IAM
CHROMATOGRAPHY
IAM Chromatography

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

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
The IAM.PC phase, developed by Dr. Charles Pidgeon of Purdue University, was the first in a line of IAM phases to be manufactured by Regis. Use of this phase has simplified the inherent difficulties of protein isolation and purification, allowing for rapid purification of membrane proteins while maintaining biological activity. The IAM.PC phase is an important tool for the pharmaceutical industry and academia alike.

The first IAM stationary phase was based on the prevalent membrane lipid, phosphatidylcholine (PC), and consists of monolayers of amphiphilic phospholipids covalently bonded to aminopropyl silica particles through a terminal amide linkage. As a result, the bulky phosphatidylcholine groups shield many of the amine binding sites on the silica surface, preventing amine interaction with the protein molecules.

The membrane nature of the IAM phase imparts surface characteristics which are useful in the chromatography of membrane proteins. These include: high protein loading, increased protein recovery, recovery of functional activity, and selectivity for membrane proteins.

Large membrane proteins can interact with any combination of polar headgroup, hydrophobic chain, or inner amine groups. The subsurface has been shown to interact with certain solutes, and may or may not contribute to the separation of a given biomolecule. The residual amines can be left unaltered on the subsurface or deactivated through an endcapping procedure, which results in increased stability of the bonded phase. The methyl glycolate endcapping, for example, converts residual amines to neutral amides and introduces a hydroxyl group (IAM.PC.MG).

**IAM.PC Applications**

Numerous applications have been developed using IAM.PC columns:

- Purification of Cytochrome P450
- Isolation of membrane proteins
- Prediction of solute transport across human skin
- Prediction of amino acid transport across the blood-brain barrier
- Binding of solutes to liposome membranes
- Immobilization of Trypsin and α-chymotrypsin for the determination of their inhibitor and substrate activity

For additional information on IAM.PC applications please contact Regis’ technical support staff.

![Figure 1. The Phosphatidylcholine is covalently bound to propylamine groups, which are in turn bound to silica. Because the bulky PC groups limit access to the unbonded amine groups, these may or may not affect the separation of a given protein.](image-url)
**Drug Discovery - Predicting Drug Membrane Permeability**

**IAM.PC.DD2**

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.

\[ K_{IAM} \] is 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.

**ODS Exhibits Poor Correlation with Intestinal Drug Absorption**

<table>
<thead>
<tr>
<th>Column:</th>
<th>C18 (ODS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile Phase:</td>
<td>0.01 M DPBS Buffer, pH 5.4</td>
</tr>
<tr>
<td>Flow Rate:</td>
<td>1.0 mL/min</td>
</tr>
<tr>
<td>Load:</td>
<td>10 µL</td>
</tr>
<tr>
<td>Detection:</td>
<td>UV 220 nm</td>
</tr>
</tbody>
</table>

1. m-Nitroaniline
2. p-Nitroaniline
3. Salicylic acid
4. p-Toluidine
5. Aniline
6. m-Nitrobenzoic acid
7. Phenol
8. Benzoic Acid
9. Acetanilide
10. Antipyrine
11. Theophylline
12. Acetylsalicylic acid

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.

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

 IAM.PC.DD2 Structure

Product information and applications are available online at: www.registech.com/iam/
**IAM.PC.DD2**

**Excellent Correlation to Traditional Methods**

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

**Intestinal Tissue Correlation**

Measuring drug permeability in the intestinal tissue, where absorption is occurring, is physiologically more relevant than measuring drug permeability in Caco-2 cells. Figure 5 and Table 1 illustrate that drug absorption in this inverted rat intestinal tissue model correlates with drug retention factors $k'_\text{IAM}$ measured on the IAM.PC.DD2 column.

<table>
<thead>
<tr>
<th>Sample</th>
<th>% Absorption of Inverted Rat Intestine</th>
<th>(k') IAM.PC.DD2</th>
</tr>
</thead>
<tbody>
<tr>
<td>m-nitroaniline</td>
<td>77</td>
<td>10.838</td>
</tr>
<tr>
<td>p-nitroaniline</td>
<td>68</td>
<td>16.086</td>
</tr>
<tr>
<td>salicylic acid</td>
<td>60</td>
<td>6.963</td>
</tr>
<tr>
<td>p-toluidine</td>
<td>59</td>
<td>4.546</td>
</tr>
<tr>
<td>aniline</td>
<td>54</td>
<td>2.069</td>
</tr>
<tr>
<td>m-nitrobenzoic acid</td>
<td>53</td>
<td>4.403</td>
</tr>
<tr>
<td>phenol</td>
<td>51</td>
<td>6.544</td>
</tr>
<tr>
<td>benzoic acid</td>
<td>51</td>
<td>2.088</td>
</tr>
<tr>
<td>acetanilide</td>
<td>42</td>
<td>5.096</td>
</tr>
<tr>
<td>antipyrine</td>
<td>32</td>
<td>3.350</td>
</tr>
<tr>
<td>theophylline</td>
<td>29</td>
<td>1.478</td>
</tr>
<tr>
<td>acetylsalicylic acid</td>
<td>20</td>
<td>0.931</td>
</tr>
</tbody>
</table>

$r$ (correlation factor) $= 0.8025$

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

**Table 1. Correlating Drug Partitioning into IAM with rat intestinal drug absorption.**

**Figure 5. IAM.PC.DD2 columns measure drug absorption in inverted rat intestinal tissue.**

**Product Table**

<table>
<thead>
<tr>
<th>Product</th>
<th>Particle Size</th>
<th>Column Length and i.d.</th>
<th>Product #</th>
<th>U.S. Price</th>
</tr>
</thead>
<tbody>
<tr>
<td>IAM.PC.DD2 Columns</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IAM.PC.DD2 12 µm, 300Å</td>
<td>3 cm x 4.6 mm i.d.</td>
<td>774010</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IAM.PC.DD2 12 µm, 300Å</td>
<td>10 cm x 4.6 mm i.d.</td>
<td>774011</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IAM.PC.DD2 12 µm, 300Å</td>
<td>15 cm x 4.6 mm i.d.</td>
<td>774014</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IAM.PC.DD2 12 µm, 300Å</td>
<td>Guard Kit*</td>
<td>774012</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IAM.PC.DD2 12 µm, 300Å</td>
<td>Guard Replacements**</td>
<td>774013</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Includes 1 holder, 1 coupler, 2 guard cartridges
** Includes 3 guard cartridges

**IAM Correlates with Intestinal Drug Absorption**

| Column: IAM.PC.DD2 10 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 µL |
| Detection: UV 220 nm |

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

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

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

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

<table>
<thead>
<tr>
<th>Method</th>
<th>Number of Compounds Evaluated</th>
<th>Correlation (r) with IAM Fast-Screen Mini Column</th>
</tr>
</thead>
<tbody>
<tr>
<td>Partitioning into liposomes</td>
<td>23</td>
<td>0.831</td>
</tr>
<tr>
<td>Intestinal drug permeability</td>
<td>12</td>
<td>0.839</td>
</tr>
<tr>
<td>Caco-2 cell permeability</td>
<td>8</td>
<td>0.909</td>
</tr>
</tbody>
</table>

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.

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

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

r (correlation factor) = 0.8385

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

Chromatographic Conditions:

<table>
<thead>
<tr>
<th></th>
<th>IAM Fast-Screen Mini Column</th>
<th>1 cm x 3.0 mm i.d.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile Phase</td>
<td>Dulbecco’s Phosphate Buffered Saline, pH 5.4</td>
<td></td>
</tr>
<tr>
<td>Flow Rate</td>
<td>0.3 mL/min</td>
<td></td>
</tr>
<tr>
<td>Load</td>
<td>10 µL</td>
<td></td>
</tr>
<tr>
<td>Detection</td>
<td>UV 254 nm, 0.1 AUFS</td>
<td></td>
</tr>
</tbody>
</table>

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

Product information and applications are available online at: [www.registech.com/iam/](http://www.registech.com/iam/).
Rapid Indication of Drug Absorption

IAM Chromatography is a more rapid alternative to other methods. In a recent study completed by Regis, $k'_{IAM}$ 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.
IAM References


Regis Technologies manufactures the IAM Fast-Screen Mini Column on-site in its manufacturing facility. This column, as well as all of our other products, must adhere to rigorous manufacturing and quality control specifications before release.

Regis’ technical support staff, with years of chromatography experience, is available to answer any questions regarding the new IAM Fast-Screen Mini Column.

Product | Particle Size | Column Length and i.d. | Product # | U.S. Price |
--- | --- | --- | --- | --- |
IAM Fast-Screen Mini Column Kit | 12 µm, 300Å | 1 cm x 3 mm i.d. | 775014 |
IAM Fast-Screen Mini Columns, Pkg of 6 | 12 µm, 300Å | 1 cm x 3 mm i.d. | 775015 |
IAM Fast-Screen Mini Columns, Pkg of 12 | 12 µm, 300Å | 1 cm x 3 mm i.d. | 775016 |

IAM FAST-SCREEN MINI COLUMN

For additional information on IAM Chromatography, check our Web site at www.registech.com/iam/. You may also request a complete IAM publication list, by contacting Regis at:

(800) 323-8144 ext. 649
(847) 967-6000 ext. 649
e-mail us at: sales@registech.com.