IAM Chromatography

Introduction

A simple, rapid method to predict drug transport across biological barriers has been a long standing objective in the pharmaceutical sciences. A major barrier to transport of the drug into the cell is the cell membrane. The prediction of drug transport through any biological membrane barrier is typically a lengthy process that requires substantial experimental work

Immobilized Artificial Membrane (IAM) chromatography phases mimic the lipid environment found in cell membranes. Since phosphatidylcholine (PC) is the major phospholipid found in cell membranes, IAM phases prepared from PC analogs are models of cell membranes. These materials model the hydrophobic and hydrophilic contribution of a drug's partitioning and can be used for elucidating drug-membrane interactions. Therefore, Regis' IAM HPLC Drug Discovery columns are useful tools for predicting drug membrane permeability.

References:
1. Regis 1998-99 Chromatography Catalog

IAM Chromatography

Immobilized Artificial Membrane (IAM) chromatography is a useful technique for analysis and purification of many biological molecules. Originally developed by Professor Charles Pidgeon at Purdue University, the IAM stationary phase consists of a monolayer of phospholipid covalently immobilized on an inert silica support. The resulting IAM surface is a chemically stable chromatographic material

which emulates the exterior of a biological cell membrane. IAM chromatography is useful for the analytical and preparative separation of membrane-associated proteins and has been used for the non-covalent immobilization of membrane associated proteins. IAM chromatography has recently gained acceptance for the chromatographic estimation of the membrane permeability of small molecule drugs.

IAM Surface Emulates the Surface of a Biological Membrane

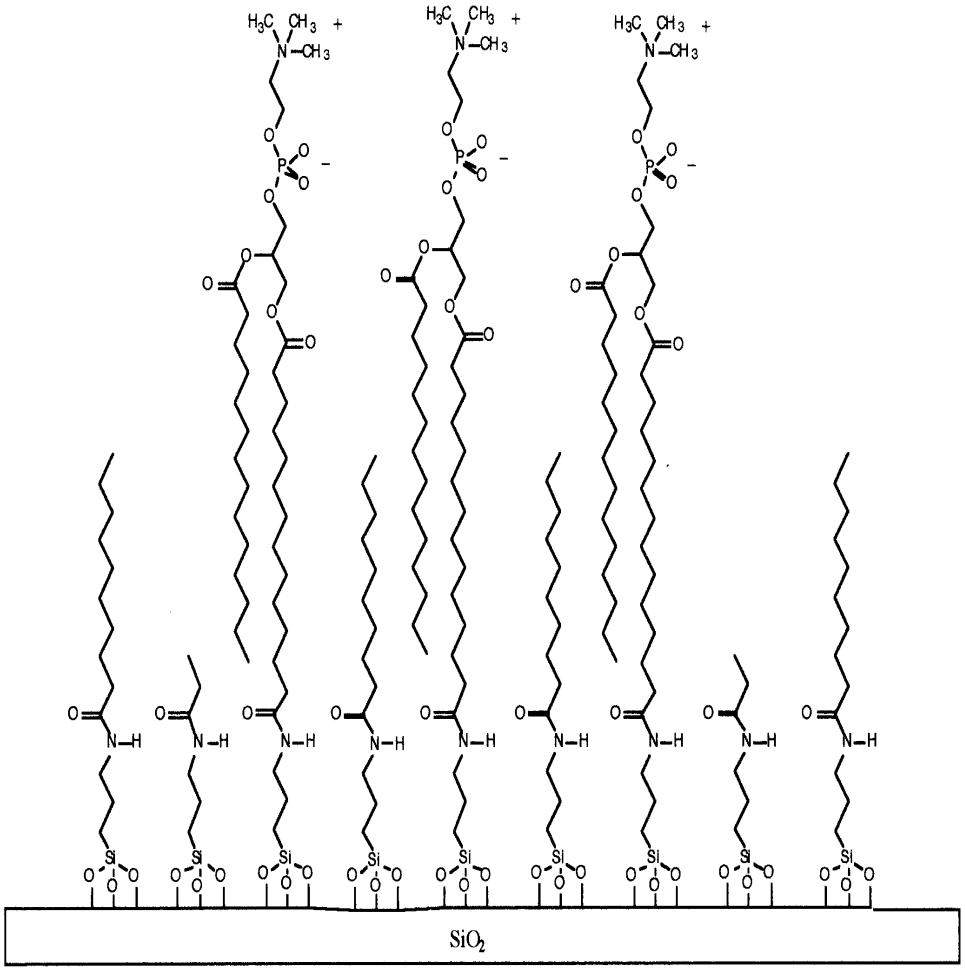


Figure 1

Estimating Drug Permeability

- IAM chromatography has recently gained acceptance for the chromatographic estimation of the membrane permeability of small molecule drugs.
- Estimation of drug permeability using IAM chromatography provides better correlation with experimentally determined drug permeability than other chromatographic techniques, such as the use of ODS silica.
- IAM retention of analytes more closely mimics the interaction of analytes with biological membranes, where a combination of hydrophobic interactions, ion pairing interactions and hydrogen bonding interactions are possible. ODS silica, however, only provides retention of analytes solely on the basis of hydrophobicity.
- IAM chromatography measures **phospholipophilicity**!

Rapid and Economical

- IAM chromatography is a rapid and economical alternative to more expensive and labor intensive methods of estimating drug permeability such as the use of liposome assays, CACO-2 cell line cultures, or intestinal tissue.

- IAM chromatography provides an estimate of drug permeability in a fraction of the time and at a fraction of the cost of conventional assay procedures.

- Data provided by the IAM chromatography method generally correlates with experimentally determined drug permeability at least as well as the data obtained from the more laborious and costly assay procedures, and much better than data obtained from computer models.

A Comparison of a C18 Drug Discovery Column and the IAM.PC.DD2

Types of Interaction	C18 Drug Discovery Column	IAM.PC.DD2
Hydrophobic Interaction?	Yes	Yes
Hydrogen Bonding?	No	Yes
Ion-Pairing?	No	Yes

References:
1. Hanlan Liu, Shaowei Ong, Louis Glunz, Charles Pidgeon; *Predicitng Drug-Membrane Interactionsby HPLC: Structural Requirements of Chromatographic Surfaces. Anal. Chem.* 1995, 67, 3550-3557

It is critical for a chromatographic surface to accurately monitor the interaction between solutes and biological membranes, that there be an ordered monolayer of immobolized lipids containing both a polar and a nonpolar region. The success of the IAM chromatography is based upon the similarities between the immobolized ligands comprising IAMs and the phospolipids comprising membrane bilayers.

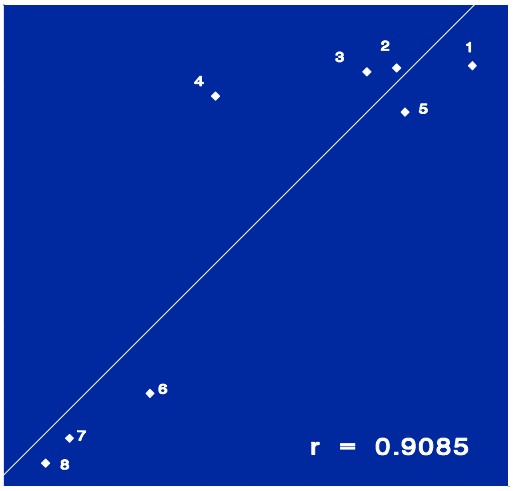
Correlation with Caco-2 Cell Permeability

The permeability of drugs through Caco-2 cells which correlates with clinical absorption of drugs is an established model for evaluating the transport properties of drugs across biological membranes. IAM drug partitioning shows excellent correlation with intestinal drug permeability (log Pm) through Caco-2 cells. The retention factors measured on reversed phase C18 columns (a commonly used model to determine drug partitioning) show extremely poor correlation with intestinal drug absorption.

Comparing IAM Chromatography to Caco-2 Cell Permeability

1. Propranolol
2. Alprenolol
3. Warfarin
4. Metoprolol
5. Hydrocortisone
6. Terbutaline
7. Atenolol
8. (AVP) Arginine-Vasopressin

Column:	IAM Fast Screen Mini Column 1 cm x 3.0 mm i.d.
Eluent:	0.01 M DPBS Buffer, pH = 7.4
Flow Rate:	0.5mL/min
Load:	10 µL
Detection:	UV 220 nm



r= 0.9085

Figure 2

Correlation of intestinal drug permeability (log P_m) through Caco-2 cells with drug partitioning into IAM Fast-Screen Mini Columns, (log k'_{IAM}).

The permeability of drugs through Caco-2 cells is an established model for evaluating the transport properties of drugs across biological membranes. Drug permeability in Caco-2 cells correlates with the clinical absorption of drugs. As shown, Drug permeability correlates well with drug capacity factors k'_{IAM} measured on the IAM Fast-Screen Mini Columns.

Initial Screening of Drug Permeability

IAM chromatography is increasingly used for an initial estimate of the drug permeability of drug candidates, produced by combinatorial chemistry. Analysis of libraries of such candidates by high throughput screening requires an assay method which is inexpensive, reproducible, rapid and dependable.

IAM.PC.DD2

- The IAM.PC.DD2 column is also used to predict drug membrane permeability. The ester bonding of the DD2 packing offers more hydrophobicity than does the DD phase. This material is a diacylated or double chain ester PC ligand, and is endcapped with C10/C3 alkyl chains.
- The IAM.PC.DD2 is more stable at higher pH.
- The increased hydrophobicity gives longer retention times to compounds not well retained on the IAM.PC.DD column.
- Like the DD it shows excellent correlation to traditional methods.

Estimation of Drug Permeability

- The IAM Fast-Screen Mini Column is a specially designed 1cm x 3mm cartridge column that contains the widely used and dependable ^{Ester} IAM.PC.C10/C3 stationary phase support.

- Developed by Regis researchers to provide a tool that can be used for a very rapid estimation of drug permeability in high throughput screening programs.

The IAM Fast-Screen Mini Column for Drug Discovery

- Like any short column, the 1cm IAM Fast-Screen Mini Column has only about 5% of the theoretical plates of a conventional analytical column. This means that the column is much less efficient for resolving the peaks of multi-component sample mixtures. However, the column can be used for accurate and reproducable determination of the retention of individual components.

- The 1cm IAM Fast-Screen Mini Column is offered not as a separation tool, but rather as a tool for characterizing the chromatographic retention factor (k') of individual analytes. The measured k' of analytes on this column can be used to estimate a value for drug permeability.

Permeability Zones for Mass Screening of Compounds

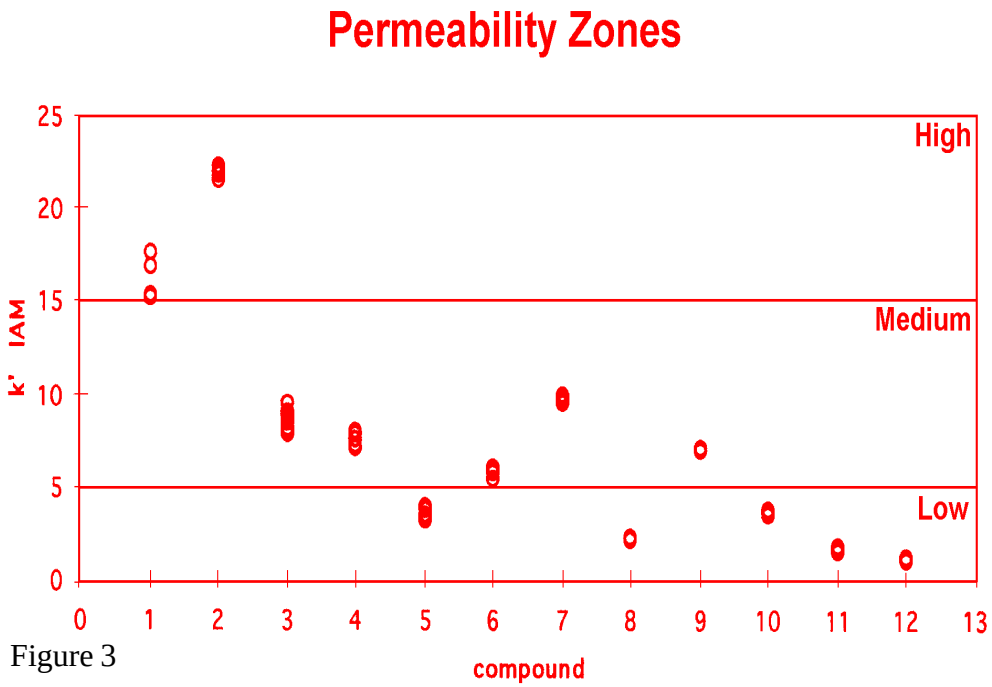


Figure 3

Permeability Zones can be determined for different analytes when performing large scale drug absorption screening. It can show whether a compound has low, medium or high permeability.

Advantages of the IAM Fast-Screen Mini Columns

- Rapid Indication of Drug Absorption
- High Sample Throughput
- Cost Effective
- Highly Reproducible Results
- Absorption Zones Established for Mass Screening

Why Use Regis' IAM Columns

Iam Chromatography allows a*:*

- Faster
- Easier
- Less Expensive
- More Reproducible Method for predicting drug ad sorption in comparison to other methods such as cell culture and animal models.

Mobile Phase

We obtain the **Dulbecco's Phosphate Buffered Solution Saline** from Sigma. The **Sigma** catalog number is **D-8537**. All the chromatographer needs to do is adjust the pH in the bottle and use the solution straight.