

IAM CHROMATOGRAPHY



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Immobilized Artificial Membrane (IAM) technology is an innovative approach to chromatography in which the chromatographic surface emulates the lipid environment of the cell membrane.^{1,2}

HPLC Separation Tools for Membrane Protein Purification and Drug Membrane Permeability Prediction

Phosphatidylcholine (PC) is the major phospholipid found in cell membranes. IAM chromatography phases prepared from PC analogs closely mimic the surface of a biological cell membrane. Consequently, IAM phases display a high affinity for membrane proteins and are useful in membrane protein purification and in the study of drug-membrane interactions. The IAM surface is formed by covalently bonding the membrane-forming phospholipids to silica.



Several different types of IAM columns are used for various applications:

Membrane Protein Purification

IAM.PC
IAM.PC.MG

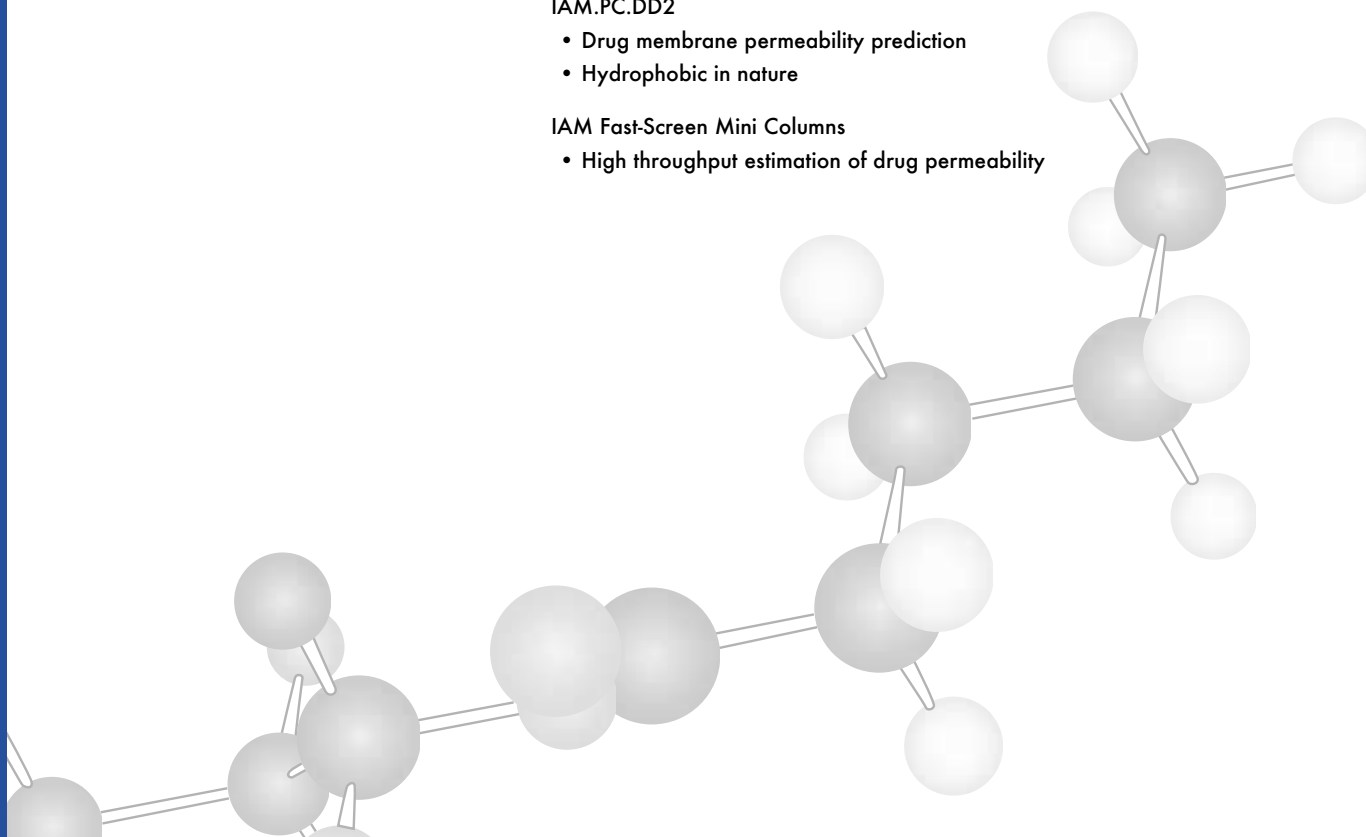
Drug Discovery

IAM.PC.DD2

- Drug membrane permeability prediction
- Hydrophobic in nature

IAM Fast-Screen Mini Columns

- High throughput estimation of drug permeability



DRUG DISCOVERY - PREDICTING DRUG MEMBRANE PERMEABILITY

IAM.PC.DD2

IAM Fast - Screen Mini Column

IAM chromatography has recently gained acceptance among drug discovery chemists for estimating the membrane permeability of small molecule drugs.

Figure 2 illustrates that the interaction between membrane bilayer and drug can be modeled by the IAM column/drug system.

K_{IAM} , the equilibrium constant describing the relative concentrations of drug in the membrane and in the external fluid, is analogous to the k'_{IAM} .

This IAM technique provides superior correlation with experimentally determined drug permeability when compared to other chromatographic methods. ODS silica, for example, retains analytes solely on the basis of hydrophobicity. IAM more closely mimics the interaction of analytes with biological membranes, where a combination of hydrophobic, ion pairing, and hydrogen bonding interactions are possible. This combination of interactions measured by the IAM column is known as phospholipophilicity.

These advances have led to the development of several new IAM phases used for predicting drug membrane permeability:

- IAM.PC.DD2
- IAM Fast-Screen Mini Column

Intestinal Drug Permeability

The retention factors measured on reversed phase C18 (ODS) columns (a commonly used model to determine drug partitioning) show extremely poor correlation with intestinal drug absorption (figure 3). For this group of compounds, hydrophobicity alone, as measured by the reversed-phase C18 column, is a poor predictor of drug absorption. Since IAM.PC Drug Discovery columns measure both hydrophilic and hydrophobic interactions between drugs and membranes, the IAM.PC Drug Discovery Column is better suited to the prediction of intestinal drug absorption.

Fluid Membrane and IAM Drug Partitioning Measurements

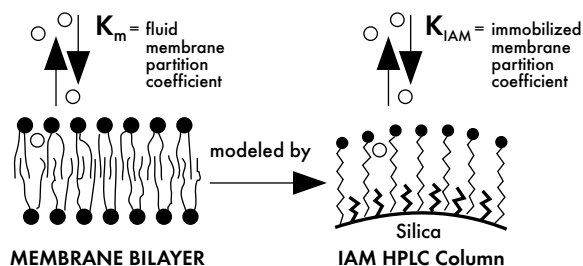


Figure 2. Fluid membrane bilayer can be modeled by IAM column.

ODS Exhibits Poor Correlation with Intestinal Drug Absorption

Column: C18 (ODS)
3 cm x 4.6 mm i.d.
Mobile Phase: 0.01 M DPBS Buffer, pH 5.4
Flow Rate: 1.0 mL/min
Load: 10 μ L
Detection: UV 220 nm

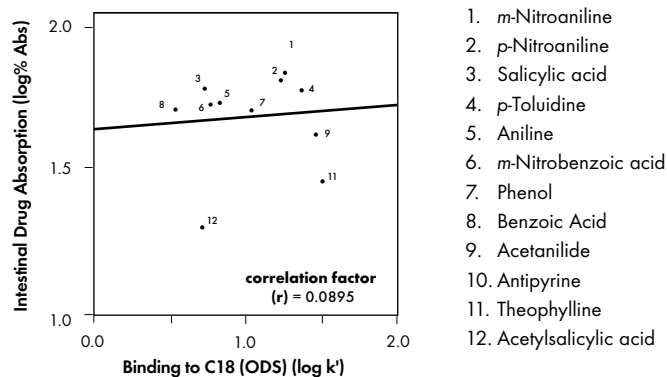


Figure 3. Drug partitioning into ODS does not correlate with intestinal drug absorption.

Product information and applications are available online at:
www.registech.com/iam/.

IAM.PC.DD2

Like the first generation IAM.PC.DD material, the IAM.PC.DD2 is used to predict drug membrane permeability. The ester bonding of the DD2 packing offers more hydrophobicity than the first generation DD phase. This material is a diacylated or double chain ester PC ligand and is endcapped with C10/C3 alkyl chains as illustrated in figure 4.

Column Advantages

The IAM.PC.DD2 material offers the following advantages:

- Hydrophobic nature
- Greater stability
- Excellent correlation to traditional methods

Hydrophobic Nature

The IAM.PC.DD2 offers more hydrophobicity than the first generation IAM.PC.DD material. This hydrophobic nature allows for longer retention times to compounds not well retained on the IAM.PC.DD material.

Greater Stability

Another distinct advantage of the IAM.PC.DD2 material is its ability to tolerate mobile phases between pH's 7.0 to 7.5, thus resulting in longer column life under these conditions.

IAM.PC.DD2 Structure

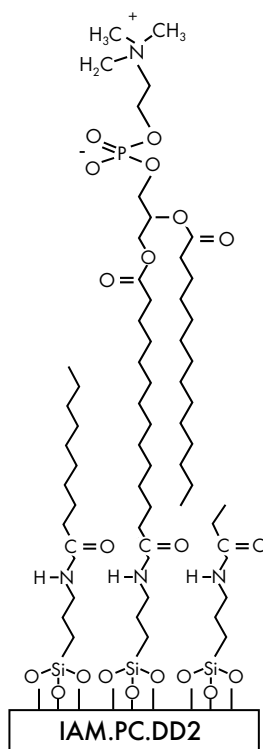


Figure 4. IAM.PC.DD2 is used to predict drug membrane permeability.

IAM FAST-SCREEN MINI COLUMN

Packed with the Ester PC Ligand phase, IAM Fast-Screen Mini columns are a rapid and economically viable screening method for the high throughput estimation of drug permeability. Their benefits include excellent reproducibility, short analysis time and low cost. This can be of great use in characterizing large libraries of compounds.

The structure of the ester IAM.PC.C10/C3 packing, selected for the Fast-Screen Mini Column, is shown in figure 6. This PC analog demonstrates superiority in retention times and stability – essential features for short columns and mass drug screening.

The IAM.PC Fast-Screen Mini Column, 1 cm in length by 3.0 mm in internal diameter, was specifically designed by Regis for rapid estimation of drug permeability in high throughput screening programs. When connected to an HPLC system with an autosampler, a single column can be used in the analysis of hundreds of samples per day with highly reproducible results.

The 1 cm 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.

IAM Fast-Screen Mini Column Structure

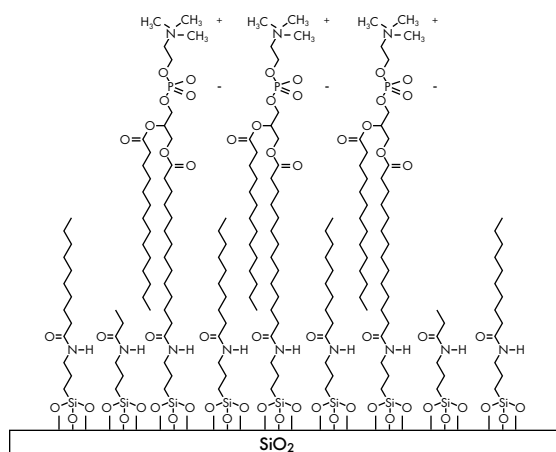


Figure 6. IAM.PC Fast-Screen Mini Column provides rapid estimation of drug permeability in high throughput screening programs.

Column Advantages

Regis Technologies' 1 cm Fast-Screen Mini Column for Drug Discovery provides the following advantages:

- Excellent correlation to traditional methods
- Rapid indication of drug absorption
- High sample throughput
- Highly reproducible results
- Durability
- Cost effectiveness
- Ability to establish absorption zones for high throughput screening

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IAM FAST-SCREEN MINI COLUMN

Excellent Correlation To Traditional Methods

The traditional means of predicting permeability include use of Caco-2 cell line cultures, intestinal tissue, or liposome assays. These are laborious and costly to perform.

Data obtained from the IAM Fast-Screen Mini Column correlate well to data obtained from traditional assays. This is summarized in table 2.

Method	Number of Compounds Evaluated	Correlation (r) with IAM Fast-Screen Mini Column
Partitioning into liposomes	23	0.831
Intestinal drug permeability	12	0.839
Caco-2 cell permeability	8	0.909

Table 2. Comparing k'_{IAM} data with other methods for estimating permeability.

Caco-2 Cell Correlation

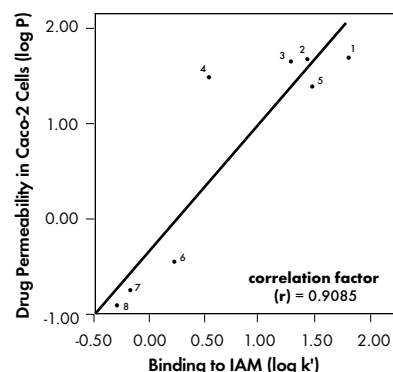
Figure 7 illustrates that drug permeability predicted by Caco-2 cells correlates well to k'_{IAM} measured on the IAM Fast-Screen Mini Columns.

Intestinal Tissue Correlation

Table 3 shows that drug permeability predicted by Inverted Rat Intestines correlates well to drug retention factors, k'_{IAM} measured on the IAM Fast-Screen Mini Columns. Note the short retention times.

IAM Fast-Screen Correlates with Drug Permeability in Caco-2 Cells

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



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

Figure 7. Correlating drug partitioning into IAM with intestinal drug permeability ($\log P$) through Caco-2 cells.

Compound	% Absorption of Inverted Rat Intestine	IAM Fast-Screen Mini Column	
		Retention Time (Sec)	k' (corrected)
m-nitroaniline	77	133.1	15.29
p-nitroaniline	68	177.9	21.84
salicylic acid	60	93.8	9.54
p-toluidine	59	79.7	7.48
aniline	54	52.1	3.45
m-nitrobenzoic acid	53	68.1	5.79
phenol	51	94.6	9.66
benzoic acid	51	43.7	2.22
acetanilide	42	76.2	6.97
antipyrine	32	51.8	3.40
theophylline	29	39.3	1.58
acetylsalicylic acid	20	36.1	1.11

r (correlation factor)* = 0.8385

* r is calculated by plotting $\log k'$ vs. \log % absorption of inverted rat intestine.

Chromatographic Conditions:

Column: IAM Fast-Screen Mini Column
1 cm x 3.0 mm i.d.
Mobile Phase: Dulbecco's Phosphate Buffered Saline, pH 5.4
Flow Rate: 0.3 mL/min
Load: 10 μ l
Detection: UV 254 nm, 0.1 AUFS

Table 3. Correlating drug partitioning into IAM with rat intestinal drug absorption.

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IAM FAST-SCREEN MINI COLUMN

Rapid Indication of Drug Absorption

IAM Chromatography is a more rapid alternative to other methods. In a recent study completed by Regis, k'_{IAM} s of 12 compounds were compared with absorption data obtained in situ using rat intestines. Retention times for the compounds tested were between 20 and 180 seconds, while retention factors correlated well to the intestinal absorption data.

High Sample Throughput

IAM chromatography is of increasing importance in combinatorial chemistry, where it is used to provide an initial estimate of a drug candidates' membrane permeability. Hundreds of samples can be injected into a single Fast-Screen Mini Column using an automated HPLC system. Recently a group of 12 test analytes was evaluated in 10 runs over the course of eight hours. Total run time for the 12 test analytes was only 42 minutes.

Highly Reproducible Results

The measured values for k'_{IAM} show excellent reproducibility, both from run to run and from day to day (figure 8).

Durability

IAM Fast-Screen Mini Columns are extremely durable. Correlation factors, r , for the original k' , and k' after 5000 column volumes were identical.

Cost Effectiveness

Because the IAM Fast-Screen Mini Column is inexpensive, has a very short analysis time, and provides drug permeability estimates for hundreds of drug candidates in a fraction of the time of conventional methods, the IAM Fast-Screen Mini Column becomes the economical alternative for high throughput screening.

Ability to Establish Permeability Zones for High Throughput Screening

Permeability zones can be determined for different analytes when performing large-scale drug absorption screening. Thus, rapid IAM analyses can characterize a drug as having low, medium, or high membrane permeability (figure 9).

Excellent Reproducibility with IAM Fast-Screen Mini Column

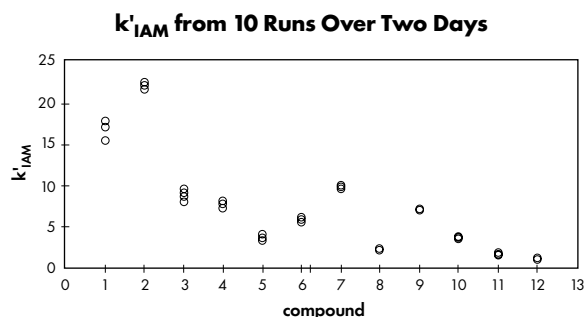


Figure 8. Highly reproducible k'_{IAM} from 10 runs over a two-day period.

k'_{IAM} Permeability Zones

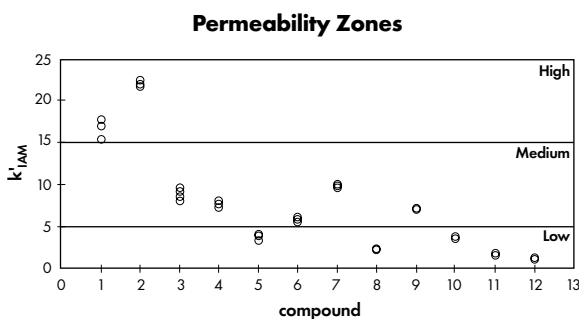
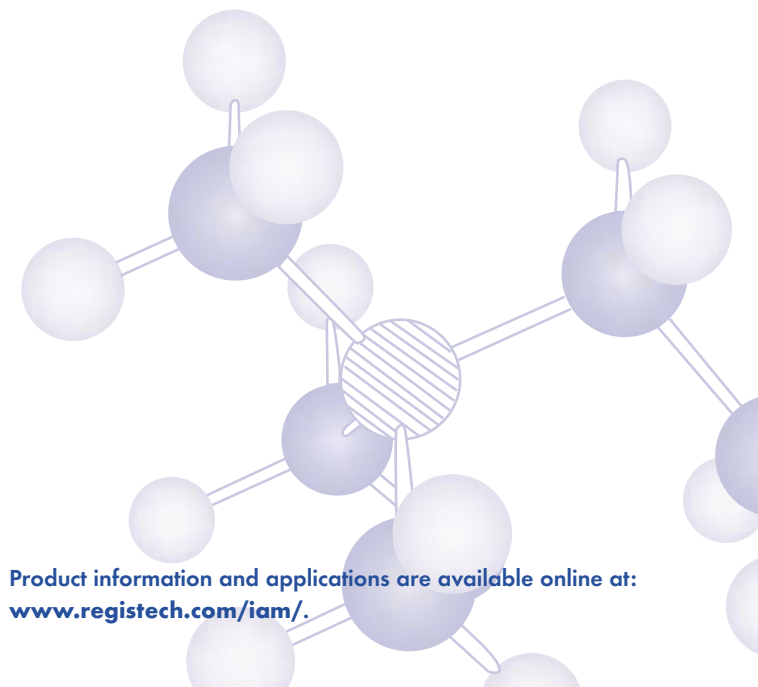


Figure 9. Permeability zones established large-scale drug absorption screening.



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