A Taste of the Pharmaceutical Sciences: Development of Labs to Support Student Learning

Alexander D Smith

Faculty of Pharmaceutical Sciences, The University of British Columbia, 2405 Wesbrook Mall, Vancouver, BC, V6T 1Z3, Canada

alexander.smith@ubc.ca

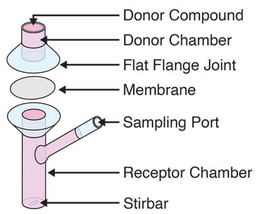
The field of pharmaceutical science requires the integration of key concepts from both chemistry and biology. As part of a new degree program in the Faculty of Pharmaceutical Sciences, I have designed a series of laboratory activities to complement lecture concepts in pharmaceutics, genetics, and nanomedicine that allow students to develop the ability to generate, interpret, and analyze data. Students begin the labs by exploring concepts of solubility, diffusion, and permeability using decalcified eggs, followed by use of a Franz diffusion chamber to see how drugs are able to transit this membrane over time. Building upon the increased familiarity with the lab, we next explore how GST metabolism is affected by both biological sex, and pH, through the use of male and female mouse liver cytosol and the UV detection of substrate metabolites. The third lab introduces concepts from pharmacogenomics with the students having the opportunity to extract their own DNA and see the correlation between their ability to taste bitter compounds and the SNPs present in their *TAS2R38* gene. The fourth lab introduces concepts from nanomedicine with students first preparing liposomes using extrusion techniques and analysing their liposomes' physical properties and loading efficiencies. The final lab has students assist with the preparation of liposome encapsulated siRNA using a microfluidic chip-based system, and the subsequent use of these particles to knock down expression of GFP expressed in HEK cells. This ties back into lecture content where they have covered the production and use of siRNA drugs like the anti-cancer medication patisiran. These labs help to introduce basic lab techniques while reinforcing lecture concepts, helping to prepare students for more advanced lab courses in their third year.

Diffusion of drugs across biological membranes

This is the first lab of the course and is meant to provide a fun introduction to the lab. It references concepts discussed in lecture allowing hands-on exploration of the movement of water across a semi-permeable membrane, and the effects of dissolved solutes on biological systems. Determining if a compound is able to pass through a biological barrier is critical in drug development. Here we use chicken egg membrane as a crude mimic of epithelium. Students also test solubility of drugs forming hypotheses about what will or will not dissolve in a particular solvent based on the drug's known physiochemical properties.

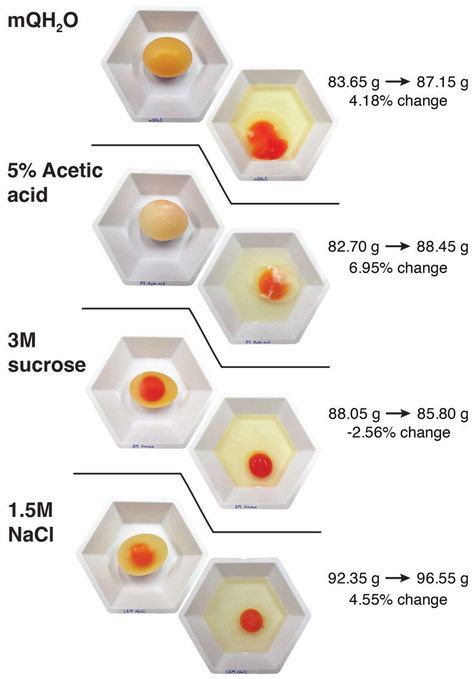
Lab outline

- ○ Prepare materials (~1 week)
- ○ Deshell eggs (~48h)
- ○ Weigh eggs
- ○ Treat eggs (~48-72h)
- ● Quiz
- ○ Mini lecture
- ● Naked Eggs
- ● Examine and weigh eggs
- ○ Drug diffusion assay[1,2]
- ○ Assemble Franz chamber



- ○ Add compound to donor chamber
- ○ Sample every 5 minutes for 1 hour
- ○ Prepare standard curve
- ○ Read absorbance in plate reader
- ○ Data analysis
- ● Drug solubility testing

Naked eggs



Key Concepts

- Calculations
- Diffusion
- Flux
- Osmolarity
- Osmolality
- Osmosis
- Reproducibility
- Semi-permeability
- Solubility

Compounds have different fluxes and solubilties

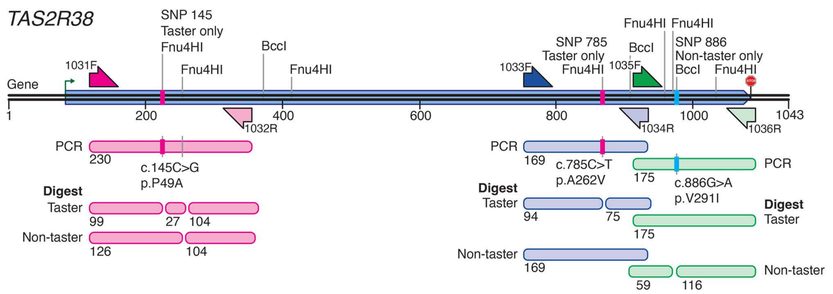
Compound	Wavelength (nm)	Mean flux ($\mu g/cm^2 min$)	Solubility* DMSO	EtOH	H_2O	Acid	Base
4-methylumbelliferone	334	5.94 ± 0.23	Yes	Part†	Yes	No	Yes
Acetaminophen	243	7.14 ± 0.84	Yes	Yes	No	No	Yes
Acetylsalicylic acid	230	5.28 ± 0.74	Yes	Yes	No	No	No
Caffeine	273	7.53 ± 0.99	No	No	Part†	Part†	Part†
Chloroquine	332	N/A	No	No	Yes	Yes	Yes
Quinidine	332	N/A	Yes	Yes	No	No	No

*20 mg/mL compound, mQH_2O, 100% EtOH, 100% DMSO, 0.1 M HCl, 0.1 M NaOH, †Fully dissolves at 10 mg/mL

Bitter taste phenotype-genotype correlation

This lab complements a series of lectures on basic genetics that lead into discussion of pharmacogenetics and pharmacogenomics. The ability to taste bitter compounds is primarily determined by three missense-coding single nucleotide polymorphisms (SNPs) in *TAS2R38* at positions 145, 785, and 886 of the open reading frame giving rise to the PAV (taster) and AVI (non-taster) diplotypes[3]. The bitter-taster phenotype is easily determined using commercially available PTC, thiourea, and PROP tasting strips.

The genotype can be determined by sequencing[4], affording a number of excellent learning opportunities, however this can be difficult with larger groups. Each SNP is located at the site of an Fnu4HI or BccI restriction enzyme recognition site, and the SNP alters this site. Amplifying these regions, digesting, and running on polyacrylamide gels allows easy determination of genotype. Students may, for any reason, decline to extract or test their own DNA and are provided with an instructor sample to use instead, allowing participation.



Key concepts

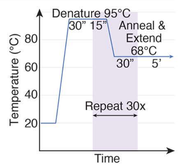
DNA codons	Genotypes	Restriction digests
DNA extractions	PCR	SNPs
Electrophoresis	Phenotypes	Translation

Lab outline

- ● Genomic DNA extraction
- ● Swab check cell DNA
- ● Extract DNA (Zymo Research Quick-DNA kit)
- ● PCR amplification of *TAS2R38* SNPs
- ● 2 μL DNA to pre-prepared tubes of PCR master mix

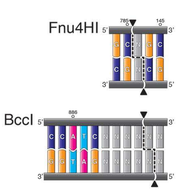
Tube	SNP	Primer	Sequence (5' - 3')
1	145	1031F	GGAGTACATTTCTGTTCATTTCAGTCCTGGAGTTTGCAGTGG
		1032R	GCTGTGGTTCAGTGGTTCACTCAACTTCTGGAAGTGG
2	785	1033F	CCAGAAACTCTCGTGACCCCAGCCTGGAGG
		1034R	GCCATTATCCCAACACAAACCATCACCCCTATTTTGTCG
3	886	1035F	GGTGATGGTTTGTGTTGGGATAATGGCAGCTTGTCC
		1036R	CCATTCTCAGCACAGTGTCCGGGAATCTGC

Component	Final concentration	Volume (μL)
pure water	—	39.75
10X ThermoPol buffer	1X	5
10 mM dNTPs	200 μM	1
10 μM Forward primer	0.2 μM	1
10 μM Reverse primer	0.2 μM	1
10 U/μL NEB Taq pol.	0.025 U/μL	0.25
Template DNA	~0.25 – 2.5 ng/μL	2
Total		50



- ○ Run PCR
- ● Restriction digestions

SNP	PCR tube	RE tube
145	17μL →	1 μL 10 U/μL Fnu4HI 2 μL rCutSmart buffer
785	17μL →	1 μL 10 U/μL Fnu4HI 2 μL rCutSmart buffer
886	17μL →	1 μL 10 U/μL BccI 2 μL rCutSmart buffer



- ● 37°C for 20 minutes
- ○ Run DNA on TBE-polyacrylamide gels
- ○ Provide gel images to students for analysis
- ● Bitter food phenotype evaluation
- ● Rate foods from 1 (not bitter) to 5 (very bitter)

Bitter melon (苦瓜)	Coffee, black	Matcha (抹茶)	Tofu
Brussels sprouts	Corn	Oranges	Tonic water
Broccoli, raw	Eggplant	Peas	Turnip
Cabbage, raw	Grapefruit	Potato	Zucchini
Carrot	Green beans	Pu'er tea (普洱茶)	
Chocolate, dark	Kale	Red radish	

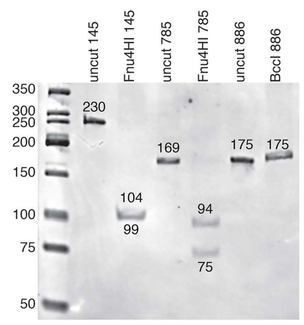
- ● Taster food phenotype evaluation

158-12V-100
165-144V-100
125-144V-100
196-12V-100
166-144V-100

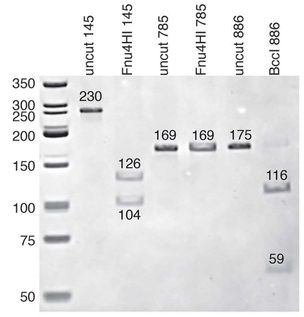
Precision laboratories

Results

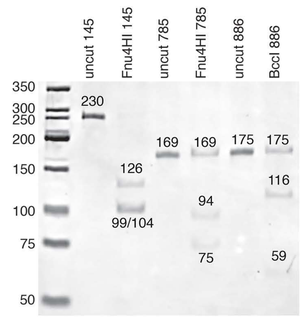
Taster (PAV/PAV)



Non-taster (AVI/AVI)



Taster (PAV/AVI)



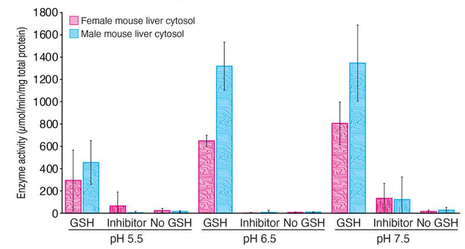
The effects of sex and pH on drug metabolism

This lab covers lab calculations, solution making, and drug metabolism and the factors affecting it such as sex and pH. Drug metabolism is known to be significantly different between men and women[5]. The cytosolic GSTs are a superfamily of enzymes that detoxify numerous endogenous and xenobiotic compounds[6]. Their activity can be determined by combining liver cytosol, buffer, a substrate, glutathione, running a kinetic assay, and then using the Beer-Lambert law to determine the concentration of the resulting metabolite[7-9].

Lab outline

- ● Quiz
- ○ Mini lecture
- ● Phosphate buffer preparation
- ● Calculations
- ● Adjust pH
- ● GST activity assay
- ● Dilute protein
- ● Prepare glutathione (GSH)
- ● Load plate with the substrate 1-chloro-2,4-dinitrobenzene, protein, buffer, and inhibitor
- ○ Incubate at 37°C, 2 min.
- ○ Add glutathione to each well
- ○ Read plate every 15 sec. for 5 min.
- ○ Data analysis

Both sex and pH affect GST metabolism



Key concepts

Beer-Lambert law	Calculations
Drug metabolism	Enzyme inhibition
Experimental controls	Henderson-Hasselbalch
pH adjustment	Sex and gender

Liposome extrusion for drug encapsulation

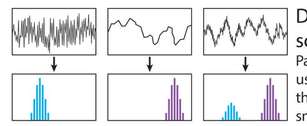
This lab occurs concurrently with a series of lectures on nanomedicine, liposomes being one of the most common nanoscale vehicles for drug delivery, and extrusion being a common method of production[10]. Here we produce liposomes using passive loading to encapsulate the fluorescent dye Nile Blue A. The encapsulation efficiency is measured by fluorescence, and particle size and uniformity evaluated by dynamic light scattering using the Malvern Instruments Zetasizer Nano ZS.

Lab outline

- ○ Thin film production
- ○ Hydrate thin film with 100 μg/mL Nile Blue A in 60°C sonicating water bath, 20 min.
- ● Quiz
- ○ Mini lecture
- ● Extrude liposomes at 60°C
- ○ 400 nm → 200 nm → 100 nm filters
- ● Purification and buffer exchange[11]
- ○ Encapsulation efficiency
- ● Lyse LNP with 1% Triton X-100
- ○ Nile blue standard curve
- ○ Read at Ex. 635 nm, Em. 680 nm
- ○ Measure particle size with Zetasizer

Extrusion apparatus

Lipid thin film: 55:45 molar ratio DSPC:cholesterol



Dynamic light scattering

Particles are sized based using Brownian motion, the more motion, the smaller the particles.

Key concepts

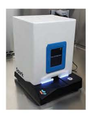
Comparisons	Dynamic light scattering
Extrusion	Hydration
Loading efficiency	Loading methods
Nanoparticle sizing	Standard curves

LNP-mediated gene knockdown by dsiRNA

Lab outline

- ○ Prepare 48-well plates of cells
- ○ Induce and treat cells (~72-96h)
- ● Quiz
- ○ Mini lecture
- ○ LNP-dsiRNA encapsulation
- ○ Encapsulation efficency via Ribogreen RNA quantification
- ○ Standard curve of dsiRNA
- ○ LNPs lysed with Triton X-100
- ○ Read at Ex. 485 nm Em. 528
- ○ Measure particle size with Zetasizer
- ● Examination of GFP knockdown
- ○ Relative knockdown quantification
- ○ Harvest cells
- ○ Lyse cells with Triton X-100
- ○ Read plate at Ex. 475 nm Em. 520

Spark NanoAssembler



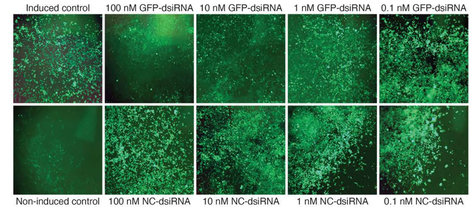
Liposomes made using a mixture of neutral, ionizable cationic lipids, cholesterol, and other modified lipids are used as carriers for RNA and are commonly produced using microfluidic chips[12]. We use the Spark NanoAssembler to produce LNPs encapsulating GFP-DsiRNA to silence GFP expression in a human cell line.

Spark microfluidic chip

Key concepts

Cell culture	Fluorescence microscopy
Gene knockdown	Microfluidics LNP production
Nanoparticle sizing	RNA quantification

DsiRNA-LNPs knockdown GFP expression



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