Delivery of proteins

Alternative routes of administration

LECTURE 5

ALTERNATIVE ROUTE OF ADMINISTRATION

- **Parenteral administration** has disadvantages (needles, sterility, injection skill) compared to other possible routes.

❖ Therefore, **systemic delivery of recombinant proteins by alternative routes of administration** (apart from the GI tract) has been studied extensively.

Delivery through nose, lungs, rectum, oral cavity, and skin have been selected as potential sites of application.

THE POTENTIAL PROS AND CONS FOR DIFFERENT RELEVANT ROUTES

I. Nasal

Advantage:

1. Easily accessible
2. Fast uptake
3. Proven track record with a number of "conventional" drugs
4. Probably lower proteolytic activity than in the GI tract
5. Avoidance of first pass effect
6. Spatial containment of absorption enhancers [osmolarity & pH] is possible (when drugs exhibits poor membrane permeability, large molecular size, lack of lipophilicity and enzymatic degradation by amino peptidases).

Nasal

Disadvantage:

1. Reproducibility (in particular under intranasal pathologies may affect or capacity for nasal absorption)
2. Safety (e.g., cilliary movement that propelled proteins into the throat where it is swallowed and destroyed by the products of the stomach).
3. Low bioavailability for proteins (Because they are large molecular weight polar drugs thus they have low membrane permeability).

II. Pulmonary (intratracheal inhalation or instillation)

Advantage:

1. Relative easy to access (aerosol or syringe).
2. Fast uptake.
3. Proven track record with “conventional” drugs.
4. Substantial fractions of insulin are absorbed.
5. Lower proteolytic activity than in the GI tract.
6. Avoidance of hepatic first pass effect.
7. Spatial containment of absorption enhancer.

Pulmonary

Disadvantage:

1. Reproducibility (in particular under pathological conditions, smoker/non-smoker).
2. Safety (e.g., inhaled human insulin [powder or liquid] has been shown to be more immunogenic than comparator insulins given by S.C. routes; however, adverse effects of antibody formation demonstrated)
3. Presence of macrophages in the lung with affinity for particulates.

III. Rectal

Advantage:

1. Easily accessible
2. Partial avoidance of hepatic first pass
3. Probably lower proteolytic activity than in the upper parts of GI tract
4. Spatial containment of absorption enhancers is possible
5. Proven track record with a number of “conventional” drugs.

Disadvantage:

Low bioavailability for proteins

IV. Buccal

Advantage:

1. Easily accessible
2. Avoidance of hepatic first pass
3. Probably lower proteolytic activity than in the lower parts of the GI tract
4. Spatial containment of absorption enhancer is possible
5. Option to remove formulation if necessary

Disadvantage:

1. Low bioavailability of proteins
2. No proven track record yet.

V. Transdermal

Advantage:

1. Easily accessible
2. Avoidance of hepatic first pass
3. Removal of formulation if necessary is possible
4. Spatial containment of absorption enhancers
5. Proven track record with “conventional” drugs
6. Sustained/controlled release possible

Disadvantage:

Low bioavailability of proteins

CONCLUSION

The nasal, buccal, rectal, and transdermal routes all have been shown to be of little clinical relevance if systemic action is required, and if simple protein formulations without an absorption enhancing technology are used.

In general, bioavailability is too low and varies too much! The pulmonary route may be the exception to this rule (because in pulmonary the absorption was strongly protein dependent, with no clear relationship with it's molecular weight).

ABSOLUTE BIOAVAILABILITY OF A NUMBER OF PROTEINS (INTRATRACHEAL VS. IV) IN RATS

Molecule	Mwt kDa	No. of AA	Absolute bioavaliability (%)
A-interferon	20	165	> 56
PTH-84	9	84	> 20
PTH-34	4.2	34	40
Calcitonin (human)	3.4	32	17
Calcitonin (salmon)	3.4	32	17
Glucagons	3.4	29	< 1
Somatostatin	3.1	28	< 1

As shown in the Table that presents the bioavailability in rats of intratracheally administered protein solutions with a wide range of molecular weights:

In human the drug should be inhaled instead of intratracheally administered.

The delivery of insulin to Type I (juvenile onset) and Type II (adult onset) diabetics has been extensively studied and clinical phase III trials evaluating efficacy and safety have been performed or are ongoing.

The first pulmonary insulin formulation was approved by FDA in January 2006 (Exubera®).

Pulmonary inhalation of insulin is specifically tested for meal time glucose control.

Uptake of insulin is faster than after a regular SC insulin injection (5-60 minutes versus 60-180 minutes).

The reproducibility of the blood glucose response to inhaled insulin was equivalent to SC injected insulin, but patients preferred SC injection over inhalation.

Inhalation technology plays a critical role when considering the prospect of the pulmonary route for the systemic delivery of therapeutic proteins.

Dry powder inhalers and nebulizers (Nebulizers are machines that transform liquid medication into a mist for inhalation) are being tested.

NEBULIZERS

NEBULIZERS

The fraction of insulin that is ultimately absorbed depends on:

1) The fraction of the inhaled/ nebulised dose that is actually leaving the device.
2) The fraction that is actually deposited in the lung.
3) The fraction that is being absorbed, i.e., total relative uptake (TO%)

TO % = % uptake from device x % deposited in the lungs x % actually absorbed from the lungs.

- TO % for estimated to be about 10%.
- The fraction of insulin that is absorbed from the lung is estimated to be around 20%.
- Insulin absorption via the lung may be a promising route; but the fraction absorbed is small.

GOAL

1- Develop a system that temporarily decreases the absorption barrier resistance with minimum and acceptable safety concerns.

2- Different approaches evaluated to increase bioavailability of the pulmonary and other non-parenteral routes of administration .

APPROACHES TO ENHANCE BIOAVAILABILITY OF PROTEINS

Classified according to proposed mechanism of action

1. Increase the permeability of the absorption barrier:

- Addition of fatty acids/phospholipids, bile salts, enamine derivatives of phyenylglycine, ester and ether type (non)-ionic detergents, saponins, salicylate derivatives of fusidic acid or glycyrrhizinic acid, or methylated β cyclodextrins
- Through iontophoresis
- By using liposomes.

2. Decrease peptidase activity at the site of absorption and along the "absorption route": aportinin, bacitracin, soybean tyrosine inhibitor, boroleucin, borovaline.

3. Enhance resistance against degradation by modification of the molecular structure.

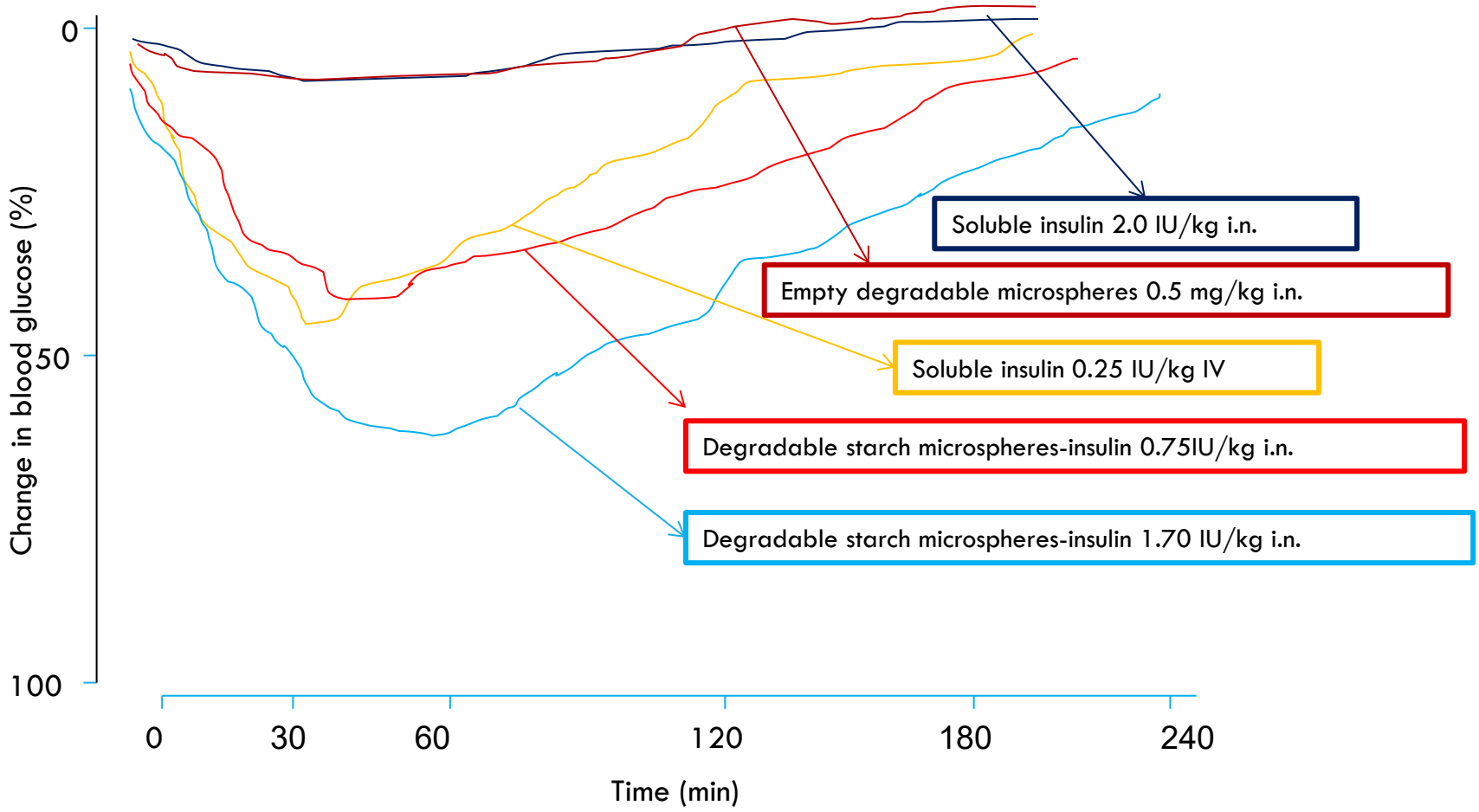
4. Prolongation of exposure time (e.g., bio-adhesion technologies).

EXAMPLES OF ABSORPTION ENHANCING EFFECTS

EFFECT OF GLYCOCHOLATE (ABSORPTION ENHANCER) ON NASAL BIOAVAILABILITY OF SOME PROTEINS AND PEPTIDES.

Molecule	No. Of AA	Bioavailability (%)	
		Without glycocholate	With glycocholate
Glucagon	29	< 1	70-90
Calcitonin	32	< 1	15-20
Insulin	51	< 1	10-30
Met-hgH	191	< 1	7-8

CHANGE IN BLOOD GLUCOSE IN RATS AFTER INTRANASAL ADMINISTRATION OF INSULIN. USING DEGRADABLE STARCH MICROSPHERES LOADED WITH INSULIN



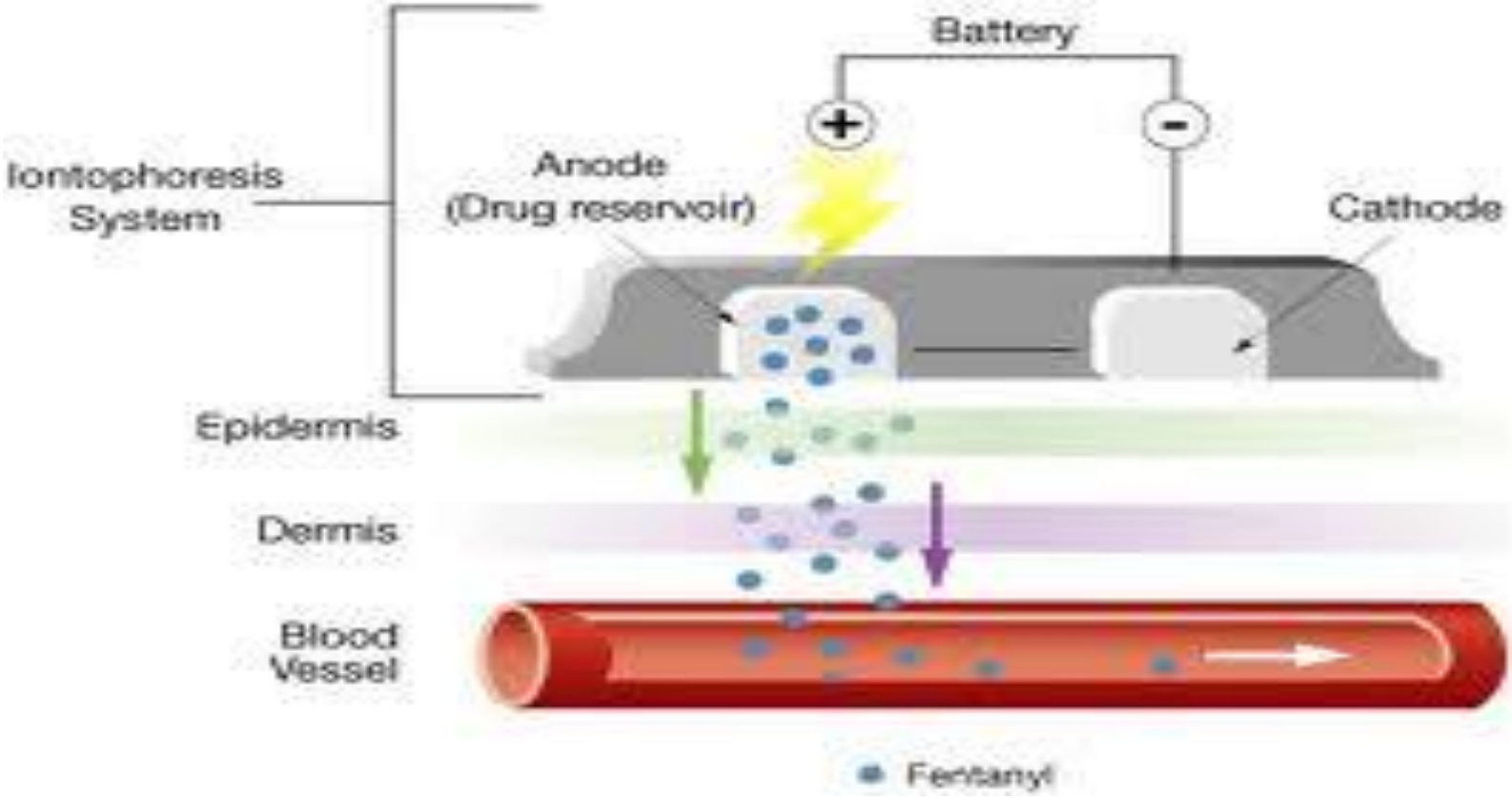
Major issues now being addressed are reproducibility, effect of pathological conditions (e.g., rhinitis) on absorption and safety aspects of chronic use.

Absorption enhancing effects were shown to be species dependent.

Pronounced differences in effect were observed between rats, rabbits, and humans.

IONTOPHORESIS

With iontophoresis a transdermal electrical current is induced by positioning two electrodes on different places on the skin.



This current induces a migration of (ionized) molecules through the skin.

Delivery depends:

1- on the current (on/off, pulsed/direct, wave shape)

2- pH

3- ionic strength

4- molecular weight

4- charge on the protein

5- temperature.

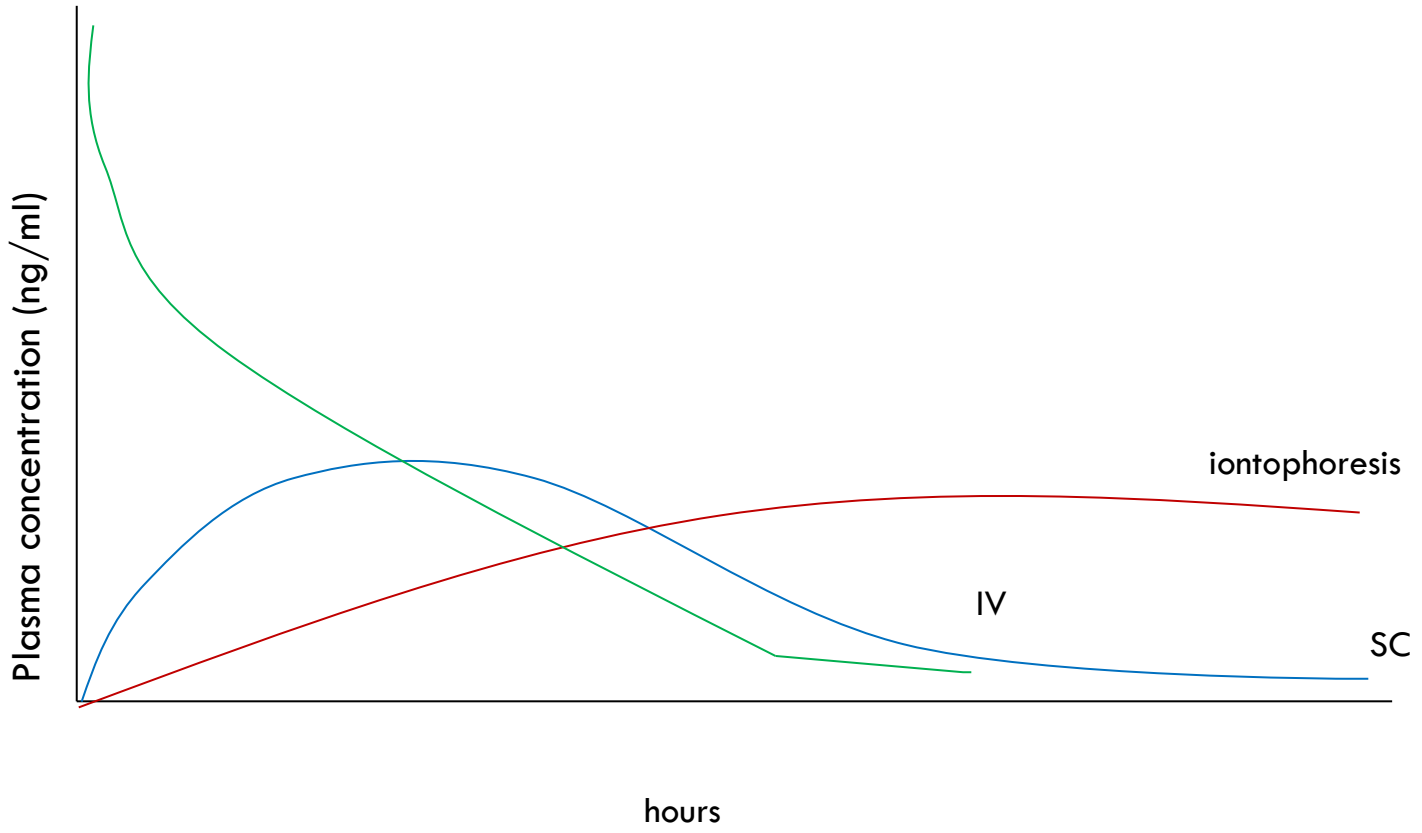
The protein should be charged over the full thickness of the skin (pH of hydrated skin depends on the depth and varies between pH 4 (surface) and pH 7.3), which makes proteins with pI values outside this range prime candidates for iontophoretic transport.

It is not clear whether there are size restrictions (protein MW) for iontophoretic transport.

Only potent proteins will be successful candidates.

With the present technology the protein flux through the skin is in the 10 $\mu g/cm^2$/hour range.

PLASMA CONCENTRATION VERSUS TIME PROFILES AFTER SC, IV AND IONTOPHORETIC TRANSDERMAL ADMINISTRATION OF GROWTH HORMONE RELEASING FACTOR (GRF)



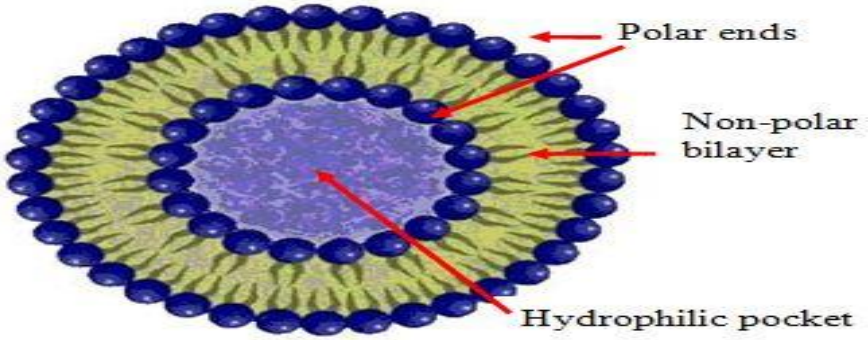
The figure presents the plasma profile of growth hormone releasing factor, GRF (44 amino acids, MW 5kDa after SC, IV, and iontophoretic transdermal delivery to hairless guinea pigs.

- A prolonged appearance of GRF in the plasma can be observed.

- Iontophoretic delivery offers interesting opportunities if pulsed delivery of the protein is required.

The device can be worn permanently and only switched on for the desired periods of time, simulating pulsatile secretion of endogenous hormones such as growth hormone and insulin.

LIPOSOMES



Liposomes are microscopic vesicles that consist of an aqueous center with a phospholipid membrane;

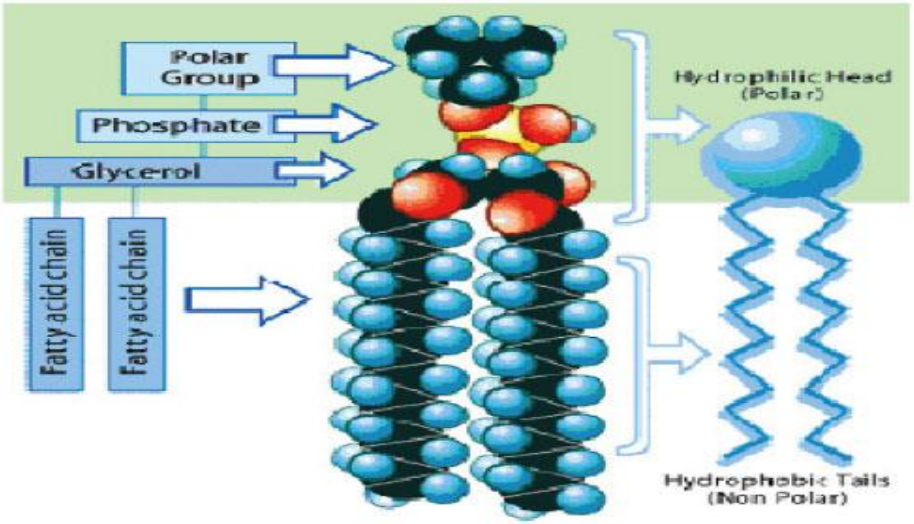
phospholipids contain a glycerol bonded to 2 fatty acids and a phosphate group with a polar head.

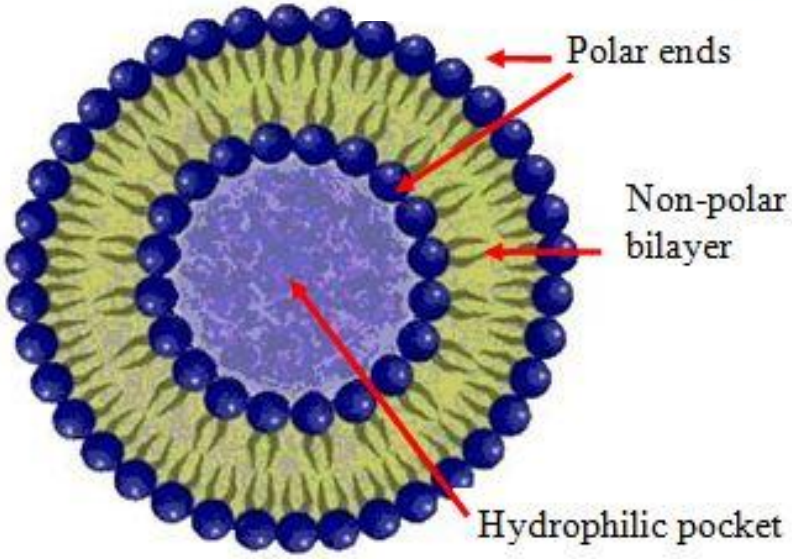
The fatty acid portion of this biomolecule is hydrophobic and is located toward the outside of the lipid bilayer whereas the phosphate group is hydrophilic and faces the aqueous interior.

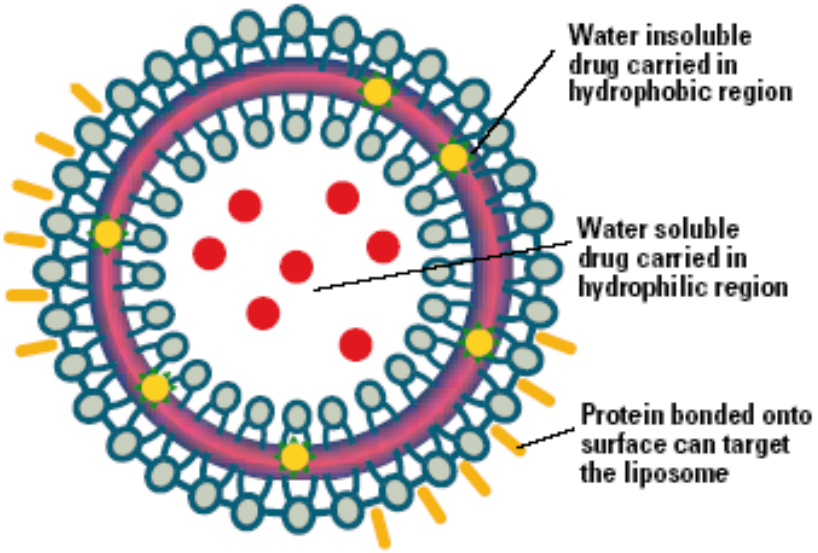
These phospholipid walls are identical to those that comprise other human cell membranes.

Liposomes can differ in size, with a range in diameter between 15-3500 nm, and they can be found in unilamellar or multilamellar forms.

LIPOSOMES


Polar Group
Phosphate
Glycerol
Fatty acid chain
Fatty acid chain
Hydrophilic Head (Polar)
Hydrophobic Tails (Non Polar)


Polar ends
Non-polar bilayer
Hydrophilic pocket


Water insoluble drug carried in hydrophobic region
Water soluble drug carried in hydrophilic region
Protein bonded onto surface can target the liposome

Insulin has traditionally been an injectable preparation which is a common barrier to its use, and as a result, since the discovery of insulin in the 1920s, new strategies for its delivery by routes other than intravenous and subcutaneous (SC) injection have been investigated.

Many pharmaceutical companies doing research in the field to develop an inhalational preparation announced the termination of product development following the poor acceptance and risk of lung cancer of the first US FDA approved inhaled insulin product, Exubera®.

This formulation produced cough, dyspnoea (difficulty in breathing), increased sputum, and epistaxis (nosebleed), and was contraindicated in patients with chronic obstructive pulmonary disease (COPD) and asthma.

TECHNOSPHERE INSULIN: A NEW INHALED INSULIN

MannKind Corporation has developed **a powdered formulation of insulin with a higher percentage of absorption from the lungs.** This product, Afrezza® (Technosphere® insulin), appears to have overcome some of the barriers that contributed to the withdrawal of Exubera® and is currently under review by the FDA.

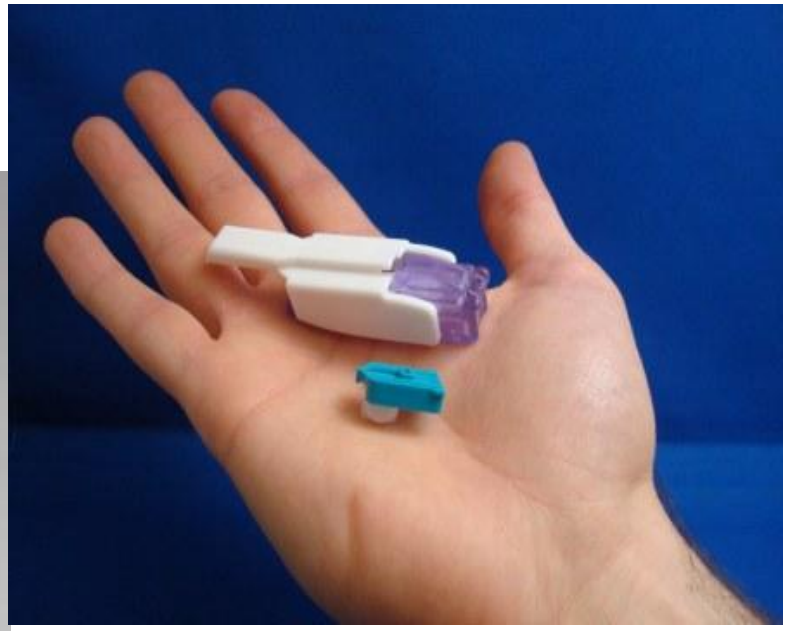
Technosphere insulin is a new inhaled insulin preparation which mimics normal prandial insulin release. It decreases post-prandial blood glucose (PPG) levels and has good glycaemic control with significantly lesser hypoglycaemia.

Current data show that this formulation has no impact on pulmonary function.

Long-term safety studies with regard to pulmonary function and risk for development of lung carcinoma need to be monitored.

The FDA is currently reviewing Technosphere insulin for use in both type 1 and type 2 diabetes.

TECHNOSPHERE® INSULIN




Thank You