo in 5 ml di esano.





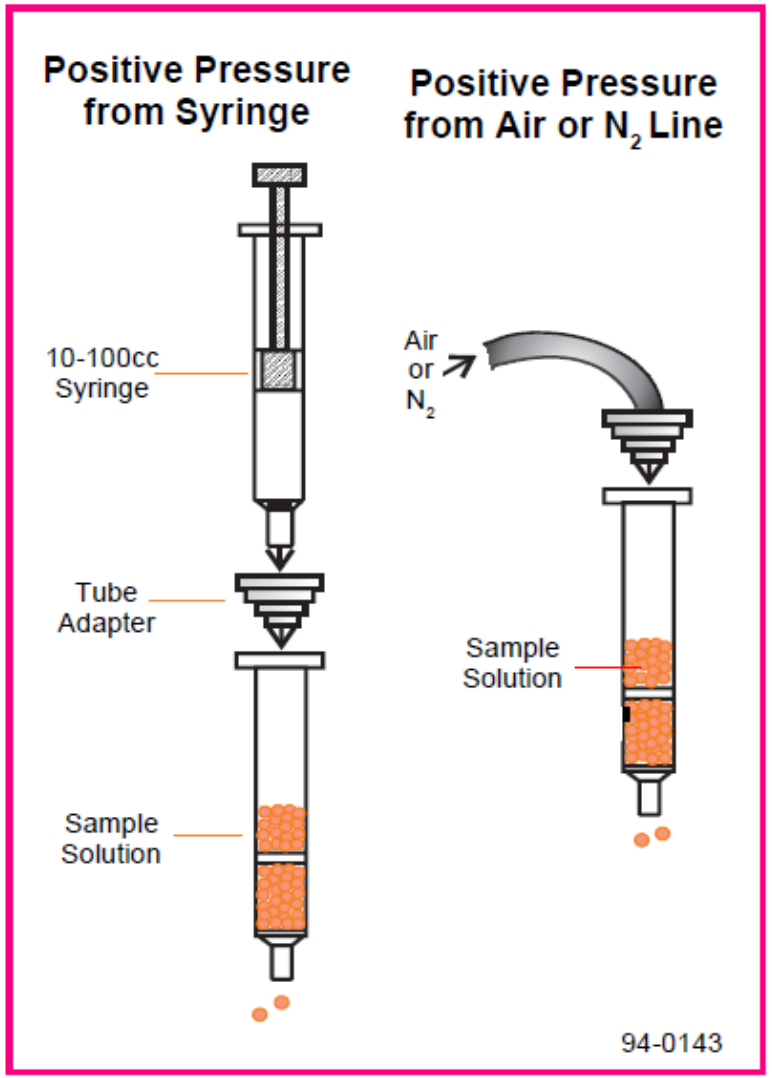
Polyphenols extraction

Solid phase extraction (SPE). Extraction form liquid sample.



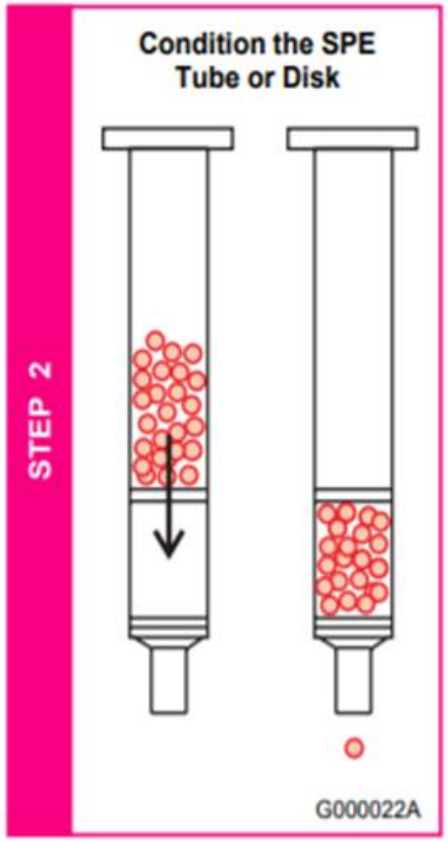
Solid phase: silica beads@C18

Figure B. Process Using Applied Pressure

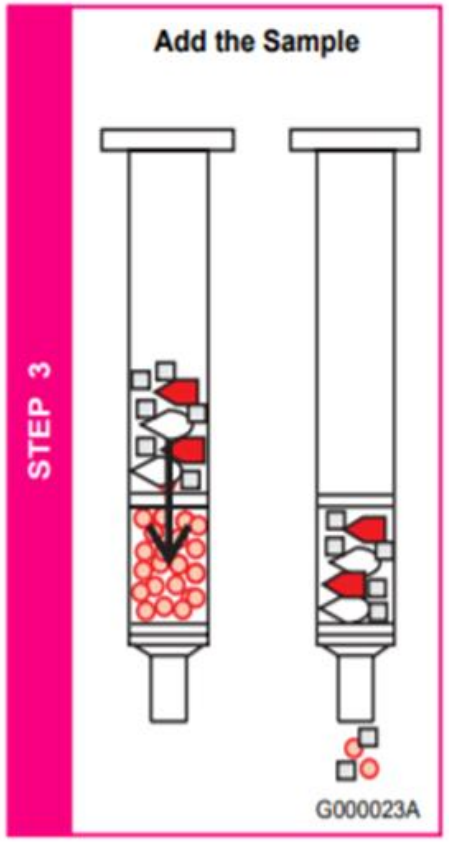


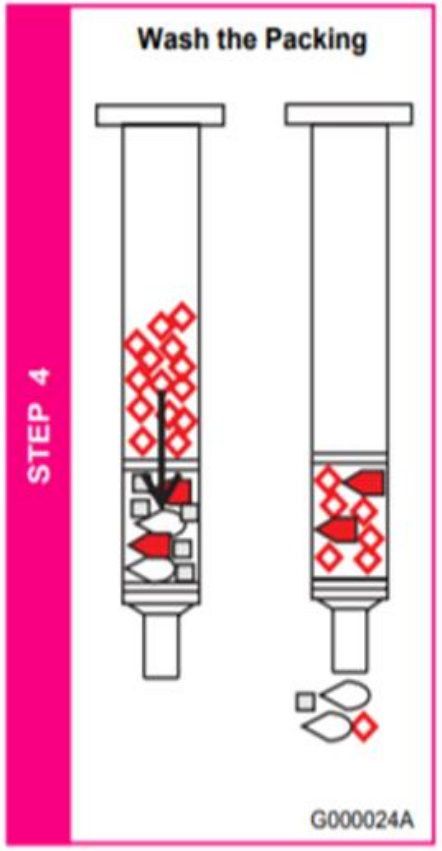
Polyphenols extraction

Solid phase extraction (SPE). Extraction form liquid sample.

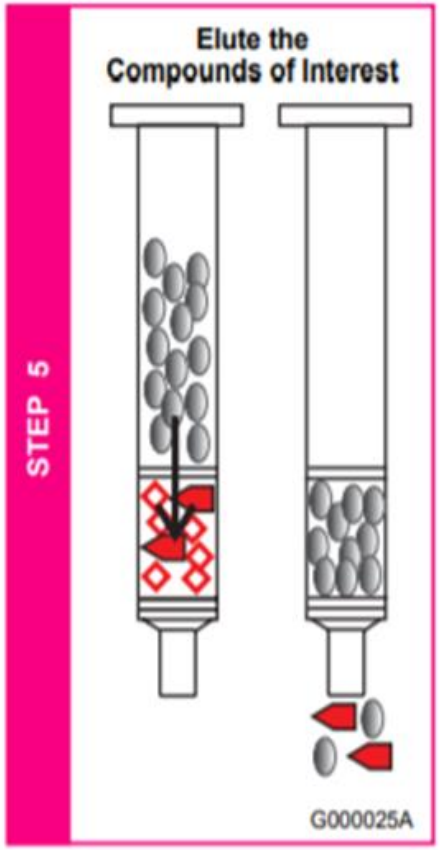


10 mL Methanol
10 mL Hexane





10 mL Hexane



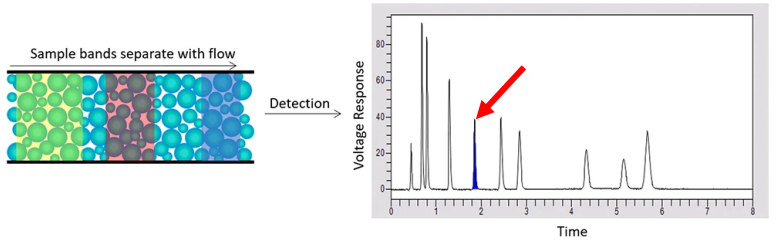
10 mL Methanol

Polyphenols extraction

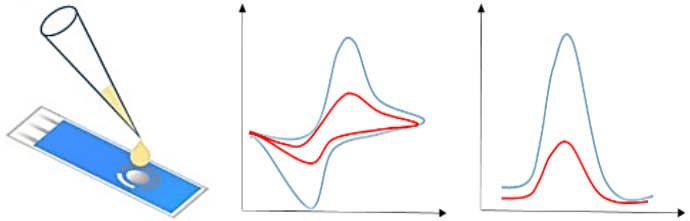
Solid phase extraction (SPE). Extraction form liquid sample.

Analysis

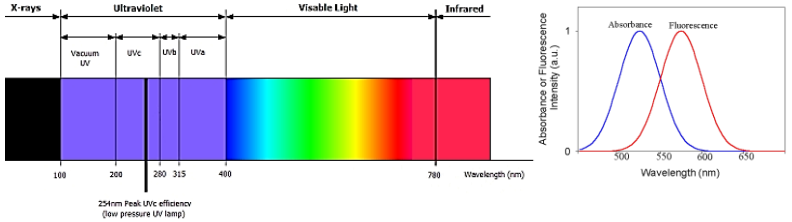
Chromatographic strategies



Electrochemical-based strategies



Optical-based strategies



Categoria oli vergini	Etil esteri degli acidi grassi (EEAG)	ACIDITA' (%)	N. PEROSSIDI (meqO2/kg)	SPETTROFOTOMETRIA			Valutazione organolettica Mediana del difetto (Md)	Valutazione organolettica Mediana del fruttato (Mf)
				K232	K270	ΔK		
VERGINE EXTRA	EEAG ≤ 35 mg/kg (campagna 2014-2016)	**≤ 0,8**	**≤ 20**	**≤ 2,5**	**≤ 0,22**	**≤ 0,01**	**Md = 0**	**Mf > 0**
VERGINE	–	0,8-2	≤ 20	≤ 2,6	≤ 0,25	≤ 0,01	Md ≤ 3,5	Mf > 0
LAMPANTE	–	> 2	> 20	> 2,6	> 0,25	> 0,01	Md > 3,5	–