Molecular Devices

molecular adsorption to polypropylene compound plate may reduce potency measurements

IONWORKS HT TECHNICAL NOTE #2

SUMMARY

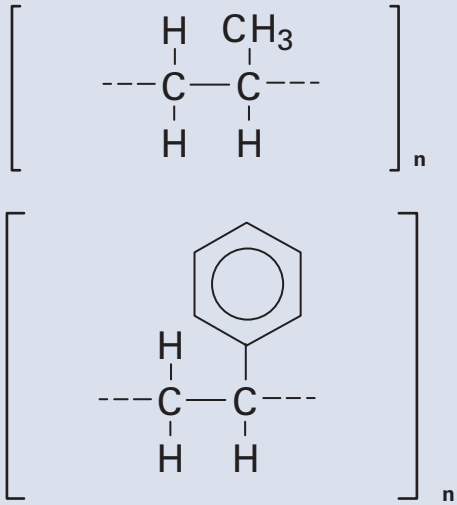
Two types of microplates that are commonly used in research laboratories are made of polypropylene or polystyrene polymers. These hydrophobic polymers (see Figure 1) disrupt the highly ordered molecular structure of water—so much so that an interface is established between the aqueous phase and the polymer surface. As a result, lipophilic compounds present in an aqueous solution are partitioned into the polymer-aqueous interface, which enables water molecules to avoid directly interacting with the hydrophobic surface. Highly lipophilic compounds are partitioned into the phase interface to a greater extent, thereby reducing the available concentration of the compound in the aqueous phase, which may be manifested as an apparent reduction in potency for some compounds in IonWorks HT assays. The use of a glass compound plate may alleviate reduced potency measurements caused by this molecular adsorption of compounds to polymeric surfaces.

ADSORPTION AND PARTITION COEFFICIENTS

Compounds interact with polymers through one or more types of molecular interactions, including van der Waals forces, donor-acceptor interactions, hydrogen bonding, inclusion complexes and dipole-dipole interactions. The polypropylene matrix contains only a carbon-hydrogen framework and lacks aromatic rings or other polar groups. As such, polypropylene is highly hydrophobic and will interact with compounds only via van der Waals interactions. The polystyrene matrix contains a carbon-hydrogen framework and aromatic rings, and is also highly hydrophobic. In addition to van der Waals interactions, the aromatic groups also permit donor-acceptor interactions if there are any π-bonds present in the compound of interest.

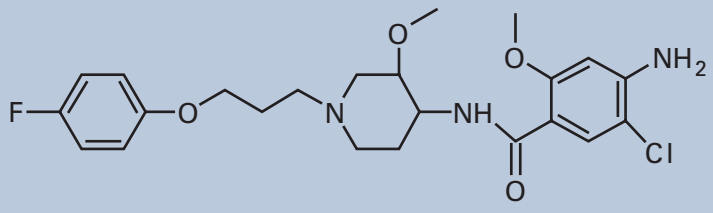
The hydrophobicity of a compound is usually expressed as the partition coefficient (P or log P) determined by mixing it with water and an

molecular structure of polypropylene and polystyrene (figure 1)



Polypropylene (top) and polystyrene (bottom) backbones.

molecular structure of the hERG inhibitor cisapride (figure 2)



cisapride

Cisapride can interact with polymers via donor-acceptor and van der Waals interactions.

immiscible organic liquid, usually octanol. Once equilibrium is achieved, the molarity of the compound in each of the phases, organic and aqueous, is determined and P is calculated as shown below:

$$P = \frac{[\text{compound}]_{\text{organic phase}}}{[\text{compound}]_{\text{aqueous phase}}}$$

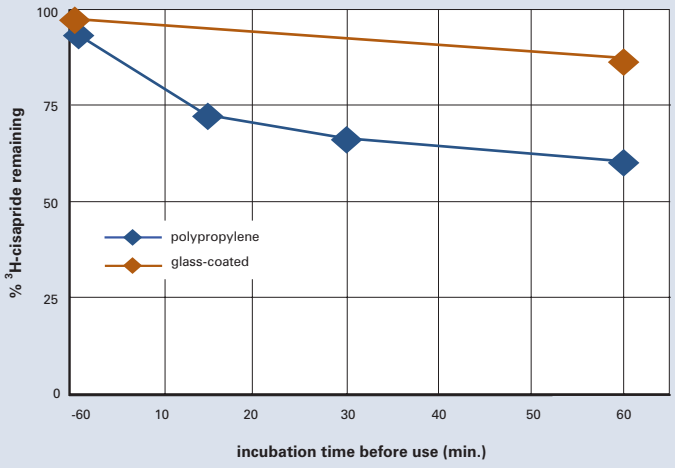
The higher the log P value, the greater the hydrophobicity, and therefore, the greater the compound adsorption to a polymeric surface. The log P values of many drug compounds are published in the scientific literature. In addition, there are many methods and software programs available for theoretical calculations of log P values. The log P value of cisapride (see Figure 2), the test compound used in this technical note, is 3.96—indicative of a highly hydrophobic substance.

This technical note describes how we are able to reduce the adsorptive losses of cisapride in IonWorks HT procedures, by reducing the hydrophobicity of the polymeric surface via the use of disposable glass-coated compound plates.

MATERIALS

- Cells: Chinese hamster ovary (CHO) cells expressing hERG
- Reagents and buffers: Amphotericin (Sigma Cat. # A-4888), DMSO (Sigma Cat. # D-2650); High K^+, high Cl^- Internal Buffer containing, in mM, 140 KCl (Sigma Cat. # 9333), 2 $MgCl_2$ (Sigma Cat. # M-2670), 5 EGTA (Sigma Cat. E-0396), 10 HEPES (Sigma Cat. # H-7523), pH 7.25 with KOH; External Buffer, in mM, 4 KCl, 137 NaCl (Fisher Cat. # S271-500), 1.8 $CaCl_2$ (Sigma Cat. # C-4901), 1 $MgCl_2$, 10 HEPES, 10 Glucose (Sigma Cat. # G-7021), pH 7.25 with NaOH; Cisapride (RDI Cat. # R51619)
- Tissue culture flasks: cells grown in T-175 flasks (Corning Cat. # 431080)
- Cell culture media: Dulbecco's Modified Eagle Medium containing L-glutamine, glucose, pyroxidine HCl, without Na pyruvate (Gibco, Cat. # 11965-092) supplemented with the following per liter: 50 ml Fetal Bovine Serum (FBS, Irvine Cat. # 3000), 5 ml Penicillin/Streptomycin (Irvine Cat. # 9366) and 5 ml Geneticin (G418, Gibco Cat. # 10131) used to grow cells; Versene™ (Gibco Cat. # 15050) used to remove cells from flasks
- PatchPlate™ consumables: Molecular Devices (Cat. # 9000-0688)
- Compound plates (96-well): Polypropylene: Costar (Cat. # 3355); Glass: Sun SRi Plate+™ glass coated microplate (Cat. # 400 058)
- ^{3}H-cisapride, custom labeled by Moravek Biochemicals, Inc.
- hERG voltage protocol: resting potential was -70 mV; 5 sec. depolarization to +40 mV; 4 sec. repolarization to -50 mV

adsorption of ^{3}H-cisapride to polypropylene and glass-coated compound plates (figure 3)



Retention of ^{3}H-cisapride to polypropylene and glass-coated microplates. Polypropylene adsorbed approximately 40% of ^{3}H-cisapride compared to 10% on glass. More adsorption to polypropylene occurred with longer incubations times.

compound plate setup for cisapride potency experiments (figure 4)

	1	2	3	4	5	6	7	8	9	10	11	12
A	PBS											
B	cisapride titration											
C	1.0E-2	3.3E-3	1.1E-3	3.7E-4	1.2E-4	4.1E-5	1.4E-5	4.6E-6	1.5E-6	5.1E-7	1.7E-7	5.6E-2
D												
E												
F												
G												
H												

Set-up of cisapride compound plate for IonWorks HT experiments. The concentrations of cisapride used in the experiment are shown (in M).

METHODS AND RESULTS

Compound plate adsorption

Adsorption of cisapride to polypropylene microplates was measured radiometrically. A concentration of 400 nM tritiated cisapride was prepared in aqueous solution (PBS) and added to polypropylene or glass-coated microplates. The plates were incubated for 1, 15, 30 or 60 minutes and samples were transferred to a scintillation vial for radiometric readout. The amount of ^{3}H-cisapride retained in the compound plate was estimated by subtracting the measured concentration of ^{3}H-cisapride remaining in the aqueous solution from the original concentration. The amount of ^{3}H-cisapride that adsorbed to the polypropylene plate increased with incubation time and approached 40%. (See Figure 3.) ^{3}H-cisapride retention in glass-coated microplates was markedly reduced in comparison to polypropylene plates—only 10% of total concentration of ^{3}H-cisapride was adsorbed by glass after 60 minutes. Polystyrene plates bind cisapride with similar affinity to that shown by polypropylene (data not shown).

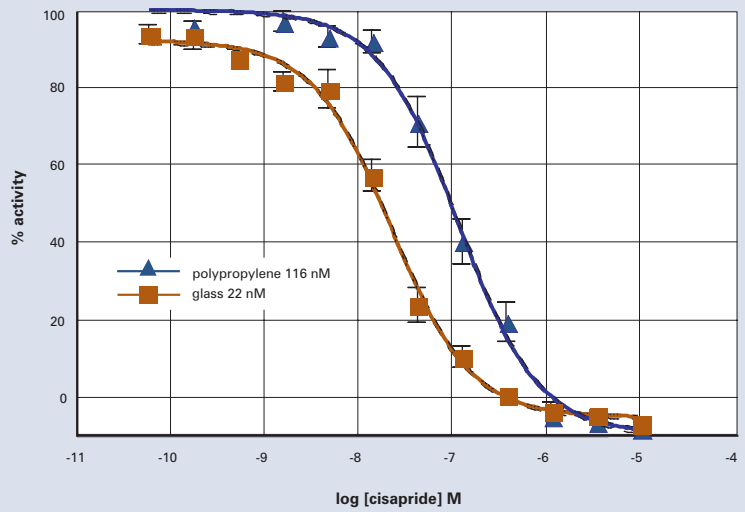
Compound plate setup for IonWorks HT experiments

Compound plates were prepared from a 10 mM stock of cisapride in DMSO. The stock solution was diluted in extracellular buffer to a concentration of 30 µM and a 12-point 1:3 serial dilution was carried out in the plates, yielding a final experimental concentration range of 10 µM to 0.06 nM. (See Figure 4.) Each concentration was replicated in three rows (n=12), each of which was replicated four times in the PatchPlate (up to n=48 per data point). Extracellular buffer was included in one row (12 wells) as a negative control. (See Figure 4.) Compound plates were incubated at room temperature for at least 60 minutes before the start of the experiments. Experiments included duplicate runs on two separate IonWorks HT machines for each compound plate condition, for a total of 8 runs (n=4 experiments for each condition, *i.e.*, polypropylene vs. glass). In each pair of runs, the cells from a single flask were lifted with Versene, resuspended and equally divided between the two instruments. The total experiment time was approximately five to six hours. Raw data sets were exported to Excel and pooled for each respective condition. The final IC_{50} determinations were fit using Prism 4 (GraphPad Software).

Effect on cisapride IC_{50}

The IC_{50} values determined for cisapride using glass-coated and polypropylene microplates are summarized in Figure 5. The IC_{50} values for cisapride using glass-coated microplates was 22 nM versus 116 nM for polypropylene plates. The 95% confidence intervals were 21.91-23.47 nM and 111.87-119.42 nM for glass-coated and polypropylene plates, respectively; the R^2 values were > 0.99 for both.

apparent cisapride potency is enhanced using a glass compound plate (figure 5)



Cisapride dose-response experiments comparing IC_{50} determinations from compounds titrated in glass-coated and polypropylene plates. The IC_{50} for cisapride was more potent with glass-compound plates. N=14-34 per data point; mean ±SEM; 95% confidence intervals plotted (dotted line). IC_{50} values are indicated.

CONCLUSIONS

The use of disposable glass-coated microplates instead of polypropylene or polystyrene plates may increase compound potency measurements in IonWorks HT experiments. The observed effect is likely to be highly dependent on the physicochemical properties of the compound in question. Researchers using compounds that are highly hydrophobic will most likely benefit from using glass-coated microplates because glass minimizes the partitioning of lipophilic compounds to the microplate surface interface.

REFERENCES

1. Lipinski, C.A., Lombardo, F., Dominy, B.W., Feeney, P (2001). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev* 46:3-26.

2. Cisapride Material Safety Data Sheet, Janssen Pharmaceutica, Titusville, NJ.

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