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Bruno Sarmento

EB PPT

BIOGRAPHY

- PhD in Pharmaceutical Technology and degree in Pharmaceutical Sciences, University of Porto, Portugal; Affiliated Researcher at Institute of Biomedical Engineering (INEB), Porto, Portugal; Assistant Professor of Pharmaceutical and Biopharmaceutical Technology at ISCS-N, Gandra, Portugal. His current research is focused on the study of nanomedicines e.g., polymer-based nanoparticles, solid lipid nanoparticles and polymeric micelles, and their application in the pharmaceutical and biomedical fields, as well as on the use of in vitro cell models as a tool to correlate the transport of biopharmaceuticals and nanoparticles across human intestinal mucosa. He is internationally recognised has an expert on oral delivery of biopharmaceuticals through nanomedicines. In this field he authors several publications, some are key references in the area, being pioneer of polymeric and solid lipid nanoparticles for intestinal delivery of insulin.
- His work has been successful, with more than 100 publications in less than 8, 3 edited books in the field of Pharmaceutical Technology and Nanomedicine, 26 book chapters and more than 150 conference proceedings/abstracts and serves as editorial board member of several international journals and evaluator of research projects form international agencies. Further is work as research scientist in various research projects and Professor, he is also supervisor of many PhD (18), postdoctoral (4) and master students (more than 15). He is also an active member of several international associations (AAPS, CRS, EUFEPS, EFSD, FIP, BRG) and collaborates in post-graduate programs at national and international level on the field of biotechnology and health. He has a strong list of national and international collaborations in the areas of controlled drug delivery, nanomedicine and tissue engineering.

RESEARCH INTEREST

- Development of new drug delivery systems using colloidal nanoparticles Therapeutic polymers for drug delivery Therapeutic polymers as new targeted cancer systems Methods of micro and nanoencapsulation of biotech drugs Biosimilars Physical and chemical characterization of pharmaceutical dosage forms (DSC, FTIR, CD, PCS, LDA, SEM, TEM, AFM, HPLC, UV) Structural characterization of proteins In vitro and in vivo models of drug bioavailability through gastrointestinal barrier Cell-culture models for drug absorption studies In vitro and in vivo models of drug bioavailability Biomaterials Cell-material interaction Advanced Light Microscopy and Flow Cytometry.

HPLC

INSTRUMENTATION

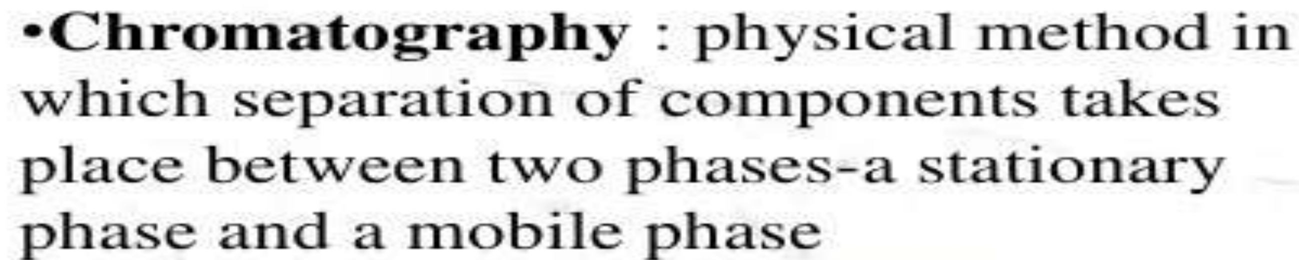
INTRODUCTION

•HPLC stands for "High-performance liquid chromatography"(sometimes referred to as High-pressure liquid chromatography).

•High performance liquid chromatography is a powerful tool in analysis, it yields high performance and high speed compared to traditional columns chromatography because of the forcibly pumped mobile phase.

•HPLC is a chromatographic technique that can separate a mixture of compounds

•It is used in biochemistry and analytical chemistry to identify, quantify and purify the individual components of a mixture.



•**Chromatography** : physical method in which separation of components takes place between two phases-a stationary phase and a mobile phase

•**Stationary phase** : The substance on which adsorption of the **analyte** (the substance to be separated during chromatography) takes place . It can be a solid, a gel, or a solid liquid combination

•**Mobile phase** : solvent which carries the analyte (a liquid or a gas)

Chromatographic techniques are divided into different types based on :

The type of chromatographic bed used
i.e. column chromatography (gas chromatography) and planar chromatography(paper and thin layer)

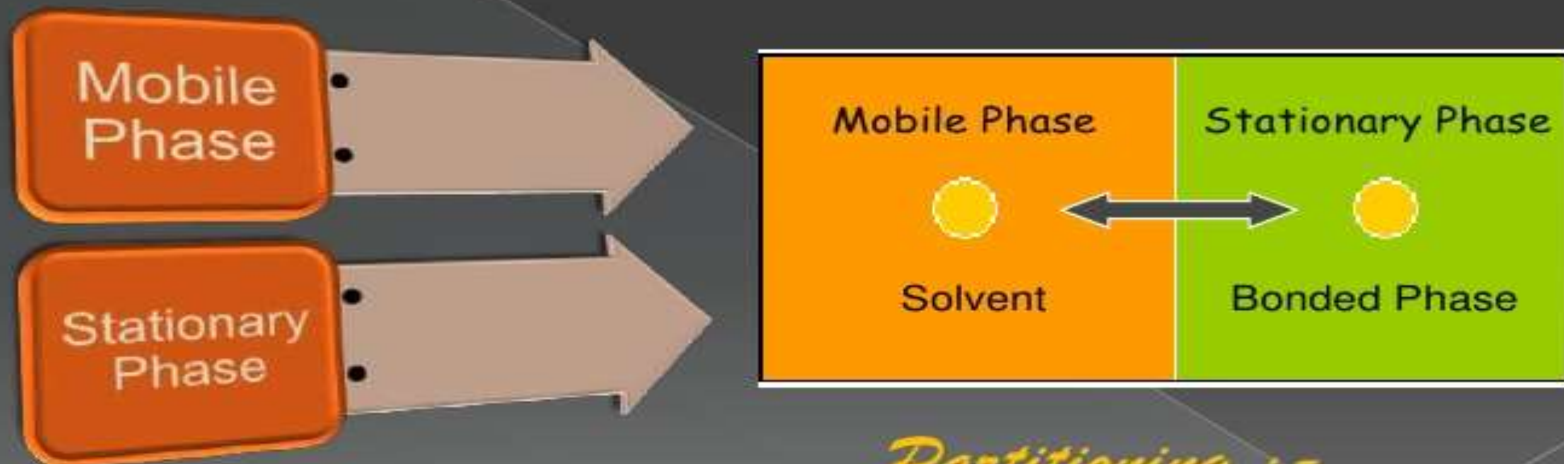
The physical state of mobile phase
i.e. gas chromatography and liquid chromatography

The separation mechanism
i.e. ion-exchange and size exclusion

HPLC is a type of **liquid chromatography** where the sample is forced through a **column** that is packed with a stationary phase composed of irregularly or spherically shaped particles, a porous monolithic layer , or a porous membrane by a liquid (mobile phase) at high pressure.

TYPES OF PHASES :-

Separation is based on the analyte's relative solubility between two liquid phases



HPLC - Modes

NORMAL PHASE.

- POLAR STATIONARY PHASE AND NON-POLAR SOLVENT.

REVERSE PHASE.

- NON-POLAR STATIONARY PHASE AND A POLAR SOLVENT.

FOUR TYPES OF HIGH PERFOMANCE LIQUID CHROMATOGRAPHY :-

1. PARTITION CHROMOTOGRAPHY
2. ION EXCHANGE CHROMATOGRAPHY
3. Size exclusion chromatography
4. AFFINITY CHROMOTAGRAPY

1. PARTITION CHROMOTOGRAPHY :-

Partition chromatography uses a retained solvent, on the surface or within the grains or fibres of an "inert" solid supporting matrix as with paper chromatography; or takes advantage of some additional coulombic and/or hydrogen donor interaction with the solid support. Molecules equilibrate (partition) between a liquid stationary phase and the eluent. Known as Hydrophilic Interaction Chromatography (HILIC) in HPLC, this method separates analytes based on polar differences. HILIC most often uses a bonded polar stationary phase and a non-polar, water miscible, mobile phase. Partition HPLC has been used historically on unbonded silica or alumina supports. Each works effectively for separating analytes by relative polar differences, however, HILIC has the advantage of separating acidic, basic and neutral solutes in a single chromatogram.

2. ION EXCHANGE CHROMATOGRAPHY

Ion-exchange chromatography is a process that allows the separation of ions and polar molecules based on their charge. It can be used for almost any kind of charged molecule including large proteins, small nucleotides and amino acids. The solution to be injected is usually called a *sample*, and the individually separated components are called *analytes*. It is often used in protein purification, water analysis, and quality control

Ion exchange chromatography retains analyte molecules on the column based on coulombic (ionic) interactions. The stationary phase surface displays ionic functional groups (R-X) that interact with analyte ions of opposite charge. This type of chromatography is further subdivided into cation exchange chromatography and anion exchange chromatography. The ionic compound consisting of the cationic species M+ and the anionic species B- can be retained by the stationary phase.

3. SIZE EXCLUSION CHROMATOGRAPHY :-

Size exclusion chromatography (SEC), also known as gel permeation chromatography or gel filtration chromatography, separates particles on the basis of size. It is generally a low resolution chromatography and thus it is often reserved for the final, "polishing" step of purification. It is also useful for determining the tertiary structure and quaternary structure of purified proteins. SEC is used primarily for the analysis of large molecules such as proteins or polymers. SEC works by trapping these smaller molecules in the pores of a particle. The larger molecules simply pass by the pores as they are too large to enter the pores. Larger molecules therefore flow through the column quicker than smaller molecules, that is, the smaller the molecule, the longer the retention time.

4. AFFINITY CHROMOTAGRAPY :-

This is the most selective type of chromatography employed. It utilizes the specific interaction between one kind of solute molecule and a second molecule that is immobilized on a stationary phase. For example, the immobilized molecule may be an antibody to some specific protein. When solutes containing a mixture of proteins are passed by this molecule, only the specific protein is reacted to this antibody, binding it to the stationary phase. This protein is later extracted by changing the ionic strength or pH.

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LIQUID CHROMATOGRAPHIC COLUMN

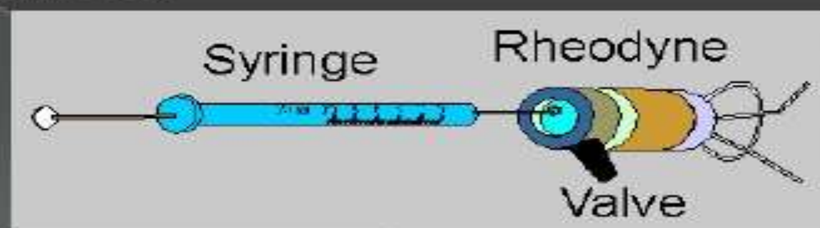
Smooth-bore stainless steel or heavy-walled glass tubing

Hundreds of packed columns differing in size and packing are available from manufacturers ($200-$500)

Add columns together to increase length

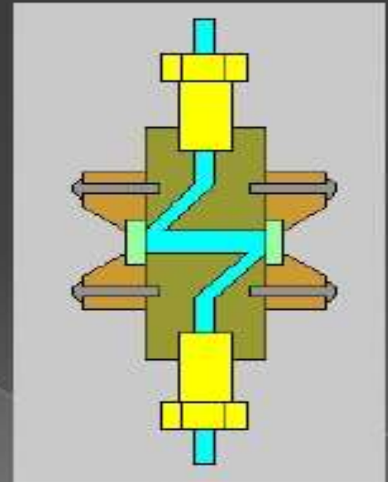
Sample Injection Systems

- For injecting the solvent through the column
- Minimize possible flow disturbances
- Limiting factor in precision of liquid chromatographic measurement
- Volumes must be small
- .1-500 μL
- Sampling loops
  - interchangeable loops (5-500 μL at pressures up to 7000 psi)



DETECTOR

- Mostly optical
- Equipped with a flow cell
- Focus light beam at the center for maximum energy transmission
- Cell ensures that the separated bands do not widen



USES OF HPLC :-

1.This technique is used for chemistry and biochemistry research analyzing complex mixtures, purifying chemical compounds, developing processes for synthesizing chemical compounds, isolating natural products, or predicting physical properties. It is also used in quality control to ensure the purity of raw materials, to control and improve process yields, to quantify assays of final products, or to evaluate product stability and monitor degradation.

2. In addition, it is used for analyzing air and water pollutants, for monitoring materials that may jeopardize occupational safety or health, and for monitoring pesticide levels in the environment. Federal and state regulatory agencies use HPLC to survey food and drug products, for identifying confiscated narcotics or to check for adherence to label claims.

SIGNATURE

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