

Biopharmaceutic Classification System and Permeability

The **Biopharmaceutics Classification System** is a system to differentiate the drugs on the basis of their solubility and permeability. This system restricts the prediction using the parameters solubility and intestinal permeability. The solubility classification is based on a United States Pharmacopoeia (USP) aperture. The intestinal permeability classification is based on a comparison to the intravenous injection. All those factors are highly important because 85% of the most sold drugs in the United States and Europe are orally administered.

Biopharmaceutics Classification System (BCS) is a regulatory mechanism through which drug developers and generic companies can obtain a waiver of clinical bioequivalence studies, also called a biowaiver. According to the 2000 FDA BCS Guidance, compounds that are classified as Class I (highly soluble, highly permeable) are eligible for BCS biowaivers. For such compounds, the rate and extent of drug absorption is unlikely to be affected by drug dissolution and/or GI residence time, and in vivo bioequivalence studies (for new formulations, etc.) may be waived based on in vitro permeability and solubility data.

Drugs are classified in BCS on the basis of solubility, permeability, and dissolution.

Solubility class boundaries are based on the highest dose strength of an immediate release product. A drug is considered highly soluble when the highest dose strength is soluble in 250 ml or less of aqueous media over the pH range of 1 to 7.5. The volume estimate of 250 ml is derived from typical bioequivalence study protocols that prescribe administration of a drug product to fasting human volunteers with a glass of water.

Permeability class boundaries are based indirectly on the extent of absorption of a drug substance in humans and directly on the measurement of rates of mass transfer across human intestinal membrane. Alternatively non-human systems capable of predicting drug absorption in humans can be used (such as in-vitro culture methods). A drug substance is considered highly permeable when the extent of absorption in humans is determined to be 90% or more of the administered dose based on a mass-balance determination or in comparison to an intravenous dose.

For dissolution class boundaries, an immediate release product is considered rapidly dissolving when no less than 85% of the labeled amount of the drug substance dissolves within 15 minutes using USP Dissolution Apparatus 1 at 100 RPM or Apparatus 2 at 50 RPM in a volume of 900 ml or less in the following media: 0.1 M HCl or simulated gastric fluid or pH 4.5 buffer and pH 6.8 buffer or simulated intestinal fluid.

BCS Divides Compounds into Four Categories:

Class I – High Solubility, High Permeability

- Example: metoprolol, paracetamol
- Those compounds are well absorbed and their absorption rate is usually higher than excretion.

Class II – Low Solubility, High Permeability

- Example: glibenclamide, bicalutamide, ezetimibe, aceclofenac
- The bioavailability of those products is limited by their solvation rate. A correlation between the *in vivo* bioavailability and the *in vitro* solvation can be found.

Class III – High Solubility, Low Permeability

- Example: cimetidine
- The absorption is limited by the permeation rate but the drug is solvated very fast. If the formulation does not change the permeability or gastro-intestinal duration time, then class I criteria can be applied.

Class IV – Low Solubility, Low Permeability

- Example: Bifonazole
- Those compounds have a poor bioavailability. Usually they are not well absorbed over the intestinal mucosa and a high variability is expected.

Different methods of determining drug absorption are:

- A. In vitro method
- B. In Vivo method
- C. In situ method

IN VITRO METHOD

In vitro methods are carried out outside of the body and are used to determine the permeability of drug using live animal tissues. In vitro models have been introduced to assess the major factors involved in the absorption process and predict the rate and extent of drug absorption.

The different in vitro methods are:

Physicochemical methods

1. Partition coefficient
2. Artificial membranes
3. Chromatographic retention indices
4. Brush border membrane vesicles (BBMV)
5. Isolated intestinal cells
6. Tissue techniques:
 - a). Everted small intestinal sac technique
 - b) Everted sac modification
 - c) Circulation techniques
 - d) Everted intestinal ring or slice techniques
7. Diffusion cell method
8. Cell culture techniques

1. PARTITION COEFFICIENT

Partition coefficient between an oil and water phase, $\log P$, is one of the easiest property of a drug molecule that can be determined. It provides a measure of the lipophilicity of a molecule and can be used to predict to what extent it will cross the biological membrane. eg. Octanol is selected as an oil phase as it has similar properties to biological membranes.

It's important to note that log P does not take the degree of ionization into consideration and hence log D is used. log D is the distribution coefficient where aqueous phase is at a particular pH and thus it takes into account the ionization of the molecule at this pH. The log D measured at intestinal pH will give a much better idea about extent of drug permeability across GI membrane than log P.

2. ARTIFICIAL MEMBRANES

Artificial membranes are very useful in studying passive membrane permeability as they are reproducible and are suitable for high throughput screening. In this method, PAMPA model is used.

Parallel artificial membrane permeability assay (PAMPA)

PAMPA is a method which determines the permeability of substances from a donor compartment, through a lipid infused artificial membrane into an acceptor compartment. The artificial membrane is like a phospholipid membranes supported by filter material. It is prepared by pipetting a solution of lipids in an inert organic solvent on a supporting filter material which is placed on 96- well microtitre plate.

A modification of this system is immobilized liposome chromatography (ILC) and on ILC, many compounds with same log P have been shown to demonstrate variable membrane partitioning based on their logs.

3. CHROMATOGRAPHIC RETENTION INDICES

Immobilized artificial membranes (IAM) chromatography along with physicochemical parameters is used for evaluation of passive intestinal absorption. IAM packings are prepared by covalently immobilizing monolayers of membrane phospholipids to silica particles. Micellar liquid chromatography (MLC) is also used for the prediction of passive drug absorption and in this system retention of drug mainly depends on hydrophobic, electronic and steric interactions. In general, chromatographic techniques are easy in operation and have high analytical sensitivity.

4. BRUSH BORDER MEMBRANE VESICLES (BBMV)

A brush border is the name for the microvilli covered surface of simple cuboidal epithelium and simple columnar epithelial cells, found in the small intestine. Both animal and human tissue can be used for this.

Procedure: intestinal tissues are treated with calcium chloride precipitation method using centrifugation. the pellets obtained after centrifugation is resuspended in buffer which results in the formation of vesicles. Vesicles are mixed with drug in buffer solution and filtered after a period of time. The amount of drug taken up by the vesicles gives an account of drug absorption. Advantage - useful for mechanistic studies of drug absorption process.

5. USING ISOLATED INTESTINAL CELLS

Here, the small intestine is perfused with enzyme solutions that release the cells and the cells are treated with chelating agents or enzymes. The freshly isolated cells are suspended in buffer solution. At the time of experiment, the cells are separated, resuspended in buffer containing the drug under O₂/CO₂ and shaken well. After a specific period of time, the cells are separated by filtration, extracted and drug absorbed is determined.

6. TISSUE TECHNIQUES

a). Everted small intestinal sac technique:

This method involves isolating a small segment of the intestine of a laboratory animal such as rat, inverting the intestine and filling the sac with a small volume of drug free buffer solution. Both the segments are tied off and the sac is immersed in an ERLLENMEYER FLASK containing a large volume of buffer solution that contains the drug. The flask and its contents are then oxygenated and the whole preparation is maintained at 37°C and shaken mildly. At predetermined time intervals, the sac is removed and the concentration of drug in the serosal fluid is determined/ assayed for drug content.

b) - EVERTED SAC MODIFICATION

In this method, the test animal is fasted for a period of 20-24 hr and water is allowed. The animal is killed and the entire small intestine is everted. Segments, 5-15 cm in length are cut from a specific region of the intestine. The distal end of the segment is tied and the proximal end is attached to the cannula. The segment is suspended in a mucosal solution which contains the drug. A drug free buffer is then placed in the serosal compartment. For determining the rate of drug transfer, the entire volume of serosal solution is removed from the sac at each time interval with the help of a syringe and replaced with fresh buffer solution. The amount of drug that permeates the intestinal mucosa is plotted against time to describe the absorption profile of drug at any specific pH.

c). CIRCULATION TECHNIQUES

In this method, small intestine may or may not be everted. This involves isolating either the entire small intestine of small lab animal or a segment and circulating oxygenated buffer containing the drug through the lumen. Drug free buffer is circulated on the serosal side of the intestinal membrane and oxygenated. Absorption rate from the lumen to the outer solution are determined by sampling both the fluid circulating through the lumen and outside.

d). EVERTED INTESTINAL RING OR SLICE TECHNIQUE

In this technique, the entire small intestine is isolated from the fasted experimental animal and washed with saline solution and dried by blotting with filter paper. The segment is tied at one end and by placing on glass rod it is carefully everted and cut into small rings. The everted intestinal rings are then incubated in drug containing buffer maintained at 37°C with constant oxygenation. Under optimal conditions, rings remain viable for up to 2 hours and the transport of drug is stopped by rinsing the rings with ice cold buffer and drying them. At selected time interval, the tissue slices are assayed for drug content and expressed as mol/gm/time.

7. DIFFUSION CELL METHOD

In this method, small segments of small intestine are mounted between two glass chambers filled with buffer at 37 C. Diffusion cell consist of two compartments :-

I- Donor compartment - which contains the drug solution and the lower end of which contains the synthetic or natural GI membrane that interfaces with the receptor compartment.

II- Receptor compartment - which contain the buffer solution.

8. CELL CULTURE TECHNIQUES

Cell culture is the complex process by which cells are grown under controlled conditions, generally outside their natural environment. In this technique, differentiated cells of the intestine,

originating from CaCo2 cells (cells of carcinoma of colon) are placed on synthetic polycarbonate membrane previously treated with an appropriate material such as collagen which on incubation aids reproduction of cells while not retarding drug permeation characteristics. These models are based on the assumption that passage of drugs across the intestinal epithelium is the main barrier for drugs to reach the circulation.

Human intestinal cell lines are generally divided into four different groups:

Type I: These cells differentiate spontaneously under normal culture conditions and hence are polarized (i.e. apical and basolateral surface), form domes, have tight junctions and brush border (eg.Caco-2 cells).

Type II: These cells differentiate into enterocytes- type cells only under specific culture conditions e.g. HT29 in presence of glucose, HT29 clone can differentiate into mucus cells.

Type III: These cells form domes but do not express any biochemical or morphological markers of differentiated cells. e.g. T84, SW116 and Coll 15 cell lines.

Type IV: These cells do not differentiate. e.g. HCA7 and SE480 cell lines. CaCo-2 is the most widely used cell line and CaCo-2 are a human colon carcinoma cell line. The CaCo-2 (colon cancer cells) cell line is a continuous line of heterogeneous human epithelial colorectal adenocarcinoma cells. Solution of drug is placed on this layer of cultured cells and the system is placed on this layer of cultured cells and the system is placed (R.C) in a bath of buffer solution. The drug that reaches the latter compartment is sampled and analysed periodically. CaCo2 cells express tight junctions, microvilli and a number of enzymes and transporters that are characteristic of enterocytes. CaCo2 monolayer is widely used across the pharmaceutical industry as an in vitro model of the human small intestine mucosa to predict the absorption of orally administered drugs.

The correlation between the in vitro apparent permeability across CaCo2 monolayers and the in vivo fraction absorbed is well established. Cell culture models have been employed in the screening of the intestinal permeability of libraries of new drug entities that have been generated through combinatorial chemistry and high throughput pharmacological screening.

IN VIVO METHODS

In vitro and in situ techniques gives us an idea about absorption, but in vivo method gives us an idea about some important factor that influence absorption such as gastric emptying, intestinal motility and the effects of drugs on the GIT can be determined.

The in vivo method can be classified into:

1. Direct method
2. Indirect method

Direct method

The drug levels in blood or urine is determined as a function of time. For this, a suitable sensitive reproducible analytical procedure should be developed to determine the drug in the biological fluid. In this method, blank urine or blood sample is taken from the test animal before the experiment. The test dosage form is administered to the animal and at appropriate intervals of time the blood or urine sample are collected and assayed for the drug content. From the data, we can determine the rate and extent of drug absorption.

In this method, the experimental animal chosen should bear some resemblance to man. It is reported that pigs most closely resemble to man but are not used due to the handling problems. The other animal that can be used are dogs, rabbits and rats. In this method, the experimental animal chosen should bear some resemblance to man. It is reported that pigs most closely resemble to man

but are not used due to the handling problems. The other animal that can be used are dogs, rabbits and rats.

Indirect method

When the measurement of drug concentration in blood or urine is difficult or not possible, but a sensitive method is available to test the activity, then absorption studies can be done by this indirect method. In this method, pharmacological response of the drug is related to the amount of drug in the body. The response is determined after the administration of a test dosage for m; LD 50 appears to be dependent on the rate of the absorption of drug.

IN SITU METHOD

It simulates the in vivo conditions for drug absorption and are based on perfusion of a segment of GIT by drug solution and determination of amount of drug diffused through it. In situ refers to those method in which the animal's blood supply remains intact in which the rate of absorption determined from these methods may be more realistic than those determined from in vitro techniques. These models are powerful tools to study the mechanistic aspects of this important process and acts as a bridge between in vivo and in vitro methods.